RESEARCH

New Sources of Resistance to Multiple Pathotypes of *Sclerospora graminicola* in the Pearl Millet Mini Core Germplasm Collection

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ABSTRACT

Downy mildew (DM), caused by *Sclerospora graminicola* (Sacc.) Schröt., is a highly destructive and widespread disease in most pearl millet [*Pennisetum glaucum* (L.) R. Br.] growing areas of Asia and Africa. Breeding for DM resistance continues to be an integral part of genetic improvement of pearl millet at ICRISAT, Patancheru, India. For the identification of new and diverse sources of DM resistance, a pearl millet mini core collection comprising 238 accessions was screened against eight pathotypes (Sg 384, Sg 409, Sg 445, Sg 457, Sg 510, Sg 519, Sg 526, and Sg 542) of *S. graminicola* collected from different geographical locations in India. Significant differences for DM reaction were observed among pathotypes, mini core accessions, and their interactions. Of the 238 accessions, 68 accessions were resistant (≤10% DM incidence) to pathotype Sg 510 followed by 40 accessions resistant to Sg 457. Resistance to pathotypes Sg 519, Sg 526, Sg 384, Sg 445, and Sg 542 was observed in 15, 27, 29, 30, and 34 mini core accessions, respectively. Resistance to two or more pathotypes was observed in 62 accessions. Several of these accessions also exhibited desirable agronomic traits. The multiple-pathotype-resistant germplasm accessions having desirable agronomic characteristics and collected from different agro-ecologies would be useful in breeding programs to develop pearl millet hybrids resistant to difficult-to-manage highly-virulent pathotypes of *S. graminicola*.

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Abbreviations: DM, downy mildew; QTL, quantitative trait loci.

Pearl millet is grown on 26 million ha in the semiarid tropics of Africa and the Indian subcontinent (Rai et al., 2009). It is a staple food for millions of poor people living in the dry areas of the world where it is difficult to grow any other crop. Pearl millet is grown both for grain and fodder. The grain is mainly used for food for human consumption and for poultry and cattle feed. Pearl millet is considered as a high quality forage crop in India, Australia, and the United States and is being recognized as a promising new forage crop of excellent quality and productivity in South America and Korea. Farmers in central Brazil have started growing pearl millet as a fodder crop in no-tillage crop farming systems. The single-cross hybrids of pearl millet have 25 to 30% grain yield advantage over open-pollinated varieties, thus it is predominantly grown in India (Rai et al., 2006). Although cultivation of single-cross F_1 hybrids have significantly contributed to increase pearl millet productivity in India, the narrow genetic base of the hybrids make them vulnerable to attack by pests and diseases (Thakur et al., 2006).

A large number of fungal, bacterial, nematode, and viral diseases are known to attack pearl millet; however, most of them are not economically important. There are only a few diseases that are considered economically important, including DM, blast, rust, smut, and ergot. Among these, DM caused by *S. graminicola* is the most damaging and dominant disease of pearl millet in India and several

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countries in West Africa. Downy mildew infects both foliage and panicles of pearl millet crops and causes about 20 to 40% grain yield losses annually worldwide (Singh, 1995; Hash et al., 1999). However, losses could be much higher if a susceptible hybrid is repeatedly grown in the same field.

Sclerospora graminicola is an obligate oomycete that has both sexual and asexual means of reproduction. Oospores are the sexual spores, and sporangia and zoospores are the asexual spores produced by the pathogen. Both heterothallism and homothallism is reported in *S. graminicola* (Michelmore et al., 1982). In oomycetes, existence of sexual mating types and their frequency greatly contribute towards the development of new recombinants and, eventually, selection of more virulent populations of the pathogen (Pushpavathi et al., 2006a). Two reference mating type isolates, Sg 018 (*Mat*-1) and Sg 019 (*Mat*-2), have been identified in *S. graminicola* and are being maintained at ICRISAT, Patancheru, India. Host-specific virulences have also been observed in *S. graminicola* (Thakur et al., 1992; Sastry et al., 2001; Pushpavathi et al., 2006b). Evolution of host-specific virulences results in the development of new pathotypes or races of the pathogen that leads to breakdown of resistance in the host cultivar within a short period (Kolmer and Hughes, 2014; Suh et al., 2009). Therefore, it is essential to monitor the virulence spectrum of *S. graminicola* populations for the management of this disease through host plant resistance. *Sclerospora graminicola* isolates are frequently collected from the major pearl millet growing areas in India and characterized for virulence diversity; representative highly-virulent isolates from different regions are then selected for greenhouse screening of breeding material at ICRISAT (Thakur et al., 2008). Although resistance sources have been identified and used in pearl millet breeding programs to combat this pathogen, evolution of new virulences of *S. graminicola* calls for the identification and utilization of new sources of DM resistance in pearl millet.

Gene banks conserve germplasm of crop species that are considered as reservoirs of traits of economic importance. The ICRISAT gene bank at Patancheru, India maintains 21,908 pearl millet accessions that have been originated from 50 countries. These accessions also include 750 accessions of 24 wild species of genus *Pennisetum* (Upadhyaya et al., 2007). This vast collection represents wide variation present in pearl millet germplasm for traits of economic significance. Therefore, it is essential to tap this variation to broaden the genetic base of pearl millet hybrid parent lines. However, it is difficult to evaluate large number of accessions in the germplasm collections, especially for the traits like DM resistance where accessions need to be screened against different pathotypes of the pathogen. This problem can be solved by selecting and evaluating subset of germplasm accessions that corresponds to variation available in the entire collection. Thus, core collections containing approximately 10% of the total number of accessions are often developed that represent the diversity available in the total germplasm collection (Frankel and Brown, 1984). A core collection comprising 2094 accessions of pearl millet has been developed (Upadhyaya et al., 2009), and an even more manageable mini core collection (10% of the core) was developed at ICRISAT, which include 238 accessions that epitomize the diversity found in the core collection (Upadhyaya et al., 2011). The objective of this study was to screen 238 germplasm accessions of the pearl millet mini core collection to identify resistance sources to different pathotypes of *S. graminicola*.

