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Evaluation of designated hybrid seed parents of pearl millet for blast resistance

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Abstract

One hundred sixty designated B-lines (maintainers of male sterile lines) of pearl millet were screened for blast resistance under greenhouse conditions along with a resistant (ICMR 06444) and a susceptible (ICMB 95444) check against five pathotype-isolates (Pg 45, Pg 53, Pg 56, Pg 118 and Pg 119) of Magnaporthe grisea. *Twenty three lines exhibited seedling stage resistance to 3-5 pathotypes. Of the 23 lines, nine (81B, ICMB 88004, ICMB 92444, ICMB 97222-P1, ICMB 02111, ICMB 06444, ICMB 07111, ICMB 09333 and ICMB 09999) were found resistant to all the five pathotypes. The identified blast resistant lines are agronomically superior breeding lines being hybrid parents designated at ICRISAT. Thus, these lines could be either used in the crossing programs to develop blast resistant hybrid parents or as one of the parents for the development of blast resistant hybrids to diversify the genetic base of blast resistance in future pearl millet hybrids.*

Keywords: Pearl millet, *Magnaporthe grisea,* designated B-lines, pathotype-isolates

Introduction

Pearl millet, [*Pennisetum glaucum* (L.) R. Br.], is an important cereal crop, grown mostly in the arid and semi-arid regions of Africa and Asia. It is primarily cultivated for grain, but is also a valuable source of fodder (both stover and green forage). In India about 60% (Sharma *et al.,* 2012) or more of this crop is sown with genetically uniform single-cross hybrids that are particularly vulnerable to downy mildew disease caused by *Sclerospora graminicola* (Sacc.) J. Schrot. During recent years, blast, also known as leaf spot caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Herbert) Barr] has emerged as another serious disease in major pearl millet growing areas in India. The disease was first reported from Kanpur, Uttar Pradesh (Mehta *et al.*, 1953) and remained as a minor disease for a long time; however, in the last 8-10 years it has become a serious threat to pearl millet grain and fodder production.

Leaf blast on pearl millet has been found to be negatively correlated with green-plot yield, dry matter yield and digestive dry matter (Wilson and Gates, 1993) thus affecting the productivity and quality of the crop (Thakur *et al*., 2011). In case of a susceptible cultivar, the entire foliage gives a burnt appearance. The pathogen has a potency to cause disease in all stages of crop growth starting from seedling to grain formation; thus can cause severe crop losses. *M. grisea* is a heterothallic, filamentous fungus, pathogenic to almost 50 plant species in 30 genera of Poaceae including economically important crops like rice, wheat, barley and millets (Ou, 1985). Most of the resistance genes in rice break down in a few years because of their race specificity and the rapid change in pathogenicity of the blast fungus (Suh *et al.,* 2009). Pathogenic variation in *M. grisea* populations adapted to pearl millet has been studied and five pathotypes of *M. grisea* infecting pearl millet have been reported in India (Sharma *et al.*, 2013).

ICRISAT has a major research focus on development of parental lines, especially diversifying the genetic base of male sterile lines, which are designated and disseminated to public organizations and private seed companies for use in developing F_1 hybrid cultivars. Although breeding for blast resistance is still in-its-infancy, the elite designated breeding lines can be screened for blast resistance and the resistant lines can be directly used in the development of blast resistant hybrids, as the earlier studies suggested blast resistance to be controlled by single dominant gene (Gupta *et al.,* 2012). The present investigation, therefore, was

undertaken to screen designated B-lines of pearl millet for resistance to different pathotypes of *M. grisea*.

Materials and methods

Plant material

One hundred sixty designated B-lines (designated till 2009 at ICRISAT's hybrid parent breeding program) of pearl millet were screened for blast resistance under greenhouse conditions along with a resistant designated restorer parent (ICMR 06444) and a susceptible designated seed parent (ICMB 95444) check. The screening was carried out against five pathotype-isolates (Pg 45 collected from Telangana, Pg 53 and Pg 56 from Rajasthan, and Pg 118 and Pg 119 from Haryana) of *M. grisea* selected from the pathogenic variability study (Sharma *et al.,* 2013).

Inoculum preparation and inoculations

Inoculum of each of the mono-conidial pathotype-isolate of *M. grisea* was multiplied on oatmeal agar plates by incubating the inoculated plates at 25°C with 12 h darkness for 7-10 days. Spores were harvested by flooding the plates with sterilized distilled water and scraping the growth with a spatula. The spore suspension was adjusted to desired concentration (1×10^5 spore mL^{-1}) with the help of a haemocytometer and a drop of surfactant (Tween 20) was added to ensure the uniform dispersal of spores.

