

Research Progress, Challenges And Future Perspectives On The Management Of Fusarium Wilt Of Banana In Malaysia: A Review

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ABSTRACT The global banana production is threatened by Fusarium wilt, a fungal soil-borne disease caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). In Malaysia, Foc Tropical Race 4 (Foc-TR4) is the most devastating disease that has completely wiped out the Cavendish plantations in 1990s. The TR4 strain attacks a diverse range of bananas and plantains, making disease management challenging. In this review, we have summarized the current status of Fusarium wilt in Malaysia, current knowledge on the epidemiology of Foc and recommended control measures outlined by the Department of Agriculture (DOA) in preventing and/or avoiding spread of the disease. The research progress on the control of Foc-TR4 in Malaysia was also presented to highlight the current findings and knowledge gaps in each approach. Recommendations for future studies were included to improve the current disease management approaches while possible challenges were provided for researchers, farmers and policy makers to consider.

Keywords: Banana, Disease management, Epidemiology, Fusarium wilt, *Fusarium oxysporum* f. sp. *cubense*

ABSTRAK Pengeluaran pisang sedunia diancam oleh penyakit layu Fusarium yang disebabkan oleh *Fusarium oxysporum* f. sp. *cubense* (Foc). Di Malaysia, Foc Tropical Race 4 (Foc-TR4) merupakan penyakit yang paling serius kerana kebanyakan ladang tanaman pisang Cavendish telah dimusnahkan pada 1990-an. Strain TR4 dapat menjangkit pelbagai jenis pisang dan plantain dan ini menyukarkan pengurusan penyakit secara efektif. Dalam artikel ini, kami merumuskan status terkini penyakit layu Fusarium di Malaysia, maklumat terbaru tentang epidemiologi Foc dan cara pengurusan penyakit yang disyorkan oleh Jabatan Pertanian Malaysia (DOA) dalam mengelakkan dan pencegahan daripada penyebaran penyakit tersebut. Kemajuan dalam penyelidikan bagi pengurusan Foc-TR4 di Malaysia juga dibentangkan untuk menunjukkan hasil kajian terkini dan juga kekurangan yang terdapat dalam setiap kajian selidik. Cadangan untuk penyelidikan seterusnya telah dijelaskan untuk menambahbaik cara pengurusan penyakit yang sedia ada dan cabaran yang mungkin dihadapi dalam penyelidikan juga dibentangkan untuk pertimbangan penyelidik, petani dan pihak berkuasa.

Kata Kunci: Epidemiologi, *Fusarium oxysporum* f. sp. *cubense*, Pengurusan penyakit, Penyakit layu Fusarium, Pisang

INTRODUCTION

In Malaysia, the National Agrofood Policy (2011-2020) has identified banana as one of the more important fruits to be developed for export markets alongside watermelon, papaya, starfruit and mango (Nik Rozana, Suntharalingam & Othman, 2017). In fact, Malaysia is ranked fourth in Asia for exporting Cavendish bananas (Truggelmann, 2013). About 15% of the locally produced bananas are exported to other countries such as Brunei, Singapore, Hong Kong and Middle Eastern countries, generating export revenue of more than US\$ 10 million (Tengku Ab. Malik, 2011). In terms of planting areas, bananas are the second most widely cultivated fruit crop, followed by pineapple and watermelon with approximately 294,000 metric tonnes produced in 2014, valued at about US\$ 24 million (Ministry of Agriculture, 2015). A total of 29,000 ha of land was allocated for banana production with major production areas located in Negeri Sembilan, Perak, Pahang, Johor, Sabah and Sarawak (Mohamad Roff, Tengku Abdul Malik & Sharif, 2012; Pauziah, Suhana, Rozeita & Maimun, 2017). However, the local banana production is often hampered by the infestation of pests and diseases, which can adversely affect its production in the long run.

Farmers often select popular cultivars for cultivation, also known as mono-cropping, to generate better profits but such action has caused low genetic variation among plantations, leading to increased susceptibility towards pests and diseases (Ghag, Shekhawat & Ganapathi, 2015). *Fusarium* wilt of banana or the Panama disease is one of the devastating soil-borne fungal pathogens that have

hampered banana production globally. This disease is caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) which specifically affects the *Musa* genus, including *Musa acuminata*, *M. balbisiana*, *M. schizocarpa* and *M. textilis* (Ploetz & Pegg, 2000). Currently, there are at least 24 vegetative compatibility groups (VCGs) identified in Foc and 21 of them are present across Asia and Australia (Mostert et al., 2017). Foc can also be divided into pathogenic races based on their pathogenicity towards the hosts. For instance, race 1 (R1) affects Gros Michel (AAA), Silk and Pome varieties (AAB) and Pisang Awak (ABB), whereas race 2 (R2) affects plantain ABB such as Bluggoe. Subtropical race 4 (SR4) causes diseases in R1, R2 susceptible varieties and R1 resistant Cavendish (AAA) in the subtropics. Tropical race 4 (TR4) affects the same varieties as SR4 but without the disease-predisposing cold temperature condition in the subtropics. Race 3 (R3) is not considered as part of Foc as it attacks *Heliconia* spp. (Ploetz, 2015).

