Plant Archives Vol. 25, No. 1, 2025 pp. 2639-2647 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.no.1.381

ASSESSMENT OF ADVANCED RICE (*ORYZA SATIVA* L.) BREEDING LINES FOR BACTERIAL BLIGHT RESISTANCE AND YIELD RELATED TRAITS

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

[1]Department of Genetics and Plant Breeding, Prof. Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad - 500 030, Telangana, India.

[2]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India.

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

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

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

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

(Date of Receiving-11-02-2025; Date of Acceptance-17-04-2025)

ABSTRACT

Bacterial blight (BB), caused by *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*), is a major threat to rice production, especially in Asia and Africa. Developing BB resistant elite rice varieties by deploying BB-resistant genes is essential for enhancing rice breeding programs is the most effective way to protect the crop and significantly elevate the yield. An experiment was conducted using fifteen IBTWGL lines developed through marker-assisted pedigree breeding from the crosses MTU1010 × RMSGM3, MTU-IL × RMSGM3, and (MTU1010 × RMSGM3) × (MTU1010 × RP5923). These lines were phenotypically screened for resistance to bacterial blight (BB) using artificial inoculation method against three virulent *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates, RPR, IXO-20, and RRC-ARI at the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, during the *Rabi* season of 2020-21. The study included parental lines and checks such as MTU1010, TN1, RMSGM3, MTU-IL and RP5923. Additionally, the lines were genotyped for the presence of the BB resistance genes *Xa21* and *xa13* using gene-specific or linked markers, pTA248 and xa13-prom, respectively and were visually assessed for agro-morphological traits. All advanced breeding lines exhibited a resistant reaction in the phenotypic screening, with an average diseased leaf area (%) ranging from 2.4% to 4.6% and a score of 1 according to the Standard Evaluation System (IRRI, 2013). Among these, seven lines demonstrated significantly higher yield per plant compared to the check variety MTU1010, carried the BB resistance genes *xa13* and *Xa21* and displayed a resistant disease reaction. These top-performing lines have the potential for advancement to multi-location yield trials and could be valuable resources in future rice breeding programs.

Key words : Bacterial blight, Artificial inoculation, Advanced breeding lines, Marker assisted selection.

Introduction

Rice (*Oryza sativa* L.) is a staple cereal crop that sustains over half of the world's population. In India, rice production was 137.82 million tonnes harvested from land area of 47.82 million hectares with productivity of 2858 Kilogram per hectare (Indiastat, 2023-2024). Worldwide, India stands first in area and second in production after China. To meet the demands of a growing population, rice production must increase by 42% from current levels by 2050 (Ray *et al*., 2013). However, yield potential of rice cultivation is highly vulnerable to various biotic and abiotic stresses. 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).

Bacterial blight (BB) is a devastating disease in India during the South-West monsoon season (*Kharif* or wet season). This vascular disease manifests as drying and yellowing of leaves, beginning at the tips and progressing

downward. The disease thrives at temperatures between 25°C and 34°C, with relative humidity exceeding 70%. The BB pathogen, *Xanthomonas oryzae* pv. *oryzae*, infiltrates the xylem vessels through natural leaf openings such as hydathodes and wounds. In field conditions, symptoms emerge at the tillering stage and intensify as the plant grows, peaking during the flowering stage. The most severe form, known as *kresek*, affects seedlings and can lead to partial or complete crop failure. The pathogen exhibits substantial genetic variation, leading to the evolution of new pathotypes and a high degree of resistance breakdown.

Studies indicate that cultural and chemical control methods are often ineffective and expensive, particularly during epidemics. Among the various management strategies, host plant resistance remains the most viable solution, as it is both environmentally sustainable and cost-effective (Yugander *et al*., 2018). Both the conventional and molecular breeding approaches for BB resistance aim at improving the genetic underpinnings of rice cultivars and the BB pathogen for host plant resistance (Li *et al*., 2020). In the present situation, it is very difficult to meet the growing challenges only with the application of conventional plant breeding techniques and tools. It would be very difficult to select rice lines possessing multiple resistance genes using the conventional approach alone, because of the several limitations in conventional breeding (Sundaram *et al*., 2014). Molecular tools of biotechnology can be helpful to meet the rice production and productivity targets by improving resistance against several diseases. Plant breeders are currently using Marker-Assisted Selection (MAS) to identify and select plants based on DNA markers associated with specific genes responsible for desirable traits. Since, these markers are located near or within the DNA sequence of the target gene, they are inherited across generations according to the standard laws of inheritance. Likewise, the genetic basis of resistance to the pathogen has been well studied in rice, and to date, approximately 45 resistance (R) genes against bacterial blight (BB) have been identified from various rice sources (Neelam *et al*., 2020). Nevertheless, only 11 of these *R* genes (*Xa1*, *Xa3*/*Xa26*, *Xa4*, *xa5*, *xa13*, *Xa21*, *Xa23*, *Xa25*, *Xa27* and *Xa41*) have been cloned and functionally assessed. 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).

