



Food and Agriculture
Organization of the
United Nations

World Banana Forum (WBF) TR4 Global Network

Working together for sustainable banana production and trade

WEBINAR

TR4-Resistant Banana Varieties Development, Introduction and Evaluation

Report of the TR4 Global Network
Wednesday, 17 May 2022

Moderator and Opening remarks:

Victor Prada, Secretary General of the World Banana Forum Secretariat (WBF), Food and Agriculture Organization of the United Nations (FAO)

Panellists:

Camilo Beltrán Montoya, International Phytosanitary Specialist, International Plant Protection Convention (IPPC), FAO

Edson Perito Amorim, Researcher, Banana and Plantain Breeding, Brazilian Agricultural Research Corporation (EMBRAPA)

Jeff Daniells, Research Horticulturist, Queensland Department of Agriculture, Australia

Monica Bettancourt Vásquez, Researcher Plant Pathologist, Colombian Agricultural Research Corporation (AGROSAVIA)

Nancy Villegas, Regional Coordinator for Risk Analysis, International Regional Organization of Plant and Animal Health (OIRSA)

Sarah Brunel, Implementation Facilitation Unit Officer, International Plant Protection Convention (IPPC), FAO

Sharl Minto, Senior Plant Pathologist, Northern Territory, Department of Industry, Tourism and Trade, Australia

Shoba Sivasankar, Head, Plant Breeding and Genetics - Joint FAO/IAEA Division of the UN · International Atomic Energy Agency (IAEA)

Yi Ganjum, Vice President, Guangdong Academy of Agricultural Sciences (GDAAS), China



Summary

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4), also known as Tropical Race 4 (TR4), is causing devastation worldwide and threatening almost all banana and plantain producers. TR4 cannot be controlled using fungicides nor eradicated from soil using fumigants. The capacity of TR4 to survive decades in the soil, along with its lethal impact and wide host range including Cavendish cultivars, are among the main reasons it was ranked as the greatest threat to banana production.

Increasing awareness and understanding of concepts related to the development of resistant varieties, proper introduction and resistance evaluation are key to confronting the disease.

This webinar is part of a series of capacity-building and awareness-raising events organized by the World Banana Forum and its TR4 Global Network. The previous events were focused on TR4 Diagnosis, Capacity Building and Awareness Raising and TR4-Resistant Varieties.

This webinar aims to provide additional information on banana cultivars and varieties with tolerance or resistance to Foc TR4 and discuss important aspects to take into consideration when introducing and evaluating those varieties.

The event will also cover the required steps for resistance assessment considering quarantine protocols for the safe introduction of foreign germplasm, indexation, planting material propagation, experimental design for resistance trials and evaluation of resistance.

The recordings of the event are available on the website: <https://www.fao.org/tr4gn/fao-in-action/webinars/>

1. Welcome by the Food and Agriculture Organization of the United Nations (FAO)

On behalf of the World Banana Forum (WBF) Secretariat, hosted by the Food and Agriculture Organization of the United Nations (FAO), **Mr Victor Prada**, Secretary General of the **World Banana Forum Secretariat (WBF)**, **Food and Agriculture Organization of the United Nations (FAO)**, welcomed participants to the webinar on TR4-resistant banana varieties: Development, Introduction and Evaluation, organized by the WBF and FAO.

The WBF is a permanent platform of assembly, where the main stakeholders of the global banana supply chain work together to achieve consensus on best practices for sustainable production and trade. It brings together governments, retailers, importers, producers, exporters, consumer associations, trade unions, civil societies and research institutions. The WBF is composed of three different working groups (WG's). The WG01 on Sustainable Production Systems and Environmental Impact, the WG02 on the Distribution of Value and the WG03 on Labour Rights.

Mr Prada mentioned that before the outbreak of TR4 in Colombia, it is necessary to support communication among various entities about how to work together against the spread of this fungal disease. The TR4 Global Network (TR4GN) is a platform for exchange and collaboration that coordinates and fills the knowledge gap for awareness and prevention of TR4, and supports proposed projects that can be regionally or nationally customized, as well as producing other resources that raises awareness and generate knowledge on how to manage Foc TR4.

This initiative is important due to the relevance of banana production for food security and poverty reduction and as an important source of income for emerging countries. More than 3 000 stakeholders are directly working against TR4, due to the economic damage that this disease can produce.



2. Current efforts at the Joint FAO/IAEA Centre for combating Fusarium Wilt TR4

Ms Shoba Sivasankar, Head of the breeding and genetics sub-programme of the **Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture**, presented the activities conducted by the centre regarding the fight against Fusarium Wilt TR4. The Joint Centre located in Vienna, Austria, advances and supports the safe and appropriate use of nuclear and related technologies in food and agriculture, aiming to contribute to global food security and sustainable agricultural development worldwide. It does so through adaptive research and development at its laboratories in Seibersdorf, Austria.

