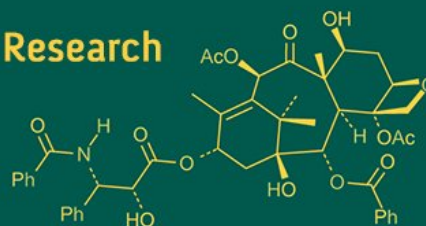


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Phenotypic evaluation of advanced breeding lines of Jagtial Vari 3 (JGL 27356) for bacterial leaf blight resistance in rice (*Oryza sativa* L.)

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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 which causes considerable yield losses in major rice-growing regions. The present investigation was undertaken during Kharif 2025 at Rice Research Unit (RRU), Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, to evaluate the bacterial leaf blight resistance of 50 advanced breeding lines (F₆ generation) derived from the cross between Jagtial Vari 3 (JGL 27356) and MTU1010NIL (IR121055-2-10-7). A total of 50 F₆ breeding lines along with its recurrent parent and two standard checks, Taichung Native 1 (TN1) and Improved Samba Mahsuri (ISM) were screened under artificial epiphytotic conditions using the leaf clipping method. Disease reaction was recorded based on lesion length and disease incidence by following IRRI Standard Evaluation System (2013). Out of 50 advanced breeding lines studied, 1 breeding line exhibited resistant reaction, 3 showed moderate resistance, 5 were moderately susceptible, 37 lines were susceptible and 4 were highly susceptible. The results indicate successful introgression of bacterial leaf blight resistance gene, *Xa21* into selected advanced breeding lines through marker-assisted pedigree breeding. Resistant breeding lines (F₆ generation) can serve as valuable genetic resources for developing high-yielding bacterial leaf blight-resistant rice varieties.

Keywords: Rice, bacterial leaf blight, *Xanthomonas oryzae* pv. *Oryzae*, *Xa21*, marker-assisted breeding, disease resistance

Introduction

Rice (*Oryza sativa* L.) is the principal staple food for more than half of the world's population and plays a vital role in global food security. India is one of the largest producers of rice, cultivating the crop over diverse agro-climatic regions. However, rice productivity is severely constrained by several biotic stresses, among which bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *Oryzae* (Xoo) is one of the most devastating diseases (Nino-Liu *et al.*, 2006) [10]. Under favourable environmental conditions, the disease can result in yield losses ranging from 20 to 80%, depending on cultivar susceptibility, crop growth stage and pathogen virulence (Ou, 1985; Mew *et al.*, 1993) [11, 9].

Though the chemical control measures provide temporary suppression of the disease, they are economically expensive, environmentally undesirable and often ineffective due to the emergence of new pathogenic races (Mew, 1987; Verdier *et al.*, 2012) [8, 16]. Therefore, host plant resistance remains the most economical, sustainable and environmentally safe approach for BLB management (Khush and Angeles, 1999) [7]. More than forty BLB resistance genes have been identified in rice (Chen *et al.*, 2020) [1], among which *Xa21* is one of the most widely utilized dominant genes because of its broad-spectrum resistance against diverse Xoo races (Song *et al.*, 1995; Ronald *et al.*, 1992) [14, 13]. Among these, several genes including *Xa1*, *xa5*, *Xa4*, *xa13*, *Xa21*, *Xa23*, *Xa25*, *Xa26* (*Xa3*), *Xa27*, *Xa10* and *xa41* have been cloned and functionally characterized (Jiang *et al.*, 2020) [5]. Marker-assisted breeding has significantly accelerated the development of disease-resistant rice cultivars by enabling precise introgression of target genes while minimizing the linkage drag.

Phenotypic validation under artificial disease pressure is indispensable for confirming the effectiveness of introgressed resistance genes, because molecular marker-assisted selection alone cannot fully assess the expression and durability of resistance under field conditions

(Kauffman *et al.*, 1973; IRRI, 2013) [6, 4]. Therefore, the present study was undertaken to evaluate advanced breeding lines of JGL 27356 for resistance to bacterial leaf blight under artificial inoculation and to identify promising resistant lines for their utilization in future rice improvement programmes.