This study was conducted to evaluate responses of 15 IBTWGL advanced generation rice breeding lines against 3 different isolates of *Xoo*, genotyped for the presence of BB resistant genes *i.e.*, *Xa21* and *xa13* as these genes offer durable resistance to BB and also to select superior resistant plants that can be forwarded further towards developing new resistant high yielding rice varieties.

Materials and Methods

Plant material

In the current study, fifteen IBTWGL lines, were derived from three different crosses *i.e.*, MTU1010 × RMSGM3, MTU-IL × RMSGM3, (MTU1010 × RMSGM3) × (MTU1010 × RP5923), which were raised along with parents and checks in *Rabi* 2020-21 (Table 1).

Phenotypic evaluation for bacterial blight resistance using artificial inoculation

Phenotypic screening for bacterial blight (BB) resistance was conducted on all advanced backcross lines (ABLs), along with their parental lines and check varieties. Three pots were prepared for each genotype, with three plants in each pot. Pot-1, Pot-2, and Pot-3 were inoculated with Isolate-1 (RPR), Isolate-2 (IX0-20) and Isolate-3 (RRC-ARI), respectively. These isolates were collected from Raipur, Chhattisgarh; Hyderabad, Telangana and Rajendranagar, Telangana. For each plant, 5-6 leaves were clipped and inoculated at the maximum tillering stage (55 days after transplanting) using the method described by Kauffman *et al*. (1973). The disease reaction was scored 14 days post-inoculation based on the Standard Evaluation System (SES) for rice, IRRI (2013), as outlined in Table 2.

Genomic DNA isolation and PCR amplification

Fresh, young leaves were collected and stored in 1.5 mL microcentrifuge tubes at - 20°C. DNA extraction was carried out following the Cetyl Tri methyl Ammonium Bromide (CTAB) method. Extracted DNA pellets were dissolved in 100 ìl of 1X TE (Tris EDTA) buffer. The quality and quantity of isolated DNA was checked through Ethidium Bromide stained Agarose gel electrophoresis (AGE). 0.8% agarose gel was prepared and the samples were electrophoresed at 75-80V for 20-30 minutes till the bromophenol dye front reached 2/3rd of the running length of the gel.

The gel was then visualized under UV light in an Alpha Innotech gel documentation system (Alpha Innotech, USA) for checking the quality and quantity of DNA. Based on comparison with intensity of DNA solution of known concentration, the concentration of samples was determined. Afterwards, the DNA samples were diluted to a final concentration of ~50 ng/µl using 1X TE buffer and stored at 4°C.

Table 1 : List of IBTWGL lines selected for raising during *Rabi* 2020-21.

S. no.	Entry used	Parentage	Gene combination in F_5
1	IBTWGL1	MTU1010 × RMSGM3	*Xa21, xa13, Gm4, Gm8*
2	IBTWGL2		*Xa21, xa13, Gm4, Gm8*
3	IBTWGL3		*Xa21, xa13, Gm4, Gm8*
4	IBTWGL4		*Xa21, xa13, Gm4, Gm8*
5	IBTWGL5		*Xa21, xa13, Gm4, Gm8*
6	IBTWGL7		*Xa21, xa13, Gm4, Gm8*
7	IBTWGL8		*Xa21, xa13, Gm4, Gm8*
8	IBTWGL9		*Xa21, xa13, Gm4, Gm8*
9	IBTWGL10		*Xa21, xa13, Gm4, Gm8*
10	IBTWGL15	MTU-IL × RMSGM3	*Xa21, xa13, Gm4, Gm8*
11	IBTWGL16		*Xa21, xa13, Gm4, Gm8*
12	IBTWGL19		*Xa21, xa13, Gm4, Gm8*
13	IBTWGL21		*Xa21, xa13, Gm4, Gm8*
14	IBTWGL22	(MTU1010 × RMSGM3) × (MTU1010 × RP5923)	*Xa21, xa13, Gm4, Gm8, gm3*
15	IBTWGL31		*Xa21, xa13, Gm4, Gm8, gm3*

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

Genotyping of rice lines was carried out using PCR analysis with gene-specific or gene-linked primers. The amplification was performed in a thermocycler (AB Veriti, USA) with a final reaction volume of 10 µl. The PCR program consisted of an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of amplification with the following conditions: denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, and primer extension at 72°C for 1 minute. A final extension step was performed at 72°C for 7 minutes.

