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Characterization of *Sclerospora graminicola* Isolates from Pearl Millet for Virulence and Genetic Diversity

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Virulence and genetic diversity were studied using 21 isolates of *Sclerospora graminicola*, the pearl millet downy mildew pathogen collected from major pearl millet growing areas of India. Variability for virulence was determined by inoculating a set of 10 differential hosts with the *S. graminicola* isolates in a greenhouse. The isolates varied for latent period (6.4 to 11 days), disease incidence (0 to 98%), virulence index (0 to 18.7) and oospore-production potential (1 to 4). Among the 21 isolates, Sg 139 (Rajasthan) was the most virulent and Sg 110 (Tamil Nadu) the least virulent. Based on virulence index (disease incidence × latent period^{-1}), the 21 isolates were classified into eight virulence groups. Genetic diversity among isolates was studied using AFLP markers. Based on similarity index of banding pattern, the 21 isolates were clustered into eight genotypic groups. The AFLP groupings, however, did not match with that of the virulence groupings, and these two were found independent. The isolate Sg 139 that remained distinct in both pathogenic and genetic groupings indicated its highly virulent nature. Implications of these results in downy mildew resistance breeding are discussed.

Keywords : downy mildew, *Pennisetum glaucum*, *Sclerospora graminicola*, virulence diversity

Downy mildew, incited by *Sclerospora graminicola* (Sacc.) Schroet, is the most destructive and widespread disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.] in major pearl millet growing areas of the world. *S. graminicola*, an obligate biotroph, reproduces asexually by means of sporangia that liberate motile zoospores, and sexually through oospores. The fungus is mostly heterothallic but homothallism may also exist (Michelmore et al., 1982), and such reproductive characteristics of the fungus make it highly variable. Sexual reproduction also provides new genetic recombination resulting in evolution of pathogen populations. Under these circumstances, utilization of host-plant resistance is the only feasible way to manage this disease. Development of stable and durable resistance against diverse pathotypes requires thorough knowledge of existence and distribution of pathotypes in a given locality which can be done through virulence surveys, using differential hosts, and molecular markers.

Variation in virulence evolves either due to the environmental and varietal differences or the selection pressure exerted by a host genotype. Isolates of *S. graminicola* from different geographic regions of Asia and Africa have been shown to differ in pathogenicity and virulence when tested on a set of host cultivars (Ball, 1983; Ball and Pike, 1984; Ball et al., 1986). Variation in virulence and aggressiveness was also demonstrated among single oospore isolates and single zoospore isolates of *S. graminicola* (Thakur and Shetty, 1993; Thakur et al., 1998a). Host-cultivar directed virulence selection in field populations of *S. graminicola* has been demonstrated (Thakur et al., 1992). The results of field surveys and other related studies have provided further evidence for the host-directed virulence selection as a mechanism for the emergence of a new virulent pathotype (Thakur et al., 1999; Thakur et al., 2003). Genetic diversity among *S. graminicola* isolates has been reported using DNA fingerprinting (Sastry et al., 1995), RAPD (Zahid, 1997) and AFLP (Sivarmakrishnan et al., 2003). This was for the first time that *S. graminicola* isolates from eight pearl millet growing states of India were assembled and characterized. The objectives of this investigation were to determine the extent of pathogenic and genetic diversity among the selected isolates of *S. graminicola* collected from commercial pearl millet cultivars being grown in several states of India, and identify the most virulent isolates that could be appropriately utilized in screening and breeding for downy mildew resistance.

Materials and Methods

Pathogen isolates and host genotypes. Of the 200 *S. graminicola* oosporic isolates collected from commercial pearl millet cultivars during on-farm surveys in major pearl millet growing areas of India, 21 representative isolates

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Table 1. Isolates of *Sclerospora graminicola* selected from collections of ICRISAT, Patancheru, Andhra Pradesh, India

