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Molecular Characterization of *Sclerospora graminicola*, the Incitant of Pearl Millet Downy Mildew Using ISSR Markers

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Abstract

The DNA polymorphism among 22 isolates of *Sclerospora graminicola*, the causal agent of downy mildew disease of pearl millet was assessed using 20 inter simple sequence repeats (ISSR) primers. The objective of the study was to examine the effectiveness of using ISSR markers for unravelling the extent and pattern of genetic diversity in 22 *S. graminicola* isolates collected from different host cultivars in different states of India. The 19 functional ISSR primers generated 410 polymorphic bands and revealed 89% polymorphism and were able to distinguish all the 22 isolates. Polymorphic bands used to construct an unweighted pair group method of averages (UPGMA) dendrogram based on Jaccard's co-efficient of similarity and principal coordinate analysis resulted in the formation of four major clusters of 22 isolates. The standardized Nei genetic distance among the 22 isolates ranged from 0.0050 to 0.0206. The UPGMA clustering using the standardized genetic distance matrix resulted in the identification of four clusters of the 22 isolates with bootstrap values ranging from 15 to 100. The 3D-scale data supported the UPGMA results, which resulted into four clusters amounting to 70% variation among each other. However, comparing the two methods show that sub clustering by dendrogram and multi dimensional scaling plot is slightly different. All the *S. graminicola* isolates had distinct ISSR genotypes and cluster analysis origin. The results of ISSR fingerprints revealed significant level of genetic diversity among the isolates and that ISSR markers could be a powerful tool for fingerprinting and diversity analysis in fungal pathogens.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fifth most important cereal crop in the world (Khairwal, 2008) and is the fourth important food crop in India, after rice, wheat and sorghum. In India, the crop is grown on approximately 8.9 million hectares with the production of 8.5 million tons and 957 kg/ha yield (Khairwal, 2008) and on more than 2 million hectares in the United States. The major states of India contributing to pearl millet grain production includes Rajasthan (Trans-gangetic plains region and central plateau), Maharashtra (Eastern and Western Plateau and Hills Region), Madhya Pradesh (Central and western Plateau and Hills Region), Gujarat (Gujarat Plains and Hills Region), Haryana (Trans-Gangetic Plains Region), New Delhi (Trans-Gangetic Plains Region), Andra Pradesh (Southern Plateau and East Coast Plains and Hills Region), Tamil Nadu (East Coast Plains and Hills Region and West Coast Plains and Ghat Region) and Karnataka (Southern Plateau and Hills region and West Coast Plains and Ghat Region).

One of the important biotic constraints to pearl millet production is the downy mildew disease caused by the Oomycetous, biotrophic organism *Sclerospora graminicola* (Sacc.) Schroet. This disease is of economic importance in all pearl millet growing regions and causes considerable yield losses, some times up to 40% (Singh, 1995).

The first evidence of pathogenic variation in pearl millet downy mildew (PMDM) was reported in 1973, when an F1 hybrid HB 3 developed on Tift 23A released in 1968 was found to be resistant at Mysore, but susceptible at some other locations in India (Bhat, 1973). Nene and Singh, 1976 interpreted this variation as being due to the existence of races in the pathogen. Shetty and Ahmad, 1981 reported that two distinct races from Mysore and Gulbarga were easily differentiated on HB3. Since then, a number of hybrids viz., NHB3, BJ 104, BK 560, GK 1004, Proagro 7701,

Pioneer 7602 and Eknath 201 (Singh, 1995; Thakur et al., 2004) have succumbed to downy mildew and have been withdrawn from cultivation.

Pathogenic and genetic variability in *S. graminicola* has been demonstrated (Sivaramakrishnan et al., 2003; Thakur et al., 2004; Pushpavathi et al., 2006). Because of the highly variable nature of the pathogen, several popular pearl millet hybrids have succumbed to the disease and withdrawn from cultivation (Singh, 1995; Thakur et al., 1999). The potential for development of new races/pathotypes makes it desirable to monitor the pathogen population regularly to detect changes in virulence. On-farm downy mildew survey data are used to estimate the relative prevalence and distribution of pathotype, to monitor the origin and spread of new virulent pathotype and to detect shift towards virulence to resistance used in commercial pearl millet cultivars (Thakur et al., 2006). The information thus developed can be used in breeding programmes before the new race/pathotype become widespread. To develop strategies for resistance gene transfer and its deployment, basic understanding of virulence diversity in the pathogen population and mechanisms of virulence development are important.

