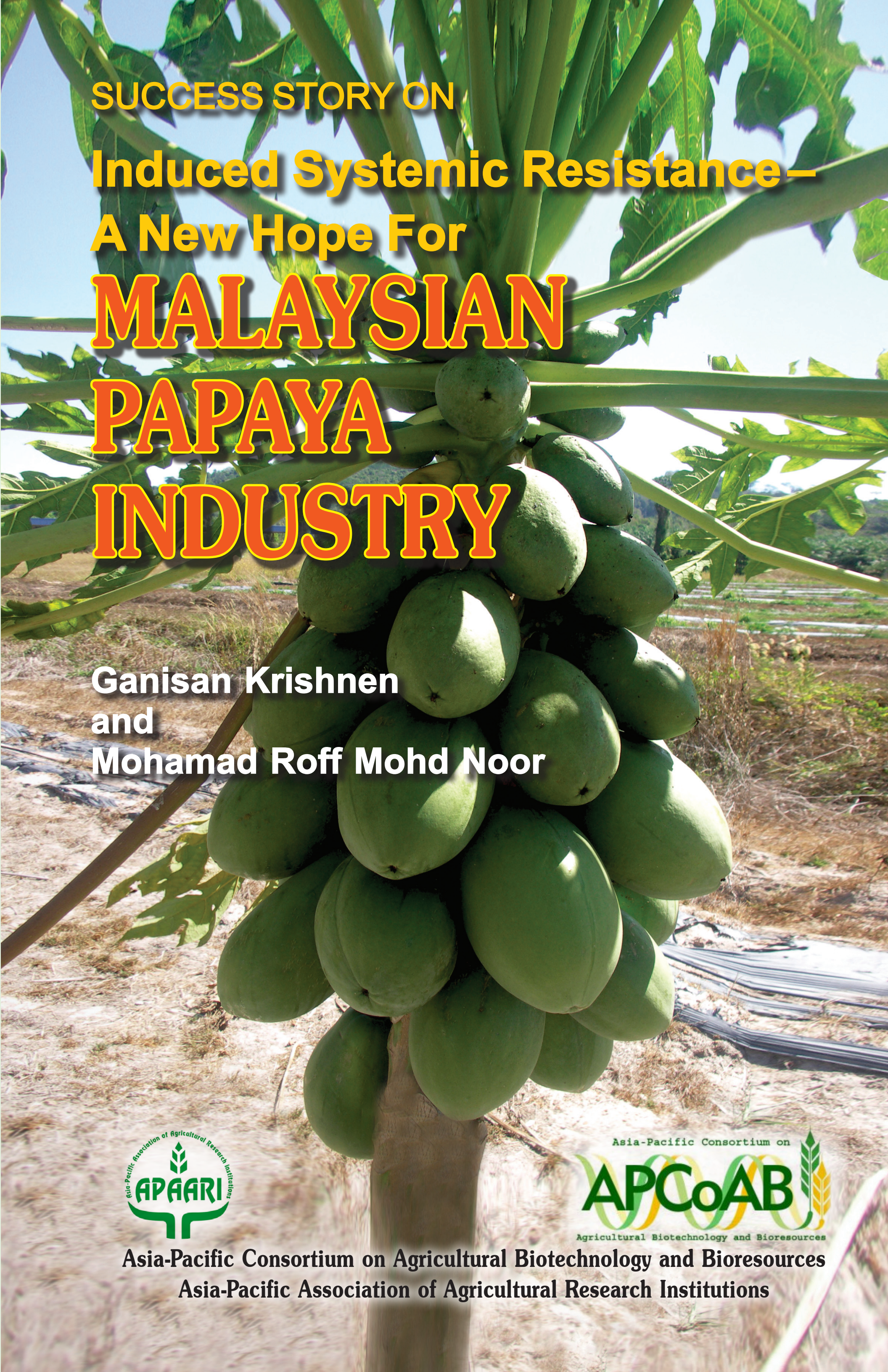

SUCCESS STORY ON

Induced Systemic Resistance – A New Hope For MALAYSIAN PAPAYA INDUSTRY

Ganisan Krishnen
and
Mohamad Roff Mohd Noor

Asia-Pacific Consortium on Agricultural Biotechnology and Bioresources
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INDUCED SYSTEMIC RESISTANCE A NEW HOPE FOR MALAYSIAN PAPAYA INDUSTRY

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Pomprab Sattrupai, Bangkok 10100, Thailand

Citation: APAARI (2021) Success Story on Induced Systemic Resistance– A New Hope for Malaysian Papaya Industry. Asia Pacific Association of Agricultural Research Institutions, Bangkok, Thailand. xiv+63p.

Published by: Asia-Pacific Association of Agricultural Research Institutions (APAARI), 182 Larn Luang Road, Klong Mahanak Sub-District, Pomprab Sattrupai, Bangkok 10100, Thailand

ISBN: 978-81-85992-47-9

Cover page photo identity: Healthy and heavy fruit bearing papaya tree with induced systemic resistance for bacterial dieback disease

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Published at Angkor Publishers Pvt. Ltd.

Printed in June, 2021

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Foreword

Papaya, a native of Mexico and South America and previously considered as exotic and rare fruit, is now grown in tropical climates across the world and is available at most times of the year. It is one of the important fruit crops cultivated in Malaysia for local consumption and export market. Malaysian papaya is highly prized for its excellent eating qualities and nutritional content, and therefore, it has been widely sought-after by local consumer and Malaysian export markets such as Mainland China, Hong Kong and Middle East. However, Malaysian papaya industry was pushed to the brink of collapse caused by the arrival of new disease known as the papaya bacterial dieback as was the case that happened due to infection by papaya crown rot in the other parts of world. This dieback disease was caused by an invasive alien bacterium species, *Erwinia mallotivora*. Since the disease arrived in 2003, the papaya cultivated area, production and papaya export of Malaysia had reduced significantly in the subsequent years. The farmers thus avoided to cultivate papaya because there was neither a resistant variety with excellent eating and nutritional qualities nor efficient chemical or biological control measures available. One of the strategies of plant disease management, which was always ignored, was the enhancement of the resistance against disease in the susceptible plant, so that it can be cultivated with reduced disease infection and yield losses. This resistance enhancement in the plant can be achieved by imparting Induced Systemic Resistance (ISR) by inoculation of Plant Growth Promoting Rhizobacteria (PGPR) on the root region. This technology was developed by Malaysian Agricultural Research and Development Institute (MARDI) by bioprospecting of the systemic resistance inducing PGPR from papaya cultivated soil and by testing it in the hotspot and up-scaling in commercial farms for disease management. Finally, this technology was launched and commercialised to make it easily available for farmers' usage. Various technical seminars and training were conducted to disseminate the knowledge and know-how about this technology to the stakeholders in order to encourage papaya cultivation. MARDI's efforts in developing this technology not only strengthen the Malaysian papaya industry, but also similar scientific endeavours. Malaysian Papaya Industry can be involved to develop effective control for other plant diseases and invigorate the Malaysian agriculture as a whole. APAARI, as an international network in Asia-Pacific region, envisions that the ISR technology developed by MARDI will be useful not only for Malaysia but also for all the other countries which were devastated by this disease. I sincerely appreciate the dedicated efforts of the authors, editor and so also MARDI's support for bringing out this publication "Success Story on Induced Systemic Resistance: A New Hope for Malaysian Papaya Industry". I would like to congratulate Dr Ganisan Krishnen and Datuk Dr Mohamad Roff Mohd Noor for bringing out this document and also to my colleague Dr Rishi Tyagi for designing the outlines and meticulous editing of this success story. APAARI believes that such a success story will inspire other member countries for adoption of ISR technology in their countries to control various plant diseases.

Ravi Khetarpal
Executive Secretary, APAARI

Preface

Advent of Eksotika varieties in Malaysia had transformed papaya industry form a backyard orchard for local consumption to monocrop plantation for export market. Since the varieties introduced, the papaya industry continuously expanded and in 2004 Malaysia had created history as world second largest papaya exporter after the Mexico with export revenue of RM 120 million. This success did not remain long because arrival of an alien invasive pathogenic bacterial disease, papaya bacterial dieback (also known as crown rot of papaya) which crippled the papaya industry. Since then, the papaya industry could not bounce back with export value about RM 31 million in 2018, a quarter of what Malaysia use to generate during its peak in 2004. Although, there was increasing demand for papaya with the existing markets in countries such as Hong Kong, China, Singapore and Middle East and new markets such as United Kingdom, Europe and United States, but Malaysia could not tap these opportunities because the papaya production could not be elevated since there is no any efficient control technique for the dieback disease. Realizing the existing chemical, bio-control and farm practices are not efficient in controlling this disease, instead we took an alternative approach of plant's resistance enhancement to disease suppression. The resistance induction was achieved by inoculating an ISR inducing Plant Growth Promoting Rhizobacteria (PGPR) inoculant.

This systemic resistance induction managed to prevent disease infection in disease-free seedlings and matured plants on the field. However, this technique is inefficient to suppress disease invasion in disease infected plants. Thus, this technique is a preventive measure instead of cure for disease infection. The ISR technology was upscaled in one ha of commercial farm and further validated in another three commercial papaya farms. One of drawback with induced systemic resistance (ISR) technology is that it is very labour intensive and increases the production cost. To address this issue, a supportive semi-mechanised ISR inoculant application technology was developed. An instrument for ISR inoculant application was developed and managed to reduce labour dependency up to 60%. The ISR inoculant was given brand name of Dieback Buster and the ISR inoculant application instrument as Adjustable Volume Liquid Gun (AVLG). This package technology of Dieback Buster and AVLG was launched by the Hon'ble Deputy Minister of Agriculture and Agro-based Industry Ministry on 1 August 2017. This package technology was commercialised to Arif Efektif Pvt. Ltd, a subsidiary of All Cosmos Pvt. Ltd on 7 October 2018. This technology was available in the market in April 2019 and business started picking up in the end of 2019 before it was halted by COVID-19 pandemic. The cross-border movement control by Singapore caused the export of papaya fruits to Singapore which remained in Malaysia only, therefore, the farm price was recorded as low as 50 cents/kg, consequently the farmers became reluctant to spend money for disease control for using the proven and effective ISR technology. Till to date, a total 393 ha of papaya farms have been growing papaya using ISR technology and expected to increase few folds after the COVID-19 pandemic brought under control and papaya export to Singapore is resumed again. ISR technology is promising and a new hope for rejuvenate back the ailing Malaysian papaya industry with a great boost. This technology has great potential to revitalize the papaya industry in countries of Asia-Pacific region and beyond also which have been affected by papaya bacterial dieback disease prevalence.

