Plant Archives Vol. 25, Supplement 1, 2025 pp. 3150-3159 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.431

MORPHOLOGICAL AND MOLECULAR EVALUATION OF ADVANCED GENERATION RICE (*ORYZA SATIVA* L.) BREEDING LINES FOR BACTERIAL BLIGHT RESISTANCE

Gaganashree. K.P.[1], K.N. Yamini[3], Karthik. R.[2], Anantha. M.S.[4], T. Kiran Babu[5] and Laha. G.S.[4]

[1]Department of Genetics and Plant Breeding, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, 500030, India

[2]Department of Genetics and Plant Breeding, University of agricultural Sciences Dharwad, Karnataka, 580005, India

[3]Institute of Biotechnology, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, 500 030, India

[4]Indian Council of Agriculture Research, Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana, 500 030, India

[5]Rice Research Centre, Agricultural Research Station, PJTSAU, Rajendranagar, Telangana, 500 030, India

*Corresponding author E-mail: kpgagana@gmail.com

(Date of Receiving : 19-11-2024; Date of Acceptance : 12-01-2025)

ABSTRACT

Bacterial blight (BB) caused by *Xanthomonas oryzae pv. oryzae (Xoo)* is a devastating rice disease in India. Given the high cost of chemical control, deploying BB-resistant genes in elite rice varieties is the most effective way to protect the crop and significantly boost yield. Thus, an experiment was conducted with seventeen F_6 generation lines developed from the cross between NILs of MTU1010 and NILs of Improved Samba Mahsuri (ISM) through marker assisted pedigree breeding method were phenotypically screened for BB resistance against three virulent isolates of *Xoo i.e.,* RPR, IXO -20 and RRC-ARI at the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, during *Rabi* season in 2020-21 along with parents and checks namely, MTU1010, TN1, ISM and ISM-Pup1 and they had been genotyped through PCR analysis for the presence of BB resistance genes *Xa21, xa13* and *xa5* using gene specific/linked markers pTA248, xa13-prom and xa5FM respectively and were analysed visually for agro-morphological traits. All lines were showed resistant reaction with average diseased leaf area (%) ranged from 2.3% to 4.9% with score1 as per Standard Evaluation System (IRRI, 2013) in phenotypic screening through artificial inoculation. Among these lines, ten were recorded with significantly superior in yield per plant than check MTU1010 and carrying BB resistance genes *i.e., xa5, xa13* and *Xa21* as well as shown resistant disease reaction. These best selected lines have the potential to be forwarded to multi-locational yield trials, could be used in further rice breeding programs.

Keywords **:** Rice, Bacterial blight, Leaf clipping, Gene specific and Gene linked markers, Agro-morphological traits.

Introduction

Rice (*Oryza sativa* L.), a monocotyledonous angiosperm, diploid ($2n = 2x = 24$), is an annual, self-pollinated crop with genome size of about 430 Mb and belongs to the *Poaceae* family. It is one of the major annual staple food crops for more than 3.5 billion people who depend on it as their day-to-day source of energy. In the world nearly 90% of the rice produced and consumed in Asia. In India, rice production was 135.75 million tonnes harvested from land area of 47.83 million hectares with productivity of 2838

Kilogram per hectare (Indiastat, 2022-2023). Worldwide, India stands first in area and second in production after China. Although significant improvement has been observed in rice production, but to satisfy the demand of the ever-growing population, productivity of rice must increase many folds. However, the yield potential is frequently threatened by various biotic and abiotic stresses. To address these problems and to increase production, developing cultivars with durable resistance is a prerequisite.

Among the biotic factors affecting rice production, bacterial blight (BB) is one of the most serious diseases caused by *Xanthomonas oryzae* pv. *oryzae (Xoo),* which is responsible for a yield loss of up to 80% depending on the severity (Kumar *et al*., 2012). Though chemicals are available to control the spread of BB, the efficiency of chemicals is not aiding the purpose as they are costly, hazardous and increases extra cost to production practices. Genetic enhancement of host plant and deployment of BB resistant rice varieties have become a viable and practical option against BB (Das *et al*., 2018) which gives durable resistance and is eco-friendly in nature.

Currently, plant breeders are utilizing Marker Assisted Selection (MAS) to select the plants based on DNA markers linked to a gene/gene specific marker responsible for the trait. As the marker is located near/ inside the DNA sequence of the desired gene, it is transmitted from one generation to the next by the standard laws of inheritance.

The genetics of resistance to the pathogen has been well characterized in rice and up to now, more than 42 resistance (*R*) genes conferring host resistance to various strains of *Xoo* have been identified among which 16 genes were recessive (*xa5, xa8, xa9, xa13, xa15, xa19, xa20, xa24, xa25, xa26b, xa28, xa31, xa32, xa33, xa34,* and *xa42*) and the rest all are dominant (Chen *et al*., 2011; Liang *et al*., 2017 and Vikal *et al*., 2017). Eleven of them were cloned and characterized, namely *Xa1, Xa3, Xa26, Xa4, xa5, Xa10, xa13, Xa21, Xa23, xa25, Xa27* and *xa41* (Ji *et al*., 2018). About 12 *R* genes (*Xa4, Xa7, Xa22, Xa30, Xa31, Xa33, xa34, Xa35, Xa39, Xa40, xa41 and xa42*) have been fine-mapped based on morphological and molecular markers (Busungu *et al*., 2016 and Zhang *et al*., 2015). It was found that the gene-pyramided lines genes *Xa21*, *xa13* and *xa5* are the most effective BB resistance genes, when evaluated against prevalent *Xoo* isolates in India (Muralidharan *et al*., 2003).

