© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 14; Issue 01; pp 001–007

Open Access
ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Phenotypic and Genotypic Assessment of Rice Germplasm for Bacterial Blight Resistance

Aparna V. S.*[1], Raji P.[2], Sumiya K.V.[3] and Vidhu Francis Palathingal[4]

[1] College of Agriculture, Vellanikkara, Thrissur - 680 656, Kerala, India
[2,4] Regional Agricultural Research Station, Pattambi - 679 306, Kerala, India
[3] Krishi Vigyan Kendra, Palakkad - 679 306, Kerala, India

Abstract

Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice. Development of resistant varieties is the most economical and environmentally friendly strategy for the management of the disease. Phenotypic screening of 50 rice germplasm accessions from South India were carried out by artificial inoculation using three virulent isolates of bacterial blight pathogen. Two accessions were moderately resistant against two Xoo isolates, (Xoo13 and Xoo63). None of the accessions tested were resistant or moderately resistant to the most virulent isolate Xoo57. Out of the 50 accessions six were moderately resistant to Xoo 63 and two accessions were moderately resistant to Xoo13. All other accessions were moderately susceptible to highly susceptible in nature. The genotypic survey was carried out using molecular markers linked to Xa4, xa5 and xa13 and Xa21 genes *viz*., MP, RM 122 xa13 prom and pTA 248. No amplicons specific to xa13 and xa21 allele were detected showing the absence of these two genes in the germplasm screened. 25 accessions amplified 150 bp size fragments specific to Xa4 gene. 20 accessions amplified 240 bp fragments specific to xa5 gene. 10 accessions carry Xa4 and xa5 genes in homozygous condition. Among the two accessions phenotypically moderately resistant to two isolates of the pathogen, one contains only xa5 gene and other does not carry any of these four genes studied. Further search for other genes contributing resistance is needed. The accessions having moderate resistance carrying Xa4 and xa5 genes could be utilized for the development of bacterial blight resistant varieties through molecular breeding.

***Key words*:** Rice, Bacterial blight, Resistance genes, Germplasm screening, *Xanthomonas oryzae* pv. *oryzae*, Molecular markers

Bacterial Blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most devastating disease of rice all over the world. The disease affects the rice crop resulting in two major symptoms viz., *kresek* (wilting) and leaf blight. The *kresek* is early systemic infection. In severe case the affected hills completely wilts. The leaf blight is the most seen symptom. The symptom starts as water-soaked lesions from the tip of the leaves and increases in length downwards. The lesions at the initial stages are pale green in colour which later turn into yellow to straw-coloured stripes with wavy margins. In severe stages the entire leaves turn whitish or straw coloured. The yield loss of 10 to 20 per cent are common. Yield losses ranging from 30 to 90 per cent have been reported depending upon the variety, stage of the crop and climatic conditions [1-5]. In India, bacterial blight is considered as a serious production constraint especially in irrigated and rainfed lowland ecosystem. The disease is major problem in *kharif* season in rice growing areas of Punjab, Haryana, Uttaranchal, Bihar, West Bengal, Tripura, Assam, Tamil Nadu, eastern Uttar Pradesh, coastal areas of Andhra Pradesh, Andaman Nicobar Islands, Kerala and parts of Maharashtra, Chhattisgarh, Gujarat, Himachal Pradesh, and Karnataka [6]. Rice is the major crop of Kerala State of India. Rice varieties predominantly cultivated such as Uma and Jyothi are highly susceptible to bacterial blight disease. In the state after the floods occurred during the years 2018 and 2019, the spread of the disease has increased in an alarming rate.

In view of the limited success of the other management practices the most effective, economical and environmentally friendly approach is the development of resistant varieties [7]. Until now forty-seven bacterial blight resistant genes conferring

Received: 25 Oct 2022; Revised accepted: 12 Dec 2022; Published online: 02 Jan 2023

Correspondence to: Aparna V. S., College of Agriculture, Vellanikkara, Thrissur - 680 656, Kerala, India, Tel: +91 9400462342; E-mail: aparnavs.13@gmail.com

Citation: Aparna VS, Raji P, Sumiya KV, Palathingal VF. 2023. Phenotypic and genotypic assessment of rice germplasm for bacterial blight resistance. *Res. Jr. Agril Sci*. 14(1): 01-07.

resistance to *Xoo* have been identified from various rice cultivars, wild relatives of rice, and mutation populations [8-18]. Large scale and long-term cultivation of rice varieties carrying a single resistance gene result in breakdown of resistance due to high pathogen variability [3], [6], [19]. Incorporation of two or more genes of bacterial blight resistance can enhance the durability of resistance [20-21].

The effectiveness of resistance genes varies over locations due to the geographical structuring of the pathogen. There is a huge potential of utilizing the untapped sources of resistance from the germplasm for development of disease resistant rice varieties with other quality parameters. Utilizing different sources of resistance for introgression of the traits will be useful for broadening the genetic base rather than utilizing the same source. Several researchers have studied the local germplasm for the identification of multiple bacterial blight resistance genes [22-25]. An attempt was made in this study to identify sources of bacterial blight resistance from the germplasm comprising local landraces and other cultivars of rice maintained at the Regional Agricultural Research Station, Pattambi, Kerala, India.

