

**REPORT**

# **WORKSHOP ON MANAGEMENT OF BACTERIAL DISEASES IN BANANA**

29 November 2022  
Virtual Workshop

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Editor:  
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International Tropical Fruits Network (TFNet)



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China

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Photo: Jane Ray, University of Queensland

## 1.0. EXECUTIVE SUMMARY

Besides diseases caused by fungal pathogens, banana diseases caused by bacteria also have an adverse impact on food security and income especially for smallholders. In recent years, there have been reports of an upsurge of bacterial diseases, mainly in cooking bananas. Observably, the impact of banana bacterial disease prevalence and seriousness differ in the different banana growing regions. Southeast Asian countries such as Indonesia, Malaysia and Philippines have reported serious incidences of banana blood disease and MOKO which are caused bacterial *Ralstonia syzygii subsp celebensis* and *Ralstonia solanacearum*, respectively. Other important banana bacterial wilt and rot diseases are caused by *Xanthomonas* sp, *Erwinia* sp., *Dickeya* sp. and *Klebsiella varicola* have been reported in Africa and China.

Researchers from Australia, China, India, Indonesia, Malaysia, Uganda and the Philippines shared information and discussed on the impact of such diseases in an online workshop on bacterial diseases in banana on 29 November 2022. The workshop was organized by the International Tropical Fruits Network (TFNet) with partners Malaysian Agricultural Research and Development Institute (MARDI) and Fruit Tree Research Institute, Guangdong Academy of Agricultural Science (FTRI, GDAAS) China.

The main aim of the workshop was to share information among researchers and stakeholders on the current status of banana bacterial diseases in Asian and African countries and ongoing research on pathogen and disease characteristics and management strategies to combat the diseases. The workshop was also intended to discuss research priorities and possibilities of joint projects and collaborations among research institutions and stakeholders.

Attended by more than 300 participants from 30 countries, the session provided some indication of the gravity and impact of bacterial diseases of banana if not well managed and contained.

While bacterial diseases on banana in Australia are manageable with strict biosecurity regulations to prevent entry, the other countries are responding to the challenges with different approaches. Dr. Nandita Pantania from the Queensland Department of Agriculture and Fisheries reported that in Australia, there are cases of corm rot caused by *Dickeya* sp. occurring in Cavendish bananas, followed by, to a lesser extent fingertip rot caused by a few associated bacteria including *Dickeya* spp. and *Klebsiella* spp. However, both diseases are manageable through good field practices.

Dr. Yunhao Sun, researcher at Zhongkai University of Agriculture and Engineering, China, highlighted the pathogen profile, comparative genomic analysis and microbial interaction network of *Klebsiella variicola*, which causes banana sheath rot, mainly on the Cavendish variety. Bacterial diseases in bananas are now a major concern in China. Investigations are currently carried out to determine the effectiveness of ethylcycin and *Bacterium subtilis* R31, currently used to control Fusarium wilt, in controlling *Klebsiella variicola*.

In Indonesia, Malaysia and the Philippines, MOKO disease (Bugtok in the Philippines) and BBD (banana blood disease) caused by *Ralstonia solanacearum* and *Ralstonia syzygii* respectively are serious diseases affecting cooking banana varieties. Disease control is mostly by field management practices. Dr. Lorna Herradurra from the Philippines Bureau of Plant Industries shared her experience in disease control through field sanitation and control of insects such as wasps, bees and thrips that visit the male inflorescence. Bagging the inflorescence and debudding it after 14 to 15 days are normally practiced to reduce infection. Dr. Catur Hermanto from the National Research and innovation Agency, Indonesia also discussed inflorescence bagging and debudding to control insect transmission, plus other field practices that include use of disease-free planting materials and field sanitation practices. Dr. Rozeita Labuh from the Malaysian Agricultural Research and Development Institute cited that even though incidences of BBD have decreased in the last few years due to better field management practices, studies on the use of induced systemic resistance (ISR) using microorganisms and the use of bacteriophage have potential in curbing disease spread. In diagnostics the development of a banana blood disease detector using LAMP (loop mediated isothermal amplification) bridged with carbon particles flocculants will be a useful tool for early disease detection.

Dr. Murugan Loganathan from National Research Centre for Banana, ICAR, India related his experience with rhizome rot/soft rot caused by *Pectobacterium caratovorum* as a serious disease affecting mostly Cavendish cultivars in Southern India. Besides the use of antibiotics, chemicals, clean planting materials and growing short-term intercrops, bioformulations (plant growth-promoting rhizobacteria) are also included as control measures.

Alliance of Bioversity International and CIAT researcher, Dr. Guy Blomme, who is based in Central Africa, illustrated the effectiveness of the innovative SDSR (Single diseased stem removal) technique to control *Xanthomonas wilt* in cooking bananas, besides other field practices such as male bud removal and sterilization of farm tools. Dr. Bloome also mentioned about the development of transgenics as an effective way to combat the disease.

Generally, the workshop sent a signal of concern that the global impact of bacterial diseases on bananas, especially on cooking bananas can be far-reaching. Research priorities to mitigate disease impact need to be streamlined by conducting surveys and identifying the causal pathogens, studying dynamics of disease spread, incorporating appropriate technologies and field practices, and using molecular approaches in the breeding of resistant varieties.

The workshop indicated that, with current breeding work to produce resistant cultivars using molecular techniques, such as CRISPR Cas-9 still in progress, the immediate and most pragmatic approach is to fine tune, strengthen and integrate current best field management practices, use of clean planting materials and proven bioformulations. Integrating and combining all control and management techniques are therefore imperative.

Regional collaboration among researchers and stakeholders in the industry is also key to resolve challenges posed by bacterial diseases in banana.

## 2.0. WORKSHOP INTRODUCTION

The workshop was introduced by Ms. Dorothy Chandrabalan, Acting CEO of TFNet who welcomed all participants. She gave a brief background on challenges faced by farmers in managing their banana crops against bacterial diseases since less focus has been given to the threat of these diseases especially on cooking varieties and plantain compared to popular dessert varieties, which involves mainly smallholders and can impact on food security and income. She added that one objective of workshop was to gauge the current status, management practices and research priorities to control banana bacterial diseases in Asia and Africa, with the overall aim of obtaining inputs for collaborative research.

## 2.0. PRESENTATIONS

The first two presentations were chaired by Dr. Li Chunyu, Vice Director of the Fruit Tree Research Institute, Guangdong Academy of Agricultural Sciences, China.

### 2.1. 'Status of bacterial diseases and their impact on the Australian banana industry' by Dr. Nandita Pathania, Queensland Department of Agriculture and Fisheries, Australia.

The session began with Dr. Nandita Pathania, Senior Plant Pathologist from the Queensland Department of Agriculture and Fisheries, Australia who emphasized that while the banana industry with Cavendish variety is the largest horticulture industry in Australia, biosecurity and pests and disease management are the key priority areas of research and development. The 2 bacterial diseases reported in Australia are the endemic corm rot caused by *Dickeya* sp. and to a lesser extent the fingertip rot caused by a few associated bacteria including *Dickeya* spp. and *Klebsiella* spp. Corm rot occurs mainly during warm and humid conditions in poorly drained areas, and transmitted by asymptomatic planting materials, contaminated equipment and machinery, prolonged survival in soil and plant debris, nematodes and weevils and a wide host range. Causal pathogens are associated bacteria of *Dickeya* sp. and *Pectobacterium* sp. Typical symptoms of bacterial corm rot include yellowing and browning of lower leaves, black discoloration and rotting within the corm and roots with 40 percent of infected plants tipping over at ground level. However, both diseases can be managed through disease free planting materials and good field practices such as disinfecting tools, improve drainage, removal of diseased plants, manage weevil borer and nematode and applying preventive sprays.

The fingertip rot disease whose symptoms are not always obvious is a postharvest malady which affects fruits in packing sheds and the supply chain thereafter. Current disease management involves removal of abnormally shaped fingers during packing and improving insect pest management.

Future research approaches include awareness of endemic, exotic and emerging pathogens, surveillance, extensive molecular screening, validating diagnostic methods on pathogenic strain diversity, disease epidemiology and management. and increased collaboration.

In response to a question on the seriousness of bacterial wilt on the Cavendish cultivars, Dr Nandita said that it is weather related, with incidences occurring more during the wet weather and higher temperatures.

To a question on why the bacterial disease occurred 25 to 40 percent more in tissue cultured plants, Dr. Nandita replied that this was due to more and smaller suckers on the plants that needed to be removed. More desuckering plus the presence of calluses resulted in more disease infection. In addition, preventive sprays of copper fungicides like Mancozeb were used to control fungal diseases, which might provide entry to bacterial. No consortium of bioformulation mix were used.

## 2.2. 'Status of banana bacterial diseases in Malaysia' by Dr. Rozeita Labuh, Deputy Director of Pest and Disease Management, Malaysian Agricultural Research and Development Institute

Dr. Rozeita Labuh confirmed that banana blood disease or BBWD (Banana blood wilt disease) caused by *Ralstonia syzygii* remains one of the major bacterial diseases of banana in Malaysia. Even though incidences of BBWD have decreased the last few years due to better overall strategy in field management practices including restriction to movement of planting materials, farm sanitation, destroying infected plants, surveillance, disease free planting materials, intercrops, capacity building and diagnostics and identification, many cooking varieties are still affected such as the popular pisang nipah. The research and development strategy for BBWD management include screening banana germplasms for disease resistance, incorporation of induced systemic resistance (ISR), biological control using lytic bacteriophage, identification of virulent genes causing pathogenicity, DNA fingerprinting for banana accessions and development of a DNA based identification kit for quick diagnostics.

Studies also include selection trials on 128 banana accessions, where 10 accessions were found to be highly tolerant, while 13 accessions were tolerant. The ISR approach also showed promise with lower infection in treated seedlings and plants exhibiting better growth as a result of bacteria suppression in infected areas. The use of lytic bacteriophage as biological control also showed promise, while the genome of locally isolated BDB has been fully sequenced to understand its virulence and pathogenicity. Furthermore, varietal accessions of bananas have been identified through SSR markers.

In diagnostics the development of a banana blood disease detector using LAMP (loop mediated isothermal amplification) bridged with carbon particles flocculants will be a useful tool for early disease detection.

The way forward according to the speaker entails the strengthening of breeding programs to develop resistant cultivars, field evaluation of lab tests and upscaling before commercialization, utilization of molecular information developed, sharing and collaboration among local and foreign researchers, confirmation of the effectiveness of bacteriophage cocktails and ISR technology in controlling banana blood wilt disease.

In response to a question on banana cultivars that are resistant to bacterial diseases in Malaysia, Dr. Rozeita replied that there are none. On the prospects of the use of bacteriophage for disease control, Dr. Rozeita responded that further studies are still being conducted in the glasshouse and it needs to be confirmed in field tests to determine the mode of delivery. Commercialization of the product is anticipated next year (2023). She added that antibiotics are not used in Malaysia, and there is no chemical control of banana blood disease. Dr. Li Chunyu added that in China antibiotics and copper fungicides are used to reduce infection.

The following session was chaired by Dr. Rozeita Labuh of the Malaysian Agricultural Research and Development Institute.



### 2.3. 'Pathogen profile of *Klebsiella variicola*, the causative agent of banana sheath rot' by Dr. Yunhao Sun, Researcher at Zhongkai University of Agriculture and Engineering, China

Dr. Yunhao highlighted that the banana sheath rot caused by *Klebsiella variicola*, is a serious problem on Cavendish banana in southern China. Through identification and isolation, he listed the many strains of the bacteria, leading to pathogenicity tests conducted using the different strains. The pathogen profile, comparative genomic analysis and microbial interaction network of *Klebsiella variicola*, which causes banana sheath rot, were also studied, which showed that 146 potential virulence factor in *K. variicola* of which 22 were carried by 12 pathogenic isolates. The studies also showed that 22,247 potential secreted proteins as potential effector with 12 present in the pathogenic isolates as core genes. He also illustrated the microbial diversity in diseased and healthy plants and the microbial interaction network of the genus *Klebsiella* in banana plants. Bacterial diseases in bananas are now a major concern in China. Dr. Yunhao also mentioned that studies are at present being conducted to elucidate the effectiveness of ethylcyn and *Bacterium subtilis* R31, currently used to control Fusarium wilt, in controlling banana sheath rot.

In response to a question on the interactions of *Klebsiella variicola* with the other microorganisms in the soil microbiome, Dr. Yunhao said that this is still under investigation.

### 2.4. 'Management of bacterial diseases of local Philippine banana cultivars' by Dr. Lorna Herradura, Agriculture Center Chief IV from the Philippines Bureau of Plant Industries.

Dr. Lorna began by mentioning that the 2 important bacterial diseases on banana in the Philippines are 'Bugtok' and Dry Rot. The causal pathogen for 'Bugtok' which means 'discolored and hard' is *Ralstonia solanacearum* similar to the banana blood disease. The disease is usually observed to infect BBB cooking cultivars Cardaba/Saba, Mundo and other BBB and ABB genomes. It has also been reported on AAB dessert bananas such as Latundan or pisang rastali (Malaysia). The mode of disease transmission is mainly via insects such as bees, wasps and thrips visiting the inflorescence. Generally, main symptoms of bugtok disease include bacterial ooze, pronounced reddish brown or black discoloration from core to whole fruit pulp and rusty brown to black discoloration on vascular tissues, fruit, peduncle, pseudostems and corm. Similarly on the latundan, there is brown to black discoloration of the fruit pulp. Main field control is bagging the mail bud at bending stage and debudding it 14 - 15 days after all fruit have set.

