Food and Agriculture Organization of the United Nations

FAO Conference on Fusarium TR4

World Banana Forum (WBF)
Emergency project on Fusarium TR4 in Latin America and the Caribbean
International Plant Protection Convention (IPPC)

Capacity Building and Awareness Raising in Response to the Threat of Fusarium Wilt of Banana, Tropical Race 4

Day 3 - "Management alternatives: agricultural practices, promising clones and procedures for their introduction"

Thursday, July 29, 2021

Moderator: Ms. Esther Peralta, Specialist in Agricultural Health of the Subregional Office for Mesoamerica of the Food and Agriculture Organization of the United Nations (FAO / SLM)

Opening Remarks: Victor Prada, Secretary General of the World Banana Forum (WBF)

Panelists:

Dr. Luis E. Pocasangre, Director of Research, EARTH University, Costa Rica
Dr. Gustavo A. Rodríguez, Fruit Tree Network-Seed Department, Headquarters-Tibaitatá, AGROSAVIA, Colombia
Ing. Antonio J. González, Head of Biosafety, Banana Administrative Services (SAB SAS), Colombia
Dr James Dale, Center for Agriculture and Bioeconomy, Australia Queensland University of Technology (QUT), Australia
Dr. Adolfo Martínez, General Director, Honduran Foundation for Agricultural Research (FHIA)
Dr. Jean-Pierre Horry, Center for International Cooperation in Agronomic Research for Development (CIRAD), France
Ing. Danilo Román (Independent Consultant)
Dra. Mónica Betancourt, Researcher PhD. Associate, Colombian Agricultural Research Corporation (AGROSAVIA)
Dr. Manuel Rodelo, Assistant Manager of Analysis and Diagnosis, Technical Directorate of Agricultural Analysis and Diagnosis, Colombian Agricultural Institute (ICA), Colombia
M.Sc. Xavier Euceda, Coordinator of Regional Plant Health Programs, International Regional Organization for Agricultural Health (OIRSA)

The recording of the event is available on the website: https://www.fao.org/tr4gn/news/news-detail/es/c/1415383/ and the starting times each session are indicated in blue.

Summary:

This webinar was oriented to members of the TR4 Global Network, the National Plant Protection Organizations of the Musaceae producing countries and related public institutions, researchers, professionals and technicians, as well as producers in Latin America and the Caribbean and other geographic regions. The third day of the Conference covered management alternatives of TR4 focusing on agricultural practices, promising clones and procedures for their introduction.

01:45 Opening Remarks

Plenary

10:19 Introductory reflections on alternatives and challenges related to the management of Fusarium wilt of Musaceae, Tropical Race 4

Dr Luis Pocasangre began his presentation by mentioning that, after the confirmation of outbreaks of Foc TR4 in Colombia and Peru, the pressure in the value chain increase in Latin America and the Caribbean (LAC). The region is at higher risk as conventional and organic bananas cultivated are susceptible Cavendish. The outbreak in Peru is more dangerous than the one in Colombia due to its occurrence in an area close to the border with Ecuador, and with a predominance of small farmers that does not have financial resources to invest in biosecurity.

Since 2000, prevention actions regarding the entry of Foc TR4 into LAC have included statements, campaigns, congresses, meetings and workshops trainings and awareness raising events were institutes, international organizations, ministries and responsible institutions, unions, multinational companies, and universities have participated. The organization of the actors involved in the value chain is key to face the TR4 problem.

According to Dr Pocasangre, it is necessary to advance on building the capacity of producers to protect their livelihoods and activate biosecurity measures on their farms. The biosecurity protocols have been designed for conventional bananas. Since the outbreaks in Peru occurred in organic bananas, their management is more challenging. The research director understands that organic product certification schemes must develop specific methodologies focused on preventing emergencies.

Dr Pocasangre pointed out the importance of using clean rubber boots given that Foc TR4 is a soil pathogen and that workers are the main route of dissemination of the disease at farm level. He also mentioned that the mapping of affected zones is essential to manage the disease at farm level and mentioned that the COVID-19 pandemic substantially reduced intercontinental travel, and phytosanitary controls in the countries.

