REPORT

VIRTUAL WORKSHOP SERIES ON

Safeguarding the Banana Industry from Fusarium Wilt: Research Updates and Opportunities in the Asia Pacific

20 Oct/27 Oct/3 Nov/10 Nov 2020

EDITORS:
Dorothy Chandrabalan
Yacob Ahmad

ORGANIZERS:
- International Tropical Fruits Network (TFNet)
- Australian Centre for International Agricultural Research (ACIAR)
- Guangdong Academy of Agricultural Sciences (GDAAS)
- Chinese Academy of Tropical Agricultural Sciences (CATAS)
- Alliance of Bioversity International & International Center for Tropical Agriculture (CIAT)

INTERNATIONAL TROPICAL FRUITS NETWORK

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International Tropical Fruits Network (TFNet)
Box 334, UPM Post Office,
43400 Serdang, Selangor,
Malaysia.
Tel: 603-89416589
Fax: 603-89416591
Website: www.itfnet.org
E-mail: info@itfnet.org

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EXECUTIVE SUMMARY

Banana wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* TR4 is one of the most devastating disease to impact the global banana industry. The dire threat to the industry has prompted research institutions to seek for solutions, including selection of resistant cultivars, best farm and cultural practices and strengthening biosecurity requirements to contain the spread of this dreaded disease.

A four-session virtual workshop on 'Safeguarding the Banana Industry from Fusarium Wilt: Research Updates and Opportunities in Asia Pacific was held in October and November 2020 to provide latest updates and explore the various research initiatives that are being carried out to combat the disease. The workshop was jointly organized by the International Tropical Fruits Network (TFNet), the Australian Centre for International Agricultural Research (ACIAR), Guangdong Academy of Agricultural Sciences (GDAAS), Chinese Academy of Tropical Agricultural Sciences (CATAS), and the Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT).

TFNet Chairperson, Honorable Datuk Haji Zainal Azman bin Abu Seman, also the Secretary General of the Malaysian Ministry of Agriculture and Food Industry (MAFI) was given the honor to officiate the workshop.

The objectives of the workshop were to:

a. Share information on current research and technologies in mitigating the impact and spread of banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* TR4;
b. Share experiences in the different countries on control and management of the disease;
c. Enhance awareness of the dangers of the disease to all participating countries to encourage regional cooperation in combating the disease;
d. Strengthen quarantine procedures among participating countries to prevent disease spread;
e. Prepare a summary and analysis of recommendations to partners and countries.

The workshop successfully gathered twenty-four researchers and experts who presented and discussed topics that are currently most relevant in the initiatives to manage the *Foc* TR4 banana wilt disease, at the same time providing focus areas that need further attention. It also managed to garner interest from both the research fraternity and industry alike with participation from governments, research institutions, universities, civil societies, NGOs, industry players, international organizations and networks. Presenters and participants were represented from more than 16 countries spanning Asia and the Pacific, Africa, Europe and Latin America, and the Caribbean.

The main themes of the workshop sessions and number of participants were:

Table 1. Workshop series and participant number

Session Date	Workshop themes		No. of participants
20 October	A.	Country reports on background and updates on *Foc* TR4 prevalence, containment measures and research initiatives	58
27 October	B.	Biodiversity, evolution and interaction mechanism between pathogen and host	65
3 November	C.	Breeding initiatives for resistance against *Foc* TR4	82
10 November	D.	Integrated control measures and sustainable field management practices	85

The first session A, began with an introduction to the World Banana Forum's *Foc* TR4 global network's initiative to encourage collaboration in the task of mitigating the impact of the disease and to sustain the global banana industry. This was followed by country presentations on disease status, management and research programs by researchers from Australia, China, Colombia, Laos, Malaysia, India, Mozambique, Indonesia and Philippines. The session gave an overall picture of the various *Foc* TR4 programs and strategies adopted in these countries, the successes achieved and setbacks faced in furthering research on the disease. The session clearly portrayed the dynamic research and the levels of efforts (and possible disparities in resources) being undertaken, some of which are still preliminary while others in more advanced stages, thus signaling the need for collective efforts to be scaled up to a higher level of integration into a global program of work. Research on containing the disease is also dependent on degree of seriousness of disease prevalence, available resources to mitigate infection or prevent spread and availability of assistance or collaboration with other entities (research institutions or projects).

Session B saw deliberations from five experts on work being done to elucidate the various aspects of interaction mechanisms between pathogen and host, mostly concerning the effect of fusaric acid and beauvericin. Discussions indicated that pathogenicity varies according to the different strains or VCGs of the fungus. At the end of this session, some key research areas were recommended for further pursuit and this included greater investment in gene editing technologies, importance of accessing germplasm diversity for screening of resistance, the need to improve access to transgenic lines, and make available affordable in-field detection methods especially for resource-poor growers.

Session C on breeding initiatives brought in four experts who provided insights into the status of activities both conventional selection of resistant cultivars and resistant breeding through genetic modification. The session highlighted the importance of durable resistance due to the evolving nature of the pathogen and environment. Recommendations suggested on durable resistance include identifying other resistance genes and stacking them.

The final session D dealt with the integrated control measures and sustainable management practices developed to suppress the level of pathogen in the soil, which includes managing functional microbiomes, use of organic fertilizers and application of cover crops and alternate hosts in banana production systems in addition to the cultivation of resistant cultivars as versatile strategies in suppressing inoculum in soil.

A panel discussion to review the insights gained from the four sessions agreed on the importance of information sharing and collaborations among country researchers that could complement and strengthen prioritized areas to better manage the disease. The workshop provided useful information which had a catalytic effect on identifying priorities for future global collaborative work on prevention of banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4.

Areas of collaborative research highlighted during the workshop were as follows:

a. More collaboration is needed to understand virulence factors and the mechanism of host-pathogen relationship, focusing on gene editing technologies, especially in banana receptor genes to improve resistance to TR4.
b. Further assessment and screening of germplasm diversity for resistance
c. The need to have more access to transgenic banana cultivars that are resistant to *Foc* TR4
d. More studies on reliable detection systems and techniques to ascertain pathogen presence and load in affected soils, water and other systems that are quick and easy to use by resource-poor smallholders.
e. More studies on the diversity and characterization of VCGs and their pathogenicity.
f. Breeding of genetically edited bananas for resistance to *Foc* TR4 is currently being carried out and there is the potential of collaboration in countries to demonstrate 'durable' resistance of the varieties in the different climatic regimes such as humid tropics and subtropical areas.
g. Using protoplast culture in the regeneration of plants with resistant traits to TR4.
h. Soil microbiome health, cover cropping, rotational and mixed cropping, alternative hosts and use of bioagents are some of the field management aspects of controlling TR4 that warrant further investigations.
i. Further studies on crop rotation using paddy to suppress *Foc* TR4

ABBREVIATIONS

1. ABGC – Australia Banana Growers Council
2. ACIAR – Australian Centre for International Agricultural Research
3. ASD – Anaerobic soil disinfestation
4. At CERK 1 – Chitin elicitor receptor kinase 1 (*Arabidopsis thaliana*)
5. BEA – Beauvericin
6. CATAS - Chinese Academy of Tropical Agricultural Sciences
7. CIAT – International Centre for Tropical Agriculture
8. CRISPR – Clustered regularly interspaced short palindromic repeats
9. EAHB – East Africa Highland Banana
10. ECS – Embryonic cell suspension
11. ETI – Ellicitor triggered immunity
12. FA – Fusaric acid
13. FAO –Food and Agriculture Organization of the United Nations
14. Foc TR4 – *Fusarium oxysporium* f. sp. *cubense* Tropical Race 4
15. Fol – *Fusarium oxysporium* sp. *lycopersici*
16. FOSC – *Fusarium oxysporium* species complex
17. FWD – Fusarium wilt disease
18. GCTCV – Giant Cavendish Tissue Culture Variant
19. GDAAS – Guangdong Academy of Agricultural Sciences
20. GFP – Green Flourescense Protein
21. HIGS – Host induced gene silencing
22. ICAR – Indian Council for Agricultural Research
23. IPPC – International Plant Protection Convention
24. IPM – Integrated pest management
25. ISR – Induced Systemic Resistance
26. ITC – International Transit Centre
27. ITFRI - Indonesian Fruits Research Institute
28. LAC – Latin America and the Caribbean
29. LAMP – Loop mediated Isothermal Amplification
30. LC-MS – Liquid chromatography mass spectroscopy
31. LyK – Lysin receptor kinase
32. LyM – Lysin motif receptor
33. Lj NFR1 – Nod factor receptor 1 (*Lotus japonicus*)
34. MAMPS – Microbe associated molecular patterns
35. MaIYK1 – Lysin motif containing receptor like kinase
36. Myc-LCOs – Mycorrhiza lipo chitin oligosaccharides
37. NBS LRR – Nucleotide binding site leucine rich repeats
38. NRCB – National Research Centre for Banana, India
39. OTU – Operational taxonomic unit
40. PTI – Pattern triggered immunity
41. PHI – Plant host interaction
42. PCR – Polymerase chain reaction
43. QUT – Queensland University of Technology
44. RGA – Resistant gene analogue
45. ROS – Reactive oxygen species

46. SDN – Site directed nuclease
47. SSR – Simple sequence repeats
48. SIX – Secreted in xylem
49. TFNet – International Tropical Fruits Network
50. TR4GN – TR4 global network
51. VCG – Vegetative compatibility groups
52. WBF – World Banana Forum
53. YAAS – Yunnan Academy of Agricultural Sciences

MEMBERS OF THE ORGANIZING COMMITTEE

1. Mr. Yacob Ahmad – TFNet (International Tropical Fruits Network)
2. Ms. Dorothy Chandrabalan – TFNet
3. Mr. Christian Cangao – TFNet
4. Dr. Yi Ganjun – GDAAS (Guangdong Academy of Agricultural Sciences)
5. Dr. Li Chunyu – Fruit Tree Research Institute, GDAAS
6. Ms. Irene Kernot – ACIAR (Australian Centre for International Agricultural Research)
7. Mr. Guanglin Wang – ACIAR
8. Ms. Tamaya Peressini – ACIAR
9. Dr. Wang Wei – CATAS (Chinese Academy of Tropical Agricultural Science)
10. Dr. Jun Peng – CATAS
11. Dr. Sijun Zheng – Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT) / YAAS (Yunnan Academy of Agricultural Sciences)
12. Dr. Rosie Godwin – ABGC (Australian Banana Growers Council)

Contributing Rapporteur

Dr. Lina Liu – YAAS

Report Layout

Mr. Christian Cangao – TFNet

20TH OCTOBER 2020

SESSION A: COUNTRY REPORTS ON BACKGROUND AND UPDATES ON FOC TR4 PREVALENCE, CONTAINMENT MEASURES AND RESEARCH INITIATIVES

SESSION CHAIR: Yacob Ahmad, International Tropical Fruits Network (TFNet)

1. **Mr. Victor Prada, Secretary, World Banana Forum, EST, Food and Agriculture Organization of the United Nations**

   Title: Fusarium Tropical Race 4 and the TR4 Global Network

   Highlights and Discussion

   a. The World Banana Forum (WBF) was established in 2009 under the FAO of the UN to look into the various issues related to the global banana industry as it is the most exported fruit, which contributes to food security and generates income. The WBF engages with players along the banana value chain including retailers, traders, governments, civil societies and NGOs, research institutes and intergovernmental organizations. WBF operates on a platform comprising 3 working groups which are W01-Working Group on sustainable production systems and environmental impact, W02 – Working Group on distribution of value, and W03 – working group on labour rights.
   b. The TR4 global network (TR4GN) was initiated upon recommendations of the WO1 task force on TR4 in August 2018, based on the disease threat to unaffected producing countries and spread in countries already affected.
   c. As a knowledge hub and platform for stakeholder collaboration, TR4GN creates basis for inclusiveness and collaboration, fosters information sharing among with WBF and stakeholders, acts as catalyst for related findings, materials and events, facilitates partnerships at the local and regional level and it acts as reference point for awareness raising and capacity development materials to promote prevention and control of TR4.
   d. Information on TR4GN can be viewed at www.fao.org/tr4gn. The network has produced 14 awareness materials and 3 guidance documents in 3 languages.
   e. To a question about the role of IPPC in the TR4GN, the presenter explained that IPPC is also a member of the working group of the WBF, which is complementary to the network.
   f. The network will also be a feature in the links to the FAO regional offices.

2. **Mr. Stewart Lindsay, Team leader, Banana production systems, Department of Agriculture and Fisheries, Government of Queensland, Australia**

Title: Fusarium wilt TR4 in Australia – status, containment measures and research initiatives.

Highlights and Discussion

a. Fusarium TR4 first appeared in Northern Territories in 1997 and was confirmed later in 2015 to have infected Cavendish bananas in Tully, Queensland (0.32 percent of total area).
b. In Australia, plant disease control and management come under the purview of State Governments, where the 3 pillars -research institutions, industry players and regulators are involved together.
c. Research work on TR4 are based on 3 strategies – prevention, containment and management.
d. Prevention involves utilizing science-based biosecurity including assessment of sanitizers, significant efforts in early detection and surveillance, and enhancing communications and extension such as developing manuals and videos on best practices for growers.
e. The containment strategy focuses on inoculum destruction, *i.e.* using urea to reduce the inoculum level. There has also been research done to reduce inoculum through planting alternate hosts.
f. Managing the already present pathogen and infected areas, is the other component of research which focuses on assessments of introduced resistant varieties conducted in multi-locational trial plots and using mutagenesis to develop new resistant varieties.
g. Breeding work with current resistant varieties is focused on varietal improvements in relation to production and taste, i.e. CJ19, GCTCV215 and 'Goldfinger'
h. The presentation indicated the importance of a multi-disciplinary approach involving relevant stakeholders to successfully confront the challenges of managing TR4 in Australia.

