# TEMPORAL VIRULENCE CHANGE AND IDENTIFICATION OF RESISTANCE IN PEARL MILLET GERMPLASM TO DIVERSE PATHOTYPES OF *SCLEROSPORA GRAMINICOLA*

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## SUMMARY

Downy mildew caused by *Sclerospora graminicola* (Sacc.) Shroet. is a major biotic constraint to pearl millet production in the semi-arid tropics. The pathogen is heterothallic and frequent recombination leads to genotypic diversity and evolution of new virulent populations. Identification of resistance to new virulent populations is a prerequisite for resistance breeding. Of 123 pearl millet germplasm accessions from 15 countries that were identified as resistant (≤10% incidence) at ICRISAT, Patancheru during 1990-93, only 21 remained resistant during 2006 under field screening indicating a temporal virulence change in Patancheru field population of *S. graminicola*. These resistant accessions when evaluated in the greenhouse against 12 diverse pathotypes, no accession showed resistance to all the 12 pathotypes. However, three accessions (IP 18295, P 1449-2 and YL 18) were resistant to 11 pathotypes, two (IP 18298 and IP 8289) to 10 pathotypes, three (IP 22396, YG 2 and YG 8) to 9 pathotypes and one (YM 16) to 8 pathotypes. Host resistance index (HRI), calculated from downy mildew incidence and latent period identified six accessions (IP 18295, -8289, P 1449-2, YG 2, -8 and YL 18) as best available resistance sources against multiple pathotypes of *S. graminicola.* The study showed temporal virulence change in *S. graminicola* population at Patancheru, emphasizing the need for closely monitoring both pathogen virulence and host resistance for effectively managing the disease through resistance breeding.

*Key words: Sclerospora graminicola*, downy mildew, pathotype, pearl millet germplasm, resistance.

## INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important cereal crop, grown on 26 million ha in the arid and semi-arid tropical (SAT) regions of Africa and Asia, with India having the largest area of 9.5 million ha (AICPMIP, 2008). It is a staple food and fodder crop for millions of poor people surviving on the most marginal agricultural lands and contributes to food security in the SAT region. Although local landraces are reasonably tolerant to a range of biotic and abiotic stresses, their yield potential is low. However, high yielding hybrid cultivars have been widely adopted by farmers for cultivation in more favorable environments. The productivity of pearl millet is severely constrained by downy mildew caused by *Sclerospora graminicola* (Sacc.) Schroet. The disease causes significant losses (10-70%) in grain yield and is more severe on genetically homogeneous single-cross pearl millet hybrids, which are grown on about 60% of the total 9.5 million ha in India, than on heterogeneous open-pollinated varieties (Thakur *et al.*, 2006). During the 1970s-80s several downy mildew epidemics occurred in India resulting in considerable yield losses and withdrawal of several hybrids from cultivation (Singh *et al.*, 1987; Thakur, 1999). Currently, over 70 different hybrids are being grown in India (Thakur *et al.*, 2006) and during our recent on-farm survey, some of them have shown downy mildew incidence up to 100% (unpublished results). The on-farm downy mildew 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 3-5 years (Thakur *et al.*, 2003; Rao *et al.*, 2005; Thakur *et al.*, 2006).

Downy mildew resistance breakdown has been common in breeding lines and hybrids. This is attributed to the evolution of new virulence in the pathogen population. Existence of mating types and their frequency greatly contribute towards the development of new recombinants in the pathogen populations (Pushpavathi *et al.*, 2006a). Evolution of host-specific virulence in pearl millet downy mildew is well documented (Thakur *et al.*, 1992; Sastry *et al.*, 2001; Pushpavathi *et al.*, 2006b). Since management of pearl millet downy mildew largely depends upon host plant resistance, evolution of new/changed virulence(s) in the pathogen population and resistance effective against new pathotypes need to be periodically monitored.

As a result of evolution of new virulent populations of

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*S. graminicola*, resistance effective in the breeding lines against old pathotypes was overcome by the new pathotypes. Therefore, there is a need to re-evaluate pearl millet germplasm lines that were identified as resistant in the past against the new pathotypes of *S. graminicola*.

In the present study, pearl millet germplasm accessions that were resistant to downy mildew at Patancheru during the early 1990s were re-evaluated in the downy mildew nursery in 2006 as well as in the greenhouse to diverse pathotypes, to determine temporal virulence change and resistance stability against new virulent pathotypes.

## MATERIALS AND METHODS

**Plant material.** A total of 123 pearl millet germplasm accessions that were found resistant to downy mildew during 1990-93 (Singh *et al.*, 1997) were used in this study. Seed of these accessions were obtained from ICRISAT Genetic Resources. These accessions originated from 15 Asian and African countries, i.e. 28 from India, 17 each from Niger and Nigeria, 15 from Mali, 12 from Cameroon, 10 each from Burkina Faso and Sudan, 4 from Senegal, 3 from Zimbabwe, 2 from Togo and 1 each from Botswana, CAR, Gambia, Namibia and South Africa. The accessions were evaluated for downy mildew resistance both under field and greenhouse conditions during 2006-2007 along with the highly susceptible line 7042S.

