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Morpho-Molecular Characterization of Rice Genotypes for Resistance to Bacterial Leaf Blight (BLB)

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ABSTRACT

Bacterial leaf blight (BLB) is one of the most devastating diseases of rice (*Oryza sativa* L.), causing substantial yield losses and posing a serious threat to food and livelihood security across rice-dependent regions of Asia and Africa. In this study, 71 rice genotypes developed through crosses among elite and improved lines, were evaluated for bacterial leaf blight (BLB) resistance using artificial clip inoculation at maximum tillering stage, with resistant (Improved Samba Mahsuri) and susceptible (Taichung Native-1, Krishnaveni) checks, under field conditions at Bapatla and Maruteru, Andhra Pradesh, India. Phenotypic screening identified nine genotypes exhibiting disease reaction towards resistance (disease scores 1–3) at both sites. Molecular screening for five BLB resistance (R) genes, *Xa21*, *xa13*, *xa5*, *Xa4*, and *Xa2*, revealed BPT-3170 carried four R genes (*xa13+xa5+Xa4+Xa2*), while eight genotypes had two genes, and 30 genotypes carried one gene. Phylogenetic analysis using 14 R gene-linked markers grouped the genotypes into three major clusters. BPT-3170 exhibited phenotypic resistance along with multiple R genes, indicating its potential to confer broad spectrum resistance and can serve as a valuable donor in BLB resistance breeding. The study also revealed the breakdown of single-gene resistance and low frequencies of *xa5*, *xa13*, and *Xa21*. These findings highlight the importance of pyramiding multiple R genes to achieve durable resistance against BLB.

Keywords: Bacterial leaf blight**,** Microsatellite markers, R genes, Genetic diversity.

INTRODUCTION

Rice (*Oryza sativa L.*) is a vital staple crop for billions of people worldwide and serves as a cornerstone of global food security and nutrition. Its productivity is challenged by as many as

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60 known rice diseases (Ou, 1972), with bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), being one of the most destructive. BLB leads to yield losses ranging from 20% to 50% (Singh *et al.*, 2011), with severity depending on the rice cultivar, environmental conditions, and management practices. *Xoo* causes wilt, yellowing, and death of rice plants. This disease has become a major concern, particularly in Asian countries, due to the congenial climatic conditions that contribute to frequent epidemics and the lack of effective control measures, making the use of resistant cultivars as the only reliable management strategy. However, the durability of resistance is often compromised due to the rapid evolution of *Xoo* races under selection pressure, necessitating continuous efforts to explore and identify new resistant resources (Xia *et al.*, 2012).

The *Oryza* base repository currently lists 44 resistance genes (*Xa1* to *Xa44*) conferring varying levels of resistance to diverse *Xoo* strains, highlighting the genetic complexity of the pathogen-host interaction. In this study, the genotypes are screened for the resistance genes *Xa21, xa13, xa5, Xa4,* and *Xa2,* exhibiting complementary resistance mechanisms. Gene *Xa21,* identified in *Oryza longistaminata,* provides broad-spectrum resistance to diverse *Xoo* strains worldwide. *xa13,* found in the variety BJ1, confers race specific resistance. xa5 imparts resistance to *Xoo* isolates from India and Nepal at all growth stages (Adhikari *et al,* 1995). Xa4 is known for its durable resistance and is widely employed in commercial breeding programs. (Ma *et al.*, 1999 and Sun *et al.*, 2003). Xa2, identified in cultivars *Tetep* and *Rantai Emas II,* offers strong resistance across diverse backgrounds (Sakaguchi, 1967).

We screened 70 diverse rice genotypes for resistance to BLB using a combination of phenotypic screening through artificial inoculation and molecular marker analysis. By integrating these complementary approaches, this study aims to identify robust resistant genotypes that can serve as valuable donors in breeding programs, ultimately contributing to the development of durable BLB-resistant rice varieties for sustainable rice production.

MATERIALS AND METHODS

Plant Material

The experimental material comprised of 74 genotypes with Improved Samba Mahsuri (ISM) as BLB resistant check, Taichung Native-1 and Krishnaveni as BLB susceptible checks and, Samba Mahsuri as yield check. These genotypes are developed and provided by ARS, Bapatla, Andhra Pradesh, India. Their pedigree is presented in Table 1.

Table 1. List and Pedigree of the Genotypes used in the study.

