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# New virulent pathotypes of *Sclerospora graminicola* and resistance sources in pearl millet for A1 zone in India

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ABSTRACT

Sixteen isolates of downy mildew (DM) pathogen (*Sclerospora graminicola*) collected during 2009 and 2010 from the A1 zone in India that comprises parts of Rajasthan, Gujarat and Haryana were characterized for virulence diversity along with five isolates collected during 1997 to 2005 from this region that are currently being used in the greenhouse screening. Based on DM incidence, three new pathotypes Sg 445, Sg 526 and Sg 519 identified as most virulent were selected for the greenhouse screening of 158 breeding lines under high disease pressure (>90% disease incidence in the susceptible checks). Twenty nine lines were resistant (=10% disease incidence) to at least one of the three pathotypes. Three lines were free from DM and five lines were resistant (=10% disease incidence) to all the three pathotypes, and seven lines were resistant to two of the three pathotypes. The multiple pathotype resistance identified in these breeding lines provides valuable genetic resources for breeding disease resistant pearl millet parental lines and hybrids for A1 zone in India.

**Key words:** A1 zone, Downy mildew, Pathotypes, Pearl millet, Virulence

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a staple cereal grown on about 29 million ha in the arid- and semi-arid tropical regions of Africa, Asia and Latin America with India having the largest area of 9.3 million ha (http://www.icrisat.org/PearlMillet/PearlMillet.htm). During the past three decades single-cross $F_1$ hybrids based on a cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly to increasing pearl millet productivity in India. Single-cross pearl millet hybrids bred by the national/state breeding programs and private seed companies are widely grown in the country, with coverage of new cultivars greater than 90% in some areas. In the arid zone, that comprises parts of Haryana, Rajasthan and Gujarat, there has been very limited adoption of new cultivars (apart from the occasional irrigated field) and yield increases have been much less than those achieved nationally. The arid zone represents about 25% of the total acreage of pearl millet area in India (>2 million of 9 million hectares), and one where farmers have no alternative to pearl millet (Bidinger *et al*. 2009). In addition, diseases such as downy mildew (DM) caused by *S. graminicola* (Sacc.) Schroet, continue to be major constraint to pearl millet production. *S. graminicola* is an obligate pathogenic oomycete and reproduces both asexually by producing sporangia and sexually by means of oospores. The fungus is largely heterothallic but homothalism has also been reported (Idris and Ball 1984, Michelmore *et al*. 1982). Existence of mating types and their frequency greatly contribute towards the development of new recombinants in the pathogen populations (Pushpavathi *et al*. 2006). These characteristics of the fungus make it a highly variable pathogen.

Pathogenic variability in *S. graminicola* studied through a collaborative International Pearl Millet Downy Mildew Virulence Nursery and on-farm DM survey has revealed differences in *S. graminicola* populations at different locations, including those within India (Thakur *et al*. 2003, 2006, Rao *et al*. 2005). Therefore, pathogen populations in the major crop growing areas need to be periodically monitored and characterized to identify new pathotypes in the target area (Sharma *et al*. 2010, 2011). Populations across the regions are compared to determine genetic similarity among them and prevalence of different pathotypes across the regions. Pathogen populations from the major pearl millet growing areas in India have been characterized and representative isolates from different regions are being maintained at ICRISAT for greenhouse screening of breeding material. However, due to evolution in the pathogen, there is a need to characterize the pathogen

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populations and identify new virulent pathotypes for use in greenhouse screening to select resistant lines for use in hybrid parent development programs. This study reports the identification of new virulent pathotypes in the A1 zone, and resistance sources in a diverse range of breeding lines of pearl millet.

## MATERIALS AND METHODS

Sixteen isolates (6 from Rajasthan, 5 from Haryana and 5 from Gujarat) of downy mildew pathogen were collected during the roving surveys of farmers' fields in 2009 and 2010 rainy seasons from different hybrids, and were established on a highly susceptible genotype, 7042S in the isolation chambers at ICRISAT, Patancheru in a greenhouse using the standard procedure (Singh *et al*. 1997). These isolates were characterized for virulence diversity to identify most virulent pathotypes. Five old isolates from these states collected during 1997 to 2005 that are currently being used in the greenhouse screening were included as reference isolates for comparison.