In the present investigation, genes of choice are *Xa21, xa13* and *xa5* as these genes offer durable resistance to BB. It is intended to carry out phenotypic, genotypic screening for 17 advanced breeding lines (ABLs) for the presence of BB resistant genes (*Xa21, xa13* and *xa5)* and also to select superior resistant plants that can be forwarded further towards developing new resistant high yielding rice varieties.

Material and Methods

Plant Material

In the present study, Seventeen F_6 lines, were derived from a single cross i.e., Near Isogenic Line (NIL) of MTU1010 (Female parent) × Near Isogenic Line (NIL) of Improved Sambha Mahsuri (ISM) (Male parent) which were raised along with parents and checks in Rabi 2020-21 (Table 1).

Phenotypic screening for bacterial blight resistance

Phenotypic screening for BB resistance was done on all ABLs along with their parents and checks. Three pots for each genotype were raised with three plants each. Pot-1, Pot-2 and Pot-3 were inoculated with Isolate-1 (RPR), Isolate-2 (IX0-20) and Isolate-3 (RRC-ARI) respectively, which were collected from Raipur-Chhattisgarh, Hyderabad-Telangana and Rajendranagar-Telangana respectively. In each plant, 5-6 leaves were clipped and inoculated at maximum tillering stage (55 days after transplanting) following the methodology of Kauffman *et al.* (1973) and disease reaction was scored after 14 days after inoculation. The disease score was calculated as per scale given by Standard Evaluation System (SES), IRRI, 2013 (Table 2).

Marker assisted selection

Genotyping for rice lines was done by using the isolated DNA for PCR analysis with the gene specific/linked primers. Amplification was performed using thermal cycler (AB Veriti, USA) in a final volume of 10 µl reaction mixture, programmed for one cycle of denaturation at 95ºC for 5 min, followed by 35 cycles of PCR amplification at the following parameters: 30 sec denaturation at 94ºC, 30 sec primer annealing at 55ºC, and 1 min of primer extension at 72ºC, followed by a final extension step at 72ºC for 7 min.

Several gene-specific/linked markers have been used for screening BB resistance alleles (Mahopatra *et al*., 2023; Kanipriya *et al.,* 2024; Sumuni *et al*., 2024). In the present study, *Xa21*, *xa13* and *xa5* genes were screened using the markers *pTA248*, *xa13-prom* and *xa5FM* respectively. The details of the markers, their sequence and allele size is given in Table 3.

Table 1 : List of F_6 plants selected for raising during *Rabi* 2020-21

S. No.	Entry used	Parentage	Gene combination in F_5
1	3-5-2-2	NIL of MTU1010 × NIL of ISM	*Xa21, xa13, xa5, Pup1, Pi1*
2	35-3-1-2		*Xa21, xa13, xa5, Pup1, Pi1*
3	56-1-3-2		*Xa21, xa13, xa5, Pup1, Pi1*
4	56-4-3-2		*Xa21, xa13, xa5, Pup1, Pi1*
5	160-2-1-2		*Xa21, xa13, xa5, Pup1, Pi1*
6	160-3-1-2		*Xa21, xa13, xa5, Pup1, Pi1*
7	251-2-2-2		*Xa21, xa13, xa5, Pup1, Pi1*
8	251-3-3-2		*Xa21, xa13, xa5, Pup1, Pi1*
9	353-3-1-2		*Xa21, xa13, xa5, Pup1, Pi1*
10	353-2-2-2		*Xa21, xa13, xa5, Pup1, Pi1*
11	382-2-2-2		*Xa21, xa13, xa5, Pup1, Pi1*
12	382-3-2-2		*Xa21, xa13, xa5, Pup1, Pi1*
13	441-1-3-2		*Xa21, xa13, xa5, Pup1, Pi1*
14	441-4-2-2		*Xa21, xa13, xa5, Pup1, Pi1*
15	450-2-3-2		*Xa21, xa13, xa5, Pup1, Pi1*
16	450-3-1-2		*Xa21, xa13, xa5, Pup1, Pi1*
17	724-4-3-2		*Xa21, xa13, xa5, Pup1, Pi1*

Table 2 : Standard Evaluation System (SES) scale for Bacterial blight disease scoring according to IRRI, 2013

Score	% Leaf area infected	Category
1	1-5%	Resistant
3	6-12%	Moderately resistant
5	13-25%	Moderately susceptible
7	26-50%	Susceptible
9	51-100%	Highly susceptible

Agro-morphological traits:

Seventeen Advanced generation (F_6) rice breeding lines including parents and checks were grown during *Rabi* season 2020-21 at college farm, PJTSAU, Rajendranagar. Three well established plants were selected for carrying out agro-morphological evaluation. The observation was recorded on days to 50% flowering (DFF), plant height (cm), number of productive tillers per plant, number of filled grains per panicle, panicle length (cm), panicle weight (g), 1000 seed weight (g) and grain yield per plant (g) and analyzed for determining the potentiality of genotypes.

Statistical analysis

The recorded data of selected three plants were averaged to obtain representative means of each genotype. The means of each character for each replication were then used for statistical analysis to calculate mean, range, standard error (SE), coefficient of variation (CV) and critical difference (CD) which have been done through OPSTAT software.

With each set of genotypes, earliness and other yield parameters of all the lines were compared with check MTU1010, the best lines with earliness and statistically significant higher yield than MTU1010 were identified and selected.

Results and Discussion

Phenotyping for bacterial blight resistance

Scoring for BB reaction was done 14 days after inoculation (DAI) using the SES (IRRI, 2013) and lesion length was recorded. According to which, the susceptible check, Rasi have shown highly susceptible reaction (HS) with a score of 9. ISM and ISM+*Pup1* for the lines of F_6 generation showed resistant reaction (R) to BB with a score of 1. All entries showed resistance against all the three isolates with the diseased leaf area ranged between 2.9% to 4.9%, 3.2% to 4.3% and 2.3% to 4.9% against Isolate-1, Isoalte-2 and Isolate-3 respectively with score of 1 (Table 4) as per SES (IRRI, 2013) scale (Figure. 1).

