

# Detection of Genetic Variability in Pearl Millet Downy Mildew (*Sclerospora graminicola*) by AFLP

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*Downy mildew, caused by Sclerospora graminicola, is an economically important disease of pearl millet in the semiarid regions of Asia and Africa. Amplified restriction fragment length polymorphism (AFLP) was used to detect the extent of genomic variation among 19 fungal isolates from different cultivars of pearl millet grown in various regions of India. Fourteen AFLP primer combinations produced 184 polymorphic bands. An unweighted pair-group method of averages cluster analysis represented by dendrogram and principal coordinate analysis separated the mildew collections into four distinct groups. Isolates having characteristic opposite mating abilities, geographic relatedness, virulence, common host cultivars, and changes through asexual generations reflected heterogeneity of the pathogen. The use of AFLP to detect genetic variation is particularly important in selecting mildew isolates to screen breeding material for identification of resistant millet and monitoring changes in S. graminicola in relation to changes in host for effective disease management.*

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**KEY WORDS:** AFLP; DNA fingerprinting; downy mildew fungus; pearl millet.

## INTRODUCTION

Downy mildew, incited by *Sclerospora graminicola* (Sacc.) Schroet., is the most widespread disease of pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Singh *et al.*,

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1993). The crop is of critical value for food security in some of the world's hottest and driest cultivated areas of Africa and Asia. In terms of annual global production, it is the sixth most important cereal crop, after wheat, rice, maize, barley, and sorghum (FAO, 1992). The *S. graminicola* reproduces by oospores and is a systemically infecting heterothallic (self-sterile) fungus. It attacks the crop at the seedling stage and transforms the panicle into a malformed ear head, called green ear. The losses can be very high under favorable conditions of disease development in a susceptible cultivar. Studies on this oomycete pathogen are difficult since it cannot be grown as an axenic culture in the laboratory.

The introduction of potentially high-yielding hybrids into India in the late 1960s led to large-scale cultivation of homogenetic material. When the first of several downy mildew epidemics occurred in 1970–71 (Andrews *et al.*, 1985), there were three consequences of resistance breakdown: withdrawal of several hybrids, yield reductions, and an increase in pathogen inoculum. Breeding for downy mildew resistance is now a key priority in pearl millet programs and all promising cultivars are routinely tested in downy mildew nurseries at ICRISAT (Williams *et al.*, 1981). The study of resistance is complicated since both the host (Brunken, 1977) and pathogen (Idris and Ball, 1984) are out-breeding, and are highly variable and exhibit non-Mendelian segregation ratios for host plant resistance. Resistance is known to be regionally variable, and therefore, new breeding material has to be tested in expensive multilocal trials (Ball *et al.*, 1986). The development of DNA markers such as RFLP and RAPDs has enabled mapping of genes contributing toward complex traits using quantitative trait locus (QTL) analysis (Jones *et al.*, 1995). Ability to breed for downy mildew resistance at the molecular level would allow strategies on specific gene exploitation and would eliminate unnecessary linkage drag and confounding environmental variation associated with conventional breeding (Jones *et al.*, 1993, 1995).

The DNA fingerprinting method of amplified fragment length polymorphism, AFLP (Vos *et al.*, 1995) combines the power of RFLP analysis with the flexibility of PCR-based technology. Technical advantages are its reproducibility, high resolution due to use of stringent PCR conditions, and portrayal on polyacrylamide gels. High annealing temperatures in the initial cycles of the PCR reaction ensure that primers differing in only one nucleotide or two nucleotides (e.g. E + A, E + AG) in the extension step produce distinct banding patterns (Majer *et al.*, 1996; Mueller *et al.*, 1996). Without prior knowledge of genomic sequences, it can differentiate highly related strains in accordance with existing taxonomic data (Janssen *et al.*, 1996). Identification of mating-type genes (Julian *et al.*, 1999), determination of sex locus in fungi to unearth the mechanism of sex differentiation (Reamon-Büttner *et al.*, 1998), clonality in fungi as a means to reproduction (Rosendahl and Taylor, 1997), and discovery of new species involved in complex diseases (Baayen *et al.*, 2000) are a few examples of the applications of AFLP among fungal pathogens.

Since the downy mildew pathogen exists as populations in a field, prediction of damage or warnings against epidemics to farmers is highly complicated. Variation in the pathogen populations contributes to the understanding of pathogenesis and could answer queries of the pearl millet–downy mildew interaction. In the study reported here, we have used AFLP as a tool to detect variability among *S. graminicola* isolates from various geographical parts of India. The objectives of this study were to (i) develop an efficient fingerprinting protocol for *S. graminicola* using AFLP, (ii) analyze the genetic variation among different isolates, and (iii) classify them into representative groups obtained by dendrogram and principal coordinate analysis (PCOA).