S. NO	GENOTYPE	PEDIGREE	S. NO	GENOTYPE	PEDIGREE
1.	BPT-1235	SARBARMATI/W-12708	38.	BPT-3129	BPT-5204/MTU-1075
2.	BPT-2231	BPT-4358/IR-64	39.	BPT-3130	BPT-5204/MTU-1075
3.	BPT-2295	BPT-1768/NLR-33641	40.	BPT-3133	BPT-5204/MTU-1001
4.	BPT-2411	BPT-5204/BPT-4358	41.	BPT-3135	BPT-5204/MTU-1001
5.	BPT-2595	MUTANT OF BPT-2270	42.	BPT-3136	RP-BIO-226/IRGC-48493
6.	BPT-2620	MTU-1061/N-22	43.	BPT-3137	RP-BIO-226/IRGC-48493
7.	BPT-2677	MTU-2077/AJAY/MTU-2077	44.	BPT-3145	RP-BIO-226/IRGC-48493
8.	BPT-2764	MUTANT OF BPT-2270	45.	BPT-3147	B-95-1/RPHR-1005/B-95-1
9.	BPT-2766	BPT-2270/NLR-145	46.	BPT-3148	RP-BIO-226/IRGC-23385/NIDHI/MTU-1081
10.	BPT-2776	BPT-2231/NLR-145	47.	BPT-3150	RP-BIO-226/JARAVA
11.	BPT-2782	NLR-145/MTU-2077	48.	BPT-3151	RP-BIO-226/JARAVA
12.	BPT-2808	BPT-2231/NLR-145	49.	BPT-3159	CULTURE-0910023/RP-BIO-226/CULTURE-0910023-8/BPT-5204/TETEP
13.	BPT-2824	MTU-7029/NLR-34449	50.	BPT-3164	B-95-1/RPHR-1005/B-95-1
14.	BPT-2846	MTU-1061/IR-78585-64-2-4-3	51.	BPT-3168	MTU-7029/IRGC-18195/MTU-1081
15.	BPT-2848	SWARNA/IRGC-18195/MTU-1081	52.	BPT-3170	RP-BIO-226/JARAVA
16.	BPT-2849	NLR-34449/MTU-5249	53.	BPT-3172	RP-BIO-226/IRGC-48493
17.	BPT-2854	MTU-1061/IR-78585-64-2-4-3	54.	BPT-3178	CULTURE-01120305/CULTURE-0910025-7
18.	BPT-2863	MTU-2077/NLR-34449	55.	BPT-3208	NLR-34449/ANNADA/NLR-34449
19.	BPT-2950	NLR-34449/BM-71	56.	BPT-3244	BPT-5204/RAMAPPA
20.	BPT-2954	NLR-34449/ANNADA/NLR-34449	57.	BPT-3260	MTU-7029/IRGC-18195/MTU-1081
21.	BPT-2958	BPT-5204/IR-50	58.	BPT-3261	MTU-70291/IRGC-18195/MTU-1081
22.	BPT-3032	BPT-5204/IR-50	59.	BPT-3262	MTU-7029/IRGC-18195/MTU-1081
23.	BPT-3033	BPT-5204/MTU-1075	60.	BPT-3263	MTU-7029/IRGC-18195/MTU-1081
24.	BPT-3061	BPT-1768/NLR-145	61.	BPT-3264	CULTURE-01120305/CULTURE-0910025-7
25.	BPT-3068	NLR-34449/RAMAPPA	62.	BPT-3269	RP-BIO-226/IRGC-23385/NIDHI/MTU-1081
26.	BPT-3074	BPT-5204/MTU-1075	63.	BPT-3270	RP-BIO-226/IRGC-23385/NIDHI/MTU-1081
27.	BPT-3081	BPT-5204/MTU-1075	64.	BPT-3274	BPT-5204/BPT-2605
28.	BPT-3086	BPT-2270/IR-64/MTU-1081	65.	BPT-3275	CULTURE-01120305/CULTURE-0910025-7
29.	BPT-3092	NLR-34449/ANNADA/NLR-34449	66.	BPT-3276	CULTURE-01120305/CULTURE-0910025-7
30.	BPT-3095	MTU-5249/IR-50	67.	BPT-3277	BPT-5204/O.-LONGISTAMINATA/B-95-1/SWARNA-SUB1
31.	BPT-3111	MTU-7029/IRGC-18195/MTU-1081	68.	BPT-3279	RP-BIO-226/JARAVA
32.	BPT-3113	BPT-2270/NLR-145	69.	BPT-3291	SONA/MAHSURI
33.	BPT-3114	BPT-2270/NLR-145	70.	BPT-4358	SONA-MAHSURI/ARC-6650
34.	BPT-3115	BPT-2270/NLR-145	71.	SAMBA MAHSURI (BPT-5204)	GEB-24/TN1/MAHSURI
35.	BPT-3118	JGL-3855/RAMAPPA	72.	IMPROVED SAMBA MAHSURI (ISM) RP BIO-226	MAS FROM BPT-5204 AND SS1113
36.	BPT-3120	JGL-3855/ANNADA	73.	KRISHNAVENI (MTU-2077)	SOWBHAGYA/ARC-5984
37.	BPT-3121	BPT-3291/RAMAPPA	74.	TAICHUNG NATIVE-1 (TN1)	DWARF CHOW WU GEN/TSAI YUAN CHUNJ

Pathogen Inoculation and Screening of Germplasm

Screening for BLB was carried out in wet season of 2020, at two locations in Andhra Pradesh, India, namely Agricultural College Farm, Bapatla (15° 54' 29.88" N; 80° 28' 7.3092" E) and RARS, Maruteru (15° 59' 12.984" N; 80° 6' 7.848" E). The rice genotypes were grown under irrigated conditions in a block with each entry planted in two rows of 2 m length, adopting a spacing of 20 x 15 cm. To ensure strong disease pressure, the block was flanked by two border rows of the susceptible check Taichung Native-1. Inoculum was prepared from BLB-infected

leaves collected from local fields. The leaves were cut into 1 cm pieces, and surface sterilized with 1% sodium hypochlorite. Smaller leaf bits of 5 x 5 mm size were placed in test tube having sterile distilled water for 15 to 20 min, to allow the bacteria to ooze out of the leaf tissue. A loopful of bacterial suspension was streaked onto nutrient agar (NA) media plates and incubated at 27 ± 1 ^{0}C for 3 days. Single yellow, round and smooth margin, non-flat, mucous colonies were picked and purified on fresh NA plates. For field inoculation, the two days old *Xoo* cultures were suspended in sterile distilled water and adjusted to ~10^8 CFU ml^{-1} (OD at 600 = 0.5) using spectrophotometer. Plants were inoculated at 75 DAS by leaf clipping method (Kauffman *et al.*, 1973). Disease severity was recorded three weeks post-inoculation and scored based on IRRI SES, 2013 (Table 2).

$$\text{Per cent diseased leaf area} = \frac{\text{Total lesion length}}{\text{Total leaf area}} \times 100$$

Table 2. Disease scoring scale for bacterial leaf blight in rice as per SES, IRRI, (2013).