Ms Sivasankar highlighted the demand-driven role of the Joint Centre in developing improved crop varieties that are adapted to climate change with a focus on food and nutrition security. She mentioned that their portfolio includes the development of varieties by induced genetic variation (obtained using physical radiation and chemical mutagenesis), genomics and genetics, precision phenotyping (focused on selection of desirable traits), speed-breeding technologies (e.g. double haploidy, rapid cycling and shuttle breeding) and support to seed systems providing farmers' access to new varieties. The innovations developed by the Centre are delivered through coordinated research — focused on fundamental research in partnership with several institutions across the globe as well as technical cooperation projects — focused on the application of the developed technologies in the field and capacity-building programmes.

Ms Sivasankar then presented the various steps required for the development of a new variety by the Joint Centre — from the development of a genetic variation to the variety release and farmers' adoption. The Centre is demand-driven and focuses on food, feed and cash crops as well as seed propagated, vegetative and perennial species. Gamma-ray, electron beam, heavy ion beam, X-ray, cosmic rays and Ethyl methanesulfonate (EMS) are the techniques implemented to induce mutagenesis and genetic variation under their breeding programme. The Centre supports member states with precision phenomics technologies from the lab to the field and performs functional genomics and speed breeding technologies such as gene-to-phenotype association, development of molecular markers and candidate genes and development of tools for gene editing.

The Head of Plant breeding and genetics briefly introduced the FAO/IAEA Mutant Variety Database (MVD) which secures voluntary contribution records from member states. According to **Ms Sivasankar**, the MVD counts on approximately 3 400 varieties of 238 species released across 72 countries among the success stories derived from varieties produced by the Centre. **Ms Sivasankar** highlighted the positive impacts of tea plant diversity, quality and resilience in Sri Lanka, the revival of ginger production in Jamaica and the development of heat-tolerant cotton varieties boosting the production in Pakistan.

The Joint FAO/IAEA Centre has been working with Fusarium wilt of bananas since 2016 when the Centre launched a coordinated research project focused on “Efficient Screening Techniques to Identify Mutants with Disease Resistance for Coffee and Banana (2016-2020)”. This year a new project called “Strengthening Member State Capacities to Combat Banana Fusarium Wilt (TR4) through Early Detection, New Resistant Varieties, and Integrated Management (2022-2026)” was launched with a focus on enhancing the technical and infrastructure capacities at the institutional level and within national, regional and global networks for the detection, surveillance, genetic resistance and management of banana Fusarium wilt (TR4). **Ms Sivasankar** then announced a new research project with a focus on “Technology development for disease management in banana and coffee using induced genetic variation (2023-2027)” to be launched next year.

She briefly presented the research and development work of the Centre on the development of TR4-resistant varieties by induced genetic variation and the screening for resistance conducted under controlled greenhouse conditions and in the field. She highlighted the work carried out in partnership with the Guandong Academy for Agricultural Sciences in the development of the ‘ZJ No.4’ Cavendish resistant variety. She then informed the audience about the regional training course for experts of Latin America and the Caribbean carried out in the Joint FAO/IAEA laboratories focused on TR4 diagnostics and screening for resistance and concluded her intervention by informing the attendants about the Symposium on Global Research in the Management of Banana Fusarium Wilt TR4 carried out on March 2022 in Ecuador.

3. Research advancements on the development of TR4-resistant varieties

Mr Yi Ganjum, Vice President of the **Guangdong Academy of Agricultural Sciences (GDAAS)**, started his intervention by providing a general overview of the banana industry in China highlighting the prevalence of the use of Cavendish cultivars across the country. The Vice President of GDAAS then presented a timeline of the spread of Foc TR4 in the South region, starting with the first report in Taiwan Province of China (1989) followed by Guangdong (1996), Fujian (2000), Hai Nan (2001) and Yun Nam and Guang Xi (2009). He highlighted the increase of infested areas from 1.4 ha in 1998 to more than 13 000 ha in 2020.

Mr Ganjum then presented the steps carried out by his laboratory for the development of Foc TR4 resistant varieties using mutagenesis techniques. The process consists in exposing multiple buds and embryogenic calluses of banana plants to sodium azide (NaN_3), ethyl methanesulfonate (EMS) and colchicine to induce chemical mutagenesis. The mutant plants are then screened for resistance to Foc TR4 in greenhouses using highly infested soils. The best candidates are then replicated for field evaluation.

The development of the 'Zhong Jiao No.4' (ZJ4) Cavendish is a successful product of the Centre's research. This variety was obtained through the selection of a somaclonal variation of 'Baxi' induced by $^{60}\text{Co-}\gamma$. According to the researchers, the variety shows resistance to Foc TR4, high yield (approximately 42.3 tons/ha), superior fruit quality and taste (total soluble solids: 22%; titratable acidity: 0.28%; soluble solid content: 18.86%) as well as good bunch, hand and finger shape.


Mr Ganjum then presented a table comparing the main agronomic traits of 'ZJ4' and 'Baxi'. Both varieties presented similar results regarding the production cycle, yield and quality of fruits. The scientist then presented the performance of the cultivars regarding the number of infected plants by Foc TR4 in heavily infested fields. In those experiments, 'Baxi' presented an infection rate superior to 50% while 'ZJ4' displayed an infection rate inferior to 5%.

