

**POLICY  
PAPER  
92**

# **Tropical Wilt Race-4 Affecting Banana Cultivation**



**NATIONAL ACADEMY OF AGRICULTURAL SCIENCES, NEW DELHI**

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## Preface

India is the largest producer of bananas (*Musa* spp.) in the world contributing 29.1% to the global production. The Cavendish clones occupy about 55% of the total area under banana cultivation and contribute 64% of the total banana production in India. It is available throughout the year and considered as a fruit loaded with nutrients. Tropical race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense*, the incitant of Fusarium Wilt of banana, is an emerging problem affecting banana industry worldwide. Recently, the disease has been reported from seventeen different countries including India and its rapid spread has raised international concern with regard to future food security in the tropics and sustainability of the international banana trade that is almost exclusively based on “Cavendish” clones. The pathogen has shown its presence on Cavendish varieties in Bihar and Uttar Pradesh. Considering serious threat of the disease to livelihood and sustainability of Indian banana farmers, the National Academy of Agricultural Sciences (NAAS) organized a Brainstorming Session (BSS) on “Tropical Wilt Race-4 Affecting Banana Cultivation” at NASC, New Delhi on September 25, 2019 with Dr Rashmi Aggarwal (Convener), Dr Rakesh Kumar Jain and Dr S. Uma (Co-Conveners). The BSS was attended by eminent scientists and experts in the relevant disciplines. The deliberations were enriched by in-depth interactions focusing on this disease.

On behalf of the Academy, I compliment the Resource Persons *viz.*, Dr Rashmi Aggarwal, Dr Rakesh Kumar Jain and Dr S. Uma and other eminent experts for their valuable inputs in developing the Policy Paper. My thanks are also due to Drs V.K. Bhatia and Kusumakar Sharma for their editorial support. I am hopeful that this document will be useful to all Fellowship, policy makers and other stakeholders.



**(Panjab Singh)**

President



# Tropical Wilt Race-4 Affecting Banana Cultivation

## INTRODUCTION

### What is TR4?

The global banana industry is under serious threat of Fusarium Wilt (FW), a soil-borne fungal disease, caused by Tropical Race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (Foc) pathogenic to Cavendish clones. Its recent rapid spread has raised international concerns with regard to food security in the tropics and sustainability of the international banana trade that is almost exclusively based on Cavendish clones (FAO, 2018). Currently, Cavendish clones contribute upto 64% of the global banana production occupying 55% of the total global area under its cultivation (Ploetz *et. al.*, 2015). In India also, Cavendish (AAA) group cultivars, Grand Naine and Robusta, are the most traded banana, which are produced on 52% of the total area under banana cultivation, and contribute upto 64% of all bananas produced. In India, the disease was first recorded in 1911 from West Bengal. The race 1 and race 2 are widespread, causing incidence of 30% in the planted crop and up to 85% in the ratoon crop. Other banana cultivars such as 'Rasthali' (syn. 'Malbhog', 'Nanjangod Rasabale', 'Amrithapani', Martaman', AAB, Silk), 'Karpuravalli' (syn. 'Kanthali', ABB, Pisang Awak), 'Monthan' (ABB) and 'Virupakshi' (syn. 'Hill Banana', AAB, Pome) are also severely affected by Fusarium Wilt (Thangavelu *et. al.*, 2019). In Karnataka, cultivation of the local cultivar 'Nanjangod Rasabale' has been reduced from 500 ha to less than 50 ha due to severe incidence of Fusarium Wilt (Narendrappa and Gowda, 1995). In Bihar, more than 55% of the area under susceptible cultivars was severely infected, and yield reduction in these areas was estimated at 50-70% (Mustaffa and Thangavelu, 2011). In Tamil Nadu, disease severity of more than 80% was reported (Sivamani, 1987). The new pathogen variant called Foc race 4 or TR4 is now affecting all the banana varieties cultivated in India (Fig. 1). Race TR4 has recently been observed infecting Grand Naine varieties in Katihar and Purnea districts of Bihar state (Thangvelu *et. al.*, 2019). This pathogen variant has shown its presence infecting Cavendish varieties in Uttar Pradesh (Damodaran *et. al.*, 2019), Madhya Pradesh (Burhanpur district) and Gujarat (Surat district) states but confirmation is still awaited.

