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Screening of coloured rice germplasm for resistance to bacterial leaf blight caused by *Xanthomonas oryzae pv. Oryzae*

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Abstract
Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*), remains one of the most destructive bacterial diseases of rice and the deployment of resistant genotypes continues to be the most economical and environmentally sound management option. Coloured (pigmented) rice landraces are widely reported to carry useful pest and disease resistance alongside their nutritional value, but the reaction of many traditional coloured genotypes to BLB is not well documented. This study screened 46 coloured rice genotypes, including six check varieties, for resistance to a virulent *Xoo* isolate obtained from ICAR-Indian Institute of Rice Research (IIRR), Hyderabad. Plants approaching panicle initiation were inoculated by the leaf-clipping method and disease severity was recorded 15 days after inoculation using the 0–9 Standard Evaluation Scale (SES) and converted to five reaction classes. Of the 46 genotypes evaluated, 4 genotypes were classified as highly Resistant, 7 Resistant, 8 Moderately Resistant, 9 Susceptible and 18 Highly Susceptible. Genotypes Bari Nellu, Kayame, Mullare and Choharto showed the lowest disease scores (SES 1) and are identified as promising sources of BLB resistance for use in breeding programmes, while a large proportion of the germplasm, including the widely grown check Jyothi, was highly susceptible. These results indicate that substantial variability for BLB reaction exists within coloured rice germplasm and that select landraces merit further genetic characterisation and use as resistance donors.

Keywords: Bacterial leaf blight, Coloured rice, Germplasm, Resistance screening, Standard evaluation scale, *Xanthomonas oryzae pv. oryzae*

Introduction
Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. It serves as the staple food for more than half of the global population, particularly in Asia, Africa and parts of Latin America. It plays a crucial role in ensuring food security and livelihoods, especially in developing countries (Muthayya *et al.*, 2014) [6]. Rice belongs to the family Poaceae and is predominantly a self-pollinated crop with a diploid genome (2n = 24). The two cultivated rice species are *Oryza sativa* L. and *Oryza glaberrima*. Among these, *O. sativa* is cultivated worldwide while *O. glaberrima* is largely confined to West Africa.

Bacterial leaf blight, caused by the Gram-negative bacterium *Xanthomonas oryzae pv. oryzae* (*Xoo*), is one of the most economically important diseases of cultivated rice (*Oryza sativa L.*) across Asia (Sundaram *et al*., 2009; Banerjee *et al*., 2018) [2, 10]. The pathogen enters the plant mainly through hydathodes at the leaf tip and margins or through wounds, colonises the xylem vessels and produces the characteristic water-soaked lesions that later turn yellow to white and extend along the leaf veins. Under favourable warm and humid conditions, severe infection can cause substantial yield reduction and losses of over fifty percent have been reported in susceptible varieties during epidemic years (Banerjee *et al*., 2018; Khannetah *et al*., 2021) [2, 5].

Traditional and landrace rice germplasm represents a valuable and largely under-exploited reservoir of resistance genes, having co-evolved with local pathogen populations over long periods of cultivation. Coloured (pigmented) rice genotypes, distinguished by anthocyanin and other flavonoid pigments accumulated in the pericarp, have attracted growing interest not only as functional foods rich in antioxidants but also as potential sources of disease resistance. Pigment accumulation in the caryopsis and leaf tissue has been proposed to act as a biochemical barrier against pathogen colonisation and comparative work on pigmented versus non-pigmented rice has shown that darker, pigmented cultivars can display measurably lower bacterial leaf blight severity than commonly cultivated white rice checks (Joko *et al*., 2019) [4]. Similar observations have also been reported in molecular characterization studies where resistant coloured rice accessions possessed combinations of major BLB resistance genes such as *Xa4*, *xa5*, *xa13* and *Xa21* (Ashiba *et al*., 2020; Khannetah *et al*., 2021) [1, 5].