## MATERIALS AND METHODS

### Description, Maintenance, and Growth of Fungal Isolates

The isolates selected belong to different geographic regions of India (Table I), and some were collected in 1997–98. Pearl millet–downy mildew pathogen isolates from Indian states of Maharashtra, Rajasthan, Gujarat, Tamilnadu, Andhra Pradesh, and Karnataka (Fig. 1) were maintained on seedlings of their respective host cultivar or on universally susceptible cultivar 7042S in isolated chambers in greenhouses at ICRISAT, Patancheru. These isolates were maintained through

Table I. *Sclerospora graminicola* Isolates

Lane no.	Isolate	Host	Geographic origin
1	Path-1	NHB3	Six host-specific genotypes maintained on respective host genotypes by repeated inoculation with asexual spore in isolation chambers in green house (Thakur et al. 1992)
2	Path-2	BJ104	
3	Path-3	MBH110	
4	Path-4	852B	
5	Path-5	700651	
6	Path-6	7042S	
7	Sg 32	HB3	Rahuri, Maharashtra
8	Sg 25	BK 560	Bhadgaon, Maharashtra
9	Sg 26	Nath 4209	Veelad, Maharashtra
10	Sg 21	MLBH 104	Ghari, Maharashtra
11	Sg 48	7042S × HB3	Mysore, Karnataka
12	Sg 110	C03	Illupanatham, Tamilnadu
13	Sg 139	Nokha local	Jodhpur, Rajasthan
14	Sg 152	Local	Durgapur, Rajasthan
15	Sg 153	7042S × HB3 (AP)	ICRISAT Patancheru, Andhra Pradesh
16	Sg 88	GK 1006	Fatiabad, Maharashtra
17	Sg 140	Local	Jamnagar, Gujarat
18	PT2	Mating-type isolates	
19	PT3		

