



## Genetic variability of *Fusarium* wilt pathogen isolates of chickpea (*Cicer arietinum* L.) assessed by molecular markers

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### Abstract

Genetic variability among 43 isolates of *Fusarium oxysporum* f.sp. *ciceri*, the chickpea wilt pathogen, collected from nine states of India including the four well-characterized races of the pathogen were assessed using the molecular markers, RAPDs and AFLP. Principal coordinate analysis of the similarity index data generated from the molecular marker studies mostly gave three different clusters: Of these two clusters represented race-1 and race-2, and the third cluster consisted of race-3 and race-4 pathogen isolates. In RAPDs a fourth cluster was seen which did not go with any of the four races of the pathogen. The molecular markers established the distinctness of race-1 and race-2 pathogen isolates and the close similarity of pathogen isolates of race-3 with that of race-4. AFLP was found to be more informative as it differentiated more number of the pathogen isolates with the known races with minimum of outliers. The high levels of DNA polymorphism observed with the molecular markers suggest the rapid evolution of new recombinants of the pathogen in the chickpea growing fields.

**Key words:** AFLP, chickpea, *Fusarium oxysporum*, genetic diversity, RAPDs

### Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes in many countries of Asia and Africa, cultivated mostly by the poor and subsistence farmers. In addition to its importance as a food crop, it is valued for its beneficial effects in improving soil fertility and thus sustainability and profitability of production systems. Yields have stagnated in comparison with other food crops, especially cereals. The major constraints to increased and stable yields are *Fusarium* wilt (*Fusarium oxysporum* f.sp. *ciceri*), *Ascochyta* blight (*Ascochyta rabiei*), dry root rot (*Rhizactonia bataticola*), *Botrytis* gray mold (*Botrytis cinerea*), *Helicoverpa* pod borer, drought, chilling temperature and soil salinity. Annual losses of 10–15% have been reported from the chickpea growing areas in these continents due to *Fusarium* wilt alone [1]. The causative agent for wilt is the seed and soil-borne fungal pathogen *Fusarium oxysporum* f.sp. *ciceri*. Several legumes are carriers of the fungus and in the absence of the

host, the fungus can survive in the soil up to 6 years [2]. The variability in the pathogen population in the chickpea growing areas pose difficulties in deploying stable varieties of chickpea as these succumb to newly evolving pathogenic races. Of the seven races of the pathogen identified, four have been reported in India [3]. To reduce the effects caused by the variability in the pathogen it is essential to know the genetic nature and pathogenic types of isolates prevailing in the different chickpea growing areas and deploy varieties that are able to resist many races. This lack of characterization is sufficiently large that 25 years of breeding for resistance have had only marginal success. Resistance is known to be variable regionally, and therefore new breeding material has to be tested in expensive multilocational trials. Fundamental information on pathogen population biology is essential to the design of an effective resistance breeding program and reduce the heavy losses inflicted by this disease.

The identification of pathogenic fungal races has been mostly by the use of differential reaction to se-

lected host genotypes. In the recent years, several biotechnological tools like RAPDs, RFLP, and SSR have been increasingly used to study the variability in the pathogenic populations especially in *Fusarium oxysporum* causing diseases in different crop species [4, 5]. RFLP (restriction fragment length polymorphism), using mitochondrial DNA has been used to detect the divergence among fungal isolates [6]. Both DNA hybridization-based markers like RFLP, SSR (simple sequence repeats) and PCR-based markers like RAPDs (random amplified polymorphic DNA), ITS (internal transcribed spacers) of rDNA, are increasingly used to study the variability in the bacterial and fungal populations [4, 7–12]. Amplified fragment length polymorphism (AFLP), which combines both the restriction digestion and PCR-based methods have been found to be a better method of detecting genetic variability among fungal and bacterial isolates [13–15]. Technical advantages are its reproducibility, high resolution due to use of stringent PCR conditions and portrayal on polyacrylamide gels. Without prior knowledge of genomic sequences, it can differentiate highly related strains in accordance to existing taxonomic data [16].

No molecular work has been carried out in the characterization of the *Fusarium* wilt pathogen of chickpea. Though some SSRs have been isolated from a genomic library of *Fusarium oxysporum* f.sp. *ciceri* they are not able to identify the races clearly [17]. The objective of the present study is to assess the genetic variability among the 43 *Fusarium* wilt pathogen isolates collected from nine chickpea growing states of India using RAPDs, and AFLP and relate with the four known prevalent races of the pathogen. This would enable us to give a pathogen map of the isolates prevalent in the chickpea growing regions of India.

