Plant Archives Vol. 26, Supplement 2, 2026 pp. 1029-1043 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.120

MOLECULAR CHARACTERIZATION OF ELITE LINES FOR BACTERIAL LEAF BLIGHT (BLB) RESISTANCE IN RICE (*Oryza sativa* L.)

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(Date of Receiving : 23-02-2026; Date of Revision : 05-04-2026; Date of Acceptance : 29-04-2026)

ABSTRACT

Most rice growing regions around the world frequently encounter with BLB disease. Host resistance is the most favored approach to manage the bacterial pathogen, *Xoo.* by developing durable BLB resistant cultivars. Eighty-eight elite lines including checks were evaluated in this study. The phenotypic screening categorized five lines as resistant (R) with the disease score of 1 and seven lines as moderately resistant (MR) with the disease score of 3. Molecular characterization was done using 25 SSR markers, specifically linked to BLB resistance genes (*Xa*2, *Xa*4, *xa*5, *Xa*7, *xa*8, *Xa*10, *xa*13, *Xa*21, *xa*24, *Xa*33). Among the lines with resistant and moderately resistant reaction at field conditions, AM865 was detected with four gene combination. The lines, AM853, AM857, AM867 and AM855 were detected with three gene combination. The markers, RM206, RM400 and RM21, were considered to be more informative and effective for differentiating the elite lines as they had higher PIC values, higher Nei's genetic diversity index and higher Shannon's informative index. Molecular diversity analysis grouped elite lines into 3 major clusters in which, all resistant and moderately resistant lines were grouped under cluster II.

The present study identified an elite line, AM865, with the multiple resistance genes (*Xa*4+*xa*5+*xa*13+*Xa*21) along with the phenotypic score of 3, could confer broad spectrum resistance for BLB.

Keywords **:** Rice, BLB, Phenotypic score, Gene combinations, leaf clipping method, SSRs, Dendrogram, DARwin, PIC value, Pop gene

Introduction

Rice (*Oryza sativa* L. 2n=2x=24) is the most important food crop that provides a significant portion of carbohydrates to the world's population. The rate of world's population growth has exceeded the rate of growth in food-grain production. The demand for rice is still increasing in Asia as the consumption rate is at least 90% and it is globally projected that the demand for rice will rise up to 650 million tons by 2050 (Chukwu *et al*., 2019a). At present, most rice growing regions around the world frequently encounter *Xanthomonas oryzae pv. oryzae* (*Xoo.*), the causal agent of bacterial leaf blight (BLB). Various chemical and cultural approaches have been used for the management of bacterial leaf blight. However, this has limited use, because it is relatively time consuming and laborious. Host resistance is the most favored approach to manage the bacterial pathogen, *Xoo.* by developing durable BLB resistant cultivars (Hasan *et al*., 2020). As this disease is prevalent almost throughout the world, the researchers are searching for available resistant genes against BLB disease. Till date, a total of 42 BLB resistance genes (*Xa*1 to *Xa*42) have been identified in rice. Plant selection for BLB resistance by conventional plant breeding method is not effective as there is a masking effect on the expression of genes. Molecular plant breeding can be used to expand genetic diversity, characterize genetic architecture, modify gene action and increase selection efficiency. An individual with BLB resistance gene(s) in a segregating population can be identified more rapidly with the help of DNA markers. Various number of tightly linked markers for BLB resistance genes were developed. These markers could be used to screen germplasm for BLB resistance genes using marker assisted selection (Arif *et al*., 2008). SSR markers are regarded as the ideal markers among several available DNA based markers, as they are abundant, multiallelic,

hyper-variable, co-dominant, flexible for automation, locus identity and suitability for high throughput analysis. The number of alleles and PIC value for markers on different chromosomes are calculated to evaluate the genetic diversity, thereby generating valid information which could be quite useful for rice breeding programmes.

Material and Methods

Plant Material

The experimental material comprised of 88 elite lines including 7 checks, national resistant check RPBIO226, local resistant check Swarna, national susceptible check TN1, local susceptible check Krishnaveni and yield checks namely, Maruteru Samba (MTU1224), Sravani (MTU1239), Maruteru Mahsuri (MTU1262) were obtained from Regional Agricultural Research Station (RARS), Maruteru, Acharya N.G. Ranga Agricultural University (ANGRAU).

Experimental design and layout

This experiment was carried out during *kharif*, 2021 at Regional Agricultural Research Station (RARS), Maruteru, West Godavari district of Andhra Pradesh located at 81.44^0 E longitude, 26.38^0 N latitude and 5 m above mean sea level, in Godavari Zone of Acharya N. G. Ranga Agricultural University (ANGRAU). All these elite lines were evaluated in alpha lattice design with two replications and were randomized in 11 blocks with block size of eight in the main field. To develop a sick plot for BLB disease screening, all the 88 lines were planted in a separate block, each entry in two rows of one-meter length, adopting a spacing of 20 x 15 cm for screening against resistance to bacterial leaf blight. The block was flanked by 3-4 border rows of susceptible cultivar,TN1.

BLB disease Screening

Plants in sick plot were inoculated artificially by leaf clipping method (Kauffman *et al.*, 1973) at 75 DAS. For inoculation, sterilized scissors dipped in bacterial suspension was used. Leaves of the plant were grasped in one hand and top 1-3 inches of the leaves were clipped off. The data was recorded 15 days after inoculation. Observation on reaction to bacterial leaf blight was recorded and categorized based on 1-9 score as per StandardEvaluation System (SES) of rice, IRRI, 2014.

$$\% \text{ Diseased Leaf Area} = \frac{\text{Total lesion length}}{\text{Total leaf area}} \times 100$$

Disease scoring scale for BLB in rice as per SES, IRRI, (2014)

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)

Molecular Profiling

DNA was extracted from the leaf tissue of all the 88 elite lines using the modified Cetyl Tri Methyl Ammonium Bromide (CTAB) method of Dellaporta *et al.* (1983). All the elite lines were analyzed using markers specifically linked to BLB resistance genes (*Xa*2, *Xa*4, *xa*5, *Xa*7, *xa*8, *Xa*10, *xa*13, *Xa*21, *xa*24, *Xa*33) to identify the lines resistant to BLB. Each PCR reaction tube contained 1 µl of Taq buffer (10 x) with $MgCl_2$, 1 µl of dNTPs mix (2.5 mM each), 1 µl of forward primer (5 pM), 1 µl of reverse primer (5 pM), 3 µl of template DNA (50 ng/µl), 1 µl of taq polymerase (1u/µl) and 2 µl of sterile distilled water and kept inside the thermocycler (Applied Biosystem's Veriti PCR machine). Amplification was carried by preheating at 94 ^oC for 5 min, followed by 35 cycles of denaturation at 94 ^oC for 30 sec, anneling at 55 ^oC for 30 sec, 1 min elongation at 72 ^oC and finale extension at 72^oC for 7 min. SSR products were separated in agarose gel of 2 % through a horizontal gel electrophoresis. DNA fragments were expressed using ethidium bromide staining procedure. The gel was run at 80 to120 V till the dye front reached 3/4th of the gel. The resolved PCR bands were documented using Syngene gel doc system. The amplified fragment size was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size *i.e.*, 100 base pair (bp) DNA ladder. To measure the informativeness of the markers, the polymorphism information content (PIC) for each marker was calculated according to the formula given by Anderson *et al.* (1993). The genetic diversity analysis parameters *viz.*, number of alleles (Na), effective number of alleles (Ne), Shannon index (I) and Nei's genetic diversity index (He) (Nei, 1973) were calculated using Popgene v 1.31. The allelic data were subjected to estimation of genetic distances among the lines using simple matching coefficients by boot strapping 1000 times and the lines were clustered using neighbor joining method based on dissimilarity matrix using Software DARwin v 6.0. (Perrier and Jacquemond, 2006).

