Evidence for Enhanced Resistance to Diverse Isolates of Pearl Millet Downy Mildew through Gene Pyramiding

CT Hash*, RP Thakur, VP Rao and **AG Bhasker Raj**
[International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India]
*Corresponding author: c.hash@cgiar.org

Introduction

Breeding for downy mildew resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.] at ICRISAT-Patancheru in India, is currently focused on developing hybrid parental lines with resistance to one or more pathogenic variants of *Sclerospora graminicola* (Sacc.) Schroet. that exist in India. Resistance incorporation in inbred lines is done through conventional pedigree and bulk-pedigree selection, pure-line selection within elite inbreds originally developed by bulk-pedigree methods, backcrossing with conventional selection, and backcrossing with marker-assisted selection (Hash et al. 1999). Stability of resistance to downy mildew in pearl millet inbred lines and hybrid cultivars has become elusive in India due to host-directed evolution of pathogenic variation in *S. graminicola* populations (Thakur et al. 1992). Average commercial life spans of popular hybrids have been reduced to only 3–5 years before they must be withdrawn due to pathogen virulence changes (Thakur et al. 2003). There are currently several pathogenic variants of *S. graminicola* prevalent in different parts of pearl millet growing regions of India, and new variants with higher virulence levels keep on appearing with deployment of new cultivars (Thakur et al. 2004; Pushpavathi et al. 2006). A number of quantitative trait loci (QTLs) for host plant resistance effective against one or more pathogen isolates of *S. graminicola* have been identified (Jones et al. 1995, Jones et al. 2002; Hash and Witcombe 2001), and resistance alleles for some of these QTLs have been transferred by marker-assisted backcrossing to elite parental lines of popular hybrids.

In this study, we evaluated 48 pearl millet inbred lines (including pairs of parental lines of mapping populations, elite hybrid parental lines and their pure-line selections, and products of conventional and marker-assisted back-crossing) against nine diverse pathogen isolates of *S. graminicola* identified from five major pearl millet growing states in India. Resistance identified from this study should be useful for utilization in resistance breeding programs in South Asia, and perhaps those in southern and eastern Africa as well.

Materials and Methods

Host genotypes. The 48 pearl millet inbred lines evaluated included

- 7 maintainer parents (B-lines) of established male-sterile lines (A-lines) on which commercial hybrids are being produced in India
- 6 restorer lines (R-lines) of such commercial hybrids
- 8 other elite hybrid parental lines
- 4 downy mildew susceptible lines and 4 resistance donors that were used in developing mapping populations
- 11 selections from within elite hybrid parent lines
- 3 products of conventional and marker-assisted backcrossing programs, and
- 5 downy mildew susceptible or resistant controls.

Pathogen isolates. Nine diverse pathogen isolates of *S. graminicola* from five pearl millet growing states of India were used to evaluate the pearl millet inbred lines. These isolates were: Sg 021 from Ahmednagar and Sg 150 from Jalna (Maharashtra state); Sg 139 from Jodhpur, Sg 212 from Durgapura, and Sg 384 from Barmer (Rajasthan state); Sg 200 from Jamnagar and Sg 445 from Banaskantha (Gujarat state); Sg 298 from New Delhi (Delhi state); and Sg 409 from Patancheru (Andhra Pradesh state).

Downy mildew screening. In a greenhouse experiment, pot-grown seedlings of each of the 48 pearl millet lines were spray-inoculated with sporangial suspensions (1×10^5 sporangia mL^{-1}) of each pathogen isolate following the standard inoculation method (Singh et al. 1997; Jones et al. 2001). The experiment was conducted in a randomized complete block design with 48 lines × 9 isolates × 3 time replications with one pot per replication and 30–45 seedlings per pot.

Data recording and analysis. Seedling counts per pot were recorded at the time of inoculation. Downy mildew incidence data were recorded two weeks after inoculation and percentage disease incidence was calculated. Disease incidence data were analyzed using the residual maximum likelihood (ReML) program of the GenStat statistical software package.