According to the director of research at Earth University, changing cultivars appears to be a solution. Research should be focus on obtaining disease resistance, through hybridization, breeding, and genetic modification. Likewise, it is essential to improve the health of the soil, through renovation of plantations, the use of short-cycle cultivars, as well as the use of biological control techniques (microorganisms and

biostimulants). The combined use of the banana clone Formosana, the soil fungus *Trichoderma spp*, and the probiotic product EM ™ appears to be a successful trinomial, but all the mechanisms of action involved need yet to be fully understood.

34:49 Biological control and management of soil health. Advances in scientific research in Colombia

Dr Gustavo Rodríguez pointed out that the research agreements between AGROSAVIA and Colombian Agricultural Institute (ICA) focuses on the management techniques for the suppression and containment of Foc TR4 and involves predisposing factors, biological control, biosecurity measures and production models, complemented by Foc TR4 genomic approaches (phylogeny and diversity studies), epidemiology (dispersion factors, predictive models and risk maps) and pre-breeding of varieties (import, evaluation, selection and healthy seed production).

The researcher informed the audience on the development of a software piloted in four countries and called *System for the Diagnosis of the Quality and Health of Soils (SiDiCaSS*), which integrates edaphic characteristics (physical, chemical and biological) and characteristics of cultivated Musaceae in indicators that supports the establishment of management recommendations. The system seeks to go beyond the evaluation of edaphic properties by integrating them into indicators sensitive to soil quality. According to the researcher, the biological properties of soil are key despite being less studied and the quantification of the health and quality of the soil is an essential starting point to manage the disease.

Dr Rodriguez then presented the results of the in-vitro evaluation of Foc TR4 antagonist products, based on beneficial microorganisms, which was carried out after observing the diametric growth of the pathogen in the presence of the biocontrol agents *Trichoderma spp, Bacillus spp and Pseudomonas spp*. He highlighted the importance of scaling these studies to nursery and field levels and integrate the study of combinations of microorganisms, the use of banks of microorganisms as alternatives, and collaborations with the private sector for its implementation.

He presented various lines of research carried out at the center, including the application of biostimulants/antagonists and its effect on the response of physiological bioprotection (rate of photosynthesis and respiration), biochemistry (sugar production), symptomatology (flaccidity, wilting, yellowness and necrosis) in the development of roots (length and biomass) and in the endophytic concentration of *Bacilus spp* and *Pseudomonas spp*. The researcher emphasized the relationship of the bioprotection response of the plant material with its physiological quality and the importance of soil management based on the understanding of the predisposing factors to Foc TR4.

The evaluation studies of the edaphic factors predisposing to Foc TR4 in the rhizosphere (physical and chemical properties, biological activity, and characterization of microorganisms) of AGROSAVIA were conducted in quarantined farms (considering affected areas, eradicated areas and not affected areas) and supplemented with foliar analysis. The relative abundance of bacteria and fungi genera showed variation between soils with and without the presence of the disease. The factors identified as predisposing for the disease were: poor drainage, density, acidity, low content of phosphorus, organic matter, and calcium;

low diversity and microbial activity; water quality, type and intensity of fertilization, salinity, temperatures and occurrence of floods.

56:03 Practical results of the use of microbial bio-inputs in areas of La Guajira

Mr Antonio González presented the practical results of the research conducted by SAB SAS with beneficial microorganisms in areas of La Guajira. The Engineer mentioned that their study process began with the identification of plants with symptoms.

After the identification of the plants, a solution containing microorganisms (*Trichoderma harzianum, Bacillus subtilis,* and *Pseudomonas sp.*) is applied through irrigation to the selected plants and their "children" covering a radius of 5 m. The child is marked and in the case of the presence of disease in the child, the productive unit is completely eradicated. In the case of susceptible materials (Cavendish - Great Nine), the plant is completely cut and removed (it is also placed in bags with urea and calcium). In the remaining hole, rice husk is applied and burned. Tolerant plants are replanted with different timeframes (after 1 month, 3 months and up to 1 year after eradication).

The Engineer informed the audience that SAB SAS in association with the international trading company Tecbaco, is looking for native isolates of *Trichoderma sp.,* whose behavior is being studied in Petri dishes (to understand the growth behavior in presence of TR4). He mentioned that the application of bio-inputs is currently carried out preventively in plantations. The company generates mixtures of *Trichoderma sp.* in rice substrate, which are dissolved and washed in water, filtered, and poured into the fertirrigation tanks (dose of 0.5kg / ha).