Dry rot disease which affects the Cardaba variety is quite similar to bugtok with symptoms of shrunken and distorted fingers with hollow chambers and rotted tissue. Immature fruits also stop filling, become necrotic and dry out.

Dr. Lorna summarised that, further studies on identification of insect species associated with bugtok or dry rot and on disease transmission and epidemiology of 'Bugtok'

disease in cooking banana cultivars, need to be prioritized as the way forward to manage banana bacterial diseases in the Philippines.

During the Q and A session, Dr. Lorna confirmed that typical 'Bugtok' dry rot symptoms are hard and dry tissues. There is no need to discard infected plants outside the planted area for fearing of disease spread. To a question whether the plants can be affected before male buds appear, Dr. Lorna said that disease spread is only through insects visits after male inflorescence open.

## **2.5. 'Current status of banana blood disease in Indonesia and possible approaches for management strategies' by Dr. Catur Hermanto, Senior Researcher from the National Research and innovation Agency, Indonesia.**

Dr. Catur showed in a recent survey that the kepuk variety, also known as saba and pisang nipah (ABB) was the main variety infected with varying incidences which caused confusion regarding the causal agent, whether fungal or bacterial. However, symptoms resembling BBD (Banana Blood Disease) such as wilting of young leaves, corm color discoloration, rotten fruit pulp and discoloration of peduncle, and shrivelling and necrotic darkening of male inflorescence were recorded. He also traced the spread and geographical distribution of the blood disease from 1990 to 2010 from North Sulawesi, to West Java, Sumatra, Kalimantan, Malaysia and West Papua. Disease spread is suspected through long distance transportation, infected planting materials, contaminated farm tools and insect and other carriers. The mode of transmission most likely if from male flower infection or infection through suckers.

Recommendations to control BBD includes disease free planting materials, bagging and debudding of inflorescence, eradication of infected plants, alternative cooking banana varieties that attract less insects, cultivate budless banana varieties, good field practices and enhanced biosecurity measures.

Research priorities suggested are breeding for resistance varieties, pathogen infectious period in infected area, physical, chemical and biological eradication of pathogen, isolation distance, rapid detection tools and regular surveillance and mapping to contain disease spread.

To a question on the decline of disease incidences after 2010, Dr. Catur responded that not much studies were done to determine disease status after that year, but the disease is still prevalent in Indonesia.

To a comment from the session chair, Dr. Catur said that it is possible for germplasm sharing of the budless pisang kapok and pisang awak species, however the mechanism of sharing has to be discussed.

The last 2 presentations were chaired by Mr. Yacob Ahmad, Advisor of the International Tropical Fruits Network (TFNet)

## 2.6. 'Bacterial diseases of banana in India: Overview on status, characterization of pathogen and management practices' by Dr. Murugan, Principal Scientist Loganathan from National Research Centre for Banana, ICAR, India.

Presenting the current status on banana bacterial diseases in bananas in India, Dr. Murugan said that MOKO, Blood disease and *Xanthomonas* wilt have not been recorded. However, Rhizome rot/soft rot caused by *Pectobacterium carotovorum* has been identified in the banana growing areas of Tamil Nadu, affecting Cavendish type Grand Naine and AB genome cultivars such as Neypoovan, where it is characterized by the presence of bacterial ooze and wet rot from infected pseudostem rhizome.

The disease has been reported in other states including Andhra Pradesh, Karnataka and West Bengal, besides more incidences (20 – 30%) in tissue cultured Grand Naine cultivars, mostly in Madhya Pradesh, Maharashtra, Gujarat, Uttar Pradesh and Bihar. The incidences were noticeable during early stages of planting (2 – months). The disease was also noticed on Naypoovan, Nendran and Grand naine sucker plants. Incidences were also higher in Fusarium wilt infected field.

In subsequent tests, it was determined that rhizome rot disease affects commercial varieties such as Grand Naine and Thellcakkakraki, (both AAAs) and Poovan and Karpurachakeli, (AAB) and Neypoovan (AB). Disease spread is through infected planting materials, wet weather, poor drainage and prior Fusarium wilt infection. Control measures include combination of the use of healthy or tissue cultured planting materials, drenching with selected combinations of bleaching powder, streptomycin and *Trichoderma viride* plus cowpea/sunhemp intercrops. Others are the use of copper hydroxide and biocontrol agents.

The way forward to reduce disease impact is through thorough analysis and characterization of pathogen, development and commercialization of bioformulation, strengthening awareness through social media, and the use of relevant diagnostic applications that utilizes the internet of things.

The presenter was asked whether the experiments were confined only to Cavendish varieties. Dr. Murugan replied that this was so because the Cavendish is a popular banana variety in South India, where he is based.

## 2.7. *Xanthomonas* wilt of banana in east and central Africa - Effective management practices by Dr. Guy Blomme, Researcher at Alliance of Bioversity International and CIAT, Central Africa.

Dr. Blomme began by illustrating that while banana bacterial diseases such as MOKO and banana blood disease (BBD) caused by *Ralstonia* sp are common in South East Asia and Central America, *Xanthomonas* wilt disease is more common in Central Africa.

Symptoms of banana *Xanthomonas* wilt include pseudostem ooze, whole leaf wilting, single leaf yellowing/wilting, fruit pulp discoloration and premature bunch ripening.

Similar to the mode of transmission for the banana blood disease, *Xanthomonas* wilt is also spread by farm tools and equipment, insect vectors, infected planting materials, large flying birds and bats and occasionally by cattle and other ruminants.

Control methods for banana bacterial diseases are early removal of male buds to prevent insect vector transmission, sterilisation of farm tools, use of cleaning planting materials, complete uprooting of diseased mats and SDSR (single disease stem removal).

Dr. Bloome illustrated the effectiveness of the innovative SDSR (Single diseased stem removal) technique to control *Xanthomonas* wilt in cooking bananas.

Dr. Bloome, said that while cultural methods can be used to minimize spread, research on transgenics is ongoing, through sourcing of highly tolerant varieties and techniques such as CRISPR Cas 9 being undertaken. He mentioned Dr. Leena Tripathi, Principal Scientist at International Institute of Tropical Agriculture (IITA), Kenya as a key researcher working on genetic engineering technologies to develop disease resistance banana cultivars.

Capacity building and training of extension agents and farmers and through the media and publications have also been carried to create awareness among stakeholders in Central and West Africa.

During the Q and A session, Dr. Mohammad T. Hussein from Bangladesh Agriculture Research Institute commented that while field management practices can reduce spread, the use of transgenic cultivars would be a better alternative. He inquired whether transgenics for banana bacterial diseases are already available for distribution. Dr. Bloome responded that transgenic cultivars have been developed by Dr. Leena Tripathi and advised Dr. Mohammad to contact her.

On another question by Dr. Catur, Dr. Bloome said that in Central Africa, bananas are more for domestic consumption and as a source on income, and largely grown as a mixed crop. While the SDSR technique is being recommended to reduce disease spread.

### 3.0. PANEL DISCUSSION

The panel discussion was moderated by Mr. Yacob Ahmad.

In Indonesia, Malaysia and the Philippines, MOKO disease (Bugtok in the Philippines) and BBD (banana blood disease) caused by *Ralstonia solanacearum* and *Ralstonia syzygii* respectively are serious diseases affecting mainly cooking bananas varieties. Disease control is mostly by field management practices.

The moderator invited all speakers to respond to a question on two research priority areas that can be collaborated among researchers to manage bacterial diseases in banana more effectively.

Dr. Nandita mentioned that research work on management strategies, including cultural techniques should be directly application to the field. This includes biocontrol, using tissue cultured materials and field adaptation of cultivars. The other that requires focus is in diagnostics and diagnostics techniques to ensure accuracy in control strategies.

Dr. Rozeita referred to MARDI's continued studies with Induced Systemic Resistance (ISR) techniques that needs to be commercialised. Other areas include studies in the use of bacteriophages and the breeding of resistant varieties.

Dr. Yunhao reiterated the potential of ethylcyn and *Bacillus subtilis* for the control of the spread of *Klebsiella* sp and diseases and he emphasized on the importance of proper sampling procedures.

Dr. Lorna related to the situation in Philippines where more in-depth studies should be conducted on bugtok, moko diseases especially on bacterial dry rot. Dr. Lorna also suggested other control strategies besides the current ones which are related to cultural practices.

Dr. Catur suggested that since Malaysia, Indonesia and Philippines are affected by similar bacterial diseases, there should be a concerted research program involving all three countries. Another area that needs to be studied is the coexistence of the different disease-causing bacteria, for example *Ralstonia* sp., *Xanthomonas* sp., and *Klebsiella* sp.

Dr. Loganathan related to the experience in India in combatting corm rot caused by *Rhizoctonia* sp, using clean tissue cultured planting materials. The demand for such materials have also increased dramatically due to its effectiveness in reducing disease incidences. Dr. Loganathan also emphasized the importance of in-depth surveys to gauge disease occurrences. Besides this, research related to climatic factors, microbioassays and genomic studies are very much needed.

Dr. Bloome referred to the *Xanthomonas* wilt situation in West Congo and emphasized the need for surveys to monitor disease spread. The screening of wild musa relatives for breeding work is another research area to look into. Dr. Bloome further stated that a study on MOKO or blood disease should be done to look at the movement of the pathogens in the stem and plant mat. This will indicate whether SDSR or single diseased stem removal can be effective in controlling MOKO or blood disease There is already a lot of knowledge and upscaling done in East and Central Africa, and Dr. Bloome also stressed the importance of knowledge transfer and scaling up of effective practices to reduce bacterial diseases of banana. There are also indications that the disease front in the Congo basin might spread to impact plantain production in West Africa.

The moderator summarize the panel discussion with three main research focus that can be developed in collaborative initiatives. These are in areas of disease distribution studies, use of molecular breeding techniques such development of transgenics and biological and cultural control, of banana bacterial diseases.

The moderator closed the workshop by thanking all speakers and all participants for their excellent presentations and participation.

## 4. APPENDICES

### 4.1. Program

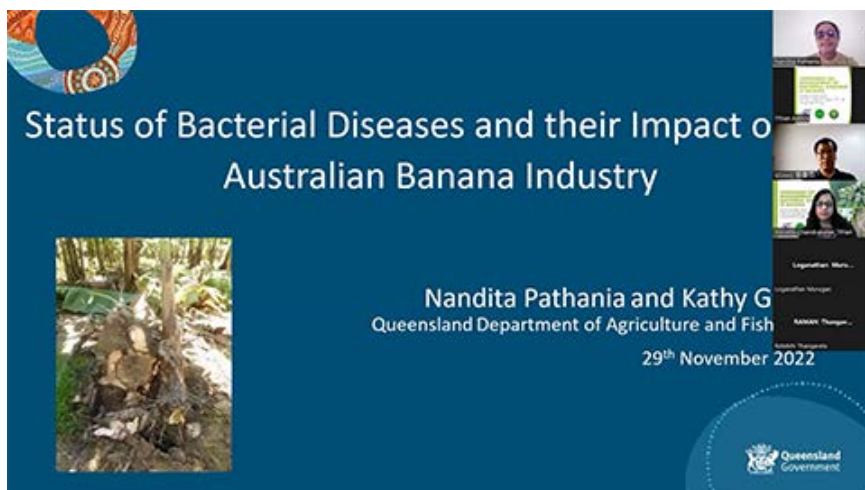
Date: 29 September 2022 (Thursday).

