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Inheritance and allelic relationship among gene(s) for blast resistance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract

Six blast-resistant pearl millet genotypes, ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1, were crossed with two susceptible genotypes, ICMB 95444 and ICMB 89111 to generate F_1s, F_2s and backcrosses, BC_1P_1 (susceptible parent × F_1) and BC_1P_2 (resistant parent × F_1) for inheritance study. The resistant genotypes were crossed among themselves in half diallel to generate F_1s and F_2s for test of allelism. The F_1, F_2 and backcross generations, and their parents were screened in a glasshouse against *Magnaporthe grisea* isolates Pg 45 and Pg 53. The reaction of the F_1s, segregation pattern of F_2s and BC_1P_1 derived from crosses involving two susceptible parents and six resistant parents revealed the presence of single dominant gene governing resistance in the resistant genotypes. No segregation for blast reaction was observed in the F_2s derived from the crosses of resistant × resistant parents. The resistance reaction of these F_2s indicated that single dominant gene conferring resistance in the six genotypes is allelic, that is same gene imparts blast resistance in these genotypes to *M. grisea* isolates.

KEYWORDS
allelism, blast, inheritance, monogenic dominance

1 | INTRODUCTION

Blast or leaf spot of pearl millet, caused by *Magnaporthe grisea* (Herbert) Barr [anamorph: *Pyricularia grisea* (Cooke) Sacc.], has become topic of discussion in scientific platforms as it has emerged as a serious disease in the recent past that challenges both forage and grain production of pearl millet across the globe (Sharma et al., 2013). In India, the disease takes an epidemic form on almost all high yielding hybrids in certain parts of middle Gujarat, north Gujarat and Saurashtra region (Joshi & Gohel, 2015). Mild to severe incidence of the disease has been recorded on a number of commercial hybrids in Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana during recent years (Anonymous 2015).

Earlier, blast was considered as a minor disease of pearl millet; therefore, breeding for blast resistance had not been a high priority as it has been for downy mildew resistance. However, due to its recent high and widespread incidence in major pearl millet growing regions of India, it is essential to breed for blast resistance to develop stable and durable varieties. Although sources of blast resistance in pearl millet have been identified and efforts have been made to incorporate resistance into improved cultivars and elite breeding lines in the USA (Hanna, Wells, Burton, & Monson, 1988), it is still in a preliminary stage in India. Breeding for resistance to a pathogen is the safest and economic approach to manage a disease in any crop, and it becomes more effective if the inheritance of resistance is well understood. Hanna and Wells (1989) discovered resistance to *M. grisea* in a weedy relative of pearl millet (*Pennisetum glaucum* spp. *monodii*) and found that resistance was controlled by three independent dominant genes. Although inheritance studies for blast resistance in pearl millet in India are in their initial stage, researchers across the globe have reported it to be generally governed by dominant gene(s). Gupta, Sharma, Rai, and Thakur (2012) used resistant restorer and susceptible maintainer lines of pearl millet for inheritance studies against foliar blast and found that

resistance in pearl millet genotype ICMR 06222 is controlled by a dominant gene.

Inheritance of resistance is always aimed towards knowing the presence of diverse resistance genes in host cultivars which is of utmost importance to manage any disease either by planting diverse resistance sources in the path of pathogen spread or by pyramiding diverse resistance genes in the elite cultivars. However, so far, no study has been reported on the diversity of blast resistance genes present in pearl millet lines being used in India. Therefore, studies discerning such relationships among resistance genes in pearl millet blast pathosystem are essential in developing varieties and hybrid cultivars with stable and durable blast resistance. Keeping this in view, this study was planned to study inheritance of blast resistance in different genotypes of pearl millet and the allelic relationship among gene(s) governing resistance in these genotypes to blast.

2 | MATERIALS AND METHODS

2.1 | *Magnaporthe grisea* isolates and inoculation of pearl millet genotypes

The monoconidial cultures of *M. grisea* isolates were obtained from culture collection being maintained in Cereals Pathology Lab, ICRISAT, Patancheru. The isolates were subcultured and maintained on oat meal agar (OMA) media at 25 ± 1°C. The pathogen isolates Pg 45 and Pg 53 representing two pathotypes of *M. grisea* adapted to pearl millet were selected for the inheritance study and test of allelism.

An inoculum of each isolate was prepared as per the procedure described by Sharma et al. (2013). The 6-mm mycelial discs of each isolate were cut from a 7-day-old culture grown on OMA medium at 25 ± 1°C. Mass multiplication of spores for inoculation was achieved by growing each isolate on OMA medium in Petri plates (3 discs/plate) incubated at 25°C with 12 hr of darkness for 7–10 days. Spores were harvested by flooding the plates with sterile distilled water, and the fungal growth containing mycelium and conidia was gently removed using a soft camel hair brush. The spore suspension was adjusted to the desired concentration (1×10^5 spore/ml) with the help of a haemocytometer, and Tween 20 @ 0.02% vol/vol was added to the suspension just before inoculation. The 12-day-old seedlings were spray-inoculated with an aqueous conidial suspension (1×10^5 spores/ml) of *M. grisea* isolates Pg 45 and Pg 53 separately and exposed to high humidity (>90% RH) under misting for 4 days. Blast severity was recorded 6 days after inoculation using a 1–9 progressive scale (Sharma et al., 2013).