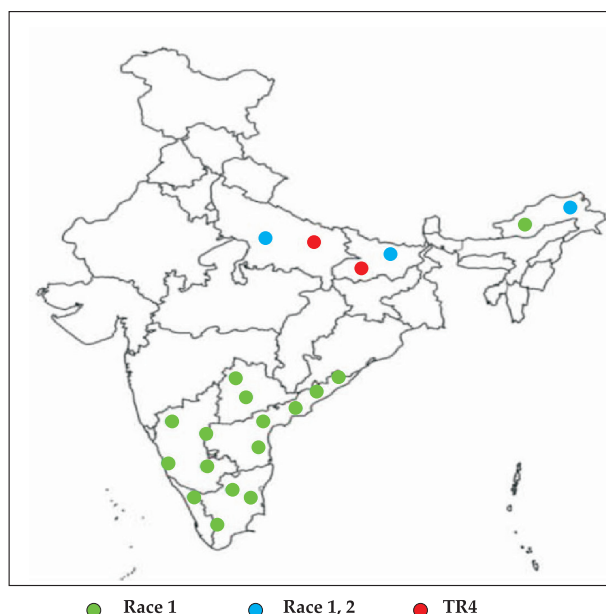


Fig. 1. Distribution profile of *Fusarium oxysporum* f. sp. *cubense* (Foc) races in different states of India

## TR4 Distribution and Impact

Since its first report from Taiwan in 1977, TR4 has dispersed to 17 countries so far. In 1977, 1,200 ha area was infected by this race, representing approximately 500,000 banana plants (Hwang and Ko, 2004). Subsequently, TR4 was recorded from Malaysia and Indonesia in the early 1990s. In Malaysia, the disease has affected 30% of banana plants. In Indonesia, more than 8 million plants were destroyed annually and banana plantations had to be abandoned with annual losses over USD 75 million. By the end of 2000, occurrence of TR4 was also recorded from China, Philippines and Australia. In Philippines, cases of TR4 incidence increased from 700 in 2005 to 15,000 in 2007. It has caused significant damage, limiting commercial production of the crop in Australia and China. Subsequently, it has been reported from several other countries like Oman, Jordan, Mozambique, Lebanon, Pakistan, Israel, Laos, Vietnam, Myanmar, India and Columbia (Fig. 2). It is estimated that banana wilt caused by TR4 could inflict heavy loss of nearly Rs. 50,000 crores to the Indian banana industry (Anonymous, 2018).

## IDENTIFICATION

### Symptomatology

Irrespective of the race, the disease symptoms are similar. Externally, the first signs of