MATERIALS AND METHODS

Plant materials

50 germplasm accessions of rice obtained from germplasm collection maintained at the Regional Agricultural Research Station, Pattambi, Kerala were used for the study. Along with these, IR24 as well as local susceptible variety Jyothi were also included.

Pathogen isolates (Xoo)

Three virulent isolates of *Xanthomonas oryzae* pv. *oryzae* viz., Xoo 13, Xoo 57 and Xoo 63 representing three different rice growing areas of the state, selected from the virulence analysis studies were used.

Phenotypic screening by artificial inoculation

Seeds of 50 germplasm accessions as well as IR24 and Jyothi, susceptible local variety were sown in pots and grown in glass house. 20 days old seedlings were transplanted to pots filled with potting mixture. Three plants were planted in each pot and three replications were maintained. Other cultural practices as per recommendations were followed. Plants were inoculated 40 days after transplanting with individual isolates of Xoo separately adopting clip inoculation [26]. The leaf tips were cut off by using sterilized scissors dipped in bacterial suspension containing 10^8 CFU/ml. The observations were recorded 15 days after inoculation as per Standard Evaluation System scale of IRRI (2013) [27]. The plants were categorised based on the diseased leaf area of top four leaves per plant as Resistant with score 1 (1-5%), moderately resistant with score 3 (6-12%), moderately susceptible with score 5 (13-25%), susceptible with score 7 (26-51%) and highly susceptible with score 9 (51-100%).

Genotypic screening for bacterial blight resistance
Plant materials

Seeds of 50 germplasm accessions along with positive resistant checks IRBB4, IRBB5, IRBB13, IRBB21 carrying Xa4, xa5, xa13, Xa21 and susceptible check IR24 were sown in pots. Plants were maintained in glass house.

Extraction of genomic DNA

Leaf samples were collected from 21 days old seedlings. DNA was extracted following CTAB method [28]. The quantity of DNA was checked performing electrophoresis using 0.8% agarose gel. DNA was quantified using spectrophotometer by measuring A260/A280. The total genomic DNA was dissolved in 100 µL sterile nuclease free water and stored at -20 °C for further use.

PCR amplification

PCR amplification was carried out using SSR/STS markers synthesized by IDT, USA to analyze the status of BB resistance genes (Table 1). Amplification was carried out in a reaction mixture of 20µL containing 30 ng of genomic DNA, 0.25 mM PCR buffer (GeNei™), 2.5µM dNTPs (GeNei™), 3U of Taq DNA polymerase (GeNei™) and 100 µM primer using a thermal cycler (Mastercycler Gradient, Eppendorf). The thermal cycling program involved an initial denaturation at 94 °C for 4 min, followed by 35 Cycles of denaturation at 94 °C for 1min, annealing at 2 °C below the Tm of the respective primers for 1min, and primer extension at 72 °C for 1min, followed by a final extension at 72 °C for 7 min. The amplified PCR products with a 100 bp DNA marker ladder (GeNei™) were size fractioned by electrophoresis in 2% agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/mL) in a gel documentation system (Bio-Rad, USA). The rimers used for the study are given in (Table 1).

Table 1 Molecular markers used for the identification of bacterial blight resistance genes

Resistance gene	Marker	Primer sequence	Reference
Xa4	MP	ATCGATCGATCTTCACGAGG TGCTATAAAAGGCATTCGGG	[29-31]
xa5	RM 122	GAGTCGATGTAATGTCATCAGTGC GAAGGAGGTATCGCTTTGTTGGAC	[29]
xa13	Xa13 prom	GGCCATGGCTCAGTGTTTAT GAGCTCCAGCTCTCCAAATG	[32]
Xa21	pTA 248	AGACGCGGAAGGGTGGTTCCCGGA AGACGCGGTAATCGAAAGATGAAA	[33]

RESULTS AND DISCUSSION

Phenotypic resistance of rice germplasm accessions to Xanthomonas oryzae pv. oryzae

50 germplasm accessions along with susceptible check IR 24 and local susceptible variety Jyothi were screened against three virulent isolates of bacterial blight pathogen viz., Xoo 13, Xoo 57 and Xoo 63 following artificial inoculation. The results are given in (Table 2). All genotypes showed the symptoms of bacterial blight upon artificial inoculation. The symptom initiated as water-soaked lesion from the cut ends of the leaf blade extending downwards. Out of 50 accessions screened against Xoo 13 isolates two were moderately resistant (score 3), 21 were moderately susceptible (score 5), 26 were susceptible (score 7) and one accession was highly susceptible (score 9). Among the 50 accessions screened against Xoo 57 none were resistant or moderately resistant. Four accessions were moderately susceptible 39 were susceptible and 7 were highly susceptible. Out of the 50 accessions six accessions showed moderate resistance, 12 were moderately susceptible and 27

accessions were susceptible and five were highly susceptible to the strain Xoo 63.

