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Phenotypic evaluation of advanced breeding lines for bacterial blight resistance in rice (*Oryza sativa* L.)

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Abstract

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most destructive diseases of rice, causing significant yield losses in major rice-growing regions. The study was conducted during the *kharif* 2025 at the Rice Research Unit (RRU), Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, under artificial epiphytotic conditions to identify advanced rice breeding lines resistant to BLB. A total of 40 near-homozygous F₆ breeding lines derived from the cross RNR 15048 × ISM NIL (*Xa21*) were evaluated along with resistant (ISM NIL) and susceptible (TN1) checks. Artificial inoculation was performed using the leaf clipping method and disease reaction was recorded 21 days after inoculation based on the Standard Evaluation System (SES) for rice. Considerable variation in disease response was observed among the evaluated lines. Eleven breeding lines (PKK-10, PKK-22, PKK-26, PKK-27, PKK-30, PKK-32, PKK-33, PKK-34, PKK-35, PKK-36 and PKK-40) exhibited a resistant reaction with disease severity ranging from 2.51 to 4.95%, while twenty-nine lines were categorized as moderately resistant (5.30% to 11.83%). The resistant check ISM NIL recorded 3.11% disease severity and the susceptible check TN1 showed 51.28% disease severity, confirming the reliability of the screening procedure. The predominance of resistant and moderately resistant genotypes indicated successful introgression of BLB resistance from the donor parent. The identified resistant breeding lines may serve as valuable genetic resources for developing high-yielding rice cultivars with durable resistance to bacterial leaf blight.

Keywords: Rice, bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*, phenotypic screening, *Xa21*, disease resistance

1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple food crops, serving as the primary source of calories for more than half of the global population and playing a vital role in ensuring food and nutritional security. Asia dominates global rice cultivation, accounting for more than 90% of the world's rice production and consumption. During 2024-25, global rice production was estimated at 520.9 million tonnes (USDA, 2024) [17]. India, the world's largest rice producer and exporter, produced 150.18 million tonnes from 51.26 million hectares, contributing nearly 28% of global rice production. In Telangana, popularly known as the "Rice Bowl of South India," rice is cultivated over 48.09 lakh hectares with a production of 174.46 lakh tonnes and a productivity of 3,627 kg ha⁻¹ (Indiastat, 2024-25) [4]. Rice productivity is constrained by numerous biotic and abiotic stresses, among which bacterial leaf blight (BLB), caused by the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the most destructive diseases affecting rice cultivation worldwide. The disease occurs at almost all crop growth stages and can cause severe reductions in grain yield. The extent of yield loss due to BLB varies with the cultivar's susceptibility, environmental conditions and disease severity. In India, yield losses range from 7% to 62%, with losses reaching up to 82% under favourable epidemic conditions (Shekhar and Kumari, 2020) [16]. Under moderate disease pressure, yield losses of 10-20% have been documented (Mew *et al.*, 1993) [11], whereas severe epidemics may cause yield reductions of up to 80% due to a substantial decline in photosynthetic leaf area, ultimately affecting grain yield (Zhang and Wang, 2013) [18].

Bacterial leaf blight is characterized by two major symptom phases: *kresek* or seedling wilt during the early crop stage and leaf blight during later growth stages (Gnanamanickam *et al.*,

1999) [5]. Initial symptoms appear as water-soaked lesions along the leaf margins that gradually enlarge, turn yellow and eventually lead to drying of the entire leaf. The pathogen survives in infected crop residues, root stubbles and weed hosts and is disseminated primarily through wind-driven rain, irrigation water and infected plant debris (Shahjahan *et al.*, 1991) [15]. Several disease management approaches, including the use of biocontrol agents, have been explored; however, their effectiveness under field conditions has been limited (Khush *et al.*, 1989) [9]. Consequently, the development and deployment of resistant cultivars remain the most economical, environmentally safe and sustainable strategy for managing BLB. The effectiveness of resistance breeding depends on the availability of diverse resistance sources and the variability of pathogen populations. The identification and incorporation of resistance genes through conventional and molecular breeding approaches, including gene pyramiding, have therefore become essential for achieving durable resistance (Chen *et al.*, 2002) [2]. Keeping these challenges in view, the present investigation was undertaken to evaluate advanced breeding lines derived from the cross RNR 15048 × ISM NIL for the identification of resistant lines against bacterial leaf blight (BLB).

2. Materials and Methods

The present investigation was conducted during *kharif* 2025 at the Rice Research Unit (RRU), Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, under artificial epiphytotic conditions to evaluate advanced breeding lines for resistance to bacterial leaf blight.

2.1 Plant Material

Forty advanced breeding lines in the near-homozygous F₆ generation were evaluated, derived from the cross RNR 15048 (Telangana Sona) × ISM NIL (*Xa21*). These were screened along with resistant (ISM NIL) and susceptible checks (TN1) to identify lines possessing durable and broad-spectrum resistance to bacterial leaf blight.