Despite this promise, the reaction of many traditional coloured rice landraces to BLB has not been systematically evaluated, particularly against locally prevalent, highly virulent *Xoo* isolates. Identifying such resistant sources within existing germplasm collections is an essential first step before they can be used as donors in resistance-breeding programmes. The present study was therefore undertaken to screen a set of 46 coloured rice genotypes, including released check varieties, for their reaction to a virulent *Xoo* isolate using the leaf-clipping inoculation method and the internationally used Standard Evaluation Scale, with the aim of identifying genotypes with useful levels of resistance to bacterial leaf blight.

Material and Methods

The present investigation was carried out during Rabi 2025-26 at ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, Telangana. The experimental material consisted of 46 coloured rice genotypes along with 6 checks. The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications. Each genotype was sown in two rows of two-metre length with a spacing of 20 cm between rows and 15 cm between plants within a row. Timely management of recommended package of practices was carried out during the crop period.

Bacterial Isolate and Culture Media

A virulent isolate of *Xanthomonas oryzae pv. oryzae* (*Xoo*) was obtained from ICAR-Indian Institute of Rice Research (IIRR), Hyderabad. The isolate was multiplied and maintained on *Xoo*-specific modified Wakimoto's medium, prepared by dissolving sucrose (20 g), sodium phosphate (0.82 g), ferrous sulphate (0.05 g), calcium nitrate (0.5 g), peptone (5.0 g) and agar
(17.0 g) in 1000 ml of distilled water. The medium was dispensed into triangular flasks, plugged with cotton, covered with kraft paper and sterilised by autoclaving at 121 °C for 30 minutes.

Inoculum Preparation and Plant Inoculation

Bacterial inoculum was prepared by suspending the pathogenic culture in sterile distilled water and adjusting the concentration to approximately 1×10^9 CFU/ml. Plants were inoculated shortly before panicle initiation, using fully expanded healthy leaves. Sterile scissors were dipped in the inoculum and used to clip three leaves per plant approximately 2–3 cm from the leaf tip, following the standard leaf-clipping technique. Characteristic BLB lesions developed on the clipped leaves and were recorded 15 days after inoculation.

Disease Scoring

Disease severity on the inoculated leaves was assessed visually using the Standard Evaluation Scale (SES) (IRRI, 2002) for bacterial leaf blight, which grades severity from 0 (no lesion) to 9 (majority of plants severely stunted, brown and dead) based on the percentage of diseased leaf area (Table 1). Each genotype was assigned a BLB score based on the predominant symptom expression and scores were converted into five reaction categories: Highly Resistant (score 1), Resistant (score 3), Moderately Resistant (score 5), Susceptible (score 7) and Highly Susceptible (score 9).

Table 1: Standard Evaluation Scale (SES) used for visual assessment of bacterial leaf blight severity.

Scale	Description	Diseased Leaf Area (%)
0	No typical susceptible lesion observed	0
1	Rapid observation does not reveal leaf lesions, but careful	<0.3
2	Scrutiny of each row reveals few lesions Rapid detection detects a few lesions	0.3-0.9
3	Several lesions are randomly scattered within a plot	1-2
4	Lesion number on an infected leaf Ranges from 1 to 4. Upper leaves are uniformly dotted with blast lesions but without necrotic (brown) leaf tips. A few to several leaves are brown.	3-7
5	Several too many lower leaves become necrotic and few dead leaves are observed. Tips of several upper leaves show brown color and begin to fold.	8-14
6	Lower leaves are uniformly exhibiting brown color and several dead leaves are visible. Tip necrosis of upper leaves is predominant.	15-24
7	Tips of most upper leaves are curling. Middle and lower leaves are brown. Several plants or tillers are stunted or dead	25-39
8	Extensive leaf curling and browning of upper and middle leaves are prevalent. Plants are generally stunted and many plants are dead.	40-65
9	Majority of plants are severely stunted, brown and dead. Only few to several plants have green leaves with heavy infection.	>65

Results and Discussion

All 46 genotypes developed typical bacterial leaf blight lesions following the leaf clipping method, confirming successful infection by the *Xoo* isolate, but the genotypes differed markedly in the severity of symptoms recorded 15 days after inoculation (Table 2). BLB scores ranged from 1 to 9 across the panel, reflecting a wide range of host response from highly resistant to highly susceptible reactions.