Results

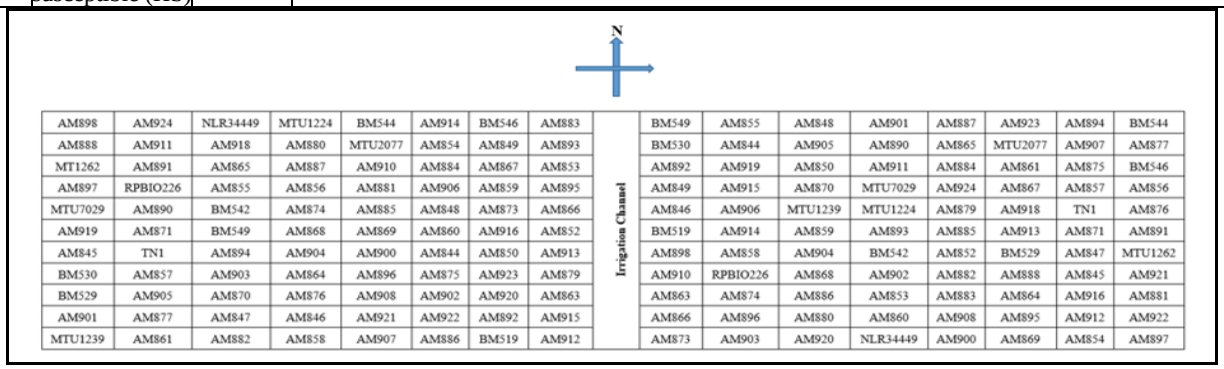
Morphological Characterization of lines for BLB disease

All the elite lines including checks were screened against BLB under field conditions by adopting leaf clipping method of artificial inoculation (Table 1). Only five of the total elite lines studied *viz.*, AM853, AM857, AM863, AM867 along with the resistant check, RPBIO226 recorded a disease score of 1. These lines were categorized as resistant because their diseased leaf area is between 1-5%. Furthermore, the disease score 3 was recorded in seven lines *i.e.*,

AM855, AM858, AM865, AM866, AM869, AM873 and AM874. These lines were considered to be moderately resistant as the diseased leaf area ranges from 6-12%. The disease score observed for the 24 lines was 5 as moderately susceptible and the per cent diseased leaf area ranged from 13-25%. Fifty lines were categorized as susceptible with a disease scoring of 7 with the range of 26-50% diseased leaf area. The lines TN1 and Krishnaveni, were categorized as highly susceptible as they recorded a disease scoring of 9 and diseased leaf area of about 51-100%.

Table 1 : Classification of elite lines according to disease scoring

BLB Score	Response to disease reaction	No. of elite lines	Elite lines
1	Resistant (R)	5	AM853, AM857, AM863, AM867 and Improved Samba Mahsuri (RPBIO226)
3	Moderately Resistant (MR)	7	AM855, AM858, AM865, AM866, AM869, AM873 and AM874
5	Moderately Susceptible (MS)	24	AM844, AM845, AM847, AM850, AM854, AM856, AM868, AM875, AM877, AM880, AM881, AM887, AM910, AM913, AM914, AM915, BM529, BM530, BM542, BM544, BM546, Nellore Mahsuri, Sravani and Swarna
7	Susceptible (S)	50	AM846, AM848, AM849, AM852, AM859, AM860, AM861, AM864, AM870, AM871, AM876, AM879, AM882, AM883, AM884, AM885, AM886, AM888, AM890, AM891, AM892, AM893, AM894, AM895, AM896, AM897, AM898, AM900, AM901, AM902, AM903, AM904, AM905, AM906, AM907, AM908, AM911, AM912, AM916, AM918, AM919, AM920, AM921, AM922, AM923, AM924, BM519, BM549, Maruteru Samba and Maruteru Mahsuri
9	Highly susceptible (HS)	2	TN1 and Krishnaveni



AM898	AM924	NLR34449	MTU1224	BM544	AM914	BM546	AM883	BM549	AM855	AM848	AM901	AM887	AM923	AM894	BM544
AM888	AM911	AM918	AM880	MTU2077	AM854	AM849	AM893	BM530	AM844	AM905	AM890	AM865	MTU2077	AM907	AM877
MT1262	AM891	AM865	AM887	AM910	AM884	AM867	AM853	AM892	AM919	AM850	AM911	AM884	AM861	AM875	BM546
AM897	RPBIO226	AM855	AM856	AM881	AM906	AM859	AM895	AM849	AM915	AM870	MTU7029	AM924	AM867	AM857	AM856
MTU7029	AM890	BM542	AM874	AM885	AM848	AM873	AM866	AM846	AM906	MTU1239	MTU1224	AM879	AM918	TN1	AM876
AM919	AM871	BM549	AM868	AM869	AM860	AM916	AM852	BM519	AM914	AM859	AM893	AM885	AM913	AM871	AM891
AM845	TN1	AM894	AM904	AM900	AM844	AM850	AM913	AM898	AM858	AM904	BM542	AM852	BM529	AM847	MTU1262
BM530	AM857	AM903	AM864	AM896	AM875	AM923	AM879	AM910	RPBIO226	AM868	AM902	AM882	AM888	AM845	AM921
BM529	AM905	AM870	AM876	AM908	AM902	AM920	AM863	AM863	AM874	AM886	AM853	AM883	AM864	AM916	AM881
AM901	AM877	AM847	AM846	AM921	AM922	AM892	AM915	AM866	AM896	AM880	AM860	AM908	AM895	AM912	AM922
MTU1239	AM861	AM882	AM858	AM907	AM886	BM519	AM912	AM873	AM903	AM920	NLR34449	AM900	AM869	AM854	AM897

Fig. 1: Experimental layout using Alpha Lattice Design

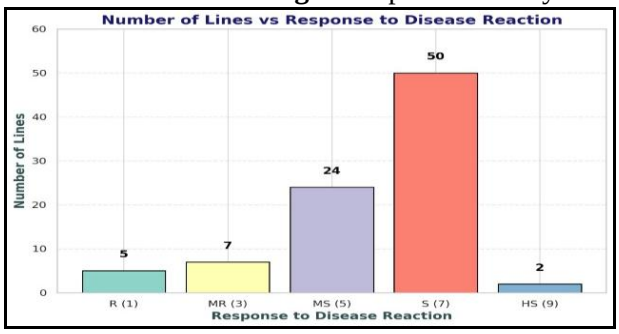


Fig. 2 : Frequency distribution graph for BLB

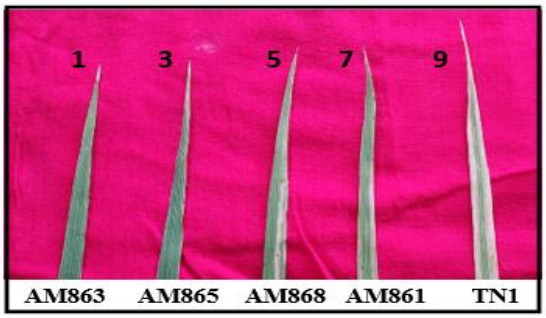


Fig. 3: Disease Scoring for BLB

Molecular Characterization of lines for BLB disease

Molecular characterization using microsatellite markers is a powerful tool for screening lines for resistance gene. In the current study, 88 elite lines were analyzed using markers specifically linked to BLB resistance genes (*Xa*2, *Xa*4, *xa*5, *Xa*7, *xa*8, *Xa*10, *xa*13, *Xa*21, *xa*24, *Xa*33) to identify the lines resistant to BLB. A total of 25 markers were used among which only 20 markers were polymorphic.

Analysis of *xa*5, *xa*13, *Xa*21 *Xa*4, *Xa*7 and *Xa*10 gene markers for BLB resistance

Identification of *xa*5 gene was done by using the marker RM122. In the current investigation 16 lines were positive for *xa*5. Resistant lines produced DNA fragment of 227 bp corresponding to RPBIO226. Out of 16 lines with positive expression for BLB resistance two lines AM857 and AM867 had phenotypic score of one (Resistant), two lines AM865 and AM873 had phenotypic score of 3 (Moderately Resistant). Thus, the presence of *xa*5 gene for BLB resistance could be confirmed in these 4 lines with the help of RM122 SSR marker. The amplification pattern of RM122 marker was shown in Plate 1.

