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Identification of novel sources of bacterial leaf blight resistance in wild species of rice

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Research Article

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Abstract

Wild species of rice possess tremendous genetic variations and harbour resistance genes for biotic stresses. Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a major disease affecting rice production globally. The current study characterized 116 accessions from 17 species of *Oryza* for BB disease during three seasons *viz*., *kharif* 2020, *rabi* 2020–21, *kharif* 2021 using an isolate of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain IX-020. A total of 40 accessions including *Oryza rufipogon*, *O. nivara*, *O. officinalis* and *O. australiensis* showed consistence resistance to the bacterial blight disease across the seasons. These accessions were further subjected to molecular characterization using 11 *Xa* genes viz., *Xa4, xa5*, *xa13*, *Xa21*, *Xa23, Xa27(t), Xa32(t), Xa33, Xa35(t)*, *Xa38* and *xa41* with gene-specific markers to ascertain the novelty. Some key resistance genes such as *Xa4*, *Xa23*, *Xa27(t)*, *Xa32(t)*, *Xa33*, *Xa35(t)* and *xa41* were detected in multiple accessions, with *O. rufipogon* and *O. eichingeri* harbouring particularly complex combinations of these genes. Notably, several accessions viz., IC521672 (*O. nivara*), EC861665 (*O. officinalis*), EC861677 (*O. latifolia*), EC861711 (*O. punctata*) and EC861738 (*O. eichingeri*) did not show the presence of any known genes indicating the possibility of novel genetic loci conferring BB resistance in these wild species. These promising accessions identified in the study are potential novel sources for bacterial leaf blight resistance in rice and will be useful for the development of durable bacterial blight resistance rice cultivars.

Introduction

Rice (*Oryza sativa* L.) ($2n = 2x = 24$) is the world's second most important cereal and a key staple food crop from the Poaceae family. It plays a crucial role both nutritionally and agriculturally, providing sustenance to around 3.2 billion people globally. Currently, the rising demand for rice emphasizes the need to enhance its production while minimizing pest and disease outbreaks. With the teeming global population, the projected rice demand is expected to reach 590 million tonnes by 2050 (Samal and Babu, 2018). Biotic stresses caused by fungi, bacteria and nematodes pose significant challenges to rice production by reducing both yield and quality. Among the bacterial diseases, bacterial blight (BB) is particularly concerning due to its widespread, destructive nature and its prevalence under favourable conditions. Caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), it is one of the most damaging diseases in both irrigated and rain-fed rice cultivation across Asia, leading to substantial losses, especially in regions with high-yielding varieties (Patil *et al*., 2017; Lu *et al*., 2022). Infection during the maximum tillering stage leads to severe leaf blight symptoms impacting the primary photosynthetic area and causing significant yield losses ranging from 20 to 30%, with 80–100% in heavily affected fields (Sombunjitt *et al*., 2017; Baliyan *et al*., 2018). Recently, the genes associated with pathogen virulence and the interactions between bacteria and plants have been closely examined (White and Yang, 2009; Ryan *et al*., 2011). The *thiG* gene, involved in thiamine biosynthesis, has been identified as essential for the virulence of *Xoo* (Yu *et al*., 2015). The protein encoded by *thiG* functions as a vital enzyme in the synthesis of the thiazole.

To minimize yield losses and prevent disease outbreaks, various management strategies have been implemented, with chemical usage being widely adopted. However, the chemical control has proven largely ineffective against this disease (Laha *et al*., 2009; Kumar *et al*., 2020), highlighting the need for cost-effective, easily adaptable and environmentally friendly solutions. One promising approach is to enhance resistance to bacterial blight by broadening the genetic base of high-yielding rice cultivars through the incorporation of resistant genes. Currently, 47 major BB resistance (R) genes have been identified in from wild relatives,

landraces, mutants and cultivated species, conferring resistance against different *Xoo* strains (Brar and Khush, 1997; Kumar *et al.*, 2020; Liu *et al.*, 2024). The emergence of new pathotypes has made existing resistance genes increasingly vulnerable, underscoring the need for novel resistance sources, as rice cultivars with single major resistance genes are more susceptible to breakdown from pathogen mutations compared to those with multi-locus resistance. Therefore, breeding programmes focused on new resistance sources along with multi-locus resistance varieties offer a more effective strategy for achieving long-term sustainability in rice production. Out of the resistance genes, few gene combinations of *xa5* + *xa13* + *Xa21* (Pradhan *et al.*, 2016), *Xa4* + *Xa21* + *xa5* + *xa13* (Chukwu *et al.*, 2019) and *Xa4* + *xa5* + *Xa7* + *xa13* + *Xa21* (Hsu *et al.*, 2020) have been shown to provide strong resistance to bacterial blight when introgressed together.