Table 2 Reaction of rice germplasm accessions against *Xanthomonas oryzae* pv. *oryzae* isolates

Germplasm accessions	Xoo 13	Xoo57	Xoo 63
Eruvakkali	7	7	5
Mandupakki	7	7	5
Mangalapuram	7	7	7
Chenkayama (Ambalapara)	5	7	5
Ponmani	7	7	7
Chettivirippu	7	9	7
Vellapokkali	7	9	7
Virippu	5	7	9
Bolamgittikayama	5	7	7
Vellakkayama	7	7	5
Mundakan	7	7	5
Anakkodan	9	7	5
Cheriya orpandy	7	9	7
Gandhasala (1)	5	7	7
Parambuvattan	7	7	7
Champan	5	5	7
Gandhasala (2)	5	7	3
Jeerakasala	5	7	5
Kokkankoli	5	9	5
Pandi Champan	7	7	9
Kalladiaryan (Red rice)	7	7	9
Kothambalarikayama (1)	5	7	3
Njavara (Black)	7	9	7
Mundon (Cheruli)	5	7	3
Veliyan (1)	7	9	7
Basmati	5	5	7
Krishnakamod	3	7	3
Karutha njavara	5	7	9
Kalluruli upland	5	7	7
Chitteni (Alathur)	7	7	7
Wayanad 2	5	7	3
Black Chitteni (Thavanur)	7	7	7
Chembavu	7	7	7
Cheruvellari	5	9	7
Ithikandan	7	7	7
Kariyadukkan	7	7	7
Kokkan	5	7	7
Kothambalarikayama (2)	7	7	5
Kunnamkulamban	5	7	7
Kuruva	5	7	7
Mallimatta	5	7	7
Mannuveliyan	7	7	5
Marathondi	5	7	5
Mullankayama	7	7	7
Mundon	7	7	7
Odiyan	7	7	7
Ottadi	3	5	3
Thondi	7	7	7
Veliyan (2)	7	5	5
Vellari	5	7	7
Jyothi	9	9	9
IR24	9	9	9

Out of the 50 germplasm accessions, six accessions viz., Gandhasala (2), Kothambalarikayama (1), Mundon (Cheruli), Krishnakamod, Wayanad 2 and Ottadi were moderately resistant against the isolate Xoo 63. Among these, two accessions Krishnakamod and Ottadi were moderately resistant to the isolate Xoo 13 too. None of these accessions were resistant to Xoo 57. Ottadi showed moderately susceptible reaction and Krishnakamod was susceptible reaction to this isolate.

Genotypic screening of germplasm accessions for bacterial blight resistance

50 landraces from germplasm collection were screened for the presence/absence of four bacterial blight resistance genes Xa4, xa5, xa13 and Xa21 using PCR based markers MP, RM 122, xa13 prom and pTA 248 linked to these genes. The resistant checks IRBB4, IRBB5, IRBB13 and IRBB21 and susceptible check IR24 were also included. PCR results were analyzed by visualization of amplicons corresponding to each gene. The amplicon size corresponding to positive and negative controls were 150 bp and 120 bp for Xa4, 240 bp and 230 bp for xa5, 450 bp and 220 bp for xa13 and 950 bp and 725 for Xa21 respectively. The results of genotypic screening of 50 germplasm accessions are presented in (Table 3) and electrophoretic patterns of molecular markers linked to bacterial blight resistance genes are shown in Figure 1a-1d and 2a-2d.

The amplicons specific to Xa21 and xa13 alleles were not detected indicating the absence of these genes in any of the germplasm accessions except in positive controls IRBB21 and IRBB13. Amplicons of size 150 bp corresponding to the marker MP linked to Xa4 gene were detected in 25 accessions indicating the presence of this gene. In five accessions showed the resistant alleles in heterozygous pattern. In 17 accessions amplicon corresponding to the linked marker was absent indicating the absence of Xa4 gene (Table 2, Fig 1a-d).

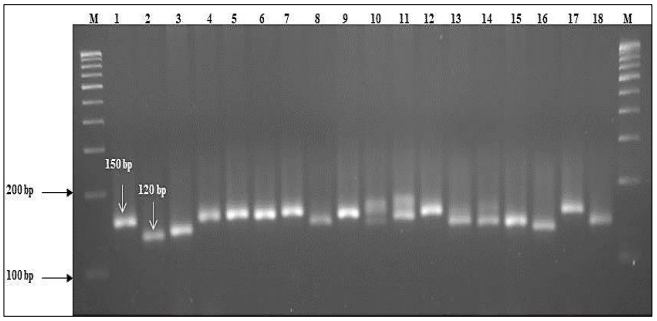

Fig 1a Banding patterns showing the presence and absence of Xa4 gene in germplasm accessions of rice amplified at 150 bp and 120 bp size fragments respectively