Time: 2.00 PM (Kuala Lumpur, Guangzhou, Manila), 7.00 AM (Rome), 9.00 AM (Kampala) 11.30 AM (Delhi), 1.00 PM (Ho Chi Minh City, Jakarta), 4.00 PM (Brisbane), 6.00 PM (Suva)

Time	Content
2.00 – 2.10 pm	Introduction Dorothy Chandrabalan (TFNet)
2.10 – 4.45 pm	Presentations Chair: Dr. Li Chunyu, GDAAS, China
Dr. Nandita Pathania Queensland Department of Agriculture and Fisheries, Australia	‘Status of bacterial diseases and their impact on the Australian banana industry’
Dr. Rozeita Laboh Horticulture Research Centre Malaysian Agricultural Research and Development Institute (MARDI), Malaysia	‘Status of banana bacterial diseases in Malaysia’
Dr. Sun Yunhao Zhongkai University of Agriculture and Engineering, PR China	‘Pathogen profile of <i>Klebsiella variicola</i> , the causative agent of banana sheath rot’
Dr. Lorna Herradura, Bureau of Plant Industries, Department of Agriculture, Philippines	‘Management of bacterial diseases of local Philippine banana cultivars’
Dr. Catur Hermanto Research Center for Horticulture and Estate Crop, National Research and Innovation Agency Jakarta, Indonesia	‘Current status of banana blood disease in Indonesia and possible approaches for management strategies’
	Chair: Yacob Ahmad, (TFNet)
Dr. Loganathan M., ICAR-NRCB, Trichy, India	‘Bacterial diseases of banana in India: Overview on status, characterization of pathogen and management practices’
Dr. Guy Blomme., Alliance of Bioversity-CIAT, Uganda	‘Xanthomonas wilt of banana in east and central Africa – Effective management practices’
4.45 – 5.00 pm	Panel discussion / Closing Moderator: Yacob Ahmad (TFNet)

## 4.2. Photos

Dr. Nandita Pathania, Queensland Department of Agriculture and Fisheries, Australia

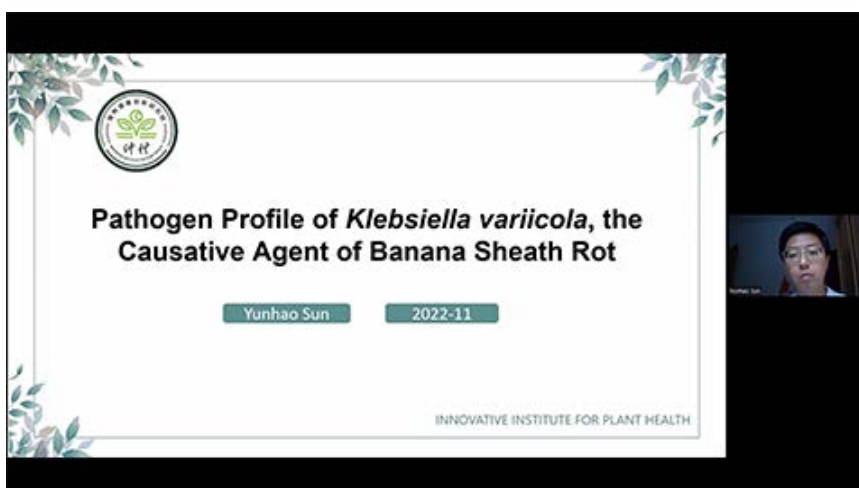


Dr. Rozeita Laboh, Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia



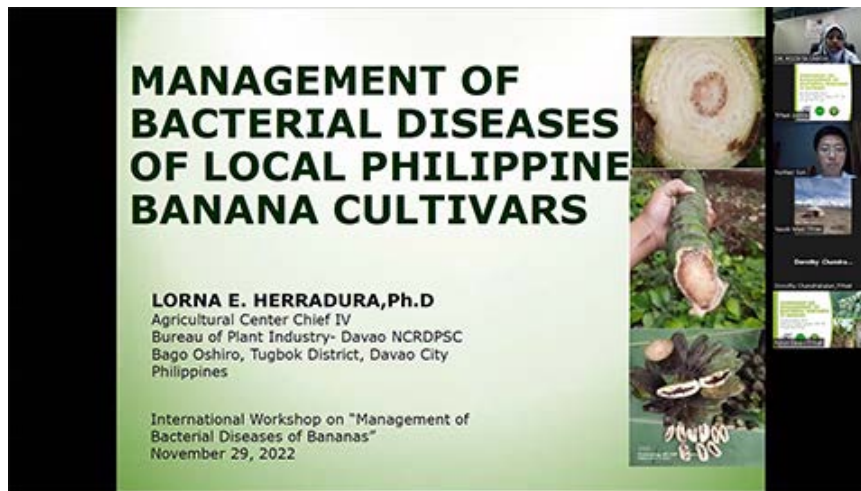


Dr. Sun Yunhao, Zhongkai University of Agriculture and Engineering, PR China





Dr. Lorna Herradura, Bureau of Plant Industries, Department of Agriculture, Philippines



Dr. Catur Hermanto, Research Center for Horticulture and Estate Crop, National Research and Innovation Agency, Jakarta, Indonesia



To be presented on international workshop on the **Management of bacterial diseases of bananas**  
Kuala Lumpur, 29 November 2022

Dr. Loganathan M., ICAR-NRCB, Trichy, India



Dr. Guy Blomme., Alliance of Bioversity-CIAT, Uganda





### Panel Discussion



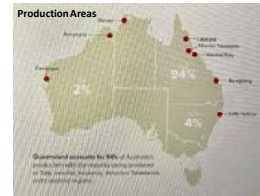
## 4.3. Powerpoint Presentations



1

### Overview : Australian Banana Industry

- Largest horticulture industry (**\$600 million**)
- Major growing area is **wet tropics of Queensland**
- Major commercial variety is **Cavendish(97%)**
- Production: **381,676 tonnes**
- Biosecurity, **pest and disease management** are key priority areas of R&D.



<https://www.horticulture.com.au/>

2

### Banana Bacterial Diseases



1. CORM rot/Rhizome rot (Jan - May)

- 1. Endemic disease**  
Affects all crop stages  
**Blts/suckers**
  - Plant crop - 25%
  - First & older ratoon - 36%
- Tissue culture raised plants**
  - 1<sup>st</sup> Ratoon (20-40%)
  - Subsequent ratoon (15-20%)
- 2. Minor disease**  
Found occasionally on 1 - 2 fingers on the hand.



2. Mokillo/Finger tip rot

3

### 1.1 Symptoms –Bacterial corm rot

- Infected plants show **yellowing and browning** of the lower leaves.
- **Black discoloration** and rotting within the corm and roots.
- **High incidence**, 40% of infected plants tip over at ground level.



- **Associated Bacteria**
  - ✓ *Pectobacterium carotovorum*
  - ✓ *P. atroseptica*
  - ✓ *Dickeya chrysanthemi*
  - ✓ *Dickeya paradisiaca*
  - ✓ *D. zoele*
  - ✓ *D. fangzhongdai*

Black discoloration between healthy and diseased tissues

4

### 1.2 Disease cycle

- **Preferred conditions**
  - Prevalent in warm (>30 °C) and humid (90 %) conditions
  - Poor Drainage
- **Transmission**
  - Asymptomatic planting material
  - Contaminated equipment and machinery
  - Prolong survival in soil, surface water and plant debris
  - Nematodes and banana weevil borer
  - Wide host range



5

### 1.3 Diagnostics *Dickeya* isolate(s)



YDC medium



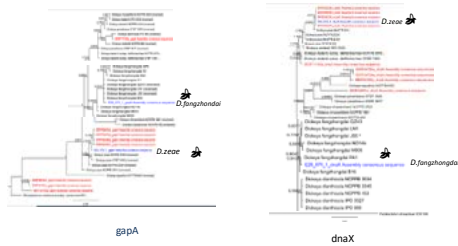
Fried egg like colonies



ADE1/ADE2  
420 bp

6

#### 1.4 Phylogeny of *Dickeya* isolate(s)/strain(s) (gapA & dnaX)



7

#### Learnings.....

#### Disease Management Strategies

- Disease free planting material (TC)
- Avoid water stress
- Disinfect de-suckering tools
- Improve drainage
- Improve soil health (organic matter/ground cover)
- Remove (burn) infected plant material
- Manage weevil borer and nematode
- Apply preventative sprays



<https://www.mybnp.com.au/>

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#### 2.1 Symptoms-Mokillo/Fingertip rot

- Infected fruit are distorted with **bulbous base** and **thinner tip**
- Straight fingers or **out of alignment**
- Internal symptoms, exhibit **rust coloured discolouration and gumming**
- **1-2 fingers** per hand



- Associated bacteria
  - ✓ *Kasalania cowanii* (Formerly *Enterobacter cowanii*)
  - ✓ *Pantoea agglomerans* (Formerly *Erwinia herbicola*)
  - ✓ *Paenibacillus solanici*
  - ✓ *Dickeya* spp. (*D. chrysanthemi*, *D. fongshangdai* and *D. zeae*) Formerly *Erwinia* spp.
  - ✓ *Erwinia amylovora*

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#### 2.2 Koch's Postulates



- Test bacteria
  - *Pantoea agglomerans*
  - *Kasalania cowanii*

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#### 2.3 Disease cycle

- **Not known**
- Likely, naturally occurring bacterium on flowers and in environment transferred by pollinating insects (bees and ants).

#### Gaps

- External symptoms not always obvious
- Sample size – low
- Occurrence – pack shed and supply chain/consumer (not always traceable to field)
- Different organisms associated with symptoms

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#### 2.4 Disease Management Strategies

- Remove abnormal shaped fingers at the pack shed.
- Improve insect pest management



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## Biosecurity Threats



Moko/bugtok  
*Ralstonia solanacearum* race 2, blower 1



Xanthomonas wilt  
*Xanthomonas wilticola* pv. *musacearum*



Blood disease  
*Ralstonia solanacearum* subsp. *celebesensis*

&  
Phytoplasmas

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Thank you

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## Future Research Approach

- Awareness of **endemic, exotic and emerging pathogens**
- Survey and Surveillance
- Extensive molecular screening
- Access and **validate diagnostic methods**
- Genetic diversity of pathogenic strains
- **Disease Epidemiology**
- **Disease management**
- **Increase collaborations**



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## Acknowledgements



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# THE STATUS OF BACTERIAL WILT DISEASE OF BANANA IN MALAYSIA

ROZEITA, L.,  
 NORLIZA ABU BAKAR, GANISAN KRISHNEN, N. SULASTRI, J., SIVANASWARY, C., & ZULHAZMI, S.  
 MALAYSIAN AGRICULTURAL RESEARCH & DEVELOPMENT INSTITUTE (MARDI)

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- 1 INTRODUCTION
  - Industry scenario
- 2 PROBLEM STATEMENT
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- 3 THE STATUS OF BBWD IN MALAYSIA
- 4 DISEASE MANAGEMENT STRATEGY
  - Government initiatives
  - R&D
- 5 CONCLUSION AND WAY FORWARD

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### 1.0 INTRODUCTION

**Industry scenario**

- Malaysia is situated in the Southeast Asia.
- Current population is estimated of over 32 million
- The country is multi races and cultural (Waly, Chinese, Indian and others).
- Contains of 13 states and 3 federal territories
- Separated by South China Sea into 2 regions- P.Malaysia and East Malaysia (Sabah & Sarawak - in Borneo Island).
- Bahasa Malaysia is country's national language and English as second language.

- Agriculture remains an important sector in Malaysia's economy.
- Presently agriculture sector contributes 7.4% to the GDP and provide 10.5% of total employment in Malaysia (2020).
- In Malaysian agriculture sector, banana (*Musa* spp.) is one of the popular and commercial fruits for local and export market.
- It is ranked the 3rd most important fruit crops cultivated in Malaysia in terms of total production and the 3rd ranked in the fruit export revenue among the other major fruit crops.

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**Industry scenario**

Planted area of the main fruits in Malaysia (ha)

Fruit	Planted Area (ha)
durian	76,972
banana	26,213
pinapple	15,316
jackfruit	14,072
watermelon	-
durian	-
artocarpus	-
durian	-

- Banana contributes around 21% of the total fresh tropical fruit production and 14% of the total fruit areas covering more than 26,000 ha of land with an estimated production volume of more than 330,000 tonnes (mt).
- There was a slight increment in the banana total production and hectares from the previous year which is in line with the Malaysian government initiatives in NAP 2.0 (National Agrofood Policy 2.0) targeting to increase the fruit sub sector production for the next 10 year (2020-2030).
- As compared to other short-term fruits, banana is the priority crop in terms of agricultural land use and this cash crop remains in the top 3 of the highest per capita consumption (9.3 kg/year) or 25.4g daily for 32 million Malaysians.

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**Industry scenario**

- Banana is cultivated as backyard crop and in small, medium and large-scale cultivation.
- Pisang Berangan and Cavendish are the most popular for commercial market whereas other local popular varieties P.Mas/Rastali/P. Nangka /Raja /Banduk/Abu/Awak to cater local market demand.
- Jhor, Pahang and Sabah served as major states producing banana and contributed nearly 70% of the total banana production in Malaysia (2021).
- The banana production was valued at around USD128 million per year with an export value around USD10 million per year (2021).
- The potential market for Malaysian banana is in the Asian region (mainly Singapore, Brunei, Vietnam and Indonesia) and the USA with an estimated amount of 32,085 tonnes (2021).

- Malaysia also imported banana from Vietnam, Philippines, Thailand, Indonesia, India, Ecuador, China and Jordan with an estimated amount of 23,701 tonnes and valued at USD12 million (2021).

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### PROBLEM STATEMENT

Major constraints of banana production

**TABLE 1: PLANTED AREAS OF BANANA IN MALAYSIA (2017-2021)**

Year	Planted Area (ha)
2017	34,894
2018	35,906
2019	34,752
2020	34,757
2021	26,213

Source: DOA Statistic, 2021

**TABLE 2: BANANA PRODUCTION IN MALAYSIA FROM 2017-2021 (TONNES)**

Year	Production (tonnes)
2017	350,493
2018	325,000
2019	315,000
2020	285,000
2021	330,253

Source: DOA Statistic, 2021

- In general, for the past 5 years, the trend of banana planted areas in Malaysia has shown to decline from 34,894 (2017) to 26,213 ha (2021) (Table 1). Similarly, the trend of banana production from 350,493 (2017) to 330,253 tonnes (Table 2)
- Despite the downward trend, there was a slight increase in both banana plantation areas and production in 2021.
- One of the contributing factors to the deterioration of this production is the major disease infection.

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## THE STATUS OF BBWD IN MALAYSIA



- Banana bacterial wilt disease (BBWD) is one of the major diseases caused by the bacterium *Ralstonia solanaceae* subsp. *celesbensis* (commonly known as Blood D disease) and *Ralstonia solanaceae* Race 2 Biovar 1 (commonly known as Moko).
- This BBWD was first detected in 2007 in Johor and resulted in remarkable yield loss and an increment in banana importation from the value of USD 1.7 million (2007) to USD 36 million (2012).
- BBWD is considered the most damaging disease of banana and has been listed as one of the top 10 invasive alien species (IAS) in Malaysia.
- The disease is easily spread by contamination of planting materials, farm tools, water and the pathogen could survive in the soil for more than 2 years.
- The infection could be up to 100% when its outbreak. Until today, no agricultural effective controls this notorious disease.



