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## IDENTIFICATION OF BACTERIAL LEAF BLIGHT-RESISTANT RICE CULTURES THROUGH ARTIFICIAL INOCULATION

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### ABSTRACT

Bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae*, is one of the most destructive diseases limiting rice productivity in Telangana and other rice-growing regions of India. Host plant resistance remains the most effective and environmentally safe strategy for BLB management. The present study was conducted to screen a diverse set of rice cultures against BLB under artificial inoculated conditions during *Kharif* 2024 at the Regional Sugarcane and Rice Research Station, Rudrur, Telangana. A total of 176 rice entries, including rice cultures and checks were evaluated using the artificial inoculation method and disease severity was recorded using the Standard Evaluation System (SES) scale. The results revealed considerable variation in disease response among the entries. Nine genotypes, namely KNM 15736, KNM 16056, KNM 16006, RDR 2747, RNRH 139, RNR 44199, E-131, KNM 12367 and KNM 12368 exhibited resistant reactions. Fifty-five entries were categorized as moderately resistant, while the majority of genotypes fell under moderately susceptible to susceptible categories. The identification of resistant and moderately resistant entries provides valuable genetic resources for BLB resistance breeding programs.

**Keywords :** Bacterial leaf blight, *Xanthomonas oryzae*, Artificial inoculation.

### Introduction

Rice (*Oryza sativa* L.) is the principal staple food crop for a large proportion of the global population and plays a crucial role in ensuring food and nutritional security in India (Khush, 2005; FAO, 2021). However, rice productivity is severely constrained by several biotic stresses among which bacterial leaf blight (BLB) is one of the most economically important diseases (Mew *et al.*, 1993; Nino-Liu *et al.*, 2006). BLB caused by *Xanthomonas oryzae* pv. *Oryzae* is prevalent in both tropical and subtropical rice-growing regions and can cause yield losses ranging from 20 to 80 per cent under severe epidemic conditions (Ou, 1985; Srinivasan and Gnanamanickam, 2005). The disease affects rice at all growth stages, resulting in leaf wilting, drying and poor grain filling, particularly under warm and humid climatic conditions and high nitrogen fertilization

(Ezuka and Kaku, 2000; Singh *et al.*, 2015). The frequent emergence of new virulent pathotypes has often led to the breakdown of resistance in released varieties, posing a major challenge to sustainable BLB management (Adhikari *et al.*, 1995; Verdier *et al.*, 2012). Although chemical control measures provide temporary suppression of the disease, their repeated use is neither economical nor environmentally sustainable due to residue accumulation and potential ecological risks (Mew, 1987; Swamy *et al.*, 2006). Breeding for host plant resistance is widely recognized as the most effective, economical and eco-friendly approach for managing BLB (Kou and Wang, 2010; Sundaram *et al.*, 2008). Continuous screening of diverse rice germplasm under artificial inoculation is essential for identifying stable and durable sources of resistance for use in breeding programs (IRRI, 1996;

Pradhan *et al.*, 2016). Therefore, the present investigation was undertaken to screen a large set of rice cultures against BLB under artificial inoculated conditions with the objective of identifying resistant and moderately resistant genotypes suitable for resistance breeding programs.

### Materials and Methods

The field experiment was carried out during the *Kharif* season of 2024 at the research farm of the Regional Sugarcane and Rice Research Station, Rudrur, Professor Jayashankar Telangana Agricultural University, Nizamabad district, Telangana. The experiment was laid out in a Randomized Block Design (RBD) with two replications. A total of 176 rice entries, comprising 175 rice cultures along with a susceptible check, were included for evaluation against bacterial leaf blight. The crop was sown on 26 June 2024 maintaining a spacing of 20 × 15 cm and all recommended agronomic practices were followed to ensure uniform crop growth.

Artificial inoculation was performed on 23 July 2024 using the standard clipping method. The bacterial inoculum was prepared from actively growing cultures of *Xanthomonas oryzae* pv. *oryzae* by suspending the bacterial growth in sterile distilled water and adjusting the concentration to an appropriate level. Inoculation was carried out by clipping the tips of fully expanded leaves (approximately 2–3 cm) with sterilized scissors dipped in the bacterial suspension ensuring uniform infection pressure across all entries. This method facilitated consistent disease development under field conditions for reliable assessment of host reactions. Disease observations were recorded at 15, 30 and 45 days after inoculation (DAI). Disease severity was assessed using the Standard Evaluation System (SES) scale (IRRI, 1996).

