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Short Communication

Inheritance of foliar blast resistance in pearl millet (*Pennisetum glaucum*)

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With 1 table

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Abstract

Foliar blast, caused by *Pyricularia grisea* (Cooke) Sacc, has recently emerged as a serious disease of pearl millet in India. To study the inheritance of resistance to this disease, two resistant restorer lines (ICMR 06222 and ICMR 07555) and two susceptible maintainer lines (ICMB 95444 and ICMB 89111) were selected on the basis of foliar blast reaction in tests conducted under field and greenhouse conditions. Each of the two resistant parents was crossed with two susceptible parents to generate four sets of F_1s, F_2s and their backcrosses with both resistant and susceptible parental lines. These were evaluated for disease reaction with artificial inoculation under both field and greenhouse conditions. The disease reaction of the F_1s, and the segregation patterns of resistance in the F_2s and backcross generations, showed that resistance to foliar blast in pearl millet is controlled by a single dominant gene.

Key words: *Pennisetum glaucum* — foliar blast — leaf spot — inheritance

Pearl millet foliar blast, also known as leaf spot caused by *Pyricularia grisea* (Cooke) Sacc. [teleomorph: *Magnaporthe grisea* (Herbert) Barr], was first reported in 1942 from Kanpur, Uttar Pradesh, India (Mehta et al. 1953). However, until recently, it had not been a disease of any economic significance in this country, which annually cultivates it on about 9.5 million ha and hence has the largest pearl millet area in the world. Leaf blast has been considered a serious disease in southern coastal plains of the USA where infection from this disease has been found to have significant adverse effects on green forage yield and digestible dry matter (Wilson and Gates 1993). It is known that host plant resistance is the most cost-effective strategy to effectively manage this disease. Thus, sources of blast resistance were identified, and efforts were made to incorporate resistance into improved hybrid parents and elite breeding lines in the USA (Hanna et al. 1988). Recently, leaf blast has emerged as a serious disease in pearl millet in India (Lukose et al. 2007, Anonymous 2009), which becomes more severe during humid weather conditions, especially in dense plant stands. Breeding for blast resistance is yet to begin in India, although field and greenhouse screening techniques have been developed and resistance sources have been identified (Thakur et al. 2009). Knowledge of the inheritance of resistance will have a direct bearing on the breeding efficiency for genetic management of this disease. We report on the results of a study of the inheritance of blast resistance to the pathogen population prevalent at ICRISAT, Patancheru research centre.

Materials and Methods

Based on the results of a previous study (Thakur et al. 2009), ICMR 06222 and ICMR 07555 were selected as resistant parents and ICMB 89111 and ICMB 95444 as susceptible parents for foliar blast disease. These selected parental lines were reconfirmed for their foliar blast reaction in the greenhouse at ICRISAT, Patancheru. Four F_1s were generated by crossing both resistant lines (P_2) on each of the two susceptible lines (P_1) in the cool post-rainy season during November–February 2008–2009. During the subsequent hot dry season, March–June 2009, in each F_1, 8–10 panicles were selfed using parchment paper bags to generate a F_2 population, and bulk pollen from 8 to 10 F_1 panicles was used to pollinate the corresponding susceptible and resistant parents to develop BCP_1 (susceptible parent × F_1) and BCP_2 (resistant parent × F_1) populations, respectively.

All parents, four F_1s, four F_2s, four BCP_1s and four BCP_2s were screened against *P. grisea* Patancheru isolate in the greenhouse in July–August 2009 in three replications. In each replication, three pots of the parents and F_1, 10 pots each of both BCP_1 and BCP_2 and 20 pots of F_2 were planted for each cross. Seeds were sown in 15-cm-diameter pots (10 seeds/pot) filled with sterilized soil–sand–FYM mix (2 : 1 : 1) and placed in a greenhouse bay maintained at 30 ± 1°C. The seedlings (12 days old) were spray-inoculated with an aqueous conidial suspension (ca. 1×10^5 spores/ml) of *P. grisea* (Patancheru isolate) and exposed to high humidity (>90% RH) under misting for 10 days. Blast severity was recorded 10 days after inoculation using a 1–9 progressive scale (Thakur et al. 2009). Following this, the plants having a score of ≤3 were rated as resistant and with a score of >5 as susceptible.