Scale	Diseased Leaf Area (%)	Description
1	1-5	Resistant (R)
3	6-12	Moderately Resistant (MR)
5	13-25	Moderately Susceptible (MS)
7	26-50	Susceptible (S)
9	51-100	Highly susceptible (HS)

Identification of R Genes

DNA was isolated from young leaves of all the genotypes using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) protocol adapted from Dellaporta *et al.* (1983). A total of 16 molecular markers, previously reported, were used to screen R genes (Table 3). PCR amplification was carried out in a 14 µl reaction mixture, consisting of 3 µl of DNA (50 ng/µl), 1.50 µl of 10X Taq buffer, 0.35 µl of dNTPs (2.5 mM), 0.75 µl of each forward and reverse primers (10 pmol), 7.40 µl of double-distilled water, and 0.25 µl of Taq polymerase (5 U/µl). The thermal cycler was set with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. PCR products were separated on 3% agarose gel, stained with ethidium bromide by gel electrophoresis. Distinct and unambiguous polymorphic bands were scored against a standard 100-bp DNA ladder.

The allelic data was subjected to estimation of genetic distances among the genotypes using DARwin v6.0 (Perrier and Jacquemond, 2006). For each SSR marker genetic diversity parameters, including the total number of alleles (Na), the effective number of alleles (Ne) were calculated using POPGENE version 1.32. (Yeh *et al.*, 2000) and polymorphic information content (PIC) was analysed using the following formula

$$PIC = 1 - \sum_{f=1}^{n} P_{ij}^2$$

Table 3. Details of the markers linked to BLB resistance.

S. No	Locus name	Linked gene	Primer	Chromosome number	Reference
1.	RM349	*Xa2*	F: TTGCCATTCGCGTGGAGGCG R: GTCCATCATCCCTATGGTCG	4	Patel *et al.* (2014)
2.	RM317	*Xa2*	F: CATACTTACCAGTTCACCGCC R: CTGGAGAGTGTCAGCTAGTTGA	4	He *et al.* (2006)
3.	RM144	*Xa4*	F: TGCCCTGGCGCAAATTTGATCC R: GCTAGAGGAGATCAGATGGTAGTGCATG	11	Patel *et al.* (2014)
4.	RM224	*Xa4*	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	11	Chen *et al.* (1997)
5.	RM39	*xa5*	F: GCCTCTCTCGTCTCCTTCCT R: AATTCAAACTGCGGTGGC	5	Subudhi *et al.* (2006)
6.	RM13	*xa5*	F: TCCAACATGGCAAGAGACAG R: GGTGGCATTCGATTCCAG	5	Panaud *et al.* (1996)
7.	RM164	*xa5*	F: TCTTGCCCGTCACTGCAGATATCC R: GCAGCCCTAATGCTACAATTCTTC	5	Amgai *et al.* (2015)
8.	RM533	*xa8*	F: AAAGGCCGTACCTTTGCCTTCC R: AGCTAGGGATCCATCCTCCAACC	7	Patel *et al.* (2014)
9.	RM254	*Xa10*	F: AGCCCCGAATAAATCCACCT R: CTGGAGGAGCATTTGGTAGC	11	Chen *et al.* (1997)
10.	RM5509	*Xa33*	F: GATGATCCATGCTTTGGCC R: TTCCAGCAGAAAGAAGACGC	6	Korinsak *et al.* (2009)
11.	RM30	*Xa33*	F: TGGGGTGGTTAGGCATCGTC R: CCTCACCACACGACACGAGC	6	Korinsak *et al.* (2009)
12.	RM206	*Xa10/Xa4*	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	11	Panaud *et al.* (1996)
13.	RM167	*Xa4/Xa10/Xa21*	F: GATCCAGCGTGAGGAACACGT R: AGTCCGACCACAAGGTGCGTTGTC	11	Wu and Tanksley 1993
14.	*Xa5*FM	*xa5*	SF: GTCTGGAATTTGCTCGCGTTCG SR: TGGTAAAGTAGATACCTTATCAAACTGGA RF: AGCTCGCCATTCAAGTTCTTGAG RR: TGACTTGGTTCTCCAAGGCTT	-	Hajira *et al.* (2016)
15.	*Xa13*prom	*xa13*	F: GGCCATGGCTCAGTGTTTAT R: GAGCTCCAGCTCTCCAAATG	8	Hajira *et al.* (2016)
16.	pTA248	*Xa21*	F: AGACGCGGAAGGGTGGTTCCCGGA R: AGACGCGGTAATCGAAGATGAAA	11	Hajira *et al.* (2016)

RESULTS

Morphological Screening for BLB

The genotype's response to BLB artificial disease screening conducted at Bapatla and Maruteru are presented in Table 4. Comparative analysis revealed that, out of 74 lines (including checks) 37 performed similarly at both locations. For the remaining genotypes, the disease scores at Maruteru exceeded those at Bapatla. Specifically, at Bapatla 7 genotypes showed resistance, 12 moderately resistance, 45 moderately susceptible and 7 susceptible. At Maruteru 9 genotypes showed moderately resistance, 32 moderately susceptible and 28 susceptible and 2 highly susceptible. Notably, 9 genotypes consistently exhibited their disease reaction towards resistance (with a score of 1-3) at both the locations, while 52 genotypes showed susceptibility (with score 5–9).

