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RESEARCH

Genetic Diversity of *Fusarium oxysporum* f.sp. *cubense* Isolates (*Foc*) of India by Inter Simple Sequence Repeats (ISSR) Analysis

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Abstract To find out the genetic diversity of Indian *Foc* isolates of banana, a total of 107 isolates of Fusarium which includes 98 *Foc* isolates obtained from different banana growing regions of India and seven *Foc* isolates belong to all known VCGs obtained from Australia and two non-pathogenic *Fusarium oxysporum* (np*Fo*) isolates were subjected to ISSR analysis. In the initial screening of ISSR primers, out of 34, 10 primers which generated more polymorphic bands were selected for further analysis. The Phylogenetic analysis carried out based on the fingerprints obtained through ISSR analysis indicated the presence of wide genetic diversity among the *Foc* isolates of India and also its polyphyletic nature. Totally, seven different clusters were obtained and these clusters differentiated the *Foc* isolates of India based on the races/VCGs. Besides, the cluster analysis clearly distinguished the freshly emerged *Foc* strain obtained from cv. Grand Naine (Cavendish-AAA) and Poovan (Mysore-AAB) from the other *Foc* isolates. The non-pathogenic *F. oxysporum* isolates which have been included for comparison purpose also clustered separately. All these above said findings indicates for the first time the discriminatory power of ISSR to clearly distinguish and separate the *Foc* isolates according to its race/VCGs and also its virulence. This study would be useful not only to design and develop effective management strategies but also useful for quarantine purposes.

Keywords ISSR analysis · Fusarium wilt of banana · Diversity analysis

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Introduction

Fusarium wilt (Panama disease), caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *cubense* (E.F. Smith) W·C. Snyd. & H.N. Hans. is one of the most catastrophic plant diseases in the world [1] and widespread in all the banana growing regions of Asia, Africa, Australia, and the tropical Americas, destroying more than 40,000 ha of banana in Central and South America over a period of 50 years. In India, the disease is present in almost all the banana growing states and causes severe yield losses in most of the commercial cultivars grown. Although the disease was recorded only in the suscepts of race 1 and race 2 of *Foc*, recent survey in Theni district of Tamil Nadu revealed the presence of Fusarium wilt strains which can attack race 4 susceptible cv. Grand Naine (Cavendish-AAA). The characterization of these isolates indicated that this new strain belongs to VCG 0124 of race 1 and an inodoratum group [2]. This finding showed that variability is occurring at faster rate within the *Foc* strains which is threatening the cultivation of important commercial cultivars of banana, particularly Cavendish group of bananas. Moreover, India is considered as one of the centers of diversity for banana. As the *Foc* is highly variable and considered to be co-evolved with banana [3, 4], there is a great chance of having great diversity among the *Foc* isolates [5, 6]. Bentley et al. [3] also stressed the importance of studying the diversity of *Foc* of Asia, the center of origin and domestication of *Musa*, for thorough understanding of genetic diversity of *Foc* worldwide.

Recently, the VCG analysis carried out for Indian *Foc* isolates using currently available 33 nit-M testers at QDPI, Australia revealed that out of 189 isolates of *Foc,* only 58 isolates have reacted to known nit-M testers and remaining were grouped as unknown VCGs. This finding also

indicates the presence of wide diversity among the Indian *Foc* isolates and also the molecular approach is the only way to characterize these unknown VCGs. But, no systematic and exhaustive works have so far been carried out to characterize these Indian *Foc* isolates. Moreover, understanding of genetic diversity/variability of *Foc* isolates of India is very much essential to design and develop effective and durable disease management strategies including resistant cultivars and also for the development of molecular markers specific to pathogenic form of *Foc*.

In the past, we have made several attempts to characterize Indian *Foc* isolates by different techniques, such as RAPD, RFLP analysis of rDNA-ITS and IGS regions, but all these methods could not yield a desired result of characterizing the Indian *Foc* isolates based on the VCGs/race and/or differentiation of virulent isolate from the normal *Foc* isolates etc. Therefore, in the present study, an attempt has been made to characterize the Indian *Foc* isolates to find out the genetic diversity of *Foc* isolates by ISSR analysis as it would be useful to develop effective management practices and also for the quarantine purposes.