Following the first outbreak of Foc-R1 in Panama and Costa Rica in 1890, the disease spread rapidly to Latin American countries where the susceptible Gros Michel bananas were widely grown for export. The Foc-R1 resistant Cavendish cultivars were soon planted but Foc-R4 soon emerged, causing massive destruction first in the subtropics and then tropics (Ploetz, 2005). Foc-TR4 is the most virulent strain reported to date, having wiped out Cavendish plantations in Indonesia, Taiwan and Malaysia in the 1990s (Ploetz, 2015). More than 50% of bananas planted in Malaysia are the highly susceptible Berangan and Cavendish which are largely exported to Indonesia, Thailand, Singapore and

Brunei (Mohamad Roff et al., 2012; Pauziah et al., 2017). Foc-TR4 also attacks other popular varieties grown locally for raw consumption such as Mas and Rastali and the plantain used for cooking or processing such as Awak, Abu and Raja (Mostert et al., 2017). Losses due to Foc-TR4 were estimated to be US\$ 14.1 million in Malaysia (Tengku Ab. Malik, 2013).

SYMPTOMS AND EPIDEMIOLOGY OF FOC

Foc-infected bananas often show typical wilting and yellowing of leaves (Figure 1A). Foc spores, present in soil, adhere to the secondary or tertiary feeder root caps one to two days post-infection (Li et al., 2017) (Figure 2A). Infection rarely occurs at the main root unless it is damaged (Dita et al., 2008). The spores then germinate upon stimulation by banana root exudates, followed by root invasion through hyphae penetration into the cell wall or wounds (Li et al., 2017) (Figure 2B, C). Lesions are often observed at infection site and the necrosis of root is observed (Figure 1B). Foc secretes various cell wall degrading enzymes such as pectin methylesterases (PMEs) to digest the root cell wall during the course of invasion (Wu et al., 2017). The hyphae first extend into the root cortex and corm tissues. Foc colonization of the vascular bundles in the corms interferes with the water and nutrient uptake to the pseudostem and leaves (Li et al., 2017). Once the rhizome is colonized, the infection becomes systemic, reaching to xylem vessels of the pseudostem.

As the Foc invades the vascular tissues, a large amount of microconidia,

macroconidia and chlamydo spores is constantly produced and rapidly distributed throughout the whole plant via the host transpiration system (Li et al., 2011) (Figure 2D,E). The hyphae continue to grow fastidiously, eventually blocking the xylem vessel and causing poor efficiency in water uptake (Yadeta & Thomma, 2013). Brown or purple strands are easily observed in corm tissues and vessels as a sign of Foc colonization (Figure 1C). As water uptake is restricted, progressive wilting of older leaves and the collapse of dead leaves form a skirt around the split pseudostem (Figure 2F). During the advanced stages of Foc invasion, corm discoloration intensifies and the plant completely withers (Figure 1D). Foc feeds saprophytically on dead tissues and produces chlamydo spores that remain viable for more than 30 years and will result in another wilt outbreak upon direct contact with susceptible banana roots (Figure 2G). Li et al. (2011) also reported that chlamydo spores are constantly produced from thickened hyphae structures during host invasion even before visible symptoms are observed. This is the reason why Foc is also known as a 'polycyclic' disease in which multiple cycles of infection can occur in Foc-infested plantations (Buddehagen, 2009).

Foc is disseminated mostly through infected rhizomes, which farmers use as planting materials in disease-free fields (Dita et al., 2018). Irrigation waters are also a source of infection and irrigating bananas from contaminated sources has caused rapid spread of the disease along river basins (Ploetz, 2005). Contaminated vehicle tires, farming tools, clothes and footwear also contribute to the Foc spread from farm to farm (Dita et

al., 2018). A recent report stated that the occurrence of nematodes, *Pratylenchus* sp. has been correlated with high levels of Foc population in banana plantations (Almeida et al., 2018). Meldrum et al. (2013) also detected TR4 propagules on the exoskeletons of banana weevils, *Cosmopolites sordidus*. Both findings suggested that nematodes and weevils could be possible vectors that transmit Foc spores. Foc was also found to colonize the roots of weeds such as *Chloris inflata*, *Euphorbia heterophylla*, *Tridax procumbens* and *Cyanthilium cinereum*, easily spreading the disease if proper weed management is not performed (Hennessy, Walduck, Daly & Padovan, 2005).

