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Molecular diversity in Indian isolates of *Fusarium oxysporum* f.sp. *lentis* inciting wilt disease in lentil (*Lens culinaris* Medik)

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For integrated management of wilt disease, foolproof knowledge about the genetic diversity among the prevalent isolates is very important. One hundred (100) isolates of *Fusarium oxysporum* f. sp. *lentis*, causing agent of vascular wilt in lentil were collected from different agro-climatic regions of India. Finally, fifteen distinct *F. oxysporum* f. sp. *lentis* isolates were selected for molecular characterization by three molecular markers. Twenty randomly amplified polymorphic DNA (RAPD) primers produced a total of 105 reproducible bands, out of which 81 (77.14%) were polymorphic and 24 (22.85%) were monomorphic. Nine simple sequence repeats (SSR) primer pairs amplified 21 alleles with 2.33 alleles per primer. Considerable length variations (561 to 668 bp) in rDNA regions were found and restriction digestion of amplified rDNA region produced forty eight different DNA bands. Three molecular markers revealed varying degree of genetic diversity in the selected isolates ranging from 54% in case of RAPD to up to 35% with ITS markers. Based on the coefficient of similarity, the isolates grouped into two major clusters in the dendrogram. Isolates from North Indian regions grouped in same cluster, whereas isolates from north east regions and eastern region fell in another cluster. This information will be helpful for pathologists and plant breeders to design effective resistance breeding programs in lentil taking into account the diversity in wilt pathogen.

Key words: Indian isolates, *Fusarium oxysporum f.sp. lentis*, vascular wilt, molecular diversity.

INTRODUCTION

Lentil (*Lens culinaris* Medikus sub sp. *culinaris*) was among the first crops domesticated and has become an important food legume crop in the farming and food systems of many countries globally (Sarker and Erskine, 2006). Among the diseases, *Fusarium* wilt caused by pathogenic fungi *Fusarium oxysporum* f.sp. *lentis* is the most important biological constraints to productivity of lentil worldwide (Bhalla et al., 1992). No physiological races of this pathogen have been reported so far. Although, *F. oxysporum* f.sp. *lentis* isolates exhibit great variability in morphology and aggressiveness (Abbas,

1995; Belabid et al., 2004), *Fusarium* vascular wilt disease can be managed by the use of resistance cultivars (Jalali and Chand, 1992) and for the development of resistant cultivars, knowledge about the existing variability within the pathogen is a pre requisite.

Accurate and rapid identification of pathogens is necessary for appropriate management of plant diseases. Identification of *Fusarium* spp. by morphological characters like size, shape of conidia and pigmentation are highly variable as all these characters are influenced by nutritional composition of the medium and cultural conditions. However, DNA-based techniques have increasingly become the tool of choice for understanding the genetic diversity and also phylogeny relationships of *Fusarium* spp. numerous workers have already worked on molecular variation in *Fusarium* spp. (O'Donnell, 2000).

RAPD analysis has been applied widely in the detection and genetic characterization of phytopathogenicy fungi, including race differentiation in several formae

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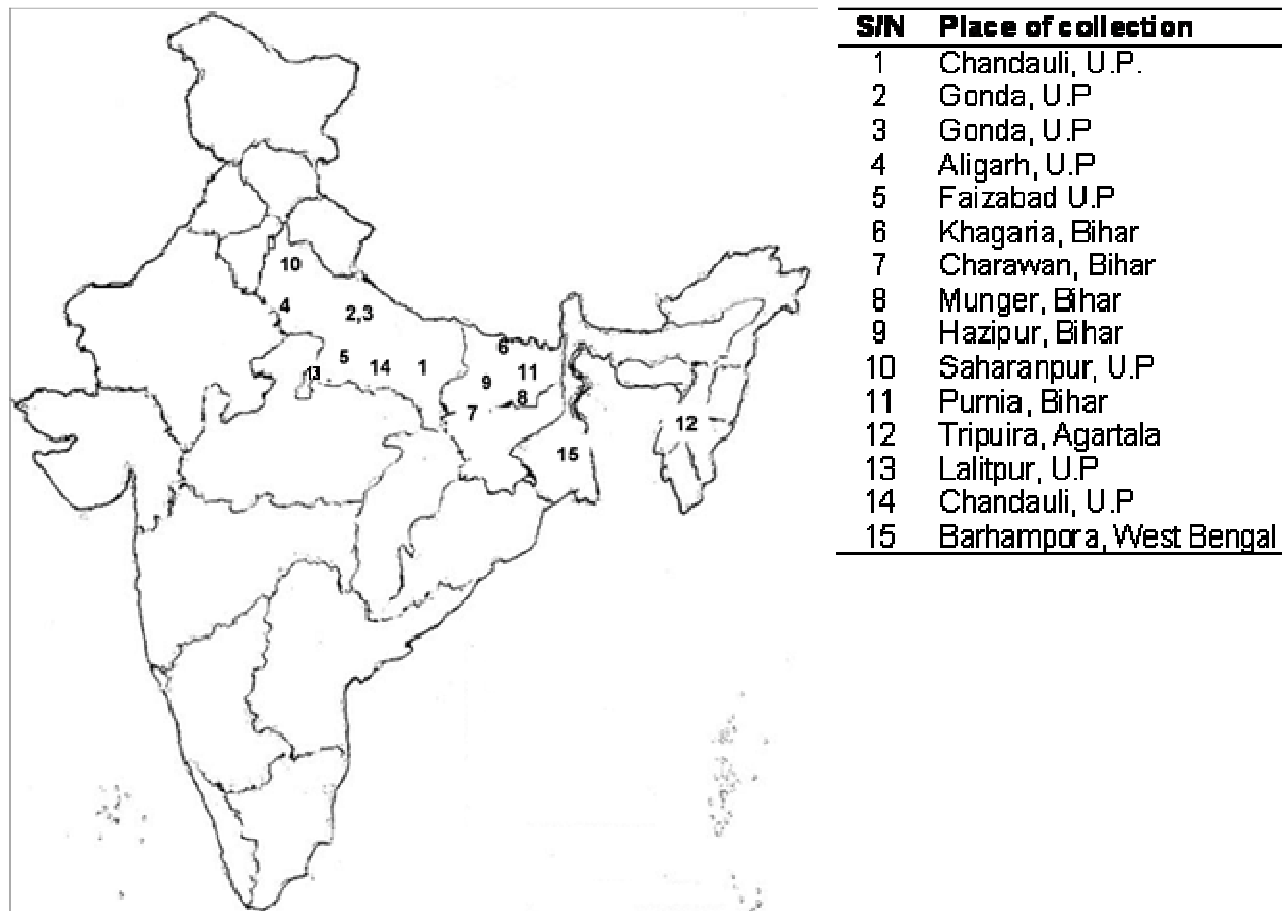| S/N	Place of collection
1	Chandauli, U.P.
2	Gonda, U.P
3	Gonda, U.P
4	Aligarh, U.P
5	Faizabad U.P
6	Khagaria, Bihar
7	Charawan, Bihar
8	Munger, Bihar
9	Hazipur, Bihar
10	Saharanpur, U.P
11	Purnia, Bihar
12	Tripuira, Agartala
13	Lalitpur, U.P
14	Chandauli, U.P
15	Barhampora, West Bengal