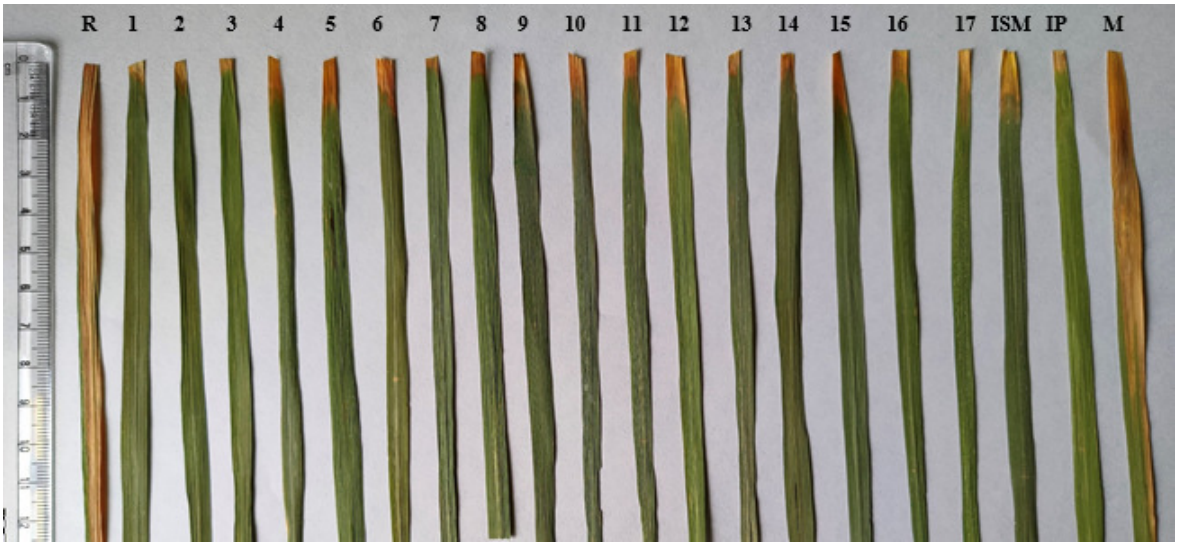

Fig. 1 : Bacterial blight reaction in F_6 lines during *Rabi* 2020-21

R: Rasi (Negative check for BB), ISM: Improved Samba Masuri (Positive check for BB), IP: ISM+ *Pup1*, M: MTU1010 (Negative check for BB),1: 382-3-2-2, 2: 724-4-3-2, 3: 450-2-3-2, 4: 441-4-2-2, 5: 441-1-3-2, 6: 353-3-1-2, 7: 382-2-2-2, 8: 160-3-1-2, 9: 251-3-3-2, 10: 353-2-2-2, 11: 56-1-3-2, 12: 251-2-2-2, 13: 160-2-1-2, 14: 3-5-2-2, 15: 35-3-1-2, 16: 56-4-3-2, 17: 450-3-1-2.

Similar to the study, Divya *et al*. (2015); ArunaKumar *et al*. (2016); Busungu *et al*. (2016); Laha *et al*. (2017); Das *et al*. (2018); Swathi *et al*. (2019) and Kotasthane A. J. and N. J. Gaikwad (2021) were used same phenotypic screening methodology *i.e*., leaf clipping method by Kauffman *et al*. (1973) for conferring disease reaction of genotypes in earlier and advanced generations. Yugander *et al*. (2018) screened backcrossed derived lines using multiple *Xoo* strains through leaf clipping method. Das *et al*. (2018) also used similar screening method for inoculating BB isolates at the maximum tillering stage to evaluate Tapaswini variety gene pyramids against the BB. Kotasthane A.J. and N.J. Gaikwad (2021) screened F_3 population produced from cross between IRBB59 and Karma Mahsuri for BB resistance. Though most researchers used single isolates to screen rice lines for BB resistance (Arunakumari *et al*. (2016); Abhilash Kumar *et al.* (2017); Nikita *et al*. (2018); Fatima *et al*. (2018); Swathi *et al.* (2019); Kotasthane and Gaikwad (2021); Duppala *et al.* 2023), few workers (Mondal *et al*. 2014; Das *et al*. 2018; Yugander *et al.* 2018; Jamaloddin *et al*. 2021; Kanipriya *et al*. 2024) have tested multiple isolates too, as in the present study.

Genotyping for bacterial blight resistance

Functional marker *xa13-prom* was used for the *xa13*, a recessive BB gene with 500 bp as resistant allele size and 300 bp as susceptible allele size and *xa5FM* consists of two sets of primer pairs that amplify a common allele of size of 424 bp in all individuals, a resistance allele of size 134 bp in resistant individuals and a susceptibility allele size of 313 bp in susceptible individuals (Hajira *et al.,* 2016). Similarly, pTA248, a gene linked marker with 990 bp as resistant allele size and 750 bp as susceptible allele size was used for dominant gene *Xa21* mapped on to chromosome 11 (Ronald *et al*., 1992). Among 17 lines all were carring *Xa21* (Figure. 2), *xa13* (Figure. 3) and *xa5* (Figure. 4) in homozygous condition as a result, the study found that combining various BB resistance genes to generate durable resistant lines with long-term steady performance is quite beneficial.

