Screening techniques and resistance sources for foliar blast in pearl millet

RP Thakur*, Rajan Sharma, KN Rai, SK Gupta and **VP Rao**

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

*Corresponding author: r.thakur@cgiar.org

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Abstract

Once considered a minor disease of pearl millet, incidence of blast disease caused by *Pyricularia grisea*, has increased at an alarming rate in the recent past, particularly on commercial hybrids in several states of India. The disease can be best managed through host plant resistance. In this study, attempts were made to develop the field and greenhouse screening techniques and screen some elite hybrid parental lines to identify resistance to this disease. The field screening technique involved the use of a highly susceptible line as an infector row grown after every four test rows, artificial spray inoculation of 30-day-old plants using *P. grisea* spore suspension (1×10^5 spores ml^{-1}) and maintaining high humidity (>90% RH) through perfo-irrigation for 2 weeks following inoculation. The greenhouse screening technique involved spray inoculation of 15-day-old potted seedlings with *P. grisea* spore suspension and maintaining moderate temperature (25±1°C) and high humidity through a misting system for 10 days after inoculation. In all, 211 elite hybrid parental lines, including 126 designated B-lines, 20 designated R-lines and 65 potential R-lines were evaluated for blast resistance in the disease nursery. Forty-five lines identified as blast resistant (score ≤3.0 on 1–9 scale) were further screened through greenhouse screening technique. Twenty-five (8 designated B-lines, 3 designated R-lines and 14 potential R-lines) of the 45 lines were found resistant to blast under greenhouse. All of these 25 foliar blast resistant lines were also resistant to downy mildew under field conditions. These resistant lines would be useful in breeding blast resistant pearl millet hybrids.

Introduction

Pearl millet (*Pennisetum glaucum*) 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). The crop is grown as a nutrient-rich food source for humans as well as a forage/fodder crop for livestock. During the past three decades, single-cross F_1 hybrids based on cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly in increasing pearl millet productivity in India. Among several diseases that affect pearl millet, downy mildew caused by *Sclerosopora graminicola* has already been a major problem of pearl millet hybrids. Now blast, also known as leaf spot caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) has emerged as another serious disease affecting both forage and grain production in pearl millet. The disease appears as grayish, water-soaked foliar lesions that enlarge and become necrotic, resulting in extensive chlorosis and premature drying of young leaves (Wilson et al. 1989b). This disease becomes more severe during humid weather conditions especially with dense plant stands. Leaf blast on pearl millet has been found to be negatively correlated with forage yield, dry matter yield and digestive dry matter (Wilson and Gates 1993) thus affecting the productivity and quality of the crop. In India, the disease was first reported from Kanpur, Uttar Pradesh (Mehta et al. 1953). Although blast was considered a minor disease of pearl millet in India, the disease incidence has increased alarmingly during the recent years (Lukose et al. 2007, AICPMIP 2009). Use of host plant resistance is the most feasible and economical means of managing this disease as the crop is mainly cultivated by resource-poor farmers. Information available on screening techniques and sources of resistance to this disease is very limited. The present investigation therefore was undertaken to develop field and greenhouse screening techniques and evaluate elite pearl millet breeding lines to identify resistance to blast.

Materials and methods

The culture of *Pyricularia grisea* was obtained from the diseased sample collected from the pearl millet fields at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The pathogen was purified through single-spore isolation and

maintained on potato dextrose-agar medium for use. Mass multiplication of fungal spores for inoculation was achieved by growing the fungus on autoclaved pearl millet leaves at 28°C for 15 days.

In all, 211 advanced breeding lines, including 126 designated B-lines, 20 designated R-lines and 65 potential R-lines were evaluated for blast resistance in the disease nursery during rainy season in 2008 and selected lines were screened in the greenhouse to confirm their field resistance.