He then briefly presented the two types of early bioassays for screening *Musa* genotypes against Foc TR4: in vitro and in greenhouses, and highlighted the importance of applying directly in vitro screening to increase the throughput of banana mutation breeding. He then informed the audience about the recently published, open source book "*Efficient Screening Techniques to Identify Mutants with TR4 Resistance in Banana*" highlighting the chapter focused on the pre-screening of banana genotypes for Fusarium wilt resistance by using an in vitro bioassay.

Mr Ganjum then concluded by highlighting the importance of additional techniques to enhance Foc TR4 resistance in bananas such as gene silencing and the growing importance of CRISPR/Cas9-mediated genome editing to the betterment of agronomic traits such as shelf life of banana fruits.

4. Development and evaluation of banana varieties with resistance to Fusarium Wilt

Mr Edson Perito Amorim, leader of the banana genetic improvement program at **The Brazilian Agricultural Research Corporation (EMBRAPA)**, started his presentation by providing an overview of EMBRAPA's current strategy to develop cultivars resistant to Fusarium wilt. He explained that *Fusarium oxysporum* race 1 is the main disease present in banana production areas in Brazil. He highlighted that Brazil produces around 7 million tonnes of this fruit per year. In this context, the development of resistant banana cultivars is a key strategy to control this fungal disease. He mentioned that their main programme is based on crossbreeding techniques. In ten years, the corporation has released more than ten hybrids, all of which are resistant to Foc race 1. **Mr Amorim** proceeded to introduce a banana hybrid resistant to Foc race 1, which has been evaluated in Brazil, Costa Rica, Colombia and in the African context. According to the scientist, EMBRAPA has efficient protocols for evaluating new hybrids in field and greenhouse environments.



Mr Amorim explained the methodology for evaluating resistant varieties in the research corporation. The methodology encompasses the steps required to compare a susceptible cultivar with the hybrid banana variety being evaluated. He explained that in the greenhouse, the banana plants are inoculated with Foc TR4, and the disease incidence and severity evolution is monitored for ninety days using the Grade Scale methodology, also developed by EMBRAPA. The results of their studies suggested that the experiments in the field and in the greenhouses are significantly correlated, confirming the efficiency of the protocol developed by the institution. Moreover, **Mr Amorim** mentioned that this comparison study is probably the only research evaluating field and greenhouse screening conditions for banana resistance to *Fusarium* TR4.

Mr Amorim concluded his presentation by introducing the six techniques used by EMBRAPA to develop banana hybrids resistant to *Fusarium oxysporum* spp., which is the result of more than 45 years of banana research conducted in Brazil. The techniques consist in the development of improved diploids, which are resistant to a number of biotic and abiotic factors that deter banana plantations from producing high-quality fruit. Also, the introduction of new cultivars by crossing improved diploids with commercial cultivars is considered, and in this way, new hybrids can be produced. Additionally, generating chromosome doubling in banana plants by using colchicine, which produces several tetraploids, from diploid plants, including Cavendish relatives is being used. Moreover, EMBRAPA works on the induction of somaclonal variation having produced 11 Cavendish and six Prata somaclones with potential resistance to TR4 – those will be evaluated in Colombia in collaboration with The Colombian Corporation of Agricultural Research (AGROSAVIA). In addition EMBRAPA applies irradiation techniques in banana plants, inducing mutations in their DNA in collaboration with IAEA, aiming to develop mutant individuals that are resistant to TR4. Finally, EMBRAPA does gene editing using CRISPR-Cas9.


Mr Amorim indicated that this work started in 2020, using resistance genes against TR1 that EMBRAPA already possessed, as well as some genes related to TR4. The banana type used in this case was Prata, due to its high importance to the Brazilian banana industry.

5. The FAO support to LAC countries on the prevention and contingency of Foc TR4

Ms Raixa Llauger, Agricultural Officer from **FAO Mesoamerica** started her presentation by introducing the roadmap of actions in Latin America and the Caribbean regarding TR4, developed with a wide participation from producing countries, World Banana Forum Secretariat and the private sector. **Ms Llauger** highlighted the specific actions involving the International Regional Organisation for Plant and Animal Health (OIRSA), such as simulation exercises for the prevention of Foc TR4, in the Mesoamerican and Caribbean region. These exercises and simulacrum were focused on strengthening the capacity of surveillance and disease management in the region.

The Agricultural Officer informed the audience regarding the preparation of a sub-regional project related to the risk management of TR4. This work is being carried out with OIRSA, focusing on innovations such as the establishment of a shared platform for TR4 prevention, taking into consideration variables that influence the outbreak of the diseases.

Moreover, **Ms Llauger** provided a brief overview of the work carried out by FAO supporting the member countries of the Mesoamerican region. She highlighted the contributes provided in the development of national action plans, as well as in the development of the Andean community project, which consists of the improvement and implementation of a common strategy for Peru, Bolivia, Colombia and Ecuador. The project is divided in five components: 1) Increase comprehensive risk management capabilities; 2) Leverage investments in infrastructure, technology and research; 3) Strengthen the resilience of the most vulnerable actors; 4) Promote the trust, concurrence and technical/financial collaboration among stakeholders; and 5) Provide supranational and subnational coordination. She then mentioned that despite the fact that the regional project is focused on the Andean countries it has the potential to be coordinated with other countries of the region.