Isolate designation	Source of host genotype	Year of collection	Collection site (Location/ District/ State)[a]
Sg 004	7042S & HB 3	1988	ARS/ Durgapura/ Jaipur/ Rajasthan
Sg 015	7042S	1992	HAU/ Hisar/ Haryana
Sg 021	MLBH 104	1993	Ghari/ Ahmadnagar/ Maharasthra
Sg 025	BK 560	1993	Bhadgaon/ Jalgaon/ Maharashtra
Sg 026	NATH 4209	1993	Veelad/ Ahmadnagar/ Maharasthra
Sg 032	HB 3	1993	MPKV/ Rahuri/ Maharasthra
Sg 040	BK 560	1994	Pachora/ Jalgaon/ Maharasthra
Sg 045	MAHYCO Hybrid	1994	Pune/ Pune/ Maharasthra
Sg 046	EKNATH 201	1994	Bandgaon/ Pune/ Maharasthra
Sg 047	BK 560	1994	Ranebennur/ Dharwad/ Karnataka
Sg 048	7042S & HB 3	1994	Univ.of Mysore/ Mysore/ Karnataka
Sg 088	GK 1006	1996	Fatiabad/ Aurangabad/ Maharashtra
Sg 110	CO-3	1996	Illupanatham/ Kovai/ Tamil Nadu
Sg 115	Hybrid	1996	Kovilpatti/ Tirunelveli/ Tamil Nadu
Sg 139	Nokha Local	1997	Jodhpur/ Jodhpur/ Rajasthan
Sg 140	7042S & HB 3	1997	Jamnagar/ Jamnagar/ Gujarat
Sg 149	Local	1997	Gwalior/ Madhya Pradesh
Sg 150	MBH 110	1997	Mahyco Farm/ Jalna/ Maharashtra
Sg 151	81 A	1997	ARS/ Durgapura/ Jaipur/ Rajasthan
Sg 152	Local	1997	ARS/ Durgapura/ Jaipur/ Rajasthan
Sg 153	7042S & NHB 3	1997	ICRISAT/ Patancheru/ Medak/ Andhra Pradesh

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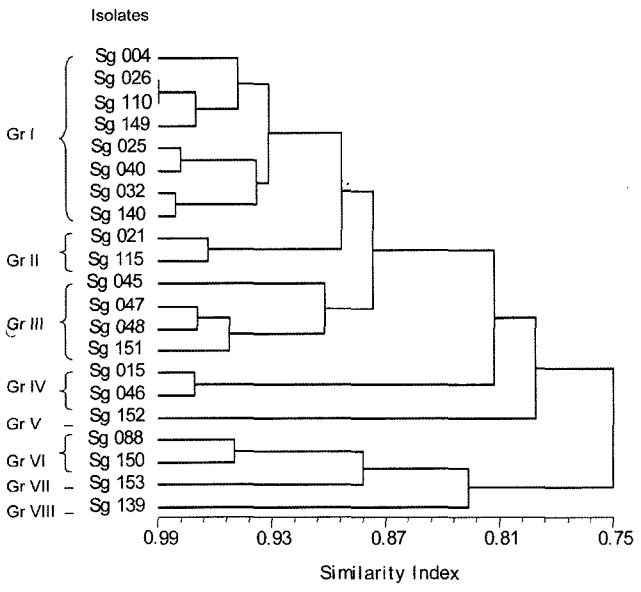

Fig. 1. Dendrogram of the 21 isolates of *S. graminicola* based on virulence index of the 10 host differential lines using NTSYSpc software package. Gr, Group.

were selected for this study (Table 1, Fig. 1). These isolates were established as asexual inocula on pot-grown seedlings of a highly susceptible genotype 7042S (Thakur et al., 1992). Each isolate was maintained separately in polyacrylic isolation chambers in a greenhouse at 25 ± 2°C. The isolates were propagated and maintained on 7042S through several asexual generations in a greenhouse.

A set of 10 inbred lines (IP 5272-1, IP 18296, IP 18297, P 536-2, P 1564, P 2895-3, P 3281-1, 700481-21-8, IP 18292, and 7042S) were used as differential hosts (Thakur et al., 1997).

Inoculation assay. Inoculum (5×10^5 sporangia ml^{-1}) was prepared from each isolate and pot-grown seedlings of the differential lines were spray-inoculated with sporangial suspension at the coleoptile to first-leaf stage using a hand sprayer (Singh et al., 1993). Inoculated seedlings were covered immediately with polyethylene sheet to provide > 95% relative humidity necessary for infection. Inoculated seedlings were incubated in dark at 20°C for 24 h. The pots for each isolate were then transferred onto greenhouse bench at 25 ± 2°C in isolation chambers. The experiment was conducted in a completely randomized design having three replications with 100 seedlings per replication for each isolate.