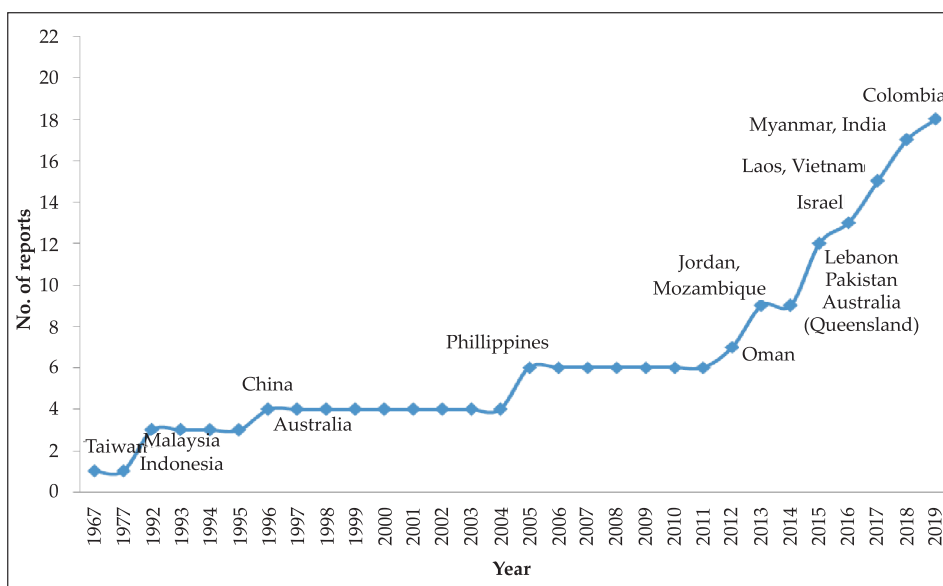


Fig. 2. *Fusarium oxysporum* f. sp. *cubense* race TR4 reports from different countries

the disease are wilted plants with yellowing of older leaves around the margins (Fig. 3). The characteristic symptoms of Fusarium Wilt are blackened, discolored and weakened vascular tissue within the stems of the plant. The discoloration varies from pale yellow in the early stages to dark red and black in the later stages. Internal symptoms initially develop in the feeder roots and rhizomes and later in the plant's pseudo stem. Sometimes the leaves remain green longer on the petiole, but as the disease progresses they eventually collapse forming a 'skirt' around the pseudo stem before falling off. New leaves may have irregular, pale margins and wrinkled blades. The disease may cause the pseudo stem to wilt or collapse. Infected suckers (used for seeding new plants) and rhizomes do not start showing signs of infection until they are around four months old. Thus, the fungus can also spread through infected suckers which may remain symptomless. Banana fruits do not show any symptom of the disease.



Fig. 3. Fusarium Wilt symptoms in banana from farmer's field of Katihar district of Bihar

## Vegetative Compatibility Groups (VCGs)

Race structure of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) was generally designated based on Vegetative Compatibility Groups (VCGs). Isolates sharing the same form (allele) of the genes that control the formation of a heterokaryon (a cell with two distinct nuclei) are designated as the same VCG. At least 24 VCGs are known to date in *Foc*. Distinct VCGs for different races are presented in Table 1. VCGs 0120, 0129, 01211, 01215, 0120/01215 and 0129/01211 have been associated with *Foc* SR4, while VCGs 01213, 01216 and 01213/01216 to *Foc* TR4. VCGs 01221, 01222, 01223, 01224 are also reported however, VCG complex and races are not known for them. In India, VCGs 0124, 0125, 0124/0125, 0128, 01220, 01218, 01212, 01211 and 01217 have been reported earlier (Mustaffa and Thangavelu, 2011). Recently, TR4 VCGs complex 01213/01216 was identified infecting Grand Naine variety in Bihar and Uttar Pradesh (Damodaran *et. al.*, 2009 and Thangavelu *et. al.*, 2019).

**Table 1. Race structure of *Fusarium oxysporum* f. sp. *cubense* based on vegetative compatibility group (VCG).**

| Races     | VCGs                             | VCG complex            |
|-----------|----------------------------------|------------------------|
| Race 1    | 0123, 01210, 01217, 01218, 01220 | 0124/0125/0128/01220   |
| Race 2    | 01214                            | -                      |
| Race 1, 2 | 0124, 0125, 0128                 | 0124/0125/0128/01220   |
| Race 4    | 0121, 0122                       | -                      |
|           | SR4: 0120, 0129, 01211, 01215    | 0120/01215, 0129/01211 |
|           | TR4: 01213, 01216                | 01213/01216            |