Table 1. Origin of *Sclerospora graminicola* pathotypes.

Pathotype	Isolate collection			Maintenance host
	Year	Cultivar	Location	
Sg 384	2003	Local	Barmer, Rajasthan	ICMP 451, susceptible at Barmer
Sg 409	2004	PMB 11571–2	ICRISAT, Patancheru, Hyderabad	PMB 11571-2, original host
Sg 445	2005	AHT-503	Banaskantha, Gujarat	Pioneer 7777, a highly susceptible hybrid in Banaskantha
Sg 457	2006	Pioneer 86 M34	Jaipur, Rajasthan	ICMP-451
Sg 510	2008	Unknown hybrid	Badaun, Uttar Pradesh	7042 S
Sg 519	2009	HHB 117	Rewari, Haryana	7042 S
Sg 526	2009	Eknath	Jodhpur, Rajasthan	7042 S
Sg 542	2010	Unknown hybrid	Aurangabad, Maharashtra	7042 S

MATERIALS AND METHODS

Seed Source

Seed of the mini core germplasm accessions (n = 238) of pearl millet was obtained from the Genetic Resources Division, ICRISAT, Patancheru, India.

Sclerospora graminicola Pathotypes

Sclerospora graminicola pathotypes used in this study were obtained from the pathogen collection being maintained at ICRISAT. Eight pathotype isolates (Sg 384, Sg 409, Sg 445, Sg 457, Sg 510, Sg 519, Sg 526, and Sg 542) of *S. graminicola* collected from different geographical locations in India were selected for this study (Table 1). A pathotype isolate of *S. graminicola* is the most virulent isolate selected from a number of isolates collected from a particular pearl millet growing area and characterized for virulence diversity. These isolates are being maintained either on the original host or cultivar from which they were collected, susceptible parental line of the original hybrid host, other equally susceptible hosts, or the highly susceptible line 7042S.

Inoculum Preparation and Inoculation

Infected leaves of each isolate were collected, cut into pieces, and washed under running tap water. The leaves were wiped clean to remove the old sporangia and placed with their abaxial

surface up in humid chambers lined with moist blotting paper. In humid chambers, these leaves were incubated in dark for 6 h at 20°C to induce sporulation. Sporangia of each isolate were harvested separately from the sporulating leaves in ice-cold, sterile, distilled water using a camel-hair brush and filtered through double-layered muslin cloth. Spore concentration in the suspension was adjusted to 5×10^5 mL^{-1} with the help of a haemocytometer before inoculation.

The experiment to screen mini core accessions was conducted in a completely randomized design with two replicates and two pots per replicate. The seeds of 238 accessions, and check lines 7042S (susceptible) and IP 18292 (differential reaction to pathotypes), were sown in 10-cm-diameter pots filled with a potting mixture (soil, sand, and farmyard manure in a 3:2:2 ratio by volume). The seeds were sown at uniform depth in holes made with the help of a dibbler stamp and kept in a greenhouse at 35°C for seedling emergence. Pot-grown seedlings of each germplasm accession and the check lines were spray inoculated at coleoptile stage (48 h old) using an atomizer and covered with a polyethylene sheet to provide >90% relative humidity. Inoculated seedlings were incubated at 20°C for 24 h and then for the next 14 d pots were transferred to greenhouse benches at 25 ± 2°C with regular misting to provide high humidity (>90%) and leaf wetness for the disease development. Numbers of total and infected seedlings were recorded in each pot 2 wk after inoculation and the percentage disease incidence was calculated as an assessment of reaction against different pathotypes of *S. graminicola*.

Evaluation for Agronomic Traits

Field evaluation of the mini core for agronomic traits was as described previously (Upadhyaya et al., 2011). The mini core was evaluated in an augmented design in the rainy season (June to October), 2007, at Patancheru, and data was recorded for agronomic traits such as days to 50% flowering, seed yield potential, green fodder yield potential, and overall agronomic score. Days to 50% flowering were recorded as days between sowing and the stage when 50% of plants in an accession exhibited stigma emergence. Seed yield potential of an accession was visually assessed based on spike number, size, density, seed setting, and seed size compared with a standard check on a 1-to-9 scale (1 = lowest, 2 = very low, 3 = low, 4 = low to moderate, 5 = moderate, 6 = good, 7 = high, 8 = very high, and 9 = excellent) at maturity. Green fodder yield potential of accessions was assessed on the basis of tillering, leafiness, and bulk at flowering, whereas overall agronomic score was based on overall agronomic desirability of accessions at dough stage. These traits were also recorded on a 1-to-9 scale, where 1 = poorest, 2 = very poor, 3 = poor, 4 = fair, 5 = average, 6 = good, 7 = better, 8 = best, and 9 = excellent. These data sets were used for the comparison of the selected DM-resistant mini core accessions for agronomic performance.

Data Analysis

Analysis of variance (ANOVA) for DM incidence was performed to determine significant differences among pathotype isolates, host genotypes, and their interactions using GENSTAT statistical package version 10.1 (Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK) (Payne et al., 2010).

Table 2. Analysis of variance (ANOVA) for downy mildew incidence.

