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Research Article

Emergence of a new *Xanthomonas oryzae pv. oryzae* (*Xoo*) pathotype breaking the earlier resistance gene combination of improved Sambha Mahsuri

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Abstract
Bacterial Leaf Blight (BLB) disease caused by *Xanthomonas oryzae pv. oryzae* (Xoo) continues to be a major threat to rice (*Oryza sativa* L.) production. The present study was conducted during *Kharif* seasons 2022-2024 to investigate the virulence of the Raipur Xoo isolate against a set of 28 Near Isogenic Lines (NILs) of IR24 introgressed with various combinations of BLB resistance gene along with local check Bamleshwari, resistant check Improved Sambha Mahsuri, and susceptible check TN-1. The results of artificial inoculation based BLB screening over the three years revealed that most single gene differentials (NILs) lines performed poorly against the Raipur *Xoo* isolate, where the most frequent resistance reactions ranged from susceptible to highly susceptible indicating the high virulence of the Raipur Xoo isolate. However, among the single gene NILs, the recessive gene xa5 showed relatively stable performance with moderate resistance across the three years. In contrast, NILs possessing two or more resistance genes exhibited improved and more stable resistance, particularly combinations involving xa5, xa13, Xa21, and Xa4. Notably, genotypes such as IRBB-60, IRBB-66, and IRBB-64 consistently displayed moderate to high resistance, emphasizing the advantage of gene pyramiding in conferring durable resistance. Interestingly, the susceptible reaction of the Improved Sambha Mahsuri having three major BLB resistance gene xa5+xa13+Xa21 during *Kharif* 2024 was against the resistance reaction during *Kharif* 2022 which further indicated the evolution of the pathogen towards virulence. This highlights the inherent dynamic nature of Xoo and its ability to overcome the existing BLB resistance genes. These findings substantiate the need for continuous monitoring of the pathogen virulence and search for new sources of BLB resistance gene.

Keywords: BLB; Marker Assisted Selection; Resistance breakdown; Artificial inoculation

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food nourishing world's half of the human population. Rice adaptation to diverse growing conditions makes it a strategic crop for ensuring food security of the growing population of the world (Fahad *et al*., 2019). The life cycle of Rice is under stress caused by several biotic and abiotic factors. Rice during its life cycle faces a multitude of challenges from various biotic and abiotic factors. Fungal pathogens cause nearly four dozen distinct diseases (Wei, 1975). Bacterial pathogens cause ten identified diseases (Goto, 1979). Viral pathogens cause fifteen different diseases and insects and nematodes, collectively are responsible for about 75 distinct diseases affecting rice cultivation (Ou, 1985).

Rice bacterial leaf blight caused by bacterial pathogen *Xanthomonas oryzae pv. oryzae* is one of the most destructive disease which can cause up to 50% yield loss (Jiang *et al*., 2020). Research has documented a substantial diversity in *Xanthomonas oryzae pv. oryzae* (*Xoo*) populations globally, with scientific literature from the late 1990s and early 2000s have reported more than three dozen distinct races (Noda *et al*., 1995; Pandey *et al*., 2014). At present there is no effective chemical identified that can manage the disease effectively. The host plant resistance offers an effective, economical and causes minimum impact on environment compared to alternative control methods used to manage the disease (Perumalsamy *et al*., 2010; Soumya and Sindhumole, 2016). Research on rice resistance to *Xanthomonas oryzae pv. oryzae* (*Xoo*) has revealed a complex genetic landscape. Scientists have identified more than 45 genes from Xa1 to Xa45 spread across ten of the twelve rice chromosomes that contribute to resistance against various *Xoo* strains (Rao *et al*., 2017; Kim and Reinke, 2019; Neelam *et al.* 2020; Biswas, 2021, Khare *et al*., 2021). Use of single major resistance gene may provide resistance to the *Xoo* but it can be easily overcome. Since several of these genes vary in the mechanism in which they impart resistance to the host plant. Therefore, introgression of a set of these genes into a single variety (i.e., pyramiding) would impart more effective and sustainable resistance that would remain effective against the pathogen for long duration. For the Raipur region the combination of three major resistance gene, xa5 +Xa13 +Xa21 have been identified as the effective resistance against the prevalent strain of *Xoo* in the region.

