Phenotypic and Genotypic Evaluation of Traditional Rice Varieties of Karnataka for Resistance to Bacterial Leaf Blight

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Abstract

Rice is one basic food crop for billions of peoples around the world. Now a days it is becoming very difficult to feed the world's population due to the drastic reduction in the productivity of rice, mainly because of production constraints such as biotic and abiotic stress. Among biotic stresses, bacterial leaf blight (BLB) is a major devastating disease which causes drastic yield loss in rice caused by bacteria Xanthomonas oryzae pv. oryzae (Xoo). To overcome this problem, identification and development of resistant varieties play a vital role. In the present study, an attempt was made to phenotypically and genotypically characterize a set of 33 traditional rice varieties (TRVs) of Karnataka for BLB resistance and presence of corresponding resistance genes, respectively. Phenotypic screening was done by artificially inoculating rice genotypes with Xoo by clipping method. Following phenotyping, other than the check variety improved samba mahsuri, Kari bhattha was the only genotype that was found to be resistant to BLB. Further, 11 TRVs were recorded as moderately resistant with lesion length ranging from 5-15 cm, whereas 21 TRVs were found to be susceptible with a lesion length of 15 cm and above. Genotyping for the presence of ten BLB resistance genes was performed using linked SSR markers for the R genes. The results of genotyping study revealed that the four broad spectrum R genes conferring durable resistance to BLB that include Xa5, Xa13, Xa21 and Xa38 were present only in check variety Improved Samba Mahsuri. Among 33 traditional rice varieties evaluated, 17 varieties harbored 8 R genes, eight varieties had 7 R genes and nine varieties had a maximum of 9 R genes. Further, among ten BLB resistance genes studied, six genes that include Xa5, Xa1, Xa33, Xa7, Xa30 and Xa38 were present in all the 33 TRVs studied.

Keywords : Bacterial leaf blight, TRVs, Phenotypic and Genotypic screening, Resistance genes

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R ICE (*Oryza sativa*) is one of the most important food crops in the world. Cultivated rice, as we know it today, was first grown about 10,000 years ago in south-east Asia, most probably in India. More than 90 per cent of the world's rice is produced and consumed in Asia. The major rice producing countries in the world are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan. In India, rice is cultivated in an area of 43.8 million hectares with the production of 116.42 million metric tonnes and productivity of 2.65 tonnes per hectare and provides food for more than 80 per cent of the population and serves as the principal energy source for most of the people. (*www.statista.com*, 2019). When compared, the rice productivity of India is less than that of world average of 3.05 tonnes per hectare. The significant difference between the rice productivity level of India and that of the world is mainly due to several production constraints that include both biotic and abiotic stress.

The major abiotic stress factors for Indian rice production include moisture stress, soil salinity, cold during reproductive stages and heat. Among biotic stress, pest and diseases problems cause major yield loss in rice production. The major diseases of rice in India are different forms of blast (leaf, neck, nodal and panicle blast), bacterial leaf blight, sheath blight and sheath rot. The Bacterial Leaf Blight (BLB) caused by the bacteria *Xanthomonas oryzae* pv. *oryzae (Xoo)* is a major and devastating diseases of rice, causing up to 50 per cent yield loss depending on the plant growth stage at the time of infection, weather condition, the genotype cultivated and the extent of application of nitrogenous fertilizers. The problem of BLB is more severe as it can affect the rice plant at all growth stages and the bacteria can spread to other uninfected plants through irrigation water, rain splash, plant-to-plant contact, tools used in transplanting and also while handling plants during transplanting (Mew et al., 1992). The pathogen causing BLB is seed-borne and hence the seed borne infection acts as a primary source of inoculum and lead to extremely high field incidence. The BLB disease is characterized by two primary symptoms of leaf blight and kresek. The leaf blight is characterized by wavy elongated lesions along one or both the leaf margins and occasionally also along the midribs generally at maximum tillering stage and onwards. On panicles, the bacteria cause grey to light brown lesions on glumes leading to infertility and low quality of the grains. The most destructive manifestation of the BLB is wilt syndrome referred to as Kresek. The wilt syndrome occurs from the seedling to the early tillering stage in which the leaves of infected plants wilt, roll up, and turn into yellow to straw-colour, wither and finally resulting in drying up of the entire plant (Naqvi, 2019).