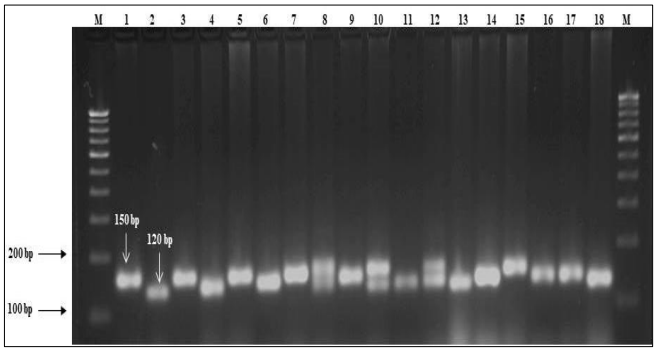

Fig 1b Banding patterns showing the presence and absence of Xa4 gene in germplasm accessions of rice amplified at 150 bp and 120 bp size fragments respectively

Lane M = 100 bp DNA ladder, lane 1 = IRBB4, lane 2. IR24, lane 3 = Eruvakkali, lane 4 = Mandupakki, lane 5 = Mangalpuram, lane 6 = Chenkayama (Ambalapara), lane 7 = Ponmani, lane 8 = Chettivirippu, lane 9 = Vellapokkali, lane 10 = Virippu, lane 11 = Bolamgittikayama, lane 12 = Vellakkayama, lane 13 = Mundakan, lane 14 = Anakkodan, lane 15 = Cheriya orpandy, lane 16 = Gandhasala (1), lane 17 = Parambuvattan, lane 18 = Champan, lane M = 100 bp DNA ladder

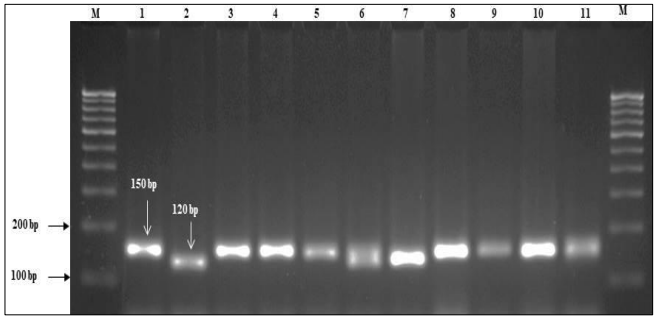

Fig 1c Banding patterns showing the presence and absence of Xa4 gene in germplasm accessions of rice amplified at 150 bp and 120 bp size fragments respectively

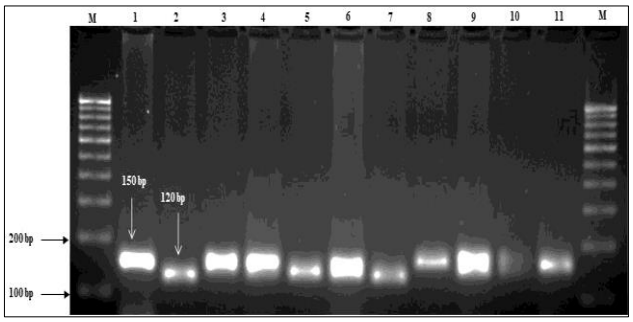

Fig 1d Banding patterns showing the presence and absence of Xa4 gene in germplasm accessions of rice amplified at 150 bp and 120 bp size fragments respectively

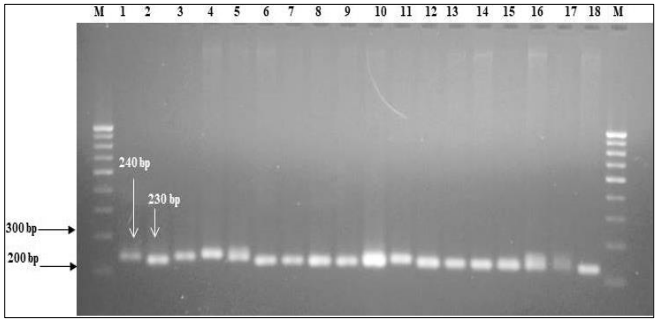

Fig 2a Banding patterns showing the presence and absence of Xa5 gene in germplasm accessions of rice amplified at 240 bp and 230 bp size fragments respectively

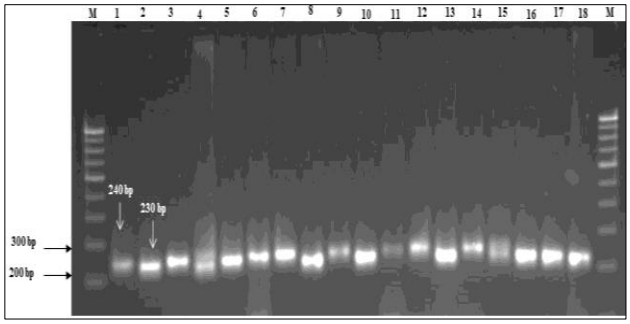

Fig 2b Banding patterns showing the presence and absence of Xa5 gene in germplasm accessions of rice amplified at 240 bp and 230 bp size fragments respectively