Source of variation	Degrees of freedom	Mean sums of square	*F* ratio	*F* probability
Replication	1	3146.73	82.42	
Genotype	239	9429.37	246.97	<0.001
Pathotype	7	164109.9	4298.25	<0.001
Genotype × pathotype	1670	836.46	21.91	<0.001
Residual	5274	38.18		
Total	7191			

RESULTS

Identification of Downy Mildew Resistance in the Mini Core Collection

Significant differences ($P < 0.001$) for the DM reaction were observed among pathotypes, mini core accessions, and their interactions (Table 2). This indicated difference in the virulence of *S. graminicola* pathotypes and resistance in the pearl millet accessions to the pathotypes. Of the 238 accessions, 68 were resistant (≤10% DM incidence) to pathotype Sg 510 followed by 40 accessions resistant to Sg 457 (Fig. 1). Pathotype Sg 409 was found highly virulent, as all the accessions except IP 13875 were susceptible to this pathotype. Eleven accessions were moderately resistant (10.1–20.0% DM incidence), 48 susceptible (20.1–50.0% incidence), and 178 accessions were highly susceptible (>50% DM incidence) to Sg 409. Resistance to pathotypes Sg 519, Sg 526, Sg 384, Sg 445, and Sg 542 was observed in 15, 27, 29, 30, and 34 mini core accessions, respectively (Fig. 1). Several accessions exhibited moderate resistance to different pathotypes of *S. graminicola*. A number of accessions having moderate resistance to pathotypes Sg 510 and Sg 457 were also high as observed for the number of resistant accessions. Sixty-nine accessions exhibited moderate resistance to Sg 457, while 61 accessions had moderate resistance to Sg 510 (Fig. 1).

None of the mini core accession were resistant to all eight pathotypes; however, IP 14537 was resistant to seven pathotypes and also exhibited moderate resistance (≤20% DM) to pathotype Sg 409. Multiple-pathotype (≥2) resistance was observed in 62 accessions (Table 3). Five accessions (IP 9645, IP 11943, IP 14542, IP 14599, and IP 21438) were resistant to any six pathotypes, and seven accessions (IP 11930, IP 12374, IP 14522, IP 20715, IP 21187, IP 21201, and IP 21244) were resistant to any five pathotypes. Nineteen accessions were resistant to three to four pathotypes, while 30 accessions were resistant to any two pathotypes.

Agronomic Performance of Downy Mildew-Resistant Mini Core Accessions

Sixty-two accessions having resistance to at least two pathotypes were compared for their agronomic performance. Several of the DM-resistant accessions exhibited

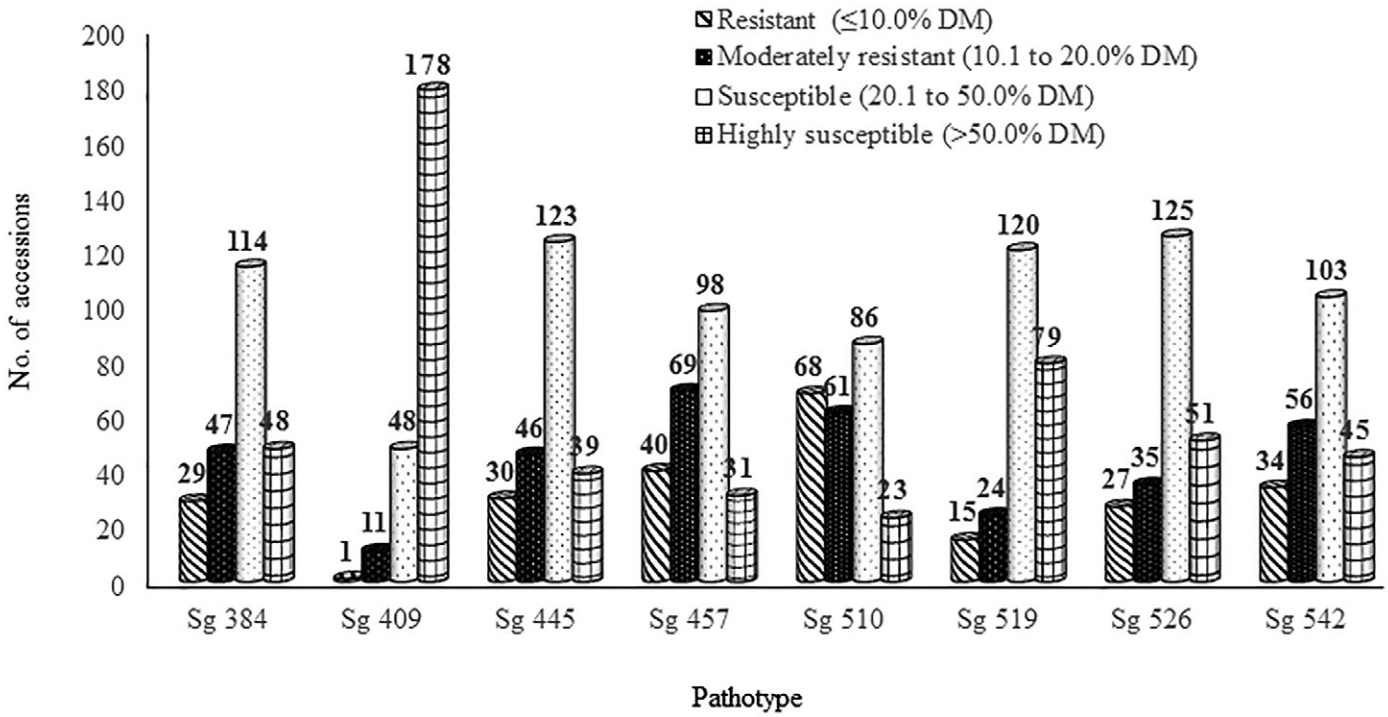

Figure 1. Summary of downy mildew reaction of mini core accessions screened against eight pathotypes of *Sclerospora graminicola*.

Table 3. Downy mildew reaction of selected mini core accessions having resistance to at least two pathotypes of *Sclerospora graminicola*.