1. Anon, 2012. Booklet Statistik Tanaman (Sub Sektor Tanaman Makanan), Department of Agriculture, Malaysia  
 2. Anon, 2003. Fruit Crop Statistics, Department of Agriculture, Malaysia  
 3. Anon, 2020. FAO Statistics

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Table 3: BBWD incidence for local banana cultivars in Malaysia (2011)

No.	BANANA CULTIVAR	AREA SURVEYED (Ha)	Ha	%
1	Meloh	1711.6	1457	85.1
2	Batangayan	574.4	273.6	47.6
3	Rastali	201.2	130.4	64.8
4	Comen	77.95	15.8	19.8
5	Karam	49.9	19.4	40.3
6	Neongka	48.7	23.6	48.5
7	Boyan	8.8	8.8	100
8	Pisang Ubiang	13.8	3.0	21.7
9	Tanjak	72.1	7.3	10.1
10	Dojan	16.4	0	0
11	Anak	4.4	2.4	54.5
12	Pisang Abu	1.0	1.0	100.0
13	Lilin	25.3	6.6	26.1
14	Lelak Mudo	7.0	2.0	28.6
15	Mesak Hias	1.0	1.0	100.0
16	Pisang Abu	2.0	2.0	100.0
	<b>Total</b>	<b>2,272.56</b>	<b>1,981.6</b>	<b>87.2</b>



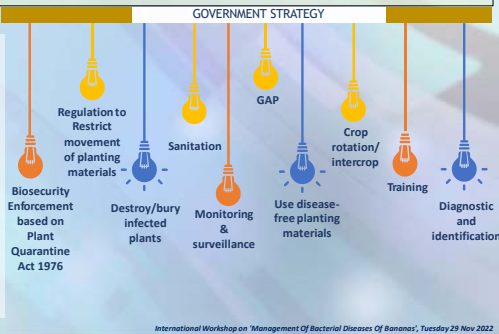
Source: Mokhtaruddin and Robert, 2011

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## DISEASE MANAGEMENT STRATEGY

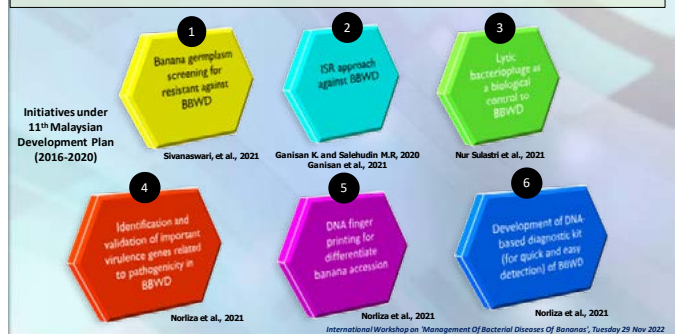
- Recent surveys done by DOA in 2022 found that out of 316 ha only 14 ha (4%) of disease infections are caused by BBWD.
- This is a very good indication that the BBWD infection and spreading have gradually decreased.
- That is also given an indication that the Malaysian Government's initiatives/strategy to contain the spreading of the BBWD is almost succeed.



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## R&D STRATEGY FOR BBWD MANAGEMENT



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**1**  
Banana germplasm screening for resistant against BBWD

Highly tolerant accessions to BBWD in MARDI banana germplasm

- 128 accessions were evaluated for *Ralstonia solanaceae* subsp. *celesbensis* at MARDI BBWD 'hotspot' field.
- 10 accessions were highly tolerant, which include Pisang Masam, THU GIA KUL 609, Jari Buaya IMTP3, Putar, CV Rose, GCTGV-215, Pisang Hijau, FHIA 01, Balbisiana KUL and Peninjau.

Siva et al., 2022

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- Tolerant accessions to BBWD in MARDI banana germplasm
- 13 accessions were tolerant, these include BURO-CEMSA, Lilin Malaysia, KRA 7730, KRA 7732, FHIA 17, Berlin IMTP3, Oter Sarawak, Kapal Bentong, pindek, Ceylan IMTP3, Pisang keladi, Pisang Serindik dan BDI UM.
- These accessions could be used for breeding improvement of banana in the future.

Siva et al., 2022

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**2** ISR approach against BBWD

**Results**

1) Isolation of bacilli in vitro

- 54 bacilli were isolated from banana grown soil using selective media from 3 varieties of banana (Berangan, Rastali x Embun and unknown).

2) Screening of bacilli on banana seedlings in the glasshouse

- 23 out of 54 bacilli have shown different disease severity against BBWD.
- From that, only 5 bacilli showed systemic resistance with below 25% disease severity against BBWD.
- From 5, 3 bacilli got total suppression against BBWD.

Ganisan et al., 2021

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The banana plants performance (treated with ISR and control) in the glasshouse were recorded after 1 and 2 months (after the banana seedlings were transferred in polybags).

- Overall, the treated banana plants have shown better growth performance (height and diameter) as compared to control in polybags.

Screening of ISR banana seedlings in the field

- The banana plants growth performance (height) were further evaluated in the field at 14, 60, 120 and 180 days after field transferred.
- From the graph, all banana plant treated with ISR showed taller than the control. The tallest banana plants with bigger leaves were treated with IB 43, followed by BIB 35 and IB 58.

Ganisan et al., 2021

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Screening of ISR banana seedlings in the BBWD 'hotspot'

- The BBWD infection was exhibited and increased in severity during the 2<sup>nd</sup> ratoon.
- Overall, ISR banana seedlings showed less mortality rate as compared to control.
- From the graph, ISR banana seedlings (BIB 10) have shown total suppression of BBWD in the hotspot either in the 1<sup>st</sup> or 2<sup>nd</sup> ratoon as compared to others.

Ganisan et al., 2021

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**3** Lytic bacteriophage is a biological control to BBWD

- Biological control approaches have received increasing interest due to environmentally friendly.
- Bacteriophage is a natural antibacterial entity a virus that targets bacteria.
- Do not affect or harm humans or other living organisms
- Ubiquitous in the environment, recognized as the most abundant biological agent on earth.
- Bacteriophages persist in high numbers in any environment as long as the host is present, without any harm.
- Generally, Phages have a narrow host range, typically being limited to strains within a particular species of bacteria (e.g., *Xanthomonas* spp., *Pseudomonas* spp., *Erwinia* spp., and *Ralstonia* spp.)

Consist of single or double stranded DNA or RNA genome encapsulated inside a protein or lipoprotein coat.

Phage: DNA

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**Isolation and evaluation of lytic bacteriophages in-vitro and in the glasshouse**

1) Isolation of bacteriophages from pseudostem and fruit of infected banana

2) Plaque assay through double agar overlay method

3) TEM visualization of X513 and X9105 both have an icosahedral head (56 and 52 nm), short and non-contractile tail, morphology belonging to the Podoviridae family.

4) Evaluation of phage treatments against *R. syzygii* subsp. celesbesensis on 2-months-old banana plants in a glasshouse

5) Thermal and pH stability test

Treatments	MOA*	Application technique	DS scoring index
T1 -ve control		Drenching of soil	1 no symptoms,
T2 -ve control (BB only)		Injection of stem	2 leaves wilted, no yellowing,
T3 -ve control (BB only)		Drenching of soil	3 leaves wilted and yellowing,
T4 X9105 + B08	1	Injection of stem	4 2 or 3 chlorotic leaves,
T5 X513 + B08	1	Injection of stem	5 4 or more chlorotic leaves,
T6 X9105 + X513 + B08	1	Injection of stem	5 plant dead.
T7 X9105 + X513 + B08	1	Injection of stem	

Nur Sukastri I. and Md. N. Khalid (2021). Bacteriofaj: Agen kawalan biologi berpotensi bagi penaktyan bakteria darab pisang. Buletin Teknologi MARDI Bil. 23 (2021) pp 65-73

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**Results**

**In vitro**

**In a glasshouse**

**In vitro evaluation of single and cocktail phage against *R. syzygii* subsp. celesbesensis in CPG liquid medium based on absorbance value (OD).**

- The bacterial suspension of *R. syzygii* infected with individual phage X9105 and X513 and, cocktail (X9105 + X513) began to show infectivity effects after 3 hr of incubation period compared to the bacterial suspension of *R. syzygii* subsp. celesbesensis without phage (control).
- The cocktail phage of X9105 + X513 showed the highest infectivity effect against *R. syzygii* subsp. celesbesensis in-vitro as compared with other treatments including control.

**Graph 3. In vitro assay of phage treatment on 2-months-old banana plants**

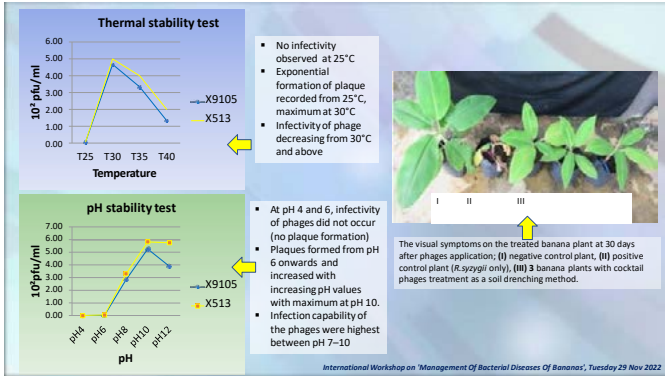
Means followed by the same letter are not significantly different (p<0.05) based on DMRT

- T7= Cocktail phage (X9105+X513) at 10<sup>7</sup> PFU/ml + *R. syzygii* subsp. celesbesensis at 10<sup>6</sup> CFU/ml as a soil drenching technique showed the lesser percentage of DS at 23.3% as compared to positive control (T3 at 63.3%) in the glasshouse evaluation.

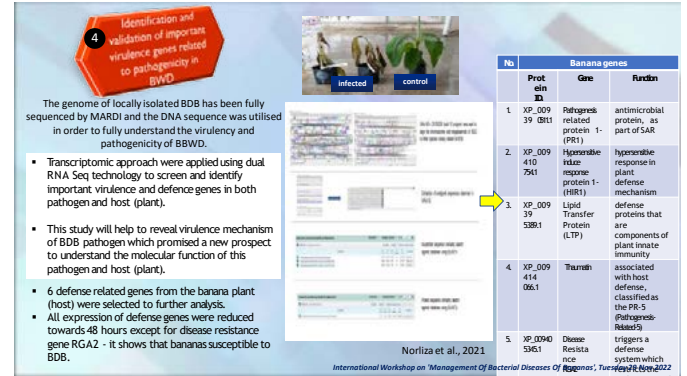
Nur Sukastri I. and Md. N. Khalid (2021). Bacteriofaj: Agen kawalan biologi berpotensi bagi penaktyan layu bakteria darab pisang. Buletin Teknologi MARDI Bil. 23 (2021) pp 65-73

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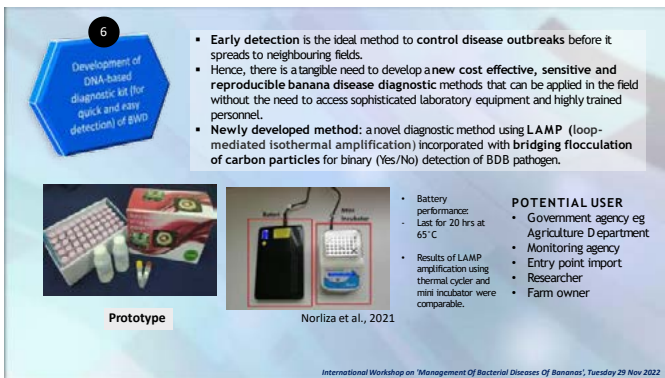
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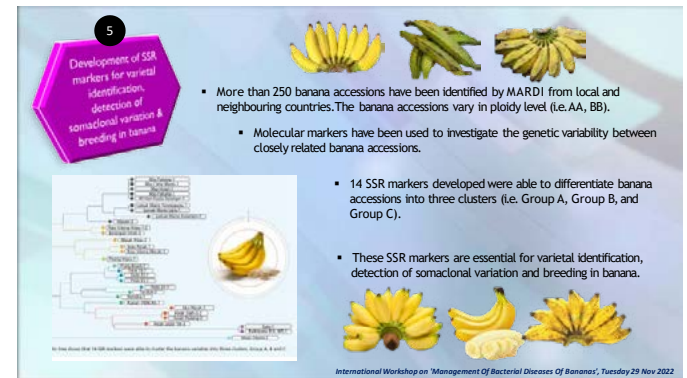
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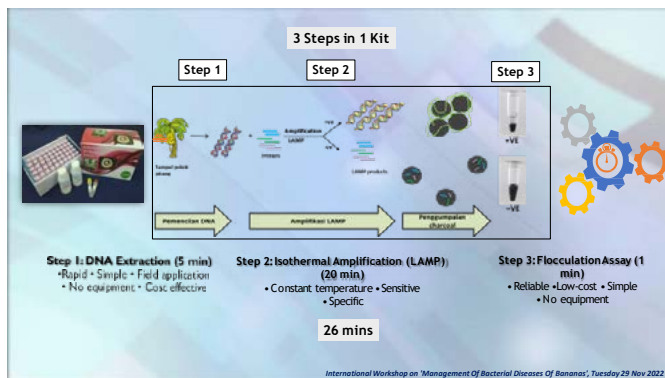
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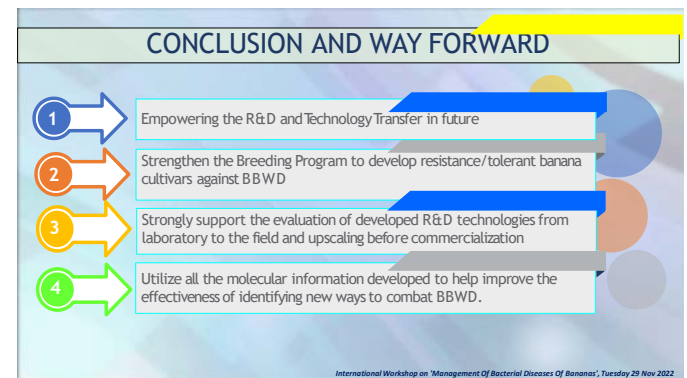
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## CONCLUSION AND WAY FORWARD

- 5 Sharing information and experiences on related issues and problems from local and foreign experts should expedite solution for the disease control.
- 6 The bacteriophage cocktail (isolates X9105+X513) is one of the potentials biological control for BBWD that will be formulated and further test in the 'BBWD hotspot' area.
- 7 ISR technology is the promised and will be the first available efficient technique in the world to control BBWD after successful in controlling papaya dieback disease (PDD).