As the use of fungicides is beyond the means of most small-scale farmers, the most practical and preferred means of PMDM management has been the use of resistant varieties. However, host resistance is difficult to maintain because the abundant virulence (isolates) diversity of *S. graminicola* renders varieties that are resistant in one location or year susceptible in another. Thus, the use of wide diversity of host resistance genes and knowledge of the pathogen's genetic diversity for virulence and other markers are useful tools in developing and deploying pearl millet varieties with durable DM resistance. Characterizing the *S. graminicola* population from India using both molecular and virulence markers will provide information on the amount of genetic variation that is maintained in this pathogen and the geographical distribution of the different pathotypes. Comparison of pathotype structure and molecular diversity may provide insights into the speed at which new virulent pathotypes will occur.

In this study, inter simple sequence repeats (ISSR) amplification was employed to assess the genetic diversity in *S. graminicola* populations. The objectives of the present study were to examine the possibility of using ISSR analysis for discerning genetic diversity in *S. graminicola* populations with respect to cultivars and geographical locations of isolate collection and to design primers for the unique/specific bands of the pathotypes and develop molecular markers linked to particular pathotype(s).

Materials and Methods

Maintenance of *Sclerospora graminicola* isolates

Downy mildew infected leaf samples from highly susceptible pearl millet cultivars were collected during on-farm surveys. Oospores contained in the infected leaf were recovered from each sample and the isolates (one from each sample) were established by infesting the autoclaved soil and sowing the seed of a highly susceptible pearl millet line. The diseased seedlings with asexual sporulation were maintained in the greenhouse isolation chamber. A total of 22 isolates collected from different pearl millet cultivars growing in different states were established (Table 1). Sporangial inoculum from single leaf was used to multiply the

Table 1
Pearl millet downy mildew isolates across the pearl millet growing areas in India

Sl. No.	Identity of *S. graminicola* Isolates	Host cultivars	Place of collection	Agroclimatic zones
1	Sg 021	7042 S	Ahmednagar, Gujarat	Gujarat Plains and Hills Region
2	Sg 048	852B	Mysore, Karnataka	West Coast Plains and Ghat Region
3	Sg 139	Nokha local	Jodhpur, Rajasthan	Trans-Gangetic plains region
4	Sg 139a	IP 18292-BP	Jodhpur, Rajasthan	Trans-Gangetic plains region
5	Sg 150	834B	Jalna, Maharastra	Eastern and Western Plateau
6	Sg151	Nokha local	Durgapura, Rajasthan	Western Dry Region and Central Plateau
7	Sg 153	7042 S	Patancheru, Andhra Pradesh	East Coast Plains and Hills Region
8	Sg 200	ICMP-451	Jamnagar, Gujarat	Gujarat Plain Region
9	Sg 212	ICMP-451	Durgapura, Rajasthan	Western Dry Region
10	Sg 298	W 504-1-1	Pusa, New Delhi	Trans-Gangetic Plains Region
11	Sg 332	MLBH-267	Aurangabad, Maharastra	West Coast Plains and Ghat Region
12	Sg 334-local	843B	Bhiwani, Haryana	Trans-Gangetic Plains Region
13	Sg 335-HHB67	843B	Bhiwani. Haryana	Trans-Gangetic Plains Region
14	Sg 348	ICMP-451	Anand, Gujarat	Gujarat Plain Region
15	Pat-K1977	7042 S	Patancheru, Andhra Pradesh	East Coast Plains and Hills Region
16	Pat-K1987	7042 S	Patancheru, Andhra Pradesh	East Coast Plains and Hills Region
17	Path-1	HB3	Gulbarga, Karnataka	Southern Plateau and Hills region
18	Path-2 (local)	Kalucombu	Mysore, Karnataka	West Coast Plains and Ghat Region
19	Path-3	MBH110	Aurangabad, Maharastra	West Coast Plains and Ghat Region
20	Path-4	7042S	ICRISAT, Andhra Pradesh	East Coast Plains and Hills Region
21	Path-5	MLBH104	Jalna, Maharastra	Eastern and Western Plateau
22	Path-6	HHB-67	Hissar, Haryana	Trans-Gangetic Plains Region

inoculum on the respective host to maintain the uniformity in the asexual spores of *S. graminicola*. Isolates were maintained on respective host cultivars in isolation chambers in greenhouses at Department of Applied Botany and Biotechnology, Mysore and ICRISAT, Patancheru (Table 1).

