

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(7): 1675-1677
www.biochemjournal.com
Received: 02-05-2025
Accepted: 06-06-2025

Sai Divya Goske
Masters's Student, Department
of Genetics and Plant
Breeding, Agricultural College,
PJTAU, Rajendranagar,
Hyderabad, Telangana, India

Dr. K Jhansi Rani
Dean of Agriculture, PJTAU,
Rajendranagar, Hyderabad,
Telangana, India

Dr. B Laxmi Prasanna
Scientist, Plant Breeding, Rice
Research Unit, Rajendranagar,
Hyderabad, Telangana, India

Dr. T Kiran Babu
Scientist, Plant Pathology,
ARI, Rajendranagar,
Hyderabad, Telangana, India

Corresponding Author:
Sai Divya Goske
Masters's Student, Department
of Genetics and Plant
Breeding, Agricultural College,
PJTAU, Rajendranagar,
Hyderabad, Telangana, India

Identification of resistant lines for bacterial leaf blight disease in rice (*Oryza sativa*. L)

Sai Divya Goske, K Jhansi Rani, B Laxmi Prasanna and T Kiran Babu

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i7u.5016>

Abstract

Bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a major biotic constraint to rice productivity particularly in irrigated and lowland ecosystems. In this study, 50 F₆ advanced breeding lines derived from a cross between RNR 15048 and MTU1010 NIL (*Xa21*) were evaluated for BB resistance under field conditions using a virulent local *Xoo* isolate. The disease severity was assessed based on lesion length following IRRI Standard Evaluation System (2013). Out of the 50 advanced breeding lines screened, 28 exhibited high resistance, 13 showed moderate resistance and 9 were moderately susceptible. The checks-ISM, TN 1, RNR 15048 and MTU1010 NIL confirmed the virulence and accuracy of the screening. The study highlights the effectiveness of incorporating the major resistance gene *Xa21*, demonstrating its potential utility in breeding programs aimed at developing BLB-resistant rice cultivars for Telangana and similar agro-ecologies.

Keywords: Bacterial leaf blight, *Xa21*, rice, resistance, breeding lines, *Xanthomonas oryzae*

Introduction

Rice (*Oryza sativa*) is a vital cereal crop that serves as a primary dietary energy and carbohydrate source for nearly 50% of the global population. In India, it is cultivated year-round across various agro-climatic zones including irrigated, rainfed uplands, deepwater regions and hill ecosystems. To sustain future food demands especially with the increasing population, rice production must increase by at least 40% by 2030 (Khush, 2005) [9]. Globally, rice is grown on approximately 165.04 million hectares with an estimated production of 520.9 million tonnes (Indiastat, 2023-24) [5]. In India, it occupies about 478.32 lakh hectares, yielding 1357.55 lakh tonnes with an average productivity of 2838 kg/ha (Indiastat, 2023-24) [5]. In the state of Telangana alone, rice spans 46.62 lakh hectares with a total output of 158.77 lakh tonnes and productivity reaching 3406 kg/ha (Indiastat, 2023-24) [5]. Rice productivity is increasingly threatened by declining natural resources, unpredictable weather patterns linked to climate change and the evolving pressure of pathogenic microbes and insect pests (Pradhan *et al.*, 2015) [11]. Among the numerous biotic stresses, bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a vascular disease known for causing substantial yield losses ranging from 74% to 81% under epidemic conditions. BB is more prevalent in irrigated and lowland rice-growing areas (Sundaram *et al.*, 2008) [14]. It can infect plants at multiple developmental stages particularly during tillering and reproductive phases resulting in systemic infection and foliar necrosis, ultimately reducing grain yield. Due to the limitations of agrochemical-based disease management primarily due to poor bactericide efficacy and high input costs, genetic resistance through host plant defense mechanisms has become the most viable and environmentally sound strategy for disease mitigation (Devadath, 1989; Sundaram *et al.*, 2008) [3, 14]. To date, over 42 major resistance loci (R loci) conferring resistance to BB have been documented, out of which 11 have been functionally characterized and cloned. These include genes such as *Xa1*, *xa5*, *Xa10*, *Xa4*, *xa41*, *Xa23*, *xa25*, *xa13*, *Xa21*, *Xa26* (also referred to as *Xa3*) and *Xa27* (Jiang *et al.*, 2020) [7]. A notable example is *Xa21*, a broad-spectrum resistance allele belonging to the NBS-LRR (nucleotide-binding site-leucine-rich repeat) gene family known for its role in pathogen-associated molecular pattern (PAMP)-triggered immunity. *Xa21* was introgressed from the wild African rice species *Oryza longistaminata* and is widely used in marker-assisted selection due to its robust effectiveness against diverse *Xoo* pathotypes.