S.No	Genotype	*Xa*4	*xa*5	*xa*13	*Xa*21	*Xa*7	*Xa*10	Total Genes	Gene Combinations	BLB reaction	
		RM144	RM122	xa13prom	pTA 248	RM251	RM206			Score	Reaction
1	RP BIO226	++	++	++	++	--	--	4	*Xa*4+*xa*5+*xa*13+*Xa*21	1	R
2	TN1	++	--	--	--	--	--	1	*Xa*4	9	HS
3	Swarna	++	--	--	--	++	++	3	*Xa*4+*Xa*7+*Xa*10	5	MS
4	Krishnaveni	--	--	--	--	++	--	1	*Xa*7	9	HS
5	AM844	++	--	--	--	--	--	1	*Xa*4	5	MS
6	AM845	++	--	--	--	--	--	1	*Xa*4	5	MS
7	AM846	++	++	--	--	++	++	4	*Xa*4+*xa*5+*Xa*7+*Xa*10	7	S
8	AM847	++	--	--	--	--	--	1	*Xa*4	5	MS
9	AM848	--	--	--	--	--	--	0	-	7	S
10	AM849	--	--	--	--	--	--	0	-	7	S
11	AM850	++	--	--	--	--	--	1	*Xa*4	5	MS
12	AM852	++	++	--	--	--	--	2	*Xa*4+*xa*5	7	S
13	AM853	++	--	++	++	--	--	3	*Xa*4+*xa*13+*Xa*21	1	R
14	AM854	++	++	--	++	--	--	3	*Xa*4+*xa*5+*Xa*21	5	MS
15	AM855	++	--	++	++	--	--	3	*Xa*4+*xa*13+*Xa*21	3	MR
16	AM856	++	++	--	--	--	--	2	*Xa*4+*xa*5	5	MS
17	AM857	++	++	++	--	--	--	3	*Xa*4+*xa*5+*xa*13	1	R
18	AM858	++	--	--	++	--	--	2	*Xa*4+*Xa*21	3	MR
19	AM859	++	--	--	++	--	--	2	*Xa*4+*Xa*21	7	S
20	AM860	++	--	--	--	--	--	1	*Xa*4	7	S
21	AM861	++	--	++	--	--	--	2	*Xa*4+*xa*13	7	S
22	AM863	++	--	--	++	--	--	2	*Xa*4+*Xa*21	1	R
23	AM864	++	--	--	--	--	--	1	*Xa*4	7	S
24	AM865	++	++	++	++	--	--	4	*Xa*4+*xa*5+*xa*13+*Xa*21	3	MR
25	AM866	++	--	--	++	--	--	2	*Xa*4+*Xa*21	3	MR
26	AM867	++	++	--	++	--	--	3	*Xa*4+*xa*5+*Xa*21	1	R
27	AM868	++	--	++	++	--	--	3	*Xa*4+*xa*13+*Xa*21	5	MS
28	AM869	++	--	--	--	--	--	1	*Xa*4	3	MR
29	AM870	++	--	--	--	--	--	1	*Xa*4	7	S
30	AM871	++	++	--	++	--	--	3	*Xa*4+*xa*5+*Xa*21	7	S
31	AM873	++	++	--	--	--	--	2	*Xa*4+*xa*5	3	MR
32	AM874	++	--	--	++	--	--	2	*Xa*4+*Xa*21	3	MR
33	AM875	++	--	+-	--	--	--	2	*Xa*4+*xa*13	5	MS
34	AM876	++	--	--	--	--	--	1	*Xa*4	7	S
35	AM877	++	++	--	--	--	--	2	*Xa*4+*xa*5	5	MS
36	AM879	--	--	--	--	--	--	0	-	7	S
37	AM880	++	--	--	--	--	--	1	*Xa*4	5	MS
38	AM881	++	--	--	--	--	--	1	*Xa*4	5	MS
39	AM882	--	--	--	--	--	--	0	-	7	S
40	AM883	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
41	AM884	++	--	--	--	--	--	1	*Xa*4	7	S
42	AM885	++	--	--	--	--	--	1	*Xa*4	7	S
43	AM886	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
44	AM887	++	--	--	--	++	--	2	*Xa*4+*Xa*7	5	MS
45	AM888	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S

S.No	Genotype	*Xa*4	*xa*5	*xa*13	*Xa*21	*Xa*7	*Xa*10	Total Genes	Gene Combinations	BLB reaction	
		RM144	RM122	xa13prom	pTA 248	RM251	RM206			Score	Reaction
46	AM890	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
47	AM891	++	--	--	--	--	--	1	*Xa*4	7	S
48	AM892	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
49	AM893	--	++	--	--	++	--	2	*xa*5+*Xa*7	7	S
50	AM894	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
51	AM895	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
52	AM896	--	--	--	--	--	--	0	-	7	S
53	AM897	--	--	--	--	--	--	0	-	7	S
54	AM898	++	++	--	--	++	--	3	*Xa*4+*xa*5+*Xa*7	7	S
55	AM900	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
56	AM901	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
57	AM902	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
58	AM903	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
59	AM904	++	--	--	--	--	--	1	*Xa*4	7	S
60	AM905	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
61	AM906	++	--	--	--	--	++	2	*Xa*4+*Xa*10	7	S
62	AM907	--	--	--	--	--	--	0	-	7	S
63	AM908	++	--	--	--	++	++	3	*Xa*4+*Xa*7+*Xa*10	7	S
64	AM910	++	--	--	--	--	--	1	*Xa*4	5	MS
65	AM911	--	--	--	--	++	--	1	*Xa*7	7	S
66	AM912	++	--	--	--	--	--	1	*Xa*4	7	S
67	AM913	++	--	--	--	--	--	1	*Xa*4	5	MS
68	AM914	++	++	--	--	--	--	2	*Xa*4+*xa*5	5	MS
69	AM915	++	--	--	--	--	++	2	*Xa*4+*Xa*10	5	MS
70	AM916	++	--	--	--	--	--	1	*Xa*4	7	S
71	AM918	++	--	--	--	--	--	1	*Xa*4	7	S
72	AM919	++	--	--	--	--	--	1	*Xa*4	7	S
73	AM920	++	--	--	--	--	--	1	*Xa*4	7	S
74	AM921	++	++	--	--	++	--	3	*Xa*4+*xa*5+*Xa*7	7	S
75	AM922	--	--	--	--	--	--	0	-	7	S
76	AM923	--	--	--	--	++	--	1	*Xa*7	7	S
77	AM924	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
78	BM519	++	--	--	--	++	--	2	*Xa*4+*Xa*7	7	S
79	BM529	++	--	--	--	++	--	2	*Xa*4+*Xa*7	5	MS
80	BM530	++	--	--	--	--	--	1	*Xa*4	5	MS
81	BM542	++	--	--	--	--	--	1	*Xa*4	5	MS
82	BM544	++	--	--	--	--	++	2	*Xa*4+*Xa*10	5	MS
83	BM546	++	--	--	--	++	--	2	*Xa*4+*Xa*7	5	MS
84	BM549	++	--	--	--	++	++	3	*Xa*4+*Xa*7+*Xa*10	7	S
85	MTU1224	++	++	--	--	--	++	3	*Xa*4+*Xa*5+*Xa*10	7	S
86	MTU1239	++	--	--	--	++	--	2	*Xa*4+*Xa*7	5	MS
87	MTU1262	--	--	--	--	--	--	0	-	7	S
88	NLR34449	++	++	--	--	--	--	2	*Xa*4+*xa*5	5	MS
No. of positive alleles		75	17	7	13	22	14				

R-Resistant; MR-Moderately Resistant; MS-Moderately Susceptible; S-Susceptible; HS-Highly Susceptible

Fig. 4 : Heatmap depicting the summary of genotyping along with the phenotypic score for BLB disease

The presence of *xa*13 gene was detected by the marker xa13-prom, using positive control RPBIO226 showing a DNA fragment of 450 bp. The susceptible lines were identified by the DNA fragment of 290 bp

corresponding to the negative control TN1. The lines, AM853, AM855, AM857, AM861, AM865 and AM868, were homozygous for the gene *xa*13 as they produced amplicon at 450 bp, while the genotype, AM875, was heterozygous and produced amplicons of 450 bp and 290 bp corresponding to the resistant and susceptible alleles, respectively. Out of the seven lines that showed positive expression for BLB resistance, two lines, AM853 and AM857, had phenotypic scores of 1 (Resistant). Two lines, AM855 and AM865, had phenotypic scores of 3 (Moderately Resistant). By using xa13-prom marker it can be understood that 4 lines had showed positive association between phenotypic and genotypic expression for BLB resistance. The amplification pattern of xa13- prom marker was shown in Plate 2.

The presence of *Xa*21 gene was detected by the pTA248. A total of 12 lines namely AM853, AM854, AM855, AM858, AM859, AM863, AM865, AM866, AM867, AM868, AM871 and AM874 showed the amplicon size of 925 bp corresponding to the resistant allele of RPBIO226 and were therefore thought to have resistant gene. The remaining lines produced amplicon sizes of 750 bp and 700 bp thus revealing the absence of *Xa*21 resistance gene. Out of 12 lines with positive expression for BLB resistance, 3 lines (AM853, AM863 and AM867) had recorded phenotypic score of 1 (Resistant). Five lines (AM855, AM858, AM865, AM866 and AM874) had recorded phenotypic score 3 (Moderately Resistant). It can be understood that 8 lines had exhibited positive compatibility between phenotypic and genotypic expression for BLB resistance using marker pTA248. The amplification pattern of pTA248 marker was shown in Plate 3.