**Evaluation in field downy mildew nursery.** For field evaluation, germplasm accessions were planted in a downy mildew-diseased plot established using oospore inoculum from the local susceptible cultivars. This diseased plot has been maintained in one field at ICRISAT, Patancheru farm for over 15 years now and has been operational during the rainy season (June-October). The soil has been infested with oospores by incorporating infected leaf debris at the end of the crop season and coating seed of infector rows of highly susceptible lines with oospores just before sowing, to provide primary inoculum (Singh *et al.*, 1993). In our study, infector rows of a mixture of two highly susceptible lines (7042S and 843B) were planted in every ninth row in the diseased plot to serve as inoculum donor to test rows. The infector rows were spray-inoculated with sporangial suspension ($1\times10^5$ sporangia $ml^{-1}$) at the 2-leaf stage. After three weeks when infector rows were heavily infected (>70% incidence), test rows of germplasm accessions were planted in between infector rows. Each test line was planted in 2 rows of 4 m length in 2 replications using a randomized complete block design. About 40 seedlings were maintained per row by thinning 2 weeks after seedling emergence. Furrow irrigation was provided soon after planting to help seed germination. Standard dosages of fertilization by NPK were supplied. High humidity (>90% RH) was maintained using overhead perfo irrigation for about 7 days, starting from the date of inoculation of infector rows, and the same was followed after planting the test rows. Total numbers of

**Table 1.** Origin of *Sclerospora graminicola* pathotypes used in the study.

| Pathotype | Isolate collection | | | Maintenance host |
|---|---|---|---|---|
| | Year | Cultivar | Location | |
| Sg 409 | 2004 | PMB 11571-2 | ICRISAT, Patancheru/Andhra Pradesh | PMB 11571-2-original host |
| Sg 021 | 1993 | MLBH 104 | Ghari/Maharashtra | 7042S- universally highly susceptible line |
| Sg 048 | 1994 | 7042S+HB#3 | Mysore/Karnataka | 852B-highly susceptible at Mysore |
| Sg 200 | 1998 | ICMH 451 | Jamnagar/Gujarat | ICMP 451-parental line of hybrid ICMH 451 |
| Sg 212 | 2002 | PG 5522 | Durgapura, Rajasthan | ICMP 451-highly susceptible at Durgapura |
| Sg 150 | 1997 | MBH 110 | Jalna, Maharashtra | 834B-parental line of hybrid MBH 110 |
| Sg 153 | 1997 | 7042S+NHB#3 | ICRISAT, Patancheru/Andhra Pradesh | 7042S-universally highly susceptible line |
| Sg 445 | 2005 | AHT-503 | Banaskantha/ Gujarat | Pioneer 7777- highly susceptible hybrid in Banaskantha |
| Sg 139 | 1997 | IP 18292 | Jodhpur/ Rajasthan | Nokha Local- susceptible at Jodhpur |
| Sg 384 | 2003 | Local | Barmer/ Rajasthan | ICMP 451- susceptible at Barmer |
| Sg 298 | 1999 | W 504-1-1 | IARI/New Delhi | W 504-1-1-original host |
| Sg 334 | 2001 | HHB 67 | Bhiwani/Haryana | 843B- parental line of hybrid HHB 67 |

plants and downy mildew-infected plants per accession were recorded at the pre-tillering stage (about 30 days after emergence) and at the soft-dough stage (about 60 days after emergence) and downy mildew incidence was calculated as percent infected plants.

**Evaluation against multiple pathotypes in greenhouse.** The germplasm accessions selected from the field screen against the Patancheru pathotype were tested against 12 different pathotypes of *S. graminicola* collected from different pearl millet-growing areas in India and maintained in the isolation chambers of the greenhouse at ICRISAT (Table 1). A pathotype-isolate is the most aggressive isolate selected among a number of isolates from a particular pearl millet-growing area. The isolate is maintained either on the original host, susceptible parental line of the original hybrid host, other equally susceptible hosts, or the universally susceptible line 7042S.

Infected leaves of each pathotype were collected from the isolation chamber, washed under running tap water and cut into pieces. The leaves were wiped with tissue paper to remove old sporangia and placed with their abaxial surface up in a chamber lined with moist blotting paper. The moist chambers were incubated in the dark at 20°C for 6 h. Sporangia from sporulating leaves were harvested in sterilized distilled ice-cold water (2-4 °C) using camel hair brush and filtered through double-layered muslin cloth. Spore concentration was adjusted to $5\times10^5$ sporangia $ml^{-1}$ for each pathotype. Inoculum concentration used in greenhouse screen was higher than that for field screen because the field had an additional oospore load in the soil. Pot-grown seedlings of the pearl millet germplasm accessions were spray-inoculated at the coleoptile stage using separate atomizer for each isolate and covered immediately with a polyethylene sheet to provide >95% relative humidity. Seedlings were incubated at 20°C for 24 h, then the pots were transferred to the greenhouse and maintained at 25±2°C, >95% RH and leaf wetness conditions for disease development during the next 2 weeks. Data for latent period and disease incidence were recorded. Latent period was expressed as number of days from inoculation to sporulation on 50% of the infected seedlings (Thakur *et al.,* 1998). Disease incidence was recorded 14 days after inoculation as percentage of infected seedlings. From the data on disease incidence and latent period we derived host resistance index (HRI) as reported by Thakur *et al.* (1997). In fact, in a systemic disease, such as downy mildew of pearl millet, where large variation occurs for percentage of disease incidence and latent period, the disease incidence alone may not provide a true measure of the resistance level of a host genotype, thus Thakur *et al.* (1997) developed the concept of HRI, which is a function of both disease incidence and latent period and is defined as:

$$HRI = [1 + (ab^{-1})]^{-1}$$

where, a = incidence (%) and b = latent period (days).

In the equation, HRI = 1, if a = 0, which corresponds to a completely resistant line; and HRI <1, if a>0, which corresponds to less than a completely resistant line, and thus the degree of resistance is directly proportional to the value of HRI.