3. **Dr. Sijun Zheng, Alliance of Bioversity International & CIAT and Yunnan Academy of Agricultural Sciences,**

Title: Background and updates on *Foc* TR4 prevalence, containment and research initiatives in China

Abstract

China is the second largest banana-producing country in the world, and has a long history of banana cultivation. Banana producing areas are mainly in Guangdong, Guangxi, Hainan, Yunnan and Fujian provinces. *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4) is one of the most destructive banana pathogen. Due to Fusarium wilt of banana and unfavorable weather, in the past three years, China's banana planting area has been shrinking. In 2017, the planting area and output

dropped to 393,000 hectares and 12.5 million tons respectively. The planting area was further reduced in 2018. Based on the FAO "capacity development on diagnostic and surveillance system of banana Fusarium wilt disease TCP/RAS/3619" project, national surveillance was carried out during 2018 and 2019. The total surveillance area was up to 3810 hectares. The average disease incidence is 4.15%. The most severe province is Guangxi, up to 9.3%. Currently, *Foc* TR4 is still one of the quarantine pests in China. Government concerns and policy on the management of *Foc* TR4 including issuance of decree or regulation has been implemented after discovery of *Foc* TR4. A national action plan to control *Foc* TR4 disease such as quarantine, domestic regulation, seedling management etc. is implemented. Until now, it has been generally accepted that *Foc* TR4 mainly colonizes the roots, rhizomes, pseudostems and leaves and spreads among plantations with infected planting material, soil residues and water. As banana Fusarium wilt is a typical vascular bundle disease, the pathogen was confirmed to possess the ability to invade into the peduncle and infect new plants, causing potential risk for spreading Foc TR4 in other regions. Currently, *Foc* TR4 is limited in 81 county-level administrative regions now. Resistant cultivars together with organic fertilizer and beneficial microorganisms will offer options to combat banana TR4.

Highlights and Discussion

a. Responding to a query on the factor contributing to the rapid increase of the banana wilt problem in China from 2010-2014, presenter linked this to the usage of suckers which were widely distributed during the period, before tissue culture plantlets became the preferred planting material.
b. Presenter further highlighted that the combination of microorganisms to control TR4 included bacillus spp. and Trichoderma sp, citing field observations in China where farmers with good facilities produce their own concoction mix with organic fertilizers, reportedly leading to better outcomes in managing the disease.
c. The Yunnan Academy of Agricultural Sciences has indicated its interest in joining a broader alliance with other entities such as the World Banana Forum.
d. The application of Allium Chinese Leek to control TR4 was reported to have some limitations.

4. Dr. Miguel Dita, Alliance of Bioversity International & CIAT

Title: Tropical Race 4 in Columbia

Highlights and Discussion

a. *Fusarium oxysporium* sp. *cubense* TR4 was first reported in the La Guajira area in Columbia in June 2019.
b. The challenge was to prevent its spread to other areas and other Latin American and the Caribbean countries which are also major banana producers.
c. Cognizant of the ominous threat of *Foc* TR4 spreading to the region, the country was well prepared to manage the situation, including carrying out simulation exercises to contain the situation.

d. The presence of the disease elicited the proclamation of a National Emergency with the setting up of a National Task Force on TR4.
e. The response to contain the disease has been largely through surveillance and detection, capacity building and biosecurity, surveillance and research and resource mobilization. One of the biosecurity strategies was to isolate infected areas by building fences and containment drainage.
f. With current efforts, some of the areas around La Guajira have been quarantined. Ninety nine (99%) of the banana areas in Columbia are still TR4-free.
g. Basically to strengthen the capacities on research in prevention, containment and management of the disease involves diagnostics, biosecurity, soil health and IPM, evaluation of promising varieties and knowledge sharing.
h. The banana production system in the LAC has to be reshaped to face TR4 with the main components – Protection, Management, Diversity and Socio-economy.
i. A query was raised on the implementation of biosecurity in Colombia especially small farms where growers did not have much financial means to afford recommended biosecurity measures.

5. Dr. Chittarath Khonesavanh, Laos Dept. of Agriculture, Ministry of Agriculture and Forestry, Laos PDR.

Title: Situation of Fusarium wilt disease in Laos PDR and laboratory studies for DNA detection in PPC

Highlights and Discussion

a. There was an estimated 19,558 ha of the total 19,811 banana areas in 12 provinces grown with Cavendish banana in 2018. Others were local varieties such as Khai, Num, Hom and Thany varieties. In 2017, export was estimated at 827,177 mtons valued at $168,023,239 with seedling imports from China, Vietnam, Thailand and the Philippines totaling $424,684.
b. *Foc* TR4 was first detected in the northern Laos provinces of Laung NamTha and Borkeo in 2016.
c. To illustrate the devastating impact of *Foc* TR4, in February 2017, less than 1 percent infection was detected in an area of 145 ha, however after 2 years (July 2019), the total banana area was wiped out.
d. Capacity building to prevent and manage the disease included training of technicians in detection and surveillance using the P tracker. Surveillance teams were also set up.
e. The disease has been detected in 7 provinces - Vientiane Capital, Vientiane Province, Xaiyaboury, Laung Pha Bang, Oudomxay, Laung Nam Tha and Borkeo.
f. A lab study on the effect of *Trichoderma haziadum* and *Baccillus* sp. to suppress the disease pathogen is currently underway. This will be later field tested.
g. In response to a query on how the disease was introduced, there is the high likelihood this occurred through planting materials which were brought in by foreign companies operating in farms, especially at the northern China-Laos border.
h. Till date, TR4 has only been found in Cavendish, while results on local varieties have not been confirmed yet as surveys are still ongoing.

6. **Dr. Uma Subbaraya, Indian Council for Agricultural Research (ICAR), National Research Centre on Bananas, India**

Title: Fusarium wilt TR4 prevalence, containment measures and research initiatives in India.

Highlights and Discussion

a. India is the world's biggest banana producer with an estimated planted area of 900,000 ha and a corresponding production of about 30 million mtons (valued at USD 800 million), almost half of the total produced in Asia in 2016. Most of the bananas are grown in 10 states from the south to central regions of the country.
b. Factors such as inaccessibility of disease free planting materials, lack of awareness, poor quarantine measures across interstate borders, planting of infected suckers, monoculture of monoclones, ratooning of susceptible varieties, nematodes and stem borer aggravation and non-adoption of management practices were cited to cause an increase in TR4 incidences in India.
c. The main variety grown is the Cavendish (63%) with the rest made of local varieties such as the Poovan (17%), Nendran, Pome, Bluggoe, Silt and others (all average of 4%).
d. The estimated loss due to *Foc* TR4 wilt is about 20 percent of total production valued at USD 160 million.
e. Fusarium *Foc* TR 4 and *Foc* Race 1 affect banana growth with the former more prevalent in the North East region (Uttar Pradesh and Bihar), and Race 1 affecting farms in the central and southern regions.
f. Capacity building programs, awareness and sensitizing campaigns were initiated in March 2016 and continues to the present day after confirmation of the first reports of TR4 infection. This includes meeting and explaining to all stakeholders the importance of biosecurity and quarantine procedures, and the launch of a survey to gauge areas that were affected.
g. Research initiatives including the characterization of *Foc* isolates showed that besides TR4 (VCG 01213/16), Race 1 isolates 01220 and 0124 (isolated from Uttar Predesh) also infected Cavendish banana.
h. Molecular markers were developed for specific identification of *Foc* TR4 in India, as known markers were not effective.
i. Loop-mediated isothermal amplification (LAMP) assay conditions were optimized for specific, rapid and accurate identification of *Foc* TR4.
j. The whole genome sequence of *Foc* TR4 was analysed to show a major difference in the *Foc* TR4 found in India. In addition, the Plant Host Interaction (PHI) search showed uniqueness compared to reference TR4.
k. Some immune and highly resistant local lines especially in the AA unique, AAA unique and AABs genome group were listed.
l. Some combinations of *Bacillus haynesii*, *Bacillus licheniformis* and others have been found to suppress *Foc* TR4 in Grand Nain. Field trials on the use of bioagents have showed good results. Other studies include using *Trichoderma asperellum* and the effect of zimmu (*Allium sativum*) leaf extract to suppress fungal growth and pathogenicity factors in plant host relationships.

m. Future objectives include survey and mapping spread of the disease, capacity development to all stakeholders, enhance extension and provide implementation packages in TR4 management.
n. A query on the application of the Loop-Mediated Isothermal Amplification (LAMP) as on-site detection or lab-based, which was then confirmed to be the latter.
o. Presence of TR4 in other cultivars other than Cavendish was not detected.
p. Rotation with paddy was recommended as a way of reducing inoculum levels.
q. Next generation tissue culture technology using bioreactors is being disseminated for meeting the demand of banana plantlets in India.

7. Dr. Riska, Indonesian Fruit Research Institute, Sumatra, Indonesia

Title: The status of Fusarium wilt disease on banana and research strategies currently developed in Indonesia.

Abstract

Fusarium wilt disease (FWD), a disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) threatened banana production globally. FWD, also known as Panama disease, is a major constraint of banana production in Indonesia. The limited knowledge of the epidemiology and lack of effective management make *Foc* an important concern to be investigated. This review summarizes the current situation of FWD in Indonesia, research of management strategies, and highlights of future research needs. First report of *Foc* was in banana plantation in Java since the 1910s. In early 1990s FWD destroyed 70% of Cavendish plantation in Southern Sumatera and significantly ruined over 1000 ha banana plantation spanning three years in North Sumatera and Halmahera significantly. In 2005, the FWD had spread to 16 banana-producing provinces. Studies on vegetative compatibility group (VCG) analysis on the isolates shown that the ones recorded were VCG 0120/15, 0121, 01219, 01213/16, 01218, 0123, 0124, 0124/5, 0126 and 0129. *Foc* VCG 01213/16 known as Tropical Race 4 (TR4) was one third of the total isolates found in almost all provinces, affecting local cultivars Barangan (AAA), Raja (AAB) and Ambon Hijau (AAA), as well as other cultivars of different ploidi and genomes. Currently the terminology for grouping of *Foc* based on race concept has shifted to VCG. The hypothesis proved throughout the investigation of virulence of several VCGs on banana varieties. The result indicated that the VCG grouped into the same race showed different levels of virulence. Recent identification of *Foc* obtained from 15 provinces also showed that 180 isolates revealed nine independent genetic lineages for *Foc*, and one novel clade in the *Fusarium oxysporum* species complex (FOSC). More than 65 % of the isolates were TR4. The *Foc*-TR4 isolates was expected is from predecessors in *Foc*-Race1. Study on microbes rhizospheral of banana affected by FWD revealed that *Foc* seen to be associated with other *Fusarium oxysporum* species complex. Another study showed that of *Foc*-infected soil contained high abundance of Xanthomonadaceae while Proteobacteria was found dominant in healthy soil. The use of beneficial microbes, particularly to *Trichoderma* sp, *Baccilus* sp, *Saccaromyces* sp, *Pseudomonas fluorescence*, endophytic and non-pathogenic of *Fusarium* to control FWD have not shown positive results yet as well as cropping system. Crop

rotation has provided promising result, where rotation with pineapple and cassava can reduce FWD incidence. To date, Indonesian Tropical Fruit Research Institute has confirmed that 2 new varieties, INA 02 and INA 03 and one indigenous banana, Ketan 01 possess potential resistance to *Foc* TR4 in field trials. Two new irradiated mutant, Pirama and Ampyang which were released by another Indonesian research institute also showed resistance to *Foc* TR4. Additionally, promising results were obtained from the application of salicylic acid which had the ability to induce disease resistance of susceptible banana. Promising further research and management strategies to control FWD include understanding and exploring potential soil microbiomes and bioagents, and introduce varieties such as Ketan 01, INA02, INA 03, Ampyang, Pirama, FHIA 17, FHIA 25 & SH 3640 (result of IMTP II) for the banana industry. In addition, there are plans to continuously introduce Kepok Tanjung (budless Kepok) and Raja Kinalun to the farmers through the Agricultural Techno Park (Ministry of Agriculture's program),

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

Highlights and Discussion

a. The CJ30 and CJ40 varieties are still under field trials and not available for distribution.
b. Biological products such as Tricostar is available commercially for *Foc* control.

8. Dr. Farah Farhanah Haron, Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia

Title: Background and Updates on *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4), prevalence, containment measures and research initiatives in Malaysia

Abstract

Banana (*Musa* spp.) is the third most important fruit crop cultivated in Malaysia in terms of its total production and ranked second in export revenue among the other major fruit crops. However, the production area and yield has been decreasing year by year since 2015. One of the main constraints in banana production worldwide is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Most popular banana varieties planted in Malaysia were reported to be susceptible to *Foc* Tropical Race 4 (*Foc* TR4) and has caused major losses in banana plantations because currently there is no effective way to control this disease. The Malaysian government has recommended all banana growers to implement Good Agricultural Practice (GAP) as a practical mean to prevent the spread of *Foc* TR4. A growing number of research activities has been conducted on molecular approaches to gain a thorough understanding on the pathogen's characterization, mode of action and pathogenicity as well as through cultural and biological control towards a sustainable and eco-friendly Fusarium wilt management in the country. This report will update more on the current status of *Foc* TR4 prevalence, containment measures and research initiatives that have been done in Malaysia to mitigate the Fusarium wilt in banana.