The host differential set used for variability study included seven pearl millet genotypes P 7-4, P 310-17, 700651, 7042R, IP 18292, IP 18293 and 852B selected from International Pearl Millet Downy Mildew Virulence Nursery (Thakur *et al*. 2004), and two known DM susceptible lines - ICMP 451 and 7042S. Sporangial inocula of the 21 isolates were raised on seedlings of a highly susceptible genotype 7042S in isolation chambers in a greenhouse at ICRISAT, Patancheru. Sporangia from sporulating leaves were harvested in ice-cold water and spore concentration was adjusted to $1 \times 10^6$/ml. Pot-grown seedlings of the differential lines and the two susceptible checks were spray-inoculated at coleoptile stage using an atomizer. The inoculated seedlings were incubated at 20ºC with >95% RH for 20 h, and then transferred to greenhouse benches maintained at 25±2°C and >90% RH for disease development for the next two weeks. The experiment was conducted in completely randomized design with three replications, and 35 to 40 seedlings per replication. The disease incidence was recorded 14 days after inoculation and percent incidence was derived based on the total and diseased seedlings. Data were subjected to analysis of variance to test the effect of isolate, host genotype and isolate × genotype interaction on DM incidence.

For identification of resistance in pearl millet breeding lines to new pathotypes, 158 lines were evaluated in a completely randomized design with two replications, 60 to 70 seedlings/replication in a greenhouse. These were inoculated with isolates Sg 519 from Haryana, Sg 526 from Rajasthan and Sg 445 from Gujarat, identified as most virulent, using standard procedure of inoculation as described above and scored for downy mildew incidence two weeks after inoculation.

## RESULTS AND DISCUSSION

The results of virulence diversity studies indicated significant variation in the virulence of different isolates on host differential lines (Table 1). The mean downy mildew (DM) incidence varied from 43% (Sg 525 and Sg 527) to 80% (Sg 526) among the six isolates from Rajasthan. Sg 526 from Osiyan in Jodhpur district was found more virulent compared to the virulent pathotype Sg 384 earlier identified from Barmer (Table 2). The mean DM incidence varied from 40% (Sg 521) to 68% (Sg 519) among the five isolates from Haryana and isolate Sg 519 from Rewari was found more virulent compared to the pathotype Sg 334 identified earlier from Bhiwani. The higher virulence of new pathotypes compared to the earlier pathotypes collected during 1997 to 2003 indicated that there was a change in the virulence in pathogen population in Rajasthan and Haryana. Temporal virulence change in the *S. graminicola* populations from India has been reported in an earlier study (Thakur *et al*. 2009). The mean DM incidence varied from 32% (Sg 560) to 69% (Sg 559) among the five isolates from Gujarat. Sg 445 collected from Banaskantha during 2005 was comparatively more virulent with 85% incidence than the five new isolates collected during 2010 (Table 2). The newly identified virulent pathotypes Sg 526 and Sg 519 have been selected for the greenhouse screening of breeding lines targeted for Rajasthan and Haryana, respectively. Sg 445 being most virulent among isolates from Gujarat was selected for greenhouse screening of lines targeted for Gujarat.

Results of the greenhouse screen showed that more than 75% of the breeding lines were susceptible (>30% incidence) to all the 3 pathotypes. Disease incidence was quite high (90 to 100%) on susceptible genotypes 7042 S and ICMP 451 indicating high disease pressure. Of the 158 lines screened, 23 were resistant (=10% incidence) to Sg 445, 14 to Sg 519 and 15 to Sg 526 which further indicated pathogenic variation among the three most virulent isolates selected from the variability study using seven host differentials (Table 3).