The wild rice species serve as valuable untapped sources of distinct alleles offering resistance to various biotic stresses (Bhasin *et al.*, 2012; Yang *et al.*, 2020), tolerance to abiotic stresses (Brar and Khush, 2006; Cao *et al.*, 2020), as well as economically important traits such as grain yield (Luo *et al.*, 2016; Balakrishnan *et al.*, 2020) and grain quality (Qi *et al.*, 2018). Regarding bacterial blight, few BB-R genes that have been identified from wild species include *Xa21 in O. longistaminata* (Khush *et al.*, 1990; Ronald *et al.*, 1992), *Xa23* in *O. rufipogon* (Zhang *et al.*, 1998; Wang *et al.*, 2014), *Xa27(t)* in *O. minuta* (Gu *et al.*, 2004), *Xa29* in *O. officinalis* (Tan *et al.*, 2004), *Xa30(t)* in *O. nivara* (Jin *et al.*, 2007), *Xa32(t)* in *O. australiensis* (Zheng *et al.*, 2009), *Xa33* in *O. nivara* (Kumar *et al.*, 2012), *Xa34* in *O. branchyantha* (Ram *et al.*, 2008), *Xa35(t)* in *O. minuta* (Guo *et al.*, 2010), *Xa38* in *O. nivara* (Kaur *et al.*, 2006; Bhasin *et al.*, 2012), *xa41 in O. barthii* and *O. glaberrima* (Hutin *et al.*, 2015), *Xa45* in *O. glaberrima* (Neelam *et al.*, 2020) and *Xa47(t)* in *O. rufipogon* (Xing *et al.*, 2021) and *Xa48* in *O. officinalis* (Sinha *et al.*, 2023). Thus, the importance of wild rice species in relation to bacterial blight resistance lies in their genetic diversity, the presence of valuable resistance genes, and their contribution to sustainable agricultural practices and food security. In light of this, the present study was undertaken to identify potential new sources of bacterial blight resistance in different species of *Oryza*, utilizing the molecular markers to enhance breeding efforts for developing resilient rice varieties.

Material and methods

Plant material

One hundred and twelve wild rice accessions, comprising of 59 accessions of *O. rufipogon*, nine of *O. nivara*, five of *O. australiensis*, five of *O. punctata*, five of *O. rhizomatis*, four of *O. officinalis*, four of *O. grandiglumis*, four of *O. alta*, four of *O. latifolia*, three of *O. eichingeri*, two of *O. minuta,* two of *O. longistaminata*, two of *O. barthii*, one of *O. glumaepatula*, one of *O. ridleyi*, one of *O. longiglumis* and one of *O. meridionalis* (online Supplementary Table S1) were utilized in the present study. In addition to these, the BB-positive checks included Improved Samba Mahsuri, PR 114, IRBB-23, IRBB-27, FBR-15 and IR-64, while Samba Mahsuri was used as the susceptible check.

Phenotypic screening of the wild accessions for BB resistance

The wild rice accessions and susceptible check Samba Mahsuri were grown in pots and screened against bacterial blight at ICAR-Indian Institute of Rice Research (IIRR), Rajendranagar, Hyderabad, during *Kharif* 2020, *Rabi* 2020–2021 and *Kharif* 2021. The isolate of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain IX-020, was used to create disease artificially. The *Xoo* strain, IX-020 was grown in modified Wakimoto's culture medium (Laha *et al.*, 2009) and using a 3-day-old culture, bacterial suspension (108 cfu/ml) was prepared and used for inoculation. The plants at maximum tillering stage were inoculated by clipping top 2–3 cm of completely developed leaves with sterilized scissors dipped in the bacterial suspension (Kauffman *et al.*, 1973). Disease reactions were recorded on five plants following the Standard Evaluation System for Rice (IRRI, 2014) by measuring the lesions length caused by BB on each inoculated leaf at 14 days and 21 days after inoculation. Accessions were classified as resistant (⩽3 cm), moderately resistant (3–6 cm), moderately susceptible (6–9 cm) and susceptible (⩾9 cm) based on the mean lesion length as per Chen *et al.* (2000).