But over the time this pathogen has displayed its remarkable adaptability to emerge as a new variant often circumventing the existing host plant resistance genes for the disease. The breakdown of resistance and the degree of virulence of the *Xoo* strain can be identified by using a set of host differentials. A set of hosts genotypes carrying different resistance genes constitutes host differentials which is used to classify and identify *Xoo* isolates based on their ability to elicit resistant or susceptible reactions. Looking in to the dynamic nature of the pathogen, researchers must constantly test the virulency of the prevalent *Xoo* strain of the region for early detection of any incidence of unexpected resistance breakdown or increased virulence of the pathogen. Additionally, it also gives opportunity to the researcher to test and identify gene combinations effective against the pathogen to employ them for breeding a disease resistant variety for the region. In this context, a set of NILs having introgression of various combinations of BLB resistance genes in the background of IR24 were used to evaluate the virulence and identify the resistance gene combination against the *Xoo* isolate of Raipur during *Kharif* 2022, *Kharif* 2023 and *Kharif* 2024.

MATERIALS AND METHODS

A set of 28 Near Isogenic Lines (NILs) with different BLB resistant gene introgression in the background of IR24 were used along with local check Bamleshwari, resistant check Improved Sambha Mahsuri and susceptible check TN-1. The details of the genotypes along with the resistance gene combination present in them have been presented in the **table 1**. The genotypes were obtained from ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad. All the experimental materials were grown in the research cum instructional farm of the Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur by following the Randomised Complete Block Design (RCBD) in two replications for three consecutive seasons *i.e. Kharif* season 2022, *Kharif* season 2023 and *Kharif* season 2024. Standard agronomic practices were adopted during the crop grown period.

Collection of BLB infected leaf samples: Samples of BLB infected rice leaves were gathered from nearby areas of Raipur and transported to the laboratory of Department of Plant Pathology, IGKV, Raipur.

Isolation and purification of the Xoo *isolates:* Isolation of the *Xoo* isolates were done following standard protocols outlined by Kotasthane (2003). Small sections from the infected leaves were taken and incubated at 22°C for about 5-6 hours. The bacterial ooze from the sliced sections were spread over the wakimoto's medium slants. After 2 days of incubation in the medium, pinhead sized smooth, convex, yellow to pale yellow colonies of *Xoo* were transferred to fresh medium and incubated. Repeated isolation on the medium were used to purify the *Xoo* isolates. The pure culture was then maintained on PSA slants at 27±2°C for further use.

Inoculation and disease scoring: Artificial inoculation of the test genotypes with the *Xoo* isolates of Raipur were done following the clip inoculation method. Disease symptoms on the inoculated leaves were recorded 15 days post inoculation and the scoring was done following the Standard Evaluation Scale (IRRI, 2013). Disease scoring was done as per following score chart:

Score	1	3	5	7	9
Reaction	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible	Highly Susceptible

Statistical Analysis: Disease scoring data were subjected for statistical analysis specifically for summary statistics, analysis of variance, and cluster analysis (ward algorithm and Euclidean distance) by using OPSTAT software.

Results: A set of hosts carrying different resistance genes constitutes a differential host system. This system is used to classify and identify *Xoo* isolates based on their ability to elicit resistant or susceptible reactions in the

