Food and Agriculture Organization of the United Nations

WORLD BANANA FORUM

World Banana Forum (WBF)
FAO Mesoamerica

Working together for sustainable banana production and trade

WEBINAR

TR4-Resistant Banana Varieties: From Selection to Market Demand

Report of the TR4 Global Network

19 and 20 January 2022

Moderator:

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

Opening Remarks:

Pascal Liu, Senior Economist and Team Leader of the Responsible Global Value Chains Team, Food and Agriculture Organization of the United Nations (FAO)

Raixa Llauger, Agricultural Officer (Tropical Fruits), Food and Agriculture Organization of the United Nations (FAO/SLM)

Panellists:

Adolfo Martínez, Director General, Honduran Foundation for Agricultural Research (FHIA)

Agustin Gus Molina, Banana R&D Consultant, Technical Advisory, Department of Agriculture, Philippines

Altus Viljoen, Plant Pathology Professor, Stellenbosch University

Anker Sørensen, Vice President New Business, Keygene & Director, Yelloway

Eli Khayat, Scientific Director, Rahan Meristem

Frédéric Bakry, Researcher, AGAP Institute, Genetic Improvement and Adaptation of Mediterranean and Tropical Plants, French Agricultural Research Centre for International Development (CIRAD)

Gert HJ Kema, Chair at the Laboratory of Phytopathology, Wageningen University and Research

James Dale, Banana Research Program Leader in the Centre for Tropical Crops and Biocommodities, Queensland University of Technology

Juhua Liu, Research Professor, Chinese Academy of Tropical Agricultural Sciences (CATAS)

Leena Tripathi, Director of the Eastern Africa Hub and Leader of the Biotechnology Program, International Institute of Tropical Agriculture (IITA)

Miguel Angel Dita Rodriguez, Senior Scientist, Plant Health for Sustainable Banana Production, The Alliance of Bioversity International and CIAT

Shouxing Wei, Research Professor, Chinese Academy of Tropical Agricultural Sciences (CATAS); Head of the Banana Germplasm Resource Nursery in Hainan Province, People's Republic of China

Summary

The webinar, co-organized by the World Banana Forum (WBF) and FAO Mesoamerica, aimed to provide an overview of banana varieties tolerant or resistant to TR4, their agronomic performance, and the challenges related to their introduction and acceptance.

Tackling TR4 and other important banana diseases in an efficient way is critical to achieving the Sustainable Development Goals (SDGs). Increasing awareness and understanding of aspects related to the use of resistant varieties, the implementation of integrated diseases management practices, and the obstacles and opportunities for successful introduction of new varieties is key to addressing those challenges.

The first day of the event focused on providing an overview on the international cooperation on breeding as well as on the experience with the introduction of the Formosana somaclone (GCTCV-218) in Asia and Africa. Panelists also presented on the breeding initiatives carried out by the Honduran Foundation for Agricultural Research (FHIA) and the French Agricultural Research Centre for International Development (CIRAD). The second day focused on provide an overview on key experiences related to the development of TR4-resistant varieties and its application in South China as well as breeding initiatives carried out by the Queensland University of Technology, The International Institute of Tropical Agriculture and Rahan Meristem. The webinar also discussed the obstacles and opportunities for banana cultivar diversification and its successful introduction.

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

Day 01

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 Pascal Liu**, Responsible Global Value Chains Team Leader, **FAO**, welcomed participants to the webinar on TR4-resistant banana varieties, organized by the WBF and FAO Subregional Office of Mesoamerica.

The WBF is a multi-stakeholder platform created in 2009 that includes all stakeholder groups of the global banana sector, including producer organizations, importers, exporters, retailers, governments, research institutes, development agencies, trade unions, consumer associations, environment associations and other civil society organizations. The Forum address the three pillars of sustainability – social, economic and environmental – through three working groups and other dedicated task forces.

Fusarium Wilt caused by Foc TR4 is a great concern for the WBF and its members. The disease has been spreading over decades from East and Southeast Asia to Middle East and Southern Africa, and has most recently reached the

Americas, which has led to increasing concerns for banana producers and exporters. In response to the spread of the disease, the WBF created a Task Force on TR4 in 2013, which is formed by experts from different institutions and constituencies. To strengthen efforts and provide a neutral platform for inclusive and open collaboration around the world regarding the disease, FAO and the WBF further established the TR4 Global Network in 2020.

Mr Liu mentioned that due to the behavior of the disease and the heavily strict and costly biosecurity measures and protocols required to prevent and contain its introduction, disease management poses a great challenge, especially for small producers that lack financial resources and organizational capacity. In this context, Mr Liu concluded that the safe implementation of resistant varieties seems to be one of the most sustainable solutions to tackle the disease worldwide.

Ms Raixa Llauger, Agricultural Officer (Tropical Fruits), **FAO**, also welcomed participants to the webinar. She states that, Fusarium wilt of Musaceae tropical race 4 is the most challenging disease faced by the banana industry in the Latin America and Caribbean region. Nevertheless, its occurrence brings opportunities for the development and implementation of innovations for biosecurity, integrated management, and development of alternatives.