**Data analysis.** The data on downy mildew incidence and latent period were subjected to analysis of variance using GENSTAT statistical package to determine signif-

**Table 2.** Pearl millet germplasm accessions from different countries screened for downy mildew resistance in a field nursery at ICRISAT, Patancheru, rainy season 2006.

| Country | No. of accessions | Downy mildew incidence (%) class[a] | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1-10 | 11-20 | 21-50 | >50 |
| Botswana | 1 | 0 | 0 | 1 | 0 | 0 |
| Burkina Faso | 10 | 0 | 6 | 2 | 1 | 1 |
| CAR | 1 | 0 | 0 | 1 | 0 | 0 |
| Cameroon | 12 | 0 | 1 | 7 | 4 | 0 |
| Gambia | 1 | 0 | 0 | 1 | 0 | 0 |
| India | 28 | 2 | 5 | 6 | 3 | 12 |
| Mali | 15 | 1 | 3 | 6 | 4 | 1 |
| Namibia | 1 | 0 | 0 | 0 | 1 | 0 |
| Niger | 17 | 0 | 1 | 7 | 1 | 8 |
| Nigeria | 17 | 0 | 0 | 13 | 4 | 0 |
| Senegal | 4 | 1 | 1 | 0 | 1 | 1 |
| South Africa | 1 | 0 | 0 | 1 | 0 | 0 |
| Sudan | 10 | 0 | 0 | 3 | 3 | 4 |
| Togo | 2 | 0 | 0 | 2 | 0 | 0 |
| Zimbabwe | 3 | 0 | 0 | 3 | 0 | 0 |
| Total | 123 | 4 | 17 | 53 | 22 | 27 |

[a]Downy mildew incidence at soft-dough stage

**Table 3.** Analysis of variance for downy mildew incidence, latent period and host resistance index (HRI).

| Source of variation | df | MS | | |
|---|---|---|---|---|
| | | Downy mildew incidence (%) | Latent period (days) | HRI |
| Replications | 2 | 14.13 | 0.08 | 0.01 |
| Pathotypes | 11 | 12697.61* | 27.81* | 1.44* |
| Genotypes | 21 | 16386.87* | 48.64* | 1.59* |
| Pathotype × genotype | 231 | 1088.27* | 7.51* | 0.16* |
| Residual | 491 | 28.26 | 1.16 | 0.01 |

*Significant at $P<0.001$.

icant differences among isolates, host genotypes and their interactions (Payne, 2002). Since HRI data is metrical and had large variation, the centroid distances method was more appropriate in measuring the distances among the genotypes. Centroid distances were obtained using Euclidian squares and these distances were subjected to hierarchical groupings using the average linkage method (Bartholomew *et al*., 2002), so that the genotypes were classified into resistant, moderately resistant and susceptible groups.

## RESULTS

**Downy mildew resistance in field screen.** In general, there were increased levels of downy mildew incidence in the test lines from the pre-tillering to the soft-dough stage under field conditions (Table 2). Therefore, disease incidence data recorded at the soft-dough stage was considered for classifying the germplasm lines into resistant or susceptible types. Of the 123 accessions screened, 4 were disease-free, 17 recorded 1-10% incidence, 53 had 11-20%, 22 exhibited 21-50%, and the remaining 27 recorded > 50% incidence.

**Downy mildew resistance to multiple pathotypes in greenhouse.** ***Disease incidence.*** Highly significant ($P < 0.001$) effects of host genotypes, pathotypes and their interactions on disease incidence were observed (Table 3). None of the 21 accessions showed resistance to all the 12 pathotypes (Table 4). However, several accessions showed resistance to more than one pathotypes;

**Table 4.** Downy mildew incidence in selected germplasm accessions against 12 pathotypes of *Sclerospora graminicola*.

| Genotypes | Downy mildew incidence (%)[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sg 021 | Sg 048 | Sg 139 | Sg 150 | Sg 153 | Sg 200 | Sg 212 | Sg 298 | Sg 334 | Sg 384 | Sg 409 | Sg 445 | Mean |
| IP 11428 | 51 | 30 | 34 | 9 | 16 | 6 | 15 | 0 | 46 | 100 | 20 | 100 | 36 |
| IP 18293 | 21 | 13 | 12 | 54 | 14 | 29 | 25 | 93 | 14 | 97 | 42 | 100 | 43 |
| IP 18294 | 7 | 17 | 0 | 97 | 0 | 92 | 2 | 90 | 14 | 100 | 23 | 100 | 45 |
| IP 18295 | 3 | 5 | 3 | 0 | 3 | 0 | 4 | 1 | 5 | 2 | 6 | 18 | 4 |
| IP 18298 | 5 | 10 | 8 | 6 | 8 | 33 | 3 | 0 | 8 | 8 | 14 | 9 | 9 |
| IP 22393 | 31 | 16 | 21 | 9 | 17 | 32 | 8 | 0 | 53 | 50 | 19 | 100 | 30 |
| IP 22396 | 6 | 7 | 0 | 3 | 0 | 10 | 3 | 0 | 6 | 16 | 11 | 54 | 10 |
| IP 8289 | 4 | 6 | 0 | 0 | 2 | 0 | 0 | 19 | 3 | 0 | 0 | 44 | 7 |
| IP 9997 | 16 | 3 | 22 | 32 | 13 | 5 | 25 | 14 | 52 | 82 | 16 | 19 | 25 |
| P 1449-2 | 4 | 2 | 5 | 1 | 3 | 4 | 6 | 0 | 9 | 11 | 4 | 3 | 4 |
| P 1591 | 27 | 5 | 5 | 6 | 1 | 24 | 6 | 49 | 23 | 13 | 16 | 37 | 18 |
| TG-4 | 19 | 10 | 1 | 10 | 5 | 21 | 4 | 8 | 23 | 33 | 44 | 67 | 20 |
| TG-8 | 10 | 6 | 0 | 0 | 5 | 11 | 1 | 20 | 15 | 83 | 27 | 78 | 21 |
| TL-5 | 13 | 8 | 6 | 8 | 0 | 8 | 7 | 2 | 19 | 22 | 18 | 33 | 12 |
| YG-2 | 6 | 0 | 4 | 0 | 9 | 0 | 0 | 0 | 8 | 47 | 32 | 18 | 10 |
| YG-8 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 17 | 80 | 1 | 17 | 10 |
| YL-18 | 5 | 10 | 7 | 0 | 5 | 0 | 23 | 0 | 0 | 9 | 0 | 6 | 5 |
| YL-34 | 9 | 8 | 23 | 17 | 16 | 0 | 23 | 0 | 32 | 66 | 24 | 16 | 20 |
| YM-16 | 12 | 0 | 7 | 8 | 5 | 0 | 8 | 4 | 26 | 32 | 3 | 100 | 17 |
| YM-19 | 15 | 12 | 0 | 93 | 19 | 0 | 25 | 0 | 89 | 43 | 17 | 100 | 34 |
| ZG-3 | 46 | 10 | 20 | 6 | 23 | 88 | 6 | 30 | 61 | 73 | 16 | 66 | 37 |
| 7042S | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean | 19 | 13 | 13 | 21 | 12 | 21 | 13 | 20 | 28 | 49 | 21 | 54 | |