Ms Llauger then provided an overview of other activities conducted by FAO in support to countries in the region such as the revision of biosecurity plans, surveillance strategies and institutional capacities in response to TR4, to ensure an up-to-date and tailored response considering the particularities and needs of each country.

Ms Llauger then announced the launching of an online course on Good Practices for Phytosanitary Risk Assessment developed by FAO, in cooperation with OIRSA, regional experts and the National Plant Protection Organisations (NPPO) of Andean countries. **Ms Llauger** also informed the audience in regards to the technical-informative exchange session carried out with the Ecuadorian National Institute of Agricultural Research (INIAP) and the Alliance of Bioversity International-CIAT, focused on resistant varieties, and how to respond to Foc TR4. FAO has also been working closely with small-scale farmers in the Andean Community delivering diagnostic and biosecurity kits. She then announced the recent publication of the *“Strategy and Regional Action Plan for the Preparation, Prevention, Detection, Response and Recovery of Latin America and the Caribbean to Fusarium Wilt of Musaceae - Tropical Race 4”* and highlighted the exchanges carried out by FAO in collaboration with private and public sectors, to ensure the participation of key stakeholders and the proper implementation of the strategy.

Ms Llauger concluded with a reflection about vegetal material being the most likely way in which TR4 can enter a country. According to the agricultural officer, preventing the entrance of TR4 through vegetal material, is a way to assure stable income to the region, as well as avoiding the entrance of other relevant pests.

6. The IPPC guidelines on TR4 prevention and preparedness


Ms Sarah Brunel and **Mr Camilo Beltrán**, **International Plant Protection Convention (IPPC)**, presented an overview of the activities of the IPPC on TR4 prevention and preparedness. **Ms Brunel** started her presentation by indicating that in a globalized world, there is an increased risk of pests moving rapidly into new countries and the IPPC needs to prevent their introduction and spread, as they are responsible for 40% of crop losses and the consequent loss of income. She continued by clarifying that IPPC deals with plants and plant products moving in international trade. They are the object of regulation by the conventions, which aim to ensure safe trade and to prevent the introduction and spread of new pests, through the National Plant Protection Organizations responsible for setting standards, legislation and regulation on quarantine pests.

Ms Brunel emphasized the dangerousness of TR4, as it is a quarantined pest in various countries. International treaties should aim for cooperation against the introduction of new pests into countries. To date, 84 countries are part of it IPPC, as well as Codex Alimentarius and the International Animal Health Organization.

She mentioned that the Convention establishes and publishes international phytosanitary measures. The new topics are defined by the contracting parties of 47 International Standards for Phytosanitary Measures (ISPMs), as well as phytosanitary treatments and diagnostic protocols. It was mentioned the fact that the IPPC unit of Implementation and Capacity Development, produces guides and training materials, including e-learning courses to improve the access to information regarding pest risk assessment.

Mr Beltrán, started his intervention by highlighting the risk of moving vegetal material internationally. He then underscored the quarantine status of TR4 and the related phytosanitary measures applied to the pest, including IPPC monitoring worldwide.

Mr Beltrán continued the presentation by introducing relevant ISPM tools for pest risk assessment and movement of plants, as well as explaining how agricultural commodities can be categorized, depending on the risk level that it poses for the pests' dispersion, their uses and the degree of industrialisation that those products have been through. For such items, the pest risk assessment should be carried out, and the associated risks can be established.



According to the specialist, the **ISPM 11** determines guidelines for pest risk analysis for quarantine pests. In this regard, each country determines if a pest, such as TR4, should be in quarantine or not.

Mr Beltrán underscored that the movement of vegetal material should be part of rigorous risk analysis, and the phytosanitary measures should be properly implemented to protect those countries that provide and receive vegetal materials, to avoid negatively affecting the commerce among countries.

Mr Beltrán then presented an overview of the process of exporting vegetal material highlighting that the exporter must certify that its products meet the regulations of the importer country and at the same time, the importer country should verify if the shipment meets the requirements and recommended criteria. He then stressed that imported plant germplasms used for sowing and propagation, should be subject of quarantine.

Mr Beltrán then provided an overview of the activities carried out by IPPC on TR4. He mentioned the Implementation and Capacity Development Committee (IC) Team on *Fusarium* TR4 established to address the emerging issue of the banana *Fusarium oxysporum f. sp. cubense*, Tropical Race 4 (TR4). The team is responsible for compiling and review documents on Foc TR4, developing global prevention, preparedness and response materials, preparing online workshops on surveillance, diagnostic, inspection and simulation exercises on this fungal disease.


Mr Beltrán then proceeded to introduce the first draft on prevention, preparedness and response guidelines for *Fusarium* TR4 created by the IC team. Several experts have participated in the peer review. In total, 49 participants will work on this document, plus the members of the implementation and development capacity committee. The guidelines will encompass different topics related to the distribution of the pathogen, biological and morphological considerations, pest risk analysis, phytosanitary regulations, and surveillance strategies, among others.

Mr Beltrán then provided an overview of the *IPPC Workshop Series on Fusarium TR4: Diagnostic, Surveillance, Inspection and Simulation Exercises*, in which virtual events were held, and several participants from various countries participate.