Table 2: Screening of coloured rice germplasm for bacterial leaf blight resistance.

S.No.	Genotype	BLB Score	Reaction
1	Ashoka	3	Resistant
2	Bara Ratna Choodi	5	Moderately Resistant
3	Banamar Vinelu	7	Susceptible
4	Bari Nellu	1	Highly Resistant
5	Belagum Mug Batta	5	Moderately Resistant
6	Bidar Local	7	Susceptible
7	Bili Doddi	3	Resistant
8	Bili Hegge	9	Highly Susceptible
9	Burma	5	Moderately Resistant
10	Chindi Batta	7	Susceptible
11	Dasari Patti	3	Resistant

12	Deva Mallige	5	Moderately Resistant
13	Dodda Gavari	7	Susceptible
14	Doddiga	9	Highly Susceptible
15	Gamand Batta	9	Highly Susceptible
16	Hakkala Sali	9	Highly Susceptible
17	Halaga	5	Moderately Resistant
18	Hola Batta	3	Resistant
19	Honasu	9	Highly Susceptible
20	Jade Batta	9	Highly Susceptible
21	Jole Kinya	5	Moderately Resistant
22	Jugal	7	Susceptible
23	Kabbiga	9	Highly Susceptible
24	Kajaya	3	Resistant
25	Kala Prasali	9	Highly Susceptible
26	Kare Batta	9	Highly Susceptible
27	Kare Jaddu	9	Highly Susceptible
28	Kari Hakkala Sali	5	Moderately Resistant
29	Kari Kagga	3	Resistant
30	Kari Kavali	9	Highly Susceptible
31	Kari Sali	9	Highly Susceptible
32	Karidari Vilai	5	Moderately Resistant
33	Kayame	1	Highly Resistant
34	Kempaithi Batta	9	Highly Susceptible
35	Kempu Madarasu	7	Susceptible
36	Kempu Nellu	9	Highly Susceptible
37	Kiruvana	9	Highly Susceptible
38	Mahakanthi	9	Highly Susceptible
39	Masale Putta Batta	9	Highly Susceptible
40	Mullare	1	Highly Resistant
41	BPT 2841 ©	3	Resistant
42	BPT 2858 ©	7	Susceptible
43	Jyothi ©	9	Highly Susceptible
44	Uma ©	7	Susceptible
45	Choharto ©	1	Highly Resistant
46	Chakhao Poireiton ©	7	Susceptible

When grouped into reaction classes (Table 3), four genotypes — Bari Nellu, Kayame, Mullare and Choharto (c) were rated Highly Resistant with the lowest possible BLB score of 1, indicating little to no visible lesion development. A further seven genotypes (Ashoka, Bili Doddi, Dasari Patti, Hola Batta, Kajaya, Kari Kagga and the check BPT 2841) were classed as Resistant and eight genotypes were Moderately Resistant. In contrast, nine genotypes were Susceptible and eighteen were Highly Susceptible, including the widely cultivated check variety Jyothi.