Figure 1. Map of India showing areas of collection of isolates of *F. oxysporum* f. sp. *lentis* from different regions.

speciales *of F. oxysporum*, such as *F. oxysporum* f.sp. *cubense* (Bentley et al., 1995). RAPDs applied to the carnation wilt pathogen *F. oxysporum* f. sp. *dianthi* and they were able to identify specific band patterns that were subsequently used as probes to distinguish between races of the pathogen (Manulis et al., 1994).

Simple sequence repeat (SSR) markers amplify small fragments of DNA in which motifs of 1 - 6 bases occur in tandem repeats. SSRs provides a powerful tool for taxonomic and population genetic studies (Britz et al., 2002). Alleles vary according to the number of repeat units present but other mutations have also been shown to be responsible for allele length variation in SSR analysis (Burgess et al., 2001; Slippers et al., 2004b). Microsatellites have been used to study polymorphism of several plant pathogenic fungi including *S. sclerotiomum* (Sirjusingh and Kohn, 2001) and *R. solani* (Mwang`Ombe et al., 2007).

Regions of ribosomal DNA (rDNA) also have been used in diversity and phylogenetic studies of several *Fusarium* spp. (Alves-Santos et al., 2002). These regions are highly conserved and can easily be investigated using PCR amplification. Out of various regions of rDNA, the internal transcribed spacer (ITS) and intergenic

spacer (IGS) of the nuclear rDNA repeat units have been reported to be evolved fast and may vary among species within a genus or among populations and hence can be used for phylogenetic studies at these taxonomic levels (O'Donnell, 2000). Molecular phylogenetic analyses have helped to clarify ambiguities in traditional classification systems of *Fusarium* species by ITS marker (LoBuglio et al., 1993).

This study was undertaken to assess the genetic variation among vascular wilt pathogen of lentil in India. The use of RAPD, SSR and ITS-RFLP markers along with morphological and pathogenic data for characterization of the *Fol* isolates could greatly enhance the understanding of the variability within this important fungus.

MATERIALS AND METHODS

Fungal Isolates and pathogenicity test

One hundred (100) representative of different morphological and cultural group of *Fol* isolates were collected from wilt infested lentil plants from India during 2006 - 2007 (Figure 1). These *Fol* isolates were multiplied on sand- maize meal medium in the glass house

Table 1. List of *Fol* isolates indicating their location, mycelium characteristics and effect of isolates on lentil variety Vidhokar local in artificially infected soil under glass house conditions.