2.2 | Plant material

Seeds of pearl millet genotypes were taken from genetic stocks being maintained at Cereals Pathology Lab, ICRISAT, Patancheru. Selection of resistant and susceptible lines for use in this study was made by screening eight pearl millet genotypes against *M. grisea* isolates in glasshouse during August–September 2014. Based on the disease reaction against *M. grisea* isolates Pg 45 and Pg 53, ICRISAT developed hybrid parental lines ICMB 95444 and ICMB 89111 were selected as susceptible parents (score ≥ 7.0 on 1–9 scale), and ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1 (score ≤ 3.0) were selected as resistant parents. The selected lines were further selfed for three consecutive generations to obtain true inbreds.

2.3 | Crossing procedure for generation of progeny

Staggered sowings of parent genotypes were carried out to get synchronization in flowering time. The seedlings of the selected resistant and susceptible genotypes were transplanted in 38-cm-diameter pots filled with sterilized soil–sand–FYM mix (3:2:1) in the glasshouse area (4–5 seedlings/pot). The plants were watered adequately, and urea and DAP fertilizers were applied to ensure healthy growth. The heads were covered with selfing bags upon emergence from the flag leaf to avoid chances of cross-pollination. Upon complete emergence of stigma, fresh pollen from the desired male parent was shed on the stigma of desired female parent during morning hours. The pollinated heads were immediately covered with selfing bags and stapled, and details of both the parents were marked on the bags with a permanent marker. The crossed heads were allowed for proper seed setting and maturity followed by single head threshing manually. The threshed seeds were stored in the well-labelled seed covers in cold stores.

For studying inheritance of resistance, six resistant lines were crossed with two susceptible lines ICMB 95444 and ICMB 89111 to generate 12 (susceptible [S] × resistant [R]) F_1s (ICMB 89111 × ICMB 93333, ICMB 89111 × ICMB 97222, ICMB 89111 × ICMR 06444, ICMB 89111 × ICMR 06222, ICMB 89111 × ICMR 11003, ICMB 89111 × IP 21187-P1, ICMB 95444 × ICMB 93333, ICMB 95444 × ICMB 97222, ICMB 95444 × ICMR 06444, ICMB 95444 × ICMR 06222, ICMB 95444 × ICMR 11003 and ICMB 95444 × IP 21187-P1). Two susceptible lines were crossed with each other to generate one (S × S) F_1 (ICMB 95444 × ICMB 89111). To carry out allelism test, six resistant lines were crossed with each other (R × R) in half diallel fashion during August–November 2014 to generate 15 F_1s (ICMR 06444 × ICMR 06222, ICMR 06444 × ICMB 97222, ICMR 06444 × IP 21187-P1, ICMR 06444 × ICMB 93333, ICMR 06444 × ICMR 11003, ICMR 06222 × ICMB 97222, ICMR 06222 × IP 21187-P1, ICMR 06222 × ICMB 93333, ICMR 06222 × ICMB 11003, ICMB 97222 × IP 21187-P1, ICMB 97222 × ICMB 93333, ICMB 97222 × ICMR 11003, IP 21187-P1 × ICMB 93333, IP 21187-P1 × ICMR 11003, ICMB 93333 × ICMR 11003). All crosses were made in the glasshouse. The hybridity of F_1s from each cross was confirmed on the basis of morphological characters as well as by screening for disease reaction against test isolate of *M. grisea*. The resistance reaction of F_1s derived from susceptible female parent and resistant male parent confirmed the hybridity. In the subsequent hot dry season during February to May 2015, F_1s from each cross were selfed using

parchment paper bags for the generation of F_2 seeds. To develop backcross populations BC_1P_1 (S parent × F_1s) and BC_1P_2 (R parent × F_1s) for inheritance study, single plant pollen of each F_1 of S × R crosses was used to pollinate the corresponding susceptible and resistant parents, respectively.

2.4 | Screening of populations for disease reaction

For inheritance study, parents, F_1s, F_2s, BC_1P_1s and BC_1P_2s of each cross were screened against Pg 45 and Pg 53 in the glasshouse during July 2015–February 2016. Seeds were sown in 15-cm-diameter pots (~15 seeds/pot) filled with sterilized soil-sand-FYM mix (3:2:1) and placed in a glasshouse bay maintained at 30 ± 1°C, whereas for allelism study, seeds of parents, F_1s and F_2s of each R × R cross were sown in plastic pots for disease screening. The 12-day-old seedlings were screened against *M. grisea* isolates Pg 45 and Pg 53 separately as described above. Blast severity was recorded 6 days after inoculation using a 1–9 progressive scale (Sharma et al., 2013). Based on disease rating, the plants having score of ≤3 were rated as resistant and those with score of ≥4 (typical blast lesions) as susceptible.

2.5 | Statistical analysis

The observed ratios of resistant to susceptible plants in the segregating generations were compared with theoretical expected ratios using a Chi square test. The Chi square test ($p \leq 0.05$) was used to test the segregation ratio of the phenotypic classes using the program GENES (Cruz, 2001).

3 | RESULTS

3.1 | Inheritance of resistance

The results of blast reaction in the different generations (F_1, F_2, BC_1P_1 and BC_1P_2) of each cross against two isolates of *M. grisea* (Pg 45 and Pg 53) are presented in Tables 1,2,3,4. Blast scores (1–9 scale) of F_2 plants derived from the crosses of susceptible parents ICMB 89111 and ICMB 95444 with resistant parents (ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1) are summarized in Figures 1,2, respectively. The plants of susceptible parents ICMB 89111 (score ≥ 7) and ICMB 95444 (score 8–9) exhibited susceptible reaction, whereas seedlings of resistant parents selected for the study showed resistance (score ≤ 3) against both Pg 45 and Pg 53. All plants of susceptible parents ICMB 95444 and ICMB 89111 and the F_1s, F_2s, BC_1P_1s and BC_1P_2s derived from them recorded disease score 6–9 against both the isolates Pg 45 and Pg 53 suggesting the presence of susceptible alleles in both the genotypes.