## Diagnostics

Different diagnostic methods for identifying *Foc* TR4 (VCG 01213/16) are currently available and these have supported the decision-making process of plant protection measures against the disease worldwide. Some of these methods are specific for VCG 01213/16, which is globally recognized as *Foc* TR4 (Dita *et. al.*, 2010; Zhang *et. al.*, 2013). At present, several molecular methods for detecting *Foc*, such as PCR, multiplex PCR, real time-PCR, LAMP, RealAmp, and iPCR, are available (Carvalhais *et. al.*, 2019). A molecular diagnostic method based on the intergenic spacer region (IGS) of the nuclear ribosomal gene cluster of *Foc* designated as *Foc* TR4-F (Table 2) is widely used for detecting TR4 (Dita *et. al.*, 2010). Lin *et. al.*, (2009, 2016) developed multiplex diagnostics for eight different VCGs, in addition to 01213/16. Specific and reproducible diagnostic assays based on conventional PCR targeting Secreted in Xylem (SIX) genes e.g. SIX6 in *Foc* race 1, SIX1 in TR4, SIX8 in subtropical race 4, SIX9/SIX10 in *Foc* VCG 0121, and SIX13 in *Foc* VCG 0122 have also been developed (Carvalhais *et. al.*, 2019).

Whole genome sequences of the isolates N2 (race 1, *Foc*1) and B2 (race 4, *Foc*4) with accession no. AMGP00000000 and AMGQ00000000, respectively, are available (Guo *et.*

*al.*, 2014). Genome analysis revealed that the genome structures of race 1 and race 4 isolates were highly syntenic with those of *F. oxysporum* f. sp. *lycopersici* strain Fol4287. A large number of putative virulence associated genes were identified in both *Foc* genomes, including genes putatively involved in root attachment, cell degradation, detoxification of toxin, transport, secondary metabolites biosynthesis and signal transductions. Importantly, relative to *Foc* race 1 (*Foc* 1), *Foc* race 4 (*Foc* 4) has evolved with some expanded gene families of transporters and transcription factors for transport of toxins and nutrients that may facilitate its ability to adapt to the host environment and contribute to pathogenicity on banana.

**Table 2. Primer sequences reported for molecular detection of TR4**

| S. No. | Primer        | Sequence                    | Region | Race identification | Reference                          |
|--------|---------------|-----------------------------|--------|---------------------|------------------------------------|
| 1      | Foc-1         | CAGGGGATGTATGAGGAGGCT       | SCAR   | ST4+TR4             | Lin <i>et. al.</i> , (2009)        |
|        | Foc-2         | GTGACAGCGTCGTCTAGTTCC       |        |                     |                                    |
| 2      | FocTR4-F      | CACGTTTAAGGTGCCATGAGAG      | IGS    | TR4                 | Dita <i>et. al.</i> , (2010)       |
|        | FocTR4-R      | GCCAGGACTGCCTCGTGA          |        |                     |                                    |
| 3      | FocSc-1       | CAGGGGATGTATGAGGAGGCTAGGCTA | SCAR   | ST4+TR4             | Lin <i>et. al.</i> , (2013)        |
|        | FocSc-2       | GTGACAGCGTCGTCTAGTTCCTTGGAG |        |                     |                                    |
| 4      | SIX1a_266_F   | GTGACCAGAACTTGCCCACA        | SIX1a  | TR4                 | Carvalhais <i>et. al.</i> , (2019) |
|        | SIX1a_266_2_R | CTTTGATAAGCACCATCAA         |        |                     |                                    |

## FACTORS DRIVING DISEASE OCCURRENCE

The pathogen is disseminated through infected seed pieces (rhizomes, aka corms or suckers), soil, surface waters used for irrigation, contaminated tools, farm tools, clothes and footwear. Once *Foc* is established in a soil, it can survive for long periods (20-40 years) in the absence of host plants (Buddenhagen, 2009). Weed hosts can act as inoculum reservoir for *Foc* without visible symptoms. *Foc* race TR4 was found in roots of *Chloris inflata*, *Euphorbia heterophylla*, *Cyanthillium cinereum* and *Tridax procumbens*, growing in infested banana plantations in Australia. Any external factor promoting root damage may facilitate *Foc* infections. Therefore, attack of plant parasitic nematodes may boost Fusarium Wilt epidemics in banana. Larger populations of *Pratylenchus* spp. were found in areas highly affected by FW (Almeida *et. al.*, 2018). Bacteria like *Chthonomonas* spp., *Pseudomonas* spp. and *Tumebacillus* spp. were significantly abundant in FW suppressive soils (Shen *et. al.*, 2015). In general, well-drained and aerated soils are assumed to reduce FW by improving root development and enhancing microbial activity (Stover and Simmonds, 1987). In India, sandy loam or sandy clay loam soils with low bulk density are more suppressive to FW of banana, while clay soils with high bulk density are more conducive (Felcy-Navajothy *et. al.*, 2012). Higher levels of FW in banana are consistently associated with lower pH values. Higher phosphorus (P) and silicon (Si) levels were also correlated with FW suppression in banana plantations (Fortunato *et. al.*, 2012).

