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Screening of Rice Genotypes for Bacterial Blight of Rice Under Artificial Inoculation Method and Monitoring Field Virulence of *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

The present study was conducted at the main rice research centre, Navsari Agricultural University, Navsari, Gujarat, India during *kharif* (July–November, 2020) to study the screening of rice genotypes for bacterial blight of rice under artificial inoculation method and monitoring field virulence of *Xanthomonas oryzae* pv. *oryzae*. Thirty genotypes along with two susceptible checks against bacterial blight pathogen under artificial inoculation condition revealed that, 3 genotypes were found moderately resistant *viz*., NVSR-466, NVSR-396 and ISM. Whereas, 12 genotypes *viz*., NVSR-411, NVSR-443, NVSR-447, NVSR-453, GNR-6, IR-28, NAUR-1, GNR-2, Gurjari, GNR-4, Krishna kamod and GAR-13 were found susceptible to *Xoo* and two checks *viz*., GR-11 and TN-1 were found highly susceptible. Observing to the disease severity, by screening 29 IRBB differentials and two checks employed for research on monitoring virulence of *Xoo*. Out of 29 differentials, 3 entries were found resistant *viz*., IRBB-53, IRBB-57 and IRBB-60. Whereas, 3 entries *viz*., IRBB-50, IRBB-52 and IRBB-61 were moderately susceptible reaction and all the single genes possessing entries were found susceptible to Navsari isolates of *Xoo*. The goal of this study provided valuable insights to determine the potential management strategies for the disease in rice crops by employing methodology of two rows of 2 meters in length for each entry, the susceptible check TN-1 and GR-11 were planted after the 10th test entry for screening of rice genotypes and monitoring the field virulence of *Xoo*.

KEYWORDS: Artificial inoculation, bacterial blight, *Oryza sativa*, screening, virulence, *Xoo*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most common staple food for more than two-thirds of the Indian population, accounting for 40% of total food grain production and so playing a critical role in the people's food and livelihood security. Despite pandemic circumstances for one year, we may be able to produce a record 118.87 mt of rice in 2019–20, with about 120 mt predicted in the subsequent years. *Xanthomonas oryzae* pv. *oryzae*, commonly referred to as *Xoo*, causes rice bacterial blight (BB) (From www.icar-iirr.org.). The continuous disease monitoring, identification of *Xoo* pathotypes and their distribution are crucial to managing BB (Yugander et al., 2022). Making use of *Xoo* isolates from four Javan areas Fatimah et al. (2014). The *Xa21* strain has been shown to be efficient and persistent against several *Xoo* isolates from Asia and Africa (Banito et al., 2012). Pyramid lines with a single gene each of *Xa5*, *Xa7* and *Xa21*, or a combination of these genes, such as IRBB64 and IRBB66 would be the most beneficial and effective Bacterial Blight resistance providers for Indonesian cultivars (Khush, 2005). The complicated R-genes including *Xa21* and *Xa4, Xa5, Xa13* and *Xa7* were substantially more resistant to BB in the field (Dinh et al., 2010). In Gujarat, major rice growing area is confined in the districts of Navsari, Valsad, Surat, Dangs, Panchmahal, Vadodara, Kheda and Ahmedabad (Verma and Shukla, 2011). Reddy et al. (1979) illustrates the difficulties of quantifying the effect of BB symptoms on yield. Along the Niger River, where farmers historically planted low-yielding native varieties, rice cultivation has a long history in Niger. Only recently introduced semi dwarf cultivars descended from the prone Taichung Native 1 showed symptoms (TN-1) (Jones et al., 1989). Srinivasan et al. (1959) from Maharashtra state in India were the first to describe the BB brought on by *Xoo*. It is a typical vascular disease with a systemic origin that infects seedlings after transplantation and subsequently at the booting or heading stage. At the seedling stage, the "Kresek" phase is the most damaging (Chahal, 2005). The biology of cereals is modelled after rice, a food that is consumed everywhere (Bennetzen and Ma, 2003, Ronald and Leung, 2002, Shimamoto and Kyozuka, 2002). Among the variety of bacterial diseases found in rice plants, bacterial leaf blight disease caused by *Xoo* is one of the most severe and most highly widespread, especially in the irrigated and rainfed lowland ecosystems of Asian tropical countries, as well as in Australia, the United States, and several other rice-growing countries (Mew, 1987).