## Materials and methods

### *Isolates of Fusarium oxysporum* f.sp. *ciceri*

Thirty-nine isolates of *Fusarium oxysporum* Schlecht. Emend Snyd. & Hans. f.sp. *ciceri* [Padwick] Snyd. & Hans. (Foc) were collected from nine chickpea growing states of India (Table 1). Pathogen isolation and production of inoculum were done as described [3]. Single spores of the pathogen isolates were obtained by subculture and the single spores of the fungus were grown in potato-dextrose broth (potato 200 g, dextrose 205, water 1 L) for 4 days at 25 °C with 12 hours

Table 1. *Fusarium* wilt pathogen isolates collected from the different chickpea growing regions in India

Isolate No.	Identification No.	Location	Total
1–13	IC-1 to IC-13	ICRISAT, Patancheru	13
14–17	KA-1 to KA-4	Karnataka	4
18–22	MA-1 to MA-5	Maharashtra	5
23	GU-1	Gujarat	1
24–25	HA-1, HA-2	Haryana	2
26–27	HP-1, HP-2	Himachal Pradesh	2
28–32	PA-1 to PA-5	Punjab	5
33–35	UP-1 to UP-3	Uttar Pradesh	3
36–40	MP-1 to MP-5	Madhya Pradesh	5
41–43	UN-1 to UN-3	Unknown	3

photoperiod [18]. Each isolate was grown in 80 mL of broth in 250 mL flasks.

The four known races of the pathogen, race-1 (isolate 1) collected from ICRISAT, Patancheru, race-2 (isolate 34 from Kanpur, Uttar Pradesh), race-3 (isolate 39 from Gurdaspur, Punjab), and race-4 (isolate 31 from Jabalpur, Madhya Pradesh) were also included in the analysis.

### DNA isolation

Fungal mycelium filtered through Mira cloth (Calbiochem, U.S.A.), and washed with distilled water was dried using paper towels. Genomic DNA was extracted from each isolate by the CTAB method [19]. Mycelial mat (3 g) was ground under liquid nitrogen in a mortar and pestle and the powdered mass was extracted with 20 mL of extraction buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl and 2% CTAB). The contents were gently mixed by inversion and incubated at 65 °C for 1 hour in a water bath. The slurry was transferred to a 15 mL plastic tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The contents of the tube were mixed gently for 5 minutes and centrifuged for 5 minutes at 10,000 × g in a Sorvall RC5 centrifuge. The aqueous phase was transferred to another tube and an equal volume of chloroform:isoamyl alcohol (24:1) was added to the aqueous phase. The contents of the tube were mixed for 5 minutes and centrifuged for 5 minutes as before. The aqueous phase was again removed and the nucleic acids were precipitated by adding 0.6 volume of isopropanol. The solution was centrifuged for 10 minutes at 12,000 × g, and after decanting the supernatant, the pellet was washed with 70% ethanol twice and

suspended in T<sub>50</sub>E<sub>10</sub> buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA). The DNA solution was treated with RNase (50 µg/ml, Sigma Co. MO, U.S.A.) at 37°C for 1 hour. An equal volume of phenol: chloroform (1:1) was added to the solution at the end of incubation, mixed well for 5 minutes and centrifuged 12,000 × g in a microcentrifuge (Eppendorf, U.S.A.). The aqueous phase was transferred to another tube and an equal vol of chloroform was added. The aqueous layer was separated and DNA was precipitated by adding 2.5 volume of absolute ethanol. The DNA pellet was washed twice in 70% ethanol, vacuum-dried, and re-dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

#### *PCR amplification conditions*

Amplification reactions were carried out in volumes of 25 µl containing 50 ng template DNA, 2.5 ul Taq Reaction buffer (10 X), 0.2 mM dNTPs, 0.4 mM primer, 2 mM MgCl<sub>2</sub>, 0.25% formamide, 1.0 unit of *Taq polymerase* (Promega, U.S.A.). Amplification was performed in a thermal cycler (MJR, U.S.A.) programmed for 35 cycles at a temperature regime of 94°C for 45 seconds, 37°C for 1 minute, 72°C for 1 minute after an initial denaturation at 94°C for 2 minutes. This was followed by a final 4-minute extension at 72°C. Reaction products were resolved by electrophoresis at 50 V for 5 hours in 1% (w/v) agarose gel in 1xTBE buffer and stained with ethidium bromide. The amplification reaction was performed 2–3 times with different batches of template, different [Mg<sup>2+</sup>] and enzymes to verify the consistency of the products. Twenty oligonucleotide primers (10-mer) from Operon Technologies Inc. (Alameda, CA, U.S.A.) were tested.