These three isolates were virulent on all the seven host differentials; however, differential reaction of pearl millet lines evaluated for disease resistance was observed against these isolates. Pearl millet lines such as (RCB-2-S1-138-1-1 × MRC)-B-5-5-2-5-3-1, Jakhrana × ESRC II S2-147-B-2-2-3-1-B, (EERC-HS-21)-B-1-1-2-2, LaGrap C2-S1-38-2-1-1-2 and MRC S1-9-1-1-B-B-B-B differentiated Sg 445 from Sg 519 and Sg 526; the lines being resistant to Sg 445 but susceptible to Sg 519 and Sg 526 (Table 4). Similarly,

Table 1 Analysis of variance for downy mildew incidence of the *Sclerospora graminicola* isolates on host differentials

| Source of variation | df | Downy mildew incidence | |
|---|---|---|---|
| | | Mean square | *F* ratio |
| Isolate (I) | 20 | 7679.19 | 483.64*** |
| Host genotype (H) | 8 | 44730.23 | 2817.12*** |
| I × H | 160 | 1392.33 | 87.69*** |
| Residual | 403 | 15.88 | |

***Significant at (P<0.001)

Table 2 Downy mildew incidence (%) on host differential lines to isolates from Rajasthan, Haryana and Gujarat

| Isolate origin | | | | Downy mildew incidence (%)[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Identity | Location | State | Year | P 7-4 | P 310-17 | 700651 | 7042R | 852B | IP 18292 | IP 18293 | ICMP 451 | 7042S | Mean |
| Sg 139 | Jodhpur | RS | 1997 | 28 | 6 | 8 | 47 | 54 | 64 | 9 | 57 | 96 | 41 |
| Sg 212 | Durgapura | RS | 1998 | 12 | 3 | 1 | 55 | 9 | 3 | 12 | 100 | 100 | 33 |
| Sg 334 | Bhiwani | HR | 2001 | 15 | 17 | 17 | 27 | 16 | 7 | 9 | 96 | 100 | 34 |
| Sg 384 | Barmer | RS | 2003 | 76 | 83 | 26 | 69 | 94 | 51 | 64 | 96 | 100 | 73 |
| Sg 445 | Banaskanta | GS | 2005 | 57 | 92 | 83 | 94 | 100 | 100 | 35 | 100 | 100 | 85 |
| Sg 519 | Rewari | HR | 2009 | 39 | 63 | 66 | 85 | 10 | 100 | 51 | 100 | 100 | 68 |
| Sg 520 | Bhiwani | HR | 2009 | 10 | 7 | 8 | 64 | 34 | 58 | 9 | 100 | 100 | 43 |
| Sg 521 | Rewari | HR | 2009 | 21 | 36 | 24 | 34 | 3 | 47 | 5 | 93 | 96 | 40 |
| Sg 522 | Rewari | HR | 2009 | 46 | 55 | 55 | 66 | 21 | 42 | 24 | 95 | 100 | 56 |
| Sg 523 | Mahendragarh | HR | 2009 | 48 | 42 | 50 | 72 | 2 | 67 | 51 | 100 | 100 | 59 |
| Sg 524 | Jodhpur | RS | 2009 | 35 | 32 | 44 | 37 | 15 | 50 | 24 | 90 | 99 | 47 |
| Sg 525 | Osiyan | RS | 2009 | 44 | 38 | 54 | 54 | 33 | 61 | 7 | 0 | 100 | 43 |
| Sg 526 | Osiyan | RS | 2009 | 62 | 77 | 69 | 59 | 93 | 100 | 57 | 100 | 100 | 80 |
| Sg 527 | Mandore | RS | 2009 | 5 | 18 | 16 | 27 | 83 | 25 | 44 | 75 | 98 | 43 |
| Sg 528 | CAZRI | RS | 2009 | 6 | 7 | 19 | 43 | 100 | 91 | 63 | 97 | 100 | 59 |
| Sg 529 | CAZRI | RS | 2009 | 40 | 41 | 65 | 51 | 98 | 70 | 38 | 99 | 100 | 67 |
| Sg 556 | Kothigaon | GJ | 2010 | 8 | 11 | 42 | 28 | 20 | 0 | 14 | 100 | 100 | 36 |
| Sg 557 | Lodhnoor | GJ | 2010 | 43 | 41 | 40 | 98 | 100 | 63 | 25 | 98 | 99 | 67 |
| Sg 558 | Gagana | GJ | 2010 | 14 | 7 | 38 | 96 | 93 | 52 | 34 | 94 | 100 | 59 |
| Sg 559 | Jamdi | GJ | 2010 | 22 | 38 | 41 | 96 | 97 | 83 | 48 | 98 | 100 | 69 |
| Sg 560 | SK Nagar | GJ | 2010 | 16 | 6 | 14 | 35 | 0 | 6 | 8 | 100 | 100 | 32 |
| Mean | | | | 30 | 33 | 36 | 59 | 49 | 52 | 29 | 90 | 100 | 53 |