Results and Discussion

Downy mildew resistance in pearl millet inbreds. With a few exceptions, the screening results for each of the host pearl millet lines were consistent across the three time replications used for each pathogen isolate. ReML analysis of the combined data set indicated highly significant differences between the 48 host genotypes across the 9 pathogen isolates, marginally significant differences between the 9 pathogen isolates across the 48 host genotypes, and highly significant host × isolate interactions (Table 1). The predicted grand mean disease incidence across the 432 host × isolate combinations was 48.3% (SE = 5.8%). The standard errors of the differences among inbreds across an isolate and among isolates across inbreds, were between 6 and 8 for downy mildew incidence (%), so a pair-wise difference of 25% in either direction (among inbreds screened against a common pathogen isolate or among isolates against screened against a common inbred) was highly significant ($P<0.01$).

In order to identify host genotypes highly resistant and highly susceptible to individual pathogen isolates, and strong differential host × isolate reactions, downy mildew incidence values of <5.0% were rated as highly resistant and those >80.0% as highly susceptible. Of the 48 inbred lines evaluated, only one (ICMB 99022) was highly resistant to eight of the nine pathogen isolates used in this study (Table 2). Four inbreds (including elite restorer line RIB 335/74 and mapping population parental lines 863B-P2, ICMB 89111-P2 and ICMB 89111-P5) were highly resistant to seven of the nine isolates, three inbreds (mapping population parents ICMB 90111-P2 and IPC 804-P6 and 81B) were highly susceptible to seven of the nine isolates, one [susceptible control 7042 (S)] was highly susceptible to eight of the nine isolates and four (mapping population parental lines ICMP 85410-P7, LGD 1-B-10, Tift 23D2B1-P1-P5 and Tift 238D1-P158) were highly susceptible to all of the nine isolates, while 17 inbred lines recorded differential reactions across these nine isolates (Table 2).

Effect of resistance gene pyramiding/stacking. Comparison of 843B (>90% incidence against six pathogen isolates Sg 021, Sg 139, Sg 200, Sg 212, Sg 298 and Sg 384), to its conventional backcross derivative ICMB 99022 (0–1% incidence to these six isolates), and the resistance donor ICML 22 (4–16% incidence across these isolates) indicated the effectiveness of pyramiding resistances from ICML 22 (Table 2). This was even clearer in case of the isolates Sg 409 and Sg 445, where neither the resistance donor ICML 22 nor the recurrent parent 843B were as resistant as their product line ICMB 99022 having pyramided resistances from both parents. The pyramided resistance genes from ICML 22 and 843B that are present in ICMB 99022 proved effective against eight of the nine pathogen isolates used in this study (Table 2). ICMB 99022, which was bred by conventional backcrossing, can be recommended as a replacement for its susceptible but commercially successful recurrent parent 843B in hybrid breeding programs targeting much of India.

Similarly, the comparison of H 77/833-2, its two marker-assisted backcross derivatives ICMR 01004 and ICMR 01007, and their common resistance donor ICMP 451-P6, also exhibited the impact of pyramiding resistances from H 77/833-2 and ICMP 451-P6. The product line ICMR 01004 was consistently as resistant or more resistant (to Sg 212 and Sg 384), than its more resistant parent. In contrast, ICMR 01007 (which appears to have one less resistance QTL introgressed from ICMP 451-P6), was as susceptible as its more susceptible parent for three of these nine isolates.

Table 1. Estimated variance components from residual maximum likelihood analysis (ReML) of disease incidence among 48 pearl millet inbred lines screened against 9 diverse pathogen isolates of *Sclerospora graminicola* in a greenhouse at ICRISAT-Patancheru, India.

Source	df	Variance (standard error)
Replication within isolate	2	4.18 (4.31)
Host genotype	47	880.25 (198.57)
Pathogen isolate	8	105.68 (60.53)
Genotype × Isolate	376	718.12 (53.78)
Error	855(7)	56.98 (2.76)

Table 2. Best linear unbiased predictions of downy mildew incidence (%) for 48 pearl millet inbreds evaluated by greenhouse screening at ICRISAT-Patancheru against 9 diverse pathogen isolates of *Sclerospora graminicola* from India.