## MANAGEMENT OPTIONS

Destruction of infected plants to reduce inoculum build up and prevent pathogen spread is a fundamental starting point. Among other practices, the use of resistant cultivars is the only effective management option available with us. However, emphasis must be given at plot, farm, country, regional, and continental levels to avoid the entrance of pathogen races. Exclusion has gained an increasing interest to prevent or delay the entrance of the highly destructive *Foc* TR4. Exclusion should also be implemented within the country and certification agencies have to play an important role to check the entrance and spread of *Foc* TR4 with a requirement for bio security measures at farm level as recently implemented by Global Gap ([https://www.globalgap.org/uk\\_en/forproducers/globalg.a.p.-add-on/tr4-biosecurity/](https://www.globalgap.org/uk_en/forproducers/globalg.a.p.-add-on/tr4-biosecurity/)).

To date, commercial cultivars having resistance to *Foc* TR4 are not available. However, after the emergence of *Foc* TR4, several Cavendish somaclones called Giant Cavendish Tissue Culture Variants (GCTCV) developed in Taiwan (Hwang and Ko, 2004) with intermediate resistance to *Foc* TR4 have been used for planting. Two clones of the first generation of GCTCV viz. GCTCV- 118 and GCTCV-119, and recurrent selection based on the first somaclones, resulted in development of GCTCV-218 and GCTCV-219. These are being used to mitigate losses caused by *Foc* TR4 in Taiwan and the Philippines and have recently been planted in Mozambique. The lack of effective TR4 control measures and the devastating impact of the disease make the deployment of resistance genes an obvious and effective strategy. Dale *et. al.*, (2017) developed a transgenic Cavendish transformed with RGA2 (a gene isolated from a TR4-resistant diploid banana *Musa acuminata* ssp *malaccensis*) and with a nematode-derived gene, *Ced9*, and so far it has remained disease free. Genome editing platform will be of considerable utility for the development of disease resistance in the future. In India, cultivars such as Nendran (AAB), Matti (AA) and Red Banana (AAA) were found to be resistant to race 1 infection (Mustaffa and Thangavelu, 2011). Some of the resistant sources available for the *Foc* race TR4 are mentioned in Table 3.

**Table 3: Resistance sources available against Fusarium Wilt of banana (*Fusarium oxysporum* f. sp. *cubense* race TR4)**

| Resistance sources | Genome              | Reaction                        | Reference                    |
|--------------------|---------------------|---------------------------------|------------------------------|
| Cultivars/Hybrids  |                     |                                 |                              |
| Rose               | AA                  | R <sup>T</sup>                  | Anonymous, 2018              |
| Ma851              | AA                  | R <sup>T</sup> and S            | Chen <i>et. al.</i> , (2019) |
| Madang Gaudelope   | <i>M. acuminata</i> | R <sup>T</sup> , R <sup>S</sup> |                              |
| FHIA-1             | AAAB, Hybrid        | R <sup>T</sup> , R <sup>S</sup> |                              |
| FHIA-25            | AAB, Hybrid         | R <sup>T</sup> , R <sup>S</sup> |                              |
| GCTCV-119          | AAA, Cavendish      | R <sup>T</sup> , R <sup>S</sup> |                              |
| FHIA-18            | AAAB, Hybrid        | R <sup>T</sup> , R <sup>S</sup> |                              |