RECOMMENDED CONTROL MEASURES IN MALAYSIA

In order to delay the disease intensity or to avoid the spread of Foc-TR4, the Malaysian Department of Agriculture (DOA) has strongly advocated the practice of good

agricultural practice (GAP) policy among banana growers (Pauziah et al., 2017). These practices include the use of disease-free planting materials derived from tissue-cultured banana seedlings, providing good water drainage system, disinfecting farming tools and, burning and burying infected plants. Regular surveillance is also carried out by DOA extension officers in banana farms registered under the Permanent Food Production Parks and Orchard Groups (TKPM) (Husain & William, 2009). At the same time, training courses, disease diagnostic services and information pamphlets are provided for farmers to identify, monitor and manage Foc-infected plants. In terms of enforcing biosecurity measures to reduce the spread of Foc, DOA imposes strict plant quarantine act 1976 to quarantine any imported planting materials and only issues the phytosanitary certificates once these materials are disease-free (Husain & William, 2009). Infected fields are also quarantined to restrict the movement of disease through humans, tools and planting materials (Pauziah et al., 2017).

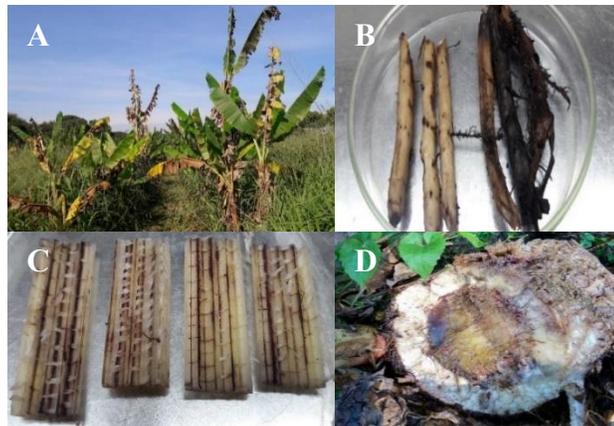


Figure 1. The disease symptoms caused by Foc-TR4 in Malaysia. Disease symptom include (A) Wilting or yellowing of leaves, (B) root necrosis, (C) vessel discoloration and (D) corm discoloration.