Several gene-specific/linked markers have been used for screening BB resistance alleles (Anik *et al*., 2022; Mohapatra *et al*., 2023; Kanipriya *et al*., 2024; Sumuni *et al*., 2024). In the present study, *Xa21* and *xa13* genes were screened using the markers *pTA248* and *xa13-prom* respectively. The details of the markers, their sequences and allele sizes are given in Table 3.

Agro-morphological traits

Fifteen IBTWGL ABLs along with 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)

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	*Xa21*	pTA248	Gene linked marker	11	F:AGACGCGAAGG GTGGTTCCCGA	R: AGACGCGGTAATC GAAGATGAAA	R-990 S-750	Ronald *et al*. (1992
2	*xa13*	xa13- prom	Gene specific marker	8	F:GGCCATGGCTC AGTGTTTAT	R:GAGCTCCAGCTC TCCAAATG	R-500 S-300	Sundaram *et al*. (2008)

R - Resistant allele S - Susceptible allele.

and analyzed for determining the yield potentiality of genotypes.

Statistical analysis

The data recorded from the three selected plants were averaged to derive representative means for each genotype. The means for each trait across replications were then subjected to statistical analysis to calculate the mean, range, standard error (SE), coefficient of variation (CV) and critical difference (CD), which were determined using the 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, TN1, MTU1010 have shown highly susceptible reaction (HS) with a score of 9. RMSGM3, RP5923 and MTUIL for IBTWGL lines 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 2.4% to 4.6% against Isolate-1, 2.6% to 4.1% against Isolate-2 and 3.1% to 4.3% against Isolate-3 with score of 1 (Table 4) as per SES (IRRI, 2013) scale (Fig. 1).

The findings of the present study align with previous research that employed the leaf clipping method of

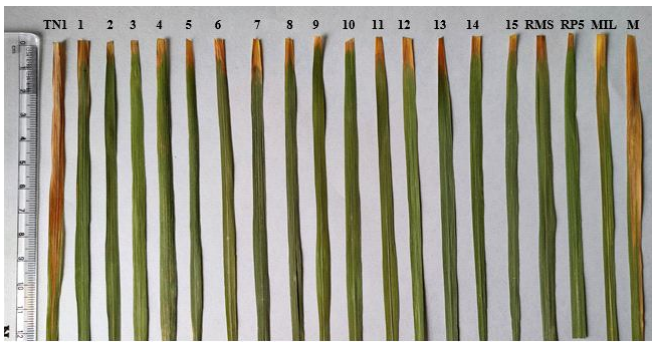

Fig. 1 : Bacterial blight reaction in IBTWGL lines during *Rabi* 2020-21. TN1: Taichung Native 1 (Negative check for BB), RMS: RMSGM3 (Positive check for BB), RP5: RP5923, MIL: MTUIL, M: MTU1010 (Negative check for BB), 1: IBTWGL1, 2: IBTWGL2, 3: IBTWGL3, 4: IBTWGL4, 5: IBTWGL5, IBTWGL7, 7: IBTWGL8, 8: IBTWGL9, 9: IBTWGL10, 10: IBTWGL15, 11: IBTWGL16, 12: IBTWGL19, 13: IBTWGL21, 14: IBTWGL22, 15: IBTWGL31.

Kauffman *et al*. (1973) for phenotypic screening of rice genotypes against bacterial blight (BB). This method has been widely utilized by various researchers across different generations of rice lines, as demonstrated in studies by Divya *et al*. (2015), Arunakumari *et al*. (2016), Busungu *et al*. (2016), Laha *et al*. (2017), Das *et al*. (2018), Swathi *et al*. (2019), Kotasthane and Gaikwad (2021), and Akter *et al*. (2022). These studies have confirmed the efficacy of the leaf clipping method in assessing BB resistance in both early and advanced generations of rice breeding programs.

Additionally, Yugander *et al*. (2018) demonstrated that backcross-derived lines can be effectively screened using multiple *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains, further validating the robustness of this methodology. Similar to our approach, Das *et al*. (2018) employed the leaf clipping technique at the maximum tillering stage to evaluate gene pyramids in the Tapaswini variety against BB. Furthermore, Kotasthane and Gaikwad (2021) successfully screened an F_3 population derived from a cross between IRBB59 and Karma Mahsuri, reinforcing the reliability of this method for resistance assessment in segregating populations.

A notable trend observed in earlier literature is the predominant use of single isolates for screening BB resistance in rice lines, as reported by 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), and Kumar *et al*. (2023). However, in contrast to these studies, a few researchers, including Mondal *et al*. (2014), Das *et al*. (2018), Yugander *et al*. (2018), Jamaloddin *et al*. (2021), and Kanipriya *et al*. (2024), have tested multiple isolates, a practice also employed in the present study. The use of multiple isolates provides a more comprehensive evaluation of resistance, as it accounts for the genetic diversity and pathogenic variability of *Xoo* strains.