Inbred	Pathogen isolate[1]									Comments[2]
	Sg 021	Sg 139	Sg 150	Sg 200	Sg 212	Sg 298	Sg 384	Sg 409	Sg 445	
Mapping population parental line pairs										
ICMP 85410-P7	98	98	99	86	98	81	97	100	100	HS to all
LGD 1-B-10	100	100	97	100	100	93	100	100	100	HS to all
Tift 23D2B1-P1-P5	100	100	98	100	100	99	100	100	100	HS to all
WSIL-P8	3	26	3	2	1	6	18	96	99	D
81B-P6	97	93	24	79	64	19	95	100	100	Mostly HS
ICMP 451-P8	35	90	12	37	58	1	90	95	48	D
ICMP 451-P6	43	35	88	93	97	3	76	54	46	D
H 77/833-2-P5(NT)	42	88	12	66	82	58	88	72	94	Mostly S
H 77/833-2	96	100	2	98	97	62	97	97	93	D
PRLT 2/89-33	6	47	96	1	13	3	10	19	74	D
W 504-1-P1	5	2	85	4	5	92	41	97	99	D
P310-17-Bk	0	2	9	4	0	2	9	37	20	Mostly R
IP 18293-P152	1	95	93	1	3	71	6	40	62	D
Tift 238D1-P158	100	100	100	100	100	94	100	100	100	HS to all
PT 732B-P2	27	6	86	4	6	95	9	99	99	D
P1449-2-P1	2	4	3	3	0	10	1	66	73	Mostly R
841B-P3	30	79	96	12	5	49	17	8	18	
863B-P2	0	6	2	0	2	0	11	2	1	Mostly HR
ICMB 89111-P2	0	3	0	0	5	3	4	9	23	Mostly HR
ICMB 90111-P2	89	88	33	97	93	61	99	86	84	Mostly HS
ICMB 89111-P5	1	7	1	0	0	3	2	7	4	Mostly HR
ICMB 90111-P5	18	9	5	3	9	30	54	7	7	
ICMB 89111-P6	98	98	32	88	97	75	94	9	45	Mostly S
ICMB 90111-P6	18	29	9	5	0	2	14	2	2	Mostly R
81B-P8	33	98	22	66	80	78	99	98	100	S
IPC 804-P6	94	96	6	93	95	51	100	89	96	Mostly HS
Elite R-lines										
H 77/833-2	96	96	5	99	98	72	96	99	98	Duplicate
ICMP 451	77	97	5	97	98	81	94	100	100	D
ICMR 01004	57	17	11	46	22	6	44	56	49	
ICMR 01007	66	89	11	22	94	11	85	74	70	
PPMI 301	11	15	7	5	20	9	17	99	99	
RIB 335/74	3	4	8	5	7	3	3	3	3	Mostly HR
RIB 3135-18	6	4	41	1	2	55	1	84	82	D
Elite B-lines										
81B	80	97	86	63	70	92	98	97	100	Mostly HS
843B	97	99	32	93	98	93	91	34	53	Mostly HS
ICMB 88004	2	1	3	0	0	3	46	43	71	Mostly HR
ICMB 89111	3	0	12	2	0	19	2	9	7	R
ICMB 90111	68	72	3	90	87	51	90	84	84	D
ICMB 92666	82	89	32	24	92	2	3	98	82	D
ICMB 95333	92	85	22	38	95	62	92	100	98	Mostly HS
ICMB 99022	1	0	26	0	0	0	1	1	1	Mostly HR

continued

Table 2. (*continued*)

Inbred	Pathogen isolate[1]									Comments[2]
	Sg 021	Sg 139	Sg 150	Sg 200	Sg 212	Sg 298	Sg 384	Sg 409	Sg 445	
Forage pollinators										
Tift 186	6	6	96	5	26	27	1	97	44	D
Tift 383	29	4	93	3	1	37	50	99	74	D
Controls										
700651 (Resistant)	19	3	92	6	35	15	4	38	42	D
7042 (S) (Susceptible)	98	98	–[3]	99	100	94	95	99	100	HS to all
ICML 22 (7042 DMR)	13	16	2	13	7	4	4	92	52	D
P7-3 (Resistant)	4	1	0	0	6	0	1	98	98	D
P7-4 (Resistant)	11	7	1	22	52	17	63	64	44	
Isolate mean	43	50	36	39	46	39	50	66	65	

1. Isolates: Sg 021 (Ahmadnagar, Maharashtra); Sg 139 (CAZRI-Jodhpur, Rajasthan); Sg 150 (Jalna, Maharashtra); Sg 200 (JAU MRS, Jamnagar, Gujarat); Sg 212 (RAU ARS Durgapura, Jaipur, Rajasthan); Sg 298 (IARI, New Delhi); Sg 384 (Barmer, Rajasthan); Sg 409 (ICRISAT, Patancheru, Andhra Pradesh); Sg 445 (Banaskantha, Gujarat).
2. HS = Highly susceptible; S = Susceptible; R = Resistant; HR = Highly resistant; D = Differential (HS vs HR) responses across isolates.
3. – = Missing.