DNA isolation

Sporangia of each isolates were harvested in a sterilized Meera cloth dispensed in cold sterile deionized water and fungal DNA was extracted from each isolate according to the method of (Sastry et al., 1995).

PCR amplification and electrophoresis

Twenty microsatellites repeat primers (ISSR), of which 17 were anchored at 3' end and three unanchored primers were used for PCR amplification (Table 2). Amplification was performed with 1× PCR buffer, 200 mM of dNTPs, 0.5 μM primer, 0.6 Units of Taq DNA Polymerase (Bangalore Genei, Bangalore, India) and 50 ng of genomic DNA in a final volume of 20 μl for 40 cycles in a thermalcycler (UNO II-BIOMETRA, Göttingen, Germany). PCR conditions included initial denaturation at 94°C for 4 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at primer specific temperature and 2 min extension at 72°C. Final extension for 10 min at 72°C was used for proper amplification of fragments.

Amplified products were mixed with loading dye (Bromophenol blue, xylene cynol and sucrose) and resolved on 1.8% agarose gel stained with ethidium bromide using 1× TBE buffer pH–8.3 at 60–65 Volts in electrophoresis unit (Maxicell, EC-360 M Apparatus). After the run, the gel was visualized under UV light for scoring the bands.

Table 2
Fingerprint patterns generated using the 20 ISSR repeat primers in 22 isolates of *Sclerospora graminicola*

Sl No	Sequence	Primer name	Annealing Temperature, (°C)	Total No. of bands generated	No. of polymorphic bands	PIC value (%)
1	$(CT)_8TC$	814	40	18	17	94.4
2	$(CT)_8AC$	844A	40	21	17	80.9
3	$(CT)_8GC$	844 B	40	19	18	94.7
4	$(CA)_6AC$	17898 A	45	24	20	83.3
5	$(CA)_6GT$	17898B	45	19	17	89.4
6	$(CA)_6AG$	17899 A	45	24	22	91.6
7	$(CA)_6GC$	17899 B	45	23	21	91.3
8	$(GA)_6GG$	HB 08	48	24	23	95.8
9	$(GT)_6GG$	HB 09	48	19	16	84.2
10	$(GA)_6CC$	HB 10	48	33	33	100.0
11	$(GT)_6CC$	HB 11	48	27	25	92.5
12	$(CAC)_3GC$	HB 12	45	29	27	93.1
13	$(GAG)_3GC$	HB 13	45	19	16	842
14	$(CTC)_3GC$	HB 14	45	21	18	85.7
15	$(GTG)_3GC$	HB 15	48	20	19	95.0
16	$(CA)_9T$	ISSR-16	48	10	06	60.0
17	$(GA)_9C$	ISSR-17	48	16	13	81.2
18	$(TAG)_4$	ISSR-18	50	00	00	–
19	$(GACA)_4$	ISSR-19	48	24	20	83.3
20	$(GGAT)_4$	ISSR-20	50	20	17	85.0
				410	365	89.0

PIC, polymorphism information content.

Scoring of amplified bands/fragments

Each fragment that was amplified using ISSR primers was treated as a unit character and scored in terms of binary code (1/0 = +/–). Amplification was repeated thrice for each primer and only reproducible bands were considered for scoring. Scoring was performed by three individuals and results were combined.

Data analysis

To better understand the patterns of variation among isolates, two multivariate analyses, clustering with unweighted pair group method of averages (UPGMA) and multidimensional scaling were performed. The data were boot strapped to get 200 replicate data sets, which were used to calculate Nei's genetic distance (Nei, 1978). Two hundred UPGMA trees were constructed based on genetic distance and strict consense UPGMA tree was generated out of 200 trees using Felsenstein's (2002) PHYLIP 3.6a3 statistical package (http://evolution.genetics.washington.edu/phylip.html). Dice similarity (Dice, 1945) matrix was calculated using ISSR binary data and multidimensional scaling was performed separately using NTYSISPC, version 2.01 statistical package (http://www.exetersoftware.com/cat/ntsyspc/ntsyspc.html).