Identification of *Xa*4 gene was done by using the marker RM144. Resistant lines produced DNA fragment of 237 bp. In the current study except 13 lines, remaining all were found positive for *Xa*4 and produced an amplicon size of 237 bp. All the lines with score 1 (Resistant) and score 3 (Moderately Resistant) had positive compatibility between phenotypic and genotypic expression for BLB resistance using marker RM144. Identification of *Xa*7 gene was done by using the marker RM251. Resistant lines produced DNA fragment of 147 bp. In the current investigation 21 lines were positive for *Xa*7 and produced an amplicon size of 147 bp. Of them 16 lines recorded the phenotypic score of 7 (susceptible). Thus, the presence of *Xa*7 gene was not found critical for resistant reaction. Identification of *Xa*10 gene was done by using the marker RM206. Resistant lines produced DNA fragment of 147 bp. In the present study 14 lines were positive for *Xa*10 and produced an amplicon size of 147 bp. The amplification pattern of RM144, RM251 and RM206 markers were shown in Plate 4, plate 5 and plate 6 respectively.

Analysis of elite lines with different combinations of BLB resistant genes

The lines AM853, AM857, AM863, AM867, AM855, AM858, AM865, AM866, AM869, AM873 and AM874 showed resistant and moderately resistant reaction in phenotypic screening were also characterized by the presence of combination of resistant genes (Table 2).

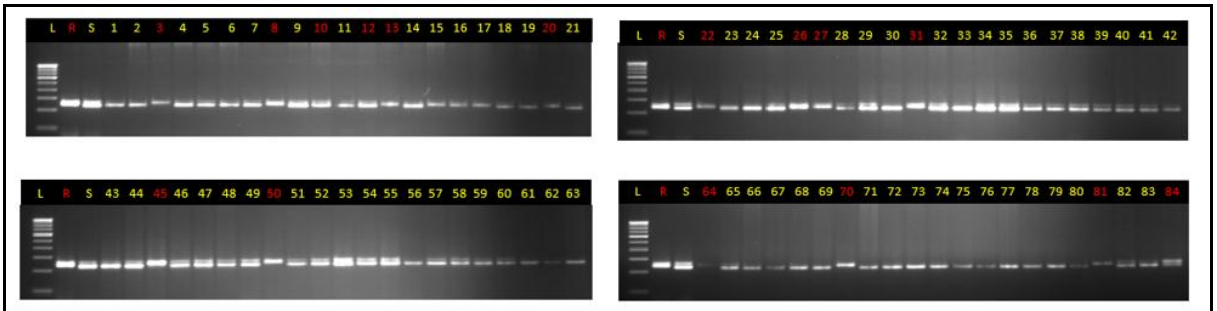


Plate 1: The amplification pattern of RM122 marker

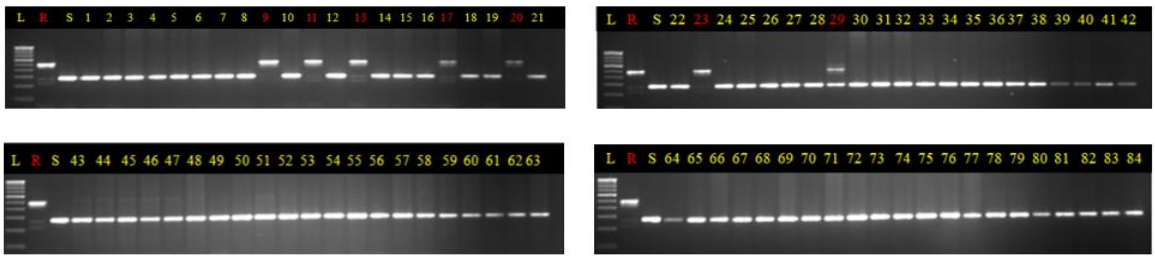
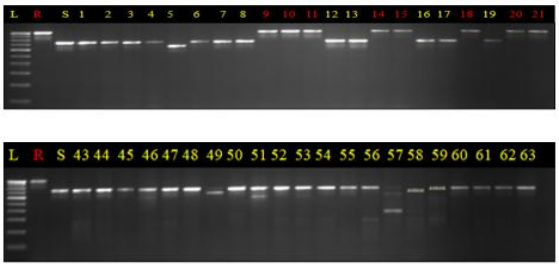
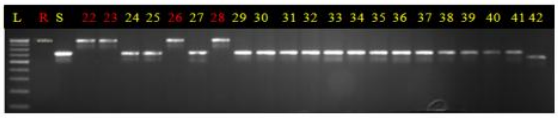


Plate 2: The amplification pattern of xa13-prom marker





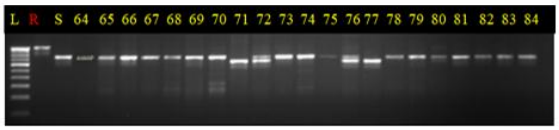
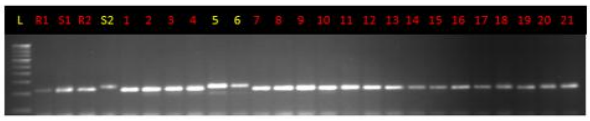
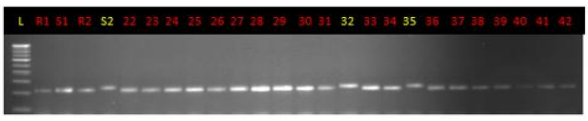


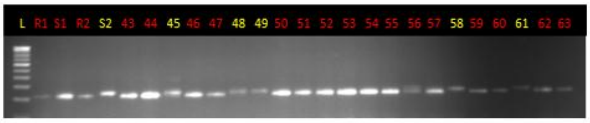
Plate 3: The amplification pattern of pTA248 marker

L: Ladder, R: Resistant check (RPBIO226), S: Susceptible check (TN1)

1.	AM 844	13.	AM 857	25.	AM 870	37.	AM 884	49.	AM 897	61.	AM 911	73.	AM 924
2.	AM 845	14.	AM 858	26.	AM 871	38.	AM 885	50.	AM 898	62.	AM 912	74.	BM 519
3.	AM 846	15.	AM 859	27.	AM 873	39.	AM 886	51.	AM 900	63.	AM 913	75.	BM 529
4.	AM 847	16.	AM 860	28.	AM 874	40.	AM 887	52.	AM 901	64.	AM 914	76.	BM 530
5.	AM 848	17.	AM 861	29.	AM 875	41.	AM 888	53.	AM 902	65.	AM 915	77.	BM 542
6.	AM 849	18.	AM 863	30.	AM 876	42.	AM 890	54.	AM 903	66.	AM 916	78.	BM 544
7.	AM 850	19.	AM 864	31.	AM 877	43.	AM 891	55.	AM 904	67.	AM 918	79.	BM 546
8.	AM 852	20.	AM 865	32.	AM 879	44.	AM 892	56.	AM 905	68.	AM 919	80.	BM 549
9.	AM 853	21.	AM 866	33.	AM 880	45.	AM 893	57.	AM 906	69.	AM 920	81.	MTU 1224
10.	AM 854	22.	AM 867	34.	AM 881	46.	AM 894	58.	AM 907	70.	AM 921	82.	MTU 1239
11.	AM 855	23.	AM 868	35.	AM 882	47.	AM 895	59.	AM 908	71.	AM 922	83.	MTU 1262
12.	AM 856	24.	AM 869	36.	AM 883	48.	AM 896	60.	AM 910	72.	AM 923	84.	NLR 34449







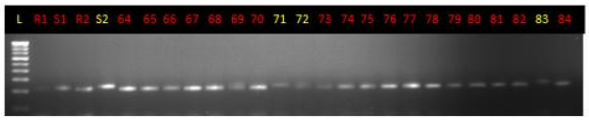
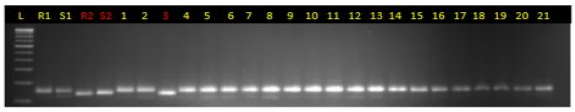
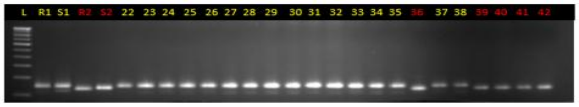
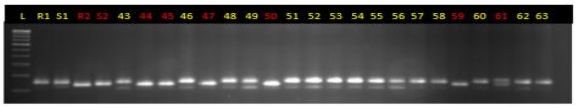