Mr Beltrán concluded by presenting some results of the Assessment of countries' Capacity in Response to *Fusarium* TR4 questionnaire carried out by the IC Team on *Fusarium* TR4. The results indicated that 53% of the countries do not have a contingency plan, 47% of the countries listed have conducted a pest risk analysis and 76% of the evaluated countries have Foc TR4 as a regulated pest. **Mr Beltrán** clarified that this numbers respond to the perception that each country has regarding the risk posed by the pest. In terms on response, not all countries have a surveillance and contingency plan, which represents an opportunity to focus on reaching a consensus on the implementation and response measures against TR4.

7. The OIRSA reference protocol for the safe introduction of Musaceae germplasm

Ms Nancy Villegas, The international Regional Organisation for Plant and Animal Health (OIRSA), introduced the product of the work developed in March 2021, '*Biosecurity Measures for the Introduction and movement of Musacea propagation material in the OIRSA region*' (Medidas de Bioseguridad para la Introducción y Movimiento de Material de Propagación de Musáceas en la Región OIRSA). OIRSA gathers 9 countries (Mexico, all countries in Central America and the Dominican Republic). This document was elaborated with the participation of FAO and involves a general approach to the agricultural innovation programs for the biosecurity regulated vegetal material introduction, promoted as resistant or tolerant against TR4. This document is based on risk assessment, in which it has been determined the types of pests that can be found in the OIRSA region. In this way, practices on how to transport vegetal material in a safe manner can be implemented. The objectives of the document are to describe the risk level that the germplasm poses, depending on its origin (different countries have different levels of pest-dissemination risk, due to the presence or absence of various pests in their territories); to describe the regulatory activities before germplasm introduction; to declare the requirements that the quarantine facilities of imported materials must meet; and to describe the procedures for the contention and manipulation of germplasm from its entry, diagnosis and its release into the fields.



Ms Villegas indicated that various pests attack *Musa spp* as their preferred host. This emphasizes how easily these plants can be negatively affected by several organisms. There are 500 pests listed when an international perspective is considered.

Ms Villegas explained that after the risk assessment in OIRSA countries, 15 pests that represent a threat to *Musa spp.* were identified. However, the list comprises both absent pests and restricted distribution pests, but the risk that they represent deserves phytosanitary measures for their mobilisation, control, contention and management. Some of the pests identified in the OIRSA region can be considered as naturalised, emergent, or re-emergent, depending on the environmental conditions that can cause the emergence of certain pests in the region.

Ms Villegas emphasised that due to vegetal materials produced in in-vitro conditions, and that are implemented in various regions, many countries have reported newly discovered pathogens, which were probably not detected in previous risk assessment studies. She then highlighted that these pests need to be monitored by the competent authorities and should be considered as dangerous as other well-known species of pathogens in countries that import vegetal material.

Among the OIRSA countries, there are regional and national risk assessments. The recommendation on the regional level is to assess the risk of each pest that could pose a threat to a country. This means that banana production could be severely affected by a previously identified pest or a newly described one.

Ms Villegas reminded the audience that OIRSA countries should regulate and, if it is the case, approve the laboratories and companies that produce vegetal materials from importer and exporter countries.

As a recommendation, **Ms Villegas** stated that the national phytosanitary authorities should visit the areas in which the plant material is produced, to determine the safety or unsafety conditions where plant material is being produced and if it should be introduced or not. In this regard, all plant material that is being moved from one country to another should go through a quarantine process, that can be done outside of the producer country, in regulated facilities, or inside the producer country, being a recommendation to keep the vegetal material in confinement and away from the economically important production areas of each country.


Ms Villegas highlighted the importance of meeting the biosecurity rules. Thus, any contingency regarding plant protection can be enacted immediately. Because of that, OIRSA disseminates protocols that help all the part-takers involved in banana production.

In addition, the Regional Coordinator for Pest Risk Analysis mentioned that in the OIRSA region, efforts have been done to produce healthy citrus, cocoa and avocado plant material. However, countries that are not part of this region tend to base their plant health protocols on activities that may not function as effectively as a protocol designed to prevent pests' outbreaks.

Ms Villegas concluded by underscoring processes recommended to countries and regions, to keep the phytosanitary status as stable as possible such as the definition of processes of importation (including phytosanitary measures and protocols), establishment of phytosanitary requirements (for production and distribution of materials), insurance of quality and traceability of mother plants, diagnostic capacity and phytosanitary surveillance among others.

8. Safe introduction and evaluation of resistant materials against Foc TR4 in Colombia

Ms Mónica Betancourt, Colombia Agricultural Research Cooperation (AGROSAVIA), started her intervention by providing an overview on how the safe introduction and evaluation of resistant materials against TR4 is conducted in Colombia. She stressed the relevance of the matter for Colombia and remembered the audience that Foc TR4 was firstly reported in the country in 2019 and immediate action regarding contention, research and cooperation among entities



was required. She then informed the audience that there are eleven banana farms in which Foc TR4 has been detected in La Guajira and 2 farms in Magdalena, comprising a total area of 2 432.01 ha.