AFLP was carried using the Gibco BRL (U.S.A.) kit as per the manufacturer's protocols with slight modifications as described below. Genomic DNA was digested with the two restriction endonucleases, *EcoRI* and *MseI* and ligated to their respective adapters. The specific DNA fragments were amplified by PCR using primers that contained the common sequences of the adapters and 1–2 arbitrary nucleotides as selective sequence. Primary template DNA was prepared in a one-step restriction-ligation reaction. Fungal genomic DNA (400 ng) was digested with *EcoRI* and *MseI* at 37°C for 2 hours and heated at 70°C for 15 minutes to inactivate the enzyme. The DNA fragments were ligated to *EcoRI* and *MseI* adapters using the Gibco BRL (U.S.A.) kit at 20°C for 2

hours. After terminating the reaction, the ligation mixture was diluted 10-fold with TE, and the fragments were preamplified in a thermal cycler (MJR, U.S.A.) using a temperature cycle of 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds in a total of 30 cycles.

The five *EcoRI* (E-AC, E-TG, E-TC, E-AA, and E-AG), and 4 *MseI* (M-CAC, M-CAA, M-CAG, and M-CTA) primers were used in six combinations for amplification. Selective primers were provided by Life Technologies (U.S.A.). The amplification was carried out as per the manufacturer's protocol. The *EcoRI* primer was labeled with [ $\gamma$ -<sup>32</sup>P]-ATP (3000 Ci/mmol) as per the protocol of Life Technologies (U.S.A.). The PCR products in 5.0 ul subsamples were separated by electrophoresis on 6% denaturing polyacrylamide DNA sequencing gel containing 7.5 M urea. Autoradiograms were obtained using Kodak X-Omat films. The dried gels were placed with the X-ray films in cassettes overnight at room temperature. Every experiment was repeated a minimum of 2 times to establish the consistency of the bands.

#### *Data analysis*

The presence or the absence of each band in the gel was scored as 1 or 0, respectively. The principal coordinate analysis on the similarity indices between isolates based on the molecular data was carried out using Genstat 5, version 4.1 and Statistica for Windows, 1995 (Statsoft, Tulsa, U.S.A.).

## **Results**

#### *Genetic variability assessment with RAPD markers*

In PCR amplification with the oligonucleotide primers, 4–16 fragments were observed on agarose gel in the region of 0.5–3 kb. With each pathogen isolate, 3–6 bands were amplified – a representative RAPD profile of the 43 – *Fusarium* wilt pathogen isolates of chickpea with OPK-15 primer is given in Figure 1. Because of the high level of polymorphism, 20 of the arbitrary primers were screened with template DNA from the 43 isolates. The level of polymorphism on the 3200 DNA fragments amplified was 98% (No. of polymorphic bands/total number of bands × 100) as very few monomorphic bands were found. The total number of bands was reproducible with the same conditions for PCR amplification in the same machine

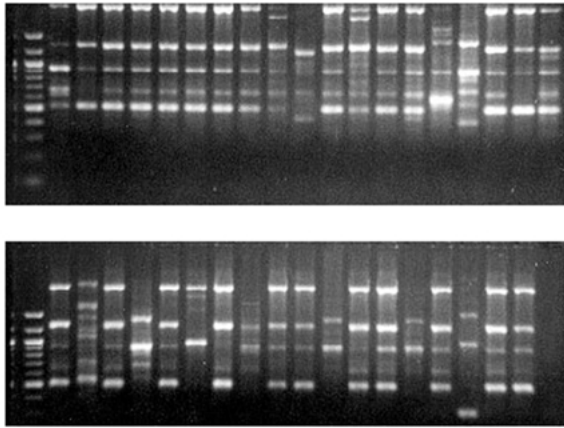


Figure 1. Agarose gel electrophoresis of DNA fragments amplified from the *Fusarium* wilt pathogen isolates of chickpea by RAPDs. The DNA fragments amplified by PCR with the primer OPK-16 in 43 *Fusarium* wilt pathogen isolates of chickpea were separated on 1.0% agarose gel and stained with ethidium bromide as described under Materials and methods. A representative agarose gel pattern with 37 pathogen isolates is given. The marker lane on the extreme left is of 100 bp ladder.

though there were some differences in the number and intensity of bands with different thermal cyclers.