Plate 4: The amplification pattern of RM144 marker







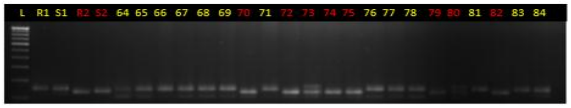
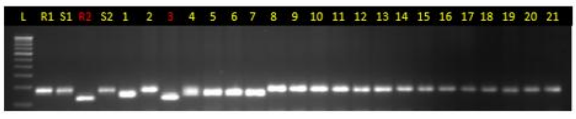
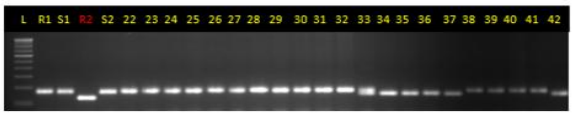
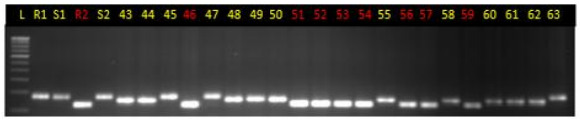


Plate 5: The amplification pattern of RM251 marker







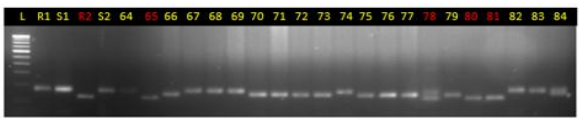


Plate 6: The amplification pattern of RM206 marker

L: Ladder, R1: Resistant check (RPBIO226), S1: Susceptible check (TN1)
R2: Resistant check (Swarna), S2: Susceptible check (Krishnaveni)

1.	AM 844	13.	AM 857	25.	AM 870	37.	AM 884	49.	AM 897	61.	AM 911	73.	AM 924
2.	AM 845	14.	AM 858	26.	AM 871	38.	AM 885	50.	AM 898	62.	AM 912	74.	BM 519
3.	AM 846	15.	AM 859	27.	AM 873	39.	AM 886	51.	AM 900	63.	AM 913	75.	BM 529
4.	AM 847	16.	AM 860	28.	AM 874	40.	AM 887	52.	AM 901	64.	AM 914	76.	BM 530
5.	AM 848	17.	AM 861	29.	AM 875	41.	AM 888	53.	AM 902	65.	AM 915	77.	BM 542
6.	AM 849	18.	AM 863	30.	AM 876	42.	AM 890	54.	AM 903	66.	AM 916	78.	BM 544
7.	AM 850	19.	AM 864	31.	AM 877	43.	AM 891	55.	AM 904	67.	AM 918	79.	BM 546
8.	AM 852	20.	AM 865	32.	AM 879	44.	AM 892	56.	AM 905	68.	AM 919	80.	BM 549
9.	AM 853	21.	AM 866	33.	AM 880	45.	AM 893	57.	AM 906	69.	AM 920	81.	MTU 1224
10.	AM 854	22.	AM 867	34.	AM 881	46.	AM 894	58.	AM 907	70.	AM 921	82.	MTU 1239
11.	AM 855	23.	AM 868	35.	AM 882	47.	AM 895	59.	AM 908	71.	AM 922	83.	MTU 1262
12.	AM 856	24.	AM 869	36.	AM 883	48.	AM 896	60.	AM 910	72.	AM 923	84.	NLR 34449

Among the above lines AM865 was detected with four R genes (*Xa*4+*xa*5+*xa*13+*Xa*21). The lines, AM853 (*Xa*4+*xa*13+*Xa*21), AM857 (*Xa*4+*xa*5+*xa*13), AM867 (*Xa*4+*xa*5+*Xa*21) and AM855 (*Xa*4+*xa*13+*Xa*21) were detected with three R genes. The lines, AM863 (*Xa*4+*Xa*21), AM858 (*Xa*4+*Xa*21), AM866 (*Xa*4+*Xa*21), AM873 (*Xa*4+*Xa*5) and AM874 (*Xa*4+*Xa*21) were detected with two R genes. The line AM869 (*Xa*4) was detected with only one R gene.

Table 2 : Multi-genic lines carrying different combinations of BLB resistant genes

S. No.	Lines	Phenotypic BLB Reaction		No. of Genes	Gene Combinations	Grain yield per plant (g)
		Score	Reaction			
1	AM853	1	R	3	*Xa*4+*xa*13+*Xa*21	11.25
2	AM857	1	R	3	*Xa*4+*xa*5+*xa*13	23.08
3	AM863	1	R	2	*Xa*4+*Xa*21	12.16
4	AM867	1	R	3	*Xa*4+*xa*5+*Xa*21	19.66
5	AM855	3	MR	3	*Xa*4+*xa*13+*Xa*21	16.54
6	AM858	3	MR	2	*Xa*4+ *Xa*21	15.81
7	**AM865**	3	MR	4	*Xa*4+*xa*13+ *xa*5+*Xa*21	12.04
8	AM866	3	MR	2	*Xa*4+ *Xa*21	18.29
9	AM869	3	MR	1	*Xa*4	13.87
10	AM873	3	MR	2	*Xa*4+*xa*5	22.89
11	AM874	3	MR	2	*Xa*4+*Xa*21	15.23

R: Resistant, MR: Moderately resistant

Average phenotypic score for different genes and gene combinations

Among all genes *xa*13 is present in 8 entries with the average phenotypic score of 3.25 followed by *Xa*21 in 13 entries with the average phenotypic score of 3.30 and *xa*5 in 17 entries with the average phenotypic score of 4.88. *Xa*4 is present in 73 entries with the average phenotypic score of 5.63, *Xa*10 in 14 entries with the average phenotypic score of 6.50 and *xa*7 in 22 entries with the average phenotypic score of 6.60. Thus, the presence of *xa*13 gene is more effective in conferring resistance, as its phenotypic score is 3.25 followed by *Xa*21, *xa*5, *Xa*4, *Xa*10 and *xa*7 (Table 3).

Table 3 : Average phenotypic scores of BLB resistant genes

S. No.	Genes	No. of entries	Average Phenotypic Score
1	*xa*5+*xa*13	**3**	1.66
2	*xa*13+*Xa*21	**5**	2.60
3	***xa*13**	8	3.25
4	***Xa*21**	13	3.30
5	*xa*5+*Xa*21	**5**	3.40
6	***xa*5**	17	4.88
7	***Xa*4**	73	5.63
8	*Xa*4+*xa*7/8	18	6.40
9	***Xa*10**	14	6.50
10	*Xa*4+*Xa*10	14	6.50
11	*Xa*10+*xa*7/8	4	6.50
12	***xa*7/8**	22	6.60

Three lines (AM857, AM865, RPBIO226) were found to possess the gene combination of *xa*5+*xa*13 with the average phenotypic score of 1.66. Thus, the presence of this gene combination was found critical for resistant reaction, followed by the combination of *xa*13+*Xa*21 in 5 lines (AM853, AM855, AM865, AM868 and RPBIO226) with the average phenotypic score of 2.60. The combination of *xa*5+*Xa*21 is present in 5 genotypes with the average phenotypic score of 3.40. Thus, the combination of two and more than two major BLB resistant genes were found to provide effective disease resistance than the presence of single gene. 18 lines were found to possess the gene combination of *Xa*4+*Xa*7 with the average phenotypic score of 6.40. The combination of *Xa*7+*Xa*10 in 4 lines with the average phenotypic score of 6.50. The combination of *Xa*4+*Xa*10 is present in 14 lines with

the average phenotypic score of 6.50. The lines with the presence of *Xa*4, *Xa*7 and *Xa*10 genes, even in the combinations were not found critical for resistant reaction and were found to be susceptible.

Molecular Diversity Analysis using Darwin

The study of genetic diversity in any breeding population is essential as it constitutes the backbone for crop improvement programme. A dendrogram grouped the total 88 elite lines into three major clusters and each was further sub grouped into two sub clusters (Fig. 2). The lines with low similarity values are more divergent.

Cluster II comprised of 30 lines and all the lines with resistant (1) and moderately resistant (3) reaction for BLB are present only in this cluster. Genotypes in this cluster have the parentage RPBIO226/NLR34449, thus implies that parental ancestry is particularly present in the cluster II. Cluster I comprised of 53 lines and Cluster III comprised of 5 lines.