[a]Mean of 3 replications, 35-40 seedlings/replication.

LSD ($P<0.05$) for pathotype means = 1.8; genotype means = 2.5, pathotype × genotype means = 8.5.

three accessions (IP 18295, P 1449-2 and YL 18) were resistant (≤ 10% disease incidence) to 11 pathotypes, two (IP 18298 and IP 8289) to 10 pathotypes, three (IP 22396, YG 2 and YG 8) to 9 pathotypes and one (YM 16) to 8 pathotypes. IP 18293 was susceptible to all 12 pathotypes with incidence range of 12-100% and the mean disease incidence of 43%. Mean downy mildew incidence in these accessions across 12 pathotypes varied between 4% (IP 18295 and P 1449-2) and 49% (IP 18294) compared to 100% in the susceptible check 7042S. Some of the accessions exhibited strong differential reactions to specific pathotypes (Table 4).

Of the 12 pathotype isolates used for screening, Sg 445 appeared to be the most virulent with 54% mean disease incidence across accessions followed by Sg 384 (49% mean incidence). None of the test accessions showed immunity (no disease) to Sg 445, while IP 8289 was immune to pathotype Sg 384. However, three accessions (IP 18298, P 1449-2 and YL 18) were resistant to Sg 445 and three (IP 18295, IP 18298 and YL 18) to Sg 384.

***Latent period.*** Large variations were observed in the latent period for different genotype x pathotype combinations, ranging from 4 to 14 days (Table 5). Mean latent period in the germplasm accessions against the 12 pathotypes was significantly higher than in the susceptible check 7042S. Maximum latent period across the isolates was observed on IP 18298 (10.5 days) followed by YG-8 (9.5 days) and IP 18295 (9.3 days), and minimum on the susceptible check 7042S (4.9 days). The mean latent period across 22 accessions, including susceptible check 7042S, varied from 6.4 days (Sg 139) to 8.9 days (Sg 153). There were significant effects of host genotypes, pathotypes and their interaction on latent period (Table 3).

***Host resistance index.*** Large and significant variations were observed for HRI among host genotypes x pathotype combinations (Table 3). HRI values ranged between 0.04 for susceptible check 7042S with 100% downy mildew incidence and 1.0 for many accessions exhibiting immunity to specific pathotypes (Table 6). Mean HRI for the test accessions across pathotypes ranged from 0.20 (IP 18293) to 0.82 (YG-8). Mean HRI across germplasm accessions showed Sg 445 to be the most virulent with 0.22 mean HRI followed by Sg 384 (0.24 mean HRI) and Sg 298, the least virulent (0.66 mean HRI) among the 12 pathotypes used in this study.

Cluster analysis of germplasm accessions based on their HRI values against test pathotypes classified them into three distinct groups (Fig. 1). Group I was desig-