[a]Based on the mean of three replications, 30 to 35 seedlings/replication. RS=Rajasthan, HR=Haryana and GJ=Gujarat
LSD (P<0.05) for isolate (I) means = 2.13; for genotype (G) means = 1.36 and for I × G means = 5.54

Table 3 Downy mildew (DM) incidence (%) in pearl millet lines screened against new pathotypes of *Sclerospora graminicola*

| DM incidence (%) | Number of lines in DM incidence classes against pathotypes | | |
|---|---|---|---|
| | Sg 445 | Sg 519 | Sg 526 |
| 0 | 13 | 6 | 8 |
| 1-10 | 10 | 8 | 7 |
| 11-20 | 8 | 10 | 7 |
| 21-30 | 4 | 5 | 4 |
| >30 | 123 | 129 | 132 |
| Total | 158 | 158 | 158 |

LaGrap C2-S1-49-2-3-3-2 and Tift 186 differentiated Sg 519 from Sg 445 and Sg 526. Sg 519 was virulent on these lines whereas, Sg 445 and Sg 526 were avirulent. Therefore, the greenhouse screen results revealed that the highly virulent isolates selected from diverse locations are pathogenically diverse as well.

Three lines [(EERC-HS-23)-B-3-1-2-4, JBV 3 S1-18-2-2-1-3-2 and IP No. 17778-1-B-2 were free from downy mildew to all the three most virulent pathotypes (one from each state), five lines- ICMR 312 S1-17-3-2-1-2-3-B-B-B-B-B; JBV 3 S1-18-2-2-2-3-1-5; (MC 94 C2-S1-3-2-2-2-1-3-B-B × AIMP 92901 S1-488-2-1-1-4-B-B)-B-2-2-3, (EERC-HS-12)-B-4-3-1-1 and LaGrap C2-S1-62-3-2-2-2 were resistant (=10% incidence) to all the three pathotypes, and seven lines were resistant to two of the three pathotypes (Table 4). Of the eight lines found resistant (=10% incidence) to all the three new pathotypes, two each were found to be derived from broad based populations like Extra-Early Restorer Composite (EERC) developed at ICRISAT and from Jawahar Bajra Variety 3 (JBV 3) developed jointly by AICPMIP-Gwalior and ICRISAT; and one from Large Grain Population (LaGraP) developed at ICRISAT. One of these identified lines again involved broad genetic base populations such as Medium Composite (MC) and an open pollinated variety (AIMP 92901) jointly developed by Marathwada Agricultural University, Aurangabad and ICRISAT. Rest of the two lines were developed involving a restorer population (ICMR 312) developed at ICRISAT and a germplasm accession, ICRISAT Pollinator line (IP 17778). Hence, the probable reason for these eight lines having resistance to all the three newly identified pathotypes, or seven lines being resistant to any two of the three pathotypes seems to be the result of involvement of broad genetic base of populations/breeding lines in the pearl millet breeding program at ICRISAT, which might have helped in accumulating DM resistance loci in these lines.