Genotypic characterization using SSR markers

The accessions showing high BB resistance after inoculating with BB strain IX-020 were selected for molecular characterization. The bacterial blight resistant accessions of wild rice were characterized for the presence or absence of 11 bacterial blight resistant genes, namely *Xa4, xa5, xa13, Xa21, Xa23, Xa27(t), Xa32(t), Xa33, Xa35(t), Xa38* and *xa41* using the gene-linked reported markers (Table 1). Resistant checks were used as positives for bacterial blight (BB) resistance genes, including Improved Samba Mahsuri (*xa5*, *xa13*, *Xa21*), PR 114 (*Xa38*), IRBB-23 (*Xa23*), IRBB-27 (*Xa27(t)*), FBR-15 (*Xa33*) and IR-64 (*Xa4*). For the remaining genes, the amplicon size corresponding to the respective original donors is considered. On the other hand, the susceptible check Samba Mahsuri was negative for all the BB resistance genes, serving as a control in the experiment. The PCR amplification using the gene-specific primers was carried out using PCR cyclers with 2 µl of diluted DNA, 0.5 µl of forward primer, 0.5 µl of reverse primer, 4 µl of Master mix and 3 µl of nuclease-free water. Marker-specific annealing temperatures ranging between 54 and 63°C were used. The PCR amplified products were then resolved in 3% agarose (3 g of agarose dissolved in 100 ml 1× TAE buffer) gel at 100 V for 2 h in gel electrophoresis unit (iLIFE Biotech). The gels stained in ethidium bromide (10 mg/ml) were placed over the UV-transilluminator and documented using GELSTAN gel documentation system (Mediccare) for documentation. The documented gels with amplified products were scored visually and allele sizes were analysed against the standard 50 and 100 bp ladder and size is expressed in base pairs (bp).

Results

Phenotypic performance of wild rice accessions for bacterial blight disease

A total of 112 wild rice accessions along with the susceptible check (Samba Mahsuri) were screened for analysing their response towards an invasive strain of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The phenotypic response of the rice accessions upon inoculation is shown in online Supplementary Table S2 and Fig. 1. In *kharif* 2020, 74 accessions were recorded as resistant with a lesion length of less than 3 cm, 18 accessions were moderately resistant with 3–6 cm lesion length, two were moderately susceptible with a length of 6–9 cm (IC581952, EC861760),

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Table 1. Bacterial blight resistance genes used for characterization of wild rice accessions

S. no.	Gene	Marker	Chr.	Primer sequence		AT	Fragment size		References
				Forward	Reverse		+ve	−ve	
1	*Xa4*	RM 224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	55	165	144	Lee *et al.* (2003)
		MP12	11	ATCGATCGATCTTCACGAGG	TGGTATAAAAGGCATTCGGG	54.3	165	144	Ma *et al.* (1999)
2	*xa5*	xa5FM-R	5	AGCTCGCCATTCAAGTTCTTGAG	TGACTTGGTTCTCCAAGGCTT	55	150	300	Hajira *et al.* (2016)
		xa5FM-S	5	GTCTGGAATTTGCTCGCGTTCG	TGGTAAAGTAGATACCTTATCAAACTCGA				
3	*xa13*	xa13promoter	8	GGCCATGGCTCAGTGTTTAT	GAGCTCCAGCTCTCCAAATG	55	480	220	Hajira *et al.* (2016)
4	*Xa21*	pTA248	11	AGACGCGGAAGGGTGGTTCCCGGA	AGACCGGTAATCGAAAGATGAAA	55	990	600	Ronald *et al.* (1992)
5	*Xa23*	RM254	11	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	55	165	146	Pai *et al.* (2003)
6	*Xa27(t)*	BDTG-19	6	GAAGCCACACACACTGAGACA	CGGAGGAGAACTAGAGAGACCA	55, 59	391	–	Gu *et al.* (2004)
7	*Xa32(t)*	RM5926	11	ATATACTGTAGGTCCATCCA	AGATAGTATAGCGTAGCAGC	55	176	–	Zheng *et al.* (2009)
8	*Xa33*	RMWR7.6	6	CAACAAACACCTCCATGGTC	GGGAATGAGCAAAAATTG	58	190	210	Kumar *et al.* (2012)
		RMWR7.1	6	TTTTATCCCCTTCTTCCTTC	CGTGTTTTGTGTGTCTTTTG	58	350	250	
9	*Xa35(t)*	RM144	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	55	237	–	Guo *et al.* (2010)
10	*Xa38*	OS04g53050-1	4	TCTTCTATTGCTAACATTGGTG	TCGCATTCCATTTTCAGAG	56	250	350	Bhasin *et al.* (2012)
11	*xa41*	OSWEET-14	11	ATTGGCACTTTCTGTCATGC	GAGACCAAAGGCGAAGGCCCA	55	489	507	Hutin *et al.* (2015)

Where, AT = annealing temperature.