Estimation of oospore production. Necrotic leaf portions collected from infected seedlings were dried under shade in brown paper bags, and stored at room temperature (approx. 25°C) until observation. Leaf pieces measuring 1 × 1 cm^2

were surface sterilized with 2% NaOCl, washed thoroughly with sterile distilled water, and cleared by incubating at 40°C in 5% NaOH for 12-16 h. Cleared leaf pieces were rinsed in distilled water and observed under microscope using a 10X objective for the presence of oospores. In each replication, for each host genotype 10 leaf pieces were observed. Oospore production rating was scored on a modified 1-4 scale, where 1 = No oospores, 2 = 1 to 100 oospores/cm, 3 = 101-1000 oospores/cm and 4 = > 1000 (numerous) oospores/cm of leaf area (Thakur and Shetty, 1993).

Assessment of genetic diversity. The molecular diversity among the 21 isolates of *S. graminicola* was assessed using AFLP markers technique (Vos et al., 1995). DNA from 21 *S. graminicola* isolates was extracted by modifying the procedures of Sivaramakrishnan et al. (2003) by increasing the four-fold concentration of RNase. The DNA pellet was washed twice with 70% ethanol, dried at room temperature and dissolved in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0) for further analysis. AFLP analysis was performed using the kit from Life Technologies, USA and assays were carried out with the manufacturer's protocol. Although a number of primer combinations were tested, the results reported in the present study were obtained with three primer combinations. The two *Eco*RI (E-TG and E-TT) primers and three *Mse*I (M-CAT, M-TAG and M-CTA) primers were used in these three combinations (E-TG/M-CAT, E-TT/M-TAG and E-TG/M-CTA) for amplification. The relatedness of the 21 isolates of *S. graminicola* was estimated by means of scoreable bands form three primer combinations used in AFLP analysis. Differences in banding pattern were scored on the basis of presence or absence of a band. Similarities between the DNA fingerprints were calculated using Nei and Lei's (1979) similarity index, given by the formula $S_{(xy)} = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of shared fragments and N_x and N_y are the number of fragments in the fingerprints x and y, respectively.

Virulence diversity. Data were recorded for latent period, disease incidence and oospore production per unit leaf area. Latent period was expressed as the number of days from inoculation to sporulation on the 50% of the infected seedlings (Thakur et al., 1998b). To record latent period, seedlings were observed daily from the 5th day after inoculation until 50% of seedlings got infected. Number of infected seedlings and total seedlings per pot were recorded 15 days after inoculation (dai) and the percent disease incidence was calculated. To determine the oospore production, necrotic leaf tissue from five infected seedlings of each genotype were collected at 30 to 45 dai. Quantitative differences in virulence levels of the isolates were determined by calculating virulence index (Thakur and Rao, 1997) as:

Virulence index = Percent disease incidence × latent period^{-1}

Statistical analysis. The data of virulence diversity were subjected to Analysis of Variance (Gomez and Gomez, 1984) using GENSTAT statistical package (Rothamsted Experiment Station, Herpenden, Herts AL 52 JQ, UK), to determine significant differences among isolates, host genotypes and their interactions. Average Linkage Cluster analysis was done using the Euclidian test to determine the similarity among the isolates and to classify isolates into virulence groups based on virulence index and a dendrogram was prepared using the NTSYSpc 2.1 software package. For AFLP banding pattern, a cluster analysis was performed using the statistical software package SYSTAT 5.1. A dendrogram showing the mean similarities between groups of different isolates was generated.

Results

Pathogenic variability. All the 21 isolates tested induced clear downy mildew symptoms on host genotypes 7042S, P 536-2, P 1564, P 2895-3 and 700481-21-8 and therefore were considered as virulent on these host genotypes. However, majority of the isolates induced traces or no symptoms on IP 18297 and IP 18292 and so these were considered as avirulent on these two genotypes (Table 2). Irrespective of the isolates used, symptoms induced on IP 18292 and P 1564 were severe stunting of the seedling, dark green foliage and lack of sporulation, while those on other host differentials include chlorosis coupled with ample sporulation. Considerable variation occurred among isolates × host differentials for disease incidence, ranging from 0.2% (Sg 046) to 97.9% (Sg 150) (Table 2). The highest mean disease incidence (49.5%) across host genotypes was recorded for isolate Sg 139 and the lowest (14.3 %) for Sg 110. Most isolates were highly aggressive on 7042S, moderately aggressive on 700481-21-8, P 1564 and P 536-2, and least aggressive on IP 18297. However, on other host genotypes, the disease incidence was highly variable. The analysis of variance indicated highly significant ($P<0.01$) effects of isolates, host genotypes and their interactions on disease incidence (Table 3).

