PROCEEDINGS

ISTF2019

INTERNATIONAL SYMPOSIUM ON TROPICAL FRUITS

24-26 September 2019
Liberty Central, Saigon Riverside Hotel
Ho Chi Minh City, Vietnam
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Organizers:
International Tropical Fruits Network (TFNet)
Ministry of Agriculture and Rural Development (MARD), Vietnam

Partners:
Fruit and Vegetable Research Institute (FAVRI), Vietnam
Southern Horticultural Research Institute (SOFRI), Vietnam

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

The 2019 International Symposium on Tropical Fruits (ISTF 2019) was held at the Liberty Central Saigon Riverside Hotel, Ho Chi Minh City, Vietnam on 24-26 September, organized by the International Tropical Fruits Network (TFNet) and the Ministry of Agriculture and Rural Development (MARD) with the support of the Fruit and Vegetable Research Institute (FAVRI) and the Southern Horticultural Research Institute (SOFRI).

Eighty participants from 11 countries attended the symposium, including Australia, China, Fiji, Germany, Indonesia, India, Malaysia, Philippines, Sri Lanka, Thailand, and Vietnam. Participants represented various stakeholders such as smallholder farmers, international organizations, government agencies, academic and research institutions, fruit experts, and the private sector.

Bearing the theme "*Recent advances and best practices to improve productivity and enhance market access for tropical fruits*," ISTF 2019 discussed the latest scientific research, technology developments, and product innovations. Moreover, the Symposium tackled how latest research can translate into practical solutions encompassing improvement of market access especially for smallholders, increasing marketing opportunities, development of supportive policies, and other economic improvements.

Twenty-five oral presentations were delivered during the two-day symposium while nine poster presentations were displayed in the exhibition area. Presentations were structured under the following themes: 1) Focus and policies on tropical fruit development; 2) Biotechnology and breeding; 3) Pests and diseases management; 4) Farm practices and recent developments to improve productivity; and 5) Markets and trade.

Two keynotes were presented by experts from the Food and Agriculture Organization (FAO) of UN and from the industry. These papers provided great oversight to the trends influencing the fruit industry including the Fourth Industrial Revolution (IR 4.0) and the opportunities that are available for industry players to tap.

The first session saw five papers delivered on the various policy directions and initiatives that are being taken by nations around the world including China, Fiji and Indonesia. Success stories and case studies were discussed as illustrations to the success and failures of policies that have been put in place to spur the tropical fruit industry. Also discussed were the implications of these policies on important beneficiaries such as smallholders, and the critical need for inclusiveness in the value chain.

Practical research is integral to the success of field initiatives. Session two involved two researchers speaking on the importance of biotechnology in breeding initiatives and also control of pest and diseases which is a major impeding factor in the productivity of tropical fruits.

To highlight the issue of pest and disease management further, three speakers in session three dwelled on the various aspects of P&D management including successful introduction of technologies which have benefitted farmers and the benefits of integrated pest management.

Session four's focus on farm practices and recent developments to improve productivity had eight papers presented. The rich knowledge shared during this session is indication that active

research is being undertaken by researchers towards productivity improvement in tropical fruits. It is also evident of the need to translate research outputs and recommendations into practical solutions for the benefit of farmers.

Session five dealt on the aspect of markets and trade. Presenters in this session who ranged from industry players to researchers highlighted the breakthrough achieved in the marketing of their commodities (i.e. the Acacha in Australia) and cautioned on the challenges that are often faced, especially in gaining market access for tropical fruits.

A final session was devoted to the briefing on the newly established Dragon fruit Network of the Food and Fertilizer Technology Center (FFTC) which is hoped to encourage knowledge exchange and greater partnership towards developing the emerging dragon fruit industry in Southeast Asia.

A panel discussion to deliberate on the readiness on the tropical fruit industry in embracing emerging technologies and adapting to disruptions created by the onset of IR 4.0 produced important recommendations. Among these recommendations include the importance of accessing technologies at a cost-effective manner and the need for an inclusive and supportive policy framework to boost the uptake and adoption of emerging technologies.

The intellectual discourse and wealth of information arising from the presentations of ISTF 2019 signals the need for 'a change in game plan' for industry players to remain relevant in the future. As disruptions continue to bombard the supply chain, all actors have to stand prepared and be able to adjust, adapt and proactively explore opportunities that await for positive growth to take place.

LIST OF ABBREVIATIONS

a.i. - active ingredient
AEZ - agroecological zone
AFP - Achacha Fruit Plantations
AIAT - Agency of Implementation Agricultural Technology
AMF - arbuscular mycorrhizal fungi
ASEAN - Association of Southeast Asian Nations
c.d. - canopy diameter
CS_2 - carbon disulphide
CTV - *C. tristeza* virus
CVPD - citrus vein phloem degeneration
DAP - Diammonium phosphate
DEG - differentially expressed genes
DGGE - denaturing gradient gel electrophoresis
DGH - Directorate General Horticulture
EBDC - ethylene bis-dithiocarbamates
EMS - ethyl methane sulfonate
FAO - Food and Agriculture Organization of the United Nations
FOC - *Fusarium oxysporium* cubense
FMCG - fast moving consumer goods
FRAP - Ferric Reducing/Antioxidant Power Assay
GA - gibberellin
GAP - good agricultural practices
GC - Gas Chromatograph
GCC - Gulf Cooperation Council
GDP - Gross Domestic Product
HLB - Huanglongbing
IAARD - Indonesian Agency for Agricultural Research and Development
IAT - Industri Asas Tani
ICSFRI - Indonesian Citrus and Subtropical Fruit Research Institute
IPM - integrated pest management
ISR - induced systemic resistance
ITFRI - Indonesian Tropical Fruits Research Institute
JC - Japanese Citroen
KEGG - Kyoto Encyclopedia of Genes and Genomes
LD - lethal dose
MD - Mekong Delta
MARDI - Malaysian Agricultural Research and Development Institute
MEYP - mannitol egg yolk polymyxin agar
NBS - nucleotide binding site
NBS-LRR - nucleotide binding site and leucine rich repeat
NCDs - noncommunicable diseases
NSFC - National Natural Science Foundation of China
OC - organochlorine

OP - organophosphorus
OTU - operational taxonomic units
PEMBA - pyruvate egg yolk mannitol bromothymol blue agar
PBDB - papaya bacterial die back
PBZ - Paclobutrazol
PCR - polymerase chain reaction
PGPR - plant growth-promoting rhizobacteria
PICs - pacific island countries
QuEChERS - quick, easy, cheap, effective, rugged, and safe
RAPD - RANDOM AMPLIFIED POLYMORPHIC DNA
RGA - resistant gene analogues
RS - resistant starch
SP - synthetic pyrethroid
SOP - standard operating procedures
SOFRI - Southern Horticultural Research Institute
STG - shoot tip grafting
SWOT - strengths, weaknesses, opportunities, and threats
UCZ - Uniconazole
TEA - twin exit apparatus
TFC - total flavonoid content
TDX - 'Tuong da xanh'
TPC - Total phenolic content

ORGANIZING COMMITTEE

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   Regional Director, CABI-Southeast Asia

SUMMARY OF THE SYMPOSIUM

DAY 1
KEYNOTE 1

To open the symposium, Food and Agriculture Organization of the United Nations (FAO) economist, Ms. Sabine Altendorf delivered the first keynote titled "*The Outlook of the Global Tropical Fruit Industry: Current and Future Trends*." Altendorf laid out possible agriculture 4.0 solutions to the challenges faced by the tropical fruit industry. Within the context of her presentation, Altendorf pointed out the application of technologies such as precision farming, nanotechnology and controlled environment cultivation for complementing existing production strategies within resource-constrained environments. Altendorf recommended for the development of supportive policy frameworks to cater to the technical, institutional, infrastructural, and capacity development demands of new technologies. Improving the digital infrastructure also goes hand in hand with capacity development for smallholder farmers. Development of such policies should be inclusive to ensure smallholders are not left behind in participating effectively in changing business models.

KEYNOTE 2

The second keynote presenter Mr. Steve Nguyen Duc Phuong Nam, Managing Director of WOWTRACE provided a comprehensive overview on the role of blockchain technology in agricultural innovation. Tropical fruits, like other agricultural produce, undergo a complex journey before reaching the market. This journey, called a supply chain, comprises of all the actors, processes, and steps involved in bringing food from farm to table. With the internet age, consumers have become more discerning about the safety and quality of food consumed, simultaneously being concerned about issues such as food safety, organic certification, food quality, and fair trade. Distributed ledger technology (DLT) allows consumers to trace the product's journey along the supply chain. Food traceability increases trust in the quality of products and is an opportunity to reward the producers who employ good agricultural practices to cultivate their produce.

SESSION 1:
FOCUS AND POLICIES ON TROPICAL FRUIT DEVELOPMENT

Kickstarting the first technical session was Dr. Yi Ganjun, Vice President of the Guangdong Academy of Agricultural Sciences, China who highlighted the status and issues faced by the fruit industry in China. Providing useful statistics on the production of various major fruit commodities of importance, he then provided an overview on the many technologies and applications existing in China. He cited some challenges such as producing sufficient quantity for the masses and ensuring high quality of produce for the market, in addition to encountering diseases such as the panama disease for banana and the Citrus Huanglongbing (HLB). He also presented some of the latest research that have been undertaken by his team including gene silencing of Foc TR4 and the use of CRISPR/Cas9 genome editing for creating a gene modified semi-dwarf banana.

Mr. Howard Hall, Program Manager from the Australian Centre for International Agricultural

Research (ACIAR), Australia spoke on the challenges faced in the integration of smallholders into the fruit value chain. The growing dependence of global food systems on smallholders should not be overlooked. He emphasized on the need to strengthen the capacities of smallholder farmers for food production. Citing examples of case studies from countries such as Philippines and Vietnam, he highlighted on the various constraints that deter the adoption of introduced technologies from R&D by smallholders. He stressed that innovations, government initiatives and even provision of free physical infrastructures will not lead to improvement in the livelihood of farmers if no effort is undertaken to engage them with market-driven and private firm-led agrifood chains, citing collaboration and inclusivity as keys towards solving the various bottlenecks existing for smallholder inclusion.

Dr. Ellina Mansyah of the Indonesian Tropical Fruits Research Institute (ITFRI), Indonesia gave an overview of the seed production distribution program introduced by the government of Indonesia to counter the insufficiency in seed supply of tropical fruits for the domestic and export markets. She also brought to fore some of the major problems plaguing the development of tropical fruits, including irregular flowering and poor fruit retention. The problem of seasonality was also linked to disruption in marketing of fruits for export. Hence the Indonesian seed production and distribution program was introduced with 1,001,180 seedlings of various tropical fruits produced and distributed within a span of three years from from 2017 to 2019.

Mr. Shalendra Prasad of the Ministry of Agriculture presented on the policies and strategies that have been put into place to enhance the Fijian tropical fruits industry. Highlighting that the lack of policy interventions specifically targeting fruits over the years had created a gap in the development of this industry, recent polices have been developed to redirect efforts towards greater focus on production of local fruits via strategies such as nationwide awareness for increasing consumption of fruits, establishments of orchards and availing elite fruit cultivars.

Prof. I Made Supartha Utama from Udayana University, Bali, Indonesia spoke on the challenges faced by small-scale agricultural systems for tropical fruits, citing the pressures placed upon these systems from the influx of tourists and a recently released regulation. He compared traditional markets with modern markets such as supermarkets frequented by tourists which often require produce of higher value which are safe, healthy and cosmetically acceptable (intrinsic and extrinsic). With the recent introduction of a regulation from the government for more produce from traditional farmers, efforts have to also be done to help these traditional systems improve their production, for example by growing better varieties, obtaining certification and improving their infrastructure. The existing value chain has to be improved to enhance inclusivity of farmers to enable them to produce which are acceptable for 'modern' consumers.

SESSION 2: BIOTECHNOLOGY AND BREEDING

Ms. Valerie Suwanseree of Kasesart University, Thailand presented the importance of mutation breeding for increasing genetic diversity in mangosteen in Thailand, given that only one cultivar exists at present in the country. The experiments indicated that some of the seedlings from the treatments showed different morphological characteristics from standard mangosteen seedlings. However further confirmation is required after genetic testing.

Dr. Sukartini of the Indonesian Tropical Fruits Research Institute spoke at length on the study undertaken to evaluate the expression of Resistant Gene Analogues (RGAs) on *Foc* infected

banana plantlets. The results of this study indicated that there were potential RGAs as candidate genes that can be obtained from Indonesian wild Musa, namely *Musa acuminata* ssp. *halabanensis* and *Musa balbisiana* from Nusa Tenggara Timur. The findings of the study will contribute to the information on the molecular aspects of genes that control the mechanism of resistance to FOC (*Fusarium oxysporium cubense*) wilt disease which is currently still scarce.

SESSION 3:
PEST AND DISEASE MANAGEMENT

Leading this session was Dr. Mohamad Roff Mohd. Noor of the Malaysian Agricultural and Research Institute (MARDI) with his paper on induced systemic resistance (ISR) as a tool for the control of bacterial transmitted papaya dieback and banana blood diseases. The technique indicated promising success in curbing the papaya dieback disease and has been commercialized and made available for farmers. For banana however, studies are still ongoing to determine the efficacy of the technique.

Dr. Siti Zaharah Sakimin of the Putra University of Malaysia spoke on the potential control of TR4 in banana through the use of aqueous neem leaves and manipulation of PH levels of media under *in vitro* conditions. Promising results were attained from the experiments indicating potential field applications in the future.

The application of the integrated pest management packages for the production of longan was discussed by Dr. Tran Thi My Hanh from the Southern Horticultural Research Institute (SOFRI), Viet Nam who also evaluated on the benefits of IPM. Her study further revealed the role of IPM packages in significantly reducing the infestations of various pests on longan trees and called for its wide-scale application in longan growing areas in Vietnam.

SESSION 4:
FARM PRACTICES AND RECENT DEVELOPMENTS TO IMPROVE PRODUCTIVITY

The first paper in this session was by Ms. Huynh Le Anh Nhi from Can Tho University, Viet Nam who presented her study undertaken to determine the effects of KClO3 doses on the diversity of bacterial communities in the soil of E-Daw longan orchards. Her study confirms and highlights the important role of KClO3 in inducing flower on 'E-Daw' longan while indicating the significance of factors such as dosage of KClO3 and age of longan tree towards the flowering percentage of 'E-Daw' longan.

The potential of grass-proof cloth covering as an alternative to reduce chemical fertilizers and pesticides in citrus production was expounded by Dr. Zhong Yun of the Guangdong Academy of Agricultural Sciences (GDAAS), China. The anti-grass covering was found to improve soil hydrothermal conditions and the overall nutrient levels in citrus orchards.

The prevalent practice of the 'mop top' system in dragon fruit cultivation in Viet Nam has often been plagued with difficulties. As an alternative to this, a new system known as the 'tee bar' has been studied and carried out by the Southern Horticultural Research Institute (SOFRI). The success of this study was presented by its researcher, Mr. Nguyen Van Son.

Ms. Nirmala Devy of the Indonesian Citrus and Subtropical Fruits Research Institute discussed in

length on the implementation of the citrus shoot tip grafting (STG) technology which has been aimed at producing virus-free mother plants especially from the HLB, *C. Tristeza* virus (CTV) and the citrus vein enation virus (CVeV). Despite achieving a low success rate, millions of virus-free citrus plants from STG mother plants have been distributed to farmers in Indonesia.

As part of efforts in increasing the production of durians plants in Indonesia, the tissue culture technique has been often favoured to address production barriers. Dr. Ir. Rd. Selvy Handayani Malikussaleh University, Indonesia spoke on her research which investigated the effects of $HgCl_2$ concentrations and the type of leaf explants on the development of durian leaf explants.

Highlighting on texture and aroma as important factors that affect the taste and consumer preferences, Ms. Warangkana Bodinthanaphat, of Kasetsart University, Thailand presented the findings of her study on the volatile substances in green fruit of four papaya cultivars.

Dr. Gao Huijun, Guangdong Academy of Agricultural Sciences (GDAAS), China delivered the findings of her investigation on the influences of hydrolases and granule structure on starch degradation in banana. These findings would shed further light into the starch-degradation mechanism in two species of bananas - Cavendish and plantain.

Given that two commonly used flower bud initiation agents, namely PBZ and Thiourea have negative implications to the environment and human health, an alternative agent, uniconazole (UCZ) was studied by Prof. Tran Van Hau, Can Tho University, Vietnam. The findings of his study suggested that for 'Tuong da xanh' mango, UCZ can be a potential replacement for PBZ.

SESSION 5: MARKETS AND TRADE

The fifth session was opened by Mr. Bruce Hill, Chief Executive Officer of the Achacha Fruit Plantations (AFP) in Australia, with the presentation titled "*Achacha: The commercialization of a tropical fruit.*" Although achacha (*Garcinia humilis*) is native to Bolivia, AFP was the first company in the world to produce the fruit on a commercial scale. Currently, they own 16,000 mature trees across 120 ha that supply fruits to markets in Australia, the EU, Canada, Middle East, and South Asia. Mr. Hill showed how the seedlings are grown in their nursery, their orchard layout, and how the fruits are harvested, graded, and packaged before shipping. While the fruit is mainly consumed fresh, its sweet, tangy, and refreshing pulp has been developed into value added products such as marmalades, juices, and alcoholic beverages. Mr. Hill concludes that while launching a new product is challenging, it has been a rewarding experience due to achacha being good, healthy, and useful.

The second presentation was "*Indonesia mango development: A research and development perspective to capture the opportunities and face the challenges in global trade era*" by Ms. Puspitasari from the Indonesian Center for Horticultural Research and Development (ICHORD). In 2016, Indonesia is the fifth largest producer of mango in the world but exports have been abysmal. The country only exported 473k tonnes, roughly 0.03% of global exports. ICHORD has been tasked to strengthen Indonesia's mango exports through their programs such as germplasm development, varietal improvement, distribution of free certified mango seedlings, off-season technology, pest and disease management, postharvest technology, mango processing, and exploration of other native *Mangifera* species. In addition to this, a Good Agricultural Practice (GAP) information system was developed to facilitate the collaboration among red mango growers, farmer groups, traders, exporters, and local governments.

The final presentation was delivered by Ms. Aimi Athirah Ahmad from the Malaysian Agricultural Research and Development Institute (MARDI) entitled "*Challenges and opportunities of exporting Malaysian tropical fruits to Gulf Cooperation Council States (GCC) market.*" While GCC markets are among the top priority of Malaysian food exports, the volume of exported fruits are still low compared to Singapore and China due to production and marketing constraints. The high cost of air freight to the GCC countries, coupled with limited supplies to fill capacity in sea freight affect the competitiveness of Malaysian fruit exports. This constraint can be reduced if there are some form of incentives by the government such as tax exemption for exporters.

SPECIAL SESSION

This session was devoted to the introduction provided by Ms. Keziah Wei Hsin-Ho of the Food and Fertilizer Technology Center (FFTC) on the Dragon Fruit Network (DFNet). The formation of the network, its vision, focus and goals were elaborated for the benefit of those present. The network is hoped to form a cross-boundary platform for future collaborative ventures in research and information sharing, towards the development of dragon fruit as a major fruit commodity in Southeast Asia.

OPENING CEREMONY

H.E. MOHD. SALLEHHUDDIN HASSAN
Chairman of TFNet's Board of Trustees,
Secretary General, Ministry of Agriculture and Agro-based Industry, Malaysia

Welcome to the International Symposium on Tropical Fruits (ISTF) 2019!

We stand at a time where we are witnessing great changes to how businesses are operated and revolutionized. Global economies are embracing change brought upon by the Fourth Industrial Revolution (IR 4.0). Digitalization has also begun to revolutionize the agriculture industry. The modern day consumer base demands for authenticity, transparency and traceability of the food or products they consume.

The tropical fruit industry is an industry which provides great opportunities for income generation and food security. Stakeholders of the tropical fruit industry need to catch up with the tremendous pace of change brought by the revolution that is taking place, especially when faced with the daunting task of feeding a nearly 10 billion people by 2050, or risked being left behind.

Despite the global economic slowdown, production of tropical fruits continues to increase around the world – in tandem with the growing demand and population growth. The industry however is still fraught with issues which demand immediate solutions and strategic actions. Tropical fruit producing countries should focus towards raising the competitiveness of produce and products and increase their technological capabilities to ensure the industry is IR 4.0-ready. There is also an urgent need to address skill gaps and skill mismatches existing in the industry.

As the host of the International Tropical Fruits Network (TFNet) - the one and only global network on tropical fruits, Malaysia is committed towards supporting international endeavours in developing the tropical fruits industry. The annual symposium on tropical fruits is an important event organized by the TFNet as part of its efforts to improve the understanding on prevailing issues while seeking evidenced-based solution; and this year's symposium theme 'Recent advances and best practices to improve productivity and enhance market access for tropical fruits' is a step in the right direction towards this. TFNet strives to ensure that collective inputs gained from events such as these are streamed towards influencing policies and directing concerted strategies that will benefit all players of the industry especially smallholders.

This year, we are thankful to the Vietnam government, especially the Ministry of Agriculture and Rural Development (MARD) and its agencies FAVRI and SOFRI for extending tremendous support in ensuring the success of this symposium.

I am certain that the deliberations of this symposium will create new knowledge and foresight for the tropical fruit industry to align its outlook for facing the future.

Wishing you a fruitful Symposium!

OPENING ADDRESS

HIS EXCELLENCY ASSOC. PROF. DR. LE QUOC DOANH
Vice Minister, Ministry of Agriculture and Rural Development (MARD), Vietnam

I wish to welcome all presenters and participants to the International Symposium on Tropical Fruits 2019 bearing the theme "Recent advances and best practices to improve productivity and enhance market access for Tropical fruits" in this lovely city of Ho Chi Minh. We are indeed honored to be the host and co-organizers of this very important symposium. I am happy that there are more than 100 participants from 15 countries attending the symposium.

Agriculture is a main activity in Vietnam with about 29 percent of total land area developed for this purpose. Besides rice which is the main export crop, there are some other crops used for export such as: fruit, coffee, cashew nuts, pepper, rubber, tea, etc. With its extensive range of tropical and subtropical fruits, the total land area for fruits in Vietnam is estimated at 989.30 thousands ha, which makes it a major fruit producer in Asia. The main fruits grown in Vietnam are citrus, banana, pineapple, mango, litchi, longan, pitaya, guava, avocado, rambutan, durian, mangosteen and sapote.

The Mekong River Delta is a major producing area of tropical fruits with a cultivated area of about 347,000 ha. The concentrated planting areas with large planting areas are followed by the Eastern provinces of the Southern region and Northern mountainous provinces. Most of the fruits produced are for the domestic market, and the rest is for export. The Government through the Ministry of Agriculture and Rural Development is now looking at avenues to increase exports of tropical fruits, through programs including improving quality and post-harvest management. Fruit crops were selected as one of the crops to be selected to replace low economic efficiency crops in the government-approved agricultural production restructuring scheme. Currently Vietnam exports pitaya, banana, mango, rambutan, durian and mangosteen to China, the European countries and other markets.

The Ministry of Agriculture and Rural Development has given much emphasis to develop tropical fruits in Vietnam through our research institutions such as the Fruit and Vegetable Research Institute (FAVRI) and the Southern Horticultural Research Institute (SOFRI), in addition to effective capacity development programs with the focus of producing high yielding, marketable good quality fruits. The Vietnam standards for Good Agricultural Practices (VietGAP) for fruits has been established and currently is being implemented in fruit growing areas. In recent years, there has been an increase in turnover of fruit exporting, with Vietnam becoming a major producer and exporter of tropical fruits in the region.

We are very grateful to the International Tropical Fruits Network (TFNet) for choosing Vietnam to host this significant symposium. With the focus on recent advances and best practices towards improving productivity and enhancing market access, the theme of ISTF 2019 is very relevant to identify and address the existing knowledge and technological gaps prevalent within the tropical fruit sector. I am certain that ISTF 2019 will provide a platform for TFNet, its partners and other industry stakeholders to discuss the contributions of research, the private sector, and policy makers on tropical fruit production and fruit security.

On behalf of the Ministry of Agriculture and Rural Development of Vietnam, I would like to wish all participants a productive and engaging symposium.

To our foreign colleagues, have a pleasant and enjoyable stay in Vietnam.

THE OUTLOOK OF THE GLOBAL TROPICAL FRUIT INDUSTRY: CURRENT AND FUTURE TRENDS

Sabine Altendorf

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EXTENDED ABSTRACT

Bananas and, particularly, tropical fruits continue to be among the fastest growing agricultural commodities. These fruits constitute a significant source of economic growth, income, food security and nutrition for the rural sectors of many developing countries. While production and consumption data for these commodities are subject to underestimation owing to extensive unreported cultivation on small household plots, the available information nevertheless indicates that their importance in global food supply has increased significantly in recent decades. This has equally been confirmed by the fast expansion in global trade flows, which reached a total of 19 million tonnes for bananas in 2018, and 7 million tonnes for the four major tropical fruits – mango, pineapple, avocado and papaya – combined.

High income growth in developing countries and a changing health perception in developed countries have underpinned these positive developments in global demand for bananas and tropical fruits, and further point to a highly favorable outlook over the medium-term. Ten-year baseline projections generated in May 2019 indicate that global aggregate trade in bananas and tropical fruits could reach close to 30 million tonnes by 2028. While this would constitute a slightly slower growth rate than seen over the previous decade – primarily on account of the near saturation levels observed in global banana trade – ample market opportunities for tropical fruits seem to be on the horizon. Particularly the producing countries of Asia, which are endowed with a large variety of highly valuable major and minor tropical fruits and, in aggregate, account for more than half the world's global supplies of bananas and tropical fruits, stand to benefit from this positive outlook.

Amidst such auspicious prospects, as the industry grows in value, already stressed natural resources will face additional pressure from climate change and faster spreading plant diseases, which threaten to reduce productivity in agriculture and global food supplies. Rapid advancements in intelligent production methods and information technologies, and their meaningful application and adaptation to the needs of smallholder farmers, will be required to meet evolving world nutrition requirements and sustain efficiency gains in production and supply chains. On the value side, the application of blockchain and distributed ledger technologies promises to improve value chains by providing transparency and traceability, thereby providing scope for increased margins and a more equitable distribution of price premia. With such technological innovations still in early implementation stages, key to their success in the agriculture sector will be the correct identification of efficacious production and marketing strategies, particularly with regard to the highly valuable and highly perishable bananas and tropical fruits.

Keywords: tropical fruits, demand, projections, trade, climate change, agriculture 4.0, blockchain

REAL-WORLD BLOCKCHAIN APPLICATIONS AND THE FUTURE OF SUPPLY CHAIN TRACEABILITY IN VIETNAM

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EXTENDED ABSTRACT

With the rapid development of blockchain in recent years, it is being demystified to most of the people across the globe. When the heat of crypto-currencies cool down, governments and enterprises pay more attention to the other applications of blockchain technologies. By making use of the decentralized architects and data immutability, more and more applications are being developed to resolve the real-world problems, including ID management, voting, food safety protection, supply-chain traceability, and so on.

In the 1986-2017 period, Vietnam achieved an average growth rate of 6.63% per year and is on track to reach over 6.7% in 2018, becoming one of the fastest growing economies in the world. Hence, to build up the sustainable supply-chain system in Vietnam, modernized technologies like warehouse management systems, robotic vision systems, supply chain planning, supply chain visibility, and more are required to apply. Combining the growing trend of traceability solutions and the significant demand from Vietnam supply-chain industry, we would suggest 'Blockchain' as one of the possible solutions that may enhance the data integrity, operation transparency, and overall traceability is to promote the technology to suppliers, distributors and exporters of fruits in Vietnam.

Keywords: blockchain, Viet Nam, supply-chain

I. INTRODUCTION : BACKGROUND AND POTENTIAL OF VIETNAM AGRICULTURE MARKET

In 2018, Vietnamese agricultural products set a record when bringing in USD 40 billion from exports. Currently, Vietnam's agricultural products are ranked 15th in the world among 150 countries. The main markets are China (accounting for 22% of Vietnam's total agricultural export market share), the US (17.9%), Japan (19.1%), Association of Southeast Asian Nations (ASEAN) (10.64%) and South Korea (6.9%). At the same time, Vietnam is also participating in many free trade agreements such as the Comprehensive and Progressive Agreement for Trans-Pacific Partnership, European Union Vietnam Free Trade Agreement, Eurasian Economic Union-Vietnam Free Trade Agreement, and European Union-Vietnam Investment Protection Agreement, which open up more opportunities for exporting Vietnamese agricultural products and achieve the goal of bringing in USD 43 billion from agricultural exports in 2019.

However, to achieve that goal, Vietnamese agricultural products face many challenges. The first is the assurance of quality, food safety, and import standards of the market, especially difficult markets like Europe, US, and Japan. These markets require very high quality, uniform, hygienic, and safe agricultural products. On the other hand, issues related to postharvest management along the supply chain, such as packaging, transport, storage, distribution, and export can also

cause deterioration in the quality of goods when it reaches the consumer market. The next challenge is to build a brand for Vietnamese agricultural products for the international market, and the issue of informing consumers of the origin of the products, for instance whether they are genuinely produced or sourced from Vietnam. Therefore there is a strong reason as to why traceability solutions are important for the agricultural industry to ensure sustainability of markets for quality produces.

2. WHY SUPPLY CHAINS NEED TRACEABILITY

Having a traceability system is not only helpful in tracing back the information, but it also brings additional value to businesses. Firstly, it helps organizations better manage risks. Through seamless data records from actual events, the supply chain manager can promptly know the current status of the moving or producing of products and anticipate potential risks which may occur and make right decisions to prevent it. Secondly, all the recorded data on consumer behaviour, in particular about how they buy products, how much, and when they buy will provide a better basis for forecasting and demand planning, thus, makes the demand forecast closer to actual demand. Another benefit of having a traceability system is cost saving as uninterrupted information flow helps to eliminate waste of production, material loss, and other opportunity costs. Hence, ethical traceability also reduces product callback costs or warranty costs by maintaining product quality and quickly implementing the returning process.

3. WOWTRACE TRACEABILITY SYSTEM AND SUPPLY CHAIN

In Vietnam, a start-up named Chain Vision has developed a blockchain-based traceability Blockchain as a Service system called WOWTRACE to enable end to end traceability in the agrifood supply chain. WOWTRACE helps businesses manage supply-chains with instant, transparent, and immutable data. It is a network that allows producers, distributors, retailers, and consumers to retrieve product information reliably and securely through blockchain technology.

Having a transparent traceability brings businesses many advantages not only in supply chain operations but also in marketing and brand building. Firstly, it provides uncorrupted and trusted data flow because of the immutable characteristics of blockchain; all stakeholders can quickly identify the root of issues when it happens and use the data for efficiency improvements. On the other hand, if consumers can access to this transparent information, they will trust and be loyal to a brand. It is a golden asset and strong brand protector in the aspect of marketing.

4. BLOCKCHAIN TECHNOLOGY

Blockchain, which is a technology for storing and handling information, is an ideal platform to perfect traceability. However, people generally mistakenly equate this term to Bitcoin, which is just an application of blockchain. Plainly explained, a blockchain is a time-stamped series of immutable records of data that is managed by a managed by a cluster of linked computers. Each of these blocks of data is secured and bound to each other using cryptographic principles. Therefore, there is no central server to execute data recording. Since it is a shared and immutable ledger, the information in it is open for anyone and everyone to see. Hence, anything that is built on the blockchain is by its very nature transparent, and everyone involved is accountable for their actions.

Unlike the centralized system, blockchain is a decentralized system, which has no central place

for data handling. In other words, blockchain is more secure, more transparent, and immutable. Once the data is updated on blockchain, it will be recorded with the exact time of updating called time-stamped, and it is unchangeable. Everyone can access to blockchain to retrieve the data easily and read the history of information updating. This makes blockchain data trustworthy and solves the weaknesses of current centralized Traceability systems.

5. CASE STUDY:

Blockchain technology traceability system has been tested and implemented in selected food production systems in Vietnam.

This paper discusses its application in 4 case studies:

- Case study of mango
- Case study of chocolate
- Case of organic vegetable
- Case of melon

CHALLENGES IN SMALL HOLDER INTEGRATION INTO THE FRUIT VALUE CHAIN

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EXTENDED ABSTRACT

An important and compelling conversation is underway about how to engage smallholder farmers in established agrifood chains. More than half a billion farmers with less than 2 hectares of land to farm are producing 80% of the food that is feeding today's world population.

The importance and timeliness of this conversation should not be undervalued. Many sources project that the world's agricultural systems will need to produce almost 60% more food to feed nearly 10 billion people, by 2050.

The challenges of engaging smallholders in tropical fruit chains are many. Public and private research and development efforts are significant, but smallholders' key constraints and the types of research and communication needed to reach them still appear unclear.

Many agencies involved in international development (including research for development) are vigorously discussing private sector engagement and inclusion, yet this discussion is rarely effectively converted to action. When a group of smallholders informs researchers that an innovation, that research identified and introduced them to, is the best way for them to improve their incomes and their livelihoods, but nevertheless stop using the innovation when the project stops, what message should it send to researchers? That there is unfinished research, another project, or finding new "methods of scaling up".

Smallholder farmers are private sector players. In almost every case, they are the smallest private entity amongst the many that they transact with to operate their farm, their business, and deliver benefits to their families: often the "minnow amongst the whales". The research community and governments, neither of which are directly engaged in the linkages to markets and to key inputs, are the only non-private sector parties in the agrifood system.

It is unlikely that adopting innovations developed through research, government initiatives like Association of Southeast Asian Nations (ASEAN) or country specific Good Agricultural Practice (GAP) training, or provision of physical infrastructure at no cost can truly improve the livelihoods of smallholder farmers, without improving their engagement with, and contribution to, market-driven and private firm led agrifood chains.

For chain captains and other downstream commercial firms, this challenge is not a research question. It is an essential part of the future, of adapting chains for improved delivery of: more volume, better quality, consistency, lower residues, traceability, lower waste and through-chain

losses, fulfillment to retailers (in-full, on-time, every-time), avoiding stock-out penalties or reputational risks, promises to consumers, and future commercial success.

Private firms including (in particular) the many local and near-farm firms, researchers, (government and private) extension / grower services / sustainability teams, governments and policy makers and men and women smallholders (micro-businesses) have an imperative to learn how to work collaboratively and inclusively (inclusiveness is not singularly about gender). To collectively identify and solve bottlenecks that are inhibiting smallholder adoption of change, for mutual benefit.

Recent supporting field experiences are shared to illustrate bottlenecks that are to be found in many areas including and not limited to: sources of finance for smallholders and chain participants, information flows and transparency, policy and regulation, understanding risks and causes of risk aversion from the smallholders' perspective, communications, private and public infrastructure (e.g. irrigation, near-farm processing, storage, roads, access to plots), collective thinking for mutual benefit, listening and asking questions.

Examples from Fast Moving Consumer Goods (FMCG) are used to discuss and understand scenarios and issues from the smallholders' point of view so as to effectively drive change for mutual gain. Some are investing heavily on farmers' education and sustainability programs with varying degrees of success.

Keywords: smallholder integration, communication, collaboration, agrifood chains

ACCELERATING INDONESIAN TROPICAL FRUITS DEVELOPMENT THROUGH SEED PRODUCTION AND DISTRIBUTION PROGRAM

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ABSTRACT

Indonesia has great potential as a major producer of tropical fruits in the region. The strengths are in genetic resources, sufficient land and labor, different harvest season among regions, and good market potential. The high population number and increasing of public awareness to consume more fruit has enhanced the demand of tropical fruits with annual increases. Until now tropical fruit supply is not sufficient for both the domestic and export markets. The main problems are caused by scattered production areas among the islands and the limited number of large-scale professionally managed fruit orchards. To overcome this problem it is necessary to develop and establish large production areas of commercial scale fruit plantations. To achieve this purpose large number of fruits seedlings are needed as planting materials. In order to accelerate the development of tropical fruits, the Indonesian government has launched a seed production distribution program throughout Indonesia. Fruit seedlings are produced in collaboration with seed growers under the supervision of the Ministry of Agriculture and seed certification institutions. The seedlings are distributed to prospective farmer groups and communities based on wide area development. Before planting the seedlings, the farmer groups were given advise and training on cultivation technology by the local government and private sector. Until 2019 about 1,000,000 seedlings consisting of mango, mangosteen, durian, papaya, banana, salacca, and breadfruit have been produced and distributed to suitable areas. The number of seedlings distributed is equivalent to 2,899 hectares of new areas. With the assumption that 50% of the seedlings will be successful, it is anticipated that in the next five years the production area will increase to about 1,450 ha and contribute to domestic and export markets.

Keywords: seedlings, production, distribution, tropical fruits, development

1. INTRODUCTION

Indonesia has great potential as a producer of tropical fruits. The strengths lies in the diversity of genetic resources, sufficient land labor for extension area, different harvest season between regions, and good market potential. There are approximately 392 fruit species found in Indonesia and only a few have been cultivated. Most of them are still growing wild in the forest (Soedjito & Uji, 1987). Another study reported that there are 266 species of indigenous fruits encountered in Indonesia and 62 species of them are cultivated. Four genera of indigenous fruits are recommended for development in Indonesia, *i.e. Durio*, *Mangifera*, *Garcinia,* and *Nephelium*. Duku (*Lansium domesticum*), salak (*Salacca zalacca*), and matoa (*Pometia pinnata*) have good

prospects to be developed in Indonesia (Uji, 2007).

Tropical fruit trees generally fruit once a year with a different harvest season based on the agroecological zone (AEZ). Indonesia has an area along 5,000,000 km from 95° to 141° East with varying AEZ. This condition gives the advantage for fruit production and availability throughout the year, including their availlability for international market. Field observations on durian show that the average durian harvest period is around 8 months each year. In some places, such as in North Sumatra and Riau province, the local durian fruits all time. These scenarios show- that durian can be produced throughout the year (Santoso, 2012).

Another strength on the fruit business in Indonesia is the adequacy of labor resources. The number of workers in February 2018 was 133,940,000 people with a participation rate about 69.20 percent (BPS, 2018). Among these workers as many as 38,875,389 people (30%) workin the agricultural sector (Sustanti & Waryanto, 2018). The Ministry of Agriculture has been supporting the initiative to increase the interest of young people in agriculture business through the "millennial farmers program". This is in accordance with the government policy in 2019 which was named the year of human resources. The goal is to build competent farmers in the future by targeting one million millennial farmers from all over Indonesia. Another program to be developed is the competency certification program in the field of expertise in the agricultural sector through professional training (Humas Kementan, 2019).

The demand for high quality tropical fruits is increasing year by year. About 90% of Indonesian fruits production is consumed domestically and 10% exported to other countries. This situation indicates high potential for both domestic and export markets. One of the biggest opportunities is the export of tropical fruits to Singapore with a capacity about 1,000 mton per day. So far, Indonesian fruit exports to Singapore is still around 6%. Papaya, banana, and avocado have great opportunity to be exported to Singapore. The objectives of Indonesian tropical fruits development are to increase quality, quantity, and continuity to fulfill domestic demand and to increase export volume.

2. PRESENT SITUATION OF FRUIT CULTIVATION IN INDONESIA

Tropical fruits are grown in the entire Indonesian islands. The availability of sufficient land provides a great opportunity in the development of fruit commodities. There are approximately 12,016,778 hectares (32.36%) unused land in Indonesia and part of them are suitable for planting tropical fruits. Most of the land available are in Sumatera and Kalimantan islands. In 2018, the total planting area for tropical and subtropical fruit accounted was about 704,860 ha, with total production 19,643,616 mton. The largest harvested area is mango (201,080 ha), followed by banana (89,615 ha), durian (63,533 ha), citrus (51,811 ha), and pineapple (20,785). The highest production commodity is banana (7,162,678 mton), followed by mango (203,789 mton), citrus (2,165,184 mton), and pineapple (1,795,982 mton) (Sustanti & Waryanto, 2018).

The most commonly consumed tropical fruits in Indonesia are banana (about 9,907 kg/capita/year), papaya (5,319 kg/capita/year), citrus (3,494 kg/capita/year), and salak (2,346 kg/capita/year). Demand for tropical fruits is increasing rapidly both for domestic and export market. The demand for tropical fruits by consumers in urban areas is very large due to lifestyle changes related to awareness in good health practices, including reducing carbohydrate consumption. According to the export data, pineapple, banana, and mangosteen are the most popular exported tropical fruits with export volume 210,045 mton, 18,177 mton, and 9,167 mton respectively. Fruit industry experts estimate about 80% of tropical fruits are consumed fresh rather than processed

or canned, with the exception for pineapple, citrus, and mango (Sustanti & Waryanto, 2018).

Generally, all Indonesian regions are suitable for tropical fruits plantation. But there are some exceptions for certain fruits such as mango which are more suitable in the dry lowlands. About 75% of Indonesian mango are produced in Java island with the largest area in East Java. The largest banana production comes from West Java and East Java, which covers about 45% of total banana production areas. Lampung contributes to 20% of banana production and the remaining 35% are grown all over Indonesia. Mangosteen is generally found more in Java, West Sumatera, North and South Sumatera. Durians are evenly scattered throughout Indonesia,with the largest production area, approximately 27%, in East Java.

3. PROBLEM IN DEVELOPMENT OF TROPICAL FRUITS

The major problem in the supply of tropical fruits is irregular flowering and poor fruit retention, while alternate bearings and small fruit sizes reduce grower returns in some districts. Trees take three to five years to come into production, and will not produce substantial crops until six or eight years. The problem with Indonesian fruit exports is quantity, quality and continuity. Indonesian exports are still dependent on the fruit season. One strategy to become an exporter of tropical fruit in the world is to develop tropical fruits such as durian, mangosteen, banana, salaca, and mango throughout Indonesia for year-long production of fruits. Development of large or industrial-scale fruit commodity is needed to make crop management, business process, application of technology, and marketing easier.

The president of the Republic of Indonesia declared 2018 as the year of "national horticulture seed production". This is in accordance with the efforts to accelerate the achievement of food self-sufficiency and to realize Indonesia as a global food source by 2045. For Indonesia to become the largest exporter in Southeast Asia in 2025 and in the world in 2045, the targets such as improving quality, quantity, and sustainability of tropical fruits have to be met. This program is part of the agenda of the 'Orange Revolution', a national program for tropical fruits production. This activities are to support the Indonesian Ministry of Agriculture program. In 2017, 971,000 tropical fruit seedlings were produced, consisting of mango, mangosteen, durian, banana, salacca, papaya, and breadfruit to distribute to farmers and growers (Mansyah *et al.*, 2017).

One of the problems in large scale fruit crop development is the limited supply of plant materials (seedlings) to be distributed, due to: 1) Limited number of parent trees as a source of seedlings; 2) Slow growth of seedlings; 3) Limited propagation techniques; and 4) Seed availability is dependent on seasonality of fruits (Mansyah *et al*, 2017).

4. ACCELERATION PROGRAM OF TROPICAL FRUITS DEVELOPMENT

The first step in tropical fruits development areas is to provide quality seedlings for increased fruit production. The seedlings are produced vegetatively by government institutions, in collaboration with seed growers, suppliers, and the seed certification institution. The seedlings distributed to prospective farmers and locations are based on regional development. Before distribution, the prospective farmers were assisted in using modern agricultural techniques to improve productivity by the private sector.

The seedling production process consists of vegetative or clonal propagation for woody fruit plants and generative propagation for apomixis plant such as mangosteen. Propagation of

banana is done through *in vitro* culture. Papaya propagation was also done through seeds. The seedlings produced could be divided into two classes: stock seedlings and extension seedlings. The stock seedlings are used as sources of planting materials for the next propagation while the extension seedlings are distributed to users. The production process of woody tropical fruits including preparation of rootstocks and scion, grafting, and certification or labeling.

4.1. Production of durian and mango seedlings

The seedlings of woody fruit plants such as durian and mango are produced by grafting between the rootstock and the scion of recommended varieties. Recomended durian varieties are 'Pelangi', 'Matahari', 'Sitokong', 'Kromo', 'Salisun', 'Tembago', and 'Sijapang'. Stock plant varieties for mango are 'Madu', 'Wajik', 'Agung', and 'Lalijiwo' while the grafted varieties are 'Garifta', 'Agrigardina45', 'Arumanis', and 'Gadung21'. (Rebin, 2017)

The scions are maintained as duplicate parent trees and are established in foundation blocks. The next step is growing the seedlings in multiplicaton block of scion as a sources of scions for extension seedlings production. Each stage of seedlings production, start from rootstock preparation, scion selection, grafting, and labeling is tightly controlled to produce true to type seedlings. Labeling is done when the plants are ready to be distributed to consumers, about 5-6 months after grafting, with a minimum height of 50 cm. Labeling process is done in collaboration with the seed certification institution.

4.2. Banana seedling production

Plant materials for mother plants (stock seeds) production are suckers in order to prevent off-type plants in *in vitro* culture propagation. The method used is conventionally by optimizing the number of shoots by peeling all the midribs and stop the growth of the apical meristem. Extension seedlings are propagated by mass propagation through *in vitro* culture. To ensure the seedlings produced are free from viruses, indexing from banana bunchy top virus (BBTV) is much recommended. BBTV virus detection or BBTV indexing is done by polymerase chain reaction (PCR) method using the leaves from suckers of prospective plants.

Extension banana seedlings are the seedlings that are directly distributed to farmers and are propagated through tissue culture. If tissue culture facilities are not available the propagation is done by modification of conventional propagation technology (conventionally optimized). The principle of this method is to remove the growing point (apical meristem) to stimulate axillary growth, and remove all the midribs to reduce the obstacle of the axillary buds growing. Labeling is carried out in collaboration with the seed certification institution.

4.3. Mangosteen seedlings propagation

Mangosteen is an apomictic plant and propagation from seeds can be considered as a clonal propagation of the parent trees. Mangosteen extension seed production in large numbers has several obstacles, including limited number of selected parent trees, the only one harvest season in a year, and slow growth of seedlings. There are several ways to accelerate mangosteen seedling growth. The use of mycorrhizal fungi is intended to overcome the slow growth of mangosteen which is caused by poor root system. Mangosteen, known as, the plant without root hairs that leads to poor uptake of water and nutrients. Arbuscular mycorrhizal fungi (AMF) is one of the obligate symbiotic fungi which is known to have beneficial effects for plant growth. The mycorrhiza acts symbiotically with plant roots to enhance nutrient uptake, stimulate growth,

and improve plant resistance to drought and soil pathogens (Muas *et al*., 2002), and enriching mangosteen seedlings environment using carbon dioxide (Jawal *et al*., 2002). Tissue culture propagation need to be improved to increase the percentage of successfully seedling growth.

4.4. Papaya Seed Production

Source seed production procedures are carried out through strict isolation and controlled pollination. As many as 300-1000 papaya mother trees are planted in one stretch. Male plants and other varieties are removed from mother trees populations. Hermaprodite mother plants which are stable and healthy with high production are selected from the middle of the orchard for selfing. It is important to avoid pollen from other flowers when the flowers are open. Paper wraps protect flowers from excessive moisture which can disturb fruit formation. At the age of 4-5 months after pollination the papaya fruit can be harvested (Budiyanti, 2017).

5. COLLABORATORION OF SEEDLINGS PRODUCTIONWITH SEED GROWERS AND PRIVATE SECTOR

In order to accelerate the development of tropical fruits, it is imperative that the Indonesian goverrnment provide and distribute the seedlings to all the different regions. In the implementation of seed production, Indonesian Tropical Fruits Research Institute (ITFRI) and Directorate General Horticulture (DGH) as executing agency, must collaborate with seed growers and the private sector. In this collaboration ITFRI contribute in providing mother plants and scions, while the Seed growers contribute in providing nursery space and labor.

Seedlings are produced through intensive supervision based on standard operating procedures. One week before grafting, the number and variety to be produced are reported to the local seed certification institution. This cooperation provides benefits to both government and seed growers. Government provides benefits in terms of accelerating process of seed production, accelerating the development of new varieties, and overcome the limitations of land and labor for large-scale seed production. This collaboration reflected good synergy between government and the communities in the development of tropical fruits. The benefits received by seed growers include opportunities for new jobs, increasing knowledge in tropical fruit seedlings production, and increasing family income.

6. SEEDLINGS DISTRIBUTION

The seedlings produced are distributed free of charge to the communities. The stock seedlings (mother plants) are distributed to government and non-government institutions that play a role in production of extension seedlings (Agency of Implementation Agricultural Technology and seed growers). Extension seedlings are distributed directly to users (farmers group and communities). Distribution activities start with selection of interested farmers and potential regions for fruit development. Some seedlings are also distributed to farmer groups that are under supervision of the private sector as satellite farms. Before seedlings distribution the technical guidance of fruit crop cultivation are given to prospective farmers. Monitoring and evaluation are needed to ensure the seedlings are planted and maintained properly.

Within three years from 2017 to 2019, about 1,001,180 of tropical seedlings of avocado, mango, mangosteen, durian, banana, papaya, salak, and breadfruit were produced and distributed. About 31,000 of them were stock seedlings (Table 1.). Mango, durian, and banana were the

most commodities with the number of seedling production. The new total area development is predicted to reach 2,899 ha. Assuming an estimated success rate of 50%, it is anticipated that in the next 4-5 years the harvest area will increaseby about 1.450 ha and will give significant impact in increasing Indonesian fruit exports. This fruit crop development program will continue until 2024. In 2020 the DGH plans to develop 4,807 ha of mango, mangosteen, durian, and banana commodities.

Table 1. Tropical fruits seedlings production and distribution from 2017 to 2019

Commodity	Seedling Production		Total seedlings production (ITFRI) 2019	Seedling production (BPTP) 2017	Seedling production (DGH) 2018	Total seedlings	Estimated new planting area (ha)
	Stock Seedling	Extension seedling					
Avocado	0	6,500	6,500		82,950	89,450	450
Mango	2,500	145,500	160,000	120,000	0	180,000	60
Mangosteen	2,500	26,500	29,000	4,500	0	33,500	335
Durian	25,000	46,890	76,890	60,000	0	136,890	1368
Banana	1,000	13,000	19,000	8,000	168,649	195,649	178
Papaya		83,000	83,000	150,000	0	233,000	155
Salacca		25,000	25,000	20,000	103,491	147,491	73
Breadfruit		19,000	19,000	40,000	0	59,000	280
Total	31,000	364,890	395,890	402,500	355,090	1,001,180	2,899

*Agricultural statistics 2018

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FIJI'S POLICIES AND FOCUS TOWARDS ENHANCING THE TROPICAL FRUITS INDUSTRY

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ABSTRACT

Fruit production in Fiji represents less than 10% of the overall horticultural output, despite favorable climate and increasing market opportunities. In part, this situation can be attributed to a prevalence of low-intensity and semi-commercial fruit production systems, poor postharvest handling practices and limited value chain development. Improving domestic fruit production and consumption in Fiji is a critical contributor to improving the nutritional status of the general population. Fiji currently has one of the highest incidences of non-communicable diseases in the world. Fiji also imports FD$17.8 million fresh fruit (USD 8.21 million) (includes; grapes, oranges, apples, pears and kiwi fruit) and an addition ofFD$3.8 million (USD 1.75 million) in processed products annually. Some of these imported fruits can be substituted with locally grown tropical fruits such as; guava, mango, avocado, oranges, mandarins and other indigenous fruits. The Fijian Government has sought to stimulate increased domestic fruit production and consumption with various levels of success. This paper will review the Fiji Government's past and present policies aimed at promoting the fruit industry and associated health outcomes, highlighting key learnings and current thinking.

Keywords: policy, fruits, industry, local, orchards

1. INTRODUCTION

The agriculture sector remains important to Fiji's prosperity providing valuable employment, income, and food security for the population. Approximately 65% of the population are directly involved in some form of agriculture where the current production is derived from approximately 65,099 small and medium sized farms (MPI, 2009). Much of Fiji's agricultural productivity is derived from subsistence-based production and small holder family-owned farms (Kumar and Kumar, 2015). These farms are found across a diverse land base that has over 300 small islands. The large population engaged in this sector, is indicative of the importance of agriculture which over-rides all other economic sectors. Until two decades ago, agriculture has remained the highest contributor to gross domestic product. The sugar industry, the mainstay of agriculture sector has substantially declined in the last decade, however provides opportunities for other agricultural subsectors. The comparatively low productivity of the sector as a whole reflects many factors including the dominance of subsistence over commercial farming (Reddy, 2003), inadequate and expensive inputs, poor or lack of low cost technologies, inadequate infrastructure (Tuqa *et al*., 2018), marketing deficiencies, and high production costs due to the lack of scale economies and expensive farm inputs. It is estimated that at present, 80% of fruits and vegetables consumed by the tourism sector are imported (Young & Vinning, 2007).

The horticulture subsector continues to contribute towards improving the standard of living of farmers in Fiji. The Fijian climate is suitable to growing a number of tropical fruits and fruit

production is now being recognized as a sustainably profitable enterprise (Tiwari *et al*., 2018). Some Pacific Island countries (PICs) have increasing occurrences of noncommunicable diseases (Snowdon *et al*., 2011). The frequent consumption of fruits can lower the risk of cancer, heart disease, hypertension, and stroke (Lako *et al*., 2007). The high rates of increasing urbanization coupled with expiry of land leases provide opportunities for domestic market of fruits. The increasing tourism market provides opportunity for fresh produce market. While the scope for increasing fruit production, exports, and processing exists, the Fijian fruit sector has challenges which remain unresolved and untapped opportunities. This paper reviews the Fijian government's past and present fruit development policies and some fruit development projects. This paper aims to identify opportunities to enhance the fruit industry in Fiji.

2. METHODOLOGY

The data for this research have been mostly extracted from secondary sources. The production, import and exports data were collected from the Ministry of Agriculture's (MOA) annual reports, bulletins and commodity reports. In addition, the national annual budget estimates from 1980 to 2010 were used to extract budget estimates. The National Development Plans have also been used to trace fruit orchard establishment policies of past governments. This study is more circumstantial than empirical on measuring performance where commodity outputs are traced over various time periods using available data.