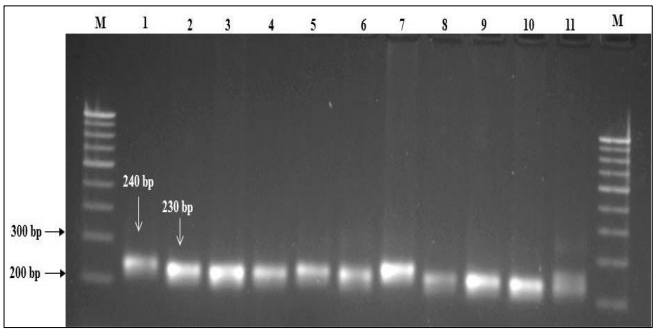

Fig 2c Banding patterns showing the presence and absence of Xa5 gene in germplasm accessions of rice amplified at 240 bp and 230 bp size fragments respectively

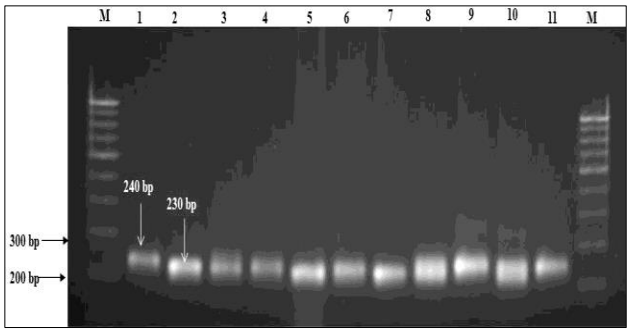

Fig 2d Banding patterns showing the presence and absence of Xa5 gene in germplasm accessions of rice amplified at 240 bp and 230 bp size fragments respectively

Lane M = 100 bp DNA ladder, lane 1 = IRBB5, lane 2 = IR24, lane 3 = Mundon, lane 4 = Thondi, lane 5 = Odiyan, lane 6 = Mannuveliyan, lane 7 = Ottadi, lane 8 = Veliyan (2), lane 9 = Vellari, lane 10 = Chembavu, lane 11 = Kunnamkulamban, lane M = 100 bp DNA ladder

The SSR marker RM122 amplified 240 bp sized fragment in positive check and 230 bp sized fragment in negative control (IR24). Out of the 50 germplasm accessions 21 accessions were positive for the presence of 240 bp amplicon indicating the presence of xa5 gene. In two accessions both resistant and susceptible alleles were present and 22 accessions showed the presence of susceptible allele (Table 2, Fig 2a-d).

10 accessions showed the presence of resistant alleles corresponding to Xa4 and xa5 genes. These include Mandupakki, Ponmani, Gandhasala (2), Kothambalarikayama 1 and Kothambalarikayama 2, Njavara (Black), Chembavu, Kunnamkulamban, Veliyan (2) and Vellari. Five accessions viz., Vellapokkali, Virippu, Bolamgittikayama, Kothambalarikayama (1), Mundon (Cheruli) carry either Xa4 or xa5 genes or both together in heterozygous condition. These germplasm accessions carrying single or combination of Xa4 and xa5 genes could be used as donors for these genes in breeding programme (Table 3, Fig 1a-d, 2a-d).

The present study was carried out to evaluate rice germplasm for bacterial blight resistance. 50 accessions of germplasm evaluated phenotypically against three virulent isolates of *Xanthomonas oryzae* pv *oryzae*. Among the 50 accessions screened, two accessions viz., Krishnakamod and Ottadi were moderately resistant to the isolate Xoo 13 of *Xanthomonas oryzae* pv *oryzae* with the score of 3. None of the accessions screened were resistant or moderately resistant to the most virulent isolate Xoo 57. The accession Ottadi showed moderate susceptibility with score 5 to this isolate. These two accessions Krishnakamod and Ottadi showed moderate resistance to the isolate Xoo 63 also. Four other accessions Gandhasala, Mundon (Cheruli), Kothambalarikayama (1) and Wayanad 2 were moderately resistant to the bacterial blight pathogen isolate Xoo 63. Many of cultivars/ landraces available in the germplasm are potential sources of resistance to diseases. Unless we know the trait qualities, we would not be able to utilize these directly for cultivation or to utilize for breeding purpose. In

the present study none of the accessions were resistant to bacterial blight phenotypically under artificial inoculation. However, the accessions with moderate resistance under artificial inoculation particularly the accessions which showed moderate resistance to more than one virulent isolate (Krishnakamod and Ottadi) could be utilized as a source for resistance to bacterial blight for the improvement of existing high yielding varieties for bacterial blight resistance or to develop new bacterial blight resistant varieties. Several researchers located landraces/ cultivars/ wild rice accessions with resistance/ tolerance to bacterial blight earlier [23-25], [34-36]. The germplasm accessions identified in this study would be of specifically useful for the state of Kerala where bacterial blight is a severe problem. By understanding the phenotypic resistance, these germplasm accessions could be utilized for cultivation directly after their performance evaluation or for conventional breeding for disease resistance.