Variation in virulence. All isolates showed susceptible (S) reaction (> 10% mean incidence) on 7042S and 700481-21-8, and resistant (R) reaction (≤ 10% mean incidence) on IP 18297 (Table 2). Except isolate Sg 152 on host genotype P 536-2 and Sg 153 on P 3281-1, all other isolates gave differential reactions on these host genotypes. However,

Table 2. Percent disease incidence[a] caused by of the 21 isolates of *S. graminicola* on the host differential lines

Isolates	Host differential lines										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	20.4	3.7	0.0	40.0	18.0	19.9	0.7	33.4	44.3	96.8	27.7
Sg 015	1.5	1.2	0.8	46.6	55.1	0.4	0.6	46.2	0.3	95.4	24.8
Sg 021	0.0	24.0	0.3	31.1	15.6	2.3	0.0	26.6	2.2	94.4	19.7
Sg 025	11.8	0.0	0.0	28.3	12.4	33.5	0.0	34.9	1.1	85.7	20.8
Sg 026	0.0	2.2	0.0	38.4	0.8	0.8	0.2	25.1	0.2	92.6	16.0
Sg 032	15.3	7.1	0.0	43.9	10.2	29.5	0.8	53.7	0.0	97.6	25.8
Sg 040	9.9	0.5	0.3	25.6	55.3	27.9	0.2	48.7	0.8	94.0	26.3
Sg 045	60.9	8.0	0.0	34.5	75.2	49.7	6.6	24.3	0.4	94.0	35.4
Sg 046	0.2	1.8	0.6	47.4	2.6	0.2	0.1	48.7	0.0	96.5	19.9
Sg 047	34.7	13.9	0.0	50.5	46.8	46.3	3.3	42.9	23.5	96.7	35.9
Sg 048	45.1	14.0	0.0	50.2	28.0	49.6	0.8	32.7	61.3	97.6	38.0
Sg 088	71.9	22.6	0.0	40.9	74.3	61.3	4.1	89.0	0.0	94.3	45.8
Sg 110	0.4	0.0	0.4	33.1	0.4	0.4	0.0	17.6	0.0	90.6	14.3
Sg 115	2.8	34.5	0.0	30.8	33.3	5.0	0.0	45.5	1.1	91.0	24.4
Sg 139	45.8	13.9	0.0	46.4	86.8	51.5	7.9	59.4	89.5	93.4	49.5
Sg 140	27.7	10.0	0.4	32.1	23.6	36.2	3.8	73.8	0.0	96.4	30.3
Sg 149	0.7	1.0	0.0	54.2	8.1	2.1	0.3	26.7	2.0	95.0	19.0
Sg 150	67.3	12.6	0.0	40.6	75.8	45.1	3.6	79.2	0.0	97.9	42.2
Sg 151	19.8	4.5	0.0	45.9	38.7	46.6	5.6	57.3	61.5	97.2	37.7
Sg 152	14.0	0.0	0.0	4.0	94.5	53.6	0.0	56.4	0.0	87.0	31.0
Sg 153	51.9	17.8	0.0	45.1	86.7	69.2	18.7	55.4	0.0	97.0	44.2
Mean	23.9	9.2	0.1	38.6	40.1	30.1	2.8	46.6	13.7	94.3	

[a]Mean of three replications. SE ± for isolates = 0.28, host differential lines = 0.19, and their interactions = 0.89. CD (1%) for isolates = 0.72, host differential lines = 0.50, and their interactions = 2.28.