The 43 isolates were classified into four clusters, based on the principal coordinate analysis of the similarity index data from RAPDs (Figure 2). The four known races of the wilt pathogen were placed in three of the clusters suggesting that two races could not be differentiated well by this marker method. Cluster I mostly comprised of pathogen isolates belonging to race-1, cluster II to race-2 and cluster III to race-3 and race-4 together. Cluster IV consisted of six pathogen isolates which did not show any similarity to the known races of the pathogen. All the 13 pathogen isolates collected from the ICRISAT fields were close to each other in cluster I along with two pathogen isolates of Karnataka (isolates 14 and 15) and one from Maharashtra (isolate 19) suggesting that these belong to race-1. Four pathogen isolates from Madhya Pradesh (isolates 36, 37, 38 and 40), three from Punjab (isolates 28, 30, and 32), two pathogen isolates of unknown origin (isolates 42, and 43), one each from Maharashtra (isolate 20), Gujarat (isolate 23), Himachal Pradesh (isolate 26), Uttar Pradesh (isolate 35) and Haryana (isolate 24) were in cluster II with the known race-2 (isolate 34) from Uttar Pradesh. Cluster III comprised of the known race-3 (isolate 31), and race-4 (isolate 39) isolates along with those from Punjab (isolate 29), Maharashtra (isolate 22), and Uttar Pradesh (isolate 33). Two pathogen isolates from

Karnataka (isolates 15 and 16), two from Maharashtra (isolates 18 and 21), one from Himachal Pradesh (isolate 27) and the isolate of unknown origin (isolate 41) were in cluster IV which had no known race of the pathogen in the group. One of the pathogen isolates from Haryana (isolate 25), which did not go with any of the clusters, came out as an outlier from all the other groups. Of the three isolates of unknown origin, two (isolates 42 and 43) were together with race-2 in cluster II whereas the third one was in clustering IV, which had no known races of the pathogen.

#### Genetic variability assessment with AFLP markers

A representative autoradiogram on the AFLP analysis of the *Fusarium* wilt pathogen isolates of chickpea with one primer combination is given in Figure 3. Principal coordinate analysis of the similarity index data of AFLP revealed the grouping of the various pathogen isolates (Figure 4). Cluster I placed all the 13-pathogen isolates of ICRISAT with race-1. In addition, two pathogen isolates from Karnataka (isolates 14 and 15), and one isolate from Maharashtra (isolate 19) were also together with race-1. Cluster II consisted of known race-2 (isolate 34) along with 16 other pathogen isolates. The two-pathogen isolates of unknown origin grouped with race-2 similar to that observed with the other two molecular markers. Similar to RAPDs, race-3 and race-4 were together in cluster III. This cluster also had other pathogen isolates from Karnataka (isolate 16), Maharashtra (isolate 21), Himachal Pradesh (isolate 27), Punjab (isolate 29) and Uttar Pradesh (isolate 33). Three pathogen isolates (isolates 18, 25, and 41) were outliers as these did not go with any of the groups mentioned above.

#### Discussion

Comparison of the two molecular methods to assess the variability among the 43 pathogen isolates of *Fusarium oxysporum* f.sp. *ciceri* reveal a few interesting facts on the race situation in the different chickpea growing states of India. From the combined analysis of the molecular markers it appears that the most predominant race of the pathogen prevalent in the chickpea growing states of India is race-1 with 17 isolates followed by race-2 with 15 isolates (Table 2). Race-3 and race-4 appear to be rare as only six pathogen isolates belong to these two together. Three of the pathogen isolates (isolates 18, 25, and 41) did