The aforementioned parents and populations were also evaluated under field conditions during the rainy season of 2009. The experiment was conducted in a randomized complete block design with three replications with one row of 4 m long for each F_1 and parents, four rows of each BCP_1 and BCP_2 and eight rows of each F_2 planted in each replication. Systematic susceptible checks (ICMB 95444, 99666 and 89111) were grown every 5th row, alternately. Plants were thinned to 20 plants/row 15 days after planting, and standard agronomic practices were followed for crop management. Plants were spray-inoculated twice, first at pretillering stage and second at flowering stage with an aqueous conidial suspension (ca. 1×10^5 spores/ml) of *P. grisea* (Patancheru isolate) High humidity was provided by perfo-irrigation twice a day on rain-free days, 30 min each between 11 and 12 h and 16–17 h, to promote disease development. Disease severity was recorded using same 1–9 progressive scale as mentioned for greenhouse screening.

The observed ratios of resistant to susceptible plants in the segregating populations in greenhouse and field experiments were compared to theoretical ratios using chi-square test after pooling of plants from all replications.

Results and Discussion

All plants of the susceptible parents were susceptible (score of > 5) under both greenhouse and field conditions. In the F_2 and BCP_1, there was a clear-cut segregation either for resistant plants (score of ≤3) or for susceptible plants (score of > 5), and no plant had a score of 4 and 5 for blast reaction under both greenhouse and field conditions. All plants of the two resistant parents were resistant under both greenhouse and field conditions. All plants in all four F_1s and their corresponding four BCP_2s were also resistant to blast under greenhouse and field conditions (Table 1). The F_2 population from cross ICMB 89111 × ICMR 06222 had a good fit to the segregation ratio of 3R : 1S in both the greenhouse and field screens, indicating dominant monogenic control of blast resistance. The BCP_1 of this cross had good fit to the 1R : 1S ratio expected for monogenic inheritance in both greenhouse and field screens. The F_2 of cross ICMB 95444 × ICMR 06222 also gave good fit to the segregation ratio of 3R : 1S in both greenhouse and field screens, and BCP_1 segregation of this cross had good fit to 1R : 1S segregation ratio in field screen but not in the greenhouse where excess of susceptible plants was observed. The resistant parent ICMR 07555 when crossed to the susceptible parents ICMB 89111 and ICMB 95444 gave a good fit to segregation ratio of 3R : 1S in the F_2 in both greenhouse and field screens, again indicating monogenic control of blast resistance. The BCP_1 ratio of these crosses had significant deviations from the expected 1R : 1S segregation ratio because of the excess of susceptible plants in both the greenhouse and field experiments. Thus, in all five cases of BCP_1 where segregation ratio had significant deviation from the expected 1R : 1S ratio, it was because of the excess of susceptible plants, which most likely could have resulted from some selfing in the susceptible parents that were used as female parents in deriving the BCP_1 generation. Such deviation from expected ratio could also result from segregation distortion caused by segregation distortion loci identified in pearl millet (Busso et al. 1995), although segregation distortion appears less likely cause of the deviation from expected ratios that almost all were found in BCP_1 and not in the F_2 generation of all crosses.

The goodness of fit to 3R : 1S segregation ratio in all four F_2s and 1R : 1S ratio in three of the eight BCP_1 populations under both greenhouse and field conditions leads us to conclude that foliar blast resistance in the pearl millet lines used for this study is controlled by a single dominant gene. In an earlier study, three independent dominant genes were reported to control blast resistance in which Tifton PS34, a weedy relative of pearl millet *Pennisetum glaucum* ssp. *monodii,* was used as resistant source and evaluated against a pathogen population from Georgia, USA (Hanna and Wells 1989). In yet other study involving Tift 85DB, a blast-resistant inbred line derived by backcrossing Tifton PS34 to cultivated pearl

Table 1: Segregation for blast-resistant (R) and susceptible (S) plants in F_1, F_2, BCP_1 and BCP_2 generations and test of goodness of fit for hypothetical Mendelian ratios in four crosses of two susceptible parents with the two resistant parents in pearl millet, in greenhouse and field experiments, rainy season 2009, ICRISAT–Patancheru

