

Genetic Resistance in Pearl Millet Seed Parent Lines Against Diverse Pathotypes of *Sclerospora graminicola*

Rajan Sharma, V P Rao, K N Rai and R P Thakur

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru-502 324, Andhra Pradesh, India.

E mail: r.sharma@cgiar.org

Abstract

Genetic resistance to downy mildew (Sclerospora graminicola) in parental lines of pearl millet (Pennisetum glaucum) is critical for successful commercial cultivation of hybrid cultivars. In this study, 23 genetically diverse B-lines were evaluated against two highly virulent as well as old and new pathotypes of S. graminicola from two agro-climatic zones in India. Significant differences were found among genotypes, pathotypes and their interactions for downy mildew incidence. The downy mildew incidence across test B-lines ranged from 0 to 100% and the reaction of the lines to different pathotypes varied considerably. Eight B-lines (ICMB 01777, -02333, -03888, -03999, -05333, -06555, -06888 and -07666) exhibited high levels of resistance across pathotypes. These resistant lines could be used in the development of F₁ hybrids with stable resistance to diverse pathotypes of downy mildew in India.

Keywords: Resistance, B-lines, *Sclerospora graminicola*, pathotypes, pearl millet

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 regions of Africa and Asia, with India having largest area of 10 million ha. Single-cross F₁ hybrids based on cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly in increasing pearl millet productivity in India. Since the release of first series of F₁ hybrids in India during the late 1960s, downy mildew has become a major production constraint for pearl millet (Thakur *et al.*, 2003). During the 1970s and 1980s several downy mildew epidemics occurred in India and a number of hybrids succumbed to this disease resulting in withdrawal of several hybrids from cultivation (Singh *et al.*, 1993).

Sclerospora graminicola, the causal agent of downy mildew reproduces both asexually by producing sporangia and sexually through oospores. Heterothallic nature of the fungus contributes towards the development of new recombinants in the pathogen populations that increase genotypic variability in the populations (Michelmore *et al.*, 1982). As a result of evolution of variants in the pathogen populations, more virulent population of the pathogen gets established and resistant cultivars lose their effective resistance within a short period (Thakur *et al.*, 1992). Circumstantial evidence exists for emergence of new virulent pathotypes specific to an F₁ hybrid when it has been grown in the same field

consecutively for 3-4 years (Thakur *et al.*, 2003).

As part of breeding for downy mildew resistance, breeding lines are screened against different pathotypes of *S. graminicola* in greenhouse under high disease pressure (>85% disease incidence in the susceptible check) and those found resistant (=10% disease incidence) to at least two pathotypes are designated and disseminated as seed parents. Evolution of more virulent populations of *S. graminicola* in the recent past has resulted in the susceptibility of pearl millet accessions hitherto resistant to earlier pathotypes (Thakur *et al.*, 2007). Therefore, monitoring stability of resistance in the breeding lines against more virulent populations is an important component of resistance breeding in pearl millet for the success of hybrids in the farmers' fields. Our objective in this study was to screen selected parental pearl millet lines that were developed and designated at International Crops Research Institute for Semi Arid Tropics (ICRISAT) during 1981-2006 against six diverse pathotypes of *S. graminicola* to identify resistance effective against more virulent populations of recent origin.

Materials and methods

Twenty-three B-lines (maintainers of male-sterile lines) along with two control B-lines, ICMB 99022 (resistant check) and 843B (susceptible check) and a highly susceptible check 7042S were selected for this study (Table 1). The

selected lines were screened against six pathotypes, two highly virulent pathotypes viz., Sg 445 (Banaskantha) and Sg 384 (Barmer); Sg 153 (old) and Sg 409 (new) from Patancheru, Sg 151 (old) and Sg 212 (new) from Durgapura (Table 2).

Pearl millet seeds of these lines were sown in plastic pots (15 cm diameter) filled with autoclaved potting mixture of soil, sand and farmyard manure (3:2:2, by volume). Sporangial inocula (spores of the fungus) of each of the six pathotypes were raised on seedlings of a highly susceptible genotype 7042S in isolation chambers in a greenhouse. Sporangia from sporulating leaves were harvested in ice-cold water and spore concentration was adjusted to 1×10^6 ml⁻¹. Pot-grown seedlings of the pearl millet lines were spray-inoculated at the coleoptile stage using an atomizer. The inoculated seedlings were incubated at 20°C and >90% RH

for 20 h, and then transferred to a greenhouse. The temperature in the greenhouse was maintained at 25±2°C and the RH greater than 95% to facilitate disease development for the next 2 weeks. The experiment was conducted in completely randomized design with three replications, and 35-40 seedlings per replication with time replications. Downy mildew incidence was recorded 14 days after inoculation as percent infected plants (Singh *et al.*, 1993).