Accession (IP No.)	Downy mildew incidence†‡									Pathotypes§
	Sg 384	Sg 409	Sg 445	Sg 457	Sg 510	Sg 519	Sg 526	Sg 542	Mean	
	%									
2322	24.2	73.2	40.6	13.2	9.4	37.7	16.5	8.3	27.9	2
3646	26.4	27.6	8.1	12.5	22.6	23.5	18.8	6.8	18.3	2
5085	18.0	64.3	12.2	9.3	3.5	28.4	12.9	11.4	20.0	2
5261	34.5	34.8	8.7	24.5	16.7	40.3	12.9	7.5	22.5	2
5719	27.0	38.1	10.6	7.8	10.2	12.9	20.8	3.0	16.3	2
5793	27.6	93.5	20.1	10.9	7.7	71.5	14.2	8.7	31.8	2
6275	44.5	11.9	9.4	22.9	6.5	47.9	27.1	10.7	22.6	2
6278	23.2	36.9	33.3	7.9	6.0	22.4	11.0	30.1	21.4	2
7422	17.4	44.0	28.1	13.1	7.6	9.4	18.9	20.0	19.8	2
7860	15.9	95.2	22.3	5.7	29.9	73.1	14.0	3.9	32.5	2
9449	8.3	21.1	11.9	9.2	18.6	21.8	39.8	21.1	19.0	2
10085	9.6	50.1	10.7	23.9	6.4	28.8	43.3	10.3	22.9	2
10263	37.4	63.4	24.5	7.2	9.2	11.3	30.1	22.2	25.7	2
10437	10.6	52.7	21.7	7.2	2.9	26.5	31.9	30.0	22.9	2
10925	15.4	72.3	12.0	13.5	2.8	42.1	28.1	5.8	24.0	2
10953	21.0	60.9	24.1	22.0	9.5	18.5	14.8	7.6	22.3	2
11010	4.4	62.8	24.5	24.0	7.1	51.0	24.8	37.0	29.4	2
11247	22.4	37.3	9.1	16.7	10.9	12.2	27.2	9.7	18.2	2
11428	31.9	26.7	17.4	39.6	11.3	44.9	6.1	6.4	23.0	2
11666	11.0	14.8	4.1	23.4	7.1	30.0	17.4	16.4	15.5	2
12221	25.4	72.9	16.3	7.5	9.9	25.4	41.3	21.0	27.5	2
12431	20.4	38.1	8.8	17.6	7.0	38.3	11.4	17.6	19.9	2
12533	41.4	95.4	40.0	6.7	11.6	60.1	14.0	6.3	34.4	2
12644	10.3	69.3	8.7	11.2	13.1	9.0	21.2	24.3	20.9	2
12650	4.3	79.3	25.6	14.2	4.5	58.9	10.8	10.9	26.1	2
14428	4.3	89.3	14.0	12.2	12.6	17.6	1.7	11.0	20.3	2
17775	10.6	63.8	23.7	5.9	5.6	45.1	14.9	40.7	26.3	2

(cont'd)

Table 3. Continued.

Accession (IP No.)	Downy mildew incidence†‡									Pathotypes§
	Sg 384	Sg 409	Sg 445	Sg 457	Sg 510	Sg 519	Sg 526	Sg 542	Mean	
19425	7.9	31.5	21.1	27.5	6.0	15.7	35.0	18.8	20.4	2
20611	7.4	52.1	10.6	6.8	10.7	21.2	21.7	11.5	17.8	2
20768	32.1	61.8	11.2	12.9	7.0	20.9	16.6	6.5	21.1	2
5153	22.2	66.0	18.5	6.0	5.1	7.8	38.2	12.2	22.0	3
5407	11.1	49.3	7.8	13.7	10.7	23.6	9.3	2.7	16.0	3
8051	7.5	25.7	28.2	5.3	5.2	30.3	35.5	17.9	19.5	3
8707	15.9	23.6	6.0	10.6	7.2	12.8	41.0	9.2	15.8	3
9692	22.0	52.7	19.1	8.4	6.0	24.2	7.9	13.6	19.2	3
9795	21.6	83.5	19.3	14.1	7.3	13.6	4.7	9.6	21.7	3
9934	9.7	24.5	5.3	11.2	11.8	5.6	33.4	55.5	19.6	3
10151	16.0	42.6	10.8	9.4	7.0	15.8	7.9	23.0	16.6	3
15273	9.6	46.1	30.8	13.8	7.7	23.6	7.7	12.3	19.0	3
15836	1.8	55.4	22.0	9.1	7.0	12.0	20.9	16.1	18.0	3
20409	16.5	46.0	5.2	8.4	12.6	28.0	23.8	2.7	17.9	3
6113	25.6	57.7	9.4	12.2	9.3	28.3	0.0	7.5	18.8	4
6193	33.6	52.9	10.2	6.1	5.1	23.8	4.2	9.6	18.2	4
8418	12.4	15.9	6.7	9.5	2.2	6.3	23.6	12.2	11.1	4
10601	28.9	45.6	12.3	6.3	5.8	26.5	7.8	7.3	17.6	4
13875	16.7	0.0	6.3	6.0	6.0	19.4	15.6	31.8	12.7	4
13991	6.2	23.2	8.1	13.7	7.9	21.7	7.2	38.3	15.8	4
17396	25.1	16.0	8.2	9.1	5.1	28.4	27.6	8.2	16.0	4
18040	7.9	58.3	6.9	5.5	32.4	14.6	8.3	13.2	18.4	4
12374	3.0	11.7	0.0	1.5	9.8	4.9	22.4	13.8	8.4	5
14522	6.5	55.8	24.8	8.5	7.2	7.3	8.4	13.1	16.5	5
11930	41.5	80.7	100.0	0.0	0.0	0.0	0.0	0.0	27.8	5
20715	9.3	15.7	7.1	7.3	6.4	13.8	7.7	25.9	11.7	5
21187	3.6	94.8	4.8	3.7	4.0	14.1	11.8	3.8	17.6	5
21201	21.0	42.1	9.4	58.4	5.3	1.5	2.1	8.6	18.6	5
21244	18.3	98.5	69.0	3.2	0.0	0.0	0.0	0.0	23.6	5
9645	5.5	20.6	6.2	0.6	3.7	12.4	0.7	3.8	6.7	6
11943	56.7	99.3	0.0	4.0	0.0	0.0	0.0	0.0	20.0	6
14542	4.5	22.0	2.1	6.6	3.8	5.2	7.2	32.6	10.5	6
14599	6.3	14.4	6.5	6.2	5.7	6.2	11.5	5.0	7.7	6
21438	2.0	77.6	1.8	8.5	2.4	0.8	67.9	0.0	20.1	6
14537	3.0	18.1	2.1	7.7	1.7	4.3	0.0	1.9	4.9	7
Checks										
7042S	100	94.3	100	100	100	100	100	100	99.3	
IP 18292	100	99.3	0.0	32.2	0.0	100	0.0	0.0	41.4	
Trial mean	32.0	65.0	31.4	26.6	23.0	42.0	35.0	30.8		

†Mean of two replicates.

