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Research Article

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

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Abstract
Pearl millet blast, caused by *Pyricularia grisea* (Cooke) Sacc, has recently emerged as a serious disease in India. Two resistant (ICMB 06444 and DHLB 10B) and two susceptible maintainer lines (ICMB 95444 and ICMB 89111) were selected and three crosses were made *viz*., ICMB 95444 x ICMB 06444 (susceptible x resistant), DHLB 10 B x ICMB 89111 (resistant x susceptible) and DHLB 10 B x ICMB 06444 (resistant x resistant) and generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 of each cross were used to study the inheritance of blast resistance in pearl millet. These were evaluated for disease reaction with artificial inoculation under field (Rahuri and Dhule) and greenhouse condition (Rahuri). 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, blast, leaf spot and inheritance

Introduction

Blast, also known as leaf spot caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*) has emerged as a serious disease affecting forage and grain production in pearl millet in India (Lukose *et al.* 2007; Anonymous, 2009). 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.*, 1989). In India, it was first reported from Kanpur, Uttar Pradesh (Mehta *et al.*, 1953). 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. Breeding for blast resistance is the only surest measure to overcome this disease. The field and greenhouse blast screening techniques have been developed and resistance sources have been identified (Thakur *et al.*, 2009). Knowledge on the inheritance of resistance will have a direct bearing on the breeding efficiency for genetic management of this disease. Hence, there is a need to study the inheritance of blast resistance in pearl millet. So far very limited research work has been carried out on these aspects. Considering the importance of the crop and the above facts, there is a need to generate information on blast inheritance pattern.

Materials and methods

Two resistant (ICMB 06444 and DHLB 10B) and two susceptible maintainer lines (ICMB 95444 and ICMB 89111) were selected for foliar blast disease. These selected parental lines were re-confirmed for their foliar blast reaction in the greenhouse available at MPKV, Rahuri. Three crosses *viz*., Cross I: ICMB 95444 x ICMB 06444 (Susceptible x Resistant), Cross II: DHLB 10 B x ICMB 89111 (Resistant x Susceptible) and Cross III: DHLB 10 B x ICMB 06444 (Resistant x Resistant) were generated during summer 2013 at Rahuri. Subsequently, during *kharif* 2013 season, F_1s were raised and 8-10 panicles in each F_1s were selfed using parchment butter paper bags to generate F_2 population. Bulk pollen from 8-10 F_1 panicles were used to pollinate the corresponding susceptible and resistant parents to develop B_1 (F_1 × susceptible parent) and B_2 (F_1 × resistant parent) populations, respectively.

All the parents, three F_1s, three F_2s, three B_1s and three B_2s were screened against *P. grisea* isolate in the field condition at Rahuri and Dhule during *kharif* 2014 in single replication with two rows each of F_1, B_1, B_2 and sixteen rows of F_2 with the row length of 5.0 m each. Test lines (different generations) were grown in the central four rows and highly susceptible line ICMB 89111 was grown on the first row and after every fifth rows. Seedlings were inoculated at pre-tillering and pre-flowering stage with aqueous conidial suspension (about 1×10^7 spores ml^{-1}) of *P. grisea*. Disease severity was recorded at hard dough stage using a 1–9 progressive scale developed by Thakur *et al.* (2009). The greenhouse screening was conducted at Rahuri during *Kharif*-2014 season. The plastic root trainer with 25 cavities/each trainer (5cm diameter each cavity cup) were filled with autoclaved soil-sand-cocopit & FYM mix (2:1:1 volume). Seeds of test lines of different generations *viz.*, P_1, P_2, F_1, F_2, B_1 and B_2 and susceptible check (ICMB 89111) were grown in root trainer (4 seed/cavity) in greenhouse and maintained at 30±1°C. Plastic root trainer was irrigated adequately and test seedlings were grown for 10-12 days. All grown seedlings (15 days old) were spray-inoculated with an aqueous conidial

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suspension (about 1×10^7 spores ml^{-1}) of *P. grisea* and exposed to high humidity (>90% RH) under misting for 10 days. Seedlings were examined visually daily for blast symptoms development. The highly susceptible genotypes ICMB 89111 and ICMB 95444 showed symptoms within 4-5 days after inoculation (Plate 1). Disease severity score was recorded 10 days after inoculation using 1-9 rating scale (Thakur *et al*., 2009). Chi-square test (χ^2 test) was applied to find out the test ratios for blast resistance inheritance.