It is crucial to strengthen the institutions responsible for pest management and ensure the involvement of government, private sector, and general public in the fight against the disease. Hundreds of thousands of families depend on the plantain and banana agroindustry worldwide. Since the confirmation of the outbreak in Colombia and Peru, the probability of introduction in other areas has increased. FAO is working with governments and National Plant Protection Organizations at regional and global levels to strengthen the prevention, preparedness, response, and recuperation regarding Foc TR4. FAO is supporting small farmers to create solutions to make agricultural systems more resilient, inclusive, and sustainable. The organization also facilitates the exchange of experiences regarding the safe and traceable introduction of indexed germplasm materials with tolerance of partial resistance to TR4 between governments and private sector.

The two-day event aims to facilitate discussions and presentations from international experts worldwide concerning the genetic improvement of Musaceae, research agendas and promissory germplasm options for TR4-resistance. FAO is strengthening its commitment to support the sector by promoting a scientific agenda of innovation to tackle the challenges imposed by the presence of Foc TR4, thereby fulfilling the needs of all actors of the Musaceae value chain and supporting the achievement of the sustainable development goals.

2. International Cooperation on Breeding

On behalf of the Accelerated Breeding Better Bananas (ABBB) project consortium, **Mr Gert Kema, Wageningen University and Research (WUR),** briefly presented the ABBB project, which focuses on the improvement of banana for smallholder farmers in the Great Lakes Region of Africa. The ongoing project, funded by the Bill and Melinda Gates Foundation and coordinated by the International Institute for Tropical Agriculture, revolves around the breeding of highland bananas, one of the main staple foods in East Africa.

Mr Kema stated that the classical breeding of bananas and plantains in the region, including of *Mattoke* and *Mchaare* plantains, is extremely important to guarantee not only high yields, but high quality. Advantages of using modern breeding technologies include the possibility of improving different characteristics simultaneously such as yield, quality and disease resistance (not only to Fusarium Wilt but also black sigatoka (*Pseudocercospora fijiensis*)). The ABBB consortium involves a large number of partners, including Wageningen University and KeyGene. Both partners recently joined the consortium to support the work under the Pre-Breeding programme, more specifically to work towards effector-based screening of banana germplasm. The switch of physical strain-based screening of Fusarium wilt to effector-based screening can substantially increase the throughput. This eventually will come down to mapping genes, and after identifying the genes and having the markers, the phenotyping process can also be avoided. KeyGene is working on mapping the populations previous screened by WUR and on the identification of molecular markers.

Mr Anker Sørensen, KeyGene & Yelloway, presented the initiatives carried out by Yelloway, a company entirely focused on the development of novel varieties for the export-oriented banana markets. There is a growing need to increase banana genetic diversity in the global market, as the use of the same clone or variety is not sustainable and the occurrence of major diseases faced by the industry is proof of that. Yelloway is formed by a consortium between KeyGene, Chiquita and MusaRadix, and the current focus is on the development of banana cultivars resistant to Black Sigatoka and Fusarium wilt. The Yelloway breeding program is based on knowledge-based breeding – through analysis of the existing gene banks of banana germplasm – to identify resistance genes and develop crossing schemes that produce elite banana varieties with excellent agronomic performance and resistance to important diseases.

He mentioned that the development of segregated populations in bananas is quite challenging. First, it is necessary to cross populations and then multiply each of the progenies in vitro to have enough plants to perform screening. Once the segregated plants are multiplied, they are exposed to the fungus in a controlled way and, depending on the resistance or susceptibility of the germplasm, the genotypes are analyzed and the locations on which the genotype corresponds to the phenotype are identified for the development of molecular markers to be used in crossing and segregation schemes. The company is also working on transcriptomics to identify the genes expressed on the plants when exposed to the fungus to narrow down the genes responsible for the resistance reaction. In order to conduct the experiments, tropical greenhouses were established in the Netherlands and a field trial location is being established in the Philippines.

3. The Formosana Experience in Asia

Mr Agustin Molina, technical advisor of the **Department of Agriculture, Philippines,** and former regional coordinator of Bioversity International for Asia and the Pacific, presented the lessons and results from the introduction and establishment of the Formosana somaclone (GCTCV 218) developed by the TBRI in Foc TR4-affected areas in the Philippines.

The presence of the disease was confirmed in the country in 2006 by Stellenbosch University and, in 2011, it spread to thousands of hectares with higher severity in small, independent farms. The use of resistant varieties was identified as the best strategy for disease management. However, initial trials with high-yield resistant varieties derived from conventional breeding programmes failed on the consumer acceptance, increasing the need for a new variety.