Score	Description (affected lesion area)	Host response
1	1-5%	Resistant
3	6-12%	Moderately Resistant
5	13-25%	Moderately Susceptible
7	26-50%	Susceptible
9	51-100%	Highly Susceptible

### Results

The screening of 176 rice entries under artificial inoculated conditions revealed a wide spectrum of

reactions to bacterial leaf blight, indicating substantial genetic variability for disease response among the evaluated materials. Typical BLB symptoms appeared within 10–12 days after inoculation and intensified progressively with crop growth, characterized by water-soaked lesions that later turned yellowish to straw-colored, leading to leaf wilting and drying under susceptible reactions. Out of the total entries evaluated, nine genotypes namely KNM 15736, KNM 16056, KNM 16006, RDR 2747, RNRH 139, RNR 44199, E-131, KNM 12367 and KNM 12368, exhibited resistant reactions with a disease score of 1 (Table 2). These genotypes showed very limited lesion development, restricted lesion expansion and minimal impact on leaf area indicating effective resistance against *Xanthomonas oryzae* pv. *oryzae* under artificial epiphytotic conditions (Table 1).

Fifty-five entries were categorized as moderately resistant, exhibiting comparatively lower disease severity with shorter lesions and restricted disease spread. These genotypes maintained greener foliage and demonstrated the ability to limit pathogen multiplication, suggesting the presence of partial or quantitative resistance mechanisms. Such moderately resistant lines are considered valuable for breeding programs due to their relatively stable performance across environments. Seventy-three entries displayed moderately susceptible reactions, showing intermediate disease severity with noticeable lesion development and partial leaf drying. In contrast, seventeen entries were classified as susceptible, exhibiting extensive lesion enlargement, coalescence of infected areas and pronounced leaf drying which are typical indicators of severe BLB infection. None of the evaluated entries showed highly resistant or highly susceptible reactions, indicating the absence of extreme phenotypes within the tested material. A few entries failed to germinate and were therefore excluded from disease assessment. The susceptible checks, TN1 and BPT 5204 consistently recorded high disease severity and extensive symptom expression thereby validating the effectiveness of the artificial inoculation technique and ensuring adequate disease pressure throughout the experiment. Overall, the identification of resistant and moderately resistant genotypes provides promising sources for their utilization in bacterial leaf blight resistance breeding and gene deployment strategies in rice improvement programs.

**Table 1 :** Disease reaction of different rice entries to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*)

S.No	Entry	15 DAI (05-09-24)	30DAI (20-09-24)	45 DAI (05-10-24)	Reaction
1	KNM 14319*	0	3	5	MS
2	KNM 13555*	0	3	3	MR
3	KNM 12362*	0	3	5	MS
4	KNM 14376	0	1	7	S
5	KNM 15970	0	3	5	MS
6	KNM 15736	0	1	1	R
7	KNM 15692	0	1	3	MR
8	KNM 14283	0	1	3	MR
9	KNM 16056	0	1	1	R
10	KNM 16036	0	1	3	MR
11	KNM 16006	0	0	1	R
12	KNM 13584	0	1	5	MS
13	KNM 12466	0	3	7	S
14	TN1	1	5	5	MS
15	ISM	0	3	7	S
16	MTU-1001	3	5	7	S
17	JGL-24423	3	5	7	S
18	NLR-34449	1	5	7	S
19	KPS-10329	0	3	5	MS
20	KPS-12657	No germination			
21	KPS-14341	3	5	7	S
22	KPS-14785	3	5	5	MS
23	KPS-14625	5	7	7	S
24	KPS-14920	5	5	5	MS
25	KPS-14758	3	5	5	MS
26	KPS-14515	3	5	5	MS
27	KPS-14932	0	5	5	MS
28	WGL 1880	1	5	5	MS
29	WGL 1969	0	3	5	MS
30	WGL 1972	1	5	5	MS
31	WGL 1977	0	3	7	S
32	WGL 2000	3	5	5	MS
33	WGL 2001	3	5	5	MS
34	WGL 2013	0	3	5	MS
35	WGL 2019	1	5	5	MS
36	WGL 2024	1	3	5	MS
37	WGL 2025	0	3	5	MS
38	WGL 2027	0	1	3	MR
39	WGL 3034	0	3	3	MR
40	IBT-WGL-17	0	3	3	MR
41	IBT-WGL-26	0	1	3	MR
42	WGL 1927	0	5	5	MS
43	WGL 1931	1	3	7	S
44	WGL 1942	0	3	3	MR
45	WGL 1957	0	3	5	MS
46	WGL 2042	0	1	3	MR
47	RDR 2742	1	3	5	MS
48	RDR 2747	0	1	1	R
49	RDR 4644	0	3	5	MS
50	RDR 4642	0	1	3	MR
51	RDR 4626	3	5	5	MS
52	RDR 4621	0	3	5	MS
53	RDR 4914	No germination			
54	RDR 5773	0	3	3	MR