The segregation ratios of crosses of ICMB 89111 and ICMB 95444 with resistant parents against Pg 45 are summarized in Tables 1,3, respectively. In the cross ICMB 89111 × IP 21187-P1, a total of 72 plants of F_1, 483 plants of F_2, 189 plants of BC_1P_1 and 226 plants of BC_1P_2 were screened against Pg 45 (Table 1). All the F_1s were resistant to Pg 45 implying the dominance of resistance over susceptibility. Among the 483 F_2 plants, 370 plants were resistant (score ≤ 3) and 113 were susceptible (score ≥ 4) with a best fit 3:1 R/S ratio (χ^2 = 0.66; p = 0.42), which is indicative of single dominant gene for resistance. The BC_1P_1 plants segregated in 93 resistant and 96 susceptible plants in a good fit of 1:1 R/S ratio, which is affirmative of monogenic dominance. This monogenic dominant inheritance of resistance was further supported by the resistant reaction of all 226 BC_1P_2 plants. Similar results were obtained in the progeny of S × R crosses of ICMB 89111 with other resistant parents (Table 1) and all crosses of susceptible ICMB 95444 with resistant parents ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003, IP 21187-P1 (Table 3), thus confirming the presence of single dominant gene for blast resistance in these resistant genotypes.

The results of inheritance study of S × R crosses against Pg 53 are summarized in Tables 2,4. Similar results were observed for Pg 53 as observed in the case of Pg 45. A total of 75 F_1 plants, 446 F_2 plants, 218 BC_1P_1 plants and 178 plants of BC_1P_2 of ICMB 89111 × ICMB 97222 were screened against Pg 53 (Table 2). All F_1s were resistant; 323 of the 446 F_2 plants were resistant and 123 were susceptible showing a good fit for segregating ratio of 3:1 R/S (χ^2 = 1.58, p = 0.21) marking the governance of resistance by single dominant gene. The segregation of BC_1P_1 into 119 resistant and 99 susceptible plants showed a good fit for 1:1 R/S, and complete resistance of 178 plants of BC_1P_2 supported the single dominant gene governance of resistance. Similar observations were made for different generations when resistant parents were crossed with another susceptible parent ICMB 95444, for example cross ICMB 95444 × ICMR 06444 showed resistant reaction in 78 F_1s, 421 F_2s segregated in a good fit ratio of 3:1 R/S (322 resistant and 99 susceptible) connoting single dominant gene for resistance, which was confirmed by segregation of 222 BC_1P_1s in the good fit ratio of 1:1 R/S (108 resistant and 114 susceptible) (Table 4). The dominant gene governance of resistance was further confirmed by resistant reaction of all 203 BC_1P_2 plants. Similar results were observed for the crosses of ICMB 89111 and ICMB 95444 with other resistant parents.

The screening of S × R crosses against Pg 45 and Pg 53 exhibited resistance in all F_1s, best fit ratio of 3:1 R/S in F_2s, a good fit 1:1 R/S ratio in BC_1P_1s and complete resistance in all BC_1P_2s, thus confirming the blast resistance to be governed by a dominant gene in all the selected resistant genotypes.

3.2 | Test of allelism

The results of allelism study are summarized in Tables 5,6 for Pg 45 and Pg 53, respectively. In the cross of ICMR 06444 and ICMR 06222, all 61 F_1 plants and 379 F_2 plants were found to be resistant against Pg 53. Similar to that, F_2s of ICMR 06222 × IP 21187-P were resistant to both Pg 45 and Pg 53. The F_2s of not only these two crosses but also from other crosses of the resistant parents did

TABLE 1 Segregation analyses for blast reaction in the different generations derived from crosses between susceptible ICMB 89111 (P_1) and resistant parents (P_2) against *Magnaporthe grisea* isolate Pg 45

Cross	Generation	No. of plants observed		No. of plants expected		Expected ratio	χ^2	*p*	R-gene
		R	S	R	S				
ICMB 89111 × ICMB 93333	ICMB 89111 (P_1)	0	65						1 dominant
	ICMB 93333 (P_2)	72	0						
	F_1	75	0	75	0	1:0	–	–	
	F_2	282	97	284	95	3:1	0.071	0.790	
	BC_1P_1	119	98	108.5	108.5	1:1	2.032	0.154	
	BC_1P_2	223	0	223	0	1:0	–	–	
ICMB 89111 × ICMB 95444	ICMB 89111 (P_1)	0	65						–
	ICMR 95444 (P_2)	0	75						
	F_1	0	70	0	70	–	–	–	
	F_2	0	421	0	421	–	–	–	
	BC_1P_1	0	144	0	144	–	–	–	
	BC_1P_2	0	197	0	197	–	–	–	
ICMB 89111 × ICMB 97222	ICMB 89111 (P_1)	0	65						1 dominant
	ICMB 97222 (P_2)	75	0						
	F_1	68	0	68	0	1:0	–	–	
	F_2	357	122	359	120	3:1	0.056	0.812	
	BC_1P_1	121	94	107.5	107.5	1:1	3.391	0.0656	
	BC_1P_2	217	0	217	0	1:0	–	–	
ICMB 89111 × ICMR 06444	ICMB 89111 (P_1)	0	65						1 dominant
	ICMR 06444 (P_2)	68	0						
	F_1	66	0	66	0	1:0	–	–	
	F_2	375	117	369	123	3:1	0.390	0.532	
	BC_1P_1	95	86	90.5	90.5	1:1	0.448	0.5035	
	BC_1P_2	192	0	192	0	1:0	–	–	
ICMB 89111 × ICMR 06222	ICMB 89111 (P_1)	0	65						1 dominant
	ICMR 06222 (P_2)	71	0						
	F_1	70	0	70	0	1:0	–	–	
	F_2	356	125	361	120	3:1	0.250	0.617	
	BC_1P_1	102	101	101.5	101.5	1:1	0.005	0.944	
	BC_1P_2	220	0	220	0	1:0	–	–	
ICMB 89111 × ICMR 11003	ICMB 89111 (P_1)	0	65						1 dominant
	ICMR 11003 (P_2)	75	0						
	F_1	66	0	66	0	1:0	–	–	
	F_2	247	80	245	82	3:1	0.050	0.823	
	BC_1P_1	79	67	73	73	1:1	0.986	0.3206	
	BC_1P_2	191	0	191	0	1:0	–	–	
ICMB 89111 × IP 21187-P1	ICMB 89111 (P_1)	0	65						1 dominant
	IP 21187-P1 (P_2)	73	0						
	F_1	72	0	72	0	1:0	–	–	
	F_2	370	113	362	121	3:1	0.663	0.415	
	BC_1P_1	93	96	94.5	94.5	1:1	0.048	0.8273	
	BC_1P_2	226	0	226	0	1:0	–	–	