2.1. Fiji fruit production, import and exports

Over the years, the government has introduced a number of fruit crops with the view of providing food and income security for farmers. As a result, a large number of tropical fruits have been cultivated in Fiji. Some are commercially cultivated while the rest grows in scattered areas. The initial problems of production, processing, and marketing still exist with many major fruit crops. In the 1930s, minor crops were largely cultivated for local consumption and with the decline in banana exports, the administration focused on exporting citrus and exporting canned pineapples (Department of Agriculture, 1931). With increased production of fresh fruits, some farmers faced problems of marketing and to solve this problem market structures were constructed in Nausori and Sigatoka (Parham, 1940). The construction of these structures not only eased the marketing of produce locally but also provided opportunities for export of fresh fruit and vegetables to the New Zealand military despite the floods of 1941 (Ackland, 1941). Despite lack of storage, poor transportation facilities and support services in place, the farmers were urged to produce fruits and vegetables to meet the demands of increasing export market. This eventually resulted in farmers being unable to maintain the quality of products after harvesting (post-harvest losses), which was complicated by poor transportation services (Jack, 1942).

Interestingly, guava was once declared a noxious weed in the colony of Fiji as it was spreading at an alarming rate infesting arable land (Mune & Parham, 1956) though it is regarded as a nutritious fruit. A continuous effort has been made by the national government to promote fruits as commercial ventures for farmers and enhancing their opportunities for exports and even local processing through 5-year Development Plans (DP). DP 6 highlighted fruit development, DP 7 focused on passion fruit, DP 8 on citrus, and DP 9 was on pineapple, mango, pawpaw, and citrus (Table 1). Thereafter, a 4-year Commodity Development Framework programme (1997 to 2000) policy direction was focused on pineapple, mango, papaya. The table is adopted and modified from (Kumar & Kumar, 2015).

Table 1. Development Plans highlights fruit development strategies.

Period	Objectives	Fruit Focus Areas/Strategies
DP6 1971 to 1975	Stimulation of agricultural sector by raising farmer's income and increasing rural employment. Creation of efficient marketing system by National Marketing Authority	Raise efficiency on existing farms by intensification, crop diversification and research, provision of subsidies, credit and financial incentives. The targeted commodities also include fruits.
DP7 1976 to1980	Increase in agricultural output to raise the rural income and employment opportunities.	Production of fruits. The export crop included was passion fruit.
DP8 1981 to 1985	Increase agricultural output, expand, diversify & increase exports, and encourage local participation in agribusiness.	The strategy was import substitution and self-sufficiency. Focus on citrus and other fruits.
DP9 1985 to 1990	Improve self-sufficiency; concentrate resources in selected commodities for export and domestic market to make agriculture more efficient by employment & income generation and extend new technologies to farmers.	Promoting self-sufficiency and exports. Fruit crop included were pineapple, mango, pawpaw and citrus.

2.2.1. Fruit Production trend from 1960 to 2014

The dynamics of total fruit production (ha) trend from 1960 to 2014 is referred in Figure 1. It is noted that initially there were just a few fruit crops. Banana was the major fruit crop and over the years more fruit crops have been introduced. The total area under fruits in early 1960s was approximately 2200 ha. The production has been reduced due to national disasters. The sharp decline in production was during 1972 due to cyclone Bebe, which devastated the banana industry. Thereafter, in terms of area under fruits, it has taken over 40 years to match the production levels of 1960s. This is despite introducing a number of fruit crops as per DPs. The trend indicates that there exists challenges and opportunities for the Fijian fruit industry.

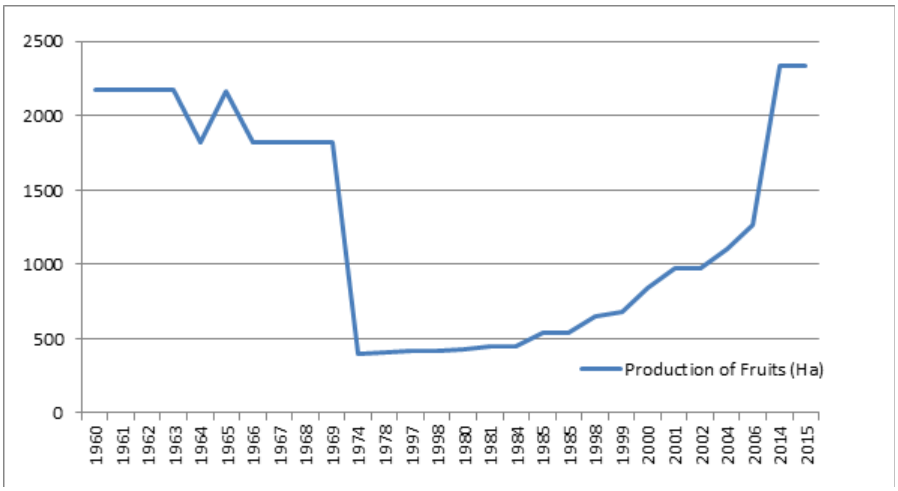

Figure 1. Fruit production (ha) trend 1960-2014

2.2.2. Import and Export Trend of Fruits from 1959 to 2015

The import and export trends of fruits (tonnes) from 1960 to 2014 is referred in Figure 2. Until around late 1970s, Fiji was self-sufficient in fruits indicating zero imports. The imports of fruits commenced in early 1980s and have significantly increased to unprecedented levels. The banana exports had ceased completely by the early 1980s, passionfruit juice and pulp exports markedly declined and production and marketing performance continued to deteriorate (Fleming & Blowes, 2003). This could be contributed to decrease in production after cyclone Bebe and increase in tourism industry. Small volumes of fresh pineapples were exported to New Zealand

during the 1980s, with the highest value reached of USD 54,000 in 1983 (Fleming & Blowes, 2003).

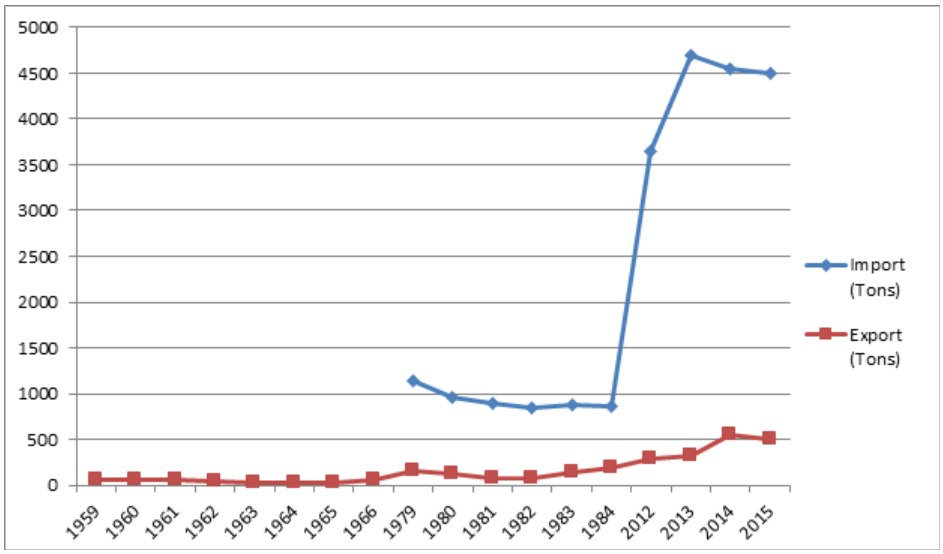

Figure 2. Import and Export Trend of Fruits from 1959 to 2015

3. PERFORMANCE OF SOME SELECTED FRUIT CROPS IN FIJI

3.1. Banana

Bananas (family *Musaceae*) existed from an early stage in Fiji. It has been exported in 1800. Banana is the first fruit that was successfully grown commercially in Fiji and exported to countries as far as Canada. Banana Licensing Board was established by MoA to promote banana production and export. Chinese farmers grew bananas along the banks of the Sigatoka River in the early 1900s exported to Australia until tariffs were increased in 1911 (Duncan & Sing, 2009). By 1931 banana was planted on the islands Tavueni, Gau, Moala, and Kadavu and large areas opened in Rewa (DOA, 1931). Despite this, banana production continued to increase and exported to New Zealand. In order to increase production there was a need for demonstration plots to be established near farms and Fijians had to be instructed use of implements (DOA, 1931). The government's incentive to value adding of banana resulted in successfully producing banana figs, chips, and flour which could be stored for longer period of time (Jack, 1942).

By early 1960's, the banana export industry became noticeable high and many smallholder farmers were involved in hope of achieving economic success (McPaul, 1960). The Banana Export and Marketing Act was implemented in 1960 and Banana Marketing Board was established to secure abundant and efficient supply of banana, develop bylaws to licence suppliers, act as middlemen to purchase bananas from producers, and arrange the export and marketing of Fijian banana brand. Around mid-1960s, under the Intensive Banana Scheme, banana farms were established in Lomaivuna. Under this scheme, banana farmers were settled on 3.64 ha farms with a house and 1.61 ha of established banana orchards (Duncan & Sing, 2009). This scheme was later extended to Waidina locality. Banana ranked as the third most valuable agricultural export after sugar and coconut products in 1970 (Fleming & Blowes, 2003). However, the banana industry was severely affected by cyclone Bebe in 1972. The industry faced further demise due to the heavy infestation of pests and diseases resulting in high rejection rates of export quality bananas. Compounded by these problems, the banana industry never recovered.

3.2. Mango

Mango, botanical name *Mangifera indica* is native to India and is one of the most popular seasonal fruit in Fiji. The mango fruits are in season from August to March, which includes both the hybrids and local varieties. Mango was first introduced in late 1800s and grows vigorously and produces well though it is not indigenous to Fiji (Iqbal, 1982). It is found almost all over Fiji and particularly grows well in extensive areas of Western Viti-Levu and Northern Vanua Levu. This widespread distribution of naturally occurring mango trees is an indication of its adaptability in Fiji. After introduction, mango was grown as scattered trees for domestic consumption. The interest for developing the mango industry was revisited in the 1980s after being prioritized in the DP9 period with prospects for exports and processing. Within this period, opportunity for export of improved varieties of mangoes to Japan was also identified as a highly remunerative investment. However, export production levels were not achieved due to devastation of the mango orchards during the cyclone. The national government acknowledged the progress and commenced the development of mango industry by establishing the mango research at Legalega and Sigatoka Research Stations. The recorded first commercial mango planting was in 1981 in Yaqara, Tavua and the second was done at the Native Land Development Commission Legalega orchard in 1983 (Abbas *et al*., 2019). Smallholder mango planting was encouraged by the Ministry of Primary Industries in collaboration with the National Marketing Authority using improved varieties produced at the Sigatoka Research Station. By the end of 1984, 37 farmers in North Western Viti Levu were involved in this programme (Iqbal, 1982). The potential to supply the domestic tourist market was recognized in the mid-1990s due to increasing tourism sector (ADB, 1996), which provided an incentive for farmers with supplementary marketing opportunities. The niche market export demand for pickled and dried local mango varieties resulted in recommendations included in the Fiji policy framework for agricultural marketing.

Mango value chain provides alternative livelihoods to a number of people. It provides food security and income. The majority of market vendors are women and trading of mangoes provides supplementary income. The increasing number of former Fijian residents living abroad (New Zealand, Australia Canada, and United States) provides market for export of dried and processed mango. The mango fruit is an important source of Vitamin A, B, and C (Nath, 1995), which contributes to nutritional health benefits. Mangoes for export are picked half ripe (color break). The yields vary depending on the varieties, age of the tree, and environmental conditions. It is suggested that harvesting be carried out in the early part of the day so as to avoid build-up of field heat (Prasad, 2008). The mangoes are largely sourced from scattered trees that have limited or no cultivation practices. This equates to no pest and diseases management with resultant poor quality fruits. These trees gradually become tall, hindering harvesting process. If not harvested and handled properly, mangoes falling from higher trees are prone to damage and brushing. Poor harvesting and postharvest handling practices with absence of cold chain increases the likelihood of mangoes to increased postharvest losses. Fijian government's intentions for processing and value addition of mango (dried and jam) considering the existing adaptability of these varieties in Fiji conditions were documented in the Fiji 2020 Agriculture Sector Policy Agenda (Bacolod, 2014).

3.3. Passionfruit

The passionfruit (*Passiflora edulus*) was introduced in Fiji from Hawaii in 1958 to broaden the export base to complement sugar which was the principal agricultural crop (Prasad & Chandra,

1980). The national government availed necessary resources to develop this industry. Around 1961 experimental plots were set up at Sigatoka Research Station and later introduced to farmers. By 1968 it was grown on commercial scale to supply to two processing factories in Sigatoka under a contract between Land Development Authority (LDA) for producing and Can-pac Limited for processing (Hampton & Thompson, 1974). Can-pac Limited was taken over by South Pacific Foods an Australian based company. The LDA was taken over by Valley Industrial Cooperative Association (VICA), a local grower's organization for organizing contracts between farmers and processors by arranging and financing passion fruit plantings. In 1967 The VICA joined with Davis Consolidated Industries and took over Cottees limited. By 1972 -73 the fruit purchase and payment system broke down and the two processors made their own contract arrangements with farmers (Hampton & Thompson, 1974).

During this period, passion fruit industry was flourishing and was supported by government through agronomic research, University of the South Pacific through social, economic, and institutional research, and commercial companies through processing and marketing research (Prasad & Chandra, 1980). During 1960s and early to mid-1970s, there was a significant value of passionfruit exports having over 400 producers and two processing firms operating in Sigatoka Valley on the main island of Viti Levu (Fleming, 1996). In terms of production practices, government initiative research included diseases and pest management, fertilizer application, vine spacing, pollination and flowering, and development of crop calendar in relation to climatic conditions. Unfortunately, passionfruit industry failed to live up to its potential and stagnated for the remainder of the 1970s. While passionfruit is found in the local markets during season, this is often through the initiatives of individual farmers.

3.4. Pineapple

Pineapple production in Fiji received various levels of support to develop into a commercial crop. The colonial administration in the 1800's was concerned of the economy driven by a single commodity (sugarcane) and was seeking other cash crops that would support the economy. Two such crops identified to provide leverage to the Fijian economy were banana and pineapples (Surridge, 1931).

Pineapples had already started as a crop in Fiji in 1870's and was planted in Levuka and sold to Europeans and the visiting steamers berthing on the island. They were also sold to passengers in the steamers leaving Levuka port for either Australia or New Zealand. Fruits were exported to Australia and New Zealand in 1880's. Export to New Zealand in 1889 according to the newspaper comprised of 380 boxes of tea, 120 cases molasses, 460 cases of pineapples, and about 4,000 bunches of banana.

Fijian pineapples in 1800's were gaining popularity amongst the local and international markets. The quality of the pineapples grown in the country was hailed as one of the best. The soil and the climatic conditions of Fiji were considered as very ideal for pineapple cultivations. While fresh pineapples were already exported, it was however dependant on the arrivals of the steamers on regular basis. The discussions on the prospects of canning pineapples had already started in 1886. The first ever policy implemented in Fiji for development of fruits was passed by the Legislative Council in 1923, providing export duty concession for 10 years on any pineapple grown and canned in Fiji.

The prospects of canning pineapples in the islands were increasing and the colonial government was very supportive of the idea since it had keen interest to diversify agriculture due to

dependence on sugar which was subject to wide fluctuations on price in the world market. However, it was not until 1923 that the Legislative Council decided that no export duty will be charged for a period of 10 years on any pineapples grown and canned in Fiji. The legislation proved to be attractive to investors and a year later, Dominion Canners Limited from Canada expressed interest in establishing pineapple growing and cannery industry in Fiji. The Colonial Sugar Refinery Company in 1936 diversified its operations from operating sugar mills to producing canned pineapples (Lal, 2015). However they had to close the cannery in 1955 after operating for 20 years (Lal, 2015; Moynagh, 1978).

Area cultivation under pineapple production drastically reduced after the closing of the cannery since there was no processing and very limited export market. Most of the pineapples produced were mainly for the local markets. Pineapples continued to be an important crop in the post-independence period. In 1974, a total of 11.5 ha of crop was established in the Western Division and 46.5 ha in Central Division with increasing number of farmers reportedly using fruiting hormone and chemical weed control (MOA, 1974).

Pineapples continue to play an important role in Fiji's agricultural productivity. The agricultural census conducted in 2009 identified 914 pineapple farmers farming a total land area of 445 hectares producing 2,800 tonnes of pineapples annually (DOA, 2009). The main markets are the local municipal markets, road side stalls, and the hotel industry. Even though exports in the last decade have been as low as at an average of 6 tonnes per annum, considerable amounts have been marketed in the tourism sector.

3.5. Citrus

Citrus of various varieties have been grown in Fiji for domestic use for a very long time. However not until the late 70's the government embarked on a commercial development program to enhance citrus production for major export oriented industry. Considerable research work was undertaken in various Government Research Stations to identify suitable varieties, and develop proper package of practices.

The major development project for citrus started in 1977 in Batiri, Vanua Levu with the objective of producing processed citrus juice for domestic consumption and for export. The processing factory was established in 1979. A total of 148 ha of land using 'Late Valencia' variety were planted with first commercial harvesting starting in 1981 (Chandra, 1983). The project ran into major financial difficulties in 1988 and was subsequently taken over by the National Marketing Authority (Jansen *et al*, 1990). Small holder farmers around Vanua Levu in 1984 were supplying 10 tonnes of fruits while 109 tonnes were supplied from Rotuma. Fruit piercing moth was the major problem in the citrus orchard resulting in 25% loss of mature fruits (DOA, 1985). Fiji currently produces 990 tonnes of citrus (MOA, 2018).

4. CHALLENGES AND OPPORTUNITIES OF FIJIAN FRUIT INDUSTRY

Tropical fruits can play a pivotal role in enhancing Fiji's agricultural contribution to the national economy. Opportunities exist into developing fresh and processed fruit industry for both domestic and export markets. Fiji currently imports USD 21 million worth of assorted fruits mainly consisting of apples, oranges, and grapes. A bulk of this can be easily replaced with increased local production of a range of tropical fruits such as guava, dragon fruit, mangoes, rambutan, and mangosteen.

Fruits and vegetables provide a diversified, flavored, colorful, low calorie, and high micro nutrient rich diet, having the ability to reduce non-communicable diseases. Globally, people are consuming less than the daily recommended requirement of fruits.

There exist enormous opportunities for developing the Fijian tropical fruit industry. In order to enhance production and consumption of fruits in Fiji, the following strategies are recommended:

- Nationwide awareness needs to be created to increase consumption of fruits.
- Increasing production through the establishment of fruit orchards.
- Making available elite fruit cultivars.
- Product development and processing fruits in season.
- Provision of cold storage facilities.
- Analysis of value chain to improve supply.
- Awareness on good postharvest practices.
- Aggressive promotion of local fruits into formal markets.

5. CONCLUSION

Fiji's tropical climatic conditions provide favorable environment for production of good quality fruits. However, the lack of policy interventions specifically targeting fruits over the years has created a gap in the development of this industry. Recently, the government has realized the importance of local fruit industry, therefore some policies are targeted to enhance local fruit production. Suitable policy measures promoting value addition and processing of fruits are encouraged to prolong shelf life of many seasonal tropical fruits.

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SMALL-SCALE AGRIBUSINESS CHAINS FOR TROPICAL FRUITS IN BALI IN FACING THE DYNAMIC DEVELOPMENT OF INSTITUTIONAL CONSUMERS

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ABSTRACT

The small-scale agricultural system for tropical fruits on the island of Bali has been challenged by the yearly increase in the number of incoming international and domestic tourists. Institutional consumers, such as hotels, restaurants, and catering services as well as modern local consumers develop values that are difficult to be fulfilled by the existing small-scale traditional agribusiness chains system. The intrinsic and extrinsic qualities, in addition to services are emerging values that have to be met for the institutional consumers. The Governor of Bali Province has released regulation (Reg. No. 99, 2018) of which indicates that the institutional consumers have to accept at least 60% of local agricultural products. The regulation causes a conflict between the traditional supply chain system, which is less value orientated; and value requirements of the institutional consumers. A concept in developing an integrated value chain system which is inclusive for small-scale holders is discussed here.

Keywords: value chains, small-scale farmers, institutional consumers, extrinsic quality

INDUCED SYSTEMIC RESISTANCE (ISR): AN EFFICIENT TOOL TO CONTROL BACTERIAL TRANSMITTED PAPAYA DIEBACK AND BANANA BLOOD DISEASES

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ABSTRACT

The Malaysian Government is focused on increasing the production of fruit types that can contribute economically to the industry. Among the fruits, are papaya and banana, which are important for local consumption and export. However, the goal to increase the production and export has been hampered by the emergence of invasive alien diseases namely papaya bacterial dieback and banana blood disease, which are transmitted by bacterial pathogens. Various approaches including chemical and biological controls have been attempted but none were successful in controlling these diseases. One of the techniques that has not been fully explored is the enhancement of plant resistance against diseases known as the Induced systemic resistance (ISR). The aim of this study was to induce systemic resistance in plants against both bacterial diseases using Plant growth-promoting rhizobacteria (PGPR). ISR technology to control both papaya bacterial dieback and banana blood disease was developed by bio-prospection and manipulation of ISR inducing PGPR from soil. For papaya, the ISR seedlings showed total recovery against the dieback infection while the Control seedlings (non-ISR) totally succumbed to the disease. When papaya seedlings (ISR and non-ISR) were produced and tested in a hotspot for dieback disease, for one crop cycle (2 years), all treatments except one survived, compared to the control where none of the plants survived. In another trial, one of the ISR treatment was selected and up-scaled in a famer's plot (hotspot). 95% of resistance against the dieback disease was recorded. This success enabled the technology to be commercialized and the product made available in the market for farmers to adopt. This success on papaya, compelled a repetition of the same approach to develop ISR technology against blood disease of banana. When the ISR banana seedlings were challenged to the pathogen, three treatments had recorded 100% suppression and another two had 75% suppression against the disease, whereas all the control plants did not survive. All these five treatments were tested in a hotspot area (area of elevated disease occurrence) where disease development was observed. During the first crop harvested from the mother plants, the ISR treatment recorded 0-8% disease infection compared to the Control which recorded three-fold higher incidences of infection than the ISR treatment (24%).

Keywords: papaya bacteria dieback, blood disease of banana, induced systemic resistance (ISR), plant growth-promoting rhizobacteria (PGPR)

1. INTRODUCTION

Papaya and banana are important Malaysian fruits grown for the domestic and export markets. Both fruits have been devastated by the emergence of bacterial transmitted diseases, specifically the papaya bacterial dieback (also known as crown rot of papaya) and blood disease of banana.

The causal bacterial pathogens for both diseases have been listed under invasive alien species.

Various approaches including chemical and biological controls have been attempted but none were successful in controlling these diseases. The possible strategy to control these diseases is through the development of resistant varieties which is time consuming and cannot offer a faster disease control solution. One technique that has never been tested for this papaya and banana diseases is the enhancement of plant resistance against the pathogens. This process of plant resistance enhancement, known as induced systemic resistance (ISR), systematically activates the plant disease resistance in the roots which then extends it to above-ground plants parts. The ISR can be activated by inoculating the plant growth-promoting rhizobacteria (PGPR) on the roots. PGPR mediated ISR has been reported on a wide range of crops, such as *Arabidopsis*, bean, cucumber, carnation, radish, tobacco, tomato, and banana against various pests and pathogens, such as viruses, bacteria, nematode, fungi, and insects (van Loon, *et al*., 1998); therefore, it would be a worthwhile effort to study the effect of PGPR induced ISR as a possible mode of control for both bacterial diseases. *Bacillus* and *Pseudomonas* are among the PGPR genera that are commonly used for ISR induction in plants (Liu, *et al*., 1995; Pieterse, *et al*., 1998; Ramamoorthy, *et al*., 2001). Therefore, this study was carried out to isolate such promising bacilli and pseudomonads from healthy papaya and banana grown soils, screening them for ISR induction against papaya bacterial dieback and blood disease before their efficacies were evaluated in infected field sites (hotspot- area of elevated disease prevalence).

2. MATERIALS & METHODS

2.1. Induced Systemic Resistance for Papaya

2.1.1. Source of Erwinia mallotivora and 'Eksotika I' papaya seeds

The pathogen, *E. mallotivora*, culture used in this study was kindly provided by Ms. Noriha Mat Amin from the Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters. The 'Eksotika I' papaya seeds were purchased from the Seed and Planting Materials Unit, MARDI, Serdang.

2.1.2. Isolation of bacilli and pseudomonads

The soil from healthy papaya plants were sampled from three locations *viz* Serdang, Selangor and Lunas and Keladi in Kedah. The soil beneath the papaya canopy was sampled by scooping. The soil samples were placed in a sterile Falcon tube and transferred to the MARDI Headquarters, Serdang in a cooler box for bacilli and pseudomonads isolation.

The bacilli were isolated using mannitol egg yolk polymyxin agar (MEYP) and polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA); and the pseudomonads were isolated using *Pseudomonas* F agar and King's media B (Atlas, 1997). Bacterial cultures with different morphology, size and color were purified. The bacilli will form red culture on MEYP and blue culture on PEMBA. The pseudomonads grown on the King's B will produce fluorescence surrounding the colony mainly for *Pseudomonas fluorescens* and *Pseudomonas* F agar does not exhibit any special feature for the pseudomonads grown on it. The pure culture of the isolates were grown on slant nutrient agar and kept stored in a refrigerator for a few months before sub-cultured again.

2.1.3. Screening of bacilli and pseudomonads for ISR induction

The 'Eksotika' papaya seeds were soaked overnight in a container containing distilled water. Floating seeds were discarded as they were considered non-viable. The submerged seeds were sown into germination trays filled with peat moss. Two weeks after that, young seedlings (3-4 leaves stage) were transferred into 15 x 23 cm perforated polybags containing a soil mixture of 2 top soil: 1 organic matter: 1 sand and kept under 50% shade in the nursery.

Two-week old seedlings were then transferred to soil media. The treatments were treated with the PGPR bacterial suspension while the control seedlings were applied with sterile distilled water. Each bacterial treatment and control had five replicates. The second application of bacterial suspension for treatments and sterile distilled water for control was conducted two weeks after the first application. The seedlings were maintained for a further two weeks before being challenged with the papaya bacterial dieback (BDB) pathogen, *E. mallotivora*. This experiment was conducted at a glasshouse in MARDI, Serdang.

The pathogen was grown in a nutrient broth with agitation for 48 hours at room temperature. The treated and control seedlings were pricked (20 pricks/seedling) with a sterile needle on the stem close to the crown. Ten mL of the *E. mallotivora* suspension was sprayed onto the pricked area using a hand held sprayer. Then, the pathogen-sprayed seedlings were covered with a plastic bag and left for two weeks. The disease symptom development was observed at 4, 7, and 14 days after pathogen application and the rate of the plants dying due to the dieback infection was recorded.

2.1.4. Evaluation of ISR seedling for disease resistance in hotspot

The best performing ISR treatments (with 100% disease resistance) were selected and tested for disease infection in a hotspot (area of elevated disease occurrence) in MARDI. Briefly, ISR seedling were prepared as mentioned above and transferred to a hotspot in MARDI and maintained for a crop cycle (24 months). After the seedlings were transferred, a routine monthly application of ISR inoculant was conducted throughout the crop cycle. Control plants were also prepared and cultivated as comparison to the ISR plants, and to evaluate the effects of disease infection. Fertilizer application and other pest and control measures were according to (Chan, *et al*., 1994). Good Agriculture Practice (GAP) was employed during the whole crop cycle. Symptoms of disease infection and plant death caused by the dieback disease were recorded.

2.1.5. Up-scaling of ISR technology for disease resistance in farmer's plot (hotspot)

One of the best performing PGPR treatment from the field study above was selected and tested in the farmer plot (also a hotspot) as up-scaling activity at Ampang Tinggi, Kuala Pilah, Negeri Sembilan. Two thousand eight hundred and fifty (2850) ISR papaya seedlings were prepared and cultivated in the field. A routine application of ISR inoculant was conducted once a month throughout the crop cycle. Fertilizer application and pest and disease management were followed according to common farmer's practices. GAP was employed during the whole crop cycle. The disease infection and plant death caused by the dieback disease was recorded.

2.2. Induced Systemic Resistance for Banana

2.2.1. Source of Ralstonia syzygii and banana plantlets (var. 'Berangan')

The pathogen, *Ralstonia syzygii* culture used in this study was kindly provided by Ms. Nursulastri Jaffar from the Horticulture Research Centre, MARDI Head Quarters. The 'Berangan' banana plantlets were purchased from Exotic Biotech Sdn. Bhd., Ampang Tinggi, Kuala Pilah, Negeri Sembilan.

2.2.2. Isolation of bacilli

The soil from healthy banana plants were sampled from three different local varieties namely 'Berangan', 'Rastali' x 'Embun', and an unknown variety from MARDI's Integrated Organic Farm's banana plot. The soil beneath the banana canopy was sampled by scooping. The soil samples were placed in a sterile Falcon tube and transferred to the bioprocessing lab in a cooler box for bacilli isolation. Based on our previous experience with papaya, we found that only the bacilli can induce systemic resistance thus only the bacilli were isolated for banana ISR study. The bacilli were isolated as explained above for papaya.

2.2.3. Screening of bacilli for ISR induction

The banana plantlets were treated with PGPR inoculant and transferred into a germination tray filled with peat moss. For control seedlings, the PGPR inoculant application was omitted. Two weeks after that, both the control and inoculant treated plantlets were transferred into 25 x 30 cm perforated polybags containing a soil mixture of 3 top soil : 2 organic matter : 1 sand and kept under 50% shade in the nursery.

Two weeks after that, the seedling were transferred to soil media. Treatments were drenched with the PGPR bacterial suspension while for the control seedlings, sterile distilled water was applied instead. Each bacterial treatment and control had five replicates. The second and third application of bacterial suspension for Treatments and sterile distilled water for control were conducted every fortnight after the first application. The seedlings were maintained for a further two weeks before being challenged with the blood disease pathogen, *Ralstonia syzygii*. This experiment was conducted at a glasshouse in MARDI, Serdang.

The pathogen was grown in a nutrient broth with agitation for 48 hours at room temperature. Each treated and Control seedlings were injected with 5 mL pathogen suspension with density of 108 cfu/ml on the banana petiole close to the crown. The disease symptom development was observed at 2, 4, and 8 weeks after pathogen application and the disease severity was recorded. The severity index of 0 denoted a healthy plant while 100% indicated that the plant has died.

2.2.4. Evaluation of ISR seedlings for disease resistance in hotspot.

The best performing ISR treatments (with lowest disease severity: 0-25%) were selected and tested for disease infection in a hotspot in MARDI, Sintok. The plants were maintained for a crop cycle (18 months). One crop cycle of banana consists of banana production from mother plants and primary and secondary ratoons. After the seedlings were transferred, a routine monthly application of ISR inoculant was conducted throughout the crop cycle. Control plants were also prepared and cultivated as comparison to the ISR plants, to evaluate the effects of disease infection. Fertilizer application and other pest and control measures were as listed in the

following references by MARDI (1990) and Department of Agriculture (2009). GAP was employed during the whole crop cycle. Developments in disease infection and plant death caused by the blood disease were recorded.

3. RESULTS

3.1. Induced Systemic Resistance for Papaya

3.1.1. Isolation of bacilli and pseudomonads

Several bacilli and pseudomonads were isolated using selective media, MEYP and PEMBA for bacilli and King's B and *Pseudomonas* F for pseudomonads and the results obtained are shown in Table 1. Nine bacilli were isolated from the Serdang sample while 42 and 36 bacilli were isolated from Keladi and Lunas, respectively. Pseudomonads isolated from Serdang, Keladi and Lunas were 20, 19, and 13, respectively. The total number of bacilli and pseudomonad isolated from these three locations were 139.

Table 1: Bacilli and pseudomonads isolated from three different locations using selective media

Media	Location		
	Serdang	Keladi	Lunas
	Bacilli		
MEYP	7	23	23
PEMBA	2	19	13
	Pseudomonad		
King's B	10	16	6
Pseudomonas F	10	3	7
Total	29	61	49

3.1.2. Screening of bacilli and pseudomonads for ISR induction

All 139 isolates of bacilli and pseudomonads were tested for systemic resistance induction in papaya seedlings. All the treatments and control were challenged with *E. mallotivora*. Out of 139 isolates tested, only 31 shown systemic resistance induction with at least 20% control against dieback disease (Figure 1).

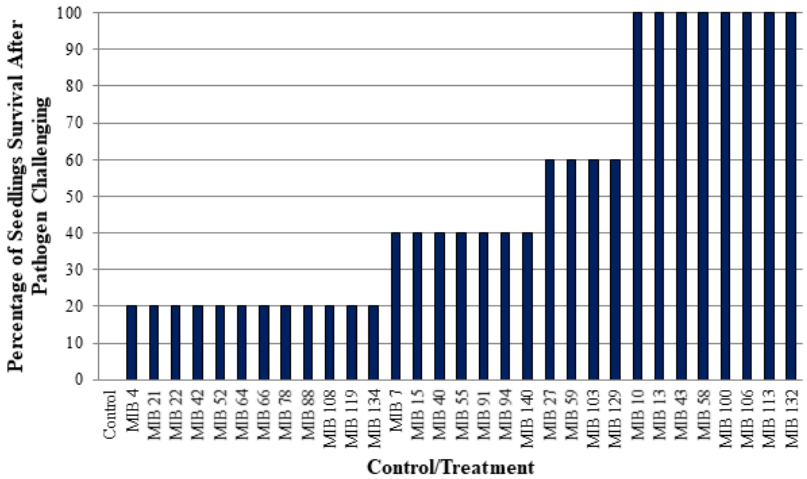

Figure 1: Effects of ISR treatment on survival against papaya bacterial dieback disease

Bacterial dieback symptom development was observed as early as 4 days after the *E. mallotivora* challenge. The seedling's shoot and stems showed watery lesions which are typical symptoms associated with papaya dieback disease. Generally, all the treatments and control exhibited these symptoms development. 108 treatments and control showed increased severity of disease symptoms after 7 days of challenging. The control and other treatments without ISR response finally started to die-off. One month after the pathogen challenge, plants with no resistance showed the crown either dried-off or snapped down. The remaining 31 treatments showed different levels of resistance against disease which ranged between 25-100% (Figure 1). The best treatment with total suppression (100%) against the disease were treatments inoculated with bacilli MIB 10, MIB 13, MIB 43, MIB 58, MIB 100, MIB 106, MIB 113, and MIB 132. The effects of pseudomonads used in this experiment were far inferior than the bacilli in resistance induction against papaya dieback disease.

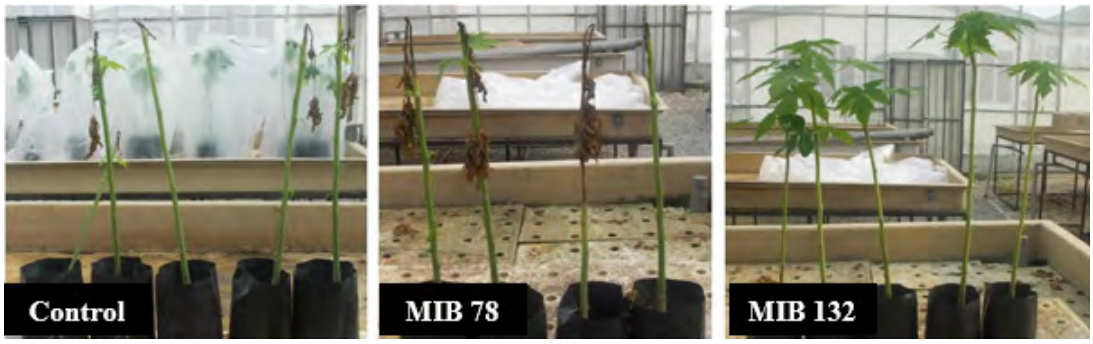

Figure 2: The effects of pathogen challenging on control, ISR-negative (MIB 78), and ISR-positive (MIB 132) plants

3.1.3. Evaluation of ISR seedling for disease resistance in hotspot

All the eight ISR positive PGPR isolates (with 100% suppression against dieback disease), MIB 10, MIB 13, MIB 43, MIB 58, MIB 100, MIB 106, MIB 113, and MIB 132 were used to produce ISR seedlings as mentioned above. Control (non-ISR) seedlings also were produced as mentioned above. Both the ISR and control seedlings performance against papaya BDB infection were evaluated in the open field in MARDI, Serdang which was considered as a hotspot (area of elevated disease prevalencee) were tested. After one crop cycle (2 years) in the hotspot and experiencing the second rainy season in Malaysia (3 months), all the control plants had died because of papaya BDB (Table 2). Almost all of the ISR treatments managed to survive 24 months without any casualty caused by the dieback disease, except one treatment (MIB 13) (Plate 2). MIB 13 had recorded 87.25% resistance against papaya bacterial dieback. All the control plants succumbed to dieback disease within 11 months after being transferred to the field.

The ISR plants showed good vegetative growth without any changes against yield and quality of fruits. The quality of the fruits produced for ISR treatments were as good as control with TSS 13-14% and red intense flesh.

Table 2: Effects of ISR seedling on plant survival against dieback disease infection on papaya grown in hotspot.

Control/Treatment	Plant survival rate after 1 crop cycle (24 months) (%)
Control	0
MIB 10	100
MIB 13	87.5
MIB 43	100
MIB 58	100
MIB 100	100
MIB 106	100
MIB 113	100
MIB 132	100

Figure 3: The destruction of control plants (above) and survival of ISR (bottom) plants after 1 crop cycle grown in the hotspot for papaya bacterial dieback.

3.1.4. Up-scaling of ISR technology for disease resistance in farmer's plot (hotspot)

Two thousand eight hundred and fifty (2,850) ISR 'Eksotika I' papaya seedlings were produced at MARDI, Serdang and transferred to another locality at Ampang Tinggi farm. This site is considered as a hotspot because the previous papaya crop was totally infected by dieback disease. Two weeks after that, all the 2,850 ISR seedlings were transferred to the field. The first incidence of infection was observed 3 months after the seedling were transferred with 85 plants succumbing to the disease. Thirteen months after this, another 61 plants were infected by *E. mallotivora* and died. No further dieback infection was observed until the termination of the project. When the up-scaling activity concluded, the total number of papaya plants which died because of the dieback infection was 5% of total plants grown on the field. The yield and quality of fruits produced in this experiment were good.

3.2. Induced Systemic Resistance for Banana

3.2.1. Isolation of bacilli

Fifty-four bacilli were isolated from bananas grown in soil using two selective media MEYP and PEMBA from three varieties of banana (Table 3). Twenty-four bacilli were isolated from the

'Berangan' variety while 13 and 17 bacilli were isolated from 'Rastali' x 'Embun' and unknown varieties, respectively. The total number of bacilli isolated from these three varieties were 54.

Table 3: Bacilli isolated from soil of three different banana varieties

Media	Variety		
	Berangan	Rastali x Embun	Unknown
	Bacilli		
MEYP	10	6	8
PEMBA	14	7	9
Total	24	13	17

3.2.2. Screening of bacilli for ISR induction

All the 54 isolates of bacilli were tested for systemic resistance induction in banana seedlings. The control and all the treatments and were challenged with *Ralstonia syzygii*. Data of disease severity of 23 isolates are shown in Figure 2, and the remaining 31 are not shown because all of these recorded 100% severity and died off. Out of 54 isolates tested, only 5 showed systemic resistance induction with least severity of 25% and below against blood disease (Figure 2). Blood disease symptoms were observed as early as 14 days after the pathogen challenge. The leaves of seedlings turned yellowish and gradually the seedlings wilted and died-off in two months. Generally, all the treatments without ISR response and control exhibited these symptoms development. The best treatment with total suppression against the disease were treatments which were inoculated with bacilli BIB 10, IB 43, and IB 58. Another two treatments, BIB 23 and BIB 35 had recorded relatively lower severity of 25% than others.

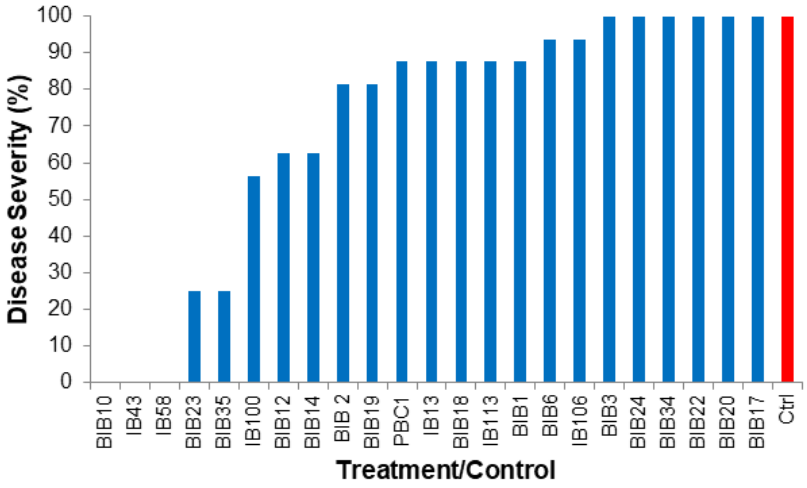

Figure 4: Effects of ISR treatment on disease severity survival against papaya bacterial dieback disease.

3.2.3. Evaluation of ISR seedling for disease resistance in hotspot

All the five ISR-positive PGPR isolates (with least severity, 25% and below), BIB 10, IB 43, IB 58, BIB 23 and BIB 35 were used to produce ISR seedlings as mentioned above. Control (non-ISR) seedlings also were produced by omitting the PGPR inoculation. Both the ISR and control seedlings performance against blood disease infection were evaluated in the open field in MARDI, Sintok, Kedah that was considered a hotspot, where the blood disease incident was recorded in the adjacent plot (20 m away from the selected plot for this experiment). After the

completion of production from the mother plants in the hotspot, 24% of the control and 8% each for treatment BIB 23 and IB 43 (Figure 3) died because the blood disease infection. Other three treatments continue to be free from blood disease infection till to date. However, this observation may change during primary (going to be completed in 3 months) and secondary ratoon production of banana. The ISR plants showed good vegetative growth with shorter time for flower induction and higher yield. The quality of the fruits produced for ISR treatment was as good as the control.

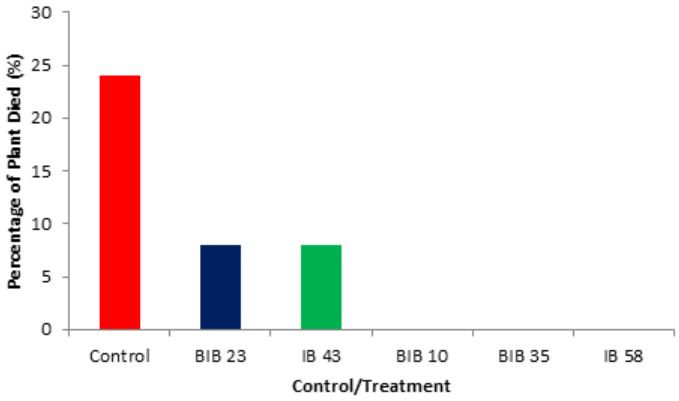

Figure 5: Percentage of plant died caused by blood disease in the hotspot after 1 year.

4. DISCUSSION

Bacilli and pseudomonads were successfully isolated from all three different locations for selection of strains that have capacities to induce systemic resistance in papaya seedlings against papaya bacterial dieback disease. Eighty-seven bacilli and 52 pseudomonads were isolated. Overall, the Keladi sample had the most diverse bacilli and pseudomonad populations followed by Lunas and Serdang. The Serdang sample however had more pseudomonads than bacilli. In contrast, Keladi and Lunas samples had relatively more bacilli than pseudomonads. Keladi has the highest number of bacilli and pseudomonads populations because it was a well managed papaya farm among the three farms, with better soil quality. In contrast, Serdang has the lowest number of bacilli and pseudomonas because the soil quality was very poor, degraded and had less organic matter. Generally, microorganisms including bacteria thrive in soil rich with organic matter.

Thirty-one isolates had shown some level of systemic resistance induction, which ranges between 20-100%. Among these, 8 isolates showed the best systemic resistance inducement in papaya seedlings with total suppression against disease infection. For all these 8 isolates, disease symptoms were observed only during the early period of pathogen challenge but totally healed after that. Although, some level of damage was observed on the plants, the seedlings survived and finally become healthy again. Molecular identification of all these 8 isolates revealed that they belong to the *Bacillus* genus and none were under the genus of *Pseudomonas*. Generally, Bacillus and Pseudomonas are among the genera that is commonly used for ISR induction in plants (Pieterse *et al.*, 1998; Ramamoorthy *et al.*, 2001; Kloepper, *et al.*). However, this study shows that only the *Bacillus* genus is involved in systemic resistance induction, possibly caused by its spore-forming ability that led to better survival capabilities compared to the non-spore forming *Pseudomonas*.

All the ISR treatments except one had recorded excellent control against the dieback disease in the hotspot areas. All the control plants succumbed to disease infection and died within

one year after cultivated. The induction of systemic resistance from the seedling stage and maintained by a periodical ISR inoculant application on mature plants made it resistant against dieback disease. The PGPR mediated ISR has been reported in a wide range of crops such as Arabidopsis, bean, cucumber, carnation, radish, tobacco, tomato, and banana against various pests and pathogens such as viruses, bacteria, nematode, fungi, and insects (van Loon, 1998) in glasshouse studies; but there have not been many studies reported success on disease control by systemic resistance against disease in the open field where there is elevated disease prevelence (hotspots).

This technology was up-scaled in 1 ha of a farmer's plot where this technology can be adapted to commercial farm practices. MIB 106, the best performing treatment in disease control, vegetative growth and yield production from the hotspot study in MARDI Serdang was selected for this up-scaling activity. After one crop cycle, this technology managed to control the dieback disease up to 95%. The 5% losses due to the dieback infection occurred when the weeds were not managed properly in the up-scaling plot. Dense weed around the root region of the papaya hindered the penetration of the ISR inoculant to the root region, therefore the efficacy of systemic resistance in the plants reduced and consequently the disease incidence increased. GAP including weed management is crucial for optimal systemic resistance induction against disease.

4.1. Induced Systemic Resistance for Banana

Based on our previous experience with the papaya's ISR, we found that only *Bacillus* can induce systemic resistance. Therefore, for the banana ISR study, we only isolated bacilli from three varieties. Fifty-four isolates were isolated, where the 'Berangan' variety was found to harbor more bacilli than the 'Rastali' x 'Embun' and an unknown varieties. This possibly was caused by the root exudates generated by the 'Berangan' variety which were more attractive for growth and survival of the bacilli in the soil within the banana root zone.

All the 54 isolates were screened for systemic resistance induction in banana against blood disease. The first disease symptom was observed 14 days after the pathogen challenge and the severity gradually increased and plants would die if they did not have resistance. Treatment of banana seedlings with bacilli had elevated the resistance against blood disease in banana seedlings. Previously the PGPR mediated ISR has been reported on a wide range of crops such as Arabidopsis, bean, cucumber, carnation, radish, tobacco, tomato, and banana against various pests and pathogens such as viruses, bacteria, nematode, fungi, and insects (van Loon, 1998); but nothing much has been reported on blood disease control by using this mechanism.

The best five treatments with disease severity not exceeding 25% were tested in the hotspots for disease resistance. Among these, three recorded total suppression and other two 92% resistance against disease on the mother plants. However, the disease severity may increase in the primary and secondary ratoons of banana in the future. At the point of writing this article, there has been no disease incidence reported in the primary ratoon. Generally the ISR treatment has shown better vegetative growth compared to the control.

5. CONCLUSION

The ISR technology was developed to control papaya bacterial dieback through the bioprospection of PGPR. For this process, 139 bacilli and pseudomonads were isolated,screened for disease resistance inducement, and the eight best ISR inducing PGPR with total control (100%) against papaya bacterial dieback in seedling level were selected for field (hotspot) evaluation. All these

eight PGPR treatments were tested in a hotspot for disease resistance for one crop cycle (2 years), where seven of these had recorded a total suppression (100%) to papaya dieback infection. One of the best performing PGPR treatment (MIB 106) in disease suppression and growth was tested in an up-scaling activity in a 1 ha plot which managed to suppress the disease up to 95%. Development of this technology with a higher disease suppression rate is a boon for the ailing Malaysian's papaya industry.

Our success in controlling the papaya bacterial dieback by ISR technology, compelled us to develop a similar ISR technology to control the blood disease of banana. As for the papaya ISR, 54 bacilli were isolated from three banana varieties. All the isolates were screened for ISR induction, of which five of exhibited least disease severity (25% and below) and were shortlisted as potential candidates to control banana blood disease. Currently, all these five PGPR treatments are being evaluated in a hotspot and at the point of writing this article, all the treatments in mother plants have recorded 92-100% suppression against the blood disease.

The ISR technology to control the papaya BD is the first available and efficient technique in the world to control this disease; thus this technology has huge potential to boost local and international papaya production. Similarly, development of ISR technology to control blood disease will be a game changer in the Malaysian banana industry.

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MUTATION BREEDING TO INCREASE GENETIC DIVERSITY IN MANGOSTEEN

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ABSTRACT

Mangosteen, a popular tropical fruit crop, is unusual in that the flowers do not produce viable pollen. There is only one cultivar of mangosteen available in Thailand, and increased genetic diversity is desirable. Traditional hybridization breeding through cross pollination is not possible so this research used mutation breeding. Mangosteen seeds were treated with 8 concentrations of the chemical mutagen ethyl methane sulfonate (EMS) and 4 concentrations of the spindle fiber inhibitor colchicine. Surviving seedlings that sprouted were grown in pots filled with planting mixture under shade. Morphological abnormalities observed included irregularly shaped leaves, asymmetrical leaves, new branches arising from the base of stem or near the top, multiple branches from one part of the stem rather than pairs, asymmetrical branching, curved stem, and recumbent growth habit. Some of the abnormalities were transient and did not persist as the plants grew new sets of leaves or branches. The maximum percentage of leaf abnormalities recorded was 50% in the EMS 0.75% group, compared to 20% in the control. The maximum percentage of unusual branching patterns recorded was 50% in the EMS 0.5% group, compared to 20% in the control. In many cases these abnormalities may not have been mutations but may have been caused by environmental conditions such as insect damage, sunburn, and crowding. For the colchicine treatment groups, 1.8% of the 0.1% colchicine group, 4.9% of the 0.25% colchicine group, 22.9% of the 0.5% colchicine group, and 15.2% of the colchicine 75% group had thick, misshapen leaves with prominent midribs, compared to 0% of the control. Ten of the thick-leaved plants had stomata guard cells that were larger than the control. Six were shown by flow cytometry to have a greater amount of DNA per cell than the control. They will be grown to maturity to observe their horticultural traits.

Keywords: apomixis, colchicine, ethyl methane sulphonate, *Garcinia mangostana*, genetic diversity, morphology, mutagen, polyploid

1. INTRODUCTION

Mangosteen is an important fruit crop in Southeast Asia. Thailand is the world's largest exporter of mangosteen, followed by Indonesia, Vietnam, Malaysia, and the Philippines (Food and Agriculture Organization of the United Nations, 2011). In Thailand, mangosteen orchards cover approximately 66,403 hectares (Office of Agricultural Economics, 2016). Mangosteen is valued for its delicious fruit and the juice is promoted as a healthy beverage. Mangosteen rind also contains antibiotic and antioxidant substances and can be used to make a wide variety of cosmetic products. It is very popular as an ingredient for soap, lotion, facial masques, scrubs, and anti-acne cream. Some manufacturers also put mangosteen rind in deodorant, feminine hygiene products and toothpaste.

Mangosteen is adapted to the climactic conditions of the humid tropics where it originated, so it is suited to a temperature range of 25-35°C. Mangosteen trees need high humidity and moist conditions most of the year but require a dry period to stimulate flowering (Chaisrichonlathan & Noomhorm, 2011; Suwanseree, 2017). The trees are very slow-growing compared to many tropical crops. The seedlings and even mature trees require shade to protect them from sunburn (Verheij & Coronel, 1992). All these constraints have limited the amount of land area used for growing mangosteens.

The main physiological disorders of mangosteen fruit that affect fruit quality are gummosis (also called Gamboge disorder), translucent flesh disorder and hardened rind. Gummosis is when yellow latex seeps from the stem or rind on to the edible aril in the fruit, giving it a yellow color and very bitter flavor. Gummosis is suspected to be related to damage from thrips or other insects (Yapwattanaphun, 2008). Most export markets (for example, Taiwan, Korea, Saudi Arabia, and United Arab Emirates) will not accept mangosteens with spots of yellow latex on the outer rind (Dorly *et al.*, 2011). Gummosis normally affects between 9-50% of fruits in Indonesia (Kurniadinata *et al.*, 2016).

Botanically, mangosteen is unusual because it is an obligate apomict, producing viable seed without pollination. Research showed that pollen does begin to develop in the staminodes of mangosteen flowers, but it degenerates either before or soon after it is completely formed (Yapwattanaphun *et al.*, 2008). New plants arise from unfertilized seeds through adventitious embryony. Because there is no pollination, all mangosteen trees should theoretically be genetically identical clones of the same mother plant. However, some genetic variation could occur through natural mutations due to UV radiation or mistakes in DNA replication. Several genetic marker studies have been done to assess the genetic uniformity among mangosteen trees from different geographical areas or parentages, and they all reported a certain degree of genetic variation (Mansyah *et al.*, 2013; Ramage *et al.*, 2014; Matra *et al.*, 2014; Mansyah *et al.*, 2010).

There are 2 cultivated varieties of mangosteen recognized by the National Seed Industry Council of the Philippines – 'UPLB Sweet' registered in 2006 and 'Roxas Purple' registered in 2007 (Namuco, 2008). Namuco (2007) states, "There is no available record regarding the origin and parentage of 'UPLB Sweet' mangosteen." It was developed from a tree growing at the University of the Philippines Los Baños. Similarly, "there is no recorded account on the origin and parentage of 'Roxas Purple' mangosteen." It was developed from a tree growing in the orchard of Mr. Marino Roxas in Laguna (Namuco, 2008). The listed fruit characteristics of these cultivars appear to be quite similar to ordinary mangosteen, so it is debatable that they should be considered cultivars.