Genetic Diversity Parameters

Genetic diversity of 88 elite rice lines was determined using 25 molecular markers among which 20 were polymorphic. The genetic diversity parameters namely, number of alleles (Na), effective number of alleles (Ne), Shannon index (I) and Nei's genetic diversity index were calculated using Pop gene v 1.31 for the 20 polymorphic markers and the results were presented in the Table 4. In the current study, a total of 47 number of alleles (Na) were detected with a maximum and minimum of 3 and 2 alleles, respectively. Major allelic frequency (MAF) ranged from 0.50 (RM164) to 0.97 (RM5509) with an average of 0.77, the Shannon's information index (I) varied between 0.13 (RM285) to 1.00 (RM5509) with an average of 0.54. Genetic diversity index or heterozygosity (**Nei**) is based on the polymorphic nature or genetic variation of each locus. A high heterozygosity value denotes high genetic variation among the individuals. The highest level of gene diversity was recorded for the marker, RM206 (0.60).

The polymorphic information content (PIC) value is a measure of polymorphism among lines for marker locus depending on the number of detectable alleles and the distribution of their frequency. Thus, it provides an estimate of the marker's discriminating ability. In the present study there were 4 highly informative markers (PIC > 0.5), eleven were moderately informative markers (0.25 < PIC < 0.5) and only 5 slightly informative markers (PIC < 0.25). High PIC markers RM206 (0.606), can efficiently distinguish the lines and are more informative. The PIC value for RM5509 (0.084) was the lowest, indicating that this marker has less ability to distinguish between lines. Polymorphic information content (PIC) of the markers ranged from 0.084 (RM5509) to 0.606 (RM206), with an average PIC value of 0.35. The markers, RM206 (0.606), RM400 (0.547), RM21 (0.553) and RM164 (0.500), had the highest PIC values. The markers RM206, RM400 and RM21 had higher PIC values along with higher number of effective alleles, high Nei's genetic diversity index and high Shannon's informative index.

Table 4 : Genetic diversity parameters of 38 polymorphic markers

S. No.	SSR Marker	Na	Ne	MAF	I	PIC	Nei	Amplicon size range (bp)
1	MP1MP2	2	1.47	0.80	0.50	0.330	0.32	150-180
2	RM224	2	1.56	0.77	0.54	0.372	0.36	160-180
3	RM144	2	1.37	0.84	0.44	0.278	0.27	230-250
4	RM164	2	2.00	0.50	0.69	0.500	0.50	250-280
5	RM153	2	1.16	0.92	0.27	0.148	0.14	180-200
6	RM122	2	1.46	0.80	0.50	0.317	0.32	210-230
7	RM13	2	1.49	0.79	0.51	0.341	0.33	140-160
8	M5	3	1.52	0.80	0.64	0.430	0.34	300-1300
9	RM500	2	1.27	0.88	0.37	0.304	0.21	260-320
10	RM263	3	1.11	0.95	0.22	0.107	0.10	180-210
11	RM254	2	1.24	0.89	0.34	0.199	0.19	150-180
12	RM206	3	2.53	0.51	1.00	0.606	0.60	150-190
13	xa13-prom	2	1.19	0.91	0.30	0.167	0.16	290-450
14	RM230	2	1.94	0.59	0.68	0.495	0.48	260-650
15	RM264	3	1.53	0.80	0.64	0.371	0.35	180-210
16	pTA248	3	1.66	0.76	0.72	0.423	0.40	680-1000

S. No.	SSR Marker	Na	Ne	MAF	I	PIC	Nei	Amplicon size range (bp)
17	RM21	3	2.19	0.62	0.93	0.553	0.54	140-190
18	RM400	3	2.09	0.62	0.87	0.547	0.52	200-500
19	RM5509	2	1.06	0.97	0.13	0.084	0.06	210-260
20	RM251	2	1.56	0.77	0.54	0.367	0.36	150-170
Minimum		**2**	**1.06**	**0.5**	**0.13**	**0.084**	**0.06**	**140-160**
Maximim		**3**	**2.53**	**0.97**	**1**	**0.606**	**0.6**	**680-1000**
Mean		**2.21**	**1.57**	**0.7745**	**0.5415**	**0.34695**	**0.3275**	

Na = Number of alleles; Ne = Number of effective alleles; I = Shannon's information index; MAF = Major allelic frequency; PIC = Polymorphic information content; Nei = Genetic diversity index; bp = Base pairs

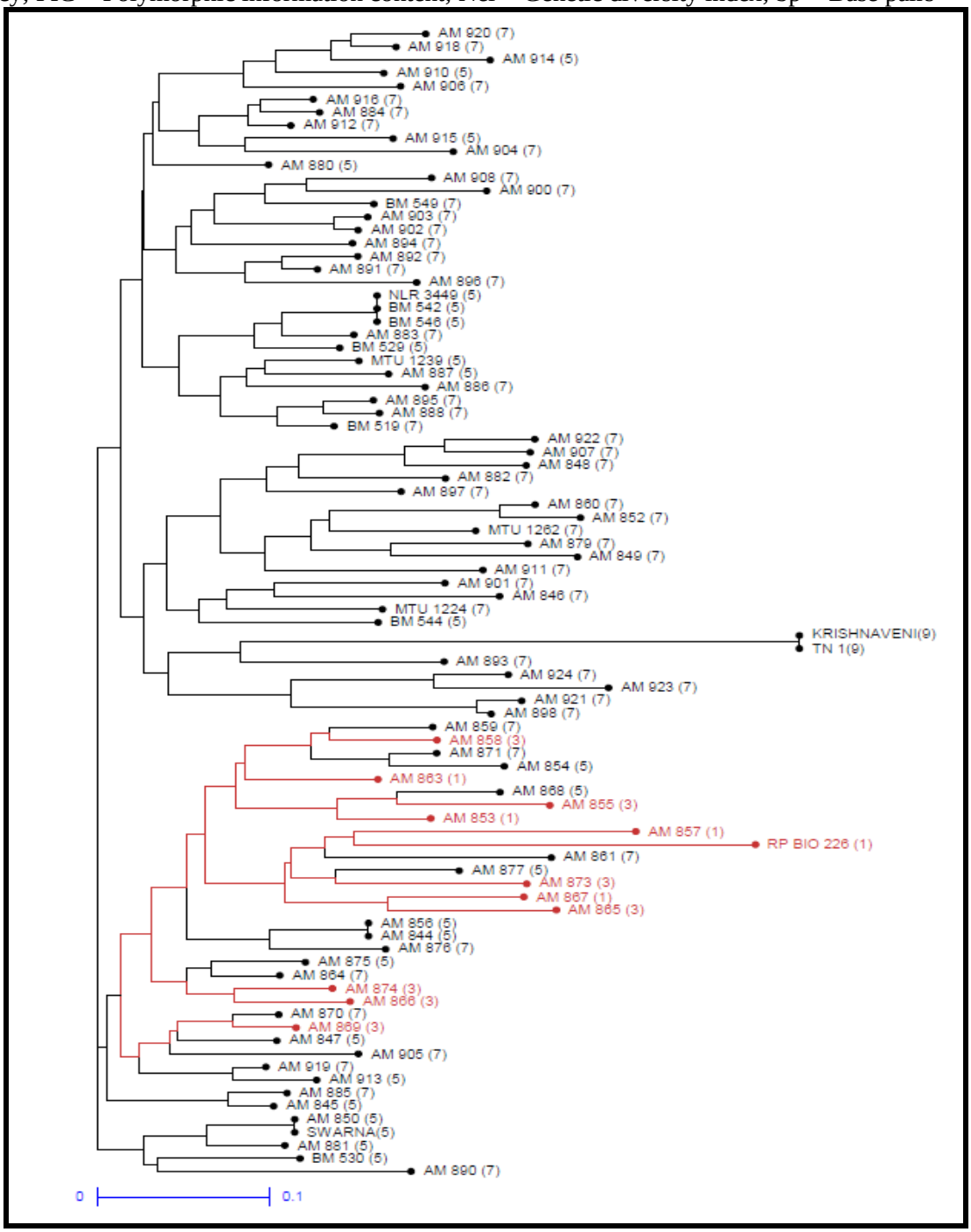


Fig. 5 : Dendrogram depicting clustering pattern of 88 rice lines using 25 SSR markers