Results and discussion

All F_1's of three crosses were resistant (score of ≤ 3) under both greenhouse (Rahuri) and field (Rahuri and Dhule) conditions. Among parents, the susceptible parents ICMB 95444 and ICMB 89111 showed susceptibility to blast (score>5), while resistant parent ICMB 6444 and DHLB 10 B showed all resistant plants (score of ≤ 3). In F_2 of both CI and CII , B_1 of CI and B_2 of CII, there was a clear-cut segregation either for resistant plants (score of ≤ 3) or for susceptible plants (score>5) and no plant had a score of above 3 and below 5 for blast reaction under both greenhouse (Rahuri) and field (Rahuri and Dhule) conditions.

The F_2 population of C-I (S x R: ICMB 95444 x ICMB 6444) had good fit in to the segregation ratio of 3R : 1S in greenhouse condition at Rahuri (308 R plants : 95 S plants), field condition at Rahuri (339 R plants : 103 S plants) and field condition at Dhule (327 R plants : 105 S plants) with chi-square value of 0.44, 0.64 and 0.11 in greenhouse condition at Rahuri, field condition at Rahuri and Dhule, respectively (Table 1). The B_1 of the cross I, S x R had good fit into the ratio of 1R: 1S in greenhouse condition at Rahuri (38 R plants: 33 S plants), field condition at Rahuri (25 R plants : 32 S plants) and field condition at Dhule (24 R plants : 28 S plants) with chi-square value of 0.35, 0.86 and 0.31 in greenhouse condition at Rahuri, field condition at Rahuri and Dhule, respectively.

The F_2 population of cross II (R x S: DHLB 10B x ICMB 89111) also gave good fit into the segregation ratio of 3R: 1S in all the three conditions *viz*., greenhouse condition of Rahuri (325R plants: 102S plants; χ^2=0.28), field condition of Rahuri (321R plants: 93S plants; χ^2=1.42) and field condition of Dhule (321R plants: 96S plants; χ^2=0.87). The B_2 segregation of this cross had good fit to 1R : 1S segregation ratio in greenhouse condition at Rahuri (32R plants : 35S plants; χ^2=0.13), field condition at Rahuri (29R plants : 26S plants; χ^2=0.16) and field condition at Dhule (27R plants : 24S plants; χ^2=0.18) (Table 1). While, in cross III (RxR : DHLB 10B x ICMB 6444) all plants were observed as resistant for F_2 and backcross generation in all the three conditions. The goodness of fit to 3R:1S segregation ratio in the two F_2s (of C-I and C-II) and 1R:1S ratio in their two backcross populations under both greenhouse and field conditions lead to conclude that foliar blast resistance in the pearl millet lines used for this study is controlled by a single dominant gene. However, no segregation was observed in F_2 and backcross population of cross DHLB 10B x ICMB 6444 (R x R) for blast resistance, which indicate that both parents might have the same resistant 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) and single dominant gene was reported by Gupta *et al.* (2012) in their studies of pearl millet foliar blast resistance. The identified blast resistant plants could be used to develop blast resistant variety/hybrid and efforts should be made to study pathogenic variability in *P. grisea* isolates from different pearl millet growing areas in India and to identify resistant sources to different pathotypes for utilizing them in breeding program to manage this disease through host plant resistance.

