Author's post-print of the paper published in the Proceedings of the International ISHS-ProMusa Symposium on Global Perspectives on Asian Challenges, Acta Horticulturae 897, ISHS 2011. Available from www.musalit.org

Status of Fusarium Wilt in India

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Keywords: Biological control, chemical control, diagnostics, endophytes, rDNA-IGS-RFLP, resistant cultivars.

Abstract

With an annual production of 27 million tonnes, India is the world largest producer of banana. Many pests and diseases cause huge economic losses to the farmers. Among these, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the most important production constraints. The disease is widespread in almost all banana-growing states of India, with disease severity as high as 80-90% in some states where susceptible cultivars are grown on large areas. The important groups of banana severely affected by this disease are: 'Silk' (AAB), 'Ney Poovan' (AB), 'Pisang Awak' (ABB), 'Pome' (AAB), 'Bluggoe' (ABB) and 'Monthan' (ABB). Recently, a virulent strain of Foc affecting Cavendish types has been identified. In addition, 'Mysore' (AAB) which was hitherto tolerant to Foc has recently been found infected by Foc vegetative compatibility group (VCG) 0124/0125. A diversity analysis was carried out on 200 Foc isolates collected from different parts of India, to find out the various pathotypes in Foc by VCG analysis. The analysis indicated the presence of six different VCG groups. Ddiversity analyses of Foc, pathogen-host resistance, biological control using endophytes, standardisation of a diagnostic kit for the identification of the pathogen present in the soil and in the plant are the major areas of Foc research in India. Recently, molecular markers for the identification of pathogenic *Fusarium* present in the soil as well as in planting material have been developed. No effective control measures are available except growing of resistant cultivars. Recently, the National Research Centre for Banana (NRCB) has identified an effective fungal antagonist, *Trichoderma viride*, which has effectively controlled the soil-borne inoculum of the *Fusarium* pathogen. A mass-production protocol at farm level using banana farm waste has been developed for the cost-effective management of the disease. Activities for the effective management of this disease are discussed.

INTRODUCTION

Banana (including plantain) is the world largest fruit crop with an annual production of 93.71 million tonnes (FAO, 2008). It is a staple food for nearly 400 million people worldwide. India produces 27 million tonnes annually, with average productivity levels ranging from 20 to 36 tonnes per hectare (IHD, 2009). Poor soil health, nutrients imbalance, diseases and nematodes are major production constraints affecting productivity. Among the diseases, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is recognised as one of the most widespread and destructive banana diseases, and a major production constraint to banana worldwide. In Asia, the disease was first recorded in 1911 in West Bengal, India, and the disease now is widespread and destructive in almost all the banana-growing states in India, causing disease incidence of 30% in the plant crop and up to 85% in the ratoon crop. The cultivars '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 severely affected by wilt (Thangavelu et al., 2001). In Karnataka, cultivation of the local cultivar 'Nanjangod Rasabale' has been reduced from 500 ha to less than 50 ha (Narendrappa and Gowda, 1995) due to severe incidence of Fusarium wilt. 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%. In Tamil Nadu, it is becoming a major threat, with disease severity as high as 80-90% (Sivamani, 1987). Cavendish cultivars were also recently affected in Tamil Nadu. In Andhra Pradesh, farmers have abandoned the cultivation of the most susceptible cultivar 'Amrithapani' for more than 15 years due to Fusarium wilt incidence. Since the introduction of the wilt-tolerant 'Martamon' (AAB, an ecotype of 'Rasthali' from West Bengal), the cultivation has been revived. However, wilt incidence of up to 2% was noticed in this cultivar also.