By incorporating multiple isolates in our study, we were able to assess the resistance spectrum of the tested genotypes more effectively. This approach is particularly crucial in breeding programs aimed at developing broad-spectrum resistance to BB, as it ensures that selected lines possess durable resistance across diverse pathogen populations. The observed results reinforce the need for continued efforts in deploying multiple isolates for resistance screening to enhance the robustness of breeding strategies targeting BB resistance in rice.

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

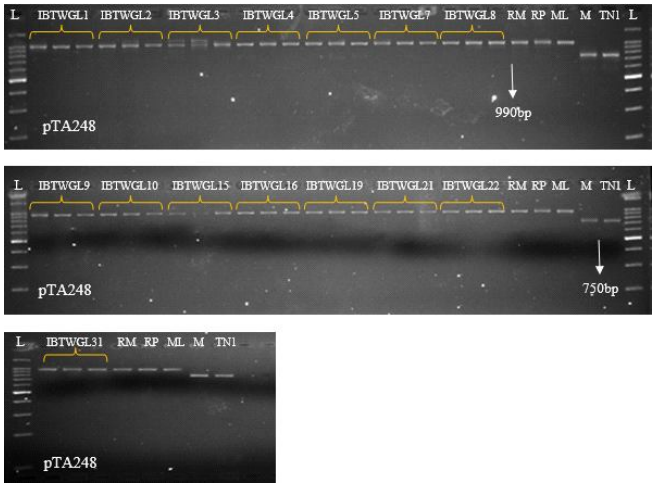

Fig. 2 : Molecular confirmation of IBTWGL lines *xa21* gene using gene linked marker pTA248. Three plants of each line of the 15 IBTWGL lines were used for PCR analysis. The marker pTA248 showed resistance allele size of 990 bp and susceptible allele size of 750 bp L: 100 bp ladder, RM: RMSGM3, RP: RP5923, ML: MTU-IL, M: MTU1010, TN1: Taichung Native 1 (Negative check).

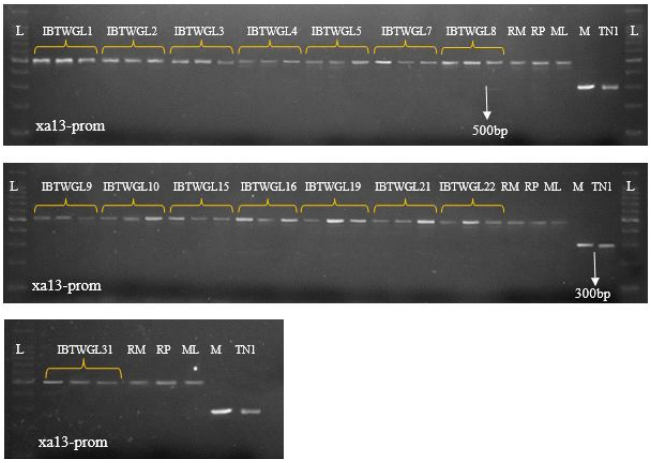

Fig. 3 : Molecular confirmation of IBTWGL lines for *xa13* gene using gene-specific marker xa13-prom. Three plants of each line of the 15 IBTWGL lines were used for PCR analysis. The marker xa13-prom showed resistance allele size of 500 bp and susceptible allele size of 300 bp L: 100 bp ladder, RM: RMSGM3, RP: RP5923, ML: MTU-IL, M: MTU1010, TN1: Taichung Native 1 (Negative check).

and 300 bp as susceptible allele size. Similarly, pTA248, a gene linked marker with resistant allele size of 990 bp and susceptible allele size of 750 bp was used for dominant gene *Xa21* mapped on to chromosome 11 (Ronald *et al*., 1992). Among 15 lines, all were carrying *Xa21* (Fig. 2) and *xa13* (Fig. 3) 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. Although several effective BB resistance genes, such as *xa13* and *Xa21*, have been identified (Hajira *et al*., 2016), although single resistant gene is usually race-specific and can be overcome as new races emerge, whereas the combination of different resistance genes gives broad spectrum durable resistance against a wide range of races.

The results of present study line up with previous findings that pyramiding multiple BB resistance genes into a single genetic background enhances the durability and broad-spectrum resistance of rice cultivars. The incorporation of two or more resistance genes (*xa5*, *xa13* and *Xa21*) has been shown to provide significant protection against diverse strains of the bacterial blight pathogen, which supports the effectiveness of this approach for developing resilient rice varieties (Sundaram *et al*., 2008; Arunakumari *et al*., 2016; Jamaloddin *et al*., 2020).

