Plant Archives Vol. 26, No. 1, 2026 pp. 24-28 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.no.1.003

ENHANCING BACTERIAL LEAF BLIGHT RESISTANCE IN RICE THROUGH *XA21* GENE INTROGRESSION AND MARKER-ASSISTED SELECTION

Sai Divya Goske[1*], Jhansi Rani K.[2], Laxmi Prasanna B.[3] and Kiran Babu T.[3]

[1]Agricultural College, Jagtial, Professor Jayashankar Telangana Agricultural University (PJTAU), Telangana, India
[2]Dean of Agriculture, Professor Jayashankar Telangana Agricultural University (PJTAU), Rajendranagar, Telangana, India
[3]Agriculture Research Institute, Professor Jayashankar Telangana Agricultural University (PJTAU), Rajendranagar, Telangana, India
*Corresponding author E-mail: saidivyagoske@gmail.com
(Date of Receiving-20-11-2025; Date of Revision-30-12-2025; Date of Acceptance-28-01-2026)

ABSTRACT

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* is a major constraint to rice production leading to severe yield losses in susceptible cultivars. Host plant resistance remains the most effective and eco-friendly strategy for managing the disease and marker-assisted selection (MAS) facilitates precise introgression of resistance genes into elite genetic backgrounds. The present study aimed to confirm the introgression of the broad-spectrum BLB resistance gene *Xa21* in advanced rice breeding lines derived from a cross between RNR 15048 and MTU 1010 NIL carrying *Xa21*. Fifty F_7 generation breeding lines were evaluated during *rabi* season of 2024-25 for the presence of *Xa21* using the functional gene-linked marker pTA248. Genomic DNA was isolated using modified CTAB method, followed by PCR amplification and agarose gel electrophoresis. Molecular analysis revealed that forty-one breeding lines were homozygous for *Xa21* resistance allele whereas nine lines were identified as homozygous susceptible. The identified *Xa21* positive lines constitute valuable genetic resources for the development of bacterial leaf blight resistant rice cultivars and can be effectively utilized in future breeding programs aimed at enhancing disease resistance and yield stability.

***Key words*:** Bacterial leaf blight, marker-assisted selection, introgression, advanced breeding lines, molecular screening.

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops and serves as the staple food for more than half of the world's population, contributing over 20% of global caloric intake (USDA-ERS, 2012). Increasing food demand necessitates a substantial rise in rice production by 2035, placing pressure on sustainable yield enhancement. Although molecular breeding has enabled development of high-yielding varieties, genetic uniformity has increased vulnerability to biotic stresses (Khush & Virmani, 1985). Among these, bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is a major disease causing yield losses up to 50%. Since chemical control is ineffective, host plant resistance is the most reliable management strategy (Devadath, 1989). Gene pyramiding through marker-assisted selection (MAS) enhances durability of resistance by combining multiple *Xa* genes (Khush *et al.*, 1989). *Xa21*, a widely used BB resistance gene confers broad-spectrum resistance and encodes an LRR receptor-like kinase (Song *et al.*, 1995). Molecular markers such as SSRs and InDels enable precise introgression and background recovery. MTU 1010, a popular high-yielding variety in Telangana and Andhra Pradesh, is highly susceptible to BB. Therefore, the present study aims to develop BB-resistant lines of MTU 1010 through MAS-based introgression of effective *Xa* gene(s).

Material and Methods

This investigation involved two rice genotypes in crossbreeding program: RNR 15048 (Telangana Sona., IET 23746) as female parent which is a high-yielding fine-grain variety and MTU1010 NIL (IR121055-2-10-7) as male parent having bacterial leaf blight resistant gene *Xa21*. The seed material for MTU1010 NIL (IR121055-2-10-7) was sourced from the ICAR- Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad. RNR 15048 was developed by Agriculture Research Institute (ARI), PJTAU, Hyderabad. A total of 50 advanced breeding lines were examined during *rabi* 2024-25 seasons at College Farm, Rajendranagar, Hyderabad. Improved Samba Mahsuri (*Xa21*) and TN1 were included as resistant and susceptible checks respectively for genotypic validation.

Isolation of Genomic DNA

Genomic DNA was isolated from fresh leaf tissue using a modified CTAB method (Doyle and Doyle, 1987-Fig. 1).