Materials and Methods

The current experiment was conducted in rice varieties from F₆ advanced lines obtained from the cross RNR 15048 (Telangana sona) as female parent and MTU 1010 NIL (*Xa21*-BB) as male parent during *kharif* 2024 at Institute of Rice Research, ARI, Rajendranagar, Hyderabad. Phenotypic screening was done in 50 advanced breeding lines for bacterial leaf blight as per SES scale, IRRI-2013 [6]. The screening was conducted using a virulent strain of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which was sourced from the infected leaf samples on modified Wakimoto's medium from ICAR-IIRR, Hyderabad, Telangana. The bacterial culture was cultivated on Hayward's agar medium at 28°C for 96 hours. After the incubation period, the bacterial cells were collected and diluted with 10 mL of sterile distilled water to achieve a final concentration of approximately 108-9 cfu/μL (Preece, 1982) [12]. The fifty advanced breeding lines along with parents and checks (2 rows of 1.5 meter length, 20 x 15 cm spacing) were inoculated with the bacterial suspension during the maximum tillering stage (55 days after transplanting) using the leaf clipping technique as outlined by Kauffman *et al.*, (1973) [8]. Inoculation was performed by clipping the tip (1-2 cm) of the fully expanded uppermost leaf using scissors dipped in the bacterial inoculum. Each plant had ten leaves inoculated and disease progression was monitored by measuring the lesion length 21 days post-inoculation. Additionally, disease severity was assessed using the IRRI Standard Evaluation System (IRRI-SES, 2013) [6] scale, as shown in Table 1.

Table 1: Standard Evaluation System, IRRI (2013) [6] scale for BLB Rating

Scale	Rating	% leaf area diseased
1	Resistant	1-5
3	Moderately Resistant	6-12
5	Moderately Susceptible	13-25
7	Susceptible	26-50
9	Highly susceptible	51-100

Results

Phenotypic evaluation of advanced breeding lines for BLB resistance

A total of 50 advanced rice breeding lines along with the parental genotypes and standard check varieties were assessed for resistance to bacterial blight (BB) caused by a virulent local strain of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The plants were inoculated at the maximum tillering stage using the leaf clipping method described by Kauffman *et al.*, (1973) [8]. Field-based evaluations also included resistant check-Improved Samba Mahsuri (ISM), RNR 15048 and MTU1010 near-isogenic line (NIL). Twentyone days post-inoculation, lesion lengths on the leaves were measured and disease reactions were rated using the Standard Evaluation System (SES) scale IRRI, 2013 [6]. Among the 50 breeding lines tested, twenty eight lines-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 and GSD-48 exhibited strong resistance each registering a disease score of 1. Thirteen genotypes including 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 moderately resistant, scoring 3. The remaining nine entries-GSD-1, GSD-2, GSD-10, GSD-14, GSD-17, GSD-18, GSD-20, GSD-37 and GSD-41 displayed moderate susceptibility with a score of 5. The susceptible control TN1 showed a severe disease reaction, reflected by a high score of 9 and the female parent RNR 15048 with a score of 7. In contrast, the resistant control ISM maintained moderate resistance scoring 3 and MTU1010 scored 5 showing moderately resistance under the same conditions (refer to Table 2).