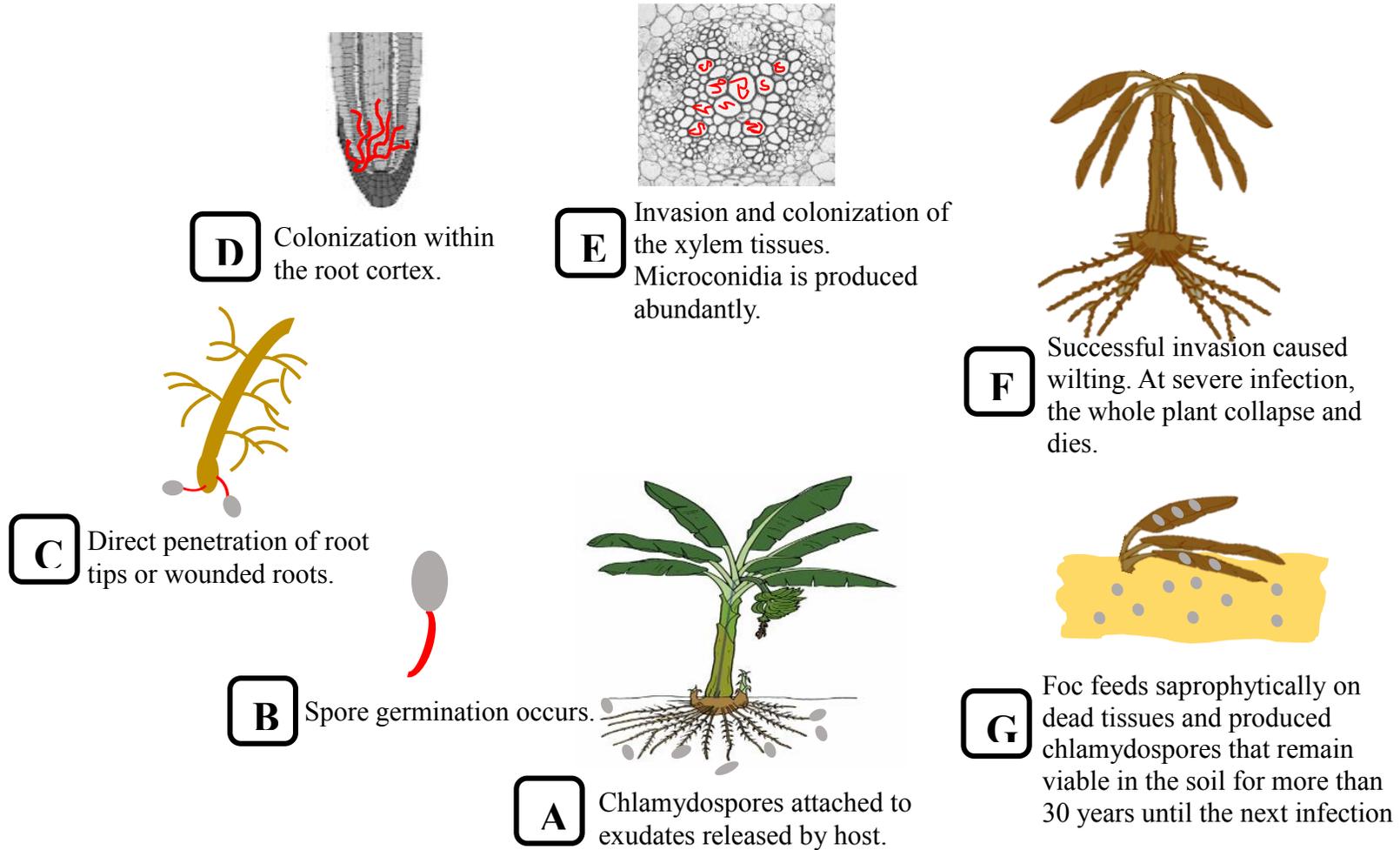


Figure 2. The disease cycle of a typical Foc infection in banana plants

CURRENT RESEARCH PROGRESS OF FUSARIUM WILT MANAGEMENT IN MALAYSIA

Practicing exclusion of diseased planting materials and farm quarantine are regular standards adopted in many parts of the world to contain the spread of a disease. However, growers might not be able to plant bananas again in the same infected field unless other crops are cultivated. Other disease management alternatives have emerged and a growing number of research has indicated promising findings in controlling Foc in Malaysia by employing these approaches (Table 1 and 2). The following discusses the current research that has been carried out in Malaysia to manage Foc.

Mutation breeding and transgenic approach

Mutation breeding involves the use of chemical mutagens, irradiation and long-term *in-vitro* culture of plant tissues to create somaclonal variants or mutants that exhibit enhanced tolerance towards Foc (Ghag et al., 2015). Bananas that survived in Foc-infested fields can also be selected as potential tolerant mutants (Mak, Mohammed, Liew & Ho, 2001). Malaysia has successfully produced a Foc-TR4 resistant mutant from Rastali (AAB), also known as Rastali Mutiara, derived from a rigorous selection of susceptible Rastali in infested field (Mak, Ho & Tan, 1998, Mak et al., 2001, Yang, Sathyapriya & Wong, 2016). Meanwhile, the first field trial of chronic gamma irradiated banana mutants in Foc hotspot around Malaysia is still ongoing (Pauziah et al., 2017). These lines have to undergo multi-location field trials in order to evaluate their disease resistance and agronomic traits. Such screening trials

may take decades before they are commercially planted and marketed.

Transgenic bananas showing improved tolerance towards Foc-R1 and TR4 have been developed but no data on field trials is available in Malaysia. The transgenic Rastali (AAB) and Nangka (AAB) genotypes have enhanced disease tolerance under glasshouse conditions but no field trial validated the consistency of these promising lines (Maziah, Sariah & Sreeramanan, 2007; Sreeramanan, Maziah, Sariah, Puad & Xavier, 2009; Mahdavi, Sariah & Maziah, 2012). Under the Malaysian Biosafety Act 2007, two field trials involving transgenic papaya and rubber tree were approved in 2013 and 2015, respectively (Andrew, Ismail & Djama, 2017). Even though transgenic technology is still at its infancy in Malaysia, the local government has invested resources in developing policies and regulations on biotechnology, in which the technology is viewed as a possible way to sustain the agriculture productivity (Pillai & Bakar, 2007; Andrew et al., 2017). Therefore, bringing transgenic bananas from the laboratory to the field is possible and crucial as the first step towards commercializing local transgenic bananas resistant to Foc-TR4.