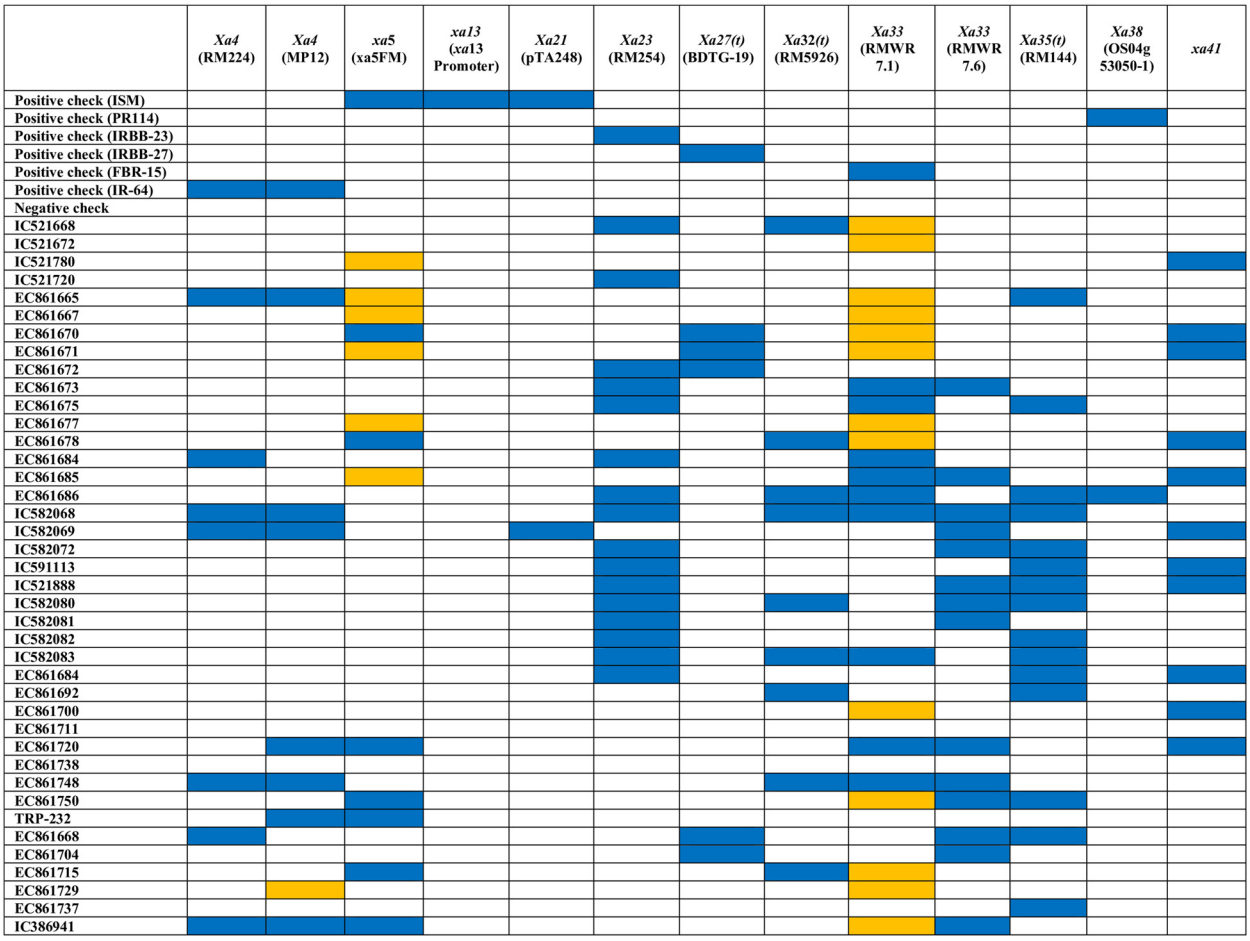

Figure 1. Colour map representing the presence or absence of reported genes in checks and 40 wild rice accessions. Blue colour indicates the presence of a gene, white colour indicates the absence of gene and yellow colour indicates no amplification.

while one accession (IC581951) was susceptible with more than 9 cm lesion length, after 14 days of inoculation. The susceptible check recorded a lesion length of 9.46 cm.

In *rabi* 2020–2021, 91 accessions showed a lesion length of less than 3 cm (resistant), 16 accessions recorded a lesion length in between 3 and 6 cm (moderately resistant), two accessions showed between 6 and 9 cm (IC521719, EC861749), rendering them as moderately susceptible at 14 days after inoculation. During the same season, at 21 days, 34 accessions showed a lesion length of less than 3 cm (resistant), 50 accessions had 3–6 cm length (moderately resistant), 14 accessions showed between 6 and 9 cm (moderately susceptible) and six accessions showed more than 9 cm (susceptible). The susceptible check recorded 10.8 and 16.92 cm at 14 and 21 days, respectively. In *Kharif* 2021, at 14 days after inoculation, 98 accessions were resistant showing a lesion length of less than 3 cm, 16 accessions were moderately resistant (3–6 cm), one accession (IC581956) was moderately susceptible with a score of 6.74 cm. At 21 days after inoculation, 42 accessions were resistant, 64 accessions were moderately resistant, eight were moderately susceptible, while one accession (EC861727) was susceptible. Samba Mahsuri was completely susceptible at both 14 (9.78 cm) and 21 days (12.8 cm) after inoculation.