ICRISAT has a major research focus on development of hybrid parental lines, which are disseminated to public organizations and private seed companies for use in developing $F_1$ hybrid cultivars. The multiple pathotype resistance identified in the advanced breeding lines would, therefore, be useful in broadening the genetic base of DM

Table 4 Selected pearl millet lines resistant to new pathotypes of *Sclerospora graminicola*

| Line | Downy mildew incidence (%)[a] | | |
|---|---|---|---|
| | Sg 445 | Sg 519 | Sg 526 |
| (EERC-HS-23)-B-3-1-2-4 | 0.0 | 0.0 | 0.0 |
| JBV 3 S1-18-2-2-1-3-2 | 0.0 | 0.0 | 0.0 |
| IP No. 17778-1-B-2 | 0.0 | 0.0 | 0.0 |
| (MC 94 C2-S1-3-2-2-2-1-3-B-B × AIMP 92901 S1-488-2-1-1-4-B-B)-B-2-2-3 | 0.0 | 5.6 | 0.0 |
| Jakhrana × ESRC II S2-11-B-1-2-1-1-B | 0.0 | 11.3 | 0.0 |
| LaGrap C2-S1-49-2-3-3-2 | 0.0 | 18.0 | 0.0 |
| JBV 3 S1-18-2-2-2-3-1-5 | 0.0 | 5.4 | 2.5 |
| (EERC-HS-12)-B-4-3-1-1 | 0.0 | 1.7 | 6.1 |
| (RCB-2-S1-138-1-1 × MRC)-B-5-5-1-2-2-B-B | 0.0 | 95.2 | 13.6 |
| (RCB-2-S1-138-1-1 × MRC)-B-5-5-2-5-3-1 | 0.0 | 98.4 | 30.9 |
| (RCB-2-S1-138-1-1 × MRC)-B-5-5-2-5-3-1-B | 0.0 | 75.6 | 46.1 |
| Jakhrana × ESRC II S2-11-B-1-3-2-1-B | 0.0 | 31.0 | 100.0 |
| Jakhrana × ESRC II S2-147-B-2-2-3-1-B | 0.0 | 100.0 | 100.0 |
| (EERC-HS-21)-B-1-1-2-2 | 1.7 | 91.3 | 95.1 |
| ICMR 312 S1-17-3-2-1-2-3-B-B-B-B-B-B | 1.7 | 8.9 | 2.3 |
| MDRRC-HS-28-3 | 1.9 | 7.9 | 14.9 |
| LaGrap C2-S1-38-2-1-1-2 | 1.9 | 98.3 | 31.0 |
| MRC S1-9-1-1-B-B-B-B | 2.5 | 98.6 | 100.0 |
| LaGrap C2-S1-62-3-2-2-2 | 2.8 | 0.0 | 0.0 |
| MDRRC-HS-55-3 | 6.2 | 10.9 | 11.8 |
| IP No. 17518-1-2-1 | 6.9 | 0.0 | 13.8 |
| (MC 94 C2-S1-3-2-2-2-1-3-B-B × ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-4-1 | 10.4 | 5.6 | 1.6 |
| HiTiP S1-7-2-1-2-1 | 10.4 | 75.7 | 75.0 |
| AIMP 92901 S1-183-2-2-1-B-B-1 | 12.8 | 0.0 | 0.0 |
| (MC 94 C2-S1-3-2-2-2-1-3-B-B × ICMR 312 S1-3-2-3-2-1-1-B-B)-B-22-2-1 | 17.2 | 13.2 | 3.0 |
| MDRRC-HS-13-1 | 18.9 | 18.4 | 10.5 |
| LaGrap C2-S1-96-2-2-1-1 | 23.5 | 5.2 | 29.7 |
| IP No. 9348-1-2 | 31.7 | 15.5 | 8.7 |
| MC 94 C2-S1-47-1-1-1-B-1-1 | 54.9 | 9.2 | 8.5 |
| *Pathology checks* | | | |
| IP18292 | 100.0 | 100.0 | 100.0 |
| RIB 335/74 | 14.5 | 42.4 | 13.2 |
| Tift 186 | 8.3 | 41.1 | 5.9 |
| ICMP 451 | 100.0 | 90.0 | 100.0 |
| 7042S | 100.0 | 100.0 | 100.0 |

[a]Based on the mean of two replications. LSD (P<0.05) for pathotype (P) means = 0.25; for genotype (G) means = 2.03 and for P × G means = 3.51

resistant pearl millet parents and consequently the hybrids for cultivation in A1 zone in India.

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