55	RDR 5288	0	3	3	MR
56	RDR 4927	0	3	3	MR
57	JGL 46164	0	3	3	MR
58	JGL 46210	0	3	3	MR
59	JGL 46224	0	3	5	MS
60	JGL 46549	1	3	5	MS
61	JGL 46298	0	3	5	MS
62	JGL 46211	0	3	5	MS
63	JGL 47849	0	3	5	MS
64	JGL 47870	1	3	7	S
65	JGL 47877	0	3	5	MS
66	JGL 47949	3	5	5	MS
67	JGL 46239	0	3	5	MS
68	JGL 46429	1	3	5	MS
69	JGL 47951	1	3	5	MS
70	JGL 47973	1	3	3	MR
71	JGL 47861	1	5	5	MS
72	JGL 44139	0	1	3	MR
73	JGLH 489	1	3	5	MS
74	JGLH 518	0	0	3	MR
75	JGLH 490	0	1	3	MR
76	JGLH 514	1	1	3	MR
77	JMS 24B	0	1	3	MR
78	RNRH 139	0	1	1	R
79	RNRH 290	0	1	3	MR
80	RNRH 310	0	1	3	MR
81	RNRH 315	3	5	5	MS
82	RNRH 320	0	1	5	MS
83	RNRH 246	0	1	3	MR
84	RNRH 329	0	1	3	MR
85	RNRH 334	0	0	3	MR
86	RNRH 427	0	0	5	MS
87	RNRH 408	0	0	5	MS
88	RNRH 414	0	0	3	MR
89	RNRH 417	0	1	5	MS
90	RNRH 418	0	1	5	MS
91	RNRH 419	1	3	5	MS
92	RMS 9 B	3	5	5	MS
93	RNR 48755	1	3	5	MS
94	RNR 44199	3	5	1	R
95	RNR 44060	0	3	3	MR
96	RNR 44059	0	3	5	MS
97	RNR 51329	0	3	3	MR
98	RNR 51436	1	3	3	MR
99	RNR 51447	1	3	5	MS
100	RNR 51464	0	1	3	MR
101	RNR 51493	0	1	3	MR
102	RNR 51569	0	1	5	MS
103	RNR 51668	1	3	5	MS
104	RNR 35008	0	0	5	MS
105	RNR 37910	No germination			
106	RNR 41653	0	1	5	MS
107	RNR 41714	0	1	3	MR
108	RNR 44197	1	1	3	MR
109	RNR 44035	1	5	5	MS
110	RNR 44177	0	3	5	MS
111	M – 78	1	1	5	MS