Materials and Methods

Foc Isolates

Totally, 98 *Foc* isolates were recovered from the Fusarium wilt infected samples of different cultivars of banana which belong to different genomic groups grown in different parts of India. Besides, seven DNA samples of different VCGs obtained from QDPI&F, Brisbane, Australia and two non-pathogenic *F. oxysporum* (np*Fo*) isolates obtained from the roots of banana cv. Dudhsagar (AAB) were used for the comparison purpose (Table 1). All the *Foc* cultures were isolated using half-strength potato dextrose agar (PDA) amended with streptomycin [7]. Single-spore cultures were then prepared from fungal colonies. Each single-spore isolate was grown on filter paper overlaid on half-strength PDA. The colonized filter paper was lifted from the agar plate, dried and stored at 4°C until further use.

Pathogenicity Testing

To test the pathogenicity of the *Foc* isolates to banana plants, a pot culture experiment was performed using tissue-cultured material from cultivars Rasthali (for race 1), Monthan (race 2), and Grand Naine (race 4). Disease-free, 3-month-old tissue-cultured plants were obtained from the tissue culture laboratory of National Research Centre for Banana, Thiruchirapalli, Tamil Nadu and were planted in mud pots (30 × 30 × 30 cm), filled with 7 kg sterilized potting mix (1:1:1 ratio of red soil, sand and decomposed farmyard manure). One month after planting, sand maize meal inoculum of putative *Foc* isolates prepared according to Ricker and Ricker [8] was applied individually in separate pots around the plants in soil at the rate of 30 g/pot. For each isolate of *Foc*, five replications were maintained. Pots were fertilized with the recommended dose of organic and inorganic fertilizers, and watered regularly. Disease severity was estimated as described by Carlier et al. [9].

DNA Extraction and Purification

For DNA extractions, all isolates of *Foc* were grown on half-strength Potato Dextrose Broth in sterile 250 ml conical flasks for 7 days at 25°C. Mycelia were collected on two layers of Cheese cloth, washed in sterile distilled water three times and dried using sterile filter paper. DNA extraction and purification were performed as described by Raeder and Broda [10] and Nel et al. [11]. In brief, the *Foc* mycelial samples were ground under liquid nitrogen into a fine powder and immersed in 1 ml of DNA extraction buffer (2% sodium dodecyl sulfate (SDS), 40 mM ethylene diamine tetraacetic acid (EDTA), 40 mM sodium chloride, 100 mM Tris–Hcl (pH 8.0), and 25 mM diethyldithiocarbamic acid) at 37°C for 2 h. The suspension was extracted twice with an equal volume of phenol (Tris equilibrated, pH 8.0) to remove proteins and then twice extracted with chloroform. The DNA was then precipitated over night at −20°C in a sodium acetate (5 M)/isopropanol solution (1:10 v/v). The sample was centrifuged (13,000 rpm, 30 min) to pellet the DNA. The DNA was then cleaned with the addition of 70% ethanol and then centrifuged (13,000 rpm, 30 min). Ethanol was removed with a pipette and the reminder was removed in vacuum. The pellet was resuspended in 100 μl of sterile TE (10 mM Tris, 1 mM EDTA, pH 8.0) buffer. RNase (100 μg/μl) was added to the DNA samples and incubated at 37°C for 3–4 h to digest any residual protein or RNA. The DNA was visualized on a 1% agarose gel (w/v) stained with ethidium bromide viewed under UV light. DNA concentration and quality was determined spectrophotometrically using a spectrophotometer (Lamda 25, Perkin Elmer, USA) and visualized on agarose gels. Working concentration of DNA was adjusted to 25 ng/μl and stored at 4°C.