TABLE 2 Segregation analyses for blast reaction in the different generations derived from crosses between susceptible ICMB 89111 (P_1) and resistant parents (P_2) against *Magnaporthe grisea* isolate Pg 53

Cross	Generation	No. of plants observed		No. of plants expected		Expected ratio	χ^2	*p*	R-gene
		R	S	R	S				
ICMB 89111 × ICMB 93333	ICMB 89111 (P_1)	0	75						1 dominant
	ICMB 93333 (P_2)	76	0						
	F_1	71	0	71	0	1:0	–	–	
	F_2	296	105	301	100	3:1	0.30	0.58	
	BC_1P_1	119	111	115	115	1:1	0.28	0.60	
	BC_1P_2	169	0	223	0	1:0	–	–	
ICMB 89111 × ICMB 95444	ICMB 89111 (P_1)	0	75						–
	ICMB 95444 (P_2)	0	68						
	F_1	0	76	0	76	–	–	–	
	F_2	0	382	0	382	–	–	–	
	BC_1P_1	0	174	0	174	–	–	–	
	BC_1P_2	0	222	0	222	–	–	–	
ICMB 89111 × ICMB 97222	ICMB 89111 (P_1)	0	75						1 dominant
	ICMB 97222 (P_2)	64	0						
	F_1	75	0	68	0	1:0	–	–	
	F_2	323	123	334.5	111.5	3:1	1.58	0.21	
	BC_1P_1	119	99	109	109	1:1	1.84	0.18	
	BC_1P_2	178	0	178	0	1:0	–	–	
ICMB 89111 × ICMR 06444	ICMB 89111 (P_1)	0	75						1 dominant
	ICMR 06444 (P_2)	74	0						
	F_1	78	0	78	0	1:0	–	–	
	F_2	280	92	279	93	3:1	0.014	0.90	
	BC_1P_1	119	110	114.5	114.5	1:1	0.35	0.55	
	BC_1P_2	184	0	184	0	1:0	–	–	
ICMB 89111 × ICMR 06222	ICMB 89111 (P_1)	0	75						1 dominant
	ICMR 06222 (P_2)	76	0						
	F_1	77	0	77	0	1:0	–	–	
	F_2	296	105	301	100	3:1	0.3	0.5838	
	BC_1P_1	105	112	108.5	108.5	1:1	0.226	0.6347	
	BC_1P_2	214	0	214	0	1:0	–	–	
ICMB 89111 × ICMR 11003	ICMB 89111 (P_1)	0	75						1 dominant
	ICMR 11003 (P_2)	82	0						
	F_1	70	0	70	0	1:0	–	–	
	F_2	314	96	307.5	102.5	3:1	0.55	0.46	
	BC_1P_1	88	97	92.5	92.5	1:1	0.44	0.51	
	BC_1P_2	195	0	195	0	1:0	–	–	
ICMB 89111 × IP 21187-P1	ICMB 89111 (P_1)	0	75						1 dominant
	IP 21187-P1 (P_2)	80	0						
	F_1	70	0	70	0	1:0	–	–	
	F_2	316	103	314.25	104.75	3:1	0.04	0.84	
	BC_1P_1	96	84	90	90	1:1	0.80	0.37	
	BC_1P_2	215	0	215	0	1:0	–	–	

TABLE 3 Segregation analyses for blast reaction in the different generations derived from crosses between susceptible ICMB 95444 (P_1) and resistant parents (P_2) against *Magnaporthe grisea* isolate Pg 45