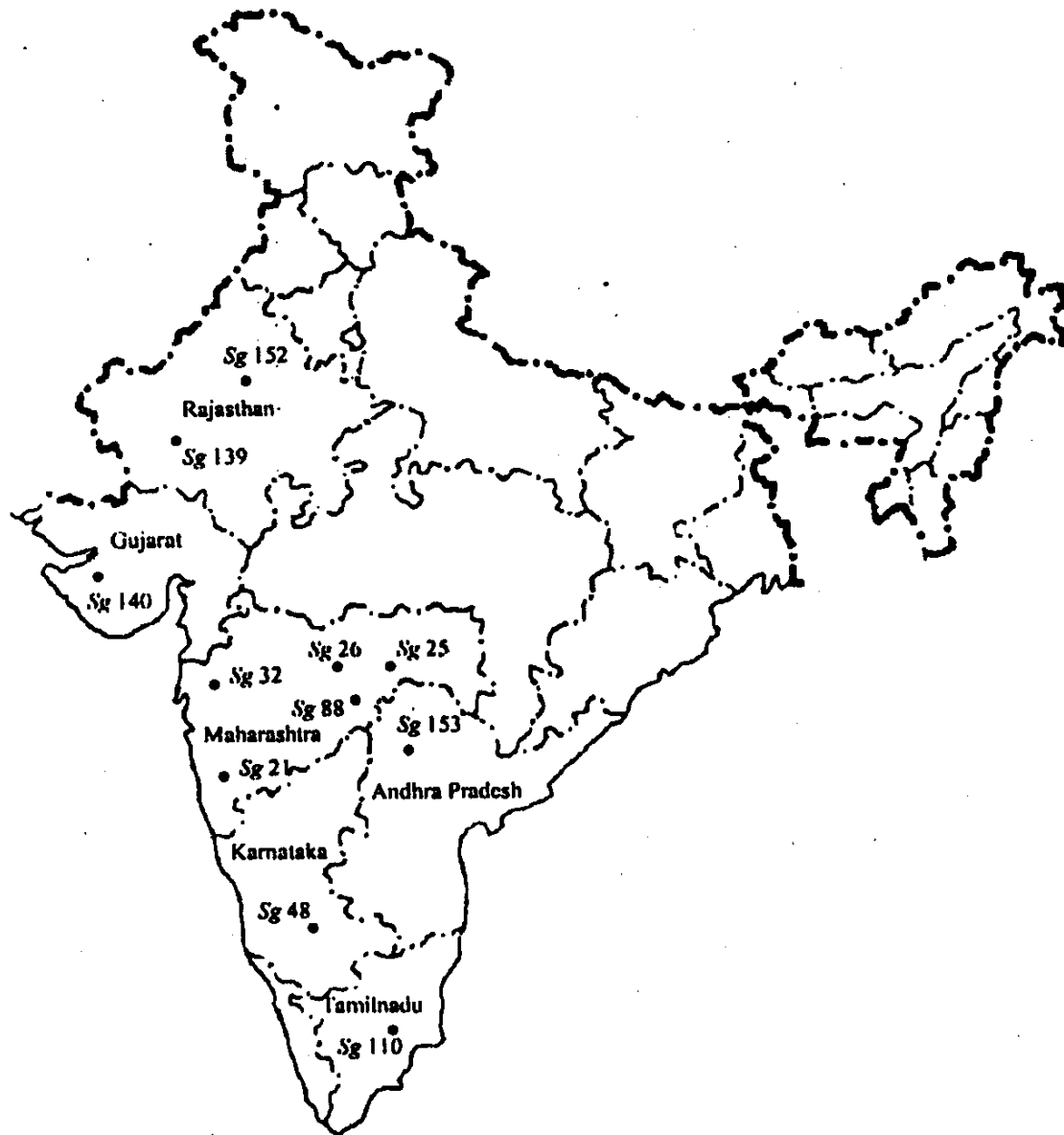


Fig. 1. Locations where *Sclerospora graminicola* (Sg) isolates were collected from the represented states of India.

asexual generations. Other pathogen isolates, Path-1 to Path-6 are differential host-specific genotypes while pathogen isolates PT2 and PT3 are compatible heterothallic-mating types (Michelmore *et al.*, 1982). The method of harvesting zoospores is as described by Singh *et al.* (1993).

### DNA Extraction

Fungal DNA extraction was essentially according to the method described by Sastry *et al.* (1995). Sporangia were harvested from sporulating leaves in ice-cold sterile deionized water and a pellet collected by centrifugation. The pellet was powdered in liquid nitrogen and incubated with prewarmed 5 volumes of extraction buffer (50 mM Tris-HCl, pH 8.0, 20 mM EDTA, 0.5 M NaCl, 1% SDS) for 20 min at 65°C. A 1:1 volume of phenol-chloroform (chloroform:isoamyl

alcohol 24:1) was added and gently mixed, followed by centrifugation for 10 min at 12,000g. The aqueous layer was further treated with an equal volume of chloroform:isoamyl alcohol (also in 24:1 proportion) and centrifuged. The nucleic acids were precipitated from the aqueous phase by adding 0.6 volumes of isopropanol. DNA was spooled, washed in 70% ethanol twice, dried, and dissolved in T<sub>50</sub>E<sub>10</sub> buffer (50 mM Tris-HCl, 10 mM EDTA, pH 8.0). RNase treatment and further purification was also carried out. DNA dissolved in T<sub>10</sub>E<sub>1</sub> buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was checked for amount (OD 260/280) and quality.

### AFLP Analysis

The protocol employed for AFLP analysis was as reported by Vos *et al.* (1995). The research kit for AFLP of plant genomic DNA was from Life Technologies, USA, and assays were carried out as described in the manual. Genomic DNA of 150–200 ng was incubated with 1 U *Eco* RI/*Mse*I mix for 90 min at 37°C with 1× Reaction Buffer. Digestion was followed by inactivation at 95°C for 2 min; the aliquot was divided in two equal parts for ligation. Solution's containing equimolar concentrations of adaptors as given in the kit and T4 DNA ligase were mixed and incubated at 20°C for 2 h. The ligated sample was diluted 1:10 and 2 μL was used for preamplification with 8 μL preamplification mixture, 1 U *Taq* polymerase (Promega Corporation, USA) and 1× buffer. Selective amplification was carried out with 1:50 diluted preamplified mix, using [ $\gamma^{32}$  – P] labeled selectively modified *Eco* RI primer, selective *Mse*I primer containing dNTP mix, 1 U *Taq* polymerase, 1× Buffer, and AFLP grade water as described in the manual. Reaction cycles were carried out on Perkin–Elmer 9600 Thermocycler. The amplified samples were mixed with 98% formamide and xylene cyanol-bromophenol blue dyes, heated at 96°C for 5 min, ice-cooled and loaded in continuation on 6% polyacrylamide gel for prerun at 80 W according to standard method described for DNA sequencing (Sambrook *et al.*, 1989). After electrophoresis, amplification products were viewed by autoradiography and scored for polymorphism.