‡Least significant difference ($P \leq 0.05$) for pathotype = 0.55; genotype = 3.03; pathotype × genotype = 8.6.

§Accession resistant to number of pathotypes.

traits considered to be agronomically desirable. There was considerable variation for days to 50% flowering in the 62 DM-resistant accessions that ranged from 41 to 120 d. Similarly, variation (4–8 score on a 1-to-9 scale) was also observed for seed yield potential and overall agronomic score. Green fodder yield potential scores ranged from 3 to 9 (Table 4). Four (IP 12644, IP 13991, IP 12650, and IP 18040) of the 62 DM-resistant accessions were early (≤50 d), 40 were medium (51–80 d), and 18 accessions were late (>80 d) for flowering. Twenty-six accessions had very good rating (score 7) for seed yield potential and overall agronomic score, and two accessions, IP 10263 and IP 20409, recorded score 8 (best) for these traits. Excellent green fodder yield potential (score 9) was observed in 21 accessions, while 13 accessions had score 8 for this trait.

Geographical Distribution of Downy Mildew-Resistant Mini Core Accessions

The pearl millet mini core encompasses germplasm accessions representing 46 countries; however, none of the accessions from 18 countries exhibited resistance to any pathotypes used in this study (Table 5). Twelve accessions

Table 4. Origin, days to 50% flowering, seed and fodder yield potential, and overall agronomic score of 62 accessions selected from pearl millet mini core collection having multiple-pathotype resistance.

Accession (IP) no.	Origin	Days to 50% flowering	Seed yield potential†	Green fodder yield potential†	Overall agronomic score†	Pathotypes‡
2322	Nigeria	53	6	6	6	2
3646	India	53	5	6	5	2
5085	Nigeria	63	6	9	6	2
5153	Niger	93	6	9	6	3
5261	Niger	66	6	6	6	2
5407	Niger	60	7	7	7	3
5719	Nigeria	57	7	8	7	2
5793	Union of Soviet Socialist Republics	65	4	3	5	2
6113	Niger	91	7	9	7	4
6193	Cameroon	90	6	8	6	4
6275	Mali	79	5	9	5	2
6278	Mali	72	5	8	5	2
7422	Tanzania	80	6	9	6	2
7860	India	51	4	3	4	2
8051	Mexico	57	5	7	5	3
8418	Nigeria	53	6	7	6	4
8707	Sudan	82	7	7	7	3
9449	Ghana	56	6	6	6	2
9645	Nigeria	60	7	8	7	6
9692	Nigeria	60	7	6	7	3
9795	USA	78	6	7	6	3
9934	Sudan	79	7	8	7	3
10085	Mali	60	7	7	7	2
10151	Mali	116	6	9	6	3
10263	Mali	62	8	8	8	2
10437	Benin	111	7	7	7	2
10601	Mali	76	7	7	7	4
10925	Sudan	69	6	8	6	2
10953	Kenya	60	6	9	6	2
11010	India	70	7	9	7	2
11247	Zimbabwe	59	6	6	6	2
11428	Burkina Faso	80	6	9	6	2
11666	United Kingdom	77	7	9	7	2
11930	Sierra Leone	57	7	7	7	5
11943	Sierra Leone	59	7	8	7	6
12221	Nigeria	88	7	9	7	2
12374	Nigeria	57	7	7	7	5
12431	Cape Verde	58	7	8	7	2
12533	India	54	6	5	6	2
12644	India	41	4	4	4	2
12650	India	50	4	5	4	2
13875	Burkina Faso	83	7	9	7	4
13991	Zimbabwe	44	5	4	5	4
14428	Cameroon	103	5	8	5	2
14522	Cameroon	89	7	9	7	5
14537	Cameroon	96	7	9	7	7
14542	Cameroon	105	7	9	7	6
14599	Cameroon	89	7	9	7	6
15273	India	56	5	9	5	3
15836	Tanzania	103	5	8	5	3
17396	Central African Rep.	120	6	9	6	4
17775	Togo	53	5	5	5	2

(cont'd)

Table 4. Continued.

Accession (IP) no.	Origin	Days to 50% flowering	Seed yield potential†	Green fodder yield potential†	Overall agronomic score†	Pathotypes‡
18040	Pakistan	50	4	5	4	4
19425	Zaire	79	6	7	6	2
20409	Nigeria	96	8	9	8	3
20611	Nigeria	91	7	8	7	2
20715	Nigeria	69	7	9	7	5
20768	Nigeria	97	7	9	7	2
21187	India	56	5	8	5	5
21201	India	53	5	7	5	5
21244	India	62	5	6	5	5
21438	India	62	7	7	7	6
Trial mean		65.7				
CV (%)		6.5				
LSD		8.5				

†Scored on 1-to-9 scale.

‡Resistant to number of pathotypes.

Table 5. Geographical distribution of peal millet mini core accessions screened against eight pathotypes of *Sclerospora graminicola*.