As part of the immediate response facing this emergency, resistant/tolerant plant materials available were identified. The materials produced by TBRI, The University of Queensland (Australia), The French Agricultural Research Centre for International Development (CIRAD), The Brazilian Agricultural Research Corporation (EMBRAPA), Rahan Meristem (Israel), and The Honduran Foundation for Agricultural Research (FHIA) were considered as a possible solution and transfer agreements between the Colombian government and the institutions were achieved. These agreements describe how these vegetal materials can be safely introduced, and for which research tests they will be evaluated.

Specifically, AGROSAVIA will receive the 'GCTCV 218' and 'GCTCV 219' somaclones from TBRI, the 'C4', 'Gal' and 'DR4' somaclones from Rahan Meristem, the Genetically modified organisms (GMO) materials from Queensland University, 18 varieties from EMBRAPA, the 'FHIA 25' from FHIA and four varieties from CIRAD, namely 'Ruby', 'CIRAD 924', 'CIRAD 931' and 'CIRAD 938'.


Ms Betancourt, explained the risks associated with the exchange of vegetal material for plant propagation among countries. She mentioned that the entry of plant material into Colombia has been regulated before the TR4 was detected for the first time on its territory. These regulations impede the entrance of any plant material that is not produced through in-vitro technologies. Due to these actions, the presence of pathogenic fungi, bacteria and nematodes can be deterred. Additionally, quarantine viruses such as Banana bunchy top – BBTv – Babuvirus, Banana streak virus – BSV – Badnavirus, among others could be introduced by infected plant material due to the lack of preventive measures, such as thermotherapy or chemotherapy.

Ms Betancourt remarked the importance of meristem size in banana plants and the possibility of virus multiplication in this vegetal material, highlighting the possibility of virus presence even in in-vitro produced material.

According to the researcher, in Colombia, there are four reported viruses of economic importance in bananas: Cucumber mosaic virus, CMV – Cucumovirus; Banana bract mosaic virus, BBrMV – Potyvirus; Banana mild mosaic virus, BanMMV; and Banana streak virus, BSV Badnavirus. Additionally, there are three viruses that affect *Musa spp.*, which are not present in the country, namely: Abaca bunchy top virus – ABTV; Abaca mosaic virus – SCMV-AB – Potyvirus; Banana bunchy top – BBTv – Babuvirus). From the previous viruses, BSV and BBTv raised alert in the country due to the devastation that can cause in banana plantations, and to its difficult diagnose.

Ms Betancourt presented the distribution of the BBTv virus worldwide, highlighting its spread in Africa, Asia and Oceania. Despite of the absence of the virus in Colombia, its vector, *Pentalonia nigronervosa*, is present as a common aphid in banana plants. On this regard, the occurrence of the virus in the vegetal material is high in the country. **Ms Betancourt** then introduced the diagnosis guidelines that AGROSAVIA developed in cooperation with ICA, which involves 10 pathogens, including Foc TR4, *Ralstonia solanacearum* race 2, and viruses.

She stated that the following activities to contain Foc TR4 involves creating a Model for the introduction of Musacea promissory materials due to their resistance to Foc TR4 in Colombia. In general, the guidelines contained in the Model are a comprehensive guide that regulates the entrance and exit of vegetal material in Colombia. She then informed that by august 2021, the banana varieties proceeding from CIRAD were introduced in Colombia. These materials spent six months in quarantine, and after two rounds of samplings, the release of the material was possible. For the germplasm coming from EMBRAPA, Brazil, eighteen materials were introduced. These plants will be tested regarding their agronomic performance and resistance to TR4 in field conditions. The trials are being conducted in the research center of Magdalena-Cariba and on the research center of Cenibanano in Uraba.



Ms Betancourt concluded her presentation with the following remarks: the biggest risk for the banana industry after Foc TR4, is the entry of BBTV; the high risk of non-described BSV species entering Colombia; and the countries that are in risk of getting the disease should establish protocols to avoid the entrance of phytopathogens through propagation materials.

9. TR4-Resistant banana trials in Australia

Mr Jeff Daniells, Queensland Department of Agriculture, commenced his presentation by explaining that 90% of banana production in Australia comes from the tropical coast of North Queensland. He stated that 370 000 tonnes of bananas are produced in about 14 000 hectares. **Mr Daniells** remarked that TR4 was firstly detected in Australia in 1997, in the Northern Territories and detected in 2015 in the main production areas of Queensland. Since then, five farms were infected and about 160 infected plants have been detected. This is associated to the relatively slow rate of spread, and it is proof of the high level of biosecurity measures implemented in Australia regarding Foc TR4.

The banana varieties ‘Williams Cavendish’ and ‘Lady Finger’, which are the most important varieties due to the volume of production that they represent in the county, are susceptible to Foc TR4. **Mr Daniells** provided a brief overview of the Banana Variety Options Paper, in which banana varieties’ breeding improvements are described. This allows researchers to identify and justify where to focus when in search of novel hybrids, and ‘Cavendish’ and ‘Lady Fingers’ varieties. He then mentioned that the Queensland Department of Agriculture, started to develop Cavendish varieties by mutagenesis based on previous success on Foc Sub Tropical Race4 in the 1990s.