In Malaysia and Singapore some vendors sell another variety of mangosteen known as 'Masta', 'Mesta', or 'Japanese mangosteen'. 'Masta' is also used instead of *manggis* for the common name of mangosteen in some areas. 'Masta' has fruit that is more ovoid than globose in shape, with more pointed ends. It is possible that it is the fruit of a closely related species, *Garcinia malaccensis*. *G. malaccensis* may soon be considered a sub-species, based on genetic and morphological data that *G. malaccensis* and mangosteen are actually so closely related that they should be combined together and considered as one species (Nazre, 2017).

Because by nature mangosteen trees do not produce viable pollen, crossing to create new hybrids is not possible. In light of this limitation, alternative methods should be used to create

greater genetic variation in mangosteen for the selection of superior cultivars. One approach is to induce random mutations using radiation or chemical treatment. This method is much less precise than genetic modification, it is not banned by governments, feared by consumers, nor criticized activists. In this research we used ethyl methanesulfonate (EMS) and colchicine as chemical mutagens to induce mutations in mangosteen seeds.

EMS is an alkylating agent. It reacts with DNA, converting guanine to alkylated guanine. This can result in base substitutions, depurination or single-strand breaks (Wongpiyasatid, 1997). EMS has successfully been used to develop new cultivars of several agronomic and horticultural crops including beans, oats (Arias & Frey, 1973; Rines, 1985), barley (Arian, 1974), rice (Augustine *et al.*, 1975), black gram (Rao & Jana, 1976), and banana (Omar *et al.*, 1989; Bhagwat & Duncan, 1998)

Colchicine is an alkaloid obtained from the root of *Colchium autumnale* L. or *Iphigenia indica* Kunth et Benth that can be used to induce mutations, especially chromosome doubling, because it binds with tubulin and thus interferes with microtubule formation during mitosis (Kingsbury, 2009). Because polyploid plants are often larger and more robust than plants with the normal chromosome number, scientists have used colchicine to induce tetraploidy in many crops, especially ornamentals. The extra set of chromosomes can stimulate the expression of a greater range of genetic variation, such as changes in leaf size, flower size or flower color (Osborne, *et al.*, 2003). Colchicine has been successfully used to induce polyploidy in fruit crops such as citrus, banana, pear, pomegranate, grape and persimmon (Zeng *et al.*, 2006).

2. MATERIALS AND METHODS

Mangosteen seeds were removed by hand from the flesh of ripe fruit obtained from a wholesale market in Bangkok. They were washed in detergent, rinsed with tap water and then agitated for 2 h (shaken on an Innova 2300 orbital shaker at 105 rpm) in an anti-bacterial and anti-fungal solution containing 0.1% Kanker-X and 0.1% Captan orthocide. Next, the seeds were removed from the anti-bacterial and anti-fungal solution in a laminar flow hood and surface sterilized in 20% Clorox solution for 20 minutes, followed by 10% Clorox solution for 10 minutes, then rinsed with autoclaved water three times. Lastly, the seeds were randomly dispensed into jars containing the different chemical mutagen treatments: aqueous solutions of EMS at the concentrations of 0 (control), 0.1%, 0.25%, 0.5%, and 0.75%; and colchicine at the concentrations of 0 (control), 0.1%, 0.25%, 0.5%, and 0.75% for 16 hours in closed jars on an orbital shaker.

For the first, second, and third EMS replications, 25 seeds were used for each treatment group, while in the fourth and fifth replications the samples sizes were 38 and 32 seeds per treatment group. For both times colchicine was used, the sample size was 34 seeds per treatment group.

After the mutagen treatment, the seeds were rinsed in autoclaved dH2O 5 times and placed, one to a jar, in tissue culture vessels containing 25-30 ml MS medium supplemented with 0.5 g l^{-1} PVP, 3% sucrose and 7 g l^{-1} agar (pH 5.7). They were maintained at 25±5°C with an 8:16 h photoperiod until leaves had emerged.

After approximately 50 days in tissue culture, surviving seedlings were planted out in seed trays filled with peat moss (Kekkilä), sprayed with benomyl fungicide and the seed trays were covered with a plastic bag and placed in the shade. After approximately 36 days the seedlings were transferred to 25 cm plastic pots filled with KU potting mixture (compost : rice husk charcoal : coconut husk chips : fine ground coconut husk, 1:2:4:4 by volume) and kept in partial shade. They were fertilized with 15-15-15 formula slow-release fertilizer every 3 months and watered

daily. Plant height and the width and length of the largest pair of leaves and the newest pair of leaves were recorded every 3 months.

Stomata guard cells of 12 month old colchicine-treated plants were compared by microscopic analysis. Impressions were made of the underside of mature leaves by applying a coat of clear nail polish over an area of about 2-3 square centimeters on one side of the leaf (around the middle of the leaf from midvein out to the margin) and waiting approximately 15 minutes for it to dry, then carefully peeling it off. The leaf impressions were viewed at 40x magnification with a Xenon light microscope and photographed with Future Winjoe software.

Selected specimens from the colchicine treatments that had thick, misshapen leaves and were presumed to be polypoid were analyzed by flow cytometry at the floriculture breeding lab of Dr. Chalermsri Nontaswatsri at the Faculty of Agricultural Production, Maejo University, in Chiang Mai, Thailand. A piece of leaf lamina from a newly expanded leaf measuring about 1.5 cm square was finely macerated with a razor blade in 1% PVP aqueous solution with Tris MgCl buffer, then filtered through a 41 µ nylon net and chilled at approximately 5° C for 15 minutes, then vortexed and Guava® Cell Cycle Reagant dye was added. The samples were analyzed in a Guava EasyCyteTM flow cytometer (EMD Millipore Corp.).

3. RESULTS

For the seed experiments, 8 batches of surface-sterilized seeds from ripe fruit were soaked in EMS or colchicine solution of varying concentrations, or in plain water for the control, for 16 hours and then after rinsing were left to sprout in vitro.

In the initial test, (sample size 25 seeds per treatment) the survival rate was 96% for control and 48% for 0.5% EMS but dropped to zero for the 1%, 1.5%, and 2% EMS treatment groups. As a result, lower concentrations were used for the subsequent experiments – 0.75%, 0.5%, 0.25%, 0.1%, and 0% EMS, and the same concentrations were used for colchicine.

A total of 130 seeds per treatment group were treated with EMS, divided into 4 replications spanning August 2016 to June 2017. As expected, the survival rate dropped with increasing concentration of EMS. The lethal dose or LD50 for EMS (the concentration at which half the seeds died) was calculated to be 0.43% (Figure 1).

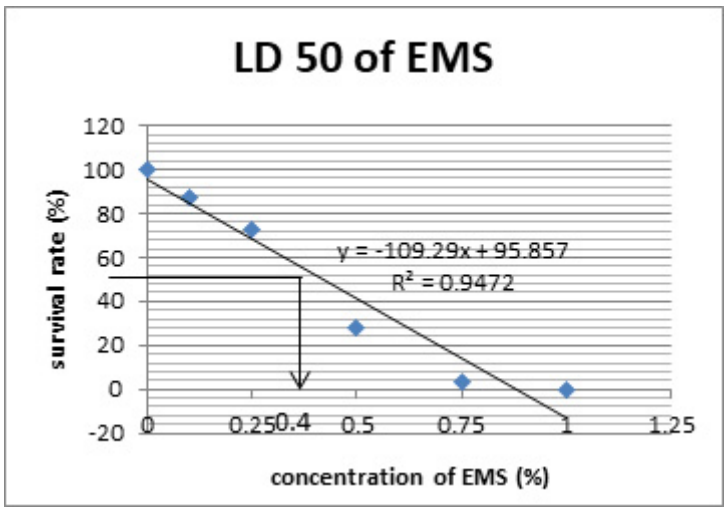

Figure 1. LD50 of EMS for mangosteen seeds exposed to aqueous solutions of EMS for 16 hours

Two batches of seeds were treated with colchicine at the same concentrations and duration as that was used for EMS. The sample size was 34 seeds per treatment. The survival rate was high for all concentrations of colchicine, but dropped slightly with increasing concentration, from 97% in the control down to 94% in the 0.1% and 0.25% colchicine groups, 92% in the 0.5% colchicine group and 85% in the 0.75% colchicine treatment group. It was not possible to determine the LD50 at the concentrations of colchicine tested because the survival rate was high for all concentrations tested.

Several morphological abnormalities were observed in mutagen-treated seedlings, as well as in some of the control seedlings, both when they were first removed from the tissue culture jars, and later when they were growing in the nursery. Many of the leaf abnormalities were no longer observable after the plants developed 3 or more pairs of leaves. However, many of the abnormalities, such as lack of a main root, almost certainly affected the later survival of the seedlings.

Out of the control group, about 6% of sprouting seeds had no root at 8-10 weeks after treatment time. Of the EMS group, the percentage of seedlings with no root increased with increasing concentration of EMS (6%, 19%, 25%, and 29% had no root in the EMS 0.1%, 0.25%, 0.5%, and 0.75% groups, respectively). For colchicine, the percentage of seedlings with no root was rather high in every treatment group at 25%, 18%, 17%, and 24% in the colchicine 0.1%, 0.25%. 0.5% and 0.75% groups, respectively (Figure 2).

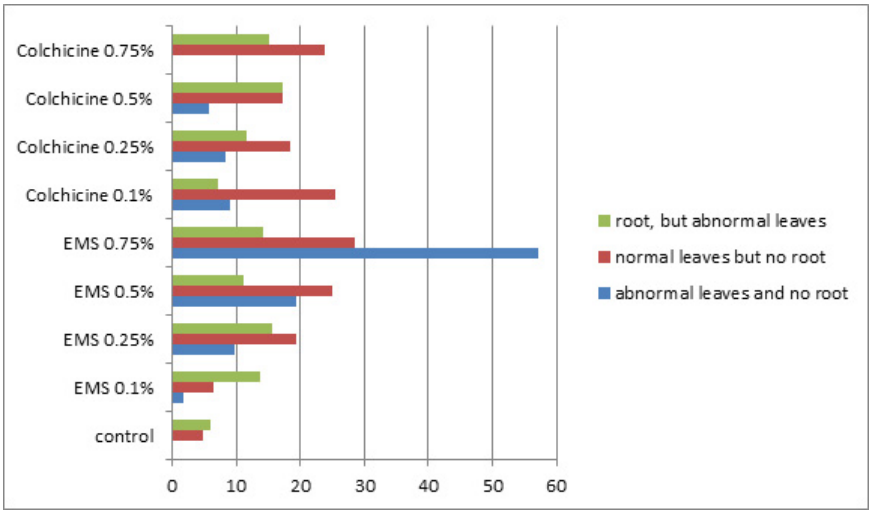

Figure 2. Percentage of abnormal seedlings 9 weeks after treatment. Note: "normal leaves, no root" means 1 or more pairs of approximately symmetrical leaves and no visible root, or root less than 3 mm in length; "root but abnormal leaves" means normal primary or main root or both with a single leaf per node, 3 leaves per node, a pair of leaves of unequal size, misshapen leaves, or leaves arising almost directly from the seed with almost no stem; "abnormal leaves and no root" means no visible root or root less than 3 mm in length and a single leaf per node, 3 leaves per node, a pair of leaves of unequal size, misshapen leaves, or leaves arising almost directly from the seed with almost no stem.

Approximately 6% of control seedlings had leaf abnormalities (one leaf larger than its pair, or misshapen, or an odd number of leaves). In the EMS group, the number of seedlings with abnormal leaves at planting out time was about double that in the control for every concentration of EMS (14%, 16%, 11%, and 14% for the EMS 0.1%, 0.25%, 0.5%, and 0.75% groups, respectively). For the colchicine group, the percentage of seedlings with leaf abnormalities was only slightly higher than in the control for the lowest concentration (7% in the 0.1% colchicine group), but

quite higher than in the control in the other groups (12%, 17%, and 15% in the 0.25%, 0.5% and 0.75% colchicine groups, respectively). This high occurrence of abnormalities in the treatment groups definitely suggests that mutations were induced by the treatments. However, because there was a 6% occurrence of root and leaf abnormalities in the control seedlings, it is possible that some of the abnormalities observed at the planting out stage may have been a result of the tissue culture process rather than the mutagens, or simply natural variation (Figure 2).

Most striking was the number of seedlings with both no root and with abnormal leaves among the EMS-treated seedlings, which was much higher than control and the percentage increased with increasing concentration of EMS. None (0%) of the control seedlings fell in this category, but 2%, 10%, 19%, and 57% of the seedlings in the EMS 0.1%, 0.25%, 0.5%, and 0.75% groups, respectively, had no root and abnormal leaves. The number of colchicine-treated seedlings that had both no root and abnormal leaves was 9%, 8%, 6%, and 0% in the 0.1%, 0.25%, 0.5% and 0.75% colchicine groups, respectively (Figure 2).

In the nursery, the main abnormalities observed over the first 2 years were in the following categories: leaf abnormalities (irregularly shaped leaves, asymmetrical leaves, missing or extra leaves, or wavy or ridged leaves), unusual branching pattern (new branches arising from the base of stem or near the top, early development of lateral branches before 27 months, multiple branches from one part of the stem rather than pairs, or asymmetrical branching), curved stem, and recumbent growth habit.

Some of the morphological abnormalities were transient in nature and did not persist as the plants grew new sets of leaves or branches. The number of samples in each treatment group also changed over time as some of the plants died for various reasons (primarily sunburn). The following data are therefore approximate percentages. The maximum percentage of leaf abnormalities recorded was 50% in the EMS 0.75% group (a group with a very small sample size because of the lethal effect of EMS), followed by approximately 31% in the EMS 0.5% group, 21% in the EMS 0.1% group, 20% in the control, and 12% in the EMS 0.25% group. The maximum percentage of unusual branching patterns recorded was 50% in the EMS 0.5% group, followed by 24% in the EMS 0.1% group, 20% in the control, and 19% in the EMS 0.25% group. The maximum percentage of curved stems was 29%, recorded in the control group, followed by 19% in the EMS 0.25% group, and 10% in the EMS 0.1% group. The occurrence of recumbent habit was 9% in both the EMS 0.1% and 0.25% groups, followed by 8% in the control. In many cases these abnormalities may not have been mutations but may have been caused by environmental conditions such as insect damage, sunburn, lack of sufficient water and lack of space, because the seedlings were spaced very closely together in plastic pots.

Morphological variations were more easily observable in the colchicine-treated groups compared to the EMS-treated groups, as several plants from the colchicine groups displayed a thick leaf phenotype that may be indicative of induced polyploidy (Figure 3). Many of the colchicine-treated plants also exhibited early or unusual branching patterns, short internodes (stunted appearance), or curved stems (Table 1).

Table 1. Percent morphological abnormalities in 12-15 month old mangosteen seedlings from colchicine-treated treatment groups and control.

Phenotype	Control (n=15)	0.1% colchicine (n=55)	0.25% colchicine (n=61)	0.5% colchicine (n=48)	0.75% colchicine (n=46)
Thick, misshapen leaves with prominent midrib	0%	1.8%	4.9%	22.9%	15.2%
Short internodes	6.7%	10.9%	13.1%	25%	17.4%
Unusual branching	20%	25.5%	26.2%	12.5%	30.4%
Curved stem	0%	3.6%	3.3%	6.3%	4.3%

Leaf impressions were taken of 12-month-old seedlings from the colchicine trial. Ten of the plants with thicker leaves and midribs as pictured in Figure 3 had stomata guard cells that were noticeably larger than the control – 2 from the 0.25% treatment group, 3 from the 0.5% treatment group and 5 from the 0.75% treatment group (Appendix A).

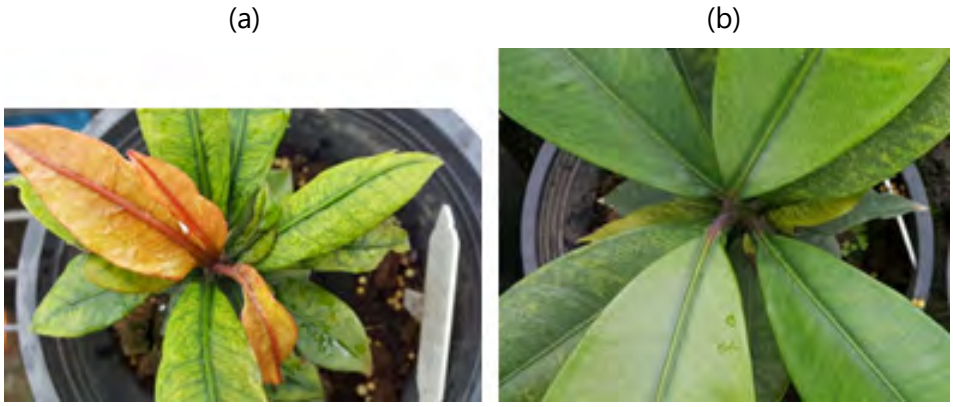

Figure 3. Example of a mangosteen seedling grown from colchicine-treated seed with thick, misshapen leaves and prominent midribs: (a) 0.75% colchicine group, 12 months; (b) control, 12 months.

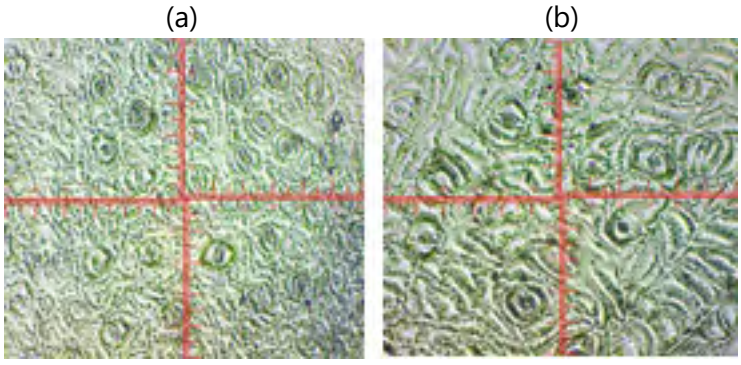

Figure 4. Example of stomata guard cells viewed under light microscope at 40x magnification; (a) control, (b) specimen #7 from the 0.5% colchicine treatment group. For additional stomata comparison photos, see Appendix A.

Flow cytometry analysis was used to determine the amount of DNA in the cells of experimental plants that were selected as putative polyploids based on morphological characteristics and examination of stomata guard cell size. Five specimens were confirmed to be polyploid based on the amount of DNA – 2 from the 0.5% colchicine treatment group and 3 from the 0.75% colchicine treatment group (for dendrograms, see Appendix B). This data affirms that treatment

with colchicine at the rate of 0.5-0.75% applied in aqueous solution on mangosteen seeds for 16 hours can induce polyploidy.

4. DISCUSSION

The LD50 for EMS was 0.43% (Figure 1). This is similar to the findings of Te-chato, who reported that the LD50 was 0.5% EMS when young leaves of mangosteen were exposed to varying concentrations of EMS in aqueous solutions for 2 hours (Te-chato, 1998). Te-chato and colleagues also reported a growth inhibition rate of 50% at the concentration of 0.5% EMS for in vitro mangosteen callus tissue when it was exposed to EMS in aqueous solutions for 2 hours (Te-chato & Phrommee, 1999).

Some of the seedlings from both the control and the mutagen-treated treatment groups were very small or abnormal at the planting out stage, and most of those tended to die off during the first few months. Some of the surviving mutagen-treated plants seemed to grow somewhat faster than the control, and this could be because they were hardier individuals that were able to withstand the mutagen treatment. Another important factor explaining much of the variation in our results is that it was not possible to make the conditions in the nursery absolutely identical for all the experimental plants. For example, some that were in pots that were at the end of a row or in the side-most rows were exposed to more sunlight than the others and may have suffered more sunburn or insect damage.

However, some of the abnormalities observed were probably due to EMS and colchicine treatment. EMS-induced mutations reported in the literature include several changes in leaf shape such as narrow, crinkled leaf phenotype in black gram beans (Rao & Jana, 1976), unspecified leaf morphology changes in oat (Chawade *et al.*, 2010), thin, elongated leaves in *Silene latifolia* (white campion flower) (Jenkins *et al.*, 2005), curly leaf phenotypes in rapeseed (Wang *et al.*, 2008), brown midribs, erect leaves and multiple tillers in sorghum (Xin *et al.*, 2008), and taller plants with more branches and more and larger leaves in mulberry (Kumar *et al.*, 2013). In the present research the occurrence of curved leaves, unequal sizes for leaf pairs, notched leaves and heart-shaped leaves were noted in the EMS-treated seedlings and also some of the control group, but none of these changes in morphology were permanent and usually the next pair of leaves that developed was normal, so they were probably not due to permanent genetic mutations.

Other EMS-induced mutations reported in the literature include changes to plant size. For instance, both dwarf and giant mutants were identified from among oat plants grown from seeds exposed to EMS (Chawade *et al.*, 2010). Other researchers reported some dwarf and also some oversized plants and decreased fruit size in tomato (Gady *et al.*, 2009), increased plant height in papaya (Santosh *et al.*, 2010), shorter internodes in asparagus (Sonoda *et al.*, 2008), dwarf plants in Indian mustard (Prem *et al.*, 2012), and short internodes in mulberry (Kumar *et al.*, 2013). In this research we did note short internodes on many plants in both the EMS-treated groups and the control and we suspect this may be due mainly to environmental factors.

Another category of EMS-induced mutations often reported in the literature is chlorophyll variations, such as albino, viridis, xantha, striata and intermediate leaves in barley seeds (Aram, 1974); albino, yellow, and variegated leaves in *Tillandsia fasciculata* (Koh & Davies, 2001); increased chlorophyll content and increased yield in yam (Sahoo *et al.*, 1986); chlorophyll deficiency and decreased yield in diploid oat (Rines, 1985); orange pericarp and short plant height in capsicum (Bhargava & Umalkar, 1989); chlorosis in oat (Chawada *et al.*, 2010); chlorophyll deficiency in

tomato (Gady *et al.*, 2009); and variegated leaves in *Silene latifolia* (Jenkins *et al.*, 2015). In the present research chlorophyll content was not tested. Some plants did have more pale green or yellowish leaves at some times, but again, this characteristic was observed in the control group as well, and the change was not a permanent one. When newer pairs of leaves developed (especially in the rainy season) they usually reverted to the normal color.

As for cases in which EMS has successfully been used to generate commercially useful mutations in crop plants, some examples include increased gum content in senna (Bhargava & Umar, 1989), abscisic acid insensitivity leading to reduced grain dormancy in wheat (Schramm *et al.*, 2012), auxin resistance in rice (Meng *et al.*, 2009), tolerance to *Fusarium oxysporum* fungus in banana (Bhagwat & Duncan, 1998), and tolerance to *Rhizoctonia solani* (damping off fungi) in wheat (Okubaka *et al.*, 2009). It was beyond the scope of the present research to test pathogen resistance and xanthone content in the mutagen-treated mangosteen plants, but it would be very lucky indeed if any of the specimens had such qualities.

Some preliminary work has been done in the past using colchicine as a mutagen on mangosteen. Te-chato and Sujaree (1999) treated shoot buds of mangosteen with colchicine at concentrations ranging from 500 up to 10,000 mg l^{-1} and durations ranging from 2 hours to 30 days. At the concentration of 10,000 mg l^{-1} and exposure time of 10 hours the survival rate of shoot buds treated with colchicine dropped to 58%, and after exposure of 30 hours the survival rate dropped to 12% (Te-chato & Sujaree, 1999). In another study, 20-day-old mangosteen callus tissue was cultured on media containing colchicine for 30 days. Morphological abnormalities such as 5 roots growing from one shoot and 3 leaves from one node were observed, but the shoots did not survive to the stage of transfer to the greenhouse (Te-chato & Sujaree, 2000). Te-chato and Sujaree (1999) also observed enlarged stomata guard cells on the leaves of mangosteen plants grown from shoot buds that were treated with colchicine at concentrations of 750 mg l^{-1}, 3,000 mg l^{-1}, and 10,000 mg l^{-1} for 2 hours (Te-chato & Sujaree, 1999). Stomatal guard cell size has been used as an indicator of ploidy level in coffee (Mishra, 1997) and rye grass (Speckman *et al.*, 1965).

We recorded 10 colchicine-treated plants with enlarged stomata guard cells, and 5 of these were confirmed by flow cytometry to contain more DNA than the control, indicating polyploidy. Our findings are consistent with the work of Blasco *et al.* (2015), who obtained polyploid loquat plants after treating the seeds with 0.5% colchicine for 24 hours (Blasco *et al.*, 2015). The results are also similar to those of Rey *et al.* (2002), who induced tetraploidy in *Ilex paraguariensis* by exposing zygotic embryos to 0.5% colchicine solution for 48 hours (Rey *et al.*, 2002). The success rate of the present research was greater, however, because Rey et al (2002) found only 2 tetraploid plants in 152 specimens whereas we detected 2 out of 48 in the 0.5% colchicine group for a polyploidy induction rate of 4.2% in that group, and 3 out of 46 in the 0.75% colchicine group, for a polyploidy induction rate of 6.5% in that group. Based on the results, we recommend using colchicine at a concentration of 0.75% to induce polyploidy in mangosteen.

The colchicine-treated plants are less than 2 years old now at the time of writing and are still very small, but if possible they will be raised to maturity to see what characteristics they exhibit. It is possible that having double or quadruple the number of chromosomes will enable the plants to express more genetic diversity.

5. CONCLUSION

The objective of this research was to increase genetic diversity in mangosteen through mutation

breeding. Some of the seedlings from the EMS treatments did exhibit different morphological characteristics from standard mangosteen seedlings but genetic testing has not yet been performed. The colchicine treatments did induce polyploidy, with a polyploidy induction rate of 4.2% - 6.5%. Hopefully, the experimental plants from both the EMS and colchicine treatment groups will be able to be grown to full size and will produce fruit in the future. Then they can be assessed to discover if their characteristics are valuable for commercial production or not. If any of them prove to be of use in the future, then they can be propagated through cloning and also used for a breeding program.

AUTHOR CONTRIBUTIONS

Conceptualization, C.Y., V.S. and S.P.; Methodology, C.Y., S.P. and V.S.; Validation, C.N., C.Y. and S.P.; Formal Analysis, V.S. and C.Y.; Investigation, V.S.; Resources, S.P., C.N. and C.Y.; Data Curation, V.S.; Writing-Original Draft Preparation, V.S.; Writing-Review & Editing, S.P. and C.Y.; Visualization, V.S. and S.P.; Supervision, S.P. and C.Y.; Project Administration, C.Y.

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APPENDIX A. STOMATA GUARD CELL COMPARISON PHOTOS

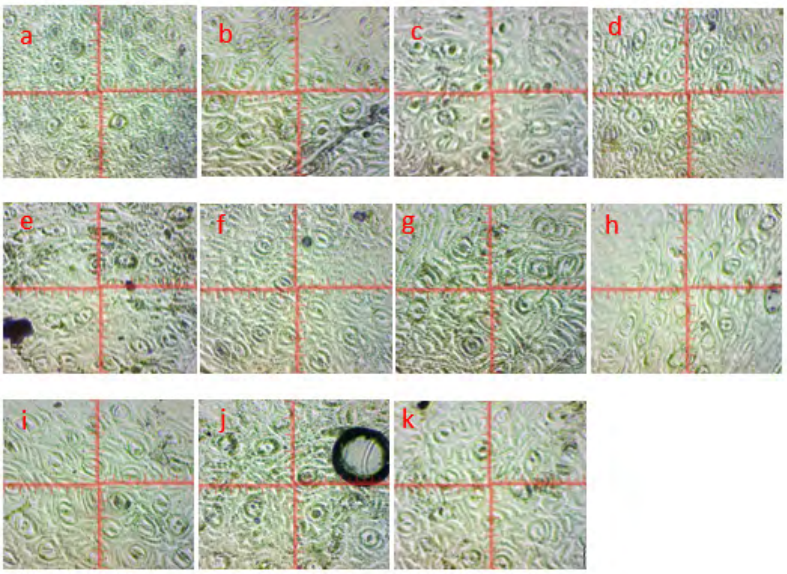

Figure A1. Stomata guard cells viewed under light microscope at 40x magnification; (a) control, (b) specimen # 19 from the 0.75% colchicine treatment group, (c) specimen # 3 from the 0.75% colchicine treatment group, (d) specimen # 9 from the 0.75% colchicine treatment group, (e) specimen # 17 from the 0.75% colchicine treatment group, (f) specimen # 4 from the 0.75% colchicine treatment group, (g) specimen #7 from the 0.5% colchicine treatment group, (h) specimen #19 from the 0.5% colchicine treatment group, (i) specimen # 20 from the 0.5% colchicine treatment group, (j) specimen #16 from the 0.25% colchicine treatment group, (k) specimen # 22 from the 0.25% colchicine treatment group.

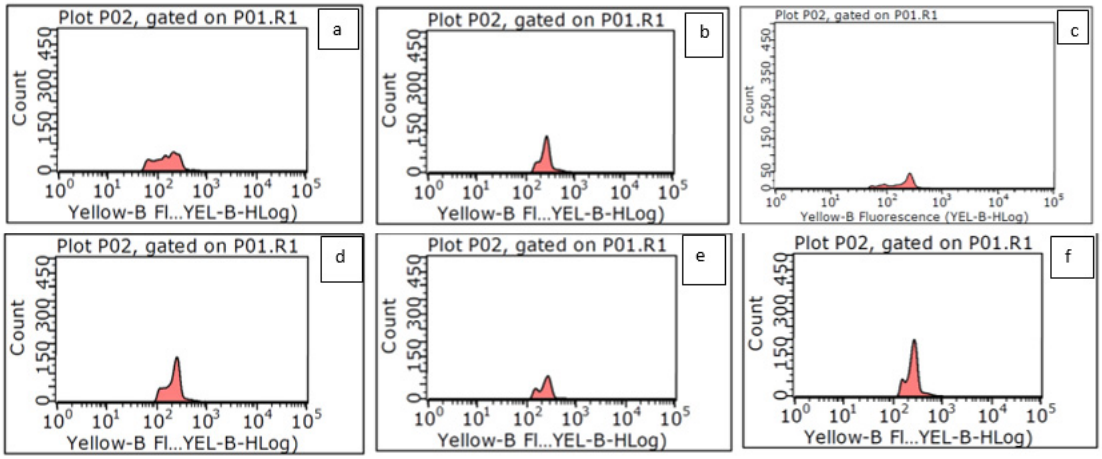

Figure B1. Flow cytometry histogram showing the frequency of cells counted with intensity of fluorescence, indicating amount of DNA detected; (a) control, (b) specimen #19 from the 0.5% colchicine treatment group, (c) specimen #20 from the 0.5% colchicine treatment group, (d) specimen #4 from the 0.75% colchicine treatment group, (e) specimen #9 from the 0.75% colchicine treatment group, (f) specimen #19 from the 0.75% colchicine treatment group.

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THE EVALUATION OF RESISTANT GENE ANALOGUES (RGAS) ON TWO WILD *MUSA* SPECIES AGAINST FUSARIUM WILT DISEASE

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ABSTRACT

Information about the molecular aspects of genes that control the mechanism of resistance to *Fusarium oxysporium cubense* (*Foc*) wilt disease is still very limited. Most of resistance genes (R genes) encode receptor proteins. About 70% of receptor proteins of R genes contain the domain nucleotide binding site and leucine rich repeat (NBS-LRR) which control resistance to pathogens. The expression of resistant gene analogues (RGAs) on *Foc* infected banana plantlets will be evaluated in this study. The two wild *Musa* species (*Musa acuminata* ssp. *halabanensis* and *Musa balbisiana* from Nusa Tenggara Timur) used in this research are native to Indonesia. Two *Foc* isolates (TR 4 or VCG 01213/16 and race 1 or 0124/5) were used for evaluation. Four RGAs were used on semi quantitative RT-PCR of *Foc* infected root samples. Two RGAs namely MNBS16 and RGC2 were expressed on only the infected plants, while MNBS5 and MNBS15 were expressed on both infected and uninfected plants. From this study it can be concluded that MNBS16 and RGC2 were involved in the resistance mechanism of banana against *Foc*.

Keywords: RGAwild, *Musa* species, *Fusarium* wilt

1. INTRODUCTION

Plant growth is strongly influenced by climate factors. In addition to influencing production, climate also influences the development of pathogens, one of which is the *Fusarium oxysporum* f.sp. *cubense* (*Foc*) fungus. *Foc* causes wilt disease on bananas. The use of disease resistant cultivars is one of the strategies to control banana plant diseases (Rowe & Rosales, 1996). Information about the molecular aspects of genes that control the mechanism of resistance to Foc wilt disease is still very limited. Most of resistance genes (R genes) encode receptor proteins. About 70% of receptor proteins contain the domain nucleotide binding site and leucine rich repeat (NBS-LRR) which are involved in resistance mechanism to pathogens such as insect pests, fungi, bacteria, viruses, and nematodes (Dangl & Jones, 2001).

The NBS-LRR receptor protein recognizes pathogen effectors proteins and produces transduction signals that will stimulate the expression of defenses against pathogens (Caplan *et al*., 2008). NBS-LRR gene composition consists of several domains, namely N-terminal, nucleotide binding site (NBS), and C-terminal LRR. The NBS domain contains *p-loop* or kinase-1, kinase-2, kinase-3a and hydrophobic (GLPL) motifs that are conserved in various ATP- / GTP-binding proteins from various organisms (Traut, 1994).

Up to 2013, National Center for Biotechnology Information (NCBI) has registered more than 180 resistant gene analogue (RGA) gene sequences from the NBS-LRR class isolated from bananas. After being reexamined based on nucleotide and amino acid sequences, it is known that from

180 RGAs only 168 RGAs have NBS-LRR characteristics (Pei *et al*., 2007; Peraza-Echeverria *et al*., 2008; Azhar & Heslop-Harrison, 2008; Sun *et al*., 2009; Sutanto *et al*., 2014).

The variety of NBS-LRR type RGAs gives a big picture of the family of NBS on *Musa*. Peraza-Echeverria *et al*. (2008) found an RGA from wild *Musa acuminata* ssp. *malaccensis* that expresses against fusarium fungal infection of race 4. Based on this, it is estimated that there are still many resistance genes to *Foc* wilt disease found in other wild Musa. This study will evaluate the expression of RGAs on two wild Musa species after infected by *Foc* wilt disease, especially for TR 4 (VCG 01213/16) and race 1 (0124/5).

2. MATERIAL AND METHOD

2.1. Materials

The two wild bananas used in the study are native to Indonesia. These were *Musa acuminata* spp. *Halabanensis* and *Musa balbisiana* from Nusa Tenggara Timur. Meanwhile, *Foc* suspension (dH20 + *Foc*) at density 106/mL was used for inoculation of samples. The *Foc* isolates used in this study were VCG 01213/16 (TR4) and VCG 0124/5 (race 1).

2.1. Method

2.1.1. Inoculation of Foc

Seeds of the two wild species were germinated and maintained until 10 cm in height (seedling). The seedling roots were dipped in *Foc* suspension for 15 minutes. The RNA extraction was carried out at 24 and 48 hours after *Foc* inoculation.

2.1.2. Transcript expression analysis

RNA Extraction

The total RNA was extracted from the roots of *Foc* treated or untreated seedlings, using Plant Total RNA Mini Kit (Geneaid, USA) as company instruction.

Semi quantitative RT-PCR

The cDNA was synthesized from total RNA using Tetro cDNA Synthesis Kit (Bioline) based on company intructions. The primers used were the primer pairs that were designed based on:

a. MNBS5: 5′-AAAAGTTCCAGTTGGCGGAC-3′ & 5′-GTCGCTGTCATGGTTGATGG-3′ (Sutanto *et al*., 2014);
b. MNBS15: 5′-CGGAGAGTTAATTCGGTGCG-3′ & 5′-CGTGCTGCTACCTACTGCTT-3′ (Sutanto *et al*., 2014);
c. MNBS16: 5′-TGGGGCACAGATGTATGGGA-3′ & 5′-CCCAATCTCAGGCTCCTCCT-3′ (Sutanto *et al*., 2014);
d. RGC2: (5′-GGGTGTGTGTGTCTGACGAT-3′ & 5′-ATGGGGCTAACAGGCTTTCC-3′ (Peraza-Echeverria *et al*., 2008);
e. 25S rRNA (AY651067) (5′ACATTGTCAGGTGGGGAGTT-3′ and 5′-CCTTTTGTTCCACACGAGATT-3′) (Van den Berg *et al*., 2007). 25S rRNA is usually used as an endogenous internal control because this gene is a housekeeping gene with stable expression in the organism (Van den Berg *et al*., 2007).

f. PCR was carried out in a volume of 25 µL containing 20 ng cDNA, 12.5 µL MyTaq™ Red Mix (Bioline), 1 µL of each 10 µM primer, and double destilled water. The PCR reactions were 3 min. of 95°C and followed by 29 cycles of 95°C for 1 min., 53°C for 10 sec., and 72°C for 10 sec. Finally the reactions were incubated at 72°C for 10 min. PCR products were separated using agarose gel electrophoresis and visualized using a gel doc. The bands of PCR products were quantified using GelQuant.net software (http://biochemlabsolutions.com/GelQuantNET.html) in order to estimate the level of expression.

3. RESULT

Analysis of RGA expression is indicated by the abundance of RNA after wild bananas were inoculated with *Foc* suspension. The abundance of RNA can be quantified based on the thickness of the electrophoresis band. Electrophoresis results on *M. acuminata* spp. *halabanensis* (Figure 1) and *M. balbisiana* (Figure 2) were diverse. MNBS16 primer on *M. balbisiana* did not produce a band. This might have been caused by human errors that occurred during the study.

The band profile in agarose gel is the result of electrophoresis from wild banana cDNA. Normalization of real-time PCR data using control genes is needed to obtain accurate and reliable gene expression results. 25S is one of the internal control genes that will be displayed very uniformly in living organisms during various phases of development and in different environmental conditions (Jain *et al.*, 2006)

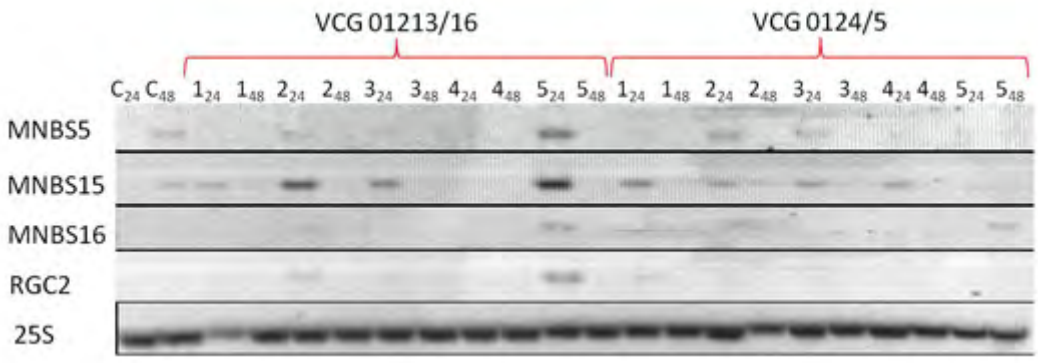

Figure 1: MNBS5, MNBS15, MNBS16, and RGC2=primers, C=without inoculation, 1-5= sample number 1...5 (*M. acuminata* ssp. *halabanensis*) were inoculated by VCG01213/16 (*Foc* TR4) and VCG0124/5 (*Foc* race 1), 24 and 48=24 and 48 hours after *Foc* inoculation.

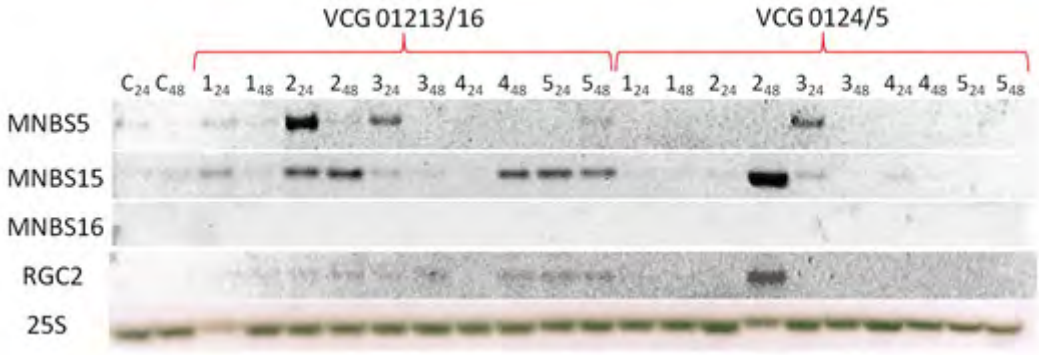

Figure 2: MNBS5, MNBS15, MNBS16, and RGC2=primers, C=without inoculation, 1-5=sample number 1...5 (*M. balbisiana*) were inoculated by VCG01213/16 (*Foc* TR4) and VCG0124/5 (*Foc* race 1), 24 and 48=24 and 48 hours after *Foc* inoculation.

In this research, the abundance of RNA was measured semi-quantitatively using Gelquant.net software. The abundance of RNA is indicated by the level of RGA expression. The presence of RGA expression indicates the activation of genes that can recognize the presence of *Foc* wilt disease (fig. 3-6= *M. acuminata* ssp. *halabanensis* with primers MNBS5, MNBS15, MNBS16, and RGC2; and fig. 7-9= *M. balbisiana* with primers MNBS5, MNBS15, and RGC2).

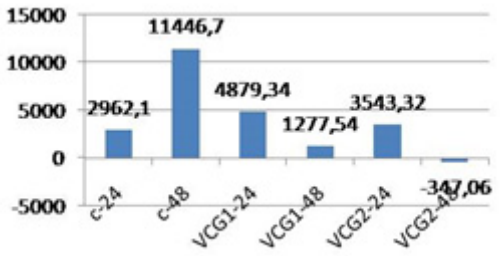

Fig. 3. RGA expression on *M. acuminata* ssp. *halabanensis* using primer MNBS5

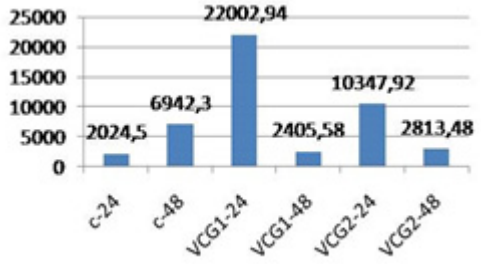

Fig. 4. RGA expression on *M. acuminata* ssp. *halabanensis* using primer MNBS15

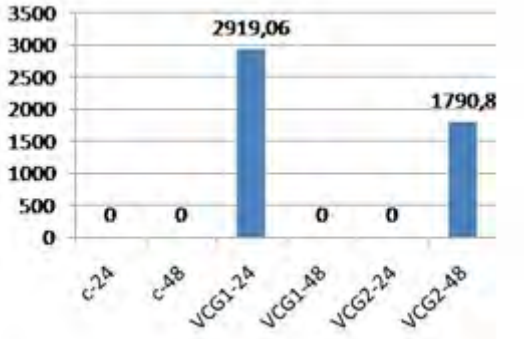

Fig. 5. RGA expression on *M. acuminata* ssp. *halabanensis* using primer MNBS16

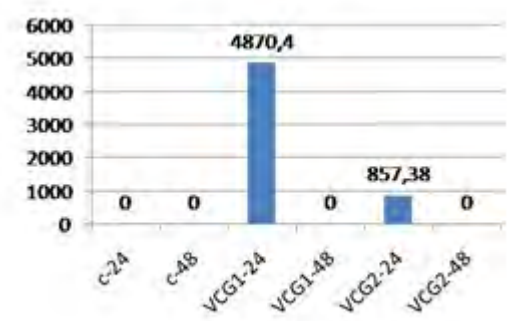

Fig. 6. RGA expression on *M. acuminata* ssp. *halabanensis* using primer RGC2

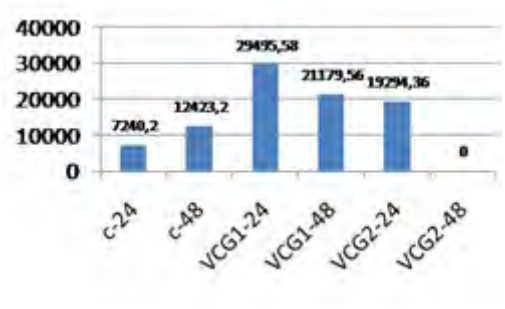

Fig. 7. RGA expression on *M. balbisiana* using primer MNBS5

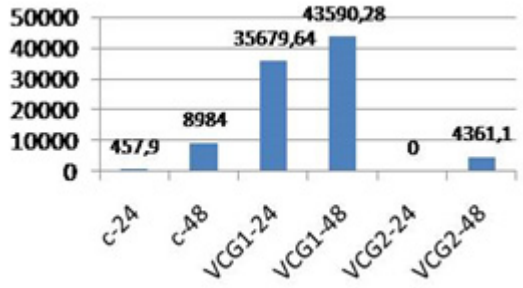

Fig. 8. RGA expression on *M. balbisiana* using primer MNBS15

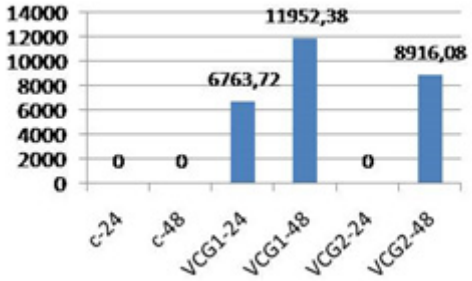

Fig. 9. RGA expression on *M. balbisiana* using primer RGC2

Based on the results of capture of RNA abundance, not all RGA expressions were related to the gene activites against *Foc* infection. In the figures 3, 4, 7, and 8, there were abundance of RNA expressions from MNBS5 and MNBS15 in the control treatment or no *Foc* infection. These data in the figures 3, 4, 7, and 8 showed that MNBS5 and MNBS15 were not involved in the resistance mechanism against *Foc*.

RGA MNBS16 was actively expressed in the roots 24 hours after being infected by *Foc* TR 4 and 48 hours after being infected by *Foc* race 1 on *M. acuminata* ssp. *halabanensis* (fig. 5). In the same wild banana, RGA RGC2 was also actively expressed 24 hours after being infected by *Foc* TR4 and race 1. RGA RGC2 was also actively expressed in the roots of *M. balbisiana* after 24 and 48 hours after infection of *Foc* TR4; and 48 hours after infection of *Foc* race 1.

4. DISCUSSION

The results of this study indicated that there were potential RGAs as candidate genes that can be obtained from Indonesian wild *Musa*, namely *Musa acuminata* ssp. *halabanensis* and *Musa balbisiana* from Nusa Tenggara Timur. The MNBS16 and RGC2 have proven to actively express against *Foc* infection both TR4 (VCG 01213/16) and race 1 (VCG 0124/5). It is likely that the data will be better if the RGA analysis using MNBS16 primer on *M. balbisiana* is repeated. Similar studies of other RGA primers and other wild Indonesian *Musa* will enrich information about RGA against *Foc* wilt disease. The next step that can be carried out is isolating the whole gene sequences. Based on the whole sequences, single-nucleotide polymorphisms can be identified for designing single nucleotide amplified polymorphism (SNAP) markers. The SNAP markers can support breeding programs for *Foc* resistance of banana.

5. CONCLUSIONS

Two RGAs namely MNBS16 and RGC2 were actively expressed in the *Foc* treated plants of *Musa acuminata* ssp. *halabanensis* and *Musa balbisiana*. It can be concluded that MNBS16 and RGC2 are potentially the R gene candidates against Foc TR4 (VCG 01213/16) and race 1 (0124/5).

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BANANA DISEASE CONTROL AS INFLUENCES BY AQUEOUS NEEM LEAVES EXTRACT AND MEDIA PH LEVEL UNDER IN VITRO CONDITION

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ABSTRACT

Banana (*Musa* spp.) is the most popular exported fruit in the world. The most ubiquitous cultivar of banana in the market is the 'Cavendish'. However, the world's most popular banana might soon go extinct due to outbreaks of *Fusarium* wilt disease. Thus, there is a need to identify the effects of different pH media and different concentrations of neem leaves extract to control the *Fusarium* fungus *in vitro*. In the first *in vitro* experiment, *Fusarium oxysporum* f. sp. *cubense* (*Foc*) fungus was grown in different pH levels (5.0, 5.5, 6.0, and 7.0) of potato dextrose agar (PDA) – pH 5.5 was designated the control pH; and in the second experiment, different concentrations of neem leaves extract (0 mL, 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL) were added into 250 mL of PDA. Results from the first in vitro experiment revealed that both pH 5.0 and pH 7.0 had the same significant effect in controlling the colony radius, growth rate, and percentage inhibition of radius growth (PIRG) of *Foc*. Moreover, after 7 days of incubation, colony growth decreased while the PIRG increased significantly with increasing neem leaves extract rate from the second experiment. However, no significant difference of all parameters was recorded for both 8 mL and 10 mL extracts. Thus, the study showed that application of liquid neem leaves extract at 8 mL and 10 mL were effective at inhibiting Fusarium growth under optimum pH (pH 5.0 and pH 7.0). Due to planting conditions of banana plants and soil environment, the best concentration of neem leaves extract, 8 mL per 250 mL media, was suggested to be applied for field applications of banana under optimum pH 7.0 of the soil media.

Keywords: 'Cavendish', outbreaks, *Fusarium* wilt, fungus, colony, growth inhibition, planting condition

APPLICATION OF INTEGRATED PEST MANAGEMENT PACKAGE IN LONGAN PRODUCTION IN THE SOUTH OF VIET NAM

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ABSTRACT

The longan has been ravaged by various pests including the longan gall mite *Eriophyes dimocarpi*, litchi stinkbug *Tessaratoma papillosa*, litchi leafminer *Conopomorpha litchiella*, Yellow peach moth *Conogethes punctiferalis*, and many others. This paper evaluated the benefits of using an integrated pest management (IPM) package composed of using fertilizers with compost inoculated with the antagonistic fungus, *Trichoderma* sp., pruning and destroying infected shoots after harvesting, setting up light and/or pheromone traps, protein bait, Southern Horticultural Research Institute (SOFRI)-ant baits; and spraying the trees with *Beauveria bassiana, Paecilomyces* sp., or *Metarhizium* sp., sulfur, and neem oil. Field trials were conducted at two longan plantations (0.5 ha each) in Tien Giang and Vinh Long provinces from January 2016 to September 2018. Data were collected from two plots from within the 2 plantations: 1) the IPM plot (applying the IPM package), and 2) the control plot (applying farmer practices). Our study revealed that, on average, the IPM package plots significantly reduced the percentage of infected fruit rot, mealybugs, oriental fruit flies, and fruit borer infestations as compared to the control plots at all development stages of the tree. Population of natural enemies of the IPM package plots were higher than the non-IPM plots. Fruit quality of the IPM plots increased as compared to the control plots. Fruit sizes, on average in the IPM package were 11.64 g/fruit and 12.51 g/fruit, bigger than the control plots at 10.15 g/fruit and 10.16 g/fruit in Tien Giang and Vinh Long, respectively. The IPM package reduced the times of pesticide spraying (3–4 times) compared to the control plot. Profit rate on the IPM package was 82.07% and 86% higher than the control at 76.26% and 79% in Tien Giang and Vinh Long, respectively. This implies a high economic benefit from the application of the IPM package and longan farmers would profit significantly if the IPM package is applied on a widescale in longan growing areas in Vietnam.

Keywords: longan, integrated pest management, IPM, pests, diseases

EFFECTS OF $KCLO_3$ DOSES ON BACTERIAL COMMUNITIES IN SOIL CROPPED TO 'E-DAW' LONGAN (*DIMOCARPUS LONGAN* L.) AT THE AGE OF 8 AND 11-YEARS-OLD

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ABSTRACT

This study was aimed to determine the effects of $KClO_3$ doses on the diversity of bacterial communities in the soil of 'E-Daw' longan orchards at the age of 8- and 11-years-old. Experiments were carried out off-season, from February 2018 to February 2019, in Dong Thap province, Viet Nam. A field trial was arranged in randomized complete block design with two factors, each of which had three replications, forty trees per replication. The two levels of tree age, *i.e.* 8- and 11-years-old comprising the first factor; while the second covering the five doses of $KClO_3$, viz. 50g, 100g, 150g, and 200g active ingredient (a.i.) m^{-1} canopy diameter (c.d.) and 2 control treatments (130 and 170 g a.i. m^{-1} c.d. for 8- and 11-years-old trees, respectively). The latter originated from the doses used by growers at the planting location. $KClO_3$ was applied by collar drenching. Fingerprints of 16S rDNA gene of bacterial diversity were amplified by polymerase chain reaction (PCR) and subjected to separation by denaturing gradient gel electrophoresis (DGGE). The latter was estimated by the number of amplified 16S rDNA bands, each of which was assumed to represent a single operational taxonomic unit. Results showed that 8-year-old trees had lower root tip damage rate than that of 11-year-old ones. In DGGE patterns, there was a reduction in the number of bands in all treatments applied with $KClO_3$ regardless of dose levels. In fact, the most significant difference was observed in the two control treatments. It is interesting that $KClO_3$ applied at 150g a.i. m^{-1} c.d. and 200g a.i. m^{-1} c.d. significantly stimulated the population of some specific groups of soil bacteria. These results indicated that the bacterial communities in soil cropped to 'E-Daw' longan were strongly affected by the $KClO_3$ application.