Table 3 Status of bacterial blight resistance genes in germplasm accessions

Germplasm accessions	Xa4	xa5	xa13	Xa21
Eruvakkali	-	-	-	-
Mandupakki	+	+	-	-
Mangalapuram	+	-	-	-
Chenkayama (Ambalapara)	+	-	-	-
Ponmani	+	+	-	-
Chettivirippu	-	+	-	-
Vellapokkali	+	+ -	-	-
Virippu	+ -	+ -	-	-
Bolamgittikayama	+ -	-	-	-
Vellakkayama	+	-	-	-
Mundakan	-	+	-	-
Anakkodan	-	+	-	-
Cheriya orpandy	-	+	-	-
Gandhasala (1)	-	+	-	-
Parambuvattan	+	-	-	-
Champan	-	+	-	-
Gandhasala (2)	+	+	-	-
Jeerakasala	-	+	-	-
Kokkankoli	+	-	-	-
Pandi Champan	-	-	-	-
Kalladiaryan (Red rice)	+	-	-	-
Kothambalarikayama (1)	+ -	+	-	-
Njavara (Black)	+	+	-	-
Mundon (Cheruli)	+ -	+	-	-
Veliyan (1)	-	-	-	-
Basmati	+ -	-	-	-
Krishnakamod	-	+	-	-
Karutha njavara	-	-	-	-
Kalluruli upland	+	-	-	-
Chitteni (Alathur)	-	-	-	-
Wayanad 2	-	-	-	-
Black Chitteni (Thavanur)	-	+	-	-
Chembavu	+	+	-	-
Cheruvellari	+	-	-	-
Ithikandan	+	-	-	-
Kariyadukkan	+	-	-	-
Kokkan	+	-	-	-
Kothambalarikayama (2)	+	+	-	-
Kunnamkulamban	+	+	-	-
Kuruva	+	-	-	-
Mallimatta	+	-	-	-
Mannuveliyan	+	-	-	-
Marathondi	+	-	-	-
Mullankayama	+	-	-	-
Mundon	+	-	-	-
Odiyan	-	-	-	-
Ottadi	-	-	-	-
Thondi	+	-	-	-
Veliyan (2)	+	+	-	-
Vellari	+	+	-	-
IR24	-	-	-	-

Conventional breeding is time consuming and laborious tool for gene pyramiding. To get broad spectrum durable resistance against bacterial blight, incorporation of more than one BB resistance genes is more desirable [20], [37-38]. To achieve this marker assisted breeding is an efficient tool. For this approach donors with known resistance genes are essential. Once the resistance genes in our landraces are identified, these can be further utilized for molecular breeding. In this study, we report the resistance spectrum of 50 germplasm accessions and the status of bacterial blight resistance genes Xa4, xa5, xa13 and Xa21.

The effectiveness of these genes in various combinations for enhancing bacterial blight resistance has already been reported by several workers. The high yielding rice variety Jalmagna was improved by incorporating three resistance genes, xa5, xa13 and Xa21 [39]. Four resistance genes Xa4, xa5, xa13 and Xa21 were pyramided to a popular variety Ranidhan and developed a variety with broad spectrum resistance to bacterial blight [40]. Improvement of rice variety Basmati 385 was done by pyramiding of Xa4, xa5, xa13 and Xa21 genes by [41].

Among the germplasm accessions of rice identified with moderate phenotypic resistance to bacterial blight pathogen isolates, the accession namely Krishnakamod carry only xa5 gene. In Ottadi none of these genes were present. This suggests the contribution of some other genes also towards the phenotypic resistance shown by these varieties. To utilize these two accessions for molecular breeding further search for other genes is required. Among the other accessions exhibited moderate resistance to other isolate Xoo 63 possess two resistance genes surveyed viz., Gandhasala (Xa4 and xa5), Mundon (Cheruli) (Xa4 (±) and xa5) and Kothambalarikayama (1) (Xa4 (±) and xa5). The accession Wayanad 2 which showed phenotypic moderate resistance to the isolate Xoo 63 does not carry any of these four genes. So as in Ottadi this also may harbour source other resistance genes. These accessions having moderate phenotypic resistance along with known resistance genes could be utilized as potential donors for bacterial blight resistance breeding. Some of the accessions which apparently did not show resistance reaction also carries Xa4 and xa5 genes such as Mandupakki, Ponmani, Gandhasala (2), Vellapokkali, Njavara (black), Chembavu, Kunnumkulamban, Veliyan (2) and Vellari. This may be due to the wide range of genetic background. Similar results of occurrence of one or more R genes in moderately susceptible germplasm accessions were earlier reported [25], [27]. None of the genotypes screened had Xa21 and xa13 genes [35]. The presence of Xa21 gene in germplasm is very rare as the gene was introgressed originally from wild rice, *Oryza longistaminata* [11].