The use of molecular markers, particularly xa13-prom and pTA248, has proven to be a reliable tool for confirming the presence of BB resistance genes *xa13* and *Xa21* (Mohapatra *et al*., 2023; Kanipriya *et al*., 2024; Sumuni *et al*., 2024). In this study, six pyramided lines were successfully developed in the genetic backgrounds of Swarna and IR64. Their evaluation across different regions suggests their potential as broad-spectrum resistant cultivars. This geographical assessment is crucial, as pathogen virulence can vary by location, and resistant lines must demonstrate effectiveness across diverse environments (Pradhan *et al*., 2015).

Moreover, the successful introgression of *xa5*, *xa13*, and *Xa21* into the salt-tolerant, high-yielding Basmati variety CSR-30 by Nikita *et al*. (2018) underscores the feasibility of combining biotic and abiotic stress tolerance. The use of Marker-Assisted Backcross Breeding (MABB) in this process has facilitated the precise selection of resistant individuals, reducing linkage drag and ensuring that desirable agronomic traits are retained. This reinforces the importance of marker-assisted selection in modern breeding programs.

While pyramiding BB resistance genes has shown promise, further long-term field evaluations are necessary to assess their stability under varying environmental conditions. Additionally, continuous pathogen monitoring is essential, as new virulent strains may emerge that could potentially overcome the resistance conferred by these genes. Future studies should explore the combination of BB resistance genes with other disease resistance genes to develop even more robust cultivars.

Evaluation of agro-morphological traits

Phenotypic data were recorded for all the rice lines

Table 4 : Disease reaction of IBTWGL rice breeding lines against three *Xoo* isolates.

S. no.	Genotypes	Isolate-1 (RPR)			Isolate-2 (IX0-20)			Isolate-3 (RRC-ARI)		
		Diseased leaf area %	BB Score*	Reaction	Diseased leaf area %	BB Score*	Reaction	Diseased leaf area %	BB Score*	Reaction Reaction
1	IBTWGL1	3.3	1	R	3.9	1	R	3.7	1	R
2	IBTWGL2	3.5	1	R	3.8	1	R	3.4	1	R
3	IBTWGL3	2.7	1	R	2.6	1	R	3.5	1	R
4	IBTWGL4	2.4	1	R	3.0	1	R	4.1	1	R
5	IBTWGL5	4.1	1	R	4.1	1	R	3.2	1	R
6	IBTWGL7	3.6	1	R	3.5	1	R	3.1	1	R
7	IBTWGL8	3.4	1	R	3.1	1	R	4.0	1	R
8	IBTWGL9	2.8	1	R	3.9	1	R	4.3	1	R
9	IBTWGL10	4.6	1	R	3.2	1	R	3.5	1	R
10	IBTWGL15	3.6	1	R	2.6	1	R	4.3	1	R
11	IBTWGL16	3.7	1	R	3.6	1	R	3.8	1	R
12	IBTWGL19	3.5	1	R	3.5	1	R	3.7	1	R
13	IBTWGL21	4.0	1	R	3.4	1	R	3.1	1	R
14	IBTWGL22	3.9	1	R	4.0	1	R	3.4	1	R
15	IBTWGL31	3.0	1	R	3.7	1	R	4.2	1	R
16	RP5923	3.5	1	R	3.3	1	R	4.4	1	R
17	MTU1010	67.7	9	HS	71.7	9	HS	69.1	9	HS
18	RMSGM3	2.7	1	R	3.8	1	R	4.1	1	R
19	MTUIL	2.5	1	R	3.8	1	R	3.5	1	R
20	**TN1**	**69.2**	**9**	**HS**	**81.2**	**9**	**HS**	**64.5**	**9**	**HS**

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

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. Five among 15 IBTWGL lines [IBTWGL4 (28.17 g), IBTWGL8 (27.65 g), IBTWGL16 (28.55 g), IBTWGL21 (27.58 g) and IBTWGL31 (28.92 g)] were recorded statistically significant superior yield than the MTU1010 whereas, two lines IBTWGL5 (26.0 g) and IBTWGL10 (26.92 g) were numerically higher in yield when compared to MTU1010 (Table 5).

The identification and development of disease-resistant rice lines with improved yield potential have been a key focus of recent breeding efforts. Arunakumari *et al*. (2016) identified three ICF3 promising lines with grain yields comparable to MTU1010, highlighting their potential for BB and blast resistance. Similarly, Abhilash Kumar *et al*. (2017) demonstrated that RPIC-16-65-125, a single ICF4 line carrying the *Xa21*, *Gm4*, and *Gm8* genes, exhibited superior panicle structure and a higher grain count per panicle compared to the recurrent parent RPHR-1005. This suggests that these genetic factors contribute to enhanced grain output per plant.