DNA sequences of *Eco* RI and *Mse*I primers were as per the kit specifications. Selective amplification was done with five *Eco* RI primers with two selective nucleotides, e.g. E + AC, E + TG, etc. and seven *Mse*I primers with three selective nucleotides, e.g. M + CAG, M + CAA etc. Fourteen combinations were screened: E + AC with M + CAT, M + CAG, M + CTC, M + CTG, and M + CAA; E + AG with M + CTG, M + CTC, and M + CAT; E + TG with M + CAG and M + CTG; E + TC with M + CAC and M + CTC; and E + AA with M + CAA and M + CTC. Only clear and unambiguous polymorphic bands ranging between 130 and 400 bp were scored. Pearl millet DNA from cultivars NHB3 and universal susceptible host 7042S were used as controls in the AFLP reactions to avoid artifactual polymorphism from the plant DNA as this is a sensitive technique and parts of leaf sections are often isolated along with sporangia.

### Data Analysis

Each AFLP fragment selected was manually scored as present (1) or absent (0) for each pathogen isolate. The calculation of genetic distances was done according to the DICE coefficient with

$$GS = \frac{2N_{xy}}{N_x + N_y}$$

where  $N_{xy}$  is the number of shared fragments between two pathogen isolates  $X$  and  $Y$  and  $N_x$  and  $N_y$  are the total number of fragments present in isolates  $X$  and  $Y$ , respectively.

On the basis of the GS (genetic similarity) values, a cluster analysis was performed using the UPGMA (unweighted pair-group method of averages) procedure using the software package NTSYS pc 2.0 (Exeter Software, East Setauket, NY) from which the dendrogram was drawn and plotted using the consecutive commands *SimQual* with coefficient DICE, *SAHN* with clustering method UPGMA, and *TreePlot*.

A graphical representation of the estimated genetic similarities between pathogen isolates was obtained by principal coordinate analysis (PCOA) from NTSYS pc 2.0 on the basis of the above calculated similarity matrix as described by Gower (1966). The consecutive commands *Dcenter* using the GS matrix as input, *Eigen*, and *2Dplot* were used to generate the two-dimensional PCOA plot.

## RESULTS

### AFLP Analysis of the Isolates

The 14 AFLP primer combinations used for the analysis produced reproducible bands, of which 184 were polymorphic. The average number of polymorphic bands detected were 13 per primer combination. Among these combinations, E + AC/M + CAA, E + AA/M + CTC, E + TC/M + CAC, and E + TG/M + CTG sets gave highly polymorphic banding patterns. All 19 pathogen isolates could be distinguished by the AFLP analysis. The marker numbers 43, 49, and 58 from primer combination E + AC/M + CAA; Markers 69, 76, and 77 from the E + TC/M + CAC set; marker 162 from E + TG/M + CTG; and markers 122, 130, and 134 from E + AA/M + CTC set exhibited notable polymorphism as they could divide the isolates in a similar fashion as the dendrogram. Furthermore, fragment numbers 9, 11, 12, 13, 19, 22, 40, 41, 42, 43, 49, 58, 69, 76, 77, 95, 101, 105, 122, 130, 132, 134, 149, 152, 162, 175, and 182 could easily distinguish the isolates. These belong to different primer combinations and the fragment sizes range between 250 and 450 bp.