Analysis of variance of disease incidence data was done using GENSTAT statistical package (Rothamsted Experiment Station, Harpenden, Herts AL52JQ, UK). In order to classify the designated B-lines into different groups based on their resistance spectrum, the data set was subjected to an average linkage cluster analysis using Euclidian distance as dissimilarity measure (SAS 9.1).

Table 1. Pedigree of pearl millet seed parent lines evaluated for resistance against diverse pathotypes of *Sclerospora graminicola*

B-line	Pedigree
ICMB 01777	(BSECBPT/91-38xSPF3/S91-529)-10-1-6x834B
ICMB 02333	(BSECBPT/91-39xSPF3/S91-116)-15-2-1-2
ICMB 02666	(ICMB89111xIP9554-9)-4-2-2
ICMB 03111	{{[843Bx(843Bx700651)-11-1-2-B] X1163B} x (ICMB89111xICMB88005)}-5-3-B
ICMB 03555	(843BxICTP8202-161-5)-17-1-3-B-2
ICMB 03777	(SPF3/S91-933xSPF3/S91-3)-8-1-1-B
ICMB 03888	[(ICMB88006xICMB88005) x (ICMB89111xICMB88005)]-1-1-3-B-9
ICMB 03999	(ICMB89111xIP9402-2-1-1-2)-31-1-B-B
ICMB 04333	(96111B x 4026-1-6-B)-4-2-1-1
ICMB 04999	(EBC-Gen-S1-40-2-2-1 x B-line bulk)-25-B-B
ICMB 05333	(MC 94 S1-30-2-B x HHVBC)-16-3-1-1
ICMB 06111	(ICMR 312 S1-8-3-3-B x HHVBC)-13-2-1
ICMB 06444	EEBC S1-407-1-B-B-B-1
ICMB 06555	[{{{[843Bx(843Bx700651)-11-1-2-B] x1163B} x (89111Bx88005B)}]-27+x B-line bulk]-3-B-B-10
ICMB 06888	[(BSECBPT/91-40 x SPF3/S91-514)-7-2-1-B x B-lines bulk]-2-B-1-1
ICMB 06999	(HTBC-48-B-1-1-1-5 x B-line bulk)-1-B-B-8
ICMB 07222	(D2BLN95-262 x EEBC C1-3)-12-B-1-B-B-4
ICMB 07333	(ICMB 97444 x (D2BLN95-98 x EEBC C1-1)-7-B-B-34-2-4-B-B
ICMB 07555	[(843B x ICTP 8202-161-5)-20-3-B-B-3 x B-lines bulk]-2-B-1
ICMB 07666	[ARD-288-1-10-1-2 (RM)-3 x B-lines bulk]-14-B-1-1
ICMB 07777	[ICMB 99555 x {78-7088/3/SER3 AD//B282/(3/4) EB x PBLN/S95-359}-19-5-B-B]-13-2-B-B-B-B
ICMB 07888	{HTBLN95-98 x (SPF3/S91-544 x SPF3/S91-5)}-5-1-2]-3-B-B-1-B-1-B
ICMB 07999	HTBC-48-B-1-1-1-5 x B-line bulk]-25-1-B-B
ICMB 99022 – R check	843-22A
843B – S Check	Selection from KSU line BKM 2068
7042S –S Check	Selection from a landrace from Chad

Table 2. Origin of *Sclerospora graminicola* pathotypes used in the study

Identity	Year	Cultivar/line	Collection location	Maintenance host
Sg 153	1997	7042S+NHB3	ICRISAT, Patancheru/Andhra Pradesh	843B
Sg 409	2004	PMB 11571-2	ICRISAT, Patancheru/Andhra Pradesh	PMB 11571-2
Sg 151	1997	81A	Durgapura/ Rajasthan	Nokha Local
Sg 212	1998	PG 5522	Durgapura/ Rajasthan	ICMP 451
Sg 384	2003	Local	Barmer/ Rajasthan	ICMP 451
Sg 445	2005	AHT-503	Banaskantha/ Gujarat	Pioneer 7777