CONCLUSION

Phenotypic screening of 50 germplasm accessions for bacterial blight resistance revealed the moderate resistance of two accessions Krishnakamod and Mundon to two isolates of the bacterial blight pathogen viz., Xoo 13 and Xoo 63. Six accessions viz., Gandhasala (2), Kothambalarikayama (1) Mundon (Cheruli), Krishnakamod, Wayanad 2 and Ottadi were moderately resistant to X00 63. None of the accessions showed resistance to the isolate Xoo 57. The genotypic survey using linked markers, to locate the bacterial blight resistance genes showed the presence of Xa4 and xa5 genes in 10 accessions. Xa4 gene alone was present in 25 accessions. 20 accessions carry xa5 gene. None of the accessions possess xa21 and xa13 genes. The information on phenotypic resistance status and the presence of R genes for bacterial blight resistance would be useful for the utilization of these germplasm pool for future breeding programmes.

Acknowledgement

The study forms a part of the Ph. D. thesis work carried out by the first author. Authors acknowledge the laboratory and field facilities and rice germplasm provided by the Regional Agricultural Research Station, Pattambi for the conduct of the study.

LITERATURE CITED

1. Raina GL, Sidhu GS, Saini PK. 1981. Rice bacterial blight status in Punjab, *India. Rev. Plant Pathology* 61: 49-62.
2. Mew TW. 1987. Current status and prospects of research on bacterial blight of rice. *Annu. Rev. Phytopathology* 25(1): 359-382.
3. Mew TW, Cruz V, Medalla ES. 1992. Changes in race frequency of *Xanthomonas oryzae pv.oryzae* in response to rice cultivars planted in the Philippines. *Plant Disease* 78: 1029-1032.
4. Srinivasan B, Gnanamanickam SS. 2005. Identification of a new source of resistance in wild rice, *Oryza rufipogon* to bacterial blight of rice caused by Indian strains of *Xanthomonas oryzae* pv. *oryzae*. *Current Science* 88(8): 1229-1231.
5. Kumar PN, Sujatha K, Laha GS, Rao KS, Mishra B, Viraktamath BC, Hari Y, Reddy CS, Balachandran SM, Ram T, Madhav MS. 2012. Identification and fine-mapping of Xa33, a novel gene for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Phytopathology* 102(2): 222-228.
6. Laha GS, Reddy CS, Krishnaveni D, Sundaram RM, Prasad MS, Ram T, Muralidharan K, Viraktamath BC. 2009. *Bacterial Blight of Rice and its Management.* Technical Bulletin No. 41, Directorate of Rice Research, Hyderabad, Andhra Pradesh, India. pp 37.
7. Patil B, Karegowda C, Narayanaswamy H, Wasimfiroz M. 2017. Biochemical variation among isolates of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight in rice. *Int. Jr. Commun. System* 5(6): 1265-1268.
8. Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q. 1996. Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. *Phytopathology* 86: 1156-1159.
9. Nagato Y, Yoshimura A. 1998. Report of the committee on gene symbolization, nomenclature and linkage groups. *Rice Genet. Newsletter* 15: 13-74.
10. Zhang S, Song WY, Chen L, Ruan DL, Taylor N, Ronald PC, Beachy R, Fauquet C. 1998. Transgenic elite indica rice varieties, resistance to *Xanthomonas oryzae* pv. *oryzae*. *Mol. Breeding* 4: 551-558.
11. Khush GS, Angeles ER. 1999. A new gene for resistance to race 6 of bacterial blight in rice, *Oryza sativa* L. *Rice Genet. Newsletter* 16: 92-93.
12. Chen H, Wang S, Zhang Q. 2002. New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. *Phytopahtology* 92: 750-754.