Highlights and Discussion

a. The common banana cultivars in Malaysia are Berangan, Mas, Rastali, Lemak Manis, and Cavendish for dessert bananas and Pisang Nipah (similar to Saba and Abu), Raja, Tanduk, Awak and Nangka for the cooking types. The total banana areas in 2017 and 2018 were 34,894 ha and 30,455 ha with production at 350,493 mton and 330,957 mton respectively.
b. The major constraints in banana production have been diseases caused by *Foc* TR4 and Bacterial wilt caused by *Ralstonia* sp. Up to 70 percent of areas planted were affected by *Foc* TR4 was reported in 2016. Besides affected Cavendish plantations, the disease also affected other dessert varieties such as Berangan, Mas and Rastali. Cooking varieties such as Awak, Abu and Masak Hijau have been reported to be free from *Foc* TR4.
c. Containment measures include Good Agricultural Practice, disease free planting materials, good irrigation system, infected plants management, monitoring and surveillance and biosecurity enhancement.
d. Research initiatives in Malaysia cover a range of areas including breeding for resistant varieties, molecular approaches for understanding plant-pathogen interactions, cultural practices for prevention of TR4 and biological control measures. Example of mutation breeding is the selection of gamma irradiated Berangan and cavendish for field evaluation, using SIX (secreted in xylem) effector genes to characterize *Foc* TR4 isolates.
e. Promising results were also obtained in a study with 3 *Allium* sp intercrops as a cultural control practice.
f. Other approaches include biological control by utilizing bio-consortium formulations, microencapsulation of *Streptomyces*, lixiviate from banana decomposition and induced systemic resistance (ISR).

9. Dr. Altus Viljoen Plant Protection Department, University of Stellenborsch, South Africa

Title: Banana Fusarium wilt TR4 in Northern Mozambique

Abstract

Fusarium oxysporum f. sp. *cubense* tropical race 4 (TR4) was first detected on a 1500-ha commercial Cavendish banana plantation (Matanuska) in northern Mozambique in 2013. The fungus was rapidly disseminated, and affected approximately half a million plants within 3 years. *Foc* TR4 was found on a nearby commercial banana farm in 2014, and in a small grower's field in 2015. There are numerous reasons to be greatly concerned about the spread of TR4 in Mozambique and into Africa. In Mozambique, as with other countries in Africa, bananas constitute part of the staple diet of families. Losses in production could, therefore, threaten food security and income to millions of Africans. Surveys in Mozambique and East Africa showed that non-Cavendish banana varieties were only affected by *Foc* race 1 strains. The testing of Cavendish banana somaclones in northern Mozambique revealed that GCTCV-119 was most resistant to *Foc* TR4, but that GCTCV-218 produced better bunches. In January 2018, Matanuska farm was declared bankrupt, and in 2019 *Foc* TR4 was detected in Mayotte Island

off the east African coast. In southern Africa, Cavendish somaclones can be used in combination with integrated disease management practices to replace susceptible Cavendish cultivars in southern Africa. A small collection of African cooking bananas and plantains had been tested for resistance to *Foc* TR4 in Asia, but comprehensive field testing in Africa is still required. A banana breeding project was also initiated to develop African cooking bananas resistant to *Foc* TR4.

Highlights and Discussion

a. Africa accounts for 26 percent of global banana production. There are 4 major types of bananas grown in Africa, namely the plantains (mainly in Western Africa), EAHB (East African highland banana), Cavendish (South and East Africa) and the local dessert/cooking bananas.
b. Studies have shown that the EAHB group of bananas (AAA) exhibit strong resistance to *Foc* TR4. Introduced somaclones such as GCTCV 119 and 218 (Formosana) have also indicated significant resistance.
c. The first detection of TR4 was in 2013 in Northern Mozambique at the Matunuska farm. The rapid increase of the disease from 2015 onwards forced its closure in 2018. Spread was caused by movement of workers and vehicles but mainly through floods, which occurred during the period.
d. In 2020, replanting efforts were undertaken in the farm (now known as the Monapo river farm) using the Formosana (GCTCV 218) which has shown strong resistance. The bananas are now being exported to the Middle east and other countries. Containment measures at the site has also been improved. 2018 saw a number of short term research projects implemented in the country aimed at reducing *Foc* TR4 inoculum in soil, determining adaptability of Formosana in the Southern regions and surveillance for the spread of TR4, among others.
e. There has been substantial capacity building and awareness programs, including disease diagnostics have been facilitated by donor agencies to mitigate the TR4 situation.
f. The focus now is to carry out early detection and surveillance, strengthen biosecurity measures, increase genetic resistance and integrated disease management.
g. A series of strategic meetings also took place to develop an African *Foc* TR4 platform for coordination and management of activities in the continent.

10. Dr. Edna Anit, Crop Research Division, DOST-PCAARD, Philippines

Title: Updates and Status on Fusarium wilt on Cavendish Banana in the Philippines

Abstract

The Philippines has 9.1 million MT annual banana production in 449,030 hectares as of 2019. It is the second largest global banana exporter, mainly Cavendish variety, in terms of volume at 3.9 million MT in 2018 (FAOSTAT). One of the major production constraints of banana is the Fusarium wilt (FW) which causes great losses to farmers. In 2005, a disease outbreak occurred in Cavendish banana farms in Mindanao, southern part of the Philippines, wherein *Fusarium oxysporum* f. sp. *cubense* (*Foc*)

Tropical Race 4 (TR4) was confirmed as the causal organism. From 2011 to 2016, *Foc* TR4 caused devastating damages in banana plantations on the island. The Department of Science and Technology (DOST) through the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) has been spearheading initiatives to develop S&T interventions to manage the FW disease. R&D programs have been funded and implemented in partnership with different SCUs, government agencies, banana growers and exporters associations, and international organizations. Management of FW were studied through different S&T-based strategies. These include adaptability trial of *Foc* TR4-resistant varieties, development of biological control strategies, and assessment of distribution and incidence of *Foc* TR4. Further research are being conducted in partnership with international organizations such as analysis of the microbiome differences existing in current banana production systems and early detection and monitoring of FW disease through smart agriculture.

Highlights and Discussion

a. The total area of bananas in 2019 is 449,030 ha with a production of 9.2 million mtons, productivity of 21.1 mt/ha and export value of USD 1.13 billion.
b. Area wise, in 2019, the Saba banana took up 41 %, Cavendish 20 %, Lakatan 10 % and other varieties 13 %. However, in volume of production, Cavendish tops the list with 53%, Saba 27 %, Lakatan 10 % and others at 10 %.
c. Fusarium banana wilt is confined mainly to the Southern Province of Mindanao. A survey showed the main areas affected are those surrounding big commercial farms. *Foc* TR4 was first observed in 2005 and in 2015 about 15,500 ha were affected. The disease was also observed to affect the local Lakatan and Saba varieties.
d. Past initiatives include assessment and distribution of *Foc* TR4, adaptability of TR4 resistance varieties and good management practices.
e. The two prominent somaclones with resistance to TR4, namely the GCTCV 219 and 218 have been widely distributed to farmers and commercial banana companies. Other measures include the use of microbial agents such as ACTICon (a mixture of actinomycetes) with Grand Nain and resistant somaclones of GCTCV 218 and 219 were observed to reduce infection up to 73 %.
f. Cover crops such as peanut, kudso and ornamental sweet potato were used in areas planted with bananas to enhance the suppression of TR4 in the soil.
g. Ongoing projects include the use of anticon as a bioagent, development of nanoformulated biopesticide, decision expert system for monitoring and management using spatial data and integrated management strategies
h. Various capacity building efforts have also been done for researchers and farmers alike for increasing their level of preparedness when facing fusarium wilt.

SESSION CONCLUSION

The session indicated the various levels of research present in respective countries for managing the TR4 problem. The different strategies and research initiatives in the countries signal a common resolve and call for collaboration to control and minimize disease spread. The Chair remarked that this first session provided a backdrop for the workshop and hoped that it would elicit useful discussion in the coming sessions.

27th OCTOBER 2020

SESSION B: BIODIVERSITY, EVOLUTION AND INTERACTION MECHANISM BETWEEN PATHOGEN AND HOST

SESSION CHAIR: Irene Kernot, Australian Centre for International Agricultural Research (ACIAR)

1. **Dr. Chun-Yu Li, Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, China**

Title: *Foc* TR4 modulates its invasion in banana by the mytoxin and effectors

Abstract

Fusarium wilt (Panama disease) is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4), which is considered to be the most destructive disease of bananas. This disease is threatening the production of banana in Asia, Africa, Australia and the tropical Americas. Chlamydospores are resistant to desiccation, resilient in unfavorable environmental conditions, and may survive in the soil for more than 30 years. Chlamydospores are stimulated to germinate and infect nearby banana roots, and enter the xylem, induces wilt and kills banana plants. The molecular mechanisms underlying *Foc* TR4 virulence remain elusive, and surely, *Foc* TR4 employs virulence factors as molecular weapons to manipulate host immunity and facilitate their colonization and invasion, *Foc* is a highly variable pathogen and comprised of a total of 24 vegetative compatibility groups (VCGs) or three pathogenic races (*Foc* races 1, 2 and 4), which was classified according to their selective impairment of banana cultivars. In order to unravel the invasion strategies of *Foc* TR4, we have sequenced and assembled its genome. From *Foc* TR4 genome, we identified some gene-clusters involving in the biosynthesis of mycotoxin, such as Fusaric acid (FSA), beauvericin (BEA) and others, including candidate effectors. FSA is a phytotoxin produced by several *Fusarium* species and has been associated with plant disease development, and mutation of key genes in the FSA bio-synthetic gene (FUB) cluster in Foc TR4 reduced the FSA production and resulted in decreased disease symptoms and reduced fungal biomass in the host banana plants. When pretreated with FSA, both banana leaves and pseudostems exhibited increased sensitivity to Foc TR4 invasion. Banana embryogenic cell suspensions (ECSs) treated with FSA exhibited a lower rate of O_2 uptake, loss of mitochondrial membrane potential, increased reactive oxygen species (ROS) accumulation, and more nuclear condensation and cell death. Consistently, Transcriptomic analysis of FSA-treated ECSs showed that FSA may induce plant cell death through regulating the expression of genes involved in mitochondrial functions. The above results demonstrated that the FSA from *Foc* TR4 functions as a positive virulence factor and acts at the early stage of the disease development before the appearance of the fungal hyphae in the infected tissues.

Except the genome sequence, we also unraveled the virulent genes expression using RNA-seq. *Foc* CP1, which belongs to the Cerato-platanin protein family, is highly expressing during *Foc* TR4 invasion. In our research, we demonstrate that functions as an effector that is required by *Foc* for full virulence. Inoculation of purified *Foc* CP1 protein could induce significant cell death and activate plant immunity, like ROS eruption, callose deposition and up-regulated defense-related genes. *Foc* CP1 transcripts accumulated during fungal growth and plant infection, and deletion of *Foc* CP1 significantly reduced virulence and fungal biomass in planta. Furthermore, scanning election microscopy observation and qRT-PCR analysis of infection process revealed that *Foc* CP1 plays an important role in penetration of banana roots.

Key words: *Fusarium oxysporum* f. sp. *cubense* TR4, fusaric acid, banana, effector

Highlights and Discussion

a. The continuous spread of *Foc* TR4, with recent reports in Columbia and Mozambique has elicited investigations into the interaction mechanism of pathogen attack and host response.
b. The invasion of the pathogen is by the primary inocula made up of macrocondia, microconidia and mycelium from infected tissue or chlamydospores from dead tissues and soil. Invasion of the pathogen is characterized first by spore germination initiated by favorable conditions (weather, pH, nutrient exudates from host etc), attachment to host roots at the elongation zone, root caps and site of wounds. This leads to penetration of cells which the host immunity system reacts with lignification or strengthened cell walls PTI (Pattern Triggered Immunity). and also produce antimicrobial chemicals such as phytotoxins and hydrolytic enzymes ETI (Effector Trigger Immunity), and finally the infection phase, where chemicals produced by the pathogen result in collapsed and distorted vessels and vascular system in the pseudostem and petiole, leading to disruptions in water, nutrients, transpiration and photosynthesis, causing wilt symptoms.
c. *Foc* TR4 infects its host through producing elicitors, secreting effectors, producing mycotoxins and enzymes to break down cell walls. Examples of effectors are ECM33 and Foc CP1 while Beauvericin (BEA) and Fusaric acid (FA) are mycotoxins. The more virulent the *Foc* TR4 isolates the more mycotoxins are produced.
d. It was observed that accumulation of Fusaric Acid (FA) in diseased tissue was more pronounced than Beauvericin (BEA). FA spreads much faster than the invasion of Foc TR4 fungus in banana. Fusaric acid is also an uncoupling agent for respiration and phosphorylation, which can affect mitochondrial activity and decrease ATP production. FA was observed to induce banana protoplasts death besides causing the accumulation of Reactive Oxygen Species (ROS) in the leaves which causes necrotic conditions and increased lesion size.
e. Discussions revolved around factors influencing amounts of mycotoxins by TR4 and if other pathogenic fusarium species were known to produce mycotoxins for infection.