PCR Amplification and Electrophoresis

Initially, to select primers which produce more number of polymorphic bands for the characterization of *Foc* isolates, 34 ISSR primers with di- or tri nucleotide repeats were screened using DNA samples of nine representative isolates of *Foc* which include *Foc* isolates of major commercial cultivars of banana such as Rasthali, Karpuravalli, Ney Poovan, Monthan, *Foc* of newly emerged isolates

Table 1 Details of the Indian *Foc* isolates used for the genetic diversity analysis

S.No	Cultivar of the isolate	Source and number of *Foc* collected		Total no. of isolates
		State	No. of isolates	
1	Rasthali (Silk-AAB)	Tamil Nadu	31	50
		Andra Pradesh	6	
		Kerala	10	
		Assam	3	
2	Monthan (cooking banana-ABB)	Tamil Nadu	13	13
3	NPF	Tamil Nadu	2	2
4	Hill banana (Pome-AAB)	Tamil Nadu	3	3
5	Poovan (Mysore-AAB)	Tamil Nadu	1	1
6	Karpuravalli (Pisang Awak-ABB)	Tamil Nadu	9	15
		Kerala	3	
		Assam	1	
		Nagaland	2	
7	Ney Poovan (AB)	Tamil Nadu	9	15
		Kerala	3	
		Karnataka	3	
8	Cavendish (AAA)	Tamil Nadu	1	1
9	VCGs	Australia	1	7
		India	1	
		Malaysia	2	
		Philippines	2	
		Thailand	1	
Total				107

which attack Cavendish and Poovan cultivars and VCGs of race 1, race 2, and race 4. From the preliminary screening, ten primers which generated more polymorphic bands were alone selected for amplification of all DNA samples (Table 2).

The PCR was performed in a total volume of 25 μl reaction containing 1X PCR buffer, 0.2 mM of dNTPs, 1.5–2.5 mM $MgCl_2$, 0.5 μM primers, 1 Unit of Taq DNA Polymerase (Bangalore Genei, Bangalore, India), and 25 ng of genomic DNA. The amplification was carried out in an eppendorf thermal cycler with the modification of annealing temperature to optimize the reaction condition for individual primers (Table 2). The reaction mixture was denatured at 94°C for 5 min followed by 40 cycles of 30 s denaturation at 94°C, 45 s annealing at 48–64°C and 1.30 min extension at 72°C and final extension for 7 min at 72°C was used for proper amplification of fragments. The PCR products were separated electrophoretically on a pre-stained ethidium bromide gel consisting of 1.5% Agarose gels at 70 V for 4 h. After the run, the gel was visualized under UV light and photographed using Alpha imager® EC for scoring the bands. All PCR amplifications were performed at least twice for each isolate.

Data Analysis

The bands generated by ISSR primers which were clearly visible with high intensity, repeatable and with the size of more than 100 bp were scored manually for presence (1) or absence (0) of bands in each isolate for the purpose of phylogenetic analysis. Data analyses were performed using Win 95/98/NT programme Free Tree (http://www.natur.cuni.cz/Cflegr/programs/freetree) [12]. The dataset of isolates and reproducible bands were used to calculate Jaccard's coefficients using the Free Tree programme. Matrices of similarity coefficients were subjected to unweighted pair group method with arithmetic mean (UPGMA) to generate a dendrogram [13].

Results

Fusarium Wilt Isolates and Pathogenicity

The inoculation of all 98 isolates of *Foc* as sand maize meal inoculum individually into the differential hosts revealed the presence of only two races viz. race 1 and