Mr Molina informed the audience that Bioversity-Asia had acquired Giant Cavendish' tissue-culture variants (GCTCVs) selected by TBRI in the framework of regional collaboration for testing the somaclones in Asia and particularly the Philippines where the problems were more serious. The farmer-participatory selection process carried out by TBRI selected plant survivors from Foc TR4 hotspot fields, which were them multiplied and planted again for field screening of Foc TR4 on infested soils. Selected lines were subjected to field verification for resistance, yield, and agronomic traits. The studies of comparison of GCTCV 119 with local cultivars (from 2006 to 2009) showed that some local varieties, such as *Carba(Saba)* (BBB) and *Kluai Namwa* (AAB), had good resistance against TR4 similar to that of GCTCV 119. However, the agronomic traits of the GCTCV showed to be unsuitable for commercialization.

The advisor mentioned that follow-up trials were conducted from 2012 to 2015 with the 218 (Formosana) and 219 GCTCVs. These studies showed the high resistance of 219 and good tolerance of the 218 somaclone. The GCTCV 218 showed good potential to be implemented commercially due to its productivity and fruit quality, and semi-commercial trials with good results were also conducted by companies such as Lapanday, Mauro, Dole and TADECO.

In 2014, due to the successful implementation of the somaclone, commercial companies started the mass production of GCTCV 218 for their rehabilitation-expansion programs in the Philippines, to cover almost the totality of rehabilitation areas in the country.

The somaclone is now being produced and selected by different companies, and has further received different names such as LFC-75, TDC-7, SF-100, UCL4, etc. It is important to highlight that when GCTCV 218 is not properly selected and managed, high phenotype variation occurs. This is observed mainly in small producer areas.

According to **Mr Molina**, TBRI is continuously improving its GCTCVs derived from Pei-Chiao. One example is the GCTCV-218-3 or TC No.8 derived from Formosana, launched in 2017, which has superior agronomic traits such as earlier harvest, better bunch configuration, and less susceptibility to corky scab, amongst other factors. It is important to highlight that the success story of GCTCV 218 was the result of a long-term partnership among industry actors and research institutes from different countries and regions.

4. Performance of Formosana in Southern Africa

Mr Altus Viljoen, Stellenbosch University, shared the experience of the implementation and trials of Formosana in Mozambique, in partnership with Jacaranda farm. Cooking bananas are the most important type of banana cultivated in the African continent. Cavendish accounts for only around 9% of the bananas produced in Africa, with most of the plantations focused on exports and located in the Southern region, Eastern coastline and West Africa.

According to the scientist, there are several strategies to improve banana resistance to TR4, which can be divided as short-, medium- and long-term strategies. The use of somaclones such as Formosana Cavendish-type is a short-term strategy based on mutation breeding, which confers partial resistance to the disease. The advantage of this method is the short time required and the absence of the need to identify resistance genes.

Mr Viljoen mentioned that the trials with Formosana started in Africa almost 20 years ago, where a previous version of GCTCV-218 was tested for resistance to the Subtropical race 4 of Fusarium wilt. Since then, the somaclone has evolved and has been planted in other countries. After the outbreak of the Tropical Race 4 in Mozambique, GCTCVs trials for Foc TR4 disease resistance were conducted in Matanuska Farm (a heavily infested site) and agronomic trails for the same somaclones were carried out in Jacaranda farm (a non-contaminated site).

The study analyzed different somaclones managed according to existing production systems in a randomized complete block design. The GCTCVs (106, 119, 218, 247) and the DPM-25 (Dwaf Parfit) were sent by TBRI and multiplied in vitro by *Du Rois* to ensure disease free-materials. The local *Nandi* cavendish variety was used as the susceptible control.

The disease development was measured over two crop cycles. The evaluation showed that the GCTCV 119 performed better against the disease followed by Formosana (GCTCV 218). Agronomic traits related to crop cycle, plant morphology and productivity were also evaluated. The *Nandi* cultivar showed a faster growth and shorter cycle compared to the somaclones. However, GCTCV 218 presented a slightly higher bunch weight.

The researcher informed the audience that at the former Matanuska farm, 750 ha have been replanted with Formosana and a further 250 ha in the Lurio river area were replanted with the same somaclone. The losses due to Fusarium Wilt ranged from 5-20% and the meristems from TBRI performed better than suckers. The national and international markets accepted Formosana very well and the continuous reselection is taking place.

The number of plants affected per hectare in Matanuska was reduced in the second and third years. He then concluded stating that the staff of the farm is convinced that this is due to the integrated management of TR4 (soil treatment with compost and effective microbes), which indicated that Formosana is not a stand-alone solution and should be considered as a part of an integrated management system.

5. Genetic Improvement of Bananas by CIRAD

Mr Frederic Bakry researcher of the **AGAP Institute**, **French Agricultural Research Centre for International Development (CIRAD),** presented the efforts on genetic improvement of bananas carried out by CIRAD**.** Many

challenges surround banana production systems worldwide such as environmental constraints (including present and emerging pests and diseases and climate change), supply chain expectations (of high productivity and high income) and consumer demand (for fruit quality, organic production, and low prices).