Keywords: 'E-Daw' longan, *Dimocarpus longan*, diversity, bacterial communities, $KClO_3$, tree age

EFFECTS OF ANTI-GRASS CLOTH COVERING ON SOIL AND CITRUS ROOT GROWTH IN ORCHARDS

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ABSTRACT

In order to reduce the use of chemical fertilizers and pesticides, while improving citrus production, it is important to study the effects of grass-proof cloth covering on soil environment, citrus root growth, and fruit yields in the orchard. The single factor experiment design was used to set up soil with the anti-grass cloth covering treatment and without covering (control) treatment. Soil hydrothermal conditions, nutrient levels, root growth, and fruit yield of both treatments were compared. Covering the ground surface with grass-proof cloth increased the soil water content and temperature; availability of phosphorus, potassium, zinc, and manganese, as well as the number of soil microbial population. Root growth and fruit yield also increased. The anti-grass covering can improve the soil hydrothermal condition and the overall nutrient level of a citrus orchard, which may be beneficial to the growth of citrus roots thus increasing productivity.

Keywords: Citrus, cover, soil environment, roots growth

1. INTRODUCTION

Surface mulching cultivation techniques have been widely used in different crop production. For the production of fruits such as apples, the use of surface mulching is also gradually becoming popular. Surface mulching can substantially reduce the occurrence of weeds in cropland and therefore reduce the use of herbicides and associated environmental pollution (Chen *et al.*, 2008) . Moreover, it plays an important role in water holding capacity in soil, modification of soil environment, and improvement of fruit quality (Balwinder *et al.,* 2011; Cook *et al.*, 2006). However, studies on the effects of surface mulching on citrus cultivation and productivity are still insufficient, especially in the high-temperature zone of southern China. There is a research gap to understand the effects of surface mulching on soil physicochemical properties, microbial population, and root growth, which may limit the application of anti-grass cloth mulching techniques in citrus cultivation. This article reports the comparative study of anti-grass cloth mulching on citrus plant growth and development. It may provide some scientific evidence for the use of anti-grass cloth in the citrus industry.

2. MATERIALS AND METHODS

2.1. Study site

The experiment was conducted in a citrus orchard at the Lemon Modern Industrial Park (114°45′E, 24°6′N) of Zhongxing Lvfeng Development Co., Ltd., located in Shuntian Town, Dongyuan County, Heyuan City, Guangdong Province. The study region has an elevation of 70 m, with an average annual temperature of 20.7°C and average annual precipitation of 1567 mm–2142.6 mm. The soil type is classified as latosolic red soil, which contains 1% organic matter with a pH of 5.14.

2.2. Experimental materials and treatments

Two year old disease-free containerized citrus seedlings, (upper trunk diameter = 1.2 cm–1.5 cm, height = 1.5 m) which were scions of the cultivar 'W-Murcott' (*Citrus reticulata* Blanco × *Citrus sinensis* Osb.) grafted onto rootstock of the cultivar 'Ziyang Xiangcheng' (*Citrus junos* Sieb. ex Tanaka) were used in this experiment. Plants were spaced 3.0 m apart within rows and rows were 4.0 m apart. Ridge cultivation was adopted, with ridges of 0.4 m height and 2.0 m width. The plants showed normal growth and comparable growth potential, with a moderate management level.

A single-factor experimental design involving two treatments was used. One treatment used mulching cultivation with grass-proof cloth, whereas the other was open-field cultivation without mulching (control). Each treatment had three replica plots and a total of six plots were involved. There were 17 trees per plot, with 102 trees in total. For all plots of the mulching treatment, the ridges were completely mulched from March 2018 using black grass-proof cloth (an 80 g/m^2 degradable material manufactured by Taizhou Xiahui Trading Co., Ltd., Zhejiang Province, China).

2.3. Measurement parameters and methods

2.3.1. Measurement of soil water content

Soil samples were randomly taken at a depth of 20 cm using a soil sampler. The samples were collected from three points at the periphery of canopy dripline in each plot. Soil water content was measured gravimetrically after oven-drying (Institute of Nanjing Soil Science, 1978) and repeatedly every 30 days.

2.3.2. Measurement of soil temperature

In March 2018, three thermometers were inserted at approximately equal intervals along the vertical projection of the canopy periphery for each treatment. Diurnal variation (average soil temperature days for a year, based on data recorded from 8:00 to 18:00 at 2-hour intervals) and annual variation (average soil temperature of the observation days) in soil temperature of 20 cm depth were observed and recorded from March 15th, twice a month.

2.3.3. Measurement of soil mineral nutrient contents

To analyze soil mineral elements, orchard soil samples were collected in August 2018 (5 months after the start of the experiment for anti-grass cloth mulching). The soil samples were taken at a depth of 20 cm. The samples were kept in zipper bags and transported to the laboratory where they were air-dried, then crushed, ground, and passed through a 2 mm sieve. The sieved samples were stored under ventilated and dry conditions before testing.

The testing of soil nutrient elements was performed using the soil nutrient status systematic approach (PPI/PPIC, 1992). Alkali-hydrolyzable nitrogen content was analyzed using the alkali-hydrolysis and diffusion method. Available phosphorus content was quantified by anti-molybdenum-antimony colorimetry after extraction using a mixture of chloride acid (HCl) and ammonium fluoride (NH_4F). Available potassium, exchangeable calcium, and exchangeable magnesium contents were extracted using an ammonium acetate ($CH_3CO_2NH_4$) solution and then tested by atomic absorption spectrometry. Available zinc, available iron, and available

manganese contents were analyzed based on diet hylene triaminepenta acetic acid extraction-atomic absorption spectrometry, whereas available boron content was analyzed via boiling water extraction-curcumin colorimetry.

2.3.4. Enumeration of soil microbial population

The type and quantity of soil microbes were analyzed using the dilution plate method. Nutrient agar, modified Gause's No. 1 medium, and rose Bengal medium were used for the culture of bacteria, actinomycetes, and fungi, respectively (Li *et al.*, 2017).

2.3.5. Diversity analysis of soil microbial community

The diversity of the soil microbial community under different cultivation modes was analyzed using the Chao1, ACE, Shannon, and Simpson indices.

Surface soil samples (0–20 cm) were collected from the anti-grass cloth mulching and control treatments in November 2018. After recording their details, soil samples were immediately placed in sterile zipper bags and kept in a box with dry ice. Then the samples were sent to the Personal Biotechnology Co., Ltd. (Shanghai, China) for DNA extraction and high-throughput sequencing. The obtained sequences were clustered into operational taxonomic units (OTU) at ≥97% similarity. For each OTU, the most abundant sequence was chosen as the representative sequence. The representative sequences of all OTUs were obtained against template sequences in the corresponding database (Silva, https://www.arb-silva.de/) to acquire the taxonomic information for each OTU.

2.3.6. Measurement of root morphology, physiology, and vigor

Three citrus trees representing average growth potential were chosen from each treatment in January 2019. The samples were prepared according to the protocols by Yang (2014). Briefly, a 40 cm cubic soil block was dug 60 cm from the trunk on the same side of the ridge.

The root samples were scanned using an EPSON root scanner (Expression 10000XL 1.0, Epson Inc., Japan) according to the manufacturer's instructions. The scan results were analyzed using WinRHIZO Pro(S) v.2004b (Regent Instrument Inc., Canada) to obtain root morphological parameters including total length, average diameter, and total surface area. After scanning was completed, the root samples were taken out and dried with absorbent paper, then weighed. Subsequently, the samples were dried in an oven at 80°C to constant weight and then weighed again.

Root vigor was determined using the triphenyl tetrazolium chloride method (Zhu *et al.*, 2018).

The following root parameters were calculated:

Specific root length = total root length (cm) ÷ root dry weight (g)
Specific root surface area = total root surface area (cm2) ÷ root dry weight (g)

2.3.7. Yield estimation

Seven fruit trees with normal growth were randomly selected for each treatment. The number

of fruit on each tree was counted by hand. Fruit yield per tree was calculated as: Yield (kg/tree) = single fruit weight × fruit number per tree.

2.3.8. Statistical analysis

All the experimental data were processed and analyzed using WPS Excel 2016 (Kingsoft Office Software Co., Ltd., Zhuhai, China) and SPSS 18.0 (SPSS Inc., Chicago, USA).

3. RESULTS

3.1. Effect of mulching on soil water content

Generally, soil water content exhibited consistent trends under the two different cultivation modes (Figure 1). The changes in soil water content were mainly affected by precipitation conditions. Higher soil water content occurred during the hot rainy period in summer, whereas soil water content decreased during the cold dry period in winter. However, the mulching treatment resulted in higher soil water content than the control across most of the experimental period. The difference in soil water content between the two treatments was especially evident during the hot rainy period in May, June, and July. In contrast, this difference gradually diminished in the winter, and soil water content was even higher under control than the mulching treatment on January 26th. The maximum difference in soil water content between the two treatments was found on May 20th (4.4%), and the maximum difference appeared on October 20th (0.7%).

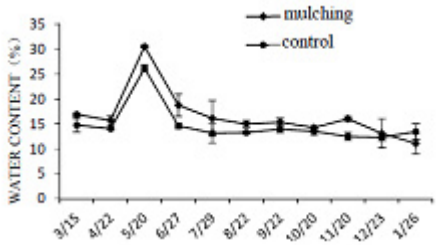

Figure 1. Effects of Covering on Dynamic Changes of Soil Water Content in Citrus Orchards

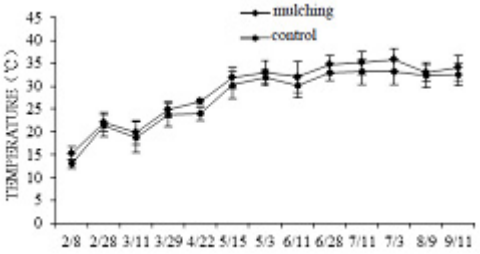

Figure 2. Effect of Coverage on Annual Change of Soil Temperature in Citrus Orchards

3.2. Effect of mulching on soil temperature

Generally, the annual variation in soil temperature exhibited consistent trends under the two cultivation modes (Figure 2). Mulching with anti-grass cloth showed an obvious warming effect, as the soil temperature of the mulching treatment was higher than that of the control across various growth periods of citrus. The maximum difference in soil temperature between the two treatments appeared on April 22th (2.7°C), with the minimum difference found on February 28th (0.7°C).

3.3. Effect of mulching on soil mineral nutrient contents

Soil mineral nutrient levels in the orchard were analyzed after five months of mulching with anti-grass cloth and the results are presented in Table 1. The use of anti-grass cloth had a significant effect on the contents of soil major elements. After mulching with anti-grass cloth, soil alkali-hydrolyzable nitrogen content decreased slightly, but not significantly, compared

with the control. However, both soil available phosphorus and potassium contents increased significantly after mulching. The available phosphorus content changed from 32.3 to 41.6 mg/kg, whereas the available potassium content increased by 5.0 mg/kg. These indicated that the cultivation mode of anti-grass cloth covering was beneficial to improve the contents of soil major elements in citrus orchard.

Table 1a. Effects of Mulching on Mineral Nutrient Content in Citrus Orchard Soil.

Treatment	Alkali-hydrolyzable N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Exchangeable Ca (mg/kg)
Mulching	56.17±2.76a	41.63±2.40a	62.80±0.30a	1310.67±58.00b
Open-field	58.87±0.35a	32.33±0.80b	57.77±1.50b	1539.00±38.74a

Note: Different lowercase letters in the same column indicate significant difference at P≤0.05. The same as other tables.

Table 1b. Effects of Mulching on Mineral Nutrient Content in Citrus Orchard Soil.

Treatment	Exchangeable Mg (mg/kg)	Available Zn (mg/kg)	Available Mn (mg/kg)	Available B (mg/kg)
Mulching	52.97±2.77b	1.13±0.11a	7.37±0.09a	0.20±0.06a
Open-field	73.63±2.25a	0.53±0.06b	6.74±0.15b	0.23±0.05a

In the case of control, soil exchangeable calcium content was approximately 1539.0 mg/kg. After mulching with anti-grass cloth, the exchangeable calcium content changed to 1310.6 mg/kg, with a decrease of 14.9%. The exchangeable magnesium content was approximately 73.6 mg/kg for the control, whereas it decreased to 52.9 mg/kg (by 28.1%) after mulching. These results indicated that the cultivation mode of anti-grass cloth mulching could decrease the contents of medium elements in soil.

With regard to trace elements, both soil available zinc and available manganese contents increased after mulching with anti-grass cloth. The most increase was found in the available zinc content which changed from 0.53 to 1.13 mg/kg; the increase rate was 113.2% and the difference between treatments was highly significant. The available manganese content changed from 6.7 to 7.3 mg/kg, with an increase rate of 8.6%. Moreover, mulching with anti-grass cloth had no significant effect on soil available boron content.

3.4. Effect of mulching on soil microbial population

The effect of mulching on soil microbial population is shown in Figure 3. The number of soil

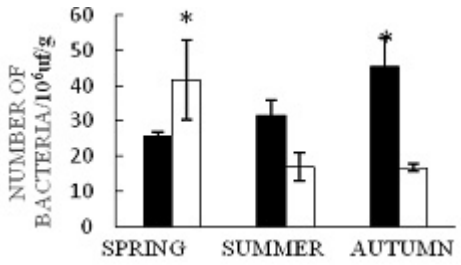

Figure 3a. Effects of Covering on Bacteria Population in Citrus Orchard (Note: Superscripts *indicate significant differences at the 0.05)

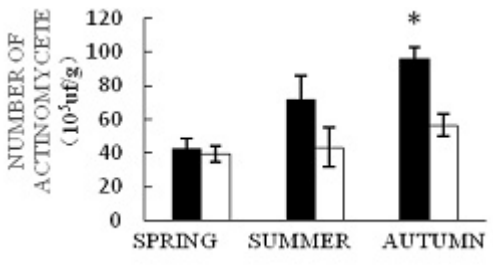

Figure 3b. Effects of Covering on actinomycetes Population in Citrus Orchard

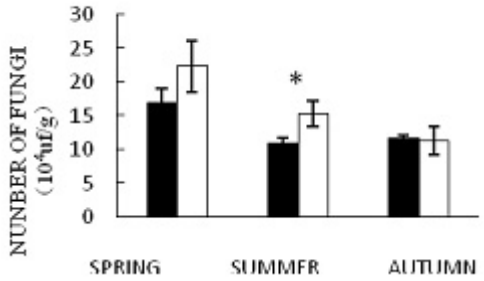

Figure 3c. Effects of Covering on Fungi Population in Citrus Orchard

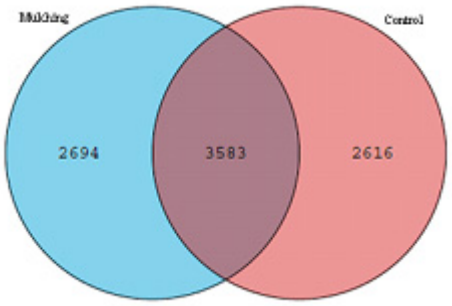

Figure 4. A total of OTU's Veen map

microbes under both cultivation modes was highest for bacteria, followed by actinomycetes and fungi. The numbers of both soil bacteria and actinomycetes increased after mulching with anti-grass cloth, and in autumn values were significantly higher compared to the control. In summer, there was significantly poor number of soil fungi in the case of mulching cultivation compared to the control,whereas for the other seasons, no significant difference between the two treatments was found.

3.5. Effect of mulching on soil microbial community richness and diversity

For both mulching and control, the detected number of OTUs gradually decreased from phylum to species levels (Table 2). This is due to the high diversity of microbial species, which may not completely cover the commonly used databases, in addition to the limitation of sequencing read lengths. Therefore, in the actual analysis, the taxonomic information cannot be obtained at the genus or species level for all representative sequences of the OTUs. However, the number of OTUs at the phylum, class, order, and species levels was higher under the mulching cultivation than control cultivation, although this difference was not significant. But at the family and genus levels, mulching cultivation resulted in a significantly lower number of OTUs compared with control.

Table 2. OTU classification and classification status identification results statistics table

Treatment	Phylum	Class	Order	Family	Genus	Species	Unknown
Mulching	3038.0±183.5a	2939.2±182.6a	2511.6±122.9a	1664.0±121.8b	978.4±45.7b	121.0±17.9a	0.6±0.5a
Open-field	3012.0±176.6a	2948.2±174.0a	2428.2±160.7a	1867.6±68.2a	1117.4±49.3a	119.6±8.6a	0.6±0.5a

The common OTUs of soil microbes shared by the two different cultivation modes were also analyzed (Figure 4). A total of 6277 OTUs were detected under the mulching cultivation mode, 3583 of which were sheared by the control mode. The remaining 2694 OTUs were unique, more than control by 78.

Both the Chao1 and ACE index values under mulching cultivation were higher than under control, whereas the corresponding Shannon and Simpson index values were lower than under control cultivation (Table 3). This result indicates that mulching cultivation resulted in higher richness but lower diversity of the soil microbial community compared with control cultivation.

Table 3. Soil microbial community diversity index table of two cultivation modes

Treatment	Simpson	Chao1	ACE	Shannon
Mulching	0.9975±0.0003b	3182.86±395.46a	3269.11±472.45a	10.4±0.1b
Open-field	0.9984±0.0005a	3012.85±176.45a	3019.61±183.44a	10.6±0.1a

3.6. Effect of mulching on citrus root growth

Mulching with anti-grass cloth had a significant positive effect on root growth and development of citrus trees (Table 4). Under mulching cultivation, the trees yielded larger total root length, total root surface area, total root volume, and average root diameter than under control cultivation. The increase rates of total root length, total root surface area, and total root volume were 37.7%, 46.0%, and 54.3%, respectively; these differences were significant compared with parameter values of the control. Moreover, mulching cultivation significantly increased the root dry weight of citrus trees, but no significant effect on specific root length or specific root surface area was observed (Table 5).

Table 4. Effect of coverage on citrus root length, surface area, volume and average diameter

Treatment	Total root length (cm)	Total root surface area (cm2)	Total root volume (cm3)	Average root diameter (mm)
Mulching	5815.1±720.3a	1198.2±112.4a	19.9±1.4a	0.66±0.04a
Open-field	4224.0±300.9b	820.9±48.0b	12.9±0.9b	0.62±0.02a

Table 5. Effects of mulching on dry weight, specific root length and specific surface area of citrus roots

Treatment	Root dry weight (g)	Specific root length (cm/g)	Specific root surface area (cm2/g)
Mulching	8.93±0.59a	649.82±39.48a	134.08±4.08a
Open-field	6.30±0.56b	671.78±36.61a	130.56±5.11a

3.7. Effect of mulching on root vigor of citrus

Under control cultivation, the root vigor of citrus trees was 91.7 μg/g/h. Mulching cultivation increased the root vigor of citrus trees to 181.5 μg/g/h, which was 89.8 μg/g/h higher than under control cultivation, with an increase rate of 97.9% (Figure 5). This result indicates that mulching cultivation improved the root vigor of citrus trees.

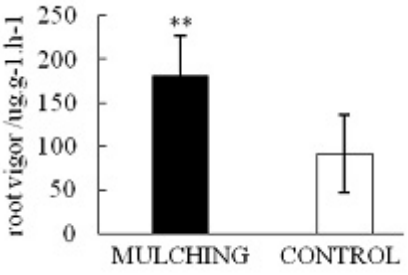

Figure 5. Effect of coverage on citrus root vigor

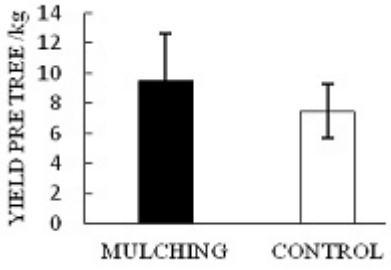

Figure 6. The effect of coverage on the yield of citrus per plant

3.8 Effect of mulching on the yield per plant of citrus

The effect of anti-grass cloth mulching on the fruit yield per plant of citrus is shown in Figure 6. The average fruit yield per plant of citrus under mulching cultivation was 12.3 kg, which was 3.8 kg higher than under control cultivation, with an increase rate of 44.7%. This result indicates that mulching with anti-grass cloth substantially increases the yield per plant of citrus.

4. DISCUSSION

Mulching cultivation is one of the most effective means to hold soil moisture. Additionally, mulching cultivation can reduce soil evaporation and improving crop transpiration and agronomic efficiency (Wang *et al.*, 2011). It increases the water reserves in the planting area via rainwater collection. During our experimental period, the soil water content under mulching cultivation was higher than under control cultivation through March to December, 2018. The difference in soil water content between the mulching and control treatments was significant in the hot and rainy summer, and the difference was reduced in the cold and dry winter. This result may be attributed to the fact that in the hot summer period, soil surface evaporation considerably increases, whereas the barrier effect of anti-grass cloth can effectively reduce the substantial soil water evaporation and drive the backflow of the evaporated water into the surface soil; these changes would stabilize the soil water content and eliminate the adverse effects of rapid soil water evaporation on the roots of fruit trees. Jiang *et al.* (2015) studied the water content and temperature in soils from mulched and open fields of orchard during summer, and they reported that mulching considerably reduced the ineffective evaporation of soil water, which is consistent with our findings.

Temperature exhibits an obvious effect on soil nitrogen mineralization and the rate of soil nitrogen mineralization increases with increasing soil temperature (Andersen & Jensen, 2001). The warming and water retention effects of anti-grass cloth can effectively raise soil temperature and thereby promote nitrogen mineralization in the soil, which in turn increases soil nitrogen content (Gen, 2010). Our results showed that soil exchangeable magnesium and exchangeable calcium contents both decreased significantly after mulching, which may be related to ionic antagonism. An increase in soil potassium content could lead to the decrease in the contents of antagonized elements including magnesium and calcium. Cui *et al.* (1998) found that mulching cultivation can increase the consumption of various zinc, copper, and manganese species in the soil. In the current study, mulching significantly increased soil available zinc and available manganese contents, along with a decrease in the available boron content. This result is inconsistent with the previous study.

Soil microbes are highly sensitive to human disturbances. In particular, agricultural management practices, such as mulching, tillage, and fertilization, can cause changes in the diversity and richness of the soil microbial community (Beauregard *et al.*, 2010; Huang *et al.*, 2019). Our experimental data showed that mulching with anti-grass cloth did not affect the number of soil fungi in citrus orchard, but it significantly increased the number of soil bacteria and actinomycetes and the total population of soil microbes. Moreover, the analysis microbial community diversity revealed that the OTUs of soil microbes detected under anti-grass cloth mulching was higher than under control cultivation. In terms of microbial community richness, the mulching treatment was also superior to the control treatment. These results are in agreement with other studies (Zhu *et al.*, 2018). The main reason is that mulching can inhibit soil water evaporation, increase surface soil temperature, and improve soil surface microenvironment.

Because it creates a root zone environment with favorable soil water content and temperature, mulching is conducive to root growth and proliferation, leading to significant increases in root morphological characteristics such as dry weight, length, surface area, and volume (Gu *et al*., 2019; Sun *et al*., 2018). However, other studies have indicated that mulching treatment can result in a decrease in the total biomass of fine roots and horizontal distribution of the roots in fruit trees, because the mulching material is not easily permeable for water and air, which affects the growth and activity of fine roots in the soil (Sun *et al*., 2016).

In this study, the total root length, total root surface area, total root volume, and root dry weight of citrus trees respectively increased by 37.7%, 46.0%, 54.3%, and 41.7% under the mulching treatment compared with the control treatment. Additionally, the root vigor of citrus trees was significantly higher under mulching cultivation than under control cultivation. These results indicate that mulching has a significant effect at promoting the root growth of citrus. Since anti-grass cloth mulching can alter the soil environment, it also has a prominent effect on the growth of crop roots. On the contrary, past studies have also indicated that mulching can lead to a decline in root function during the later stages of crop growth (Zhao, 2013).

5. CONCLUSIONS

The anti-grass covering can improve the soil hydrothermal condition and the overall nutrient level of the citrus orchard, which is beneficial to the growth of citrus roots.

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DEVELOPMENT OF THE TEE BAR PRODUCTION SYSTEM AND TECHNICAL TRANSFER ON DRAGONFRUIT (*HYLOCEREUS UNDATUS*) IN VIET NAM

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ABSTRACT

Dragonfruit (*Hylocereus undatus*) is a major important fruit crop in Viet Nam with a planting area of 53,899 ha and a production of more than one million mton of fruits per year. The fruit has been exported to over 60 countries in the world, with an exported value of USD 1.1 billion in 2018. Many farms practice dragonfruit cultivation using a concrete post called the "Mop top" system. However, this system is plagued with many difficulties such as pruning, pests and diseases management, machinery application, and low yield. This study incorporates a new "Tee bar" system and was carried out from 2014 to 2018 at Farm A of the Southern Horticultural Research Institute (SOFRI), Tien Giang with two experiments. Results showed that the Tee bar system gave a higher yield than the Mop top and single wall systems. The Tee bar system with 60 cm spacing between two plants showed higher yields with the best profit compared to the system with 40 cm and 100 cm spacing. The Tee bar system is currently applied by dragonfruit farmers in the South of Vietnam.

Keywords: Dragonfruit, mop top, single wall, Tee bar, spacing

EVALUATION AND CHALLANGES IN IMPLEMENTATION OF CITRUS SHOOT TIP GRAFTING (STG) TECHNOLOGY IN INDONESIA

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ABSTRACT

For the past three decades, the main problem of citrus development in Indonesia has been the Huanglongbing (HLB), Citrus Tristeza Virus (CTV) andand other virus diseases. Due to the severity, the government since 1987 has started to produce virus-free mother plants through Shoot Tip Grafting (STG) followed by indexing. This present research aims to evaluate the implementation of STG technology in Indonesia from 2008 to 2018. The activity was carried out in the tissue culture laboratory of ICSFRI by grafting 0.14-0.18 mm shoot tips as scions onto a 2-week-old JC rootstocks *in vitro*. To accelerate the growth, a one-month old micro grafted plant was re-grafted onto an 6-month old rootstock at the nursery house. Four to six months later, they were indexed using indirect ELISA and RT-PCR. The total number of micro grafting activities was 10,882, consisted of 9 citrus species (71 cultivars) with 9.8% success rate. The highest was on *C. microcarpa* (15%), whereas the lowest was on *C. grandis* Osbeck (3.4%) and *C. sinensis* (5.7%). Among 391 indexed plants, 75.2% of them were virus-free; 21.7% and 4.1% were still infected by CTV and HLB respectively, and 1% infected by both of the diseases. Although the success rate achieved is low, this technology is still an effective way to produce virus-free mother plants. From its propagation, in the period 2009-2014 at least more than 5 million virus-free plants have been produced by citrus farmers.

Keywords: Citrus, Huanglongbing, CTV, STG, Indexing

1. INTRODUCTION

In Indonesia, citrus is one of the horticulture commodities that has high economic value. Although it is not a native tropical plant, it has a wide genetic diversity and is grown in many developing areas (Martasari *et al*., 2012; Devy & Hardiyanto, 2017; Yulianti *et al*., 2016). According to the commercial harvested area in the year of 2018, from about of 46,922 ha, 91.9% of them were mandarin and tangerine types areas with an average production of 55.86 tons/ha (Ministry of Agriculture, 2019). Almost all of them are grown exclusively on Japanese Citroen (JC) rootstock.

In the 1980s, Huanglongbing (HLB), Citrus Tristeza Virus (CTV) and others diseases were common and this had an impact through severe crop damage that caused loss. To restore them, the Indonesia government launched the Citrus Rehabilitation Program in 1987, with the aim to produce disease-free plants, especially from HLB and CTV using the STG method (Supriyanto *et al*., 2017). This technique is also widely adopted in the world's citrus producing countries, including China, Pakistan, Italy, Cyprus, and India (Ruilin *et al.*, 1996; Naz *et al.*, 2007; Continella *et al*., 1997; Kapari-Isaia *et al*., 2002; Chand *et al.,* 2013).

To date, *in vitro* shoot tip grafting has been cited as the most reliable method to obtain virus free citrus mother trees from infected parental source (Fifaei *et al*., 2007; Sharma *et al*., 2007; Meziane *et al*., 2009). According to Carimi *et al.* (2001), it can effectively eliminate all citrus diseases originating from pathogens carried during the grafting process, although the success rate varies between 60% (Tatterleaf, Psorosis) to 100% (Citrus viroid).

The implementation of this technique in Indonesia is carried out by the Indonesian Agency for Agriculture Research and Development (IAARD) and assigned to the Indonesian Citrus and Subtropical Fruit Research Institute (ICSFRI). The plant materials are cleaned from viruses of HLB and CTV based on protocols described by past researches (Navarro *et al*., 1974) with little modification (Devy, 2014; Supriyanto & Whittle, 1991; Devy *et al*., 2014). The virus free STG plant acts as a nucleus plant and becomes the source materials for foundation seeds at Foundation Blocks (FB). These are then propagated to be Budwood Multiplication Block (BMB) plants. BMB itself is a source of scions for certified commercial citrus seeds. To prevent re-infection in the field, the FB and BMB parent plants are managed in an insect proof screen house. The purpose of this study is to evaluate the STG activities that have been carried out from 2008 to 2018.

2. MATERIALS AND METHODS

Evaluation was performed on activities carried out from January 2008 to December 2018 at the ICSFRI Tissue Culture Laboratory. The number of cultivars and total of in vitro grafted plants via the STG method are listed in Table 1.

Table 1. Total cultivars and plants derived from STG year 2008-2018

No	Year	Total Cultivars	Total of in vitro grafted plants
1	2008	18	1372
2	2009	10	809
3	2010	7	343
4	2011	13	1281
5	2012	12	810
6	2013	14	1265
7	2014	19	1223
8	2015	15	1046
9	2016	6	849
10	2017	16	1043
11	2018	13	841
Total			10,882

Principally, the STG method done was based on the technique adopted by the researchers as in (Navarro *et al*., 1974). It consisted of grafting in vitro 0.1 - 0.2 mm long shoot tips composed of the apical meristem plus three leaf primordia on to a 2-week-old seedling rootstocks. The Japanese Citroen (JC) was used as rootstocks to graft all scion shoot tips. The cultivars were generally identified as a potential citrus varieties originating from various regions in Indonesia.

2.1. Preparation of *in vitro* rootstocks

Seeds of JC were extracted from fresh or stored fruit. Before planting, the outer shell of the seed was peeled and sterilized with a 1% fungicide. Inside the Laminar Air Flow Cabinet (LAFC),

the seeds were peeled and re-sterilized, and planted in vitro on solid MS media. The test tube containing seed culture was incubated in a dark cupboard at 27 °C for 2-3 weeks until seedlings grew to 5-7 cm in height, with an ideal diameter of 1-1.5 mm.

2.2. *In vitro* grafting

Grafting was done on 2-3 week-old seedlings of JC. Shoot tips were obtained directly from the field-grown trees or from potted plants established in the glasshouse. The shoots were sterilized. Under a stereomicroscope, lateral leaves were removed from the shoot, and the meristem tip with two primordia leaves (about 0.2 mm in size) was excised. The shoot tip was inserted into an inverted-T incision made on an in vitro JC seedling. Grafted plants were placed into a test tube containing a MS liquid medium and incubated at 27 °C under 16- hour light exposure.

2.3. Acclimatization and re-grafting

About one month after grafting, plants were grown in greenhouse conditions for acclimatization, after which were re-grafted in vivo onto six-month-old JC seedlings. Four to five months after re-grafting, the leaves of re-grafted plants were ready to be used as indexing material, which is a process to ensure that the result of STG is free from CTV and HLB by using ELISA and PCR methods. Observations were made on the number of in vitro grafted plants, survival of grafted plants, duration of plants to grow, and number of re-grafted plants that were free from virus.

3. RESULTS

3.1. Citrus species, cultivars, and number of STG plants

To determine the graft union in the STG method, the number of grafted and surviving plants from year 2008 - 2018 was recorded. There were 10,882 grafted plants derived from 71 cultivars belonging to 9 species of citrus with an average 9.8% of survival rate (Table 2).

Table 2. Species, cultivars, and survival rate of STG

Species	Cultivar	Σ STG plants	Survival rate (%)
I. Mandarins (*C. reticulata* Blanco)	22	4,272	10.0
II. Lemon (*C. limon* L.)	12	1,761	7.2
III. Sweet Oranges (*C. sinensis* L. Osbeck)	4	474	5.7
IV. Calamondin (*C. microcarpa*)	1	419	15.0
V. Pummelo (*C. grandis* Osbeck)	21	2,592	3.4
VI. Citron (*C. medica*)	2	188	14.4
VII. Tangerine (*C. nobilis* Lour)	4	275	11.3
VIII. Kaffir Lime (*C. hystrix*)	2	238	10.1
IX. Key Lime (*C. aurantifolia*)	3	663	10.9
Total	71	10,882	9.8

Among all species, the STG method was done mostly on mandarin (*Citrus reticulata* Blanco), followed by pummelo (*C. grandis* Osbeck), and lemon (*C. limon* (L.) Burm. f.). This corresponded with the distribution of local varieties of citrus in Indonesia in which the major species are mandarin and pummelo (Martasari *et al*., 2012; Devy & Hardiyanto, 2017; Yulianti *et al*., 2016).

In general, pummelo is relatively difficult to graft in vitro compared to others. For this species,

there were 143 grafted plants derived from four cultivars that failed to grow. This is consistent with the results of the 1997-2007 activities (Devy *et al.*, 2015). During that period, the percentage of survival rate was 7.82%. The low percentage is thought to be caused by the relatively large size of the meristem tip compared to the others, so the joining process between meristem and the rootstock is relatively more difficult. In addition, it is suspected that their compatibility is low with the JC rootstock, as also stated in the research of (He *et al.*, 2018).

The ability of survival rate between cultivars among a species is also not significantly different ($p<0.05$) (Table 3). This illustrates that the low percentage of survival is due to conditions that are not favourable for the graft union process using very small material.

Table 3. Percentage survival rate of STG on four main citrus species

Sp. Mandarin		Sp. Lemon		Sp. Pummelo		Sp. Sweet Orange	
Local name	**%**	**Local name**	**%**	**Local name**	**%**	**Local name**	**%**
'Brasil'	1.8^{ns}	'T.Varigata'	17.2^{ns}	'Jeruk Besar'	2.5^{ns}	'Kisar'	7.2^{ns}
'Kisar'	16.5	'Swanggi'	4.6	'Jeruk Kelapa'	5.1	'M. Variegata'	2.7
'Madu'	16.0	'Bali'	4.2	'Nambangan'	0.6	'S O. Local'	5.8
'Santang'	12.0	'Jumbo'	18.0	'Baco'	2.8		
'Soe'	4.1	'Nipis Kecil'	6.4	'Bona'	3.6		
'Terigas'	6.4	'UB'	5.2	'India'	2.8		
'Topo Hitam'	7.1			'Pekalongan'	1.9		
'Topo Putih'	8.2			'Pamindo'	2.3		
p	**0.05**		**0.34**		**0.95**		**0.45**
R2	**53.2**		**34.3**		**11.0**		**23.6**

3.2. Acclimatization and regrafting

The duration required for STG plants to reach the ideal size and to be ready for acclimatization and regrafting is highly varied. After in vitro micro grafting, almost all plants (83.8%) required between 2-8 weeks to grow, and the rest do more than 9 weeks (Figure 1).

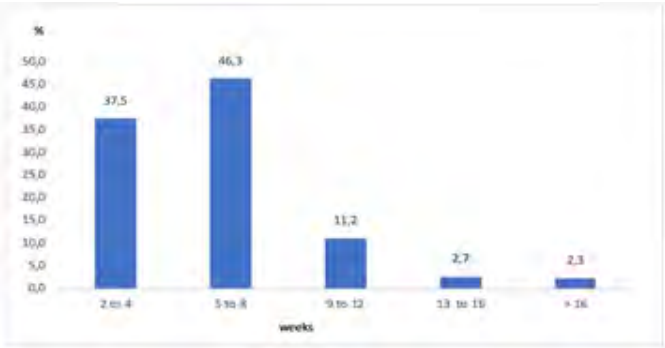

Figure 1. Percentage of in vitro grafted plants acclimated at 2-16 weeks

The differences in cultivars also affect their growth abilities. Among 37 cultivar samples, pummelo cv. 'Pekalongan' is the slowest. It took about 10.4 weeks to acclimatize, followed by lime cv. 'Kutai', lemon cv. 'UB', and 'Jari Budha'. They are significantly different from Lemon cv. 'Swanggi' and 'Manis', and lime cv. 'N. Red Center' which only took between 3.7-4 weeks ($p < 0.05$; R2 = 81.3%) (Table 3).

Table 3. Time needed of *in vitro* grafted plant to be acclimated

Species	Cultivars	Duration (weeks)	Species	Cultivars	Duration (weeks)
C. reticulata	'K. Madu'	8.4 abcd*)	*C. grandis*	'P. Pekalongan'	10.4 a
	'K. Kacang'	6.8 abcde		'P. Nambangan'	6.8 abcde
	'K. Chokun'	6.6 abcde		'P. Baco'	5.6 bcde
	'K. SoE'	6.5 abcde		'P. Kelapa'	5.6 bcde
	'K. Thay Ayam'	6.5 abcde		'PA Chuan'	5.1 bcde
	'K. Dekopon'	6.4 abcde	*C. aurantifolia*	'Nipis Kutai'	9.1 ab
	'K. Kisar'	6.3 abcde		'Jr Nipis Kecil'	6.2 abcde
	'K. Komun'	7.3 abcde		'N. Red Center'	3.7 e
	'K. Terigas Bsr'	5.8 bcde		'Lemon UB'	8.7 abc
	'K. Selwasa'	5.7 bcde	*C. limon*	'L. Tea Varigata'	7.4 abcde
	'K. Santang'	5.6 bcde		'Lemon Kuit'	6.9 abcde
	'K. Emperor'	5.4 bcde		'Lemon Jumbo'	5.6 bcde
	'K. Topo Putih'	5.1 bcde		'L. Swanggi'	4.0 e
	'K. G. Lebong'	5.0 bcde		'Lemon Manis'	4.0 e
	'K. Kendari'	4.9 bcde		'L. Cina Lamo'	5.3 bcde
	'K. Topo Hitam'	4.2 bcde	*C. microcarpa*	'Calamansi'	4.9 bcde
C. medica	'Jari Buda'	8.5 abcd	*C. histryx*	'Monte Hondu'	4.1 de
C. sinensis	'M. Varigata'	5.1 bcde			
C. nobilis Lour	'S. Mahang'	5.1 bcde			
	'S. Gn Omeh'	4.8 bcde			

***) Means that do not share a letter are significantly different (p<0.05)**

3.3. Indexing

The time interval required by in vitro grafted plants to be indexed varies greatly between cultivars. All the stages of that process needs about 20-89 weeks or 5-22 months. Cultivars of 'S. Mahang', 'K. Kendari', 'L. Jumbo', and 'K. Dekopon' were faster than others, they needed only 20-23 weeks. While 'K. Brasil', 'M. Variegata', 'L. Tea Variegata', and 'N. Red Center Lime' needed more than 1.5 years (Table 4).

Table 4. Duration up to indexing phase of *in vitro* grafted plant

Species	Cultivar	Duration (weeks)	Species	Cultivar	Duration (weeks)
C. reticulata	'K. Brasil'	73.7 ab *)	*C. grandis*	'P. Pamindo'	27.5 cde
	'K. SoE'	46.3 abcde		'P. Nambangan'	34.8 bcde
	'K. Santang'	31.5 bcde	*C. limon*	'L. Bali'	28.5 cde
	'K. Dekopon'	22.5. cde		'L.T.Varigata'	85,5 a
	'K. Terigas Bsr'	34.3 bcde		'L. Jumbo'	22.3 cde
	'K. Santang'	88.4 abcde	*C. nobilis*	'S. Mahang'	20.5 e
	'K. Kendari'	21.6 de	*C. sinensis*	'M. Varigata'	83.3 a
	'K. Topo Hitam'	61.2 abcde		'SO. Local'	64.8 abc
C. aurantiifolia	'Jr Nipis Kecil'	53.5 abcde		'M. Kisar'	51.8 abcde
	'N. Red Center'	88.4 a			

***) Means that do not share a letter are significantly different (p<0.05)**

Among 391 re-grafted plants that were indexed during2008-2018, 75.2% of them were virus free; 21.7% and 4.1% were still infected by CTV and HLB respectively, and 1% were infected by both of diseases (Table 5). A relatively similar result was obtained by (Muharam & Whittle, 1992), 80% and 60% of the STG plants indexed were CTV-free for mandarin and sweet oranges, respectively. The STG plants without preliminary heat treatment consisted negative CTV of mandarins and sweet oranges were 80% and 60% respectively. The percentage of virus free plants seemed to increase if an addition of thermotherapy treatment was performed before micrografting (Tianmiao, 1996).

Table 5. Total re-grafted plants infected by CTV, HLB, and both of CTV & HLB

Species	Σ Cultivar	Σ re-grafted plants	Σ CTV		Σ HLB		Σ CTV & HLB	Σ Infected	Σ Virus-free
			-	+	-	+	+		
C. medica	1	5	5	0	5	0	0	0	5
C. reticulata	22	156	109	47	150	4	0	49	105
C. limon	6	35	25	10	30	5	2	13	22
C. sinensis	4	14	8	6	12	2	2	6	8
C. histryx	2	12	12	0	12	0	0	0	12
C. aurantifolia	3	15	15	0	14	1	0	1	14
C. grandis	13	48	43	5	43	4	0	9	39
C. nobilis	4	14	11	3	11	0	0	3	11
Others	23	92	78	14	78	0	0	14	78
total	78	391	306	85	355	16	4	95	294
%				21.7		4.1	1.0		75.2

4. DISCUSSION

The challenges of implementing STG in Indonesia are various. The skills of the technicians are the most important. During the decade of 1997-2007, the total grafted plants in vitro was 2,616 with an average survival rate of 21.5% (Devy *et al*., 2010). In the following decade, the decline of success was due to limited skilled technicians. The relatively low result also occurred in Cyprus and others (Kapari-Isaia *et al*., 2002); the average success was 17% and 2.5 - 50 % respectively, depending on the cultivars of scion and rootstocks and the size of the shoot tip (Chand *et al*., 2013).

Low rates of success can also be due to the very minute size of the grafted meristem. A rough sliced surface will cause problems in that the two sections cannot fuse properly (Hussain *et al*., 2014). Besides this, the slice made on the seedling usually stimulates rapid callus growth, thus inhibiting the growth of the meristem in it (Navarro *et al*., 1974).

The high percentage of dead grafted plants could be due to low incompatibility between the two parts; as reflected by their vascular systems that affects the flow of the growth hormones and others. This disrupts cellular growth and development (Wang *et al*., 2016; Thomas & Frank, 2019).

Another problem is the duration needed for grafted plants to be re-grafted and indexed. Almost 84% of grafted plants can be acclimated at 2-8 weeks and require approximately 20-89 weeks to be indexed after STG process. Various ways have been done to induce the growth, including by using some tested rootstocks (Navarro *et al*., 1974) and increasing the ambient temperature on the re-grafted plants (Hussain *et al*., 2014).

However, during the years 2008 to 2018, a total of 78 virus-free citrus cultivars were produced. Meristematic tissue from the apical shoots can be free of pathogens because the vascular system of the stem is not yet interconnected. One virus-free mother plant is very valuable for the continuity of the citrus development program in Indonesia. During the period of 2009-2014, ICSFRI produced and distributed 4,437 and 27,647 plants of FB and BMB respectively derived from 31 virus-free cultivars to most Indonesian provinces. There were at least 5,529,400 virus-free citrus plants produced by farmers with a planted area of 11,058 hectares.

5. CONCLUSION

The success rate of STG method implemented in Indonesia is relatively low (9.8%). However, until now this method is still used because it is considered feasible and effective to produce disease-free mother plants. By using this, at least 78 cultivars have been produced as virus-free mother citrus plants during the last decade.

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EFFECTS OF UNICONAZOLE ON THE FLOWERING OF 'TUONG DA XANH' MANGO (*MANGIFERA INDICA* L.) IN THE MEKONG DELTA, VIET NAM

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ABSTRACT

'Tuong Da Xanh' (TDX) is a mango cultivar (also known as 'Ba Mau' or 'Dai Loan') grown widely in the Mekong Delta, Viet Nam particularly in Gieng islet, Cho Moi district, An Giang province. Similar to other cultivars, off-season flowering of TDX is implemented by collar drenching of Paclobutrazol (PBZ) which is used as a flower bud initiation agent, followed by foliar application(s) of Thiourea or KNO_3 to induce flowering. However, PBZ residue has been reported to be able to remain for a long time in both soil and leaf. In addition, Thiourea has been long labelled as a carcinogen, thus banned from use in the USA and Australia. Therefore, it is of utmost importance to look for alternatives for these two chemicals. The aim of this study was to investigate the effects of Uniconazole (UCZ), as a replacement for PBZ, on the flowering of TDX mango. Results showed that UCZ applied either as collar drenching or foliar application is a good replacement for PBZ in terms of flowering rate and yield. Trees which were collar drenched with UCZ (1.0 g a.i. m^{-1} canopy diameter [c.d.]) followed by two sprays of KNO_3 (2.5% twice in one week) after 75 days, had flowering rates like those treated with PBZ 1.5 g a.i. m^{-1} c.d. Similarly, for foliar application, the flowering rates of trees sprayed with 1,000, 1,500, and 2,000 ppm UCZ followed by bud breaking treatment using KNO_3 (2.5%, twice in one week) after 75 days was not significantly different to that of those treated with PBZ 1.5 g a.i. m^{-1} c.d.

Keywords: bud break, Tuong da xanh (TDX) mango, paclobutrazol, uniconazole

1. INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruit trees grown in many countries worldwide. According to the general statistics office of Viet Nam , mango growing area in Viet Nam is about 99,641.5 ha in 2018, primarily distributed in the Mekong Delta (MD), including An Giang, Dong Thap, Tien Giang, Vinh Long, and Hau Giang provinces. The most popular mango cultivars are 'Cat Hoa Loc' (Tien Giang and Dong Thap province) and 'Cat Chu' (Dong Thap province). Furthermore, mango cultivars originating from other countries, e.g. 'Nam Dok Mai' (Thailand), 'Keo' (Cambodia), and 'Tuong Da Xanh' (TDX) (originally from Taiwan) have also been grown in the MD. The latter is now one of the most favorite cultivars, originating from Taiwan where it is called 'Jin-Hwung'. It is reported to be one of the six most popular mango cultivars in Taiwan (Shu, 2010). 'TDX' mango can adapt well to the growing conditions of the MD as well providing high yields (Ve, 2011). Under good care, the cultivar starts to bear fruits from the second year with stable yield and no alternative bearing. An advantage of 'TDX' mango is its high fruit set rate despite low flowering rate. In addition, its fruits, suitable for consuming at unripen stage, are large in size with an average weight of 1.2-1.5 kg with thick, firm, crunchy fruit flesh.

[1]https://www.gso.gov.vn/Default.aspx?tabid=217

Various plant growth regulators and chemicals are used widely for off-season and year-round production of mango. Gibberellin (GA)-inhibitors, e.g. Paclobutrazol (PBZ), Uniconazole (UCZ), and Prohexadione-Ca, play an important role in the procedures of floral induction of not only mango but also many other fruit trees. Chemicals used for breaking bud dormancy, i.e. KNO_3, Thiourea, and NH_4NO_3 are also important for such procedures (Silva *et al.*, 2013). Particularly, in the Mekong Delta, a floral induction procedure used for mango had been proposed by Hau (2013). Accordingly, PBZ (1-2 g a.i. m^{-1} canopy diameter [c.d.]) and Thiourea (0.3-0.5%) were the two key agents. The procedure has been applied widely in the Cao Lanh district - Dong Thap province (Hau *et al.*, 2010), Cai Be district - Tien Giang province (Hau *et al.*, 2014), and Chau Thanh A district - Hau Giang province (Hau *et al.*, 2016). To date, PBZ has been the only GA-inhibitor used for mango flowering bud initiation in the MD. However, according to Litz (2009), PBZ has not been cleared for use in the USA. Similarly, Thiourea has been classified as a carcinogen (WHO, n.d.). While these two key agents are still utilized in some countries, e.g. Thailand, Indonesia and the Philippines, in the long term both PBZ and Thiourea will likely be withdrawn from usage in Viet Nam as a result of their notorious impacts on the environment as well as on human health. Thenceforth, it is of urgency to seek alternatives for the two mentioned chemicals. Krämer *et al.* (2007) reported that UCZ is safe for the environment. Besides, early studies showed that UCZ can be very persistent in retarding plant growth without causing phytotoxicity (Davis *et al.*, 1988). Consequently, the aim of this study was to investigate the effect of UCZ, as a replacement for PBZ, on flowering of 'TDX', which is a popular mango cultivar in the MD. Besides, suitable UCZ dosages as well as concentrations and the times for floral induction after UCZ application would be determined.

2. MATERIALS AND METHODS

'TDX' mango trees at the age of 7-year-old were used in this study. These trees belong to commercial orchards located in the three communes of Cho Moi district, An Giang province, i.e. My Hiep, Binh Phuoc Xuan, and Tan My. After harvesting, the trees were pruned and fertilized to help recovery and concentrated flush. Urea and diammonium phosphate (DAP) (18-46-0) in 2:1 ratio, 1 kg/tree were applied to induce flushing. Subsequently, pesticides were used to protect new flushes from diseases and pests, i.e. anthracnose, thrips, bugs, and other insects, depending on the circumstances. After two completed flushes without damage, when young leaves turned reddish or yellowish (15-day-old), the trees were applied PBZ/UCZ by either collar drenching or foliar sprays. Dosages and concentrations of PBZ/UCZ were adjusted in accordance with the experimental treatments. Afterwards, depending on the treatments of times for bud break (45, 60, 75, and 90 days after PBZ/UCZ treatment - DAPUT), the trees were sprayed with KNO_3, 2.5% to induce bud break and flowering (Hau, 2008).

Experiments were arranged in completely randomized factorial design with two factors, i.e. dosages/concentrations of PBZ and UCZ, and times for bud break after PBZ/UCZ application. For the experiment to test UCZ in collar drenching, the first factor included the three doses of UCZ, i.e. 1.0, 1.5 and 2.0 g a.i. m^{-1} c.d. Similarly, in the other experiments using UCZ as foliar applications, the first factor comprised the three concentrations, *viz*. 1,000, 1,500 and 2,000 ppm. The positive control treatment, PBZ 1.5 g a.i. m^{-1} c.d., was proposed by Hau (2008) (Hau, 2008). The second factor consisted of the three times for floral induction with KNO_3 (2.5%), i.e. 60, 75, and 90 days after PBZ/UCZ applications (DAPUT). For the trial investigating the leaf age at the time of UCZ/PBZ application, the three concentrations of UCZ, *viz*. 1,000, 1,500 and 2,000 ppm were considered as the first factor; and the second one includes the three leaf ages, *viz*. 45, 60, and 75 days. Floral induction was implemented at 75 DAPUT with KNO_3 (2.5%). All treatments were repeated six times. Each replication equaled to one tree. The observed parameters included flowering and fruit set rate, yield, and fruit quality. Flowering rate was

estimated by enumerating the number of vegetative and reproductive shoots appearing in a 1 m^2 frame. Average flowering rate was calculated from the 4 counts implemented evenly on the canopy. After emergence, 10 panicles per tree were labelled to determine the fruit set rate. Total fruit yield was obtained by weighing all fruits available on the tree. Yield of normal and seedless fruit was determined separately for each kind of fruit.

3. RESULTS

3.1. Uniconazole applied as collar drenching

3.1.1. Flowering rate

Results in Table 1 showed that the flowering rates observed at the three sites of experiment were relatively different. This could be linked to the different times of PBZ/UCZ treatment occurring in July, August, and September, which are the wettest months of the year in the MD. At site 1 (My Hiep commune), the flowering rates of PBZ/UCZ treatments were significantly different ($P<0.05$); meanwhile, those observed at sites 2 and 3, averaged at 29.3% and 45.7% respectively and were not significantly different among the PBZ/UCZ treatments. Particularly, at site 1, flowering rate of UCZ 2 g a.i. m^{-1} c.d. (61.6%) was significantly higher than that of the positive treatment - PBZ 1.5 g a.i. m^{-1} c.d. (41.0%); while those of treatments using lower UCZ doses (1 and 1.5 g a.i. m^{-1} c.d) showed no significant difference to that of the control. On the one hand, the effect of times of flowering induction treatment after PBZ/UCZ application were consistent throughout the three study sites. Accordingly, flowering rate was the highest (site 1: 82.7%; site 2: 47.5%; and site 3: 62.6%) when trees were induced for flowering at 75 days after PBZ/UCZ treatment DAPUT (days after PBZ and UCZ treatment). The flowering rates of 45 and 60 DAPUT treatment were mostly not different.

At sites 1 and 2, there was significant interaction ($P<0.05$) between the two experimental factors, i.e. PBZ/UCZ doses and times of floral induction. At site 1 (My Hiep commune), '45 DAPUT' and PBZ 1.5 g a.i. m^{-1} c.d. resulted in the lowest flowering rate (3.3%), significantly lower than these of the UCZ treatments with flowering rates varied from 31.0 – 44.5%. For 60 DAPUT, flowering rate reached to the highest level (68.2%) when trees were treated with UCZ 2.0 g a.i. m^{-1} c.d.; however those of the other two UCZ doses, viz. 1.0 and 1.5 g a.i. m^{-1} c.d., were low (37.5 and 34.8%, respectively) and not significantly different to that of PBZ (41.7%). It is noteworthy that, for 75 DAPUT, flowering rates of trees treated with either PBZ or any tested UCZ doses were high, from 77.9 – 90.0% with no significant difference. In short, these results suggested that for the trees treated with KNO3 2.5% to induce flowering at '75 DAPUT', either PBZ (1.5 g a.i. m^{-1} c.d.) or UCZ (1.0 – 2.0 g a.i. m^{-1} c.d.) application brought about high flowering rates.

3.1.2. Fruit set

Fruit set rate was significantly different among the treatments of both experimental factors. At all three sites of study, the highest fruit set was obtained with UCZ 1.0 g a.i. m^{-1} c.d., viz. 6.8% (site 1), 9.7% (site 2), and 6.8% (site 3). Similarly, for the times for floral induction, 45 DAPUT treatment resulted in the highest fruit set rate consistently at three places, i.e. 5.4% (site 1), 9.5% (site 2) and 6.6% (site 3).