Region and country	Total accessions	No. of accessions resistant to pathotype								Resistant to ≥2 pathotypes
		Sg 384	Sg 409	Sg 445	Sg 457	Sg 510	Sg 519	Sg 526	Sg 542	
Americas										
Brazil	1	0	0	0	0	0	0	0	0	0
Mexico	1	1	0	0	1	1	0	0	0	1
United States of America	3	0	0	0	0	1	0	1	1	1
North Africa										
Algeria	1	0	0	0	0	0	0	0	0	0
Morocco	1	0	0	0	0	0	0	0	0	0
Tunisia	1	0	0	0	0	0	0	0	0	0
Southern Africa										
Botswana	1	0	0	0	0	0	0	0	0	0
Mozambique	1	0	0	0	0	0	0	0	0	0
Namibia	11	1	0	0	1	3	0	0	1	0
South Africa	1	0	0	0	0	0	0	0	0	0
Zambia	2	0	0	0	0	0	0	0	0	0
Zimbabwe	10	1	0	2	0	1	0	1	1	2
Central Africa										
Cameroon	10	5	0	3	5	5	4	6	3	6
Central African Republic	2	0	0	1	2	1	0	0	1	1
Chad	2	0	0	0	0	1	0	0	0	0
Congo	1	0	0	0	0	0	0	0	0	0
Zaire	1	1	0	0	0	1	0	0	0	1
East Africa										
Ethiopia	1	0	0	0	0	0	0	0	0	0
Kenya	2	0	0	0	0	1	0	0	1	1
Malawi	3	0	0	0	0	0	0	0	0	0
Somalia	1	0	0	0	0	0	0	0	0	0
Sudan	8	1	0	2	0	2	1	0	2	3
Tanzania	3	1	0	0	1	2	1	0	0	2
Uganda	1	0	0	0	0	0	0	0	0	0
West Africa										
Benin	1	0	0	0	1	1	0	0	0	1
Burkina Faso	6	0	1	1	1	2	0	1	1	2
Cape Verde	1	0	0	1	0	1	0	0	0	1
Gambia	2	0	0	1	0	0	0	0	0	0
Ghana	4	1	0	0	1	3	0	0	0	1

(cont'd)

Table 5. Continued.

Region and country	Total accessions	No. of accessions resistant to pathotype								Resistant to ≥2 pathotypes
		Sg 384	Sg 409	Sg 445	Sg 457	Sg 510	Sg 519	Sg 526	Sg 542	
Mali	12	1	0	1	5	6	0	2	1	6
Mauritania	1	0	0	0	0	0	0	0	0	0
Niger	15	0	0	3	1	6	1	3	5	4
Nigeria	21	4	0	6	11	10	2	3	6	12
Senegal	4	0	0	0	0	0	0	0	0	0
Sierra Leone	2	0	0	1	2	2	2	2	2	2
Togo	2	0	0	0	1	2	0	0	0	1
Europe										
France	1	0	0	0	0	1	0	0	0	0
Germany	1	0	0	0	0	0	0	0	0	0
United Kingdom	1	0	0	1	0	1	0	0	0	1
Oceania										
Australia	1	0	0	0	0	0	0	0	1	0
South Asia										
India	87	11	0	6	6	12	4	7	7	11
Myanmar	1	0	0	0	0	1	0	0	0	0
Pakistan	1	1	0	1	1	0	0	1	0	1
East Asia										
Russia	1	0	0	0	0	1	0	0	1	1
West Asia										
Lebanon	1	0	0	0	0	0	0	0	0	0
Yemen, Republic of	3	0	0	0	0	0	0	0	0	0
Total	238	29	1	30	40	68	15	27	34	62

from Nigeria were resistant to at least two pathotypes of *S. graminicola* followed by 11 accessions from India and six accessions each from Cameroon and Mali. Four of the 15 mini core accessions from Niger were found to have multiple-pathotype resistance. Similarly, two of the three mini core accessions from Tanzania and three of the eight accessions from Sudan were resistant to at least two pathotypes. The mini core accessions, IP 11930 and IP 11943 collected from Sierra Leone, were resistant to five pathotypes: Sg 457, Sg 510, Sg 519, Sg 526, and Sg 542. The accession IP 11943 was also found to be resistant to pathotype Sg 445.

DISCUSSION

Downy mildew continues to be the number one biotic constraint to pearl millet cultivation around the world. However, no large-scale epidemic of the disease has been reported in India since the 1990s (Thakur et al., 2006). This has been achieved mainly through breeding for disease resistance that resulted in the development and deployment of a large number of DM-resistant hybrids (Khairwal et al., 2004). Nevertheless, on-farm surveys in the major pearl millet growing states of India have revealed that several commercial F_1 hybrids being grown in different states become susceptible to the disease within 3 to 5 yr (Rao et al., 2005; Thakur et al., 2003, 2006). Several pathogenic variants of the pathogen are prevalent in different pearl millet growing areas in India, and new variants with higher levels of virulence keep on evolving due to selection pressure imposed by the deployment of new DM-resistant cultivars (Thakur et al., 2008). Therefore, to cope up with the evolving virulences of the pathogen, it is important to diversify the genetic base of pearl millet breeding lines. We selected eight highly virulent pathotypes of *S. graminicola* that are currently being used in the greenhouse screening of breeding lines at ICRISAT, Patancheru, India, for the screening of mini core accessions for DM resistance. Greenhouse screening of pearl millet mini core led to the identification of 109 accessions that are resistant to at least one pathotype, whereas 62 accessions were resistant to two or more pathotypes of *S. graminicola*. Most of these accessions also possess desirable agronomic traits and thus, could be used in pearl millet improvement programs.

Downy mildew-resistant germplasm accessions and breeding lines have been generally found to be morphologically, genetically, and geographically quite diverse (Singh, 1995; Singh et al., 1997). The DM-resistant mini core accessions identified in this study originated from 28 countries, and most of them are of African origin. At least 50% of the mini core accessions collected from Nigeria, Mali, and Cameroon were found to have multiple-pathotype resistance. In addition, the two accessions representing Sierra Leone in the mini core collection exhibited resistance to five to six pathotypes. Therefore, remaining accessions in the pearl millet core collection (Upadhyaya et al., 2009) selected from these countries need to be screened to identify additional sources of resistance to new virulent pathotypes of *S. graminicola*. As the mini core accessions

have been collected from different countries and regions, this geographical diversity could be representing diversity for resistance genes as well (Caicedo, 2008). Therefore, use of resistance sources of diverse origin in the breeding programs can help to diversify and broaden the genetic base for DM resistance in pearl millet.