Fifteen seeds of each line were planted in 10 cm diameter pots filled with sterilized soil-sand-FYM mix (2:1:1 by volume) and seedlings were thinned to 10 per pot after germination and placed in a greenhouse bay maintained at 30±1°C. Twelve-day old seedlings maintained in the pots were spray-inoculated with spore suspension of each isolate separately, covered with polythene bags and incubated at 25 °C for 48 h to prevent cross contamination. After 48 h bags were removed and inoculated seedlings were exposed to >90% relative humidity (RH) under misting for 6 days in a greenhouse. The experiment was conducted in a completely randomized design (CRD) with three replicates (one pot per replicate) and 10 seedlings were maintained per replicate.

Disease scoring

Leaf blast severity on each line was recorded 6 days after inoculation using 1-9 progressive rating scale (Sharma *et al.,* 2013). Pearl millet lines exhibiting score 1.0-3.0 were considered resistant, with 3.1-5.0 score as moderately resistant, 5.1-7.0 score as susceptible while those showing >7.0 score were considered highly susceptible.

Data analysis

Analysis of variance (ANOVA) for blast scores was done using GENSTAT statistical package (14th Edition) to determine significant differences among pathotypes, host genotypes and their interactions (Payne *et al.,* 2011).

Results and discussion

Disease severity in the susceptible check ICMB 95444 was quite high (≥7.0) against all the five pathotypes which indicated a reliable disease screen. As expected, the resistant check ICMR 06444 included in the study was found resistant to all the five pathotypes. Significant differences for blast severity were observed for pathotypes, genotypes (B-lines) and their interaction which indicated different levels of virulence in the pathotypes and resistance in the host lines (Table 1). Maximum 24 lines were found resistant (1.0-3.0 score) to isolate Pg118 followed by 23 to Pg45. Similarly, 22 lines were resistant to Pg56 followed by 19 to Pg53 and 15 to Pg119 (Fig. 1). Twenty-eight lines showed moderate resistance (3.1-5.0 score) to Pg45, 24 to Pg119, 9 to Pg118, seven to Pg53 and four to Pg56.

Table 1. Analysis of variance (ANOVA) for disease severity of 160 designated B-lines of pearl millet against five pathotypes of *Magnaporthe grisea*

Source of variation	Degrees of freedom	Sum of square	Mean sum of square	Variance (F-value)	F probability
Replication	2	0.15	0.07	0.69	
Pathotype (P)	4	389.21	97.30	884.24	<.001
Breeding lines (B)	159	7262.07	45.67	415.05	<.001
P×B	636	1218.78	1.92	17.41	<.001
Residual	1598	175.84	0.11		
Total	2399	9046.07			

Most of the B-lines exhibited susceptible to highly susceptible reaction against five pathotypes used in this study. Based on mean disease score across pathotypes, 63 lines were found susceptible (score 5.1-7.0) and 70 lines were highly susceptible (score >7.0) to blast. Thus, these lines are not expected to develop blast resistant hybrids unless crossed with blast resistant pollinators. However, we could identify 23 lines having resistance to 3-5 pathotypes. Nine lines (81B, ICMB 88004, ICMB 92444, ICMB 97222-P1, ICMB 02111, ICMB 07111, ICMB 06444, ICMB 09333 and ICMB 09999) were found resistant to all the five pathotypes (Table 2). Eight lines had resistance against four pathotypes and six lines were found resistant to any three pathotypes. The diverse parentage of the blast resistant lines suggests that the resistance in these lines has been derived from diverse blast resistant germplasm used in the hybrid-parent breeding program. However, a few lines shared common pedigree; e.g., ICMB 97222 and ICMB 02111, and ICMB 00111 and ICMB 01777 have same parents. In fact, resistance in ICMB 97222 and ICMB

Table 2. Disease severity (on 1-9 scale) in selected B-lines showing resistance to 3-5 pathotypes of *Magnaporthe grisea*