Discussion

The present investigation was carried out for the identification of elite lines with bacterial leaf blight resistance by using molecular markers. Development of resistant lines is the most efficient and economical method for mitigating bacterial leaf blight disease in rice. A total of 88 lines, including the checks RPBIO226, Swarna, TN1 and Krishnaveni, were evaluated for BLB resistance and categorized them into different groups. The results showed that only 5 lines were resistant, 7 were moderately resistant, 24 were moderately susceptible and 50 were susceptible, while TN1 and Krishnaveni were highly susceptible. The results of present study were in accordance with the earlier findings of Rizwan *et al*. (2017), who analyzed 24 lines using artificial inoculation. Their study identified seven completely resistant lines and 12 lines exhibiting moderate resistance. Among the remaining five susceptible genotypes, three showed low susceptibility, while two were completely susceptible. This similarity suggests that resistance levels across different genetic backgrounds remain comparable under similar screening conditions. Furthermore, the findings of Qudsia *et al*. (2019), who investigated 66 Near Isogenic Lines (NILs), support our result. Their study included 31 NILs carrying the BLB-resistant genes *Xa4, xa5, Xa7, xa13,* and *Xa21*, along with 34 locally developed rice lines and a susceptible check. These lines were evaluated under natural field conditions at three BLB hotspots. They found that 31 lines were resistant, 28 were moderately resistant, 6 were moderately susceptible, and the susceptible check was classified as susceptible. The higher proportion of resistant and moderately resistant lines in their study compared to ours could be attributed to the presence of resistant genes in the 31 NILs and differences in genetic background. Additionally, Alekya *et al*. (2021) evaluated 50 F4 breeding lines derived from a three-way cross (*Pranahitha/Improved Samba Masuri/ MTU1010 NIL*) in a glasshouse for BLB resistance. Among these, 29 lines exhibited a resistant reaction, 10 were moderately resistant, 7 were susceptible and 4 were highly susceptible. The higher proportion of resistant and moderately resistant lines in this subset suggests the successful introgression of BLB-resistant genes from donor parents, particularly *Improved Samba Masuri*, which is known for its resistance to BLB. The differences in resistance levels observed across studies could be influenced by several factors, including the genetic background of the lines, the number and combination of resistance genes and environmental conditions during phenotyping.

Among the recessive genes *xa*5 is an important BLB resistance gene located on chromosome 5 and the SSR marker, RM122 is located 0.4 cM away from *xa*5 gene (Blair and McCouch, 1997). The gene originally identified in DZ192 and provides resistance to *Xoo.* races (Suh *et al.,* 2013). The present study aimed to confirm the presence of the *xa5* gene in selected rice lines using the SSR marker RM122. The molecular analysis revealed that 16 lines exhibited positive amplification for the *xa5* gene, producing a DNA fragment of 227 bp corresponding to the resistant allele. Among these lines, four showed a strong correlation between molecular and phenotypic resistance responses, which suggests their potential utility in breeding programs aimed at developing durable BLB-resistant rice cultivars. Similar polymorphic survey using forty traditional rice accessions was conducted earlier by Soumya *et al.* (2016), in which thirteen rice lines amplified 230 bp size fragments indicating the presence of *xa*5 gene.

The present study focused on the identification of the recessive resistance gene, *xa*13, which confers resistance to many Indian isolates and Philippines race of *Xoo*. It is the major resistance gene, discovered in rice variety, BJ1 and mapped on the long arm of rice chromosome 8 (Ogawa *et al.,* 1987, Khush and Angeles, 1999 and Sanchez *et al.,* 1999). The *xa*13 gene is fully recessive and confers resistance only in the homozygous states, making its identification crucial for the development of BLB-resistant rice varieties. The presence of *xa*13 gene was detected by the marker xa13-prom, using positive control RPBIO226 showing a DNA fragment of 450 bp. The results revealed that six lines were homozygous for *xa13* gene. The presence of a strong positive association between genotypic and phenotypic expression was observed in four lines, showing the effectiveness of xa13-prom as a reliable molecular marker. The findings of this study align with previous research conducted by Ashwini *et al*. (2021) evaluated 176 rice lines for two bacterial leaf blight resistant genes i.e., *Xa*21 and *xa*13 and reported that out of 25 phenotypically highly resistant genotypes, WGL1150 and WGL-1131 were observed to be positive for *xa*13 gene using the marker xa13-prom, with an amplicon size of approximately 500 bp.

The dominant gene *Xa*21 confers broad spectrum resistance to many Indian isolates of *Xoo.* and was originally introgressed from an African wild species *Oryza longistaminata* into *Oryza sativa* cultivar IR24 and mapped on chromosome 11 (Ronald *et al*, 1992 and Song *et al*, 1995). The gene *Xa*21 is the first resistant gene in rice that was cloned in 1996

(Mubassir *et al*., 2016). In this study, the presence of *Xa*21 was identified using the marker pTA248, which produced a characteristic amplicon size of 925 bp corresponding to the resistant allele of RPBIO226. Among the 88 elite lines analyzed, 12 lines were found to be positive for *Xa*21 and among them, three lines exhibited a phenotypic score of 1, while five lines recorded a phenotypic score of 3. These results suggest that the presence of *Xa*21 was strongly associated with enhanced resistance, as evidenced by the correlation between genotypic detection using pTA248 and phenotypic resistance expression. Previous studies have also demonstrated the effectiveness of *Xa*21 in conferring resistance to BLB. Sinha *et al*. (2018) studied 12 lines for four BLB resistance genes *Xa*4, *xa*5, *xa*13 and *Xa*21 and found that genotype Sudha had *Xa*21 gene in heterozygous condition by using the marker pTA248. Panwar *et al*. (2018) conducted a similar screening of 25 rice lines for detecting major BLB resistance genes *Xa*4, *xa*5 and *Xa*21 and reported that only IET18483 was positive for *Xa*21 when analyzed using the STS marker pTA248. This genotype produced an amplicon of approximately 950 bp, corresponding to the resistant lines. These studies, along with the current findings, reaffirm the significance of *Xa*21 in breeding programs aimed at improving BLB resistance in rice.

The gene *Xa*4 is most widely used BLB resistant gene in rice breeding programs because it confers durable resistance to many commercial rice cultivars. In the present study, the identification of *Xa*4 was conducted using the marker RM144, which produced a characteristic DNA fragment of 237 bp in resistant lines. Among the 88 elite lines analyzed, all except 13 tested positive for *Xa*4, indicating a widespread presence of this gene in the studied genotypes. All the lines with score 1 (Resistant) and score 3 (Moderately Resistant) had positive compatibility between phenotypic and genotypic expression for BLB resistance using marker RM144. This suggests that *Xa*4 is actively contributing to resistance in many cases. However, a significant number of lines that exhibited phenotypic susceptibility were also found to possess *Xa*4. This indicates that the gene alone may not be sufficient to confer effective resistance against all strains of *Xoo*. The widespread use of *Xa*4-containing varieties has likely exerted selection pressure on *Xoo* populations, leading to the evolution of virulent races capable of overcoming this resistance gene. Previous studies support this observation, as Ma *et al.* (1999), Mew *et al.* (1992) and Sun *et al.* (2003) reported that prolonged and extensive cultivation of *Xa*4-based resistant varieties in India and Southeast Asia has contributed to the emergence of resistant *Xoo.* races. Additionally, Lore *et al*. (2011) found that *Xa*4 alone was not effective against several pathotypes of *Xoo.* in Punjab, further emphasizing the limitations of relying solely on this gene for resistance.

In the current investigation, the identification of the *Xa7* gene was done using the RM251 marker. A total of 21 lines tested positive for *Xa7*, out of which 16 lines recorded a phenotypic score of 7, indicating susceptibility. Similarly, the *Xa10* gene was identified using the RM206 marker, where 14 lines were found to be positive, and 11 of them recorded a phenotypic score of 7. Similar to the results of the present study, Acharya *et al*. (2021) analyzed 60 rice lines using the resistant check IRBB 60 and reported that lines carrying only the *Xa7* gene were susceptible under greenhouse conditions. This suggests that the *Xa7* gene alone may not provide effective resistance, highlighting the need for further investigation into gene interactions or additional resistance factors.