Cross	Generation	No. of plants observed		No. of plants expected		Expected ratio	χ^2	*p*	R-gene
		R	S	R	S				
ICMB 95444 × ICMB 93333	ICMB 95444 (P_1)	0	75						1 dominant
	ICMB 93333 (P_2)	72	0						
	F_1	67	0	67	0	1:0	–	–	
	F_2	400	140	405	135	3:1	0.247	0.619	
	BC_1P_1	95	85	90	90	1:1	0.556	0.456	
	BC_1P_2	192	0	192	0	1:0	–	–	
ICMB 95444 × ICMB 97222	ICMB 95444 (P_1)	0	75						1 dominant
	ICMB 97222 (P_2)	75	0						
	F_1	67	0	67	0	1:0	–	–	
	F_2	394	122	387	129	1:0	0.056	0.477	
	BC_1P_1	105	80	92.5	92.5	1:0	3.378	0.066	
	BC_1P_2	179	0	179	0	1:0	–	–	
ICMR 95444 × ICMR 06444	ICMB 95444 (P_1)	0	75						1 dominant
	ICMR 06444 (P_2)	68	0						
	F1	70	0	70	0	1:0	–	–	
	F2	393	127	390	130	3:1	0.092	0.761	
	BC1P1	100	108	104	104	1:1	0.308	0.579	
	BC1P2	174	0	174	0	1:0	–	–	
ICMB 95444 × ICMR 06222	ICMB 95444 (P_1)	0	75						1 dominant
	ICMR 06222 (P_2)	71	0						
	F1	74	0	74	0	1:0	–	–	
	F2	386	142	396	132	3:1	1.01	0.315	
	BC1P1	88	98	93	93	1:1	0.538	0.463	
	BC1P2	182	0	182	0	1:0	–	–	
ICMB 95444 × ICMR 11003	ICMB 95444 (P_1)	0	75						1 dominant
	ICMR 11003 (P_2)	75	0						
	F1	74	0	74	0	1:0	–	–	
	F2	212	67	209.25	69.75	3:1	0.172	0.679	
	BC1P1	78	68	73	73	1:1	0.685	0.408	
	BC1P2	117	0	117	0	1:0	–	–	
ICMB 95444 × IP 21187-P1	ICMB 95444 (P_1)	0	75						1 dominant
	IP 21187-P1 (P_2)	73	0						
	F1	72	0	72	0	1:0	–	–	
	F2	428	130	418.5	139.5	3:1	0.863	0.353	
	BC1P1	107	93	100	100	1:1	0.980	0.322	
	BC1P2	152	0	152	0	1:0	–	–	
ICMB 95444 × ICMB 89111	ICMB 95444 (P_1)	0	75						–
	ICMB 89111 (P_2)	0	65						
	F1	0	70	0	70	–	–	–	
	F2	0	490	0	490	–	–	–	
	BC1P1	0	204	0	204	–	–	–	
	BC1P2	0	180	0	180	–	–	–	

TABLE 4 Segregation analyses for blast reaction in the different generations derived from crosses between susceptible ICMB 95444 (P_1) and resistant parents (P_2) against *Magnaporthe grisea* isolate Pg 53

Cross	Generation	No. of plants observed		No. of plants expected		Expected ratio	χ^2	*p*	R-gene
		R	S	R	S				
ICMB 95444 × ICMB 93333	ICMB 95444 (P_1)	0	68						1 dominant
	ICMB 93333 (P_2)	76	0						
	F_1	98	0	98	0	1:0	–	–	
	F_2	309	104	310	103	3:1	0.007	0.93	
	BC_1P_1	104	88	96	96	1:1	1.33	0.25	
	BC_1P_2	197	0	197	0	1:0	–	–	
ICMB 95444 × ICMB 97222	ICMB 95444 (P_1)	0	68						1 dominant
	ICMB 97222 (P_2)	64	0						
	F_1	68	0	68	0	1:0	–	–	
	F_2	312	107	314	105	1:0	0.064	0.80	
	BC_1P_1	115	111	113	113	1:0	0.071	0.79	
	BC_1P_2	223	3	223	0	1:0	–	–	
ICMR 95444 × ICMR 06444	ICMB 95444 (P_1)	0	68						1 dominant
	ICMR 06444 (P_2)	74	0						
	F1	78	0	78	0	1:0	–	–	
	F2	322	99	316	105	3:1	0.49	0.48	
	BC1P1	108	114	111	111	1:1	0.16	0.69	
	BC1P2	203	0	203	0	1:0	–	–	
ICMB 95444 × ICMR 06222	ICMB 95444 (P_1)	0	68						1 dominant
	ICMR 06222 (P_2)	76	0						
	F1	88	0	88	0	1:0	–	–	
	F2	367	131	373.5	124.5	3:1	0.45	0.50	
	BC1P1	89	109	99	99	1:1	2.02	0.16	
	BC1P2	198	0	198	0	1:0	–	–	
ICMB 95444 × ICMR 11003	ICMB 95444 (P_1)	0	68						1 dominant
	ICMR 11003 (P_2)	82	0						
	F1	90	0	90	0	1:0	–	–	
	F2	278	94	279	93	3:1	0.01	0.91	
	BC1P1	106	89	97.5	97.5	1:1	1.48	0.22	
	BC1P2	223	0	223	0	1:0	–	–	
ICMB 95444 × IP 21187-P1	ICMB 95444 (P_1)	0	68						1 dominant
	IP 21187-P1 (P_2)	80	0						
	F1	73	0	73	0	1:0	–	–	
	F2	368	114	361.5	120.5	3:1	0.47	0.49	
	BC1P1	106	108	107	107	1:1	0.019	0.89	
	BC1P2	187	2	187	0	1:0	–	–	
ICMB 95444 × ICMB 89111	ICMB 95444 (P_1)	0	68						–
	ICMB 89111 (P_2)	0	75						
	F1	0	69	0	69	–	–	–	
	F2	0	426	0	426	–	–	–	
	BC1P1	0	226	0	226	–	–	–	
	BC1P2	0	204	0	204	–	–	–	