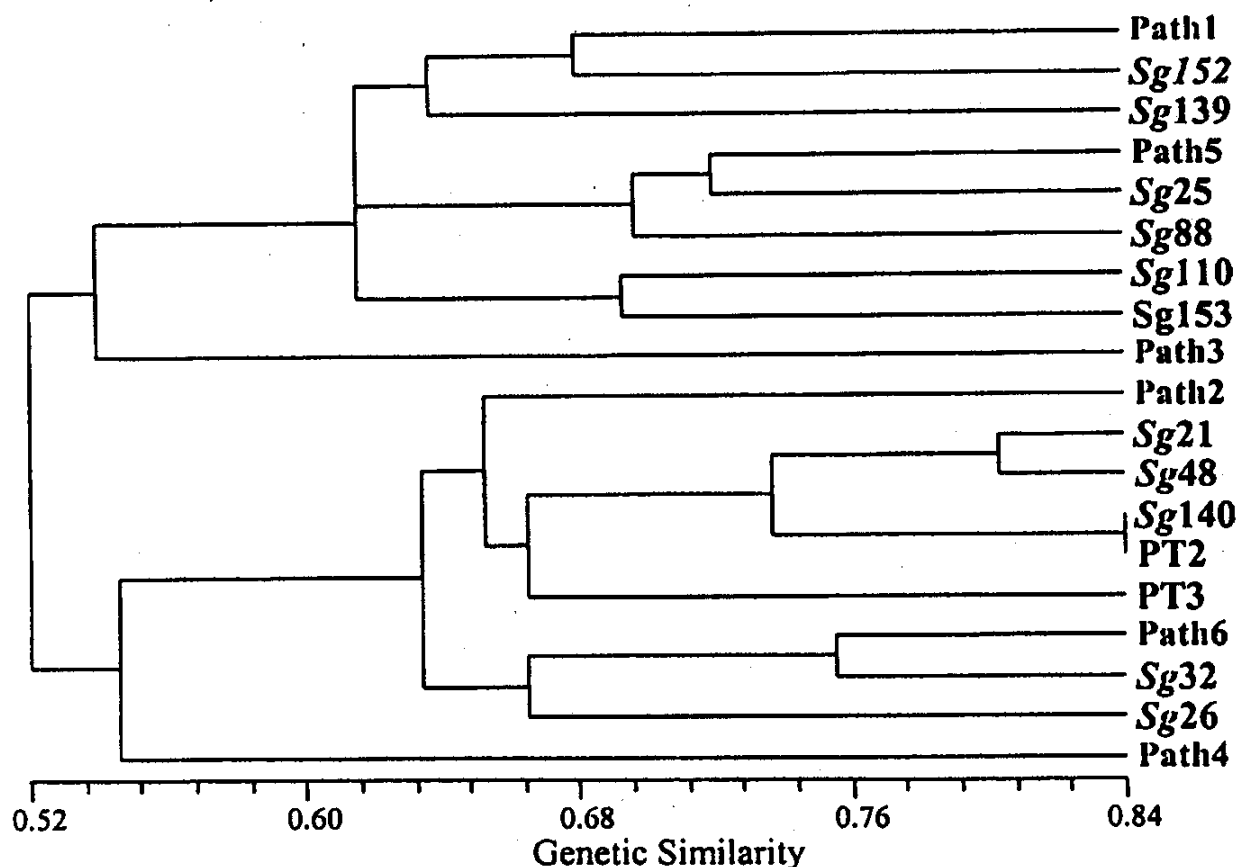


Fig. 2. Dendrogram based on AFLP polymorphisms in 19 pathogen isolates of *Sg* of pearl millet by the unweighted pair-group method of averages (UPGMA) cluster analysis. Genetic similarities were calculated using DICE coefficient in the *SimQual* module of the NTSYS-pc 2.0, based on the 184 band positions observed for 14 AFLP primer pairs.

### Dendrogram Analysis

A dendrogram produced from the similarity matrix determined by the DICE coefficient suggests that the selected Indian isolates could be divided into two main clusters separated by a threshold genetic distance of 0.54 (Fig. 2). The first cluster includes three subclusters with Path-3 distantly clustered with them, while the second cluster includes two subclusters with Path-4 distantly clustered with them. Within the subcluster, the isolates *Sg* 140 and PT2 show high genetic similarity (GS = 0.84).

### Principal Coordinate Analysis

The PCOA plot shows that the various isolates form two distinct groups (I and II) identical to the main two subclusters in dendrogram analysis with subgroups A and B in each (Fig. 3). In the PCOA plot, the first coefficient classified the host-genotypes-specific isolates into two groups while the second coefficient separated the isolates into two mating types. Geographically distant populations were also separated; for example, the Rajasthan population of Group IIA was separated from the Maharashtra isolates spanning groups IA, IB, and IIB. Genetic variation was in