Biological control

Biological control (biocontrol) refers to the use of microbial antagonists or biological control agents (BCAs) to suppress plant diseases. BCAs are free-living microorganisms found in soils and rhizospheres; some even live within the host plants (Gray & Smith, 2005). Promising results were frequently reported after the application of BCAs to manage Foc-TR4 (Table 1). The non-pathogenic *F. oxysporum* (Fo), a common

endophyte in banana plants, is known for its antagonistic effect towards Foc-TR4 (Ting, Meon, Kadir, Radu & Singh, 2008; Ting, Meon, Kadir, Radu & Singh 2009b, Ting, Sariah, Kadir & Gurmit, 2009c). However, the use of Fo should be carefully evaluated because some strains of Fo could enhance the severity of Fusarium wilt and the possibility of horizontal gene transfer may transform Fo into pathogens (Forsyth, Smith & Aitken, 2006; Ma et al., 2010). Other microbial genus including *Pseudomonas*, *Bacillus*, *Herbaspirillum*, *Streptomyces* and *Serratia* were widely applied as single strain or as a consortium to reduce the disease severity caused by Foc-TR4 (Table 1). Though some BCAs are potential antagonists, it is advisable to validate if they are potential human pathogens. For instance, *Serratia marcescens* are found ubiquitously in soil and water but at the same time, they are reported to cause clinical infections (Mahlen, 2016).

Most biocontrol research carried out in Malaysia are often confined to lab and glasshouse conditions. A single seven-month field trial indicated that a single application of BCAs resulted in only 3-11% of banana plants that survived in a Foc-TR4 hotspot (Ting et al., 2009c). Biocontrol efficacy of BCAs under field conditions has to be assessed and other influencing factors including dosage, time of application, formulating BCAs and environmental changes have to be taken into account in order to validate their control efficacy under glasshouse conditions. To further understand the complexity of soil-pathogen-plant pathogen after a BCA application, recent developments in the next generation sequencing (NGS) platforms such as metagenomics has enabled the

understanding of microbial changes that lead to disease suppression as well as identifying specific microbial genus with antagonistic properties (Dita, Barquero, Heck, Mizubuti & Staver, 2018). Shen et al. (2015) isolated *Bacillus amyloliquefaciens* strain NJN-6 from a suppressive soil from which the metagenomics data revealed that the *Bacillus* genera was the most abundant in the suppressive soil. Such discovery has led to the production of microbial fortified compost that has shown positive results in Foc-TR4 management in China (Xue et al., 2015; Fu et al., 2017). In other words, isolating potential BCAs should be based on microbial community profile instead of targeting specific microorganisms. Such strategy allows more effective disease management by improving microbial persistence and recovering the diversity of indigenous microbiota in the soil (Dita et al., 2018).

Soil amendment

The effect imposed by nitrate (NO_3^-) resulted in the reduction of disease severity of Foc whereas the application of ammonium (NH_4^+) increased disease severity (Ploetz, 2015). Since Foc thrives in acidic soil especially when the NH_4^+ level is high, amending soil with nitrate is suggested to make the soil less acidic and thus, minimizing root penetration by Foc (Mur, Simpson, Kumari, Gupta & Gupta, 2016). Amending soil with NO_3^- based salt such as calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) reduced disease severity of Foc-TR4 by up to 40% under glasshouse conditions but the soil pH was not measured before and after treatment (Ting, Meon, Jugah & Anuar, 2003). Therefore, it is essential to further understand the interaction between soil pH and the application of NO_3^- which leads to disease suppression

under field conditions. Since disease suppressive soil is the result of complex host-microbial interactions in nature (Alabouvette, Olivain, Migheli & Steinberg, 2009), it is also interesting to understand the effect of different nitrogen (N) sources on the soil microbiome community and how such changes directly affect disease-controlling efficacy.