Table 4. Phenotypic and genotypic screening for bacterial leaf blight resistance in 74 rice genotypes.

S. No.	Genotypes	Disease reaction at		*Xa21*	*xa13*	*xa5*	*Xa4*	*Xa2*	Total R genes expressed	Positive compatibility
		Bapatla	Maruteru							
1.	BPT-1235	S	S	--	--	--	++	--	1	X
2.	BPT-2231	MS	S	--	--	--	++	--	1	X
3.	BPT-2295	S	S	--	--	--	--	--	0	✓
4.	BPT-2411	MS	S	--	--	--	--	--	0	✓
5.	BPT-2595	MS	S	--	--	--	--	--	0	✓
6.	BPT-2620	MS	MS	--	--	--	--	++	1	X
7.	BPT-2677	MS	MS	**0**	--	--	--	--	0	✓
8.	BPT-2764	S	S	--	--	--	--	++	1	X
9.	BPT-2766	MS	MS	--	--	--	--	--	0	✓
10.	BPT-2776	MS	S	--	--	--	--	--	0	✓
11.	BPT-2782	S	S	--	--	--	--	--	0	✓
12.	BPT-2808	MS	MS	--	--	--	--	++	1	X
13.	BPT-2824	MS	S	--	--	--	--	--	0	✓
14.	BPT-2846	MR	MR	**0**	--	--	--	--	0	X
15.	BPT-2848	R	MS	--	--	+-	++	++	2	X
16.	BPT-2849	MS	S	--	--	--	++	--	1	X
17.	BPT-2854	MS	HS	--	--	--	++	--	1	X
18.	BPT-2863	MS	S	--	--	--	--	--	0	✓
19.	BPT-2950	MS	S	--	--	--	++	--	1	X
20.	BPT-2954	MS	S	--	--	--	--	--	0	✓
21.	BPT-2958	MR	MR	--	**0**	**0**	++	--	1	✓
22.	BPT-3032	MR	MS	--	**0**	--	++	--	1	X
23.	BPT-3033	MS	S	**0**	**0**	--	--	--	0	✓
24.	BPT-3061	MR	MS	**0**	+-	+-	--	--	0	X
25.	BPT-3068	R	MR	+-	+-	+-	--	++	2	✓
26.	BPT-3074	R	MR	**0**	--	--	++	--	1	✓
27.	BPT-3081	MS	MS	--	--	--	++	--	1	X
28.	BPT-3086	MS	MS	--	--	--	--	--	0	✓
29.	BPT-3092	R	MR	--	--	--	++	--	1	✓
30.	BPT-3095	MS	MS	--	--	--	--	--	0	✓
31.	BPT-3111	MS	MS	--	--	--	++	--	1	X
32.	BPT-3113	MS	HS	**0**	+-	--	++	--	1	X
33.	BPT-3114	MS	S	--	--	**0**	++	--	1	X
34.	BPT-3115	MR	S	--	--	--	--	--	0	X
35.	BPT-3118	MS	MS	--	--	--	--	--	0	✓
36.	BPT-3120	MS	MS	**0**	--	--	--	--	0	✓
37.	BPT-3121	MS	S	--	--	--	++	++	2	X
38.	BPT-3129	MS	MS	--	--	--	--	--	0	✓
39.	BPT-3130	MS	MS	--	--	--	++	--	1	X
40.	BPT-3133	MS	MS	**0**	**0**	--	++	++	2	X
41.	BPT-3135	R	MS	--	--	--	--	--	0	X
42.	BPT-3136	MR	MS	--	++	--	--	++	2	X
43.	BPT-3137	R	MR	--	--	--	++	++	2	✓
44.	BPT-3145	MS	S	--	--	++	++	--	2	X
45.	BPT-3147	MS	MS	--	--	--	++	--	1	X
46.	BPT-3148	MS	S	--	--	--	++	--	1	X
47.	BPT-3150	S	S	--	--	+-	--	--	0	✓
48.	BPT-3151	MS	MS	--	+-	--	--	++	1	X
49.	BPT-3159	MR	MR	+-	--	--	++	--	2	✓
50.	BPT-3164	MS	MS	--	--	--	--	--	0	✓
51.	BPT-3168	MS	MS	--	**0**	--	++	--	1	X
52.	BPT-3170	R	MR	--	++	++	++	++	4	✓
53.	BPT-3172	MR	MS	**0**	--	--	++	--	1	X
54.	BPT-3178	MR	MR	--	**0**	--	--	++	1	✓