GENETIC DIVERSITY

Studies on pathogenic diversity are essential, not only to have durable resistance of a cultivar deployed but also to establish quarantine procedures to prevent the spread of the pathogen through the movement of infected suckers and soil (Moore et al., 1993). Southeast Asia is one of the centres of diversity of banana (Valmayor, 2000) and of its pathogens, including Foc. The Foc pathogen co-evolved with its banana host in this region (Moore et al., 1995). A genetic diversity study was conducted in India by two different methods, vegetative compatibility grouping (VCG) and DNA fingerprinting through RFLP analysis of rDNA-IGS regions. The VCG studies conducted with *nit-M* testers received from Queensland Department of Primary Industries (QDPI), Australia revealed the presence of nine known VCGs, i.e. 0124, 0125, 0124/0125, 0128, 01212, 01217, 01218, 01220 and 01221, belonging to race 1 and race 2 (Table 1). However, the *nit-M* testers of QDPI were not very exhaustive since two third of the Indian Foc isolates tested did not form heterokaryons with any of these *nit-M* testers. Therefore, *nit-M* testers were developed locally from the Indian Foc isolates, and VCG studies were conducted for all the Foc isolates obtained from different banana-growing regions of India. The test results indicated the presence of nine VCGs, since the Foc isolates reacted with at least one of the *nit-M* testers developed locally (Thangavelu, 2008). During the VCG studies, it was also interesting to note that race 1 members formed heterokaryons with members of race 2, there by indicating a cross-reaction between race 1 and race 2, and this was observed in many cases. The race 2 'Monthan' Foc isolates (44 MT, 45MT, 181MT) isolated from Tamil Nadu reacted with *nit-M* testers of 'Rasthali' Foc isolates (19b) of race 1. Similarly, race 1 Foc isolates (such as 14 RT, 107RT, 132 RA, 127 RKa, 188RN) obtained from Tamil Nadu, Andhra Pradesh, Karnataka and North eastern states of India reacted with 'Monthan' isolates of race 2. The Foc isolates of 'Hill Banana' reacted with both race 1 and race 2 isolates (Thangavelu, 2008) (Table 2).

Besides VCG analysis, molecular characterisation by RFLP analysis of rDNA-IGS region was carried out using six restriction enzymes, namely *Hae*III, *Rsa*I, *Hha*I, *Hinf*I, *Taq*I and *Msp*I. In addition to Foc isolates, other fungal isolates such as non-pathogenic *Fusarium*, *F. oxysporum* f. sp. *ricini*, *Colletotrichum musae* and *Lasiodiplodia theobromae* were included for comparison. The results of the analysis indicated that the universal primer set specific for the amplification of the IGS region produced bands ranging from 1391 bp to 2306 bp. The PCR products of the IGS-amplified region digested with six restriction enzymes produced unique banding patterns. Enzyme *Hae*III produced

seven bands, *Rsa*I (six), *Hha*I (four), *Hinf*I (seven), *Taq*I (seven) and *Msp*I (six). In total, 18 IGS genotypes were observed. Each IGS genotype was designated with a six-letter code. For pathogenic *Fusarium* wilt isolates alone, 13 IGS genotypes were observed. AAAAAA (group 1) was the most common and consisted of 44 isolates of pathogenic *Fusarium*. Other isolates of banana pathogens, castor and non-pathogenic *Fusarium* isolates were grouped in to separate IGS genotype (Thangavelu, 2008).

In India, although race 4 has not been recorded so far, a Foc strain infecting Cavendish groups was recently identified (Thangavelu and Mustaffa, 2010). The characterisation of the new strain by VCG and volatile production indicated that the strain belongs to the 'inodoratum' group of VCG 0124 of race 1. A pot culture experiment confirmed that this particular strain is highly virulent with a maximum disease score of 6 in the majority of the commercial cultivars of India (Thangavelu and Mustaffa, 2010).

EARLY DIAGNOSIS

The early and correct diagnosis of plant-pathogenic fungi is a crucial component of any crop management system. Plant diseases can be managed most effectively if control measures are introduced at an early stage of disease development. Besides, it helps to reduce the number and amount of crop protection chemicals, thus reducing environmental pollution.