Table 1. Details of experimental materials used in the experiment

S. No.	Genotypes	Gene combination	Source
1	IRBB-1	Xa1	ICAR-Indian Institute of Rice Research
2	IRBB-3	Xa3	ICAR-Indian Institute of Rice Research
3	IRBB-4	Xa4	ICAR-Indian Institute of Rice Research
4	IRBB-5	xa5	ICAR-Indian Institute of Rice Research
5	IRBB-7	Xa7	ICAR-Indian Institute of Rice Research
6	IRBB-8	xa8	ICAR-Indian Institute of Rice Research
7	IRBB-10	Xa10	ICAR-Indian Institute of Rice Research
8	IRBB-11	Xa11	ICAR-Indian Institute of Rice Research
9	IRBB-13	xa13	ICAR-Indian Institute of Rice Research
10	IRBB-14	Xa14	ICAR-Indian Institute of Rice Research
11	IRBB-21	Xa21	ICAR-Indian Institute of Rice Research
12	IRBB-50	Xa4+xa5	ICAR-Indian Institute of Rice Research
13	IRBB-51	Xa4+xa13	ICAR-Indian Institute of Rice Research
14	IRBB-52	Xa4+Xa21	ICAR-Indian Institute of Rice Research
15	IRBB-53	xa5+xa13	ICAR-Indian Institute of Rice Research
16	IRBB-54	xa5+Xa21	ICAR-Indian Institute of Rice Research
17	IRBB-55	xa13+Xa21	ICAR-Indian Institute of Rice Research
18	IRBB-56	Xa4+xa5+xa13	ICAR-Indian Institute of Rice Research
19	IRBB-57	Xa4+xa5+Xa21	ICAR-Indian Institute of Rice Research
20	IRBB-58	Xa4+xa13+Xa21	ICAR-Indian Institute of Rice Research
21	IRBB-59	xa5+xa13+Xa21	ICAR-Indian Institute of Rice Research
22	IRBB-60	Xa4+xa5+xa13+Xa21	ICAR-Indian Institute of Rice Research
23	IRBB-61	Xa4+xa5+Xa7	ICAR-Indian Institute of Rice Research
24	IRBB-62	Xa4+Xa7+Xa21	ICAR-Indian Institute of Rice Research
25	IRBB-63	xa5+Xa7+xa3	ICAR-Indian Institute of Rice Research
26	IRBB-64	Xa4+xa5+Xa7+Xa21	ICAR-Indian Institute of Rice Research
27	IRBB-65	Xa4+Xa7+xa13+Xa21	ICAR-Indian Institute of Rice Research
28	IRBB-66	Xa4+xa5+Xa7+xa13+Xa21	ICAR-Indian Institute of Rice Research
29	Improved Sambha Mahsuri	xa5+xa13+Xa21	ICAR-Indian Institute of Rice Research
30	Bamleshwari	-	IGKV, Raipur
31	TN1	-	IGKV, Raipur

differential hosts. Xanthomonas oryzae pv. oryzae (Xoo) isolates are categorised according to their virulence using a differential host system, which consists of a collection of hosts with distinct resistance genes. The virulence of Xoo isolates collected from Raipur during *Kharif* 2022, 2023, and 2024 was evaluated in this study using 28 near-isogenic lines (NILs) with various resistance gene introgressions, the released variety Bamleshwari, the resistant check Improved Sambha Mahsuri (ISM), and the susceptible check TN 1. Following artificial inoculation, disease responses were graded and are shown in **Fig. 1**. Rice cultivation is seriously threatened by bacterial leaf blight (BLB), which is brought on by Xoo and can result in yield losses of up to 50%. The most efficient, cost-effective, and ecological method of managing BLB is still host plant resistance (Perumalsamy *et al.,* 2010). Selecting efficient resistance genes for breeding programs requires an understanding of the pathogenicity pattern of regional isolates.

ANOVA of pooled data of BLB scoring for three years: Replicated data of the BLB Reaction over the three years (*Kharif* season 2022, *Kharif* season 2023 and *Kharif* season 2024) were subjected to Pooled analysis of variance based on the Randomized Complete Block Design (RCBD) which has been presented in the **Table 2**. Based on the pooled analysis of variance over the three years of BLB reaction, significant differences were observed for the factor A (Year) for reaction to BLB. However, factor B (Genotypes) were not showing any significant differences among the genotype over the years. Similarly, interactions between year and genotype

Table 2. Pooled analysis of variance over the three years for BLB reaction

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	1	3,204.22			
Year (A)	2	366.66	183.328	10.616**	< 0.001
Genotypes (B)	30	89.89	2.996	0.174	1
Interaction A X B	60	1,011.01	16.85	0.976	0.535
Error	92	1,588.79	17.269		
Total	185	6,260.56			

** Significant at 1% level of significance

were also non-significant over the year which represents that the reaction of genotypes to the BLB isolate of Raipur were not stable and they were showing variable reaction to the BLB isolate.

Descriptive parameters for BLB reactions for three consecutive years: Mean data of the BLB reaction of the genotypes under test over the three years were subjected to descriptive analysis (**Table 3**). The mean BLB reaction scores was 6.52 during *Kharif* 2022 which decreased to 5.97 in *Kharif* 2023 and then increased to 6.65 during *Kharif* 2024 indicating towards the changing virulence of the pathogen. The standard deviation ranged from 1.68 in *Kharif* 2023 to 2.08 in *Kharif* 2022. The skewness values based on the BLB scores revealed a varied pattern of distribution across the years. Where, during *Kharif* 2022 negative skewness value (-0.89) was observed, indicating that there was higher frequency of resistance BLB scores, while in positive skewness (0.23) during *Kharif* 2023 suggests a towards higher frequencies of susceptible BLB scores. While *Kharif* 2024 showed a slight negative skewness value (-0.12), indicating a relatively uniform distribution of BLB scores. This trend observed in the skewness value over the years suggests that there is increase in the virulence of the BLB isolate of Raipur. Over the year pathogenicity of the BLB isolate of Raipur and susceptibility of the genotypes have been increased. Observed skewness value indicates that there could be new isolate available at Raipur centre which has potential to break the resistance reaction of multiple resistant genes which were earlier effective for managing the BLB disease in the region. This necessitates the search for new sources of the resistance for the disease.