The two investigated factors showed significant interaction at site 1 ($P<0.01$) and site 3 ($P<0.05$). At site 1, UCZ 1.0 g a.i. m^{-1} c.d. treatment combined with floral induction implemented at 45 DAPUT resulted in the highest fruit set rate (10.2%); in contrast, the lowest was observed when PBZ 1.5 g a.i. m-1 c.d. was used and subsequently induced flowering at 45 DAPUT (1.2%). At site

Table 1. Flowering rate (%), fruit set (%) and yield (kg/tree) of 'Tuong da xanh' mango under the effect of PBZ/UCZ and periods of flowering induction

Treatment	Site 1 (My Hiep commune)1					Site 2 (Tan My commune)2					Site 3 (Binh Phuoc Xuan commune)3				
	Flowering rate (%)	Fruit set (%)	NFe yield (kg/tree)	SFf yield (kg/tree)	TFg yield (kg/tree)	Flowering rate (%)	Fruit set (%)	NFe yield (kg/tree)	SFf yield (kg/tree)	TFg yield (kg/tree)	Flowering rate (%)	Fruit set (%)	NFe yield (kg/tree)	SFf yield (kg/tree)	TFg yield (kg/tree)
PBZ/UCZ doses (A)															
Controla	41.0b	3.3c	13.5b	12.0b	25.4b	24.5	7.9ab	7.2	4.6	11.8	43.6	5.1b	10.8	14.4	25.2
UCZ 1.0b	53.1ab	6.8a	12.6b	12.9ab	25.5b	28.6	9.7a	4.0	3.7	7.7	44.4	6.8a	10.8	16.8	27.5
UCZ 1.5c	53.8ab	5.2ab	18.4a	18.2ab	36.6a	36.5	6.9b	6.5	7.1	13.6	46.6	4.6b	8.7	19.5	28.2
UCZ 2.0d	61.6a	4.0b	14.5b	19.6a	34.1a	27.7	8.2ab	4.5	3.9	8.4	48.3	3.4c	11.2	20.5	31.8
Mean (A)	-	-	-	-	-	29.3	-	5.6	4.8	8.4	45.7	-	10.4	17.8	28.2
Times of flowering induction treatment (B) (days after PBZ/UCZ treatment)															
45 days	28.8c	5.4a	7.9a	17.1	25.0a	18.3b	9.5a	3.71	0.8c	4.5c	34.9b	6.6a	6.5b	19.5	26.1c
60 days	45.6b	5.2b	11.1b	13.5	24.6a	22.1b	9.1a	4.86	4.2b	9.1b	39.7b	5.6b	9.1b	14.8	23.8b
75 days	82.7a	3.9c	25.3c	16.3	41.6b	47.5a	6.0b	8.08	9.5a	17.6a	62.6a	2.8c	15.5a	19.1	34.6a
Mean (B)	-	-	-	15.7	-	-	-	5.6	-	-	-	-	-	17.8	-
F(A)	*	**	**	*	**	ns	*	ns	ns	ns	ns	*	ns	ns	ns
F(B)	**	**	**	ns	**	**	**	ns	**	**	*	*	*	ns	*
F (A*B)	*	**	*	*	*	ns	ns	ns	ns	ns	*	*	*	*	*

1Trees were treated with PBZ/UCZ on 4/7/2016; 2Trees were treated with PBZ/UCZ on 21/8/2016; 3Trees were treated with PBZ/UCZ on 11/9/2016
a Control: Paclobutrazol, 1.5 g a.i. m-1 canopy diameter; bUCZ 1.0: Uniconazole, 1.0 g a.i. m-1 canopy diameter; c UCZ 1.5:Uniconazole, 1.5 g a.i. m- canopy diameter; d UCZ 2.0:Uniconazole, 2.0 g a.i. m-1 canopy diameter; eNF yield: Normal-fruit yield; fSF yield: Seedless-fruit yield; gTF yield: Total-fruit yield.

Within one column, identical letters implied non-significant difference at $\alpha = 0.05$. ns: non-significant difference; **: significant difference/interaction at $P<0.01$

3, UCZ 1.0 g a.i. m^{-1} c.d. combined with floral induction at 45 DAPUT was again the treatment bringing about the highest fruit set rate.

3.1.3 Fruit yield

As compared with the other mango cultivars grown in the Mekong Delta, e.g. 'Cat Hoa Loc' and 'Cat Chu', fruit set characteristic of 'TDX' mango is relatively different. During the fruit set and development process, there is a certain ratio of seedless fruits. The size of seedless fruit was smaller than that of normal fruits. The size of normal and seedless fruits at maximum growth was 18,1 ± 0,5 cm length - 9.5 ± 0.5 cm width, and 9.5 ± 0.6 cm length – 5.5 ± 0.3 cm width, respectively (Hieu *et al.*, 2018).

Total fruit yield at sites 2 and 3 were at averages of 8.4 and 28.2 kg/tree, respectively. At site 1, total fruit yield of trees treated with PBZ/UCZ doses was significantly different ($P<0.01$). The highest yield was obtained with the UCZ 1.5 and 2.0 g a.i. m^{-1} c.d. treatment (34.1 - 36.1 kg/tree). For the treatments relating to the times of floral induction, 75 DAPUT brought about the highest yield 41.6 kg/tree, while those of the other two treatments (45 and 60 Days after bud initiation treatment - DABIT) were relatively low. The two factors showed significant interaction ($P<0.05$). The highest yield was obtained with PBZ and UCZ at the dose of 1.5 g a.i. m^{-1} c.d. (50.3 kg/tree), and subsequently implementing floral induction at 75 DABIT.

3.2. Foliar application

3.2.1. Concentrations of Uniconazole and times for floral induction

Results presented in Table 2 showed that when compared to the control treatment, foliar application of UCZ at all concentrations did not result in significant difference in terms of flowering rate varying from 47.5 to 59.4%. It is similar for the other parameters, i.e. fruit set (averagely 14.6%), fruit weight (941.1 g), number of fruits per tree (30.1), and yield (48.3 kg/tree). As for the treatments relating to the times of flowering induction after PBZ/UCZ application, 75 DAPUT predominated over the other two treatments (60 and 90 DAPUT) in most of the observed parameters. Flowering (74.2%) and fruit set (19.8%), and yield (48.3 kg/tree) of the 75 DAPUT treatment were significantly higher than those of the other two treatments.

3.2.2. Leaf age at the time of PBZ/UCZ application

Results in Table 3 reflected that the UCZ concentrations, 1,000 – 2,000 ppm showed no significant difference to the control treatment (PBZ 1.5 g a.i. m^{-1} c.d.) in terms of flowering, fruit set rate, and yield. Particularly, for the flowering rate, despite the significant difference observed at site 2 with the highest flowering rate changing from 51.8 – 56.3% (control, UCZ 1,000 ppm, and UCZ 2,000 ppm), the mean flowering rates at site 1 (56.2%) and 3 (58.3%) were not significantly different among the treatments. The latter results were consistent with those in the previous section for testing UCZ in foliar application (1,000 – 2,000 ppm) (Table 2). Similarly, although lower than the results of the previous trial, fruit set rates and fruit yield were not significantly different among the treatments at all three sites with averages from 1.8 – 3.2% for the fruit set rate, and 10.1 – 14.6 kg/tree for the total fruit yield. The effects of leaf ages at the time of UCZ/PBZ application, the three leaf ages (45, 60 and 75 days after emergence - DAM) generally did not show significant difference in flowering rate, fruit set and fruit yield. The only difference was observed on seedless fruit yield at site 2, with the highest yield of the 75 DAM treatment (7.9 kg/tree).

Table 2. Effect of Uniconazole concentrations and periods for floral induction with KNO3 (2.5%) on flowering and fruit set of 'Tuong da xanh' mango

Treatment	*Flowering (%)*	*Fruit set (%)*	*Fruit weight(g)*	*No. of fruits. tree-1*	*Yield (kg/tree)*
Paclobutrazol/Uniconazole (A)					
Control	59.4	16.1	936.2	25.2	23.6
U 1,000	47.5	13.8	944.8	32.0	31.5
U 1,500	51.5	14.1	937.6	31.0	29.8
U 2,000	52.3	14.5	945.7	32.0	31.7
Mean	52.7	14.6	941.1	30.1	29.2
Times of floral induction after PBZ/UCZ application (B)					
60 DAPUT	48.2b	13.0b	947.4	25b	23.3b
75 DAPUT	74.2a	19.8a	936.2	47a	48.3a
90 DAPUT	28.9c	9.7c	944.5	23b	21.4b
Mean	-	-	942.7		-
F(A)	ns	ns	ns	ns	ns
F(B)	**	**	ns	**	**
F(A*B)	ns	ns	ns	ns	ns

Control: Paclobutrazol 1.5 g a.i.m-1 canopy diameter; U 1,000: Uniconazole (UCZ) 1,000 ppm; U 1,500: UCZ 1,500 ppm; U 2,000: UCZ 2,000 ppm; DAPUT: Days after Paclobutrazol/Uniconazole treatments. Within one column, identical letters implied non-significant difference at $\alpha = 0.05$ identified by Duncan multi range test; *: significant difference at $\alpha = 0.05$; ns: non-significant difference.

Table 3. Flowering characteristics and yield of 'Tuong da xanh' mango under the influence of different concentrations of Uniconazole and leaf ages at the time of Uniconazole treatment

Treatment	Site 1 (My Hiep commune)1					Site 2 (Tan My commune)2					Site 3 (Binh Phuoc Xuan (commune)3				
	Flower-ing rate (%)	Fruit set (%)	NFe yield (kg/ tree)	SFf yield (kg/ tree)	TFg yield (kg/ tree)	Flowering rate (%)	Fruit set (%)	NFe yield (kg/ tree)	SFf yield (kg/ tree)	TFg yield (kg/ tree)	Flowering rate (%)	Fruit set (%)	NFe yield (kg/tree)	SFf yield (kg/ tree)	TFg yield (kg/ tree)
PBZ/UCZ doses (A)															
Controls	59.7	1.8	4.3	6.4	10.7	56.3a	2.2	4.6	6.9	11.5	55.8	6.6	6.7	6.9	13.6
U 1,000b	56.7	2.0	3.2	7.0	10.2	51.8a	2.2	3.5	6.4	9.8	58.0	2.1	9.2	5.7	14.8
U 1,500c	51.6	1.7	3.5	8.6	12.1	44.8b	2.2	1.9	6.5	8.4	61.3	2.2	7.0	7.4	14.4
U 2,000d	56.6	1.8	4.0	7.0	11.0	54.9a	2.2	4.1	6.5	10.6	57.9	2.0	7.6	8.0	15.6
Mean (A)	56.2	1.8	3.8	7.3	11.0	-	2.2	3.5	6.6	10.1	58.3	3.2	7.6	7.0	14.6
Times of flowering induction treatment (B) (days after PBZ/UCZ treatment)															
45 days	58.1	1.9	3.8	7.7	11.4	53.0	2.2	3.1	5.9b	9.0	60.2	5.1	8.5	5.8	14.3
60 days	54.8	1.8	3.2	6.6	9.8	52.2	2.2	3.8	5.9b	9.8	57.8	2.4	6.8	7.6	14.4
75 days	55.6	1.8	4.2	7.6	11.8	50.6	2.2	3.7	7.9a	11.5	56.6	2.2	7.6	7.6	14.2
Mean (B)	56.2	1.8	3.7	7.3	11.0	51.9	2.2	3.5	-	10.1	58.2	3.2	7.6	7.0	14.3
F(A)	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
F(B)	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
F (A*B)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

a Control: Paclobutrazol, 1.5 g a.i. m-1 canopy diameter; bU 1,000: Uniconazole, 1,000 ppm; c U 1,500: Uniconazole, 1,500 ppm; d U 2,000: Uniconazole, 2,000 ppm diameter; eNF yield: Normal-fruit yield; fSF yield: Seedless-fruit yield; gTF yield: Total-fruit yield.
Within one column, identical letters imply non-significant difference at $\alpha = 0.05$. ns: non-significant difference; **: significant difference/interaction at $P<0.01$

4. DISCUSSION

The main aim of this study was to evaluate and determine the best dose and/or concentration of UCZ to replace PBZ, which is about to be banned from use in Viet Nam. The results obtained from the two separate trials to test the possibility of using UCZ as collar drenching or foliar application have confirmed that UCZ is a good replacement for PBZ. In regard to flowering rate, there was no significant difference between PBZ and UCZ applied by either collar drenching or foliar spray (Table 1, Table 2). As for the fruit set rate, despite the non-significant difference when UCZ was tested as foliar applications, collar drenching UCZ at 1.0 g a.i. m^{-1} c.d. resulted in the highest fruit set rate across the three experimental sites. Therefore, without considering fruit yield, which is governed by various factors, 1.0 g a.i. m^{-1} c.d. could be recommended when UCZ is applied by collar drenching. For foliar application, while there was no clear difference with regard to flowering rate, fruit set, yield and fruit quality, the lowest concentration of UCZ tested in this study, 1,000 ppm, is a good choice.

For the treatments relating to the times of floral induction after PBZ/UCZ application, the flowering rates of 75 DAPUT treatment in both trials (collar drenching and foliar application) were consistently higher than those of the other treatments. In the trial testing UCZ as collar drenching, the times for floral induction varied from 45 – 75 days after PBZ/UCZ application. Such intervals were increased to 90 days (ranging from 60 to 90 days) in the trial using UCZ as foliar sprays. However, the increase, from 75 to 90 days, even reduced the flowering rate enormously, from 74.2% (75 DAPUT) to 28.9% (90 DAPUT). Therefore, within the time frame of 45 to 90 days after PBZ/UCZ application, 75 days was determined to be the best interval to induce flowering using KNO3 2.5%, either when UCZ is applied as collar drenching or foliar application. These results suggest that flowering induction conducted on 'TDX' mango can be commenced from the 75th day after the application of either PBZ (1.5 g a.i. m^{-1} c.d.) or UCZ (1.0 – 2.0 g a.i. m^{-1} c.d.). Hau (2013) reported similar results showing that flowering induction with thiourea 0.5% on 'Cat Hoa Loc' mango conducted at 75 – 90 days after PBZ application led to high flowering rates. However, for 'Cat Chu mango', according to Hau and Dien (2009), floral induction with thiourea 0.5% implemented at the 60th day after collar drenching with PBZ 1.5 g a.i. m^{-1} c.d. resulted in higher flowering rate than that of the other two times, viz. 75 and 90 days after PBZ application. Therefore, the suitable times for floral induction after PBZ/UCZ application vary with the cultivars.

In the Mekong Delta, leaf age is an important factor to determine the time for PBZ application. However, the latter also changes depending on the cultivar. For 'Cat Hoa Loc' mango, leaf flushes turning gradually to light green, with soft and flexible lamella are suitable for PBZ application. For 'Chau Hang Vo', 'Buoi', and 'Thanh Ca' cultivars, the best time for PBZ application is when leaves turn to dark green, 4 to 5-month-old ~ 120 – 150 days (Hau, 2008). That means the leaf age of these cultivars must be older than that of 'cat Hoa Loc'. For 'Cat Hoa Loc' mangoes, Hau and Thuy (2008) conducted a study in which PBZ 1.0 g a.i. m^{-1} c.d. was applied at the leaf ages of 15, 30 and 60 day-old, followed by floral induction with thiourea (0.5%). It was reported that PBZ applications when leaves were at 15, 30, and 60 day-old brought about higher flowering rates than the untreated control treatment. Among the three leaf-age treatments, there was no significant difference in flowering rates observed before thiourea application with the highest flowering rate obtained with the 15-day-old leaf age (23.7%). However, the flowering rate recorded after thiourea application was not significantly different among the three leaf ages, varied from 53.8 (60-day-old) to 55.6% (15-day-old). In the present study, the flowering rate observed after floral induction with KNO_3 (2.5%) was similar, with no significant difference among the three leaf age (45. 60 and 75-day-old), varying from 50.6 to 60.2% (Table 3). In another trial of the present study investigating the effect of UCZ concentrations applied as foliar

application and time for floral induction on flowering rate (Table 2), PBZ/UCZ was sprayed when leaf was 15-day-old. The mean flowering rate was 52.7%, varying from 47.5 to 59.4% (Table 4). Therefore, it is likely that the flowering rates obtained when PBZ/UCZ was applied on leaves at the age of 15 to 60-day-old were not different. Consequently, it is possible to commence PBZ/UCZ application as early as the leaf flushes reach to 15-day-old.

5. CONCLUSIONS

The results in this study suggested that for 'Tuong Da Xanh' mango, UCZ can totally be a good replacement for PBZ. For flowering rate, there was no clear or significant difference between PBZ and UCZ applied by either collar drenching or foliar spray. In addition, collar drenching UCZ at 1.0 g a.i. m^{-1} c.d. consistently resulted in the highest fruit set rate across the three experimental sites, thus it could be recommended for application on 'TDX'. For foliar application, while there was no clear difference about flowering rate, fruit set, yield and fruit quality, the lowest concentration of UCZ tested in this study (1,000 ppm) can be recommended. As for the time of floral induction undertaken with KNO_3 2.5% (two times in one week), the best time was 75 days after PBZ/UCZ application.

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VOLATILE SUBSTANCES IN GREEN FRUIT OF FOUR PAPAYA CULTIVARS

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ABSTRACT

Texture and aroma of the fruit are important factors that directly affect the taste and consumer preferences. One of the factors that may affect the smell of green papayas is the amount of latex produced due to its unclear harvesting period. Therefore, this study aimed to evaluate its smell by a sensory panel and determine the amount of latex produced from 8 cultivars of green papayas at 75, 95, and 125 days after flowering. The highest amount of latex was observed in 'Khak Nuan', 'Khak Dam Watpleng', 'Rang Nai Roi', and yellow flesh papayas while the lowest amount of latex produced was observed in 'Krang Daeng'. Furthermore, the highest amount of latex was observed in green papayas at 75 days after flowering and decreased at 95 and 125 days after flowering. The highest smell scored was observed in 'Khak Dam Watpleng' and green papayas at 95 and 125 days after flowering while the lowest smell scored was observed in 'Krang Daeng', yellow flesh, and green papayas at 75 days after flowering. Additionally, volatile substances and their amount in green papaya fruits at 75 days after flowering of the four cultivars ('Khak Dam Kaset', 'Khak Nuan', 'Krang Lueng', and 'Khak Dam Damnoen') were studied using Gas chromatography-Mass spectrometry. Fifteen volatile compounds, i.e., ethanol, benzyl alcohol, nonanal, methyl octanoate, benzyl isocyanate, decanal, eugenol, benzyl isothiocyanate, dihydropseudoionone, butylated hydroxytoluene, isopropyl myristate, methyl palmitate, dibutyl phthalate, isopropyl palmitate, and methyl heptadecanoate were found in green fruits of 'Khak Nuan', 'Krang Lueng', and 'Khak Dam Damnoen' papayas. Among all identified volatile substances, benzyl alcohol was the most abundant substance in all four cultivars. As for the green papaya fruit of 'Khak Dam Kaset', all volatile substances except isopropyl myristate and benzaldehyde were found that was not observed in other cultivars.

Keywords: papaya salad, fruit smell, Gas chromatography-Mass spectrometry, papaya latex, papaya smell

DIFFERENCE ANALYSIS OF RESISTANT STARCH ACCUMULATION IN BANANA FRUITS

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ABSTRACT

Bananas are well known as good sources of starch. Resistant starch (RS) is a functional component having health care function. 'Cavendish' and plantain bananas were used to investigate the influences of hydrolases and granule structure on starch degradation. The levels of RS, non-resistant starch (non-RS), total starch, and amylose content during the fruit-ripening process were determined. For starch-synthesis, yeast one-hybrid assay, Real Time q-PCR, double luciferase experiment, banana genetic transformation were used to screen and verify the transcription factors regulating the synthesis of RS in developing banana fruit. For banana starch processing, the physicochemical properties of starch were analyzed from 3 banana cultivars and potato as comparison. Compared to 'Cavendish', plantain had a higher content of total starch and RS, a faster starch-degradation rate, and a lower decrease in the ratio of RS/total starch. Two a-amylases, one starch phosphorylase, and one starch debranching enzyme were specifically upregulated in plantain, which might hydrolyze more non-RS compared with Cavendish; *MaGBE4* was specifically upregulated during banana fruit developing, 5 transcription factors were selected, which might regulate starch synthesis by binding *MaGBE4*; Banana starch had higher gelatinization temperature than potato starch, which corresponded to the rich resistant starch in banana and in conclusion there are differences in the synthesis and degradation of resistant starch among different banana varieties. Transcription factors can regulate the synthesis of resistant starch by regulating the activity of *MaGBE4*. The resistant starch in banana has great potential for processing.

Keywords: banana; resistant starch; transcription factors; processing

1. INTRODUCTION

Starch is a product of photosynthesis and is present as a semicrystalline form in plant cell plastids. In animals, starch is digested at different rates according to the structure of the starch in the starch granules. There are three categories: 1) Rapidly digestible starch, which can be digested and absorbed orally and in the small intestine rapidly (digestion time < 20 min); 2) Slowly digestible starch, which can be digested in the small intestine (digestion time between 20 and 120 min); 3) Resistant starch (RS), which refers to the starch that cannot be digested or absorbed in the small intestine (digestion time > 120 min). The existence of resistant starch was first proposed by Englyst *et al.* (1982). The physiological function of RS is similar to dietary fiber, as in that it can delay blood glucose and insulin response, and reduce the concentrations of cholesterol and triglycerides. Resistant starch is fermented and degraded to short-chain fatty acids that acidify the intestine contents and may play a role in the prevention of colon cancer (Fuentes-Zaragoza *et al.*, 2010). As more attention is being paid to healthy diets, RS has become a focus for research activity.

2. MATERIALS AND METHODS

Fruit fingers were selected for uniformity of shape, size, and color. After ethylene treatment (500mL L^{-1}) for 16 hours, fruit was stored at 24°C and 90% moisture. The samples were collected at 0, 2, 3, 4, and 5 days in storage time, and subsequently frozen in liquid nitrogen and stored at -80°C prior to further analysis. Five banana fingers were selected for each replicate. All samples were prepared with at least three biological replicates.

Fruit flesh (0.5 g fresh weight) frozen with liquid nitrogen was ground into powder using mortar and pestle, then treated with successive washes of 80% alcohol, 50% alcohol, and water to remove soluble sugar and other soluble substrates. Resistant starch and non-RS contents were analyzed using a Resistant Starch Assay Kit (Megazyme, Bray Business Park, Bray, Wicklow, Ireland) according to the manufacturer, Park, Bray, Wicklow, Ireland). Assayrc is the sum of RS and non-RS.

Starch granules were isolated from the fruit pulp at five ripening stages using a reported protocol (Soares *et al.*, 2011) with the following modifications. Frozen flesh pulp was ground into powder then a 5 g sample was directly suspended in 0.005 L of pectinase solution (15 g L^{-1}, pH 4.0, Sigma Chemical Co.) for enzymic removal of cell walls. The homogenate was laid in a shaking water bath at 45°C for 2 h, then filtered using Miracloth membrane (Calbiochem). After being centrifuged at 3000g for 10 min, the pellet was washed with distilled water three times. The pellet was dried in a drying oven, and stored at room temperature for SEM and amylose content analysis.

Amylose content was determined using a two-wavelength method with potassium iodide (Zhu *et al.*, 2008). Dry starch granules (0.1 g) were completely dissolved in 0.01 L potassium hydroxide solution (0.5 mol L^{-1}), then diluted to 0.6 L with distilled water; 0.03 L were titrated to pH 3.5 with hydrochloric acid (0.1 mol L^{-1}), then 0.002 L iodine solution was added. After color development, samples were scanned at 624 and 472 nm using a NanoDropTM 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Results were evaluated using the method described by Zeng *et al.* (2012).

The dried starch granules were fixed onto stubs using doublesided tape and coated with a 10 nm thick platinum layer using the JEOL JFC-1600 (JEOL, Tokyo, Japan) coating system. The samples were examined on a JEOL JSM-6360LV (JEOL, Tokyo, Japan) scanning electron microscope. Scanning electron microscopy was performed in secondary electron mode at 15 kV. After SEM analysis, the images were loaded in SmileView (JEOL Ltd, Tokyo, Japan) software. The length of starch granules was measured using "apan" software. The length of starch granules was measured for each sample and the average particle size of the starch nanoparticles was determined.

3. RESULTS

3.1. Changes in starch content during fruit ripening

The total starch content decreased gradually in both Cavendish and Plantain during the ripening process (Table 1). At 0 day, 91% of total starch was RS in Plantain, which dropped to 83% at 5 days. Meanwhile, the proportion of RS in total starch decreased from 87% to 69% in Cavendish. That means the ratio of RS in total starch declined at 8% and 18% in Plantain and Cavendish, respectively. This suggested that the ratio of RS in total starch decreased more slowly in Plantain

than in Cavendish, and there was obviously a different preference in starch degradation between Cavendish and Plantain.

Table 1. Resistant (RS) and non resistant starch (non-RS) content in Cavendish and Plantain at five ripening stages

Starch	Species	0 d	2 d	3 d	4 d	5 d
RS	Cavendish	0.81±0.007 b	0.114±0.008 c	0.075±0.004 d	0.057±0.003 e	0.027±0.002 f
	Plantain	0.253±0.011 a	0.201±0.026 b	0.140±0.015 c	0.080±0.008 d	0.053±0.012 e
Non-RS	Cavendish	0.028±0.001 a	0.028±0.003 a	0.017±0.001 c	0.020±0.003 c	0.012±0.002 d
	Plantain	0.024±0.004 b	0.017±0.002 c	0.017±0.002 c	0.012±0.001 d	0.011±0.001 d
Total	Cavendish	0.209	0.142	0.092	0.077	0.039
	Plantain	0.277	0.218	0.157	0.092	0.064

3.2. Starch-degradation-related gene expression analysis

Compared with b-amylases, a-amylase had different expression patterns (Figure 1). Two genes (GSMUA_Achr3T07130_001 and GSMUA_Achr3T07190_001) encoding a-amylases were downregulated during the whole ripening process in both Cavendish and Plantain, while two (GSMUA_Achr5T10560_001 and GSMUA_Achr8T04140_001) were up-regulated. Specifically, a-amylase (GSMUA_Achr7T18920_001) was down-regulated in the Cavendish fruit-ripening process but up-regulated in Plantain, especially at later stages. This suggested that GSMUA_Achr7T18920_001 might play a vital role in the differential starch degradation between Cavendish and Plantain. Isoamylase is one type of debranching enzyme, which preferentially removes short branches and participates in amylopectin synthesis. Interestingly, one gene encoding isoamylase (GSMUA_Achr7P12210_001) was strongly induced gradually during the Plantain ripening process, reaching a 49-fold increase at 5 days compared with 0 day.

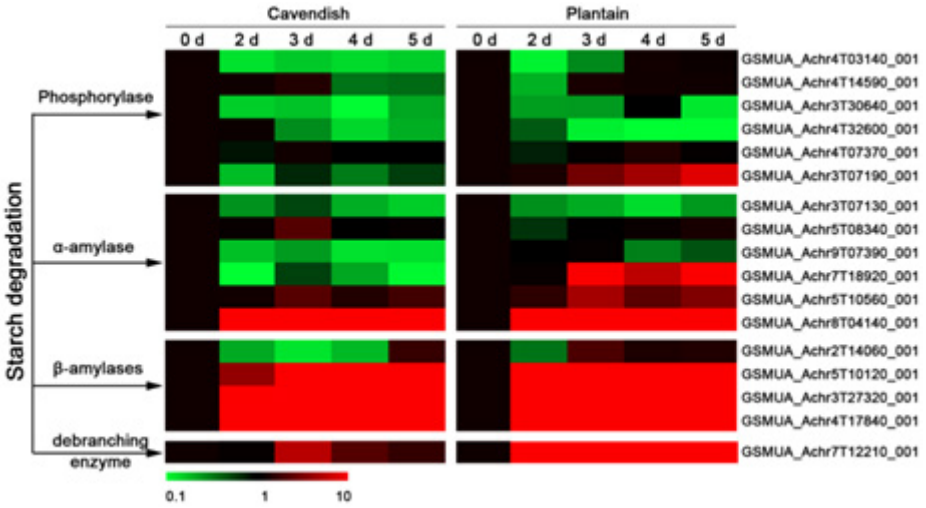

Figure 1. Starch degradation-related genes in fruit ripening

3.3. MaGBE4 expression analysis and transcription factors selected

Five MaGBEs genes were cloned from banana, which belong to three different subtypes. Among them, subtype 2 is reported to be the most functional family. The results of Real Time q-PCR

showed that the expression of *MaGBE4* was significantly higher than other homologous genes during banana fruit development. Therefore, we indicate that *MaGBE4* is the key gene regulating the synthesis of resistant starch in banana fruit. The promoter region of *MaGBE4* was then cloned and constructed as bait vectors. Five candidate transcription factors were screened by the yeast one-hybrid system. Then we verified that these 5 transcription factors could interact with *MaGBE4* by double luciferase assay, subcellular localization and Point-to-Point Y1H.

3.4. Physicochemical Properties of Different Starches

Starch physicochemical properties were analyzed in 3 banana cultivars and potato (Table 2). Pasting temperature in 3 banana cultivars was higher than in potato, while peak viscosity, cool viscosity and final viscosity of starch in 3 banana cultivars were lower than in potato. Banana starch had higher gelatinization temperature, which corresponded to the rich resistant starch in banana.

Table 2. Starch physiochemical properties for 3 banana cultivars and potato.

	Dajiao	Guang Fen	Baxi	Potato
Gelation initiation temperature (°C)	67.60	76.80	79.20	63.00
Gelation peak temperature (°C)	71.70	79.70	82.90	68.60
Gelation termination temperature (°C)	77.10	85.10	88.50	77.50
Pasting temperature (°C)	84.00	82.35	82.90	67.20
Peak viscosity (cP)	114.00	88.00	89.92	371.17
Cool viscosity (cP)	83.50	76.08	88.67	129.83
Final viscosity (cP)	126.75	98.00	123.67	172.67
Setback (cP)	43.25	21.95	35.00	42.84

Note: Starch was extracted from Dajiao (AAB gene group), Guang Fen (ABB gene group), Baxi (AAA gene group), and potato.

To explore the regulatory mechanism of starch degradation in banana fruit, 17 starch degradation-related genes were selected for quantitative real-time PCR analysis. The genes were classified into four groups based on their reaction products, including six starch phosphorylases, six a-amylases, four b-amylases, and one debranching enzyme. The ratios of the other samples compared to the 0 day samples were calculated and the Logs of ratios base 2 are presented in both 'Cavendish' and plantain. Up-regulated genes are shown in red and down-regulated genes are in green.

'Cavendish' and plantain fruits were harvested at the green maturity stage. After ethylene treatment, fruits were stored at 24°C and 90% moisture, and sampled at 0, 2, 3, 4, and 5 days. Resistant starch (RS) and non-resistant starch (non-RS) contents were determined using a Resistant Starch Assay Kit. Data presented are the average (kg kg^{-1} fresh weight) with SD of three replicates. Letters a, b, c, d, and e represent the significant differences with $P < 0.05$.

4. DISCUSSION

In commercial production, banana fruits are harvested at an unripe state and requires undergoing ripening in order to be edible after harvest. In the commercial process, fruits are harvested, and physiological and biochemical changes are controlled in ripening chambers/facilities to form unique flavors and quality. Starch, as the main carbon source stored in the fruit, is dramatically degraded during the ripening process for flavor substance synthesis and energy supply. In past decades, there have been two models of starch metabolism: one is based on the germinating cereal seed (Beck & Ziegler, 1989) and the other on the diurnal metabolism of leaves (Zeeman

et al., 2007). The banana fruit is likely to incline towards the seed model. In this process, starch is hydrolyzed into soluble sugars catalyzed mainly by four kinds of amylases, including b-amylases, a-amylases, starch phosphorylases, and debranching enzymes (Smith *et al.*, 2007).

5. CONCLUSIONS

This initial analysis of the difference in starch degradation in the fruit-ripening process between plantain and 'Cavendish' banana focuses on the role of starch structure and function of amylase enzymes in RS degradation. Both total starch and RS contents decreased in the two cultivars during ripening. Compared to 'Cavendish', plantain had a higher content of total starch and RS, a faster starch-degradation rate, and a lower decrease in RS proportion. Starch granules of 'Cavendish' were larger and more rounded, while smaller and ellipsoidal starch granules were observed in plantain, which degraded more easily. Also, the analysis of gene expression suggested that b-amylases had a key role in starch degradation in both cultivars. A number of genes were specifically up-regulated in plantain, including two a-amylases, one starch phosphorylase, and one starch debranching enzyme. These genes may be involved in the degradation of greater amounts of non-RS starch in plantain than in 'Cavendish' banana fruit.

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EVALUATION OF HETEROSIS VALUE AND POTENTIAL RATIO ON FRUIT CHARACTERS OF MANGO HYBRIDS

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ABSTRACT

Crossing is one of the ways to improve mango cultivars. Identification of heterosis and heterobeltiosis values are needed because not all crosses show desired effects of heterosis and heterobeltiosis. Selection of mango hybrids is expected to produce better progenies characteristically than their parents. The research was conducted at Cukurgondang Experimental Station, Indonesian Tropical Fruits Research Institute (ITFRI) from 2011 to 2018 with the objectives to determine the degree of heterosis, heterobeltiosis, and potence ratio in mango hybrids. Twenty three mango hybrids were used and originated from crossing of 'Arumanis 143' × 'Haden', 'Haden' × 'Arumanis 143', 'Arumanis 143' × 'Irwin', 'Irwin' × 'Arumanis 143', 'Arumanis 143' × 'Li'ar', 'Arumanis 143' × 'Gedong Gincu', 'Arumanis 143' × 'Keitt', 'Delima' × 'Arumanis 143', and 'Arumanis 143' × 'Saigon'. The parameters observed were fruit weight, fruit length, fruit width, flesh thickness, stone weight, and edible portion for heterosis and potence ratio calculations. Results showed that high diversity were observed in fruit weight (34.42%) and stone weight (31.27%) of the mango hybrids. Positive heterosis and heterobeltiosis values were detected on F1-02, F1-18, and F1-51 at the fruit weight characteristic. Positive heterosis and heterobeltiosis values were observed in F1-27, F1-01, F1-02, F1-35, F1-87, F1-61, F1-18, and F1-33 hybrids on the flesh thickness and portion of edible fruit characteristics. Meanwhile, negative heterosis and heterobeltiosis were spotted on stone weight parameters. F1-02 and F1-18 hybrids can be used as candidates for new superior mango varieties with greater fruit weight, thicker fruit flesh, smaller stone weight, and higher portions of edible fruit.

Keywords: mango, hybrids, crossing, cultivar improvement

ACHACHA – THE COMMERCIALISATION OF A TROPICAL FRUIT

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ABSTRACT

This paper describes how a little-known fruit from the Amazon Basin, the Achacha, was grown for the first time in a commercial plantation environment in tropical north Queensland, Australia. The fruit has been sold in Australia and exported since 2009. The paper describes the marketing and selling approach taken to launch a fruit new to the world, packaging details, research activities carried out to date, and an issue for the future – namely, to find a viable method of separating skin from pulp and seed.

Keywords: Achacha, *achachairú*, *Garcinia*, certified organic, biodynamic

1. THE FRUIT

Starch is a product of photosynthesis and is present as a semicrystalline form in plant cell plastids. In animals, starch is digested at different rates according to the structure of the starch in the starch grAs more attention is being paid to healthy diets, RS has become a focus for research activity.

The Achacha (*Garcinia humilis*, *achachairú*) is a tropical fruit from the Amazon Basin of Bolivia. It is orange in color, the size and shape of an egg, with a white edible pulp on a cling seed. Taste experts describe its unique flavour as "sweet, tangy, refreshing – like a sorbet". Its thick skin can be made into a tea which is used traditionally in Bolivia as a hunger suppressant. The pulp is low in sugar – one third that of fruits such as its cousin the mangosteen (*Garcinia mangostana*), and other tropical fruits including lychee, longan and rambutan, with which it is often compared, but it has a sweet taste. It has excellent health characteristics – rich in folate, riboflavin, and Vitamin C. The skin contains hydroxycitrate, betacarotene, and arginine (for the heart).

The fruit grows inside the foliage of a tree which has a dense tangle of branches and would normally reach 10m in height. Achacha Fruit Plantations (AFP) has 16,000 mature trees at its certified organic Palm Creek Plantation just south of Townsville, Queensland; biodynamic methods are used to grow the fruit.

AFP was the first commercial grower of the fruit and remains the largest. Over the last couple of years a Guatemalan company has joined the market, and it is believed that a Taiwanese grower may now have fruits available.

2. GROWING THE TREES

Highly prized in and around the city of Santa Cruz de la Sierra in the foothills of the Andes, the fruit was little known elsewhere until recently. In 2002 a Bolivian-Australian who had grown up with the fruit managed to convince a few friends that it should be grown in tropical north Queensland. At the time there was nothing on the web or in the readily available literature about the fruit, so a few kilos were imported in order to enable interested parties to see and taste it. Australia's notoriously strict plant quarantine rules meant that the fruit was frozen on arrival, but even so the unique flavour was appreciated.

Arrangements were made with Bolivian and Australian government agencies and seeds were imported and planted. After some months, the seeds germinated and grew into healthy plants needing a home. A former sugar cane farm of 123 ha was purchased, completely regraded, an irrigation plant installed, and over the next three years in excess of 16,000 trees were planted in 17 blocks separated every 150 m by rows of fast-growing African mahogany (*Khaya senegalensis*) as windbreaks.

Prior to its voyage half-way around the world to Australia, the fruit had generally been grown in small holdings of up to a few hundred trees in forest clearings under semi-shade conditions; there was no experience of plantation-style irrigated operations, with kilometer-long rows and no shade, so there was a lot to learn. A year or so was lost due to insufficient prior hardening of the young plants. Then the trunks grew rapidly, probably as a result of too much care and attention – bamboo supports were replaced by fibreglass ones, progressing to wooden stakes and eventually steel pickets. Eventually the root and trunk structures developed and now they are very robust and defy strong winds. As the trees are hand-picked from the ground, they are trimmed to a height of 3m, which reduces significantly their exposure to cyclones. They are also skirted in order to reduce the ability of vines to grow up and onto the trees, and to facilitate sprinkler inspection.

3. MARKETING AND SELLING

With a new fruit that looked good, tasted good, and had a long shelf life, first expectations were that marketing and selling would be relatively simple tasks. We soon learnt that an independent grower with a new product in a market replete with good fruit had to educate a conservative public in order to achieve sales.

The first issue faced was how to name the fruit, given that the common name in Bolivia, *achachairú* – from an indigenous word meaning honey kiss – would probably suffer from Australians' notorious habit of abbreviating long words. At the same time, as Bolivians were very proud of the fruit, it was felt that the name should reflect its origins. "Achacha" maintained the Latin rhythm.

Given the limited budget available, social media became the obvious and most effective method of communicating the virtues of the fruit; a decade later we still use social media wherever possible. Whilst there are many people in home markets who have never heard of the fruit, the web is alive with information on it for those who are curious enough to investigate and likely to buy. Initially social media was supported by in-store demonstrations which gave us the chance to meet with potential customers; we continue this process through growers' markets in selected locations.

As a new product on the market the fruit has attracted the attention of chefs, food writers,

bloggers, media outlets, schools, charities, and service groups. We have worked with all of these providing information, brochures, and speakers when requested. We encouraged visits to the plantation for local residents, and provided samples generously in season. It has been a long, hard but rewarding road.

In order to show how to open the fruit, we commissioned a young film maker to put together a 20 second YouTube video; he rallied his friends who borrowed top equipment from their employers and came back with a two and a half minute masterpiece called "The Chase" which continues to amuse viewers. When it first appeared a cinema advertising company in Sydney was so taken by its originality that it screened it in a variety of cinemas at rock bottom prices.

Although we had not planned to export until we had established a solid Australian market, an opportunity arose when the former Horticulture Australia Limited took a few boxes of the fruit to the huge Berlin Fruit Logistica 2011. This was the first time the fruit had been seen in Europe; it was so popular it was selected the following year as a finalist in the Innovation Award, and took out third place. This led to interest from European wholesalers, in particular the supplier to a major upmarket retail chain, which has become a regular customer. The EU sales helped raise the profile of the fruit in Australia and elsewhere resulting in regular exports to Asia, the Middle East and Canada. Other potentially profitable markets, such as China, USA, India, and Japan, are not accessible for political or other reasons, in spite of frequent requests from wholesalers. One important factor which restricts growth in the export market is the economic shipment size; air freight from Australia is sold by volume, with an LD3, which can hold about 1,350 kg of fruit, the smallest shipment container. It's a brave importer who will take on such a volume of an unknown fruit.

4. PACKAGING AND SHIPPING

Initially a 5 kg box containing about 100 pieces of fruit was designated as the standard packaging. We have added a 12-piece presentation box, and 2 kg and 10 kg boxes. Each box is supplied with an A6 flyer which describes how to use the fruit; another A6 flyer gives information on storage temperatures.

In order to persuade customers to take a kilo of fruit rather than one or two pieces, an attractive paper carry bag with handles was designed. The bag became a collector's item and helped sales when the fruit was not well known.

5. RESEARCH

Although funding has been difficult to find, some work has been done. The University of New South Wales carried out an initial analysis of the fruit, comparing its properties with the mangosteen, lychee, and rambutan. The University of Western Sydney has carried out several studies into the properties of the skin, with particular emphasis on a drink made from it. The University of Southern Queensland has studied the Achacha along with other garcinias with respect to their potential to combat metabolic syndrome.

6. THE FUTURE

Demand continues to rise for the first quality fruit. There is also strong demand for pulp. However in spite of significant effort a viable commercial method of taking the skin off the fruit has not been found. If it could, the skin would be used for drink manufacture, the pulp for sorbets, ice-

cream, sauces, drinks and so on, and the seeds for their oil. Work in this area is ongoing.

APPENDIX - IMAGES

Figure 1. Achacha fruit

Figure 2. Fruit on the tree

Figure 3. Five kg box

Figure 4. Overview – Palm Creek Plantation, Giru, North Queensland, Australia

INDONESIA MANGO DEVELOPMENT: A RESEARCH AND DEVELOPMENT PERSPECTIVE TO CAPTURE THE OPPORTUNITIES AND FACE THE CHALLENGES IN GLOBAL TRADE ERA

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ABSTRACT

Indonesia is ranked fifth in the world as a mango producing country. Trade balance of Indonesian mangoes have always been at a surplus, although only less than 1% of mangoes are exported. Global as well as local markets for mangoes have remained robust, thus it is important to produce quality mangoes to be accepted into those markets. This paper will discuss the development of mangoes in Indonesia in order to capture the opportunities and face the challenges in the global market era, especially from the research and development perspectives. Currently, mangoes produced that meet the standards of the global market are still limited. Most mango farming is carried out on an extensive small home garden scale. Farmers' awareness of good production practices as well as the application of technology, are still low although many research results have been disseminated. Due to these problems, the trend of mango production has declined owing to the decreasing productivity of mango trees. In 2017, national mango productivity was 10.96 mton/ha, a decrease of 2.32% from the previous year (11.22 mton/ha). To increase the performance of national mango competitiveness, the Indonesian government has massively facilitated the development of mango regions in several regencies. The program becomes an opportunity in disseminating research results because it includes the free distribution of improved and certified seeds, assistance in mango farming technology packages, and also post-harvest technology. Under this program, Indonesia's mango competitiveness is expected to increase especially in productivity, quality, and the capacity to enter the global market.

Keywords: mango, Indonesia, research and development

1. INTRODUCTION

Mango is one of the important tropical fruits in the world. More than 80 countries cultivate mango with a global planting area of more than 2.7 million hectares (Jahurul *et al.*, 2015). The five biggest mango producers in the world are India, China, Thailand, Indonesia, Pakistan, and the Philippines (Trivedi, 2012; Saave, 2013; Wandschneider *et al.*, 2013; Evans *et al.*, 2017). Mango is not only consumed domestically but also exported by these countries. The top five largest importers of mango together with mangosteen and guava in the world are the USA, the Netherlands, the Kingdom of Saudi Arabia, the UK, and Germany with a total export of 1189.10 mton in 2013, an increase of 91.23% from 2010 (Evans *et al.*, 2017). The increase in mango commercialization and also the importance of the commodity reinforces its position as one of the most popular tropical fruits in the world.

In Indonesia, mango is an important tropical fruit that contributes to the national horticulture

development and with the most significant production number after bananas (Indonesian Statistics, 2017). In 2017, production reached 2,203,789 mton. The Indonesian Ministry of Agriculture also states the importance of mango in its 2014–2019 strategic plan, by developing mango production areas under the concept of agro-industry (Indonesian Ministry of Agriculture, 2014). Mango has also been identified as a potential commodity for increasing farmers' income and can support the development of industry and export (Supriatna, 2005). In some regions in Indonesia, mango has been identified by the local and central governments as a competitive commodity (Supriatna, 2005; Anugrah, 2009; Setyawati, 2012; Yuniastuti & Purbiati, 2016; Kusumo *et al.*, 2018; Mukti *et al.*, 2018).

Despite its growing prominence domestically, Indonesia is still left behind from other mango producing countries in the international market. Based on the statistical data from FAO, Indonesia is not in the top 10 mango exporters in the world. Indonesia is also not a significant exporter in Asia (Arifin, 2013). From the perspective of competitiveness, Indonesian mango has a smaller comparative advantage compared to other mango producers in the ASEAN region (Hanani *et al.*, 2009; Pradipta & Firdaus, 2014); with only less than 1% of the Indonesian mango being exported (Wandschneider *et al.*, 2013; Qanti, 2014).

Many factors contribute to the low export and competitiveness of Indonesian mango (Purnama *et al.*, 2014). Problems such as disparity in maturity (Ropai *et al.*, 2013), fruit fly infestation (Hasbullah *et al.*, 2009), the high level of damage due to rapid ripening (Sari *et al.*, 2004; Ropai *et al.*, 2013), seasonal production patterns (Anugrah, 2009; Arifin, 2013), and use of calcium carbide for ripening (Per *et al.*, 2007) are some of the common causes affecting competitiveness of Indonesian mango. Furthermore, mango cultivation in Indonesia is predominantly undertaken by smallholders (Wandschneider *et al.*, 2013), who lack awareness of good production practices, as well as the application of technology. Good maintenance and management practices to produce quality fruits are still lacking, especially in the home gardens of Indonesian mango farmers (Purnama *et al.*, 2014; Qanti *et al.*, 2017; Mukti *et al.*, 2018). Due to these problems, mango production has declined because of the decreasing productivity of mango trees. In 2017, national mango productivity was 10.96 mton/ha, a decrease of 2.32% from the previous year (11.22 mton/ha).

The development of mangoes in Indonesia has several opportunities for improving its competitiveness in the global market. From the market perspective, the value of world mango export showed an annual 26% average growth rate, with a total value of about USD1.69 billion in 2013, rising more than 200% compared with the year 2000 (Evans *et al.*, 2017). There is still a great opportunity to increase mango exports as so far there has not been limitations by importing countries (Saptana *et al.*, 2018). Preferred characteristics in the global market are orange to red, with a slight sour taste and fresh aroma, hence, indicating that the future trend of Indonesian mango exports could be for red mangoes (Rebin *et al.*, 2014).

In addition, most markets now require a certification of Good Agricultural Practices or the GLOBALGAP (Galán Saúco, 2015). It is very important to identify opportunities for the application of innovation and technology in mango development policies to improve the competitiveness of Indonesian mangoes in the global market. Therefore, this paper will discuss the development of mango in Indonesia in order to capture the opportunities and face the challenges in the global market era, especially from the research and development perspective and how to formulate it into developmental policies.

2. METHODS

The method of study was carried out by reviewing various literature studies, especially primary scientific journals and other data and information from the Ministry of Agriculture's Data Statistics and FAOStats.

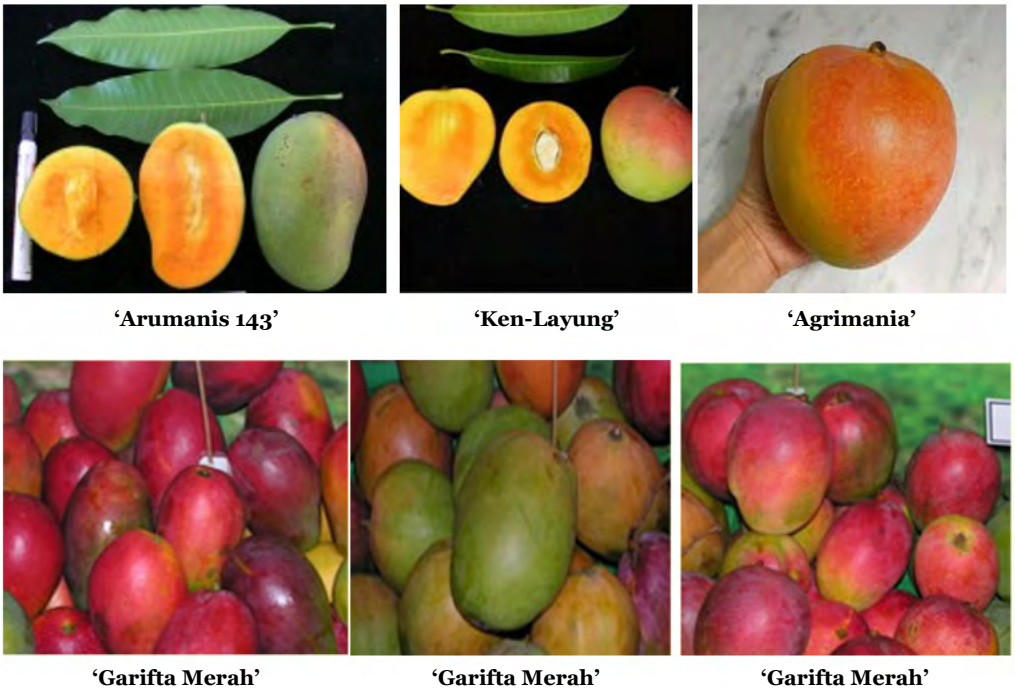
3. RESULTS

3.1. Mango cultivation technology that has been generated

3.1.1. Variety

Indonesia has more than 30 species and numerous varieties of mango, which makes it a centre of diversity for mangoes (Kostermans & Bompard, 1993). There are some common commercial mango varieties in Indonesia such as the 'Arumanis', 'Manalagi', 'Golek', 'Lalijiwo', 'Gedong', and 'Indramayu' (Yuniarti & Santoso, 2000), and also some varieties that grow wild in the community forest (Kiloes *et al.*, 2016) which are not maintained but harvested when there are fruits (Kiloes *et al.*, 2015).

Cukurgondang Experimental Garden, an experimental garden under the supervision of the Indonesian Centre for Horticultural Research and Development (ICHORD), Indonesian Ministry of Agriculture in Pasuruan, East Java has a collection of more than 200 commercial and local mango cultivars (Yuniarti & Santoso, 2000; Tasliah *et al.*, 2016) with market potential. 'Arumanis' and 'Gedong' are two primary varieties of mango in Indonesia. 'Arumanis', or the "sweet green mango" (Natawidjaja *et al.*, 2014) is the variety that is commonly produced and consumed more in the local market rather than the export market (Wandschneider *et al.*, 2013). 'Arumanis' and 'Gedong' are also the varieties that are usually exported (Yuniarti & Santoso, 2000).



'Arumanis 143' **'Ken-Layung'** **'Agrimania'**

'Garifta Merah' **'Garifta Merah'** **'Garifta Merah'**

Figure 1. New improved Indonesian mango varieties from ICHORD

Some new improved varieties of mango developed from research and breeding programs possess favorable characteristics with good market potential. Varieties such as 'Garifta', 'Ken-Layung' (Rebin *et al.*, 2016) and 'Agrimania' (Karsinah & Rebin, 2018) have a red skin color that is different from the 'Arumanis', which is a green variety. Other varieties such as 'Gadung 21' can be eaten like an avocado (Karsinah *et al.*, 2017). Hybrid mango varieties like the 'Agri-Gardina 45' have a unique characteristic, in that it can be peeled like a banana (Karsinah *et al.*, 2014).

Figure 2. 'Gadung 21' variety (Avocado mango)

Figure 3. 'Agri Gardina' variety (Banana mango)

3.1.2. Off-season technology

During the off-season, mango trees can be induced to flower by physical or chemical manipulation (Yuniastuti & Purbiati, 2016). By using off-season technology, fruits can be harvested outside the usual season, decreasing risk of post-harvest loss when there is an excessive supply during the mango season (Maloba *et al.*, 2017). However, less than 20% mango farmers are currently applying this technology (Natawidjaja *et al.*, 2014).

3.1.3. Pest and disease management

The other factors that can affect the productivity of mango in Indonesia are pests and diseases. Some pests and diseases have a different preference for each variety of mango. The hairy caterpillar is one of the pests that can cause loss of leaves in mango trees up to 100% (Baliadi *et al.*, 2012). Fruit flies also cause a decrease in quality and quantity in mango (Soemargono *et al.*, 2011; Ruswandi, 2017). Stem borers are one of the crucial pests especially in wet lowland production centres and have an average attack rate of up to 10.36% (Muryati *et al.*, 2010).

3.1.4. Post-harvest technology

There also some post-harvest technology that can support the development of Indonesian mangoes, such as controlled atmosphere storage, modified atmosphere packaging, waxing, irradiation, and packaging (Broto, 2011).

3.2. Mango development policies related to research and development

3.2.1. Development of mango agribusiness area

Based on the Indonesian agricultural land suitability mapping, there are potential areas for the expansion of mango cultivation such as in South Sulawesi, East Nusa Tenggara, and East Kalimantan (Yuniarti & Santoso, 2000). The Republic of Indonesia's Ministry of Agriculture has issued a decree to establish a national mango development area in 22 provinces in Indonesia, within 110 regencies divided by priority areas in the National Agriculture Zone. The total potential land area that can be used for mango cultivation including existing areas is 25,734,199 ha (INAagrimap, 2018).