According to **Mr Bakry**, The objectives of banana breeding are to introduce genetic diversity into the cropping systems through new dessert varieties and cooking types derived from crossbreeding. The objectives for dessert bananas for export (AAA/mono) and domestic markets (AAB/interspecific genomic constitution) are varieties with disease resistance, fruit quality and productivity. For cooking bananas there is a need for robustness, drought tolerance, pest and disease resistance, fruit quality and cooking ability.

The banana genetic improvements by CIRAD have a holistic approach, including scientific and development activities. On the upstream research, CIRAD investigates genomics and genetics considering the reproductive barriers and seed sets. CIRAD carries out activities of breeding and selection of progenies, scaling up and developing adapted production systems, and fine tuning their findings with retailers and buyers, considering post-harvest traits and market adaptation.

For breeding bananas with success, it is required to acquire knowledge in various aspects of the banana species complex considering its diversity, organization, and domestication in order to optimize breeding strategies and parent choices. It is also required to integrate the knowledge of the genome organization and dynamics considering structural variations, mosaic genomic structure and their impact on recombination and chromosome distribution taking into account the genetic basis, transmission traits and the difficulty to produce segregated population on cultivated varieties.

Mr Bakry stated that cultivated bananas nowadays are hybrid clones derived from a multitude of wild ancestors, involving in many cases several generations of meiotic recombination. It is important to identify the contributions of wild subspecies to the genome architecture of hybrid parthenocarpic accessions and to understand the hybridization processes leading to the different cultivar groups to reproduce these processes in reconstructive breeding.

The efforts of CIRAD on breeding for resistance to Fusarium Race 1 and TR4 started with the screening of genitors under controlled conditions. An access from Indonesia (IDN 110) was used as a diploid or double-diploid parent in numerous dessert hybrids from CIRAD due to its resistance to nematodes, yellow sigatoka, black leaf streak disease, Foc Race 1 and TR4. This access is being used in a reconstructive breeding scheme developed by CIRAD.

The researcher mentioned that CIRAD has been conducting early screening of selected hybrids for Fusarium wilt in partnership with WUR, and the studies have shown that the interspecific hybrids AAA developed by CIRAD have different levels of resistance for Fusarium R1 and Fusarium TR4, being highlighted the CIRAD931 (R1: HR[1] – TR4: HR), CIRAD938 (R1: R – TR4: R), PRAM01 (R1: HR – TR4:HR), while the AAB X17 (R1: HR – TR4: HR). Studies conducted in the field in Australia also showed a high resistance for the CIRAD924 variety. He them concluded by saying that CIRAD has developed a partnership network to evaluate several aspects of its hybrids such as pest and disease resistance, agronomic performance, different production systems and acceptability. The Centre is partnering with WUR and Stellenbosch University for TR4 early screening and have ongoing field trials in Martinique, Guadeloupe, Australia and La Réunion, with preparation sites in Jamaica and Colombia. CIRAD has also launched the collaborative World Musa Alliance initiative focused on the development of resistant dessert banana varieties.

6. Breeding activities of FHIA in Musaceae

Mr Adolfo Martinez, Director General of the **Honduran Foundation for Agricultural Research (FHIA)**, Honduras, presented the Musaceae breeding activities carried out by the Foundation. The Honduran breeding programme started in 1959 with a focus on the development of Gros Michel hybrids with resistance to Foc Race 1. The programme, led by Chiquita, brought to Honduras a collection from Southeast Asia with more than 1,000 varieties to

[1] HR: High resistance: R: Resistance

study its genetics and to develop diploids to use in the breeding programme of Gros Michel. During this process, Cavendish was rediscovered and adopted broadly due to its resistance to Fusarium TR1. However, due to its high susceptibility for Black Sigatoka, the programme changed its focus to develop enhanced Cavendish hybrids with resistance to the disease. This process ended in 1984, when Chiquita decided to stop its breeding activities in Honduras and ultimately donated the programme to what is now the Honduran Foundation for Agricultural Research (FHIA), a non-profit private organization funded by private companies.

The Director General mentioned that for almost 20 years, international donors funded the FHIA programme with a focus on bananas and plantains for the domestic market. In 2003, FHIA started a new programme with Chiquita for the development of special bananas beyond Cavendish. Eight hybrids were developed under this programme and two patents derived from the project were registered by Chiquita. These varieties produced small bananas with resistance to FoC 1 and Black Sigatoka and good flavour that proved to have high acceptability in the international markets.

In 2016, FHIA started a consortium with Agroamerica (Guatemala), Dole PLCand Mackays Marketing (Australia) for the development of TR4-resistant bananas. The first step of the programme was the development of improved diploids (2n) to be crossed with Cavendish tetraploids mothers (4N) in order to obtain hybrids (3n). These hybrids are being evaluated for agronomic performance in experimental trails and sent to the companies of the consortium for field trials, commercialization evaluation and patent development.

He stated that FHIA tetraploid and diploid materials are currently being evaluated for TR4-resistance. Initial results of the studies show that the hybrid development by FHIA (such as FHIA-01, FHIA-02, FHIA-03, FHIA-18 and FHIA-25) have resistance for Black Sigatoka, TR1 and TR4.