1. About 2-3 g of leaf material was collected and stored at -20 °C until extraction.
2. Leaf tissue was finely chopped and homogenized with pre-warmed CTAB buffer.
3. The homogenate volume was adjusted to 700 µl with CTAB buffer.
4. Samples were transferred to micro-centrifuge tubes.
5. An equal volume of chloroform was added and mixed gently.
6. Phase separation was achieved by centrifugation at 13,000 rpm for 15 min.
7. The aqueous phase containing DNA was carefully transferred to fresh tubes.
8. DNA was precipitated using chilled isopropanol.
9. Samples were incubated at -20 °C and centrifuged to pellet DNA.
10. The DNA pellet was washed with 70% ethanol.
11. Pellets were air-dried and resuspended in TE buffer.
12. Extracted DNA was stored at 4 °C for further molecular analysis.

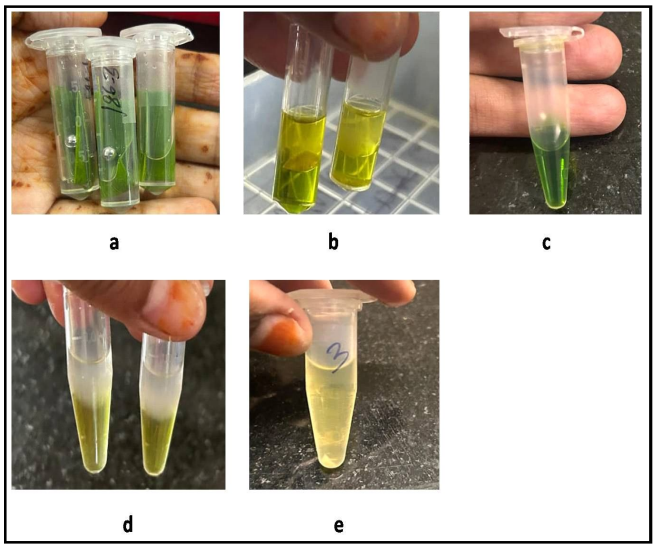

Fig. 1: Isolation of DNA through CTAB method. [a: Tissue lysing-chlorophyll rich plant extract; b: phase separation through centrifugation; c: extracted DNA; d: addition of isopropanol; e: DNA pellet]

DNA Quality Assessment and Quantification

The quality and integrity of the extracted genomic DNA were evaluated using agarose gel electrophoresis following standard molecular biology protocols (Sambrook *et al*., 1989; Green and Sambrook, 2012). DNA samples were resolved on a 0.8% agarose gel prepared in 1X TAE buffer which provides suitable resolution for high-molecular-weight genomic DNA. Agarose was dissolved by heating and allowed to cool before the addition of ethidium bromide for DNA staining. The molten gel was poured into a casting tray fitted with combs and allowed to solidify at room temperature. After solidification, gel was placed in an electrophoresis tank containing 1X TAE buffer. Approximately 2 µl of each DNA sample was mixed with loading dye and carefully loaded into the wells. Electrophoresis was performed at 100 V for 20-30 minutes to allow adequate separation of DNA fragments. Following electrophoresis, DNA bands were visualized under ultraviolet light using a gel documentation system. The presence of intact, high-molecular-weight bands with minimal smearing indicated good quality and integrity of genomic DNA suitable for downstream molecular analyses (Sambrook *et al*., 1989; Ausubel *et al*., 2002).

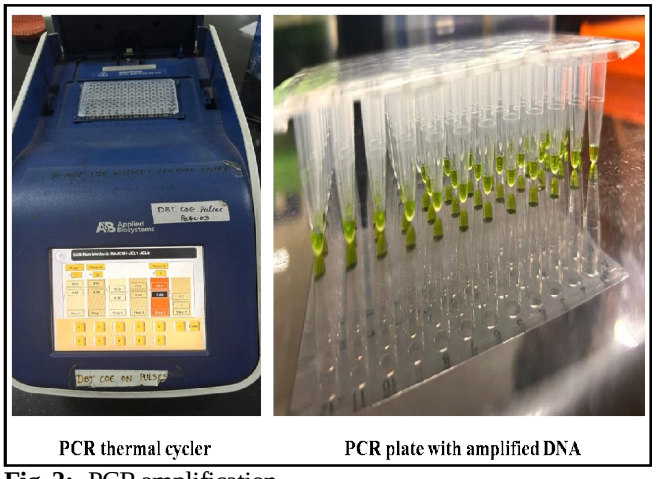

Fig. 2: PCR amplification.