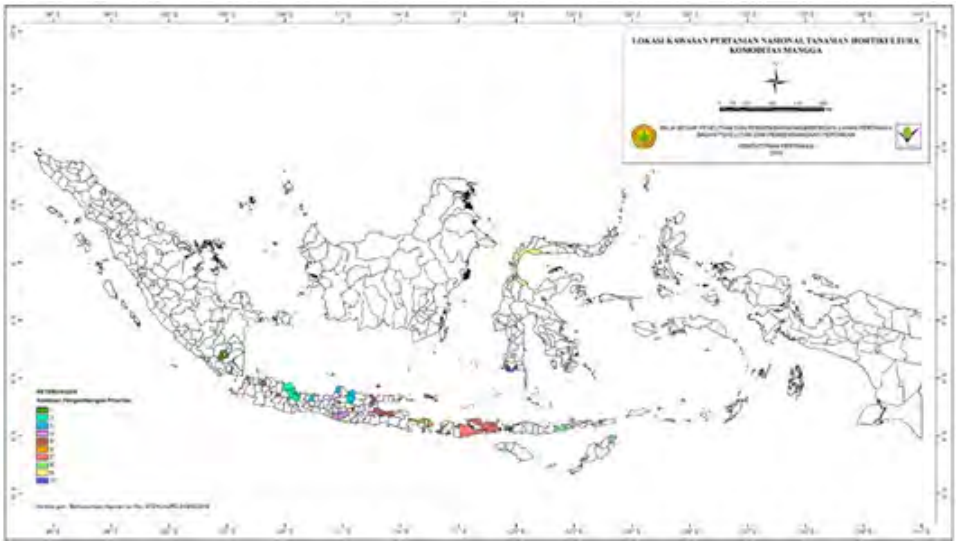


Figure 4. Potential mango cultivation areas.

3.2.2. Distribution of free certified mango seedlings

To increase the competitiveness of exported mangoes, the government needs to intensify mango production intended for export. One of the varieties that has been prioritized for export is the 'Garifta' variety. Since 2009, ICHORD through the Indonesian Tropical Fruit Research Institute has produced large quantities of 'Garifta' seedlings and distributed to communities and farmers for free in various regions in Indonesia. From 2018–2019, about 55,000 seedlings has been distributed in several districts, especially in the mango production centers in East Java, which are 'Probolinggo', 'Pasuruan', and 'Situbondo'. The spread of 'Garifta' varieties can be seen in Figure 5.

Other than to increase the area of mango cultivation, this distribution program was also an effort to regenerate old mango trees and was an endorsement to establish mango tourism areas.

Farmers are interested in developing 'Garifta' varieties, especially 'Garifta Merah' and 'Garifta

Orange' varieties, because some mango growers already knew the superior characteristics of both varieties. Based on information from farmers in Pasuruan and Situbondo who have produced Garifta mangoes, the varieties are: (1) favored by urban consumers; (2) fetches a higher price compared to other varieties; (3) have a good shelf life, which is 7–10 days after harvesting; and (4) high potential to be exported.

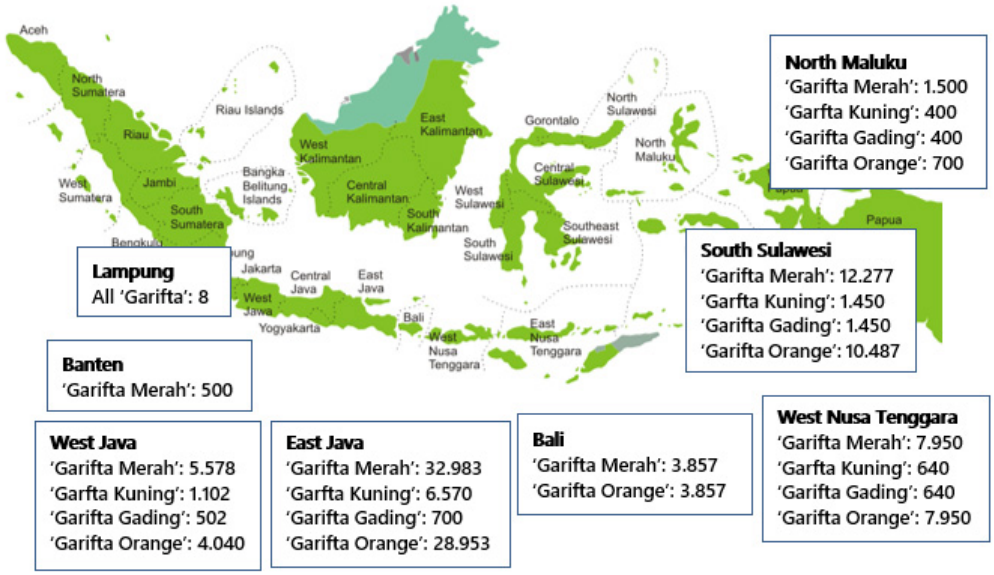


Figure 5. Distribution of Garifta varieties in Indonesia since 2009

3.2. Further development agenda

In order to increase exports, achieve competitive ability in the global market, and to provide sustainable benefits for farmers or community, the government must provide support programs, such as: (1) the assistance of export mango cultivation technology, up to post-harvest handling technology including the support of packing houses for sorting, grading, and packing; (2) the development of the agribusiness system from upstream to improve the performance of mango production, increasing the efficiency of distribution and marketing of fresh mangoes (domestic and export markets) while enriching the industry through the development of processed products and product promotion to support sustainability; (3) a program from local government agencies to support marketing of mangoes, especially for exports. A Standard Operational Procedure based on the planting region and farm registration must be prepared; and (4) establishment of marketing institutions by inviting exporters in the partnership scheme.

4. CONCLUSION

From a research and development perspective, Indonesia has a great opportunity to become a leading producer of mangoes in the world to meet global market demands due to the various innovations and technologies produced. These innovations and technologies need to be applied to support export-oriented mango development policies.

Aside from supporting the export-oriented mango development program, policies to support the adoption of new technologies by farmers also need to be formulated. Researchers and policy makers need to work together to ensure that these new innovations can be applied to further improve Indonesian mango's global competitiveness.

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CHALLENGES AND OPPORTUNITIES OF EXPORTING MALAYSIAN TROPICAL FRUITS TO GULF COOPERATION COUNCIL (GCC) MARKET

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ABSTRACT

The Gulf Cooperation Council (GCC) market is growing over time. It consists of six member countries which include Bahrain, Kuwait, Oman, Saudi Arabia, and the United Arab Emirates (UAE). The GCC was formed in 1981 in order to strengthen the members' economic, social, and political ties by harmonizing regulations in various fields including economy, finance, trade, and customs. Food and beverage imports were the third highest sector share of the GCC market after manufacturing and machinery and transport, an increase of 6.12% from USD 40.6 billion (2016) to USD 43.1 billion (2017). Food import is projected to rise to USD 53.1 billion by 2020, due to an increase in supply dependency on imported food products. The high share of food and beverage imports is due to the lack of agricultural land, cost of production, and an increasing population of expatriates and locals. Fruits and vegetables (including tropical fruits) are among the main sectors in imported agricultural products listed by the GCC. Therefore, this study aims to identify the issues, challenges, and opportunities of exporting tropical Malaysian fruits to the GCC (particularly UAE, Oman, and Qatar). SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis was carried out to determine the overall market structure. The analysis revealed huge opportunities to meet the demand and supply from the market, and Malaysia is one of the suppliers. However, freight charges, inconsistent supply, quality, and post-harvest handling are hurdles for exporters to fulfill the demand. These findings can potentially assist exporters to understand the market structure as well as increase their exports in the near future.

Keywords: Malaysian tropical fruits, challengers, opportunities, SWOT Analysis

1. INTRODUCTION

Exports of Malaysian tropical fruits to the Middle East began in the early 2000s. However, the trade and business relationship were relatively small compared to their 'traditional' trading partners (Asmak & Mohd Fauzi, 2009). With the exception of oil and gas commodity, Middle East countries have evolved significantly to be the fastest-growing region in the world. The Middle East refers to 15 countries namely, Yemen, Syria, the United Arab Emirates (UAE), Turkey, Saudi Arabia, Qatar, Oman, Lebanon, Kuwait, Jordan, Iraq, Iran, Egypt, and Bahrain. Meanwhile, the Gulf Cooperation Council (GCC), is a political, economic, social, and cultural coalition comprising six Persian Gulf Arab nations namely Saudi Arabia, the UAE, Qatar, Oman, Bahrain, and Kuwait. The GCC is well-known in the world economy for having a more dynamic market and politics than any other Arab country (Encyclopaedia Britannica, 2019). The Free Trade Agreement between Malaysia and the GCC was signed in 2011 but was postponed due to a number of issues and challenges including political uncertainty during the Arab Spring, the global financial crisis, and crude oil prices (Rupa, 2017).

Agricultural trade has played an important role in strengthening food security and safety to consumers across the globe. Being a neutral, modern, and prominent progressive Muslim state, Malaysia has been very active in the advancement of her trade by encouraging collaborations with numerous GCC countries (Irwan Shah & Muhammad, 2018). In January 2011, a framework agreement on economic, commercial, and technical cooperation between Malaysia and the GCC was sealed in Abu Dhabi (Abdurabb, 2011; Tajul *et al*., 2011). Malaysia is also involved in trade agreements with the Organization of Islamic Conference countries which comprises the GCC since the 1990s. This agreement is expected to benefit all partners in order to spur investment but also to produce growth in trade through the removal or reduction of customs barriers, the encouragement of contact between, and the establishment of economic, trade, and investment partnership (Abdurabb, 2011; Tajul *et al*., 2011).

In 2010, the Malaysian government introduced the National Agrofood Policy (NAP4) 2011–2020, as a masterplan to implement programs and projects for the development of the agricultural sector (Rozhan, 2019). One of the key focuses is to increase the export value of Malaysian agricultural produce including fruits (Chubashini, 2015). The Middle East market particularly the GCC countries is one of the main target export market of Malaysian tropical fruits in the NAP4 strategic plan. In 2017, the Middle East market contributed 4.02% (USD 8.696 million) from Malaysia's total global agriculture export (USD 216.428 million). A part of that 0.12% or USD 10.551 million is from edible fruit and nuts; peels of citrus or melons. All types of fruits, vegetables, cut flowers, and foliage are allowed to be exported to the Middle Eastern countries with approved phytosanitary certificate endorsement. No special treatments are required but it must be free from any plant pest and disease.

Dubai, a metropolis and popular city of the UAE is renowned as a transshipment hub or gateway for re-export activity to other Middle East countries and the African region. The UAE is home to over 200 nationalities, composed of 20% native residents, with the remaining 80% comprising of immigrants from India (accounting for 51%) (World Population Review, 2019). The UAE will be the second fastest growing food market at a compound annual growth rate of 4.9% by 2019 after Qatar's, 5.5% on the back growth of the younger population (International Trade Centre, 2015). The changes in preference and rising health consciousness among the younger generation has led to higher consumption of nutritional foods as well as tropical fruits. South Africa, Latin America, and the Asia Pacific region, including Malaysia are among the main exporters of tropical fruits to the Middle East. The UAE is the leading export destination of Malaysian tropical fruits with 44.3% market share, followed by Egypt (11.3%), Yemen (8.9%), and others (35.5%). Pineapple, watermelon, jackfruit, rambutan, star fruit, and papaya are the top fruits exported to the UAE. Despite a good relationship with the UAE, there is still room for improvement with the new emerging markets, Oman and Qatar.

Qatar is a country located east of the Arabian Peninsula next to the countries of Saudi Arabia, Bahrain, and the UAE. Known as the world's richest country in 2017 by the International Monetary Fund, Qatar's economic resources are from petroleum, oil, and natural gas; with 50% of Gross Domestic Product (GDP), 85% of export revenue, and 70% of government revenue (International Trade Centre, 2015). The country of Oman is located on the southeast coast of the Arabian Peninsula in West Asia and is an important route along the Persian Gulf estuary. Oman shares its border with the UAE in the Northwest, Saudi Arabia in the West, and Yemen in the southwest; making this Arab nation a fast developing country and categorized as a high-income country and listed as one of the 70 safest countries in the world. Oman's economic resources are from oil and gas production, which accounts for 45% of GDP, services (38.5%), manufacturing (16.6%), and agriculture and fisheries (1.3%). Examples of agricultural activities are camel, goat, and lamb breeding; dates and vegetables (Fanack, 2018).

The findings of this study provided market information that can assist the government in developing local tropical fruit marketing strategies in the UAE market and to embark on exports to new emerging markets. Therefore, the objectives of this study were:

i) To analyze the export trend of Malaysian tropical fruits to the UAE market.
ii) To employ SWOT analysis in identifying the issues, challenges, and opportunities in exporting tropical Malaysian fruits to the GCC (mainly to Oman and Qatar markets).

2. MATERIALS AND METHODS

2.1. Data Collection

Primary and secondary resources were used in the study. At the start of the study, a focus group discussion was held among Malaysian fruits exporter and related government agencies to identify export issues and challengers mainly for Dubai's export chain. Findings gathered from the focus group discussion were also used as a base for the structured questionnaire developed for interviews with industry players. A purposive sampling was applied to select the industry players among importers, distributors, procurement managers, and retailers to determine the distribution structure and the cost price of imported fruits in the Dubai market. Other than that, in-store observation at various categories of grocery outlets (retail stores, supermarkets and hypermarkets including Lulu Hypermarkets, Carrefour, Spinneys, Waitrose, and Union Coop) was also used to generate more information on the tropical fruits distribution network in the Dubai market. Secondary data of fruit trade statistics (import and export) from TradeMap's online database (2012–2016) was also used in the study.

2.2. SWOT Analysis

To explore new markets in Oman and Qatar, a SWOT analysis was conducted taking into account the overall relationship between internal and external market factors. This analysis is one of the frameworks in developing business and market strategies to evaluate strengths, weaknesses, opportunities and threats. Strengths and weaknesses are internal factors while opportunities and threats are external factors in the SWOT analysis.

3. RESULTS

3.1. Established Market, the Middle East

Exports of Malaysian tropical fruits to the Middle East market showed an increase with an average export growth of 38.62% per annum from RM12.2 million (2012) to RM35.7 million (2016) (MOA 2018). The sharp increase was in 2015 where exports increased by 59% from the previous year. Exports to the Middle East comprise of 14 countries as shown in Figure 1. The Middle East's primary market is the UAE (Dubai) which is the largest market (44.3%), followed by Egypt (11.3%) and Yemen (8.9%). Oman's and Qatar's market presence is less than 1%, but they are expected to potentially expand exports of fresh agricultural produce.

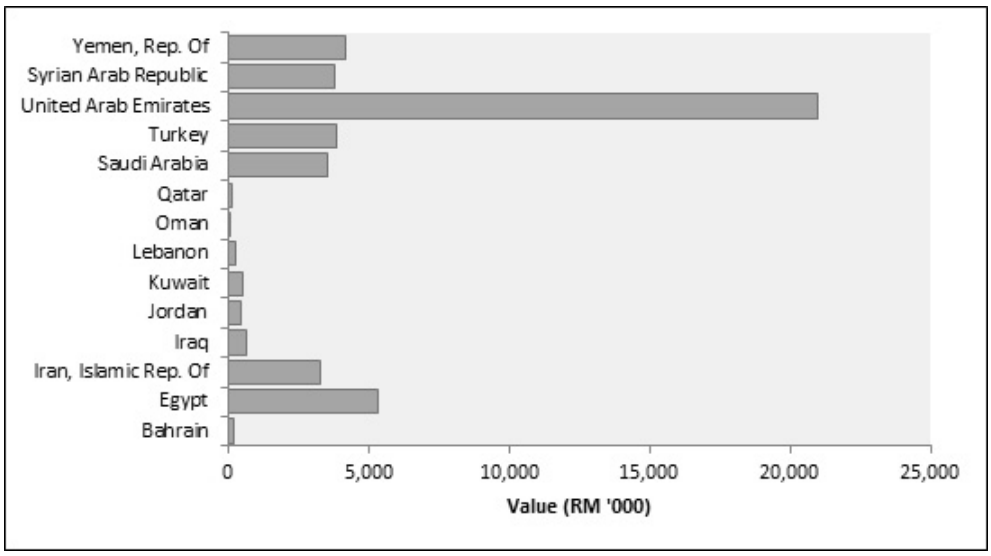

Figure 1. Export value of tropical fruit to the Middle East (2012–2016) Source : MOA, 2018

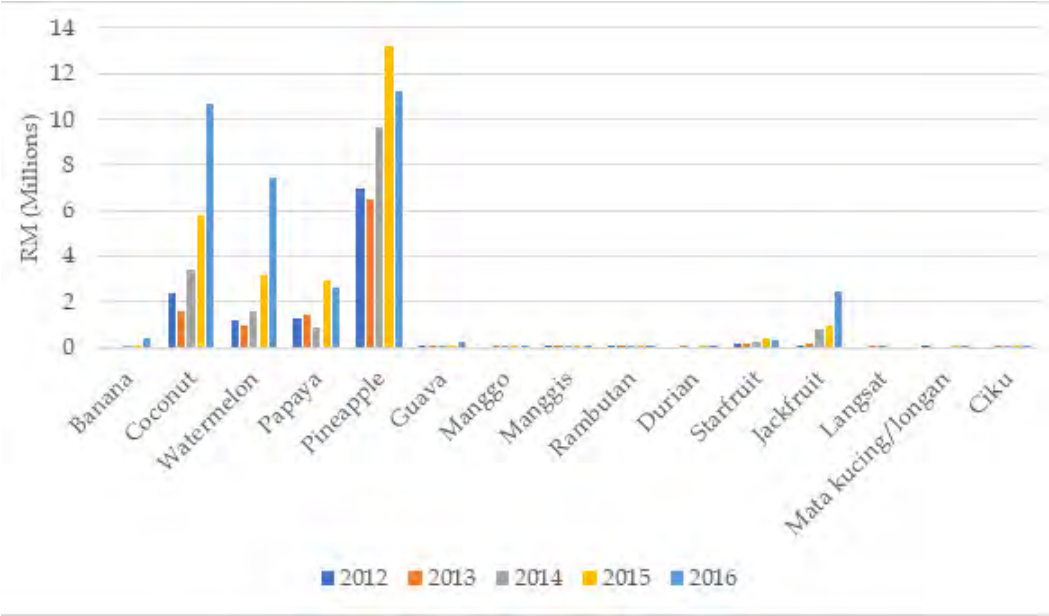

Figure 2. Total export value of tropical fruits export to the Middle East (2012–2016)[13].

3.2. Potential New Market, Oman and Qatar

The issues and challenges of exporting fruit to the Middle East especially to the UAE were identified based on interviews and focus group discussions with experienced Malaysian exporters with more than 10 years of exporting tropical fruits to this market. Some of the issues facing exporters are as follows:

i) The high cost of air transport is almost double the purchase price of the farm. For example, jackfruit is priced at RM 3.50/kg and air transport costs RM 7/ kg. These charges are from international air carriers such as Emirates, Qatar Airways, Oman Air, and others. There is no local air transport from Malaysia Airlines to the Middle East. Transportation charges continue to increase depending on the terms set by the airline. This will result in less profits for the exporters having to bear the rising costs, while the importers want cheaper

prices. An alternative to air transportation is by sea which freight charges are cheaper. However, sea transport requires larger quantities. The travel distance is also long (9–14 days) while these fresh fruits have a short shelf life. In order to provide fresh fruit on the shelves, quality care and post-harvest handling are important practices.

ii) Exporters also face the problem of shortage of quality fruit and a consistent sale price. Purchase prices at the farm vary based on the fruit supply at the farm. Prices will increase if the supply of quality products is limited and exporters have to procure the products at a higher price. Exporters also face problems with shortage of supply despite ordering fruits from farmers. This issue is especially prevalent during the rainy season as production faces short supply.

3.3. SWOT Analysis

Figures 3 and 4 show the SWOT analysis conducted on Oman and Qatar markets.

Figure 3. Summary of finding from SWOT Analysis for Oman market.

Figure 4. Summary of finding from SWOT Analysis for Qatar market.

4. DISCUSSION

4.1. Trend Analysis for UAE market

The total value of fruit exports to the UAE increased by 90% from RM7.5 million (2012) to RM14.3 million (2017) (MOA, 2018). Figure 5 shows Malaysian fruit exports in the UAE market for 2012–2016. Three types of fruit, watermelon, starfruit and jackfruit showed an increase in export value. Meanwhile, export value of desiccated coconut and guava declined from 2012 to 2016. Among all of the fruits, jackfruit showed the most potential because its export value increased significantly by RM 278,460 a year.

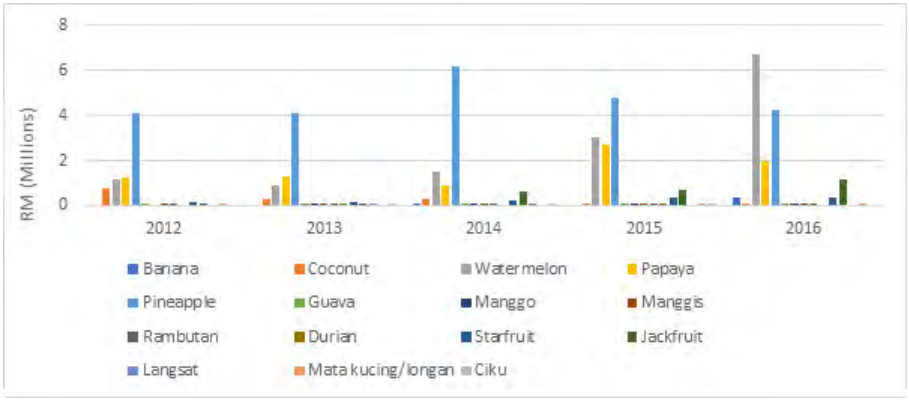

Figure 5. Total export value of tropical fruits export to UAE (2012–2016) Source: MOA, 2018

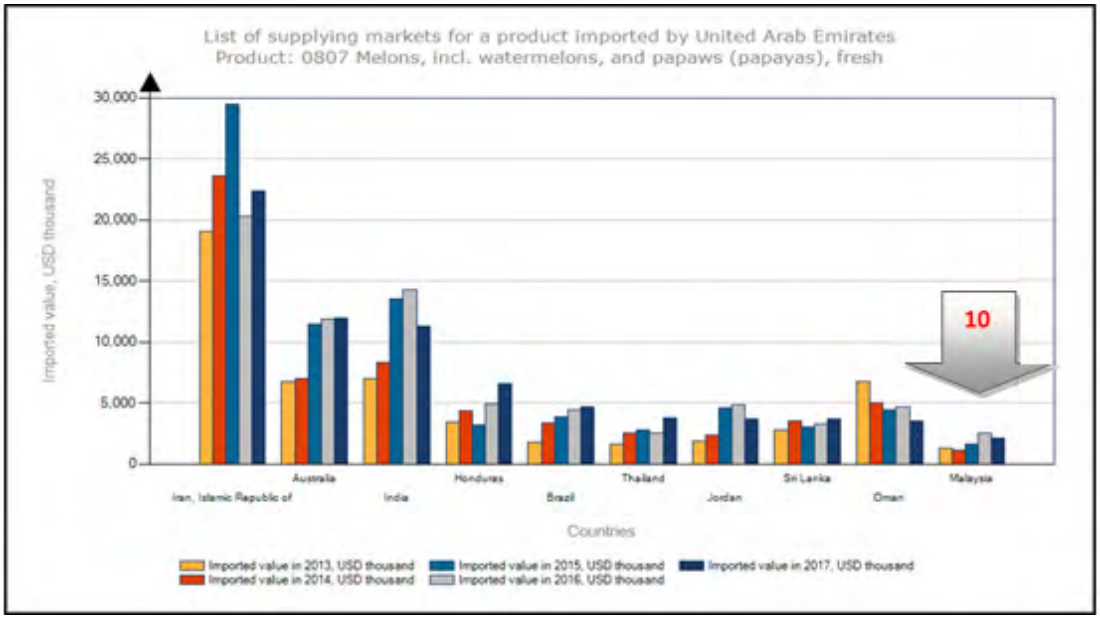

Figure 6. Malaysia's watermelon export rankings and competitors' market share in the UAE market (2013–2017). Source : Trade Map, 2018

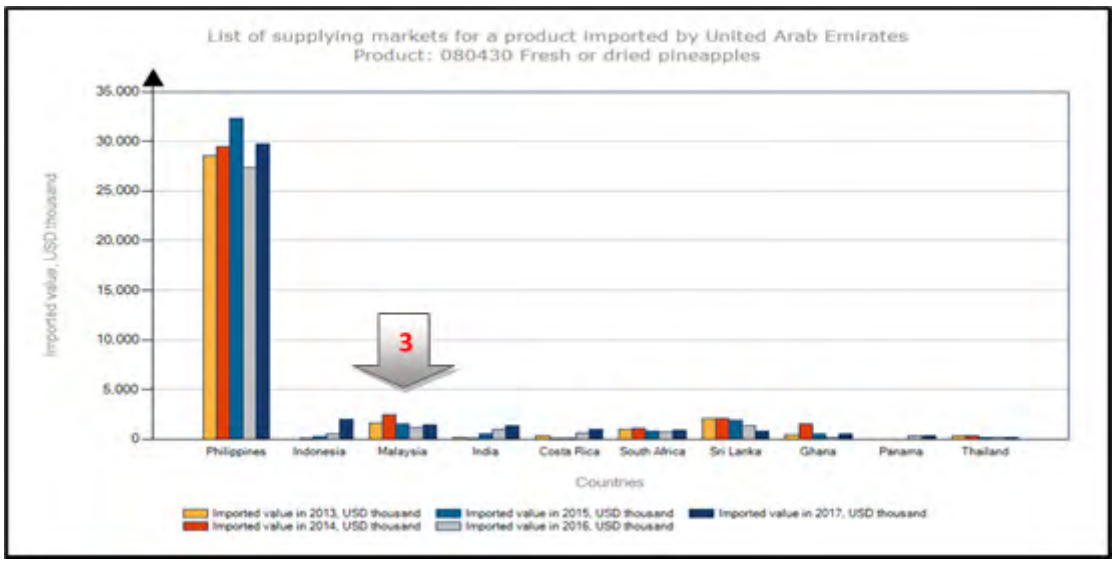

Figure 7. Malaysia's pineapple export rankings and competitors' market share in the UAE market (2013–2017). Source : Trade Map, 2018

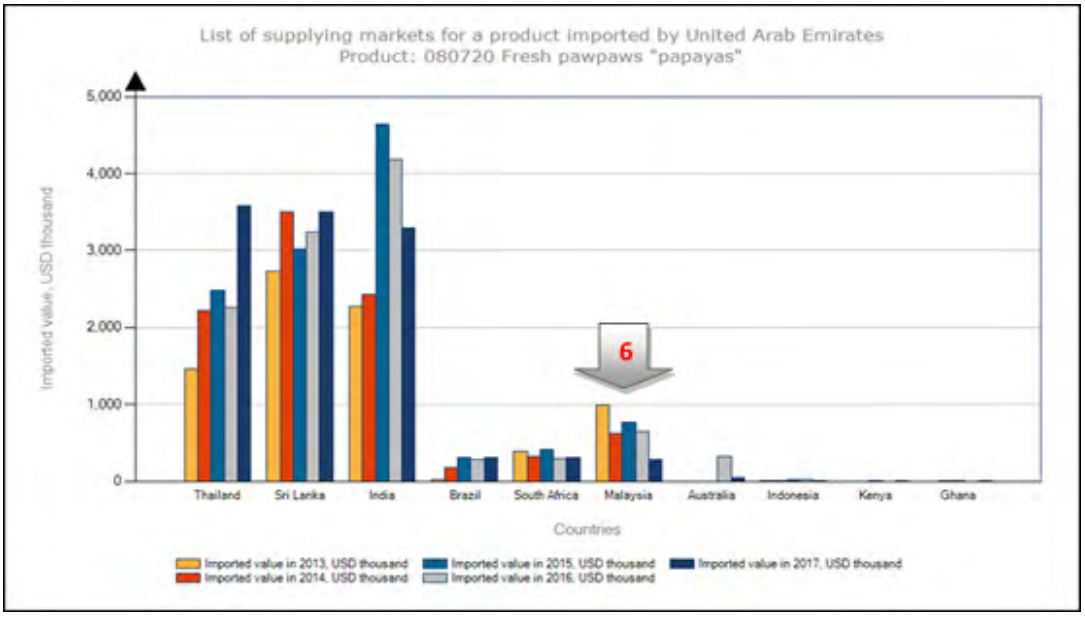

Figure 8. Malaysia's papaya export rankings and competitors' market share in the UAE market (2013–2017). Source : Trade Map, 2018

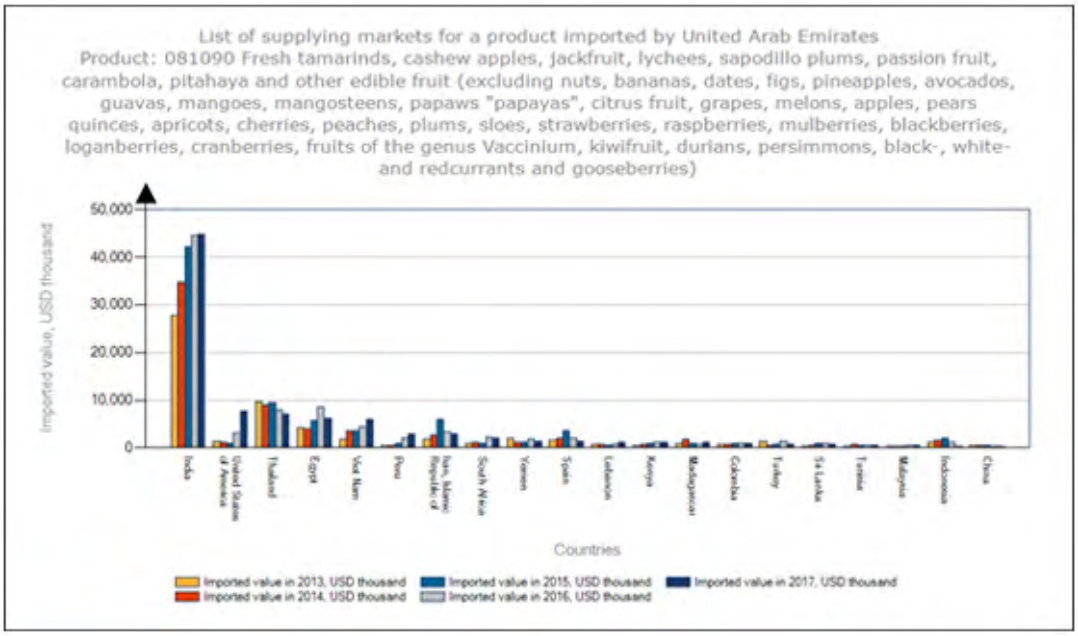

Figure 9. Total exports for Malaysia and competitor countries of other tropical fruits in the UAE market (2013–2017). Source: Trade Map, 2018

Figures 6–9 show the export status of selected Malaysian fruits and competitor countries. The countries exporting watermelons to the UAE are Iran, Australia, India, Honduras, Brazil, Malaysia, and others. Malaysia ranked tenth after Oman (Figure 6). In terms of pineapple exports, Malaysia was ranked third while the Philippines dominated pineapple exports with the highest number of exports compared to competing countries (Figure 7). MD2 pineapple varieties produced by the Philippines are well known among consumers in the UAE and the Middle East. Indonesia is a close competitor to Malaysia as it entered the UAE pineapple export market in 2015.

Overall, Malaysia ranks sixth in exports of papaya to the UAE after South Africa (Figure 8). The

exported varieties are of the Sekaki type. Thailand, Sri Lanka, and India are the three largest exporting countries to the UAE market. The Sri Lankan and Indian varieties are as small as the Solo papaya. Figure 9 shows Malaysia's export position for other tropical fruits, such as jackfruit. Although Malaysia is in the eighteenth position, exports of jackfruit are increasing every year, signaling a promising upward trend. . India is the largest exporter of other tropical fruits in the UAE market followed by the USA, Thailand, and Egypt. This calculation is cumulative for several types of fruits including jackfruit, starfish, ciku (sapodilla), and passion fruit.

4.2. SWOT Analysis for Oman Market

The Strength of exporting Malaysian fruits to Oman is based on a history of good relations between the two countries. In addition, Oman's open policy is to diversify the country's economic resources by opening up more foreign investment opportunities. The duty-free port, Duqm, has been a driving force in Malaysia–Oman trade relations. The port is being upgraded with the best facilities for trade. In terms of the distance from Europe and the Middle East, Oman is the first port of the Persian Gulf. This makes the journey shorter (7–10 days) compared to the Jebel Ali Port in the UAE which takes 9–14 days.

The Weakness, on the other hand, is air transport charges which have become a major issue for exporting fresh fruits to the Middle East including Oman. High charges are imposed by foreign airlines causing the cost of sales to rise and thus increasing prices in the retail market. Sea shipment services are an alternative but deliveries need to be in greater quantities and take longer delivery times. There is a risk of delays and damage during operation on board. Therefore, to minimize losses, exporters should minimize risks in trading.

The Opportunity can be seen in the long run, since Oman market is viewed favorably as a good investment over the next 10–15 years. The Duqm port is currently being upgraded and expected to provide a strong rivalry to the port at Jebel Ali. Competition in business will provide opportunities and positive impact for the future of Oman market. In addition, Oman's state government wants diversification of economic power in line with the increasing number of locals and expatriates. Another opportunity that Malaysian exporters can tap is by targeting or capturing seasons of reduced competition when competitors from other countries cannot fulfill supply obligations. For example, by being an alternative supplier to the Philippines from September to December due to the monsoon season in the country.

The Threat of competition from other competing countries is one of the factors to consider. Excessive selling prices will reduce demand in the retail market. However, this can happen only if the quality and quantity of supply is observed for a particular market segment; for an example a caterer market or an agro-based product manufacturing institution. In order to ensure quality throughout the value chain, post-harvest management is very important and this can also be a threat to exporters if this factor is not taken seriously. In addition, the promotion and marketing strategies need to be continued in the Oman market so that the introduction of Malaysian products is known to local consumers.

4.3. SWOT Analysis for Qatar Market

The Strength can be seen as Qatar is a very potential and well-developed market. Malaysia–Qatar's bilateral alliance opens up opportunities for international trade especially for export of local tropical fruits. Trade history has shown that Qatar is Malaysia's largest trading partner after the UAE and Saudi Arabia for electrical products, appliances, and processed foods; and this is a strength factor for this market. Qatar is one of the countries rich in oil and natural gas resources. The small local population with high national income provides the people of Qatar with a high

purchasing power. According to a report from Mohammad, The Peninsular (2017), the people of Qatar have assets of less than QR 100 million or equivalent to USD 102 million, which makes its population the wealthiest in the Middle East.

Similar Weakness as in the Oman market, which is the issue of air transport service charges resulting in export deficits in the country. Alternative transportation by sea requires a larger shipment and a greater risk of damage and loss. However, the strategy of minimizing losses can be devised by implementing good post-management practices.

The Opportunity. While products from Malaysia's closest tropical fruit competitor such as Thailand are still lacking, the strategy of expanding Malaysian agricultural produce market as a market leader is a great opportunity. In addition, with Qatar hosting the 2020 FIFA World Cup, which is expected to be watched by approximately 400,000 people, the need for food will increase. This will be an opportunity for Malaysia to become a major supplier of local fruits. and indirectly boost export performance while increasing the country's global reputation. Sanctions faced by Qatar from the allies of Saudi Arabia, the UAE, Bahrain, Yemen, and Egypt have not shaken Qatar's efforts to obtain food supply. In fact, they are aggressively upgrading trade infrastructure and logistics to import their own food supply from source countries without relying entirely on the UAE as previously practiced. This economic restriction once again provides an opportunity for Malaysia to establish closer trade relations with Qatar.

The Threats in terms of exports and imports abroad are from other competitors in terms of price, supply, quality, and service. Quality and consistent supply enable a country to meet the demand of local people. The management of post-harvest handling must be taken seriously by all exporters to ensure the quality of the exported fruits is maintained. In addition, continuous promotional programs for consumers should be continued so that Malaysian fruits are recognized and appreciated by Qatari consumers.

4.4. Recommendation

The main issue of exports of Malaysian fruits to the Middle East is air transport. Air transport charges increase every year up to 70% of total production cost in Malaysia. Despite this, air transportation is seen as a more efficient way of exporting Malaysian tropical fruits to the Middle East due to the short travel time (one day), low risk of damage, and quality is guaranteed despite transporting a limited supply. In this regard, it is proposed that a policy of incentives be targeted at exporters using air transportation based on their merits and annual export performance. The proposed incentive is to provide business tax exemptions collected as government revenue.

The second suggestion is to strengthen sea transport. Marine transport services are an alternative to air transport, but the challenge is to meet the demand in large quantities besides the risk of delays along with damage and loss to the shipment. In terms of service charges, marine logistics can reduce overall production costs. To help solve this problem, the government is advised to reduce the risk of export loss in the event of a malfunction or delivery delay that could result in a loss. The government should help cover 50% of the losses with business tax exemptions to exporters using sea transport.

5. CONCLUSIONS

Exports of Malaysian tropical fruits to the Middle East market showed an increase of 38.62% growth from 2012 to 2016. Pineapple was the main source of Malaysian exports. The UAE is the largest trading partner of Malaysian fruits among the other middle eastern countries (44.3%). Jackfruit is the most viable and competitive product to export as its value and quantity have

increased significantly by RM278,460 a year. Malaysia is competing with other countries in the UAE in the tropical fruit market, ranking third for pineapple exports, sixth for papaya exports, tenth for exports of watermelons and eighteenth for other tropical fruits including jackfruit, starfruit, squash, and passion fruit.

The markets of Oman and Qatar are potential export markets to penetrate and expand. Through the SWOT analysis conducted, Malaysian exports will continue to grow in the Middle East if the demand in Oman and Qatar can be fulfilled. However, promotional programs need to be continued to raise awareness and increase consumer interest in Malaysian fruits.

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ECONOMIC POTENTIAL OF SABAH DURIAN MERAH (*DURIO GRAVEOLENS*)

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ABSTRACT

Durio graveolens is one of the edible *Durio* species endemic to the island of Borneo. It is found either in the wild or in semi-cultivated areas in Sabah. *Durio graveolens* can be divided into two subgroups, namely 'Dalit' and 'Durian Merah'. 'Dalit' is also known as 'Durian Kuning' in Sarawak and Brunei. It has a sweet taste with a wide array of aril colours ranging from yellow and orange to red, which is commonly found in the interior regions of Sabah. 'Durian Merah' on the other hand, is concentrated in the Sandakan area. It has an attractive crimson red coloured aril, and because of its bland taste, it is often processed into a popular local delicacy called *tempoyak*. *Tempoyak* can fetch a good price which can reach as high as RM 100/kg. The Department of Agriculture Sabah had introduced 'Durian Merah' cultivation in 2012 as it has an excellent prospect in the downstream processing industry. It is also introduced as one of the crops in the agricultural development program to improve the socio-economic status of farmers in rural areas. This paper discusses the distributions and the market potential of 'Durian Merah' in Sabah.

Keywords: 'Durian Merah', *Durio graveolens*, Sabah, Borneo

PESTICIDES RESIDUE ANALYSIS OF FRUITS FOR FARM ACCREDITATION SCHEMES IN SABAH, MALAYSIA

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ABSTRACT

The Department of Agriculture Sabah initiated pesticide residue analysis in fruits for myGAP and myOrganic farm accreditation schemes since 2004 and 2010. Pesticides residue analysis is one of the requirements in the farm certification process. The aim of this project is to ensure the safety and quality of local agricultural produce. The approach is not only beneficial to consumers but also to the environment while ensuring no health implications on farmers and workers. From 2014 to 2018, a total of 176 samples of fruits out of 1,053 samples of agricultural produce have been analyzed for pesticides residue. Fruit samples were analyzed for ethylene bis-dithiocarbamates (EBDC), organophosphorus (OP), organochlorine (OC), and synthetic pyrethroid (SP) pesticides; commonly used by farmers in Sabah. The headspace method was used to analyze EBDC pesticides and the "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method for OP, OC, and SP pesticides. Of the 176 fruit samples analysed, 161 samples were for myGAP and 15 samples for myOrganic certifications. The violation rates over the five-year period for myGAP are 2% and for myOrganic, 7%. Of the 176 samples analyzed, 2% of the samples were found exceeding the maximum residue limit based on the 16th Schedule, Food Act 1983. Cypermethrin, chlorfenapyr, and dimethoate pesticides were detected in the 2% violated samples. To date, a total of 58 fruit orchards have been certified for farm accreditation schemes which covers an area of approximately 650 hectares. Seventy-one farms with an area of 243 hectares are at the auditing stage while 50 farms covering an area of 118 hectares are at the land inspection stage. The Department of Agriculture Sabah will continue to promote farm certification to educate farmers on the importance of Good Agricultural Practices (GAP) to enhance market acceptability of local agricultural produce to neighboring countries.

Keywords: pesticide residue, myGAP, myOrganic, maximum residue limit, cypermethrin, chlorfenapyr, dimethoate

1. INTRODUCTION

In Sabah, Malaysia and other tropical countries, pesticides are used extensively in agriculture for improving the quality of agricultural products and also increasing the yields. Pesticides play an important role in modern agriculture and are irreplaceable. However, most pesticides are toxic, environmentally stable, and are mobile substances in the environment, contaminating not only food crops but also soil, water, and air. Thus, good agricultural practices (GAP) were introduced with the aim to reduce excessive usage of pesticides in agriculture to ensure the safety and quality of agricultural produce.

In Malaysia, certification schemes such as GAP were introduced as one of the strategies not only to increase quality, safety, and environment-friendly products but also to satisfy consumer preferences and to enhance market acceptability of importing countries. The scheme is based

on the Malaysian Standard MS 1784:2005 *Crop Commodities – Good Agriculture Practices (GAP)* (Ministry of Agriculture Malaysia, 2014). The Malaysian Good Agricultural Practice (myGAP) is a voluntary scheme and is free of charge, where interested farms can register and undergo a series of steps before the farm can be accredited; e.g., site inspection by a soil team, pre-audit and sampling by internal auditors, follow-up audit by external auditors, recommendations for certification by a technical committee, and finally the awarding of myGAP or myOrganic certificate (Ministry of Agriculture Malaysia, 2014). The government provides free courses and trainings to the farmers. On the other hand, Malaysian Organic (myOrganic) is a rebranding of the organic certification scheme introduced by the Ministry of Agriculture to recognize farms that practice organic farming based on Malaysian Standard MS 1529:2015 *Plant-based organically produced foods-requirements for production, processing, handling, labelling and marketing (Ministry of Agriculture Malaysia)*. The implementation of myGAP and myOrganic schemes in agriculture has resulted in increased quality and safety of agricultural products and has provided consumers with better choices of products. Farm certification provides advantages not only to the consumers, but also to the farmers, workers, and the environment.

The Department of Agriculture Sabah has initiated the pesticide residue analysis in agricultural produce for the farm certification of myGAP and myOrganic since 2004 and 2010. Farmers are required to apply for farm certification through their respective district agricultural office before the food crops samples can be collected for analysis. All relevant documents are evaluated and the farms are inspected. Eligible farmers are given assistance and guidance while the farms are inspected by the soil survey and conservation teams. Pre-audit by the internal auditor at the district level are conducted once the initial inspections are accepted and passed. Food crops samples are harvested for pesticide residues and heavy metal analysis at the chemistry laboratory. After the pre-audit stage, external audit is carried out to ensure that the farmers are practising and complying with GAP in accordance to the specified standards. The final stage is the recommendation for farm certification by the technical committee at the national level (Ministry of Agriculture Malaysia, 2014). The aim of this project is to analyze pesticide residue in fruit samples for farm accreditation to ensure the safety and quality in local agricultural produce. In this paper, the analysis of pesticide residue in fruit samples received from 2014 to 2018 are discussed.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

Pesticides standard (purity 98% and above) for organophosphorus (OP), organochlorine (OC), and synthetic pyrethroids (SP) were obtained from Dr. Ehrenstorfer, Augsburg, Germany. Analytical grade of anhydrous magnesium sulfate, anhydrous sodium acetate; and gas chromatography grade of acetone, acetonitrile, and hexane for OP, OC and SP analysis were obtained from Merck, Darmstadt Germany. Dispersive solid phase extraction kits were obtained from Agilent Technologies, USA. For the ethylene bis-dithiocarbamates analysis, 99% purity of carbon disulphide (CS_2), 5M hydrochloric acid and stannous (II) chloride were also obtained from Merck, Darmstadt Germany.

Pesticide stock solutions (500 ppm) were prepared by dissolving the appropriate amount of pesticide standards in acetonitrile for OP pesticides (18 active ingredients), hexane for OC (10 active ingredients), and SP (6 active ingredients) pesticides. Intermediate standard solutions with certain concentrations were prepared for each group of pesticides.

2.2. Sampling

Following the approval of myGAP and myOrganic certification schemes of the farmers whom applied through the district agricultural office, fruit samplings would take place. Samplings were carried out by the agricultural district staff after the pre-auditing stage and the samples were sent to the pesticide residue and foliar laboratory for analysis.

2.3. Analysis

The fruits samples were analyzed for EBDC, OP, OC, and SP pesticides which are commonly used by farmers in Sabah. The headspace method was used to analyze EBDC pesticides. EBDC in fruits is determined by the carbon disulfide formed during the heating of dithiocarbamates with stannous (II) chloride and hydrochloric acid in the head-space by gas chromatograph with flame photometric detector in the sulphur mode (McLeod & McCully, 1969). Fifty millilitre of distilled water and 50 mL of 2% $SnCl_2$/5M HCl mixture were placed in a 250 mL laboratory bottle containing 30 g fruit samples. The mixtures were thoroughly shaken by hand and placed in a water bath at 80 °C for one hour. The bottles were shaken every 30 minutes. The bottles were kept in an oven at 30 °C. The upper phase (gas form) was injected manually to Gas Chromatograph (GC-FPD) using gas tight syringe.

Analysis of OP, OC, and SP pesticides were carried out using the Official QuEChERS method by Lehotey *et al*. (2005). Fifteen grams of homogenized samples were weighed into 50 mL Teflon centrifuge tubes. Fifteen millilitres of acetonitrile containing 1% acetic acid were added into the samples and shaken vigorously for 30 seconds by hand followed by vortex mixing for 2 minutes. Extraction kit containing 6 g anhydrous magnesium sulphate and 1.5 g anhydrous sodium acetate were added and shaken vigorously for 30 seconds by hand followed by vortex mixing for 2 minutes. The extract was later centrifuged at 3000 rpm for 2 minutes. One milliliter of extract was transferred to d-SPE tubes and shaken vigorously for 30 seconds by hand followed by vortex mixing for 2 minutes. The extract was centrifuged again at 3000 rpm for 2 minutes. The supernatant at 200–300 µL was transferred into 2 mL vials with low-volume-inserts for the determination of pesticides using GC.

2.4. Apparatus and Instrumentation

A sample homogenizer (GM300, Retch Germany) was used to homogenize the fruit samples and a multi speed vortex mixer (Vortexer, Heathrow Scientific) was used for sample extraction. Centrifugation of the fruit extracts was performed using Kubota 4200 (for 50 mL tubes) and Gyrozen Micro Centrifuge (for 2 mL tubes). Waterbath (Memmert) and oven (Memmert) were used for EBDC sample preparation.

A gas chromatograph (Agilent Technologies 7890A & 7890B) equipped with Flame Photometric Detector (FPD) was used for the determination of OP pesticides. This instrument was equipped with a non-polar, fused-silica capillary column, HP5 (30 m × 0.32 mm × 0.25 µm) and polar fused-silica capillary column, DB1701 (15 m × 0.53 mm × 1.0 µm), obtained from Agilent Technologies USA. The HP5 column temperature was maintained at 110 °C for 0.5 min, and then programmed at 200 °C min^{-1} to 170 °C for 0 min followed by another temperature ramp of 5 °C to 230 °C for 0 min and a final temperature ramp of 15 °C to 280 °C for 12 min. The DB-1701 column temperature was maintained at 130 °C for 0.5 min, and then programmed at 30 °C min^{-1} to 190 °C for 3 min followed by another temperature ramp of 5 °C to 240 °C for 3 min and a final temperature ramp of 10 °C to 260 °C for 12 min. The injector and detector temperature were

maintained at 260 °C and 250 °C, respectively.

A gas chromatograph (Agilent Technologies 7890B) equipped with micro Electron Capture Detector was used for the determination of OC and SP pesticides. This instrument was equipped with a polar fused-silica capillary column, SPB-608 (30 m × 0.53 mm × 0.5 µm) obtained from J&W Scientific, USA and Ultra-1 (25 m × 0.32 mm × 0.52 µm) obtained from Agilent Technologies, USA. The SPB-608 column temperature was maintained at 150 °C for 0.5 min, and then programmed at 30° C min^{-1} to 210°C for 2 min followed by another temperature ramp of 5 °C to 285 °C for 14 min. The Ultra-1 column temperature was maintained at 130 °C for 0.5 min, and then programmed at 30 °C min^{-1} to 160 °C for 0 min followed by another temperature ramp of 5 °C to 280 °C for 5 min. The injector and detector temperature were maintained at 260 °C and 300 °C, respectively.

A gas chromatograph (Agilent Technologies 6890N) equipped with FPD with sulphur mode was used for the determination of EBDC pesticides in CS2 form. This instrument is equipped with a non-polar, fused-silica capillary column, HP5 (10 m × 0.53 mm × 2.65 µm). The HP5 column temperature is maintained at 60 °C for 0.5 min, and then programmed at 30 °C min^{-1} to 230 °C. The injector and detector temperatures were maintained at 220 °C and 200 °C, respectively.

3. RESULTS AND DISCUSSION

A total of 1,053 samples of agricultural produce such as vegetables, fruits, and other food crops have been analyzed for myGAP and myOrganic farm certification schemes from 2014 to 2018. Of the 1,053 samples analyzed, 176 are fruits samples. A total of 18 districts in Sabah were involved in the pesticide residue analysis of fruit samples for farm certification schemes over the five-year period. The fruit samples were analyzed for EBDC, OP, OC, and SP pesticides, which were commonly used by farmers in Sabah. The headspace method was used to analyze EBDC pesticides and the official QuEChERS method (AOAC 2007.01) for OP, OC, and SP pesticides.

The summary of pesticides residue analysis results for myGAP and myOrganic samples categorized by types of fruits are shown in Table 1. The results of the analysis over the five-year period are summarized in Table 2. Of the 176 fruit samples analyzed, 161 (91%) samples were for myGAP and 15 (9%) samples for myOrganic certifications. The violation rates over the five-year period for myGAP were 2% and 7% for myOrganic. Overall, 2% (3 samples) of the 176 samples exceeded the MRL (Maximum Residue Limit) as stipulated in the 16th Schedule, Food Act 1983 (Ministry of Health, Malaysia). Dimethoate (OP pesticide), chlorfenapyr (OC pesticide) and cypermethrin (SP pesticide) were detected in the 2% violated samples. The violated samples were guava, 'Chok Anan' mango and salacca (*Salaca zalacca*). Guava and mango samples were from myGAP certification and salacca was from myOrganic certification. Dimethoate and chlorfenapyr were detected in guava samples meanwhile cypermethrin was detected in Chok Anan mango and salacca.

The total estimated hectarage of fruit farms in Sabah are approximately 18,100 ha with a producing area of 10,350 ha and a production of 108,150 tonnes (DOA/2018). To date, a total of 58 fruit farms have been certified for farm accreditation schemes which covers an area of approximately 650 hectares. Another 71 farms which covers an area of 243 hectares are still at the auditing stage while another 50 farms which covers an area of 118 hectares are at the land inspection stage. The 58 certified fruit farms only accounted for about 6% of the total producing area of fruits in Sabah.

The number of certified fruit farms in Sabah are still very low due to its voluntary basis for certification at the time being. The awareness on the importance and benefits of farm certification among the farmers and consumers are low. The Department of Agriculture Sabah needs to encourage food crop farmers to adopt GAP to ensure that their agricultural produce are of good quality and safe for consumption. Besides that, farm certification will improve the confidence of domestic and foreign markets on Sabah fruits and vegetables. This will also cater to the demand of importing countries for certified agricultural produce.