112	M – 79	1	1	5	MS
113	M – 80	0	3	5	MS
114	M – 81	0	1	5	MS
115	M – 83	0	0	3	MR
116	M – 84	0	3	5	MS
117	M – 85	0	1	3	MR
118	M – 86	0	1	3	MR
119	M – 87	0	1	3	MR
120	M – 88	1	3	3	MR
121	M – 89	1	5	5	MS
122	M – 90	No germination			
123	M – 92	0	3	3	MR
124	M – 94	0	3	5	MS
125	E - 113	0	3	3	MR
126	E – 114	1	3	3	MR
127	E – 115	0	3	5	MS
128	E – 116	0	3	5	MS
129	E – 117	1	3	5	MS
130	E – 119	1	1	3	MR
131	E – 120	0	3	3	MR
132	E – 121	1	1	3	MR
133	E – 122	0	1	3	MR
134	E – 124	1	1	3	MR
135	E – 125	0	1	3	MR
136	E – 126	1	1	5	MS
137	E – 127	0	1	5	MS
138	E – 128	0	0	5	MS
139	E – 129	1	1	5	MS
140	E – 130	0	1	5	MS
141	E – 131	1	1	1	R
142	E – 132	0	1	3	MR
143	E – 134	0	1	3	MR
144	E – 135	3	3	3	MR
145	E – 136	0	3	5	MS
146	E – 137	3	3	5	MS
147	E – 138	0	3	3	MR
148	WGL 1789	0	3	5	MS
149	WGL 1790	1	3	3	MR
150	WGL 1792	1	3	3	MR
151	KNM 12367	0	3	3	R
152	KNM 12368	0	3	3	R
153	KNM 12472	0	1	5	MS
154	KNM 12510	0	1	5	MS
155	KNM 14445	3	5	5	MS
156	WGL 1719	0	5	7	S
157	WGL 1837	0	3	5	MS
158	WGL 1841	0	3	7	S
159	WGL 1843	0	1	5	MS
160	WGL 1909	0	1	7	S
161	WGL 1945	0	1	7	S
162	WGL 1537	1	3	5	MS
163	WGL 1742	0	1	5	MS
164	JGL 41240	0	1	5	MS
165	JGL 39913	0	0	5	MS
166	JGL 41274	No germination			
167	JGL 38917	0	1	5	MS
168	JGL 41652	0	3	5	MS

169	RNR 37986	0	0	5	MS
170	RNR 31753	0	1	5	MS
171	KPS 10642	1	3	5	MS
172	TN1	1	3	5	MS
173	PTB 33	0	1	7	S
174	RNR 15048	1	3	5	MS
175	ISM	1	3	5	MS
<b>Checks</b>	TN 1	3	5	7	S
	MTU 1001	1	1	3	MR
	MTU-1010	0	1	5	MS
	BPT 5204	0	3	7	S

**Table 2 :** Classification of rice entries based on bacterial leaf blight disease scores

S.No	Reaction	Score	No. of entries	Name of the entries
1	HR	0	0	-
2	R	1	9	KNM 15736, KNM 16056, KNM 16006, RDR 2747, RNRH 139, RNR 44199, E – 131, KNM 12367, KNM 12368
3	MR	3	55	KNM 13555, KNM 15692, KNM 14283, KNM 16036, WGL 2027, WGL 3034, IBT-WGL-17, IBT-WGL-26, WGL 1942, WGL 2042, RDR 4642, RDR 5773, RDR 5288, RDR 4927, JGL 46164, JGL 46210, JGL 47973, JGL 44139, JGLH 518, JGLH 490, JGLH 514, JMS 24B, RNRH 290, RNRH 310, RNRH 246, RNRH 329, RNRH 334, RNRH 414, RNR 44060, RNR 51329, RNR 51436, RNR 51464, RNR 51493, RNR 41714, RNR 44197, M – 3, M – 85, M – 86, M – 87, M – 88, M – 92, E – 113, E – 114, E – 119, E – 120, E – 121, E – 122, E – 124, E – 125, E – 132, E – 134, E – 135, E – 138, WGL 1790, WGL 1792
4	MS	5	73	KNM 14319, KNM 12362, KNM 15970, KNM 13584, TN1, KPS-10329, KPS-14785, KPS-14920, KPS-14758, KPS-14515, KPS-14932, WGL 1880, WGL 1969, WGL 1972, WGL 2000, WGL 2001, WGL 2013, WGL 2019, WGL 2024, WGL 2025, WGL 1927, WGL 1957, RDR 2742, RDR 4644, RDR 4626, RDR 4621, JGL 46224, JGL 46549, JGL 46298, JGL 46211, JGL 47849, JGL 47877, JGL 47949, JGL 46239, JGL 46429, JGL 47951, JGL 47861, JGLH 489, RNRH 315, RNRH 320, RNRH 427, RNRH 408, RNRH 417, RNRH 418, RNRH 419, RMS 9 B, RNR 48755, RNR 44059, RNR 51447, RNR 51569, RNR 51668, RNR 35008, RNR 41653, RNR 44035, RNR 44177, M – 78, M – 79, M – 80, M – 81, M – 84, M – 89, M – 94, E – 115, E – 116, E – 117, E – 126, E – 127, E – 128, E – 129, E – 130, E – 136, E – 137, WGL 1789, KNM 12472, KNM 12510, KNM 14445, WGL 1837, WGL 1843, WGL 1537, WGL 1742, JGL 41240, JGL 39913, JGL 38917, JGL 41652, RNR 37986, RNR 31753, KPS 10642, TN1, RNR 15048, ISM
5	S	7	17	KNM 14376, KNM 12466, ISM, MTU-1001, JGL- 24423, NLR-34449, KPS-14341, KPS-14625, WGL 1977, WGL 1931, JGL 47870, WGL 1719, WGL 1841, WGL 1909, WGL 1945, PTB 33, BPT 5204
6	HS	0	-	-
Not germinated				KPS-12657, RDR 4914, RNR 37910, M – 90, JGL 41274
<b>Total</b>				<b>150 MRST + 25 SMRST + 4 checks</b>