1:14:07 Resistant clones obtained by biotechnological methods

Dr. James Dale (QUT) began his presentation by mentioning that Foc TR4 is currently endemic in northern Australia with a very limited distribution in northern Queensland. He then presented the necessary stages for the development of resistant varieties in his institution. In QUT the development is based on the selection of tolerant material, conventional breeding, as well as genetic modification and editing. According to the professor, in order to stop the disease, it is necessary: (i) a new variety of banana (different from Cavendish) generated conventionally with resistance to the disease, high yield, portability and excellent flavor and texture; or (ii) a genetically edited or modified Cavendish with multiple disease resistances. While genetic modification involves adding new "foreign" DNA, gene editing is precise and targeted,

The researcher provided an overview on the efforts of the University of Queensland from 2000 to date. Efforts include: the search for TR4 resistance genes in wild bananas; the isolation of a resistance gene from *Musa acuminata ssp malaccensis*; the development of a genetically modified Cavendish Grand Nain with resistance to TR4 (by transferring the *Musa acuminata ssp malaccensis* gene), implementation of field trails, selection of the best gene line, and checking its incorporation into the genome. The DNA extracted from the plants with symptoms is analyzed in laboratories of the University. According to Dr Dale, the 4 lines developed with the resistance gene had significant reductions in infection rates, which were accentuated up to 3 years of age.

After the success of the initial experiments, field trials were expanded for 5 years, considering phenology and yield. Additionally, work has been done on genetic editing of Cavendish (AAA) and other bananas for resistance to TR4 (through CRISPR/Cas9 technology), from the donor genome of *M. malaccensis* (AA) resistant to Foc R1 and TR4, as well as Sigatoka. This research has been funded by public funds from Australia and other sources, including transnational corporations.

The Researcher highlighted that in the face of the TR4 threat in the Americas, the options for resistance in the medium term are: (i) the development of a new and different export banana through conventional breeding; (ii) a Cavendish GMO already available; (iii) a gene-edited (non-transgenic) Cavendish, in development. According to him, GMOs are still politically challenging, but are already accepted in the US, Canada, Australia, Japan and the Philippines.

1:45:48 Traditional genetic improvement of Musaceae in the FHIA

Dr Adolfo Martínez started his presentation by explaining that the Honduran Foundation for Agricultural Research was established in 1959 to develop hybrids of Gros Michel resistant to Foc 1. In the 1960s, the foundation focused on the development of diploids and after Cavendish's discovery, the institution focused on the development of resistant hybrids. Since the mid-eighties the foundation has been working on the improvement of Musaceae for the local market, and more recently the focus has shifted to the development of niche bananas.

The director of the foundation highlighted the work of the Musaceae Genetic Improvement Corporation (MBC) that began in 2012, as a commercial partnership between FHIA and banana exporting companies (such as AgroAmerica, Dole Tropical Fruits and Mackays Banana Marketing).

The traditional breeding strategy for Musaceae in Honduras considered various techniques such as: the development of tetraploids (4N), crossing with improved diploids (2n) and development of triploid hybrids (3n), which have been evaluated regarding the agronomic behavior and resistance to TR4. The FHIA developed a banana “grape” which was result of selection of several generations considering agronomic aspects such as the culture cycle, the number and size of leaves, the height of the plant, the weight of the bunch and the quality of the fruits. Patent for hybrids obtained in the foundation has been processed along the years (eg: Chiquita CQB 115 and 114).

The director concluded by mentioning that the development of varieties resistant to TR1, TR4 and Black Sigatoka in the foundation are focuses on different types of bananas: a new Cavendish, a Gros Michel type, a Date type, a sweet Lady Finger type and on Plantains. The institution is also focusing on the improvement of agronomic performance of bananas and on increasing its beta-carotene content.

2:03:19 Genetic improvement of Musaceae at CIRAD

Dr. Jean-Pierre Horry (CIRAD) highlighted the challenge of improving varieties for sustainable production, integrating environmental constraints (including pests and diseases), value chains (productivity) and consumers (fruit quality). CIRAD is focusing on the development and selection new dessert varieties (for export and local markets) and for cooking (with focus on robustness, tolerance, and quality).

Dr Horry presented the stages of development of resistant varieties, highlighting the importance of fundamental genetic research (on wild subspecies, genomes, genetic bases, heritability, and promising genes), crosses (pre-crossing between diploids, chromosome duplication and triploid generation), progeny selection, scaling, development of adapted production systems, adjustments and adaptation of post-harvest and marketing systems.