| Wild relatives                                                                                                                        |                     |                |                                  |
|---------------------------------------------------------------------------------------------------------------------------------------|---------------------|----------------|----------------------------------|
| <i>M. nagensium</i>                                                                                                                   | Unknown             | R <sup>T</sup> | Li <i>et. al.</i> , (2015)       |
| <i>M. ruiliensis</i>                                                                                                                  | Unknown             | R <sup>T</sup> |                                  |
| <i>M. velutina</i>                                                                                                                    | Unknown             | R <sup>T</sup> |                                  |
| <i>M. basjoo</i>                                                                                                                      | Unknown             | R <sup>T</sup> |                                  |
| <i>M. itinerans</i>                                                                                                                   | Unknown             | R <sup>T</sup> |                                  |
| Somaclonal variants                                                                                                                   |                     |                |                                  |
| Somaclonal variants (Giant Cavendish) GCTCV-215-1 and GCTCV-218 (Taiwan)                                                              | AAA                 |                |                                  |
| RT                                                                                                                                    | Hwang and Ko (2004) |                |                                  |
| Somaclonal variants (Gros Michel) IBP 5-61, IBP 5-B and IBP 12 (Cuba)                                                                 | AAA                 | R <sup>T</sup> | Bermudez <i>et. al.</i> , (2002) |
| Transgenics                                                                                                                           |                     |                |                                  |
| Grand Naine banana transformed with RGA2-3 gene of <i>Musa acuminata</i> ssp <i>malaccensis</i> and nematode derived <i>ced9</i> gene |                     | R <sup>T</sup> | Dale <i>et. al.</i> , (2017)     |

R: resistant; S: and *Foc* sub-tropical race 4 (SR4) and T: *Foc* tropical race 4 (TR4).

Based on the current understanding of FW epidemiology, management practices are oriented towards soil health and suppressiveness, such as crop rotation, use of cover crops, application of organic amendments and biocontrol agents, as well as, the use of appropriate inorganic fertilizers and agronomic practices, which can help suppress *Foc* inoculum, reduce disease intensity and enhance yields (Dita *et. al.*, 2018). In this direction, crops with immediate market opportunities, such as cassava (*Manihot esculenta*), pineapple (*Annanas squamosa*) or plant species with different uses or purposes like Chinese leek (*Allium tuberosum*), have been used. Pinto peanut (*Arachis pinto*) as ground cover reduced the intensity of the disease (Pattison *et. al.*, 2014).

Managing the soil microbiota has an enormous potential to control soil-borne diseases like FW and suppressive soils may hold key answers to better understand and explore microbes and develop these as efficient management tools. Metagenomics have provided significant advances in understanding functional plant and soil microbiomes and identifying promising microbes against *Foc* (Cha *et. al.*, 2016). While applying “suppressive” organisms as bio pesticide has largely failed, the combination of promising microorganisms with organic amendments has shown better results (Mazzola and Freilich, 2017). Acidobacteria were reported to be more abundant in suppressive soils, while bacteroidetes were more abundant in conducive soils. *Bacillus amyloliquefaciens* strain (NJN-6) combined with compost has shown positive results to reduce epidemic caused by *Foc* TR4 in China (Dita *et. al.*, 2018).

## GLOBAL INITIATIVES

In December 2013, a task force on TR4 was set up within the framework of the World Banana Forum. In December 2014, the FAO held consultations with a group of international experts to agree on the framework for a global programme. The plan works on three main fronts: preventing future outbreaks, managing existing cases, and strengthening international collaboration and coordination among institutions, researchers, governments and producers. The Wageningen University and Research Centre in the Netherlands is leading the three projects on TR4: INREF, KNAW-SPIN and Promo Banana.

### Asia

The Banana Asia-Pacific Network (BAPNET) is coordinating a number of TR4-related projects and activities in various Asian countries. In India, Indian Council of Agricultural Research and State Agricultural Universities are working in this direction. A brain storming session on TR4 race was organized on 16.12.2017 at New Delhi.