The future of the programme will continue to be based on crossings diploids with tetraploid mother plants. So far, the majority of the 38 hybrids developed by FHIA have shown resistance to Black Sigatoka, minimizing the need for spraying and therefore reducing the costs of production. The hybrids are also suitable for organic production.

Mr Martinez highlighted that 16 hybrids were evaluated for TR4-resistance and 6 were considered highly resistant while 2 were considered resistant. Additional studies are being conducted in South Africa and FHIA hybrids are already being planted in commercial farms in Australia.

He concluded by stating that FHIA has the capacity to develop Musaceae from different tetraploid mothers. Some of the possibilities from the banana and plantain breeding programme of the institution include the: 1) development of a new Cavendish variety; 2) development of a new Gros Michel variety, 3) development of datil banana, 4) development of banana *manzano* type, 5) development of plantains; 6) development of cooking bananas and 7) development of plantains with high level of β-carotene.

7. Closing remarks of Day 1

Ms Raixa Llauger, FAO Mesoamerica, presented a summary of the discussions held on the first day of the event and provided the closing remarks. According to the Agricultural Officer, the session had high value due to the scientific results presented and topics of high relevance related to the improvement of technologies for the development of banana resistant varieties, including resistance against Fusarium wilt TR4. She concluded mentioning that the event highlighted various experiences on the use of promising germplasm materials in different countries and contexts, considering its adaptations, agronomic traits, fruit quality, selection process and inclusion in integrated disease management programmes.

Day 02

8. Breeding TR4-resistant cultivars and its application in South China

Ms Juhua Liu, with contributions from **Mr Shouxing Wei,** presented on behalf of the **Chinese Academy of Agricultural Sciences (CATAS)** and the **Chinese Agriculture Research System (CARS)** information about the Chinese TR4-resistant cultivars and their use in southern China.

To tackle Fusarium wilt TR4 in China, the government has created the Chinese Banana Research System that is responsible for the promotion and implementation of resistant cultivars and production systems. The system has 17 leading scientists responsible for germplasm collection and evaluation, breeding techniques, variety improvement and development of integrated disease management systems. It further comprises 9 integrated testing stations that are scattered around the main banana production regions.

The breeding and selection of TR4-resistant cultivars in the country comprises different techniques and the main resistant cultivars implemented in China are the Zhongre No.1 (selected from radiation mutagenesis), the Baodao, Reke No.2, the Nantianhuang (selected from natural mutations) and the Fenza No.1 (bred by hybridization).

Natural mutations are the main source of variation for obtaining new varieties. The essence of natural mutations resides on somatic mutation. However, the main shortcomings are the time needed and the low efficiency. After 12 years of research, CATAS developed a new technique for radiation mutagenesis and directional breeding, improving the efficiency of somatic mutations in a 4% increase of beneficial mutation rate.

The process described by the scientists consists of the regeneration of immature male flowers of Cavendish in embryonic calli and adventitious shoots that are irradiated with Cobalt 60. The recovered culture is then infected with Foc TR4 crude toxin and directly screened. The infection is made through a liquid culture device designed for tissue culture of plantlets and the directed screening is done by using the TR4-GFP maker ensuring homogeneity, controllability, and high efficiency during the process.

Ms Liu mentioned that for the development of Zhongre No.1, a total of 44 beneficial mutations were planted and screened in field trials in locations with high incidence of TR4 and compared with the *Baxi* Cavendish cultivar as control. Zhongre No.1 had a very low rate of disease (4%) compared with Baxi (93.30%) and high fruit quality with similar nutrient composition as Baxi. In 2021, the Ministry of Agriculture and Rural Affairs of the People's Republic of China provided the official cultivar authorization following the positive results of the field trials.

She then stated that the resistant cultivars are not a stand-alone solution and must be part of an integrated management system. CATAS has developed a five-in-one integrated control system that comprises: soil pathogen detection (indication of cultivar production system according to the spore concentration on soil), soil regulation (pH regulation, soil disinfection and increase on organic matter), resistant cultivars, bio-fertilizers (inoculation of *Lactococus, Bacillus, Strptomyces* and *Pseudomonas* and the elimination or use of minimum tillage (to avoid cross infection and root damage).

Ms Liu concluded highlighting that the integrated management system was successfully implemented in the Guangdong, Guanxi, Yunnan and Hainan provinces in southern China, where 213 training courses were conducted that benefitted 14 199 enterprises and/or farmers. As a result, the resistant cultivars were implemented in 30 000 ha with a disease rate below 10%.

9. Genetically modified and gene edited Cavendish with TR4 resistance

Mr James Dale, Queensland University of Technology (QUT), presented the efforts of QUT with regards to the development of Cavendish resistant varieties through gene modification and editing. The reality of the future of

banana varieties for export will be either a conventionally bred new banana cultivar different to Cavendish but with disease resistance, high yields, transportability and excellent taste and texture, or a genetically modified or gene edited Cavendish with multiple disease resistances.