According to the pyramiding of two or more resistant genes should result in more robust resistance in rice Huang et al. (1997). Pyramiding lines with two, three, or four resistance genes shown greater resistance across a wider range. The majority of single genes were moderately to highly sensitive to *Xoo* at the majority of the locations (Anonymous, 2006). The monitoring of field virulence's in *Xoo* studied at 22 locations. They reported that among the single genes, *Xa13* and *Xa21* were found resistant to moderately resistant in most of the locations. Another gene *Xa8* was also found resistant in some of the locations (Anonymous, 2007). Rice farmers all around the world suffer enormous output losses as a result of it. Every year, the endemic rice bacterial blight that is prevalent in Gujarat's southern region manifests itself. Various experts from N.A.U., Navsari have documented an annual bacterial blight outbreak in South Gujarat in their survey reports. (Anonymous, 2018).

2. MATERIALS AND METHODS

The screening trial was conducted during *kharif* (July–November, 2020) at Main Rice Research Centre, NAU, Navsari, Gujarat, India. Bacterial blight of rice (*Xoo*) is one of the most aggressive plant diseases of rice, causing yield and grain quality reductions as well as considerable economic losses. Every growing season, the pathogen destroys millions of hectares of rice, especially in the environment. As a result, Use of high yielding and identification of resistant/tolerant genotypes/entries is the most viable, environmentally safe and economically sound technique for the management of the disease. Hence, the present investigation was undertaken to find out resistant sources against bacterial blight of rice under artificial condition. Thirty genotypes+two checks were screened under field condition.

Two rows of 2meter length for each entry, the susceptible check TN-1 and GR-11 were planted after 10th test entry. The local susceptible check GR-11 was planted around the screening nursery. The artificial inoculation of *Xoo* was done by standard clip inoculation technique after 30 days of transplanting.

The formula for calculating per cent disease intensity

PDI=(Sum of score/No.of observation×Highest number of rating scale)×100

Ten hills from each genotype were randomly selected and considering for grading the severity of disease on standing plants. The labelled plants were observed for disease rating by using 0–9 scale, by following the standard evaluation system for rice (Anonymous, 2013).

The experiment was conducted at Main Rice Research Centre, Navsari Agricultural University, Navsari, Gujarat, India during *kharif* (July–November, 2020) with 31 genotypes of seeds received from IIRR, Hyderabad. Rice is impacted by among the most dangerous plant diseases, bacterial blight (*Xoo*), reduces output and grain quality as well as costs growers a significant amount of money. Millions of hectares of rice are destroyed each growing season by the pathogen, particularly in the environment. As a consequence, 29 IRBB differentials were screened and two checks were employed for *Xoo* virulence monitoring study.

After the tenth test entry, two rows of each entry measuring 2 metres in length were planted with the susceptible checks TN-1 and GR-11. The screening nursery was surrounded by the regional susceptible check GR-11. Following a transplant that lasted 30 days, various isolates of *Xoo* were artificially inoculated using the conventional clip inoculation approach.

Ten hills from there were a random selection of each genotype and considering for grading the severity of disease on standing plants. Following the normal assessment technique for rice, the tagged disease-related plant inspections using a 0–9 scale (Anonymous, 2013).

3. RESULTS AND DISCUSSION

Thirty genotypes along with two susceptible check GR-11 and TN-1 were screened against *Xoo* under field condition by artificial inoculation method. The results presented in Table 1 and figure 1, 2, 3 revealed that among all the tested genotypes none was found immune and resistant against the disease. Out of them, 3 genotypes were found moderately resistant *viz.*, NVSR-466, NVSR-396 and ISM. Whereas, 15 genotypes *viz.*, NVSR-467, NVSR-494, NVSR-418, NVSR-496, GR-5, GR-8, NVSR-392, NVSR-395, GNR-3, GNR-5, GNR-7, GR-15,GR-17, Masuri and GR-101, were found moderately susceptible.12 genotypes *viz.*, NVSR-411, NVSR-443, NVSR-447, NVSR-453, GNR-6, IR-28, NAUR-1, GNR-2, Gurjari, GNR-4, Krishna kamod and GAR-13 were found susceptible to *Xoo,* as well as 2 genotypes were found highly susceptible *viz.*, susceptible check GR-11 and TN-1. Among the thirty-two genotypes screened, highest per cent disease severity (81.0%) was recorded in international susceptible check TN-1, Which was followed by; genotype