Although there are several chemical management practices recommended for the management of BLB, deployment of resistance genes in rice breeding programs and their incorporation into the susceptible rice varieties is the most economical, eco-friendly and sustainable way. In this direction, enormous work has been done in the past with the identification of several host resistance genes against Xanthomonas oryzae pv. Oryzae. Among, 40 host resistance genes identified in rice to date (Xia et al., 2012; Kim, 2018; Dilla-Ermita et al., 2017 and Ji et al., 2018) Xa5, Xa13 and Xa21 are most effective and commonly used BLB resistance genes in rice breeding programs (Chukwu et al, 2019; Hajira et al., 2016 and Pradhan et al., 2015). Advance breeding methods such as marker assisted selection aid in precise and quick transfer of these resistance genes from a donor to susceptible rice variety.

Traditional Rice Varieties (TRVs) have proven to be a treasure of several resistance genes. Despite identification of a number of BLB resistance genes in several varieties of rice, their presence and identification in TRVs of Karnataka will be of great importance with several uses. Although one or more resistance genesare present in a rice variety, in certain cases, their use from that particular host background may be limited due to linkage drag. Hence, it is always beneficial to identify the presence of these resistance genes in more than one background. In the cases of undesirable linkage drag, one will have more options to deploy the same gene from a different background. Due to this reason, screening of TRVs for the presence of different BLB resistance genes is of great importance.

Hence, in the present investigation an effort was made to phenotype a set of TRVs of Karnataka for BLB by artificial inoculation with *Xanthomonas oryzae* pv. *oryzae*. Further, the same set of TRVs was also genotyped and BLB resistance genes identified using linked SSR markers.

MATERIAL AND METHODS

Plant Materials

A set of 33 TRVs of Karnataka were obtained from germplasm collection maintained at Division of Rice Breeding, All India Co-ordinated Research Project (Rice), Zonal Agricultural Research Station, V.C. Farm, Mandya. The list of TRVs involved in the present study and checks used are furnished in Table 1. While the rice variety, Improved Samba Mahsuri (RP Bio-226, ISM), harboring BLB resistance genes *Xa5*, *Xa13* and *Xa21*, was used as a resistant check, a red rice variety Jyothi (PTB 39) was used as a susceptible check.

Phenotyping for BLB

The plants were inoculated with bacterial suspension of *Xanthomonas oryzae* pv. *oryzae*. The mother culture of *Xoo* strain was maintained at Division of Rice Pathology, All India Co-ordinated Research Project (Rice), Zonal Agricultural Research Station, V.C. Farm, Mandya. A small quantity of mother culture

TABLE 1
List of TRVs of Karnataka used
in the present study

Name of TRV	Name of TRV / Rice variety
Adikanne Bhattha	Gidda Raja
Asundi	Gulwadi Sannakki
Sanna Bhattha-2	Kachadi Samba
Akalu-1	Akalu
Jeerige Sanna	Kempudoddi Gidda
Theerthalli	Duddoge
Gudda Bhattha	Karidoddi Budda
Mallige-1	Sanna Mallige
Anandhi-1	Bangara Sanna-1
Sona Masuri	Nellur Black
Kari Bhattha	Mysore Sanna-2
Nellur Sona	Sanna Bhattha-1
Nati Bhattha	Mysore Mallige
Doddalur	Kari Mundaga
Raichur Sanna	Kage Sale-1
Bilijaddu Alneran Bhattha	Improved Samba Mahsuri
Bangara Sanna-3	(Resistant Check)
Gowri Sanna	Jyothi (Susceptible Check)

was sub-cultured in 250 ml conical flask containing 100 ml nutrient broth and incubated with shaking at 80 rpm at 30 °C for 48 hours. After 48 hours, clear solution of nutrient agar became turbid, confirming the growth of bacteria.

40-50 seeds of each TRV and check were sown in plastic protrays. Seedlings were transplanted to field after 25 days of sowing with the spacing of 15 cm between plants and 20 cm between rows in three replications. The leaves of rice plants, aged about 50-55 days, were inoculated with the bacteria by dipping the sterilized surgical scissor in the bacterial suspension and clipping the tips of the seedlings. The disease reaction on inoculated plants was recorded 15 days post inoculation by measuring affected leaf area.