The researcher mentioned that QUT's Cavendish TR4-resistance programme has identified the RGA2 resistance gene[2] from wild banana (*Musa acuminata ssp malaccensis*) and transferred it using genetic modification (through agrobacterium) into Cavendish Grand Nain. The modified variety has been tested in small-scale field trials at the Darwin Fruit Farm field trial site. The trial site, which has been severely affected by TR4 for over 10 years, is adjacent to commercial plantations. At the site, a complete history of every plant is collected and all infections are confirmed by PCR (and sequencing, in many instances). The results of the first phase of the trial — after 3 years and 5 production cycles — showed that the RGA2-2, RGA2-3, RGA2-4 and RGA2-5 lines had high resistance to the disease when compared to the controls and the RGA-2-3 performed better with no infections observed.

He stated that the second phase of the programme focused on large scale field trials over five years in the Northern Territory designed to *mimic* a commercial plantation. The trials were conducted on randomised block design and evaluated the four original Grand Nain RGA2 lines. The results were almost identical to Phase 1 and data regarding yield, cycle time and history of each plant was collected. The results after 3 years of planting indicated that the lines with RGA2 resistance gene QCav-4 and QCav-3 had the lowest rate of infection (0% and 6% respectively) and the Williams and Grand Nain non-GM controls (68% and 58% respectively). After six crop cycles, the QCav-4: had only two harvests out of 300 which were lost due to TR4 infection; its bunch weight was equivalent; and, the cycle time was shorter than the Grand Nain control. The QCav-4 was transformed with a single banana gene, RGA2, demonstrating that Cavendish bananas with high immunity against Foc TR4 are a reality with no impact on yields.

According to the researcher, the genetic modification (transgenics, cisgenics) consists in the addition of new DNA into a plant through transformation that almost always include "new or foreign" DNA. Gene editing (non-GM) is a precise, targeted modification of a plant's DNA, including deletions, substitutions and the addition of entire genes. For non-GM editing, there must be no "foreign" DNA added.

QUT has developed an efficient non-GM gene editing system for Cavendish banana using CRISPR/Cas9 which was able to produce stably transformed transgenic lines with very high proportion of lines contained an edited sequence. The next stage of the research is focusing on the development of a platform technology for regenerating Cavendish bananas that have been edited. The non-GM editing platform for Cavendish bananas consists in four steps: (1) editing and plant regeneration; (2) confirmation of edits by Sanger sequencing; (3) PCR confirmation for the absence of integrated DNA; and (4) whole genome sequencing and mapping.

The next phase of the programme has identified a panel of 24 candidate genes to be edited for TR4 resistance. It is also currently editing Cavendish cells with up-regulating and down-regulating identified genes, regenerating the cells as plants and screening it. **Mr Dale** then concluded by stating that in mid- to late 2022, the promising non-GM edited lines will be evaluated at field trials.

10. CRISPR/CAS9 Technique in the Development of TR4-Resistant Bananas

Ms Leena Tripathi, International Institute of Tropical Agriculture, provided an overview of the CRISPR/CAS9 technique in the development of TR4-resistant bananas. Genome editing is a technique used to precisely and efficiently make specific changes to the DNA of a cell or organism. An enzyme cuts the DNA at a specific sequence, and when this is repaired by the cell, a change or 'edit' is made to the sequence. Genome-editing can be used to add, remove, or alter the DNA of the genome.

Ms Tripathi mentioned that not all types of gene editing (GE) can be considered as non-GM. For this reason, is important to understand the type of editing to comply with the regulations that differs across countries. After the

[2] The gene RGA2 occurs naturally in virtually all domesticated bananas

break of the double-stranded DNA by the site-directed nucleases (SDN), there are two possible pathways: the non-homologous end joining (NHEJ) — a pathway that repairs double-strand breaks in DNA that can create some mutation —, or the Homology Directed Repair (HDR) where a gene insertion can happen.

The NHEJ can target random mutations that allow gene silencing, gene knockout or a change in the activity of a gene (SDN1). The HR pathway on is turn can allow the introduction of the mutation(s) at the target site (SDN2) or the introduction of a gene or genetic elements at the target site (SDN3), which can be considered GM plants.

The IITA uses different crop improvement tools for bananas. The researcher mentioned that the institute has a strong conventional breeding programme that has the advantage of being low cost and without regulatory issues, the seatbacks of the conventional techniques are the long time for develop a cultivar (10 to 15 years) and the impossibility of adding a trait that is not present in the germplasm. The genetic modification allows the introduction of a trait that is not present in the genome, requires a medium time for development of cultivars, has a high cost and is subject to safety regulations. Genome editing also allows the development of a new trait and requires short time for development, low-to-medium cost, and no regulatory issues. However, the regulations depend upon on country polices.

IITA applies genetic modification for the development of cultivars with resistance to Banana Xanthomonas Wilt, nematodes and control of banana bunchy top virus. The institute uses gene editing techniques for the development of banana varieties with resistance to banana streak virus, Xanthomonas wilt, Fusarium wilt and black sigatoka.