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## Pathogen Profile of *Klebsiella variicola*, the Causative Agent of Banana Sheath Rot

Yunhao Sun     2022-11

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### Banana Sheath Rot

Shengtang County (112°36'E, 22°29'N), Eping City, Guangdong Province, China.

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### Isolation and identification of pathogenic bacteria

Strain	Sampling sites	Sampling time	Host cultivars
KV1	ShiuiCity, Guangdong, China(112°73' E, 23°33' N)	2020.09.11	Feniza No. 1
KV2	YunfuCity, Guangdong, China(112°01' E, 22°56' N)	2020.09.14	Jirfen No. 1
KV3	TaishanCity, Guangdong, China(112°75' E, 22°22' N)	2020.09.15	Feniza No. 1
KV4	YangchuanCity, Guangdong, China(111°79' E, 22°17' N)	2020.09.14	Feniza No. 1
KV5	EpingCity, Guangdong, China(112°31' E, 22°18' N)	2020.09.13	Feniza No. 1
KV6	EpingCity, Guangdong, China(112°31' E, 22°18' N)	2020.09.13	Feniza No. 1
KV7	FoshanCity, Guangdong, China(113°05' E, 23°16' N)	2020.07.28	Feniza No. 1
KV8	FoshanCity, Guangdong, China(112°50' E, 23°16' N)	2019.12.07	Feniza No. 1
KV9	DongguanCity, Guangdong, China(113°75' E, 23°02' N)	2020.01.02	Jirfen No. 1
KV10	DongguanCity, Guangdong, China(113°75' E, 23°02' N)	2020.01.02	Fendajiao
KV11	DongguanCity, Guangdong, China(113°75' E, 23°02' N)	2020.01.13	Fendajiao
KV12	GuangCity, Guangdong, China(109°57' E, 23°11' N)	2021.05.21	Baodajiao

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### Isolation and identification of pathogenic bacteria

Strain	Biochemical tests											
	Sucrose	Sorbitol	Rhamnose	Inositol	Esculin	Melanose	Raffinose	Gelatin	Mannose	Amylum	Other 1	Other 2
KV1	+	+	+	+	+	+	+	+	+	+	+	+
KV2	+	+	+	+	+	+	+	+	+	+	+	+
KV3	+	+	+	+	+	+	+	+	+	+	+	+
KV4	+	+	+	+	+	+	+	+	+	+	+	+
KV5	+	+	+	+	+	+	+	+	+	+	+	+
KV6	+	+	+	+	+	+	+	+	+	+	+	+
KV7	+	+	+	+	+	+	+	+	+	+	+	+
KV8	+	+	+	+	+	+	+	+	+	+	+	+
KV9	+	+	+	+	+	+	+	+	+	+	+	+
KV10	+	+	+	+	+	+	+	+	+	+	+	+
KV11	+	+	+	+	+	+	+	+	+	+	+	+
KV12	+	+	+	+	+	+	+	+	+	+	+	+

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### Pathogenicity tests

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### Klebsiella variicola

(Pinto-Tomás et al., 2009)

**Complete genome sequence of endophytic nitrogen-fixing *Klebsiella variicola* strain DX120E**

J. Li<sup>1</sup>, C. Chen<sup>1\*</sup>, M. Qiu<sup>1</sup>, H. Wang<sup>1</sup>, Y. Wang<sup>1</sup>, J. Tang<sup>1</sup>, L. Song<sup>1</sup>, S. Gao<sup>1</sup> and Q. An<sup>1\*</sup>

[www.nature.com/naturegenetics](https://doi.org/10.1093/nar/nkab221)

**New Disease Reports**

**First report of bacterial soft rot of carrot caused by *Klebsiella variicola* in India**

S. S. Choudhary<sup>1</sup>, M. K. Prasad<sup>1</sup>, V. K. Prasad<sup>1</sup>, S. K. Prasad<sup>1</sup>, B. S. Prasad<sup>1</sup> and R. S. Prasad<sup>1</sup>

**First Report of Plantain Soft Rot Caused by *Klebsiella variicola* in Haiti**

J. S. F. L. de Souza<sup>1</sup>, J. S. F. L. de Souza<sup>1</sup>, R. A. S. de Souza<sup>1</sup>, P. P. de Souza<sup>1</sup>

[Published Online: 7 Apr 2020](https://doi.org/10.1093/nar/nkab221)

**First Report of Rhizome Rot of Banana Caused by *Klebsiella variicola* in India**

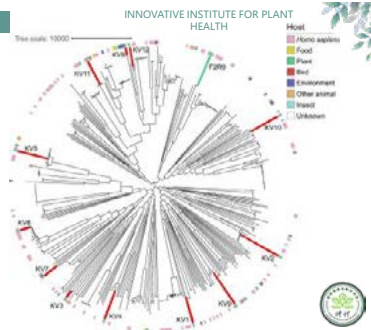
M. Loganathan<sup>1</sup>, R. Thangavelu<sup>1</sup>, P. Pushpakanth<sup>1</sup>, K. Muthukumarthi<sup>1</sup>, K. Ramesh<sup>1</sup>, R. Selvarajan<sup>1</sup>, and S. Uma<sup>1</sup>

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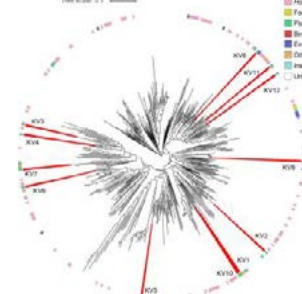
Comparative genomic analysis

Strain_name	ST_type	Allele_1
KV7	new	leuS(23)
KV12	365	leuS(9)
KV1	new	leuS(1)
KV10	unknown	leuS(1)
KV11	183	leuS(1)
KV2	114	leuS(1)
KV3	210	leuS(22)
KV4	25	leuS(9)
KV5	61	leuS(1)
KV6	new	leuS(16)
KV8	unknown	leuS(1)
KV9	new	leuS(3)



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Comparative genomic analysis



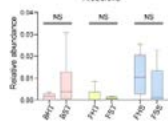
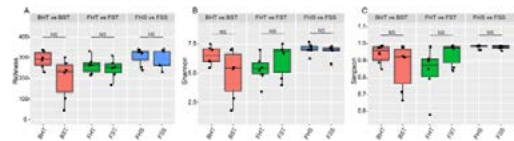
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**Potential virulence factors**  
146 potential VFs in *K. variicola*,  
22 of which were carried by the 12  
pathogenic isolates

**Potential effector**  
2,247 potential secreted proteins  
12 were present in the pathogenic  
isolates as core genes

8

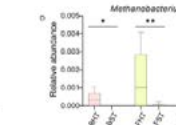
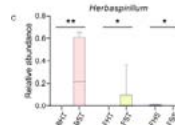
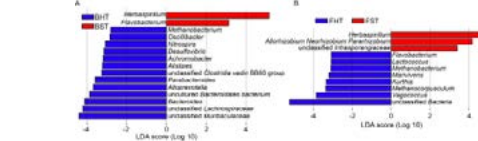
Microbial diversity in diseased and healthy plants



BHT, tissue from healthy 'Brazil' banana plants; BST, healthy tissue from diseased 'Brazil' banana plants; FHT, tissue from healthy 'Fenza No. 1' banana plants; FST, healthy tissue from diseased 'Fenza No. 1' banana plants; FHS, rhizosphere soil from healthy 'Fenza No. 1' banana plants; FSS, rhizosphere soil from diseased 'Fenza No. 1' banana plants.

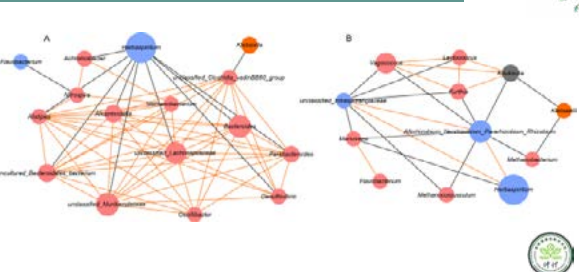
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Microbial interaction network of the genus Klebsiella in banana plants



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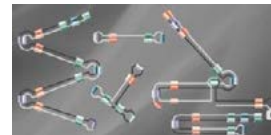
Microbial interaction network of the genus Klebsiella in banana plants



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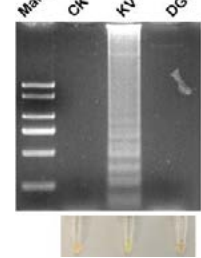
Rapid detection of disease agent

Loop-mediated isothermal amplification, LAMP



Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. Nucleic acids research, 28(12), e63-e63.

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Screening of agents for disease treatment

INNOVATIVE INSTITUTE FOR PLANT HEALTH



Ethylcin



*Bacillus subtilis* R31



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Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering




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15

# MANAGEMENT OF BACTERIAL DISEASES OF LOCAL PHILIPPINE BANANA CULTIVARS




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 Philippines

International Workshop on "Management of Bacterial Diseases of Bananas"  
 November 29, 2022

1

## TOPIC OUTLINE




- Bacterial diseases on banana
  - Bugtok
  - Dry Rot
- Management
- Way forward

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## BUGTOK DISEASE

Causal Organism- *R. solanacearum*




- First observed in the Philippines by Roperos, et. al. 1965; Zher and Davide 1969; San Juan, 1977
- Local term in the southern Phil to describe the infected fruit which are discolored and hard

3




4

- Usually observed with B genome
  - Cooking cultivars  
 Cardaba/Saba, Mundo, etc)



**Name of Cultivar:** Cardaba or Saba  
**Origin:** Philippines  
**Genome:** BBB (Cardaba type)  
**Type:** Cooking  
**Uses:** alternative staple crop, exported semi processed, fresh or chips

**Synonyms**  
 Philippines: Dippig (Ilocos Region)  
 Cadianan (Northern Mindanao)  
 Southeast Asia: Pisang Chematu (Malaysia), Pisang Kopok Besar (Indonesia), Chuoi Mat (Vietnam)  
 Cardaba (International)



**Name of Cultivar:** Mundo  
**Origin:** Philippines  
**Genome:** BBB  
**Type:** Cooking

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- Usually observed with B genome
  - Other cultivars infected observed at the Banana Genebank at BPI
    - Mundo, Turangkog, Paa Dalaga, Biguihan, Inabaniko and Java (ABB/BBB genome), Gubao, Katsila, Pelipita, Madurangga (ABB) and Giant Kalapua (ABBB)

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**Dessert type (Latundan)**

Name of Cultivar: Latundan  
 Origin: Philippines  
 Genome: AAB  
 Type: Dessert  
 Uses: consumed in the domestic market as dessert, exported in niche markets

**Synonyms**

Philippines: Tundan (Cebu, Davao, Negros Or., Turdan (Tagalog Regions, Cantong Surig (Ilocos and Cordillera Regions)

Southeast Asia: Pisang Rastali (Malaysia)  
 Pisang Raja Serih (Indonesia)  
 Kuai Nam (Thailand),  
 Chuoi Goong (Vietnam)  
 Silk, Manzana (International)




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**BUGTOK DISEASE**

Causal Organism- *R. solanacearum*

- Phylogenetic analysis of endoglucanase gene sequence data from the Moko and Bugtok disease causing strains from the Philippines shows that these strains cluster with sequevar 3 (MLG24) strains (Figure 1 & 2) (<https://www.planthealthaustralia.com.au>)



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**SIGNS ON INFECTED PLANTS**

- Bacterial Ooze coming from infected plant parts




Source: Soguilon et. al. 1995

10

**MODE OF TRANSMISSION**


- Transmitted by insects (bees, wasps and thrips) through the inflorescence



9

**SYMPTOMS**


- Bracts covering the male bud are dry, slightly rolled up, and loosely attached
- Differentiates healthy from infected plants



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**SYMPTOMS ON CARDABA**

- Pronounced reddish-brown or black discoloration starting from the core and progressing towards the whole pulp of the fruit



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### SYMPTOMS ON CARDABA

- Rusty brown to almost black discoloration of the
  - vascular tissues
  - fruit
  - peduncle
  - central portion of the pseudostem
  - corms (in advanced cases)



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### SYMPTOMS ON LATUNDAN

- Rusty brown to almost black discoloration of the fruit pulp



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### CONTROL

- Manual bagging
- Manual bagging of closed male bud (puso) using bagging material



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### CONTROL

- Bagging male bud at bending stage
- Bagging materials:
  - Plastic (PE) bags, muslin cloth, jute sacks
- HOW?
- Using improvised fruit bagger
  - Bag the still closed male bud using improvised fruit bagger (3meters height) with bagging material (perforated plastic bag with open bottom) for 14-15 days.