Table 1: Components used in PCR for amplification of *Xa21* associated markers.

Components	Stock concentration	Quantity required for 1 Reaction (µl)
Template DNA	50 ng/µl	3.0
PCR buffer	10X	1.0
d NTP's	2.5 mM	0.5
Forward primer	2.5 pmole	0.5
Reverse primer	2.5 pmole	0.5
Taq DNA Polymerase	1U/µl	0.3
Sterile Distilled Water	-	4.2

PCR Amplification of *Xa21* Gene

Following agarose gel electrophoresis, concentration of genomic DNA was estimated by comparing band intensity with a standard DNA ladder and all samples were diluted to a uniform concentration of 25-50 ng μl^{-1} using TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0; Sambrook *et al*., 1989). The standardized DNA samples were subsequently used for PCR amplification. Polymerase chain reaction (PCR) was carried out using a gene-linked microsatellite marker specific to the bacterial blight resistance gene *Xa21*, following standard PCR protocols (Ronald *et al*., 1992; Fig. 2). PCR reactions were performed in a final volume of 10 µl containing diluted genomic DNA, 10X PCR buffer with MgCl, dNTPs, forward and reverse primers, Taq DNA polymerase and sterile double-distilled water. Amplification was carried out in a thermal cycler under optimized cycling conditions. The amplified products were later resolved by agarose gel electrophoresis to confirm the presence of *Xa21* gene. This standardized PCR-based marker assay enabled reliable detection of gene-specific amplification suitable for downstream molecular analysis (Sambrook *et al*., 1989; McCouch *et al*., 2002). PCR amplification was performed using a programmable thermal cycler (G-Storm and Eppendorf). Details of

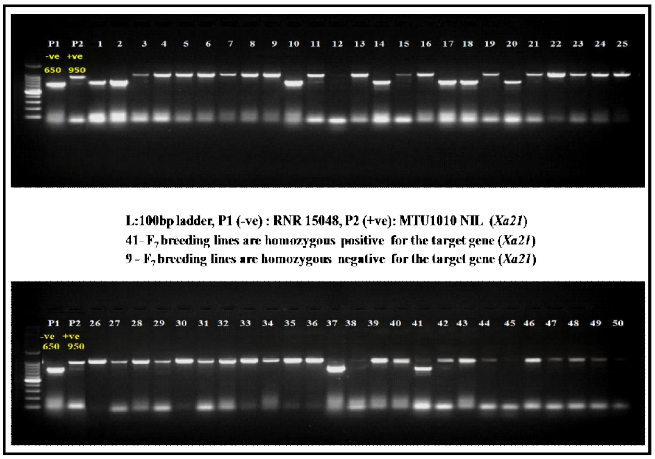

Fig. 3: Confirmation of advanced breeding lines (F7 generation) for *Xa21* gene with pTA248 marker

Table 2: The PCR protocol adopted for amplifying the Functional/gene-linked markers pTA248 MAS.

Initial denaturation	94°C		5 minutes
Denaturation	94°C	Repeated	30 seconds
Annealing	55°C	for 30	30 seconds
Extension	72°C	cycles	1 minute
Final extension	72°C		7 minutes

components and PCR protocol used for amplifying *Xa21* gene-linked marker are provided in Tables 1 and 2.

Gel Electrophoresis and Marker Scoring

The amplified PCR products were resolved on 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet light using a gel documentation system. Genotypes exhibiting a band size of approximately 950 bp were scored as resistant while those showing a 650 bp fragment were classified as susceptible as described by Ronald *et al*., (1992) in Table 3. Marker data were used to confirm the presence of *Xa21* gene in the advanced breeding lines.

Results

Genotypic evaluation of advanced rice breeding lines at F_7 generation was carried out during *rabi* season of 2024-25 to verify successful introgression of bacterial blight resistance gene *Xa21*. Molecular screening was conducted on fifty advanced breeding lines (Table 4) at the Institute of Biotechnology (IBT), PJTAU, Hyderabad with the aim of confirming the presence of *Xa21* gene responsible for resistance against bacterial blight.

For molecular characterization, three randomly selected plants from each breeding line were analyzed. Genomic DNA from these samples were subjected to PCR amplification using functional marker pTA248, following a foreground selection strategy as described by Ronald *et al*., (1992). The pTA248 marker is tightly linked to *Xa21* gene and is widely used for precise identification of resistant genotypes through marker-assisted selection.