Table 2 Features of ISSR primers

S.No	Primer sequence	Annealing temperature (°C)	Amplified bands		Polymorphism (%)
			Total no of bands generated	Polymorphic bands	
1	$(CA)_8$ RG	48	3	2	66.67
2	$(GA)_8$ YG	48	12	3	25
3	$(CA)_8$ RC	48	6	2	33.3
4	BDB$(CA)_7$	48	3	1	33.3
5	DBD$(AC)_7$	48	12	4	33.3
6	VHV $(GT)_7$	48	10	5	50
7	VDV$(CT)_7$	48	3	1	33.33
8	DVD$(TC)_7$	48	–	–	–
9	$(AC)_8$YG	48	11	4	36.36
10	GGA$(GAG)_2$ACG AGA	48	11	2	18.2
11	$(AG)_8$YA	48	5	3	60
12	GCA$(AC)_7$	48	6	2	33.3
13	$(AC)_8$ YA	50	6	3	50
14	$(AC)_8$G	50	5	2	40
15	$(GA)_8$C	50	6	2	33.3
16	$(GT)_8$YA	50	–	–	–
17	$(GA)_8$ YC	50	3	1	33.3
18	CCA$(TG)_7$T	50	13	4	30.8
19	$(ACC)_6$	50	12	8	66.7
20	$(AG)_8$T	50	14	3	21.4
21	$(AC)_8$G	50	5	2	40
22	$(GA)_8$YT	52	4	1	25
23	$(AG)_8$YT	52	6	2	33.3
24	$(AG)_8$YC	52	–	–	–
25	$(ACC)_6$CC	52	6	2	33.3
26	CCA$(TGA)_5$TG	52	–	–	–
27	$(CA)_8$T	52	1	–	–
28	$(AG)_8$C	52	6	2	33.3
29	$(AG)_8$G	52	5	2	40
30	BHB $(GA)_7$	53	–	–	–
31	$(ATG)_6$	55	–	–	–
32	$(CAG)_5$	58	4	1	25
33	$(GAC)_5$	60	10	2	20
34	$(GTG)_5$	64	10	10	100

race 2 in India. Although the *Foc* isolates of cv. Grand Naine produced symptoms of wilt disease in cv. Grand Naine as well as in race 1 and race 2 suscepts, the characterization by volatile production and VCG analysis showed that this particular Cavendish strains belongs to race 1 (data not shown). The inoculation of two isolates of np*Fo* did not produce any symptoms of wilt disease in any of the above said differentials which confirms its non-pathogenic nature.

Primer Selection

To select primer which gives more polymorphic bands, totally 34 ISSR primers were screened using nine representative isolates of *Foc* which belongs to race 1 and 2 (Table 2). These primers were of di- or tri nucleotide repeats. Among the 34 ISSR primers, only 27 ISSR primers generated good bands and remaining seven primers did not produce any band. Among the 27 ISSR primers, 10 ISSR