Table 1: Screening of rice genotypes for bacterial blight of rice under artificial inoculation method

Sl. No.	Cultivar/genotypes	Disease severity (%)	Disease index (0-9 scale)	Disease reaction
1.	NVSR-411	29.0	7	S
2.	NVSR-443	27.4	7	S
3.	NVSR-447	35.7	7	S
4.	NVSR-467	15.7	5	MS
5.	NVSR-494	13.4	5	MS
6.	NVSR-418	16.8	5	MS
7.	NVSR-466	8.2	3	MR
8.	NVSR-496	15.2	5	MS
9.	NVSR-453	43.4	7	S
10.	GNR-6	40.2	7	S
11.	IR-28	35.7	7	S
12.	GR-5	19.4	5	MS
13.	GR-8	20.1	5	MS
14.	NVSR-392	23.4	5	MS
15.	NVSR-395	17.5	5	MS
16.	NVSR-396	7.2	3	MR
17.	NAUR-1	34.5	7	S
18.	GNR-2	40.1	7	S
19.	GNR-3	22.3	5	MS
20.	GNR-5	13.4	5	MS
21.	GNR-7	21.2	5	MS
22.	GR-15	13.4	5	MS
23.	GR-17	23.4	5	MS
24.	Masuri	15.2	5	MS
25.	Gurjari	48.6	7	S
26.	GNR-4	27.7	7	S
27.	GR-101	14.5	5	MS
28.	Krishna kamod	42.1	7	S
29.	GAR-13	27.0	7	S
30.	ISM	8.6	3	MR
31.	Check State- GR-11	72.2	9	HS
32.	Check international-TN-1	81.0	9	HS

Figure 1: Nursery rising of different genotypes of rice under varietal screening

Figure 2: Screening of rice genotypes under artificial inoculation condition

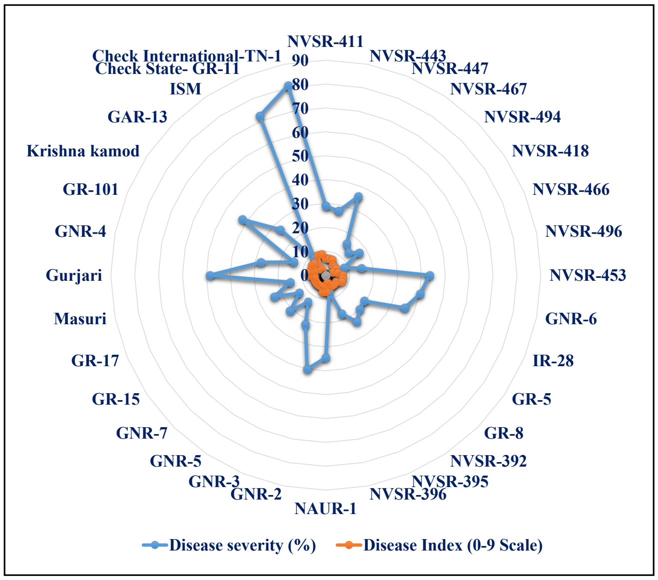

Figure 3: Screening of rice genotypes for bacterial blight of rice under artificial inoculation method

susceptible check GR-11 (72.2%) and Gurjari (48.6%). Whereas, lowest disease severity (7.2%) was recorded in genotype NVSR-396.

From the study, it was revealed that out of 30 genotypes tested along with 2 susceptible check, 3 genotypes were found moderately resistant. Whereas, 15 genotypes were showed moderately susceptible and 12 genotypes were found susceptible, as well as 2 genotypes were found highly susceptible to *Xoo* under artificial inoculation method.