2. **Dr. Elizabeth Aitken, University of Queensland, Australia**

Title: Diversity in Fusarium oxysporum f. sp. cubense

Abstract

The fungus *Fusarium oxysporum* exists in many different forms including host specific pathogenic forms, endophytes and saprophytes. The host specific form *F. oxysporum* f.sp. *cubense* (*Foc*) is the causal agent of Fusarium wilt in banana. *Foc* can be divided up into different pathogenic races including Race 1, subtropical race 4 (SR4) and tropical race 4 (TR4). Each race is comprised of one or more different vegetative compatibility groups (VCGs). Initially, *Foc* races were identified by VCGs; this is a reliable but a time consuming process. More recently PCR based methods have been used but those based on core gene analysis have occasionally given unexpected results. A PCR based diagnostic has now been developed based on secreted in xylem (SIX) gene analysis. SIX genes were originally identified by reverse genetics of peptides present in the xylem of tomato plants infected with *F. oxysporum* f.sp. *lycopersici* (*Fol*). A profile of and presence or absence of specific SIX genes correlated with races within *Fol*. Using the SIX gene sequences from Fol as a template for studies on *Foc*, a fingerprint of presence/absence of SIX genes was established for *Foc* races. When studying SIX gene sequence, specific homologues were identified in TR4 specific to that strain; indications are that such homologues have been horizontally acquired spanning different formae speciales of *F. oxysporum*. *Foc* is a complex fungus and has been proposed to comprise more than one species, whether this is the case or not, caution is advised when deploying genetic resistance in the host as it may not encompass all the pathogenic variants of the fungus.

Highlights and Discussion

a. In plants infected with *Foc*, growth of fungus starts from the roots to the xylem, cortex and pseudostem. *Foc* is confined to xylem in healthy looking leaves and as the leaves senesced it colonized extensively as a saprophyte.
b. Once present, *Foc* cannot be eradicated due to long lasting spores and its ability to remain persistent as a saprophyte on roots of alternate hosts. It is also observed that sporulation also occurs in decaying leaves and not in the soil only.
c. *Foc* can also occur asymptomatically on wheat, while pathogenic forms attack banana. It also is a saprophyte as well as an endophyte.
d. VCGs or vegetative compatibility groups are fusions between hyphae of related fungal strains or alternatively inhibits fusion between not-related strains. VCGs are traditionally used to group and identify fusarium species or races. VCGs are identified by complementation of mutants in the nitrate reductase pathway. The Chlorate pathway is used as a means to select mutations in the Nitrate reductase pathway.
e. Though VCGs are useful for distinguishing between races of *Foc*, screening process takes up to 6 weeks and requiring expertise. It also does not tell everything about the pathogenicity nor the relationships of different VCG's to each other.
f. In Australia, TR4 is more confined to the Northern Territory, TR4 and Race 1 in

Northern Queensland and SR4 and Race 1 in Southeast. Queensland / Northern New South Wales.

g. Effectors (pathogenicity factors) involve SIX (secreted in xylem) genes encode small protein secreted by the fungus in the vascular tissue of infected plants. Using this SIX genes diagnostic comparison, STR4 can be distinguished with the TR4.
h. Researchers are also looking at genetic resistance to *Foc* races using *Musa acuminata* ssp, *malaccensis* (AA)
i. During discussions, there was interest from participants on the prediction of vegetative compatibility, the sufficiency of using SIX genes for confirming virulence of TR4 and STR4, and production of different SIX gene patterns from isolates in same VCGs.

3. Dr. Tingting Bai, Yunnan Academy of Agricultural Sciences, China

Title: The population variations of *Foc* TR4 under different types of field soil and different banana growing stage

Abstract

Banana (*Musa* spp.) is the most exported fruit and also a major food crop for millions of people around the world. But global banana production is now severely threatened by Fusarium wilt disease. The cause is a special soil-born fungal pathogen, *Fusarium oxysporum* f.sp. *cubense* also known as Tropical Race 4 (*Foc* TR4). The population of *Foc* TR4 pathogen in different types of field in Yunnan was analysed by real time quantitative PCR (q-PCR). The average population of *Foc* TR4 pathogen in the heavily infected field was 4,745 copy numbers/g, while the average population of *Foc* TR4 pathogen in field started showing *Foc* TR4 symptoms was 1,306 copy numbers/g. *Foc* TR4 with less than 500 copy numbers/g still could be detected in some fields even without *Foc* TR4 symptoms. Later we investigated the population of *Foc* TR4 by qPCR over one year in a heavily infected banana field in Xishuangbanna Prefecture of Yunnan province in Southwest China. The *Foc* TR4 population in soil was found to initially increase and then decrease at the vegetative growth phase of banana. When the banana grew into reproductive bud formation stage, the *Foc* TR4 population in field significantly increased again. Meanwhile bio-organic fertilizer with beneficial microbe's application into field showed a transient suppression effect on *Foc* TR4 in the same trial field. The finding will have value in other studies related with *Foc* TR4 control and banana-*Foc* TR4 interaction.

Highlights and Discussion

a. In 2018 there were a total of 100,000 ha of banana areas in Yunnan Province. Banana Fusarium wilt was first discovered 2009 and in 2018 has been found in all countries. The areas affected is estimated about 1.89 % of the total area
b. Eco-friendly management methods such as use of anaerobic soil disinfestations (ASD) and bio-organic fertilizers have been investigated as alternatives to suppress the disease.

c. The qPCR technique was used to determine *Foc* TR4 population in the experiments that involves applications of ASD and bio-organic fertilizers to control the disease.
d. The dynamic distribution pattern of *Foc* TR4 is related to the different growth stages of the plant and prevailing climatic conditions.
e. Responding to a query on the sampling method to determine the copy numbers of the fungus in the soil, the presenter explained that samples were taken using a probe from the soil rhizosphere area at a depth of 0.5 to 20 cm; clarification was sought on how much of the inoculum was viable. There were also queries on the factors for fluctuation of TR4 population in soil, and effective control strategies to counter these fluctuations at the different growth stages.

4. Dr. Wang Wei, Chinese Academy of Tropical Agricultural Sciences, China

Title: Lysin motif containing receptor-like kinase 1 protein of banana required for perception of pathogenic and symbiotic signals

Abstract

Lysine motif (LysM) receptor kinases (LYK) recognize microbe-associated molecular patterns (MAMPs) to activate defense or symbiotic signaling. How plants can distinguish pathogens and arbuscular mycorrhizal (AM) fungi remain largely unknown. Here, we report that MaLYK1 (LYSIN MOTIF-CONTAINING RECEPTOR-LIKE KINASE 1) of banana (*Musa acuminata*), has a dual function in the associations with pathogenic and AM fungi. Live cell imaging and analysis of microsomal fractions revealed that MaLYK1 is a plasma membrane protein. MaLYK1 expression was induced by the pathogen *Fusarium oxysporum* f. sp. *cubense* race 4 (*Foc*4) and diverse MAMPs. MaLYK1-silenced banana lines showed a reduction of chitin-triggered defense responses, increased *Foc*4-induced disease symptoms, and reduced AM colonization. Ligand affinity experiments indicated that the ectodomain of MaLYK1 can bind to chitin and Myc-LCOs. Ligand binding rapidly induces complex formation and phosphorylation of MaLYK1 *in vivo*. Finally, we analyzed the effects of chimeric receptors on defense responses in *Arabidopsis thaliana* and nodulation responses in the legume *Lotus japonicus.* The kinase domain of MaLYK1 could functionally replace the kinase domains of the chitin elicitor receptor kinase 1 (AtCERK1) in A*rabidopsis thaliana* and the Nod factor receptor 1 (LjNFR1) in *Lotus japonicus*. These results suggest that MaLYK1 represents a central molecular switch that controls defense- and symbiosis-related signaling.

Highlights and Discussion

a. Presenter expressed hope that by understanding microbe interactions, resistant cultivars will be obtained by transfer of this receptor gene into susceptible cultivars, which will assist in management of the disease and resistance breeding.
b. Arbuscular Mycorrhizae (AM) fungi can form a symbiotic and mutually beneficial relationship with more than 80% of terrestrial plants, improving stress resistance and mineral exchange.

c. There is the question of how the plant can recognize both pathogens and AM fungus but reacts differently for both, one for defense and the other for symbiotic relationship.
d. LysM proteins which have a hydrolase function exist in diverse organisms. It was also found to participate in symbiosis signaling pathway in legumes and defense signaling in non-legumes.

5. Dr. Diane Mostert, Stellenbosch University, South Africa

Title: Evolution of and diversity in the Fusarium wilt fungus, *Fusarium oxysporum* f.sp. *cubense*

Abstract

Fusarium wilt of banana is caused by the soil-inhabiting fungus *Fusarium oxysporum* f. sp. *cubense* (Foc). Foc is a member of the *F. oxysporum* species complex (FOSC), which is a morphologically related group of fungi that includes both pathogenic and non-pathogenic strains. Pathogenic isolates of *F. oxysporum* are recognized by host specificity and called formae speciales. The pathogen is further divided into races and vegetative compatibility groups (VCGs), based on the ability to cause disease to a particular set of host cultivars and to exchange genetic material through heterokaryosis, respectively. The race designation of *Foc* is still unresolved due to the small number of differential cultivars used and the effect of temperature on host susceptibility. While VCGs were useful to resolve uncertainties about the origin and global spread of the fungus, it is tedious and does not indicate genetic distance among *Foc* isolates. DNA-based methods have therefore been used to study the relatedness of individuals and the evolution of the banana wilt fungus. The polyphyletic nature of *Foc* suggests that at least two host speciation events occurred after the domestication of bananas. One event lead to the development of *Foc* races 1 and 2 strains (pathogenic to non-Cavendish bananas), and the other to the development of *Foc* race 4 strains (pathogenic to Cavendish bananas. An understanding of the evolution and diversity in *Foc* assist quarantine authorities to prevent the introduction of foreign strains into new banana-producing areas and allow the rapid screening of banana varieties for resistance against *Foc*.

Highlights and Discussion

a. Dr. Mostert highlighted how the large scale production of Cavendish indirectly influenced the spread of the disease as production areas increased to other countries, suggesting that the type of banana variety planted influences the diversity of *Foc* populations in an area.
b. *Foc* distribution can be attributed to the host cultivar planted especially in monoculture production.
c. *Foc* VCGs are particularly diverse, can indicate global spread and vary in virulence. Currently there are 24 VCGs with some novel ones (18) being investigated. The strains have evolved on at least two occasions, which are the Cavendish and non-Cavendish clades and evolution had been through vertical and horizontal gene transfer.

d. More research is needed to understand how pathogenesis towards banana have evolved.
e. Discussions evolved around the influence of production systems on presence and dominance of VCGs and the possibility of varying cultivars per growing season to reduce dominance of a specific VCG.
f. There was concern that blanket recommendations through use of resistance cultivars will not be effective if there is already evidence pointing that different VCGs exhibit different virulence capacities.

SESSION CONCLUSION

The Session Chair initiated a brief discussion with the presenters on key research areas which could be prioritized for future collaboration in Foc research, by identifying gaps and addressing key research questions. Dr. Chun Yu Li proposed greater investment for understanding virulence factors and their mechanisms of actions, especially focusing on gene editing technologies to edit receptor genes in banana for improving resistance to TR4.

a. Dr. Liz Aitken called for greater collaboration for assessing germplasm diversity for resistance, while also highlighting the importance of germplasm screening.
b. Dr. Wang Wei emphasized the need for accessing transgenic banana cultivars with resistance to TR4 (transgenic plants), which are currently difficult to obtain.
c. Dr. Diane Mostert called for detection methods which are quick and easy to use, especially for resource-poor farmers. She also expressed keenness to collaborate on diversity studies, especially related to the characterization of VCGs and populations, studies of which are currently limited. The recommendations of Dr. Mostert were agreed by Dr. Altus Viljoen who reiterated the importance of affordable in-field detections and reliable detection systems for inoculum present on soil, water and other systems.
d. The session chair called for organizers of the workshop to facilitate linkages and collaborations among researchers. She concluded by stating that the presentations and discussions presented clear opportunities for the future where TR4 can be effectively managed by greater understanding to the interactions, diagnostics and building on resistance.

3rd NOVEMBER 2020

SESSION C: BREEDING INITIATIVES FOR RESISTANCE AGAINST FOC TR4

SESSION CHAIR: Dr. Chunyu Li, Fruit Tree Research Institute, Guangdong Academy of Agricultual Sciences, China

1. **Prof James Dale, Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, Queensland, Australia**

   Title: Genetically modified and potentially gene edited bananas with resistance to Fusarium wilt tropical race 4

   Abstract

   Cavendish bananas and many other banana cultivars are highly susceptible to Fusarium wilt tropical race 4 (TR4). Genetic modification and gene editing provide a platform for developing TR4 resistance in current popular but susceptible bananas such as Cavendish. We have developed TR4 resistant Cavendish by genetic modification by transferring an NB-LRR gene, *RGA2*, from a resistant diploid *Musa acuminata* spp *malaccensis* into Grand Nain. In our Phase 1 small field trial, one GM line appeared to be immune with a further three lines with high resistance levels after three years. These four lines have been progressed to a much larger Phase 2 field trial. After 2.5 years of this trial, one line has no infected plants and virtually no yield penalty. Non-GM gene editing provides the opportunity to develop and commercialize TR4 resistant bananas such as Cavendish without regulation and labelling in many of the major banana producing and consuming countries. The challenges are to generate such bananas without the introduction of foreign DNA and to identify the banana gene or genes to be edited for resistance. We are developing a protoplast platform for editing Cavendish without the integration of foreign DNA. In parallel, we have sequenced and are assembling the Cavendish and *M. acuminata* ssp *malaccensis* genomes to finalize our selection of genes to be edited. Importantly, the genes used for TR4 resistance via genetic modification or gene editing will be able to be utilized in any banana cultivar with the potential to expand the range of commercial bananas.