Field screening. The experiment was conducted in a randomized complete block design with two replications, one row of 2 m length/entry in each replication. Systematic susceptible checks (ICMB 95444, ICMB 99666 and ICMB 89111) were planted on every 5th row alternately. Plants were thinned to 20 plants/row 15 days after planting and other agronomic practices were followed as per local practices. Plants were spray-inoculated at pre-tillering and flowering stage with an aqueous conidial suspension (about 1×10^5 spores ml^{-1}) of *P. grisea*. High humidity was provided by perfo-irrigation twice a day on rain-free days, 30 min each to promote disease development. Disease severity was recorded at the hard-dough stage using a 1–9 progressive scale developed at International Rice Research Institute (IRRI), Philippines for rice blast (1 = no lesion to small brown specks of pinhead size; 2 = larger brown specks; 3 = small, roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter with a brown margin; 4 = typical blast lesions, elliptical, 1–2 cm long, usually confined to the area between main veins, covering <2% of the leaf area; 5 = typical blast lesions covering <10% of the leaf area; 6 = typical blast lesions covering 10–25% of the leaf area; 7 = typical blast lesions covering 26–50% of the leaf area; 8 = typical blast lesions covering 51–75% of the leaf area and many leaves dead; 9 = all leaves dead).

The experiment was conducted in the downy mildew sick plot and thus downy mildew incidence was also recorded in the test lines at the soft-dough stage as percent infected plants (Singh et al. 1993).

Greenhouse screening. Pearl millet lines found resistant to blast under field screen were further evaluated for blast resistance under greenhouse conditions. Seed of test lines along with susceptible checks (ICMB 95444 and ICMB 89111) was planted in 15-cm diameter pots (10 seeds/pot) filled with sterilized soil-sand-FYM (farmyard manure) mix (2:1:1) and placed in a greenhouse bay maintained at 30±1°C. The pot-grown seedlings (15 days old) were spray-inoculated with an aqueous conidial suspension (about 1×10^5 spores ml^{-1}) of *P. grisea* and exposed to high humidity (>90% RH) under misting for 10 days. Blast severity was recorded 15 days after inoculation using a 1–9 scale.

Results and discussion

Field resistance to blast. In the 126 designated B-lines, blast scores ranged from 2.0 to 9.0 on a 1–9 scale compared to a score of 7.0 to 9.0 in the susceptible checks. Nine lines (ICMB 93222, -97222, -01333, -01777, -02111, -02444, -02777, -03444 and -03999) were found resistant (score 2.0–3.0), 55 moderately resistant (score 3.1–5.0), 34 susceptible (score 5.1–7.0) and the remaining 28 highly susceptible (score >7.0) (Table 1). The above nine blast resistant lines were also resistant to downy mildew incidence (≤10%) in the downy mildew sick field. In the 20 designated R-lines, blast scores varied from 1.0 to 6.5. One line (ICMR 06222) was highly resistant, five (ICMR 06111, -06444, -06666, -07555 and -356) resistant, 13 moderately resistant, and one susceptible to blast. Six lines that were resistant to blast were also resistant to downy mildew. Of the 65 potential R-lines, two were highly resistant, 28 resistant, 23 moderately resistant, nine susceptible and remaining three were highly susceptible to blast. Most of these lines (57) were resistant to downy mildew as well. Resistance of these lines to downy mildew is not unexpected as these were earlier selected for resistance to this disease.

Table 1. Performance of advanced hybrid parental lines of pearl millet for resistance to leaf blast under field conditions during the rainy season 2008 at ICRISAT, Patancheru, India.

		No. of lines with blast severity (1–9 scale)[1]				
Material	Total lines	1.0 (HR)	2.0–3.0 (R)	3.1–5.0 (MR)	5.1–7.0 (S)	7.1–9.0 (HS)
Designated B-lines	126	0	9	55	34	28
Designated R-lines	20	1	5	13	1	0
Potential R-lines	65	2	28	23	9	3
Total	211	3	42	91	44	31

1. Mean of two replications; 20 plants/replication.
HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

Confirmation of field blast resistance. The lines found resistant to blast (9 designated B-lines, 6 designated R-lines and 30 potential R-lines) in the field screen were further evaluated under greenhouse condition to confirm their resistance. The blast severity scores (on a 1–9 scale) in the 9 designated B-lines ranged from 1.4 to 6.2, in 6 designated R-lines from 1.0 to 6.2 and in 30 potential R-lines from 1.0 to 7.4 compared to 7.0 and 8.0 score on susceptible checks ICMB 89111 and ICMB 95444, respectively (Table 2). All the designated B-lines (except ICMB 03444), three designated R-lines (ICMR 06222, -06444 and -07555) and 14 potential R-lines were

Table 2. Selected B- and R-lines of pearl millet with resistance to blast and downy mildew.