Table 1: Pesticide residue analysis for myGAP and myOrganic categorized by types of fruits from 2014-2018

Types of Fruits	myGAP		myOrganic		Overall	
	No. of Samples Analyzed	No. of Samples > MRL	No. of Samples Analyzed	No. of Samples With Residue (Violation)	No. of Samples Analyzed	No. of Samples > MRL / Violation
Custard Apple	1	0	0	0	1	0
Avocado	1	0	0	0	1	0
Papaya	9	0	0	0	9	0
Durian	6	0	3	0	9	0
Soursop	2	0	0	0	2	0
Sweet Corn	11	0	1	0	12	0
Water Apple	1	0	1	0	2	0
Seedless Lemon	0	0	2	0	2	0
Guava	24	2 (8%) Dimethoate (2) Chlorfenapyr (1)	0	0	24	2 (8%) Dimethoate (2) Chlorfenapyr (1)
Sweet Orange cv. Madu	2	0	0	0	2	0
Mango cv. Chok Anan	10	1 (8%) Cypermethrin	0	0	10	1 (8%) Cypermethrin
Mango cv. Harumanis	7	0	0	0	7	0
Mangosteen	2	0	2	0	4	0
Passionfruit	1	0	0	0	1	0
Pineapple	3	0	0	0	3	0
Pamelo	18	0	4	0	22	0
Banana cv. Berangan	7	0	0	0	7	0
Plaintain Banana cv. Saba	27	0	0	0	27	0
Plaintain Banana cv. Sekaki	1	0	0	0	1	0
Pitaya	8	0	1	0	9	0
Rambutan	4	0	0	0	4	0
Rockmelon	1	0	0	0	1	0
Salacca	3	0	1	1 (100%) Cypermethrin	4	1 (25%) Cypermethrin
Watermelon	10	0	0	0	10	0
Jackfruit	2	0	0	0	2	0
Total	161	3 (2%)	15	1 (7%)	176	4 (2%)

Table 2: Pesticide residue analysis for myGAP and myOrganic in fruit samples from 2014 to 2018

Types of Fruits	myGAP		myOrganic		Overall	
	No. of Samples Analyzed	No. of Samples > MRL	No. of Samples Analyzed	No. of Samples With Residue (Violation)	No. of Samples Analyzed	No. of Samples > MRL / Violation
2014	14	0	7	1 (14%)	21	1 (5%)
2015	16	1 (6%)	1	0	17	1 (6%)
2016	29	0	5	0	34	0
2017	23	0	1	0	24	0
2018	79	2 (3%)	1	0	80	2 (3%)
Total	161	3 (2%)	15	1 (7%)	176	4 (2%)

4. CONCLUSIONS

From 2014 to 2018, the overall violation rate for fruit farms certification in Sabah was at 2%. At present, the number of fruit farms in Sabah with myGAP and myOrganic certification are still very low with only 58 farms are certified. This achievement only accounted for about 6% of the total producing area of fruits in Sabah. The Department of Agriculture Sabah will continue to promote and to educate farmers on the importance of GAP in agriculture to ensure the quality and safety of our agricultural produce. Apart from that, more awareness campaigns on the advantages and benefits of farm certification will be organized. Technical support, training, and extension services to the farmers will also be provided.

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PREFERENCE OF SCIRTOTHRIPS DORSALIS (THYSANOPTERA : THRIPIDAE) HOOD ON SEVERAL PHENOLOGICAL STAGES OF MANGO 'ARUMANIS 143': IMPLICATION FOR CONTROL

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ABSTRACT

The production and quality of Indonesia's 'Arumanis 143' mango have been on the decline due to the serious infestation of *Scirtothrips dorsalis* Hood. Laboratory studies were done to determine the preference of *S. dorsalis* on several phenological stages of mango. Collection of mango samples was conducted in a mango plantation in Situbondo, East Java, Indonesia, between June to August 2015. Subjecting *S. dorsalis* to different food sources, a series of choice tests (based on choice and no-choice tests) on growth stages of mango were undertaken to determine the host preference on growth stage. T-test analysis was performed to determine the significant difference between the two choices of host. Results showed that *S. dorsalis* preferred flushes to flower, flushes than dormant leaves, and favored flower over dormant leaves. The host preference was determined by color, water and nutrient contents; diameter of stomata, and morphological surface of the host. The research implies that control strategies should be applied in the early emergence of young shoots to avoid initial population build up.

Keywords: mango, phenological stages, *Scirtothrips dorsalis*, preference

1. INTRODUCTION

Regular insecticide spray is the most common practice farmers use to keep their orchard free from pests. This activity does not only increase pesticide cost but also labor. Reducing input cost such as pesticides and labor is one of the ways to increase farmer's benefit. Hence, effective and efficient pest control strategies should be developed based on insect abundance and behavioral observation associated with the growth stage of the host. Insect host selection sequence includes habitat location, host location, host acceptance, and host use. Several sensory cues were utilized in host selection including visual, olfactory, gustatory, and tactile stimuli as well as humidity and light intensity (Bernays & Chapman, 1994). These cues stimulate receptors, generating sensory input, and finally behavioral responses.

Recently, the production and quality of mango as Indonesia's national commodity has declined due to the infestations of *Scirtothrips dorsalis* Hood. The thrips rasp and suck up cell content that cause in discoloration of young shoots due to the cell's exposure to air. Heavy incursions lead to curly shoot leaves, a result of unbalanced development among healthy and damage cells. Furthermore, the flush will dry and wither leaving only twigs. Twigs will never produce flowers and fruits. Attacks on flowers will prohibit the pollination process due to the pollen being consumed by thrips as a source of protein (Tsai *et al.,* 1996). It also causes a decline in fruit setting as the young fruit drops. The effects of *S. dorsalis* infestation on immature and mature fruits are peel scarring. Affandi *et al.* (2018) revealed that economic losses on 'Arumanis 143' mango caused by *S. dorsalis* was estimated at USD 1.040–USD 1.300/ha.

S. dorsalis has been reported to prefer buds, tender leaves, as well as flowers (Seal *et al.*, 2010; Kumar *et al.*, 2013; Mannion *et al.*, 2013; Mannion *et al.*, 2014). However, there is no information yet about its preference on several phenological stages associated with 'Arumanis 143' mango. Different insect species express high specificity at different stages in the host selection (Heard, 2018). Hence, investigation on partiality and all factors which support greater liking over another is an inevitable need for preventive strategic control programs.

The objective of the research was to identify the preference of *S. dorsalis* on several phenological stages of 'Arumanis 143' mango.

2. MATERIALS AND METHOD

Using the choice and no-choice tests, S. dorsalis was subjected to food at different growth stages of 'Arumanis 143' mango to determine its preference towards growth stage. The methodology of Bora *et al.* (2012) on host plant selection of Muga silkworm, *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae) was modified and used in this study. In the choice test, *S. dorsalis* was subjected to choose between two growth stages of mango (dormant and young shoots). A Petri dish (9 cm in diameter) was used as an arena for testing. It was padded at the bottom with a moistened Whatman filter paper. A rounded dormant leaf and young shoot of mango leaves (3 cm in diameter) were placed in opposite sides of the Petri dish, maintaining a 3 cm space between them. Ten female *S. dorsalis* were placed at the center of the petri dish. The number of *S. dorsalis* that moved and chose a certain growth stage versus those without any movement was counted every four hours until all of them died. In the no-choice test, a similar procedure was repeated, however providing only one growth stage of mango per Petri dish. This choice test was done using a combination of flush leaves and flower, flush leaves and dormant leaves, as well as dormant leaves and flower. Ten (10) trials were done for each of the tests.

Complimentary data such as number of leaf pores and its diameter was observed on flush and dormant growth stages. Surface characters of the host were also observed descriptively. T-test analysis was used to determine significant difference between the two growth stages.

3. RESULTS AND DISCUSSIONS

The choice tests indicated that the order of preference of *S. dorsalis* on the phenological stages of mango was as follows: flush>flower>matured. Given two choices at a time, flush stage was preferred over flower; flush stage was preferred over dormant stage of leaf; and flower was preferred over dormant stage. Variation in the number of adult thrips moving to a certain phenological stage in a time series of observation is presented in Figures 1, 2, and 3.

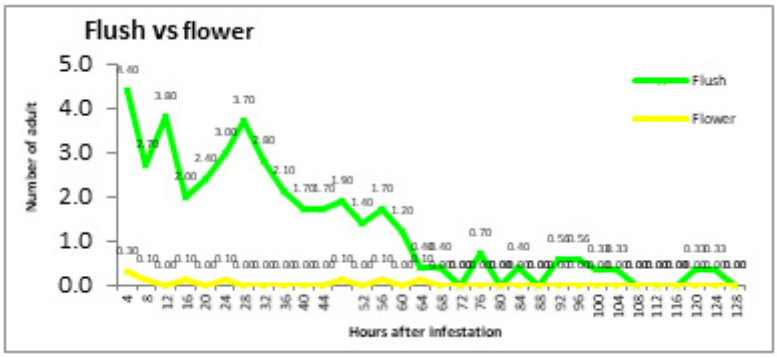

Figure 1. Preference of *S. dorsalis* on flower and flush growth stage of mango in two choices test.

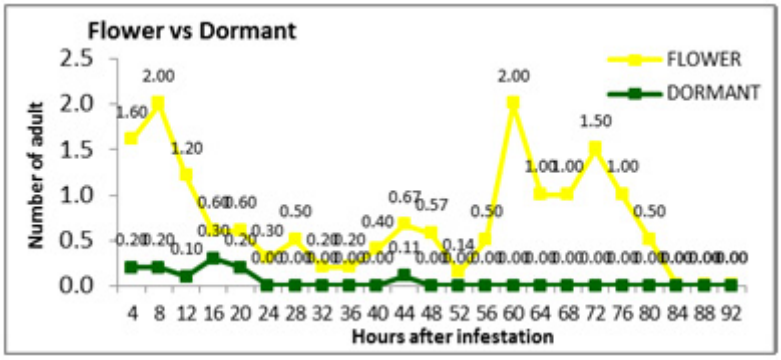

Figure 2. Preference of *S. dorsalis* on flower and dormant growth stage of mango in two choices test.

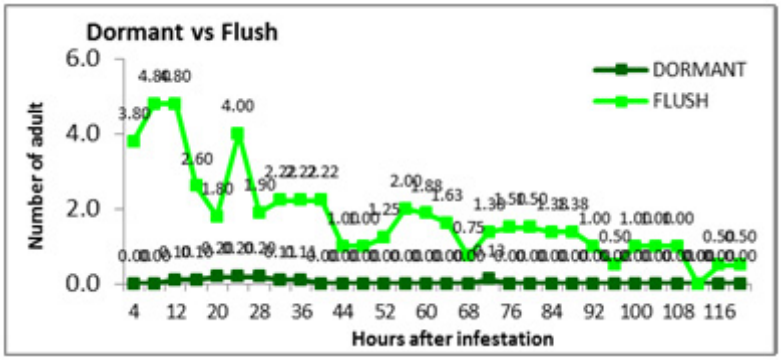

Figure 3. Preference of *S. dorsalis* on flush and dormant growth stage of mango in two choices test.

Thrips tended to move from one food choice to another in the duration of the study. The trend of their preference seemed to be observed 8–12 hours after infestation where about 50% of the test population remained on the phenological stage of their choice. The rest of the test population was either on the other choice (10%–20%) or on the surface of the Petri dish.

Results from the no-choice test further supported the result of the choice tests (Figure 4). The behavior of the population was the same in both choice and no-choice tests. Without any choice, the number of *S. dorsalis* on flowers and matured leaves did not increase. They were not forced to feed on the non-preferred stages even in the absence of an alternative. The proportion of feeding on flushes was higher than either flowers or matured leaves when offered alone. Similar to that of the no-choice test, the choice of most individuals in the test population became apparent around 8–12 hours after infestation.

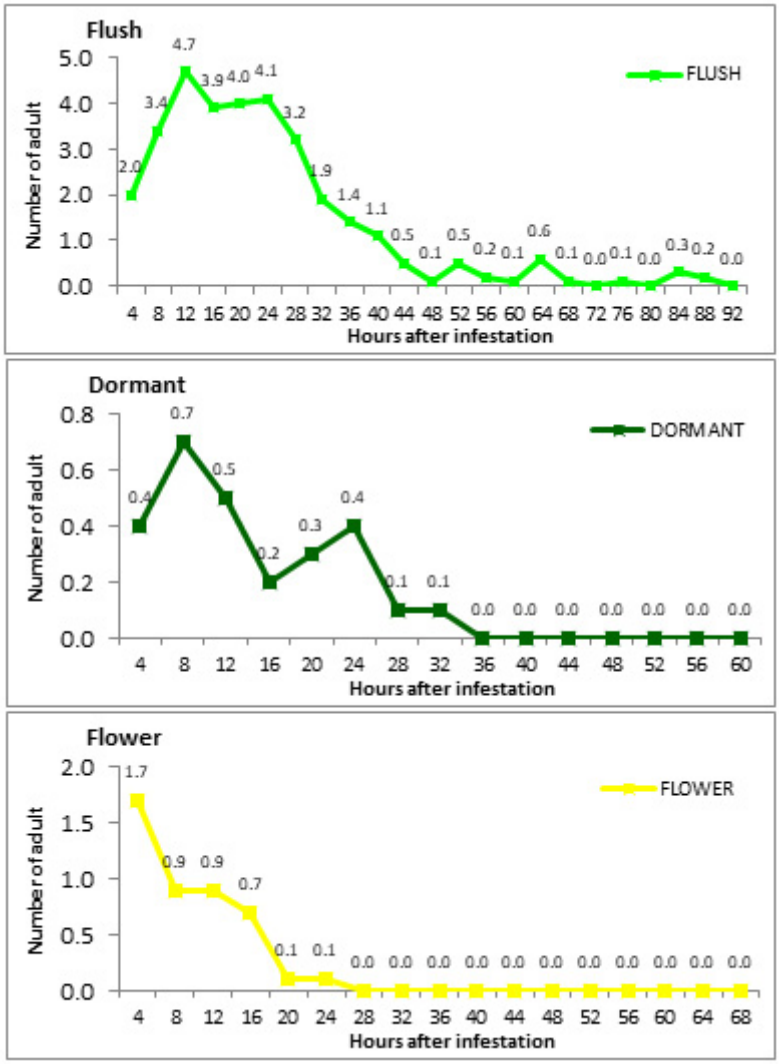

Figure 4. Mean number of *S. dorsalis* occupying the flush, flower, and dormant leaves in the no-choice test.

Preference is a behavior shaped by evolution. Understanding the behavioral processes involved in selection of a host plant can be used to improve the accuracy of host preference. The sequence of steps in host selection includes habitat location, host location, host acceptance, and host use. Insects use a number of sensory cues in host selection including visual, olfactory, gustatory, and tactile stimuli as well as humidity and light intensity (Bernays & Chapman, 1994). The food choices of insects are affected by factors like nutrition, freedom from natural enemy threat, host plant cues, and availability that promote their development, survival and reproduction. Both flush and flowers of mango became matured almost at the same time (10 days) as shown in Figures 1 and 2. The flushes nevertheless must be a more stable unit of food and habitat than the flowers considering that most parts of the florets fall off after anthesis and are transformed into a fruit. Also, there are other insect pests and diseases that attack the flowers than the leaves, hence more competitors. The occupation time of the chilli thrips, *S. dorsalis* Hood, associated with flush and flower growth stages of mango indicated that *S. dorsalis* started to move into the mango canopy during early shoot emergence (Affandi *et al.*, 2017). Field observations presented that population fluctuation of *S. dorsalis* associated with mango increased in the early dry season during flushing stage (143 thrips/sampling unit), and density declined when the plant enters the flowering stage and is dormant (Affandi *et al.*, 2018).

Color of the host play an important role in host selection of *S. dorsalis*. We observed that *S. dorsalis* prefer mangoes which are yellowish green (Appendix 1). Similar research on mandarin oranges revealed that yellowish-green, green, and yellow color sticky trap board with spectral reflectance (SR) 400–450, 480–540, and 540–580 nm, respectively, were favored by *S. dorsalis* and attracted more adult thrips. Shoot leaves and young fruits of mandarin oranges were more attractive to adult *S. dorsalis* due to the spectral reflectance of mature leaves (Tsuchiya *et al*., 1995, Prema *et al*., 2018b).

The various growth stages of mango must have met the suitability of nutrients required by *S. dorsalis* in terms of availability and balanced composition (Nation, 2001). Nutrient content analysis indicated that flush had high water, nitrogen, and protein content including cellulose as well as lignin (Table 1). The rasping-sucking mouth type of *S. dorsalis* requires high water content to acquire soluble nutrients and this is fulfilled by flush leaves than flower and dormant growth stages. Affandi *et al.* (2018a) stated that mango flush supported the development and survivability of *S. dorsalis* which develops from egg to pupa at 12.55±0.41 days with a survival rate of 47 %. High water and nutrition content on flush growth stages apparently influence the feed suitability. Runagall-McNaull, Bonduriansky, & Crean (2015) narrated that sufficient protein in the neriid fly, *Telostylinus angusticollis* affected the short larval life-span. Larvae also depended on high moisture content in its diet for survival. The presence of lignin was crucial to maintain physiological activities of the lower termite, *Coptotermes formosanus* Shiraki, and increased the survival rate significantly (Tarmadi *et al.*, 2017). Moisture content on leaves is mostly affected by the spacious circle of the stomata and thickness of the leaves. Laboratory observation showed that flush has a higher spacious circle of stomata compared to dormant leaves (Appendix 1) as well as thickness of the leaves (Appendix 3). Therefore, the high moisture content provided a more suitable environment for *S. dorsalis* to survive, develop, and reproduce.

Table 1. Analysis of nutrients content in several mango growth stages

MANGO GROWTH STAGES	WATER	NITROGEN	PROTEIN	CELULLOSE	LIGNIN
Dormant	60,39	0,51	3,19	13,24	14,43
Flush	79,64	0,77	4,82	18,86	18,98
Flower	79,86	0,71	4,42	14,68	18,84

Note: The analysis was done at Laboratorium Chem-Mix Pratama, Jl. Kretek, Jambitan, Banguntapan, Bantul, Jogyakarta, Indonesia.

Physical conditions such as the presence of hairs on flower stalks lessened the preference of *S. dorsalis* on the flower stage due to difficulties or inconvenience in reaching the pollen as an additional source of protein. Similar research on cotton discovered that much more damage was caused by *T. palmi* on cultivars with low densities of hairs than those with many hairs (Bournier, 1983; Gopichandran *et al*., 1992). Kirk (1997) revealed that hairiness is beneficial to plants, barring insects access to parts of the plant as source of food and oviposition as the hairs trap or injure the insect.

CONCLUSIONS

Host preference of *S. dorsalis* was determined by growth stages of the host. Flush growth stage was the most preferred, followed by flower, and dormant leaves. Determination of the preferences was deeply influenced by color, water and nutrient contents, diameter of stomata, thickness of the leaves, and morphological surface of the host.

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APPENDIX

Appendix 1. Population of *S. dorsalis* on several color of mango flush leaves.

No.	Clone	Replication	Color of flush leaves	Number of thrips		No.	Clone	Replication	Color of flush leaves	Number of thrips	
1.	Agrigardina 45	1	Greyed-Orange Group 177 A	0	0.00	6.	Garifta orange	1	Greyed-Orange Group 166 A	1	1.00
		2	Greyed-Orange Group 165 A	0				2	Greyed-Orange Group 166 B	1	
		3	Greyed-Orange Group 177 B	0				3	Greyed-Orange Group 165 A	1	
2.	O	1	Greyed-Orange Group 165 A	2	2.33	7.	Mang-gasari	1	Greyed-Orange Group 164 A	3	2.33
		2	Greyed-Orange Group 166 A	2				2	Greyed-Orange Group 166 C	3	
		3	Red Group 51 B	2				3	Greyed-Orange Group 165 B	3	
3.	Keitt	1	Yellow-Green Group 152 C	2	2.00	8.	Ken-layung	1	Greyed-Orange Group 164 A	1	1.00
		2	Yellow-Green Group 144 A	2				2	Greyed-Orange Group 164 A	1	
		3	Yellow-Green Group 144 A	2				3	Greyed-Orange Group 164 A	1	
4.	Garifta kuning	1	Greyed-Red Group 180 B	1	1.00	9.	Marifta	1	Yellow-Green Group 152 A	1	1.00
		2	Greyed-Red Group 180 B	1				2	Yellow-Green Group 152 A	1	
		3	Greyed-Orange Group 175 A	1				3	Yellow-Green Group 152 A	1	
5.	Arumanis 143	1	Yellow-Green Group N144 A	1	1.33	10.	Saigon kuning	1	Greyed-Purple Group N 186 C	3	2.67
		2	Yellow-Green Group 152 B	1				2	Greyed-Purple Group N 186 C	3	
		3	Yellow-Green Group 152 B	1				3	Greyed-Purple Group N 186 C	3	
No.	**Clone**	**Replication**	**Color of flush leaves**	**Number of thrips**		**No.**	**Clone**	**Replication**	**Color of flush leaves**	**Number of thrips**	
11.	Garifta merah	1	Red Group 38 A	2	1.33	14.	Kraton	1	Greyed-Orange Group 177 A	2	2.00
		2	Red Group 38 A	2				2	Greyed-Orange Group 195 A	2	
		3	Red Group 38 A	2				3	Greyed-Orange Group 174 A	2	

Appendix 1. Population of *S. dorsalis* on several color of mango flush leaves. (continued)

No.	Clone	Repli-cation	Color of flush leaves	Number of thrips		No.	Clone	Repli-cation	Color of flush leaves	Number of thrips	
12.	Durih	1	Greyed-Orange Group 165 A	5	5.00	15.	Garifta gading	1	Yellow-Green Group 15 B	1	3.00
		2	Greyed-Orange Group 165 A	5				2	Yellow-Green Group 152 C	1	
		3	Grey-Brown Group N199 C	5				3	Yellow-Green Group 152 B	1	
13.	Gadung	1	Yellow-Green Group 152 B	1	1.00						
		2	Yellow-Green Group 152 B	1							
		3	Yellow-Green Group 152 B	1							

Appendix 2. Length, width and spacious stomata on flush and dormant leaves of 'Arumanis 143' mango

No. of stomata	Flush leaves			Dormant leaves		
	Length (micron)	Width (Micron)	Spacious (½P x ½ L) x π	Length (micron)	Width (Micron)	Spacious (½P x ½ L) x π
1	19	18	268.5	25	14	274.8
2	17	10	133.5	12	10	94.2
3	20	10	157.0	12	10	94.2
4	18	15	212.0	15	10	117.8
5	20	18	282.6	14	10	109.9
6	17	14	186.8	25	25	490.6
7	25	10	196.3	13	10	102.1
8	20	10	157.0	20	10	157.0
9	15	12	141.3	19	10	149.2
10	18	10	141.3	20	15	235.5
Total number	189	127	1876.2	175	124	1825.1
Average	18.9	12.7	187.6	17.5	12.4	182.5
1	25	27	529.9	20	15	235.5
2	18	14	197.8	18	15	212.0
3	27	24	508.7	19	15	223.7
4	22	19	328.1	18	16	226.1
5	20	15	235.5	18	15	212.0
6	21	15	247.3	18	15	212.0
7	15	14	164.9	19	16	238.6
8	20	20	314.0	15	13	153.1
9	20	15	235.5	15	14	164.9
10	15	10	117.8	19	15	223.7
Total number	203	173	2879.4	179	149	2101.4
Average	20.3	17.3	287.9	17.9	14.9	210.1

Appendix 2. Length, width and spacious stomata on flush and dormant leaves of 'Arumanis 143' mango

No. of stomata	Flush leaves			Dormant leaves		
	Length (micron)	Width (Micron)	Spacious (½P x ½ L) x π	Length (micron)	Width (Micron)	Spacious (½P x ½ L) x π
2	20	15	235.5	17	15	200.2
3	20	16	251.2	19	12	179.0
4	20	20	314.0	19	17	253.6
5	20	21	329.7	30	20	471.0
6	20	19	298.3	20	20	314.0
7	20	15	235.5	20	15	235.5
8	15	13	153.1	20	16	251.2
9	20	15	235.5	19	10	149.2
10	20	15	235.5	21	15	247.3
Total number	193	164	2500.2	218	170	3078.0
Average	20.3	17.3	287.9	17.9	14.9	210.1
Total average	19.5	15.5	236.7	19.1	14.9	221.02

Appendix 3. Thickness of flush and dormant leaves of 'Arumanis 143' mango

No. of leaves	Flush leaves (micron)	Dormant leaves (micron)
1.	1.63	1.51
2.	1.89	1.5
3.	1.7	1.59
4.	1.85	1.29
5.	1.58	1.38
6.	1.82	1.38
7.	1.50	1.45
8.	1.83	1.64
9.	1.85	1.60
10.	1.58	1.5
Total	14.2	14.77
Average	1.72	1.48

PHYLOGENETIC RELATIONSHIPS AMONG SEVERAL SALACCA SPECIES (*SALACCA* SPP.) USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

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ABSTRACT

Of 23 salacca species found in the world, 15 species are found in Indonesia. The high salacca diversity is caused by open pollination systems, frequent usage of seeds as planting material, and breeding practices. This research which was conducted at the Molecular Laboratory of the Indonesian Tropical Fruit Research Institute, aimed to determine the level of primer polymorphism and phylogenetic relationships among salacca species. Five species of salacca (10 accessions) and 9 progenies from their crossing were used as samples. Eleven Rapid Amplified Polymorphism DNA markers were utilized for molecular analysis. All primers were given ranges of 57.1-100% polymorphic level. The range of genetic similarity coefficients were 0.512 - 0.937. Observed accessions could be separated into four groups. The highest genetic similarity coefficient was determined between PH-K and PH-MJ accessions (0.937), meanwhile the lowest value was indicated on MW-AFN hybrid and *S. wallichiana* (0.512). For ensuring the efficiency in germplasm management, only accessions which showed high genetic similarities are chosen as representation of the group while eliminating others. On the contrary, accessions which showed low genetic similarity are used as crosses between parents to obtain wide genetic variability and high heterosis effects.

Keywords: *Salacca* spp., Phylogenetic relationship, RAPD

1. INTRODUCTION

Salacca plants in the world are known to be very diverse. 15 out of the 23 species of salacca in the world are found in Indonesia (Zumaidar & Miftahuddin, 2018). Kalimantan has the largest diversity (11 species) of salacca (Ramella *et al.*, 2005). Natural open pollination environment, use of seeds as material for planting, and breeding programs contribute to the high diversity in salacca (Suskendriyati *et al.*, 2000). Inter and intra-species diversity are genetic sources for germplasm collections and producing new superior varieties. Phenotypic and genotypic variabilities and phylogenetic relationship information are needed to determine the parental crossing. Progenies with high heterosis can be obtained from parents with a wide genetic distance to a certain extent (Bruel, 2006; Nandariyah, 2010).

Genetic diversity for breeding programs can be obtained through the use of molecular markers. Random amplified polymorphic DNA (RAPD) is one way to know genetic diversity based on Polymerase chain reaction (PCR) (Rauf *et al.*, 2010). RAPD techniques have been used to study genetic diversity, genetic distance and species identification of salacca (Nandariyah *et al.*, 2004; Nandariyah, 2010; Budiyanti *et al.*, 2015; Elly *et al.*, 2018). However, the number of primers used in past study is still limited (3-6 primers). The number of primers determine the pattern of DNA bands.

Varied and many DNA bands will increase the accuracy of phylogenetic analysis (Das *et al.*, 2009). Polymorphisms resulting from RAPD amplification are random (Kumar & Gurusubramanian, 2011) and more suitable for the purpose of characterization and determination of genetic diversity (Das *et al.*, 2009). Genetic diversity is useful as a reference in breeding programs (Kelly & Miklas, 1998). The reference can be used before crossing to assemble new varieties, or to evaluate the results of crossing. One such reference is genetic information obtained from phylogenetic analysis (Theanphong *et al.*, 2016). The purpose of this study was to determine the level of primary polymorphism used and grouping between species and varieties of salacca (phylogenetic) based on RAPD analysis.

2. MATERIAL AND METHODS

2.1. Material

The sample for this research consisted of 5 species (10 accessions) and 9 progenies (Table 1). The morphological characters of the accessions can be seen in Appendix 1.

Table 1. List of salacca accessions used as research materials

No.	Accession	Individual plant codes	Species
1.	Sidempuan Merah	SDM (44-3)	Salacca sumatrana
2.	Sidempuan Putih	SDP (31-26)	Salacca sumatrana
3.	Pondoh	PH	Salacca zalacca var. zalacca
4.	Sanjung	SJG (18-11)	Salacca zalacca var. zalacca
5.	Mawar	MWR (27-18)	Salacca zalacca
6.	Gading Bali	GB	Salacca zalacca var. amboinensis
7.	Gula Pasir	GP (23-3)	Salacca zalacca var. amboinensis
8.	Affinis	AFN	Salacca affinis
9.	Wallichiana	WLC	Salacca wallichiana
10.	Glabrescens	GBC	Salacca glabrescens
11.	Sidempuan Merah x Sanjung	SDM x SJG (36-15)	S. sumatrana x S. zalacca
12.	Pondoh x Sanjung	PH x SJG (41-7)	S. zalacca x S. zalacca
13.	Pondoh x “M”	PH x “M” (49-19)	S. zalacca x S. zalacca
14.	Pondoh x “K”	PH x “K” (48-21)	S. zalacca x S. zalacca
15.	Pondoh x “MJ”	PH x “MJ” (17-4)	S. zalacca x S. zalacca
16.	Pondoh x Mawar	PH x MWR (6-28)	S. zalacca x S. zalacca
17.	Mawar x S. affinis	MWR x AFN (12-4)	S. zalacca x S. affinis
18.	Mawar x Sidempuan Putih	MWR x SDP (35-13)	S. zalacca x S. sumatrana
19.	Bali x Pondoh (Sari Intan 541)	B x PH	S. zalacca var. aboinensis x S. zalacca var. zalacca

2.2. Methods

Eleven primers namely AST2 L, AST2 R, AST9 L, AST9 R, AST12 R, OPA17, OPA 18, OPX 17, RAPD3, RAPD4, and RAPD6 were used for amplification in the RAPD technique. Genomic DNA was extracted using the CTAB method. Total volume (12,5 µl) for amplification consisted of 4,5 µl dH2O; 1,25 µl 10 pM primer; 6,25 µl Green go taq; and 0,5 µl 20 ng genomic DNA. PCR machines were set according to 45x cycles; preheating = 95 °C (2 min); denaturation = 95 °C (1 min); annealing = 36 °C (1 min); elongation = 72 °C (2 min); and final elongation = 72 °C (10 min). An amplification product (3 µl) was electrophoresed on 1.2% agarose gel with 1x SB buffer (50 volt; 60 min). Furthermore, the gel was immersed in a solution of ethidium bromide (40 µl 1%

ethidium bromide/litre dH2O) for 10 minutes and dH2O for 15 minutes. A UV transilluminator was used for viewing DNA band profile images. Binary data was obtained from scoring based on the presence or absence of the band. Scored binary data was used to calculate:

a. Polymorphism/primer: (∑ polymorphic bands/∑ total bands)x100%
b. Phylogenetic analyzed using *NTSYSpc* versi 2.1 and *UPGMA*.

3. RESULT

The amplification results showed the diversity of the number of polymorphic and monomorphic DNA bands (Table 2).

Table 2. Polymorphism levels of 11 primers based on the salacca DNA banding patterns

No.	Primer	Sequences 5' - 3'	Total bands	Polymorphic bands	Monomor-phic bands	Polymor-physm level (%)
1.	AST2 L	ATATGGTTGCAGAGCGGATG	5	4	1	80
2.	AST2 R	GCAAAACAGTGCTTGCTTCC	5	4	1	80
3.	AST9 L	ACATCGCAGGGGTCTTGA	13	10	3	76,9
4.	AST9 R	CAACCATTGTGGGGATGTG	8	6	2	75
5.	AST12 R	TGAATCCCATTCCTGTCAGC	7	4	3	57,1
6.	OPA17	GACCGCTTGT	8	8	0	100
7.	OPA 18	AGGTGACCGT	9	9	0	100
8.	OPX 17	GACACGGACC	7	5	2	71,4
9.	RAPD3	GTAGACCCT	4	3	1	75
10	RAPD4	AAGAGCCCGT	10	9	1	90
11	RAPD6	CCCGTCAGCA	7	6	1	85,7
	Total		83	68	15	

UPGMA cluster analysis showed that the 19 salacca accessions were divided into four major groups at 0.71 genetic similarity coefficient (Figure 1).

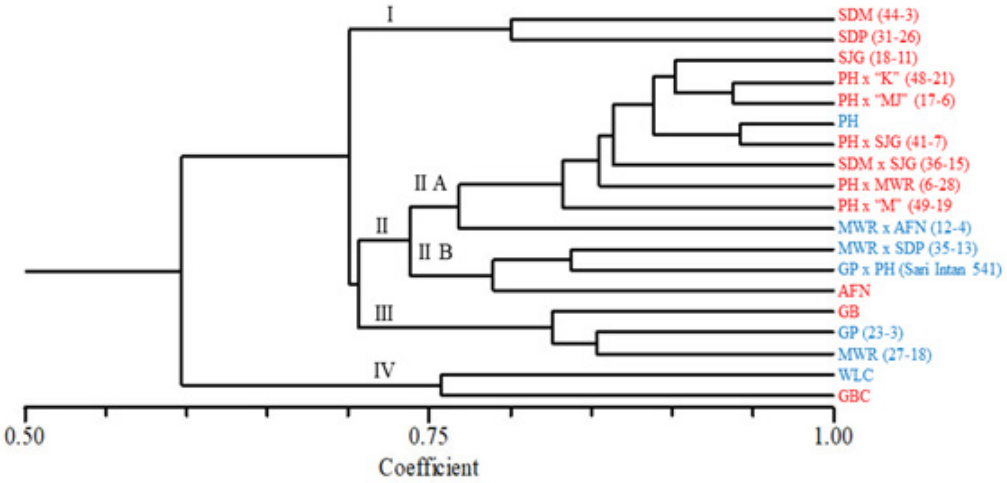

Fig. 1: Dendrogram of 19 salacca accessions based on UPGMA cluster analysis using 11 primers)

Notes : SDM='Sidempuan Merah', SDP='Sidempuan Putih', PH='Pondoh', SJG='Sanjung', MWR='Mawar', GB='Gading Bali', GP='Gula Pasir', AFN=*S. affinis*, WLC= *S. walliciana*, GBC= *S. glabrescents*. The name of the red accession=astringent taste, the name of the blue accession=non-astringent taste.

The genetic similarity of 19 salacca based on 11 RAPD primers ranged from 0.512 to 0.937. The greatest genetic similarity coefficient (0.937) was between the PHxK accession and the PHxMJ accession, while the smallest was between MWRxAFN accession and WLC (*S. wallichiana* (0.512)) (Table 3).

Table 3. Matrix of genetic similarities between species or accessions of salacca

Acc	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1,000																		
2	0,800	1,000																	
3	0,725	0,682	1,000																
4	0,747	0,808	0,848	1,000															
5	0,681	0,687	0,848	0,855	1,000														
6	0,706	0,710	0,903	0,865	0,942	1,000													
7	0,723	0,703	0,835	0,804	0,824	0,813	1,000												
8	0,707	0,711	0,889	0,871	0,851	0,905	0,860	1,000											
9	0,674	0,681	0,915	0,876	0,895	0,929	0,845	0,937	1,000										
10	0,737	0,714	0,738	0,716	0,716	0,742	0,667	0,729	0,711	1,000									
11	0,630	0,667	0,691	0,652	0,674	0,698	0,619	0,699	0,690	0,805	1,000								
12	0,730	0,732	0,756	0,688	0,71	0,736	0,706	0,714	0,727	0,846	0,853	1,000							
13	0,683	0,667	0,828	0,816	0,857	0,891	0,844	0,876	0,86	0,699	0,675	0,741	1,000						
14	0,595	0,609	0,739	0,738	0,816	0,784	0,737	0,766	0,755	0,636	0,729	0,698	0,813	1,000					
15	0,732	0,684	0,785	0,711	0,689	0,738	0,756	0,790	0,776	0,693	0,722	0,767	0,718	0,747	1,000				
16	0,747	0,723	0,771	0,787	0,745	0,773	0,698	0,776	0,764	0,734	0,710	0,779	0,756	0,759	0,838	1,000			
17	0,720	0,699	0,699	0,723	0,660	0,705	0,698	0,776	0,719	0,683	0,715	0,675	0,732	0,667	0,784	0,795	1,000		
18	0,629	0,590	0,590	0,562	0,584	0,602	0,519	0,600	0,571	0,676	0,620	0,667	0,571	0,512	0,609	0,658	0,685	1,000	
19	0,647	0,605	0,605	0,574	0,552	0,593	0,532	0,615	0,561	0,583	0,551	0,571	0,587	0,550	0,597	0,648	0,676	0,758	1,000

Notes: 1).SDM; 2) SDP; 3). SJG; 4). SDMxSJG; 5). PH; 6). PHxSJG; 7). PHxM; 8). PHxK; 9). PHxMJ; 10). GB; 11). GP; 12). MWR; 13). PHxMWR; 14). MWxAFN; 15). MWRxSDP; 16). Sari Intan 541 PNG; 17). AFN; 18). WLC; 19). GBC

4. DISCUSSION

The main target expected is polymorphic bands. Polymorphism can be caused by several factors including: a primary mismatch, the modification or change of the primary, and differences in the amplified genomic DNA region (Kumar & Gurusubramanian, 2011). The presence of monomorphic bands shows the high conserved region of the genome (Singh *et al.*, 2006).

Based on Figure 1, Group I consisted of accessions of the species *S. sumatrana*, namely red-fleshed (SDM) and white-fleshed (SDP). *S. sumatrana* has superior characters (thick fruit flesh and large fruit), but astringent fruit taste (Hadiati *et al.*, 2012). In addition, it has a larger plant size compared to the Javanese salak and Balinese salak (Hadiati *et al.*, 2008). Group II was divided into two sub groups. Sub-group IIA consisted mainly of *S. zalacca* var. *zalacca* (Javanese Salak) and the results of the crossing. In this group, most of them produced astringent fruits, except for 'Pondoh; (PH) and MWRxAFN. Group IIB consisted of MWRxSDP; GPxPH and AFN (*S. affinis*). In this group, most of them had non-astringent fruit taste, except for AFN. Groups IIA and IIB could not be categorized explicitly based on astringency. Group III consisted of accessions belonging to the species *Salacca zalacca* var. *amboinensis* (GP and GB). The inclusion of MWR in group III was acceptable because one of the MWR's parental is GP. This group had specific leaflet characteristics. The curled leaflet edge and the smaller leaflet size are clear differentiators compared to *S. sumatrana* and *S. zalacca* var. *zalacca* (Appendix 1). Group IV consisted of two species namely *S. wallichiana* and *S. glabrescens*. Based on morphological characters, the two

species are different. The two species should be separated into different groups. *S. wallichiana* had a spineless petiole and the number of fruits per bunch can reach > 300 pieces per bunch.

The inaccuracy of salacca grouping in this study can be improved by increasing the diversity of the band (polymorphism of the amplification results). Addition of primers is one way to increase polymorphism (Sall *et al.*, 2000). Another way that can be taken is the use of specific primers for species/variety differentiation (Ramella *et al.*, 2005). The genetic similarity of 19 salacca based on 11 RAPD primers ranged from 0.512 to 0.937 (Table 2). The similarity coefficient above 50% shows the relationship between accessions is quite close (Zumaidar *et al.*, 2015). Except for MWR x SDP, progenies have a large genetic similarity coefficient to one of their parents.

5. CONCLUSION

All primers were given a range between 57.1-100% polymorphic level. The range of genetic similarity coefficients was between 0.512 - 0.937. Observed accessions could be separated into four groups. The highest genetic similarity coefficient was determined between PH-K and PH-MJ accessions (0.937), meanwhile the lowest value was indicated on the MW-AFN hybrid and *S. wallichiana* (0.512).

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Appendix 1. Morphological characters of salacca

No.	Accession	Curled leaflet edge	Petiole	Fruit skin	Flesh color	Astringent taste
1.	Sidempuan Merah/ SDM (44-3) (*S. sumatrana*)	absent	spiny	spiny	Reddish white (red 45 D, yellow 4 D)	present
2.	Sidempuan Putih/ SDP (31-26) (*S. sumatrana*)	absent	spiny	spiny	Cream (Yellow white 158 A)	present
3.	Pondoh/PH *S. zalacca*	absent	spiny	spiny	White (White NN 155 B)	absent
4.	Sanjung/SJG (18-11) (*S. zalacca*)	absent	spiny	spiny	Light cream (Yellow white 158 B)	present
5.	Mawar/ MWR (27-18) (S. zalacca)	present	spiny	spiny	White (White NN 155 A)	absent
6.	Gading Bali/GB (*S. zalacca* var *amboinensis*)	present	spiny	spiny	Cream (Yellow white 158 A)	present
7.	Gula Pasir/GP (23-3) (*S. zalacca* var *amboinensis*)	present	spiny	spiny	White (White NN 155 A)	absent
8.	*S. affinis*/AFN	absent	spiny	spineless /smooth	Cream (Yellow white 158 A)	present
9.	*S. wallichiana*/WLC	absent	spineless	spiny	Yellow orange 22 C	absent

10.	*S. glabrescents*/GBC	absent	spiny	spiny	Yellow orange 20 D	present
11.	SDM x SJG (36-15) (Sidempuan Merah x Sanjung)	absent	spiny	spiny	Cream (Orange white 159 B)	present
12.	PH x SJG (41-7) (Pondoh x Sanjung	absent	spiny	spiny	Cream (Yellow white 158 A)	present
13.	PH x "M" (49-19) (Pondoh x "M")	absent	spiny	spiny	Cream Yellow white 158 A	present
14.	PH x "K" (48-21) (Pondoh x "K")	absent	spiny	spiny	Cream (Yellow white 158 A)	present
15.	PH x "MJ" (17-6) (Pondoh x "MJ")	absent	spiny	spiny	Cream (Yellow white 158 A)	present
16.	PH x MWR (6-28) (Pondoh x Mawar)	absent	spiny	spiny	Cream (Yellow white 158 B)	present
17.	MW x AFN (12-4) (Mawar x *S. affinis*)	absent	spiny	spiny	White (White NN 155 C)	absent
18.	MWR x SDP (35-13) (Mawar x *S. sumatrana*)	absent	spiny	spiny	White (White NN 155 A)	absent
19.	Sari Intan 541 (GP x Pondoh)	absent	spiny	spiny	White (White NN 155 A)	absent

HUANGLONGBING (HLB) RAPID DETECTION KIT DISSEMINATION TO BUILD COMMUNITY-BASED EARLY WARNING SYSTEM: CASE STUDY IN KOTO TINGGI, WEST SUMATERA, INDONESIA

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ABSTRACT

Huanglongbing (HLB) is a disease of citrus that can lead to plant death. Most farmers are unaware of the symptoms of HLB from the beginning of plant growth, and normally they suspect the condition as a result of nutrient deficiency. The usual detection of HLB conducted is through expensive and complicated laboratory tests using PCR. A quick detection method is necessary in order to formulate a strategy to solve the HLB outbreak appropriately. The HLB Rapid Detection Kit is an innovation that can identify the presence of HLB easily and inexpensively. Even farmers can do the test by themselves with realtime result. An observation has been done to test the effectiveness of the HLB Rapid Detection Kit in a citrus plantation area at Koto tinggi, Agam district, West Sumatera. The results on productive citrus crops revealed that the number of plants infected by HLB was between 19.22%-41.84% of the citrus population. The use of the HLB Rapid Detection Kit in Koto Tinggi, Agam district, West Sumatera was successful in detecting the level of HLB infections at the citrus plantation. The kit was also able to predict the cause of infection, which was found to be from the use of citrus seedlings that were already infected with HLB. The strategies for restoration of citrus areas infected by HLB are eradicating HLB vectors and replanting citrus plants using certified healthy planting materials. The implementation of the program should be integrated in one area.

Keywords: citrus, HLB, rapid detection kit

1. INTRODUCTION

Citrus is one of the fruits that is a source of vitamin C and popular among consumers everywhere.. However, currently the national citrus supply has not been able to meet domestic needs. Although the production and area of citrus continue to increase, but its productivity has declined, especially in West Sumatera area where in 2016, the productivity was 60.45 kg/Ha, but has decreased to 55.87 kg/Ha in 2017. Another problem is the quality of citrus still does not meet consumers' preferences, and also cannot compete in the global market. Indonesia's citrus trade balance has constantly been on a deficit whereby in 2018 Indonesian citrus exports amounted to 1,220 mton while imports were much higher reaching 85,273 mton.

One of the main causes of the declining productivity and quality of citrus is due to the Huanglongbing (HLB) outbreak. Huanglongbing disease (HLB), also known as CVPD (citrus vein phloem degeneration) is a disease caused by the bacterium *Liberobacter asiaticus*, which causes damage to the phloem vessels in citrus plants. Plants affected by this disease will have photosynthetic translocation disorders. In the initial stages even though the plant has been infected with HLB, it will still show healthy growth. At a later stage, the visible symptoms will

manifest in the form of yellowing or chlorosis, leading to stunted canopy, leaves becoming stiff and slender, small and asymmetrical fruits, with branch and twig dieback, eventually causing the plants to die (Wijaya, 2007; Himawan, *et al.*, 2010; Nurhadi, 2015; Burhansyah, 2014).

HLB disease can infect citrus plants at the seeding phase, because infection can occur through grafting by using buds or cuttings taken from infected mother trees. The spread of the HLB disease can occur through the planting of infected seedlings, even though the seedlings look healthy, because the incubation period is 3-5 months. It is therefore, necessary to strengthen the domestic quarantine system in preventing the spread of HLB from endemic areas to disease free areas (Rustiani *et al.*, 2015; Zamzani & Arsanti 2014; Wijaya 2007). This systemic disease spreads rapidly through a vector, the Asian citrus psyllid known as *Diaphorina citri*. A HLB epidemic can reach, more than 95% within 3-13 years after the first symptoms appear (Wijaya 2007; Nurhadi 2015; Rustiani *et al.*, 2015). It can be said that the HLB epidemic is a threat to the sustainability of citrus farming in Indonesia.

Upon observing initial chlorosis symptoms, farmers are often led to generally suspect that the plants suffer from nutrient deficiency, prompting more intensive fertilization. But what happens within a period of 2-4 years after the initial symptoms appear is that plants fail to develop and produce. Decreased fruit production and quality due to HLB attacks cause farming to be economically unfeasible within 7-10 years after planting (Nurhadi, 2015), compounded with the fact that even citrus fruits produced by HLB-infected plants experience nutrient deficiency and poor quality (Wirawan, *et al.*, 2017). However, because the plants are still in production, farmers object to destroying their citrus plants. Whereas in terms of epidemiology, this condition will increase the availability of inoculum sources in the farm which in turn leads to high risk conditions for citrus plants that are still healthy (Nurhadi, 2015). Misdiagnosis of the disease causes errors in decision making, which in turn leads to ineffective and inefficient management, and ultimately causing greater losses for farmers.

The detection of HLB is usually conducted using expensive and complicated laboratory tests through PCR (Polymerase Chain Reaction) analysis (Wijaya, 2007; Rustiani, 2015). The Indonesian Citrus and Subtropical Fruit Research Institute (ICSFRI) under the Ministry of Agriculture (MoA) has created innovations to detect HLB diseases rapidly. The HLB Rapid Detection Kit is an innovation that can identify the presence of HLB easily and inexpensively. Even farmers can perform the test themselves with realtime results obtained. The HLB Rapid Detection Kit has been disseminated and tests conducted at various citrus centers in Indonesia, including in Koto Tinggi Village, Agam Regency, West Sumatra. Dissemination of this kit is expected to help farmers and the local government detect the possibility of an HLB outbreak rapidly in citrus growing areas, so that strategic steps can be taken immediately to handle the outbreak effectively.

2. METHODOLOGY

This research was carried out in November 2018 in Koto Tinggi Village, Baso District, Agam Regency, West Sumatra. The location was chosen purposively because it's one of the locations of citrus development programs from the government. The selection of respondents was also conducted purposively consisting of 15 respondents who were representatives of three farmer groups. The number of respondents was determined based on the consideration of limited funds and time and the amount was considered to be sufficiently representative.

Primary data collection was done through farmer interviews in groups, commonly called Focus Group Discussions (FGD) regarding the development of the citrus area and current conditions.

While secondary data were obtained from the observations conduct by the research team from ICSFRI of the possibility of HLB outbreak on citrus plants using HLB Rapid Detection Kit.

3. RESULT AND DISCUSSION

Based on information from the FGD results, the citrus area in Koto Tinggi village, Agam district, West Sumatra began to be developed since 2008 which was a government program. From the initial citrus area of 20 hectares, there are only 30% left, while the others are no longer in production and have been replaced by other commodity crops. Based on farmer information, in 2011 citrus plantations produced well with an average productivity reaching 50 kg/tree, and peak production of up to 60-70 kg/tree in 2013-2015, but at the end of 2016 to 2017 production began to decline, plants began displaying symptoms of nutrient deficiency such as leaf chlorosis, shoots and stems turning yellow, abnormally small leaves and erect stems (Figure 1).

Figure 1. Citrus Plants infected by HLB in Kototinggi village, Agam District, West Sumatera

At the end of 2017, the affected plants were not able to recover even though fertilizing was carried out. These conditions positively reflect HLB symptoms, but most of the farmers were not alerted and assumed that their plants were damaged and under developed due to nutrient deficiency or have reached the peak of physiological age. Generally, plants that are not infected with HLB and well cared for can continue producing for more than 20 years (Nurhadi, 2014).

Those affected citrus plants could still produce fruit which were abnormal, low quality, sour, small sized and misshapened which were generally sold for fresh juice consumption at low prices. Therefore, many citrus trees were cut down, and replaced with other commodity crops. However, there were farmers who preferred maintaining their citrus plants as these were grown as polyculture with vegetable commodities such as caisim, cabbage, eggplant, tomatoes, and chillies for additional income.

Based on the information from farmers, pests and citrus plant diseases that were most feared were fruit fly and powdery mildew whose effects were seen directly on the citrus fruits produced, especially fruit damage, such as rotten fruit. Farmers were not yet aware of the symptoms and deadly consequences of HLB disease.

The results of HLB disease testing using the HLB rapid detection kit in the citrus area in the Koto Tinggi, Agam district are presented in Table 1. From ± 3500 plants originating from citrus plantations in 3 farmer groups, there were 19.22%-41.84% plants positively infected with HLB. Moreover, in one of the farmers' citrus farm, based on initial symptoms which appeared, plants

were 100% infected with HLB. However, the infectious vector *Diaphorina citri* was not found, indicating the possibility that a location of higher altitude could affect the presence of the vector. From the results of this observation, the absence of vectors indicates that the number of citrus plants that have been infected with HLB was because the source of planting materials or the seedlings used were already infected.

Table 1. Test results of the HLB Rapid Detection Kit in Koto Tinggi citrus development area, Agam District, West Sumatera

No.	Farmer Groups	Plant Age	Number of Citrus Plants	Number of Citrus plants infected with HLB	Vector existence	% infected with HLB
1.	Tunas Baru	3 - 8 years	951	243	0	25
2.	Bumi harapan	5 - 8 years	1336	559	0	41,84
3.	Amanah	4 -15 years	1321	254	0	19,22

Data source: ICSFRI (2018)

The advantages of the HLB rapid detection kit are 1) Quick detection process (60-75 minutes) from preparation to interpretation of results, 2) Easy, simple protocols and the application does not require special expertise, 3) Inexpensive, does not require special laboratory facilities and less sophisticated. The HLB rapid detection kit can help control HLB disease in endemic areas where infrastructure, facilities and resources are minimal. Test results can be observed visually by the occurrence of discoloration that can be seen with the naked eye. The advantages of the HLB Rapid Detection Kit compared to other detection methods is presented in Table 2.

Table 2. The advantages of the HLB Rapid Detection Kit compared to the PCR and RT-PCR methods

No.	Parameter	PCR	Kit Detection	RT-PCR	Validation
1.	Affordable	+	+++	-	Respondent
2.	Sensitivity	ND	+++	+++	Genie II
3.	Spesivity	ND	+++	+++	Genie II
4.	User-friendly	-	+++	+	Referensi
5.	Rapid and robust	+	45´	++	Genie II
6.	Economic	+	+++	+	Economy Analysis
	Laboratoium	+++	-	+++	Economy Analysis
	Equipment	+++	+/-	+++	Economy Analysis
7.	Deliverable to end user	-	+++	-	Economy Analysis
8.	False negative	< 10	< 5	< 1	Participant
9.	False positive	< 10	< 5	< 1	Participant

Data source: ICSFRI (2018)

Based on the results of the FGD and the results of observations of the HLB outbreak rate, the following are recommendations that require follow through:

1. It is important to provide farmers with knowledge and understanding of the specific HLB disease – its symptoms and its effects.
2. Strategy to rehabilitate citrus areas that have been infected can be done by eradicating infected plants and replanting with certified healthy citrus seedlings. Handling of HLB and improving plantations must be carried out in one area through an integrated manner.
3. The use of healthy seeds is important to ensure disease free plants. However, currently the number of certified citrus seeds is very limited, so it is necessary to establish seedling institutions at the farm level. The acceleration of technology transfer of disease-free citrus

seedlings to farmer/breeder groups needs to be done to ensure the availability of healthy citrus seeds..

4. At present, ICSFRI is the only institution for citrus research which has the responsibility in producing and distributing disease-free mother trees. In the context of developing citrus agribusiness in Indonesia through the development of citrus areas, and realizing the independence of seeds at the farm level, the ICSFRI can arrange to provide technological assistance to farmers to produce healthy citrus seeds. The seeds produced by farmers can be used to revitalize the citrus farmers' own estate, or can be sold so that it can be an additional income.
5. Optimizing the role of quarantine institutions between regions to prevent the spread of HLB through citrus seedling deployment.
6. The local government should play a role in assisting the procurement of HLB Rapid Detection Kits for farmers and field officers in citrus centers. This would enable the farmers and field officers to detect HLB attacks early, so they can immediately make decisions and execute rapid and accurate actions to minimise the impact of the disease.

4. CONCLUSION

The application of the HLB Rapid Detection Kit in Koto Tinggi, Agam district, West Sumatera was successful in detecting the levels of HLB infection in citrus plantations, and in predicting the cause of the infection which was from the use of citrus seedlings that had already been infected with HLB. Strategic steps could then be immediately determined to overcome the HLB outbreak in the area.