Screening of rice genotypes against *Xoo* was carried out on different varieties by earlier workers. Mahajan et al. (2020) screened sixteen germplasm among them, not a single germplasm was found immune or resistant to the disease, four germplasm were showed moderately resistant. Senthilvel et al. (2022) screened improved recombinant lines by using functional markers and resistance reaction was confirmed through phenotypic and genotypically screening confirmed twenty-seven RILs, (AD(Bio)13056, AD(Bio)13060, ACM18089, ACM18091, ACM18097, ACM18068 and ACM20015 were found to be promising for agronomic traits.

The *Xoo* monitoring nursery consisted of 29 almost identical lines (IRBB lines) carrying different bacterial blight resistance genes. Out of the 31 entries tested alongside the susceptible checks, the results shown in Table 2 and figure 4 and 5 showed that 3 entries were observed on scale-1 as well as evaluated as resistant, 12 entries with disease scale-3 were assessed as moderately resistant, while 3 entries displayed a moderately susceptible reaction and the remaining entries were susceptible to extremely prone to BB. This shows that the *Xoo* of South Gujarat has different levels of virulence.

Table 2: List of bacterial blight differentials used for study on virulence of isolates

Sl. No.	Designation	Gene combinations	Disease scale (BB)	Disease reaction
1.	IRBB-1	*Xa1*	7	S
2.	IRBB-3	*Xa3*	7	S
3.	IRBB-4	*Xa4*	7	S
4.	IRBB-5	*Xa5*	7	S
5.	IRBB-7	*Xa7*	7	S
6.	IRBB-8	*Xa8*	7	S
7.	IRBB-10	*Xa10*	7	S
8.	IRBB-11	*Xa11*	7	S
9.	IRBB-13	*Xa13*	7	S
10.	IRBB-14	*Xa14*	7	S
11.	IRBB-21	*Xa21*	7	S
12.	IRBB-50	*Xa4+Xa5*	5	MS
13.	IRBB-51	*Xa4+Xa13*	3	MR
14.	IRBB-52	*Xa4+Xa21*	5	MS
15.	IRBB-53	*Xa5+Xa13*	1	R
16.	IRBB-54	*Xa5+Xa21*	3	MR
17.	IRBB-55	*Xa13+Xa21*	3	MR
18.	IRBB-56	*Xa4+Xa5+Xa13*	3	MR
19.	IRBB-57	*Xa4+Xa5+Xa21*	1	R
20.	IRBB-58	*Xa4+Xa13+Xa21*	3	MR
21.	IRBB-59	*Xa5+Xa13+Xa21*	3	MR
22.	IRBB-60	*Xa4+Xa5+Xa13+Xa21*	1	R
23.	IRBB-61	*Xa4+Xa5+Xa7*	5	MS
24.	IRBB-62	*Xa4+Xa7+Xa21*	3	MR
25.	IRBB-63	*Xa5+Xa7+Xa13*	3	MR
26.	IRBB-64	*Xa4+Xa5+Xa7+Xa21*	3	MR
27.	IRBB-65	*Xa4+Xa7+Xa13+Xa21*	3	MR
28.	IRBB-66	*Xa4+Xa5+Xa7+Xa13+ Xa21*	3	MR
29.	ISM	*Xa5+Xa13+Xa21*	3	MR
30.	TN-1 (SC)		9	HS
31.	GR-11 (SC)		9	HS

From the results, it is cleared that entries IRBB-53 (*Xa5+Xa13*), IRBB-57 (*Xa4+Xa5+Xa21*) and IRBB-60 (*Xa4+Xa5+Xa13+Xa21*) were showed resistant