Despite the fact that many effective BB resistance genes (*xa5*, *xa13*, and *Xa21*) have been identified against BB (Hajira *et al*., 2016) whereas resistance conferred by a single gene have been observed to break down in many places (Yoshimura *et al*., 1995), and thus pyramiding two or three genes into a single genetic background has been advocated (Sundaram *et al*., 2008), which would be a viable strategy to develop durable resistance against multiple genes.

The same markers *xa13-prom*, *xa5FM* and *pTA248* were used by many of the workers for confirming the presence of BB resistance genes *xa13*, *xa5* and *Xa21* (Mahopatra *et al*., 2023; Kanipriya *et al.,* 2024; Sumuni *et al*., 2024). About six pyramided lines having the above mentioned were developed in the background of Swarna and IR64, which were evaluated using the markers *xa13-prom*, *xa5FM* and *pTA248*, across the different regions of the country to test the broad spectrum, durable resistant lines to consider as cultivar and donor for future breeding program

(Pradhan *et al.,* 2015). The same three BB resistance genes *xa5, xa13 and Xa21* were introgressed by Nikita *et al*. (2018) using MABB from the donor variety IRB60 into a popular salt-tolerant high yielding Basmati variety CSR-30.

To boost TNG82's resistance to BB disease, Yu-Chia Hsu *et al*. (2020) pyramided BB resistance genes in Tainung82 for broad-spectrum resistance utilizing MAS. Five BB resistance genes (*Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*) were obtained from a donor parent, IRBB66, and transferred into TNG82 through MABB.

Evaluation of agro-morphological traits

Phenotypic data were recorded for all the rice lines along with parents and checks which were grown with two replications in Randomised Complete Block Design (RCBD) at college farm, PJTSAU, Rajendranagar during *Rabi* 2020-21. The traits recorded were days to 50% flowering (DFF), plant height (cm), number of tillers, number of productive tillers, panicle length (cm), panicle weight (g), number of grains per panicle, 1000 seed weight (g), grain yield per plant (g) and were statistically analysed using software OPSTAT. Based on the phenotypic data, best performing lines with BB resistance were selected and forwarded to next generation.

All the lines were observed early compared to MTU1010 in duration. Nine F_6 lines [382-3-2-2 (34.5 g), 724-4-3-2 (36.4 g), 441-1-3-2 (28.92 g), 353-3-1-2 (29.17 g), 382-2-2-2 (30.17 g), 251-3-3-2 (29.92 g), 251-2-2-2 (29.5 g), 160-2-1-2 (29.83 g) and 3-5-2-2 (31.08 g)] recorded statistically significant superior yield per plant whereas one 56-4-3-2 (27.25 g) was numerically superior than best check MTU1010 (Table. 5).

As in the present study, several workers have attempted combining phenotypic selection for agro-morphological traits along with genotypic screening of rice lines (Arunakumari *et al*., 2016; Dash *et al*., 2016; Abhilash Kumar *et al*., 2017 and Nguyen *et al*., 2018; Yugander *et al*., 2018 and Jamaloddin *et al*., 2020).

About 43 lines with four BB resistance gene combination (*Xa4*, *xa5*, *Xa21* and *xa13*) were evaluated for agronomical and quality traits by Dash *et al*. (2016), where the results showed that most of the traits were identical and yield range were comparable to those of recurrent parent. Based on BB and blast resistance, Arunakumari *et al*. (2016) identified three ICF_3 promising lines that had grain yields comparable to MTU1010. Abhilash Kumar *et al*. (2017) discovered that RPIC-16-65-125, a single ICF_4 line (including *Xa21*, *Gm4* and *Gm8* genes), had a higher quantity of grains per panicle and a better panicle than recurrent parent RPHR-1005, which could be responsible for enhanced grain output per plant. Nguyen *et al.* (2018) found 11 BC_3F_3 plants, of which eight were chosen based on agronomical characteristics. Five of the best plants with BB resistance and high agronomic performance were chosen to produce pure lines for evaluating the potential of BB resistant LT2. Dixit *et al*. (2020) have done extensive phenotypic selection for the improvement of introgressed lines (ILs) after genotypic screening for BB resistance genes *Xa21*, *xa13*, *Xa4* and *xa5*. Seven ILs were high yielding under various biotic stresses such as BB, blast, GM and Brown plant hopper.

Ten F_6 lines (382-3-2-2, 724-4-3-2, 441-1-3-2, 353-3-1-2, 382-2-2-2, 251-3-3-2, 251-2-2-2, 160-2-1-2 and 3-5-2-2) lines which have shown BB resistance in both phenotypic and genotypic evaluations, had confirmed the presence of resistance genes *xa13*, *Xa21* and *xa5* with superior grain yield than the best check, MTU1010 and which were early or on par with MTU1010 in duration.

These identified 10 BB resistant and high yielding advanced generation rice breeding lines hold good potential to be forwarded for further. Since these lines contain additional genes too, for resistance to gall midge/ tolerance to low soil phosphorus, these could also be screened further and best lines with multiple stress resistance could also be identified.

Conclusion

In conclusion, from the present study homozygous lines with multiple BB resistance genes, showing resistance reaction against different BB isolates coupled with higher yield have been identified which can be forwarded further towards development of high yielding BB resistance varieties for the benefit of rice farming community.