Results and discussion

Significant ($P < 0.001$) differences were found among genotypes, pathotypes and their interactions for downy mildew incidence. The downy mildew incidence across test B-lines ranged from 0 to 100% and the reaction of the genotypes to different pathotypes varied considerably (Table 3). Of the 23 B-lines, ICMB 03888, -3999 and -06555 were resistant (=10% downy mildew incidence) to all the 6 pathotypes, ICMB 02333, -04999, -06888, -07222 and -07666 to 5 pathotypes and ICMB 01777, -05333 and -07333 to 4 pathotypes and the remaining lines showed differential reaction. Mean downy mildew incidence in the test lines across pathotypes ranged from 2% (ICMB 03999) to 64% (ICMB 03777). Seven lines - ICMB 01777, -02333, -03888,

-03999, -05333, -06555 and -06888 recorded =10% mean downy mildew incidence indicating resistance effective against multiple pathotypes.

Mean downy mildew incidence across the lines including checks revealed Barmer (Sg 384) and Banaskantha (Sg 445) to be more virulent with 44% mean downy mildew incidence. In general, new pathotypes of *S. graminicola* from Patancheru and Durgapura were more virulent on the test lines than the old pathotypes. Of the 23 B-lines screened, eight lines (ICMB 02666, -03111, -03555, -03777, -04333, -06111, -06999 and -07888) could differentiate the virulence of Sg 153 and Sg 409 pathotypes from Patancheru as these lines were resistant to Sg 153, but susceptible to Sg 409. Similarly, ICMB 02333, -04333, -06111, -06888, -07333, -07666, -07777 and -07999 (8 lines) were resistant to Sg 151, but susceptible to Sg 212, old and new pathotypes from Durgapura, respectively. The significantly high disease levels in these lines due to Sg 212 indicated temporal virulence change in the pathogen populations.

Three B-lines - ICMB 04999, 07222 and 07333 could clearly differentiate the virulence of two highly virulent pathotypes - Sg 384 (Barmer) and Sg 445 (Banaskantha) that are being used at ICRISAT for screening of breeding lines. ICMB 04999 was resistant to Barmer pathotype (no disease) and susceptible to Banaskantha pathotype (97% disease incidence), whereas, ICMB 07222 and 07333 were resistant (no disease) to Banaskantha and susceptible to Barmer pathotype (96-99% disease incidence) indicating pathotype specific resistance in pearl millet against *S. graminicola*. The pathotype specific resistance has been reported in pearl millet and quantitative trait loci (QTL) for downy mildew resistance effective against specific-pathotypes of *S. graminicola* have been identified (Hash *et al.*, 2001). Differential reaction of some of the B-lines against two highly virulent pathotypes - Sg 384 (Barmer) and Sg 445 (Banaskantha) revealed that different resistance genes/QTLs are effective against these pathotypes.

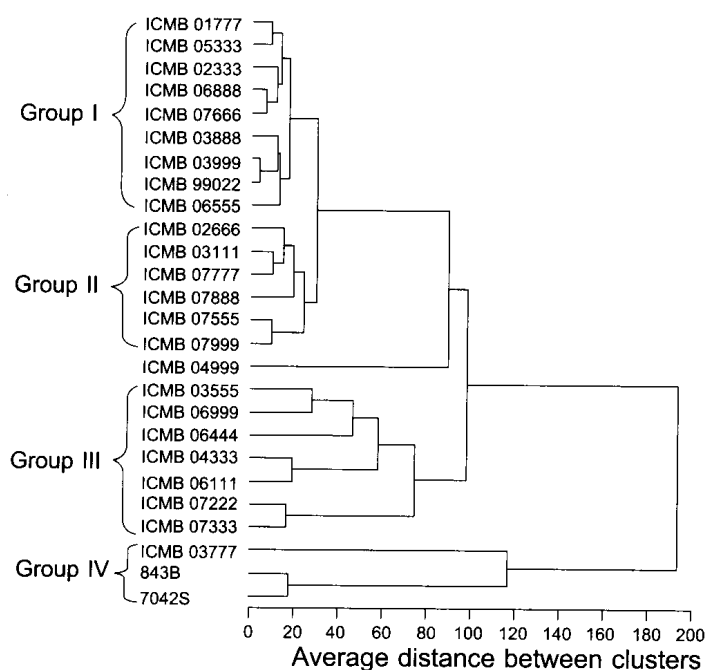


Figure 1. Grouping of pearl millet lines based on their reaction to six *S. graminicola* pathotypes: Group I = resistant, Group II = moderately resistant, Group III = susceptible, Group IV = highly susceptible.