Results

Fingerprinting of *Sclerospora graminicola* isolates by ISSR

The microsatellite primers used included 13 dinucleotide repeats, five trinucleotide and two tetranucleotide repeats. For scoring, thick and prominent bands were considered; however, bands of lower intensity, but with high reproducibility were also included in the analysis (Table 2). Out of the 20 primers screened, trinucleotide primer $(TAG)_4$ failed to amplify even at different annealing temperatures after repeated trials. A total of 410 bands were obtained with 365 bands (89%) polymorphic from PCR amplification with 19 primers using genomic DNA from 22 *S. graminicola* isolates. The results indicated that ISSR fingerprints of *S. graminicola* detected a significant level of diversity among the isolates and that ISSR markers could be a powerful alternative for fingerprinting and diversity analysis. Dinucleotide repeat $(GA)_6CC$ produced a maximum of 33 bands showing 100% polymorphism (Fig. 1), followed by 29 bands by trinucleotide repeat $(CAC)_3GC$ (Fig. 2) and 27 bands by dinucleotide repeat $(GT)_6CC$. $(GA)_9T$ primer amplified a total of 10 bands of which four were monomorphic among all the isolates studied.

Clustering of isolates Sg 200 from Jamnagar, Gujarat (Gujarat Plain Region) and Sg 212 from Durgapura, Rajasthan (Western Dry Region), Path-6 from Hissar, Haryana (Trans-Gangetic Plains Region) and Sg 335 from Bhiwani, Haryana (Trans-Gangetic Plains Region) and Sg 151 from Durgapura, Rajasthan (Central Plateau region) and Sg 139 from Jodhpur, Rajasthan (Trans-Gangetic Plains Region) having the same host, but different origin in the separate sub-groups, support the host-specific nature of the

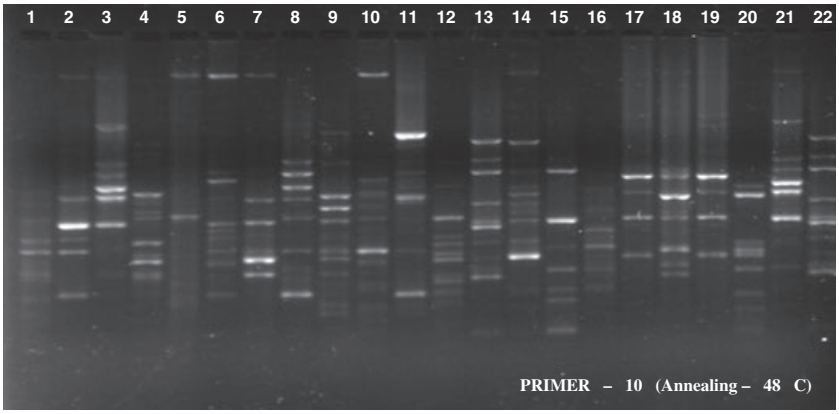

Fig. 1 DNA fingerprint (one of the three replicates) of 22 *S. graminicola* isolates amplified with dinucleotide repeat ISSR-10 (GA)6 CC produced maximum 33 bands with 100% polymorphism

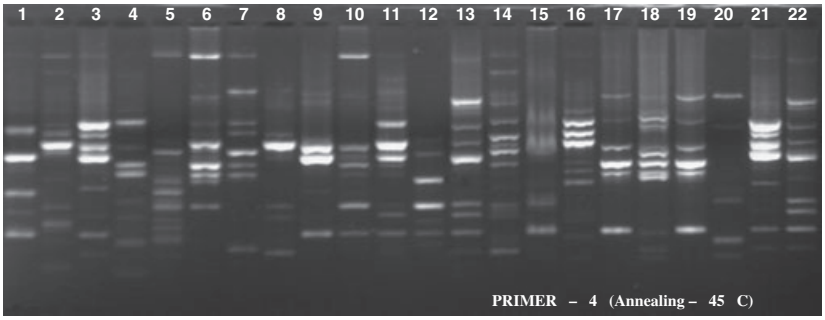

Fig. 2 DNA fingerprint (one of the three replicates) of 22 *S. graminicola* isolates amplified with trinucleotide repeat $(CAC)_3$ GC showing 29 bands with 93.1% polymorphism

pathogen. The combined influence of new cultivars, stepwise selection of virulence and unstable mechanisms of genetic reassortment may have contributed to the development of new races. This high virulence can be used to test new pearl millet breeding lines and hybrids.