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### CONTROL

- Debudbing
- Cut the male bud immediately after removing the bagging material after 14- 15 days or all fruits have set.

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### CONTROL

- Early Debudbing



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## CONTROL

- Field Sanitation
- Deleafing of dried leaves



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## DRY ROT DISEASE

- First observed in the cooking banana cultivar "Cardaba" in backyard farms in Davao City, Philippines in 1994



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## DRY ROT DISEASE

- The disease is similar in many respects to "Bugtok" caused by the bacterium *Ralstonia solanacearum* (Soguilon, 1995) and blood disease (Eden-Green, 1994)



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## DRY ROT DISEASE

- Initial studies indicate that when DNA from the bacterium is used as template in the polymerase chain reaction with oligonucleotide primer pairs OLI1/Y2 or T3A/T5, similar amplification products are produced when *R. solanacearum* DNA is used as template (Herradura et. al 2000)



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## SYMPTOMS ON CARDABA

- Cutting open the still green but shrunken and distorted fingers reveals hollow chambers where the flesh has rotted.
- Immature fruits are observed to stop filling and become distorted and then turn necrotic and dry out.



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## WAY FORWARD: RESEARCH NEEDS

- Further study on the identification of insect species associated with bugtok and dry rot disease
- Further study on the transmission and epidemiology of the "Bugtok" disease on banana cultivars with B genome



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## RESEARCH NEEDS

- Survey on banana bacterial diseases in the present millenium



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**THANK YOU VERY  
MUCH!**



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## CURRENT STATUS OF BANANA BLOOD DISEASE BACTERIUM IN INDONESIA, AND ITS POSSIBLE APPROACHES FOR MANAGEMENT STRATEGIES

Catur Hermanto  
Research Center for Horticulture and Estate Crop  
National Research and Innovation Agency

To be presented on international workshop on the **Management of bacterial diseases of bananas**  
Kuala Lumpur, 29 November 2022

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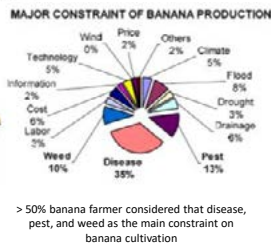


**Abstract. Catur Hermanto. Current status of banana blood disease bacterium in Indonesia, and its possible approaches for management strategies.** After a century banana blood disease bacterium initially recognized in Indonesia as reported by Gauman in 1921, the disease still relevant to the national problem on banana production, and now worrying regionally. With flashy of reddish fruit rot, dry-shrank male flower, and wilted, the etiology has been progressively changing the name from *Pseudomonas celebensis* (Gauman in 1921 in Wardlaw, 1972), to *Pseudomonas* spp. (Jones, 1997), to *Ralstonia* species complex (Taghavi et al., 1996), to *R. solanacearum* (Thwaites et al., 2000), to *R. solanacearum* phylotype IV (Fegan and Prior, 2006), and finally became *R. syzygii* sub sp. *celebensis* subsp. nov. (Safni et al., 2014), where the genome assembly was 3,568,564 bp, with 92x genome coverage for the chromosome and 1,614,128 bp, with 102x genome coverage for the plasmid (Prakoso et al., 2022). The disease was reported initially from Selayar Island - South Sulawesi in 1921, then appear in West Java in the late 80's, and was found in Moluccas in 1996, from where the disease move westward to Sumatera, eastward to eastern part of Java, and northward to Kalimantan. A massive survey conducted in 2006 - 2008 under two ACIAR's project found the disease in Aceh, North Sumatera, West Sumatera, Lampung, East, Central and West Java, South and Central Kalimantan, Southeast and North Sulawesi, and Irian Jaya. Human was believed as the main actor in distributing and transmitting the disease in long and short distance through the contaminated cloths, vehicle, agriculture tools, and infected plant materials. The next important transmitting agents are flower-visiting insects that were contaminated by infected plants. The other possible transmission was happened through banana pests such as weevil borers and nematodes. Disease management should be addressed comprehensively at macro level by implementation of quarantine procedures and disease incursion, farmer education in handling diseased plants, and micro level of disease-free planting material,

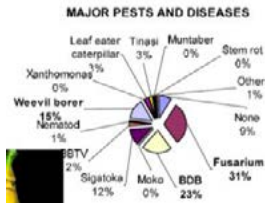
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## BANANA FARMER PERCEPTION



Fusarium wilt and blood disease are the major constraint, because they are massively existed and deadly

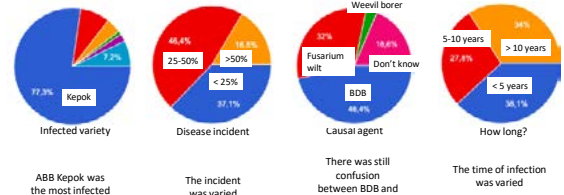


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## Current Situation of Blood Disease in Indonesia

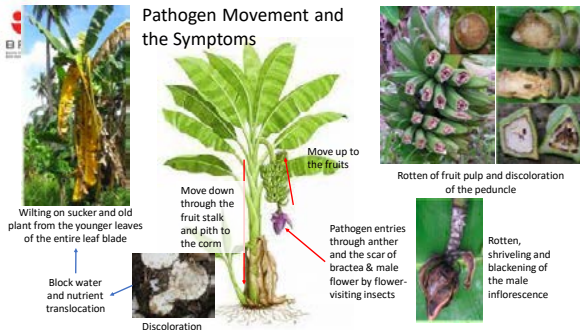
- A quick google survey was conducted from Oct 28<sup>th</sup> - Nov 1<sup>st</sup>, 2022, followed by 97 respondents from 22 provinces represented Sumatera, Java, Kalimantan, Sulawesi, Papua, Nusa Tenggara, Moluccas
- Respondent's profession: researcher, extension worker, agricuturist, farmer, and others
- The survey attached photo of BDB symptoms but did not mention the name of the disease
- 92.8% of the respondent saw the symptoms in their provinces;



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## Pathogen Movement and the Symptoms



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## Etiology of Blood Disease Pathogen

*Ralstonia syzygii* subsp. *celebensis* is a non-fluorescent Gram-negative bacterium, rod shaped, not spore forming, slow growing, and does not have a capsule structure

<i>Pseudomonas celebensis</i> by Gauman in 1921	Wardlaw (1972)
<i>Pseudomonas</i> spp	Jones (1997)
<i>Ralstonia</i> species complex	Taghavi et al (1996)
<i>Ralstonia solanacearum</i>	Thwaites et al. (2000)
Phylotype IV of the <i>R. solanacearum</i> species complex.	Fegan and Prior (2006)
<i>Ralstonia syzygii</i> subsp. <i>celebensis</i> subsp. nov.	Safni et al. (2014)

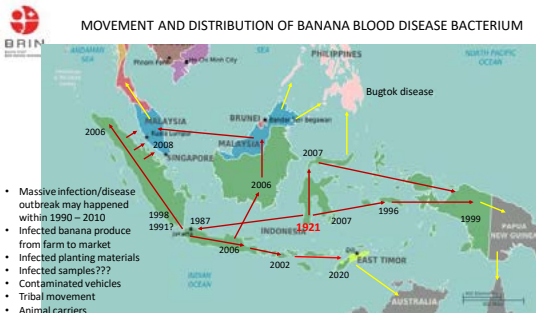
Table 1. Genome comparison of *Ralstonia syzygii* subsp. *celebensis* UGMSS\_D001 and blood disease bacterium A2-HR MAR01

Parameters	UGMSS_D001	A2-HR MAR01*
Genome length	5,185,188	5,089,680
Genome coverage		
Chromosome	92x	98.4x
Plasmid	102x	Unavailable
GC content (%)	66.5	66.4
Chromosome length	3,598,564	3,603,919
Plasmid length	1,614,128	1,480,041
Total genes	4,719	4,826
Total coding sequences	4,651	4,581
Transfer RNAs	54	55

\* Blood disease bacterium A2-HR MAR01 (Badrian et al. 2017)

Prakoso et al., 2022

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**GEOGRAPHICAL DISTRIBUTION**

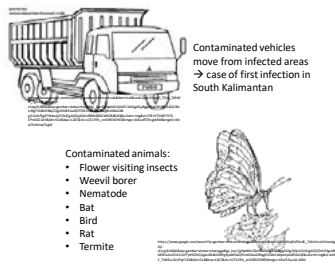
Year	Area(s)	Report
1921	Selayar Island by Gauman in 1921 and Sulawesi	Warldlaw (1972), Roesmiyanto and Hutagalung (1989), Stover and Espinoza (1992), Baharuddin (1994), Edison et al. (1996), Hermanto and Setyawati (2002), Hermanto (2009), Sutanto and Sirajudin (2008), Ray et al. (2021)
1987	Java	Subianto (1989), Eden Green (1990), Hermanto and Setyawati (2002), Hermanto (2009)
1996	Moluccas	Edison et al. (1996)
1998	Sumatera	Hermanto et al. (1996), Hermanto et al. (1998), Setyobudi and Hermanto (1999), Hermanto (2000), Hermanto and Setyawati (2002), Jumjundang et al. (2008), Kusumoto et al. (2004), Hermanto (2009), Hermanto et al. (2013), Ray et al. (2021)
Or 1991?		
2000	Irian Jaya	Davis et al (2000), Hermanto and Setyawati (2002), Hermanto (2009), Ray et al (2021)
2002	West Nusa Tenggara	Hermanto and Setyawati (2002), Hermanto (2007), Ray et al. (2021)
2006	Kalimantan	Jumjundang et al. (2006), Sutanto and Aida (2007), Buddenhagen (2009), Hermanto and Setyawati (2002), Hermanto (2009), Ray et al. (2021)
2017	East Nusa Tenggara (Sumba Island)	Ray et al. (2021)
2018	Bali	Ray et al (2021)
2020	Kupang – East Nusa Tenggara	Henuk et al. (2020)

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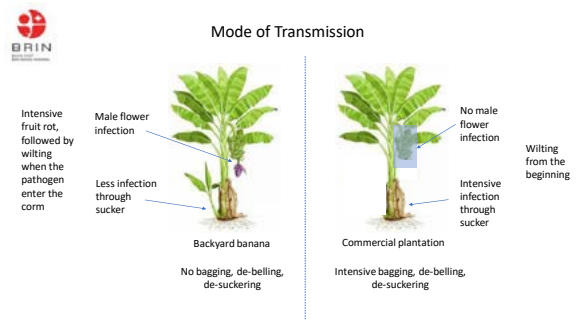
The pathogen systemically distributed in all part of the plants, and all sources of inoculum were infectious (Hadiwiyono, 2011)

- Long and short distances
- Infected planting material → new planting
- Infected banana fruit → trading
- Contaminated farming tools during orchard management

**Mode of Transmission and Disease Movement**



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**Most infected banana varieties**

**Table 1. Incidence of BDB infestation in Sumatera Island.**

Distance from the first endemic location (km)	Average between symptom (km)	Cultivar	Level of Infestation	Notes
(1)	(2)	(3)	(4)	(5)
0	-	Kepok	-	First reported in 1992
15-15	1.29	Kepok	+	
80-100	6.67	Kepok	++	
123-179	5.60	Kepok, Candi	++ to +++	Endemic since 1998. Symptom also found on P. Nangka, Raja Sere and Anson (Local farmers informants) at the border of Lampung and South Sumatera Provinces
188-200	6.60	Kepok	++	
294-310	2.29	Kepok, Omb*	++ to ++	
338-459	24.20	Kepok, Omb*	+	
994-1024	375	Kepok, Omb*	++ to +++	Border of South Sumatera and West Sumatera provinces.
1059	-	Kepok	+	Reported in 1996
1120-1124	1.25	Kepok	+	Reported since 1998. Sokik district where BDB located.
1374-1424	± 50	Kepok	+	Border of Riau and West Sumatera provinces. Mad 1999

\* Unknown cultivars

Setyobudi and Hermanto, 1999

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**Most infected banana varieties**

**Table 4. Farm- or yard-grown banana varieties (Musa spp.) identified during this study with symptoms of Blood disease (BD) confirmed to be caused by *Ralstonia solanaceae* subsp. *cefebensis***

Species	Variety/common name	Genotype	Number confirmed BD
<i>Musa × paradisiaca</i> Linn.	Kepok*	ABB	110
<i>Musa × paradisiaca</i> Linn.	Kepok "Tanjung"	ABB	9
<i>Musa × paradisiaca</i> Linn.	Kepok "Sayang"	ABB	7
<i>Musa × paradisiaca</i> Linn.	Raja Nangka	ABB	1
<i>Musa × paradisiaca</i> Linn.	Awak	ABB	5
<i>Musa × paradisiaca</i> Linn.	Raja	AAB	4
<i>Musa × paradisiaca</i> Linn.	Goosho	AAH	1
<i>Musa acuminata</i> Colla	Anson	AAA	4
<i>Musa acuminata</i> Colla	Barangan	AAA	1
<i>Musa acuminata</i> Colla	Sana	AAA	5
<i>Musa acuminata</i> Colla	Tembaga Hija	AAA	1
<i>Musa acuminata</i> Colla	Cavendish	AAA	5
<i>Musa acuminata</i> Colla	Gros Michel	AAA	1
<i>Musa acuminata</i> Colla	Acuminata var. Acuminata	AA	1
<i>Musa acuminata</i> Colla	Tomontona	AA	2
<i>Musa acuminata</i> Colla	Mas	BB	1
<i>Musa balbisiana</i> Colla	Rata	BB	1
<i>Musa senilis</i> Née	Ahsak	I	1
<i>Musa</i> spp.	Unknown	I	1

\*Variation exist within Kepok. only Kepok. \*\*Tan Ray et al. (2021)

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### Role of Flower Visiting Insects in the Epidemiology of Blood Disease

- Sahetapy, 2013
- 16 insect families visited banana flower
  - Blood disease incidence was correlated with the population density of *Drosophila* – *Drosophilidae*
  - Stinkles bee (*Trigona* sp. - Aphidae)??