On the whole, based on the mean performance of three seasons at 14 days and 21 days after inoculation, 40 accessions (Table 2, Fig. 1) were consistently resistant with a mean lesion length of less than 3 cm. These 40 BB resistant accessions included 22 accessions of *O. rufipogon,* two accessions *of O. nivara,* three accessions *of O. officinalis,* two accessions *of O. latifolia,* two accessions *of O. australiensis,* one accession of *O. minuta,* two accessions of *O. punctata,* three of *O. eichingeri,* one accession of *O. rhizomatis* and two accessions of *O. alta.* These 40 resistant accessions were selected for further molecular characterization studies.

Molecular characterization of the BB resistant accessions

The molecular profiling of wild rice accessions for bacterial blight resistance revealed the presence of several *Xa* genes linked to resistance. The results are detailed in Fig. 1 and online Supplementary Fig. S1. The dominant *Xa4* gene which is linked to RM224 marker was found in seven resistant accessions, namely, *O. officinalis* (EC861665, EC861668), *O. rufipogon* (EC861684, IC582068, IC582069), *O. australiensis* (IC386941) and *O. alta* (EC861748). *Xa21* gene located on chromosome 11, linked to the pTA248 marker, was present only in one *O. rufipogon* accession (IC582069). RM254 marker flanking the *Xa23* gene got validated in 16 of the wild rice accessions, viz., *O. nivara* (IC521668), *O. rufipogon* (IC521270, EC861672, EC861673, EC861675, EC861684, IC582068, IC582072, IC591113, IC521888, IC582080, IC582081, IC582082, IC582083), *O. eichingeri* (EC861686) and *O. alta* (EC861748). Accessions *O. officinalis* (EC861668) and *O. rufipogon* (EC861670, EC861671, EC861672, EC861704) were found to be having *Xa27(t)*, flanked by BDTG-19 marker.

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Table 2. Reaction of promising BB resistant accessions of wild species of rice against IX-020 isolate across seasons

S. no.	Accession no.	Species	Mean lesion length (cm) at 14 DAI	Mean lesion length (cm) at 21 DAI
1	IC521668	*O. nivara*	1.44	1.98
2	IC521672	*O. nivara*	0.85	1.76
3	IC521780	*O. rufipogon*	1.05	1.61
4	IC521720	*O. rufipogon*	1.10	2.17
5	EC861665	*O. officinalis*	1.06	1.99
6	EC861667	*O. officinalis*	0.55	1.04
7	EC861668	*O. officinalis*	1.51	1.40
8	EC861670	*O. rufipogon*	0.63	2.07
9	EC861671	*O. rufipogon*	1.45	2.47
10	EC861672	*O. rufipogon*	1.62	2.06
11	EC861673	*O. rufipogon*	0.93	1.56
12	EC861675	*O. rufipogon*	1.07	1.72
13	EC861677	*O. latifolia*	1.32	1.48
14	EC861678	*O. latifolia*	1.31	1.37
15	EC861684	*O. rufipogon*	1.37	1.90
16	EC861685	*O. eichingeri*	0.93	1.48
17	EC861686	*O. eichingeri*	0.97	2.38
18	IC582068	*O. rufipogon*	0.97	2.06
19	IC582069	*O. rufipogon*	1.27	1.64
20	IC582072	*O. rufipogon*	1.38	2.82
21	IC591113	*O. rufipogon*	1.79	1.69
22	IC521888	*O. rufipogon*	0.80	0.98
23	IC582080	*O. rufipogon*	1.36	2.79
24	IC582081	*O. rufipogon*	0.91	2.45
25	IC582082	*O. rufipogon*	1.36	2.55
26	IC582083	*O. rufipogon*	0.86	2.54
27	EC861684	*O. rufipogon*	1.27	1.54
28	EC861692	*O. rufipogon*	1.35	1.62
29	EC861700	*O. rufipogon*	1.38	1.56
30	EC861704	*O. rufipogon*	0.67	1.22
31	EC861711	*O. punctata*	2.39	1.97
32	EC861715	*O. rhizomatis*	1.48	1.38
33	EC861720	*O. australiensis*	1.65	1.87
34	EC861729	*O. punctata*	0.75	2.01
35	EC861737	*O. minuta*	0.86	1.31
36	EC861738	*O. eichingeri*	0.85	1.51
37	IC386941	*O. australiensis*	0.79	1.60
38	EC861748	*O. alta*	0.91	1.74
39	EC861750	*O. alta*	1.86	2.22
40	TRP232	*O. rufipogon*	0.77	1.72