According to the researcher, alliances, and partnerships are key for the development of new varieties. He mentioned that the cooperation with Wageningen University was key to identify parents with resistance to Foc R1 and TR4 and to generated triploid hybrids; and the partnership with Queensland University supported field trials.

To advance in the evaluation of selected hybrids in Latin America and the Caribbean (in relation to tolerance to pests and diseases, agronomic performance, production systems and acceptability), CIRAD is partnering with entities present in Cuba, Guadeloupe, Martinique and Colombia.

2:15:43 Behavior and considerations of the Formosana somaclone against the FoC TR4

Eng. Danilo Román (Independent Consultant) mentioned that, between 1967 and 2021, it went from 1 to 20 the number of countries with occurrence of Foc TR4. In the Philippines, the pathogen was officially reported in 2005. Since the banana expansion was carried out with traditional clones, the proliferation of the pathogen significantly affected the national territory, mainly, in lowlands and small farms. Large companies implemented selection, improvement, and propagation processes, applied new prevention and containment protocols, and adjusted their agricultural practices. The 2013 floods accelerated the conversion, replacement, and expansion of areas.

The commercial clone GCTCV 218 developed by the Taiwan Banana Research Institute (TBRI) is a somaclonal variant of the Giant Cavendish "Pei Chiao" with less susceptibility to Foc TR4. This clone was registered as "Formosana" in 2002. Initial field results showed an incidence of the disease of 4% (vs 25-29% for "Pei Chiao"). It was made available to the industry through Bioversity International and Banana Asia Pacific Network (BAPNET), through public-private agreements. Large companies sourced vegetative material and selected and reproduced their own material. The clone showed reinfection levels ranging between 5-20%. In some markets, complaints about the rounded fingertips are reported. Productivity of the clone is lower than traditional Cavendish.

TBRI has other clones, some of them resistant but with poor phenotypic characteristics and low productivity. The GAL C4 clone of *Rahan meristem*, is in semi-commercial process, and can be considered as another option.

For containment purposes, although it seems ideal to implement eradication strategies (such as performed in La Guajira), it is not productively sustainable. Inoculating soils with *Trichoderma spp.* and other microorganisms (eg, *Bacillus spp.*) can support the prevention and reduction of inoculum.

2:57:35 Key elements for the introduction of promising Musaceae plant materials

Dr Monica Betancourt presented AGROSAVIA's efforts in the search for promising resistant banana materials, in conjunction with AUGURA and ASBAMA. The researcher mentioned the focus on materials that present similarities to the Cavendish type. Enhanced clones with levels of resistance to Foc TR4 and/or R1 under study include GCTCv 218/Formosana and C4 (Taiwan), Gal (Israel), CJ19 and Transgenic (Australia), GCTCV119 (Taiwan-Australia), BRS Princesa and others (Brazil), FHIA 25 (Honduras), Ruby and CIRAD 924, 931 and 938 (France).

Colombia is considering the introduction, in phases, of materials under evaluation in other countries, through alliances with Brazil (Embrapa-AUGURA), France (CIRAD-Australia), Israel (Rahan Meristem-ASBAMA) and Honduras (Formosa-Dole). The risk of exchanging propagation material between countries is related to the presence of pathogens (fungi, bacteria, viruses, and nematodes) in countries of origin and their absence in countries of destination.

In the case of Colombia, the importation of materials is only authorized in-vitro conditions. However, since the use of the apical meristem does not guarantee the absence of viruses, eradication procedures such as thermotherapy and chemotherapy must be applied to both in-vitro and in vivo plants. The effectiveness of these procedures is verified through diagnostic techniques.

Dr Betancourt mentioned that AGROSAVIA and ICA have generated a Guide to importing plantain and banana germplasm and propagation material into Colombia and fourteen protocols for the qualification of quarantine (covering topics such as contingency, biosecurity, wastewater, handling, packaging, diseases, quarantine pests, tools and materials, infrastructure, substrates, weeds, contingencies, samples, footwear and foot baths).