Ordo Diptera	Jumlah (individu) per Desa				
	Simpang Betang 1	Simpang Betang 2	Capah Patch 1	Capah Patch 2	Pante Cerem
Drosophilidae	110	70	50	81	25
Muscidae	10	2	8	9	6
Calliphoridae	7	3	0	0	3
Micropezidae	8	0	0	0	8
Richardsiidae	5	0	0	0	0
Platyperidae	0	15	0	0	0
Cypselosomatidae	0	5	0	0	0
Tephritidae	8	7	8	6	0
Tetritidae	0	0	5	0	0
Dryomyzidae	0	0	0	5	0
Milichidae	0	0	0	4	0
Luxamiidae	0	0	0	2	0
Cnecopidae	0	0	0	0	4
Phoridae	0	0	0	0	2
Phopliidae	0	0	0	0	4
Noridae	0	0	0	4	0

Table 3. Sugar content of male flower nectar from some different bananas. Gramine.

Genome	Sample (n)	Average (%)	SE	95% CI
AA	36	18.87	0.42	17.70-20.24
BB	20	17.17	1.70	18.04-24.30
AAA	4	17.00	0.43	17.76-16.90
AAB	18	17.44	0.47	18.51-20.85
ABB	18	22.09	3.69	14.50-30.48
(Kepek ABB)	8	30.17	0.86	24.61-35.71

High sugar content of ABB Kepek might influence insect preference to and the most infected variety

Setyobudi and Hermanto, 1999

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### DISEASE MEASUREMENT

- ✓ Disease-free planting material: especially for cooking banana
- ✓ Bunch management: bagging and de-belling
- ✓ Cultivate budless banana
- ✓ Explore and cultivate other cooking banana (alternative for Kepok/Saba type) that less preferable by flower visiting insects: Pisang Jantan, possibility to introduce and conduct field test for varieties from ITC
- ✓ Good Agriculture Practices: sterile farming tools, protocol for healthy planting, local quarantine for infected plants
- ✓ Eradication of the infected plants
- ✓ Quarantine: nationally for the disease-free areas, regionally between countries

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### Disease measurement: Bunch Management

Code	Treatment	Disease incidence (%)	Reduction of Disease Incidence (%)	Percentage of Disease Incidence Decline (%)
A	Fruit bagging and debudding on Kepok Kuning	3.7 c <sup>1)</sup>	61.0	94.3
B	Debudding on Kepok Kuning	9.1 c	55.7	86.0
C	Fruit bagging on Kepok Kuning	32.6 b	32.1	49.6
D	Control-1 (untreated Kepok Kuning)	64.7 a	0.0	0.0
E	Fruit bagging on Kepok Tanjung	0.0 c	0.0	0.0
F	Control-2 (untreated Kepok Tanjung)	0.0 c	0.0	0.0

Hermanto et al., 2013

- Bagging for BDB control was actually only needed for 1 – 1.5 months along the emergence of female flower of each hand
- De-budding prevented the emergence of male inflorescence for > months
- Kepok Tanjung is budless, with no male inflorescence
- The effectiveness of the treatments proved the hypothesis of infection by flower-visiting insects

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### Disease measurement: Budless Varieties



Pisang Tanduk (AAB plantain)

Budless Kepok (ABB Saba)

Budless Awak (ABB)

Pisang Tongkat Langit (Musa fe'i)

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### RESEARCHABLE AREAS

- Induce or varietal development for resistance (empowered by the knowledge of the pathogen's genome)
- Pathogen survival in the soil, and in the infected areas
- Pathogen eradication: physical, chemical, biological (biocontrol for the pathogen)
- Distance for isolation of infected areas: consider the movement of contaminated animals: what the treatments for isolation are
- Rapid detection of infected materials
- Regular regional mapping and quarantine strategies

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18

**Education:**

- o Doctoral on Plant Pathology at the University of the Philippines Los Banos
- o Master on Plant Protection at the Andalas University – Padang
- o Bachelor on Agronomy at the Muhammadiyah University - Malang

**Working experiences:**

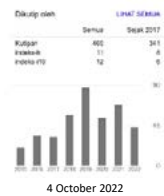
- o Researcher at the Research Center for Horticulture and Estate Crop – Research Organization for Agriculture and Food – National Research and Innovation Agency of Indonesia (June 2022 – to date)
- o Researcher at the Indonesian Agency for Agriculture Research and Development (1995 – 2022)
- o Head of East Java Assessment Institute for Agriculture Technology (2020 - 2022)
- o Head of Indonesia Vegetable Research Institute (2-16 – 2020)
- o Head of North Sumatera Assessment Institute for Agriculture Technology (2013 – 2016)
- o Head of Indonesian Tropical Fruit Research Institute (ITFRI) (2010 – 2013)
- o Coordinator for several research collaboration with BioBiodiversity International, and ACIAR funded Bioiversity International Project (2006 – 2013)

**Papers, varieties, and patents:**

- o 85 papers, reports, book chapters
- o 35 varieties (local and breeding result)
- o 10 patents and its regime



Catur Hermanto  
081374589020



**BACTERIAL DISEASES OF BANANA IN INDIA:  
OVER VIEW ON STATUS, CHARACTERIZATION OF  
PATHOGEN AND MANAGEMENT PRACTICES**

**Murugan Loganathan, Raman Thangavelu,  
and Subbaraya Uma**

ICAR-National Research Centre for Banana,  
Tiruchirappalli, India

1

**INTRODUCTION**

- India
  - World leader of banana
  - Cultivated in 0.86 mha with production of 30 m.t
  - Host diversified bananas



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**PRODUCTION HURDLES**

- Biotic stresses
  - Fusarium wilt
  - Leaf spot
  - Viral diseases
  - Bacterial diseases- rhizome/soft rot




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**STATUS OF BACTERIAL DISEASES IN INDIA**

**Survey**


- Tamil Nadu
- Karnataka
- Andhra Pradesh
- Maharashtra
- Madhya Pradesh
- Gujarat
- West Bengal
- Uttar Pradesh
- Bihar



4

**REPORTS ON BACTERIAL DISEASES IN INDIA**

- Moko wilt ✗
- Except few not thoroughly confirmed reports on bacterial wilt (race 2 of *Ralstonia solanacearum*) of banana in West Bengal, India (Chattopadhyay and Mukhopadhyay, 1968; Gnananickam et al. 1979; Mondal et al 2012)
- Blood disease ✗
- Xanthomonas wilt ✗
- Rhizome rot/soft rot ✓



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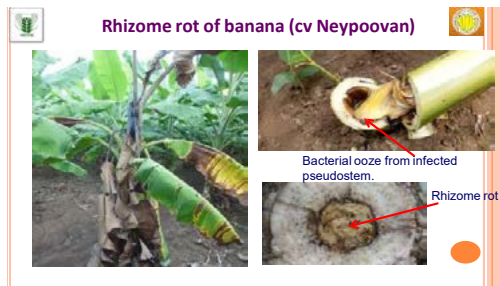
**Status of rhizome rot disease in different banana growing regions (TN)**

S.No	Place	Cultivar (Planting material)	Incidence (%)
1.	Lalgudi	Poovan (Suckers)	3.9
		Neypoovan (Suckers)	6.3
	Koppu	Neypoovan (Suckers)	2.5
		Nendran (Suckers)	6.3
	Thottiyam	Neypoovan (Suckers)	10-15
	NRCB	Grand Naine(Suckers)	3.8
	Nachalur	Grand Naine (TC)	20-30

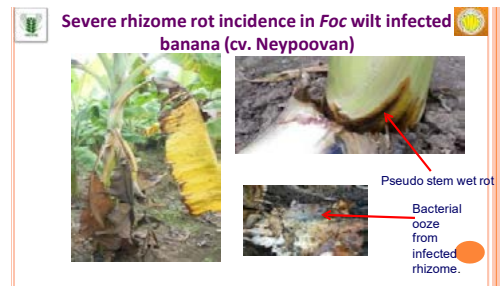
TC: Tissue culture

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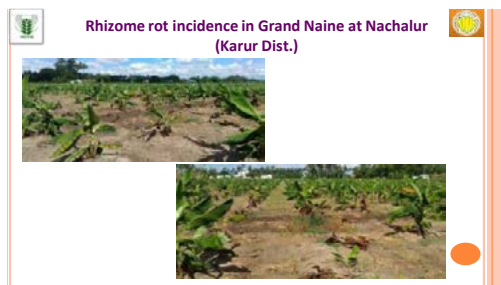




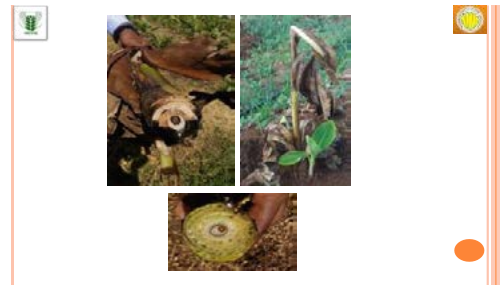
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**Status of rhizome rot disease in different banana growing states**

S.No	Place	Cultivar (Planting material)	Incidence (%)
2.	Andhra Pradesh	Grand Naine (Tissue culture plants)	10-20
		Kovvur, West Godavari Karpurachakkarakeli (Suckers)	10-15
	Pulidindi East Godavari	Tellachakkarakeli (Sucker)	5-8
3.	Karnataka	Chikkabalapura Neypoovan (Suckers)	8.0
		Grand Naine (Suckers)	3.6
4.	West Bengal Chinsura	Champa (Poovan type)	1-3

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### Status of rhizome rot

S.No	Place	Cultivar (Planting material)	Incidence (%)
5	Madhya Pradesh	Grand Naine (TC)	10-15
6	Maharashtra	Grand Naine (TC)	10
7	Gujarat	Grand Naine (TC)	<20
8	Uttar Pradesh	Grand Naine (TC)	5.5
9	Bihar	Grand Naine (TC)	6.2

TC: Tissue culture

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- ### Summary on Status of rhizome rot of banana
- ❑ Rhizome rot incidence was more in tissue culture banana cv. Grand Naine (20-30%).
  - ❑ The incidence was noticed during early stage of planting (2-4 months after planting).
  - ❑ Sucker plants: Neypoovan was more susceptible (8-10%) than Nendran (6.0%) and Grand Naine (3-5%).
  - ❑ Rhizome rot incidence was high (10-15%) in Foc wilt infected field (cv. Neypoovan).

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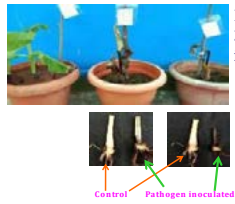
### CHARACTERIZATION

- Rhizome rot/soft rot is caused by *Pectobacterium carotovorum* (Snehalatharani and Khan 2010; Rajamanickam et al. 2018; Loganathan et al. 2019; Kalaivanan et al. 2020, 2021).
- CVP (57/60 cultures)
- NA (3 samples; Ney Poovan (Kulithalai, Tamil Nadu), Grand Naine (Thayanur, Tamil Nadu) and Thellachkarakeli (Pulitinddi village, A.P).
- Isolates: NPK-3-48, GTC-5 and 1-1B-3.



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### PATHOGENICITY TEST



Rhizome rot developed 35 days after inoculation.

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### Pathogenicity for rhizome rot pathogen in banana

Rhizome rot in Ney Poovan (suckers)

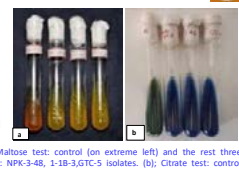


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### BIOCHEMICAL

- Gram-
- Catalase+
- Oxidase-
- Utilized Glucose, Maltose and Citrate

(Chandrashekar et al. 2018; Ajaysree and Borkar 2018)

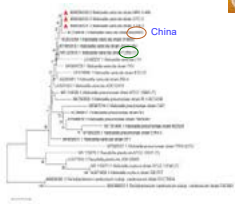


(a): Maltose test: control (on extreme left) and the rest three tubes: NPK-3-48, 1-1B-3, GTC-5 isolates. (b): Citrate test: control (on extreme left) and the rest three tubes: NPK-3-48, 1-1B-3, GTC-5 isolates.

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**MOLECULAR ANALYSES**

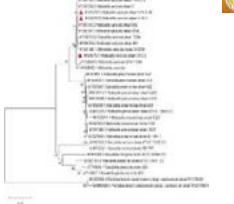
- PCR : **16S rDNA** - 3 isolates with universal primers 27F (5' - AGAGTTTGATCTGGCTCAG - 3') and 1492 R (5' - GGTTACCTGTTACGACTT - 3')
- NCBI (Accession Nos. MW036529; MW036530; MW036531).