Similarly, seven accessions (*O. nivara* (IC521668), *O. latifolia* (EC861686), *O. eichingeri* (EC861686), *O. rufipogon* (IC582068, IC582080) and *O. rhizomatis* (EC861715) were recorded to be having the dominant *Xa32(t)* gene flanked by the RM5926. RMWR7.1 and RMWR7.6 markers flanking the *Xa33* gene on chromosome 6, was identified in 14 accessions, viz., *O. officinalis* (EC861668), *O. rufipogon* (EC861673, IC582068, IC582069, IC582072, IC521888, IC582080, IC582081, EC861704), *O. eichingeri* (EC861685), *O. australiensis* (EC861720, IC386941) and *O. alta* (EC861748, EC861750). Fifteen accessions of wild rice, including *O. officinalis* (EC861665, EC861668), *O. eichingeri* (EC861686), *O. rufipogon* (EC861676, IC582068, IC582072, IC591113, IC521888, IC582080, IC582082, IC582083, EC861684, EC861692), *O. minuta* (EC861737) and *O. alta* (EC861750) were found to be having positive alleles for RM144 linked to *Xa35(t)* on chromosome 11. The dominant *Xa38* gene on chromosome 11, flanked by the marker Oso4g53050-1, was found only in one accession of *O. eichingeri* (EC861686). Ten accessions, namely, *O. rufipogon* (IC521780, EC861670, EC861671, IC582069, IC591113, IC521888, EC861684), *O. latifolia* (EC861678), *O. eichingeri* (EC861685) and *O. australiensis* (EC861720) were positive for the presence of *xa41,* flanked by Osweet14 marker. On the other hand, the recessive *xa5* gene, located on chromosome 5 and linked to the xa5FM marker, was identified in six accessions, viz., *O. rufipogon* (EC861670), *O. latifolia* (EC861678), *O. rhizomatis* (EC861715), *O. australiensis* (EC861720, IC386941) and *O. alta* (EC861750). Likewise, another recessive gene, *xa13* on chromosome 8, linked to the xa13promoter marker, was absent in all the resistant accessions.

Out of the 40 BB-resistant accessions of wild rice, 30 accessions had more than one resistant gene, while five accessions, viz., IC521672 (*O. nivara*), EC861665 (*O. officinalis*), EC861677 (*O. latifolia*), EC861711 (*O. punctata*) and EC861738 (*O. eichingeri*) did not show any BB resistance genes validated. Notably, the accessions of *O. rufipogon*, namely, EC861670 (*xa5* + *Xa27(t)* + *xa41*), EC861675 (*Xa23* + *Xa33* + *Xa35(t)*), IC582072 (*Xa23* + *Xa33* + *Xa35(t)*), IC591113 (*Xa23* + *Xa35(t)* + *xa41*), EC861684 (*Xa4* + *Xa23* + *Xa33),* EC861684 (*Xa23* + *Xa35(t)* + *xa41*) had three genes, while accessions IC582068 (*Xa4* + *Xa23* + *Xa32(t)* + *Xa33* + *Xa35(t)*), IC582069 (*Xa4* + *Xa21* + *Xa33* + *xa41*), IC521888 (*Xa23* + *Xa33* + *Xa35(t)* + *xa41*), IC582080 (*Xa23* + *Xa32(t)* + *Xa33* + *Xa35(t)*) and IC582083 (*Xa23* + *Xa32(t)* + *Xa33* + *Xa35(t)*) showed even greater genetic complexity with more than three resistance genes (Table 3). Similarly, *O. australiensis* with EC861720 (*Xa4* + *xa5* + *Xa33* + *xa41*) and IC386941 (*Xa4* + *xa5* + *Xa33*) accessions, *O. officinalis* (EC861668- *Xa4* + *Xa27(t)* + *Xa33* + *Xa35(t)), O. nivara* (IC521668- *Xa23*, *Xa32(t)*) and *O. latifolia* (EC861678- *xa5*, *Xa32(t)*, *xa41*) species presented valuable gene combinations. Moreover, *O. eichingeri* accession (EC861686) was particularly noteworthy for its unique combination of five resistance genes, viz., *Xa23*, *Xa32(t)*, *Xa33*, *Xa35(t)* and *Xa38.* On the other hand, *Xa21* was detected in only one *O. rufipogon* accession (IC582069). Overall, the gene combinations, namely, *Xa23* + *Xa33* + *Xa35(t)* + *xa41* in IC521888, *Xa23* + *Xa32(t)* in IC521668, *xa5* + *Xa32(t)* + *xa41* in EC861678 and *xa5* + *Xa32(t)* in EC861715 were found to be highly effective as these genotypes exhibited high resistance.

Discussion

Developing disease-resistant cultivars is an effective and resource-efficient approach for attaining durable, environmentally sustainable and broad-spectrum resistance to BB (Sundaram

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Table 3. List of BB resistant accessions of wild species rice with different gene combinations