Materials and Methods

The present investigation was conducted during *Kharif* 2025 at Rice Research Unit (RRU), Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, to evaluate the advanced breeding lines of rice (*Oryza sativa* L.) for resistance to bacterial leaf blight (BLB). The experimental material is comprised of 50 advanced breeding lines (F₆ generation) developed from the cross between JGL 27356 (Jagti Vari 3) and MTU1010NIL (IR121055-2-10-7). For phenotypic screening, the breeding lines were evaluated along with its recurrent parent (JGL 27356), Taichung Native 1 (TN1) as the susceptible check and Improved Samba Mahsuri (ISM) as the resistant check. A virulent isolate of *Xanthomonas oryzae* pv. *oryzae* (Xoo), which was obtained from the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad, was cultured on Hayward's agar medium and incubated at 28°C for 96 hours. The bacterial cells were harvested and suspended in sterile distilled water to obtain an inoculum concentration of approximately 10⁸-10⁹ CFU mL⁻¹. Artificial inoculation was performed at the maximum tillering stage (approximately 55 days after transplanting) by following the leaf clipping method as described by Kauffman *et al.* (1973) [6], wherein the terminal 1-2 cm portion of fully expanded uppermost leaves was clipped using scissors, which were dipped in the bacterial suspension. Approximately ten leaves per plant were inoculated to ensure uniform disease development. Disease severity was assessed 21 days after inoculation by recording lesion length and total leaf length. The percentage of disease incidence was calculated using the formula:

$$\text{Disease incidence (\%)} = (\text{Lesion length} / \text{Leaf length}) \times 100$$

The disease reaction was classified according to the IRRI Standard Evaluation System (SES, 2013) [4] as follows (Table-1)

Table 1: Standard Evaluation System, IRRI-2013 scale for bacterial leaf blight (BB)

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 response of advanced breeding lines to bacterial leaf blight

Significant variation for bacterial leaf blight resistance was observed among the advanced breeding lines, which indicate the successful segregation of disease resistance in the breeding population. Among the 50 F₆ advanced breeding

lines tested, one line namely, VPN-31 exhibited the highest level of resistance with disease score of 1, which was comparable to the resistant check Improved Samba Mahsuri. Three advanced breeding lines, namely VPN-29, VPN-43 and VPN-50 exhibited moderately resistant reaction with disease score of 3. Five breeding lines- VPN-25, VPN-26, VPN-27, VPN-30 and VPN-49 were categorized as moderately susceptible with disease score of 5. The majority of the breeding population i.e., about 37 entries, namely VPN-1, VPN-3, VPN-4, VPN-5, VPN-6, VPN-7, VPN-8, VPN-9, VPN10, VPN-11, VPN-12, VPN-13, VPN-14, VPN-15, VPN-16, VPN-17, VPN-18, VPN-19, VPN-20, VPN-21, VPN-22, VPN-23, VPN-24, VPN-28, VPN-32, VPN-33, VPN-35, VPN-36, VPN-37, VPN-38, VPN-39, VPN-40, VPN-42, VPN-44, VPN-45, VPN-47 and VPN-48 exhibited susceptible reactions with disease score of 7, while four entries (VPN-2, VPN-34, VPN-41 and VPN-46) were highly susceptible, recording a disease score of 9. The recurrent parent JGL 27356 showed disease score of 5 which indicates moderate susceptibility to bacterial leaf blight. The susceptible check TN1 recorded disease score of 9 which showed severe disease incidence, whereas the resistant check Improved Samba Mahsuri (ISM) exhibited a score of 1, which confirms the successful disease establishment and effective screening conditions (Table 2).

Table 2: Screening and scoring details of advanced breeding lines of rice as per IRRI-SES scale

Parents, checks	Score	R/MR/MS/S/HS
JGL 27356	5	MS
TN1	9	HS
ISM	1	R

ABLs	Score	R/MR/MS/S/HS	ABLs	Score	R/MR/MS/S/HS
VPN-1	7	S	VPN-26	5	MS
VPN-2	9	HS	VPN-27	5	MS
VPN-3	7	S	VPN-28	7	S
VPN-4	7	S	VPN-29	3	MR
VPN-5	7	S	VPN-30	5	MS
VPN-6	7	S	VPN-31	1	R
VPN-7	7	S	VPN-32	7	S
VPN-8	7	S	VPN-33	7	S
VPN-9	7	S	VPN-34	9	HS
VPN-10	7	S	VPN-35	7	S
VPN-11	7	S	VPN-36	7	S
VPN-12	7	S	VPN-37	7	S
VPN-13	7	S	VPN-38	7	S
VPN-14	7	S	VPN-39	7	S
VPN-15	7	S	VPN-40	7	S
VPN-16	7	S	VPN-41	9	HS
VPN-17	7	S	VPN-42	7	S
VPN-18	7	S	VPN-43	3	MR
VPN-19	7	S	VPN-44	7	S
VPN-20	7	S	VPN-45	7	S
VPN-21	7	S	VPN-46	9	HS
VPN-22	7	S	VPN-47	7	S
VPN-23	7	S	VPN-48	7	S
VPN-24	7	S	VPN-49	5	MS
VPN-25	5	MS	VPN-50	3	MR