Table 3. Analysis of variance for disease incidence, latent period, virulence index and oospore production by the *S. graminicola* isolates on the host differentials

Source of variation	df	Disease incidence		Latent period		Virulence index		Oospore production	
		MS	F-value	MS	F-value	MS	F-value	MS	F-value
Isolates (I)	20	1997.6	1698.3[a]	28.4	600.35[a]	70.46	833.16[a]	6.05	1150.16[a]
Host genotypes (H)	9	30494.5	25924.9[a]	231.2	4892.67[a]	1364.30	16132.19[a]	83.53	15893.09
I × H	180	451.5	383.9[a]	22.5	475.69[a]	15.07	178.20[a]	2.05	390.95[a]
Residual	420	1.2		0.05		0.08		0.005	–

[a]Significant at ($P < 0.01$).

isolates had clear differential disease reactions on IP 5272-1, IP 18296, P 1564, P 2895-3 and IP 18292.

Variation in disease incidence. Virulence index of the 21 isolates ranged between 0.02 and 18.69 (Table 4). Virulence index was generally greater for most isolates on 7042S, moderate on 700481-21-8, P 536-2 and P 1564, and lower on IP 18297. Across host genotypes, isolate Sg 139 had greatest virulence index (7.55) and Sg 110, the lowest (2.04), while across isolates it was highest on 7042S (16.09) and lowest on IP 18297 (0.02). F-values were highly significant ($P < 0.01$) for isolates, host genotypes and their interaction (Table 3).

A dendrogram generated by the average linkage cluster analysis based on virulence index (disease incidence) classified the 21 isolates at the 90% similarity level into eight major pathotype groups (Fig. 1). Isolates Sg 004, Sg 032, Sg 047, Sg 048, Sg 151, Sg 040 and Sg 140 were in group I; Sg 015 and Sg 149 in group II; Sg 021 and Sg 115 in group III; Sg 025, Sg 026, Sg 046 and Sg 110 in group IV; Sg 045, Sg 153, Sg 088 and Sg 150 in group V. Three isolates, Sg 152, Sg 153 and Sg 139 were not clustered at the 90% similarity level and thus formed separate groups V, VII and VIII, respectively.

Table 4. Virulence index[a] (percent disease incidence × latent period^{-1}) of the 21 isolates of *S. graminicola* on the host differential lines

Isolates	Host differential lines										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	2.2	0.4	0.0	5.0	1.7	2.1	0.1	3.5	5.2	17.4	3.8
Sg 015	0.1	0.1	0.1	7.0	7.0	0.0	0.1	6.6	0.0	16.9	3.8
Sg 021	0.0	2.8	0.0	3.3	1.5	0.2	0.0	3.3	0.2	16.7	2.8
Sg 025	1.4	0.0	0.0	3.5	1.3	3.9	0.01	3.9	0.1	12.9	2.7
Sg 026	0.0	0.2	0.0	4.8	0.0	0.1	0.0	2.8	0.0	14.6	2.3
Sg 032	1.7	0.8	0.0	4.9	1.2	3.5	0.1	7.0	0.0	16.3	3.5
Sg 040	1.5	0.0	0.0	3.5	5.2	4.2	0.0	5.8	0.1	14.5	3.5
Sg 045	8.3	0.9	0.0	4.3	9.6	5.8	0.9	2.6	0.0	15.3	4.8
Sg 046	0.0	0.2	0.1	6.2	0.3	0.0	0.1	5.9	0.0	16.5	2.9
Sg 047	4.4	1.6	0.0	7.2	5.3	5.4	0.3	5.1	2.6	16.1	4.8
Sg 048	5.6	1.7	0.0	7.0	3.2	6.7	0.1	3.8	8.2	16.3	5.3
Sg 088	11.2	3.1	0.0	5.8	9.3	8.1	0.5	12.7	0.0	17.9	6.9
Sg 110	0.0	0.0	0.0	3.4	0.0	0.0	0.0	1.6	0.0	15.1	2.0
Sg 115	0.3	3.6	0.0	4.1	3.3	0.7	0.0	5.5	0.1	14.1	3.2
Sg 139	6.1	1.7	0.0	6.6	13.2	7.2	0.1	8.1	12.8	18.7	7.6
Sg 140	3.1	1.1	0.0	4.3	2.5	4.2	0.4	10.1	0.0	17.0	4.3
Sg 149	0.1	0.1	0.0	7.1	0.9	0.3	0.0	2.8	0.2	15.9	2.7
Sg 150	8.8	1.4	0.0	5.8	10.1	5.6	0.4	9.8	0.0	16.6	5.9
Sg 151	2.5	0.5	0.0	5.8	4.5	5.8	0.7	7.4	7.7	17.2	5.2
Sg 152	1.7	0.0	0.0	0.5	13.9	7.2	0.0	7.3	0.0	14.5	4.5
Sg 153	6.8	2.2	0.0	7.0	11.0	9.2	2.3	6.4	0.0	17.5	6.2
Mean	3.15	1.1	0.0	5.1	5.0	3.8	0.3	5.8	1.8	16.1	