Acknowledgements

The authors would like to thank the Asia-Pacific Association of Agricultural Research Institutions (APAARI) for the opportunity given to write the Success Story on Induced Systemic Resistance: A New Hope for Malaysian Papaya Industry. Special thanks to Ms Aniadila Kamaruddin, the Deputy Director of International Network, Corporate Communication Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia, for communicating with APAARI and relevant departments in MARDI in getting approval for this document. The authors would like to thank APAARI for the opportunity given to us to share our experience in developing ISR technology to combat the papaya bacterial dieback disease. We also like to convey our gratitude to Dr Rishi Kumar Tyagi, Coordinator, Asia-Pacific Consortium on Agricultural Biotechnology and Bioresources (APCoAB), APAARI, Bangkok, Thailand, for his constructive insights in development of the outlines of the document, critical editing and being patient with us during the documentation of this success story. I also wish to express my gratitude to Datuk Dr Abdul Shukor Abdul Rahman, the chairman of Arif Efektif Pvt. Ltd and All Cosmos Pvt. Ltd for allowing me to expose the ISR technology through this booklet.

We also like to thank Mr Mohd Zaffrie Mat Amin for economic analyses of the Induced Systemic Resistance (ISR) technology in comparison to the conventional method used for papaya production in Malaysia. Finally, we would like to express our appreciation to Ms Nor Fadilah Abd. Halim, the Deputy Director for Fertiliser Technology Programme and Mr Zulkafli Ismail, the Director of Soil Science, Water and Fertiliser Research Centre, MARDI, Malaysia, for their support in preparation of this success story.

Authors

Abbreviation and Acronyms

AVLG	Adjustable Volume Liquid Gun
BCR	Benefit Cost Ratio
BD	Bacterial Dieback
CIRP	Christmas Island Rock Phosphate
DB	Dieback Buster
EPP	Entry Point Project
FAMA	Federal Agricultural Marketing Authority
IRR	Internal Return Rate
ISR	Induced Systemic Resistance
MARDI	Malaysian Agricultural Research and Development Institute
MNAFP	Malaysian National Agro Food Policy
MOA-ABI	Ministry of Agriculture and Agro-based Industry
NKEA	National Key Economic Area
NPV	Net Present Value
PGPR	Plant Growth Promoting Rhizobacteria
PP	Payback Period
TSP	Triple Superphosphate
RM	Malaysian Ringgit (currency)
TSS	Total Soluble Solids
WHO	World Health Organisation

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Executive Summary

Realizing the existing chemical, bio-control and farm practices that failed to control the bacterial dieback (BD) infection on papaya, a new strategy to induce the plant's resistance to manage this disease was developed. Some 139 isolates of bacilli and pseudomonad were isolated from the papaya cultivated soil. All these beneficial bacteria were screened for systemic resistance induction against papaya BD disease by using papaya seedlings. A total of 31 of those bacterial isolates shown an induced systemic resistance (ISR) induction with disease control scoring ranged from 20-100%. Eight isolates among them had recorded complete (100%) disease suppression. These isolates were further tested on other disease hotspots in Malaysia. After one crop cycle of 2 years, seven out of eight isolates shown best disease control capacity (100%) against BD disease. One of best performing isolates in terms of disease control i.e. MIB 106 was selected for upscaling in 1 ha of farmer's plot which is also a hotspot for BD disease. In all, 2,850 ISR seedlings were prepared and transplanted to farmer's plot. After one crop cycle, only 5% of the plants succumbed to BD infection. Further, this technology was validated in three commercial farms which were affected by BD disease. The application of MIB 106 inoculant manage to induce resistance in disease-free plants, however, all the disease-infected plants before the beneficial bacterial application could not be saved. This observation clearly indicating that this technology is a preventive measure, not a cure for disease-infected plants. To ease the labour intensive ISR inoculant application, a semi-mechanised application technique was developed. As a positive result from the upscaling and technology validation with three farms, both the ISR inoculant MIB 106 (brand name: Dieback Buster) and ISR inoculant application instrument [brand name: Adjustable Volume Liquid Gun (AVLG)] package was launched and commercialised to a biotechnology company. In order to disseminate the technique and know-how of this technology, a series of seminar and training were

conducted for the technology buyers and extension officers from various departments and agencies and the papaya farmers. The well-trained company's technical representatives and government extension officials managed to introduce this technology to the farmers. Most of the farmers who were using ISR technology as a prevention against papaya BD disease had given good testimonies about this technology. However, more training, awareness, advocacy and publicity campaigns are needed to be conducted to outreach ISR technology to the Malaysian papaya farmers, thus, the papaya productivity can be increased to fulfil the demands of domestic and export markets. ISR technology has been tested with desirable results and may be applicable to all papaya growing countries in Asia-Pacific region and beyond to prevent BD disease in papaya.

Introduction

Papaya was first introduced to Malaysia (Malacca) as early as 1550 from the seeds brought in from the Philippines by the Portuguese (Sekeli *et al*., 2018). Since then, the papaya has been cultivated as a backyard crop for personal consumption and to be sold in local market. Papaya is a fast-growing plant and have an early maturation. Papayas in tropics can be harvested 8 to 9 months after sown and generated bounty of yields which ranged from 60-100 tonnes/ha/year for improved varieties (Chan and Sim, 2019). Papaya is rich in nutrients such as vitamins A and C. One medium-sized papaya have 3000 IU for vitamin A and 90 mg for vitamin C which exceeds the Dietary Reference Intake (DRI) as established by the US Food and Nutrition Board (OECD, 2005). Papaya consumed fresh when fully ripen when the flesh is soft and succulent. Papaya also can be eaten raw as salad or cooked as vegetable or processed into various edible product as candy, jam, pickle and puree. Other usage of papaya is latex from unripe fruit and leaves which contains proteolytic enzyme papain, which can be used for meat tenderising, tanning leather, chill-proofing beer and making chewing gum (Chan and Sim, 2019). In pharmaceutical industry, papain is used as remedy for suppression of inflammation, treatment of gangrenous wounds and for various digestive ailments (Chan and Sim, 2019). The proteolytic enzyme from papaya latex has exfoliating property that removes the dead surface cells of the skin therefore used in soaps (Figure 1), creams, shampoo and lotions in the cosmetic industry (Chan

Figure 1: *Soaps made from papaya were displayed in the shelf of pharmacy in Malaysia*

and Sim, 2019). Recently, the extract from papaya leaves was used as cure for dengue in Malaysia.

Papaya in Malaysia

Papaya is one of the important fruits cultivated in Malaysia with 3218 ha cultivated area in 2017 and production value of RM 120 million. Currently, Sekaki (Figure 2a) is the main cultivated variety and Eksotika (Figure 2b), Hawaiian Solo, Subang and Batu Arang were also cultivated in a relatively small acreage. Previously, it was Eksotika dominated the industry which had lost its prominence after arrival of an invasive alien disease in 2003.

Figure 2: (a) *Sekaki* and (b) *Eksotika papayas*

Malaysia papaya industry had gone through a long transformation process from a cottage industry to a commercial plantation set up to fulfil the domestic and export demands. Chronologically, Malaysian papaya industry can be divided into four eras, namely:

i) Pre-Eksotika Era (pre-independence to 1986)

ii) Eksotika Era (1987-2003)

iii) Papaya Bacterial Dieback Era: Destruction of Malaysian Papaya Industry (2003-2017)

iv) Induced Systemic Resistance Technology Era: New hope for the Malaysia papaya industry (2017 onwards)

Pre-Eksotika Era

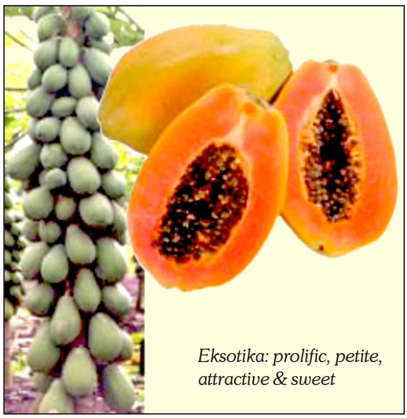
Before a papaya breeding programme was initiated by MARDI, there was no any superior papaya variety available in the country. The available varieties such as Sitiawan, Batu Arang, Subang and Sekaki were cultivated on a small scale or in backyard for own consumption. Among them, the popular variety was Subang, a variety bearing female fruits which are bigger and rounder. This variety locally known as Malaysia Dolly Parton – recognised for its big fruit characteristic. During 1972-1975, MARDI had recommended Subang 6 and Sitiawan for fresh consumption and canning, respectively. Generally, these varieties had inferior eating quality with big fruit size, therefore, disqualified for export. The papaya export in 1986 valued RM 3 million, mainly to export Sekaki, Sitiawan and Subang 6 varieties to the neighbouring countries Singapore and Brunei.

Eksotika Era: Golden Era of Malaysian Papaya Industry

Eksotika varieties breeding programme

Realising the potential of papaya for local consumption and export market, MARDI initiated a breeding programme in 1972 to develop superior varieties with few selected characteristics such as: i) sexually hermaphrodite; ii) small size and uniform fruits; and iii) good eating quality.

The papaya breeding in MARDI was initiated by Dr Chan Ying Kwok in 1972 by crossing the Sunrise Solo from Hawaii, which has excellent eating qualities but with poor yield and small fruit size in comparison to locally adapted large-fruited Subang 6. The subsequent progenies went through a series of self-pollination and backcrossing to develop Sunrise Solo. After 15 years of breeding and selection, a line known 'Backcross Solo' with features of Sunrise Solo and local adaptability and bigger size of Subang 6 was selected in 1987 which was released as Eksotika. The introduction of a high-quality papaya variety Eksotika, by MARDI in 1987 (Figure 3), had dramatically boosted the acreage of papaya, local papaya production and fruit export revenues to Malaysia.



Eksotika making headlines of Newspapers

Time is ripe for papaya farmers to think big

Figure 3: *The news published in the local daily about the Eksotika papaya after the variety was released in 1987 by MARDI*

However, the Eksotika was found susceptible to fruit freckles, thrips attacks, soft texture and sensitive against environmental stress. Thus, the cosmetic appearance and keeping quality of Eksotika was quiet poor. The Eksotika was crossed with its sister line (Line 19) which was resistant against freckles and had better keeping qualities. The resultant F_1 hybrid was more robust, high yielding and has much improved cosmetic and keeping quality. This was the first F_1 hybrid papaya developed in the world by MARDI. This hybrid was named as Eksotika II which was released in 1991.

The development and release of high-quality papaya varieties, Eksotika and Eksotika II, respectively, in 1987 and 1991 had revolutionised the Malaysian papaya industry. The papaya industry had transformed from a backyard orchard to a monocrop plantation which had generated copious amount of papaya for local consumption and also for export. These two varieties have dramatically boosted the papaya fruit export revenue to Malaysia. The papaya export revenue valued RM 3 million in 1986 (before the advent of Eksotika) reached its peak at 2004 to RM 120 million and Malaysia was ranked as second most important papaya exporting country in the world after the Mexico (Chan, 2010). This time period is considered as Golden Era of Malaysian papaya where Malaysian Eksotika papaya were exported to Mainland China, Hong Kong and Middle East. However, this achievement did not sustain for very long time when the industry was devastated by a new invasive alien disease known as papaya bacterial dieback (BD).