**Table 5.** Latent period for *Sclerospora graminicola* pathotypes on 21 germplasm accessions under greenhouse screen.

| | Latent period (days)[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Sg 021 | Sg 048 | Sg 139 | Sg 150 | Sg 153 | Sg 200 | Sg 212 | Sg 298 | Sg 334 | Sg 384 | Sg 409 | Sg 445 | Mean |
| IP 11428 | 6 | 6 | 5 | 5 | 9.7 | 7 | 5 | -[b] | 6 | 4.7 | 5.3 | 5 | 5.5 |
| IP 18293 | 6 | 6 | 7 | 8 | 10.0 | 6 | 5 | 6 | 6.3 | 5 | 5 | 5 | 5.9 |
| IP 18294 | 8 | 6 | - | 8 | - | 6 | 6.9 | 6 | 6 | 5 | 5 | 5 | 6.9 |
| IP 18295 | 12 | 14 | 7.5 | - | 7.0 | - | 10 | 6.1 | 9 | 9.7 | 8 | 10 | 9.6 |
| IP 18298 | 6 | 14 | 7 | 12 | 11.3 | 14 | 7.3 | - | 6 | 10 | 14 | 14 | 10.4 |
| IP 22393 | 6.3 | 6.7 | 8.7 | 5 | 8.7 | 6 | 7 | - | 6 | 6 | 5 | 5 | 6.2 |
| IP 22396 | 5.7 | 8.3 | - | 12 | - | 9 | 7.7 | - | 9 | 9 | 6 | 7 | 8.2 |
| IP 8289 | 14 | 6 | - | - | 9.0 | - | - | 6 | 6 | - | - | 7 | 7.8 |
| IP 9997 | 6.7 | 11 | 5 | 6 | 11.0 | 8 | 6.7 | 6 | 7 | 5 | 6.3 | 6.7 | 6.7 |
| P 1449-2 | 8 | 9.5 | 7 | 12 | 9.0 | 9 | 7.3 | - | 6 | 6.7 | 5.3 | 11.9 | 8.3 |
| P 1591 | 6 | 5 | 6 | 8 | 6.0 | 6 | 9.3 | 6 | 6 | 5 | 6 | 6 | 6.3 |
| TG-4 | 8 | 7.3 | 5 | 9 | 8.3 | 6.7 | 11 | 9.4 | 8.7 | 6.3 | 5 | 8 | 7.7 |
| TG-8 | 10 | 14 | - | - | 11.5 | 8 | 14 | 5.7 | 8.3 | 5 | 7 | 6.3 | 8.7 |
| TL-5 | 8 | 5 | 5 | 6.7 | - | 6.7 | 6 | 9.8 | 7 | 8.3 | 5 | 6.7 | 6.7 |
| YG-2 | 8 | - | 5 | - | 11.3 | - | - | - | 14 | 7 | 8 | 6 | 8.0 |
| YG-8 | 10.2 | - | - | - | - | - | 10 | 9.1 | 8.9 | 9 | 9 | 10 | 9.5 |
| YL-18 | 10 | 8.7 | 8 | 8.6 | 7.0 | 7.8 | 6 | 7.2 | 7.6 | 5 | 7 | 9 | 7.7 |
| YL-34 | 8 | 10 | 7.5 | 9 | 7.6 | - | 11 | - | 6 | 6 | 14 | 9 | 8.9 |
| YM-16 | 6.3 | - | 8 | 9 | 7.6 | - | 6.9 | 5 | 5.9 | 6 | 7.5 | 8.9 | 7.1 |
| YM-19 | 8 | 12 | - | 9 | 10.0 | - | 6.9 | - | 8.7 | 8 | 8.7 | 8.9 | 8.8 |
| ZG-3 | 8 | 5 | 5.7 | 8 | 10.7 | 6 | 6.7 | 6 | 6 | 8 | 5 | 6 | 6.4 |
| 7042S | 5 | 5 | 5 | 4 | 5.0 | 6 | 5 | 5 | 6 | 4 | 5 | 4 | 4.9 |
| Mean | 7.9 | 8.4 | 5.7 | 8.2 | 9.0 | 7.5 | 7.8 | 6.7 | 7.3 | 6.7 | 7.0 | 7.5 | 7.6 |

[a]Mean of 3 replications: [b]No disease symptoms: SEM for pathotype means = ±0.07; for genotype means = ±0.19 and for pathotype x genotype means = ±0.62: LSD ($P$<0.05) for pathotype means=0.21; genotype means=0.52 and pathotype x genotype means =1.71.

