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Inheritance and allelic relationship of foliar blast resistance gene(s) in pearl millet

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ABSTRACT

Foliar blast disease in pearl millet (*Pennisetum glaucum* (L.) R. Br.) caused by *Magnaporthe grisea* once considered as a minor disease, has become major threat for pearl millet cultivation during recent decade in almost all pearl millet growing areas. The present study is an attempt to understand the inheritance of foliar blast resistance in pearl millet against the natural inoculum of *Magnaporthe grisea* and also against artificial inoculum of its devastating strain PMg_DI. The experiment was conducted at ICRISAT, Hyderabad and IARI, New Delhi during 2017–19. F_2 and backcross populations derived from a susceptible genotype ICMB 95444 and two resistant lines PPMI-10018 and PPMI-2464 were used. Screening of individual plant in F_1s, F_2s, BCP_1s (susceptible parent × F_1) and BCP_2s (resistant parent × F_1) under field and controlled conditions indicated that the resistance is governed by a single dominant gene. The test of allelism was conducted by crossing the resistant parents. No segregation for resistance was observed in F_2 generation when screened against the natural inoculum of *Magnaporthe grisea* and its strain PMg_DI. This indicates that both the resistant parents possess the same gene.

Keywords: Climate change, Inheritance, *Magnaporthe grisea,* Pearl millet

Pearl millet (*Pennisetumg laucum* (L.) R. Br.) is one of the most extensively grown millet, mainly cultivated in arid and semi-arid regions of India and Africa. India is the largest producer of pearl millet in the world with 7.4 million ha area and average production of 9.13 million tonnes (Satyavathi 2019). Pearl millet utilizes soil moisture very efficiently and is highly tolerant to heat stress (Singhal *et al.* 2018, 2019). Foliar blast caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) was once considered as a minor disease but now it has emerged as a serious disease affecting both grain and forage production of pearl millet (Lukose *et al.* 2007). It can cause yield loss up to 80% amounting to 800 kg/ha (Chandra *et al.* 2017). Hence, now it has become essential to develop stable and durable blast resistant varieties in pearl millet. Success of this will depend mainly on availability of diverse genes against the pathogen. But the breeding for blast resistance is still in nascent stage and very limited information is available on this aspect. Gupta *et al.* (2012), Pawar *et al.* (2016) and Singh *et al.* (2018) reported that the resistance against blast is controlled by single dominant gene.

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Pearl millet blast, *Magnaporthe grisea* Delhi strain (PMg_DI) is one of the devastating strains of the pathogen (Prakash *et al.* 2019). It severely affects the crop in Delhi and surrounding areas. The strain can also infect wheat, oats and barley which are grown in *rabi* after pearl millet (Rajashekara *et al.* 2016, Prakash *et al.* 2019). The best and cheapest method to manage the disease is development and cultivation of resistant varieties. Success of this approach is related to the information about the inheritance of the resistance genes from various sources and also allelic relationship among them. Hence the present study was carried out to understand the inheritance of blast resistance and allelic relationship among genes providing resistance in two genotypes of pearl millet.

MATERIALS AND METHODS

The material was comprised of one highly susceptible parent ICMB 95444 with blast score ≤9 and two highly resistant parents PPMI-10018 and PPMI-2464 with blast score ≤3. ICMB 95444 is one of the designated early seed parent bred at International Crops Research Institute for the Semi-arid Tropics (ICRISAT) [pedigree: (81-1164 DB/85-1856 LR-16-B × 843DMR1)-14-6-3], PPMI-10018 is a blast resistant line developed at ICAR-Indian Agricultural Research Institute, New Delhi through pedigree breeding and PPMI-2464 is the early derivative selected from the induced mutagenesis of ICMR 06444 at ICAR-IARI, New Delhi.

These parental lines were screened against blast pathogen in field as well as under artificial epiphytotic conditions (Prakash *et al.* 2016, Singh *et al.* 2018).

Development of segregating populations: The F_1 plants developed by the crosses ICMB 95444 × PPMI-10018 (susceptible × resistant), ICMB 95444 × PPMI-2464 (susceptible × resistant) and PPMI-2464 × PPMI-10018 (resistant × resistant) were raised and selfed during summer 2017 at ICAR-IARI, New Delhi to develop F_2 populations. Backcross populations BCP_1 (susceptible parent × F_1s) and BCP_2 (resistant parent × F_1s) were also developed.