Importance of Papaya

Papaya is very popular fruits among Malaysian and also has a big export potential. The papaya consumption per capita in 2002 was just 3.69 kg/person had increased to almost double to 7.0 kg/person in 2010 (FAMA, 2014) which indicating that papaya was one of most-sought-after fruit by Malaysians. The export value of papaya in 1986 was just RM 3 million had bloated to RM 120 million, thanks for the excellent Eksotika and Eksotika II varieties developed and launched by MARDI in 1987 and 1991, respectively. Realising the importance of papaya for local consumption and export market, the government had listed this fruit under the Entry Point Project (EPP) of the National Key Economic Area (NKEA). However, the strategy to develop the papaya industry in this country was hampered by the emergence of an invasive alien disease known as BD disease which is affecting all the commercially important varieties.

Nutritional Content and Health Benefits of Papaya

Papaya is highly prized for its nutritional value (Table 1) (Chan and Sim, 219) and the World Health Organisation (WHO) declared papaya as King of Fruits for two consecutive years, 2018 and 2019 for recognising its nutritional and health benefits. Papaya has 89.3 g of water (from 100 g of papaya) and 123 kJ of energy. Papaya rich with vitamins and minerals that are required for the normal function of the body. Various vitamins such as vitamin B and C and minerals exists in abundance in papaya compared to other fruits. As an example, the vitamin C contents in papaya is much higher than the tomatoes or oranges (Chan and Sim, 2019) and totally free from cholesterol.

Table 1: Nutritional content of papaya fruits (for 100 g edible portion)

Nutrient	Value of Nutrient
Water	89.3 g
Energy	123kJ/29 kcal
Protein	0.4 g
Fat	0.1 g
Carbohydrates (total)	6.9 g
Carbohydrates (sugar)	6.9 g
Dietary fibre	2.3 g
Cholesterol	Nil
Sodium	7 mg
Potassium	140 mg
Calcium	28 mg
Magnesium	14 mg
Iron	0.5 mg
Zinc	0.3 mg
Beta-carotene	910 μg
Thiamine	0.03 mg
Riboflavin	0.03 mg
Niacin	0.3 mg
Vitamin C	60 mg (171% RDI)
Pyridoxine	0.038 mg
Folates	37 μg
Vitamin A Eq	150 μg

Source: *Chan and Sim (2019)*

Papaya Cultivation Practices in Malaysia

Seedling Preparation

Papaya seedlings are prepared by germinating over-night immersed seeds into a germination tray or directly into soil mixture (2 top soil:1 organic matter: 1 sand) packed in polybag. One seed was sown in 1 cm depth of each cell of germination tray or in soil mixture in a polybag. If the seeds were sown in germination tray, the young seedlings (4-5 leaves stage; approximately 2 weeks after sowing) were transferred in a polybag containing soil mixture as mentioned above. The seedlings in the polybag were maintained in the nursery for 2 months before transplanting the same to the farm. Alternatively, papaya seedling can be produced by sowing 3-4 seeds in a polybag containing soil mixture, expecting that 2-4 seedlings will be established in a seedling polybag and then to be transplanted into the fields.

Land Preparation

The vegetation in the land should be cleared before plough. One pass of disc plough followed by rototiller is sufficient for papaya cultivation. Alternatively, two runs of disc plough and a disc harrow also suitable for papaya production. Application of lime (3-4 tonnes/ha) during land preparation is crucial to nullify the residual effects of earlier crops and higher yields because the papaya does well under pH of 6.0 – 6.5, where most tropical soils are acidic and below this optimum range. It is advisable to blanket spray the ploughed area with pre-emergent herbicide *e.g.* s-metalachlor (Dual G) to ensure the sowing area are free from weeds during the first 6-8 weeks after the seedlings were transferred (Chan *et al*, 2011; Chan and Sim, 2019).

The optimum distance for papaya cultivation is 2.7 m apart between row and 1.8 m apart within row which can allow to maintain about 2000 plants/ha. The size of hole is 10 cm diameter for friable soil and should increase to 30 cm if the soil is compacted or has hard laterite (Chan *et al*, 2011; Chan and Sim, 2019). Two-month-old papaya seedlings from the nursery and transferred to the field. Each planting hole loaded with 2 kg of composted chicken manure (other compost also can be used) and 200 g of Triple Superphosphate (TSP) or Christmas Island Rock Phosphate (CIRP) and mix well with soil. The polybag is carefully torn and the seedling planted into the hole and the soil is pushed around the collar where the soil level covers approximately 2 cm of the collar (Chan *et al*, 2011; Chan and Sim, 2019). The double or triple point planting system implemented where two or three seedlings planted to a point, spaced 30 cm apart to maximise hermaphrodite trees in the field. Alternatively, a single polybag containing 2-4 seedlings can be cultivated in a single hole (Figure 4). Two months after transplanting, the seedlings should flower and the sex can be determined by the shape of the flower (Chan and Sim, 2019). The female flower has rounded shape compared to the hermaphrodite with elongated flower. The female trees are culled out which generate nearly 100% hermaphrodite plants. The elongated fruits generated from the hermaphrodite tress enjoyed higher price than the female one.

Figure 4: *Establishment of 2-3 young papaya plants on the farm where only one hermaphrodite plant maintained after the flowering*

Papaya as a fast-growing plant and provides fruits all the year around, abundant supply of nutrients at regular intervals is necessary to sustain good growth and production. During the early stage, adequate supply of nitrogen and phosphate required for foliage, trunk and root development. However, during the fruiting stage, higher level of potassium required for improving the quality of the fruits in term of colour, sweetness and firmness of the flesh. Generally, in Malaysia, 50-100g/tree of NPK 15:15:15 applied monthly until the plant flowering (4-month-old) and followed by 180g/tree of 12:12:17:2 (NPK+TE) formulation applied monthly (Chan *et al*, 1991; Chan *et al*, 2011). Adequate water supply is essential for production of bounty of yield.

Each tree required 60-90 litres/week immediately after transferred and 120-240 litres/week during the dry season. Lack of water especially during dry season generally retard the plant growth, abortion of flowers and fruitlet leading to sterile phases of fruiting skips (Chan and Sim, 2019). Adoption of irrigation in the farm results in an increase of number and size of marketable fruits (Chan and Sim, 2019). Irrigation by flooding between row or micro-sprinklers, jets or drip emitter are suitable for papaya production. However, utilisation of overhead sprinklers is not recommended because it constantly keep the leaves and trunk wet, which has high risk for disease infection.

Various pest and diseases devastating the papaya production in Malaysia (Chan *et al*, 2011). Pest such as mites, thrips, fruit flies, scale insects and nematodes are attacking the plant and fruits, thus, the yield reduces. All these pests can be efficiently controlled by spraying appropriate insecticide and implementation of good agriculture practices in the farm. Weeds infestation is another major problem in papaya cultivation. This issue can be addressed by blanket spraying of pre-emergent during land preparation and field spraying when there is weed infestation. Various diseases such as BD, papaya ringspot virus, collar and root rot, bunch/malformed top and anthracnose are important diseases that plaguing the Malaysian papaya industry (Chan *et al*, 2011). Among all, BD is the most notorious one which causes huge economical losses to the farmers and the industry. There are various chemical and farm practices tested to control the BD disease without any success. However, MARDI managed to develop a biological approach to efficiently control the disease which will be described subsequently in this document.

The fruits will be matured and ready to harvest in 5 months after the flowering. The yield will be harvested when the fruit attained colour indices 2 and 3 (index 2 - green with traces of yellow, index 3 - more green than yellow) (Chan and Sim, 2019). The fruits need to be handled carefully during harvesting and transporting to avoid bruises. The harvested fruits transported to packing house for sorting, sanitising, chemical deep or hot vapour treatment (for export purpose) and ripening induction before marketing.

Supply and Demand of Papaya in Malaysia

Malaysia papaya production showing an increasing trend after the introduction of Eksotika and Eksotika II, mainly to cater local and international markets. The closest competitor to Malaysia in papaya export was Mexico. In 2004, Malaysian ranked as second largest global papaya exporter after the Mexico. Therefore, Malaysia always placed Mexico as bench mark to develop its papaya industry. In 2003 and 2004, Mexico had slightly higher production of papaya compared to the Malaysia (Figure 5). However, after that Mexico had recorded tremendous increase in papaya production while Malaysia shown a decreasing pattern until 2011 and, thereafter, slight increase was recorded. This decline in production caused by the emergence of papaya BD disease in Malaysia which had reduced the cultivated acreage and crippled the production.

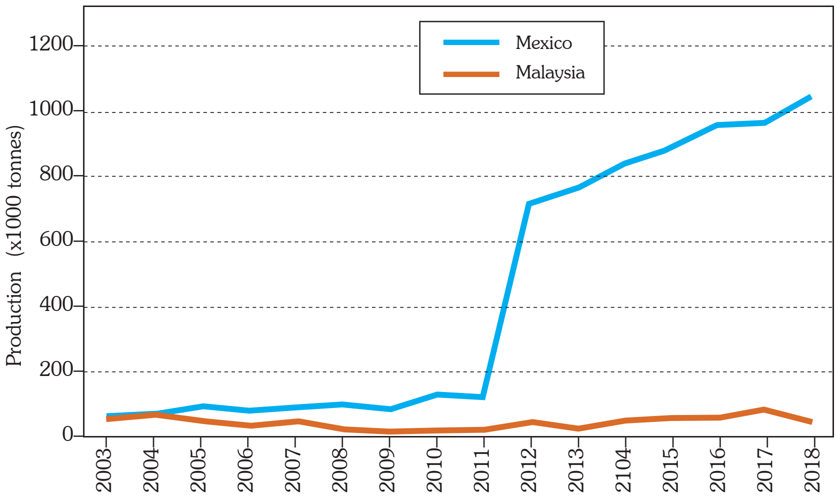


Figure 5: *Papaya production trend for Malaysia and Mexico (2003-2018)*

Import and Export of Papaya in Malaysia

The Malaysia papaya export had continuously increased after the release of both Eksotika varieties. The export value of RM 3 million before the varieties release had continuously increased to RM 120 million in 2004 and dropped after that (Figure 6). Malaysian papaya was exported to Mainland China, Hong Kong, Singapore and Middle East. This drop was caused by the arrival of a new disease which caused massive damage to the papaya farm and productivity reduced. Although, there is slight increase in export after 2013, but still very far to fetch the value of RM 120 million.