S/N	Isolate	Host	Mycelium characteristic	Place of collection	Pathogenicity
1	*Fol* 83	Lentil	Pigmented, greenish-black mycelia	Chandauli, U.P., India	High
2	*Fol* 95	Lentil	Non pigmented, white mycelia	Gonda, U.P., India	Moderate
3	*Fol* 100	Lentil	Pigmented, greenish-black mycelia	Gonda, U.P., , India	Moderate
4	*Fol* 115	Lentil	Pigmented, greenish-black mycelia	Aligarh, U.P., India	High
5	*Fol* 137	Lentil	Non pigmented, white mycelia	Faizabad U.P., India	Moderate
6	*Fol* 140	Lentil	Non pigmented, white mycelia	Khagaria, Bihar, India	Moderate
7	*Fol* 141	Lentil	Pigmented, greenish-black mycelia	Charawan, Bihar, India	High
8	*Fol* 156	Lentil	Pigmented, greenish-black mycelia	Munger, Bihar, India	High
9	*Fol* 167	Lentil	Non pigmented, white mycelia	Hazipur, Bihar, India	High
10	*Fol* 208	Lentil	Pigmented, greenish-black mycelia	Saharanpur, U.P., India	Moderate
11	*Fol* 235	Lentil	Non pigmented, white mycelia	Purnia, Bihar, India	Moderate
12	*Fol* 242	Lentil	Non pigmented, white mycelia	Tripuira, Agartala, India	Moderate
13	*Fol* 248	Lentil	Non pigmented, white mycelia	Lalitpur, U.P., India	Moderate
14	*Fol* 262	Lentil	Pigmented, greenish-black mycelia	Chandauli, U.P., India	Moderate
15	*Fol* 265	Lentil	Pigmented, greenish-black mycelia	Barhampora, West Bengal, India	Moderate

condition in completely randomized design. The isolates were identified according to the identification key of *F. oxysporum* (Nelson et al., 1983) and their pathogenicity was tested on wilt susceptible cultivar Vidhokar local under artificially inoculated condition. Lentil plants were grown in pots containing sand-maize meal and 10% w/w fungus inoculums (Miller, 1946). Control plants were grown in a comparable mixture of non-infested sand, sand-maize meal medium with autoclaved soil. After emergence of seedlings, progression of disease was recorded and expressed as percentage (%) wilting. Isolates which caused > 50% wilt incidence were scored as highly pathogenic and which caused 30 - 50% wilt was scored as moderately pathogenic isolates.

Genomic DNA Isolation and RAPD Analysis

Genomic DNA was isolated from single spore culture of each isolates (Murray and Thompson, 1980). DNA concentration and purity were measured using a spectrophotometer (BioRad Smart-spec Plus) at 260 nm and 280 nm. For RAPD analysis, 20 random primers (17 primer of 10 mer, 1 primer of 17 mer and 2 primers of 20 mer) were selected after screening of 100 RAPD primers (Table 2). Amplification was performed in a 25 µl reaction volume containing buffer (10 mM Tris-HCl pH 8.0), 0.2 mM of each dNTPs, 0.6 U of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., India), 1 µl of RAPD primer (15 pmol) and 25 ng of template DNA. Amplification was performed using Biometra thermal cycler (USA), programmed for initial denaturation at 94°C for 2 min and 45 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 5 min. The amplification was completed with a 5 min final extension at 72°C. Amplified products was resolved in 1.5% agarose gel at 60 V cm^{-1} using in tris boric acid EDTA (1X TBE) buffer.

Amplification of DNA with SSR Primers

A total of 9 SSR primers were synthesized from Operon Technologies, USA (Table 2b) and used for the amplification of individual microsatellite loci (Bogale et al., 2005). PCR amplification was performed in 25 µl total volume containing 2.5 µl of 10X *Taq* Buffer, 0.2 mM of dNTPs mix (100 mM of each dNTPs), 1 µl of each forward and reversed primer (25 ng each primer), 0.6 U of *Taq* polymerase with 25 ng of template DNA. PCR conditions for SSR were as follows; the PCR programme had one initial denaturation step at 94°C for 4 min followed by 35 cycles of 94°C for 30 s, annealing for 30 s (appropriate annealing temperature were used for each primers set, Table 2a). The thermal cycles were terminated by a final extension of 10 min at 72°C. Amplified products were resolved in 2.0% agarose gel @ 60 V cm^{-1} using 1X TBE buffer.

rDNA region amplification and PCR-RFLP analysis

An internal transcribed spacer (ITS) region of rDNA was amplified using the primers ITS-1(5`TCCGTAGGTGAACCTGCGG3`) and ITS-4 (5`TCCTCCGCTTTATTGATATG3`) according to White et al. (1990). Amplification was performed in total volume of 50 µl containing 0.7 U *Taq* DNA polymerase, 0.2 mM each dNTPs, 1 µl of each ITS-1 and ITS-4 (25 pmol) primers and approximately 50 ng template genomic DNA with the following condition; an initial denturation for 1 min at 94°C followed by 30 s denaturation at 94°C, 30 s annealing at 55°C, 30 s elongation at 72°C repeated 31 times with the final elongation step of 5 min at 72°C. The PCR products were precipitated by adding 0.1 volume of 3 M sodium acetate and 2 volume of absolute ethanol to remove excess dNTPs and primers and resuspended in sterilized distilled water. Five restriction enzymes; *Eco*RI, *Eco*RV, *Sma*I, *Pst*I and *Hind*III were used to digest the amplified products according to manufacturers instruction. The restricted fragments were electrophoreses on 2.5% gel in 1X TBE buffer at 60 V cm^{-1}. The molecular size of each fragments were estimated using a standard curve of migration verses the log of molecular size of the 100 bp ladder and λ DNA cut with *Eco*RI + *Hind*III (MBI Fermentas, USA). Agarose gel were stained with ethidium bromide (0.5 µg/ml) and photographed under UV light using gel documentation system (Alpha Digi Doc).

Analysis of RAPD, SSR and ITS-RFLP data

All amplification reactions were repeated at least thrice and only clear and reproducible bands were scored. The polymorphic DNA bands were scored as binary digit code of "0" (for absence) and "1"

Table 2a. RAPD primers used in this study and % polymorphism detected by 20 selected primers.