Entry	Pedigree	Blast severity (1-9 scale)[a]						
		Pg 45	Pg 53	Pg 56	Pg 118	Pg 119	Mean	Pathotypes[b]
81B	Induced downy mildew resistant selection from Tift 23D2B	2.0	1.0	1.0	1.0	1.0	1.2	5
ICMB 88004	Togo-11-5-2 selection	3.0	2.0	2.0	2.0	2.0	2.2	5
ICMB 92444	(843B x ICMPS 1500-7-4-1-6)-23-1-B-1-4	3.0	2.0	2.0	1.0	2.0	2.0	5
IICMB 97222-P1	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88004)]-28-2-B	3.0	2.0	2.0	1.0	3.0	2.2	5
ICMB 02111	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88004)]-99-B	3.0	3.0	3.0	3.0	2.0	2.8	5
ICMB 07111	(ICMB 96111 x 4038-4-2-B)-2-1-5-4	2.0	1.0	1.0	1.0	2.0	1.4	5
ICMB 09333	[(SRC II C3 S1-103-1-1 x HHVBC)-20 x (81B x ICMP 451)-5-4-2-3]-5-2-1-B-B-3-B	2.0	1.0	1.0	1.0	1.0	1.2	5
ICMB 09999	(81B x 4025-3-2-B)-8-1-B	2.0	1.0	1.0	1.0	2.0	1.4	5
ICMB 06444	EEBC S1-407-1-B-B-B-B-1	2.8	2.8	2.5	3.0	1.7	2.6	5
ICMB 92666	[ICMPES 34 x (843B x ICMPES 34)]-155-4-2	2.0	3.0	3.0	1.0	4.0	2.6	4
ICMB 92777	[843B x (ICMPS 500-4-4-3 x ICMPS 1800-3-1-2-C3-4)]-7-1-3	3.0	3.0	3.0	3.0	4.0	3.2	4
ICMB 93222	(26B x 834B)-11-2-B-B	3.0	4.0	3.0	3.0	3.0	3.2	4
ICMB 93333	(843B x ICMPS 900-9-3-8-2)-21-8-4	2.9	2.0	1.8	3.6	2.7	2.6	4
ICMB 00111	(BSECBPT/91-40 x SPF3/S91-3)-1-2-2-4	2.0	3.0	1.0	1.0	4.0	2.2	4
ICMB 01777	(BSECBPT/91-38 x SPF3/S91-529)-10-1-6	2.0	3.0	3.0	3.0	4.0	3.0	4
ICMB 04222	(843B x EEBC S1-407)-12-3-B	5.0	2.0	3.0	2.0	3.0	3.0	4
ICMB 05777	(D2BLN/95-98 x EEBC C1-1)-7-B-B	4.0	3.0	3.0	2.0	2.0	2.8	4
ICMB 00222	{[(81B x SRL 53-1) x 843B]-3-5-2 x (843B x 834B)-25-B-B-1}-84-6-B-B	3.0	5.0	3.0	3.0	4.0	3.6	3
ICMB 00999	(ICMB 89111 x 863B)-65-8-B-B	2.0	2.0	4.0	2.0	4.0	2.8	3
ICMB 03333	9035/S92-B-3	3.0	5.0	3.0	3.0	6.0	4.0	3
ICMB 03888	[(ICMB 88006 x ICMB 88005) x (ICMB 89111 x ICMB 88005)] -1-1-3-B-9	4.0	2.0	5.0	2.0	2.0	3.0	3
ICMB 03999	(ICMB 89111 x IP 9402-2-1-1-2)-31-1-B-B	2.0	4.0	3.0	2.0	4.0	3.0	3
ICMB 04111	(81B x 4017-5-4-B)-12-3-1-3	4.0	3.0	2.0	2.0	4.0	3.0	3
ICMR 06444	[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-B	2.0	3.0	3.0	1.0	2.0	2.2	5
ICMB 95444	(81-1164 DB/85-1856 LR-16-B x 843DMR1)-14-6-3	7.0	8.0	8.0	9.0	8.0	8.0	0

[a]=Mean of 3 replicates; LSD (P<0.01): Pathotype = 0.04; Breeding line = 0.23, Pathotype × Breeding line = 0.53

[b]=Resistant to number of pathotypes

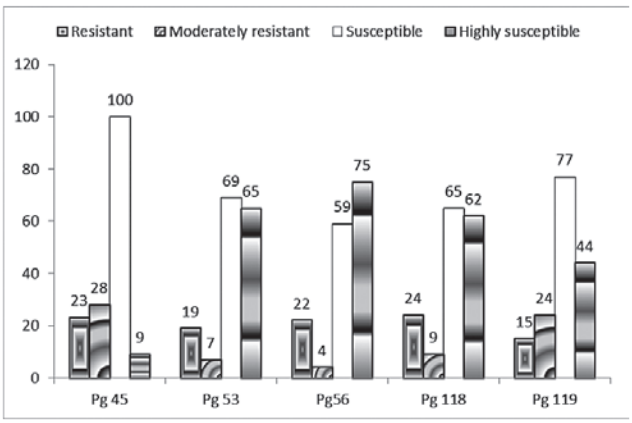

Figure 1. Number of designated B-lines (n=160) exhibiting resistant to highly susceptible reaction against five pathotypes of *Magnaporthe grisea*

02111 appears to be derived from another blast resistant (resistant to all pathotypes) line ICMB 88004 involved in the parentage of these lines as two (ICMB 88006 and ICMB 89111) of the remaining three lines used to develop ICMB 97222 and ICMB 02111 are highly susceptible to blast.