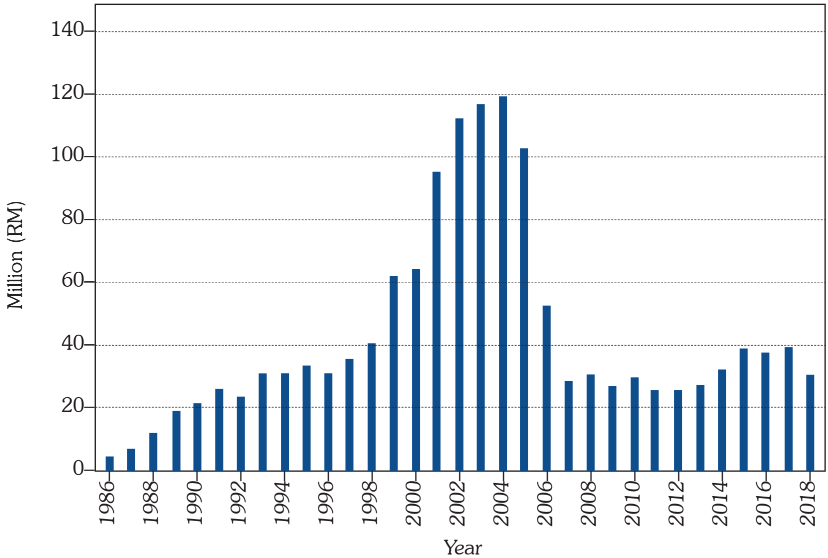


Figure 6: *Malaysian papaya export trend for past 33 years (1986-2018)*

Constraints to Enhance Production and Productivity of Papaya

Papaya bacterial dieback or crown rot of papaya, an invasive alien disease was first reported at Java in 1930. Since then, it had spread to 23 countries in the world without any efficient control methods. The BD disease was first detected at Malaysia in 2003 and within five years it caused destruction to an estimated 800 ha of planting, which caused loss of 200,000 tonnes of papaya amounting to USD 58 millions (Figure 5). The country's papaya export revenue dropped sharply from RM 120 millions in 2004 (just after the disease was discovered) to only RM 28 million in 2012; however, slight increase was recorded after that (Figure 6). The spread of this disease is rapid and the causal agent was initially identified as *Erwinia papayae* (*E. papayae*) (Makhtar *et al*, 2008), latter re-classified as *Erwinia mallotivora* (*E. mallotivora*) (Figure 7) by MARDI scientists using molecular tools (Mat Amin

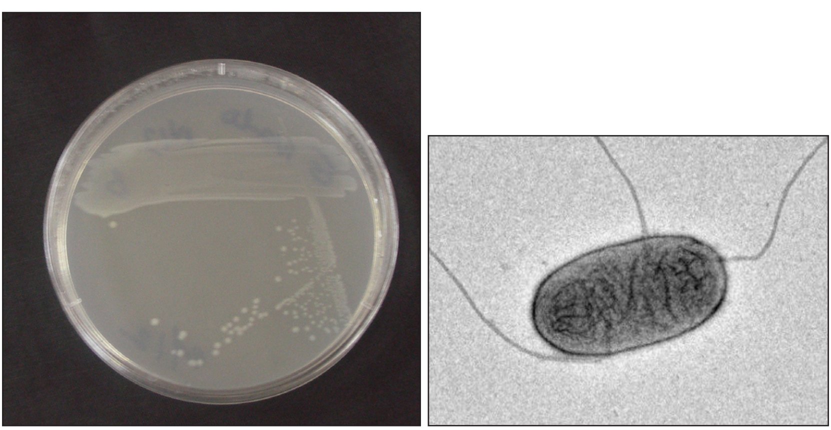


Figure 7: *The culture of Erwinia mallotivora grown on agar media and electron microscopy photo of the bacterial cell. (Source: Mat Amin et al. 2010)*

et al, 2010). Rapid spread of this disease put the industry under threat by infecting all the commercial varieties including important export varieties such as Eksotika, Solo, and Sekaki. Eksotika was the most susceptible to BD infection. This disease reportedly exists in all the Malaysian states except Sarawak. This has been attributed to stringent control measures in exportation of papaya seeds and seedling in entry point quarantine implemented in Sarawak (Lily Eng, pers.comm., ARC Sarawak). The Department of Agriculture, Malaysia, has classified this disease as invasive alien species without any efficient control measure available to date, not only in Malaysia but also in all other 22 affected countries in the world (Table 2).

Table 2: List of countries affected by papaya bacterial dieback (crown rot of papaya) disease in the world

1. Anguilla	13. Montserrat
2. Antigua	14. North Mariana Islands
3. Barbados	15. Philippines
4. Barbuda	16. St Croix
5. Brazil	17. St Kitts & Nevis
6. Dominica	18. St Lucia
7. Grenadines	19. Taiwan
8. Guadeloupe	20. Tonga
9. Indonesia	21. Trinidad & Tobago
10. Japan	22. Venezuela
11. Malaysia	23. Virgin Islands
12. Martinique	

Early symptoms of infection include yellowing, necrosis along leaf edge, bending of water-soaked leaf stalks and followed by necrotic water-soaked area on the stems and spread to the internal tissues (Figure 8). In advance stages, the terminal area of the crown will be bent or broken leading to dieback and death of the trees. This disease was found to infect seedlings, young and matured trees and fruits. The pathogen also has higher possibilities to infect the stem on the terminal top/crown of the papaya trees (Figure 8). Infection of the terminal top considered very vulnerable because this pathogen can soften the stem tissues and killed the plant. Infection of disease to the edge of leaf, petiole and fruits which is considered as mild infection and not considered as vulnerable because it will not kill the plant. However, failure to contain the disease

Figure 8: *Mild infection (a, b, c & d) and chronic infection (e, f, g & h) of BD disease on various part of papaya plants*

which consequently will spread to other parts of plant and become deadly to the papaya plants. The papaya plant cannot be saved once the pathogen is infecting the young seedlings, stem, and plant's crown, which is considered as chronic infection.

The seeds also found to carrying this pathogen especially in the newly prepared or wet seeds. However, longer storage of dried seeds was found free from the pathogen (Lily Eng, pers. comm., ARC Sarawak).

Development of Induced Systemic Resistance (ISR) Technology for Papaya Bacterial Dieback Control

What is Induced Systemic Resistance (ISR)?

Various approaches including chemical and biological controls were tested but none of them was successful in controlling this disease. One of the techniques that was not explored much for this papaya disease control is the enhancement of plant resistance which may increase its resistance against the disease causal pathogens. This process of plant resistance enhancement known as ISR which is defined as an enhancement of the plant's defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation which can be triggered by Plant Growth Promoting Rhizobacteria (PGPR); which systematically activates the plant disease resistance on the roots and further extends it to above-grounds plants parts (van Loon *et al*, 1998; Ramamoorthy *et al*, 2001). 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, nematodes, fungi, and insects (van Loon *et al*, 1998).

Induced Systemic Resistance Era: A New Hope for the Malaysian Papaya Industry

Induced Systemic resistant seedling technology establishment

ISR technology to control papaya dieback disease was developed by bioprospection and manipulation of ISR inducing PGPR obtained from papaya cultivated soil. This study involved isolation and screening for selection of potential PGPR strains, testing and up-scaling of the ISR seedlings in open field (hotspot) for papaya BD disease. The process involved works very well which is illustrated as below:

The selected PGPR, bacilli and pseudomonads were isolated from the papaya cultivated soils (Figure 9). The soil for this isolation sampled from MARDI Headquarters (Serdang, Selangor) and Keladi and Lunas, both from the northern state of Kedah. Nine bacilli were isolated from the Serdang sample while 42 and 36 bacili were isolated from Keladi and Lunas, respectively. Pseudomonads isolated from Serdang, Keladi and Lunas were 20, 19 and 13, respectively. Eighty-seven bacilli and 52 pseudomonads were isolated. The total number of bacilli and pseudomonad strains isolated from these three locations were 139 (Table 3).

Figure 9: *Sampling of papaya cultivated soils for ISR inducing bacilli and pseudomonads isolation at Lunas, Kedah*

Table 3: Bacilli and pseudomonads isolated from papaya roots sampled from three different locations in Malaysia

PGPR	Location		
	Serdang	Keladi	Lunas
Bacilli	9	42	36
Pseudomonads	20	19	13
Total	**29**	**61**	**49**

All the isolated PGPR were tested by inoculating them on the roots for systemic resistance induction in papaya seedlings. For control plants, root inoculation was omitted. All the treatments and controls were challenged with the pathogen, E. *mallotivora* (Figure 10).

Figure 10: *Challenging of pathogen E. mallotivora on ISR and non-ISR papaya seedlings (2 months), (a) pricking in crown area of papaya seedling to create wound, (b) spraying of E. mallotivora bacterial suspension and (c) pathogen challenged seedlings were covered with plastic bag to maintain the high humidity to create favourable conditions for disease infection*

Bacterial dieback symptom development was observed as early as in 4 days after *E. mallotivora* inoculation. The seedling's shoot and stems showed watery lesions and gradually died-off, which are typical symptoms associated with papaya dieback disease (Figure 8c, f and g). Generally, all the treatments and control exhibited development of such symptoms. The seedlings survival rate after 14 days of pathogen challenging were recorded. Among the isolates tested, thirty-one of them shown at least a single plant survival which was caused by healing of the BD disease. Eight of the treatments were found to record 100% disease healing and survival, which were inoculated with isolates MIB

10, MIB 13, MIB 43, MIB 58, MIB 100, MIB 106, MIB 113 and MIB 132. All the control were succumbed to BD disease (Figure 11).