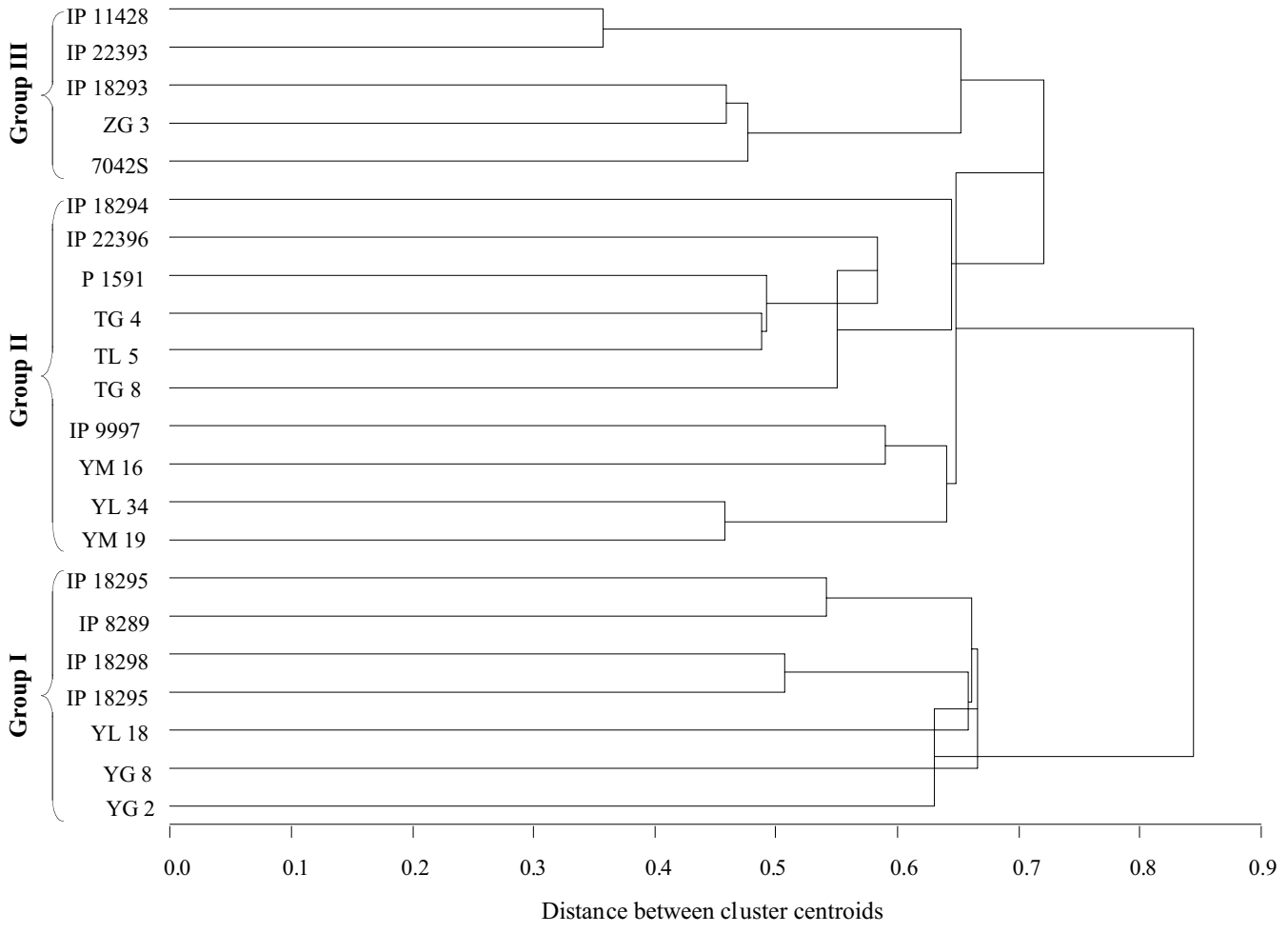


**Fig. 1.** Classification of pearl millet germplasm accessions based on hierarchical groupings using average linkage cluster analysis of centroid distances of host resistance index (HRI) values: group I = resistant; group II= moderately resistant; group III = susceptible.

nated as resistant and consisted of 7 accessions with more than 0.7 mean HRI, except for one accession IP 18298 with 0.59 mean HRI. Group II included 10 germplasm accessions with moderate level of resistance across pathotypes, and four accessions with ≤ 0.30 HRI were clustered in group III, together with the susceptible check 7042S.

## DISCUSSION

Host plant resistance is the most economic and efficient strategy for the management of downy mildew of pearl millet. Effective resistance breeding programmes require close monitoring of virulence change in the pathogen and identification of new resistance sources to the new virulent strains. Virulence change in *S. graminicola* populations is monitored through a collaborative pearl millet downy mildew nursery, on-farm surveys for downy mildew incidence and by characterizing pathogen isolates collected from highly susceptible cultivars in the farmers' fields on a set of putative differential hosts (Sivaramakrishnan *et al.,* 2003; Thakur *et al.,* 2004; Sharma *et al.*, 2008). On-farm surveys in the hybrid-intensive states of India during the past several years have indicated increased susceptibility of a hybrid when grown in the same field for more than three consecutive crop seasons suggesting emergence/selection of new virulence traits over time at the same location (Thakur *et al.*, 2003, 2006). The major change in disease incidence (%) of a pearl millet line over time at the same location was considered as reflection of virulence shift in the pathogen population. This is based on the basic assumptions that variables, such as environmental factors and inoculum load, were optimal for disease development and that the seed of each pearl millet line was genuine at both times of testing.

From amongst a number of isolates collected from susceptible cultivars in a particular pearl millet growing area, a highly virulent isolate, which infects most differential lines and causes high disease incidence, is selected as a representative isolate, called a pathotype. Currently,

there are 12 such pathotypes that exist in different pearl millet growing areas in India (unpublished results).

Large numbers of germplasm accessions and breeding lines have been screened at ICRISAT, and a number of resistant germplasm/lines have been identified (Singh *et al.,* 1997). However, virulence change has been reported in *S. graminicola* and more virulent pathotypes have evolved in the recent past (Thakur and Shetty, 1993; Thakur and Rao, 1997; Thakur *et al.,* 2007). Therefore, monitoring stability of resistance in the germplasm accessions/lines against newly evolving virulent populations is of great importance for downy mildew resistance breeding in pearl millet.

In the present study, most of the 123 germplasm accessions that proved resistant in the downy mildew nursery at Patancheru in the 1990s, lost their resistance in the same nursery in 2006, so that only 21 accessions remained resistant. This clearly indicates the temporal virulence change in the pathogen population at Patancheru. The mean downy mildew incidence across tested accessions was significantly higher for the new Patancheru pathotype Sg 409 (21%) than the old Patancheru pathotype Sg 153 (12%). Temporal virulence change was also evident from the disease incidence levels in IP 18293 and IP 18294, which were reported to be resistant during 1996 and found susceptible to new pathotypes of *S. graminicola* used in this study. In an earlier study, based on disease incidence on a set of pearl millet lines, temporal virulence change in *S. graminicola* populations was also reported in two other Indian locations, Durgapura and Anand, between 2001 and 2006 (Thakur *et al.,* 2007).

A number of germplasm accessions that exhibited strong differential reaction to specific pathotypes could be used as a new set of host differentials. For example, IP18294 had 90% disease incidence against Sg 150, Sg 200, Sg 298, Sg 384 and Sg 445 and showed no disease against pathotypes Sg 139 and Sg 153. Similarly, IP 8289 clearly differentiated Sg 384 (no disease) and Sg 445 (44% incidence).