   Highlights and Discussion

a. Dr. Dale suggested that the mid to long term solution for TR4 is through the use of new or improved cultivars and among the various ways for developing resistance include tolerant selections, conventional breeding leading to new cultivars, and of interest to his presentation, genetic modification and gene editing. After providing some initial background to the generic approaches used, he dwelled on Queensland University of Technology's (QUT)'s research program that has already developed GM Cavendish with resistance to TR4 and are now in advanced field trials.
b. Dr. Dale distinguished gene modification (genetic engineering, transgenics) as the addition of new DNA into a plant through transformation that almost always includes

'foreign' DNA, while gene editing (non-GM) refers to precise, targeted modification of a plants DNA including deletions, substitutions to additions of entire genes, with not 'foreign' DNA added. There are 3 types of gene editing, SDN 1 (Site Directed Nuclease), which employs 'knockout' or substitution, SDN 2 is oligonucleotide directed nuclease and SDN3 involves insertion of entire genes from the same plant.

c. For somaclonal variation, genes are obtained within banana cultivars, in conventional breeding genes are sourced form banana accessible gene pool. Genes for genetic modifications come from nature, while for gene editing genes come from within bananas.

d. Queensland University of Technology's TR4 R&D program began with the identification of a single dominant TR4 resistance gene in wild banana *Musa acuminata* sp. *malaccensis*, (native to Malaysia and Indonesia), and transferring it into Cavendish (Grand Nain) by genetic modification. Field trials were conducted in a severely affected site (10 years) in Darwin from 2012 to 2015. Among the transgenics, *RGA2* line 3 was found to be highly resistant compared to other *RGA2* lines. The *RGA2* gene occurs in all non-transgenic Cavendish, however with low gene expression compared to those with the *RGA2-3* gene. Further expanded field trials using *RGA2-3* and new *RGA2* lines together with other new resistance genes were conducted from early 2018 confirmed that generally particular lines of GM plants performed better compared to non-GM plants in the control.

e. To summarize QUT's current approach, CRISPR Cas-9 is the editing technology of choice for non-GM gene editing techniques. Work on the ECS (Embrogenic Cell Suspension) -derived protoplasts technology, which later can be developed into embryos and plantlet, is progressing. Some banana genomes have been sequenced and annotated, The SDN1 or 'knockout' strategy is progressing, and Gros Michel modification and editing is in program. Plantains could also be included in the research program.

f. Among the questions from participants included the crop cycling time, specifically the harvest time for the different GM lines in comparison to the controls; and overcoming the possibility of the breakdown of single gene resistance and obtain durable resistance. Presenter explained that given the asexual reproductive nature of *Fusarium oxysporum*, the fungus does not go through the same amount of genetic recombination as a sexually produced fungus and the breakdown process maybe be slow. Presenter agreed on the need to not depend on single resistance genes and highlight that his team are identifying new resistance genes, with the plan to stack as many resistance genes as possible.

g. Clarification was sought on the terminologies 'highly tolerant' versus 'less resistant'. Prof Dale clarified that for his work, the TR4 resistant by GM is classified as 'very resistant' which he linked to the plant not getting infected and performing well (producing high yields).

h. In relation to a question on the existence of different VCGs, different strains and different pathogenicity which can complicate conditions, presenter emphasized on the need for multilocational field trials (not glass house trials) in other countries to replicate the various environmental factors and different soils.

i. Other discussion highlights included the insertion position of *RGA2* to achieve resistance, and the number of years before a gene edited resistant Cavendish could be made available to growers.

2. **Dr. S. Uma, Indian Institute of Banana Research, India**

Title: Breeding Initiatives for Resistance against TR4 in India

Highlights and Discussion

a. Dr. Uma described in length the progress of work carried out in clonal selections, conventional breeding, mutation breeding (mutants with resistance to both Race 1 and Race 4) and molecular breeding. Genetic transformation was described to be the next step, once more leads are available.
b. Phenotyping in sick plot for indigenous varieties have been completed, however, the same task for ITC introductions will be taken up in 2021. Phenotyping for mapping population (Math x cv Rose) is in progress.
c. The development to improved diploids and tetraploid is being continued.
d. Mass multiplication of identified Grand Nain replacement among Cavendish clones for large scale evaluation in Bihar State. Ten Cavendish mutants resistant to TR4 identified through pot culture screenings will be mass multiplied and tested on infected plots.
e. Candidate R gene has been identified to express five-fold upon TR4 inoculation..
f. Further evaluation using CRISPR 'knockdown' or RNAi approach will be used for resistance validation.
g. Genic SSR marker associated with TF4 resistance has been identified which will be validated in mapping population.
h. There has also be research done in using protoplasts culture to regenerate new plants.
i. Among discussions highlighted included greenhouse evaluation for TR4 resistance on accessions received from ITC, the number of field trials and number of plants used in a TR4 evaluation, and determining the density of TR4 in soil.
j. To a question on characteristics used for classification of what comprises 'immune', 'highly resistant' and 'resistant', presenter responded that classifications follow a Bioversity International-developed protocol which consisted of scores and pictorial representations.

3. **Dr. Sheng Ou, Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, China**

Title: Establishment of resistance evaluation methods of banana to Foc TR4 and field trials

Abstract

Fusarium wilt is seriously threatening the sustainable development of banana industry in China. Breeding varieties with high resistance is a very effective way to control such notorious disease. We established an evaluation system based on the incidence index of the symptoms occurred inside the banana rhizome. Using this system, we evaluated the resistance levels of the collected germplasm and the new varieties.

Collaborating with Bioversity International, we confirmed that all of the 7 varieties of East African highland banana (EAHB, AAA) and 5 varieties of Plantain (AAB) are highly resistant to *Foc* TR4, which could eliminate the panic caused by the spreading of *Foc* TR4 to Mozambique. We also established the physical and chemical mutagenesis breeding systems, and through this system we selected Zhongjiao No.8 and No.4, which have moderate resistance or tolerance to Foc TR4. Besides, our research group also developed the CRISPR/Cas9 mediated gene editing technology for the first time in banana, and albino plants have obtained from target gene MaPDS editing, and some candidate susceptible genes in transcriptome data are now being studied. At present, several resistant varieties selected by our group have been widely growing in the regions where fusarium wilt seriously occurred, which greatly contribute for alleviating the economic losses caused by this disease.

a. Dr. Sheng Ou shared his experience working on the resistance evaluation methods developed for *Foc* TR4. Among the methods used were a GFP (Green Flourescence Protein) tracing protocol, greenhouse assays, PCR detection and through field evaluations.
b. The invasion process of the fungus in the plant can be traced using the green fluorescence protein (GFP) tracing protocol. Susceptible banana varieties show more GFP incorporated in *Foc* TR4 genes, which indicates the invasion pattern of the fungus. There were more germinated *Foc* TR4 spores in the susceptible varieties than in resistant varieties. The exudates in resistant varieties play a role in reducing the number of germinated spores in the root zone.
c. In greenhouse assays, resistance is evaluated by assigning a disease index (0 – 5) based on symptoms on the leaves and corms. Molecular detection using PCR can also confirm the level of resistance of the varieties.
d. Field evaluations were conducted in different locations using six major cultivars to confirm their resistance to the fungus.
e. In a field evaluation, East African Highland banana and plantain were found to be highly resistant, including the diploid varieties.
f. Chemical mutagenesis is used to produce resistant somaclones. Some examples of clones produced are Zhongjiao No.3 (tolerant), Zhongjiao No. 4 (highly resistant), Zhongjiao No. 6 is moderately resistant.
g. A cross breeding program using Pisang Awak as the female parent, was also conducted, resulting in some highly resistant tetraploid hybrids, which are now under evaluation.
h. For molecular breeding, an Agrobacterium mediated genetic transformation system based on ECS has been established, also using Host Induced Gene Silencing (HIGS) technique for high *Foc* TR4 resistance. Currently CRISPR/Cas 9 gene editing system is becoming a useful tool develop resistant cultivars.
i. Questions from participants centered on the details of methodologies used in the different evaluation methods *i.e.* the methodology for collection of root exudate and concentrations of exudates needed for media, and time taken for hybridization of species. Banana varieties were cultivated in liquid medium, and final concentration of root exudes determined by using 10g of the root system, then the primary root exudates were used to treat spores.
j. To a question on the time taken to produce the hybrids, the presenter responded that it took more than 10 years.

k. On the commercial planting of ZJ3 and ZJ4, currently 100 ha of Zhongjiao No 4 is grown by farmers.
l. Participants also inquired on the availability of these improved materials outside China, to which presenter expressed hope that these materials will soon be available for exchange and future collaboration, especially in undertaking evaluations in other countries.

4. Dr. Sharl Mintoff, Department of Industry, Tourism and Trade, Northern Territory Government, Australia

Title: Screening for TR4 resistance: Banana variety field trials in the Northern Territory of Australia

Abstract

Fusarium wilt (Panama disease), caused by the pathogen *Fusarium oxysporum* f.sp. *cubense* (*Foc*) Tropical Race 4 (TR4) is considered one of the largest threats to the national and global banana industries. TR4 is known to infect and often kill the economically and globally important Cavendish varieties. Research work in the Northern Territory of Australia, includes the field trials in order to identify banana varieties that show some, if not complete, resistance to *Foc* TR4. Since 2016, two varietal screening field trials have been completed which assessed 54 varieties for their resistance or susceptibility to *Foc* TR4. Trial plants were inoculated with *Foc* TR4 colonized millet at planting and were assessed for the first appearance of external disease symptoms which is commonly leaf yellowing and wilting and pseudostem splitting, while internal symptoms were assessed following plant death or crop harvest. Agronomic characteristics were also examined, such as bunch emergence, bunch weight and plant height. Several varieties including some FHIA, selected hybrid, Cavendish and CIRAD lines have displayed a robust resistance or at least strong tolerances to TR4 with little to no disease symptoms observed.

Highlights and Discussion

a. Dr. Mintoff highlighted some of the TR4 research done in the Northern Territory, particularly results from the variety resistance screening work being done.
b. *Foc* TR4 was first discovered in Northern Territories, Australia in 1997 and was confirmed endemic in 2012. The TR4 research program in NT focused on alternate hosts, mutagenesis screening, crop rotation and variety resistance screening.
c. In a variety screening study conducted from 2016 to 2018, 25 banana varieties were assessed, together with reference varieties Williams (susceptible), GCTCV 218 (intermediate), FHIA 01 (resistant) and FHIA 25 (very resistant). After 2 years, some FHIA parents and hybrids such as FHIA 25, FHIA 01 and some SH lines were found without TR4 symptoms. For the Cavendish and other varieties, GCTCV 215, and Gajih Merah did not show symptoms as with FHIA 25 and 01 after the ratoon crop cycle. Generally taking into account resistance to TR4 and market quality, FHIA 25, FHIA 02, GCTCV 215 and FHIA 01 are ranked the top 4. Cavendish varieties of interest include CJ19, GCTCV 215 and GCTCV 247.

d. In a follow-up variety screening from 2018 to 2020 involving 32 Cavendish varieties, novel and other varieties including 3 reference varieties – Williams (very susceptible), Formosana (intermediate) and Goldfinger (resistant) were identified. This time however, some symptoms were observed during the plant crop in the Formosana and Goldfinger, whereas varieties such as AP1, CIRAD 03, 04 and 05 did not show symptoms. However in the 1st ratoon crop only CIRAD 03, 04 and AP1 did not show symptoms. The others showed various degrees of disease severity including Formosana and Goldfinger. To summarize, some agronomic issues were noted in the unaffected varieties – AP1 (small bunch, slow cycle) and CIRAD-4 (prone to snapping), and some Cavendish varieties performed better than Formosana – GCTCV105, GCTCV 217 and AP3. However, fruit quality and economic viability need to be investigated.

e. The next stage is to finalize the trial results with new variety trials expected to begin in December 2020 which will include CIRAD lines, EMBRAPA lines, various other varieties and 3 mutant Goldfinger lines.

f. Responding to a query on the 'opposite' results in the decreased performance of resistant varieties in the ratoon crop, compared to the plant crop, the presenter noted that this needs to be investigated further.

g. Questions were raised on the differences in results for susceptibility for Formosana between the previous study presented by Dr. James Dale and the present study. Presenter attributed this to the higher levels of the pathogen being inoculated on Formosona.

h. Another question was posed on the disease score used which did not match the reaction, to which presenter mentioned scores were adjusted and were based on mortality rates and symptom presence in plants of a particular cultivar.

i. Trials conducted in China on GCTCV 218 and 219 were not consistent in greenhouse experiments compared to field trials, which might explain some of the inconsistencies in field trials.

j. Efforts are being done to see whether weather pattern had an impact on disease susceptibility especially in the field trial results.