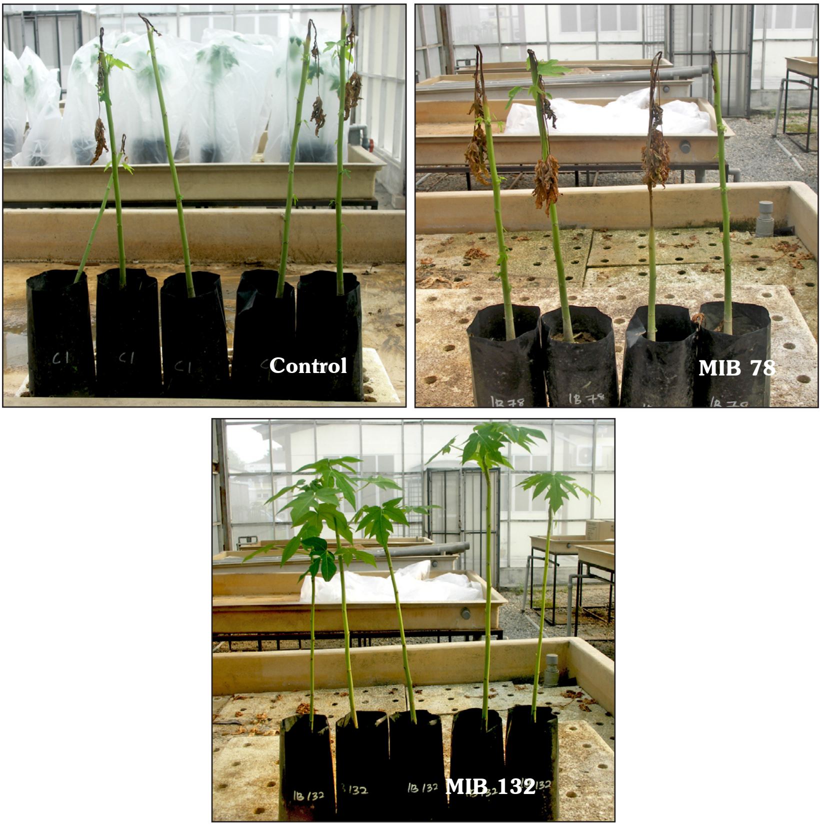


Figure 11: *Effects of pathogen challenging on control, ISR negative (MIB 78) and ISR positive (MIB 132) seedlings*

Field evaluation (hotspot) of the selected ISR inducing PGPR in Papaya bacterial dieback control

Eksotika ISR seedlings were produced by using all the selected eight PGPR and their capacities in controlling papaya BD were tested on papaya plants grown in open field in MARDI Farm at Serdang, which is considered as hotspot for BD disease. This site was selected because for seven years, before this study conducted, none of the Eksotika varieties cultivated on this site survived up to 11 months due to BD disease. The Eksotika, which was MARDI's variety, was selected because this variety is highly prized for its excellent eating quality and this variety monopolised the Malaysian papaya export mainly to Mainland China, Hong Kong and Middle East markets. However, this variety also was one of the highly susceptible variety available in our country, therefore, the supply of this variety was declined significantly caused by papaya BD disease and subsequently the export value also declined.

Papaya seedlings with Induced Systemic Resistance (hereafter called as ISR seedlings or ISR plants) were produced by inoculating with PGPR individually in the roots zone of each seedling. Non-ISR (Control) seedlings were raised by omitting the PGPR inoculation. Both the control and ISR seedlings (total eight treatment representing inoculation of eight bacterial isolates; MIB 10, MIB 13, MIB 43, MIB 58, MIB 100, MIB 106, MIB 113 and MIB 132) were tested on hotspot at MARDI Farm in Serdang. Generally, all the ISR seedlings have shown better early establishment than the control in the nursery (Figure 12). When the seedlings were transferred to the field, both the control and ISR seedlings did not differ each other because of the rainy season continued up to three months (first rainy season). ISR inoculant application was conducted once a month with 5 ml concentrated bacterial suspension diluted into 350 ml of water (dilution 1:70). The diluted bacterial suspension was drenched surrounding the active root zone below the periphery of papaya crown. Due to the wet weather, 17.5% of the control plants succumbed to BD infection, however, all the ISR seedlings (100%) survived.

After one crop cycle (24 months) on the hotspot and experienced second rainy seasons (3 months), all the control plants died due to papaya BD infection, however, all the ISR seedlings except seedlings raised with MIB 13 inoculation, had recorded 100% survival (Table 4; Figure 13). The treatment MIB 13 had recorded 12.5% plants death caused by this disease. The quality of the fruits produced for treatment were as good as control with 13-14% higher total soluble solids (TSS) and red intense colour (Figure 13). However, TSS up to 16% were recorded for both control and treatments during the dry season.

Generally, MARDI recommends Eksotika papaya cultivation season up to 24 months. All the plants with ISR treatments except MIB 13 survived for 24 months without disease during this period (Figure 14). Most of the farmers has been forced to cease Eksotika papaya cultivation and forbidden for recultivation for 2 years because the severity of this disease. This drastically reduces the country's papaya production and the export revenue in 2012 declined sharply to about 17.5% from the peak export values of 2004, the best ever achieved as an exporter by Malaysia.

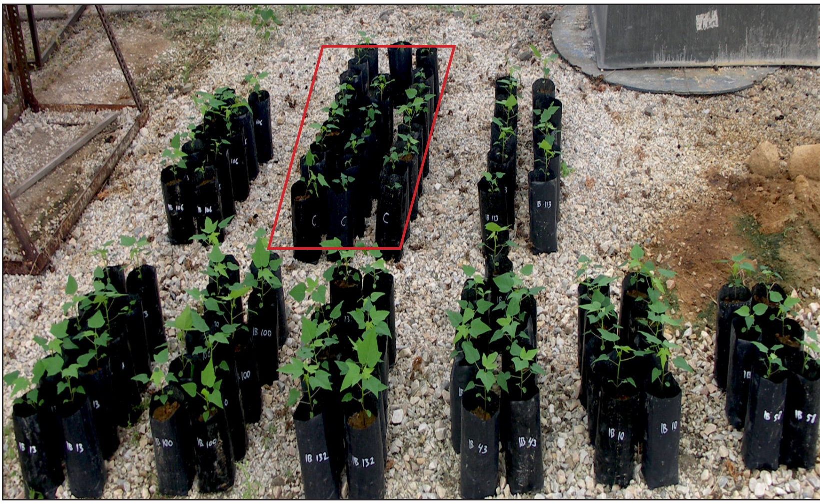


Figure 12: *Effects of PGPR on vegetative growth of ISR and non-ISR (control -in red quadroon) seedlings (30 days after sowing)*

Figure 13: *High quality Eksotika papaya with intense red flesh and TSS of 14% harvested from (a) a non-ISR tree and (b) an ISR tree*

Table 4: Effects of ISR plants on the survival against papaya BD in 24-month-old papaya trees grown on a hotspot in Serdang

Control/Treatment	Survival Rate (After 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 14: *Effects of ISR plants (a) resistance against papaya BD compared to control (b) for 24 months which were grown on a hotspot in Serdang, Malaysia*

Supporting Technology for Efficient Application of Dieback Buster

The Dieback Buster diluted suspension application in the papaya field is a very laborious and time consuming. Each individual plant needs to be drenched with ISR bacterial suspension; therefore, a semi-mechanised application system was developed. An instrument, Adjustable Volume Liquid Gun (AVLG) was developed for ISR bacterial suspension application to the plants in the field (Figure 15). This semi-mechanised application system managed to reduce the labour usage for the bacterial suspension application as low as 40%. Therefore, the labour cost for this process can be reduced and this is in line with government policy to decrease the labour force in agricultural sector. Both the manual and semi-mechanised ISR inoculant application is shown in Figure 16. This instrument also can be used for other purposes such as application of biofertiliser, pesticide and hormones in the field.

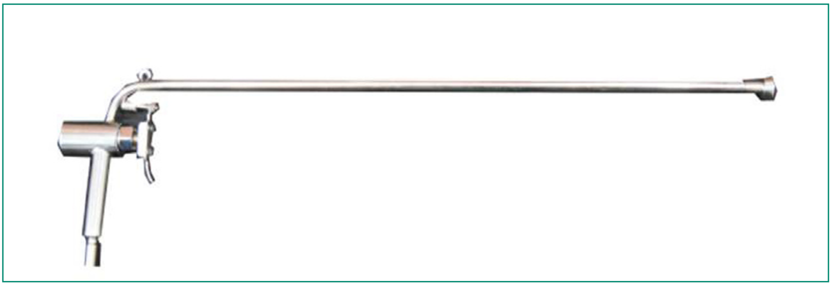


Figure 15: *Adjustable Volume Liquid Gun, an instrument developed specially for diluted ISR inoculant suspension application*

Figure 16: (a) *Manual application of ISR inoculant, compared to (b) application using AVLG*

Major Protocols

A. ISR Induction in Seedlings

Best results for BD control can be obtained by cultivating ISR seedlings in the farm and followed by periodical application (once a month) of ISR inoculant in the field. The ISR seedlings production is varied from the conventional technique where the ISR inoculant treatment on the seeds before sowing and followed by two-time application of diluted inoculant on young seedlings in the nursery. Step-wise protocol for ISR seedling production is given in following section:

1. The papaya seeds were soaked in water for overnight (Figure 17). After overnight soaking, those floating seeds need to be discarded. Only the submerged seeds were used for sowing.

Figure 17: *Overnight soaked papaya seeds. There are floating and submerged papaya seeds*

2. The submerged seed were rinsed 2-3 times using clean water. Then the rinsed seeds were soaked in the ISR bacterial suspension for 1-2 hours (Figure 18).

Figure 18: *The rinsed papaya seeds were soaked with un-diluted ISR bacterial suspension*

3. After one to two hours soaking in ISR bacterial suspension, papaya seeds were sown in a germination tray containing peat moss. The remaining Dieback Buster bacterial solution was dripped on the peat moss where the seeds were sown. The peat moss needs to be kept moist by spraying with a very fine droplets of water. After two weeks (4-5 leaves stage), the young seedlings were transferred into polybag containing soil mixture of 2 top soil:1 organic matter:1 sand (2:1:1 ratio).

Figure 19: *The Dieback Buster treated seeds were sown into germination tray containing peat moss*

4. Follow-up drenching (booster application) of diluted ISR inoculant (30 ml) (Figure 20). The ISR inoculant was diluted with water (1:50 ratio) and the suspension application was conducted after 2 or 4 weeks after the young seedlings were transferred to soil mixture in polybag.

Figure 20: *Follow-up drenching of Dieback Buster into polybag, two and four weeks after the young seedlings transferred to the soil mixture*

6. After two months, the ISR inoculant-treated young seedlings transferred to polybags (two weeks after the follow-up drenching completed), the seedlings are ready to be transferred to field/farm (Figure 21).

Figure 21: *Two-month-old ISR papaya seedlings are ready to be transferred to field*

B. ISR Induction at Farm – ISR Inoculant Application on Trees Planted in the Farm

The recommended dose of the ISR inoculant was 5 ml un-diluted ISR inoculant/tree. The ISR bacterial concentrated inoculant need to be diluted. The recommended dilution is 1 ISR inoculant: 70 water to 1 ISR inoculant: 100 water. For the dilution purpose, any farm water without severe contamination with pesticides or hazardous chemical can be used. The application process of diluted ISR inoculant can be achieved by manual or partially mechanised process.

Manual application of Dieback Buster

1. The ISR inoculant is a concentrated bacterial suspension that has capacity to induce systemic resistance against papaya BD disease. The best dilution of this product for field application is 1:70 or 1:100. The 1:70 dilution is suitable to be used during wet season or in the farm that has good irrigation system. The 1:100 dilution is mainly for plants grown in dry season or on farm with poor irrigation system.

Figure 22: *ISR inoculant (Dieback Buster) in 5-litre container*

2. 150 ml of ISR inoculant measured using a measuring cylinder. If there is no measuring cylinder, the farmers can use the measuring apparatus supplied together with the pesticide products. The farmers need to make sure that the pesticide measuring apparatus never been used to measure any pesticide before.