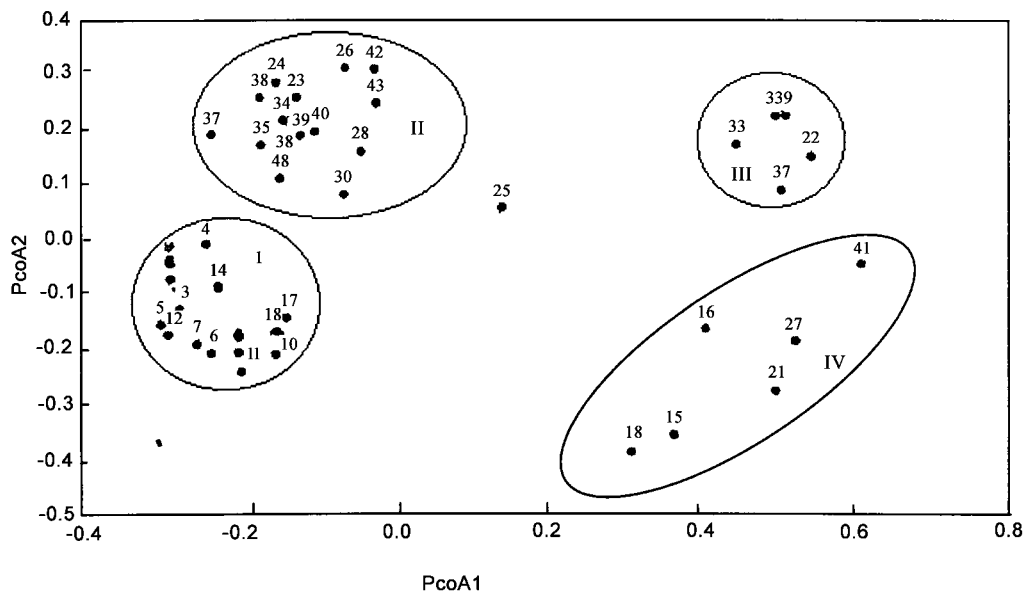
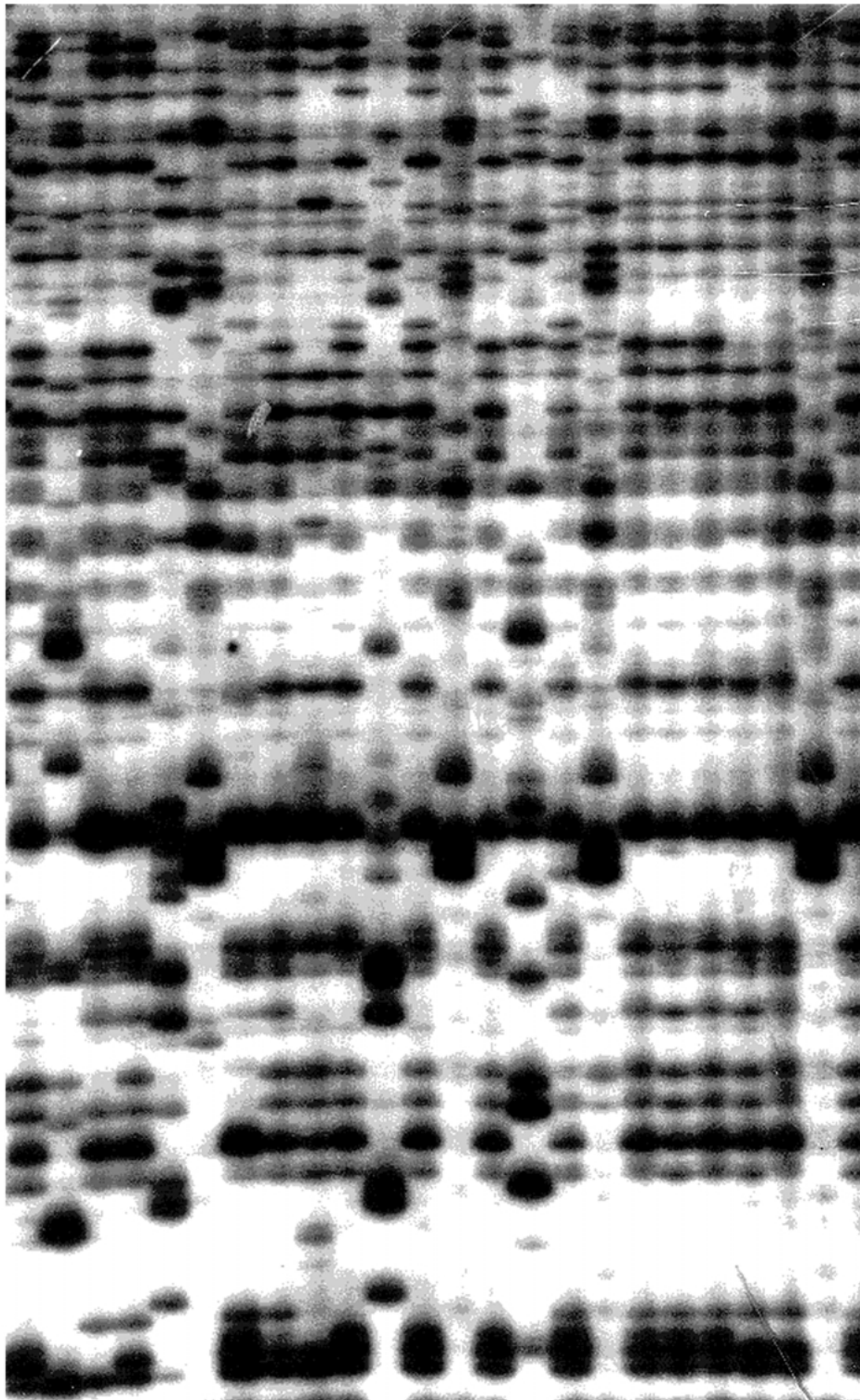