Latent period is one of the important parameters for host plant resistance as longer latent period delays spore production and secondary spread of the disease, and reduces disease severity. Therefore, germplasm accessions with lower disease incidence and longer latent period would be useful sources of resistance against highly variable populations of *S. graminicola*. Host resistance index, which is a function of latent period and disease incidence, provided useful indication of resistance stability of pearl millet lines across pathotypes, i.e. higher the HRI values, better the resistance stability of a line (Table 6). The clustering of pearl millet accessions based on HRI values clearly indentified resistant, moderately resistant and susceptible groups (Fig. 1), which

**Table 6.** Host resistance index of 21 pearl millet germplasm accessions caused by 11 pathotypes of *Sclerospora graminicola.*

| | Host resistance index[a] | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Sg 021 | Sg 048 | Sg 139 | Sg 150 | Sg 153 | Sg 200 | Sg 212 | Sg 298 | Sg 334 | Sg 384 | Sg 409 | Sg 445 | Mean |
| YG-8 | 0.98 | 1.00 | 1.00 | 0.98 | 1.00 | 0.98 | 0.98 | 0.98 | 0.35 | 0.01 | 0.98 | 0.37 | 0.80 |
| IP 8289 | 0.85 | 0.52 | 1.00 | 1.00 | 0.87 | 1.00 | 1.00 | 0.45 | 0.65 | 1.00 | 1.00 | 0.14 | 0.78 |
| IP 18295 | 0.84 | 0.76 | 0.77 | 1.00 | 0.74 | 1.00 | 0.72 | 0.87 | 0.66 | 0.82 | 0.57 | 0.36 | 0.76 |
| YL-18 | 0.71 | 0.45 | 0.52 | 1.00 | 0.78 | 1.00 | 0.21 | 1.00 | 1.00 | 0.38 | 1.00 | 0.61 | 0.72 |
| YG-2 | 0.77 | 1.00 | 0.77 | 1.00 | 0.56 | 1.00 | 1.00 | 1.00 | 0.64 | 0.13 | 0.23 | 0.25 | 0.71 |
| P 1449-2 | 0.68 | 0.81 | 0.60 | 0.94 | 0.85 | 0.68 | 0.55 | 1.00 | 0.43 | 0.38 | 0.55 | 0.86 | 0.68 |
| IP 22396 | 0.47 | 0.54 | 1.00 | 0.82 | 1.00 | 0.49 | 0.74 | 1.00 | 0.67 | 0.37 | 0.36 | 0.11 | 0.60 |
| IP 18298 | 0.65 | 0.58 | 0.45 | 0.68 | 0.59 | 0.32 | 0.69 | 1.00 | 0.44 | 0.55 | 0.51 | 0.62 | 0.59 |
| YM-16 | 0.36 | 1.00 | 0.53 | 0.52 | 0.64 | 0.98 | 0.47 | 0.63 | 0.19 | 0.16 | 0.73 | 0.08 | 0.52 |
| TG-8 | 0.54 | 0.71 | 1.00 | 1.00 | 0.75 | 0.41 | 0.94 | 0.24 | 0.36 | 0.06 | 0.21 | 0.08 | 0.50 |
| YL-34 | 0.59 | 0.53 | 0.47 | 0.35 | 0.35 | 1.00 | 0.31 | 1.00 | 0.17 | 0.08 | 0.41 | 0.38 | 0.48 |
| YM-19 | 0.51 | 0.60 | 1.00 | 0.09 | 0.36 | 1.00 | 0.22 | 1.00 | 0.09 | 0.16 | 0.34 | 0.08 | 0.46 |
| TL-5 | 0.40 | 0.39 | 0.46 | 0.47 | 1.00 | 0.47 | 0.47 | 0.87 | 0.31 | 0.27 | 0.22 | 0.17 | 0.41 |
| TG-4 | 0.31 | 0.41 | 0.86 | 0.47 | 0.64 | 0.24 | 0.77 | 0.64 | 0.30 | 0.16 | 0.11 | 0.11 | 0.40 |
| P 1591 | 0.19 | 0.54 | 0.55 | 0.60 | 0.89 | 0.20 | 0.61 | 0.11 | 0.21 | 0.28 | 0.27 | 0.14 | 0.34 |
| IP 9997 | 0.33 | 0.86 | 0.19 | 0.18 | 0.49 | 0.60 | 0.21 | 0.31 | 0.12 | 0.06 | 0.29 | 0.26 | 0.31 |
| IP 18294 | 0.53 | 0.26 | 1.00 | 0.08 | 1.00 | 0.06 | 0.83 | 0.06 | 0.32 | 0.05 | 0.20 | 0.05 | 0.31 |
| IP 22393 | 0.18 | 0.30 | 0.30 | 0.37 | 0.34 | 0.20 | 0.47 | 1.00 | 0.11 | 0.11 | 0.21 | 0.05 | 0.29 |
| IP 11428 | 0.11 | 0.17 | 0.13 | 0.35 | 0.43 | 0.58 | 0.25 | 1.00 | 0.12 | 0.04 | 0.22 | 0.05 | 0.28 |
| ZG-3 | 0.14 | 0.34 | 0.22 | 0.59 | 0.32 | 0.06 | 0.51 | 0.17 | 0.09 | 0.10 | 0.24 | 0.08 | 0.23 |
| IP 18293 | 0.23 | 0.32 | 0.38 | 0.13 | 0.42 | 0.17 | 0.17 | 0.06 | 0.33 | 0.05 | 0.11 | 0.05 | 0.18 |
| 7042S | 0.05 | 0.05 | 0.05 | 0.04 | 0.05 | 0.06 | 0.05 | 0.05 | 0.06 | 0.04 | 0.05 | 0.04 | 0.05 |
| Mean | 0.47 | 0.55 | 0.60 | 0.58 | 0.64 | 0.57 | 0.55 | 0.66 | 0.35 | 0.24 | 0.40 | 0.22 | 0.47 |

Mean of 3 replications, based on $[1+(\text{DM incidence\%} \times \text{latent period}^{-1})]^{-1}$: SEM for pathotype means = ±0.01; for genotype means = ±0.02 and fo pathotype x genotype means = ±0.07; LSD ($P$<0.05) for pathotype means = 0.04; genotype means = 0.06 and pathotype × genotype means = 0.19.

will be useful for identifying lines to be used in breeding for resistance. Disease incidence, latent period and HRI were all highly influenced by pathotype, genotype and their interaction, but the individual influence of pathotype or genotype was relatively greater than that of their interaction (Table 3). This is true for an obligate host-pathogen system, where host genotype directs the pathogen evolutionary process and their pathogenicity.