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THE POTENTIAL APPLICATION OF SABA BANANA FLOUR IN BAKERY PRODUCTS

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ABSTRACT

'Saba' banana is a triploid hybrid (ABB) cultivar that is normally marketed as fresh produce and the downstream processing is limited to local dishes or chips. Bananas are rich in potassium and unripe bananas contain high resistant starch that is essential for healthy digestion and has moderate glycaemic index. Processing 'Saba' banana into flour provides a more stable storage form while reducing wastage of unmarketable fruits. The aim of this study was to examine consumers' acceptance of bakery products produced using banana flour. Green (unripe) 'Saba' bananas were obtained from local farmers and developed into banana flour. The bananas were cleaned, peeled, sliced, and dried in a fan-forced oven at 75°C. The drying process was stopped once water activity and moisture content were below 0.5 aw and 10%, respectively. The dried bananas were then ground, sieved manually, and sealed in a plastic bag. The flour was analysed for its nutritional content and used as the main ingredient in bakery products to test for consumers' acceptance. Five bakery products (layered cake, mini cake, biscuit, crepe, *bahulu*) were developed and served during Sabah's state level Farmers Day in 2017. Acceptance of the bakery products was based on a 5-points hedonic scale, where 49 respondents who tested all five products and completed the evaluation form were included in the analysis. The nutritional analysis showed that green 'Saba' banana flour contains energy (367kcal/100g), carbohydrates (87.7g/100g), protein (3.4g/100g), fat (0.3g/100g), and potassium (994mg/100g). The acceptance test showed that 'Saba' banana flour was well accepted (most scored 4 and 5) as an ingredient in bakery products. The mean score of acceptance for crepe, layered cake, biscuit, mini cake, and *bahulu* were 4.47±0.92, 4.43±0.61, 4.20±0.76, 4.14±0.82, and 4.02±0.85, respectively. Significant difference ($p<0.05$) was observed between the five tested products, where the respondents preferred the layered cake and crepe made of 'Saba' banana flour. These results demonstrated the potential of incorporating 'Saba' banana flour as the ingredient in bakery products as a healthier alternative. This healthy replacement meets the demand of consumers who seek health and taste in their food.

Keywords: 'Saba' banana, green banana flour, bakery products

1. INTRODUCTION

Banana is the fourth major fruit crop in the world and one of the most consumed fruits in tropical and subtropical regions (Alkarkhi *et al.*, 2011; Bello-Perez, 2012). There are more than 1000 varieties of bananas around the world with Cavendish being the most commercialized (about 45% of global market) and widely exported (Falcomer *et al.*, 2019; Food and Agriculture Organization, 2015). This is due to its high production per hectare and resilience to damage from environmental changes (Falcomer *et al.*, 2019). The other large variety group of bananas is the plantain which has upwards of 100 cultivars (Falcomer *et al.*, 2019).

'Saba' banana is a triploid hybrid (ABB) cultivar which originates from the Philippines. 'Saba'

is the given name for cooking bananas in the Philippines, but they are also known as 'Pisang Kepok' in Indonesia, 'Pisang Abu/Nipah' in Peninsular Malaysia and 'Kluai Hin' in Thailand (Van den Bergh, 2017). Sabah is one of the main producers for 'Saba' banana in Malaysia with the annual production of around 35,000 tonnes in 2017 (Department of Agriculture Sabah, 2017). The export of 'Saba' banana has also increased in the past five years from 6,529.47 metric tonnes with the value of RM 5,148,000 in 2014, to 15,373.55 metric tonnes with the value of RM 15,784,810 in 2018 (Federal Agricultural Marketing Authority, 2019).

Bananas are highly convenient and affordable snacks and are well known for their abundant source of health benefits such as potassium, dietary fibre, and vitamin B6 (Hark & Deen, 2007). Unripe (green) bananas seem to be a good source of fibres, vitamins (Vitamin C, B6, and provitamin A), minerals (potassium, phosphorus, magnesium, zinc), bioactive compounds such as phenolic compounds, and high resistant starch (Falcomer *et al.*, 2019). Resistant starch is not digested in human small intestines and is fermented by bacterial microflora in the large bowel (Juarez-Gracia *et al.*, 2006). This affects a number of physiological functions and thus having effects on health such as reduction in glycaemic and insulinemic response to food, hypocholesterolemic action, and protective effects against colorectal cancer (Juarez-Gracia *et al.*, 2006).

Bananas are typically marketed as fresh produce and the downstream processing is limited to local dishes or chips. The new economic strategy in increasing the utilisation of banana includes production of banana flour using unripe fruits and incorporating the flour into various innovative food products (Alkarkhi *et al.*, 2011). Additionally, the conversion of fresh bananas into flour provides a more stable storage form and avoids wastage of unmarketable fruits. Banana is a climacteric fruit, prone to mechanical damage when ripe and perishable during the maturation process (Falcomer *et al.*, 2019). Furthermore, almost 20% of banana production is not commercialized due to appearance flaws leading to the increase in wastage. Thus, banana processing can reduce the production of the waste and improve bioavailability and utilization of nutrients available in this fruit (Falcomer *et al.*, 2019).

There are vast studies on unripe banana flour conducted for plantain (Agama-Acevedo *et al.*, 2012; Juarez-Garcia *et al.*, 2006) and 'Cavendish' (Campuzano *et al.*, 2018; Loong & Wong, 2018; Alkharkhi *et al.*, 2011; Bezerra *et al.*, 2013) bananas. As one of the main producers of 'Saba' banana in Malaysia, there is a big potential for developing products from a locally grown food source. Additionally, promotion is also required since food products made from unripe 'Saba' banana flour is quite new to Sabahan consumers. Therefore, the aims of this study were to develop unripe 'Saba' banana flour and its bakery products; and to promote and examine their acceptance among local consumers.

2. MATERIALS AND METHODS

Green (unripe) 'Saba' bananas were obtained from local farmers in Tenom, Sabah and processed into flour in the Agriculture Research Station, Lagud Seberang, Tenom.

2.1. Sample Preparation and Flour Production

The bananas were cleaned, peeled, and sliced using an automated slicer. The sliced bananas were then arranged on stainless-steel trays, before being loaded into a fan-forced oven and dried at 75°C. The drying process was stopped once the water activity and moisture content were below 0.5 aw and 10% respectively. The water activity was determined with a water activity meter (Pawkit, decagon Devices Inc. Pullman, WA, USA), while moisture content was determined

using oven method at 105 °C until a constant weight is reached. The dried bananas were then ground using an automated grinder and sieved manually before being sealed in a plastic bag and placed in the refrigerator until further processing.

2.2. Preparation of Bakery Products

'Saba' banana flour was used as the main ingredient in the development of five bakery products, namely layered cake, mini cake, biscuit, crepe, and *bahulu*. The recipes of all bakery products, except *bahulu* were developed by the Agro-Based Industry Development Section (Industri Asas Tani – IAT). 'Saba' banana *bahulu* were produced by one of the IAT entrepreneurs. The recipes used in developing bakery products are listed in Table 1. All products were baked at the IAT testing kitchen, Tuaran. The baking ingredients were purchased from the local supermarket, while existing utensils and equipment such as measuring spoons, bowls, sifter, weighing scale, mixer, baking tins, trays, and oven were used for baking.

Table 1: Recipes of four bakery products made from unripe 'Saba' banana flour

Ingredients	Preparation Method
A. Layered Cake	
1. Unripe banana flour (280g) 2. Butter (250g) 3. Castor sugar (230g) 4. Egg (3) 5. Milk (1 tablespoon) 6. Mashed banana (200g) 7. Sliced banana (2) 8. Salt (1/4 teaspoon) 9. Baking Powder (1/4 teaspoon)	1. Mix salt and mashed banana 2. Sieve banana flour and baking powder, place aside 3. Beat butter and sugar until fluffy, and add eggs gradually 4. Add in milk and mashed banana 5. Add in sieved banana flour gradually and mix well 6. Pour batter into baking tin, alternate with sliced banana 7. Steam for 7 minutes
B. Mini Cake	
1. Unripe banana flour (250g) 2. Egg (3) 3. Sugar (100g) 4. Corn oil (200 ml) 5. Mashed banana (200g) 6. Bicarbonate soda (1 tablespoon) 7. Baking powder (1 tablespoon)	1. Sieve banana flour, bicarbonate soda, and baking powder, place aside 2. Beat sugar and egg 3. Add in corn oil, mashed banana and mix well 4. Add in sieved banana flour and mix well 5. Pour batter into cupcake tins 6. Bake in an oven at 150°C to 180°C for 15 to 20 minutes
C. Biscuit	
1. Unripe banana flour (180g) 2. Egg (2) 3. Icing sugar (150g) 4. Butter (130g) 5. Corn flour (20g)	1. Beat butter and icing sugar 2. Add in eggs and mix well 3. Add in banana flour, corn flour and mix well 4. Roll the dough into even thickness and cut out using cookie cutter 5. Bake in an oven at 150°C to 180°C for 20 minutes
D. Crepe	
1. Unripe banana flour (80g) 2. Tapioca flour (80g) 3. Corn flour (40g) 4. Custard flour (20g) 5. Egg (4) 6. Corn oil (4 tablespoon) 7. Castor sugar (200g) 8. Milk (160mL) 9. Water (600mL)	1. Mix all ingredients in a bowl 2. Coat the pan with butter to avoid crepe from sticking 3. Pour in the batter and swirl the pan into an even thin layer at the bottom of the pan 4. Apply low heat and until the crepe is cook 5. Fill the crepe with any desired filling, fold and serve cold

2.3. Nutritional Analysis

'Saba' banana flour was sent to the accredited laboratory for nutritional content analysis. Analysis conducted include energy, carbohydrate, protein, fat, and potassium.

2.4. Promotion and Acceptance Test

The promotion of unripe Saba banana flour bakery products was conducted during the launching of Sabah's state level Farmers Day 2017 in Tuaran. In order to determine the acceptance of the products, visitors who tested all five bakery products were given forms for evaluation.

The acceptance of the products was based on 5 points hedonic scale, ranging from 1 – dislike extremely to 5 – like extremely. Respondents who completed the evaluation forms were included in the analysis. Mean (± S.D.) scores for the acceptability of all 5 bakery products were presented. Analysis of variance (ANOVA) was performed using IBM SPSS version 26, where statistical significance was defined at $p<0.05$.

3. RESULTS AND DISCUSSION

The nutritional values of unripe Saba banana flour are presented in Table 2. The responses received during the promotion of bakery products made of Saba banana flour were overwhelming. Forty-nine (49) respondents who tested all five products and completed the evaluation forms were included in the analysis. The majority of the respondents were female and within the age group of 26 to 35 years old and 46 to 55 years old.

Table 2: Nutritional content of unripe Saba banana flour

Nutrient	Amount (per 100g)
Energy	367 kcal
Carbohydrates	87.7 g
Protein	3.4 g
Fat	0.3 g
Potassium	994 mg

Based on the acceptance test results presented in Figure 1, the products were well accepted by the respondents during the state level's Farmers Day. Most of the respondents (> 80%) scored 4 (like slightly) and 5 (like extremely) on all products except for *bahulu* (< 80%). Only 6.1% respondents scored 2 (dislike slightly) for mini cake and *bahulu* respectively, 2% respondents for biscuit and 4.1% respondents for crepe; while 2% respondent scored 1 (dislike extremely) for crepe product.

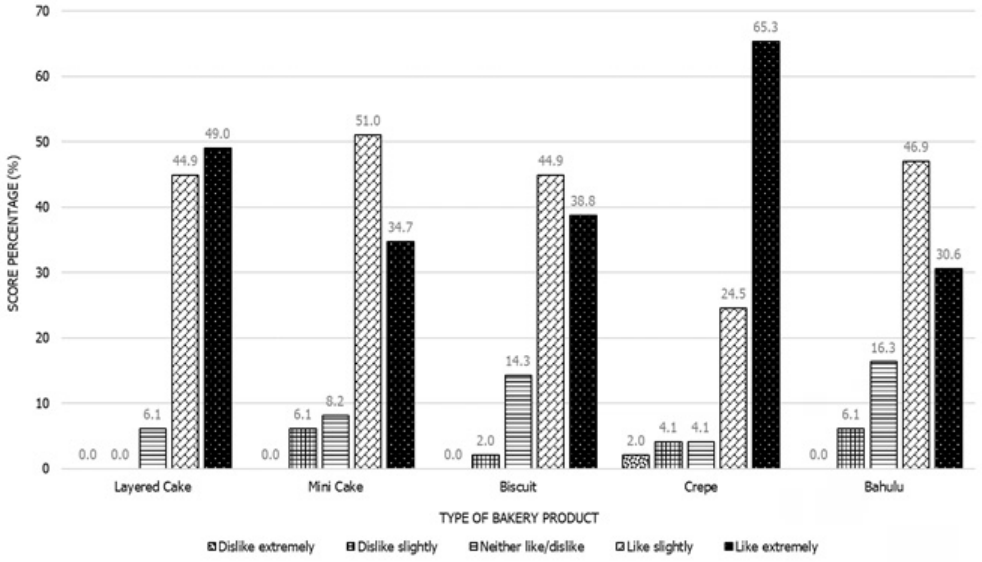

Figure 1: Percentage of consumers' acceptance score for five bakery products made of Saba banana flour (n = 49)

The mean scores for all five bakery products made of 'Saba' banana flour are displayed in Table 3. There was a statistically significant difference in the overall acceptability of the products as determined by one-way ANOVA ($F(4,240) = 2.808$, $p = 0.026$). A Tukey post-hoc test revealed that there is a statistical significance between the preference for crepe and *bahulu*. The respondents favored 'Saba' banana crepe followed by layered cake, biscuit and mini cake, while *bahulu* is the least favored among the five tested products. This was due to the reported dry and brittle texture.

Table 3: . Mean scores for the acceptability of all five bakery products made of Saba banana flour

Type of Product	Mean Score
Crepe	4.47 ± 0.92 [a]
Layered cake	4.43 ± 0.61 [ab]
Biscuit	4.20 ± 0.76 [ab]
Mini cake	4.14 ± 0.82 [ab]
Bahulu	4.02 ± 0.85 [b]

- **Acceptability scores are based on a 5 -point hedonic scale where: 5 = like extremely, 4 = like slightly, 3 = neither like nor dislike, 2 = dislike slightly, 1 = dislike extremely**
- **Value represented the mean ± S.D, where n = 49**
- **Different superscripts indicated significant differences ($p<0.05$) among samples at the 5% by Tukey HSD**

This preliminary acceptability test showed that utilization of unripe 'Saba' banana flour in bakery products create promising value addition that could potentially compete with other wheat-based products in the market. Furthermore, the high content of functional ingredients in unripe bananas can provide health benefits for humans. The data from this project can help in guiding the alternative replacement of wheat flour and diversifying the uses of 'Saba' banana in food product development. This diversification will continue to enhance its utilization and market potential. However, higher number of respondents and detailed sensory evaluation are required in the future to further verify this preliminary findings, and more detailed nutritional analyses on 'Saba' banana flour are required to further understand the health benefits of this product.

CONCLUSIONS

The overall purposes of this study were to develop green (unripe) Saba banana flour and its products, evaluate its nutritional properties and test their acceptance among local consumers. The study indicates that nutritious flour can be produced from green (unripe) Saba bananas and bakery products can be developed utilizing the flour. Additionally, acceptance test results demonstrate good acceptability among consumers. Therefore, there is a potential of incorporating Saba banana flour as an ingredient in bakery products as a healthier alternative. This healthy replacement meets the demand of consumers who seek health and taste in their food consumption.

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BIOLOGY, CULTIVATION, AND PRODUCTION OF GIANT PASSION FRUIT (*PASSIFLORA QUADRANGULARIS* L.)

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ABSTRACT

Passion fruits are widely known for its unique flavour, fruity aroma, and desirable organoleptic properties. It belongs to the family Passifloraceae comprising more than 500 species. There are only two forms of *Passiflora edulis*; i.e., purple and yellow passion fruits which are widely cultivated on a commercial scale. In Malaysia, only less than eight *Passiflora* species were recorded. Out of this, *Passiflora quadrangularis* L. also known as the giant passion fruit, has received rising attention by growers in recent years due to its phytotherapeutic properties and ethnobotanical uses. There is great potential of expanding this species on a commercial scale in Malaysia with a market focus both at national and international levels. However, information on its cultivation, adaptability, and nutritional properties are scarce. Therefore, this paper aims to shed some light on the giant passion fruit. Our research revealed that the giant passion fruits' first flower blooms 6 months after transplanting, followed by fruiting at two months after anthesis. This pattern was similar to the purple passion fruit. Giant passion fruit flowers require a slightly longer period (16.8±0.84 days) to bloom after visible appearance. Flowers of this species started to open early in the morning at 06:52±0.17 hour followed by anthesis at 08:06±0.23 hour and remained open until sunset. Contradictory to purple passion fruit which flowers all year round, this species only exhibits two peaks with minor peaks recorded in January–March and major peaks in September–November. Good fruit yields were observed throughout the year which was attributed to its ability for self-pollination. The production of *P. quadrangularis* which produced bigger fruits, was 18,800.62kg ha-1 (9,585 fruits) with fruit weight ranged from 774.2g–3034.4g.

Keywords: giant passion fruit, mesocarp, *Passiflora quadrangularis*, phenology, yield

FEASIBILITY OF TWIN EXIT APPARATUS (TEA) TO FACILITATE STINGLESS BEE (*HETEROTRIGONA ITAMA*) POLLINATION OF FRUITS UNDER NET-ENCLOSED CULTIVATION

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ABSTRACT

This experiment evaluated twin exit apparatus (TEA) to facilitate the use of stingless bee (*Heterotrigona itama*) pollination to produce fruit grown under plastic roof net house. The stingless bee hive was placed just outside the net house with the TEA attached; with one entrance hole to the inside and an exit hole to the outside. The entrance and exit holes are opened at 10:00 am and closed at 7:00 pm. The TEA innovation has enabled apiculture for sustainable enclosed farming. The stingless bee colony is no longer subjected to high temperatures inside the net house and crop yield is maximized. Closing the exit hole to the outside until 10:00 am directs the bees into the net house for pollination and at latter part of the day for resin and pollen collections from the wild. Our results showed that there was a significant increase in yield when the bees were directed into the net house for pollination. The yield obtained was as good as under open conditions. The TEA method was cheap and simple to apply.

Keywords: stingless bee, *Heterotrigona itama*, enclosed cultivation, pollination, sustainable

PHYSICOCHEMICAL PROPERTIES OF THE TERAP FRUIT (*ARTOCARPUS ODORATISSIMUS* BLANCO)

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ABSTRACT

The genus *Artocarpus* belongs to the family of Moraceae with *Artocarpus altilis* (Breadfruit), *Artocarpus integer* (Chempedak) and *Artocarpus heterophyllus* (Jackfruit) being the common species in this genus. *Artocarpus odoratissimus* also known as Terap in Sarawak or Marang in English is found to be indigenous to the Borneo Island. This fruit is presently also being introduced to other Southeast Asian countries such as Philippines and Thailand. Terap is well-known for its sweet tasty flesh which is eaten raw and the seeds are normally steamed and used in some local dishes. The fruits are highly valued as a potential food source for local communities. However, their nutritional attributes and phytochemical properties are not fully explored particularly in Sarawak, Malaysia. Thus, the present study aims to evaluate the nutritional compositions and phytochemical properties of the *A. odoratissimus* fruit from Sarawak. Proximate analysis results obtained showed that the flesh had 75.90±0.75% moisture, 82.70±0.36% carbohydrate, 14.59±1.49% protein, 3.87±0.42% ash and 0.03±0.01% crude fiber. Terap fruit also possessed good mineral compositions with the trend of K > Ca > Na > Mg > P > Cu. Potassium was the major mineral component of the fruit ranging between 1237.16-1654.17 mg/100g. Terap is the sweetest fruit among all other *Artocarpus* species as fructose was found to be the most abundant sugar in the flesh (26.7±0.70 g/100 g) followed by glucose (25.38±0.45 g/100 g) and least amount being sucrose (4.38±0.21 g/100 g). Terap flesh was found to be an excellent source of vitamin B complex, with thiamine content (11.07±0.31 mg/100 g) being the highest. For the phytochemical attributes in Terap flesh, total phenolic content was 2.11-2.70 mg GAE/g, total flavonoid was 0.75-1.44 mg QUE/g and total antioxidant activity was 187.25-189.48 mg/mL. The present study supports the ethno-botanical uses of Terap by the local communities and the study sheds initial evidence on the potential for this fruit to be further explored and developed by nutraceutical and pharmaceutical industries, leading to enhancing the downstream application and product development of this indigenous fruit crop.

Keywords: Antioxidant, *Artocarpus odoratissimus*, indigenous, terap, marang

1. INTRODUCTION

Borneo is recognized as a land of diversity and rich heritage of flora as it is the widest island in the world. Sarawak, Malaysia, one of the parts in Borneo Island, is enriched with bountiful biodiversity consisted of approximately 3000 plant species (Soepadmo, 1995; Soepadmo & Saw, 2000). This tropical rainforest is abundant in diversity of beneficial indigenous plants and some of them are endemic species to the state of Sarawak. There are about 200 species are reported as endemic plants.

Generally, indigenous plant are fruits and vegetables that originate and naturally grow on the land or were introduced a long time ago from one place to another through natural processes or

human domestication (Abd Rahman, 2018). Indigenous plants have been exploited traditionally by various ethnics and indigenous people around the world. The knowledge on indigenous plants has been transferred over generations by the elders verbally thus local people gain benefits from its uses (Runi *et al.*, 2018). Local populace especially those living in the rural area highly depend on the indigenous plants to serve as good sources of nutrition, energy, minerals and medicine. Through exchanges of ethno-botanical knowledge and information among the communities in Sarawak, locals and tourists alike can now enjoy the goodness of many of these plants (Runi *et al.*, 2018).

Among these indigenous species, *Artocarpus odoratissimus* Blanco is one of the indigenous fruit species that is popular for its odour, hence gaining visibility in the local fruit industry. There have been increasing efforts made by several researchers to explore the potential of this fruit. *Artocarpus odoratissimus* species belongs to the genus *Artocarpus* from the Moraceae family. The synonyms of *A. odoratissimus* are *Artocarpus tarap* Becc., and *Artocarpus mutabillis* Becc. This fruit is known as Marang as its English name or Terap by the local communities in Sarawak and has various common names according to different regions and localities including *terap* (Malaysia), *pi-ien* (Bidayuh), *pingan* (Iban), and *keiran* (Kelabit). In other countries, the fruit is known as and *marang* (Sulu), *madang* (Lanao), *loloi* (Tagalog), and *khanun sampalor* (Thailand) (Subhadrabandhu, 2001). It is an indigenous crop to the Borneo Island states of Sabah and Sarawak (Malaysia), Kalimantan (Indonesia), and Brunei. Indigenous crops are defined as crops that are endemic and locally grown in a specific region (Haq, 2006). The plant is endemic to Borneo Island and also being cultivated at other countries such as Philippines (Soepadmo, 1995).

Artocarpus odoratissimus is an evergreen tree that is wild crafted and can be found in secondary forests. This mid-canopy tree can grow up to 40 m tall and has a diameter of 40-45 cm low buttresses trunk. The bark produces white sticky latex. The twigs are long, and has yellowish to reddish colour hair with 4-10 mm thickness (Lim, 2012). The fruit is large, with a length averaging about 16-17 cm, a diameter of approximately 13 cm, weighing almost 1 kg. The young fruit is green and turns yellowish brown as it ripens. The fruit shape is roundish oblong, covered with short, brittle spines. The flesh is white coloured, and has a strong aromatic smell with juicy flesh (Coronel, 1998; Lim 2012). The middle core of this fruit has flesh clinging on it, containing seed sized at 8 mm x 15 mm (Galang, 1955; Lim, 2012).

This entire tree and its parts have been found to be useful to the local consumers. The ripen fruit flesh are juicy, has a strong odour and aromatic taste which is suitable to be eaten raw (Tang *et al.*, 2013). Besides, the seeds of *A. odoratissimus* have a nutty flavour and can be consumed after it is roasted and boiled to soften the texture (Lim, 2012). Apart from the fruit, the roots are boiled to extract the water which is believed by the Ibans in Sarawak, Malaysia to cure diarrhea, while the leaves can treat venomous stings of centipedes and scorpions (Lim, 2012). These ethno-botanical features of *A. odoratissimus* as well as the nutritional values of this fruit have induced the interest to study this valuable fruit.

Due to the increasing demand for the fruit of *A. odoratissimus*, the state government of Sarawak, Malaysia believes that the fruit has great potential for commercialization. This is in line with the State's mission in popularizing indigenous crops for commercial cultivation, while tapping into the tremendous scope for accessing national and international markets. However, there has been limited documentation on the nutritional and phytochemical investigations of *A. odoratissimus* fruit particularly in Sarawak. Despite the potential importance of *Artocarpus odoratissimus* especially in its nutritional and pharmaceutical values, documented details on the nutrient and

phytochemical attributes are still lacking. Therefore, the objective of this study is to evaluate the nutritional compositions and phytochemical properties of the flesh of *A. odoratissimus*.

2. MATERIALS AND METHODS

2.1. Survey and sampling collection

The fruits of *A. odoratissimus* were collected and bought at the local market Pasar Utama Bintulu (3.1705° N, 113.0405° E), Bintulu, Sarawak, Malaysia. The fruits were collected from the months of June 2018 to January 2019. The chosen fruits were healthy and free from diseases and pests. The fruits were brought to the laboratory and immediately inspected and cleaned with distilled water to remove the debris.

2.2. Sample preparation

The fruits were cleaned and the flesh, seed, skin, and pedicel parts were separated. All the separated parts were divided into three divisions. Firstly, fresh samples were used directly for the determination of moisture content. Secondly, the oven dried samples were dried at 50°C until content weight were obtained and homogenized for analyses of proximate and mineral composition. The freeze dried samples were used for analysis of phytochemical compositions. All the processed samples were kept in an air-tight container prior to analyses.

2.3. Proximate composition analysis

The moisture content was determined by the method of oven drying based on AOAC (2000) official method 934.06. Samples weighing 2.0 g were cut into smaller pieces and spread evenly across a pre-weighed drying dish. Flesh samples were dried to a constant mass at 50°C. Ash content was determined by incinerating air dried samples in muffle furnace (method 930.05, AOAC, 2000). Crude fiber was determined in accordance to the (AOAC, 2000) method 993.19 by using Fibertec Hot Extraction Unit (FOSS Fibertec 2010 Hot Extractor). Crude protein was determined by the Kjeldahl method following the AOAC (2000) method 955.04. Soxhlet extraction was done via the Foss Tecator Soxtec 2055 Manual Extraction Unit for the determination of crude fat of *A. odoratissimus* based on the AOAC official method 920.39 Available carbohydrate was calculated according to (James, 1995) using the subtracting method. The calculation was performed based on the following formula:

Carbohydrate (%) = 100% – [Ash (%) + Crude protein (%) + Crude fat (%) + Crude fiber (%)

2.4. Mineral content analysis

Th mineral contents of *A. odoratissimus* in fruit parts for Potassium (K), Calcium (Ca), Sodium (Na), Magnesium (Mg), Phosphorus (P), Copper (Cu) and Ferum (Fe) were determined using atomic absorption spectrum (AAS) (AA800 Perkin-Elmer, Germany) according to the AOAC method 975.03 (AOAC, 1990).

Ash was used to extract the minerals. Two milliliters of concentrated HCl was added and left to evaporate on hot plate in a fume chamber. After the samples were totally evaporated, 10 mL of 20% HNO_3 was added and evaporated until the volume became half. The sample solutions were

then filtered using Whatman Paper No. 2 and marked up to 100 mL with distilled water. The extracted sample solutions were used for the analyses.

2.5. Vitamin analysis

The vitamin content in the flesh of *A. odoratissimus* fruit for vitamins A, B1 (thiamin), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folicacid), B12 (cyanocobalamin), D, E and K was determined according to the AOAC method 974.29 (AOAC, 2000). Vitamin C in the flesh was determined according to AOAC method 967.21. The flesh samples were dried at 45°C and grounded prior to analysis. Ascorbic acid was determined by the Indophenol titration method with a Vitamin C solution of known concentration as a standard. The samples were sent to the BP Food and Environment Industrial Testing Laboratory, Malaysia for determination of the other vitamin content.

2.6. Sugar analysis

The glucose, fructose and sucrose contents in A.odoratissimus flesh were determined using HPLC system according to AOAC method 923.09 (AOAC, 2000). The samples were dried at 45°C and grounded prior to analysis. The samples were sent to the BP Food and Environment Industrial Testing Laboratory, Malaysia for determination of the sugar content. The mobile phase for the determination of sugar content was 75% acetonitrile (HPLC grade) and 25% ultra-pure water. The sugar content was identified by comparing the retention time of standard and samples and further quantified by peak area measurement using software.

2.7. Phytochemical analysis

2.7.1. Determination of total phenolic content (TPC)

The total phenolic content in dried extracts of methanol obtained from freeze dried samples was determined spectrophotometrically using the Folin-Ciocalteu's reagent (Shukla *et al*., 2012). 1 mL methanolic solution of extract (1 mg/mL) or standard solution of gallic acid (10, 20, 40, 60, 80, 100 µg/mL) was added to 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in distilled water and incubated for 5 min. After the 5 min incubation, 2 mL of 7.5% Na_2CO_3 solution was added to the mixture, incubated in dark for 30 min at 24°C, and the absorbance was measured at 765 nm with an UV-VIS spectrophotometer. A Blank reagent containing 1 mL methanol, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2 mL of 7.5% Na_2CO_3 solution was also prepared. The results were calculated using the calibration curve of gallic acid and the total phenolic content was expressed as milligrams of gallic acid equivalents per gram extract (mg GAE/g dried extract).

2.7.2. Determination of total flavonoid content (TFC)

The total flavonoid content was also determined spectrophotometrically at 510 nm with slight modifications (Zhishen *et al.*, 1999). 1 mL of methanolic solution of extract (1 mg/mL) or standard solutions of quercetin (10, 20, 40, 60, 80, 100 µg/mL) was added into 0.5 mL of 5% $NaNO_2$ solution and 0.5 mL of 10% $AlCl_3$ solution. After 5 min, 2 mL of 4% NaOH solution was added and incubated for 15 min at room temperature and the absorbance was measured against the blank at 510 nm with an UV-VIS spectrophotometer. A Blank reagent was prepared by adding the entire reagent without adding sample or standard. A calibration curve was constructed using

standard quercetin and the total flavonoid content was expressed as milligrams of quercetin equivalents per gram extract (mg QE/g dried extract).

2.7.3. Total antioxidant activity

- Ferric Reducing/Antioxidant Power Assay (FRAP)

  The FRAP assay was performed according to (Benzie & Strain, 1999) with slight modifications. The working FRAP reagent consisted of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, 20 mM $FeCl_3$.6H2O and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio prior to use in water bath at 37°C. 100 µL of extract solution was mixed with 900 µL of FRAP reagent. The mixture was left to stand for an incubation period of 4 minutes at 37°C. After that, the reading of absorbance was taken spectrophotometrically at 593 nm against the blank. The calibration standard curve was determined using Trolox with standard series from 1 to 4 µg and the volume was made up to 300 µL with distilled water. FRAP values were calculated and expressed as milligrams of Trolox equivalent per gram extract (mg TE/g).

- Free - radical scavenging assay (DPPH)

  The scavenging acidity of the *A. odoratissimus* fruit extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical assay. Scavenging activity on DPPH was assessed according to the method reported by (Shukla *et al.*, 2012) with a slight modification. 1 mL methanolic solution of each extracts (20, 25, 50, 100, 150, 200 µg/mL) was added to 3 mL working solution of DPPH (0.1 mM DPPH in methanol) and incubated in the dark. After 30 min, the absorbance of all extracts were read at 517 nm with an UV-VIS spectrophotometer and compared to that of standard Trolox with similar concentrations. 1 mL of methanol with 3 mL working solution of DPPH served as blank. The % inhibition was calculated as:

  Inhibition = [(Absorbance of blank − Absorbance of sample or standard after 30 min) / (Absorbance of blank after 30 min)] x 100

  The EC50 value in methanol extracts of freeze dried samples and standard Trolox were calculated by plotting a graph of % inhibition versus concentration.

3. RESULTS AND DISCUSSION

3.1. Proximate content of *Artocarpus odoratissimus* flesh

Fruits have been a part of human diets over the years. They contain high water content, carbohydrates, sugars, vitamins, minerals and organic acids which are required by the body to function well (Onibon *et al.*, 2007). Apart from commercial fruits, indigenous fruits also possess ample nutrition which is useful for local consumers especially those from rural areas. The fruit of *A. odoratissimus* has always been used for its juicy flesh. The results of proximate compositions of *A. odoratissimus* fruits are presented in Table 1.

The proximate content of flesh was categorically represented as carbohydrate > moisture > crude protein > ash > crude fat > crude fibre. The present moisture content of flesh was slightly higher than the reported values (67.5-73.4%) by (Tang *et al.*, 2013) in Brunei. Relatively higher moisture content is important in fruits for their stability and quality. The flesh contained

14.59±0.19% of protein, indicating that this fruit can be an alternative source of protein for rural communities. The present protein values in flesh were relatively higher than the previous observation by (Tang *et al.*, 2013) with values of 1.31-1.51%.

For the flesh, the crude fat content in the present study was similar to the crude fat in *A. heterophyllus* (0.2%). Whereas flesh of *A. champeden* (2.4-3.5%) possessed higher crude fat content as compared to flesh of *A. odoratissimus* in this study. The amount of fats present in the flesh and seeds of *A. odoratissimus* is available for the use of the body to generate body cells, regulate normal body temperature and aid the body system in the absorption of vitamin A, D, E and K.

The present fiber content in the flesh was relatively lower (0.03%) than the reported values (0.9-1.13%) (Tang *et al.*, 2013). Crude fiber in the flesh of *A. champeden* (4.6-7.6%) and *A. heterophyllus* (5.6%) were relatively higher compared to flesh of *A. odoratissimus* as recorded in previous studies (0.90-1.13%) and the present (0.03±0.01%) study. Additionally, higher carbohydrate content was recorded in the flesh at the ranges of 82.27-83.41%. The carbohydrate content in the flesh was relatively higher compared to previous studies (12.0-25.2%) in *A. odoratissimus* and other *Artocarpus* species including *A. champeden* (16.2-28.3%) and *A. heterophyllus* (9.4-25.4%). The higher carbohydrate in the flesh shows that this fruit contains higher sugar compostion and can potentially serve as an energy source for consumers.

Table 1. Proximate constituents (%) of Artocarpus odoratissimus flesh and comparison with other commercial *Artocarpus* species

Species	Moisture	Ash	Crude protein	Crude fat	Crude fibre	Carbohydrate	Trend
A. odoratissimus	75.90±0.75 (74.65-77.23)	3.87±0.42 (3.03-4.30)	14.59±1.49 (13.13-17.51)	0.26±0.09 (0.10-0.40)	0.03±0.01 (0.02-0.04)	82.70±0.36 (82.27-83.41)	C > M > P > A > F > Fi
*A. odoratissimus**	67.9-73.4	0.6-0.8	1.31-1.51	-	0.90-1.13	12.0-25.2	M > C > P > Fi > A
*A. champeden***	62.3-73.4	2.5-3.9	4.9-5.8	2.4-3.5	4.6-7.6	16.2-28.3	M > C > Fi > P > A> F
*A. heterophyllus****	83	2.2	1.6	0.2	5.6	9.4-25.4	M > C > Fi >A > P > F

*(Tang *et al.*, 2013), **(Lim *et al.*, 2011), ***(Janick & Paull, 2008)

3.2. Minerals composition of *Artocarpus odoratissimus* flesh

Minerals are important constituents in the determination of nutritional value in the fruit. The value of macronutrients and micronutrients of the flesh are presented in Table 2. The edible portion of *A. odoratissimus* which is the flesh contains a relatively higher value of ash (3.03-4.30%). The ash content of the flesh confirms that it contains high mineral values. The *A. odoratissimus* fruits possess appreciable quantities of K, P, Na, Ca, Mg, and Cu which are useful for human health. The trend of nutrients in the flesh of *A. odoratissimus* was K > Ca > Na > Mg > P > Cu. However, the trend of the elements may vary even in similar *Artocarpus* species. *Artocarpus champeden* and *A. heterophyllus* were reported to have lower Na content than Ca in flesh (Lim *et al.*, 2011; Tiwari & Vidyarthi, 2015; Sy Mohamad *et al.*, 2019). The species composition and locality may also have effects on the nutritional attributes of the plant (Wardlaw, 2003).

As in many other fruits, K is the most abundant mineral in the A. odoratissimus fruit parts. In the edible portion of the fruit, the flesh contains high concentrations of K with 1210.40±28.00 mg/100 g. The K concentration in the present study was six times higher for the flesh than

the reported value for flesh (176-298 mg/100 g) by (Tang, 2013). This higher K content in *A. odoratissimus* fruit can potentially serve as a significant source of K which is vital in controlling blood pressure and body fluid balance (Wardlaw, 2003). K also is relatively important for heart health. Numerous studies have also reported the potential of K in reduction of blood pressure, thus mitigating the risks of other non-communicable diseases including hypertension, cardiovascular diseases and stroke (Whelton, 2014).

The second most abundant mineral content in *A. odoratissimus* fruit is Ca (916.81±23.70 mg/100 g). The fruit can provide appreciable amount of Ca content which is important for bone and teeth formation and strength, muscle contraction and blood clotting (Hadji, 2015). The Mg content in *A. odoratissimus* was found to be 150.93±2.78 mg/100 g. The values of Mg obtained from the present study for the flesh were comparatively higher than the reported study by (Tang *et al.*, 2013)which was 14.8-31.3 mg/100 g. Mg is an essential constituent for building a healthy diet for the body. The importance of Mg includes regulating optimum biochemical activity in human bodies and is required as a co-factor in enzymatic reactions (Schwalfenberg & Genuis, 2017).

The P level was recorded low in the fruit flesh. Phosphorus is known to be an essential element for the metabolic process and healthy bone development (Wardlaw, 2003; Penido & Ulon, 2012). The Na value obtained for the flesh was 182.33±15.53 mg/100 g. An adequate intake of Na is vital for cellular homeostasis and balancing body fluids (Maron *et al.*, 2015).

Cu was found in trace quantity in all parts of the *A. odoratissimus* fruit. The present study shows that Cu in the flesh was 0.89±0.03 mg/100 g. The concentrations of Cu in *A. odoratissimus* fruits were found to be within the limits of the recommended maximum level allowed in food based on the Malaysian Food Regulations (Akta Makanan, 1985) limit of 4.00 mg/ 100 g. No traces of Fe were found in the flesh of the fruit.

Table 2. Mineral composition (mg/100 g) of Artocarpus odoratissimus flesh

Mineral composition (mg/100 g)						References
K	P	Na	Ca	Mg	Cu	
1210.40±28.00 (1237.16-1654.17)	98.12±2.51 (93.15-101.15)	182.33±15.53 (68.46-184.91)	916.81±23.70 (586.80-1156.66)	150.93±2.78 (107.58-184.91)	0.89±0.03 (0.63-1.00)	Present study
176-298	-	1.15-1.70	0.48-1.35	14.8-31.3	0.39-0.59	[11]

3.3. Sugar composition and content in flesh of *Artocarpus odoratissimus*

Basically *A. odoratissimus* is the sweetest fruit of the *Artocarpus* genus. The sweet juicy flesh is a favourite for many people in Borneo Island. The flesh also consists of higher carbohydrate content (82.70±0.36%) which is related to higher sugar composition. The major source of energy comes from carbohydrate which can be further categorized into three main sugars. The three main sugars are sucrose, glucose and fructose which were all found to be the prime constituents in the flesh of *A. odoratissimus*. The sugar content of *A. odoratissimus* is presented in Table 3 and Figure 1. Quantitative measurements of sugar content influences total sweetness. In the sweetness category, fructose has been reported to be at least 1.73 times sweeter than sucrose (Hanover & White, 1993). From the analysis of the study, fructose was found to be the most abundant sugar composition in the flesh with a range of 26.7±0.70%. The fructose composition in this study was comparatively higher than reported values (6.9-13.7%) by (Tang *et al.*, 2013). The flesh consisted of higher fructose than the *A. heterophyllus* flesh which was 4.53% (Chowdhury *et al.*, 1997).

Fructose comprised the larger portion of total sugars ranging from 26.0-27.4 g /100 g followed by glucose ranging from 25.4-26.3 g/100 g. A positive correlation was observed for both fructose and glucose values.The fructose and glucose composition in *A. odoratissimus* flesh ranged ~47% and ~45% respectively while sucrose consisted of 8% of total sugars (Figure 1). Sugar compositions in other *Artocarpus* species varied from *A. odoratissimus*. A similar composition of sugar content was recorded in *A. heterophyllus*. On the contrary, the trend of sugar compositions in *A. champeden* showed that it possessed higher sucrose than the fructose and glucose contents. As for non-reducing sugar composition (sucrose), the level of sucrose in *A. odoratissimus* (4.38±0.21 g/100) was lower than in *A. champeden* (20.02±1.88 g/100 g). The trend of sugar content in *A. odoratissimus* for the present study was fructose > glucose > sucrose. A similar trend was reported for *A. odoratissimus* fruit sugar content by (Shahrir *et al.*, 2013) in Sarawak. Generally, the glucose/fructose ratio is the key indicator for determining the palatability of the fruit flesh (Kelebek *et al.*, 2011). In the *A. odoratissimus* fruits studied, the measured glucose/fructose ratio was 0.95 and this is in line with the reported ratio by (Tang *et al.*, 2013) in Brunei. This value is important to determine the correct amount of sugar accepted by our body whereby excess of fructose may cause fructose malabsorption.

Table 3. Sugar content (g/100 g) in flesh of *Artocarpus odoratissimus* and comparison with other commercial Artocarpus species

Species	Non-reducing Sucrose	Reducing		G/F ratio	Total sugar
		Glucose	Fructose		
A. odoratissimus	4.38±0.21 (4.60-5.00)	25.38±0.45 (25.40-26.30)	26.7±0.70 (26.00-27.40)	0.95	57.35±1.91 (56.00-58.70)
A. odoratissimus [11]	0.3-11.2	5.8-13.7	6.9-13.7	0.95	13.00-38.6
A. champeden [28]	20.02±1.88	5.52±0.46	6.12±0.28	0.90	31.66±2.48
A. heterophyllus [25]	1.49	2.06	4.53	0.45	8.08

Values are expressed as mean ± standard deviation and values in bracket are the range.

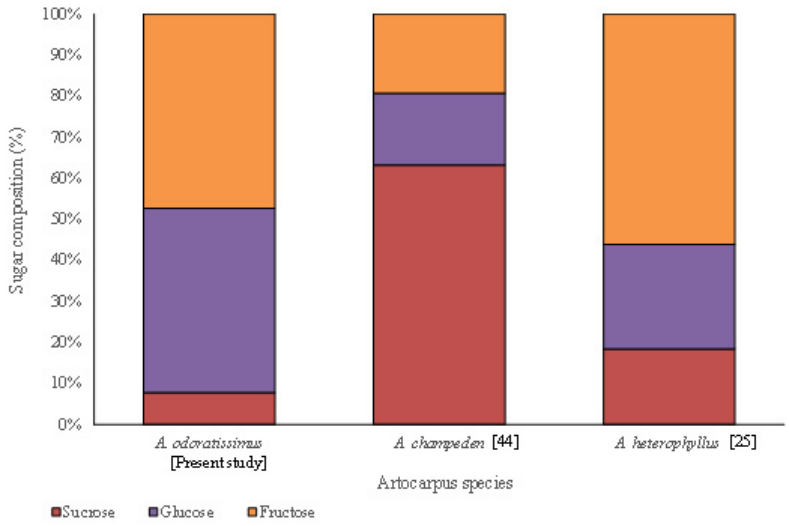

Figure 1. Sugar composition (%) in *Artocarpus* species

3.3. Vitamin composition in flesh of *Artocarpus odoratissimus*

The flesh of *Artocarpus odoratissimus* is an excellent vitamin source to the human diet especially in supplying the human body with B vitamins. This study shows that the flesh of *A. odoratissimus* consist predominantly of vitamin B1 (Thiamin) (11.07±0.31 mg/100 g), followed by vitamin B3

(Niacin) (0.93±0.06 mg/100 g), vitamin B9 (Folic acid) (0.50±0.00 mg/100 g) and the least amount of vitamin B2 (Riboflavin) (0.27±0.06 mg/100 g) and vitamin C (0.33±0.06 mg/100 g). This trend and composition were different from other *Artocarpus* species where higher vitamin C has been recorded in *A. champeden* (90.33 mg/ 100 g) (Abdullah *et al*., 2013) and *A. heterophyllus* which is rich in Vitamin A (175-540 mg/100 g) (Arora & Parle, 2016). As for other vitamins analysed such as vitamin A, B5, B6, B7, B12, D, E, and K, the values were not detected in the flesh of *A. odoratissimus*. This is the first documentation of vitamin composition in *A. odoratissimus* flesh. The variation of vitamin content may be due to the flesh colour and composition of the *Artocarpus* species fruit. Basically the flesh of *A. champeden* and *A. heterophyllus* are yellow while *A. odoratissimus* is white.

Table 4. Comparison of vitamin contents (mg/100 g) in the flesh of Artocarpus odoratissimus to other commercial Artocarpus species

Vitamins	*Artocarpus odoratissimus* (Present study)	*Artocarpus champeden**	*Artocarpus heterophyllus***
Vitamin A	n.d.	-	175-540
Vitamin B1 (Thiamin)	11.07±0.31 (10.80-11.40)	-	0.03-0.09
Vitamin B2 (Riboflavin)	0.27±0.06 (0.20-0.30)	-	133
Vitamin B3 (Niacin)	0.93±0.06 (0.90-1.00)	-	-
Vitamin B9 (Folic acid)	0.50±0.01 (0.50-0.52)	-	-
Vitamin C	0.33±0.06 (0.30-0.40)	90.33 ± 28.01	7.0-10.0

Values are expressed as mean ± standard deviation and values in bracket are the range
***Abdullah *et al*., 2013; **Tiwari & Vidyarthi, 2015; Arora & Parle, 2016**

3.4. Phytochemical properties of different parts of the Artocarpus odoratissimus fruit

3.4.1. Total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolic content of *A. odoratissimus* was determined by the reaction of oxidation-reduction using Folic-Ciocalteu reagent. The values of TPC were expressed in milligrams gallic acid equivalent to 1 gram dried sample (mg GAE/ g). The TPC of the fruit was 2.35±0.18 mg GAE/ g. The previous study by (Bakar *et al*., 2015) reported that a higher value of TPC was found in the skin (42.38±0.20 mg GAE/ g) and the least value of TPC was found in the flesh (3.53±0.33 mg GAE/ g). The results of both studies support that the A. odoratissimus fruit is rich in phenolics that might contribute to high antioxidant activities (Jagtap & Bapat, 2010). Thus, as the fruits reach maturity stage, the amount phenolic also increases (Duenas *et al*., 2009).

TFC content observed in the flesh of the fruit was 1.14±0.20 mg QUE/g. The TFC content in the skin part was reported by (Bakar *et al*., 2015) to be 36.78±0.28 mg GAE/ g) which is higher than in the flesh (1.23±0.09 mg GAE/g). Bakar *et al.* (2009) reported that bambangan also possessed a high phytochemical content in the skin and seed compared to the flesh part. Soong and Barlow (2004) also stated that the by-product of *A. heterophyllus* (jackfruit) such as the skin and seed showed higher phytochemical contents compared to the flesh of the fruit. Flavonoids have been reported for their effectiveness against cancer, and the ability to act as cardio protective agents, antioxidants, possess antibacterial properties, and protect the skin from UV radiation. It also has a great potential for application in pharmaceutical and medical industries (Ahmed *et al*.; Andreu *et al*., 2018; Meng *et al*., 2018).

3.4.2. Ferric Reducing/Antioxidant Power Assay (FRAP) properties

The FRAP value in the flesh was 40.84±0.12 µM TE/g. The trend of the previous study by Bakar *et*

al. (2015) report that the skin had higher FRAP properties which is 378.93±20.25 µM/g, followed by the seed (68.06±2.93 µM/g) and the flesh (17.92±0.74 µM/g).

3.4.3. DPPH properties

DPPH assay is used to analyse the potential of *A. odoratissimus* fruit extracts to scavenge free radicals. The antioxidant activity of fruit extracts of *A. odoratissimus* is presented in Table 5. The free-radical scavenging activity was measured through conversion of DPPH into stable DPPH-H formation that occur after the hydrogen radical or electron was accepted (Hatano, 1995). The present study shows that the sample extracts generated antioxidant colour from pale purple to dark purple due to the ability of hydrogen to donate electron. The present result indicated that A. odoratissimus fruit possess free radical scavenging activity. A lower EC50 value shows that the fruit extracts have greater antioxidant activity. The results of the present study are in line with the reported study.

Table 5. TPC, TFC, FRAP and DPPH properties of Artocarpus odoratissimus flesh

TPC (mg GAE/g)	TFC (mg QUE/g)	FRAP(µm TE/g)	DPPH (mg/mL)	References
2.35±0.18 (2.11-2.70)	1.14±0.20 (0.75-1.44)	40.84±0.12 (40.66-40.79)	188.48±0.65 (187.25-189.48)	Present study
3.53±0.33	1.23±0.09	17.92±0.74	-	[30]

Values are expressed as mean ± standard error (n=4) and values in bracket are the range

4. CONCLUSIONS

Artocarpus odoratissimus is an economically important indigenous crop that highly possesses nutritional and medicinal benefits. The demand for this fruit among consumers has increased largely due to its excellent and aromatic flavor and various ethnobotanical attributes. The increase in demand opens up opportunities for commercialization and is in line with the Sarawak state's mission in popularizing indigenous crops for commercial cultivation while tapping into the tremendous scope for access into national and international markets. The findings show that the fruit exhibited good proximate compositions. The flesh (82.70±0.36%) of *A. odoratissimus* contained high carbohydrate in line with a high total soluble solid value of 18.13°Brix. *Artocarpus odoratissimus* contained good mineral constituents with a varied trend of minerals according to the fruit parts. The trend was K > Ca > Na > Mg > P > Cu. Potassium was the major mineral component found in the fruit (905.61±18.89 mg/100g). The flesh of *A. odoratissimus* contained the highest level of sweetness as compared to other *Artocarpus* species. There were three main sugars observed to be present in this fruit which were fructose, glucose and sucrose with the total sugar content of 57.35±1.91 g/100 g. The flesh predominantly consist of reducing sugars with fructose at 26.7±0.7 g/100 gfollowed by glucose (25.38±0.45 g/100 g), while sucrose was the lowest amount of non-reducing sugars4.38±0.21 g/100 g. The flesh was also rich in vitamin B complex particularly vitamin B1, thiamine (11.07±0.31 mg/100 g).

From a broader perspective, the information gathered in this study highlights the potential uses of indigenous fruit crops such as Terap in food and health sectors due to its nutritional and phytochemical attributes. In future, more studies and research can be conducted to provide detailed information and documentation on the phytochemicals and physicochemicals of *A. odoratissimus*. Therefore, this could enhance the downstream application or product development of the *A. odoratissimus* fruit.

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PANEL DISCUSSION

The term Agriculture 4.0 has been bandied around by policymakers, researchers and technology prospectors in seeking ways to utilize the ubiquitous availability of communication networks and digital devices to improve productivity and marketability of agricultural produce.

Recent digital and communications advancements have been very exciting with digital instrumentation permeating in every aspect of everyday life. Hand held devices are ubiquitous and communication protocols have developed exponentially with 5G touted as the most advanced technology nowadays. With these advancements come applications that capitalize on massive unorganized information such as big data analytics, artificial intelligence, internet of things and robotics which are then conveniently amalgamated into terms such as digitalization and Industry 4.0. Following this the equivalent term Agriculture 4.0 was coined to describe digitalization of agriculture, and this seems to be aggressively pursued by Governments in the form of policies to 'modernize' agriculture. There are various degrees of adoption for all these technologies, depending on the financial status, technical competencies and availability of all the various technologies. Smart Agriculture, Agri-technology, Precision farming, geospatial applications and use of GPS and sensors have been around for quite some time and have been adopted by companies or individuals who can afford and use them to improve agricultural productivity. Examples of recently popular technologies are those utilizing geospatial applications, new ledger distribution applications using blockchain technology and the use of drones are becoming more popular, while for some, the use of hand held devices are common.

This topic was discussed in a panel discussion during the International Tropical Fruits Network's (TFNet) International Symposium on Tropical Fruits (ISTF 2019) in Ho Chi Minh City, Vietnam from 24 – 26 September 2019. All the panellists agreed that Agriculture 4.0 or the digitalization of agriculture is at an early stage of development and its adoption depends on the socio-economic status, cultural values and immediate needs of the adopters. It was also agreed that eventually, disruptive technologies will be inevitable and have to be adopted in some way or another, however its affordability and selected usage are limited to the digital advancement and socio-economic development status of countries concerned. This is in view of the challenges faced when dealing with smallholders including small land holdings, practice of mixed farming, level of literacy and inefficient production, that would have an impact in advancing digital agriculture.

Noting the fact that most of tropical fruit producers are smallholders, is tropical fruit production ready for Agriculture 4.0?

On the plus side, it was pointed out that with hand held devices such as mobile phones, younger smallholders are more receptive to new technologies, especially with the increasing popularity of online marketing. Currently online information on latest technologies and information on prices and market are readily available. With improved and expanding telecommunications coverage areas, mobile phones are effective tools that can be utilized in enhancing agricultural practices.

It is also apparent that Agriculture 4.0 is easily adopted by multinationals or companies that can afford the technologies to improve efficiency of production, improve quality and marketability, while smallholders are still stuck in the quagmire of low productivity, lack of financial support, poor market access and therefore earn low incomes. The higher initial costs of new technologies are a big concern for smallholders.

The panellists concurred that Governments would have to look at the target crop types to be developed based on economic and export values, and initiate selected digital applications for both the commercial players and smallholders to adopt. Another approach is to focus and prioritize components of the value chain that require improvement. Smallholders also need to be categorized according to their adoption response to new technologies. For instance, some smallholders can be trained to familiarize themselves with applications to identify and control pests and diseases using hand held devices. This also includes implementation of popular modern technologies, such as, the use of devices to measure produce maturity and ripeness, computer controlled fertilizer applications, soil moisture sensors and use of drones.

The role of Governments in drawing up policies and private sector to encourage the use of new technologies, including training, is imperative to get the smallholders on board. Sharing of information and cooperation on affordable and applicable technologies among countries, the private sector, and international organizations are also precursors for the introduction of Agriculture 4.0 in tropical fruits. International Tropical Fruits Network supports the various initiatives in the use of innovative and disruptive technologies to improve agriculture, particularly the tropical fruit industry.

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