Figure 2. Principal coordinate analysis of the RAPDs marker data of *Fusarium* wilt pathogen isolates of chickpea. Principal coordinate analysis on the similarity index data from with 20 random oligonucleotide primers on 43 *Fusarium* wilt pathogen isolates of chickpea was carried out as given under the Materials and methods.

not go with any of the known races by these molecular markers implying that these could be new races or possible contaminants in the isolates which need to be further tested. Of these three, isolate 25 appeared to be unique in that both the molecular markers used could not group this pathogen isolate with any of the other isolates. On the other hand, pathogen isolates 18 and 41 though were grouped together with other pathogen isolates by RAPDs, these also did not belong to any known race of the pathogen. All the pathogen isolates collected from ICRISAT fields appear to belong to race-1. A maximum number of three races are prevalent in the chickpea growing areas of Karnataka and Maharashtra whereas in all the other Indian states there appears to be predominantly two races of the pathogen. It is however, surprising to know that ICRISAT field has isolates belonging to race-1 but not the other three races, namely races, 2, 3 and 4. Among the three isolates of unknown origin, two belonged to race-2 whereas the third one did not group with either race-3 or race-4 in any of the molecular markers used for analysis.

RAPDs and oligonucleotide fingerprinting has been successfully used to assess the genetic and pathogenic diversity within *Ascochyta rabiei* populations in Pakistan [20]. Our present study could be used to draw the pathogen diversity map in the chickpea growing areas in India for the *Fusarium* wilt patho-

gen, which will be taken up with the inclusion of more number of pathogen isolates from the various states of India. However, both the markers were not quite successful in differentiating all the four races of the pathogen isolates without much ambiguity. Only two races could be distinguished clearly by the marker technologies. The limitation of RAPDs was that it could only differentiate between race-1 and race-2 but not race-2 and race-3. Again, AFLP was able to separate race-1 and race-2 but not race-3 and race-4 similar to that observed in RAPDs. The grouping of six isolates in cluster IV with no known races of the pathogen (Figure 2) would suggest the detection of genetic variants of the other known races using RAPDs. However, the placing of some of these pathogen isolates along with the known races using the other molecular markers like AFLP also reflect the limitation of this marker more than the possibility of genetic variants mentioned above (Table 2). Though RAPDs could be used for a quick screening of *Fusarium* isolates in the laboratory there are still some inherent problems of reproducibility associated with the technology that makes the comparison of results from one lab to another rather difficult [21]. Though the two markers gave somewhat similar groupings, AFLP was more effective in bringing out the polymorphism among the pathogen isolates and placing these in different race-specific clusters in contrast to RAPDs. It is also supported by



*Figure 3.* Autoradiogram showing the AFLP analysis of the *Fusarium* wilt pathogen isolates of chickpea. AFLP analysis was carried out using the primer combination E-TC and M-CAG as given under Materials and methods. A representative autoradiogram with 24 pathogen isolates is shown.