The observations were recorded and scoring was done following Standard Evaluation System (SES, 2013)

TABLE 2							
Standard evaluation system (SES, 2013) scale							
ot rice for BLB							
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	Lesion length (cm)	Score
	0-5	Resistant (R)
	5-15	Moderately Resistant (MR)
	>15	Susceptible (S)
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Isolation of genomic DNA and quantification

scale of rice for BLB (Table 2), developed by International Rice Research Institute (*http://www.clrri.org/ver2/uploads/ SES_5th_edition.pdf*) Briefly, the lesion length was recorded in centimetre using measuring scale and grouping was done using SES scale.

Isolation of Genomic DNA and Quantification

Leaf samples for DNA isolation were collected from 20 days old seedlings and stored immediately at - 20 °C until DNA isolation. The sample was prepared for DNA isolation by surface sterilization with 70 per cent ethanol. DNA was extracted from the frozen leaf sample using CTAB protocol of Wen-Yue *et al.* (2006). The DNA purity was analysed by estimating the ratio between 260 nm and 280 nm by using advanced automated DNA quantifier, Multiskansky High plate reader (Thermofisher). Quality of genomic DNA was checked by gel electrophoresis using 0.8 per cent agarose.

Polymerase Chain Reaction

The genotyping was done by polymerase chain reaction with linked SSR markers for BLB resistance genes. The primer sequences were obtained from www.graminae.org and the oligos were synthesized from Sigma Inc. PCR was performed following standard molecular biology practices and using TAKARA PCR mix. The list of BLB resistance genes studied, the associated markers used and their primer sequences are furnished in Table 3.

BLB resistance gene	Liked SSR e Marker	Primer name	Primer sequence (5 - 3)	Annealing temperatur (°C)	g Reference re
Xa l	XalP	Xa1PF	F- ACTGCCCTCTTGCACACGCCTTTGG	60	Yoshimura et al., 1998
		Xa1PR	R- CCGGTACATCAGTATTGTCCATCGG		
Xa4	MP1+MP2	MP1+MP2F	F- ATCGATCGATCTTCACGAGG	60	Sun et al., 2003
		MP1+MP2R	R- TCGTATAAAAGGCATTCGGG		
Xa5	xa5S	xa5SF	F- GTCTGGAATTTGCTCGCGTTCG	57.5	Iyer-Pascuzzi and McCouch., 2007
		xa5SR	R- TGGTAAAGTAGATACCTTATCAAACTGGA	A	
Xa7	M5	M5F	F- CGATCTTACTGGCTCTGCAACTCTGT	55	Porter et al., 2003
		M5R	R- GCATGTCTGTGTCGATTCGTCCGTACGA		
Xa 1 3	xa13prom	xa13promF	F- GGCCATGGCTCAGTGTTTAT	57.5	Chu et al., 2006
		xa13promR	R- GAGCTCCAGCTCTCCAAATG		
Xa21	pTA248	pTA248F	F- AGACGCGGAAGGGTGGTTCCCGGA	57.5	Huang <i>et al.</i> , 1997
		pTA248R	R- AGACGCGGTAATCGAAAGATGAAA		
Xa23	RM206	RM206F	F- CCCATGCGTTTAACTATTCT	55	Wang et al., 2005
		RM206R	R- CGTTCCATCGATCCGTATGG		
Xa38	Oso4g53050-1	Oso4g53050-1F	F- TCTTCTATTGCTAACATTGGTG	56	Bhasin et al., 2011
		Oso4g53050-1R	R- TCGCATTCATTTTCAGAG		
Xa30	LOC_Os04g53060	LOC_Os04g53060F	F- TGGAAACAAGGAAGGTTTCG	57	Cheema et al., 2008
		LOC_Os04g53060R	R- TGGACTGAGATGAGGTGCTG		
Xa33	RMWR7.1	RMWR7.1F RMWR7.1R	F- TTTTATCCCCTTCTTCCTTC R- CGTGTTTTGTGTGTGTCTTTTG	55	Kumar <i>et al.</i> , 2012

TABLE 3 List of BLB resistance genes studied, linked SSR markers, primers used in genotyping and their annealing temperatures