The application of organic amendments derived from agriculture wastes holds great promise in managing Foc-TR4. The oil palm and rice industries in Malaysia are actively producing agriculture by-products such as empty fruit bunches and rice straw which could be effectively used for composting. Composts are commonly enriched with specific microorganisms or BCAs to achieve increased suppressive and consistent effect in disease control (Dita et al., 2018). It is also vital that composts are completely matured or decomposed to prevent adverse effects on the roots and soil pH changes which could predispose plants to diseases (Nasir, Pittaway & Pegg, 2003). In fact, BCA-fortified composts produced from rice straw and oil palm fruit bunch showed effective control on fungal pathogens while improving the overall vegetative growth and crop yield albeit most studies were conducted in glasshouses (Kausar et al., 2012; Ili Nadhrah, Nulit, Nurrashyeda & Idris, 2015; Mukhlis et al., 2017; Ng, Sariah, Radziah, Zainal Abidin & Sariam, 2017). In China alone, the continuous application of *B. amyloliquefaciens*-fortified composts increased microbial diversity in soil thereby suppressing Foc-TR4 in the long run under field conditions (Xue et al., 2015; Fu et al., 2017). Hence, future research should be driven towards the use of oil palm and rice straw-based

composts supplemented with BCAs to evaluate their disease controlling efficacy against Foc-TR4 under local field conditions.

Intercropping

Intercropping is a cultural method to reduce the accumulation of host-specific pathogens in the soil by planting non-susceptible host plants (Huang et al., 2012). Compared to monocultures, mixed plantings with bananas grown together with other crops lead to moderate losses and are suitable for small-scale growers (Ploetz, 2005). Plants from the *Allium* family were largely utilized in intercropping due to their production of anti-fungal volatile compound (Zhang, Mallik & Zeng, 2013). Nadarajah, Sreeramanan & Zakaria (2016) reported that the disease incidence of Foc-TR4 was reduced when banana cultivar 'Grand Naine' cultivar was intercropped with Chinese chives (*Allium tuberosum*) but the disease was not suppressed when intercropped with bananas cultivar 'Lemak Manis' and 'Berangan Intan'. Pauziah et al. (2017) further evaluated three *Allium* sp. but the disease suppression in field was not as effective as the glasshouse trials. Such findings did not agree with previous successful intercropping instances in managing Foc in Indonesia and China (Huang et al., 2012; Wibowo et al., 2015). Perhaps, the release of antifungal compounds from *Allium* sp. could be dependent on other factors such as pathogen strain, soil type, fertilization, banana genotypes, and environmental changes which require further investigation. The colonization capacity of Foc on other crops should also be taken into consideration as a precautionary measure to the disease spread. Other *forma speciales* of Fo were

reported to colonize and infect commonly grown rotation crops (Scott, McRoberts & Gordon, 2014; LaMondia, 2015).

FUTURE PERSPECTIVES AND CHALLENGES AHEAD

Malaysia is one of the center of banana diversity, providing a genepool for breeding of resistant cultivars towards Foc-TR4 (Heslop-Harrison & Schwarzacher, 2007). Unfortunately, most banana cultivars are genetically polyploid, pathernocarpic and sterile in nature, making conventional breeding a technically challenging and time-consuming task (Ortiz & Swenen, 2014). Furthermore, wild diploids have extremely poor agronomic traits and the introgression of disease resistance traits into present cultivars require several generations of screening and selection. To overcome long cropping cycle and large cultivation area for banana breeding, molecular tools such as genomic-assisted breeding could simplify screening of resistant genotypes by employing molecular markers that target specific traits of interest (Friedmann et al, 2018). With the release of genomic sequence of *Musa acuminata* subspecies *malaccensis* 'DH-Pahang', comparative genomic studies with other banana subspecies have led to the discovery of novel alleles which could greatly facilitate banana breeding (Friedmann et al., 2018). So far, banana breeding in Malaysia is still largely unheard of. Realizing the importance of the local banana diversity, global collaborations can be easily established to rapidly screen for potential Foc-TR4 resistant cultivars for future breeding programmes.

Biotechnological approach of breeding for disease-resistant bananas remains as an attractive approach compared to plant breeding. Foc-TR4 resistant *M. acuminata* cv. Rastali Mutiara (AAB) was first developed from mutation breeding in 1999 (Muhammad & Othman, 2005). Since then, there has been no release of new resistant cultivars for about two decades in Malaysia. Other emerging biotechnology approach such as CRISPR/Cas9 genome editing has been applied in agricultural crops to improve agronomic traits including disease resistance; these crops do not fall under the classification of genetically modified organisms (GMO) in many countries (Jaganathan, Ramasamy, Sellamuthu, Jayabalan & Venkataraman, 2018). As more disease-resistance traits are discovered in bananas following the release of the banana genome sequence, producing resistant bananas using CRISPR is encouraging and time-saving as it bypasses the regulations of GMO crops. However, the regulatory framework for genome-edited crops has yet to be established in Malaysia. It is unknown whether field trial is permissible. The glasshouse experiments on CRISPR crops can still be conducted and such findings will be a significant breakthrough in banana breeding.