Figure 23: *Measuring of ISR inoculant using measuring cylinder*

3. 150 ml of ISR inoculant was diluted in a pail filled with 10 litres of water (dilution factor: 1 ISR inoculant: 70 water). For 1:100 dilution, 100 ml ISR inoculant were diluted into 10 litres of water.

Figure 24: *Dilution of ISR inoculant into pail containing 10 litres of water*

4. 350 ml (1:70 dilution) or 500 ml (1:100 dilution) of diluted ISR inoculant were drench surrounding the roots region follow the periphery of plant's canopy.

Figure 25: *Drenching of diluted ISR inoculant to root region using a liquid filler*

Partially mechanised application of Dieback Buster

A detailed explanation of ISR inoculant application by using a semi-mechanised system is explained below. For the partially mechanised application of ISR inoculant, there are few apparatuses are needed as listed below:

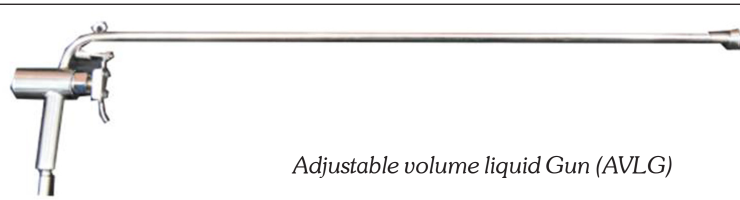
Petrol engine 2L

Pump (2Hp)

The water pump system photo source:
https://www.engine-specs.net/honda/g200.html

Polyurethane

150 L water



Adjustable volume liquid Gun (AVLG)

(i) 2.15 litres of ISR inoculant were diluted in a water barrel filled with 150 litres of water (dilution factor: 1 Dieback Buster: 70 water). For 1:100 dilution *i.e.* 1.5 litre Dieback Buster was diluted into 150 litres of water. The mixture was mixed thoroughly by using a long pole or stick (Figure 26).

Figure 26: *Dilution of ISR inoculant into water barrel containing 150 litres of water*

(ii) 3-4 water barrels (150 litres) can be arranged in a trailer together with petrol engine and pump. The pump fixed with hose which was attached with Adjustable Volume Liquid Gun (AVLG) (Figure 27). Each time after the engine and pump were on, the AVLG apparatus need to be calibrated for a desired application volume. For a dilution of 1:70, the discharge volume will be adjusted to 350 ml. While for 1:100 dilution, the discharge volume set to 500 ml.

Figure 27: *Complete set-up of four barrels, engine, pump, hose and AVLG*

(iii) 350 ml or 500 ml of diluted ISR inoculant can be drenched on the soil by pressing the trigger once. Two workers are needed for this process where one person is in-charging the AVLG while the second person holding the hose to avoid the tangling of the hose. By using four 150 litres barrels, 1700 plants can be inoculated with diluted ISR inoculant if 350 ml discharged for each tree. For 500 ml application, four barrels with 150 litres each, 1200 plants can be inoculated with this semi-mechanised system (Figure 28).

Figure 28: *Application of ISR inoculant using semi-mechanised system; two workers needed for this process*

Up-scaling of ISR Technology at Farmers' Fields

The efficacy of this technology in controlling the papaya BD disease in farmers' fields was determined by implementing an up-scaling project. The up-scaling activity was conducted with Exotic Biotech Sdn. Bhd. (subsidiary of Exotic Star Sdn. Bhd) on a farm (hotspot) of 1 ha at Ampang Tinggi, Kuala Pilah, Negeri Sembilan, Malaysia. A total of 2,850 ISR seedlings were prepared at MARDI headquarters and transferred to up-scaling farm at Ampang Tinggi. After one crop cycle (2 years), the efficacy of this technology in controlling papaya BD disease was recorded as 95% (Figure 29). About 5 per cent casualties in plants were observed during wet seasons because the root zone was heavily infested by weeds (Figure 30a). It was observed that weed infestation prohibited efficient application of ISR inoculant to the root zone of papaya and reduces the efficacy of the inoculant to induce resistance against BD disease. Therefore, for an efficient control of this disease, a good agriculture practice is compulsory to be followed, especially removal of weeds (Figure 30b).

Figure 29: *Up-scaling plot of ISR technology at Ampang Tinggi, Negeri Sembilan, Malaysia*

Figure 30: *Infestation of weed in up-scaling plot of ISR technology at (a) Ampang Tinggi, Negeri Sembilan, Malaysia and (b) manual weeding after recommendation by MARDI*

This technology also was tested in both conventional and organic plots in MARDI farm which both recorded 100% control of disease. Good agriculture practice was implemented, therefore, we could maintain the plots without any casualties. Other than that, this technology also was tested in two commercial farms which managed to stop the spread of the disease from infected to healthy plants in the farm.

Launching of ISR Technology — Dieback Buster and AVLG Package

'Dieback Buster' was the given commercial name to the systemic resistance inducing bacterial inoculant. The name was selected because this bacterial suspension can induce resistance and bust the dieback disease efficiently. The name chosen for the instrument developed for Dieback Buster (ISR inoculant) application was Adjustable Volume Liquid Gun (AVLG). Package technology of Dieback Buster and AVLG, was launched by the Hon'ble Datuk Nogeh Gumbek, the Deputy Minister of Agriculture and Agro-based Industry (MOA-ABI) Ministry on 1 August 2017 (Figure 31). The launching location was

Figure 31: *Launching of (a,b,c) ISR technology package (Dieback Buster and AVLG) by the Hon'ble Datuk Nogeh Gumbek, the Deputy Agriculture and Agro-based Industry (MOA-ABI) Deputy Minister*

Dengkil papaya farm which was owned by Exotic Star Sdn. Bhd. This technology launching was reported in various local dailies.

Commercialisation of ISR Technology

The ISR technology to control the papaya BD disease was commercialised to Arif Efektif Pvt. Ltd. , a subsidiary of All Cosmos Pvt. Ltd (a public listed company with BioNexus status) on 7 October 2018. The agreement exchange ceremony between MARDI and Arif Efektif Pvt. Ltd was witnessed by the Hon'ble Agriculture and Agro-based Industry (MOA-ABI) Minister (Figure 32). Since then, the company managed to produce and sell the Dieback Buster worth RM 130,000. Three hundred ninety three ha of papaya cultivated area had benifitted from this technology since the product was available in the market since the April 2019. The company together with the MARDI had initiated many activities such as seminar, hands-on training and presentation in various scientific and technical fora to promote the technology to the extension agents and farmers (Figure 33). The company is also conducting many promotional activities through their dealers, using social media and print media by publishing its benefits in local agriculture magazines.

Figure 32: *Commercialisation agreement exchange ceremony*

Figure 33: *Promotion of Dieback Buster product through (a) seminar to papaya farmers and (b) by placing it in dealer's shop*

Challenges

Production and Distribution of Planting Material

Production of systemic resistance induced planting material by treating the papaya seeds with Dieback Buster and followed by booster application of diluted inoculant on young seedlings (4 and 6 weeks after transferred to polybag) is one of the major challenges. Generally, in the big farms with own nursery facility, the owners produce the ISR seedlings at their farms. Small- and medium-farm holders had to request for purchase of ISR seedlings but the demand could not be fulfilled. Although, there are demand for ISR seedlings, but lack of private nurseries to produce ISR seedlings had hampered the availability of the seedling to meet the demand of the farmers. Realizing this issue, recently MARDI and All Cosmos Pvt. Ltd. had decided to produce ISR seedlings and distribute the same to interested farmers. This effort was temporarily was halted due to the COVID-19 pandemic. However, both the parties will initiate the seedlings production once the pandemic is brought under control.

Field Validation of ISR Technology

This technology was validated in three commercial farms in the state of Negeri Sembilan (2 farms) and Selangor (1 farm). This technology was tested on plants already grown using non-ISR seedlings, therefore, does not has resistance against dieback disease. Two of the farms in Negeri Sembilan already has dieback infection and had requested MARDI to control it with ISR technology. The farm in Selangor, started using the Dieback Buster 2 months after seedlings were transferred to the field. The suitability of ISR technology for organic production of papaya was tested in MARDI headquarters where the ISR seedlings were used. The effects of Dieback Buster application in these three farms and an organic plot are given below:

i) *Testing of ISR technology on matured papaya plants grown using non-ISR seedlings at Gunong Pasir, Negeri Sembilan*

The ISR-AVLG technology package was tested on fully grown 10-month-old fruit bearing papaya trees on two farms at Gunong Pasir, Negeri Sembilan. The ISR inoculant was applied when the farmer had requested to eradicate the disease from his farms (the farms were already infected by disease by then). All the papaya plants were drenched with diluted Dieback Buster bacterial suspension. All the disease-free plants in the farms were managed to be rescued from the disease infection after the application of ISR inoculant. Most of the plants with mild infection on the leaves edge, petioles and fruits were also saved. However, all those plants with chronic infection in the crown of papaya were succumbed to the disease. This observation indicating that the ISR technology managed to prevent the BD infection in the disease-free as well as in the plants with mild infection (Figure 34).

ii) *Testing of ISR technology on papaya plants grown using non-ISR seedlings at Dengkil, Selangor*

The ISR technology was tested on papaya trees grown on a farm at Dengkil, Selangor (5 acres) just two months after the seedlings

Figure 34: *The papaya farm in Gunong Pasir shows that disease spread was contained from mild infection and the plants' recovery after 1 month of Dieback Buster application. Notably, yellow and dried leaves (flagging leaves) were the pathogen infected leaves which died. The disease was not spread to other parts of the plant.*

were transferred to the field. The ISR inoculant was applied once a month. All the papaya plants were drenched with diluted ISR bacterial suspension. The disease incident in the farm was maintained as low as 3%. However, the trees in neighbouring farm (5 acres owned by the same farmer) which were not treated with the ISR inoculant, affected badly by BD disease.

iii) *Testing of ISR technology on organic papaya production*

The suitability of ISR technology for organic papaya production was also tested at MARDI headquarters. The size of plot was 0.5 acre with 400 ISR seedlings of Eksotika variety. After one crop cycle (2 years), all the plants had shown good growth (Figure 35) with no report of dieback disease incidence. However, since there is no pesticide or insecticide was used, there are few incidents of insects infestation and collar rot disease infection were recorded. Nevertheless, the pest and insect attacks were controlled by organic pesticide and insecticide. For the collar rot disease, removing the whole plant (together with roots)

Figure 35: *The organic papaya production in MARDI headquarters; notably there was not any dieback infection reported throughout the whole crop cycle*

and cultivation point applied with lime was sufficient to contain the disease spread to other healthy plants.