To assess the relatedness between the isolates, genetic distance was calculated using ISSR fingerprints (Table 3). The maximum distance index was 0.0206 between Pathotypt-5 and Pathotype-2, which originated from Jalna, Maharashtra (Western Plateau region) and from Mysore, Karnataka (West Coast Plain Region) respectively. The minimum distance index of 0.0050 was obtained between Pathotype-1 of Gulbarga, Karnataka (Southern Plateau region) and Sg153, a field isolate from a disease nursery at ICRISAT, Patancheru, Andhrapradesh (East Coast Plains and Hills Region); both locations are geographically close. Cluster analysis of ISSR banding patterns revealed a high degree of genetic variability among the isolates examined in this study.

The dendrogram consisted of four groups with two major clusters containing 10 and seven isolates respectively (Fig. 3). The first major Group I was further divided into three sub clusters and the second major Group-II was divided into two sub clusters containing three and four isolates. Groups III and IV were minor groups having three and two isolates respectively. Isolates Sg 200 and Sg 212 having the common host ICMP-451, but from different locations namely Jamnagar, Gujarat (Gujarat Plain Region) and Durgapura, Rajasthan (Western Dry Region) were clustered together as a separate sub-group within group I. Similarly, Path-6 and Sg 335 with common host HHB 67 from the same location Bhiwani and Hissar, Haryana (Trans-Gangetic Plains Region) were also grouped together supported by a very high bootstrap value of 100. Isolates Pat-1977 and Pat-1987 with common host 7042S collected from same location Patancheru, Andhra Pradesh (East Coast Plains and Hills Region) were placed in the separate sub-groups within Group I. However, other isolates from this host like Sg 021 and Path-4 but from different regions were included in group II and Sg 153 in group III. Isolates Sg139a, Sg334, Sg332 and Path-5 all having different host cultivars and belonging to nearby locations were also grouped in Group 1. Seven isolates were clustered together in Group II. Sg 151 and Sg 139 having a common host Nokha Local, collected from different locations namely Durgapura, Rajasthan (Western Dry Region) and Jodhpur, Rajasthan (Trans-Gangetic plains region) were clustered together within group II. Isolates Sg 139 and Sg 139a were grouped in different clusters because both isolates have host specificity for pathogenicity. Other isolates included in group II were Sg 048, Path-4, Sg 021, Sg 348 and Sg 298 having different host cultivars and are from widely disbursed geographical locations. Cluster III consisted of Path-3, Path-1 and Sg 153. These isolates have different host cultivars and originated from Aurangabad, Maharashtra, Gulbarga, Karnataka and Patancheru Andhra pradesh are grouped together. Cluster IV formed a monophyletic group consisting Sg 150 from Jalna, Maharashtra (Eastern and Western Plateau) and Path-2 from Mysore, Karnataka (West Coast Plains)

Table 3
Genetic Distance of 22 isolates of *Sclerospora graminicola* collected from different parts of India