Other alternative approaches such as biological control, soil amendment and intercropping have been conducted locally and proven effective against Foc-TR4. Additionally, more field trials must be carried out to evaluate the disease-controlling efficacy of these methods in the long run. Though longer time is required to achieve the desired effect, these practices could improve plant productivity, reduce disease severity of

Foc and improve the control of other pest and diseases (Haddad et al., 2018).

On the other hand, the presence of nematodes was found in areas with high Foc density. The nematodes were suggested to cause wounding in banana roots and facilitate in the colonization of Foc as previously described (Almeida et al., 2008). Banana weevil borers might be vectors of Foc by carrying spores on their exoskeletons (Meldrum, Daly, Tran-Nguyen, & Aitken, 2013). The presence and putative role of weevil borers and nematodes in spreading Foc requires further validation in Malaysian farms. Biocontrol of these pests is an alternative for researchers to identify potential BCAs that show antagonistic effects towards nematodes, weevils and Foc at the same time. The entomopathogenic fungus, *Beauveria bassiana* was reported to possess nematicidal, fungicidal and pesticidal properties which might be a suitable BCA candidate (Ownley, Pereira, Klingeman, Quigley & Leckie, 2004; Kepenekei, Saglam, Oksal, Yanar & Yanar, 2017).

Integrated management is recommended to manage a complex agro-ecosystems. Dita et al. (2018) suggested that management strategies could be deployed according to the disease intensity within a farm. Briefly, plot quarantine and exclusion of planting materials are suitable when Foc is first detected. Planting resistant cultivars, crop rotation or plot eradication are proposed for farms with severe Foc infestations. Nevertheless, evaluating a farm's disease intensity and providing suitable management tactics are challenging as they require the cooperation from farmers, agriculture extension officers and policy makers.

In Malaysia, banana cultivation is mostly carried out in small and unorganized farms. They often adopt conventional farm management, leading to disease outbreak, and low yield and fruit quality (Kayat, Mohammad Amizi, Idris, Ibrahim, & Soon, 2018). Some farmers may still use traditional planting methods by using suckers which are the main source of Foc spread. Some may lack the knowledge to identify disease, manage infected plants and sanitize farming tools. Most importantly, they may not be aware of the recent technology that can assist them in sustaining banana production when Foc is present. The farm would most likely be abandoned or planted with other crops. Compared to large plantations or farms registered under TKPM, small farms are often neglected. Frequent surveillance, farm data collection and dissemination of information should be carried out to reach out to these smallholders. In a way, the cause of disease spread as a result of negligence can be easily managed.

Finally, the emergence of new and unknown Foc VCGs or strains have been reported in Malaysia (Mostert et al., 2017). Any future disease management strategies should consider these new strains before they turn into a major outbreak. Biosecurity measures should also pay special attention to other strains than TR4 to prevent the spread of more virulent Foc to and from other countries. Researchers and farmers should not be easily contented because a resistant variety is available. Strict quarantine procedures must be followed to ensure clean planting materials are used.

Table 1. Biological control research and their effectiveness in controlling Foc-TR4 in Malaysia

BCAs	Outcomes	Field/Glasshouse/Others	Reference
<i>Bacillus subtilis</i>	Soil application of <i>B. subtilis</i> reduced DS by more than 80% under severe water stress condition. DS increased in mild water stress and well-watered conditions.	Rain shelter	Din et al. (2018)
<i>Streptomyces griseus</i>	Kaolin-formulated <i>S. griseus</i> cells reduced Foc spores in soil compared to alginate beads. Both kaolin and alginate based crude extract from <i>S. griseus</i> did not reduce Foc spores.	n.d.	Zacky & Ting (2015)
<i>Streptomyces griseus</i>	Soil application of <i>S. griseus</i> cells was more effective in reducing Foc spores in soil compared to using crude extract with 6 log ₁₀ and 7 log ₁₀ CFU/mL enumerated respectively.	n.d.	Zacky & Ting (2013)
<i>Penicillium citrinum</i>	Pre-inoculation of plantlets with <i>P. citrinum</i> resulted in slower disease progression and reduced DS at 58%	n.d.	Ting et al. (2012)
<i>Herbaspirillum</i> sp. and <i>Pseudomonas</i> sp.	The presence of three volatile compounds such as 2-pentane 3-methyl, methanethiol and 3-undecene in both BCAs showed <i>in-vitro</i> antifungal properties.	n.d.	Ting et al. (2011b)
<i>Serratia marcescens</i>	<i>S. marcescens</i> formulated with bentonite was more efficient in inhibiting Foc spores in soil compared to kaolin formulation with 4.06 and 4.28 log ₁₀ CFU/mL enumerated respectively.	n.d.	Ting et al. (2011a)
<i>Serratia marcescens</i>	Pre-inoculation of plantlets with <i>S. marcescens</i> showed a reduction of DS at 50%.	Glasshouse	Ting et al. (2010b)
Unidentified fungal endophytes	Several important antifungal volatile compounds were detected from fungal endophytes. Isolates that produced lesser metabolites tend to have higher inhibitory activity.	n.d.	Ting et al. (2010a)