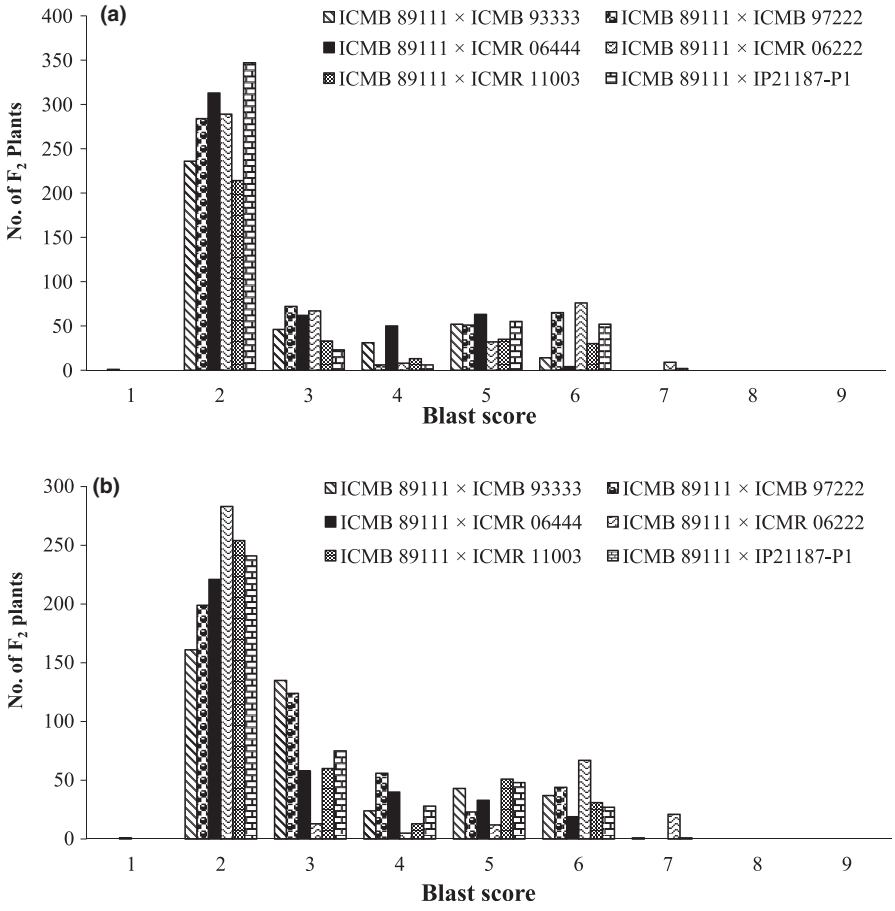

FIGURE 1 Blast score (1–9 scale) of F_2 plants derived from susceptible ICMB 89111 × resistant ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1 parents against *Magnaporthe grisea* isolates Pg 45 (a) and Pg 53 (b)

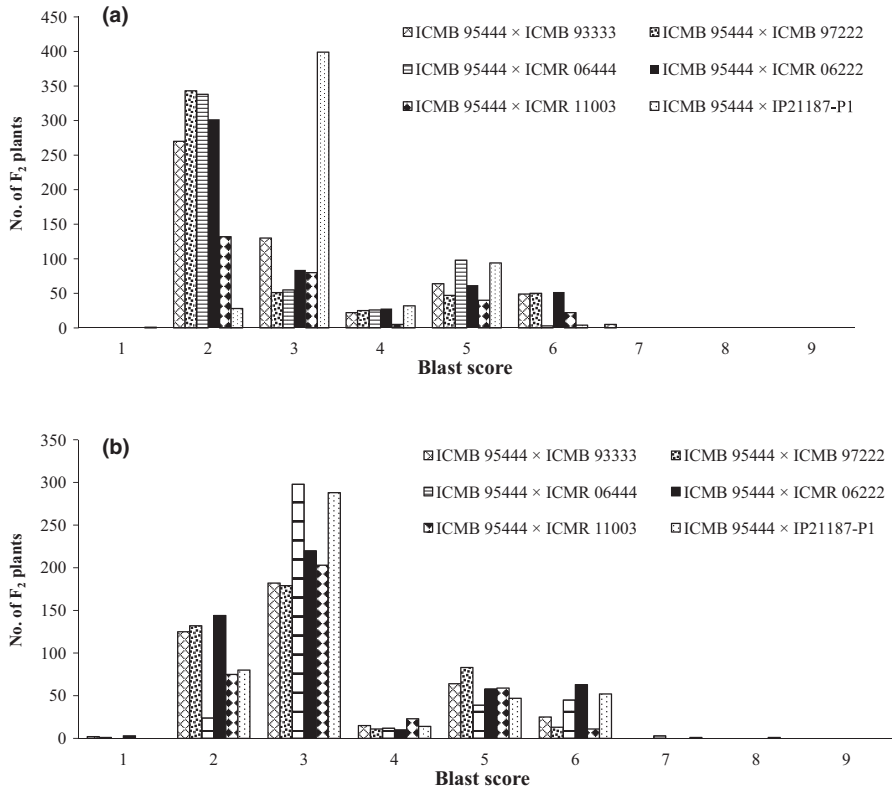

FIGURE 2 Blast score (1–9 scale) of F_2 plants derived from susceptible ICMB 95444 × resistant ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1 parents against *Magnaporthe grisea* isolates Pg 45 (a) and Pg 53 (b)

TABLE 5 Test of allelism for genes governing blast resistance in pearl millet lines to *Magnaporthe grisea* isolate Pg 45