Technology Transfer

The technology was transferred to various government agencies and commercial farmers. Few of the technology transfer activities carried out are listed as below:

i) Workshop on ISR technology for Federal Agricultural Marketing Authority (FAMA) contract farmers. This workshop was held at Simpang Renggam, Johor on 9 April 2018. This workshop consisted of seminar and followed by hands-on training on ISR inoculant application on field-grown papaya (Figure 36 a, b).

ii) Workshop on ISR technology for papaya farmers and extension officers from the Department of Agriculture (DOA). This workshop was held at MARDI Kluang, Johor, on 7 October 2018 in conjunction with 2018 Johor's MARDI Day and ISR technology commercialisation. This workshop consisted of seminar and followed by hands-on training on ISR inoculant application on field grown papaya (Figure 36 c, d).

iii) Workshop on ISR technology for the staff members of All Cosmos Pvt. Ltd., the company which bought over this technology from MARDI. This workshop was held at Pasir Gudang, Johor, on 29 January 2019. This workshop consisted of seminar and hands-on training on ISR papaya seedlings production.

iv) An online seminar was held for the selected extension officers from Department of Agriculture those were involved in plant disease control. This seminar was held at Headquarters of Department of Agriculture, Federal Administrative Centre, Putrajaya, on 8 September 2020.

v) Various hands-on trainings on farmers' fields were conducted whenever technical assistance or trainings were sought by the farmers.

Adoption of Technology by Farmers

Once ISR technology was released and commercialised, there were few farmers who had been successful in using this technology to efficiently controlling the papaya BD disease. Farmers from various agro-climatic

Figure 36: *Seminar and hands-on training conducted for (a, b) FAMA's contract farmers and (c, d) ISR technology training in conjunction with MARDI Johor Day*

regions were selected and interviewed through farm visit or through phone calls. Visit to papaya farms were very much restricted because there was movement control order implemented in certain part of Malaysia due to COVID-19 pandemic. The experiences of few famers, who had used Dieback Buster as prevention against BD disease at their farms, are discussed in following section:

i) *Semarak Agro Pvt. Ltd , Baling, Kedah (Northern region – West Coast of Peninsular Malaysia)*

Mr Farhaan started using Dieback Buster when his one and half year-old trees were infected with the dieback disease. After application, he noticed that the disease did not spread to other healthy trees, although, the infected plants could not be saved. Since then, he started using this product for his papaya plants and no BD infection reported so far. However, due to current price of papaya dropping which was caused by COVID-19 spread and consequently fruits could not be exported to neighbouring Singapore and the farmer postponed usage of the Dieback Buster to save the cost of production. Most of the old trees were removed by the farmer as the disease infected again when he stopped using the Dieback Buster. Once, he realised that the infection peaked up without the Dieback Buster, he started again to use it for younger plants (11-month-old) in the same farm of 6 acres size. The farmer was satisfied with this product and he will continue to use this product including the new farm which will be opened in the mid of 2021.

By using this product continuously, the farmer realised that beside the BD disease control, there was no any other soil-borne disease infection occurred, which is otherwise very common during wet season. The papaya plants also produced more flower with very less flower

Figure 37: *The farm manager Mr Farhaan (a) holding the Dieback Buster bottle in his farm and (b) bounty of yield harvested on the previous day*

abortion (drop), therefore, the yield also increased with attractive fruits (Figure 37 a, b). The quality of the fruit is sweeter and firmer. Other than that, he also observed that the soil texture was also improved.

ii) ***Lim Seng Koi Farm, Slim River, Perak (Middle region – West Coast of Peninsular Malaysia)***

Mr Lim Seng Koi cultivated papaya in 15 acres in Slim River Perak and the plants approaching almost three years of age. A part of the farm was infected with dieback disease and he had tested Dieback Buster for disease control. Two small plots approximately contain 100 disease-free plants which situated side by side were selected. Dieback Buster was applied for one of the plots once every fortnight for 2 months and another was applied with copper-based bactericide. After two months, all the Dieback Buster-treated plants were infection free and survived (Figure 38a) but the copper-based bactericide treated plants were infected with the disease and succumbed (Figure 38b). However, the farmers ceased the production of papaya because the plants already exceed its production period of three years and he temporarily does not want to start new planting of papaya because the fruits price is very low. Before the COVID-19 pandemic, the farm price for papaya could fetch as high as RM 2.80/kg and now the price dropped as low as 50 cents/kg. The farmer wants to continue the usage of ISR technology to re-cultivate the papaya when the fruit import restrictions lifted by the Singaporean government, thus, the demand and the price for papaya will increase.

iii) ***Mr Han Farm, Pengkalan Kempas, Negeri Sembilan (Middle region – West Coast of Peninsular Malaysia)***

Mr Han used Dieback Buster when the plants were 1.5 year-old and until the age of 2.5 years. He observed no dieback disease incidence. However, he already cut down the tree foliage and left the trunk (2-3 feet from the soil) for emergence of new shoots and the plants will be maintained for one more year for seeds production purpose. This process is recommended to be applied if the plants produce the excellent fruits in term of quantity and quality.

For the second batch of the papaya plantation, which was already two months old after the seedlings were transferred, he has been using Dieback Buster two times since the seedlings were transferred and very prolific fruit bearing was noticed by the farmer (Figure 39a). After 38

Figure 38: *The effects of (a) Dieback Buster on BD disease control compared to (b) conventional chemical control*

days, the plants were applied with Dieback Buster to initiate the early flowering (Figure 39b) compare with plants without Dieback Buster treatment at same farm which started flowering only after on 60-65 days of transfer. There is no BD or soil-borne diseases were observed in second batch of plants. The farmer had decided to continue to use

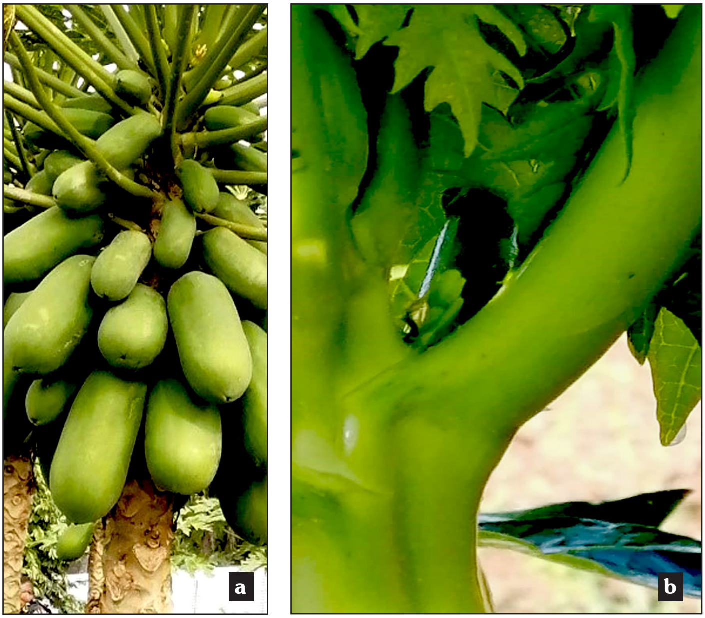


Figure 39: *(a) Prolific fruit bearing papaya plant treated with Dieback Buster before cut down (2.5 years) and (b) early flowering new batch of ISR plants*

the Dieback Buster and will recommend it to other papaya growing farmers in the vicinity. He has also already started using this product on banana plants where the reduction in *Fusarium* wilt infection has also been observed.

iv) *Waybest Pvt. Ltd. Farm, Mersing Johor (Southern region – East Coast of Peninsular Malaysia)*

Mr Manimaran started using Dieback Buster as early as during seedling production. He treated the seeds with Dieback Buster (Figure 40a) before sowing into a germination tray. Two months after sowing, the ISR seedlings were transferred to field. The plants showed vigorous and uniform vegetative growth (Figure 40b, c). Two months after transfer, there was no incidence of dieback or any other soil-borne diseases was observed.

v) *Agro Acres Farm, Desaru Johor (Southern region – East Coast of Peninsular Malaysia)*

Mr Danni has been using Dieback Buster inoculant together with fermented fruit juice, generated by himself which is rich in enzymes

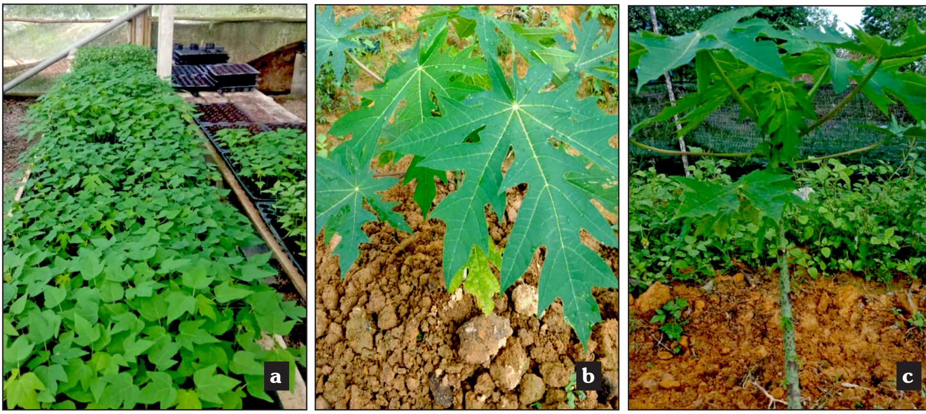


Figure 40: *Production of (a) papaya seedlings using Dieback Buster treatment and (b and c) young ISR papaya plants two months after transferred to the field*

and other nutrients for plant growth. Mostly, the farmer will place all the rotten and un-marketable fruits in a 150 litres container and leave the fruits to get fermented for one month. The mixture of Dieback Buster and fermented fruit juice will be diluted and drenched on the root region and also applied on the foliage. So far there is no any dieback disease incidence was reported and the plant have shown better growth and yield (Figure 41).

Figure 41: *Effects of Dieback Buster and fermented fruit juice application on plant growth and yield of papaya plants*

vi) *Sun Zone Pvt. Ltd. Farm, Kuala Lipis, Pahang (Middle region – East Coast of Peninsular Malaysia)*

Mr Lim Hap Ping started using Dieback Buster since the seedling were transplanted to the field for almost 1 year; no any soil-borne disease or BD infections were observed. The papaya plants showed good vegetative growth and better yield (Figure 42) compared to the

Figure 42: *(a) Healthy papaya plants and (b, c) prolific fruit bearing papaya plants treated with Dieback Buster and (d) the good quality papaya harvested*

conventional technique. Mr Lim realised that some of his papaya plants' foliage turn yellow which is a very common symptom for collar rot disease that caused by *Phytophtra palmyvora* infection especially during wet season. The farmer applied Dieback Buster once every fortnight for 2 months and all the plants completely revived from the yellowing caused by collar rot disease. The farmer mentioned that he will continue to use it once a month and will increase the application frequency of the same to twice a month during wet season.