Overall performance of the NILs against the Raipur isolate in BLB during *Kharif,* 2022; *Kharif,* 2023 and *Kharif* 2024: Among the 28 NILs evaluated during *Kharif* 2022 , the Raipur isolate of BLB was able to produce moderately resistant reaction on 3 NILs (IRBB-59, IRBB-60 and IRBB-66); moderately susceptible reaction on 11 NILs (IRBB-4, IRBB-51, IRBB-52, IRBB-53, IRBB-55, IRBB-58, IRBB-61, IRBB-63, IRBB-64, IRBB-65 and Bamleshwari), susceptible reaction on 8 NILs (IRBB-7, IRBB-11, IRBB-13, IRBB-14, IRBB-50, IRBB-54, IRBB-56 and IRBB-57); and highly susceptible reaction on 7 NILs (IRBB-7, IRBB-11, IRBB-13, IRBB-14, IRBB-50, IRBB-54, IRBB-56 and IRBB-57) (**Table 4 and Fig. 1**).

From the 28 NILs used for the disease evaluation using the new isolate of BLB at Raipur centre in *Kharif* 2023 (**Table 4 and Fig. 1**), 2 NILs produced moderately resistant reaction (IRBB-53 and IRBB-64); 15 NILs produced moderately susceptible reaction (IRBB-5, IRBB-50, IRBB-51, IRBB-52, IRBB-54, IRBB-55, IRBB-56, IRBB-57, IRBB-58, IRBB-60, IRBB-61, IRBB-62, IRBB-63, IRBB-65 and IRBB-66); 9 NILs produced susceptible disease reaction (IRBB-1, IRBB-4, IRBB-7, IRBB-10, IRBB-11, IRBB-13, IRBB-21, IRBB-59 and Bamleshwari); and 3 NILs produced highly susceptible disease reaction (IRBB-3, IRBB-8 and IRBB-14).

During *Kharif* 2024, the BLB scoring on the 28 NILs at Raipur centre shows that, 12 NILs exhibited moderately resistant reaction (IRBB-5, IRBB-21, IRBB-51, IRBB-52, IRBB-54, IRBB-56, IRBB-57, IRBB-58, IRBB-60, IRBB-64, IRBB-65 and IRBB-66); 6 Nils produced susceptible reaction (IRBB-7, IRBB-8, IRBB-50, IRBB-53 and IRBB-

Table 3. Descriptive parameters for BLB reactions for three consecutive years.

Parameters	*Kharif* season 2022	*Kharif* season 2023	*Kharif* season 2024
Mean	6.52	5.97	6.65
Standard Error	0.37	0.30	0.35
Standard Deviation	2.08	1.68	1.92
Kurtosis	0.25	-0.32	-1.62
Skewness	-0.89	0.23	-0.12
Minimum	2.00	3.00	4.00
Maximum	9.00	9.00	9.00
CV (%)	31.91	28.20	28.96

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Table 4. Disease scoring obtained during evaluation in the tested lines with Raipur *Xoo* isolate during *Kharif* 2022, *Kharif* 2023, *Kharif* 2024 based on SES (IRRI, 2014).