Foc generally spreads through infected corms/planting materials, and symptoms appear 5 to 6 months after planting or at the time of flowering. Application of available chemical and biocontrol measures after appearance of the symptoms is not effective, leading to huge losses to the farmers. If disease-free planting materials are planted in pathogen-free soil and/or if diseased plants are eliminated at the early stage of pathogen multiplication, the disease can be effectively managed by arresting the spread of the disease. Hence, pathogen diagnosis in the soil, in planting materials and also in growing plants at an early stage of pathogen infection is essential for effective control of the disease. To fulfil these objectives, a PCR-based diagnostic assay was developed to specifically detect Foc race 1 and race 2 by exploiting the variations in the rDNA-ITS regions of several Foc isolates obtained from different banana-growing regions of India (Thangavelu, 2008). Although this marker works well under in-vitro conditions, further validation in vivo is to be carried out so as to put it to practical purpose for the benefit of the farmers.

DISEASE MANAGEMENT

The options for the control of Fusarium wilt are limited, since no chemical control measures are effective so far. Hence, disease management is possible only by integrating different effective control strategies which are mentioned below.

Resistant Cultivars

Among all the disease management practices available, planting of resistant cultivars is the most effective and economical approach, particularly in endemic areas. Many commercial cultivars like 'Rasthali', 'Karpuravalli', 'Ney Poovan' (AB), 'Pachanadan', 'Virupakshi', 'Poovan' (AAB, Mysore), 'Tella Chakkerakeli' (AAA), 'Robusta' (AAA, Cavendish) and 'Monthan' (ABB) are highly susceptible to this disease. Among the commercial varieties available in India, 'Nendran' (AAB), 'Matti' (AA) and 'Red Banana' (AAA) are found to be unaffected by Fusarium wilt. The diploid AA cultivars 'Matti', 'Anaikomban', 'Ambalakadali', 'Nivedia Kadali', 'Pisang Lilin' and

'Tongat' are resistant (Anon., 2009). Some of the hybrids developed involving these parents, like 'H-201', 'H-61' and 'H-65', are being screened under in-vitro and in-vivo conditions and are being used to improve some of the commercial triploid cultivars. Field evaluation of exotic cultivars under sick plot condition indicated that 'Yangambi Km 5' (AAA, Ibota) among dessert cultivars and 'FHIA-03' among cooking cultivars were resistant to Fusarium wilt. Recent screening of 11 parents and 22 hybrid progenies against Foc under sick plot condition indicated that eight parents, namely 'Pisang Jajee', 'Cultivar Rose', 'Burro Cemsa', 'H3', 'Hatidat', 'Pisang Mas', 'Kanai Bansi' and 'Tongat', and 13 of their progenies were resistant (Anon., 2009).

Cultural Methods

Generally, cultural practices are recommended to prevent the introduction of the pathogen into the field and to reduce the inoculum level of the pathogen. These practices are economical and easy to adopt. Some of the recommended cultural practices are: i) inclusion of paddy, sugarcane, maize, elephant foot, yam or sweet potato in the banana cropping system; ii) flooding infested fields for at least 6 months; iii) planting disease-free suckers; iv) eradication of infected plants and application of lime in the infested pits; and v) application of organic amendments with or without bio-agents. Narendrappa and Gowda (1995) reported that urea with sugarcane trash at 250 g/pit gave satisfactory control. Among different organic amendments, maximum reduction in mycelial growth (80.15%) and lowest mycelial weight (47.39%) was observed with neem cake. Neem cake was also found to be compatible with *Pseudomonas fluorescens* and *Trichoderma* spp. (Saravanan et al., 2003). But Prasadji and Smith (2002) reported that flood fallowing, crop rotation and use of organic amendments were not effective in managing the disease. They also reported that in some farmers' field, cultivation of rice under wet land conditions for 3-5 years followed by planting of disease-free suckers of susceptible cultivar 'Amrithapani' resulted in negligible incidence of disease. However, the incidence increased with continued cultivation of the susceptible cultivar.

Chemical Control

In India, chemical control is widely followed for the control of the disease. Among different fungicides Carbendazim application is adapted on a large scale. Different methods of application are: i) dipping a pared corm in 0.1% Carbendazim for 30-45 min; and ii) soil drench with 0.2% Carbendazim at 5, 7 and 9 months after planting. A field trial conducted at NRC for Banana also indicated that dipping suckers in Carbendazim (0.1%) for half an hour at the time of planting + drenching the soil with Carbendazim (0.1%) around the pseudostem + injection of the pseudostem with Carbendazim (0. 1%) at 2, 4 and 6 months after planting drastically reduced wilt severity (score 1.3) as compared to untreated control plants (score 4.0) (Anon., 2009).