Further advancements in BB-resistant rice breeding were reported by Nguyen *et al.* (2018), who identified 11 BC3F3 plants, selecting eight based on agronomic traits. Among these, five high-performing plants with strong BB resistance were chosen to develop pure lines for evaluating the potential of BB-resistant LT2. Similarly, Dixit *et al*. (2020) employed a rigorous phenotypic selection process following genotypic screening for BB resistance genes *Xa21, xa13, Xa4*, and *xa5*. Their study successfully identified seven high-yielding introgressed lines (ILs) that performed well under various biotic stresses, including BB, blast, gall midge (GM), and brown planthopper (BPH). Varanasi *et al.* (2023) reported that

Table 5 : Agro-morphological data of IBTWGL lines during *Rabi* 2020-21.

S. no.	Entry No	Days to 50% flowering (DFF)	Plant height (cm)	No. of tillers	No. of productive tillers	Panicle length (cm)	Panicle weight (g)	No. of filled grains/ panicle	1000 seed weight (g)	Grain yield/plant (g)
1	IBTWGL1	107	80.17	9	9	21.81	2.42	122	18.30	19.22
2	IBTWGL2	107	81.67	11	10	22.08	2.18	132	18.40	22.93
3	IBTWGL3	108	80.92	10	9	22.51	2.22	121	18.45	20.47
4	IBTWGL4	108	95.83	14	12	22.25	2.24	122	15.50	28.17*
5	IBTWGL5	109	95.67	11	10	22.00	2.38	119	16.95	26.00
6	IBTWGL7	107	90.81	11	10	21.51	2.11	124	15.70	19.08
7	IBTWGL8	108	81.83	12	11	21.69	2.31	128	12.60	27.65*
8	IBTWGL9	110	72.67	12	11	21.64	2.08	121	16.85	21.03
9	IBTWGL10	108	78.33	12	10	20.92	1.84	116	16.70	26.92
10	IBTWGL15	106	77.00	11	9	21.53	1.87	121	19.20	22.33
11	IBTWGL16	107	75.17	14	11	21.93	2.16	120	16.50	28.55*
12	IBTWGL19	110	80.50	10	8	21.43	1.93	118	17.30	22.33
13	IBTWGL21	109	90.00	14	12	22.43	2.05	126	17.70	27.58*
14	IBTWGL22	108	83.50	13	11	21.80	1.88	126	18.60	21.08
15	IBTWGL31	109	98.00	14	12	21.99	2.19	134	16.90	28.92*
16	RMSGM3	109	96.33	11	9	21.32	2.07	119	14.80	19.15
17	RP5923	110	104.83	10	9	22.34	1.81	119	15.35	21.50
18	MTUIL	110	93.83	14	12	22.39	2.10	129	15.05	22.33
19	**MTU1010**	**112**	**80.33**	**12**	**11**	**20.36**	**1.60**	**118**	**14.15**	**25.27**
	Mean	108	86.18	11	10	21.79	2.08	122	16.58	23.71
	CV		1.66	8.76	8.54	3.53	17.13	5.16	1.58	4.77
	CD		2.59	1.86	1.58	1.39	0.64	11.49	0.47	2.04

among the RP1-ILs, seven recorded higher grain yield than Krishna Hamsa, while the remaining 15 had similar grain yield. Similarly, among the RP2-ILs, 14 recorded higher grain yield than WGL 14, whereas the remaining five had comparable grain yields with resistance to bacterial blight.

These studies collectively emphasize the significance of integrating resistance genes into elite rice varieties to enhance both yield and resilience. The identification of high-yielding and disease-resistant lines offers promising prospects for future breeding programs aimed at improving rice productivity under biotic stress conditions.

In this study, all 15 IBTWGL lines carrying the BB resistance genes (*Xa21* and *xa13*) exhibited resistance to three *Xoo* isolates in phenotypic screening. Agro-morphological analysis indicated that these lines had an earlier maturity duration compared to MTU1010. Among them, five ABLs (IBTWGL4, IBTWGL8, IBTWGL16, IBTWGL21, and IBTWGL31) demonstrated significantly higher yields, while two (IBTWGL5 and IBTWGL10) recorded numerically superior yields than MTU1010. These seven high-yielding, BB-resistant advanced-generation rice breeding lines show strong potential for further development. Additionally, as these lines carry genes for gall midge resistance, further screening could help identify those with multiple biotic stress resistance.

Conclusion

Sustaining rice production and productivity is increasingly challenging with conventional breeding alone due to its limitations in phenotype-based selection. Additionally, the high variability of the

BB pathogen and the constant evolution of virulent *Xoo* isolates necessitate the continuous identification and integration of new BB resistance genes into popular varieties. Marker-assisted backcross breeding has proven effective in incorporating major resistance genes against BB and other biotic stresses, enhancing both yield and disease resistance. The identification, characterization, mapping, tagging, and introgression of BB resistance genes into elite rice varieties have played a crucial role in establishing durable resistance worldwide. The adoption of molecular approaches to combat BB has shown promising results and is expected to significantly improve rice productivity, quality, and overall production in the coming years. The present study was an attempt to evaluate advanced generation rice breeding lines and to identify the best performing high yielding lines with durable BB resistance. These lines could be evaluated further and promising ones could be forwarded towards release as BB resistant high yielding varieties that would be useful to the rice farming community.