S. No.	Genotypes	2022 (BLB Score)	Reaction	2023 (BLB Score)	Reaction	2024 (BLB Score)	Reaction
1	IRBB-1	9	HS	7	S	9	HS
2	IRBB-3	9	HS	9	HS	9	HS
3	IRBB-4	5	MR	7	S	9	HS
4	IRBB-5	9	HS	5	MR	5	MR
5	IRBB-7	7	S	7	S	7	S
6	IRBB-8	9	HS	9	HS	7	S
7	IRBB-10	9	HS	7	S	9	HS
8	IRBB-11	7	S	7	S	9	HS
9	IRBB-13	7	S	7	S	9	HS
10	IRBB-14	7	S	9	HS	9	HS
11	IRBB-21	9	HS	7	S	5	MR
12	IRBB-50	7	S	5	MR	7	S
13	IRBB-51	5	MR	5	MR	5	MR
14	IRBB-52	5	MR	5	MR	5	MR
15	IRBB-53	5	MR	3	R	7	S
16	IRBB-54	7	S	5	MR	5	MR
17	IRBB-55	5	MR	5	MR	9	HS
18	IRBB-56	7	S	5	MR	5	MR
19	IRBB-57	7	S	5	MR	5	MR
20	IRBB-58	5	MR	5	MR	5	MR
21	IRBB-59	3	R	7	S	7	S
22	IRBB-60	3	R	5	MR	5	MR
23	IRBB-61	5	MR	5	MR	9	HS
24	IRBB-62	9	HS	5	MR	9	HS
25	IRBB-63	5	MR	5	MR	9	HS
26	IRBB-64	5	MR	3	R	5	MR
27	IRBB-65	5	MR	5	MR	5	MR
28	IRBB-66	3	R	5	MR	5	MR
29	Improved Sambha Mahsuri	3	R	7	S	9	HS
30	Bamleshwari	5	MR	7	S	7	S
31	TN1	9	HS	9	HS	9	HS

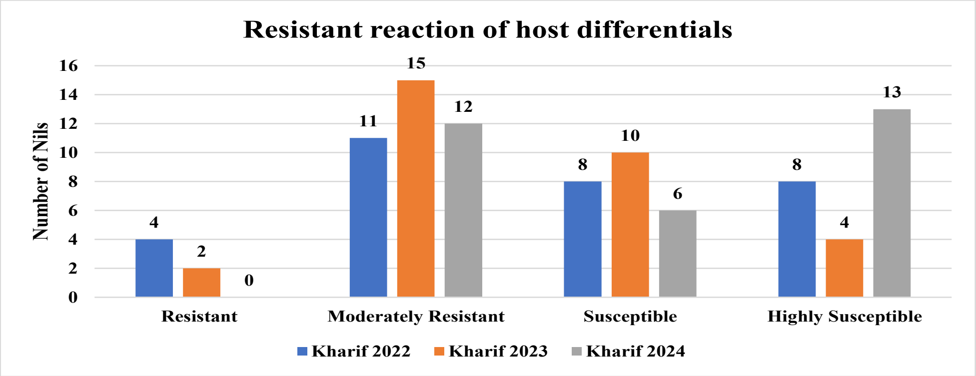

Fig. 1. Number of test entries showing different reactions to the inoculation of *Xoo* isolate during *Kharif* 2022, *Kharif* 2023 and *Kharif* 2024

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59); while 13 NILs showed highly susceptible disease reaction (IRBB-1, IRBB-3, IRBB-4, IRBB-10, IRBB-11, IRBB-13, IRBB-14, IRBB-55, IRBB-61, IRBB-62 and IRBB-63). None of the tested NILs showed resistant and moderately resistance disease reaction indicating towards increased virulence of the pathogen over the three year. A comparison of the performance of various NILs tested over the three years indicates stable performance and durable resistance of NILs IRBB-51, IRBB-52, IRBB-54, IRBB-56, IRBB-57, IRBB-58, IRBB-60, IRBB-64, IRBB-65 and IRBB-66.

Performance of NILs with single resistance gene during Kharif 2022, Kharif 2023 and Kharif 2024: The *Xanthomonas oryzae pv. oryzae* (*Xoo*) isolates from Raipur were evaluated against 11 rice differential hosts containing a single *Xoo* resistance gene during the *Kharif* 2022, *Kharif* 2023 and *Kharif* 2024. The results showed that the NILs exhibited reactions ranged from moderately resistant to highly susceptible and none of the BLB resistant gene tested was found resistant to the *Xoo* isolate of Raipur. Comparing resistance reaction results of the different genotypes across years, the genotype IRBB-3 (Xa3) consistently showed highly susceptible reaction throughout all three years, while the genotype IRBB-7 (Xa7) exhibited susceptible reaction consistently across the three years. The genotype IRBB-4 (Xa4) showed a progressive decline in resistance, from moderately resistant during *Kharif* 2022 to susceptible during *Kharif* 2023 and finally to highly susceptible in *Kharif* 2024. The genotypes IRBB-1 (Xa1) and IRBB-10 (Xa10) showed fluctuating reactions, changing from highly susceptible in *Kharif* 2022 to susceptible in *Kharif* 2023, and then reverting to highly susceptible in *Kharif* 2024. The genotype IRBB-8 (xa8) exhibited highly susceptible reaction in the first two years which improved slightly to susceptible in *Kharif* 2024. The genotype IRBB-11 (Xa11) and IRBB-13 (xa13) maintained susceptible reaction for the first two years but deteriorated to highly susceptible by *Kharif* 2024. The genotype IRBB-14 (Xa14) showed a decline from susceptible in *Kharif* 2022 to highly susceptible in both *Kharif* 2023 and 2024. The most notable changes were observed in genotypes IRBB-5 (xa5) and IRBB-21 (Xa21). IRBB-5 with single BLB resistance gene xa5 improved from highly susceptible in *Kharif* 2022 to moderately resistant in both *Kharif* 2023 and 2024, demonstrating consistent effectiveness against the Raipur isolate in later years. IRBB-21 (Xa21) showed a variable response, changing from highly susceptible in *Kharif* 2022 to susceptible in *Kharif* 2023, and finally improving to moderately resistant in *Kharif* 2024.