The results of this study indicate the importance of regularly monitoring virulence shift in the pathogen populations and identifying stable resistance for an effective downy mildew resistance breeding in pearl millet. The resistance identified in the past must be re-evaluated against the new populations of the pathogen before using it in the resistance breeding programmes. Although most of the accessions used in this study have lost their resistance to the new virulence(s), some of the accessions have exhibited good levels of resistance to individual and multiple pathotypes and these could be strategically utilized in resistance breeding to effectively manage the disease and enhance productivity of pearl millet.

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## REFERENCES

AICPMIP (All India Coordinated Pearl Millet Improvement Project). 2008. Coordinator's Review 2007-2008.

Bartholomew D.J., Steele F., Moustaki I., Galbraith J.I., 2002. The Analysis and Interpretation of Multivariate Data for Social Scientists. CRC Press, Boca Raton, FL, USA.

Payne R.W., 2002. The Guide to GenStat (R) Release 6.1. Part 2: Statistics. VSN International, Oxford, UK

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

Pushpavathi B., Thakur R.P., Chandrashekara Rao K., Rao V.P., 2006b. Characterization of *Sclerospora graminicola* isolates from pearl millet for virulence and genetic diversity. *Plant Pathology Journal* **22**: 28-35.

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

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

Sharma R., Rao V.P., Varshney R.K., Prasanth V.P., Kannan S., Thakur R.P., 2008. Characterization of pathogenic and molecular diversity in *Sclerospora graminicola*, the causal agent of pearl millet downy mildew. *Archives of Phytopathology and Plant Protection* (in press).

Singh S.D., Gopinath R., Pawar M.N., 1987. Effects of environmental factors in asexual sporulation of *Sclerospora graminicola*. *Indian Phytopathology* **40**: 186-193.

Singh S.D., King S.B., Werder J., 1993. Downy mildew disease of pearl millet. Information Bulletin No. 37. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, A.P. 502 324, India.

Singh S.D., Wilson J.P., Navi S.S., Talukdar B.S., Hess D.E., Reddy K.N., 1997. Screening techniques and sources of resistance to downy mildew and rust in pearl millet. Information Bulletin No. 48. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, A.P. 502 324, India.

Sivaramakrishnan S., Thakur R.P., Kannan S., Rao V.P., 2003. Pathogenic and genetic diversity among Indian isolates of *Sclerospora graminicola* from pearl millet. *Indian Phytopathology* **56**: 392-397.

Thakur R.P., 1999. Pathogen diversity and plant disease management. *Indian Phytopathology* **52**: 1-9.

Thakur R.P., Pushpavati B., Rao V.P., 1998. Virulence characterization of single-zoospore isolates of *Sclerospora graminicola* from pearl millet. *Plant Disease* **82**: 747-751.

Thakur R.P., Rao V.P., 1997. Variation in virulence and aggressiveness among pathotypes of *Sclerospora graminicola* on pearl millet. *Indian Phytopathology* **50**: 41-47.

Thakur R.P., Rao V.P., Amrutesh K.N., Shetty H.S., Datar V.V., 2003. Field surveys of pearl millet downy mildew: effects of hybrids, fungicide and cropping sequence. *Journal of Mycology and Plant Pathology* **33**: 387-394.

Thakur R.P., Rao V.P., Singh S.D., Navi S.S., 1997. Characterization of downy mildew resistance in pearl millet. *Journal of Mycology and Plant Pathology* **27**: 6-16.

Thakur R.P., Rao V.P., Wu B.M., Subbarao K.V., Shetty H.S., Singh G., Lukose C., Panwar M.S., Sereme P., Hess D.E., Gupta S.C., Dattar V.V., Panicker S., Pawar N.B., Bhangale G.T., Panchbhai S.D., 2004. Host resistance stability to downy mildew in pearl millet and pathogenic variability in *Sclerospora graminicola*. *Crop Protection* **23**: 901-908.

Thakur R.P., Shetty K.G., 1993. Variation in pathogenicity among single-oospore isolates of *Sclerospora graminicola*, the causal organism of downy mildew in pearl millet. *Plant Pathology* **42**: 715-721.

Thakur R.P., Shetty K.G., King S.B., 1992. Selection for host-specific virulence in asexual populations of *Sclerospora graminicola*. *Plant Pathology* **41**: 626-632.

Thakur R.P., Shetty H.S., Khairwal I.S., 2006. Pearl millet downy mildew research in India: progress and perspectives. *International Sorghum and Millets Newsletter* **47**: 125-130.

Thakur R.P., Rao V.P. and Sharma R., 2007. Evidence for temporal virulence change in the pearl millet downy mildew pathogen, *Sclerospora graminicola* in India. *Journal of SAT Agricultural Research* **3** (www.icrisat.org/journal/index.htm).

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