Table 1: Advanced breeding lines, parents and Checks used for Screening for BLB resistance

Sl. No.	Advanced Breeding Lines	Sl. No.	Advanced Breeding Lines
1	PKK-1	21	PKK-21
2	PKK-2	22	PKK-22
3	PKK-3	23	PKK-23
4	PKK-4	24	PKK-24
5	PKK-5	25	PKK-25
6	PKK-6	26	PKK-26
7	PKK-7	27	PKK-27
8	PKK-8	28	PKK-28
9	PKK-9	29	PKK-29
10	PKK-10	30	PKK-30
11	PKK-11	31	PKK-31
12	PKK-12	32	PKK-32
13	PKK-13	33	PKK-33
14	PKK-14	34	PKK-34
15	PKK-15	35	PKK-35
16	PKK-16	36	PKK-36
17	PKK-17	37	PKK-37
18	PKK-18	38	PKK-38
19	PKK-19	39	PKK-39
20	PKK-20	40	PKK-40
PARENTS		CHECKS	
P1	RNR 15048	Resistant	ISM NIL
P2	ISM NIL	Susceptible	TN1

The seedlings of all advanced breeding lines, along with parents and checks were raised in a nursery on 15 July 2025, following standard agronomic practices. Transplanting was done on 7 August 2025. Each advanced breeding line was transplanted in two rows of 1.5 m length, with a spacing of 20×15 cm. In the screening plot, ten plants of each advanced breeding line were randomly selected and tagged for recording observations on the severity of BLB. The individual plants were inoculated 30-35 days after transplanting at the maximum tillering stage. The highly virulent Xoo isolate of RRU, ARI, Rajendranagar was first cultured on Modified Wakimoto’s Agar (MWA) at 28 °C for 96 hours. The resulting bacterial growth was harvested and suspended in 10 ml of sterile distilled water to achieve a final concentration of approximately 10⁸-10⁹ CFU/ml (Preece *et al.*, 1982) [14]. Inoculation of the forty advanced breeding lines was carried out at the maximum tillering stage (around 45 days after sowing), following the leaf-clipping method described by Kauffman *et al.* (1973) [8]. The tips (1-2 cm) of fully expanded uppermost leaves were clipped using scissors dipped in the bacterial suspension; ten leaves per plant were inoculated. Disease development was recorded by measuring lesion length at 21 days after inoculation and disease reaction was classified based on the IRRI Standard Evaluation System (SES, 2013) scale as presented in Table 2.

Table 2: Standard Evaluation System (SES) for bacterial leaf blight (IRRI, 2013)

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

3. Results

Screening of the 40 advanced breeding lines against bacterial leaf blight under artificial epiphytotic conditions revealed disease severity ranging from 2.51 to 11.83% leaf area damaged (Tables 2 and 3). Based on the IRRI Standard Evaluation System (SES), all the breeding lines were classified into two reaction categories, namely resistant (R, scale 1) and moderately resistant (MR, scale 3), with no genotype falling under the moderately susceptible (MS), susceptible (S) or highly susceptible (HS) classes. Among the evaluated lines, eleven genotypes, namely PKK-10, PKK-22, PKK-26, PKK-27, PKK-30, PKK-32, PKK-33, PKK-34, PKK-35, PKK-36 and PKK-40, exhibited a resistant reaction with disease severity ranging from 2.51% to 4.95%. The lowest disease severity was observed in PKK-26 (2.51%), followed by PKK-36 (2.90%), while PKK-10 recorded the highest disease severity (4.76%) within the resistant group. These lines showed disease levels comparable with the resistant check, ISM NIL (3.11%), indicating successful introgression of the *Xa21* resistance gene. The remaining twenty-nine genotypes, namely PKK-1, PKK-2, PKK-3, PKK-4, PKK-5, PKK-6, PKK-7, PKK-8, PKK-9, PKK-11, PKK-12, PKK-13, PKK-14, PKK-15, PKK-16, PKK-17, PKK-18, PKK-19, PKK-20, PKK-21, PKK-23, PKK-24, PKK-25, PKK-28, PKK-29, PKK-31, PKK-37, PKK-38 and PKK-39, were classified as moderately resistant, with disease severity ranging from

5.30% to 11.83%. Although these lines exhibited slightly higher disease severity than the resistant group, all remained within the moderately resistant category and below the susceptible threshold, indicating an acceptable level of resistance. The susceptible check TN1 recorded 51.28% leaf area damaged and was classified as highly susceptible, whereas the recurrent parent RNR 15048 recorded 18.67% disease severity and was rated as moderately susceptible. In contrast, all advanced breeding lines exhibited lower disease severity than the recurrent parent and susceptible check, confirming successful introgression of bacterial leaf blight

resistance. The clear differentiation between the resistant donor, susceptible check and breeding lines further validated the effectiveness of the artificial inoculation and disease evaluation. The disease reactions of the parents, checks and advanced breeding lines are presented in Tables 3.1 and 3.2.