Primer	Sequence	Monomorphic band	Polymorphic band	Total number of band	% Polymorphism	Size range of band (bp)
K_1	5' TGCGTGCTTG 3'	1	6	7	85.71	300 - 2635
K_2	5'ACTTCGCCAC 3'	1	3	4	75.00	300 - 1264
K_3	5' GGCTCATGTG 3'	1	4	5	80.00	700 - 2400
K_4	5' CAAACGTGGG 3'	2	4	6	66.67	300 - 1600
K_5	5'CGAGGTCGACGGTATCG3'	2	2	4	50.00	300 - 1000
K_6	5'CACCGCCCCAAAATGGCCAC 3'	1	4	5	80.00	550 - 2300
K_7	5'GTCCTCAGTCCCCCAATCCC 3'	2	3	5	60.00	500 - 2900
P_1	5' CGTTGGATGC 3'	1	4	5	80.00	500 - 2350
P_2	5' TACGGCTGGC 3'	1	5	6	73.33	350 - 1750
P_3	5' GCGGCATTGT 3'	1	6	7	85.71	400 - 2200
P_8	5' CAGGCCCTTC 3'	2	4	6	66.66	450 - 2200
P_{17}	5' TACGGCTGGC 3'	2	2	4	50.00	430 - 1000
P_{19}	5' GCGGCATTGT 3'	1	3	4	75.00	350 - 2150
P_{21}	5' CCAGACAAGC 3'	1	3	4	75.00	300 - 1400
OPD 11	5'AGCGCCATTG 3'	1	3	4	75.00	700 - 2700
OPD 16	5' AGGGCGTAAG 3'	1	5	6	83.33	750 - 2400
OPA 11	5' CAATCGCCGT 3'	0	4	4	100.00	700 - 2700
OPF 01	5'ACGGATCCTG3'	1	5	6	83.33	450 - 1500
OPF 05	5'CCGAATTCCC 3'	1	4	5	80.00	350 - 1100
OPI 07	5'CAGCGACAAG3	1	2	4	50.00	600 - 2200
Total		24	81	105	Average % polymorphism 65.82	

(for presence), respectively. Genetic similarity between pairs was estimated using Jaccard`s similarity coefficient with the SIMQUAL option. Data were used for similarity based analysis using the NTSYS-PC (version 2.02) software. Similarity coefficients were used for the construction of UPGMA (Unweighted Pair Group Method with Arithmetic Average) dendrogram (Rolhf, 1990). The polymorphic information content (PIC) value of SSR markers were calculated using the formula:

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

Where, k is the total number of alleles detected for a microsatellite; Pi is the frequency of the ith allele in the set. The genetic variation was measured in terms of genetic diversity and was computed by averaging PIC estimates over all loci (Nei and Li, 1979).

RESULTS

Fifteen distinct *Fol* isolates were selected for molecular characterization. All pathogenic isolates were pigmented with greenish black mycelia, while one pathogenic isolate *Fol* 167 was non-pigmented, with white mycelia. Two moderately virulent isolates (*Fol* 262 and *Fol* 265) were pigmented with greenish black mycelia, while all other moderate isolates were white in colour (Table 1). Isolates

Fol 83, *Fol* 141, *Fol* 156 and *Fol* 167 were more virulent caused > 50% wilt incidence and remaining ten isolates were moderately pathogenic caused 30 - 50% wilt.

RAPD analysis

By using 20 RAPD primers, a total of 105 reproducible bands were amplified, out of which 81 (77.41%) were polymorphic and 24 (22.85%) were monomorphic. On an average of 4.37 bands per primer were amplified and size of bands were varied from 0.3 to 3.0 kb. Primers K_1, P_3, OPF and OPA-11 were the most informative (Figure 2a). A dendrogram based on UPGMA analysis indicated that 15 isolates formed two major clusters (Figure 3a). Major cluster 'A' comprised of eight isolates from central north region and one isolates (*Fol* 140) from north region. Maximum similarity of 72% was found in between *Fol* 95 and *Fol* 183, both from central north region. In the cluster A, isolate *Fol* 100 was most dissimilar from other seven isolates. The second cluster 'B' comprised of seven isolates from north and north east regions. Isolates *Fol* 141 and *Fol* 167 showed maximum similarity (67%) in the cluster B. Genetic similarity coefficient among the 15 isolates of *Fol* varied from 0.28 - 0.72 with RAPD

Table 2b. Allele amplification of 15 *F. oxysporum* f. sp. *lentis* with nine SSR primers.