Ms Tripathi informed the audience regarding the protocols for genome editing of various genomic groups of banana developed by IITA and about the field trials expected to take place within this year for the genome edited bananas with resistance to bacterial wilt in Kenya. She then concluded mentioning that IITA is developing a TR4-resistant variety by knocking down the candidate susceptible gene using CRISPR/Cas9 and integrating CRISPR/Cas9 based genome editing into the breeding programme to obtain durable resistance to TR4.

11. Induced Mutation Breeding for TR4-resistance

Mr Eli Khayat, Rahan Meristem, presented the GAL variety and the mutation breeding techniques used for TR4 resistance by Rahan Meristem. The varieties of Raham Meristem passed through different levels of validation to ensure its resistance against Foc TR4. First there was a molecular proof of induced mutagenesis, followed by ex vitro greenhouse inoculation experiments. The next stages of validation were the whole genome sequencing comparing the wild type with the GAL variety followed by field trials in three heavily infected sites in Philippines and a field trial in an infected plantation is expected to take place in La Guajira, Colombia in 2022**.**

According to **Mr Khayat**, Raham Meristem performs mutagenesis by activation of retro transposable elements that exist in the genome, the activation and amplification of these elements are achieved by the demethylation of the DNA. Raham Meristem created a library of mutants that shows that each individual plant is different to its siblings due to the different levels of amplifications and positions of the transposable elements in the cromossomes.

The scientist mentioned that mutations proved to not affect yield components (bunch weight, number of fingers and cycle time) and quality components (proper ripening, shelf life and brix value). The development of the new varieties started with the GAL clone where in-vitro mutagenesis for tissue culture was performed with the addition of TDC (a strong cytokinin) to activate cell division and mutations. In the final step of the tissue culture, the populations were divided in clusters and screened for inoculation and selection in the laboratory and for the whole genome analysis.

He informed the audience that around 10 000 mutated plants were screened for resistance against Fusarium wilt Race 1 and TR4 and around 4% of the plants showed to be asymptomatic after inoculation. Those asymptomatic clones where then selected and propagated by the Wageningen University and tested in the Philippines field trial with a high percentage of asymptomatic plants (> 96%).

During the selection process of Raham Meristem, to avoid tolerant plants, only the clones that had 0% of infection were multiplied. If one plant derived from the clone showed any symptoms, the whole clone was discarded from the programme. The next step of the selection process was to identify, select and multiply only the clones that were surrounded by plants that died due to TR4. He then concluded by mentioning that other mutants were selected, and all promising germplasm will be soon evaluated in field trials in la Guajira.

12. Fusarium wilt TR4: Challenges and opportunities for banana cultivar diversification

Mr Miguel Dita, The Alliance of Bioversity International and CIAT, presented the obstacles and opportunities for banana cultivar diversification. The main topics to be considered for cultivar diversification could be summarized in: cultivar development and opportunities beyond an improved Cavendish; intellectual property, including the negotiation process and access for small farmers; movement of germplasm and post-entry quarantine, facilities and diagnostic capacities; field evaluations in multi-sites applying reliable, comparable and biosecurity protocols; large-scale multiplication; and distributions considering certifications and seed systems.

According to the scientist, the first and main challenge for large-scale banana cultivar diversification through exporting channels is a globally Cavendish-oriented market. A series of aspects should be taken in consideration during the cultivar development such as tools, needs and regulations and the need for better understanding of the available varieties with tolerance of partial resistance for the disease. A diversified portfolio of Cavendish bananas is coming to the market. Nevertheless, there are opportunities to think outside of the box and promote different banana types to the exporting markets, ensuring that no farmer is left behind during the process. Regarding TR4 resistance, it is important to understand that most of the clones present quantitative resistance, which means that they are not immune and can get infected. Tailored training programs on how to deal with this type of resistance are therefore required, bearing in mind the risk for pathogen spreading by infected planting material.

Another challenge faced for cultivar diversification is related to the intellectual property. The initial process can be difficult due to language barriers, need for legal support and clarity regarding national and international genetic resources laws. The National Plant Protection Organizations capacities and agility to meet producers' needs and desires are also an important theme that should be taken into consideration for cultivar diversification. The access of the patented germplasm for small farmers should be discussed from the beginning.

Another important point, is related to the process of importing promising TR4-resistant cultivars. Five main aspects can be considered in Latin America and the Caribbean: the post entry quarantine and the institutional roles (of national research institution and the national plant protection organization); need for adequate national facilities for acclimatization and quarantine of plant materials; the national capacities for multi-pest diagnosis and plant indexing; the sampling methods for audits and quality control; and the protocols for cultivar release for field phenotyping.

Mr Dita mentioned that there are successful experiences for the safe movement of Musa germplasm, developed by Bioversity International and MusaNet that can be adapted. There is an international transit centre in Belgium where the materials are sent following biosecurity protocols. The Alliance of Bioversity International and CIAT are also planning to launch the *future seeds* facility in LAC focused on the safe movement and trade of germplasm.