S. No.	Genotypes	Disease reaction at		*Xa21*	*xa13*	*xa5*	*Xa4*	*Xa2*	Total R genes expressed	Positive compatibility
		Bapatla	Maruteru							
55.	BPT-3208	MS	S	--	--	--	++	--	1	X
56.	BPT-3244	MS	S	--	**0**	--	--	--	0	✓
57.	BPT-3260	S	S	--	--	--	--	--	0	✓
58.	BPT-3261	MR	S	++	--	--	--	--	1	X
59.	BPT-3262	MR	MS	--	--	--	--	--	0	X
60.	BPT-3263	MS	MS	--	+-	--	--	--	0	✓
61.	BPT-3264	MR	MS	--	--	--	++	--	1	X
62.	BPT-3269	MS	MS	--	--	--	++	--	1	X
63.	BPT-3270	MS	MS	--	--	--	--	--	0	✓
64.	BPT-3274	MS	MS	--	--	--	--	--	0	✓
65.	BPT-3275	S	S	--	--	--	--	--	0	✓
66.	BPT-3276	MS	MS	--	--	--	++	--	1	X
67.	BPT-3277	MS	S	**0**	**0**	--	++	--	1	X
68.	BPT-3279	MS	S	--	--	--	++	--	1	X
69.	BPT-3291	MS	MS	--	--	--	--	--	0	✓
70.	BPT-4358	MS	S	--	--	--	--	--	0	✓
71.	Samba Mahsuri	MS	MS	--	--	--	--	--	0	✓
72.	ISM	R	R	++	++	++	--	--	3	✓
73.	Krishnaveni	S	S	--	--	--	--	--	0	✓
74.	Taichung Native-1	HS	HS	--	--	--	--	--	0	✓
	TOTAL			**4**	**3**	**3**	**31**	**12**	**53**	**38**

R- Resistant; MR- Moderately Resistant; MS- Moderately Susceptible; S- Susceptible; HS- Highly Susceptible; ++ Resistant; +- Heterozygous; -- Susceptible**; 0-** Null allele; ✓ - Positively compatible; X- Positively not compatible

Molecular Characterization of Rice

Among the 16 markers used in this study, two SSR markers *i.e.*, RM144 and RM13 markers were not amplified, hence these markers were excluded from the analysis.

Polymorphism and Marker Efficiency

Analysis of the 74 rice genotypes with 14 polymorphic SSR markers revealed 47 alleles, averaging 3.22 per locus (Table 5). Alleles per locus ranged from 2 to 7, with effective allele counts from 1.05 (RM167) to 2.58 (RM39) with an average of 2.03. Polymorphism information content (PIC) ranged from 0.06 (RM167) to 0.65 (RM349, RM39), averaging 0.48. Eight were deemed highly informative, with PIC value exceeding 0.5. The maximum and the minimum allele sizes observed markers was 982 bp (pTA248) and 130 bp (RM206) respectively.

Table 5. Genetic diversity parameters of 14 BLB resistance linked markers.

S.No.	SSR marker	Na	Ne	PIC	Amplicon size range (bp)
1.	RM349	7	2.57	0.65	190-871
2.	RM317	4	1.98	0.48	154-174
3.	RM224	3	2.55	0.61	129-163
4.	RM39	3	2.58	0.65	120-820
5.	RM164	4	1.52	0.33	240-290
6.	RM533	2	1.62	0.39	250-270
7.	RM254	3	1.82	0.46	140-170
8.	RM5509	3	2.30	0.57	270-290
9.	RM30	4	2.19	0.54	200-250
10.	RM206	3	2.56	0.61	130-170
11.	RM167	2	1.05	0.06	250-260
12.	*xa13* Prom	2	1.16	0.18	270-470
13.	*xa5*FM-SR	3	2.18	0.55	134-424
14.	*Xa21* pTA248	4	2.51	0.61	639-982
	Maximum	**7**	**2.58**	**0.65**	**982**
	Minimum	**2**	**1.05**	**0.06**	**130**
	MEAN	**3.31**	**2.03**	**0.48**	

Na- Number of alleles; Ne- Number of effective alleles; PIC- Polymorphic information content; bp- base pairs

Marker-Assisted Selection (MAS) for BLB Resistance Genes

Molecular data for the genes *Xa21, xa13, xa5, Xa4,* and *Xa2* are presented in the Table 4.

Xa21-Linked STS Marker Analysis for BLB Resistance

The presence of *Xa21* gene in germplasm was detected by the STS marker pTA248, which amplified four alleles of 982bp, 737bp, 715bp and 639bp. The 982bp fragment, associated with resistance, was observed in the positive control, ISM, whereas the 715bp fragment was detected in the negative control, Taichung Native-1. Other variants, 737bp and 639bp, were also linked to susceptibility. Among the 71 genotypes, three genotypes namely, BPT-3068, BPT-3159 and BPT-3261 showed the 982bp amplicon, indicative of the presence of the Xa21 gene. Notably, BPT-3261 possessed the gene in a homozygous condition (982bp), while BPT-3068 and BPT-3159 displayed the gene in a heterozygous state (982bp and 737bp). The remaining genotypes produced amplicons of 737bp, 715bp, or 639bp, confirming the absence of the Xa21 resistance gene. The amplification pattern of pTA248 marker was represented in the (Fig. 1).