Mr Daniells underscored the importance of development of resistant varieties to ensure production in affected areas without neglecting the implementation of quarantine measures and the risk of introduction of exotic insect pests and diseases.


He then presented the quarantine facility of Brisbane and mentioned that after importation, quarantine, and in-vitro multiplication, the research institute commence the agronomic assessment and screening of TR4. As a security measure, trials in greenhouses and fields that involve the management of TR4 are only permitted in the northern territories. This is to lessen the proliferation of disease inoculum in Queensland.

When a variety is imported, it needs to be tested under the production situation in which it is intended to be established, to see how it responds to climate, crop management procedures and edaphic factors, which can be very different from where the plant is originally from. As a recommendation, **Mr Daniells** indicated that is important to verify the plant material that is received: it should not be mixed with another variety. These issues may come from unreliable information provided or poor transferability of the overseas results.

Mr Daniells then presented the results of a large agronomic study, in which a comparison of varieties was done. In this study, resistant/tolerant Cavendish varieties (‘GCTCV 218’, ‘GCTCV 217’, ‘GCTCV 105’, ‘GCTCV 215’, ‘GCTCV 247’, ‘GCTCV 119’ and ‘CJ19’) were compared to Williams (industry control standard) variety. The ‘Giant Cavendish’ tissue-culture variants presented intermediate resistance. The cumulative yield for the first two crops ranged from 63% – 92% of the yield of ‘Williams’, with the ‘GCTCV’ 217 being the most productive.

According to the scientist, most of the varieties tested are significantly taller than ‘Williams’, which can make crop management difficult, and often leads to crop loss due to wind. He mentioned that Australia has tested and identified TR4 resistant varieties in several hybrids such as ‘FHIA-25’, ‘FHIA-18’, CIRAD hybrids as well as in cooking bananas such as ‘Pisang Gajih Merah’. However, according to the scientist, they are less productive than ‘Cavendish’ varieties, and not always accepted by the markets.

Following the first evaluations, the field trials continue with the best performing varieties from the first agronomic TR4 studies onto trials inside production farms. These trials are an opportunity to test the varieties in a range of environments



and under commercial conditions. This also gives the opportunity to obtain producers' insights, which includes feedback from the market and supply chain stakeholders.

The scientist informed the audience about on farm trials, which were established in North Queensland, and one in the Northern Territory with the varieties 'GCTCV 215' and 'GCTCV 247' compared to the standard 'Williams'. The trials had between 200 – 300 plants for each variety. A grower group demonstrated healthy TR4 disease-free banana plants from these varieties ('GCTCV 215' and 'GCTCV 247'). Nevertheless, the expected productivity was significantly lower than 'Williams' when it grows in the absence of TR4. Additionally, these selections tend to bend the pseudo stem when exposed to strong winds.

Mr Daniells explained that his department works with its own banana mutagenesis program, following the TR4 incursion in North Queensland. In one of their experiments, four Cavendish selections with disease resistance were chosen as candidates, along with the 'Goldfinger' variety that is also resistant. The objective was to improve the agronomic characteristics of the 'Cavendish' selections and the organoleptic characteristics of the 'Goldfinger', whilst retaining TR4 resistance. From the 'Cavendish' work, 18 varieties were selected and were reproduced for future tests.

For 'Goldfinger', a high number of variations was obtained by mutagenesis, mainly on the bunch's morphological characteristics. **Mr Daniells** also informed the audience that improvements were obtained in the organoleptic properties of the mutants. The team reduced the number of the obtained promising varieties to five, based on large-scale taste test and sensory evaluation. Further confirmation on TR4 resistance is planned to be carried out before the conduction of on-farm trials.


The scientist holds that resistant varieties are just part of the solution to TR4 in Australia. As part of an integrated production and management system, the research for new TR4-resistant varieties of banana that could replace 'Cavendish' in the supply chain is considered. **Mr Daniells** then highlighted the example of the implementation of 'Formosana' in the Philippines, where it observed yield and fruit quality penalties associated with resistance to the disease. Therefore, he considers that the adoption of such varieties will be greatly delayed due to the different yield and fruit quality of resistant varieties compared to susceptible varieties such as 'Cavendish' grown in the absence of TR4. **Mr Daniells** emphasized that even if there are some promising varieties of banana in the market, it is imperative to carry out activities that can make a positive difference. In particular, activities that reduce disease and inoculum levels in the field.

Mr Daniells concluded his presentation by asking what would happen if a new race such as TR5 appears in the world. He suggested that the application of an integrated approach will allow farmers to be better prepared for this and other potential future threats.

10. Field screening for resistance of Foc TR4

Mr Sharl Mintoff, Northern Territory Government of Australia, started his presentation by explaining that TR4 was first detected in Australia in June 1997 in the Northern Territory of Australia. This finding triggered a biosecurity response. However, more properties were found to be infected with TR4 and the containment of the pathogen failed. This has produced a decline in the banana industry in the Northern Territory, and in 2012, TR4 was declared endemic in the Northern Territories. As a result, this part of the country is the only place in Australia in which researchers can work with TR4.