[a]Mean of three replications. SE ± for isolates = 0.08, host differential lines = 0.10, and their interactions = 0.47. CD (1%) for isolates = 0.19, host differential lines = 0.13, and their interactions = 0.61.

Variation in latent period. The isolates varied significantly for latent period, ranging from 5.0 to 11.0 days (data not shown). The mean latent period across the isolates was longest on IP 18292 (9.4 days) and shortest on 7042S (5.9 days), while across the host genotypes it was longest for isolate Sg 110 (9.5 days) and shortest for Sg 139 (7.08 days). There were significant ($P < 0.01$) effects of host genotypes, isolates and their interactions on latent period (Table 3). A significant negative correlation ($r = -0.77$ at $P < 0.01$) was found between latent period and disease incidence across the host genotypes and isolates.

Variation in oospore production. Oospore production was highly variable among isolates × host genotype combinations. There was no oospore production in P 1564 and IP 18292, and it was highest (3.79 on a 1-4 scale) for most of the isolates on 7042S (Table 5). However, considerable variation was observed for oospore production ratings among the isolates on the remaining host genotypes, and it ranged between 1.47 and 4.00. Across the isolates, the highest mean oospore production was on 7042S (3.79) and the lowest on P 1564 and IP 18292 (1.00). Across the host genotypes, it was highest for Sg 153 (3.25), and the lowest in Sg 115 (2.02). Oospore production was highly influenced by host differential lines, isolates and their interactions (Table 3).

Genetic diversity. A high level of polymorphisms was obtained with AFLP analysis using three primer combinations (E-TG/M-CAT, E-TT/M-TAG and E-TG/M-CTA) among the 20 isolates. A total of 185 bands were scored for the three primer combinations, of which about 80% was found polymorphic (Table 6). With each of the three primer combinations, isolate-specific bands were obtained. Cluster analysis of the similarity index data classified the 20 isolates into eight groups at 70% similarity level (Fig. 2). Isolates Sg 015, Sg 046, Sg 047, Sg 149, Sg 045, Sg 048, Sg 088 and Sg 040 were in group I; Sg 021, Sg 115 and Sg 153 in group II; Sg 110, Sg 150, Sg 152, and Sg 140 in group III. The isolates Sg 004, Sg 026, Sg 025, Sg 139 and Sg 151 were not clustered at the 70 % similarity level and thus formed separate groups, group IV, V, VI, VII and VIII, respectively.

Table 5. Oospore production ratings[a] (on 1-4b rating scale) of the 21 isolates of *S. graminicola* on the host differential lines