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Table 1. Segregation for blast resistant (R) and susceptible (S) plants in P_1, P_2, F_1, F_2, B_1 and B_2 generations and χ^2 test in pearl millet

Cross	Environment	Generation	No. of plants observed			Expected Ratio		No. of plants expected		χ^2	P
			R	S	Total	R	S	R	S		
C-I (S x R) ICMB 95444 x ICMB 6444	Greenhouse (Rahuri)	P_1	0	69	69	-	-	-	-	-	-
		P_2	64	0	64	-	-	-	-	-	-
		F_1	69	0	69	-	-	-	-	-	-
		F_2	308	95	403	3	1	302.25	100.75	044	0.51
		BCP_1	38	33	71	1	1	35.5	35.5	0.35	0.55
		BCP_2	65	0	65	-	-	-	-	-	-
	Field (Rahuri)	P_1	0	41	41	-	-	-	-	-	-
		P_2	39	0	39	-	-	-	-	-	-
		F_1	52	0	52	-	-	-	-	-	-
		F_2	339	103	442	3	1	331.5	110.5	0.68	0.41
		BCP_1	25	32	57	1	1	28.5	28.5	0.86	0.35
		BCP_2	56	0	56	-	-	-	-	-	-
	Field (Dhule)	P_1	0	32	32	-	-	-	-	-	-
		P_2	38	0	38	-	-	-	-	-	-
		F_1	47	0	47	-	-	-	-	-	-
		F_2	327	105	432	3	1	324	108	0.11	0.74
		BCP_1	24	28	52	1	1	26	26	0.31	0.58
		BCP_2	57	0	57	-	-	-	-	-	-
C-II (R x S) DHLB 10B x ICMB 89111	Greenhouse (Rahuri)	P_1	70	0	70	-	-	-	-	-	-
		P_2	0	65	65	-	-	-	-	-	-
		F_1	72	0	72	-	-	-	-	-	-
		F_2	325	102	427	3	1	320.25	106.75	0.28	0.60
		BCP_1	51	0	51	-	-	-	-	-	-
		BCP_2	32	35	67	1	1	33.5	33.5	0.13	0.71
	Field (Rahuri)	P_1	37	0	37	-	-	-	-	-	-
		P_2	0	24	24	-	-	-	-	-	-
		F_1	53	0	53	-	-	-	-	-	-
		F_2	321	93	414	3	1	310.5	103.5	1.42	0.23
		BCP_1	45	0	45	-	-	-	-	-	-
		BCP_2	29	26	55	1	1	27.5	27.5	0.16	0.69

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Table 1. Contd.,

Cross	Environment	Generation	No. of plants observed			Expected Ratio		No. of plants expected		χ^2	P
			R	S	Total	R	S	R	S		
C-II (R x S)		P_1	33	0	33	-	-	-	-	-	-
		P_2	0	29	29	-	-	-	-	-	-
DHLB 10B	**Field (Dhule)**	F_1	51	0	51	-	-	-	-	-	-
x		F_2	321	96	417	3	1	312.75	104.25	0.87	0.35
ICMB 89111		BCP_1	46	0	46	-	-	-	-	-	-
		BCP_2	27	24	51	1	1	25.5	25.5	0.18	0.67
		P_1	68	0	68	-	-	-	-	-	-
		P_2	71	0	71	-	-	-	-	-	-
	Greenhouse	F_1	69	0	69	-	-	-	-	-	-
	(Rahuri)	F_2	407	0	407	-	-	-	-	-	-
		BCP_1	59	0	59	-	-	-	-	-	-
		BCP_2	64	0	64	-	-	-	-	-	-
		P_1	48	0	48	-	-	-	-	-	-
C-III (RxR)		P_2	51	0	51	-	-	-	-	-	-
		F_1	54	0	54	-	-	-	-	-	-
DHLB 10B	**Field (Rahuri)**	F_2	418	0	418	-	-	-	-	-	-
x		BCP_1	51	0	51	-	-	-	-	-	-
ICMB 6444		BCP_2	52	0	52	-	-	-	-	-	-
		P_1	49	0	49	-	-	-	-	-	-
		P_2	46	0	46	-	-	-	-	-	-
		F_1	51	0	51	-	-	-	-	-	-
	Field (Dhule)	F_2	415	0	415	-	-	-	-	-	-
		BCP_1	53	0	53	-	-	-	-	-	-
		BCP_2	47	0	47	-	-	-	-	-	-