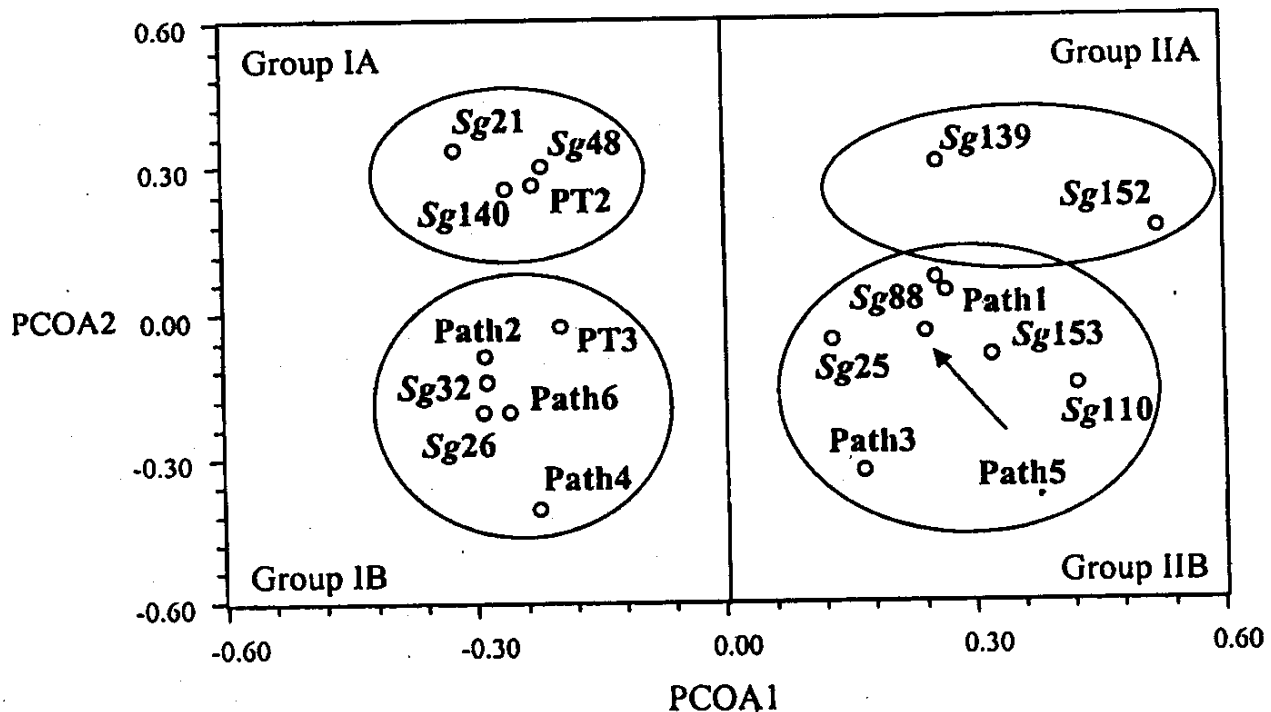


Fig. 3. Differentiation of 19 *Sg* isolates by two-dimensional principal coordinate analysis (PCOA) based on genetic similarity calculation from 14 AFLP primer combinations.

accordance with the cultivars from which the isolates were collected and linkage between their virulence qualities.

## DISCUSSION

Biological pathotyping indicates diversity in the fungal pathogenic populations of *S. graminicola* in aspects such as host specificity, adaptation, and virulence (Thakur *et al.*, 1992). When supported by genetic similarity, precise information about the variation in aggressiveness of the isolates in relation to each other and to the host can be secured. Previous studies by our group have explored adaptation of pathotypes to alternate hosts over asexual generations using RAPD (Sastry *et al.*, 2001), repeatable elements using restriction digestion (Sastry *et al.*, 1997), and RFLP fingerprinting of the six host-specific pathotypes (Sastry *et al.*, 1995). The advantage of AFLP over other markers like Sequence tagged site analysis, RAPD and RFLP has been described by Vos *et al.* (1995) and Mueller *et al.* (1996). This study provided an effective method to find AFLP-related markers for investigating the genetic diversity among *S. graminicola* isolates.

### Heterothallism Supported by AFLP Analysis

Downy mildew causes significant economic losses of pearl millet. Previous reports and reviews have presented many details of breeding for disease resistance in pearl



millet and have suggested the need of knowledge of genetic structure of the host and pathogen populations for durable resistance against downy mildew (Andrews *et al.*, 1985; Hash *et al.*, 1997; Jeger *et al.*, 1998). *S. graminicola* is heterothallic and the existence of two sexually compatible mating types, PT2 and PT3, has been established (Ball and Pike, 1983; Michelmore *et al.*, 1982). Mating type is a genetically regulated sexual compatibility phenotype in which gamete nuclei must come from parents of different mating type (Kronstad and Staben 1997). In our analysis using AFLP, the mating types fall in two distinct groups IA and IB as seen in the PCOA plot (Fig. 3) and subclusters of the dendrogram (Fig. 2). Heterogeneity in terms of virulence and aggressiveness has been previously reported among these isolates (Thakur and Shetty, 1993). It would be interesting to discover whether any other isolates have a similar genetic basis for mating-type ability or virulence. Three isolates (namely *Sg* 21 collected from the south central part of India, *Sg* 48 from the south-western part of India, and *Sg* 140 from the western part of India) cluster with the PT2 mating-type isolate in Group IA. Among these, Idris and Ball (1984) have shown that *Sg* 140 is sexually compatible and it will be interesting to see if the virulence peculiarities of *Sg* 21 and *Sg* 48 isolates resemble PT2 (work in progress, Thakur *et al.*, ICRISAT). The fine level subclustering observed among these isolates, however, may be due to their dissimilar host origins (Table I): *Sg* 21 is isolated from MLBH 104, a popular high-yielding pearl millet hybrid released in India; *Sg* 48 is from 7042S and HB3 hosts maintained in the downy mildew nursery at Mysore; and *Sg* 140 is from a local variety.