13. Lee KS, Rasabandith S, Angeles ER, Khush GS. 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology* 93: 147-152.
14. Tan GX, Ren X, Weng QM, Shi ZY, Zhu LL, He GC. 2004. Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. *Acta Genetica Sinica* 431: 724-729.
15. Xiang Y, Cao YL, Xu CQ, Li XH, Wang SP. 2006. Xa3, conferring resistance for rice bacterial blight and encoding a receptor kinase–like protein, is the same as Xa26. *Theor. Appl. Genetics* 113: 1347-1355.
16. Busungu C, Taura S, Sakagami JI, Ichitani K. 2016. Identification and linkage analysis of a new rice bacterial blight resistance gene from XM14, a mutant line from IR24. *Breeding Science* 66(4): 636-645.
17. Chen S, Wang C, Yang J, Chen B, Wang W, Su J, Feng A, Zeng L, Zhu X, 2020. Identification of the novel bacterial blight resistance gene Xa46 (t) by mapping and expression analysis of the rice mutant H120. *Science Reporter* 10(1): 1-11.
18. Xing J, Zhang D, Yin F, Zhong Q, Wang B, Xiao S, Ke X, Wang L, Zhang Y, Zhao C, Lu Y. 2021. Identification and fine-mapping of a new bacterial blight resistance gene, Xa47 (t), in G252, an introgression line of Yuanjiang common wild rice (*Oryza rufipogon*). *Plant Disease* 105(12): 4106-4112.
19. Shanti LM, Kumar VM, Premalatha P, Devi GL, Zher U, Freeman W. 2010. Understanding the bacterial blight pathogen-combining pathotyping and molecular marker studies. *Int. Jr. Plant. Pathology* 1: 58-68.
20. Sundaram RM, Vishnupriya MR, Biradar SK, Laha GS, Reddy AG, Rani NS. 2008. Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite *Indica* rice variety. *Euphytica* 160: 411-422.
21. Hajira SK, Yugander A, Balachiranjeevi CH, Pranathi K, Anila M, Mahadevaswamy HK. 2014. Development of durable bacterial blight resistant lines of Samba Mahsuri possessing *Xa33*, *Xa21*, *Xa13* & *Xa5*. *Prog. Research* 9: 1224-1227.
22. Amgai RB, Niroula RK, Pantha S, Hamal SS, Tamang BG, Sah BP, Bhatta MR. 2015. Marker assisted screening of nepalese rice for bacterial leaf blight (BLB) resistance. *Nepal Jr. Biotechnology* 3(1): 35-39.
23. Yadav S, Singh A, Goel N, Singh AK. 2013. Identification of Indian rice germplasm lines with bacterial leaf blight (BLB) resistance genes. *Indian Jr. Genet Plant Breeding* 73(3): 310-313.
24. Banerjee A, Roy S, Bag MK, Bhagat S, Kar MK, Mandal NP, Mukherjee AK, Maiti D. 2018. A survey of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in rice germplasm from eastern and northeastern India using molecular markers. *Crop Protection* 112: 168-176.
25. Zhao C, Yin F, Chen L, Li D, Xiao S, Zhong Q, Wang B, Ke X, Fu J, Li X, Chen Y. 2022. Identification of bacterial blight resistance genes in rice landraces from Yunnan Province, China. *Aust. Plant Pathology* 51(1): 59-69.
26. Kauffman HE, Reddy APK, Hsieh SPY, Merca SD. 1973. An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae* pv. *oryzae*. *Plant Dis. Rep*. 57: 537-541.
27. IRRI. 2013. *Standard Evaluation System for Rice*. (5th Edition). International Rice Research Institute (IRRI). Manila, Philippines.
28. Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 39-40.
29. Chen X, Temnykh S, Xu Y, Cho YG, Mc Couch SR. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genetics* 95(4): 553-567.
30. Ma BJ, Wang WM, Zhao B, Zhou YL, Zhu LH, Zhai WX. 1999. Study on the PCR marker for the rice bacterial blight resistance gene Xa4. *Hereditas* 21(3): 9.
31. Mc Couch SR, Teytelman L, Xu Y, Lobos KB, Clare K, Walton M, Fu B, Maghirang R, Li Z, Xing Y, Zhang Q. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research* 9(6): 199-207.
32. Zhang G, Angeles ER, Abenes MLP, Khush GS, Huang N. 1996. RAPD and RFLP mapping of the bacterial blight resistance gene *xa13* in rice. *Theor. Appl. Genetics* 93: 65-70.
33. Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. *Mol. Gen. Genetics* 236(1): 113-120.
34. Majumder K, Mondal SI, Mallick R, Dasgupta T. 2020. Identification of BLB resistant genes in some rice varieties for development of high yielding bacterial leaf blight tolerant types. *Jr. Environ. Biol*. 41(1): 85-91.
35. Singh AK, Dharmraj E, Nayak R, Singh PK, Singh NK. 2015. Identification of bacterial leaf blight resistance genes in wild rice of eastern India. *Turk. Jr. Botany* 39(6): 1060-1066.
36. Thimmegowda PR, Ambika DS, Manjunatha L, Arun RS, Prasad PS, Chandrashekar M. 2011. Screening germplasm for resistance to bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*. *Int. Jr. Sci. Nat.* 2(3): 659-661.
37. Rajpurohit D, Kumar R, Kumar M, Paul P, Awasthi A, Osman Basha P, Puri A, Jhang T, Singh K, Dhaliwal HS. 2011. Pyramiding of two bacterial blight resistance and a semi dwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* 178(1): 111-126.
38. Dasari A, Vemulapalli P, Gonuguntla R, Thota DK, Elumalai P, Muppavarapu K, Butam LP, Kulkarni SR, Sinha P, Gunukula H, Kale RR. 2022. Improvement of bacterial blight resistance of the popular variety, Nellore Mahsuri (NLR34449) through marker-assisted breeding. *Journal of Genetics* 101(1): 1-11.
39. Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E, Lenka S, Anandan A. 2015.Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deep water rice variety, Jalmagna. *Rice* 8(1): 1-14.
40. Pradhan KC, Pandit E, Mohanty SP, Moharana A, Sanghamitra P, Meher J, Jena BK, Dash PK, Behera L, Mohapatra PM, Bastia DN. 2022. Development of broad spectrum and durable bacterial blight resistant variety through pyramiding of four resistance genes in rice. *Agronomy* 12(8): 1903. https://doi.org/10.3390/agronomy12081903
41. Ullah I, Ali H, Mahmood T, Khan MN, Haris M, Shah H, Mihoub A, Jamal A, Saeed MF, Mancinelli R, Radicetti E. 2022. Pyramiding of four broad spectrum bacterial blight resistance genes in cross breeds of Basmati rice. *Plants* 12(1): 46. https://doi.org/10.3390/plants12010046