Isolates	Host differential lines										Mean
	IP 5272-1	IP 18296	IP 18297	P 536-2	P 1564	P 2895-3	P 3281-1	700481-21-8	IP 18292	7042S	
Sg 004	3.9	3.0	–	3.4	1.0	4.0	3.2	4.0	1.0	4.0	2.9
Sg 015	2.9	1.9	2.8	2.0	1.0	2.0	1.9	3.2	1.0	3.1	2.2
Sg 021	– c	2.7	3.9	3.2	1.0	3.5	–	3.9	1.0	4.0	2.9
Sg 025	3.9	–	–	3.5	1.0	3.1	–	4.0	1.0	4.0	2.9
Sg 026	–	1.9	–	3.1	1.0	3.5	2.0	3.9	1.0	4.0	2.6
Sg 032	2.9	1.8	–	1.9	1.0	2.0	1.9	2.8	–	3.9	2.3
Sg 040	3.9	1.6	4.0	3.5	1.0	3.1	2.6	3.9	1.0	4.0	2.9
Sg 045	4.0	2.0	–	3.0	1.0	3.3	2.0	3.9	1.0	4.00	2.7
Sg 046	3.6	2.0	3.8	3.1	1.0	3.0	2.8	4.0	–	1.00	3.0
Sg 047	4.0	3.3	–	3.5	1.0	3.6	3.0	4.0	1.0	4.00	3.1
Sg 048	3.9	3.4	–	3.7	1.0	3.7	3.1	4.0	1.0	4.00	3.1
Sg 088	4.0	2.7	–	3.4	1.0	3.8	2.1	3.9	–	4.00	3.1
Sg 110	2.1	–	2.8	2.5	1.0	1.9	–	3.4	–	3.3	2.4
Sg 115	2.0	1.7	–	2.2	1.0	1.6	–	3.1	1.0	3.5	2.0
Sg 139	4.0	3.5	–	3.6	1.0	3.1	3.2	4.0	1.0	4.0	3.1
Sg 140	2.7	1.6	2.1	2.5	1.0	1.9	1.6	3.1	–	3.6	2.2
Sg 149	3.0	2.0	–	2.4	1.0	1.8	2.2	2.8	1.0	3.3	2.2
Sg 150	4.0	3.2	–	3.1	1.0	3.3	3.1	4.0	–	4.0	3.2
Sg 151	4.0	3.1	–	3.5	1.0	2.8	3.0	3.9	1.0	4.0	2.9
Sg 152	2.8	–	–	1.8	1.0	1.5	–	2.9	–	2.9	2.2
Sg 153	4.0	3.4	–	3.5	1.0	3.1	3.0	4.0	–	4.0	3.3
Mean	3.4	2.4	3.2	3.1	1.0	2.8	2.4	3.6	1.0	3.8	

[a]Mean of three replications. SE ± for isolates = 0.02, host differential lines = 0.03, and their interactions = 0.12. CD (1%) for isolates = 0.05, host differential lines = 0.03, and their interactions = 0.15.
[b]1, no oospores/cm^2; 2, 1-100 oospores/cm^2; 3, 101-1000 oospore/cm^2; 4, >1000 oospore/cm^2.

Table 6. Number of polymorphic and monomorphic bands in three primer combinations of AFLP analysis

Primer combination	Number of bands		
	Total	Polymorphic	Monomorphic
E-TG/M-CAT	58	49(84)[a]	9(16)
E-TT/M-TAG	63	51(81)	12(19)
E-TG/M-CTA	64	49(77)	15(23)
Total	185	149(81)	36(19)

[a]Percent of bands.

Discussion

The results indicated variations in disease incidence, latent period, disease incidence and oospore production potential among the 21 *S. graminicola* isolates. Significant variation of virulence on differential hosts indicated the existence of specificity in the host-pathosystem (Vanderplank, 1984). These results further support the findings on pathogenic variation among the sporangial (Thakur and Rao, 1997) and oospore (Thakur and Shetty, 1993) isolates of *S. gramini-*

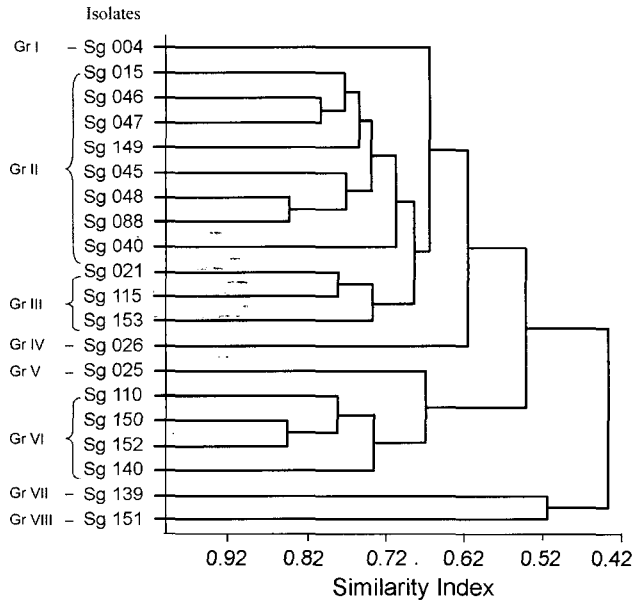

Fig. 2. Dendrogram of 20 isolates of *Sclerospora graminicola* based on AFLP data with three primer combinations using the software package SYSTAT 5.1. Gr, Group.