Primer	Repeat motif	Primer sequence (5`-3`)	Tm (°C)	Number of alleles	Amplified alleles size (bp)	PIC value
MB 2	$(GT)_{11}(GA)_6$	TGCTGTGTATGGATGGATGG CATGGTCGATAGCT	57	2	250, 225	0.780
MB 5	$(TG)_9$	ACTTGGAGAAATGGGCTTC GGATGGAGTTTAATAAATCTGG	54	2	150,100	0.633
MB 9	$(CA)_9$	TGGCTGGGATACTGTGTAATTG TTAGCTTCAGCCCTTTGG	51	2	175, 150	0.818
MB 10	$(AAC)_6$	TATCGAGTCCGGCTTCCAGAAC TTGCAATTACCTCCGATCCAC	48	1	300	0.966
MB 11	$(GGC)_7$	GTGGACGAACACCTGCATC AGATCCTCCACCTCCACCTC	68	5	500, 400, 300, 225, 150	0.918
MB 13	$(CTTGGAAGTGGTAGCGG)_{14}$	GGAGGATGAGCTCGATGAAG CTAAGCCTGCTACACCCTCG	68	5	500, 350, 300, 250, 150	0.935
MB 14	$(CCA)_5$	CGTCTCTGAACCACCTTCATC TTCCTCCGTCCATCCTGAC	57	1	700	0.923
MB 17	$(CA)_{21}$	ACTGATTCACCGATCCTTGG GCTGGCCTGACTTGTTATTCG	57	1	325	0.876
MB 18	$(CAACA)_6$	GGTAGGAAATGACGAAGCTGAC TGAGCACTCTAGCACTCCAAAC	57	2	300, 275	0.802
Mean PIC value						0.850

markers.

SSR analysis

The SSR markers developed by Bogale et al. (2005) for species of *F. oxysporum* complex were tried against Indian isolates of *Fol* and all primers showed good polymorphism. Nine SSR primers were amplified a total of 21 alleles. Maximum of 5 alleles were amplified by primer pairs of MB 11 and MB 13 followed by 2 alleles from MB 2, MB 5, MB 9 and MB 18 (Figure 2b). Size of the amplified alleles varied between 100 and 850 bp. On an average, 2.33 alleles per locus were amplified by the *Fol* population. The mean number of PIC value of SSR primer pairs in this study was 0.80 (Table 2b). The SSR primers clustered all 15 *Fol*
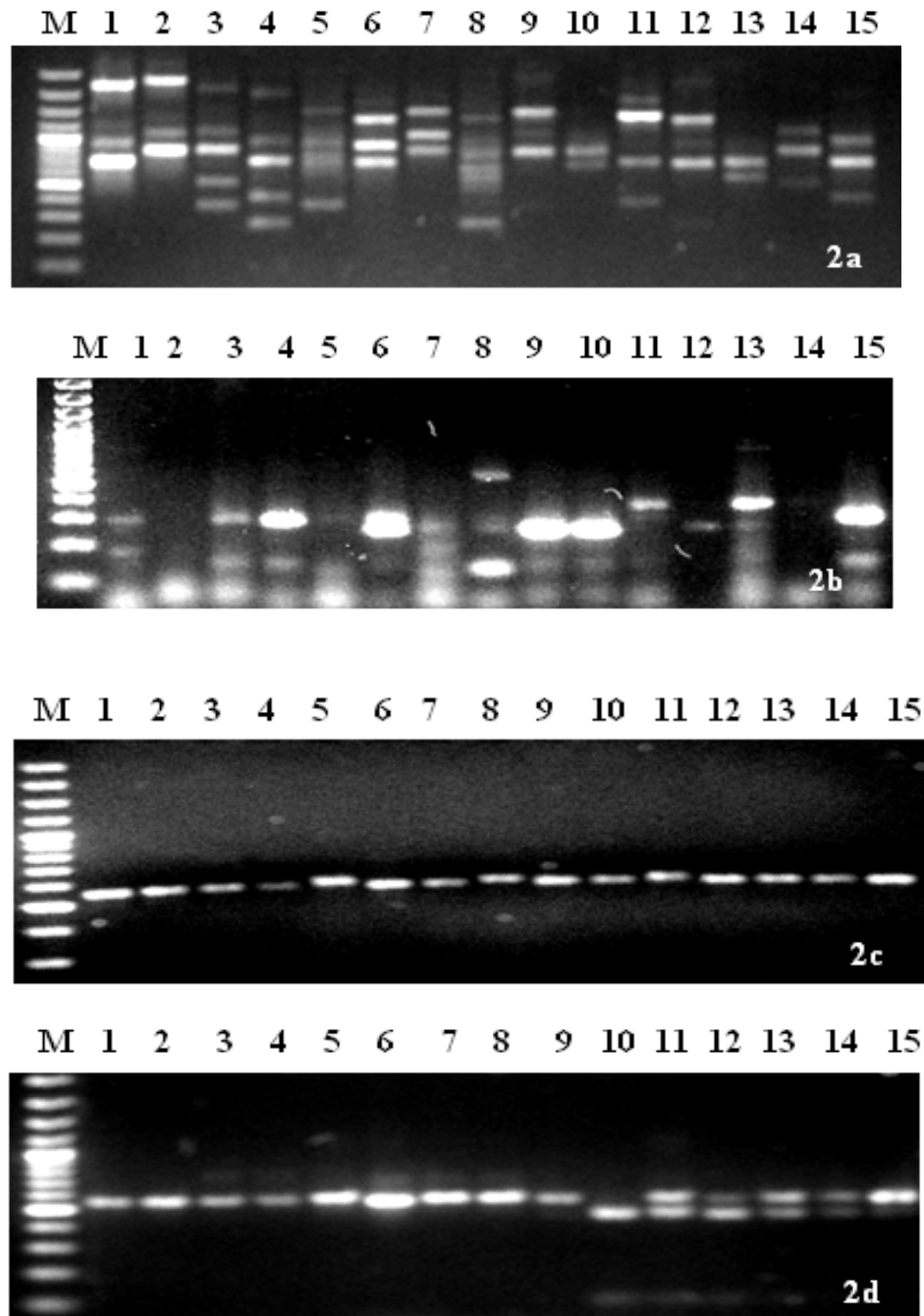
Figure 2. (A) Random amplified DNA polymorphisms of *Fol* isolates with random primer OPA-11; **(B)** Simple sequence repeats polymorphisms of *Fol* isolates with MB 13 primer pair; **(c)** Internal transcribed spacer region (ITS) amplification of *Fol* isolates; **(D)** Restriction digestion of rDNA region of *Fol* isolates with *Eco*RI restriction enzyme. Lanes 1 – 15: *Fol* isolates (ordered as per Table 1). M: Molecular weight marker of 100 bp ladder (Fermentas).