Many pathogenic races have been identified in *M. grisea* infecting rice and this variability has been cited as the principal cause for the frequent breakdown of resistance in rice varieties (Suh *et al.*, 2009). Pathogenic variation in the pearl millet infecting populations of *M. grisea* has also been reported (Sharma *et al.,* 2013). Evidence also exists for the intra-population variability in the pathogenicity of mono-conidial cultures of *P. oryzae* from single lesions (Bonman *et al.,* 1987). The migration of pathotypes within the same field from one phenological stage to the other is quite common in *M. oryzae*-rice pathosystem (Chen *et al.*, 1995; Silva *et al.*, 2009). Under such conditions, it is essential to deploy multiple-pathotype resistance in the crop cultivars. We could identify nine B-lines having resistance to all the five pathotypes of *M. grisea* in India. Therefore, these lines can serve as potential source of multiple-pathotype resistance in the development of blast resistant pearl millet hybrids and hybrid parents.

Sources of blast resistance in pearl millet were identified in the 1980s and efforts have been made to incorporate resistance into improved cultivars and elite breeding lines by Hanna *et al.,* (1988), and Wilson and Hanna (1992). Though sources of blast resistance have also been identified against *M. grisea* populations in India (Sharma *et al.,* 2013), concerted efforts are required to use these resistance sources in the breeding programs to develop blast resistant hybrid parent lines for use in the development of hybrid cultivars. However, the lines screened and identified as blast resistant in the present study are designated B-lines that are ready for use in hybrid development; thus, these lines can be directly used in the hybrid development programs to manage blast of pearl millet through host plant resistance.

References

Bonman J M, Vergel de Dios T I, Bandong J M and Lee E J 1987. Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. *Plant Disease* **71 :** 127-130.

Chen D, Zeigler R S, Leung H and Nelson R J 1995. Population structure of *Pyricularia grisea* at two screening sites of Philippines. *Phytopathology* **85 :** 1011-1020.

Gupta S K, Sharma R, Rai K N and Thakur R P 2012. Inheritance of foliar blast resistance in pearl millet (*Pennisetum glaucum* L. (R.) Br.). *Plant Breeding* **131 :** 217-219.

Hanna W W, Wells H D, Burton G W and Monson W G 1988. Registration of 'Tifleaf 2' pearl millet. *Crop Science* **28 :** 1023.

Mehta P R, Singh B and Mathur S C 1953. A new leaf spot disease of bajra (*Pennisetum typhoides* Staph and Hubbard) caused by a species of *Pyricularia. Indian Phytopathology* **5 :** 140-143.

Ou S H 1985. Rice Diseases 2nd ed. Commonwealth Mycological Institute, Kew, UK.

Payne R W, Murray D A, Harding S A, Baird D B and Soutar D M 2011. An Introduction to GenStat for Windows (14th Edition). VSN International, Hemel Hempstead, UK.

Sharma R, Gate V L, Kishore Babu B, Rao V P and Ghughe S S 2012. Prevalence and pathogenic diversity in pearl millet downy mildew pathogen populations in Maharashtra, India. *Indian Journal of Plant Protection* **40 :** 306-311.

Sharma R, Upadhyaya H D, Manjunatha S V, Rai K N, Gupta S K and Thakur R P 2013. Pathogenic variation in the pearl millet blast pathogen, *Magnaporthe grisea* and identification of resistance to diverse pathotypes. *Plant Disease* **97 : 1**89-195.

Silva G B, Prabhu A S, Filippi M C C, Trindade M G, Araujo L G and Zambolim L 2009. Genetic and phenotypic diversity

of *Magnaporthe oryzae* from leaves and panicles of rice in commercial fields in the State of Goias, Brazil. *Tropical Plant Pathology* **34 :** 71-76.

Suh J P, Roh J H, Cho Y C, Han S S, Kim Y G and Jena K K 2009. The Pi40 gene for durable resistance to rice blast and molecular analysis of Pi40-advanced backcross breeding lines. *Phytopathology* **99 :** 243-250.

Thakur R P, Sharma R and Rao V P 2011. Screening Techniques for Pearl Millet Diseases. *Information Bulletin* No.89. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru PO - 502 324, Andhra Pradesh, India, 48 pp.

Wilson J P and Gates R N 1993. Forage yield losses in hybrid pearl millet due to leaf blight caused primarily by *Pyricularia grisea*. *Phytopathology* **83 :** 739-743.

Wilson J P and Hanna W W 1992. Effects of gene and cytoplasm substitutions in pearl millet on leaf blight epidemics and infection by *Pyricularia grisea. Phytopathology* **82 :** 839-842.

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