Screening of population for blast disease reaction: The populations were screened under three different conditions i.e field conditions using natural inoculum, artificial epiphytotic conditions in net house and phenotypic chamber using artificial inoculum of PMg_DI. F_2 and back cross populations were grown during *kharif* 2018 at farm of ICAR-IARI, New Delhi for evaluation under field conditions. After every five rows of segregating population, four rows of susceptible parent and two rows each of resistant parents were planted. The populations were screened under natural conditions at Delhi because it is a hot spot for pearl millet blast. Blast severity was scored at 0-9 scale at dough stage.

During summer 2018, the F_2 and back cross populations were sown in net houses of ICAR-IARI, New Delhi. Four rows of susceptible parent and two rows each of resistant parents were grown after every five rows of segregating population. Aqueous conidial suspension of PMg_DI with concentration 35×10^5 conidia/ml was sprayed twice, at pre-tillering and flowering stage. Blast severity was scored on 0-9 scale at dough stage. For the disease evaluation in phenotypic chamber inoculum of PMg_DI at concentration of 35×10^5 conidia/ml was sprayed thoroughly over the leaves of 15 days old seedlings by using glass atomizer. The inoculated plants were kept under dark for initial 24 h in phenotypic chamber. The temperature was maintained at 25±1ºC and RH above 90%. The plants were irrigated regularly for maintaining high humidity inside the chamber and also with proper illumination of light duration of 10 h light (10000 Lux units) & 14 h darkness (Prakash *et al.* 2016). After seven days of inoculation blast severity was scored using 0-9 scale. The plants having score of ≤3 were rated as resistant and those with score of ≥4 as susceptible.

Statistical analysis: Foliar blast reaction scored over individual populations was statistically analysed using the Anderson-Darling test for normality. Since the distribution was skewed, the Hartigans' dip test was conducted to find the modality (unimodality/multimodality). Anderson-Darling normality test and Hartigans' dip test for unimodality or multimodality were carried out using statistical package R version 3.6.1. Chi square test ($P\leq0.05$) was used to compare the ratio of observed resistant to susceptible plants in the segregating populations in field conditions and artificial epiphytotic conditions.

RESULTS AND DISCUSSION

Inheritance of blast resistance: The blast reaction scale significantly deviated from normal distribution ($p = 0.000$) when analysed using the Anderson-Darling test for normality. The normal curve was skewed to the left showing that most of the lesions were scored ≤5. Since the distribution was skewed, the Hartigans' dip test was conducted to find the modality (unimodality/multimodality). The result of Hartigans' dip test showed non-unimodal i.e. at least bimodal (multimodal) in all the populations. This result indicated that the blast resistance was controlled by one or two genes. In all three conditions the susceptible parent ICMB 95444 was severely infected, showing 7-9 rating of severity. The resistant parent PPMI-10018 showed 0-1 rating whereas another resistant parent PPMI-2464 showed 2-3 rating indicating the possibility of two different genes conferring the resistance (Table 1).

In the cross, ICMB 95444 × PPMI-10018, 112 F_1, 465 F_2, 452 BCP_1, and 447 BCP_2 plants were screened under field conditions. In net house, 105 F_1, 480 F_2, 460 BCP_1, 438 BCP_2 and in phenotypic chamber 113 F_1, 535 F_2, 532 BCP_1 and 526 BCP_2 plants were screened, respectively. All of the F_1 and BCP_2 plants exhibited resistance under all three conditions, which indicated that resistance in PPMI-10018 is governed by dominant gene(s). In the F_2 population, 338 plants were resistant and 127 were susceptible under field condition. Similarly, 351 resistant and 129 susceptible individuals in net house condition and 395 resistant and 140 susceptible plants in phenotypic chamber were observed. Ratios in all the three conditions well fitted into the segregation ratio of 3:1 (resistant: susceptible) (Table 1). Among the BCP_1 population, 220 plants were resistant and 232 plants exhibited susceptible reaction, 236 resistant and 224 susceptible plants, 269 resistant and 263 susceptible plants were observed in field, net house and phenotypic chamber, respectively indicating 1:1 (resistant:susceptible) segregation ratio (Table 1). These ratios confirm that the resistance in PPMI-10018 is governed by a single dominant gene.