RESULTS AND DISCUSSION

Phenotyping for Bacterial Leaf Blight

In an effort to identifyresistant genotypes, a set of 33 traditional rice varieties of Karnataka were phenotypically evaluated for bacterial leaf blight disease. The red rice variety Jyothi, identified as highly susceptible variety for bacterial leaf blight, was used as susceptible check. Improved Samba Mahsuri, a BLB resistant and improved rice variety harbouring most widely known BLB resistance genes viz., Xa5, Xa13 and Xa21 was used as a resistant check (Shaik et al., 2014). The disease incidence was ensured by artificial inoculation of the bacterial suspension following leaf clip method and the disease reaction was recorded by measuring the length of the blight lesions on the leaves. The genotypes were further grouped in to different categories of resistance or susceptibility, following SES

scale for rice by International Institute of Rice Research (2013). Following 15 days after artificial inoculation, the susceptible check Jyothi recorded the average lesion length of 21.17 cm and found to be susceptible for BLB (Table 4). In contrast and according to our expectation, the resistant check variety, Improved Samba Mahsuri, harbouring Xa5, Xa13 and Xa21 recorded the lesion length of 1.50 cm and found to be resistant to BLB. While the highest lesion length of 21.17 cm was recorded by Jyothi (susceptible check) and a TRV Adikanne Bhattha, the lowest lesion length of 1.50 cm was recorded by resistant check variety Improved Samba Mahsuri (Table 4). Among 33 traditional rice varieties screened, only one variety Kari Bhattha was found to be resistant to BLB. However, the lesion length of Kari Bhattha (3.5 cm) was slightly higher than that of the resistant check Improved Samba Mahsuri. Further, 12 TRVs that include Kempudoddi Gidda, Akalu, Sanna Mallige,

Name of the TRV/Rice		Lesio	n length (cm)		Disease reaction				
variety	R1	R1	R1	Average	R1	R1	R1	Average		
Sanna Bhattha - 2	8.5	11	13	10.83	MR	MR	MR	MR		
Akalu - 1	21	16	18.5	18.50	S	S	S	S		
Jeerige Sanna	19	16.5	17	17.50	S	S	S	S		
Theerthalli	18	17	17.5	17.50	S	S	S	S		
Gudda Bhattha	16	19.5	17	17.50	S	S	S	S		
Mallige - 1	20	21	18.5	19.83	S	S	S	S		
Anandhi - 1	19.5	20.5	17.5	19.17	S	S	S	S		
Sona Masuri	10	11	14	11.67	MR	MR	MR	MR		
Kari Bhattha	4	3	3.5	3.50	R	R	R	R		
Nellur Sona	16.5	18	17	17.17	S	S	S	S		
Nati Bhattha	18	19.5	18.5	18.67	S	S	S	S		
Doddalur	17	19	20.5	18.83	S	S	S	S		
Raichur Sanna	18	18.5	20	18.83	S	S	S	S		
Bilijaddu Alneran Bhattha	11	12.5	10	11.17	MR	MR	MR	MR		
AdikanneBhattha	21	22.5	20	21.17	S	S	S	S		
Asundi	18	17.5	19	18.17	S	S	S	S		
Gidda Raja	20	20.5	21	20.50	S	S	S	S		
Gulwadi Sanakki	16	18	17.5	17.17	S	S	S	S		
Kachadi Samba	13	13.5	16	14.17	MR	MR	S	MR		
Akalu	10	8	9.5	9.17	MR	MR	MR	MR		
Kempudoddi Gidda	7.5	6	8.5	7.33	MR	MR	MR	MR		
Duddoge	16.5	18	17	17.17	S	S	S	S		
Karidoddi Budda	9.5	12.5	11	11.00	MR	MR	MR	MR		
Sanna Mallige	12	8	9	9.67	MR	MR	MR	MR		
Bangara Sanna-1	11	9.5	10	10.17	MR	MR	MR	MR		
Nellur Black	17	19.5	18	18.17	S	S	S	S		
Mysore Sanna-2	19.5	16.5	20	18.67	S	S	S	S		
Sanna Bhattha-1	18	19	21	19.33	S	S	S	S		
Mysore Mallige	12.5	13.5	10	12.00	MR	MR	MR	MR		
Kari Mundaga	22.5	21	19	20.83	S	S	S	S		
Kage Sale-1	13	10.5	11	11.50	MR	MR	MR	MR		
Bangara Sanna-3	22	21	19	20.67	S	S	S	S		
Gowri Sanna	11	10.5	13.5	11.67	MR	MR	MR	MR		
Jyothi	22	20.5	21	21.17	S	S	S	S		
Improved Samba Mahsuri (ISM)	1.5	1	2	1.50	R	R	R	R		

TABLE 4 Lesion length and disease reaction of the TRVs for bacterial leaf blight disease

Bangara Sanna-1, Sanna Bhattha-2, Karidoddi Budda, Bilijaddu Alneran Bhattha, Kage Sale-1, Sona Mahsuri, Gowri Sanna, Mysore Mallige and Kachadi Samba were found to be moderately resistant to BLB. The remaining 20 TRVs recorded the lesion length greater than 15 cm and found to be susceptible to BLB.