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.

Conflict of interests : The authors have declared no conflict of interests exist.

References

Abhilash Kumar, V., Balachiranjeevi C.H., Bhaskar Naik S., Rekha G., Rambabu R., Harika G., Pranathi K., Hajira S.K., Anila M., Kousik M. and Kale R. (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. *Mole. Breed.*, **37**, 1–14. https://doi.org/10.1007/s11032-017-0677-5

Akter, A., Nihad S.A.I., Hasan M.J., Hassan L., Robin A.H.K., Quddus M.R., Tabassum A. and Latif M.A. (2022). Evaluation of hybrid rice parental lines against bacterial blight disease and detection of resistant gene(s) by gene specific, linked markers. *J. Phytopathol.*, **170(6)**, 382–390. https://doi.org/10.1111/jph.13137

Anik, T.R., Nihad S.A.I., Hasan M.A.I., Hossain M.A., Rashid M.M., Khan M.A.I., Halder K.P. and Latif M.A. (2022). Exploring of bacterial blight resistance in landraces and mining of resistant gene(s) using molecular markers and pathogenicity approach. *Physiol. Mole. Biol. Plants*, **28(2)**, 455–469. https://doi.org/10.1007/s12298-021-01066-6

Arunakumari, K., Durgarani C.V., Satturu V., Sarikonda K.R., Chittoor P.D.R., Vutukuri B., Laha G.S., Nelli A.P.K., Gattu S., Jamal M. and Prasadbabu A. (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. https://doi.org/10.1016/j.rsci.2016.09.004

Busungu, C., Taura S., Sakagami J.I. and Ichitani K. (2016). Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24. *Breed. Sci.*, **66(4)**, 636–645. https://doi.org/10.1270/jsbbs.16021

Das, G., Rao G.J., Varier M., Prakash A. and Prasad D. (2018). Improved Tapaswini having four BB resistance genes pyramided with six genes/QTLs, resistance/tolerance to biotic and abiotic stresses in rice. *Scientific Reports*, **8(1)**, 1–16. https://doi.org/10.1038/s41598-018-26344-x

Divya, D., Madhavi K.R., Dass M.A., Maku R.V., Mallikarjuna G., Sundaram R.M., Laha G.S., Padmakumari A.P., Patel H.K., Prasad M.S. and Sonti R.V. (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. https://doi.org/10.1186/s12284-018-0216-2

Dixit, S., Singh U.M., Singh A.K., Alam S., Venkateshwarlu C., Nachimuthu V.V., Yadav S., Abbai R., Selvaraj R., Devi M.N. and Ramayya P.J. (2020). Marker assisted forward breeding to combine multiple biotic-abiotic stress resistance/tolerance in rice. *Rice*, **13**, 1–15. https://doi.org/10.1186/s12284-020-00407-3

Fatima, P., Mishra A., Khan N.A. and Singh K.N. (2018). Towards developing bacterial leaf blight (BB) resistance rice varieties of eastern Uttar Pradesh by using marker assisted selection. *Int. J. Curr. Microbiol. Appl. Sci.*, **7**, 1090–1097.

Hajira, S.K., Sundaram R.M., Laha G.S., Yugander A., Balachandran S.M., Viraktamath B.C., Sujatha K., Balachiranjeevi C.H., Pranathi K., Anila M. and Bhaskar S. (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. https://doi.org/10.1016/j.rsci.2016.02.002

Indiastat (2023–2024). *Agriculture production statistical database*. http://www.indiastat.com

Jamaloddin, M., Durga Rani C.V., Swathi G., Anuradha C., Vanisri S., Rajan C.P.D., Krishnam Raju S., Bhuvaneshwari V., Jagadeeswar R., Laha G.S. and Prasad M.S. (2020). Marker Assisted Gene Pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety "Tellahamsa." *PLOS ONE*, **15(6)**, e0234088. https://doi.org/10.1371/journal.pone.0234088

Kanipriya, R., Natarajan S., Gopalakrishnan C., Ramalingam J., Saraswathi R. and 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. https://doi.org/10.1016/j.pmpp.2023.102286

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

Kotasthane, A.J. and Gaikwad N.J. (2021). Marker Assisted Selection of xa5, xa13 and Xa21 gene in breeding populations derived from Karma Mahsuri × IRBB 59. *Plantae Scientia*, **4(1)**, 108–116.

Kumar, D.M., Srinivas T., Rao L.S., Suneetha Y., Sundaram R.M., Kumari V.P., Bhuvaneswari V. and Ganesh B. (n.d.). Screening of recombinant inbred lines for resistance to bacterial leaf blight pathotypes in rice (*Oryza sativa* L.). *HORIZON*, **10(3)**, 343–353.