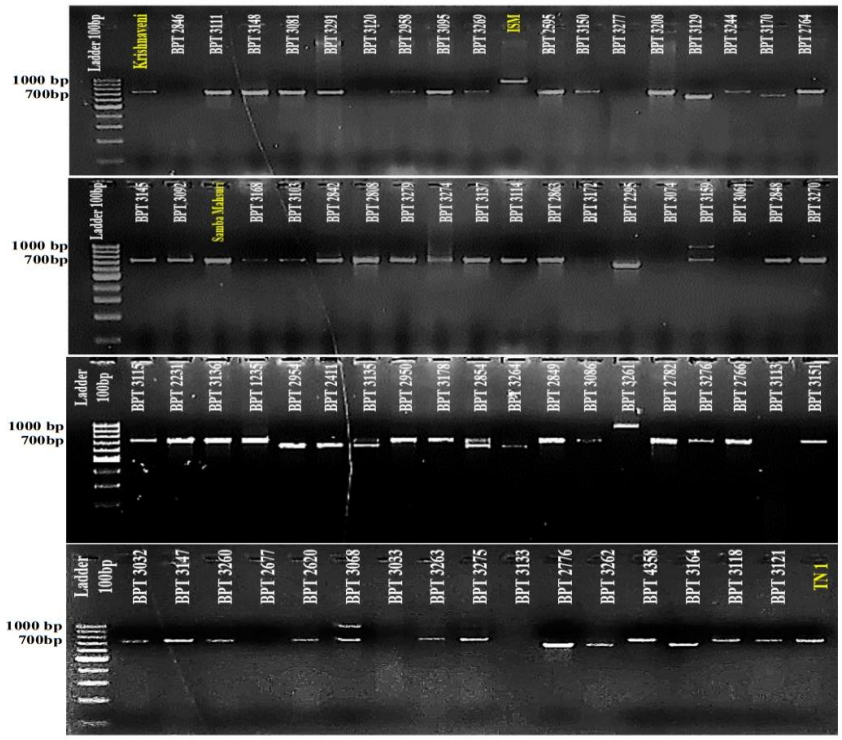

Fig. 1. Expression of *Xa21* gene amplification using marker pTA248.

Xa13-Linked STS Marker Analysis for BLB Resistance

The *xa13* gene in germplasm was detected by the marker *xa13*prom. The positive control, ISM, showed a 470 bp fragment, while the negative control, Taichung Native-1, exhibited a 270 bp fragment. BPT-3136 and BPT-3170 produced amplicon of 470bp, carried *xa13* in homozygous condition. The genotypes BPT-3061, BPT-3068, BPT-3113, BPT-3151 and BPT-3263 produced heterozygous bands of 470bp and 270bp, where *xa13* the recessive gene was not expressed. While, remaining 64 genotypes produced amplicon of 270bp (similar to Taichung Native-1).

Xa5-Linked STS Marker Analysis for BLB Resistance

The STS marker *xa5*FM-SR linked to recessive *xa5* gene amplified 424bp (common), 134bp (resistance specific) and 313bp (susceptibility specific) fragments. Genotypes BPT-3145 and BPT-3170, showed of 424bp and 134bp fragments (similar to the ISM) indicating the presence of the resistant gene. BPT-2848, BPT-3061, BPT-3068, and BPT-3150 were heterozygous, producing 424 bp, 134 bp, and 313 bp fragments. The remaining 65 genotypes produced only 313bp and 134bp bands.

Xa4-linked SSR marker analysis for BLB resistance

The Xa4 gene was identified using the marker RM224, with resistant genotypes producing a 160bp fragment and susceptible genotypes a 150bp fragment (Panwar *et al.*, 2018; Chen *et al.* 1997). In this study, 31 genotypes carried Xa4, producing a 160bp amplicon.

Xa2-Linked SSR Marker Analysis for BLB Resistance

The *Xa2* gene was detected using the SSR marker RM317. with a 154bp DNA fragment indicating resistance (Hasan *et al.*, 2020; He *et al.*, 2006). In this study, 12 genotypes namely BPT-2620, BPT-2764, BPT-2808, BPT-2848, BPT-3068, BPT-3121, BPT-3133, BPT-3136, BPT-3137, BPT-3151, BPT-3170 and BPT-3178 amplified the154bp fragment, and considered to possess *Xa2*.

R Genes Expressed

A total of 53 R genes were expressed from all the cultivars, for the five genes *Xa21, xa13, xa5, Xa4* and *Xa2* studied. The gene *Xa4* was found to be most frequent, carried by 31 genotypes, followed by *Xa2* (12) and *Xa21* (3), while *xa5* and *xa13* were found in only two genotypes. BPT-3170 possessed combination of four multiple resistance genes (*xa13+xa5+Xa4+Xa2*). Two gene combinations were found in eight genotypes, namely BPT-2848, BPT-3068, BPT-3121, BPT-3133, BPT-3136, BPT-3137, BPT-3145 and BPT-3159. Single genes were detected in 30 genotypes, and, 32 genotypes did not express any R genes.

Compatibility between Genotypic and Phenotypic Expression of BLB Resistance

Positive compatibility between phenotypic and genotypic expression of BLB resistance was observed in 38 rice lines, including checks of which nine exhibited resistance (score 1-3), and 29 showed susceptibility (score 5-9) (Table 4). Among the resistant genotypes namely, BPT-3068, BPT-3074, BPT-3092, BPT-3137, BPT-3170, BPT-2846, BPT-2958, BPT-3159, and BPT-3178, all except BPT-2846 showed positive compatibility with R gene expression. BPT-3170 carried four resistance genes (*xa13+xa5+Xa4+Xa2*), while BPT-3068 (*Xa21+Xa2*), BPT-3137 (*Xa4+Xa2*), and BPT-3159 (*Xa21+Xa4*) carried two genes. BPT-3074 (*Xa4*), BPT-2958 (*Xa4*), BPT-3178 (*Xa2*), and BPT-3092 (*Xa4*) carried a single gene.