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.0000																					
2	0.0080																					
3	0.0103	0.0071																				
4	0.0109	0.0099	0.0096																			
5	0.0185	0.0145	0.0119	0.0118																		
6	0.0132	0.0109	0.0085	0.0116	0.0097																	
7	0.0128	0.0107	0.0117	0.0094	0.0086	0.0114																
8	0.0096	0.0088	0.0090	0.0073	0.0150	0.0125	0.0125															
9	0.0110	0.0095	0.0118	0.0135	0.0142	0.0150	0.0119	0.0074														
10	0.0099	0.0113	0.0096	0.0122	0.0167	0.0104	0.0127	0.0103	0.0144													
11	0.0106	0.0068	0.0094	0.0085	0.0163	0.0126	0.0123	0.0066	0.0077	0.0115												
12	0.0116	0.0085	0.0079	0.0078	0.0115	0.0132	0.0109	0.0095	0.0091	0.0125	0.0070											
13	0.0127	0.0099	0.0127	0.0093	0.0161	0.0169	0.0135	0.0082	0.0093	0.0115	0.0085	0.0106										
14	0.0107	0.0089	0.0119	0.0115	0.0147	0.0138	0.0134	0.0093	0.0085	0.0119	0.0069	0.0106	0.0095									
15	0.0115	0.0121	0.0090	0.0089	0.0159	0.0174	0.0130	0.0080	0.0080	0.0090	0.0085	0.0083	0.0077	0.0097								
16	0.0104	0.0086	0.0088	0.0122	0.0174	0.0189	0.0117	0.0097	0.0089	0.0105	0.0098	0.0092	0.0076	0.0110	0.0071							
17	0.0117	0.0120	0.0113	0.0109	0.0116	0.0155	0.0050	0.0133	0.0134	0.0113	0.0148	0.0112	0.0122	0.0166	0.0119	0.0101						
18	0.0176	0.0147	0.0149	0.0169	0.0094	0.0140	0.0172	0.0189	0.0167	0.0139	0.0164	0.0180	0.0187	0.0139	0.0158	0.0178	0.0135					
19	0.0134	0.0141	0.0122	0.0145	0.0119	0.0120	0.0058	0.0146	0.0125	0.0119	0.0136	0.0138	0.0130	0.0143	0.0128	0.0116	0.0056	0.0167				
20	0.0077	0.0068	0.0096	0.0088	0.0118	0.0099	0.0106	0.0098	0.0109	0.0088	0.0108	0.0121	0.0123	0.0098	0.0134	0.0112	0.0123	0.0124	0.0152			
21	0.0110	0.0102	0.0134	0.0104	0.0175	0.0139	0.0100	0.0072	0.0081	0.0113	0.0092	0.0109	0.0074	0.0116	0.0099	0.0073	0.0123	0.0206	0.0107	0.0126		
22	0.0113	0.0099	0.0107	0.0096	0.0132	0.0169	0.0136	0.0079	0.0085	0.0113	0.0083	0.0091	0.0022	0.0094	0.0070	0.0083	0.0119	0.0195	0.0137	0.0118	0.0078	0.0000

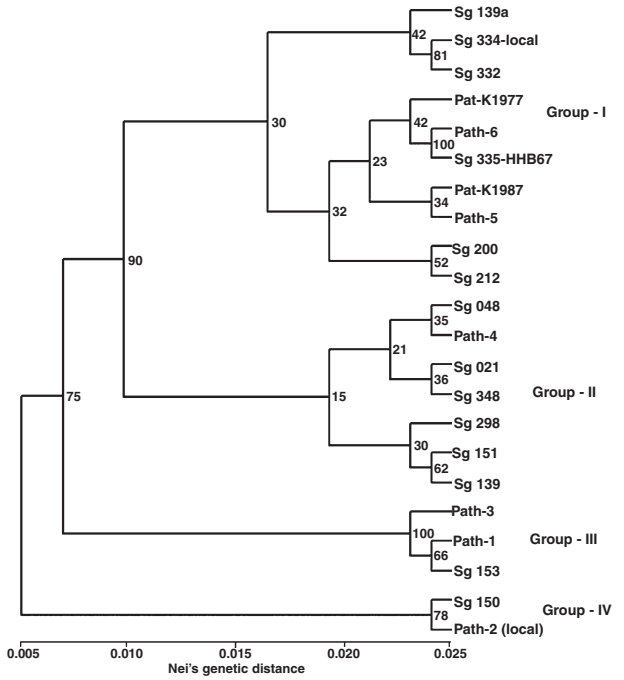

Fig. 3 Dendrogram based on ISSR polymorphisms in 22 isolates of *Sclerospora graminicola* of pearl millet by unweighted pair group method of averages (UPGMA) cluster analysis (numbers inside the branches are bootstrap values)

locations and which was found to be profoundly distinct from the other clusters.

Three-dimensional scaling

The 3D scaling is one of the multivariate approaches of grouping based on the similarity coefficient values of the component traits of the entities. The 3D scale shows that the various isolates form 4 distinct groups (1, 2, 3 and 4) supported the UPGMA results and clarified relationships among *S. graminicola* isolates (Fig. 4).