Standard errors of differences for same level of entry: mean = 6.1, maximum = 6.8, minimum = 6.1; standard errors of differences for same level of isolate: mean = 6.1, maximum = 7.4, minimum = 6.1.

Differential reactions of pearl millet lines to pathogen isolates. Among the 48 inbred lines evaluated, ICMB 99022 was highly resistant to all of the nine isolates except Sg 150 (Jalna) (Table 2). Similarly, four lines were highly resistant to seven isolates, three lines were highly resistant to six isolates, two lines were highly resistant to five isolates, and three lines were highly resistant to four isolates. Four lines were highly resistant to three isolates. Three lines were highly resistant to two isolates, and ten lines were highly resistant to just one of the nine isolates.

Several host × isolate interaction results stand out including

- the differences in reaction of sister-line resistant controls P7-4 and P7-3
- the differences between single-plant selections of 81B, ICMP 451, ICMB 89111 and ICMB 90111 that were used as mapping population parents and their respective bulks
- strong differential responses of WSIL-P8 and P7-3 that are susceptible to Sg 409 and Sg 445
- weaker differentially susceptible responses of P310-17-Bk, P1449-2-P1, RIB 3135-18, ICMB 88004 and ICML 22 to Sg 409 and Sg 445
- differences in disease reactions between tall forage pollinator Tift 186 and its d_2 dwarf derivative Tift 383.

We identified 17 lines that provided strongly differential reactions to the nine pathogen isolates (Table 3). Eight of these lines have been used as parents of pearl millet mapping population progeny sets used to map host-plant resistance to *S. graminicola* (Hash and Witcombe 2001). As the genetic basis of resistance of these lines has been partially characterized, some of them can be utilized as host differentials, in addition to the existing ones, to study pathogenic variation in *S. graminicola*.

Downy mildew resistance donor parent ICML 22, which was used in breeding ICMB 99022, has been susceptible to downy mildew in the 'sick plot' at RAU ARS, Durgapura for several years. During the 2006 rainy season, ICMB 99022 exhibited severe susceptibility to downy mildew in the breeding nursery at that location. Pyramiding of additional downy mildew resistances in this genetic background will be necessary to provide resistance that both stable and durable.

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Table 3. Differential downy mildew reactions[1] of 17 pearl millet inbred lines to nine diverse pathogenic isolates of *Sclerospora graminicola* of Indian origin in a greenhouse experiment, ICRISAT-Patancheru, India, Apr–Jun 2006.

	Isolate								
Inbred	Sg 021 Ahn	Sg 139 Jdp	Sg 150 Jln	Sg 200 Jmn	Sg 212 Dup	Sg 298 Ndl	Sg 384 Bmr	Sg 409 Pat	Sg 445 Bnk
700651	–	R	S	–	–	–	R	–	–
H 77/833-2	S	S	R	S	S	–	S	S	S
ICMB 90111	–	–	R	S	S	–	S	S	S
ICMB 92666	S	S	–	–	S	R	R	S	S
ICML 22	–	–	R	–	-	R	R	S	–
ICMP 451	–	S	R	S	S	S	S	S	S
ICMP 451-P6	–	–	S	–	S	R	–	–	–
ICMP 451-P8	–	S	–	–	–	R	S	S	–
IP 18293-P152	R	S	S	R	R	-	–	–	–
P7-3	R	R	R	R	-	R	R	S	S
PRLT 2/89-33	–	–	S	R	-	R	–	–	–
PT 732B-P2	–	–	S	R	-	S	–	S	S
RIB 3135-18	–	R	–	R	R	–	R	S	S
Tift 186	–	–	S	–	–	–	R	S	–
Tift 383	–	R	S	R	R	–	–	S	–
W 504-1-P1	R	R	S	R	–	S	–	S	S
WSIL-P8	R	–	R	R	R	–	–	S	S

1. R = Highly resistant, <5.0% downy mildew (DM) incidence; S = Highly susceptible, >80.0% DM incidence; – = Intermediate reaction.

resistance in rice and pearl millet.' The views expressed are not necessarily those of DFID or USAID. The contributions of A Ganapathi, P Om Prakash, PJ Mohan Rao and R Narasinga Rao for producing the seed samples and conducting the disease screens are gratefully acknowledged.

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