### Africa

Following the announcement that the race TR4 has been detected in Mozambique, the African Consortium for TR4 (AC4TR4) was launched at a workshop held at the University of Stellenbosch on April, 2014.

### Australia

Following the first confirmed case of TR4 in Queensland, Biosecurity Queensland, in partnership with the Australian Banana Growers' Council, set up a programme of surveillance and containment. A system of certification was put in place for TR4-infested banana plantations that meet the requirements for inter-state and intra-state quarantine regulations. The system allows the accredited businesses to certify their fruit consignments without putting the wider industry at risk. Accredited farms will be visited by bio security officers to audit the fruit inspection process and ensure that bio security requirements are being met.

### Latin America and the Caribbean

OIRSA, a regional organization for plant and animal health, produced a contingency plan specific to TR4 for its nine member countries (Belize, Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Mexico, Nicaragua and Panama). The banana research network for Latin America and the Caribbean, MUSALAC, has been organizing trainings on quarantine pests, with a special emphasis on TR4. In 2014, the Caribbean Agricultural Research and Development Institute (CARDI) organized a seminar and a training workshop to raise awareness about the potential threat of TR4 as a key step to prevent its introduction into the Caribbean.

## RECOMMENDATIONS

Realizing the emerging threat of TR4 race to banana cultivation in India, the National Academy of Agricultural Sciences organized a brainstorming session on September 25, 2019 at NASC Complex, New Delhi on the topic “Tropical Wilt Race-4 Affecting Banana Cultivation”, so as to understand etiology of the virulent strain and devise suitable management strategies against Fusarium Wilt of banana. There were 35 participants including eminent scientists like Prof C. D. Mayee (Former Chairman, ASRB), Prof Anupam Varma (Former Joint Director, Education, IARI), Dr P. K. Chakraborty (Member, ASRB), Prof B. L. Jalali (Former Director of Research, CCSHAU, Hisar) and Dr D. V. Singh (Former Head, Division of Plant Pathology, IARI). Dr Rashmi Aggarwal (Dean and Joint Director, Education and Head, Division of Plant Pathology, IARI) was the convener, and Dr Rakesh Kumar Jain (Emeritus Scientist and Former Joint Director (Education), IARI) and Dr S. Uma (Director, NRCB, Trichy) were co-conveners. The recommendations of the session are as follows:

### Urgent

1. A networking project needs to be formulated on Fusarium Wilt of banana to assess distribution profile of the disease, to develop methodology for race identification and to devise suitable management options including GM technology. For this, funding from National Agricultural Science Fund or DAC, Govt. of India may be explored (Action: NRCB / IARI).
2. Policy guidelines to contain the disease need to be formulated, such as,
  - (i) Take only one crop of banana in disease prone areas
  - (ii) Field sanitation by burning diseased plant debris
  - (iii) Use of certified planting material and hardening by the producer company in coco- pit or solarite with amendment (Action: NPPQ).
3. Field sanitation must be strictly implemented. Destroy the harvested plants. Diseased plant debris should also be burnt.
4. Soil amendments used for disease management should be tested at multi locations.

### Long term

1. Regular disease monitoring in banana growing areas involving tissue culture companies and Krishi Vigyan Kendras. Remote sensing using drones could also be deployed for disease monitoring.
2. Race identification and distribution in banana growing areas to demarcate disease free areas. Training courses on advanced technologies for race identification need to be arranged in well equipped labs for the scientists engaged in *Foc* wilt research.

3. ICAR-FUSICONT technology (bio-formulation using the eco-friendly effective antagonistic microbes on a unique IPR protected bio-media with the defined protocol of application) needs to be validated in larger area. For managing *Foc* TR4, major emphasis needs to be given on the development of resistant cultivars with International collaboration. Develop suitable integrated disease management options including genetically modified technologies.
4. Epidemiological study should be conducted on pathogen survival in the soil, role of irrigation on the disease spread in scientific manner.
5. Pathological determinants for *Foc* TR4 need to be identified.

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