Discussion

Continuous monitoring of *S. graminicola* population is necessary because they are characterized by high rates of recombination and extreme virulence shifts leading to breakdown of resistance in hitherto unknown hybrids (Hess et al., 2002). Such a situation is alarming for the farmers who rely solely on host resistance as the chemical control measure is expensive and beyond the reach of marginal farmers (Zarafi et al., 2005). Diversity analysis of *S. graminicola* has been carried out over the years using various methods like host specificity, virulence, molecular methods like RAPD, AFLP and DNA fingerprinting (Sastry et al., 1995; Singru et al., 2003) and serological techniques (Shishupala, 1994). However, the precise nature of the genetic changes involved cannot be perfectly measured in all the techniques mentioned above. Additionally, the banding patterns can be difficult to interpret and the resultant data are dominant in nature. Furthermore, very little is known about the individual loci that make up the fingerprint (Cooke and Lees, 2004).

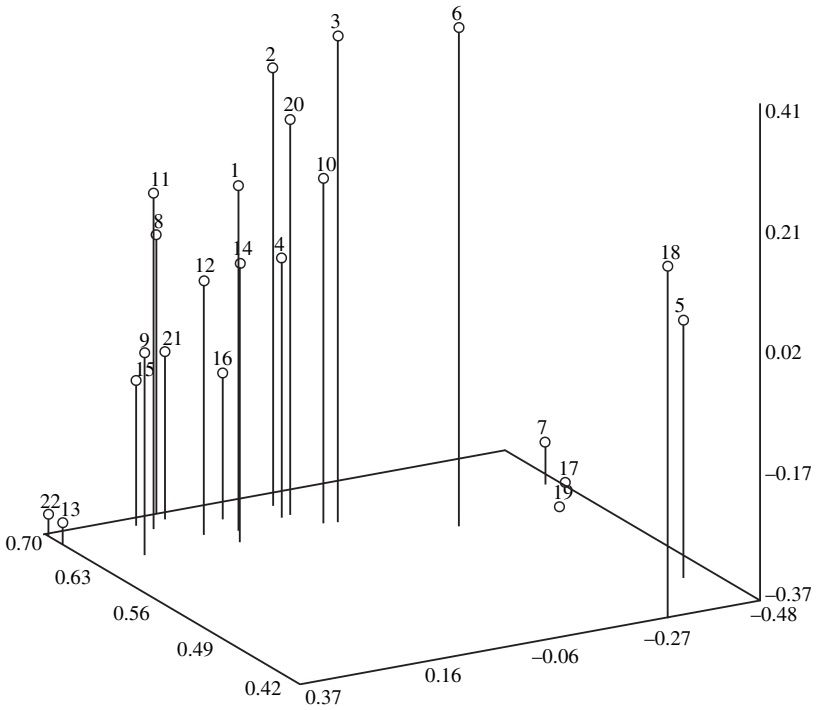

Fig. 4 Three-dimensional scaling generated by NTSYS of principal coordinate analysis (PCA) of 22 isolates of *Sclerospora graminicola* based on the combination of data obtained with 19 ISSR primers. Axes values represent eigenvalues

In this study we could obtain extremely high degree of polymorphism amounting to 89% variability among the isolates. This was mainly due to the use of a number of randomly selected prescreened highly informative primers. In the present study, 13 di, four tri and tetra nucleotide repeats each, were used as ISSR primers (Table 2). Except for tetra nucleotide repeats, all other primers were anchored at 3′ region with one or two bases to prevent incidental annealing of primers within SSRs, which may lead to smear formation on agarose gels. We did not consider primers anchored at 5′ region since these may impose selection of long simple sequence repeats within the amplified region (Blair et al., 1999). Only four di nucleotide repeats (CT, CA, GA and GT) were used with different base combination at 3′ anchored region. Number of amplified fragments varied within the primers having same repeat motifs, but differed only in anchored bases. This indicated that ISSR-amplified products mainly depended on the anchored base, rather than the di-nucleotide repeat motifs.