A dendrogram of resistance spectrum against six pathotypes grouped them into four clusters (Figure 1). Group I included 8 B-lines (ICMB 01777, -02333, -03888, -03999, -05333, -06555, -06888 and -07666) and the resistant check ICMB

99022 having high level of resistance across pathotypes. Group II represented 6 lines with moderate resistance across pathotypes. Mean downy mildew incidence in these lines ranged between 11 to 19% against six pathotypes. Group

Table 3. Downy mildew incidence of designated B lines against six pathotypes of *Sclerospora graminicola*

Designation	DM incidence (%) ^a						Mean
	Sg 153	Sg 409	Sg 151	Sg 212	Sg 384	Sg 445	
ICMB 01777	5	6	14	13	7	4	8
ICMB 02333	1	10	6	13	0	1	5
ICMB 02666	9	15	2	6	15	20	11
ICMB 03111	10	15	4	10	23	15	13
ICMB 03555	10	22	18	17	46	43	26
ICMB 03777	10	20	78	81	100	96	64
ICMB 03888	0	1	7	5	0	6	3
ICMB 03999	0	8	1	1	0	0	2
ICMB 04333	10	19	6	13	100	96	41
ICMB 04999	0	7	6	0	0	97	18
ICMB 05333	2	1	13	13	1	4	6
ICMB 06111	1	21	10	15	100	80	38
ICMB 06444	12	10	8	10	100	44	31
ICMB 06555	3	0	5	1	10	1	3
ICMB 06888	5	6	2	12	3	9	6
ICMB 06999	3	13	12	9	69	48	26
ICMB 07222	4	0	0	0	96	0	23
ICMB 07333	3	0	6	15	99	0	37
ICMB 07555	13	21	9	10	2	21	11
ICMB 07666	8	9	5	15	2	9	11
ICMB 07777	10	12	10	16	23	12	15
ICMB 07888	10	23	0	1	27	23	19
ICMB 07999	12	15	5	13	3	26	12
ICMB 99022 - Check	0	6	1	0	2	1	2
843B - Check	83	96	100	100	100	98	96
7042S - Check	100	100	100	99	100	100	100
Mean	18	22	16	20	44	44	27

^aMean of 2 runs, 3 replications/isolate/run

SE (m) for isolate means = ± 0.3 ; for genotype means = ± 0.6 ; for isolate \times genotype means = ± 1.6

LSD ($P < 0.05$) for isolate means = 0.8; for genotype means = 1.8 for isolate \times genotype means = 4.3

III comprised seven lines and was designated as susceptible. The constituent lines of this group were highly susceptible to at least 2 pathotypes. Highly susceptible checks, 7042S and 843B appeared separately and only one designated B-line ICMB 03777 could be clustered with them in the group IV. ICMB 04999 could not be assigned to any group. However, it was close to group I being resistant to 5 of the 6 pathotypes, but susceptible to Banaskantha pathotype.

It is evident from this study that more virulent populations of *S. graminicola* have evolved over time and resistance effective against more virulent new pathotypes of *S. graminicola* is available in some of the B-lines. Since these are designated B-lines, the lines with high level of resistance against multiple pathotypes would be useful in the development F_1 hybrids with stable resistance to diverse pathotypes of downy mildew in India.

References

Hash C T and Witcombe J R 2001. Pearl millet molecular marker research. *International Sorghum and Millets Newsletter* **42** : 8-15.

Michelmore R W, Pawar M N, Williams R J 1982. Heterothallism in *Sclerospora graminicola*. *Phytopathology* **72** : 1368-1372.

Singh S D, King S B and Werder J 1993. Information Bulletin no. 37. International Crops Research Institute for the Semi-Arid Tropics, Patancheru - 502 324, Andhra Pradesh, India. 36 pp.

Thakur R P, Rao V P, Amrutesh K N, Shetty H S and 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 and Sharma R 2007. Evidence for temporal virulence change in the pearl millet downy mildew pathogen, *Sclerospora graminicola* in India. *SAT e-journal* **3** : 1-3.

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

Received : 14-10-08

Accepted : 15-12-08