The tree generated by MEGA-X software using the NJ and the algorithm of Maximum composite likelihood with 1000 bootstrap re-samplings. Numbers at each branch indicate bootstrap value. Scale bar represents 50 nucleotide substitutions per 1000 nucleotides.

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- PCR : **rpoB gene**
- rpoB CM7: AACCAGTTCGCGTTGGCCTGG
- rpoB CM31b: CCTGAACAACACGCTCGGA
- NCBI (Accession Nos. MW497572-MW497574).



The tree generated by MEGA-X software using the NJ and the algorithm of Maximum composite likelihood with 1000 bootstrap re-samplings. Numbers at each branch indicate bootstrap value. Scale bar represents 20 nucleotide substitutions per 1000 nucleotides.

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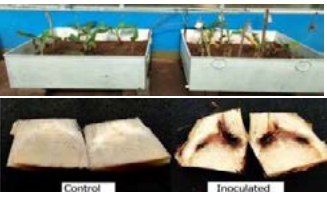
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**SUMMARY OF CHARACTERIZATION**

- Rhizome rot disease affects the most commercial cultivars: Grand Naine (AAA), Thellachakrakeli (AAA), Karpurachakrakeli (AAB), Poovan (AAB), Neypoovan (AB) etc.
- Besides *Pectobacterium*, *K. variicola* also causes the rot.

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**RAPID PATHOGENICITY TEST**



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**Validation of quick rhizome rot pathogenicity test**

- Repeated assays using fabricated soil heating unit for pathogenicity of rhizome rot bacterial isolates showed consistent results.
- Specific soil temperature was standardized.



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**Etiology**

- Spreads through infected planting materials.
- Hot weather followed by high rainfall favour the disease.
- Ill drain soil aggravates the disease.
- Foc* and *Corm borer* infestation favour secondary infection by rhizome rot pathogen.

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**MANAGEMENT**

- Planting of healthy suckers/TC plants.
- Drenching of streptomycin @ 500 ppm at 2<sup>nd</sup> and 4<sup>th</sup> months after planting + growing cowpea/sunhemp in the interspaces till 6 month (Patel et al. 2016).
- Bleaching powder @ 6 g/plant at monthly intervals for five times + Drenching of 500 ppm of streptomycin @ 1-2 litres/plant a month after planting + *Trichoderma viride* @50 g/plant at 2<sup>nd</sup> and 4<sup>th</sup> months after planting + Growing cowpea/sunhemp in the interspaces up to 6 month after planting (Patel et al. 2016).

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**Union agriculture ministry prohibits use of TB antibiotics on crops**

The most water-soluble agents, tetracycline or tetracycline hydrochloride and tetracycline dihydrate are prohibited from 2020 onwards in crops like banana, mango, guava, papaya, etc.

The Union Ministry of Agriculture and Farmers Welfare (Government of India) notified the draft order on prohibition of Tetracycline and Tetracycline hydrochloride for use in agriculture from 2020 onwards.

The draft order will come into force on the date of its final publication in the official gazette.

The draft order covers growing concerns over antimicrobial resistance observed in various crops, particularly in tetracycline, which is used in the treatment of tetracycline (TB). Tetracycline antibiotics find application in the treatment of bacterial infections.

Tetracycline is a critically important antimicrobial which has a long history in the class of widely important antimicrobials, according to the World Health Organization.

The draft order is complete but on the date of this prohibition is applicable (2020) onwards.

A detailed order will be published in the next few days after the necessary clearance from the Union Ministry of Agriculture and Farmers Welfare.

The draft order is the result of the deliberations within the Central Insecticide Board and Registration Committee (CIBRC) before the registration committee (RC) in August 2021 had approved the recommendation of the group on the use of tetracycline and tetracycline hydrochloride in agriculture (2020).

The draft order was considered "according to the availability of alternatives".

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**ALTERNATIVE CHEMICALS FOR RHIZOME ROT**

- Copper hydroxide (0.2%) against *Pectobacterium carotovorum*
- COC (0.2%) against *K. varicosa*

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**BIOCONTROL AGENTS**

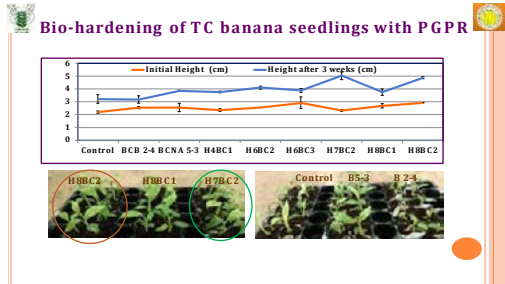
- In India, 455 million TC seedlings are produced annually
- Bio-priming of TC seedlings with PGPR found effective against rhizome rot/soft rot (Rajamanickam et al. 2018; Loganathan et al. internal communication).
- Hitherto no bio-formulation has been registered under CIBRC, Gol against rhizome rot disease of banana in India.

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**CHARACTERIZATION OF THE EFFECTIVE PGPR ISOLATES**

- 16s rDNA sequencing
- Priestia aryabhatai*
  - BCNA5-3 (Accession: MNS24145)
  - H7BC2 (Accession: OM188392.1)
  - H0BC1 (Accession: OM188390.1)
  - H6BC3 (Accession: OM188389.1)
  - H4BC1 (Accession: OM188387.1)
  - H8BC2 (Accession: OM188391.1)
- P. megaterium*
  - H6BC2 (Accession: OM188388.1)

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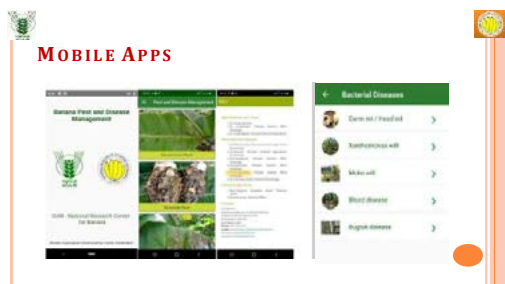
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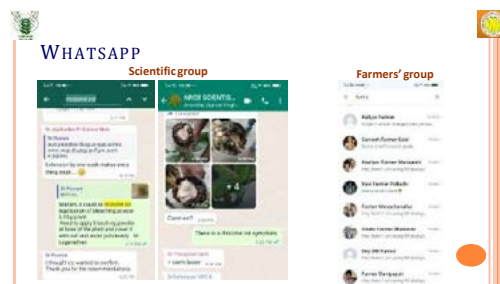
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**CONCLUSION AND WAY FORWARD**

- o Thorough analyses (cultural, morphological, biochemical and molecular bases) for characterization of bacterial pathogens are must before reporting.
- o Development of low volume and high shelf life bioformulations.
- o Validation of the formulations under multi-locations.
- o Registration of the formulations under CIBRC, GoI.
- o Commercialization of technology for adoption by all stakeholders.

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**CONTINUE...CONCLUSION AND WAY FORWARD**

- o Strengthening Awareness



- o Use of advance technologies
  - IoT enabled diagnostics
  - Nano-biopesticides

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**WORKING TEAM**



39

**ACKNOWLEDGEMENT**

**Funding and supports**



Dr B.V.K.Bhagwan, Dr Snehalatharani, Dr.V.Devappa, Dr. Das,  
Scientific and technical staff from KVKs of Burhanpur (Madhya Pradesh) and Surat (Gujarat).  
Dr K.B. Patil (Jain Irrigation Ltd.)

40



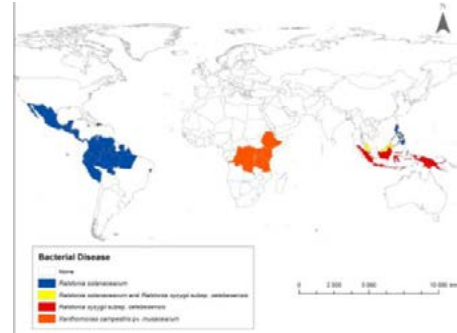
**THANK YOU VERY MUCH**

भास्करानुपुय - राष्ट्रीय कोला जलसुधारण केंद्र  
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**Xanthomonas wilt of banana in east and central Africa - Effective management practices.**

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• First reports of Xanthomonas wilt in African countries.  
(Source: Blomme et al. 2013).



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**Banana blood disease** caused by *R. syzygii* subsp. *celebensis*

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### Mode of transmission

- Garden tools
- Insect vectors
- Infected shoots/planting materials
- Large flying birds
- Bats
- Browsing cattle and small ruminants
- ....



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### Control strategy

- Early removal of male buds (to prevent insect vector transmission)
- Sterilisation of garden tools (fire, soap and water)
- Use of clean planting materials
- Complete uprooting of diseased mats
- SDR



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### SDSR, builds on incomplete systemicity and latent infections

- This method is based on:

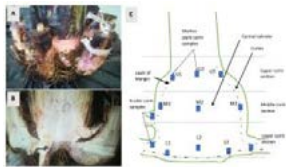
- On farm observations (where farmers only cut visibly diseased stems and did not uproot complete mats as had been advised).
- Incomplete systemicity work carried out in on station trials in Uganda (Bioversity and IITA).



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Lower Xvm recoveries occurred in the lower corm sections to which most suckers were attached relative to the middle and upper corm sections.

The position of suckers, predominantly at the bottom of corms also protects them from infection.

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- The SDRS technique comprises:
- i) regular (at least once a week) cutting, at soil level, of all visibly diseased stems,
  - ii) the removal of the apical meristem of cut plants to prevent re-growth of diseased stems,
  - iii) sterilize the cutting tools after all visibly diseased stems have been cut (cutting tool sterilization is hence only done once),
  - iv) leaving cut stems intact in the field or at the edge of the plantation/a compost heap in a corner of the farm during annual crop cultivation/intercropping
  - v) minimizing farm tool use for e.g. de-leafing and de-suckering during the initial 3-4 months of SDRS application.
- Farmers are also advised to continue with the practice of male bud removal using a forked stick.

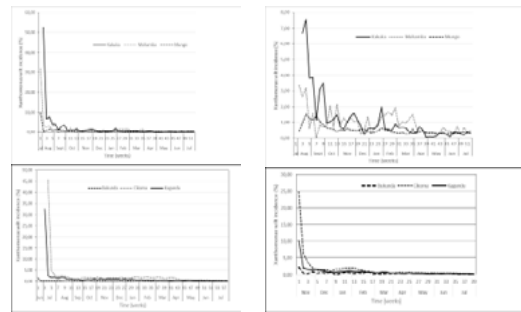
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Incidence levels drop to below 2% within 3 months, and to below 1% within 10 months.

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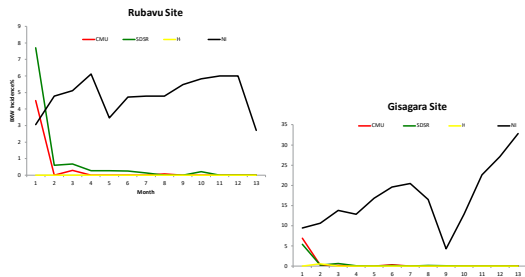
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Research results suggest that initial application of SDR preferably needs to be carried out at regular intervals (i.e. at least weekly) during the first 3–4 months of application, to remove most of the Xvm inoculum from a field or landscape.

After this phase of rigorous removal of inoculum, frequency of application could be reduced (e.g. to bi-weekly applications), in case e.g. labour is a constraint.

For SDR, the continuous appearance of diseased plants, even when numbers are very low, over an often large time period, is discouraging small-scale farmers who often want quick results. This is calling for continuous support and training of affected farm communities.

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Sources of resistance, transgenic and cis-genic approaches and CRISPR/Cas-based gene editing. See e.g.:



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The SDR technique can transform/revive a Xanthomonas wilt devastated plantation into a productive one within a year.

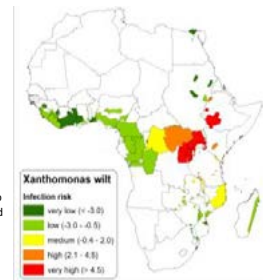
This method has strong scaling potential, with significantly reduced labour and income loss for farmers.

The SDR technique can be applied in all production systems (AAA-EAH, ABB), however in well-managed and commercially driven systems e.g. in South-Western Uganda or in fields where the disease has just appeared farmers could opt for complete diseased mat removal.

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### The risk posed by XW, mapping of hotspots, disease fronts, and vulnerable landscapes

- o Africa-wide maps highlighting XW hotspots and disease fronts within ECA and vulnerable landscapes across Africa.
- o High risks observed for the plantain production zones in north and northwestern DR Congo (due to connectivity, short distance and trade), Malawi and Zambia (connectivity and trade) and Mozambique (presence of susceptible ABB types).
- o A proactive approach is needed at frontline and vulnerable sites/landscapes [quarantine efforts, information dissemination on XW diagnosis, epidemiology, and control].



Infection risk of XW in tropical Africa

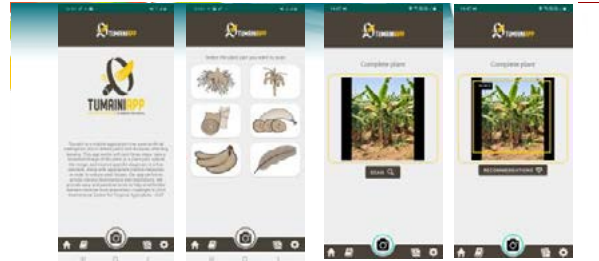
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-The Alliance of Bioversity and CIAT, together with partner organisations from east DR Congo, India, Indonesia,...

www.cgiar.org

-Focus only on banana, currently covers 5 banana diseases and 1 pest.

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