*Performance of NILs with two BLB resistance gene combination during Kharif 2022, Kharif 2023 and Kharif 2024:*The NILs with two BLB resistance gene combination in general performed better than the single resistance gene NILs. The genotypes IRBB-51 (Xa4+xa13) and IRBB-52 (Xa4+Xa21) consistently showed moderately resistant reaction across all three years, demonstrating stable resistance against the Raipur isolate. The resistance response of the genotype IRBB-53 having resistance gene xa5+xa13 improved from moderately resistant in *Kharif* 2022 to resistant in *Kharif* 2023, however, it deteriorated to susceptible in *Kharif* 2024. The genotype IRBB-54 (xa5+Xa21) showed improved resistance from susceptible in *Kharif* 2022 to moderately resistant in both *Kharif* 2023 and 2024. Similarly, the resistance reaction of the genotype IRBB-50 (Xa4+xa5) improved from susceptible in *Kharif* 2022 to moderately resistant in *Kharif* 2023, but reverted to susceptible in *Kharif* 2024. The genotype IRBB-55 with gene combinations xa13+Xa21 maintained moderately resistant reaction for the first two years, but showed a significant decline to highly susceptible reaction in *Kharif* 2024, indicating that this gene combination has been overcome by the evolving pathogen population.

Performance of NILs with three BLB resistance gene combination during Kharif 2022, Kharif 2023 and Kharif 2024: Among the NILs having combination of three BLB resistance genes, the genotype IRBB-58 (Xa4+xa13+Xa21) consistently showed moderately resistant reaction across all three years. The genotypes IRBB-56 with resistance gene combination Xa4+xa5+xa13 and IRBB-57 with resistance gene combination Xa4+xa5+Xa21 exhibited improvement from susceptible reaction during *Kharif* 2022 to moderately resistant in *Kharif* 2023, and maintained moderately resistant reaction in *Kharif* 2024. The genotype IRBB-61 with gene combinations Xa4+xa5+Xa7 and IRBB-63 with gene combination xa5+Xa7+xa13 maintained moderately resistant reaction during *Kharif* 2022 and *Kharif* 2023, but both deteriorated to highly susceptible in *Kharif* 2024. Similarly, the genotype IRBB-62 (Xa4+Xa7+Xa21) showed significant improvement from highly susceptible reaction in *Kharif* 2022 to moderately resistant reaction in *Kharif* 2023, but reverted to highly susceptible in *Kharif* 2024. Notably, the genotype IRBB-59 (xa5+xa13+Xa21) showed a progressive increase in susceptibility to the *Xoo* isolate of Raipur, changing from resistant reaction in *Kharif* 2022 to susceptible reaction in *Kharif* 2023, and maintaining susceptible reaction in *Kharif* 2024. The Improved Samba Mahsuri with gene combination xa5+xa13+Xa21 also displayed a similar pattern, showing resistant reaction in *Kharif* 2022, deteriorating to susceptible in *Kharif* 2023, and further declining to highly susceptible in *Kharif* 2024.

Performance of NILs with four BLB resistance gene combination during Kharif 2022, Kharif 2023 and Kharif 2024: Total 3 NILs with four resistance gene combination were evaluated against the *Xoo* isolate of Raipur. The resistance reaction ranged from resistance to moderately resistance. In general, the four gene combination performed better than other gene combinations. The genotype IRBB-65 (Xa4+Xa7+xa13+Xa21) showed a consistent moderately resistant reaction across all three years of evaluation. The genotype IRBB-60

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(Xa4+xa5+xa13+Xa21) showed resistant reaction in *Kharif* 2022 but maintained a moderately resistant reaction in both *Kharif* 2023 and *Kharif* 2024. Whereas, the reaction of genotype IRBB-64 with gene combination Xa4+xa5+Xa7+Xa21 exhibited increased resistance to the strain from moderately resistant reaction in *Kharif* 2022 to a resistant reaction during *Kharif* 2023, but reverted to moderately resistant in *Kharif* 2024. Despite minor fluctuations in the level of resistance, all four-gene combination NILs maintained at least moderately resistant reaction against the *Xoo* isolate throughout the three-year evaluation period, indicating their stable performance against the evolving pathogen population in Raipur.