S. no.	Accession	Species	Combination of genes
1	IC521668	*O. nivara*	*Xa 23 + Xa32(t)*
2	EC861665	*O. officinalis*	*Xa4 + Xa35(t)*
3	EC861668	*O. officinalis*	*Xa27(t) xa33 + Xa35(t)*
4	EC861670	*O. rufipogon*	*xa5 + Xa27(t) + xa41*
5	EC861671	*O. rufipogon*	*Xa27(t) + xa41*
6	EC861672	*O. rufipogon*	*Xa23 + Xa27(t)*
7	EC861673	*O. rufipogon*	*Xa23 + Xa33*
8	EC861675	*O. rufipogon*	*Xa23 + Xa35(t)*
9	EC861678	*O. latifolia*	*xa5 + Xa32(t) + xa41*
10	EC861685	*O. eichingeri*	*Xa4 + Xa33 + xa41*
11	EC861686	*O. eichingeri*	*Xa23 + Xa32(t) + Xa33 + Xa35(t) + Xa38*
12	IC582068	*O. rufipogon*	*Xa4 + Xa23 + Xa32(t) + Xa33 + Xa35(t)*
13	IC582069	*O. rufipogon*	*Xa4 + Xa21 + Xa33 + xa41*
14	IC582072	*O. rufipogon*	*Xa23 + Xa33 + Xa35(t)*
15	IC591113	*O. rufipogon*	*Xa23 + Xa35(t) + xa41*
16	IC521888	*O. rufipogon*	*Xa23 + Xa33 + Xa35(t) + xa41*
17	IC582080	*O. rufipogon*	*Xa23 + Xa32(t) + Xa33 + Xa35(t)*
18	IC582081	*O. rufipogon*	*Xa23 + Xa33*
19	IC582082	*O. rufipogon*	*Xa23 + Xa35(t)*
20	IC582083	*O. rufipogon*	*Xa23 + Xa32(t) + Xa35(t)*
21	EC861684	*O. rufipogon*	*Xa23 + Xa35(t) + xa41*
22	EC861720	*O. australiensis*	*xa5 + Xa33 + xa41*
23	EC861748	*O. alta*	*Xa4 + Xa23 + Xa33*
24	EC861750	*O. alta*	*xa5 + Xa33 + Xa35(t)*
25	EC861704	*O. rufipogon*	*Xa27(t) + Xa33*
26	EC861715	*O. rhizomatis*	*xa5 + Xa32(t)*
27	IC386941	*O. australiensis*	*Xa4 + xa5 + Xa33*

et al., 2008; Kanipriya *et al.*, 2024). In particular, creating and utilizing rice cultivars with multiple BB-R genes has proven to be a successful strategy for controlling the diverse range of *Xoo* strains (Kottapalli *et al.*, 2007; Kumar *et al.*, 2020). In the present study, the phenotypic evaluation of wild rice accessions across multiple seasons revealed significant insights into their potential for bacterial blight resistance. The consistent performance of several accessions, particularly their resistance to the invasive strain of *Xanthomonas oryzae pv. oryzae* (*Xoo*), underscores the value of wild rice germplasm in breeding programmes aimed at enhancing disease resistance in cultivated rice varieties. In *Kharif* 2020, the majority of the accessions exhibited strong resistance, with 74 accessions showing lesion lengths of less than 3 cm, indicating high levels of resistance. This trend was similarly observed in subsequent seasons, with an even greater number of accessions demonstrating resistance during *Rabi* 2020–2021. Notably, in *Kharif* 2021, 98 accessions were resistant at 14 days post-inoculation, further confirming the robustness of these accessions against bacterial blight. These results are particularly significant when considering that the susceptible check, Samba Mahsuri, consistently recorded much larger lesion lengths, highlighting the enhanced resistance in the wild rice accessions. Species, *O. longistaminata, O. barthii, O. ridleyi, O. longiglumis, O. grandiglumis, O. meridionalis* and *O. glumaepatula* were not resistant to the bacterial blight. Similar studies on the screening for BB resistance were taken up by Singh *et al.* (2015), classifying 11 wild rice accessions as moderately resistant, 21 as moderately susceptible, and three as susceptible accessions.

The resistance observed in 40 wild rice accessions across three seasons, underscores the significant potential of these accessions in breeding programmes aimed at enhancing bacterial blight resistance. The prominence of *O. rufipogon* species with 22 accessions showing consistent resistance reinforces its importance as a key source of resistance genes. The diverse species represented in this resistant group, including *O. rufipogon*, *O. nivara*, *O. officinalis*, *O. latifolia*, *O. australiensis*, *O. minuta*, *O. punctata*, *O. eichingeri*, *O. rhizomatis* and *O. alta*, highlights the broad genetic base which is crucial for breeding efforts aimed at combating the evolving threat of bacterial blight. The selection of these 40 accessions for further molecular characterization aims to identify the underlying genetic factors contributing to their resistance.