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Plate 1. Screening of pearl millet genotypes against blast disease under field conditions

C-I (S x R): ICMB 95444 x ICMB 6444)

C-II (R x S): DHLB 10B x ICMB 89111

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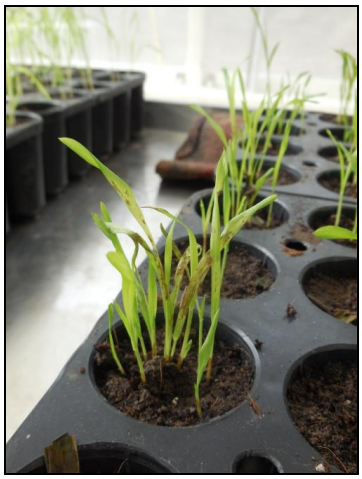
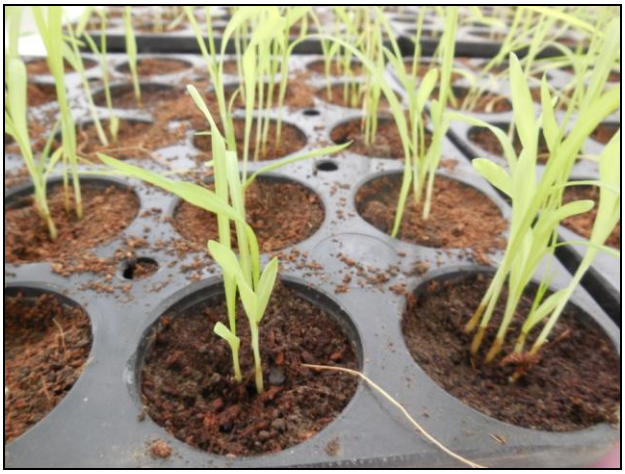
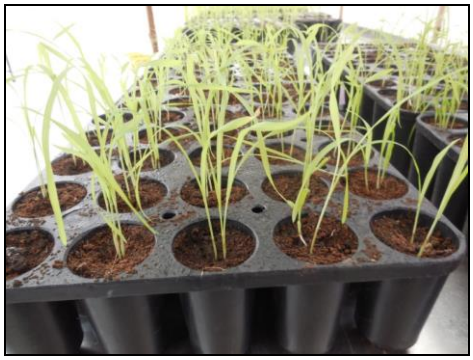
Plate 2. Screening of pearl millet genotypes against blast disease under glass house conditions



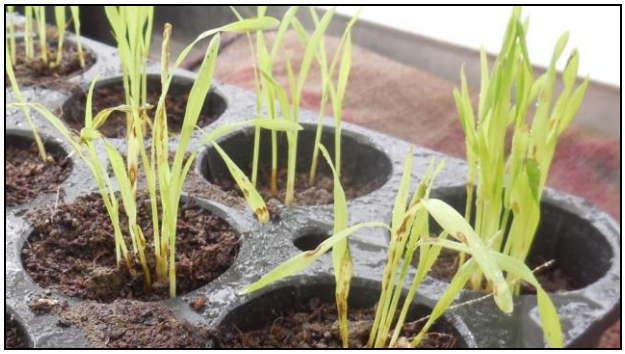
CI-P_1: ICMB 95444 (Susceptible Parent)



CI -P_2: ICMB 06444 (Resistant Parent)



CII-P_1: DHLB 10 B (Resistant Parent)



CII-P_2: ICMB 89111 (Susceptible Parent)