Resistant × resistant (P1 × P2)	Generation	No. of plants observed		Allelic relationship
		Resistant	Susceptible	
ICMR 06444 × ICMR 06222	F_1	115	0	Allelic
	F_2	205	0	
ICMR 06444 × ICMB 97222	F_1	130	0	Allelic
	F_2	510	0	
ICMR 06444 × IP 21187-P1	F_1	126	0	Allelic
	F_2	514	0	
ICMR 06444 × ICMB 93333	F_1	97	0	Allelic
	F_2	490	0	
ICMR 06444 × ICMR 11003	F_1	105	0	Allelic
	F_2	512	0	
ICMR 06222 × ICMB 97222	F_1	101	0	Allelic
	F_2	498	0	
ICMR 06222 × IP 21187-P1	F_1	102	0	Allelic
	F_2	497	0	
ICMR 06222 × ICMB 93333	F_1	94	0	Allelic
	F_2	452	0	
ICMR 06222 × ICMR 11003	F_1	105	0	Allelic
	F_2	586	0	
ICMB 97222 × IP 21187-P1	F_1	89	0	Allelic
	F_2	481	0	
ICMB 97222 × ICMB 93333	F_1	71	0	Allelic
	F_2	479	0	
ICMB 97222 × ICMR 11003	F_1	75	0	Allelic
	F_2	426	0	
IP 21187-P1 × ICMB 93333	F_1	61	0	Allelic
	F_2	434	0	
IP 21187-P1 × ICMR 11003	F_1	76	0	Allelic
	F_2	473	0	
ICMB 93333 × ICMR 11003	F_1	75	0	Allelic
	F_2	403	0	

not show any segregation for resistance against Pg 45 and Pg 53. No segregation in the F_2s derived from crosses of R × R parents indicated that the same gene is conferring resistance in the selected resistant genotypes to Pg 45 and Pg 53.

4 | DISCUSSION

Studies on genes conferring resistance to individual pathogen races have been very well defined in many plant species, in particular cereals, where resistance to rusts, mildews and other fungal pathogens is well known (Knogge, 1991). The pathogen causing blast on pearl millet is highly variable making it essential to comprehend gene(s) conferring resistance to different races/pathotypes of the pathogen. The parents used in this study exhibited differential disease response; resistant genotypes showed high resistance (score ≤ 3) and susceptible plant demonstrated high susceptibility (score ≥ 7) to two isolates of *M. grisea* (Pg 45 and Pg 53). The resistant lines (ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1) selected for this study are of diverse genetic background and have been developed at ICRISAT over past several years. The germplasm accession IP 21187-P1 and the R-line ICMR 06222 are direct selections from IP 8695-1 and SDMV 90031-S1-3-3-2-1-3-2-2-1-B, respectively. Another line, ICMB 93333 ([843B × ICMPS 900-9-3-8-2]-21-8-4), was derived from the selection of a single cross. The remaining three lines were derived from selections of double cross (ICMB 97222), three-way cross (ICMR 06444) and bulk seed of multiple cross (ICMR 11003). Besides this, the *M. grisea* isolates Pg 45 and Pg 53 selected for screening were also diverse and represented two pathogenic groups/pathotypes (Sharma et al., 2013); Pg 45 was isolated in 2010 from infected leaf samples of pearl millet

TABLE 6 Test of allelism for genes governing blast resistance in pearl millet lines to *Magnaporthe grisea* isolate Pg 53

Resistant × resistant (P1 × P2)	Generation	No. of plants observed		Allelic relationship
		Resistant	Susceptible	
ICMR 06444 × ICMR 06222	F_1	61	0	Allelic
	F_2	379	0	
ICMR 06444 × ICMB 97222	F_1	70	0	Allelic
	F_2	420	0	
ICMR 06444 × IP 21187-P1	F_1	61	0	Allelic
	F_2	410	0	
ICMR 06444 × ICMB 93333	F_1	68	0	Allelic
	F_2	404	0	
ICMR 06444 × ICMR 11003	F_1	98	0	Allelic
	F_2	372	0	
ICMR 06222 × ICMB 97222	F_1	84	0	Allelic
	F_2	371	0	
ICMR 06222 × IP 21187-P1	F_1	59	0	Allelic
	F_2	846	0	
ICMR 06222 × ICMB 93333	F_1	90	0	Allelic
	F_2	413	0	
ICMR 06222 × ICMR 11003	F_1	71	0	Allelic
	F_2	554	0	
ICMB 97222 × IP 21187-P1	F_1	61	0	Allelic
	F_2	375	0	
ICMB 97222 × ICMB 93333	F_1	70	0	Allelic
	F_2	382	0	
ICMB 97222 × ICMR 11003	F_1	91	0	Allelic
	F_2	239	0	
IP 21187-P1 × ICMB 93333	F_1	44	0	Allelic
	F_2	418	0	
IP 21187-P1 × ICMR 11003	F_1	77	0	Allelic
	F_2	316	0	
ICMB 93333 × ICMR 11003	F_1	73	0	Allelic
	F_2	395	0	

inbred ICMB 95444 from Patancheru and Pg 53 was collected from infected leaf samples of hybrid cultivar '86M64', DuPont Pioneer, in 2010 from Jodhpur, Rajasthan, India. As all six resistant lines have been developed from diverse sources, it was assumed that the lines could differ in their genetics of blast resistance. For instance, nature of resistance genes was different in pearl millet landrace accessions of Burkina Faso and Tift 85DB (Senegal) to one isolate of *P. grisea* due to difference in background (Wilson, Wells, & Burton, 1989). In addition, as the isolates used to screen different crosses represented two different pathotypes and locations, pattern of inheritance of resistance in the pearl millet genotypes was speculated to be different.