For field evaluations of resistant cultivars, it is important to conduct multi-site trials in different climatic conditions and in TR4-infected and TR4-free sites. As mentioned previously, it is crucial to have reliable and comparable protocols for field trials ensuring that the "survivors" were in contact with the fungus, and that the biosecurity measures are in place (in countries where the affected areas are under official control).

In the past, the MusaNet had a global collaborative framework for Musa-related research aiming to ensure the long-term conservation and increased use of Musa diversity. This could provide lessons for potential collaborative regional programmes for the evaluation and distribution of TR4-resistant varieties. Regarding field trials, a guideline was

published last year focused on providing information and comparable protocols for field evaluation of Banana against Fusarium wilt, *Pseudocercospora* Leaf Spots, and drought.

After the development of a resistant cultivar there are challenges related to the large-scale multiplication and distribution. The tissue culture must be incentivized for the safe multiplication of plants. However it is not a guarantee*, per se,* of disease-free planting material. Other factors such as water, substrate and transport must be taken into consideration. For the informal exchange of plant materials, it is crucial to collect ratoon from areas without symptoms. **Mr Dita** concluded by mentioning that after the consolidation of a resistant cultivar adapted to the site and to the market, there are additional challenges regarding the production systems that must be considered.

13. Discussions

Mr Adolfo Martinez, **FHIA,** mentioned that most of the varieties presented during the webinar will be patented and most probably under royalty payments, which can make difficult their availability for small-scale farmers, unless governments and/or international entities decide to provide support. It appears that the only ones that will be available for the small-scale farmers are the ones presented by IITA. For FHIA, this will not be possible, and most probably the same occurs with Israel, France, and Australia, since it is needed to recover the investment costs for its development. The cost to develop a new variety in FHIA is around USD 3 million (considering their broad experience and the low cost of labour in Honduras).

Mr Miguel Dita mentioned that private consortiums are currently working on the development of resistant or improved varieties and underscored the opportunities for the development of public-private consortiums that could ensure the access of small-scale farmers to the improved materials. The banana cultivation demands labour force, and it is important for the economic development of many countries, so it is important to discuss these aspects including the legal aspect of intellectual property in a way to not leave no one behind. It is crucial to find win-win situations where the researchers and developers can profit out of their investments and including small-scale producers in their distribution strategies.

Mr Adolfo Martinez, **FHIA,** mentioned that regarding the FHIA consortium, the companies are allowed to multiply and use the materials in their plantations without restrictions, and in case they want to supply to third parties, they will charge a royalty's fee for box of banana produced – justified by the reduction of costs of production derived from the Sigatoka resistance, for covering the costs of the development of the improved hybrid and for reinvestment in order developments.

Mr Eli Khayat, Rahan Meristem, stated that, as any other technology it is important to protect the developer and breeder's rights, since improved varieties will benefit both, companies, and growers. For its TR4-resistant variety, Raham Meristem has utilities patents worldwide ensuring the protection of the breeding method.

Mr Anker Sørensen, Keygene, commented that the banana sector can learn from other agricultural sectors where breeders' rights have provided constant innovations and are the best guarantee of a sustainable deliverable of innovations for a diversified supply of bananas in the market.

Mr Frederic Bakry, CIRAD, highlighted the need of funding breeding activities, and the different arrangements that can be put in place. Due to the easy vegetative propagation of banana plants, it is very difficult to ensure the banana breeders' rights in many producing countries around the world. One possible scenario would be the establishment of a system of partnership — with many stakeholders from the industry and research institutes — to fund the development of resistant cultivars and once the materials are developed, the members of the partnership have priority on accessing it, having commercial advantage.

14.Closing remarks

Ms Raixa Llauger, Agricultural Officer (Tropical Fruits), **FAO** thanked all the panelists and attendees for the participation in the event, and underscored the continuous work carried out by FAO in partnership with the World Banana Forum for the strengthening of capacities regarding the disease. The two-days webinars covered topics under the priorities of the banana agroindustry including gene-editing technologies, and the avant-garde research and development of banana resistant varieties. It is important to highlight the importance of the safe introduction of germplasm protecting countries for the introduction of quarantine plant pests and diseases at global level and the importance of training and capacity building for producers regarding the production systems and management of partially resistant and/or tolerant varieties to TR4.

It is important to align the scientific agendas with the work carried out by different public and private institutions to develop a common strategy on the management of the disease. Opportunities were presented on the diversification of banana varieties beyond Cavendish and the access to improved varieties by all stakeholders. Institutions and organizations must strengthen their role on the safe movement and exchanges of germplasm following biosecurity protocols. For the countries that still do not have the presence of the disease it is crucial to continue all the efforts carried out so far regarding the prevention and the ones that already have it, must continue the efforts on the contention.

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World Banana Forum Secretariat
Trade and Markets Division

Food and Agriculture Organization of the United Nations
Viale delle Terme di Caracalla
00153 Rome, Italy

WBF@fao.org | www.fao.org/wbf | @FAOwbf