Genotyping for Bacterial Leaf Blight

Having done the phenotypic evaluation, we were interested to determine the underlying reason for the phenotypic resistance or susceptibility of the TRVs. Therefore, we performed genotyping of the TRVs to check for the presence of different ten BLB resistance genes using linked SSR markers. The list of genes for genotyping and the linked SSR markers are furnished in Table 3.

Following genotyping, out of 10 BLB resistance genes looked for, the susceptible check Jyothi was found to harbour eight genes and lacked Xa13 and Xa21 (Table 5 and Fig. 1). In continuation with this, the phenotypic evaluation had indicated susceptibility of Jyothi rice variety. In contrast, the resistant check (Improved Samba Mahsuri) had all the 10 BLB resistance genes under study and was phenotypically characterized to be resistant to BLB. In fact, Improved Samba Mahsuri (resistant check) was the only variety under study that had all the BLB resistance genes (Fig. 1). There are several reports that have demonstrated the ability of Xa5, Xa13, Xa21 and Xa38 BLB resistance genes in providing durable



Fig. 1 : Bacterial leaf blight resistance genes in check varieties Jyothi and Improved Samba Mahsuri (ISM). Briefly, PCR was performed to check the presence or absence of the indicated genes using primer pairs for linked SSR markers as indicated in materials and methods. Shown in arrow marks are the size of the DNA bands. The letter R or S withing the parenthesis represents resistance or susceptible alleles, respectively.

resistance to Indian strains of Xoo (Sundaram et al., 2008, Goel et al., 1998; Joseph et al., 2004, Shanti et al., 2001, Yugander et al., 2018, Bhasin et al., 2012 and Ellur et al., 2016). Therefore, in our study also, the presence of these four resistance genes in Improved Samba Mahsuri proved to provide resistance to BLB. The absence of Xa13 and Xa21 could be the potential reason for the susceptibility of Jyothi rice variety. Although Xa5 and Xa38 are present in Jyothi rice variety, it is possible that the mere presence of these two genes may not be sufficient to provide resistance to the prevalent race (s) of *Xoo* in our study. Among 33 traditional rice varieties evaluated, 17 varieties were found to have 8 R genes, seven varieties had 7 R genes and nine varieties had a maximum of 9 R genes (Table 5 and Fig. 2). Among these nine varieties that had maximum of nine BLB resistance genes, five varieties viz., Sanna Bhattha-2, Kachadi Samba, Akalu, Kempudoddi Gidda and Mysore Mallige were phenotypically characterized to be moderately resistant to BLB and one TRV named Kari Bhattha as resistant (Table 4). Interestingly, despite having nine R genes, the remaining three varieties viz., Theerthalli, Mallige-1 and Doddalur were phenotypically susceptible to BLB and all these three varieties were found to lack Xa13 (Table 4, Fig. 2). Hence, Xa13 being the only difference between the resistant check



Fig. 2 : Profile of Bacterial leaf blight resistance genes in traditional rice varieties of Karnataka. SSR markers are indicated within parenthesis below the gene name. Shown in arrow marks are the size of the DNA bands. The letter R or S within the parenthesis represents resistance or susceptible alleles, respectively. The lanes of DNA ladder were removed from the figures for the sake of simplicity and accommodating all the gene profiles in a single figure. However, the allele size is indicated as explained earlier.

TABLE 5
Genotypic screening of TRVs for the presence or absence of bacterial blight resistance
genes using linked SSR markers