In the inheritance study, the F_1s of all S × R crosses and all plant of BC_1P_2s (backcross with resistant parent) exhibited complete resistance to both Pg 45 and Pg 53. This complete resistance in F_1 and BC_1P_2 generations indicated dominant nature of resistance in all the resistant lines used in this study. Similar to that, dominance of resistance in pearl millet to *Pyricularia* has been reported by Hanna and Wells (1989) in a weedy relative of pearl millet (*P. glaucum* [L.] R. Br. subspecies *monodii* [Maire] Brunken) obtained from Senegal. The F_2 generation of all S × R crosses and corresponding BC_1P_1 generations (backcross with susceptible parent) showed clear segregation for resistant and susceptible plants to both the isolates. The resistance and susceptible plants in F_2 generations of all the S × R crosses showed best fit ratio of 3:1 R/S to both the isolates, suggesting dominant monogenic control of blast resistance in all the resistant lines. It was further confirmed by the corresponding BC_1P_1 generations in which resistant and susceptible plants segregated into a good fit of 1:1 R/S ratio against both the isolates. Similar results have been reported earlier by Gupta et al. (2012) in pearl millet resistant lines ICMR 06222 and ICMR 07555 against Patancheru (Pg 45) isolate of *M. grisea*. The genotype ICMR 06222

was also included in the present study and screened against two diverse pathotype isolates Pg 45 and Pg 53 of *M. grisea*. Six resistant lines were used in this study, and results of inheritance study revealed that resistance in these genotypes against Pg 45 and Pg 53 is governed by single dominant gene. These resistance sources would be of much use in the breeding programmes if they carry different genes for resistance that could be combined in the same genetic background to breed for durable resistance. Hence, test of allelism was conducted by crossing all the resistant genotypes with each other. Segregation in the F_2 generation of a cross of two resistant parents indicates that genes imparting resistance in the parent genotypes involved in that cross are nonallelic, that is different genes govern resistance in the test genotypes against a particular race/pathotype of the pathogen. However, in the present study, no segregation was observed in the F_2 generation of all the R × R crosses involving resistant parents ICMB 93333, ICMB 97222, ICMR 06444, ICMR 06222, ICMR 11003 and IP 21187-P1 when screened against Pg 45 and Pg 53. This indicated that same gene governs resistance to Pg 45 and Pg 53 in these diverse genotypes. Similar results have been reported by Gupta et al. (2012); 150 plants of a F_2 population derived from the cross ICMR 06222 × ICMR 07555 exhibited resistance reaction when tested against Pg 45 in the glasshouse indicating common gene for resistance in both the lines. In case of pearl millet downy mildew as well, same gene for resistance in two resistant genotypes, PPMI 519 and PPMI 517, has been reported (Deswal & Govila, 1994). In contrast, nonallelic nature of blast resistance genes in pearl millet landrace accessions from Burkina Faso and Tift 85DB has been reported by Wilson et al. (1989).

This study undertakes to drive breeding efforts in pearl millet for blast resistance. The resistant breeding lines used in this study were not intentionally bred for blast resistance indicating natural occurrence of resistance. Although six resistant lines used in this study were of diverse genetic background, they were found to carry same gene for blast resistance against equally diverse pathotype isolates Pg 45 and Pg 53 of *M. grisea*. However, there is a possibility of these resistant lines having additional genes for blast resistance because of their differential reaction to different isolates of *M. grisea*. IP 21187-P1 was found to be resistant to most of the isolates when screened under glasshouse conditions at ICRISAT (unpublished). The germplasm accession IP 21187 was found to be susceptible to Pg 45 in the initial screen with mean score 6 on 1–9 scale (Sharma et al., 2013); however, there were some resistant plants as well. As this accession was resistant to other four pathotypes (Pg 53, Pg 56, Pg 118 and Pg 119), the resistant plants were selected and selfed, and further screened to develop a stable line (IP 21187-P1) resistant to many pathotype isolates including Pg 45.

For an effective breeding programme, it is imperative to identify diverse resistance genes existing in crop species. This could be achieved by screening the genotypes against diverse pathotypes of the pathogen. Identification of diverse genes for blast resistance in pearl millet has important implications in breeding programmes aimed at pyramiding race/pathotype specific resistance genes into elite breeding lines. Pyramiding of genes is a strategy to develop varieties with durable resistance; accumulation of resistance genes with major effects delays the appearance of new races of the pathogen due to decreased pathogen fitness as more virulence genes would be required to overcome the resistance of the host (Thakur, Rai, Khairwal, & Mahala, 2008; Vanderplank, 1984). There is a strong consensus across the globe that growing genetically resistant cultivars is the most appropriate and cost-effective means of managing pests and diseases, which is one of the key components of crop improvement (Allen, 1983; Russell, 1978). Therefore, a potential strategy to maintain disease resistance for a long period of time would be the introgression of several resistance genes in a single cultivar. The results of this study lay the foundation for identification of diverse resistance sources and resistance genes present in them so as to allow for utilization of different resistance genes in the development of effective and durable blast-resistant cultivars.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHORS' CONTRIBUTION

Conception and design of study: Rajan Sharma, Shweta Singh, B. Pushpavathi and S K Gupta; acquisition of data: Shweta Singh and Chandramani Raj; analysis and/or interpretation of data: Rajan Sharma, Shweta Singh, S K Gupta and Ch. V. Durgarani; drafting the manuscript: Shweta Singh and Rajan Sharma; revising the manuscript critically for important intellectual content: S K Gupta, B. Pushpavathi, Ch. V. Durgarani and Chandramani Raj; reading and approval of the final manuscript: all authors.

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