Group IB (Fig. 3) includes the second known mating type, PT3; host-genotype-specific isolates Path-2 (parent BJ 104, hybrid cultivated on a large scale for its agronomic traits, resulting in disease pressure, subsequent removal from cultivation); Path-4 (parent 852B); Path-6 (the most susceptible parent 7042S, a landrace originated from Africa); isolates from relatively adjoining geographic regions *Sg* 26 and *Sg* 32 (Fig. 1). The aggregation of these isolates in Group IB could probably be attributed to their analogous fertility to cross breed and magnitude for rapid virulence change (Thakur *et al.*, 1998). It has been demonstrated that although populations differ in pathogenicity, like Path-2, Path-4, and Path-6, such isolates can cross breed (Idris and Ball, 1984) and cause rapid proliferation of pathogen genotypes in the absence of appropriate resistance factors in the host. The isolate, *Sg* 26 has been reported to have intrapopulation pathogenic variability like Path-3 and *Sg* 21 which were pandemic in the past leading to withdrawal of the hybrids (Thakur *et al.*, 1998). *Sg* 26 has also been found to be aggressive on the hosts of Path-4 and Path-6 of this group. Michelmore *et al.* (1982) and Idris and Ball (1984) tried categorizing single oospore isolates according to their mating-type compatibility and found homothallism frequent among these isolates. The relative frequencies of the mating types could be affected if oospores were inadvertently transported from one continent like Asia (India) to Africa presenting a risk to the pearl millet crop. Ascertaining of sexual compatibility of isolates will

be of paramount importance (Idris and Ball, 1984) in determining the nature of the ability of pearl millet to resist downy mildew.

### Geographic Relatedness Among the Isolates

Geographic relatedness is a frequent mode of classifying pathogen populations and this set could be termed as a gene pool (Stappen *et al.*, 2000). In our analysis, group IIA of the PCOA plot (Fig. 3) signifies the isolates from a neighboring geographic areas, for example Sg 139 from Jodhpur is an aggressive isolate from a cultivar bred locally at Nokha and Sg 152 from NHB3 in a disease nursery at Durgapur. It is further reported that both have host specificity for pathogenicity (Singh and King, 1988). Group IIB comprises host-genotype-specific isolates Path-1; Path-3 and Path-5; Sg 25 from Bhadgaon, central India; Sg 88 from Fatiabad, central India; Sg 110 from south India; and Sg 153, a field isolate from a disease nursery at ICRISAT, Patancheru. A similar observation has been made by Pei *et al.* (2000), where they have discovered that collection sites of rust isolates divide them into common groups on the basis of cluster analysis and outbreaks of new pathotypes from these populations. Furthermore, isolates Sg 32 and Sg 26 from Group IA (Fig. 3) and Sg 25 and Sg 88 from Group IIB belong to nearby locations of south western and central India (Fig. 1). Closer physical distance accounts for the partial genetic homogeneity within the group, as oospores can spread rapidly (Williams, 1984). The genetic separation of these isolates probably suggests diversification of the pathogen coupled with the out-breeding nature of host giving rise to corresponding changes in pathogen genotypes.

### Demonstration of Variation in Populations

AFLP demonstrated a high level of genetic variation in natural populations of *S. graminicola*. From the breeding point of view, this could indicate rapid selection of isolates that are resistant to fungicides or virulent on pearl millet varieties having pathotype-specific resistance. Selection of pyramided virulence, by targeting specific traits to pearl millet populations in planned crossing programs, has been manifested to be more efficient where the pathogen is an obligate biotroph, can reproduce sexually, and the disease can spread rapidly by air-borne sporangia (Day, 1974), traits which *S. graminicola* satisfies. Separation of host-genotypes-specific pathotypes, Path-1, Path-2, Path-3, Path-4, Path-5, and Path-6 into two distinct clusters, IA and IIB, is indicative of the adaptable pathosystem to match the changes in host. These isolates are sustained on specific genotypes at ICRISAT (Thakur, personal communication) to find the virulence change from Path-6 to different isolates through several asexual generations in the greenhouse studies (Sastry *et al.*, 2001). The host genotypes of Path-1 and Path-2 share a common male parent, HB3 (Mehta and Thakur, 1985) and both are isolated from high-yielding varieties. However, Path-1 is much more aggressive than Path-2 (Thakur, 1995), and this could account for their grouping to different clusters. Path-3 and