Tillering stage view of monitoring field virulence of *Xoo*

Maturity stage view of monitoring field virulence of *Xoo*

Figure 4: Monitoring field virulence of *Xoo*

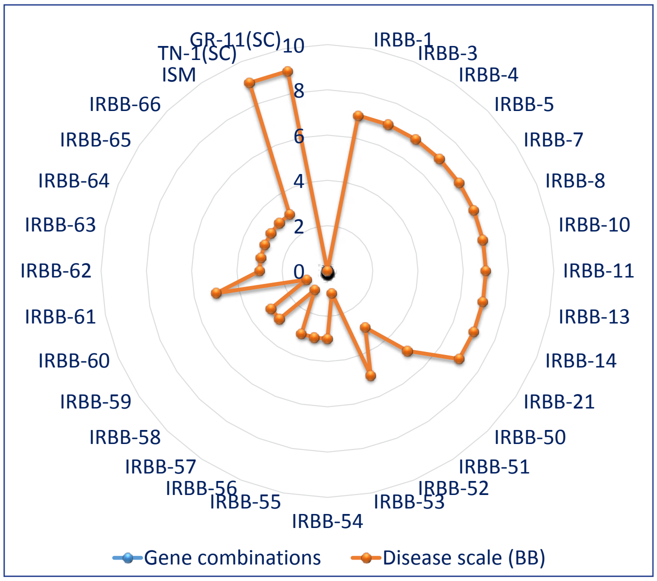

Figure 5: List of bacterial blight differentials used for study on virulence of isolates

reaction to prevailing *Xoo* strains. Among them, IRBB-51 (*Xa4+Xa13*), IRBB-54 (*Xa5+Xa21*), IRBB-55 (*Xa13+Xa21*), IRBB-56 (*Xa4+Xa5+Xa13*), IRBB-58 (*Xa4+Xa13+Xa21*), IRBB-59 (*Xa5+Xa13+Xa21*), IRBB-62 (*Xa4+Xa7+Xa21*), IRBB-63 (*Xa5+Xa7+Xa13*), IRBB-64 (*Xa4+Xa5+Xa7+Xa21*), IRBB-65 (*Xa4+Xa7+Xa13+Xa21*), IRBB-66 (*Xa4+Xa5+Xa7+Xa13+Xa21*) and ISM (*Xa5+Xa13+Xa21*) were found moderately resistant against *Xoo.* while, IRBB-50 (*Xa4+Xa5*), IRBB-52 (*Xa4+Xa21*) and IRBB-61 (*Xa4+Xa5+Xa7*) were found moderately susceptible. Whereas, all the single genes possessing entries viz., *Xa1, Xa3, Xa4, Xa5, Xa7, Xa8, Xa10, Xa11, Xa13, Xa14* and *Xa21* were found susceptible to Navsari isolates of *Xoo*. TN-1 and GR-11 checks showed the highly susceptible reaction.

The results obtained in this experiment are close with the work of others findings and BB resistance was noted in the RP Bio-226 type. Disease scale 3 is seen in IRBBs 21, 50, 55, 56 and 60 (Anonymous, 2008). Arshad (2016) assessed the resistance ability of 26 IRBB lines against 29 prevalent *Xoo* pathotypes, 54 mutated lines in M6-M7 generation and 72 in M3-M4 generations were all discovered by analyzing the pathogenicity profile with 300, *Xoo* isolates on 6 rice differentials against *Xoo*. All gene pyramids but one did not offer total defense. Xa21 alone expressed resistance up to 93% against *Xoo* pathotypes. The rest of the Xa genes in pyramid was moderately susceptible to susceptible. Yugander et al. (2017) assessed virulence and found that with the exception of IRBB-13, which exhibited sensitivity to as many as 35% of the isolates, 22 close NILs of IR 24 with different *Xoo* resistance genes as well as various mixtures sensitivity was discovered in between 59 and 94% of the *Xoo* isolates. IXoPt-1 and IXoPt-2 were the least virulent of the 22 pathotypes, but IXoPt-18 and 22 were quite virulent. All individual BB resistance genes, with the exception of Xa13, were vulnerable to pathotype *IXoPt-19*'s virulence.

4. CONCLUSION

Screening of 30 genotypes along with 2 susceptible checks against *Xoo* under artificial inoculation condition revealed that, 3 genotypes were found moderately resistant.12 genotypes were found susceptible to *Xoo* and 2 checks were found highly susceptible. 29 IRBB differentials and two checks used for virulence of *Xoo*. Three entries, were resistant out of the 29 differentials. The Navsari isolates of *Xoo* were susceptible to all of the single gene entries, with exception of 3 entries, which showed moderately susceptible reaction.

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