<i>Pseudomonas</i> sp. and <i>Burkholderia</i> sp.	Pre-inoculation of plantlets with <i>Pseudomonas</i> sp. reduced DS by 51% while <i>Burkholderia</i> sp. only showed 38% reduction. Application of both BCAs reduced DS by 39%.	Glasshouse	Fishal et al. (2010)
<i>Serratia marcescens</i>	No significant differences in <i>in-vitro</i> inhibition of Foc-TR4 when bentonite-formulated and non-formulated <i>S. marcescens</i> were applied.	n.d.	Ting et al. (2009a)
Non-pathogenic <i>Fusarium oxysporum</i> and <i>Serratia marcescens</i>	Single application of one or combination of BCAs resulted in the survival of 3-11% of plants at the end of week 28.	Field (7 months)	Ting et al. (2009c)
Non-pathogenic <i>Fusarium oxysporum</i>	Soil application of <i>F. oxysporum</i> resulted in reduction of DS by up to 80% with induced systemic response of banana plantlets	Glasshouse	Ting et al. (2009b)
Non-pathogenic <i>Fusarium oxysporum</i> and <i>Serratia</i> sp.	Pre-inoculation of single or a combination of BCAs resulted in improved vegetative growth when challenged with Foc-TR4.	Glasshouse	Ting et al. (2008)
<i>Streptomyces</i> sp.	Soil application or root drenching of plants reduced disease severity by about 53%.	Glasshouse	Getha et al. (2005)
<i>Streptomyces violaceusniger</i>	<i>S. violaceusniger</i> showed <i>in-vitro</i> antagonism activity towards Foc.	n.d.	Getha & Vikineswary (2002)

*DS, disease severity; n.d., not determined

Table 2. Other management approaches employed and their outcomes in Foc management in Malaysia

Management approach	Outcomes	Foc strain	Field/Glasshouse/ Others	Reference
Mutation breeding	Successfully produced Foc-TR4 resistant <i>M. acuminata</i> cv. Rastali Mutiara (AAB) through continuous selection in infested field.	TR4	Field	Mak et al. (1998)
	First field trial conducted on chronic gamma irradiated banana mutants in Foc hotspots. Results are pending.	n.d.	Field	Pauziah et al. (2017)
Transgenic technology	Transgenic <i>M. acuminata</i> cv. Rastali (AAB) harbouring either single or both β -1,3-glucanase and chitinase genes showed enhanced disease tolerance. No reduction of DS estimated.	R1	Glasshouse	Sreeramanan et al. (2006)
	Transgenic <i>M. acuminata</i> cv. Rastali (AAB) harbouring β -1,3-glucanase gene showed enhanced disease tolerance. No reduction of DS estimated.	R1	Glasshouse	Maziah et al. (2007)
	Transgenic <i>M. acuminata</i> cv. Nangka (AAB) harbouring thaumatin-like protein gene reduced DS by up to 70%.	TR4	Glasshouse	Mahdavi et al. (2012)
Soil amendment	Application of Ca(NO ₃) ₂ reduced disease severity by up to 49%.	TR4	Glasshouse	Ting et al. (2003)
Intercropping	Control of Foc-TR4 in field intercropped with <i>Allium tuberosum</i> was genotype-dependent. The 'lemak manis' exhibited the lowest DS at 2.8% followed by Grand Naine at 27.8% and lastly <i>M. acuminata</i> cv. Berangan Intan at 87.5%	TR4	Field	Nadarajah et al. (2016)
	Intercropping with <i>A. tuberosum</i> , <i>A. cepa</i> var. <i>aggregatum</i> and <i>A. ampeloprasum</i> var. <i>porrum</i> showed positive disease control in glasshouse but subsequent field trial was not satisfactory. No revealed DS.	TR4	Glasshouse and field	Pauziah et al. (2017)

*DS, disease severity; n.d., not determine

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