isolates into two main clusters (Figure 3b). In the cluster A₁, the maximum similarity (73%) was found in between two isolates from central north region of India, *Fol* 83 and *Fol* 100. The second group B₁ subdivided into two sub groups. In the first sub group, three isolates *Fol* 140, *Fol*
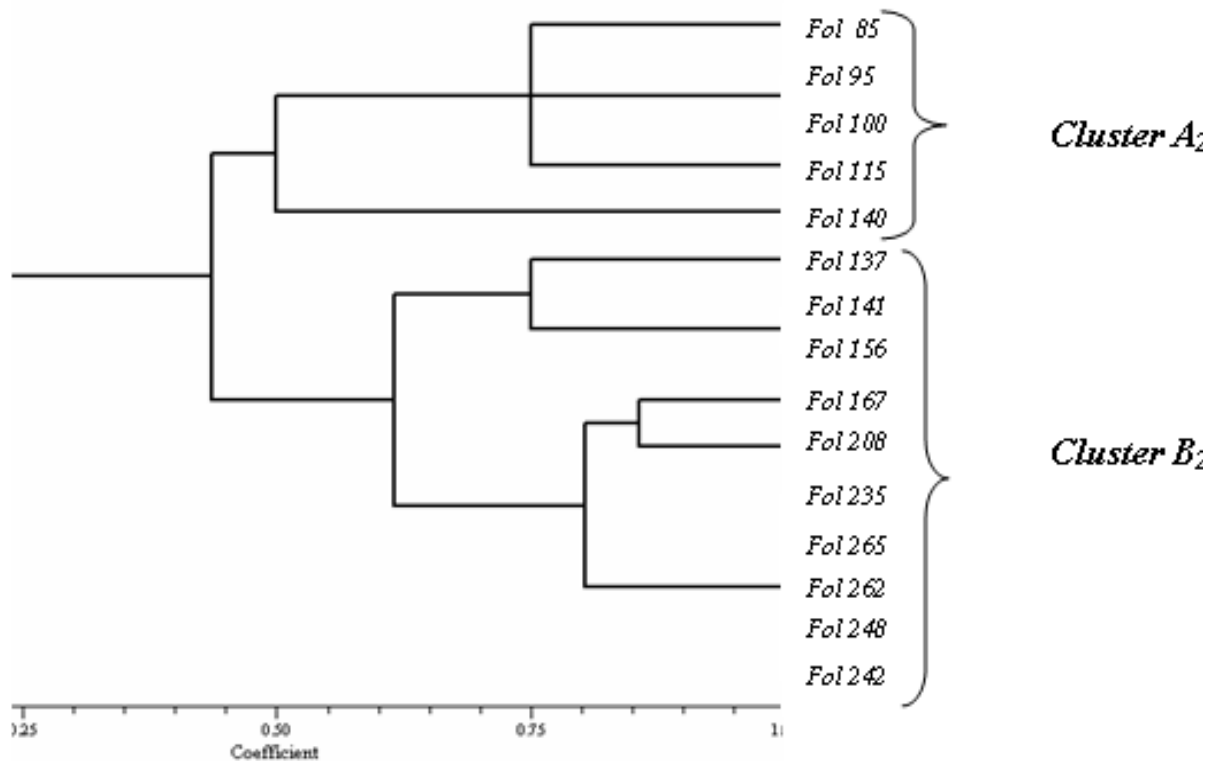