Molecular Diversity Analysis

The phylogenetic tree was constructed using 14 markers linked to BLB resistance based on neighbour-joining method (Fig. 2). The genotypes were grouped into three major clusters

(Table. 6). Cluster I, containing 32 genotypes, was further subdivided into four sub-clusters (IA, IB, IC, ID) and possessed the highest number of resistance genes (21), of which only 3 were positively compatible (Tables 7, and 8). Cluster II, the smallest with 7 genotypes and resistant check, was divided into sub-clusters IIA and IIB. Sub-cluster IIA contains exclusively resistant genotypes exhibiting multiple resistance genes namely, BPT-3068, BPT-3170 and resistant check ISM. This cluster expressed 11 R genes, 9 of which were positively compatible. Cluster III was the largest with 32 genotypes mostly showing susceptibility reaction (with score 5-9) including both the susceptible checks (Taichung Native-1 and Krishnaveni). It was divided into four sub-clusters (IIIA, IIIB, IIIC, IIID) and possessed highest number of R genes (21) with only 5 being positively compatible.

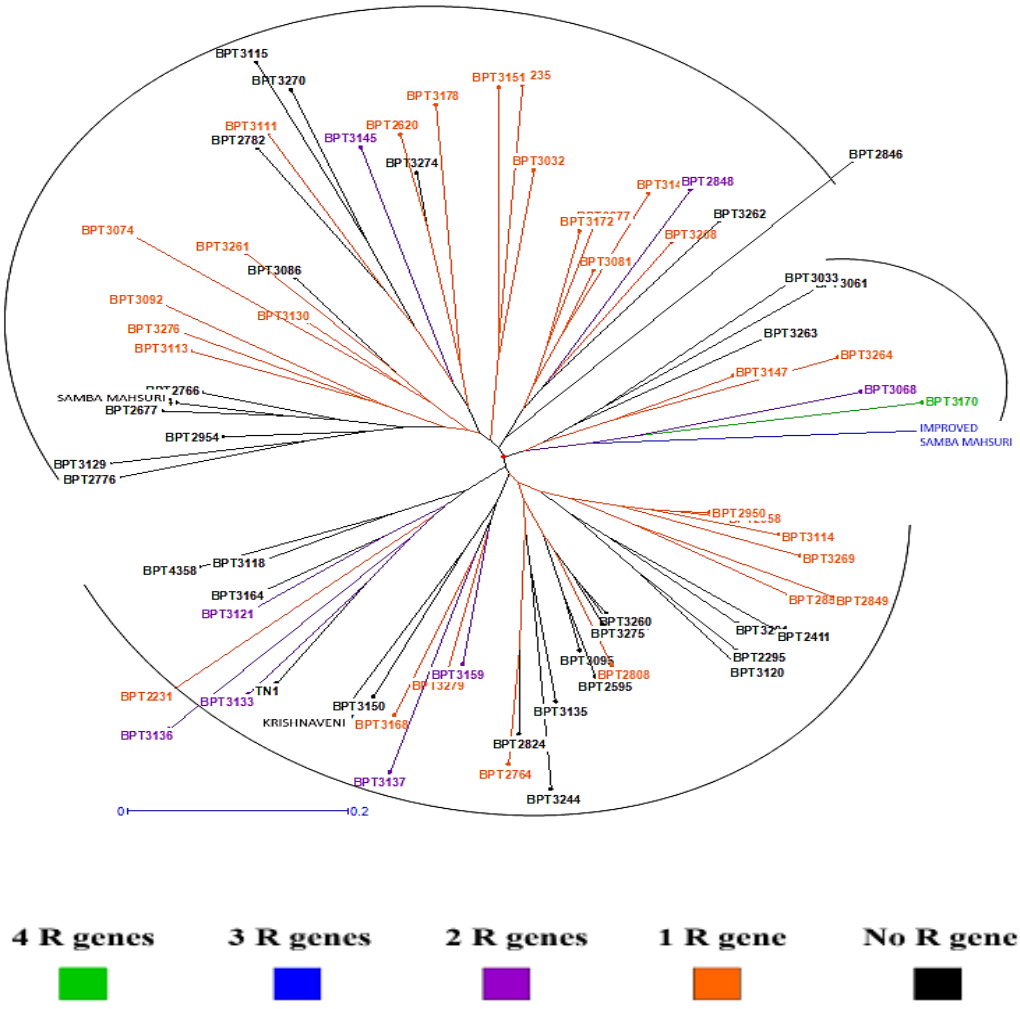

Fig. 2. Phylogenetic tree constructed based on BLB linked markers representing resistance genes.

Table 6. Grouping of genotypes into different clusters based on molecular diversity.

Name of the cluster	Name of the sub-cluster	Number of Genotypes	Name of the Genotypes
I	IA	13	BPT-2776, BPT-3129, BPT-2954, BPT-2677, Samba Mahsuri, BPT-2766, BPT-3113, BPT-3276, BPT-3092, BPT-3074, BPT-3130, BPT-3261, BPT-3086
	IB	8	BPT-2782, BPT-3111, BPT-3115, BPT-3270, BPT-3145, BPT-2620, BPT-3274, BPT-3178.
	IC	3	BPT-3151, BPT-1235, BPT-3032
	ID	9	BPT-3172, BPT-3277, BPT-3081, BPT-3148, BPT-2848, BPT-3208, BPT-3262, BPT-2846
II	IIA	5	BPT-3033, BPT-3061, BPT-3263, BPT-3147, BPT-3264
	IIB	2	BPT-3068, BPT-3170, **ISM**
III	IIIA	10	BPT-2950, BPT-2958, BPT-3114, BPT-3269, BPT-2849, BPT-2854, BPT-2411, BPT-3291, BPT-2295, BPT-3120
	IIIB	10	BPT-3260, BPT-2863, BPT-3275, BPT-2808, BPT-2595, BPT-3095, BPT-3135, BPT-3244, BPT-2824, BPT-2764
	IIIC	5	BPT-3159, BPT-3279, BPT-3137, BPT-3168, BPT-3150, **Krishnaveni**
	IIID	7	**Taichung Native-1**, BPT-3133, BPT-3136, BPT-2231, BPT-3121, BPT-3164, BPT-3118, BPT-4358

Table 7. Clustering of R genes for bacterial leaf blight resistance.