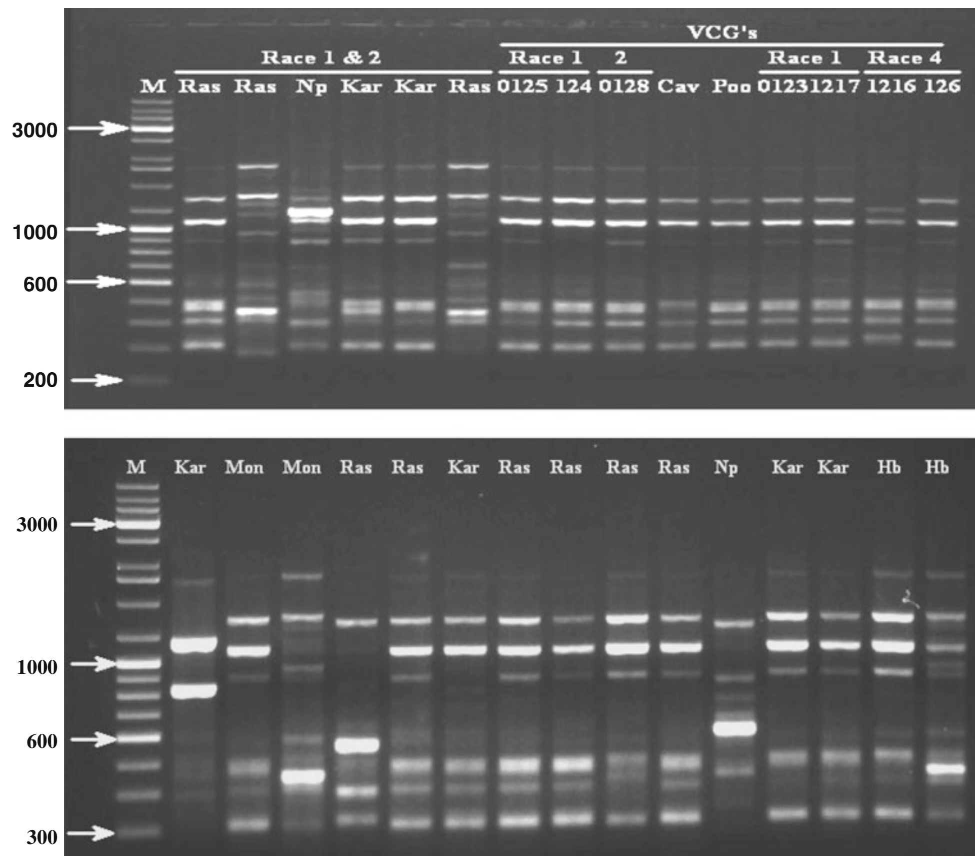

Fig. 1 The ISSR fingerprinting of *Foc* isolates of different cultivars of banana and VCGs generated by $(GTG)_5$ Primer. *M* Medium range DNA ruler (100−5000 bp) Marker, *Ras Foc* isolate from cv. Rasthali (Silk-AAB), *Mon Foc* isolate from cv. Monthan-(cooking banana-ABB), *Np Foc* isolate from cv. Ney Poovan (AB), *Cav Foc* isolate from cv.Cavendish (Silk-AAA), *Poo Foc* isolate from cv. Poovan (Mysore-AAB), *Kar Foc* isolate from cv. Karpuravalli (Pisang Awak-ABB), *Hb Foc* isolate from cv. Hill banana (Pome-AAB)

primers generated more polymorphic bands which ranged from 2 to 10 with the maximum of 100% polymorphism (Table 2).

ISSR Analysis

The ISSR amplification of 107 isolates of *F. oxysporum* using 10 primers viz. $(AC)_8YG$, VHV $(GT)_7$, $(ACC)_6$ $(GTG)_5$, $(GA)_8$ YG, DBD$(AC)_7$, GGA$(GAG)_2$ACGAGA, CCA$(TG)_7$T, $(AG)_8$T, and $(GAC)_5$ produced 3–14 bands and out of which the number of polymorphic bands were 2–10. The size of PCR fragments generated was ranged from 100 to 4500 bp. In order to assess the genetic relatedness between the *Foc* isolates of banana, distance matrix was calculated based on the fingerprints obtained (Fig. 1) and it was ranged from 0.27 to 1.00. This indicates that there is a wide genetic diversity among the *Foc* isolates of India indicating its polyphyletic nature.

The dendrogram consisted of two major clusters A and B (Fig. 2). The cluster "A" contains four *Foc* isolates of cv. Rasthali and one *Foc* isolate each from cv. NeyPoovan and cv. Karpuravalli (sub-cluster I) and cluster "B" contains all the remaining 101 *Foc* isolates including seven VCGs of foreign origin and two np*Fo* isolates. The genetic similarity of Indian *Foc* isolates was ranged from 27 to 100%. In cluster "B" six sub-clusters (sub-clusters II to VII) were observed. The sub cluster II contains two np*Fo* isolates obtained from cv. Dudhsagar. The VCGs 0126 and 01216 of race 4 were grouped in sub-cluster III. The sub-cluster IV contains majority of the *Foc* isolates (94 out of 107) and VCGs which belong to race 1 and race 2 with the genetic similarity of 46 to 100%. The sub-cluster V contains *Foc* isolate of cv. Karpuravalli, and sub-clusters VI and VII contain one *Foc* isolates each of cv. Rasthali. Interestingly, in the major sub cluster IV, the newly emerged virulent strains identified first time in India from cvs. Grand Naine (Cavendish-AAA) and Poovan (Mysore-AAB) were clustered separately. The genetic distance between the newly emerged virulent strains and other *Foc* isolates is 56%. In this ISSR analysis, it is very obvious