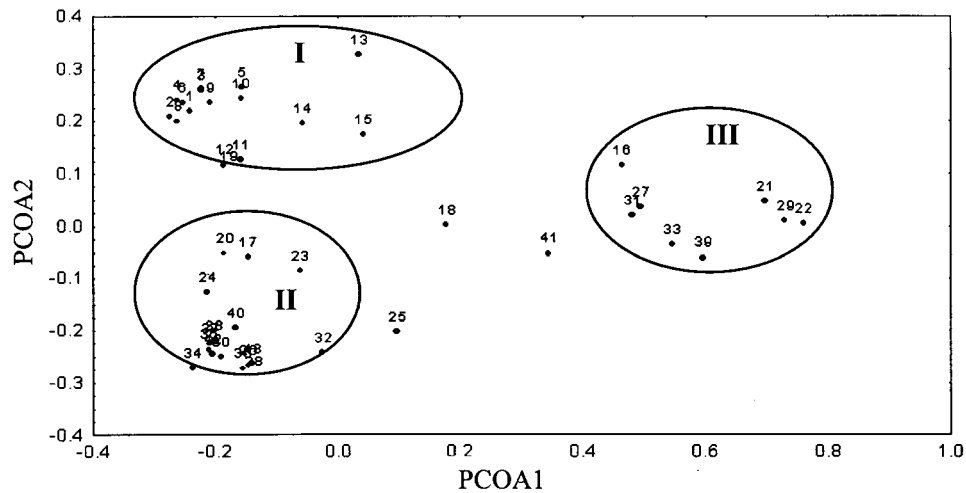


Figure 4. Principal coordinate analysis of the AFLP marker data of *Fusarium* wilt pathogen isolates of chickpea. Principal coordinate analysis on the similarity index data of the AFLP markers with four primer combinations on 43 *Fusarium* wilt pathogen isolates of chickpea was carried out as given under Materials and methods.

Table 2. Identification of *Fusarium oxysporum* f.sp. *ciceri* races in chickpea using different the molecular markers

Race	RAPDs (isolates)	AFLP (isolates)	Combined markers (isolates)
Race 1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 19 (16)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 19 (17)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 19 (17)
Race 2	20, 23, 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 43 (15)	20, 23, 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 43 (15)	20, 23, 24, 26, 28, 30, 32, 34, 35, 36, 37, 38, 40, 42, 43 (15)
Race 3/4	22, 29, 31, 33, 39 (5)	16, 21, 22, 27, 29, 31, 33, 39 (8)	16, 21, 22, 27, 29, 31, 33, 39 (8)
Not known	15, 16, 18, 21, 25, 27, 41 (7)	18, 25, 41 (3)	18, 25, 41 (3)

The total number of pathogen isolates in a particular group (race) is indicated in parenthesis in bold.

the fact that the two coordinates in the principal coordinate analysis accounted for 32%, and 45% of the genetic variability in the pathogen isolates by RAPDs, and AFLP, respectively. Though we used the UPGMA method for data analysis, the dendrograms did not reveal clear groupings compared to PcoA (data not shown). Similar results were obtained with *Fusarium* wilt pathogen isolates of pigeonpea where the race situation has not been still established either by using host differentials or other molecular methods (Sivaramkrishnan et al., in press). Further, AFLP seem to be more efficient as it had the minimum number of outliers that were not grouped with any of the known races of the pathogen. The close similarity between race-1 and race-2 and race-3 and race-4 was revealed by the different molecular markers. This could either be due to the closeness of these two sets of races for many of the other morphological, pathological, in

vitro growth characteristics or other evolutionary relationships. The inability to differentiate these two sets of races by the different methods also may reflect the limitation of these molecular markers or there is a need to try more number of primers. AFLP has been used to characterize Mexican isolates of *C. lindemutianum* [22] The study by Barve et al. [17] using microsatellites also did not clearly differentiate the four races. It is important to target the molecular markers closer to the avirulence gene to differentiate the races. The extensive genetic diversity seen with these molecular markers could also be contributed by the in vitro culture conditions of the isolate, the high mutation rate in the organism under field conditions or both.