SESSION CONCLUSION

The chair highlighted that in resistance breeding, durable resistance is of importance due to the evolving nature of the pathogen citing farm examples, where previous resistant cultivars provided to farmers saw high incidences of TR4 a few years later. He then requested session speakers on means of producing varieties with durable resistance to TR4.

a. Prof James Dale suggested two ways, the first is to stack resistance genes as it is still not clear on the number of genes which control resistance. Referring to his work, the transgenic lines produced is a single gene, which he added may also be the case for many conventionally-bred or selected cultivars. He emphasized on the importance of seeking for additional resistance genes (different from *RGA2* etc.). Prof Dale shared his own observations, that there were differences in resistance between crop cycles with increasing levels of infection from the initial cycle. He hence recommended looking at long-term field trials since most farmers plant three, four and even five ratoons, to confirm durable resistance. To the suggestion on the possibility of GM lines (Line B) to be tested in other places, Prof Dale agreed that this is in the pipeline, however work has to be done to fully characterize and deregulate the line, while stating willingness for further collaboration with interested partners for screening the lines in different environments to provide consistent level of resistance as those grown in NT under extreme conditions.

b. Dr Uma reiterated on the dangers of depending on single dominance genes which may experience breakdown due to the evolvement of new virulence strains, and stressed on developing horizontal resistance. She too agreed on the need to stack R-genes for developing new varieties. For conventional breeding, she highlighted on the importance of understanding the genetics of inheritance, thus enabling breeding a variety with more resistance to a different spectrum of pathogens.
c. Dr. Ou Sheng suggested continuous selection from somaclonal variation and cross breeding as ways of achieving durable resistance.
d. Dr. Sharl Mintoff recommended long term screening trials and screening of parental lines.

10th NOVEMBER 2020

SESSION D: INTEGRATED CONTROL MEASURES AND SUSTAINABLE FIELD MANAGEMENT PRACTICES

SESSION CHAIR: Dr. Sijun Zheng, Alliance of Bioversity International and CIAT/ Yunnan Academy of Agricultural Sciences, China

1. **Dr. Paul G. Dennis, School of Earth and Environmental Sciences, The University of Queensland, Brisbane, Queensland, Australia**

Title: A roadmap to improve banana production via microbiome management

Abstract

While plant microbiomes are known to influence host fitness, management of these communities to optimize crop productivity remains an intractable problem. Some of the key challenges faced by those attempting to solve this issue, are associated with the fact that plant microbiomes are extremely diverse, and often differ in composition between host genotypes, and geographic location due to edaphic and climatic conditions. These issues make it difficult to identify management interventions that have predictable consequences for host-microbe interactions between production systems. Importantly, despite a preponderance of transient host-microbe interactions, a subset of habitually associated 'core' taxa are typically present, and are inferred to be important for host fitness. By identifying, and learning how to influence these taxa, it may be possible to accelerate the development of microbiome management practices that help to optimize agriculture. Here, I present our work on identifying a core microbiome of *Musa* spp., and the progress that we are making in understanding the functions and ecological preferences of each population.

Highlights and Discussion

a. Dr. Dennis' presentation clearly highlighted the importance of microbiomes in sustaining plant and soil health. Microbiomes are so complex and diverse as their effects are profound that they can cause deleterious as well as beneficial effects. Dr. Dennis also rendered crucial insights to the complexities of managing these microbiomes which are diverse and different according to conditions.
b. One way to analyze microbiome complexity in soils is to use gamma radiation to neutralize it. With no microbiomes, 'dead' soil inoculated with TR4, showed an increase of 2,500 times after 21 days, compared to living soil at only 0.6 times.
c. Even though managing the microbiomes is difficult due to their diversity and complexity, fortunately, many important populations, known as core taxa are ubiquitous. Core taxa are persistently associated with the host and inferred to be important to host fitness.
d. For banana, the roadmap to identify core taxa involves identifying core *Musa* spp. microbiome, assign function to core taxa and to learn how to control their abundances.

e. It has been observed that diversity of microbes decrease from the soil to leaves and they are compartmentalized according to the species and composition. But no difference was observed between genotypes.
f. In defining candidate core microbial taxa 47 core taxa have been identified
g. Validating in field trials that core taxa are significantly more important than none core taxa. Populations in the field – core taxa dominated the key constituents of field microbiomes. *e.g.* in endorhizosphere, more than 90% represented by core taxa.
h. Core bacterial OTUs (Operational Taxonomic Units) have close relatives globally, for example, 38 core OTU's) have close relatives of the core taxa found across 4 continents. Similarly for fungi 21 core taxa including *Foc*, it has been recorded that 75 % of key OTUs in 20 banana farms were core taxa.
i. The research identified 38 core bacterial OTUs and 22 fungal OTUs which were relevant in pot and field grown plants, across multiple soils and continents.
j. Field management influences microbiome by enhancing or reducing its benefits.
k. Clarifying the function of ground cover in reducing the abundance of *Foc*, Dr. Dennis explained that having other plants (rather than bare soil) provided microbes with other alternative hosts, with some of these releasing compounds suppressing soil pathogens, providing competition for soil nutrients which are otherwise accessed by Fusarium.
l. Dr. Dennis further elaborated why genotypes were not relevant in field experiments. In field experiments, genotypes were not replicated and basically used as a germplasm collection with physical and agronomic attributes checked by experts, and no experiments conducted to analyze differences between genotypes. However, of relevance were the distribution of the core taxa which showed throughout the genotypes.
m. The presenter also confirmed that no temporal studies have so far been conducted on the stability of microbe populations in the field. However in a study on banana-associated soils, banana tissues were collected from 20 farms under different managements. Through characterization of the banana microbiome and tissues in each of the farms importance of microbes was considered if presence is observed in majority (half) samples. Findings indicated 75% to be of the core taxa. Information on collected samples were also inputted into the SILVA database for analysis and confirmation.
n. Dr. Dennis summed the discussion saying that despite the gargantuan challenge in managing thousands of taxas in nature and the impossibility in studying all of these, obtaining understanding on a smaller amount (such as from his study) and its functions provides hope for future work.

2. Dr. Rong Li, Nanjing Agricultural University, China

Title: Biofertilizers and banana plant health

Abstract

Use of bio-organic fertilizers (organic fertilizers containing biocontrol microorganisms) has proven to be a promising approach for environmentally friendly control of plant pathogens. However, we still have surprisingly little insight into the mechanisms that

dictate effective disease protection, hampering future developments. In the lecture, we used a unique experimental design to disentangle the roles of specific components of bio-fertilizer, including the organic substrate, the biocontrol agent (*Bacillus amyloliquefaciens* W19) and the compost microbiome. This experiment followed Fusarium wilt disease in banana over three growing seasons and linked the level of disease to changes in the soil microbiome. We found that the biocontrol agent was critical to the disease suppressive activity, while the organic substrate and compost microbiome had no significant impacts, and this activity was due to both the biocontrol agent as well as changes in the microbiome resident to the soil. Most strikingly, one specific *Pseudomonas* spp. OTU was highly responsive to treatments containing the biocontrol agent, sparking us to examine this genus in greater depth. Via a series of additional cultivation-dependent and independent experiments, we could establish a link between *Pseudomonas* spp. density and the abundance of *Bacillus*, demonstrate enhancement of *Bacillus* biofilm formation by specific *Pseudomonas* isolates and validate synergistic effects between responsive *Pseudomonas* population and *B. amyloliquefaciens* W19 in suppressing Fusarium wilt disease. In total, our results provide the mechanistic underpinnings explaining the disease-suppressive action of bio-organic fertilizer treatment, thereby paving the way for more informed future strategies for sustainable disease control.

Highlights and Discussion

a. Field experiments using bio-organic fertilizer and organic fertilizer were conducted in newly reclaimed fields. Bio-organic fertilizer application induced significantly variation of bulk soil bacterial community which further affected to bacterial composition in the rhizosphere.
b. In another long term experiment in monoculture fields, disease incidence was observed to have occurred less in fields treated with bio-organic fertilizers compared to chicken manure organic fertilizer, organic fertilizer and chemical fertilizer control. Different soil amendments induced different soil microbial composition.
c. In a 4 season pot experiment using infected soil with treatments comprising bio-organic fertilizer, sterilized bio-organic fertilizer, organic fertilizer and sterilized bio-organic fertilizer plus NJN -6 bacteria. It was observed that for all treatments, disease incidence declined over the 4 seasons.
d. Organic matter and Bacillus could significantly induce the increase in *Pseudomonas* numbers and generate biofilm. In general, stable abundance of bio-control agent, the increased abundance of Pseudomonas and their interaction contributed to the enhanced disease suppression of *Fusarium oxysporium* sp. *cubense*.
e. Questions were raised on the effectiveness of bio-organic fertilizers on disease suppression levels in the field.
f. The session chair proposed combining the bio-organic fertilizer approach with resistant and tolerant varieties for a more effective outcome. Presenter agreed that in experiments with the Guangdong Academy of Agricultural Sciences, positive results were obtained with the resistant varieties combined with use of bio-organic fertilizer.
g. There was also interest on if any source of soil from the farm can be used as the

source for bioorganic fertilizer, to which presenter responded that in China most soil types were not very different.

3. Dr. Tony Pattison, Department of Agriculture and Forestry, Queensland, Australia

Title: The role of non-banana plants in the Management of Banana Fusarium wilt

Abstract

Banana Fusarium Wilt caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), causes devastating losses to banana production and threatens livelihoods around the world. The Tropical Race 4 (TR4) strain continues to spread to more banana producing countries with potentially severe consequences for the global export banana trade. Banana growers are faced with three scenarios in relation to the banana Fusarium wilt; being free from the disease, having the first incursion onto their farms, with low disease incidence or having being devastated by the disease and requiring methods to re-establish profitable banana production. The strategies used to manage the disease in each of the scenarios changes, moving from biosecurity, to management and finally to cultivar replacement. However, in each of the three different scenarios, the management of non-banana plants is a critical component of the overall disease management. Non-banana plants can be viewed as having three different roles within the banana plantation, either; alternative hosts that provide refuge for the pathogen and carry the disease over, as rotation crops that can be grown between successive banana crops or as ground covers and intercrops growing alongside banana plants. Management of non-banana plants can have long term consequence for subsequent banana production where banana Fusarium wilt is present. Depending how non-banana plant species host *Foc* and organisms that are antagonistic to *Foc*, they can be viewed as either being desirable or undesirable.

Highlights and Discussion

a. The 3 main scenarios to combat *Foc* TR4 are to strengthen biosecurity measures in the absence of the pathogen, inoculum management and plant tolerance when there is initial incursion and low incidence of the disease and working on resistance varieties when there is a serious disease incursion and epidemic.
b. The presenter focused on the inoculum and plant management through studies on non- banana and alternative host plants. In following the growing pattern of bananas, infected areas could include alternative hosts, rotation crops incorporated during fallow period and ground cover use after replanting and during ratooning. Based on earlier experiments disease symptoms were observed in sterile soil inoculated with *Foc* TR4 rather than in non-sterilized soil, indicating some form of antagonism in fresh soil. There has also been very little research work in alternative host, rotation crops and ground cover as management tools to suppress *Foc* TR4.
c. A field surveys on alternative plant species was conducted in Northern NSW, North Queensland and Northern Territories. These results have been published as a 'Guide to Alternative hosts of panama disease in Australia'.
d. All plants are potential alternative hosts to Foc. Host status depends on inoculum

level, with some hosts better than others. It is rather difficult to isolate *Foc* from field samples and when plants senescence they are colonized by *Foc*.

e. For rotational crops, the cavalcade did seem to reduce symptoms of the disease.

f. Compared to bare soil, ground covers have been shown to significantly reduce pseudostem discoloration, even though all plants were affected. There was also more fungal richness in the areas with ground cover which was correlated to the increase of beta gluscosidase activity.

g. Non-banana plants have an important role as alternative hosts, rotation crops and ground covers in the management of banana Fusarium Wilt, but they may either be undesirable or desirable.

h. Responding to a query on the efficacy in using paddy rice for crop rotation for suppressing *Fusarium* inoculum, the presenter responded that other research has indicated that paddy rice as a rotated crop can act as a suppressant. Dr. Pattison also linked this to functioning microbiome (as discussed by Dr. Dennis) which will then lead to greater suppression. However, if this functioning microbiome is lost under paddy cultivation chances of the recurrence of the disease is high. This relationship he suggested is an avenue for future research, especially in Southeast Asia.

i. On the yield of bananas in ground cover versus non-ground cover, Dr. Pattison highlighted that through collection of agronomic data in field trials, yields were lower under ground cover, and what is being recommended now is to establish the ground cover after the banana has been established to avoid early competition and yield reduction.

j. Providing a possible explanation to the role of nematodes in Fusarium wilt, Dr. Pattison stated that his studies where nematode diversity was measured, the types of nematodes identified seemed to indicate that conditions which favor plant parasitic nematodes tend to also be conditions which favor *Fusarium*. However, it is still unclear if nematodes are the cause of fusarium wilt, but underlying conditions such as high nitrogen, lack of diversity and lack of competition tend to favor both root pathogens.

4. Dr. Xu Shengtao, Yunnan Academy of Agricultural Sciences, China

Title: Effect of cover crops on soil chemical properties and microbial diversity under banana cropping system in a dry hot valley area.