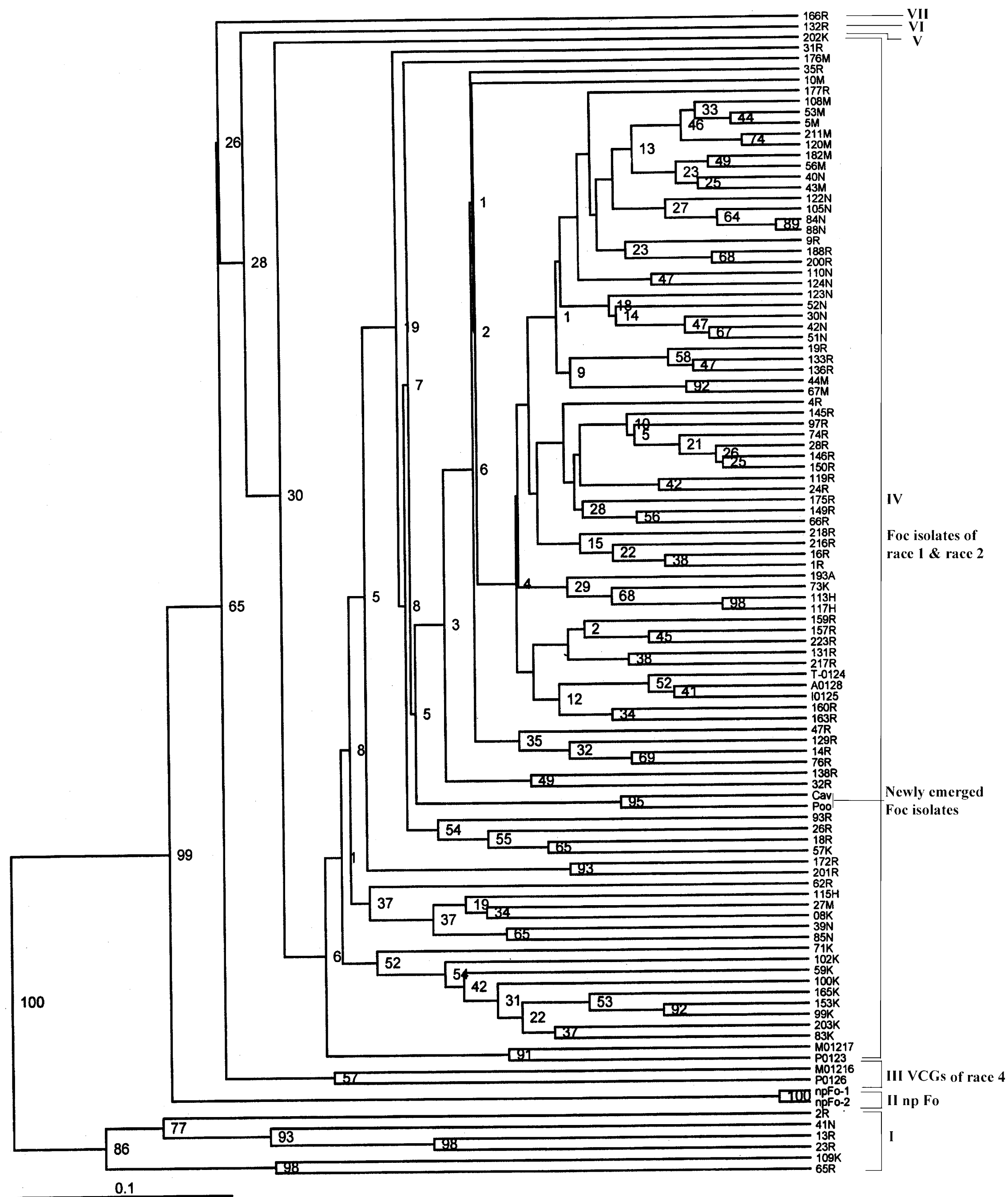

Fig. 2 Dendrogram based on ISSR polymorphisms for 107 isolates of *Fusarium oxysporum* f.sp. *cubense* by UPGMA cluster analysis. Sub-clusters: *I Foc* isolate each from cv. Neypoovan and Karpuravalli, and 4 *Foc* isolates from cv. Rasthali, *II* 2 np*Fo* isolates from cv. Dudhsagar, *III* VCG;s of Tropical race 4 (VCG 01216, VCG 0126), *IV* 94 *Foc* isolates from different cultivars & 5 VCG's of race 1 & race 2. *V* One *Foc* isolate from cv. Karpuravalli, *VI* One *Foc* isolate from cv. Rasthali, *VII* One *Foc* isolate from cv. Rasthali

that (i) majority of the *Foc* isolates of India (89 out of 94) which belong to race 1 and race 2 were grouped in one cluster (sub-cluster IV), (ii) the *Foc* isolates and VCGs of race1 and race 2 (Sub cluster IV) are clearly distinguished from the VCGs of race 4 (sub-cluster III), (iii) the pathogenic form of *Foc* was clustered separately from the non-pathogenic *Foc* (Sub cluster II), and (iv) the virulent *Foc* strains of cvs. Grand Naine (VCG 0124) and Poovan (VCG 0124/5) which belong to race 1, were grouped separately within the major cluster IV which contains majority of the *Foc* isolates of India which belong to race 1 and race 2 and also the VCGs of race 1 and race 2.