Kumar, P.N., Sujatha K., Laha G.S., Rao K.S., Mishra B., Viraktamath B.C., Hari Y., Reddy C.S., Balachandran S.M., Ram T. and Madhav M.S. (2012). Identification and fine-mapping of Xa33, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology*, **102(2)**, 222–228. https://doi.org/10.1094/PHYTO-04-11-0116

Li, L., Mo X., Li T., Zhang L. and Dong H. (2020). Effector *XopN* of *Xanthomonas oryzae* pv. *oryzae* plays a virulent role in rice varieties harboring *OsSWEET11* homologs. *Chinese J. Rice Sci.*, **34(4)**, 368. https://doi.org/10.16819/j.1001-7216.2020.9046

Mohapatra, S., Barik S.R., Dash P.K., Lenka D., Pradhan K.C., Raj K.R.R., Mohanty S.P., Mohanty M.R., Sahoo A., Jena B.K. and Panda A.K. (2023). Molecular breeding for incorporation of submergence tolerance and durable bacterial blight resistance into the popular rice variety 'Ranidhan'. *Biomolecules*, **13(2)**, 198. https://doi.org/10.3390/biom13020198

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

Neelam, K., Mahajan R., Gupta V., Bhatia D., Gill B.K., Komal R., Lore J.S., Mangat G.S. and Singh K. (2020). High-resolution genetic mapping of a novel bacterial blight resistance gene xa-45(t) identified from *Oryza glaberrima* and transferred to *Oryza sativa*. *Theoretical and Applied Genetics*, **133**, 689–705. https://doi.org/10.1007/s00122-019-03506-6

Nguyen, H.T., Vu Q.H., Van Mai T., Nguyen T.T., Vu L.D., Nguyen T.T., Nguyen L.V., Vu H.T.T., Nong H.T., Dinh T.N. and Toshitsugu N. (2018). Marker-assisted selection of Xa21 conferring resistance to bacterial leaf blight in indica rice cultivar LT2. *Rice Science*, **25(1)**, 52–56. https://doi.org/10.1016/j.rsci.2017.10.003

Nikita, B., Malik R., Rani R., Mehta K., Vashisth U., Dhillon S. and 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. https://doi.org/10.1016/j.crvi.2017.11.002

Pradhan, S.K., Nayak D.K., Mohanty S., Behera L., Barik S.R., Pandit E., Lenka S. and Anandan A. (2015). Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice*, **8**, 1–14. https://doi.org/10.1186/s12284-015-0051-7

Ray, D.K. and Foley J.A. (2013). Increasing global crop harvest frequency: Recent trends and future directions. *Environ. Res. Lett.*, **8(4)**, 044041. https://doi.org/10.1088/1748-9326/8/4/044041

Ronald, P.C., Albano B., Tabien R., Abenes L., Wu K.S., McCouch S. and 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. https://doi.org/10.1007/BF00277122

Sumuni, S.M., Kaur R., Kaur R., Khanna R., Kaur K., Lore J.S., Singh G., Chahal R.K. and 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. https://doi.org/10.1007/s42976-023-00429-6

Sundaram, R.M., Chatterjee S., Oliva R., Laha G.S., Cruz C.V., Leach J.E. and Sonti R.V. (2014). Update on bacterial blight of rice: Fourth international conference on bacterial blight. *Rice*, **7**, 1–3. https://doi.org/10.1186/s12284-014-0024-z

Sundaram, R.M., Vishnupriya M.R., Biradar S.K., Laha G.S., Reddy G.A., Rani N.S., Sarma N.P. and Sonti R.V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite indica rice variety. *Euphytica*, **160**, 411–422. https://doi.org/10.1007/s10681-007-9564-6

Swathi, G., Durga Rani C.V., Md J., Madhav M.S., Vanisree S., Anuradha C., Kumar N.R., Kumar N.A.P., Kumari K.A., Bhogadhi S.C. and Ramprasad E. (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. https://doi.org/10.1007/s11032-019-0927-2

Varanasi, Y.V.P., Isetty S.R., Revadi P., Balakrishnan D., Hajira S., Prasad M.S., Laha G.S., Perraju P., Singh U.M., Singh V.K. and Kumar A. (2023). Molecular and morphological characterization of introgression lines with resistance to bacterial leaf blight and blast in rice. *Plants*, **12(16)**, 3012. https://doi.org/10.3390/plants12163012

Yugander, A., Sundaram R.M., Singh K., Ladhalakshmi D., Subba Rao L.V., Madhav M.S., Badri J., Prasad M.S. and 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. https://doi.org/10.1371/journal.pone.0198260.