Of all the primers tested, di-nucleotide GA repeat primers detected 100% polymorphic bands; this was supported by previous reports where GA repeat produced excellent banding pattern in mulberry (Vijayan and Chatterjee, 2003). Even though primers having tetra-nucleotide as repeat motifs were not anchored at 3‘ region, these primers also detected a considerable number of polymorphic bands. This may be due to lower copy number of the tetra-nucleotide repeat motifs present within amplifiable distance and thus can be easily picked by the unanchored primers. Tri and tetra nucleotide repeats are reported to be less frequent in plant genomes (Reddy et al., 2002). Non-anchored tetra nucleotide primers were successively used to study polymorphism in tomato.

The amount of polymorphism obtained and the polymorphic/discriminant bands recorded in the study is much higher than many of the previously reported variability studies on *S. graminicola* using RAPDs (Sastry et al., 2001), UP-PCR (Amruthesh, 2000), AFLP (Singru et al., 2003) and DNA fingerprinting (Sastry et al., 1995). This is true in most cases because ISSRs have a high capacity to reveal polymorphism and offer a great potential to determine variability in *Rhizoctonia solani* isolates as compared with other techniques that use arbitrary primers (Sharma et al., 2005).

Molecular markers such as RAPDs are widespread in their distribution throughout the genome having higher degree of polymorphism (Cramer et al., 2003). However, ISSR markers span short chromosomal sequence between SSRs and hence are easily assayed than the RAPD markers (Parsons et al., 1997). In addition, ISSR markers also mark gene rich regions as in the case of virulence of a pathogen especially with respect to pathogenic oomycetes. Most of the ISSR markers identified are randomly distributed in their respective genomes and high level of polymorphism observed in their study using ISSR primers is due to variation in the number of tandem repeat motif at a specific locus (Davierwala et al., 2000).

Clustering of isolates Sg 200 and 212, Path-6 and Sg 335 and Sg 151 and Sg 139 having the same host, but different origin in the separate sub-groups, support the host-specific nature of the pathogen. The combined influence of new cultivars, stepwise selection of virulence and unstable mechanisms of genetic reassortment may have contributed to the development of new

races. This high virulence can be used to test new pearl millet breeding lines and hybrids. Similarly, *Sg* 021, Sg 153, Pat-K 1977, Pat-K 1987 and Path-4 have a common host (7042S) but group in different subclusters. The variation shown by these isolates shows that ISSR can detect differences at a very fine scale.

In addition to the dendrograms, the results from multi dimensional scaling using eigenvalues for correlation of genetic similarity show that individual units can be grouped into four different principal components amounting to 70% variation among each other. However, the two methods show that subclustering and multidimensional scaling plots are slightly different. Both methods, however, used the same number of 410 amplicons, but differed markedly in showing relatedness of individual geographical isolates. Various studies in downy mildews of pearl millet (Sastry et al., 1995; Thakur et al., 1998; Singru et al., 2003) have also shown that multi dimensional scaling plots and dendrograms differ in clustering of the isolates. Therefore, it is desirable to use different fingerprinting methods used in tandem to remove the differences and address the problem has arisen.

Among all the methods tested, ISSRs appear to offer the greatest potential across a wide range of applications and should be developed further. ISSR markers will clearly be useful for further population studies in *S graminicola* as the application of these markers, which facilitates for analysis of multiple alleles at a single locus, is less time consuming, requires less DNA and can be scored more precisely (Sexton and Howlett, 2004). ISSR fingerprinting has the potential for large scale tracking of isolates and understanding origin and spread of strains with novel specificities. This is mainly possible because the microsatellites are highly variable and densely present throughout the genome (Kohn et al., 2000). Such isolate tracking can be used to effectively determine primary sources of inoculum (Zwankhuizen et al., 2000) which can have important implications for developing new hybrids resistant to highly aggressive and variable populations of *S. graminicola*. In general, when a comparison is carried out between various marker systems, it has been evident that ISSRs represent an efficient and suitable marker system for discriminating among closely related pathogenic isolates like *Phytophthora* and *Pythium* (Cooke and Lees, 2004). Our study also demonstrates the discriminatory power of ISSR to clearly distinguish and separate a related oomycetes pathogen *S. graminicola*.

Further research comparing genetics and virulence across populations from different isolates differing in geographical locations would be of great interest and will be valuable for disease management strategies and screening for resistance to this highly destructive pathogen.

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