Consequently, the molecular characterization revealed a high level of variability in the combination of BB resistance genes across the accessions. The presence of the *Xa4* gene in eight accessions, *xa5* in six accessions, *Xa23* in 17 accessions, *Xa27(t)* in five accessions, *Xa32(t)* in eight accessions, *Xa33* in 18 accessions, *Xa35(t)* in 15 accessions and *xa41* in 11 accessions highlights a broad spectrum of bacterial blight resistance across wild rice species. This distribution indicates a rich genetic diversity that is valuable for breeding programmes aimed at developing durable and effective resistance. The recessive *xa13* gene was absent in all resistant accessions, indicating a possible lack of contribution to resistance in these wild rice varieties. One of the most promising aspects of this study is the identification of accessions harbouring multiple resistance genes. The wild rice species *O. rufipogon*, a key progenitor of cultivated rice (Barbier *et al.*, 1991; Khush, 1997), exhibited significant variation in its bacterial blight resistance gene combinations. Several accessions contained more than three resistance genes, exhibiting greater genetic complexity, making them highly valuable for gene pyramiding strategies to enhance resistance in cultivated rice. Similarly, species like *O. australiensis*, *O. officinalis*, *O. nivara* and *O. latifolia* showed valuable gene combinations, contributing further to the diversity of resistance genes available for breeding. Two standouts were an *O. eichingeri* (EC861686-*Xa23 + Xa32(t) + Xa33 + Xa35(t) + Xa38*) and *O. rufipogon accession* (IC582068-*Xa4 + Xa23 + Xa32(t) + Xa33 + Xa35(t)*), with a unique set of five resistance genes, highlighting their high potential for durable resistance and effectiveness against *Xoo*, especially given the rarity of *Xa38*. Notably, the combinations of *Xa23 + Xa33 + Xa35(t) + xa41* in IC521888, *Xa23 + Xa32(t)* in IC521668, *xa5 + Xa32(t) + xa41* in EC861678 and *xa5 + Xa32(t)* in EC861715 demonstrated a high degree of resistance against the pathogen. These multiple gene combinations could be essential for maintaining stability in resistance against the pathogenic strains, potentially mitigating the risk of resistance breakdown, as stated by Nath *et al.* (2022). On the other hand, the detection of *Xa21* in a single *O. rufipogon* accession, pointed its rarity and significant potential for providing resistance. These accessions provide crucial genetic resources for developing rice varieties with enhanced and durable resistance.

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Xa23 was originally mapped in *O. rufipogon* (Zhang *et al.*, 1998; Wang *et al.*, 2014). In our study, the presence of *Xa23* was confirmed in several *O. rufipogon* accessions (IC521720, EC861672, EC861673, EC861675, EC861684, IC582068, IC582072, IC591113, IC521888, IC582080, IC582081, IC582082, IC582083, EC861684), highlighting its potential as a valuable genetic resource for improving bacterial blight resistance in rice. While both *Xa35(t)* and *Xa27(t)* have been identified in *O. minuta* (Gu *et al.*, 2004; Guo *et al.*, 2010), our current study found the *O. minuta* accession EC861737 carrying only the *Xa35(t)* gene. Likewise, *Xa32(t)* which was identified in *O. australiensis* species (Zheng *et al.*, 2009) was not found in any of the resistant *O. australiensis* accessions, including EC861720 and IC386941. In the case of *O. nivara*, two genes, namely *Xa33* (Kumar *et al.*, 2012) and *Xa38* (Cheema *et al.*, 2008) which have been previously reported were not found in the *O. nivara* accession IC521672, in spite of being resistant. Similar validation of reported genes was performed by Chen *et al.* (2022) and Singh *et al.* (2015) in wild rice accessions. Additionally, five accessions belonging to *O. nivara*, *O. officinalis*, *O. latifolia*, *O. punctata* and *O. eichingeri* species did not show any of the validated BB resistance genes despite their phenotypic resistance, suggesting the presence of novel resistance mechanisms or genes that were not covered by the markers used. This highlights the necessity of further inheritance and mapping studies to ascertain the novelty of the sources. Additionally, integrating advanced genomic tools such as whole-genome sequencing and transcriptome analysis could provide deeper insights into the genetic basis of resistance in these novel sources. Also, we reported the resistance of the accessions based on screening with a single virulent strain of the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolate. While our findings provide valuable insights into the resistance profiles, screening them against multiple *Xoo* isolates with varying virulence levels as a future line of work would strengthen the reliability of the resistance assessments. This approach will enhance understanding of the genetic basis of resistance and offer a more comprehensive evaluation of the accession's resilience to different *Xoo* strains.

Conclusion

The wild rice accessions identified in this study represent a rich source of genetic diversity for bacterial blight resistance. The combination of phenotypic and molecular characterization has provided a comprehensive understanding of their resistance potential, paving the way for their use in breeding programmes aimed at developing robust, disease-resistant rice varieties. The identification of accessions with complex gene combinations offers a promising avenue for marker-assisted selection and gene pyramiding in breeding programmes. These findings emphasize the value of conserving and utilizing wild rice germplasm to address the ongoing challenges of bacterial blight in rice production. Future research should focus on the functional validation of these genes in various genetic backgrounds and environments to confirm their utility in resistance breeding.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262124000601.

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