In the other cross, ICMB 95444 × PPMI-2464, all 108 plants of F_1 and 493 of BCP_2, 110 F_1 and 455 BCP_2, 108 F_1 and 538 BCP_2 plants were screened in field, net house and phenotypic chamber and exhibited resistant reaction. This indicates dominant nature of the resistance gene(s) in PPMI-2464. F_2 and BCP_1 segregated into resistant (367, 354, 389 plants in F_2 and 220, 250 and 269 plants in BCP_1) and susceptible (107, 102, 134 plants in F_2 and 254, 225 and 249 plants in BCP_1) when screened in field, net house and phenotypic chamber, respectively. These ratios revealed best goodness of fit ratio of 3:1 and 1:1 in F_2 and BCP_1 (Table1), respectively. These results showed that resistance in PPMI-2464 is also monogenic. Similar observations were also made by Hanna and Wells 1989 in a weedy relative of pearl millet (*P. glaucum* [L.] R. Br. sub-species *monodii* [Maire] Brunken), Gupta *et al.* (2012), Pawar *et al.* (2016) and Singh *et al.* (2018). In F_2 generations of both susceptible × resistant crosses showed goodness of fit for 3:1 (resistant: susceptible) in field, net house and phenotypic chamber. This indicates that resistance against natural inoculum of

Table 1 Segregation pattern for blast reaction in the P_1, P_2, F_1, F_2, BCP_1 and BCP_2 generations derived from crosses between susceptible parent (P_1) and resistant parents (P_2) against *Magnaporthe grisea* under different conditions

Cross	Conditions	Generation	No of plants observed		No of plants expected		Expected ratio	χ^2	P	R-gene
			R	S	R	S				
ICMB 95444 × PPMI-10018	Field (Natural inoculum)	ICMB 95444 (P_1)	0	102						1 dominant
		PPMI-10018 (P_2)	132	0						
		F_1	112	0	112	0	1:0	-	-	
		F_2	338	127	349	116	3:1	1.33	0.23	
		BCP_1	220	232	226	226	1:1	0.32	0.57	
		BCP_2	447	0	447	0	1:0	-	-	
ICMB 95444 × PPMI-2464		ICMB 95444 (P_1)	0	102						1 dominant
		PPMI-2464 (P_2)	126	0						
		F_1	108	0	108	0	1:0	-	-	
		F_2	367	107	355	118	3:1	1.69	0.19	
		BCP_1	220	254	237	237	1:1	2.44	0.12	
		BCP_2	493	0	493	0	1:0	-	-	
ICMB 95444 × PPMI-10018	Net house (PMg_DI strain)	ICMB 95444 (P_1)	0	96						1 dominant
		PPMI-10018 (P_2)	92	0						
		F_1	105	0	105	0	1:0	-	-	
		F_2	351	129	360	120	3:1	0.90	0.34	
		BCP_1	236	224	230	230	1:1	0.31	0.58	
		BCP_2	438	0	438	0	1:0	-	-	
ICMB 95444 × PPMI-2464		ICMB 95444 (P_1)	0	96						1 dominant
		PPMI-2464 (P_2)	97	0						
		F_1	110	0	110	0	1:0	-	-	
		F_2	354	102	342	114	3:1	1.68	0.19	
		BCP_1	250	225	237.5	237.5	1:1	1.32	0.25	
		BCP_2	455	0	455	0	1:0	-	-	
ICMB 95444 × PPMI-10018	Phenotypic chamber (PMg_DI strain)	ICMB 95444 (P_1)	0	92						1 dominant
		PPMI-10018 (P_2)	89	0						
		F_1	113	0	113	0	1:0	-	-	
		F_2	395	140	401	134	3:1	0.4	0.53	
		BCP_1	269	263	266	266	1:1	0.07	0.79	
		BCP_2	526	0	526	0	1:0	-	-	
ICMB 95444 × PPMI-2464		ICMB 95444 (P_1)	0	92						1 dominant
		PPMI-2464 (P_2)	87	0						
		F_1	108	0	108	0	1:0	-	-	
		F_2	389	134	392	131	3:1	0.11	0.74	
		BCP_1	269	249	259	259	1:1	0.77	0.38	
		BCP_2	538	0	538	0	1:0	-	-	

χ^2 (0.05,1) = 3.84; R= Resistant; S= Susceptible

Magnaporthe grisea and PMg_DI strain of pearl millet blast pathogen is controlled by single dominant gene in the resistant parents. It was further confirmed by the respective BCP_1 generations in which resistant and susceptible plants segregated into a goodness of fit of 1:1 in all the conditions. Similar results have been reported by Gupta *et al.* (2012), Pawar *et al.* (2016) and Singh *et al.* (2018).