The presence of multiple resistance genes in certain lines, particularly AM865 (*Xa4+xa5+xa13+ Xa21*) and AM846 (*Xa4+xa5+Xa7+Xa10*), suggests a broad-spectrum resistance potential against resistance to BLB. Phenotypic screening confirmed that resistant and moderately resistant lines were characterized by the presence of multiple resistance genes, supporting the concept that gene pyramiding enhances resistance levels. The above results of association between phenotypic and genotypic screening for BLB resistance were similar with the findings of Mallikarjun *et al*. (2017) who reported two gene pyramids namely *Xa*7+*Xa*21 in 5 lines and *Xa*4+*Xa*7 in 1 line. Genes in combinations were found to provide high levels of resistance. Khannetah *et al*. (2021) investigated 100 rice accessions for four major BLB resistance genes viz., *Xa*4, *xa*5, *xa*13 and *Xa*21 using analyses of 13 BB resistance genes linked with polymorphic microsatellites markers indicating one, two, three and four-gene combinations of *xa*5, *xa*13, *Xa*4 and *Xa*21 were present. Kotasthane and Gaikwad (2021) introgressed three resistance genes (*xa*5, *xa*13, *Xa*21) and revealed that three lines were found to have three gene (*xa*5, *xa*13 and *Xa*21), 23 lines possessed combination of *xa*5 and *xa*13 while only one line was detected with *xa*5 and *Xa*21. Further, eight lines were detected with *xa*5 gene, 17 lines with *xa*13 gene. Overall, the association between genotypic and phenotypic screening in this study confirms that single genes alone may not provide effective resistance, whereas pyramided genes, particularly combinations of *Xa4, xa5, xa13,* and *Xa21*, offer broader resistance against multiple *Xoo* races. This highlights the importance of marker-assisted selection and gene

pyramiding strategies for developing durable BLB-resistant rice varieties. The resistance gene combinations *Xa*4, *xa*5, *xa*13 and *Xa*21 provided a broad spectrum of BLB resistance against many *Xoo.* races (Bharathkumar *et al.,* 2008).

The genetic diversity analysis of 88 elite rice lines using 20 polymorphic markers showed moderate variation, with 47 alleles detected and an average PIC value of 0.35. RM206 was the most informative marker. Compared to previous studies, such as Khan *et al*. (2015) and Ashiba *et al*. (2020), the genetic diversity indices, effective alleles and Shannon's information index were lower, suggesting a relatively narrow genetic base among the tested lines. The study highlights the importance of highly polymorphic markers like RM206 and RM400 for better differentiation of rice genotypes and suggests further investigations with a broader set of markers to enhance genetic diversity analysis.

Conclusion

The results of this study emphasize the importance of phenotypic screening in identifying resistant lines for breeding programs. The resistant and moderately resistant lines identified here can serve as valuable genetic resources for developing high-yielding, BLB-resistant rice varieties.

The elite line, AM865, was detected with the multiple resistance genes (*Xa*4+*xa*5+*xa*13+*Xa*21) along with the phenotypic score of 3, could confer broad spectrum resistance. Hence, AM865 can be used as a donor in BLB resistance breeding programs to deploy bacterial blight resistant genes into elite rice cultivars. Further, the lines AM857 (*Xa*4+*xa*5+*xa*13) and AM873 (*Xa*4+*xa*5) that exhibited resistance and moderately resistance reaction under field screening were detected with resistant gene combinations. Also these lines, AM857 (23.08g) and AM873 (22.89g) showed high yield potential when compared to the superior yield check Sravani (21.22g) Table 2. Hence, these lines may be utilized in development of varieties with high yielding ability along with BLB resistance.

The markers RM206, RM400 and RM21 had higher PIC values along with higher number of effective alleles, high Nei's genetic diversity index and high Shannon's informative index. Therefore, these markers can be regarded as more informative and effective at differentiating elite lines.

Table 6 : Details of SSR markers used in the study for BLB resistance

S. No.	Gene	Marker	Chr.	Primer	Mottif	Exp bp.	Distance (cM)	References
1	*Xa*2	RM317	4	F: CATACTTACCAGTTCACCGCC R: CTGGAGAGTGTCAGCTAGTTGA	(GC)4(GT)18	155 bp	29.0609-29.0611	[28]
2	*Xa*2	RM349	4	F: TTGCCATTCGCGTGGAGGCG R: GTCCATCATCCCTATGGTCG	(GA)16	136 bp	32.4994-32.4996	[28]
3	*Xa*4	MP1MP2	4	F: ATCGATCGATCTTCACGAGG R: TCGTATAAAAGGCATTCGGG	-	150 bp	-	[1]
4	*Xa*4	RM224	11	F: ATCGATCGATCTTCACGAGG R: TGCTATAAAAGGCATTCGGG	(AAG)8(AG)13	157 bp	27.6732-27.6733	[7]
5	*Xa*4	RM144	11	F: TGCCCTGGCGCAAATTTGATCC R: GCTAGAGGAGATCAGATGGTAGTGCG	(ATT)11	237 bp	28.2816-28.2819	[28]
6	*xa*5	RM164	5	F: TCTTGCCCGTCACTGCAGATATCC R: GCAGCCCTAATGCTACAATTCTTC	(GT)16TT(GT)4	246 bp	19.1964-19.1967	[7]
7	*xa*5	RM153	5	F: GCCTCGAGCATCATCATCAG R: ATCAACCTGCACTTGCCTGG	(GAA)9	201 bp	0.18978-0.18998	[42]
8	*xa*5	RM122	5	F: GAGTCGATGTAATGTCATCAGTGC R: GAAGGAGGTATCGCTTTGTTGGAC	(GA)7A(GA)2A (GA)11	227 bp	0.31105-0.31121	[1]
9	*xa*5	RM13	5	F: TCCAACATGGCAAGAGACAG R: GGTGGCATTCGATTCCAG	(GA)6-(GA)16	141 bp	8.19492-8.19498	[41]
10	*Xa*7	RM251	3	F: GAATGGCAATGGCGCTAG R: ATGCGGTTCAAGATTCGATC	(CT)29	147 bp	9.94972-9.94978	[4]
11	*Xa*7	M5	6	F: CGATCTTACTGGCTCTGCAACTCTGT R: GCATGTCTGTGTCGATTCGTCCGTACGA	-	294 bp	-	[1]
12	*xa*8	RM533	7	F: GCAACTGCTCTACGCCTCTC R: CCTGAGGCTTCACCTACTCG	(CT)16	257 bp	17.5135-17.5134	[40]
13	*xa*8	RM500	7	F: GAGCTTGCCAGAGTGGAAAG R: GTTACACCGAGAGCCAGCTC	(AAG)9	259 bp	15.9106-15.9107	[40]
14	*xa*8	RM263	2	F: CCCAGGCTAGCTCATGAACC R: GCTACGTTTGAGCTACCACG	(CT)34	199 bp	25.8713-25.8714	[4]
15	*Xa*10	RM254	11	F: AGCCCCGAATAAATCCACCT R: CTGGAGGAGCATTTGGTAGC	(TC)6ATT(CT)11	165 bp	24.2303-24.2304	[40]

16	*Xa*10	RM206	11	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	(CT)21	147 bp	22.0146-22.0148	[4]
17	*xa*13	xa13 -prom	8	F: GGCCATGGCTCAGTGTTTAT R: GAGCTCCAGCTCTCCAAATG	-	450 bp	-	[12]
18	*xa*13	RM230	8	F: GCCAGACCGTGGATGTTC R: CACCGCAGTCACTTTTCAAG	(AGG)4(GA)9A (AG)13	257 bp	25.8372-25.8371	[7]
19	*xa*13	RM264	8	F: GTTGCGTCCTACTGCTACTTC R: GATCCGTGTCGATGATTAGC	(GA)27	178 bp	29.7763-29.7762	[7]
20	*Xa*21	pTA248	11	F: AGACGCGGAAGGGTGGTTCCCGGA R: AGACGCGGTAATCGAAGATGAAA	-	925 bp	-	[1]
21	*Xa*21	RM21	11	F: ACAGTATTCCGTAGGCACGG R: GCTCCATGAGGGTGGTAGAG	(GA)18	157 bp	19.6391-19.6392	[7]
22	*xa*24	RM138	2	F: AGCGCAACAACCAATCCATCCG R: AAGAAGCTGCCTTTGACGCTATGG	(GT)14	233 bp	35.6801-35.6800	[33]
23	*Xa*33	RM400	6	F: ACACCAGGCTACCCAAACTC R: CGGAGAGATCTGACATGTGG	(ATA)63	321 bp	28.4315-28.4319	[42]
24	*Xa*33	RM5509	6	F: GATGATCCATGCTTTGGCC R: TTCCAGCAGAAAGAAGACGC	(TC)31	255 bp	27.8282-27.8284	[42]
25	*Xa*3/4/ 10/21	RM167	11	F: GATCCAGCGTGAGGAACACGT R: AGTCCGACCACAAGGTGCGTTGTC	(GA)16	128 bp	4.0730-4.0733	[4]

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