A number of quantitative trait loci (QTLs) for DM resistance have been detected in pearl millet that are effective against one or more pathotypes of *S. graminicola* (Hash and Witcombe, 2001; Jones et al., 1995, 2002). Resistance alleles for some of these QTLs have been introgressed in the elite parental lines of popular pearl millet hybrids following marker-assisted backcrossing. Furthermore, pyramiding of QTL alleles for DM resistance in the parental lines of pearl millet is well documented; comparison of DM-susceptible pearl millet line 843B, to its conventional backcross derivative ICMB 99022 and the resistance donor ICML 22 has clearly demonstrated the effectiveness of pyramiding resistance alleles from ICML 22 (Hash et al., 2006). Parental line 843B had >90% DM incidence against six isolates (Sg 021, Sg 139, Sg 200, Sg 212, Sg 298, and Sg 384) of *S. graminicola*; whereas, ICMB 99022 recorded 0 to 1% incidence, and the resistance donor ICML 22 had 4 to 16% incidence across these isolates. Thus, ICMB 99022 was recommended as a replacement for its susceptible but commercially successful recurrent parent 843B in hybrid breeding programs in India (Hash et al., 2006). Similarly, multiple-pathotype (6–7) resistant accessions (IP 9645, IP 11943, IP 14542, IP 14599, IP 21438, and IP 14537) identified in this study could be used as donor parents for pyramiding resistance alleles in the parental lines of commercially successful pearl millet hybrids.

Some of the DM-resistant mini core accessions have also been found resistant to another important disease, pearl millet blast caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*). Mini core accessions IP 11247 and IP 12650, having resistance to two pathotypes of *S. graminicola*, also carry resistance to pathotype Pg 118 of *M. grisea* (Sharma et al., 2013). The DM-resistant accessions IP 3646, IP 9692, and IP 17396 have been reported to be resistant to *M. grisea* pathotype Pg 45. Resistance to multiple pathotypes of *M. grisea* has also been observed in some of the selected DM-resistant accessions. Mini core accession IP 11010 was found resistant to two pathotypes (Pg 118 and Pg 119), and IP 21187 was resistant to four pathotypes (Pg 53, Pg 56, Pg 118, and Pg 119) of *M. grisea* (Sharma et al., 2013). Once considered a minor disease of pearl millet, blast has now emerged as a serious threat to pearl millet production in India. Since DM and blast are the two major diseases of pearl millet, mini core accessions such as IP 11010 and IP 21187, having resistance to multiple pathotypes of both DM and blast would be very useful in the pearl millet breeding program.

The results of this study clearly indicate that the mini core collections can be used as a starting point to screen for desirable traits in a crop species. Mini core collections have been successfully used in the past to identify sources of disease resistance (Sharma et al., 2010, 2012), salinity (Serraj et al., 2004), drought tolerance (Kashiwagi et al., 2005; Upadhyaya, 2005), and multiple traits of economic importance (Upadhyaya et al., 2014). The agronomically desirable multiple-pathotype-resistant germplasm accessions identified in this study have originated from different agroecologies and are therefore expected to be highly divergent and useful in breeding programs to develop pearl millet hybrids having resistance against difficult-to-manage, highly-virulent pathotypes of *S. graminicola*.

References

Caicedo, A.L. 2008. Geographic diversity cline of *R* gene homologs in wild populations of S*olanum pimpinellifolium* (Solanaceae). Am. J. Bot. 95:393–398. doi:10.3732/ajb.95.3.393

Frankel, O.H., and A.H.D. Brown. 1984. Plant genetic resources today: A critical appraisal. In: J.H.W. Holden and J.T. Williams, editors, Crop genetic resources: Conservation and evaluation. Allen and Unwin, Winchester, MA. p. 249–268.

Hash, C.T., S.D. Singh, R.P. Thakur, and B.S. Talukdar. 1999. Breeding for disease resistance. In: I.S. Khairwal, K.N. Rai, D.J. Andrews, and G. Harinarayana, editors, Pearl millet breeding. Oxford & IBH, New Delhi, India. p. 337–379.

Hash, C.T., R.P. Thakur, V.P. Rao, and R.A.G. Bhaskar. 2006. Evidence for enhanced resistance to diverse isolates of pearl millet downy mildew through gene pyramiding. Int. Sorghum and Millets Newsl. 47:134–138.

Hash, C.T., and J.R. Witcombe. 2001. Pearl millet molecular marker research. Int. Sorghum and Millets Newsl. 42:8–15.

Jones, E.S., W.A. Breese, C.J. Liu, S.D. Singh, D.S. Shaw, and J.R. Witcombe. 2002. Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. Crop Sci. 42:1316–1323. doi:10.2135/cropsci2002.1316

Jones, E.S., C.J. Liu, M.D. Gale, C.T. Hash, and J.R. Witcombe. 1995. Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor. Appl. Genet. 91:448–456. doi:10.1007/BF00222972

Kashiwagi, J., L. Krishnamurthy, H.D. Upadhyaya, H. Krishna, S. Chandra, V. Vadez, and R. Serraj. 2005. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica 146:213–222. doi:10.1007/s10681-005-9007-1

Khairwal, I.S., K.N. Rai, O.P. Yadav, and S.K. Bhatnagar. 2004. Pearl millet cultivars. All India Coordinated Pearl Millet Improvement Project, Indian Council of Agricultural Research. Mandor, Jodhpur, India.

Kolmer, J.A., and M.E. Hughes. 2014. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2012. Plant Dis. 98:1145–1150. doi:10.1094/PDIS-12-13-1267-SR

Michelmore, R.W., M.N. Pawar, and R.J. Williams. 1982. Heterothallism in *Sclerospora graminicola*. Phytopathology 72:1368–1372. doi:10.1094/Phyto-72-1368

Payne, R.W., D.A. Murray, S.A. Harding, D.B. Baird, and D.M. Soutar. 2010. GenStat for Windows introduction, 14th ed. VSN International, Hemel Hempstead, UK.