Performance of NILs with five BLB resistance gene combination during Kharif 2022, Kharif 2023 and Kharif 2024: The only genotype with combination of five BLB resistant gene i.e., IRBB-66 (Xa4+xa5+Xa7+xa13+Xa21) showed resistant reaction in *Kharif* 2022 but maintained a moderately resistant reaction in both *Kharif* 2023 and *Kharif* 2024. Despite the slight decline in resistance level after the first year, this five-gene pyramid continued to provide effective protection against the *Xoo* isolate of Raipur across all three years of evaluation.

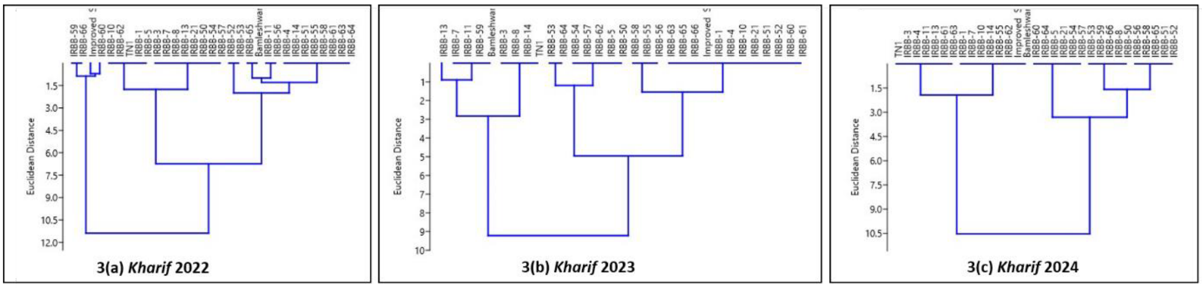
The susceptible check TN-1 showed consistently a highly susceptible reaction during all three years. Whereas the resistant check Improved Sambha Mahsuri with three gene combination (xa5+xa13+Xa21) exhibited a resistant reaction in *Kharif* 2022 but during *Kharif* 2023 it displayed a susceptible reaction, and further deteriorated to highly susceptible in *Kharif* 2024, indicating the increasing virulence of the pathogen population against this previously effective gene combination.

Cluster analysis of the data of BLB Scoring for three years: Based on the BLB reaction of genotypes for all three years, cluster analysis was performed by following the ward algorithm and Euclidean distance (**Fig 2**). The cluster diagram for the year *Kharif* 2022 (Fig 2(a)) reveal that ISM, IRBB-60, IRBB-66 and IRBB-59 were distantly related with the rest 27 genotypes. Similarly, in *Kharif* season 2023 (**Fig 2(b)**), TN-1, IRBB-14, IRBB-8, IRBB-3, Bamleshwari, IRBB-59, IRBB-11, IRBB-7 and IRBB-13 are distantly related with rest of the 22 genotypes. Furthermore, during *Kharif* season 2024 (**Fig 2(c)**), two distant clusters were formed, making TN-1, IRBB-3, IRBB-4, IRBB-11, IRBB-13, IRBB-61, IRBB-63, IRBB-1, IRBB-7, IRBB-10, IRBB-14, IRBB-55, IRBB-62, ISM and Bamleshwari from rest of the genotypes.

Most of the single gene have shown poor performance for the Raipur *Xoo* isolate in all the years. However, the recessive BLB resistance gene xa5 (Present in IRBB-5) exhibited increased resistance from Highly susceptible reaction in *Kharif* 2022 to moderately resistance reaction during *Kharif* 2023 and *Kharif* 2024, while the dominant gene Xa21 (Present in IRBB-21) have shown some potential as evident from the improved resistance over the years from Highly susceptible reaction in *Kharif* 2022 to susceptible reaction during *Kharif* 2023 and moderately resistance reaction during *Kharif* 2024. These two resistance genes show potential for their use in future breeding programs for developing resistance genotypes for the Region. Raipur *Xoo* isolate has been regarded as highly virulent strain as it produced more than 7 LSI (Location Severity Index) on single gene introgression differentials (ICAR-IIRR, 2024). The ineffectiveness of tested NILs carrying single resistance genes against the evolving BLB isolate of Raipur, highlights the limitations of mono-genic resistance in managing the disease.