## Discussion

The present investigation demonstrated considerable variability in the reaction of rice genotypes to bacterial leaf blight (BLB) under artificial inoculated conditions, reinforcing that resistance to BLB is predominantly quantitative in nature rather than absolute. Among the screened entries, the absence of highly resistant genotypes and the predominance of moderately resistant (MR) and moderately susceptible (MS) reactions align with findings from other large-scale phenotypic screenings showing limited sources of strong resistance in diverse germplasm panels (Ashwini *et al.*, 2025). This pattern suggests that BLB

resistance often involves multiple genes and minor effect loci that cumulatively confer partial resistance rather than single major effect genes conferring complete immunity. The identification of nine resistant genotypes that consistently exhibited minimal lesion development even under high disease pressure is significant for breeding programs. Similar limited but promising resistant sources have been documented in recent field screens, underscoring the importance of continuing germplasm evaluation for durable BLB resistance (Goske *et al.*, 2025). Moderately resistant entries also deserve attention as quantitative resistance has been shown to offer more stable performance across seasons and locations compared to single major



gene resistance, which is often overcome by evolving pathogen populations (Qi *et al.*, 2025). The frequent breakdown of resistance due to virulent pathotypes has been documented in recent pathotype studies, highlighting the dynamic nature of *Xanthomonas oryzae* pv. *oryzae* populations (Tiwari *et al.*, 2025). This underscores the need for continuous screening and diversification of resistance sources. The predominance of moderately susceptible and susceptible reactions among the evaluated entries further emphasizes the vulnerability of many currently cultivated genotypes to BLB. This observation is consistent with other regional reports documenting high BLB incidence and yield losses in rice ecosystems due to susceptibility of leading cultivars (Verma & Tiwari, 2025). Mechanistic studies on BLB resistance pathways suggest that host resistance involves complex networks of defense responses, including phytohormone signaling and upregulation of pathogenesis-related genes which may underlie the partial resistance observed in MR genotypes (Zhong *et al.*, 2024). Such insights into defense mechanisms are valuable for integrating phenotypic resistance with molecular markers in breeding programs. Moreover, recent advances in BLB resistance gene discovery, including novel genes like *Xa50(t)* expand the molecular toolkit available for breeding durable resistance (Li *et al.*, 2025). The consistent high disease severity in susceptible checks reaffirmed the reliability of the artificial inoculation method and established uniform disease pressure across the trial. This methodological robustness is critical for accurate discrimination of genotype responses and has been emphasized in several recent screening protocols (Pradhan *et al.*, 2016; Ashwini *et al.*, 2025). Overall, the results underscore the importance of systematic and ongoing germplasm screening for BLB resistance. The resistant and moderately resistant genotypes identified in this study constitute valuable donors for BLB resistance breeding programs their integration into breeding pipelines, potentially in combination with marker-assisted selection and gene pyramiding approaches could enhance the development of rice cultivars with more durable and broad-spectrum resistance suited to Telangana and similar agro-climatic regions.

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