Cross	Environment	Generation	No. of plants observed		Expected ratio		No. of plants expected		χ^2	P
			R	S	R	S	R	S		
ICMB 89111 × ICMR 06222	Greenhouse	F_1	145	0	–	–	–	–	–	–
		F_2	338	107	3	1	334	111	0.21	0.64
		BCP_1	117	140	1	1	128.5	128.5	2.05	0.15
		BCP_2	220	0	–	–	–	–	–	–
	Field	F_1	52	0	–	–	–	–	–	–
		F_2	494	149	3	1	482	161	1.14	0.28
		BCP_1	206	201	1	1	203.5	203.5	0.06	0.80
		BCP_2	202	0	–	–	–	–	–	–
ICMB 95444 × ICMR 06222	Greenhouse	F_1	142	0	–	–	–	–	–	–
		F_2	561	189	3	1	563.5	187.5	0.016	0.89
		BCP_1	109	156	1	1	132.5	132.5	8.33	0.003
		BCP_2	314	0	–	–	–	–	–	–
	Field	F_1	55	0	–	–	–	–	–	–
		F_2	544	164	3	1	531	177	1.27	0.26
		BCP_1	90	106	1	1	98	98	1.30	0.25
		BCP_2	180	0	–	–	–	–	–	–
ICMB 89111 × ICMR 07555	Greenhouse	F_1	130	0	–	–	–	–	–	–
		F_2	396	161	3	1	418	139	4.52	0.03
		BCP_1	25	167	1	1	96	96	105.0	< 0.001
		BCP_2	103	0	–	–	–	–	–	–
	Field	F_1	46	0	–	–	–	–	–	–
		F_2	570	165	3	1	551	184	2.55	0.11
		BCP_1	38	95	1	1	66.5	66.5	24.4	< 0.001
		BCP_2	170	0	–	–	–	–	–	–
ICMB 95444 × ICMR 07555	Greenhouse	F_1	93	0	–	–	–	–	–	–
		F_2	736	234	3	1	727.5	242.5	0.39	0.53
		BCP_1	36	202	1	1	119	119	115.7	< 0.001
		BCP_2	352	0	–	–	–	–	–	–
	Field	F_1	53	0	–	–	–	–	–	–
		F_2	550	159	3	1	532	177	2.5	0.11
		BCP_1	28	77	1	1	52.5	52.5	22.86	< 0.001
		BCP_2	214	0	–	–	–	–	–	–

millet, resistance to blast was reported to be under dominant monogenic control (Wilson et al. 1989). Thus, only one of the three resistant genes from Tifton PS34 got introgressed into Tift 85DB during backcrossing programme, and it was as effective for resistance as the three genes. However, Tift 85DB was found to be susceptible to the Patancheru isolate used in our study, indicating that the pathotype used in our study is different from the one used in the above study. We also observed that all 150 plants of a F_2 population derived from cross ICMR 06222 × ICMR 0755 when tested for blast reaction in the greenhouse were resistant to the disease, indicating that both parents carried the same common gene for resistance. It is significant to note that the resistant parents used in this study are of very diverse origin: ICMR 06222 (SDMV 90031-S1-3-3-2-1-3-2-2-1-1-B) is derived from an *iniari* landrace-based open-pollinated variety developed by ICRISAT in southern Africa, and ICMR 07555 (ICMS 8511 S1-17-2-1-1-4-1-B-3-3-2-2-B) is derived from a non-*iniari*-based synthetic developed at ICRISAT in India. A blast-resistant seed parent composite has been constituted from the intercrosses of eight blast-resistant seed parental lines of diverse origin developed at ICRISAT. About 500 plants of this composite were evaluated during the 2009 rainy season under field conditions using artificial inoculation. Interestingly, all plants were found resistant to moderately resistant with no segregation for susceptible plants, indicating that all lines involved in this composite carried a common resistance gene. Considering the severity and wider occurrence of this disease in India, extensive efforts should be made to identify additional sources of resistance to the pathotype used in our study as well as to other more virulent pathotypes recently identified and being studied for virulence diversity (R. Sharma unpublished data).

Magnaporthe grisea infecting rice had shown large pathogenic variability. Thus, a preliminary assessment of the pathogenic variability for virulence was conducted in pearl millet using 20 isolates from different locations in India. The most resistant line ICMR 06222 used in this study was found susceptible to four isolates, indicating pathogenic variability in the pathogen and suggesting the use of different pathotypes for the identification of resistance sources. In rice, about 50 blast resistance genes have been identified, and several of them have been incorporated into rice cultivars. However, most of these resistance genes have broken down to blast disease because of their race specificity and also because of the rapid changes in pathogenicity of the blast fungus (Suh et al. 2009). Various potential mechanisms, including sexual recombination, heterokaryosis, parasexual recombination and aneuploidy, have been proposed to explain frequent race changes in the rice blast fungus (Kang and Lee 2000). Therefore, efforts should be made to study the pathogenic variability in *P. grisea* isolates from different pearl millet-growing areas in India and to identify the resistant sources to different pathotypes for utilizing them in breeding programme to manage this disease through host plant resistance.

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