235 and *Fol* 265 separated in one cluster with 58% similarity. The second sub group consisted of virulent isolates, *Fol* 141, *Fol* 156 and *Fol* 242 with 55% similarity. Genetic similarity coefficient among the 15 isolates of *Fol* varied from 0.26 - 0.73 with SSR markers. The highly
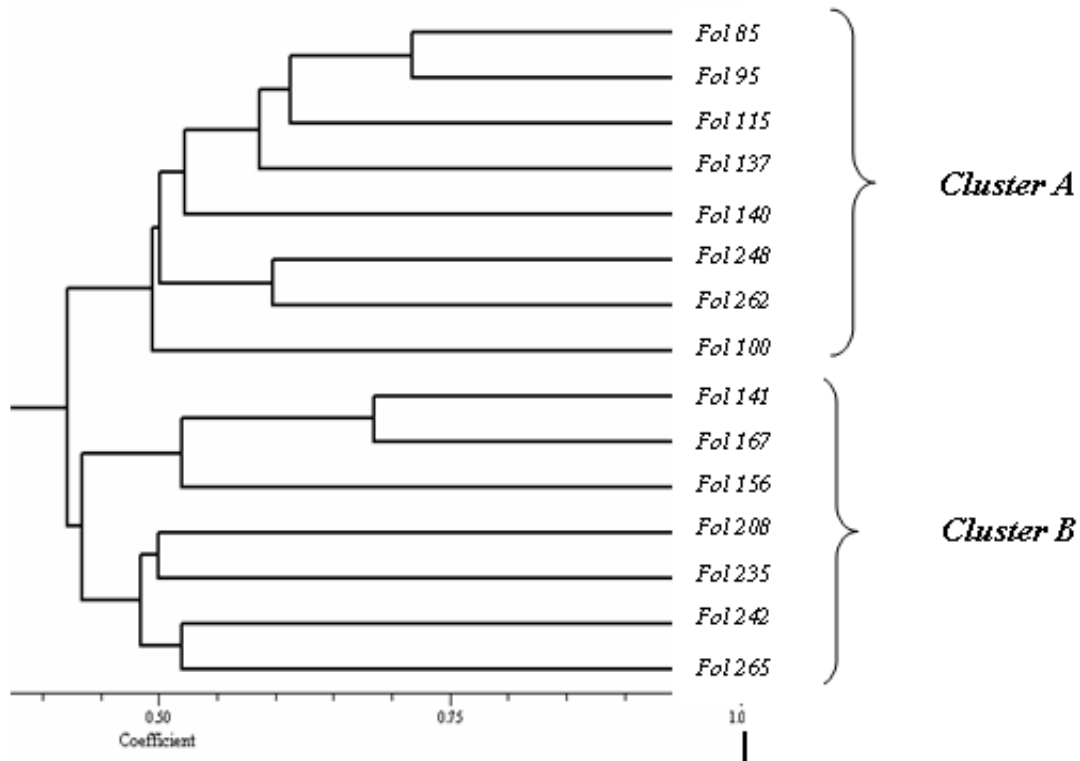
A
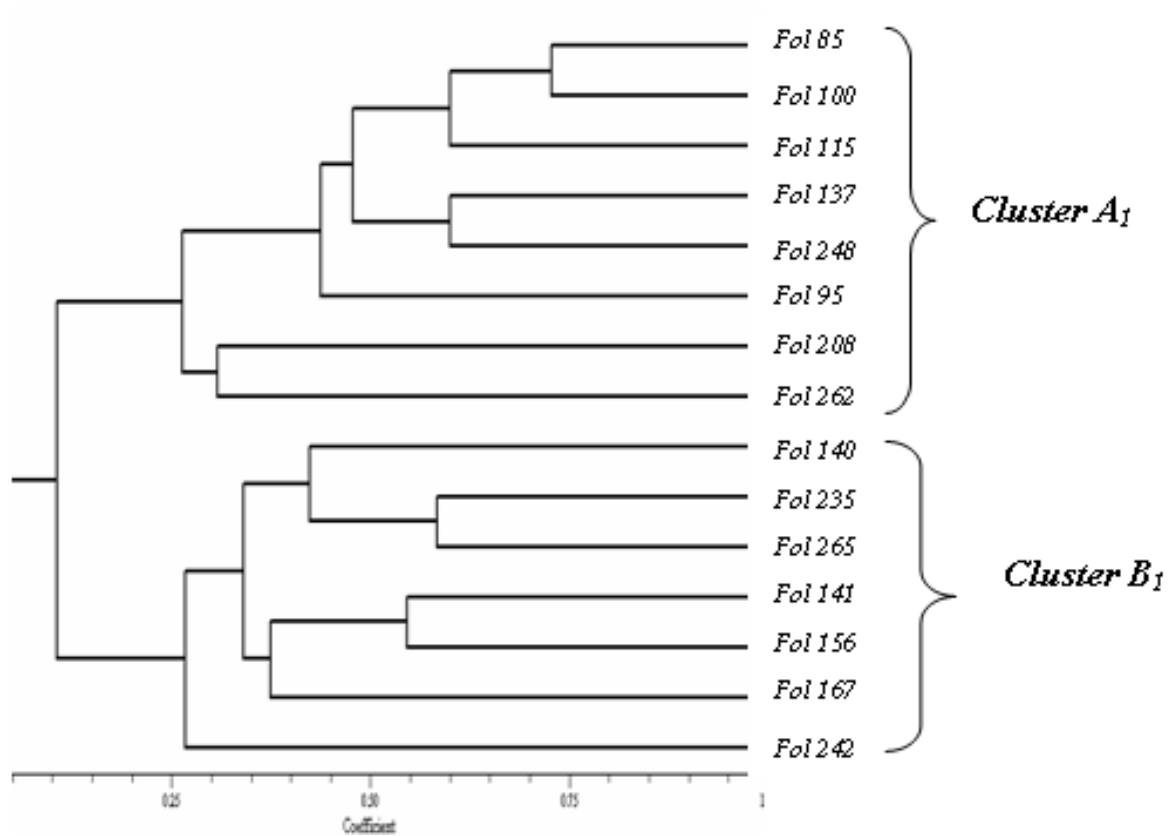
B