ABLs- Advanced Breeding Lines, R- resistant, MR- moderately resistant, S- Susceptible, MS- Moderately Susceptible and HS- Highly Susceptible

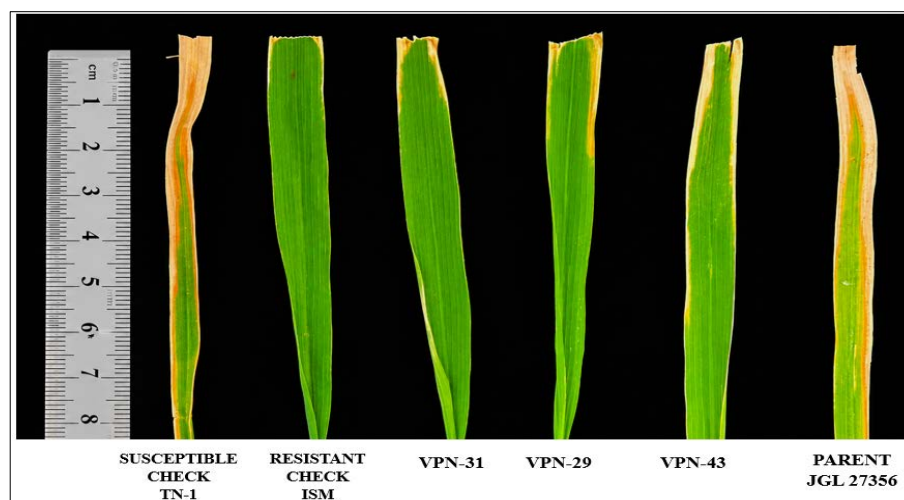


Fig 1: Phenotypic screening for bacterial leaf blight. [TN1 (Taichung Native 1): susceptible check; ISM (Improved Samba Mahsuri): resistant check; VPN-31, VPN-29 and VPN-43: advanced breeding lines showing resistant reaction.]

Discussion

The development of rice cultivars possessing durable resistance to bacterial leaf blight (BLB) through marker-assisted breeding has become an effective strategy for minimizing the yield losses caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). Among the identified BLB resistance genes, *Xa21* has been widely utilized because of its broad-spectrum resistance against diverse pathogen races (Song *et al.*, 1995) [14]. Several studies have demonstrated that introgression of *Xa21*, either alone or in combination with other resistance genes will provide an effective protection against bacterial leaf blight under both artificial and natural field conditions. Similar observations were reported by Sundaram *et al.* (2008) [15], who demonstrated the marker-assisted pyramiding of bacterial blight resistance genes, including *Xa21*, into an elite rice variety Samba Mahsuri, which resulted in broad-spectrum and durable resistance against diverse races of *Xanthomonas oryzae* pv. *oryzae*. Hari *et al.* (2013) [3], who successfully introgressed *Xa21* into elite rice cultivars and observed enhanced resistance under artificial screening. Similar results were also documented by Laxmi Prasanna *et al.* (2018) [12], who reported that breeding lines carrying *Xa21*, consistently exhibited lower lesion lengths and reduced disease severity, when compared with susceptible parental lines. Gautam *et al.* (2023) [2] demonstrated that pyramiding of *Xa21*, *xa13*, *xa5* and *Xa4* through marker-assisted backcross breeding produced rice lines, that exhibited durable bacterial blight resistance across multiple environments and also highlighted the continued effectiveness of *Xa21* in combination with other resistance genes.

The recovery of resistant and moderately resistant individuals in the advanced generation suggests the successful incorporation of the *Xa21* gene into selected breeding lines through marker-assisted pedigree breeding. Nevertheless, the predominance of susceptible lines indicates segregation of resistance loci and highlights the importance of combining molecular marker-assisted selection with phenotypic screening to identify true resistant recombinants.

Conclusion

The present investigation demonstrates a substantial variability among advanced breeding lines for bacterial leaf blight resistance under artificial inoculation. Among the 50

advanced F₆ breeding lines, VPN-31 exhibited a resistant reaction comparable to the resistant check Improved Samba Mahsuri, whereas VPN-29, VPN-43 and VPN-50 expressed moderate resistance. These breeding lines of F₆ generation represent valuable genetic resources for the development of high-yielding rice cultivars with durable resistance to bacterial leaf blight. Further multilocation evaluation, molecular confirmation and agronomic assessment are recommended before their utilization in varietal release programmes or as donor parents in future rice improvement programmes.

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