Table 2: Disease reaction of parents and checks for bacterial leaf blight during *kharif* 2025.

Sl. No.	Entry	Damage (%)	Scale	Reaction
P1	RNR 15048	18.67	5	MS
P2/C1	ISM NIL	3.11	1	R
C2	TN1	51.28	9	HS

Table 3: Disease reaction of advanced breeding lines for bacterial leaf blight during *kharif* 2025.

Sl. No.	Advanced Breeding Lines	Damage (%)	Scale	Reaction	Sl. No.	Advanced Breeding Lines	Damage (%)	Scale	Reaction
1	PKK-1	11.6	3	MR	21	PKK-21	5.96	3	MR
2	PKK-2	8.68	3	MR	22	PKK-22	4.10	1	R
3	PKK-3	10.98	3	MR	23	PKK-23	5.37	3	MR
4	PKK-4	8.95	3	MR	24	PKK-24	5.72	3	MR
5	PKK-5	10.27	3	MR	25	PKK-25	5.30	3	MR
6	PKK-6	5.58	3	MR	26	PKK-26	2.51	1	R
7	PKK-7	11.15	3	MR	27	PKK-27	3.15	1	R
8	PKK-8	9.54	3	MR	28	PKK-28	6.56	3	MR
9	PKK-9	7.5	3	MR	29	PKK-29	5.99	3	MR
10	PKK-10	4.76	1	R	30	PKK-30	4.95	1	R
11	PKK-11	5.63	3	MR	31	PKK-31	10.70	3	MR
12	PKK-12	6.74	3	MR	32	PKK-32	4.76	1	R
13	PKK-13	11.51	3	MR	33	PKK-33	3.73	1	R
14	PKK-14	11.45	3	MR	34	PKK-34	3.47	1	R
15	PKK-15	11.73	3	MR	35	PKK-35	4.69	1	R
16	PKK-16	10.03	3	MR	36	PKK-36	2.90	1	R
17	PKK-17	11.38	3	MR	37	PKK-37	11.83	3	MR
18	PKK-18	8.03	3	MR	38	PKK-38	5.43	3	MR
19	PKK-19	6.16	3	MR	39	PKK-39	6.92	3	MR
20	PKK-20	5.72	3	MR	40	PKK-40	3.51	1	R

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

4. Discussion

The present investigation revealed considerable variation in bacterial leaf blight (BLB) resistance among the forty advanced breeding lines evaluated under artificial epiphytotic conditions. Disease severity ranged from 2.51 to 11.83%, and all breeding lines were classified as either resistant (R) or moderately resistant (MR) according to the IRRI Standard Evaluation System (SES). None of the lines exhibited moderately susceptible or susceptible reactions, indicating a marked improvement over the recurrent parent, RNR 15048 (18.67% disease severity), while the susceptible check TN1 recorded 51.28% disease severity. These results suggest successful introgression of BLB resistance from the donor parent into the advanced breeding population. Among the evaluated lines, PKK-10, PKK-22, PKK-26, PKK-27, PKK-30, PKK-32, PKK-33, PKK-34, PKK-35, PKK-36 and PKK-40 exhibited resistant reactions comparable to the resistant check ISM NIL, whereas the remaining twenty-nine lines showed moderately resistant reactions. The observed variation in resistance among the breeding lines may be attributed to genetic background and the influence of minor genes governing disease response. The effectiveness of the leaf clipping inoculation method was evident from the clear differentiation between the resistant and susceptible checks, thereby validating the phenotypic screening procedure under uniform disease pressure. These findings are consistent with those reported by Laxmi Prasanna *et al.* (2018) ^[10], Biswas *et al.* (2021) ^[1]

and Goske *et al.* (2025) ^[6], who also identified BLB-resistant breeding lines through artificial inoculation under field conditions. The agreement of the present results with these reports confirms that phenotypic screening under epiphytotic conditions is an effective approach for identifying BLB-resistant breeding material. The resistant breeding lines identified in the present study constitute valuable genetic resources for bacterial leaf blight resistance breeding and may be utilized in future varietal improvement programmes. However, further validation through molecular marker analysis and multi-location evaluation is required to confirm the stability of resistance under diverse environmental conditions and pathogen populations.

6. Conclusion

The present investigation identified eleven advanced breeding lines with resistant reactions and twenty-nine lines with moderately resistant reactions to bacterial leaf blight under artificial epiphytotic conditions. The predominance of favourable disease reactions confirmed successful introgression of resistance from ISM NIL into the RNR 15048 background. The resistant lines identified in this study may serve as valuable donor lines for bacterial leaf blight resistance breeding and could be utilized in future marker-assisted selection (MAS) and gene pyramiding programmes aimed at developing durable disease-resistant rice cultivars.

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