Distinct and consistent amplification patterns of pTA248 marker were detected through agarose gel electrophoresis. Out of fifty breeding lines evaluated, forty-one genotypes namely GSD-3, GSD-5, GSD-6, GSD-7, GSD-8, GSD-11, GSD-12, GSD-15, GSD-16, GSD-19, GSD-22, GSD-23, GSD-25, GSD-26, GSD-27, GSD-29, GSD-31, GSD-32, GSD-34, GSD-35, GSD-38, GSD-39, GSD-40, GSD-42, GSD-45, GSD-46, GSD-47, GSD-48, GSD-4, GSD-9, GSD-13, GSD-21, GSD-24, GSD-28, GSD-30, GSD-33, GSD-36, GSD-43, GSD-44, GSD-49 and GSD-50 were confirmed as homozygous for *Xa21* resistance allele. Conversely, nine genotypes

Table 3: Target gene and its functional marker along with its physical position, annealing temperature and amplicon size of the molecular marker used for foreground selection.

S. No	Target gene	Chr. No.	Primer name	Primer sequence	Amplicon size	Reference
1	*Xa21*	11	pTA 248	F AGACGCGGGAAGGGTGGTTCCCGGA R AGACGCGGGTAATCGAAAGATGAAA	P- 950(resistant) N- 650(susceptible)	(Ronald *et al.*, 1992)

(GSD-1, GSD-2, GSD-10, GSD-14, GSD-17, GSD-18, GSD-20, GSD-37 and GSD-41) did not possess the resistance allele and were categorized as homozygous susceptible lines.

The presence of *Xa21* gene was characterized by an amplification product of approximately 950 bp whereas susceptible genotypes yielded a fragment of nearly 650 bp as depicted in Fig. 3. The reliability and precision of pTA248 marker for *Xa21* detection have been consistently demonstrated in previous studies (Aruna Kumari *et al*., 2016; Abhilash Kumar *et al*., 2016; Rekha *et al*., 2018), highlighting its effectiveness as a robust tool for marker-assisted selection in rice breeding programs.

Table 4: List of advanced breeding lines (F_7 generation) used for genotypic screening of BB resistant gene, *Xa21*.

S. No.	Advanced breeding lines (F_7 generation)	S. No.	Advanced breeding lines (F_7 generation)
1	GSD 1	**26**	GSD 26
2	GSD 2	**27**	GSD 27
3	GSD 3	**28**	GSD 28
4	GSD 4	**29**	GSD 29
5	GSD 5	**30**	GSD 30
6	GSD 6	**31**	GSD 31
7	GSD 7	**32**	GSD 32
8	GSD 8	**33**	GSD 33
9	GSD 9	**34**	GSD 34
10	GSD 10	**35**	GSD 35
11	GSD 11	**36**	GSD 36
12	GSD 12	**37**	GSD 37
13	GSD 13	**38**	GSD 38
14	GSD 14	**39**	GSD 39
15	GSD 15	**40**	GSD 40
16	GSD 16	**41**	GSD 41
17	GSD 17	**42**	GSD 42
18	GSD 18	**43**	GSD 43
19	GSD 19	**44**	GSD 44
20	GSD 20	**45**	GSD 45
21	GSD 21	**46**	GSD 46
22	GSD 22	**47**	GSD 47
23	GSD 23	**48**	GSD 48
24	GSD 24	**49**	GSD 49
25	GSD 25	**50**	GSD 50
P1	RNR 15048 (Female Parent)	**P2**	MTU 1010NIL (Male Parent)

Discussion

The present study confirmed the effectiveness of marker-assisted selection (MAS) for introgressing bacterial leaf blight resistance gene *Xa21* into advanced rice lines, consistent with other reports on MAS-mediated BLB resistance improvement in rice cultivars (Nguyen *et al*., 2017). The use of functional marker pTA248 enabled accurate discrimination of resistant and susceptible alleles in agreement with earlier validation studies employing this marker for *Xa21* detection (Swamy *et al*., 2020). High recovery of homozygous *Xa21* lines at F_7 stage indicates stable inheritance and effective fixation of the resistance allele, similar to patterns observed in backcross breeding programs for BLB genes (Edukondalu *et al*., 2007). The presence of *Xa21* was widely reported to confer broad-spectrum resistance across diverse *Xoo* races making it a valuable gene in rice breeding programs (Nguyen *et al*., 2018). Furthermore, comparisons with studies that combine multiple BLB resistance genes suggest that pyramiding resistance loci may further enhance durability and spectrum of resistance in varied agro-ecologies (Pradhan *et al*., 2015). These results reinforce the utility of MAS and functional markers like pTA248 in accelerating the development of BLB-resistant rice cultivars.