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## References

- Singh BK, Dahiye BS. Breeding for wilt resistance in chickpea. Proceedings of the National Symposium on Wilt Problems and Breeding for Wilt Resistance in Bengal Gram, September 1973. Indian Agricultural Research Institute, New Delhi, India (abstract), 1975: 13–14.
- Haware MP, Nene YL, Natarajan M. Survival of *Fusarium oxysporum* f.sp. *ciceri* in soil in absence of chickpea. In: Management of soil-borne diseases of crop plants. Proceedings of National Seminar, 8–10 January 1986. Tamil Nadu Agricultural University, Coimbatore, India, 1986: 1–2.
- Haware MP, Nene YL. Races of *Fusarium oxysporum* f.sp. *ciceri*. Plant Disease 1982; 66: 809–810.
- Assigbetse KB, Fernandez D, Subois MP, Geiger JP. Differentiation of *Fusarium oxysporum* f.sp. *vasinfectum* races on cotton by RAPD analysis. Phytopathology 1994; 84: 622–626.
- Appel DJ, Fordon TR. Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. Experimental Mycologia 1995; 19: 120–128.
- Kim DH, Martyn RD, Magill CW. Restriction fragment length polymorphism groups and physical map of mitochondrial DNA from *Fusarium oxysporum* f.sp. *niveum*. Phytopathology 1992; 82: 346–353.
- Yao CL, Magill CW, Frederiksen RA, Bonde RR, Wang Y, Wu PS. Detection and identification of *Peronosclerospora sacchari* in maize by DNA hybridization. Phytopathology 1991; 81: 901–905.
- Grajal-Martin MJ, Simon CJ, Muelbauer FJ. Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f.sp. *pisi*. Phytopathology 1993; 83: 612–614.
- Rehner SA, Uecker FA. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the coelomycete *Phomopsis*. Canadian J Botany 1995; 72: 1666–1674.
- Sastry JG, Ramakrishna W, Sivaramakrishnan S, Thakur, RP, Gupta VS, Ranjekar PK. DNA fingerprinting detects genetic variability in the pearl millet downy mildew pathogen (*Sclerospora graminicola*). Theoret Appl Genet 1995; 91: 856–861.
- Geistlinger J, Maqbool S, Kaiser WJ, Kahl G. Detection of microsatellite fingerprint markers and their Mendelian inheritance in *Ascochyta rabiei*. Mycologia Res 1997; 101: 1113–1121.
- Munaut F, Hamaide N, Van der Stapper J, Maraitte H. Genetic relationships among isolates of *C. gloeosporioides* from *Stylosanthes* spp. in Africa and Australia using RAPDs and rDNA markers. Plant Pathology 1998; 47: 641–648.
- O'Neill NR, Van Berkum P, Lin JJ, Kwo J, Ude GN, Kenworthy W, Saunders JA. Application of amplified fragment length polymorphism for genetic characterization of *Colletotrichum* pathogens of alfalfa. Phytopathology 1997; 87: 745–750.
- Restrepo S, Duque M, Tohme J, Verdier V. AFLP fingerprinting: an efficient technique for detecting genetic variation of *Xanthomonas axonopodis* pv. *manihotis*. Microbiology 1999; 145: 107–114.
- Thyssen A, van Eygen S, Hauben L, Goris J, Swings J, Ollevier F. Application of AFLP for taxonomic and epidemiological studies of *Photobacterium damsela* subsp. *piscicida*. Inter J Systemat Evolut Microbiol 2000; 3: 1013–1019.
- Janssen P, Coopman R, Huys G, Sings J, Bleeker M, Vos P, Zabeau M, Kersters K. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacterial taxonomy. Microbiology 1996; 142: 1881–1893.
- Barve MP, Haware MP, Sainini MN, Ranjekar PK, Gupta VS. Potential of microsatellite to distinguish four races of *Fusarium oxysporum* f.sp. *ciceri* prevalent in India. Theoret Appl Genet 2001; 102: 138–147.
- Gerlach W, Nirenberg H. The Genus *Fusarium* – a pictorial atlas. Berlin and Hamburg: Kommissionsverlag Paul Parey, 1982: 355–358.
- Murray M.G, Thompson WF. Rapid isolation of high molecular weight DNA. Nucleic Acids Res 1980; 8: 4321–4325.
- Jamil, FF, Sarwar N, Sarwar M, Khan JA, Geistlinger J, Kahl G. Genetic and pathogenic diversity within *Ascochyta rabiei* (Pass.) Lab. Populations in Pakistan causing blight of chickpea (*Cicer arietinum* L.). Physiol Molec Plant Pathol 2000; 57: 243–254.
- Harris SA. RAPDs in systematics – a useful methodology? In: Hollingsworth, PM, Bateman, RM, Gornall, RJ, eds. Molecular systematics and plant evolution. London: Taylor & Francis Ltd. 1999: 211–228.
- Gonzalez M., Rodriguez R, Zavala ME, Jacobo JL, Hernandez F, Acosta J, Martinez J, Simpson J. Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. Phytopathology 1998; 88: 292–299.

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