In collaboration with the Queensland Government, the Northern Territory has been conducting trials concerning TR4 since 2016. The experiments included reference banana varieties: 'Williams' (very susceptible), 'GCTCV 218' (intermediately susceptible), 'FHIA 01' (resistant), and 'FHIA 25' (highly resistant). Assessments to this trial were on external and internal symptoms on banana plants at harvest and death, symptoms at harvest, and agronomic assessments. After the trial, a disease severity score was assigned to each variety tested that ranged from 0 – 2, where 0 means that banana plants do now show disease symptoms under high inoculum pressure, and 2 means that symptoms of the disease are severe and high mortality rates are present due to the disease, in which more than 70% of plants were affected.



The first variety screening period was from June 2016 – 2018, in which all trials were artificially inoculated with approximately 200 ml of TR4 inoculum. Specifically, 24 banana varieties were assessed, which were predominantly ‘Cavendish’ (‘GCTCV 106’, ‘GCTCV 215’, ‘GCTCV 247’, ‘GCTCV 218’, ‘CJ19’), FHIA hybrids (‘FHIA-18’ and ‘FHIA-25’) and parental lines (‘SH-3436’, ‘SH-3656’, ‘SH-3748’ and ‘SH-3217’). The plants were assessed during two cropping cycles that finalised in March 2018. The results in this experiment show that in 50% of banana plants tested were resistant. Additionally, some of the Chinese varieties of Cavendish analysed were rated as highly resistant. The main results of the experiment are available in the paper *“Banana Cultivar Field Screening for Resistance to Fusarium oxysporum f.sp. cubense Tropical Race 4 in the Northern Territory”*.

Mr Mintoff mentioned that by 2018, another screening trial was carried out, in which 32 varieties were tested. The planting and inoculation occurred in December 2018. This evaluation of banana plants was divided into two experimental designs: main trial and sub-trial. The experiment ended in August 2020, in which the disease was assessed over two crop cycles and lasted approximately 20 months. In addition to the previous trials, new varieties such as the ‘Cavendish GCTCV 217’ and ‘GCTCV 105’, the novel hybrids from CIRAD (‘03’, ‘04’, ‘05’ and ‘06’), ‘PKZ’, ‘High Noon’ (‘Lady Finger’ hybrid) and cooking bananas were also evaluated.

According to the scientist, the CIRAD varieties ‘03’ and ‘04’ did not showed symptoms of disease. On the other hand, the ‘Goldfinger’ variety that was the resistance control presented some symptoms at the beginning of the experiment. The ‘Cavendish’ varieties showed high susceptibility to TR4. Interestingly, the ‘High Noon’ variety displayed recovery from the disease, which outperformed banana varieties that remained with less symptoms throughout the experiment. In general, the lines ‘GCTCV 105’, ‘GCTCV217’, ‘Dwarf French Plantain’, and CIRAD ‘03’, ‘04’, ‘05’ performed better than ‘Formosana’. **Mr Mintoff** then mentioned that some agronomic issues were noted in some varieties such as long cropping cycles, tendency to snapping before harvest in ‘CIRAD 04’. The reason for that is still unknown, but it could be due to a trait of the variety or due to the environment of the Northern Territories in Australia. There should be further work to conclude if any of the lines tested in this study are commercially viable in the Australian market.

Mr Mintoff then briefly presented the experiment carried out in 2021, on which was structured as one experiment. In this study, 23 varieties including 3 reference varieties were analysed and the data collected was for the plant crop only. This trial contained CIRAD lines (‘01’, ‘02’, ‘07’ and ‘08’), EMBRAPA lines (‘JV 42.41’, ‘PA 03.22’, ‘PA 12.03’, ‘PV 03.44’), 3 mutant Goldfinger lines (‘1/44 GMS’, ‘4/17 GMS’ and ‘5/44 GMS’) – that are part of a separate mutagenesis programme in Australia, in which they are trying to improve the agronomics of resistant banana varieties among other traits.

The scientist said that some Goldfinger mutants (‘1/44 GMS’ and ‘4/17 GMS’) demonstrated resistance against TR4 together with ‘CIRAD 08’, ‘Calcutta’ and ‘M61’. Other varieties such as ‘Goldfinger’, ‘5/44 GMS’, ‘2390-2’ and ‘PA12.03’ showed better results than ‘Formosana’. **Mr Mintoff** mentioned that resistant varieties are not necessarily adequate or accepted by the market in Australia and internationally. In this context, resistance against TR4 and market acceptability are two areas that do not necessarily overlap. He then concluded by saying that the Queensland group has conducted pre-commercialisation trials for resistant lines identified in the Northern Territory, but that those have not yet been adopted as a replacement for the current varieties in the market.

10. Closing remarks

Mr Victor Prada, Secretary General of the **World Banana Forum Secretariat (WBF)**, **Food and Agriculture Organization of the United Nations (FAO)**, provided the closing remarks of the webinar. He mentioned that the information concerning the work that the work being carried out by the World Banana Forum can be found in the website of the **TR4 Global Network**. Finally, **Mr Prada** thanked the participation of all the panellists and attendees in the event, as well as mentioning that the **World Banana Forum** is continuously offering support on matters concerning the banana production worldwide.



CONTACT

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