Cluster No	*Xa21*	*xa13*	*xa5*	*Xa4*	*Xa2*	Total
Cluster I	1	-	1	15	4	**21**
Cluster II	2	2	2	3	2	**11**
Cluster III	1	1	-	13	6	**21**
Total	**4**	**3**	**3**	**31**	**12**	**53**

Table 8. Clustering of positively compatible R genes for bacterial leaf blight based on phenotypic and genotypic marker data.

Cluster No	*Xa21*	*xa13*	*xa5*	*Xa4*	*Xa2*	Total
Cluster I	-	-	-	2	1	**3**
Cluster II	2	2	2	1	2	**9**
Cluster III	1	-	-	3	1	**5**
Total	**3**	**2**	**2**	**6**	**4**	**17**

DISCUSSION

This study evaluated the genetic resistance to bacterial leaf blight (BLB) in 71 rice genotypes developed from diverse elite and improved breeding lines across multilocation and revealed significant variability in disease response. Disease severity was higher at Maruteru, a known BLB hotspot, compared to Bapatla, likely due to Maruteru's favorable environment for pathogen spread. This variation can be attributed to the polygenic nature of BLB resistance, governed by over 40 R genes, and strongly influenced by environment. Genotypes showed a wide range of responses, from resistant to highly susceptible, consistent with findings by Rahman *et al.* (2017), Qudsia *et al.* (2019), and Majumder *et al.* (2019).

Using 14 gene-linked markers, 47 alleles including rare and null alleles were detected across 74 rice genotypes. Rare alleles identified with markers RM349, RM30, and RM164 likely result from structural variations or mutational events (Victoria et al., 2007). The

informativeness of SSR markers in assessing genetic diversity aligns with Ashiba *et al.* (2020), and Khan *et al.* (2015).

Molecular characterization of five resistance genes *Xa21*, *xa13*, *xa5*, *Xa4* and *Xa2* was conducted with ISM (*Xa21+xa13+xa5*) as a positive control. BPT-3170 carrying *xa13+xa5+Xa4+Xa2* may confer broad spectrum resistance and serve as a valuable donor for BLB resistance breeding. BPT-3061 and BPT-3068 possess the recessive resistance genes *xa13* and *xa5 in* heterozygous condition, requiring homozygosity for expression. Advancing these lines through selfing can facilitate allele fixation and enhance resistance to BLB.

Among the 74 genotypes screened, 38 showed concordance between phenotypic and genotypic resistance to BLB, while 36 showed discrepancies. The genotypes showing resistant reaction without detected R genes may harbor additional resistance genes not included in this study. Conversely, the susceptibility observed in some *Xa4*-carrying genotypes suggests pathogen adaptation, likely driven by wide spread use of *Xa4*-based cultivars in India and Southeast Asia (Ma *et al.*, 1999 and Sun *et al.*, 2003). This study shows that genotypic screening correlates with phenotypic screening in most genotypes and it also underscore the importance of combining genotypic and phenotypic data for accurate resistance evaluation.

Cluster analysis based on 14 R-gene-linked markers grouped genotypes into three major clusters, with the resistant check (ISM) and susceptible checks (Taichung Native-1 and Krishnaveni) occupying distinct clusters. The eight genotypes, BPT-3068, BPT-3074, BPT-3092, BPT-3137, BPT-3170, BPT-2958, BPT-3159 and BPT-3178 that exhibited phenotypic disease reaction towards resistance (score 1-3) for BLB at both the locations and demonstrated positive R gene compatibility were distributed across clusters, highlighting their genetic diversity. These resistant genotypes are promising resources for developing durable BLB-resistant varieties. Similar studies on phylogeny analysis in rice using SSR markers were reported by Ashiba *et al.* (2020), Khan *et al.* (2015) and Khannetah *et al.* (2021).

CONCLUSIONS

This study assessed genetic resistance to bacterial leaf blight (BLB) in 71 rice genotypes through multilocation screening and molecular characterization. BPT-3170, exhibiting phenotypic resistance and carrying multiple resistance genes (*xa13+xa5+Xa4+Xa2*), shows strong potential as a donor for broad-spectrum BLB resistance. Additionally, genotypes BPT-3068, BPT-3074, BPT-3092, BPT-3137, BPT-3170, BPT-2958, BPT-3159, and BPT-3178 showed consistent phenotypic resistance (disease score 1–3) across locations and positive

compatibility with R-gene expression, making them valuable genetic resources for BLB resistance breeding. The findings underscore the ineffectiveness of single gene resistance and emphasizes the need for pyramiding multiple R genes to achieve durable and broad-spectrum resistance. Deploying diverse R-gene combinations in elite cultivars will enhance the resilience of rice crops against evolving BLB pathotypes, ensuring sustainable production and food security particularly in areas where BLB is a persistent threat to rice cultivation.

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