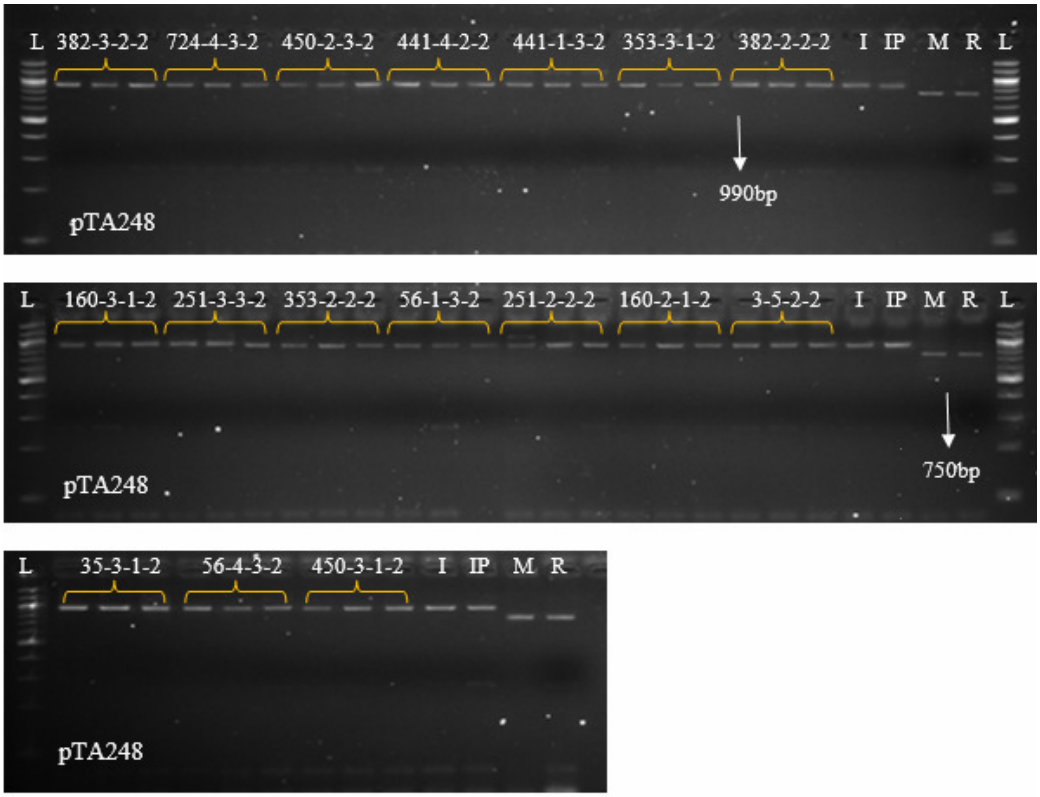

Fig. 2 : Molecular confirmations of F_6 lines for bacterial blight resistance for *Xa21* gene using gene linked marker (pTA248)

Amplification of three plants each of 17 F6 lines along with parents and checks using pTA248 marker for *Xa21* gene with resistance allele size of 990 bp and susceptible allele size of 750 bp. L:100 bp ladder, I: Improved Samba Mahsuri (Positive check for *Xa21* gene), IP: ISM+*Pup1* (Positive check for *Xa21* gene), M: MTU1010 (Negative check for *Xa21* gene), R: Rasi (Negative check for *Xa21* gene).

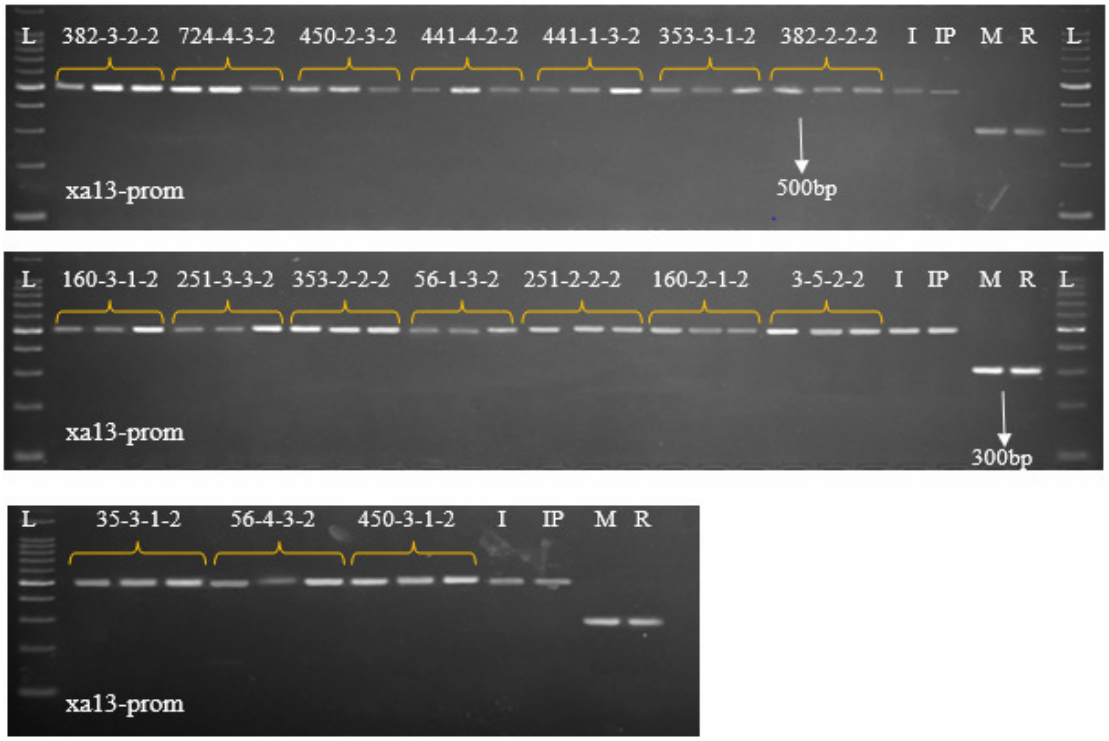

Fig. 3 : Molecular confirmations of F_6 lines for bacterial blight resistance for *xa13* gene using gene-specific marker (xa13-prom)

Amplification of three plants each of 17 F6 lines along with parents and checks using xa13-prom marker for *xa13* gene with resistance allele size of 500 bp and susceptible allele size of 300 bp. L:100 bp ladder, I: Improved Samba Mahsuri (Positive check for *xa13* gene), IP: ISM+*Pup1* (Positive check for *xa13* gene), M: MTU1010 (Negative check for *xa13* gene), R: Rasi (Negative check for *xa13* gene).