Name of the TRV/ Rice variety	Xa5	Xa13	Xa21	Xa10	Xa23	Xal	Xa33	Xa7	Xa30	Xa38	Total
Sanna Bhattha-2	✓	Х	\checkmark	✓	✓	✓	✓	\checkmark	✓	✓	9
Akalu-1	\checkmark	Х	Х		\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Jeerige Sanna	\checkmark	Х	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	7
Theerthalli	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	9
Gudda Bhattha	\checkmark	Х	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	7
Mallige-1	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	9
Anandhi-1	\checkmark	Х	\checkmark	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Sona Masuri	\checkmark	Х	Х	~	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	7
Kari Bhattha	\checkmark	Х	~	~	 Image: A second s	~	\checkmark	\checkmark	\checkmark	\checkmark	9
Nellur Sona	\checkmark	Х	1	X	-	1	~	\checkmark	\checkmark	\checkmark	8
Nati Bhattha	~	X	1	~	Х	1	\checkmark	\checkmark	\checkmark	\checkmark	8
Doddalur	~	Х	Н		1	~	~	\checkmark	\checkmark	\checkmark	9
Raichur Sanna	v	Х	X		~	~	1	✓	\checkmark	\checkmark	8
Bilijaddu Alneran Bhattha	1	X	~	~	Х	~	~	✓	\checkmark	\checkmark	8
Adikanne Bhattha	~	Х	~	Х	~	✓	~	✓	\checkmark	\checkmark	8
Asundi	~	Х	~	Х	~	~	1	✓	\checkmark	\checkmark	8
Gidda Raja	v	Х	\checkmark	~	1	~	~	✓	\checkmark	\checkmark	8
Gulwadi Sanakki	1	Х	1	Х	Х	~	\checkmark	~	\checkmark	\checkmark	7
Kachadi Samba	1	Х	1	~	1	1	~	\checkmark	\checkmark	\checkmark	9
Akalu	1	Х	~	~	~	~	~	\checkmark	\checkmark	\checkmark	9
Kempudoddi Gidda	\checkmark	Х	~	~	~	~	1	\checkmark	\checkmark	\checkmark	9
Duddoge	\checkmark	X	Х		-	~	\checkmark	\checkmark	\checkmark	\checkmark	8
Karidoddi Budda	\checkmark	Х	Х	Х	~	1	\checkmark	\checkmark	\checkmark	\checkmark	7
Sanna Mallige	\checkmark	Х	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Bangara Sanna-1	\checkmark	Х	\checkmark	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Nellur Black	\checkmark	Х	\checkmark	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Mysore Sanna-2	\checkmark	Х	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Sanna Bhattha-1	\checkmark	Х	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	7
Mysore Mallige	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	9
Kari Mundaga	\checkmark	Х	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	7
Kage Sale-1	\checkmark	Х	\checkmark	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Bangara Sanna-3	\checkmark	Х	\checkmark	\checkmark	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Gowri Sanna	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Jyothi	\checkmark	Х	Х	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	8
Improved Samba Mahsuri (ISM)	✓	√	√	√	✓	✓	√	~	√	√	10

and Theerthalli, Mallige-1 and Doddalur rice varieties, Xa13 is most likely to provide resistance to races of Xoo present in the locality. Therefore, based on the present investigation it can strongly be suggested to always include Xa13 in BLB resistance breeding programs for the present location and races. Although it is possible that there may be genes, other than the ten genes studied, contributing to the resistance of Improved Samba Mahsuri, Xa13 is most likely to be an important R gene that should be considered while breeding for BLB resistance. This viewpoint is further supported by our finding that Xa13 is present only in Improved Samba Mahsuri, the resistant check variety under the study and absent in all the remaining rice varieties that are either susceptible or moderately resistant. The only exception to this notion is Kari Bhattha that does not have Xa13, yet resistant to BLB. This suggests that there may be other novel resistance genes present in Kari Bhattha that need to be studied in future. Further, among ten BLB resistance genes studied, six genes viz., Xa5, Xa1, Xa33, Xa7, Xa30 and Xa38 were present in all the 33 TRVs studied. While Xa21 was present in 27 TRVs, Xa10 and Xa23 were found in 22 and 20 TRVs, respectively.

Among the set of 33 traditional rice varieties of Karnataka, Kari Bhattha was the only variety found to be resistant to the prevalent race (s) of Xoo. Hence, Kari Bhattha may be used as a donor for imparting BLB resistance in otherwise BLB susceptible varieties. As Kari Bhattha was found to provide strong resistance to BLB despite lacking Xa13, it needs to be studied in detail to look for the presence of novel BLB resistance genes present if any. The present investigation has also proved the importance of Xa13 which should be invariably used in addition to Xa5, *Xa21* and *Xa38* in BLB resistance breeding programs. Further, it is learnt from this investigation that mere presence of maximum number of resistance genes does not provide resistance to BLB. However, it is the presence of specific genes that plays greater role in conferring resistance to BLB.

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