cola. All isolates were highly aggressive and virulent on the susceptible host genotype 7042S. However, majority of the isolates were avirulent on IP 18297; and had variable virulence on other genotypes (Table 2), suggesting the likely presence of a range of resistance genes in the host genotypes to the corresponding virulence genes in the isolates. This suggests the presence of diversity in virulence factor(s) specific to host resistance factor(s) in the pearl millet-downy mildew system. Evidences from this study and those of earlier findings (Appadurai et al., 1975; Ball, 1983; Bal and Pike, 1984; King et al., 1989; Thakur et al., 1992; Thakur and Shetty, 1993) indicate the high degree of genetic variation in virulence of *S. graminicola* populations that enable them to match resistance genes rapidly in the host genotypes.

Shorter latent periods were generally observed with the more aggressive isolate on highly susceptible host genotypes. However, the isolates varied greatly for latent period (7.1-10.0 days) even on a highly resistant host genotype IP 18297. A host genotype, such as IP 18297, having fewer infected plants with shorter latent period could contribute more towards disease spread than those having a greater number of infected plants with longer latent period. It has been suggested that the lower disease incidence alone cannot be taken as a true measure of resistance in a highly variable pearl millet-downy mildew system, and thus virulence index provides a better measure of resistance (Thakur et al., 1997). However, highly significant negative correlations between disease incidence and latent period provided a reasonable basis of understanding the stability of resistance in the host genotypes.

In the present study, virulence index was used to indicate the relative potential of individual isolates by combining the two independent pathogenicity parameters, disease incidence and latent period. It is quite variable among the isolates across the host genotypes indicating the presence of different virulence genes in the pathogen populations. Host genotypes on which the virulence index was lower across a number of isolates would probably be more stable than those with higher virulence index. Of the 21 isolates tested, Sg 139 had the highest virulence index indicating its highly virulent nature, while Sg 110 with lower virulence index could be less fit and eventually be eliminated from the populations in the successive generations.

All isolate-genotype combinations supported oospore production, except on P 1564 and IP 18292. In addition, these two genotypes developed similar disease symptoms, which included dark green foliage with severe stunting and no asexual sporulation on leaf lamina. The inability to support oospore production and expression of stunting reaction by these two genotypes indicate that they probably have a common resistance gene(s). The higher oospore production observed with some isolates (Sg 046, Sg 047, Sg 048, Sg 088, Sg 139, Sg 150 and Sg 153), irrespective of their virulence levels, could be attributed to the existence of differential mating types, MatA and MatB in equal proportions and it was demonstrated in case of isolates Sg 048, Sg 139 and Sg 153 (Pushpavathi, 2003). These findings are in conformity with the observations made with *B. lactucae* on lettuce (Michelmore and Ingram, 1980). In contrast, IP 18297 that showed resistant reaction to all the isolates surprisingly supported relatively more oospore production. This unusual phenomenon needs further investigation.

DNA fingerprinting pattern from AFLP analysis showed more diversity than virulence analysis based on pathogenicity using differential hosts. The cluster composition also varied in both virulence and AFLP analysis. This is expected because gene(s) controlling a particular character is most likely to be present in a small fraction across the genome, whereas the molecular banding pattern obtained from the total DNA reflects diversity within the entire genome (Andebrhan and Furtek, 1994). Similar observations were made by Chen et al. (1993) with isolates of *Puccinia striiformis* that had the same virulence phenotype and concluded that the molecular polymorphism observed was largely independent of virulence polymorphism. The inability of isolates Sg 004, Sg 025, Sg 026, Sg 139 and Sg 151 to form into clusters could be due to the presence or absence of few unique bands in these isolates.

No significant geographical distribution of isolates was found either in pathogenic or molecular analysis and isolates from the same state were assigned to different clusters. However, Sg 139 remained distinct from other clusters in both analyses indicating its highly virulent nature. Virulence analysis based on pathogenicity using host differentials have been found more useful than the molecular analysis in determining race structures of plant pathogens in several other host-pathogen systems (Ouellet and Seifert, 1993; Casela and Ferreira, 1995; Sivaramakrishnan et al., 2002). As isolates from the major pearl millet growing areas of the country were included in the study, screening of breeding material against the representative isolates of these pathotype groups would provide useful resistance for a successful pearl millet breeding program in India.

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