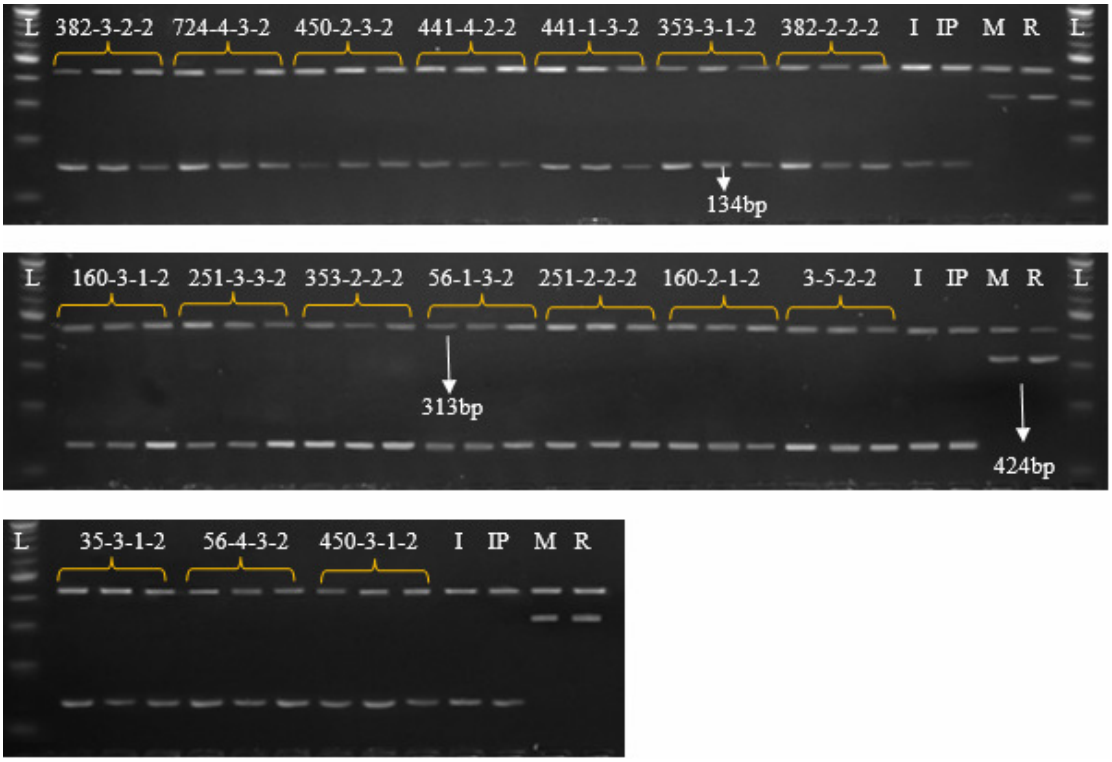

Fig. 4 : Molecular confirmations of F_6 lines for bacterial blight resistance for *xa5* gene using gene-specific marker (xa5FM)

Amplification of three plants each of 17 F_6 lines along with parents and checks using xa5FM marker for *xa5* gene with resistance allele size of 134 bp, susceptible allele size of 313 bp and common allele size of 424 bp for all genotypes. L:100 bp ladder, I: Improved Samba Mahsuri (Positive check for *xa5* gene), IP: ISM+*Pup1* (Positive check for *xa5* gene), M: MTU1010 (Negative check for *xa5* gene), R: Rasi (Negative check for *xa5* gene).

Table 3 : Details of gene specific and gene linked markers for bacterial blight resistance

S. No	Target gene	Molecular marker	Type of marker	Chromosome location	Forward primer	Reverse primer	Allele size (bp)	References
1	*xa5*	xa5S (Multiplex) xa5SR/R (Multiplex)	Gene specific marker	5	F:GTCTGGAATTTGCTCGCGTTCG F:AGCTCGCCATCA AGTTCTTGAG	R:TGGTAAAGTAGAT ACCTTATCAAACTGGA R:TGACTTGGTTCTC CAAGGCTT	R-134 S-313	Hajira *et al*., 2016
2	*Xa21*	pTA248	Gene linked marker	11	F:AGACGCGAAGG GTGGTTCCCGA	R: AGACGCGGTAATC GAAGATGAAA	R-990 S-750	Ronald *et al*., 1992
3	*xa13*	xa13-prom	Gene specific marker	8	F:GGCCATGGCTCAGTGTTTAT	R:GAGCTCCAGCTC TCCAAATG	R-500 S-300	Sundaram *et al*., 2008

R - Resistant allele
S - Susceptible allele

Table 4 : Disease reaction of F_6 generation rice breeding lines inoculated with three *Xoo* isolates:

S. No	Genotypes	Isolate-1			Isolate-2			Isolate-3		
		Diseased leaf area %	BB Score*	Reaction	Diseased leaf area%	BB Score*	Reaction	Diseased leaf area%	BB score*	Reaction
1	382-3-2-2	3.4	1	R	3.8	1	R	4.3	1	R
2	251-2-2-2	4.1	1	R	3.4	1	R	2.5	1	R
3	450-3-1-2	3.4	1	R	3.7	1	R	2.3	1	R
4	35-3-1-2	3.2	1	R	4.3	1	R	4.5	1	R
5	441-4-2-2	3.6	1	R	3.3	1	R	4.9	1	R
6	450-2-3-2	3.6	1	R	3.7	1	R	4.7	1	R
7	441-1-3-2	4.4	1	R	3.8	1	R	4.1	1	R
8	724-4-3-2	2.9	1	R	3.2	1	R	4.4	1	R
9	56-1-3-2	3.6	1	R	3.7	1	R	3.7	1	R
10	382-2-2-2	3.4	1	R	3.7	1	R	3.1	1	R
11	3-5-2-2	4.5	1	R	3.9	1	R	4.6	1	R
12	353-2-2-2	3.2	1	R	3.9	1	R	4.6	1	R
13	251-3-3-2	3.0	1	R	4.0	1	R	4.3	1	R
14	160-2-1-2	3.7	1	R	3.9	1	R	3.7	1	R
15	160-3-1-2	3.4	1	R	4.0	1	R	4.8	1	R
16	353-2-2-2	3.4	1	R	4.0	1	R	4.8	1	R
17	56-4-3-2	4.9	1	R	4.1	1	R	4.9	1	R
18	ISM+*Pup1*	4.4	1	R	4.1	1	R	5.0	1	R
19	MTU1010	69.0	9	HS	64.7	9	HS	61.9	9	HS
20	ISM	3.7	1	R	3.9	1	R	4.0	1	R
21	Rasi	59.8	9	HS	67.7	9	HS	58.8	9	HS

*Bacterial blight reaction scoring was done as per SES (IRRI, 2013)
HS: Highly Susceptible, R: Resistant

Table 5 : Agro-morphological data of F_6 lines for season *Rabi* 2020-21

S. No.	Entry No	Days to 50% flowering (DFF)	Plant height (cm)	No. of Tillers	No. of Panicles	Panicle length (cm)	Panicle weight(g)	No. of grains /panicle	1000 Seed weight (g)	Grain yield /plant (g)
1	382-3-2-2	107	79.17	13	11	21.95	1.97	125	15.30	34.5*
2	724-4-3-2	108	85.33	14	11	21.76	1.80	120	17.05	36.4*
3	450-2-3-2	105	69.83	13	11	21.16	1.74	107	15.40	21.75
4	441-4-2-2	107	67.67	13	12	22.50	1.85	120	16.45	21.67
5	441-1-3-2	108	68.50	12	10	21.31	2.00	121	16.00	28.92*
6	353-3-1-2	108	77.00	12	11	21.41	1.85	115	17.00	29.17*
7	382-2-2-2	107	87.50	10	10	21.61	1.77	129	16.35	30.17*
8	160-3-1-2	110	80.33	12	10	21.11	1.80	123	15.00	24.8
9	251-3-3-2	110	80.83	11	9	21.97	1.88	120	16.00	29.92*
10	353-2-2-2	105	84.5	14	12	21.80	1.74	115	17.00	22.58
11	56-1-3-2	108	84.00	13	12	21.57	2.02	123	15.70	20.83
12	251-2-2-2	110	80.17	13	11	22.40	2.13	123	15.10	29.5*
13	160-2-1-2	109	80.33	11	9	22.01	2.03	124	15.80	29.83*
14	3-5-2-2	108	62.50	11	11	21.23	1.82	117	15.75	23.42
15	35-3-1-2	110	75.50	11	9	22.60	2.05	135	15.75	31.08*
16	56-4-3-2	107	74.50	11	10	22.02	1.93	124	17.00	27.25
17	450-3-1-2	105	77.00	12	11	21.60	1.94	131	15.70	22.17
18	ISM	108	68.67	12	10	21.41	1.86	125	15.55	28.65*
19	ISM+*Pup1*	110	78.67	12	11	22.06	1.82	108	15.15	31.67*
20	**MTU1010**	**112**	**80.33**	**12**	**11**	**20.36**	**1.60**	**118**	**14.15**	**25.27**
	Average	108	77.12	12	10	21.69	1.88	121	15.86	27.48
	CV		4.07	11.47	9.10	4.0	10.77	8.71	1.94	6.69
	CD		5.55	2.47	1.72	1.5	0.35	18.67	0.54	3.25

Acknowledgements

The authors are thankful to Prof. Jayashankar Telangana State Agricultural University, Hyderabad for providing the facilities and financial assistance for carrying out the present study. We wish to present our special thanks to ICAR-IIRR, Hyderabad, Agricultural Research Station, Rajendranagar, for guiding me throughout the research.

References

Abhilash Kumar, V., Balachiranjeevi, C. H., Bhaskar Naik, S., Rekha, G., Rambabu, R., Harika, G., ... & Sundaram, R. M. (2017). Marker-assisted pyramiding of bacterial blight and gall midge resistance genes into RPHR-1005, the restorer line of the popular rice hybrid DRRH-3. *Molecular Breeding*, ***37***, 1-14.

Arunakumari, K., Durgarani, C. V., Satturu, V., Sarikonda, K. R., Chittoor, P. D. R., Vutukuri, B., ... & Sundaram, R. M. (2016). Marker-assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into Indian rice variety MTU1010. *Rice Science*, ***23***(6), 306-316.

Busungu, C., Taura, S., Sakagami, J. I., & Ichitani, K. (2016). Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24. *Breeding science*, ***66***(4), 636-645.

Dash, A. K., Rao, R. N., Rao, G. J. N., Verma, R. L., Katara, J. L., Mukherjee, A. K., ... & Bagchi, T. B. (2016). Phenotypic and marker-assisted genetic enhancement of parental lines of Rajalaxmi, an elite rice hybrid. *Frontiers in plant science*, ***7*,** 1005.