Table 3: Distribution of 46 coloured rice genotypes according to BLB reaction category

BLB Score	Reaction	Number of genotypes	Genotypes
1	Highly Resistant	4	Bari Nellu, Kayame, Mullare, Choharto©
3	Resistant	7	Ashoka, Bili Doddi, Dasari Patti, Hola Batta, Kajaya, Kari Kagga and BPT 2841
5	Moderately Resistant	8	Bara Ratna Choodi, Belagum Mug Batta, Burma, Deva Mallige, Halaga, Jole Kinya, Kari Hakkala Sali and Karidari Vilai
7	Susceptible	9	Banamar Vinelu, Bidar Local, Chindi Batta, Dodda Gavari, Jugal, Kempu Madarasu, BPT 2858, Uma and Chakhao Poireiton
9	Highly Susceptible	18	Bili Hegge, Doddiga, Gamand Batta, Hakkala Sali, Honasu, Jade Batta, Kabbiga, Kala Prasali, Kare Batta, Kare Jaddu, Kari Kavali, Kari Sali, Kempaithi Batta, Kempu Nellu, Kiruvana, Mahakanthi, Masale Putta Batta, Jyothi

The consistent identification of Bari Nellu, Kayame, Mullare and Choharto as highly resistant, together with a further cluster of resistant genotypes, indicates that useful levels of BLB resistance are present within this coloured rice germplasm collection despite the high virulence of the *Xoo* isolate used. Similar observations have been reported by Banerjee *et al.* (2018) [2], who screened 210 rice germplasm lines and identified several resistant and moderately resistant genotypes carrying different combinations of BLB resistance genes. This is consistent with earlier observations that pigmented rice genotypes can show improved resistance to bacterial leaf blight relative to commonly cultivated non-pigmented checks, an effect that has been linked to flavonoid and anthocyanin accumulation acting as a biochemical barrier to pathogen colonisation (Joko *et al.* 2019) [4].

The presence of highly resistant genotypes in the present investigation also agrees with the findings of Qudsia *et al.*

(2019) [8], who reported that rice lines carrying multiple BLB resistance genes, particularly *Xa4* and *xa13*, expressed stable resistance under natural hotspot conditions. Likewise, Sirohi *et al.* (2019) [9] observed that backcross-derived lines possessing *Xa4*, *xa5*, *xa13* and *Xa21* showed strong resistance under both molecular and artificial screening, highlighting the effectiveness of pyramiding multiple resistance genes for achieving durable resistance. Sundaram *et al.* (2009) [10] successfully introgressed *xa5*, *xa13* and *Xa21* into the elite rice variety Samba Mahsuri through marker-assisted backcross breeding, resulting in improved resistance without compromising desirable agronomic traits. Similarly, Pradhan *et al.* (2015) [7] reported that rice lines carrying multiple BLB resistance genes exhibited significantly enhanced resistance against diverse pathogen isolates, thereby confirming the value of gene pyramiding in resistance breeding programmes.

The wide range of disease reactions observed in the present study indicates that pigmentation alone does not confer resistance, since a substantial number of coloured landraces were highly susceptible; resistance in this panel is therefore more likely governed by specific resistance (*Xa*) genes segregating within the germplasm rather than pigmentation *per se*. Similarly, Khannetah *et al.* (2021) [5] reported substantial phenotypic variation among 100 rice accessions and identified resistant donor lines carrying *Xa4*, *xa5*, *xa13* and *Xa21*, confirming the usefulness of these genes in BLB resistance breeding. The high proportion of susceptible and highly susceptible reactions, including in the released check Jyothi, also illustrates the virulence of the *Xoo* isolate used and reinforces the value of continued screening to identify durable resistance donors before this isolate's associated pathotype becomes more widespread.

Further validation of the highly resistant genotypes through molecular characterization of major bacterial leaf blight resistance genes such as *Xa4*, *xa5*, *xa13* and *Xa21* would facilitate their effective utilization in marker-assisted breeding programmes.

Conclusion

Screening of 46 coloured rice genotypes against a virulent *Xoo* isolate revealed considerable variability in reaction to bacterial leaf blight, ranging from highly resistant to highly susceptible. The genotypes Bari Nellu, Kayame, Mullare and Choharto were identified as highly resistant and represent valuable donor parents for bacterial leaf blight resistance breeding programmes, while the high proportion of susceptible genotypes, including the check variety Jyothi, underlines the continuing threat posed by virulent *Xoo* populations to widely cultivated rice varieties.

Referance

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