Conclusion

The superior breeding lines identified in the present study, comprising resistant genotypes will be advanced for further evaluation through multilocation trials within Telangana and at the national level under the All India Coordinated Research Improvement Project (AICRP). Following comprehensive field evaluation, promising lines may be recommended for release as varieties to meet the needs of farmers in the state. In addition, breeding lines exhibiting bacterial leaf blight resistance coupled with desirable yield potential can serve as valuable donor parents for the targeted introgression of major resistance genes into elite rice varieties cultivated in Telangana.

Acknowledgement

The authors sincerely acknowledge ARI, IBT and University authorities of Professor Jayashankar

Telangana Agricultural University (PJTAU), Telangana, India for providing the necessary facilities and support to carry out this research.

References

Abhilash, K.V., Balachiranjeevi C.H., Bhaskar N.S., Rambabu R., Rekha G., Harika G., Hajira S.K., Pranathi K., Vijay S., Anila M., Mahadevaswamy H.K., Koushik M., Yugander A., Aruna J., Hariprasad A.S., Madhav M., Laha G.S., Balachandran S.M., Ravindra Babu V., Sundaram R.M. and Prasad M.S. (2016). Marker-assisted improvement of the elite restorer line of rice, RPHR-1005 for resistance against bacterial blight and blast diseases. *Journal of Genetics*. **95**, 400-409.

Aruna kumari, K., Durgarani C.V., Satturu V., Sarikonda K.R., Chittoor P.D.R., Vutukuri B. and 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.

Ausubel, G. (2002). *Theory and problems of adolescent development*. iUniverse.

Devadath, S. (1989). Chemical control of bacterial blight of rice. *International Rice Research Institute*, Manila, Philippiness. 89-98.

Doyle, J.J and Doyle J.L. (1987). A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin*. **19**, 11-15.

Edukondalu, B., Shankar V.G., Revathi P., Naik D.S. and Chary D.S. (2020). Evaluation of BC_1 F_1 plants developed by MAS for BLB resistance in rice.

Green, M.R. and Sambrook J. (2012). Molecular cloning. *A Laboratory Manual 4th*. 448.

Khush, G.S. and Virmani S.S. (1985). Breeding rice for disease resistance. *Progress in Plant Breeding-I, London*. 239-279.

Khush, G.S., Mackill D.J. and Sidhu G.S. (1989). Breeding rice for resistance to bacterial blight from *O. longstaminata*. *Rice Genetics Newsletter*. **16**, 92-93.

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.

Pradhan, S.K., Nayak D.K., Mohanty S., Behera L., Barik S.R., Pandit E., Lenka and Anandan A. (2015). Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice*. **8(1)**, 19.

Rekha, G., Kumar V. A., Viraktamath B.C., Pranathi K., Kousik M.B.V.N., Prasanna B.L. and Sundaram R.M. (2018). Improvement of blast resistance of the popular high-yielding, medium slender-grain type, bacterial blight resistant rice variety, Improved Samba Mahsuri by marker-assisted breeding. *Journal of Plant Biochemistry and Biotechnology*. **27(4)**, 463-472.

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

Sambrook, J. (1989). Molecular cloning: a laboratory manual. *Cold Spring Habor Laboratory*.

Song, W.Y., Wang G.L., Chen L.L., Kim H.S., Pi L.Y., Holsten T., Gardner J., Wang B., Zhai W.X., Zhu L.H., Fauquet C. and Ronald P.C. (1995). A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*. **270**, 1804-1806.

Swamy, H.M., Anila M., Kale R.R., Rekha G., Bhadana V.P., Anantha M.S., Brajendra P., Balachiranjeevi C.H., Hajira S.K., Prasanna B.L. and Pranathi K. (2020). Marker assisted improvement of low soil phosphorus tolerance in the bacterial blight resistant, fine-grain type rice variety, Improved Samba Mahsuri. *Scientific reports*. **10 (1)**, 21143.