Biological Control

Biological control methods involving use of various fungal and bacterial isolates are becoming popular among the farmers. Several workers have reported that application of biocontrol agents effectively controlled the disease. Thangavelu (2002) reported that application of *Trichoderma harzianum* Th-10 as dried banana leaf formulation at 10 g/plant as basal + top dressing at 2, 4 and 6 months after planting recorded the highest reduction of disease incidence (51.16%), followed by *Bacillus subtilis* or *Pseudomonas fluorescens* (41.17%) application as talc-based formulation both under glass-house and

field conditions. Application of these bio-control agents in the field as basal + top dressing also completely eliminated nematodes. Saravanan et al. (2003) reported that either basal application of neem cake at 0.5 kg per plant + sucker dipping in spore suspension of *P. fluorescens* for 15 min. + soil application of *P. fluorescens* at 10 g per plant at 3, 5 and 7 months after planting or basal application of neem cake at 0.5 kg/plant + soil application of *P. fluorescens* at 10 g per plant at 3, 5 and 7 months after planting recorded the greatest suppression of Fusarium wilt under field conditions. Recent studies at NRCB on the bio-control of Fusarium wilt indicated that soil application of *T. viride* NRCB-1 as rice chaffy grain formulation significantly reduced the external (78%) and internal (80%) symptoms of Fusarium wilt in tissue cultured and sucker plants of 'Rasthali' and significantly increased plant growth parameters as compared to talc powder formulation under both pot culture and field conditions (Thangavelu and Mustaffa, 2010).

Thangavelu and Jayanthi (2009) reported that soil application of two potential non-pathogenic *F. oxysporum* isolates Ro-3 and Ra-1 in both tissue-cultured and sucker plants of banana registered reduction of Fusarium wilt severity (89%) and also increased plant growth parameters significantly when compared to inoculated control plants. The Ro-3 isolate performed better in reducing the wilt disease than the Ra-1 isolate. Field evaluation of these isolates using 'Rasthali' also significantly reduced Fusarium wilt severity (84%). The endophytic bacterial isolates obtained from roots, corms, leaf lamina and mid-rib portions of 17 resistant banana accessions were screened for their ability to inhibit spore germination and mycelial growth of Foc. The result indicated that only three isolates, namely *Actinomycetes* sp. (17Ra), *Klebsiella* sp. (17Rb) obtained from roots of 'Yangambi Km 5' and *Staphylococcus* sp. (15Cb) from corm of 'Pisang Seribu' (AAB), exhibited 90-100% inhibition of spore germination, 49-54% reduction in mycelial growth by volatile production and 60-74% reduction of mycelial growth by dual culture plate methods (Anon., 2008). Further studies on the compatibility of these effective endophyte isolates with the fungicides Propiconazole and Carbendazim at different concentrations (1-0.01%) indicated that these endophytes were compatible up to 0.1% concentration with the fungicides tested. This indicated the potential of using these effective endophytic bacterial isolates with fungicides for the effective management of Fusarium wilt in banana (Anon., 2008).

FUTURE THRUSTS

- Diversity map of Foc isolates for different banana-growing states of India for a durable, effective and economical management of Foc in India;
- Marker development for early diagnosis of pathogenic *Fusarium*, both in planting material and in soil;
- Biological control methods, including biotisation of planting material such as suckers and tissue-culture plants with endophytes and non-pathogenic forms of *Fusarium*;
- Integrated Disease Management (IDM) technologies involving bio-agents, botanicals, plant activators and resistant cultivars;
- Pyramiding of defence genes including biocontrol agents for effective transgenic plants production.

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Tables

Table 1. The vegetative compatibility groups of Fusarium wilt pathogen of banana identified in different states of India using nit-M testers of QDPI, Australia.