Interestingly these resistance genes when present in combination with other resistance genes (i.e., pyramiding) has showed increased proportions of moderately resistant to resistant reactions. As the effectiveness of single genes reduced from 2022, 2023 and 2024 there has been improved effectiveness of the resistance for multiple resistance gene lines.

In the study NILs with two gene combinations have shown slightly better performance than the NILs with single genes, but still have not been able to maintain a consistent resistance reaction against the pathogen. However, specific combinations like Xa4 + xa13 (IRBB-51) and Xa4 + Xa21 (IRBB-52) showed stable moderate resistance across the years, indicating towards presence of a good gene synergies among them. NILs carrying combinations



3(a) *Kharif* 2022

3(b) *Kharif* 2023

3(c) *Kharif* 2024

Fig. 2: 2(a): Cluster diagram *Kharif,* 2022, 2(b): Cluster diagram *Kharif*, 2023 2(c): Cluster diagram *Kharif,* 2024

of three or more resistance genes have demonstrated a consistent (durable) broad resistance towards the pathogen over the tested years. NILs like IRBB-58, IRBB-60, IRBB-64, IRBB-65 and IRBB-66 have shown consistently moderately resistant reaction, while the NILs IRBB-56 and IRBB-57 have shown improvement in terms of resistance over the years. Its, noteworthy to say that IRBB-58 with three gene combination (Xa4+xa13+Xa21) performed better for most of the strains of *Xoo* in the AICRP BLB resistance trials of *Kharif* 2023 (ICAR-IIRR, 2024). The gene combinations present in these NILs have proven their potential for their employment in gene pyramiding program for breeding high yielding disease resistant genotype for the region. The findings of the experiment suggests that multiple gene resistance is more effective and stable against bacterial leaf blight when compared to single gene resistance. Therefore, pyramiding multiple resistance genes in high yielding varieties is a good strategy to impart a durable resistance against which can be difficult to break for a rapidly evolving bacterial leaf blight pathogen (Singh ***et al***., 2003; Sundaram ***et al***., 2008; Raghunandana ***et al***., 2023).

One of the important finding of this study is the unexpected susceptibility of the resistance check Improved Sambha Mahsuri (ISM) to the existing *Xoo* isolate of Raipur. ISM having the resistance gene combination xa5+xa13+Xa21, showed a resistant reaction in 2022, but became susceptible in 2023 and 2024. The NIL IRBB-59 having same gene combination as ISM, also shows the similar trend over the years. It is notable that this gene combination was earlier identified as highly effective resistance gene combination for the Raipur *Xoo* isolate. The current data suggests that the pathogen has potentially developed a virulence towards this gene combination. This highlights the dynamic nature of the pathogen their ability to evolve and overcome the existing resistance genes. Multiple studies by researchers across the globe have documented *Xoo* strains ability to evolve and develop virulence against the known resistance genes (Adhikari ***et al***., 1999; Mishra ***et al***., 2013; Triplett ***et al***., 2014; Suryadi ***et al***., 2016; Tekete ***et al***., 2020). Therefore, it further warrants for a perpetual search of new sources and combination of resistance genes to impart an effective control of the disease. Further sequencing based characterization of the *Xoo* isolates of Raipur centre will be done.

The present study highlights a critical challenge imposed by the increased virulence of *Xanthomonas oryzae pv. oryzae* (Xoo) isolate of Raipur region to the known BLB resistance gene. It was observed that single resistance genes offered limited and unstable protection. However, pyramiding multiple resistance genes proved far more effective in managing the severity of the disease. Combinations like Xa4 + xa13 and Xa4 + Xa21 showed stable moderate resistance, while combination of three or more genes consistently provided broad, durable resistance (e.g., IRBB-58, IRBB-60). The superior performance of IRBB-58 highlights the importance of gene pyramiding in managing the disease. The pathogen's ability to overcome even established resistance combinations (like those in Improved Sambha Mahsuri) underscores its dynamic nature. Therefore, continuous exploration for new resistance sources and gene combinations is vital. Future efforts will include sequencing Raipur Xoo isolates to better understand their virulence, ultimately guiding the development of robust, disease-resistant rice varieties.

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