Pushpavathi, B., R.P. Thakur, and K. Chandrashekara Rao. 2006a. Fertility and mating type frequency in Indian isolates of *Sclerospora graminicola*, the downy mildew pathogen of pearl millet. Plant Dis. 90:211–214. doi:10.1094/PD-90-0211

Pushpavathi, B., R.P. Thakur, K. Chandrashekara Rao, and V.P. Rao. 2006b. Characterization of *Sclerospora graminicola* isolates from pearl millet for virulence and genetic diversity. Plant Pathol. J. 22:28–35. doi:10.5423/PPJ.2006.22.1.028

Rai, K.N., I.S. Khairwal, C.J. Dangaria, A.K. Singh, and A.S. Rao. 2009. Seed parent breeding efficiency of three diverse cytoplasmic-nuclear male-sterility systems in pearl millet. Euphytica 165:495–507. doi:10.1007/s10681-008-9765-7

Rai, K.N., V.N. Kulkarni, R.P. Thakur, B.I.G. Haussmann, and M.A. Mgonja. 2006. Pearl millet hybrid parents research: Approaches and achievements. In: C.L.L. Gowda, K.N. Rai, B.V.S. Reddy, and K.B. Saxena, editors, Hybrid parents research at ICRISAT. ICRISAT, Patancheru. p. 11–73.

Rao, V.P., R.P. Thakur, K.N. Rai, and Y.K. Sharma. 2005. Downy mildew incidence on pearl millet cultivars and pathogenic variability among isolates of *Sclerospora graminicola* in Rajasthan. Int. Sorghum and Millets Newsl. 46:107–110.

Sastry, J.G., S. Sivaramakrishnan, V.P. Rao, R.P. Thakur, R.S. Singru, V.S. Gupta, and P.K. Ranjekar. 2001. Genetic basis of host specificity in *Sclerospora graminicola*, the pearl millet downy mildew pathogen. Indian Phytopathol. 54:323–328.

Serraj, R., L. Krishnamurthy, and H.D. Upadhyaya. 2004. Screening chickpea mini-core germplasm for tolerance to salinity. Int. Chickpea and Pigeonpea Newsl. 11:29–32.

Sharma, R., V.P. Rao, H.D. Upadhyaya, V.G. Reddy, and R.P. Thakur. 2010. Resistance to grain mold and downy mildew in a mini-core collection of sorghum germplasm. Plant Dis. 94:439–444. doi:10.1094/PDIS-94-4-0439

Sharma, R., H.D. Upadhyaya, S.V. Manjunatha, K.N. Rai, S.K. Gupta, and R.P. Thakur. 2013. Pathogenic variation in the pearl millet blast pathogen, *Magnaporthe grisea* and identification of resistance to diverse pathotypes. Plant Dis. 97:189–195. doi:10.1094/PDIS-05-12-0481-RE

Sharma, R., H.D. Upadhyaya, S.V. Manjunatha, V.P. Rao, and R.P. Thakur. 2012. Resistance to foliar diseases in a mini-core collection of sorghum germplasm. Plant Dis. 96:1629–1633. doi:10.1094/PDIS-10-11-0875-RE

Singh, S.D. 1995. Downy mildew of pearl millet. Plant Dis. 79:545–550. doi:10.1094/PD-79-0545

Singh, S.D., J.P. Wilson, S.S. Navi, B.S. Talukdar, D.E. Hess, and K.N. Reddy. 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Information Bulletin no. 48. ICRISAT, Patancheru.

Suh, J.P., J.H. Roh, Y.C. Cho, S.S. Han, Y.G. Kim, and K.K. Jena. 2009. The *Pi40* gene for durable resistance to rice blast and molecular analysis of *Pi40*-advanced backcross breeding lines. Phytopathology 99:243–250. doi:10.1094/PHYTO-99-3-0243

Thakur, R.P., K.N. Rai, I.S. Khairwal, and R.S. Mahala. 2008. Strategy for downy mildew resistance breeding in pearl millet in India. SAT eJournal 6:1–11.

Thakur, R.P., V.P. Rao, K.N. Amrutesh, H.S. Shetty, and V.V. Datar. 2003. Field surveys of pearl millet downy mildew: Effects of hybrids, fungicide and cropping sequence. J. Mycol. Plant Pathol. 33:387–394.

Thakur, R.P., H.S. Shetty, and I.S. Khairwal. 2006. Pearl millet downy mildew research in India: Progress and perspectives. Int. Sorghum and Millets Newsl. 47:125–130.

Thakur, R.P., K.G. Shetty, and S.B. King. 1992. Selection for host-specific virulence in asexual populations of *Sclerospora graminicola*. Plant Pathol. 41:626–632. doi:10.1111/j.1365-3059.1992.tb02463.x

Upadhyaya, H.D. 2005. Variability for drought resistance related traits in the mini-core collection of peanut. Crop Sci. 45:1432–1440. doi:10.2135/cropsci2004.0389

Upadhyaya, H.D., S.L. Dwivedi, V. Vadez, F. Hamidou, S. Singh, R.K. Varshney, and B. Liao. 2014. Multiple resistant and nutritionally dense germplasm identified from mini core collection in peanut. Crop Sci. 54:2120–2130. doi:10.2135/cropsci2014.01.0040

Upadhyaya, H.D., C.L.L. Gowda, K.N. Reddy, and S. Singh. 2009. Augmenting the pearl millet core collection for enhancing germplasm utilization in crop improvement. Crop Sci. 49:573–580. doi:10.2135/cropsci2008.06.0378

Upadhyaya, H.D., K.N. Reddy, and C.L.L. Gowda. 2007. Pearl millet germplasm at ICRISAT genebank—Status and impact. J. SAT Agric. Res. 3:1–5.

Upadhyaya, H.D., D. Yadav, K.N. Reddy, C.L.L. Gowda, and S. Singh. 2011. Development of pearl millet mini core collection for enhanced utilization of germplasm. Crop Sci. 51:217–223. doi:10.2135/cropsci2010.06.0336