Identity	Pedigree	Blast severity[1]		Downy mildew incidence (%)[2]
		Field	Greenhouse	
ICMB 01333	HHV-S1-64-3-2-3-2-1	2.0	1.8	6
ICMB 01777	(BSECBPT/91-38 × SPF3/S91-529)-10-1-6	2.0	1.5	7
ICMB 02111	{(ICMB 89111 × ICMB 88004) × (ICMB 88006 × ICMB 88005)-2-1-1-4}-101-B-3	2.0	2.2	0
ICMB 02444	(BSECBPT/91-38 × SPF3/S91-529)-2-1-B-2	2.0	1.6	0
ICMB 02777	HHVBC-II HS-9-1-1-2-7-1	2.0	2.0	5
ICMB 03444	HHVBC-II D2 HS-456-1-2-5-1	3.0	6.2	10
ICMB 03999	(ICMB 89111 × IP 9402-2-1-1-2)-31-1-B-B	2.0	2.0	2
ICMB 93222	(26B × 834B)-11-2-B-B	2.0	1.8	3
ICMB 97222	{(ICMB 88006 × ICMB 88005) × (ICMB 89111 × ICMB 88004)}-28-2-B	2.0	1.4	9
ICMR 06111	MC 94 C2-S1-3-1-3-3-2-2-B	3.0	6.2	4
ICMR 06222	SDMV 90031-S1-3-3-2-1-3-2-2-1-B	1.0	1.0	0
ICMR 06444	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-B	2.0	2.1	0
ICMR 06666	MRC HS-219-2-1-2-B-B-B-B-B	3.0	5.6	0
ICMR 07555	ICMS 8511 S1-17-2-1-1-4-1-B-3-2-2-B	2.0	2.2	0
ICMR 356	Yet to be provided	3.0	5.1	4
K-08-18343	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-1	3.0	4.9	0
K-08-18344	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-3-1	3.0	5.3	0
K-08-18345	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-3-1	3.0	4.7	13
K-08-18346	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-4-3-1-1	3.0	4.4	0
K-08-18347	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-4-3-2-1	3.0	4.2	10
K-08-18348	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-5-4-1-2	3.0	4.0	6
K-08-18349	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 × HHVBC)-1-3-3))-B-10-1-1-5-4-2-2	3.0	4.2	0
K-08-18350	(RCB-2-S1-138-1-1 × MRC)-B-1-1-2-B	3.0	6.0	0
K-08-18353	[(((ICMV-IS 94206-15) × -B-lines)-B-6) × (MRC S1-156-2-1-B)]-B-13-1-3-3-2-B	3.0	6.1	23
K-08-18354	[(((IP 12322-1-2) × B-14)-B-6) × (MRC S1-156-2-1-B)]-B-3-3-B-B	2.0	2.9	0
K-08-18356	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-1-1-2-3-2-B	2.0	2.6	4
K-08-18357	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-18-2-2-4-1-B	2.0	1.9	0
K-08-18358	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-28-2-2-3-1-2	2.0	2.8	0
K-08-18359	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-34-1-1-2-2	2.0	2.3	0
K-08-18360	[((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-2	2.0	2.1	5
K-08-18369	ICMR 312 S1-3-2-1-2-4	2.0	4.3	0
K-08-18372	ICMS 7704 S1-52-3-1-2-1-2-1-3-B-1	2.0	1.9	0
K-08-18375	ICMS 7704 S1-80-2-1-1-2-2-1-B-B-B-B	2.0	4.2	0
K-08-18382	JBV 3 S1-22-1-2-3	2.0	2.0	0
K-08-18383	JBV 3 S1-2-3-2-2-B-1	2.0	1.6	0
K-08-18384	JBV 3 S1-2-3-2-2-B-3	2.0	2.4	0
K-08-18390	JBV 3 S1-35-2-1-2-B	3.0	6.9	0
K-08-18391	MC 94 C2-S1-3-1-1-1-2-4-B-B-B-B	3.0	5.7	0
K-08-18392	MC 94 C2-S1-3-1-1-1-2-1-B-B-B-B-B	3.0	7.4	0
K-08-18393	MC 94 C2-S1-3-1-1-1-4-2-2-B-B-1	3.0	5.9	0
K-08-18394	MC 94 C2-S1-3-1-1-1-4-B-B-1-B-B	2.0	5.6	0
K-08-18395	MRC HS-130-2-2-1-B-B-1-B-B-B	2.0	1.3	0
K-08-18400	MRC S1-191-2-1-5-B-B-B-B-B	1.0	1.0	4
K-08-18402	RCB-2 S1-19-2-2-1-2-3-2-1-B-B-B	2.0	2.0	10
K-08-18404	SDMV 90031-S1-3-3-2-1-3-2-2-3-B	1.0	1.2	0
Checks				
ICMB 89111	{843B × (GNS × SS-48-40-4)-1-9-8}-30-B-B-1	8.0	7.0	29
ICMB 95444	(81-1164 DB/85-1856LR-16-B × 843DMR1)-14-6-3	9.0	8.0	0
ICMB 99666	(BSECBPT/91-38 × SPF3/S91-529)-10-1-5-2	7.0	–	0
Mean		2.7	3.6	3.2
SE(m)±		0.79	0.44	1.5
LSD (P <0.05)		2.21	1.24	2.8