c

Figure 3. (a) UPGMA dendrogram showing relationship among 15 isolates *Fol* based on RAPD markers; (b) UPGMA dendrogram showing relationship among 15 isolates *Fol* based on SSR markers; (c) UPGMA dendrogram showing relationship among 15 isolates *Fol* based on PCR-RFLP of the rDNA ITS region.

pathogenic isolates *Fol* 161, *Fol* 167 and *Fol* 156 were grouped in one clad in clusterA_1 and B_1.

rDNA region amplification and PCR-RFLP analysis

ITS-1 and ITS-4 primers amplified a single band ranging from 561 to 668 bp (Figure 2c). The amplified ITS region was restricted with the five different hexa cutter restriction enzyme and only two restriction enzymes, *Eco*RI and *Hind*III were cleaved in ITS region (Figure 2d). These two enzymes revealed the extensive polymorphism in ITS regions in three isolates *Fol* 140, *Fol* 167 and *Fol* 208. With the enzyme *Eco*RI, all isolates showed three bands, which ranged 668 - 242 bp. Restriction enzymes *Hind*III could also detect variation in the restriction sites in another isolates *Fol* 208, *Fol* 235, *Fol* 242, *Fol* 248 and *Fol* 262, as these isolates were restricted and others were not. The dendrogram constructed from ITS-RFLP data also grouped all 15 isolates in two major clusters with 65.49% of average similarity (Figure 3c).

In cluster A_2, almost all isolates from north central region were clustered and others were in cluster B_2. The dendrogram also supported the restriction digestion data of the isolates *Fol* 140, *Fol* 167 and *Fol* 208, which showed the different banding patterns by PCR-RFLP patterns and were separated from all other isolates.

DISCUSSION

The prevalence of different *Fol* isolates in lentil growing regions makes it essential to identify region specific pathogen to devise strategies for conferring resistance against them in the respective agro-climatic regions. RAPD markers grouped the *Fol* isolates into two major groups based on their geographical location as earlier reported by Belabid et al. (2004). Highly pathogenic isolates, *Fol* 83 and *Fol* 115, clustered in one cluster, while the remaining three isolates, *Fol* 141, *Fol* 156 and *Fol* 167, grouped in second subcluster. Similar studies on other plant pathogenic fungi have emphasized the importance of molecular approaches to characterize genetic diversity within and between isolates (Sivaramakrishnan et al., 2002; Bentley et al., 1995). The pathogen can be divided in two genetic subpopulations and can be characterized by a low polymorphism in same subgroups subpopulations. Similar results were also obtained with *F. oxysporum* f. sp. *erythroxyli* and by *F. oxysporum* f. sp. *lentis* (Belabid et al., 2004). By SSR

markers, different allele sizes were recorded in all the loci in the genome of *Fol*, the smallest allele size was detected as 100 bp and the largest one was 850 bp. The polymorphic character of SSRs produces highly discri-minating fingerprints that often allow characterization of fungi at a strain level (Migheli et al., 1998; Barre`s et al., 2006). The variable numbers of alleles per loci is an indication of high level of polymorphism and was reported by Mwang`Ombe et al. (2007) in Kenyan *R. solani* isolates. The mean number of PIC value of SSR markers in this study was 0.80. Primer MB-10 gave maximum PIC value with 0.97, whereas MB-5 gave minimum PIC value of 0.63. This PIC value indicates that the Indian isolates had a high degree of biodiversity. Polymorphism has been observed in other fungi as a direct record of genetic evolution (Sanders, 2002; Schardl and Craven, 2003). SSR markers placed all the *Fol* isolates in two major groups which were separated from 27% of genetic simi-larity. Earlier, Dubey and Singh (2008) reported the clus-tering of 64 *F. oxysporum* f. sp. *ciceris* isolates into three categories at 25% genetic similarity and into two major categories at 30% genetic similarity with ISSR and RAPD markers. Thus, they were found suitable for the study of genetic diversity in the pathogen. MB 13, MB 11 and MB 05 were given the maximum allelic variation in *Fol* isola-tes. Earlier, MB 05, MB 14 and MB 17 were also given the good allelic variation in *F. oxysporum* f. sp. *ciceris* isolates from Indian isolates (Dubey and Singh, 2008).

ITS-RFLP analysis has been used extensively to distinguish many *Fusaium* species and remains an important tool for species identification in ECM fungal communities (Joshi et al., 2006). Isolates from central north region grouped in first cluster A$_2$, except four isolates *Fol* 137, *Fol* 208, *Fol* 248 and *Fol* 262 which did not group in first cluster. All isolates from north east regions grouped in the second major cluster B$_2$. The dendrogram constructed from ITS-RFLP data also grouped all 15 isolates in two major clusters with 65.49% of average similarity.

Considerable variability was also found in some isolates belonging to the same agro climatic regions like two isolates (*Fol* 137 and *Fol* 208) from north India. All the three molecular techniques used in this study separated the isolates into two major clusters. Similar results were also reported in *F. oxysporum* f.sp. *phaseoli* by Woo et al. (1996) and in *F. oxysporum* f. sp. *ciceris* by Jimenez-Gasco et al. (2001).

The three methods provided similar resolution, a-though, there were differences in the distribution of iso-lates and the ratio of genetic similarity in the dendrogram. Similar findings have also been reported in the Ethiopian *F. oxysporum* isolates by AFLP, SSR and ITS sequence analysis by Bogale et al. (2006). Genetic variation in *Fol* detected by molecular marker indicates the ability of a pathogen to adapt to different life cycle condition according to different climatic regions, cultural practices and crop rotation. This has far reaching consequences on

breeding programs. Resistance genes against all major pathogenic races should be pyramided according to their agro climatic regions for effective management of the wilt disease.

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