Abstract

Exposed soil degradation is one of the most constrains for banana production. Developing the adaptable farming system is becoming an urgent issue for the sustainability of banana industry. Cover plants as an effective method of conservation tillage could improve soil quality, which would increase the absorption and utilization of nutrients by crops. In this study, banana is chosen as an important cash crop in Yunnan Province. We used the traditional cropping system (bared soil) as the control, and the two treatments were natural weed cover and cultivated clover (*Macroptilium atropurpureum* (DC.) Urb.) as cover plant. The effects of cover plant on soil quality were analyzed by evaluating soil chemical properties and microbial diversity. The results showed that natural weed cover and cultivated clover significantly affected

and increased the content of organic matter and alkali nitrogen in coverage zone soil, and had less effect on the banana planting zone soil physical and chemical properties. Both natural weed cover and cultivated clover significantly affected coverage zone soil enzyme activity, only significantly affected the soil invertase, catalase and phosphatase activities in the banana planting zone soil, but natural weed cover and cultivated clover significantly improved coverage zone soil invertase and catalase activities compared to the soil before banana was planted. Both natural weed cover and cultivated clover affected the soil microbial diversity index in planting and coverage zone, but the soil microbial diversity index of banana plantation showed a downward trend compared with the soil before banana was planted. Both natural weed cover and cultivated clover significantly affected the carbon-nitrogen functional gene copies of coverage zone soils, and it had less impact on planting zone soil. Results showed that legume cover plant is superior to natural weed coverage. Our research indicated that cover plant effectively increases the soil potential production under banana cropping system in the perspective of soil properties. However, due to the short period of cover plant, the impact of cover plant on the whole ecosystem of banana cropping system needs further systematic research.

Keywords: cover plant, banana, soil chemical properties, soil microbial diversity, soil enzymatic activity

Highlights and Discussion

a. The presenter suggested that further research has been planned to study the effect of cover on the incidence of pest and diseases, the screening of functional cover plants for banana cropping system and to study the relationship between the soil microbial community and TR4 incidence in heavily infected soils.
b. Responding to a query on the method for measuring microbial diversity, the presenter mentioned that the bacteria and fungi were sequenced using the Illumina MiSeq platform. The planned study on the relationship between the soil microbial community and TR4 incidence will be slightly different than the one conducted by Dr. Tony Pattison in Australia, in that there would be screening of functional cover plants first. This is in response to the possibility of achieving similar results related to less pseudostem discoloration caused by TR4 in covered soil compared to bare soil, as reported in the Australian study.

5. **Dr. T. Damodaran, ICAR-Central Soil Salinity Research, Lucknow India**

Title: Field management strategy to control Fusarium wilt disease

Highlights and Discussion

a. Dr. Damodaran provided insights to the findings from work undertaken for the past three years on field management strategies for controlling TR4. His presentation started with an introduction to the outbreak of the TR4 which began in the Northeast subtropical region in India in 2017, and has since spread through planting materials, irrigation canals and floods. The main strategy was to map and stop the disease from spreading to the central or southern regions which are main banana growing areas. Among the strategies adopted for control included survey and mapping, identification and confirmation, and management through the use of biocontrols, induction of host tolerance, rhizosphere microbiome and resistant/tolerant cultivars. He also introduced the ICAR-FUSICONT, a bio-fungicide using the novel strain of *Trichoderma reesei* on a patent protected media designed for controlling TR4.

b. The different phases in containment were community sensitization, identification of hotspots, identification of antagonist, pot and field efficacy evaluation and formulation of antagonist and large scale testing in communities.

c. In a pot study, *Trichoderma reesei* was found to have antagonistic properties as well as plant growth enhancement potential. In a field trial hotspot farmers field, *Trichoderma* was recorded to have reduced disease incidence as compare to the control plants. Important metabolites such as Iturin C, Fenigycin and Peptabiols have also been identified using LC-MS analysis. It was observed that *Trichoderma reesei* induces a mechanism which antagonizes the mytoxins produced by the pathogen. A bio-fungicide was then formulated using the novel strain of *Trichoderma reesei* for the control of *Foc* TR4 in bananas (ICAR FUSICONT)

d. In trials with farmers, there was a significant decrease in disease incidences and higher yields in plots belonging to adopter farmers compared to the non-adopters. This intervention showed positive results because the targeted bio-fungicide application is in accordance to the pathogen load with is dependent on weather conditions being in a sub-tropical environment.

e. This has also encouraged development of a technique to induce host tolerance through *in-vitro* immunization technology. The *in-vitro* immunized plantlets are now being tested in farmers' plots.

f. The ongoing research includes identification of a tolerant line (Malbhog) type (MBB-1), large scale evaluation of immunized plantlets and supplementation with mutualist microbes that have antibiosis effects with *Foc* TR4.

g. Discussion covered a range of interests from the participants including the number of growing seasons using the product for assessing the percentage of infected plants. So far 2 growing seasons and currently the third growing season are being assessed. On a query about FUSICONT, the presenter explained that FUSICONT is a consortium containing primarily *Trichoderma reesei* and *Lactobacillus* sp. In response to the paddy crop rotation with bananas, the presenter confirmed that this is true of the trial plots next to those treated with FUSICONT. On another query about the effectiveness of *in-vitro* immunization of plantlet, the presented mentioned that this is still being assessed as it is in the trial stage.

Panel Discussion and Workshop Conclusion

Chairperson for the panel discussion, Mr. Yacob Ahmad of TFNet congratulated all speakers and participants for providing rich knowledge and input during the four series of the workshop. He called for greater collaboration as it was evident there are varied levels of research work carried out (some very advanced, while some still preliminary) which are also possibly linked to availability of resources, disease infection status, the state of readiness to combat the disease and linked to the various conditions on the ground.

Ms. Irene Kernot of ACIAR once again reminded that the aim of the workshop was to create awareness of ongoing research. She expressed support for countries that are only now beginning the journey in managing TR4, and thus called for strengthening collaboration. She was also amazed at the wealth of research going in the field, some of which have not been published and are new and innovative, and called for continued discussion to take place while challenging one another to conduct better research in this field. Besides receiving participant feedback through direct emails, Ms. Kernot proposed that a survey be conducted to focus on the research areas that need to be prioritized and pursued.

Dr. Chun Yu- Li of GDAAS recommended the establishment of a collaborative platform for all experts with coordination from international agencies such as FAO-UN, the Alliance of Bioversity-CIAT, ACIAR and TFNET, with the purpose of designing international collaborative projects involving country level experts to collectively solve the TR4 challenge. In addition, he also suggested organizing visits, workshops and other activities of relevance from time to time.

Dr. Sijun Zheng of the Alliance of Bioversity-CIAT agreed on the recommendation for greater collaboration and suggested developing a knowledge product from this workshop in the form of a publication (i.e. booklet) as a first step to kickstart other efforts such as developing project proposals.

Participants generally were in favor of the calls by the panelists for greater collaboration and accelerating the process of obtaining practical solutions.

Finally, participants were invited to communicate with organizers via email to provide their suggestions on potential areas which could be taken up as a research effort in the future.

Areas of collaborative research highlighted during the workshop were as follows:

a. More collaboration is needed to understand virulence factors and the mechanism of host-pathogen relationship, focusing on gene editing technologies, especially in banana receptor genes to improve resistance to TR4.
b. Further assessment and screening of germplasm diversity for resistance
c. The need to have more access to transgenic banana cultivars that are resistant to *Foc* TR4
d. More studies on reliable detection systems and techniques to ascertain pathogen presence and load in affected soils, water and other systems that are quick and easy to use by resource-poor smallholders.

e. More studies on the diversity and characterization of VCGs and their pathogenicity.
f. Breeding of genetically edited bananas for resistance to *Foc* TR4 is currently being carried out and there is the potential of collaboration in countries to demonstrate 'durable' resistance of the varieties in the different climatic regimes such as humid tropics and subtropical areas.
g. Using protoplast culture in the regeneration of plants with resistant traits to TR4.
h. Soil microbiome health, cover cropping, rotational and mixed cropping, alternative hosts and use of bioagents are some of the field management aspects of controlling TR4 that warrant further investigations.
i. Further studies on crop rotation using paddy to suppress *Foc* TR4

Other proposed research focus among countries will be based on a on a post-workshop survey sent to all participants and further discussion with the workshop organizers and relevant research institutions.

List of Presenters and Participants

#	Name	Organization	Country
Presenters			
1	Dr. Elizabeth Aitken	The University of Queensland (UQ)	Australia
2	Dr. Tony Pattison	Department of Agriculture and Fisheries Queensland	Australia
3	Dr. James Dale	Queensland University of Technology	Australia
4	Dr. Sharl Mintoff	Biosecurity and Animal Welfare Department of Industry, Tourism and Trade, Australia	Australia
5	Dr. Paul Dennis	University of Queensland Australia	Australia
6	Stewart Lindsay	QLD DAF Australia	Australia
7	Dr. Chunyu Li	Fruit Tree Research Institute, Guangdong Academy Of Agricultural Sciences (GDAAS)	China
8	Dr. Ou Sheng	Fruit Tree Research Institute, Guangdong Academy Of Agricultural Sciences (GDAAS)	China
9	Dr.Wei Wang	Chinese Academy of Tropical Agricultural Sciences (CATAS)	China
10	Dr. Rong Li	Nanjing Agri. University	China
11	Dr. Sijun Zheng	Alliance Bioversity CIAT/YAAS	China
12	Dr. Xu Sheng Tao	Yunnan Academy of Agricultural Science (YAAS)	China
13	Dr. Tingting Bai	Yunnan Academy of Agricultural Science (YAAS)	China
14	Dr. Miguel Dita	Alliance of Bioversity International & CIAT	Colombia
15	Mr. Victor Prada	Food and Agriculture Organization	FAO
16	Dr. S. Uma	National Research Centre for Banana (ICAR - NRCB)	India
17	Dr. Damodaran Thukkaram	Central Soil Salinity Research Institute (ICAR - CSSRI)	India
18	Dr. Riska	Indonesian Tropical Fruit Research Institute (IFTRI)	Indonesia
19	Dr. Chittarath Khonesavanh	Department of Agriculture	Laos
20	Dr. Farah Farhanah Haron	Malaysian Agricultural Research and Development Institute (MARDI)	Malaysia
21	Dr. Edna A. Anit	Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development. (DOST-PCAARRD)	Philippines
22	Dr. Altus Viljoen	University of Stellenborsch South Africa	South Africa
23	Dr Diane Mostert	Stellenbosch University	South Africa

#	Name	Organization	Country
Participants			
1	Irene Kernot	Australian Centre for International Agricultural Research (ACIAR)	Australia
2	Ryan Orr	James Cook University	Australia
3	Stephen Spear	Australian Banana Growers Council	Australia
4	Tamaya Perressini	Australian Centre for International Agricultural Research (ACIAR)	Australia
5	Wayne O'Neil	Department of Agriculture and Fisheries Queensland	Australia
6	David East	Department of Agriculture and Fisheries Queensland	Australia
7	Rosie Godwin	Australian Banana Growers Council	Australia
8	Hazel Gaza	Department of Agriculture and Fisheries Queensland	Australia
9	Pratyush Ravichandra	Department of Agriculture and Fisheries Queensland	Australia
10	Ceri Pearce	Department of Agriculture and Fisheries	Australia
11	Paul Nelson	James Cook University	Australia
12	Tuan Nguyen	Department of Agriculture and Fisheries Queensland	Australia
13	Jim Pekin (CEO)	Australian Banana Growers Council	Australia
14	Robert (Bob) C. Williams		Australia
15	Mr. Jun Peng	Chinese Academy of Tropical Agricultural Sciences (CATAS)	China
16	Rainbow Kuang (GSS)	Guangdong Academy Of Agricultural Sciences (GDAAS)	China
17	Li Zheng	Zhongkai Uni. Of Agri. & Engineering	China
18	Qia Song Yang	Guangdong Academy Of Agricultural Sciences (GDAAS)	China
19	Ms. Liu Lina	Yunnan Academy of Agricultural Science (YAAS)	China
20	XY Yang	Yunnan Academy of Agricultural Science (YAAS)	China
21	Li Shu	Yunnan Academy of Agricultural Science (YAAS)	China
22	Zeng Li	Yunnan Academy of Agricultural Science (YAAS)	China
23	Fan Huacai	Yunnan Academy of Agricultural Science (YAAS)	China
24	He Ping	Yunnan Academy of Agricultural Science (YAAS)	China
25	Jua Cai Fan	Yunnan Academy of Agricultural Science (YAAS)	China
26	Kesuo Yin	Yunnan Academy of Agricultural Science (YAAS)	China
27	Gao Huijun	Yunnan Academy of Agricultural Science (YAAS)	China
28	Yongfen Wang	Yunnan Academy of Agricultural Science (YAAS)	China
29	Jing Liu	Yunnan Academy of Agricultural Science (YAAS)	China
30	Momo	Yunnan Academy of Agricultural Science (YAAS)	China
31	Weidi He	Yunnan Academy of Agricultural Science (YAAS)	China
32	Zhang	Yunnan Academy of Agricultural Science (YAAS)	China