Discussion

Among the various diseases of banana, Fusarium wilt is becoming very serious threat to banana production in most of the banana growing regions of India. Although several management practices have been evolved from time to time, all these methods are not much effective except growing of resistant varieties and use of antagonistic microbes. Comparatively, these methods are becoming popular among the researchers and banana growers as these are found effective in managing the disease [11, 14–18]. But, to sustain the durable nature of resistant variety as well as effective bio-control strains, thorough knowledge and continuous monitoring of the genetic diversity of *Foc* strains present in a particular locality are very much essential. The recent survey conducted by National Research Centre for Banana, Thiruchirapalli, India revealed the emergence of new strains with high virulence within the race structure present in India [2]. Although the Fusarium wilt was recorded in India in 1911 in the state of West Bengal, no systematic and exhaustive research has so far been carried out to understand the genetic diversity within the pathogenic isolates of *Foc*. In 2008, an attempt was made by the senior author to characterize the *Foc* isolates of India by VCG analysis, using currently available nit-M testers (33nos) at QDPI, Australia. The results showed that out of 160 isolates of *Foc*, only 58 isolates have formed heterokaryon with any one of nit-M testers subjected. This indicated that the nit–M testers currently available are not exhaustive. Therefore, the present study was undertaken to characterize the Indian *Foc* isolates by molecular approach particularly by ISSR analysis as the method is very much useful to precisely measure the genetic changes, simple in operation, high stability and low cost compared to other markers such as RAPD, AFLP, DNA fingerprinting etc. [19, 20]. In the present study, totally 107 DNA samples of *Fusarium oxysporum* were subjected to genetic diversity analysis. Before carrying out the genetic diversity analysis, all the 98 isolates of *Foc* obtained from different banana growing regions of India were proved for their pathogenicity and identified the presence of only race 1 and race 2. Among the 98 *Foc* isolates, the *Foc* isolate from cv. Grand Naine (Cavendish-AAA) caused wilt disease in differentials of race 1, race 2, and race 4, but it was confirmed as VCG 0124 of race 1 and belongs to inodoratum group [2]. Similar observation of race 1 attacking race 4 differential host of Cavendish group of bananas has also been observed in Australia (Henderson Juliane, Plant pathologist DPI &F, Brisbane, Queensland, 2010 Pers. communication). For carrying out genetic diversity analysis of Indian *Foc* isolates by ISSR marker, 10 ISSR primer sets which generated more polymorphic bands compared to other primers were selected for further ISSR analysis. The genetic diversity analysis carried out with these primer sets for all the 107 *Foc* isolates indicated the presence of wide genetic variation among the Indian *Foc* isolates**.** Similar studies conducted to find out the genetic variation within the *Foc* isolates using different markers such as RFLP, rDNA-ITS RFLP and DNA fingerprinting by various researchers also indicated wide genetic variation among the *Foc* isolates [3, 21, 22]. In the present study, the cluster analysis obtained from the DNA fingerprinting of ISSR analysis conducted indicated the presence of six different genotypes within the Indian *Foc* isolates. The genetic similarity among the Indian *Foc* isolates was ranged from 27 to 100%. Besides, out of 98 Indian *Foc* isolates of race 1 and race 2 subjected, 90.8% of the isolates were grouped in one cluster along with the VCGs of race 1 and race 2. The genetic similarity of these majority of Indian *Foc* isolates was from 46–100%. Koenig et al. [21] analyzed 165 isolates of *Foc* of world wide collection for the genetic diversity by RFLP and found that there were 72 RFLP haplotypes and nearly half the isolates were represented by the five most common haplotypes. They have also observed that all isolates within the same VCG were in the same clade. Bentley et al. [3] studied genetic variation of 208 isolates of *Foc* which belong to 20 different VCGs and 133 isolates which do not belong to any of the reported VCGs of *Foc* by modified DNA amplification finger printing and found that there were totally 33 different genotypes and among which 19 genotypes were identified among the isolates that belonged to the 20 reported VCGs and 14 new genotypes were identified among the isolates that did not belong to any of the existing VCGs. Leong et al. [4] also studied the genetic diversity of *Foc* isolates of Malaysia by three methods such as enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), random amplified micro satellites (RAMS), and restriction fragment length polymorphism of intergenic spacer (RFLP-IGS). These three molecular techniques showed 35.7–100% genetic similarities among the *Foc* isolates. These techniques showed intraspecific variation and banding patterns generated using each technique were highly variable. They also found that out of 29 ERIC banding pattern identified among 59 isolates of *Foc* from different banana cultivars, none of the banding pattern could differentiate the *Foc* isolates according to cultivars. The IGS-RFLP conducted also could not differentiate the *Foc* isolates based on cultivars. Kumar et al. [23] characterized only six isolates of *Foc* from India by isozyme analyses and found the presence of three major groups indicating high genetic diversity. Leong et al. [22] conducted molecular characterization of 13 *Foc* isolates from Malaysia and three *Foc*