According to the researcher, after Foc TR4, the greatest risk for the banana sector in the region is the entry of the Banana bunchy top virus (BBTV). There is also a significant risk related to Banana streak virus (BSV) species. Dr Betancourt mentioned that countries must generate protocols that prevent the entry of pathogens into their borders and guarantee quality assurance schemes for their seedlings and concluded presenting the main components of the FONTAGRO project "Regional Strategy for Capacity Building and Research in prevention, containment and management of tropical Fusarium race 4 "[1].

3:17:27 Official diagnostic platform for the introduction of Musaceae plant material in Colombia

Dr Manuel Rodelo indicated that ICA's mission is agricultural health and food safety. The institute has a plant quarantine station and a National Laboratory for Phytosanitary Diagnosis (LNDF) with a platform for Musaceae developed within the framework of the mitigation and containment project of Foc TR4 in Colombia covering 8 viruses, 1 fungus (Foc TR4) and 1 bacterial disease. The ICA uses this structure to

[1]Its components are: (i) diagnosis (standardize diagnostic methodologies in the region; evaluate molecular diagnostic methods); (ii) biosecurity and soil management (predisposing factors; epidemiological approaches; evaluation of disinfectants; use of microorganisms with emphasis on suppression); (iii) evaluation of materials (import and evaluation of materials under nursery and field conditions; evaluation of national bananas); and (iv) management and transfer (regional training workshops; virtual platform with specific courses).

perform analyzes during quarantine, prior to the development of field trials. In the Laboratory, biosecurity practices associated with the detection of Foc TR4 are implemented (including clothing for handling, single use supplies and proper waste disposal). The institute's procedures consider:

- For the analysis of viruses with a DNA or RNA genome: sample preparation; DNA extraction (+ amplification) or RNA extraction (+ reverse transcription); detection by screening, confirmatory analysis of other regions of the genome and sending samples to other NPPOs and specialized laboratories.
- Foc TR4: DNA extraction from symptomatic and asymptomatic plant tissue; fungal isolation and monitoring of mycelial growth, in which case DNA is extracted from the mycelium and detection is carried out (PCR, confirmatory test). Three biosecurity phases are considered: sample transit; containment of the pathogen in the laboratory; and detection process.
- *Ralstonia solanacearum* Race 2 bacteria: tissue preparation, disinfection, and maceration; sowing in growing medium; purification; colony extraction, cooking and centrifugation. Duplex PCR amplification is performed for different strains (pathogenic, non-pathogenic).

3:35:18 The reference procedure proposed by OIRSA for the introduction of Musaceae plant materials in its member countries

The M.Sc. Xavier Euceda mentioned that the determining factors for the emergence of pests in plant populations are mainly related to introductions (anthropic factor), climatic conditions, cultivation techniques and changes in vector populations. He highlighted the main transmissible pathogens in Musaceae propagation material as: Banana bunchy top virus (BBTV); Banana streak caused by the Banana streak virus species complex; Banana mosaic by Cucumber mosaic virus (CMV); Bract mosaic caused by Banana bract mosaic virus (BBrMV); Bacterial wilt by *Xanthomonas vasicola pv. Musacearum* (Xvm); *Fusarium oxysporum* sp. cubense Tropical race 4; Soft rots caused by *Dickeya paradisiaca*; Soft rot of the rhizome caused by *Pectrobacterium carotovorum* s. sp. Carotovorum; Banana wilt associated with phytoplasma wilt (BWAP), and Elephantiasis of banana and plantain associated with *Candidatus Phytoplasma*.

He indicated that for the exclusion of quarantine and endemic pests linked to the movement of germplasm, it is important that the plant materials is obtained from safe sources, that it passes through intermediate quarantine, that a sampling and analysis is made to detect the presence of viruses, bacteria and other pathogens, that the risk of pest occurrence is analyzed, the phytosanitary measures for shipment (certificate) are ensured and in place, and that the material introduced to the country is monitored (surveillance and diagnosis).

Mr Euceda acknowledged that the citrus, cocoa and avocado cultures are among the few examples in the region that have healthy plant production systems (free of important pathologies), with comprehensive and rigorous processes, including import stages and regulations, production, distribution, traceability, certification and surveillance. He concluded by mentioning that the confrontation of phytosanitary threats during the exchange of genetic material ensures its genetic and phytosanitary quality, contributing to the productive base, food security and the economy of the countries.

4:10:21 Closing remarks.

To discover the benefits of becoming a member of the World Banana Forum and to take an active role towards a sustainable banana sector, please visit:

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