Path-4 show large differences in virulence ability, phenotypic changes over asexual generations (Singh and Thakur, 1995), and logically have large genetic distance between them as per our AFLP analysis. The virulence of Path-3 has been linked to Path-1, but not to Path-4 (Thakur *et al.*, 1992), which is very well supported by our present data. Path-5 is isolated from a highly resistant host genotype, 700651, an accession that originates from Nigeria; in contrast, Path-6, the most aggressive downy mildew isolate known, is collected from a universal susceptible host (Singh and Gopinath, 1985). These two isolates belong to two different groups. Agronomic concern about Path-2 and Path-5 has grown as their hosts which have desirable crop qualities, and exhibit drought tolerance, are being implemented in a major program to incorporate downy mildew resistance in these pearl millet lines (Govila, 1993). Considering the causes of breakdowns of resistance from the epidemics of 1984–85 (Singh and King, 1988), Path-2 and Path-5 were checked for their virulence and aggressiveness and were found to have variable disease incidence (Thakur, 1995). In our work, we have shown that these isolates are genetically distant from each other, grouping in separate clusters of the PCOA plot.

#### Variation in Isolates With Similar Host Origins

In the dendrogram, it is important to note that both Path-1 and Sg 152 have a common host, NHB3 (Thakur, 1995) (Table I, Fig. 2), and share genetic similarity. The combined influence of new cultivars, step-wise selection for virulence, and mutable mechanisms of genetic reassortment may have contributed to the development of new virulent races. Path-6 and Sg 153 are ICRISAT, Patancheru populations and have a common host 7042S. Their high virulence capacity is used to test new pearl millet releases in breeding programs. Similarly Path-2 and Sg 25 have an identical male sterile parent (5141A) and Sg 32 and Sg 48 have a common host (HB3) but group in different subclusters of the PCOA plot. The variation shown by these isolates shows that AFLP can detect differences at a very fine scale even in clonal populations. From the evidence of Ball *et al.* (1986), who found inconsistent disease incidence of the two isolates, and the present evidence that these are genetically dissimilar, one can explain why the isolates do not conform to the general pattern. The pattern refers to differences in disease incidence to establish consistency in pathogen behavior to fit host–pathogen cross relationships. In other host–pathogen systems also, namely *Magnaporthe grisea*–rice (Ziegler *et al.*, 1995), perfect correlation between host and genotype is seldom observed (Gonzalez *et al.*, 1998).

#### Advantages of Using Dendrogram and PCOA Plot to Represent Diversity

In our analysis, the combined strategy of representations of dendrogram and PCOA plot has allowed calculation of genetic similarity to specific units and easy visualization of differences, respectively, amidst collected sporangial populations to two

main groups. Comparing the two methods of data presentation, the subclustering of the dendrogram slightly differs from the PCOA plot. Both methods used data generated from 184 amplicons wherein the PCOA plot revealed marked features of the isolates such as geographic relatedness, linking Sg 139 to Sg 152 in group IIA while separating mating types PT2 and PT3 to form individual groups. The dendrogram shows that Path-3 and Path-4 are notably different from each other as well as from the other isolates in the same groups and this has been pathotypically observed (Thakur *et al.*, 1998). Sastry *et al.* (1995) have demonstrated Path-3, Path-4; Path-5, and Path-6 in distinct groups with Path-2 clustering closer to Path-6 which is well supported by our PCOA plot. They have also shown Path-1 and Path-2 to be genetically closer. On the whole, however, Path-1 and Path-2 fall in different groups as revealed by the dendrogram (Fig. 2) and PCOA plot (Fig. 3) of our analysis, which demonstrates the importance of choosing correct primer pairs to reveal polymorphisms among pathotypes that are related. This strengthens the use of different fingerprinting techniques like RFLP and AFLP to support each other. In summary, DNA fingerprinting by AFLP has facilitated the molecular description of the genetic differences between sporangial isolates providing a basis for mechanisms that generate variation in the downy mildew fungus.

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