IPR Issues

The ISR technology for induction of resistance against papaya DB disease was protected by patent filing with Intellectual Property Corporation of Malaysia (MyIPO). The process of systemic resistance induction in seedlings and field-grown papaya plants likely to be protected by the filed patent. Currently, the patent agency is evaluating the application for patent granting. Since the technology was patented and sold to Arif Efektif Pvt. Ltd, nobody allowed to produce the inoculum prior written consent from the company. The farmers who intended to use this technology can purchase both the Dieback Buster and AVLG from any of All Cosmos Pvt. Ltd. dealers in Malaysia. However, the farmers can produce their own ISR seedling by using Dieback Buster which is already available in the market. However, the quality of ISR seedling produced varied based on the individual experience in inducing systemic resistance using Dieback Buster. MARDI, Arif Efektif Pvt. Ltd. and All Cosmos Pvt. Ltd. as the Malaysian entities who strongly support the Sustainable Development Goal, we are pleased to offer this technology to be benefited by papaya growers in various part of globe including the Asia-Pacific countries. The papaya ISR technology can be transferred to other countries by communicating with MARDI, Arif Efektif Pvt. Ltd and All Cosmos Pvt. Ltd. Any correspondence regarding the technology transfer to foreign countries can be sent to aniadila@mardi.gov.my (MARDI) or wan_azha@allcosmos.com (All Cosmos Pvt. Ltd).

Capacity Development

Various training and workshop were conducted to train the staff members of All Cosmos Pvt. Ltd. Trainings were also imparted to extension officers in Department of Agriculture (DOA) and Federal Agricultural Marketing Authority, thus in turn, they can train other staff members and famers under their guidance. At MARDI level, we regularly organise in-house training to train MARDI staff members with basic skill of ISR technology. However, extensive trainings need to be conducted at large scale to outreach the papaya growing farmers in Malaysia to harvest the maximum benefits of ISR technology.

Potential Benefits to Papaya Farmers by adopting ISR technology over Conventional Planting

Cost of planting material

In Malaysia, production cost of a 1-month-old non-ISR seedlings ranged between RM 1.20 to RM 1.50 which are mostly sold at RM 2.00 to RM 2.50. But 2-month-old seedling is more expensive which is sold for RM3.50-RM4.50, which contains 1-3 seedlings per polybag. For the ISR seedling, the production cost is approximately RM 1.80 to RM 2.10. The extra cost incurred for treating the seeds with Dieback Buster during sowing of seeds in tray and two booster application at 4th and 6th weeks after the young seedlings were transferred to polybags. Therefore, the retail price for ISR seedlings is expected more expensive in the range of RM5.00 to RM5.50 for 2-month-old seedlings; generally, the ISR seedling are bigger in size and more resistance against BD disease (Figure 43).

Figure 43: *2-month-old healthy and vigorous ISR papaya seedlings grown in polybags*

Economic benefit

The Malaysian National Agrofood Policy (2011-2020) (MNAFP) emphasises to increase export value of Malaysian fruits (Suntharalingam *et al*, 2015). Papaya is also one of the fruits listed in the MNAFP for export expansion. Malaysian papaya, especially our flagship varieties of Eksotika and Eksotika II, are already popular in countries such as Hong Kong, China, Singapore and Middle East where the consumers in those country appreciate the quality and taste of fruits of above varieties. In 2004, when Malaysian papaya export was on the peak (RM120 million), both Eksotika varieties contributed 60% of the export value. There is great demand for papaya from new markets of countries such as United Kingdom, Europe and USA, which are offering new opportunities for local farmers to increase the production and income. Therefore, a feasibility analysis was conducted to determine the economic viability of ISR technology compared to the conventional technology (non-ISR) (Table 5). This feasibility analysis was conducted to determine the viability of papaya production based on the ISR and conventional technologies for 10 years, where the papaya BD disease is badly affecting the papaya industry.

Table 5: Cost of production and feasibility analysis of papaya using ISR compared with conventional (non-ISR) techniques

Indicator	Conventional Technology (Non-ISR)	ISR Technology
Papaya survival period	10-12 months	24 months
Damage caused by Dieback disease	85-100%	0-5%
*Fruit production period	3-4 months	16 months
Exotica papaya yield/ ha / crop cycle	10 ton/ha	56.25 ton/ha
Price/kg	RM 4.00	RM 4.00
Production cost /ha	RM 20,065	RM 58,978
Gross revenue/ ha / crop cycle	RM 40,000	RM 225,000
Net revenue / ha / crop cycle	RM 19,935	RM 166,023
Net income/month	RM 1,661	RM 13,835
Financial analysis		
Net Present Value (NPV) @10%	7,439.72	700,529.82
Internal Return Rate (IRR) @ 10%	12%	65%
Benefit Cost Ratio (BCR) @10%	5.37	0.20
Payback period (PP)	7.95 year	3.52 year

Indicator	Conventional Technology (Non-ISR)	ISR Technology
Break-even point		
Fixed Cost (RM)	12,860.95	34,305.45
Average price/kg	4.00	4.00
Average of variable cost/kg	0.64	0.42
Break even point (tonne)	3.8	9.6
Break even point (sale)	15,302.68	38,286.83

Eksotika papaya is highly prized for its nutritional values and quality, and mainly cultivated for export purpose. The price of Eksotika papaya had fetched as high as RM 6.00/kg, however, the average farm price in 2018 was RM 4.00. Among the commercially important varieties available in Malaysia, Eksotika were the most vulnerable to diseases and pest.

Generally, the economic period for Eksotika papaya production is 2 years with 20% of the yield harvested in the first year and the remaining 80% in the second year (Chan *et al*, 2011; Chan and Sim, 2019). However, the emergence of papaya BD disease had shortened the survival period of Eksotika papaya to 10-12 months, mostly after that the whole plantation likely to be affected by this disease. The losses caused by the disease to the plantation can amount to 85-100%, thus, causes significant reduction in the yield. However, usage of ISR technology manage to maintain the productivity period of 2 year with 95% survival and produce bounty of yield as much as 56.25 tonne/ha/season of Eksotika papaya. The production cost for ISR technology is 2.5 times higher than the conventional but more profitable because usage of technology had generated more income contributed by higher yield harvested. The conventional production technique generated net income of RM 1,661/month, while for the ISR technology, the net income can go as high as RM 13, 835/month.

The Net Present Value (NPV) for ISR technique for 10 years was as high as RM 700,529.82 while NPV value for conventional technique was just RM 7,439.72. The positive NPV analysis value indicate that usage of ISR technology is profitable and sustainable. The IRR value for ISR technology was 5 times higher than the conventional production technique. The payback period (PP) is the time needed for capital repayment which funded for the year of project start and the project's capital return period was 3.52 years for ISR technology compared to conventional technique which needed 7.95 years. The project is more profitable and sustainable if the payback period is shorter, therefore, the ISR technology is more profitable than the conventional one.

Eksotika papaya production using ISR technology is more profitable than that with the conventional practices. Among the Malaysia fruits exported to Singapore, Hong Kong and Mainland China, papaya is one of the fruits with highest competitiveness compared to the other papaya exporting countries to above countries (Suntharalingam *et al*, 2011). Other than three countries mentioned above, the demand for papaya for new markets such as United Kingdom, USA, Europe and Middle East also very encouraging but the local papaya production is insufficient for local and export demand which was caused by the spread of dieback disease. The Malaysian papaya had lost it competitiveness to its competitor in world market mainly caused by the BD disease. The invention of ISR technology is only available efficient technique to control this disease in Malaysia, thus, the production and export can be increased. This technology will also increase the competitiveness of Malaysian papaya in global market.

Perception of Farmers

The ISR technology developed as a preventive measure against papaya BD disease. This technique is not efficient in curing the disease-infected plants, especially with the chronic infection. Many of the papaya producers only seek for Dieback Buster when the plants got infected with BD disease expecting that this product will cure their plants from disease infection. Since this product for preventive purpose, the best results in disease control can be only obtained when Dieback Buster was applied during seed sowing, booster application during nursery stage and continuous monthly application on the field until the production *i.e.* up to 2 years.

Generally, the farmers who used it as a preventive measure to control the disease had experienced good results and had given good testimonies about Dieback Buster and ISR technology as an efficient strategy to control the BD disease which generated better yield. All these farmers are willing to continuously use the ISR technology to maintain high papaya productivity at their farms and generate high income.

Conclusions

Malaysian papaya industry was pushed to the brink of collapse by emergence an alien invasive papaya DB disease. The papaya production and exports affected by the emergence of this disease. To resolve this issue, MARDI had developed an ISR technology to combat this deadly papaya disease. This technology was developed by bioprospecting of systemic inducing PGPR. The eight PGPR isolates with best disease control (100%), were used to produce seedling and tested on the open field (hotspot) in MARDI, where seven of them had demonstrated total control against papaya bacterial dieback disease. One of the isolates, MIB 106 was chosen for up-scaling activities. This technology was also validated again on three commercial farms (conventional production using chemical inputs) and for organic papaya production in MARDI. This technology doing well for organic as well as non-organic papaya production. ISR technology was protected by filing patent with Intellectual Property Corporation of Malaysia (MyIPO).

To ease the ISR inoculation application, a semi-mechanised ISR inoculation technique was developed by inventing an instrument which the diluted inoculant volume discharged can be adjusted. The ISR inoculant containing ISR inducing bacteria isolate MIB 106 was given brand name of ‘Dieback Buster’ and the ISR inoculant application instrument as Adjustable Volume Liquid Gun (AVLG). Both of this technology was launched in one of the farm where the validation was conducted. This package technology was commercialised to Arif Efektif Pvt. Ltd, a subsidiary of All Cosmos Pvt. Ltd. and the products available since April 2019 in the market for farmers usage. Since the technology commercialised, 393 ha of papaya had been benefitted from this technology. However, the COVID-19 had slowed down the usage of this technology.

Various seminars and trainings were conducted to disseminates the knowledge and technology transfer of this technology to stakeholders, farmers and extension agents.

Those farmers using this technology as prevention against papaya dieback disease had given good testimonies and interested to continuously using it. For time being this is only available solution to produce papaya with least losses due to papaya BD disease. ISR-AVLG technology package is the only available economically viable strategy to produce papaya in Malaysia. The government's goal to increase the papaya production and export only can be achieved by using this ISR technology. Therefore, the Department of Agriculture had developed an extensive programme to increase the papaya production by using this ISR-AVLG technology package.

The papaya production and export will be boosted with this new technology. ISR technology is the only available hope to rejuvenate the ailing Malaysian papaya industry to increase the production and export. It is hoped that one day Malaysia will bounce back as world's main papaya exporter when papaya can be produced successfully with eradication of papaya BD disease. It is also strongly recommended that ISR technology should be applied in papaya growing countries to prevent the BD disease in the Asia-Pacific region and beyond.

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