#	Name	Organization	Country
33	Dandan Xiang	Yunnan Academy of Agricultural Science (YAAS)	China
34	Guangdong Zhou	Yunnan Academy of Agricultural Science (YAAS)	China
35	Shu Sheng	Yunnan Academy of Agricultural Science (YAAS)	China
36	Xundong Li	Yunnan Academy of Agricultural Science (YAAS)	China
37	Guanglin Wang	Australian Centre for International Agricultural Research (ACIAR)	China
38	Jorge Vargas	Cenibano Augura	Columbia
39	Carlos Arturo Berben	PROMUSA	Costa Rica
40	Gustavo Gandini	Bananaos Ecologison de la Linea Noreste Banelino	Dominican Republic
41	Mr. Ignacio Antonio Sotomayor Herrera	Universidad Tecnológica Empresarial de Guayaquil (UTEQ)	Ecuador
42	Bo Zhou	Food and Agriculture Organization	FAO
43	Dr. BK Pandey	Indian Council of Agricultural Research (ICAR)	India
44	Dr. Prakash Patil	Indian Council of Agricultural Research (ICAR)	India
45	Backiyarani N.	National Research Centre for Banana (ICAR - NRCB)	India
46	Raman Tangavelu	National Research Centre for Banana (ICAR - NRCB)	India
47	Dr. Ramajayam D.	National Research Centre for Banana (ICAR - NRCB)	India
48	Prashanti Patel	Bhabha Atomic Research Centre	India
49	R. Selvarajan	National Research Centre for Banana (ICAR - NRCB)	India
50	M. Loganathan	National Research Centre for Banana (ICAR - NRCB)	India
51	Dr. M. Muthukumar	National Research Centre for Banana (ICAR - NRCB)	India
52	R. Selvarajan	National Research Centre for Banana (ICAR - NRCB)	India
53	Dr. Anup Karwa	National Research Centre for Banana (ICAR - NRCB)	India
54	KN Shiva		India
55	Arjun Singh		India
56	Nidhi		India
57	Dinesh Kumar - Agarwal		India
58	Siddesh Ghag		India
59	Kavita (Akanksha) Yadav		India
60	Niimi		India
61	Giribabu		India
62	MS Saraswathi	National Research Centre for Banana (ICAR - NRCB)	India
63	Suresh Kumar Paramasiram		India

#	Name	Organization	Country
64	Dr. A. Snehalatha Rani	HRS Kovvur	India
65	Keerthana Sasikumar (KNMS 4B)		India
66	Dr. Shailendra Rajan	Central Institute for Subtropical Horticulture (CISH) Lucknow	India
67	Agnes Mukherjee		India
68	Dr. Ellina Mansyah	IFTRI, Indonesia	Indonesia
69	Ms. Anik Kustayarti	Department of Horticulture, Ministry of Agriculture Indonesia	Indonesia
70	Intan M. Fajarsari	Department of Horticulture, Ministry of Agriculture Indonesia	Indonesia
71	Nelly Saptayanti	Department of Horticulture, Ministry of Agriculture Indonesia	Indonesia
72	Lia Hapsari	Purwododi Botanic Garden Indonesia Inst of Science	Indonesia
73	Yacob Ahmad	International Tropical Fruits Network (TFNet)	Malaysia
74	Dorothy Chandrabalan	International Tropical Fruits Network (TFNet)	Malaysia
75	Hariyatul Asni Abdul Rani	International Tropical Fruits Network (TFNet)	Malaysia
76	Christian Cangao	International Tropical Fruits Network (TFNet)	Malaysia
77	Noor Ba'ah Abdol Said	International Tropical Fruits Network (TFNet)	Malaysia
78	Dr. Rozeita Laboh	Malaysian Agricultural Research and Development Institute (MARDI)	Malaysia
79	Suhana Ahmad	Malaysian Agricultural Research and Development Institute (MARDI)	Malaysia
80	Mr. Aiman Takrim Zakaria	Universiti Putra Malaysia	Malaysia
81	Sathis	CABI	Malaysia
82	Dr. Sivapragasam	CABI	Malaysia
83	Sabariah Khamis	Biosecurity Department of Agriculture	Malaysia
84	Yasmin Othman	Universiti Malaya	Malaysia
85	Sivanaswari Chalaparmal	Malaysian Agricultural Research and Development Institute (MARDI)	Malaysia
86	Salehudin Md. Radzuan	Hort. Research Center, MARDI Sintok	Malaysia
87	Dr. Ganisan	Malaysian Agricultural Research and Development Institute (MARDI)	Malaysia
88	B. Biosecurity		Malaysia
89	Daw Poe Ei Win	Agri Microbiology	Myanmar
90	Khun Nay Mio Lwin	Department of Agricultural Research (DAR)	Myanmar
91	Cho Cho Din	Department of Agricultural Research (DAR)	Myanmar
92	Luud Cercx	Agrofair	Netherlands
93	Olawale Arogundade	National Horticultural Research Institute (NIHORT)	Nigeria
94	Jessa Blesilda Antero	Bureau of Plant Industry	Philippines
95	Kristine Panaligan	Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development. (DOST-PCAARRD)	Philippines

#	Name	Organization	Country
96	Dr. Lilia Portales	Bureau of Plant Industry	Philippines
97	Dr. Lorna Herradurra	Bureau of Plant Industry	Philippines
98	Benny Mendoza Corcolon	Fasum Agri. Dev. Company Inc.	Philippines
99	Maricar Salacinas Niez	Dole Inc. Philippines	Philippines
100	Aaron Shipman	Hawaii University	USA
101	Achyut Adikari	Hawaii University	USA
102	Dr. Nguyen Quoc Hung	Fruit & Vegetable Research Institute (FAVRI)	Vietnam
103	Minh Tran	Fruit & Vegetable Research Institute (FAVRI)	Vietnam
104	Vu Dang Toan	Fruit & Vegetable Research Institute (FAVRI)	Vietnam
105	Vu An Binh	Fruit & Vegetable Research Institute (FAVRI)	Vietnam
106	VRQ	Fruit & Vegetable Research Institute (FAVRI)	Vietnam
107	Christobal Fabrega		
108	Edwin Raj		
109	Mr. Kavino		
110	J. Sandoval		
111	Kodjo Tomekpe		

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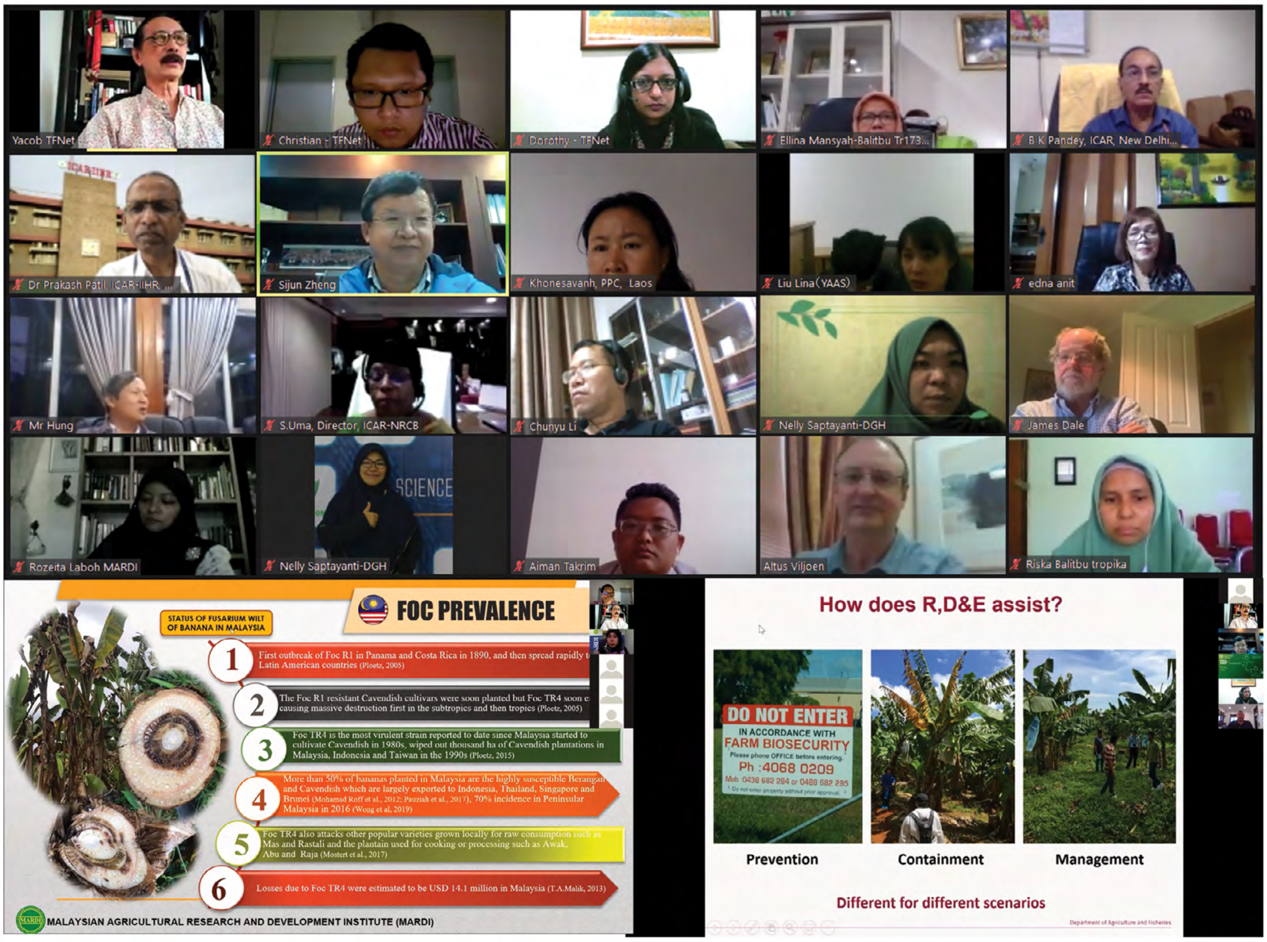

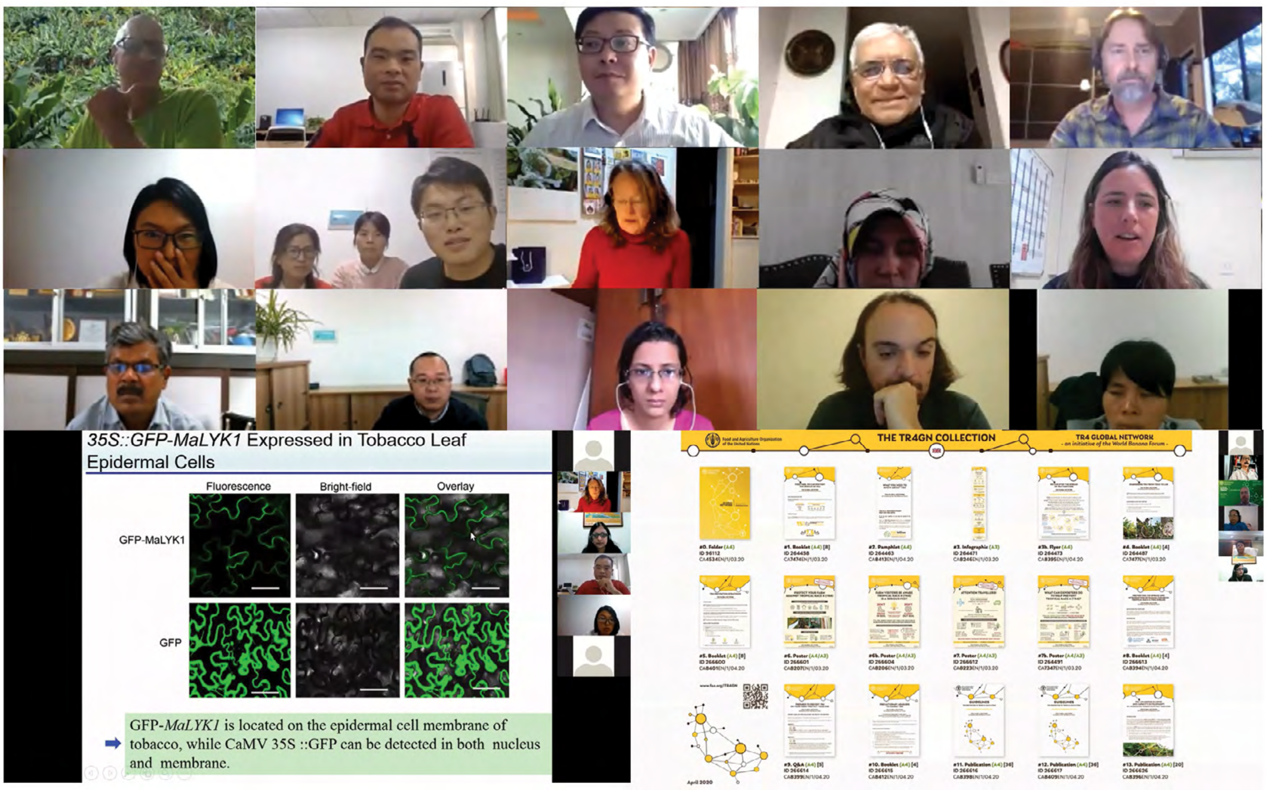

INTERNATIONAL TROPICAL FRUITS NETWORK
P.O. Box 334, UPM Post Office
(Block C8, MARDI Headquarters)
43400 Serdang, Selangor
Malaysia

Tel. No.: 603-8941-6589
Fax No.: 603-8941-6591
Email: info@itfnet.org
Website: www.itfnet.org

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