1. Mean of two replications using 1–9 scale, where 1 = no infection and 9 = >75% leaf area with lesions.
2. Mean of two replications under field conditions.

resistant to blast (<3.0 score) confirming their field resistance to *P. grisea*. All the lines found resistant to blast in greenhouse screen also had field resistance (≤10% incidence) to downy mildew (Table 2). The inoculated plants were observed till the dough stage for neck and/or head blast. However, no neck/head blast was seen in this experiment (neck blast was seen in few pearl millet fields in Gujarat during the rainy season 2008 – YS Verma, Metahelix Life Sciences Pvt Ltd, Ahmedabad, personal communication). It would be useful to confirm the observation on the occurrence of neck blast and study the host-pathogen interactions leading to foliar and neck blast.

In this study, we developed the field and greenhouse screening techniques for pearl millet leaf blast whereby pearl millet breeding material can be effectively screened in the field and resistance confirmed through greenhouse screen. Field screening has been reported (Wilson and Hanna 1992) to identify blast resistant pearl millet lines under natural infection, and no artificial inoculation was made. Singh and Pavgi (1974) screened pearl millet varieties for blast resistance in field following artificial inoculation under natural conditions. Screening under natural infection condition may provide escapes and the true resistance may not be identified. However, in this study, both field and greenhouse screening methods involved artificial inoculation of plants at appropriate stages of the plant and favorable conditions (temperature and RH) were provided for disease development that greatly minimized any chances of escape from infection. Wilson et al. (1989a) screened pearl millet landraces under greenhouse conditions using 0–4 infection-type scale. However, we have followed a more precise rating scale of 1–9 developed at IRRI for rice blast screening to categorize pearl millet lines into different reaction types such as highly resistant, resistant, moderately resistant, susceptible and highly susceptible.

Sources of blast resistance have been reported in pearl millet, and efforts have been made to incorporate resistance into improved cultivars and elite breeding lines (Hanna et al. 1988, Singh et al. 1997). Singh and Pavgi (1974) observed blast resistance in some of the local pearl millet varieties; however, all the improved hybrids tested for blast resistance were found susceptible to the pathogen. In this study, resistance was identified in advanced breeding lines that were not intentionally bred for blast resistance indicating the natural occurrence of resistance in pearl millet. Resistance to blast disease in pearl millet sub-species *P. glaucum* ssp *monodii* was characterized as three independent, dominant genes (Hanna and Wells 1989), although Tift 85DB with resistance derived from *monodii*, was shown to have a single resistance gene (Wilson et al. 1989b). Race pathogenicity has been reported in rice blast system; however, no host-pathogen race interaction has been reported for pearl millet and *P. grisea* as yet. It would therefore be useful to identify diverse sources of blast resistance in pearl millet in anticipation of race interactions to prevent disease outbreaks in future. It would also be desirable to test the blast resistance stability of these lines through multilocational testing in India and elsewhere.

The pathogen is highly variable, with many strains that are specialized in their host range and thus strains from rice (*Oryza sativa*) cannot infect pearl millet and vice versa. However, unlike the pathogens of rust and downy mildew, this pathogen does not pass through a sexual stage in order to survive from season to season, implying there is less chance of developing new genetic recombinants. Thus, breeding for durable resistance to blast in pearl millet might be easier than that to rust or downy mildew.

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 dual-resistance for downy mildew and blast identified in the advanced hybrid parental lines would, therefore, be useful in developing disease resistant pearl millet hybrid parental lines and hybrids in India.

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