State	Varieties	VCGs identified
Assam	Malbhog, Martamon, Athiakol	0125, 0124/0125,0128, 01220, 01218, 01212, 01211, 01217
Nagaland	Karpuravalli	0125
Meghalaya	Karpuravalli	0124
Kerala	Rasthali	01212
Karnataka	Ney Poovan	0124/0125, 0125, 01212,
Andhra Pradesh	Martamon, Karpuravalli	0124
Tamil Nadu	Ney Poovan, Rasthali, Monthan, Karpuravalli	0124, 0124/0125, 0125, 0128

Table 2. The reaction of nit-1 mutants of *Fusarium oxysporum* f. sp. *cubense* (Foc) isolates of India with nit-M testers developed from wild Foc isolates.

Nit-M testers generated from Indian Foc isolates*	Nit-1 mutants generated from Indian Foc isolates*	Location from which the Foc isolate was obtained
3R from 'Rasthali'	93RT, 94RT, 34NT, 39NT, 122NKa, 123NKa, 124NKa, 190RN	Tamil Nadu, Karnataka, North Eastern region
19R from 'Rasthali'	32RT, 35RT, 93RT, 94RT, 177RT, 179RT, 180RT, 9RT, 19RT, 133RA, 134RA, 136RA, 137RA, 138RA, 130RA, 131RA, 82RKe, 145RKe, 160RKe, 165RKe, 188RN, 190RN, 149RKe, 29NT, 34NT, 39NT, 40NT, 41NT, 51NT, 143NT, 44MT, 45MT, 181MT,117HT, 122NKa, 191KN, 72KT	Tamil Nadu, Andra Pradesh, Kerala , North Eastern region, Karnataka
130R from 'Rasthali'	35RT, 93RT, 94RT, 177RT, 179RT, 180RT, 19RT, 133RA, 134RA, 136RA, 137RA, 138RA, 130RA, 131RA, 145RKe, 160RKe, 188RN, 117HT, 40NT, 41NT, 51NT, 92NT, 143NT, 122NKa, 123NKa, 45MT, 181MT, 167KKe	Tamil Nadu, Andra Pradesh, Kerala , North Eastern region, Karnataka
152K from 'Karpuravalli'	4RT, 14RT, 93RT, 94RT, 131RA, 132RA, 129RKa, 187RN, 205RN, 193AN, 104NT, 144NT, 124NKa, 126NKa, 22MT, 44MT, 178MT, 182MT, 184MT, 50KT, 72KT,	Tamil Nadu, Andra Pradesh, Kerala , North Eastern region, Karnataka

	173KT, 59KT, 141KA, 142KA, 152KKe, 164KKe, 167KKe, 168KKe, 203KN	
50K from 'Karpuravalli'	14RT, 94RT, 107RT, 9RT, 132RA, 127RKa, 129RKa, 187RN, 205RN, 190RN, 193AN, 124NKa, 126NKa, 5MT, 22MT, 111MT, 178MT, 182MT, 184MT, 139MA, 50KT, 89KT, 173KT, 59KT, 142KA, 152KKe, 164KKe, 167KKe, 168KKe, 203KN	Tamil Nadu, Andra Pradesh, Kerala , North Eastern region, Karnataka
30NP from 'NeyPoovan'	32RT, 133RA, 137RA, 190RN, 29NT, 34NT, 41NT, 51NT, 104NT, 89KT	Tamil Nadu, Andra Pradesh, North Eastern region
86NP	32RT, 34NT, 39NT, 122NKa, 123NKa	Tamil Nadu, Karnataka
111M from 'Monthan'	14RT, 107RT, 132RA, 127RKa, 188RN, 117HT, 193AN, 104NT, 144NT, 124NKa, 5MT, 22MT, 27MT, 111MT, 178MT, 185MT, 184MT, 139MA, 50KT, 72KT, 173KT, 59KT, 167KKe, 168KKe	Tamil Nadu, Andra Pradesh, Kerala, North Eastern region, Karnataka
6M from 'Monthan'	5MT, 72KT, 167KKe	Tamil Nadu, Kerala

* The name for nit-M testers and nit-1 mutants of Indian isolates was given after the cultivars and state of the country from which the wild Foc isolates were obtained.