isolates from Indonesia by ERIC-PCR and ITS-RFLP. The cluster analysis of combined data showed that all the *Foc* isolates were grouped into two clusters with 42.9–100% similarity.

The phylogenetic analysis of this study also indicated the presence of wide genetic variation within each of the major cluster indicating the polyphyletic nature of the Indian *Foc* isolates. The polyphyletic origin of the *Foc* isolates have been previously indicated by other molecular methods such as RAPD [24], DNA fingerprinting analysis [3], and sequence analysis of TEF-1α gene [4]. In the present investigation, the ISSR marker has clearly distinguished the *Foc* isolates and VCGs of race 1 and race 2 from the VCG of race 4. Similarly, Groenewald et al. [25] identified seven genotypic groups from 35 isolates of *Foc* which belong to 16 different VCGs by high throughput AFLP's. This technique has clearly distinguished the VCGs of race 1 from VCGs of race 4.

Recently, the commercial cultivars Grand Naine (Cavendish-AAA) and Poovan (Mysore-AAB) which are being grown widely in India hitherto considered as resistant to Fusarium wilt disease probably because of absence of race 4 has succumb to Fusarium wilt attack. The characterization of *Foc* isolates obtained from these cultivars indicated that these *Foc* isolates belong to VCG 0124 and 0124/5, respectively, of race 1. As the *Foc* isolate of Cavendish was found to inflict disease in all the other cultivars as well, this isolate (VCG 0124) is considered as more virulent [2]. The present ISSR study has grouped these *Foc* isolates of cv. Grand Naine (VCG 0124) and Poovan (VCG 0124/5) separately within the major cluster IV which contains majority of Indian *Foc* isolates of race 1 and race 2 and also the VCGs of race 1 and race 2. Therefore, this study indicates that the ISSR analysis can also able to identify the emergence of new strains of *Foc* which would in turn be helpful for quarantine purposes. The present ISSR study has also clearly grouped the non-pathogenic *Foc* isolates separately (sub-cluster II) from the pathogenic *Foc* isolates of India.

To conclude, the ISSR analysis which has been conducted first time for *Foc* isolates of banana indicated the presence of six different genotypes in India and clearly distinguished the Indian *Foc* isolates based on the races it belongs to. Besides, this ISSR study has differentiated the VCG of tropical race 4 from the VCGs of race 1 and race 2, and also the non-pathogenic *Fusarium* from the pathogenic *Fusarium*. This study also demonstrated the discriminatory power of ISSR to clearly distinguish the emergence of new strains from the other *Foc* strains of India. All these characters emerged from this study would help in evolving effective management strategies including developing and deploying durable resistant cultivars and also for identifying effective bio-agents. Besides, this study is also useful for effective quarantine purposes both within and outside India.

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