Divya, D., Madhavi, K. R., Dass, M. A., Maku, R. V., Mallikarjuna, G., Sundaram, R. M., ... & Bentur, J. S. (2018). Expression profile of defense genes in rice lines pyramided with resistance genes against bacterial blight, fungal blast and insect gall midge. *Rice*, ***11***, 1-13.

Dixit, S., Singh, U. M., Singh, A. K., Alam, S., Venkateshwarlu, C., Nachimuthu, V. V., ... & Kumar, A. (2020). Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/tolerance in rice. *Rice*, ***13***, 1-15.

Duppala, M. K., Srinivas, T., Rao, L. S., Suneetha, Y., Sundaram, R. M., Kumari, V. P., ... & Ganesh, B. (2023). Screening of recombinant inbred lines for resistance to bacterial leaf blight pathotypes in rice (Oryza sativa L.). *Plant Science Today*, ***10***(3), 343-353.

Hajira, S. K., Sundaram, R. M., Laha, G. S., Yugander, A., Balachandran, S. M., Viraktamath, B. C., ... & Rekha, G. (2016). A single-tube, functional marker-based multiplex PCR assay for simultaneous detection of major bacterial blight resistance genes Xa21, xa13 and xa5 in rice. *Rice Science*, ***23***(3), 144-151.

Indiastat. Agriculture production statistical database. 2022-23. http://www. indiastat.com.

Kanipriya, R., Natarajan, S., Gopalakrishnan, C., Ramalingam, J., Saraswathi, R., & Ramanathan, A. (2024). Screening for disease resistance and profiling the expression of defense-related genes contributing to resistance against bacterial blight (Xanthomonas oryzae pv. oryzae) in rice genotypes. *Physiological and Molecular Plant Pathology*, ***131***, 102286.

Kauffman, H. E., Reddy, A. P. K., Hsieh, S. P. Y., & Merca, S. D. (1973). An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae.

Kotasthane, A. J., & Gaikwad, N. J. (2021). Marker Assisted Selection of xa5, xa13 and Xa21 Gene in Breeding Populations Derived from Karma Mahsuri x IRBB 59. *Plantae Scientia*, ***4***(1), 108-116.

Kumar, P. N., Sujatha, K., Laha, G. S., Rao, K. S., Mishra, B., Viraktamath, B. C., ... & Sundaram, R. M. (2012). Identification and fine-mapping of Xa33, a novel gene for resistance to Xanthomonas oryzae pv. oryzae. *Phytopathology*, ***102***(2), 222-228.

Muralidharan, K., Krishnaveni, D., Laha, G. S., Reddy, C. S., Srinivasprasad, M., & Sridher, R. (2003). Appraisal of bacterial blight resistance genes in India. *Rice Genetic Newsletter*, ***20***, 96-98.

Mohapatra, S., Barik, S. R., Dash, P. K., Lenka, D., Pradhan, K. C., Raj K. R, R., ... & Pradhan, S. K. (2023). Molecular Breeding for Incorporation of Submergence Tolerance and Durable Bacterial Blight Resistance into the Popular Rice Variety 'Ranidhan'. *Biomolecules*, ***13***(2), 198.

Hue Thi Nguyen, H. T. N., Quang Hong Vu, Q. H. V., Tan Van Mai, T. V. M., Thu Thi Nguyen, T. T. N., Lam Duc Vu, L. D. V., Tung Thanh Nguyen, T. T. N., ... & Liet Van Vu, L. V. V. (2018). Marker-assisted selection of Xa21 conferring resistance to bacterial leaf blight in indica rice cultivar LT2.

Baliyan, N., Malik, R., Rani, R., Mehta, K., Vashisth, U., Dhillon, S., & Boora, K. S. (2018). Integrating marker-assisted background analysis with foreground selection for pyramiding bacterial blight resistance genes into Basmati rice. *Comptes rendus biologies*, ***341***(1), 1-8.

Pradhan, S. K., Nayak, D. K., Mohanty, S., Behera, L., Barik, S. R., Pandit, E., ... & Anandan, A. (2015). Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice*, ***8***, 1-14.

Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Wu, K. S., McCouch, S., & Tanksley, S. D. (1992). Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. *Molecular and General Genetics MGG*, ***236***, 113-120.

Sumuni, S. M., Kaur, R., Kaur, R., Khanna, R., Kaur, K., Lore, J. S., ... & Mangat, G. S. (2024). Multivariate analysis for morpho-physiological and milling traits along with molecular profiling of known bacterial blight resistance genes in advanced breeding lines of rice. *Cereal Research Communications*, ***52***(2), 759-775.

Sundaram, R. M., Vishnupriya, M. R., Biradar, S. K., Laha, G. S., Reddy, G. A., Rani, N. S., ... & Sonti, R. V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, ***160***, 411-422.

Swathi, G., Durga Rani, C. V., Md, J., Madhav, M. S., Vanisree, S., Anuradha, C., ... & Jagadeeswar, R. (2019). Marker-assisted introgression of the major bacterial blight resistance genes, Xa21 and xa13, and blast resistance gene, Pi54, into the popular rice variety, JGL1798. *Molecular Breeding*, ***39***, 1-12.

Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S. R., Abenes, M. L., Baraoidan, M. R., ... & Nelson, R. J. (1995). Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Molecular breeding*, ***1***, 375-387.

Yugander, A., Sundaram, R. M., Singh, K., Ladhalakshmi, D., Subba Rao, L. V., Madhav, M. S., ... & Laha, G. S. (2018). Incorporation of the novel bacterial blight resistance gene Xa38 into the genetic background of elite rice variety Improved Samba Mahsuri. *Plos one*, ***13***(5), e0198260.