Test of allelism: Data of blast screening of F_2 generation derived from resistant parents PPMI-2464 × PPMI-10018 was analysed (Table 2). No segregation was observed in F_2. All the 478, 483 and 536 F_2 plants showed highly resistant reaction with rating of 0-1 in field, net house and phenotypic chamber, respectively. These results confirm that both the parents have same resistance gene.

The blast pathogen is highly variable so it is necessary to develop a cultivar with different resistance genes for stable and durable resistance. Recently wild pearl millet subspecies *violaceum* found in Niger and Chad has resistance to blast (Major 2019). The test of allelism was carried out by crossing both the resistant parents. Since the resistant parents showed different degree of resistance under all three conditions there was a doubt that different genes might be controlling the disease resistance in these two resistant parents. However, no segregation was observed in the F_2 generation of the resistant × resistant cross under any condition. This indicated that same gene governs resistance in both the parents. The difference in the degree of disease severity might be due to any post transcriptional or post translational modification of the gene products which needs further investigation. The results obtained are in accordance with the results reported by Gupta *et al.* (2012) and Singh *et al.* (2018). In contrast, Wilson *et al.* (1989) reported non-allelic nature of blast resistance genes in pearl millet landraces from Burkina Faso and Tift 85DB.

In rice, more than 100 blast resistance genes have been identified and many have been incorporated into rice lines. But, most of these resistance genes have been broken down to the blast pathogen because of rapid changes in pathogenicity of the blast fungus and also due to the race specificity (Suh *et al.* 2009). Various potential mechanisms, including aneuoploidy, parasexual recombination, heterokaryosis and sexual recombination have been proposed to explain frequent race changes in the rice blast fungus (Kang and Lee 2000). Therefore, efforts should be made to study pathogenic variability in *Magnaporthe grisea* isolates from different pearl millet growing areas in India and resistant sources to different pathotypes should be identified. Therefore, for success of any disease breeding programme, identification of diverse resistance genes against the pathogen is of prime importance, especially in a pathogen which is highly variable like pearl millet blast pathogen. If many major genes are available then genes can be pyramided into elite breeding lines and also to ruling varieties, which will result into development of stable and durable resistance against blast. The appearance of new races of the pathogen is delayed by accumulation of resistance genes with major effects due to decreased pathogen fitness as many virulence genes would be required to break down the resistance of the host (Vanderplank 1984). The present investigation lays foundation for identification of different genes from diverse source to use the host plant resistance in an effective way. However, more studies are required to identify different resistance genes especially for their spatial and temporal deployment.

The foliar blast resistance against natural inoculum of *Magnaporthe grisea* under field conditions and against PMg_DI under net house and growth chamber conditions is controlled by a single dominant gene, as both F_2 and backcross populations exhibited 3:1 (resistant : susceptible) and 1:1 ratios (resistant : susceptible), respectively. The test

Table 2 Test of allelism for genes governing blast resistance in resistant parents

Cross	Growing condition	Generation	No of plants observed		Allelic relationship
			R	S	
PPMI-2464 × PPMI-10018	Field (Natural inoculum)	PPMI-2464 (P_1)	126	0	Allelic
		PPMI-10018 (P_2)	132	0	
		F_1	118	0	
		F_2	478	0	
	Net house (PMg_DI strain)	PPMI-2464 (P_1)	97	0	Allelic
		PPMI-10018 (P_2)	92	0	
		F_1	115	0	
		F_2	483	0	
	Growth chamber (PMg_DI strain)	PPMI-2464 (P_1)	86	0	Allelic
		PPMI-10018 (P_2)	84	0	
		F_1	106	0	
		F_2	536	0	

R= Resistant; S= Susceptible

of allelism showed that the gene governing the resistance in PPMI-10018 and PPMI-2464 are the same. This resistance gene should be mapped so that it can be used in marker assisted foliar blast resistance breeding.

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