Table 2: Screening and scoring details of advanced breeding lines of rice as per IRRI-SES scale (IRRI 2013) [6]

Parents, checks	Score	R/MR/MS/S/HS	Parents, checks	Score	R/MR/MS/S/HS
RNR 15048	7	S	HR 12	7	S
MTU 1010	3	MR	NLR 34449	5	MS
ISM	3	MR	Kavya	5	MS
TN1	9	HS	Swarna	5	MS
JGL 24423	5	MS	MTU 1001	3	MR
Improved breeding lines	Score	R/MR/MS/S	Improved breeding lines	Score	R/MR/MS/S
GSD 1	5	MS	GSD 26	1	R
GSD 2	5	MS	GSD 27	1	R
GSD 3	1	R	GSD 28	3	MR
GSD 4	3	MR	GSD 29	1	R
GSD 5	1	R	GSD 30	3	MR
GSD 6	1	R	GSD 31	1	R
GSD 7	1	R	GSD 32	1	R
GSD 8	1	R	GSD 33	3	MR
GSD 9	3	MR	GSD 34	1	R
GSD 10	5	MS	GSD 35	1	R
GSD 11	1	R	GSD 36	3	MR
GSD 12	1	R	GSD 37	5	MS
GSD 13	3	MR	GSD 38	1	R
GSD 14	5	MS	GSD 39	1	R
GSD 15	1	R	GSD 40	1	R
GSD 16	1	R	GSD 41	5	MS
GSD 17	5	MS	GSD 42	1	R
GSD 18	5	MS	GSD 43	3	MR
GSD 19	1	R	GSD 44	3	MR
GSD 20	5	MS	GSD 45	1	R
GSD 21	3	MR	GSD 46	1	R
GSD 22	1	R	GSD 47	1	R
GSD 23	1	R	GSD 48	1	R
GSD 24	3	MR	GSD 49	3	MR
GSD 25	1	R	GSD 50	3	MR

R-resistant, MR-moderately resistant, S-susceptible, MS-moderately susceptible, HS-highly susceptible

Discussion

Pyramiding of multiple resistance alleles through marker-assisted selection has proven to be an effective strategy for enhancing durable resistance against bacterial blight (BB) as demonstrated in the studies by Sundaram *et al.* 2008 [14]. Monogenic resistance conferred by a single dominant locus such as *Xa21* has also exhibited robust phenotypic resistance against a wide spectrum of *Xanthomonas oryzae* pv. *oryzae* isolates. Comparable results indicating strong host resistance governed by a single gene introgression were reported by Hari *et al.* 2013 [4], Balachiranjeevi *et al.* 2018 [2], Abhilash *et al.* 2016 [1] and Laxmi Prasanna *et al.*, 2018 [10]. In the current investigation, elite rice lines incorporating the *Xa21* allele are anticipated to confer high-level

resistance under field conditions in Telangana and other southern agro-ecological zones. The functional efficiency of *Xa21*, a receptor-like kinase gene involved in pathogen recognition and defense signal transduction has been validated in these regions indicating its continued relevance in local pathotype-host interactions.

Conclusion

Results from mean disease score show similar trend for resistant, moderately resistant, moderately susceptible and susceptible genotype as obtained through SES scale visual scoring. In final, this study shows out of 50 breeding lines; 41 breeding lines are resistant to bacterial leaf blight. These breeding lines can be utilized in various breeding programmes for bacterial blight resistance.

Acknowledgement

I am sincerely thankful to my advisory committee for their continuous support and invaluable guidance throughout this research journey. I extend my profound appreciation to Dr. K. Jhansi Rani, Dean of Agriculture, PJTAU, Rajendranagar, Hyderabad for her pioneering ideas, thoughtful mentorship and consistent motivation which skillfully bridged scientific accuracy with field-level applicability. I gratefully thank Dr. B. Laxmi Prasanna, Scientist (Plant Breeding), Rice Research Unit, Rajendranagar, Hyderabad for granting access to crucial laboratory infrastructure during the molecular phase of my study and for her constructive inputs. My warm thanks to Dr. T. Kiran Babu, Scientist, Plant Pathology, RRU Rajendranagar for his expert advice and for facilitating the use of specialized research facilities. Lastly, I extend my heartfelt gratitude to Professor Jayashankar Telangana Agricultural University (JTASU) for the generous financial assistance that played a pivotal role in the successful execution of this work.

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