Short Research Article

SCREENING FOR BACTERIAL LEAF BLIGHT RESISTANT GENES IN SOME RICE GENOTYPES USING SSR MARKERS

ABSTRACT

Bacterial leaf blight disease plays a major detrimental effect in the yield and quality of Rice (*Oryza sativa*). To overcome the problem, finding bacterial leaf blight resistant gene is very crucial. In this study, identification of bacterial leaf blight resistant genes was attempted among 27 rice genotypes including two control genotypes using three Simple Sequence Repeats markers tagged with Bacterial leaf blight genes which are *Xa4, xa5* and *xa13*. As a result, six genotypes, MTU1010, IR 68144-2B-2-2-3-1-127, ARC-10086, Mali 4, IR 64 and Kalinga-2, were classified as resistant to the disease due to the presence of bands (150bp, 160bp and 190bp), of MP1, RM13 and RM264 markers which were tagged with *Xa4, xa5* and *xa13* genes respectively. Among the genotypes, 12 genotypes which were considered as susceptible and the other 7 genotypes showed moderate resistance or susceptible results. The identified resistant genotypes can be utilized as a donor for developing Bacterial leaf blight resistant rice varieties in breeding programme.

Keywords: Bacterial leaf blight, Marker, Rice, Resistant, Simple Sequence Repeats.

1. INTRODUCTION

Rice is the most popular cereal crop because of its extensive consumption as a food crop for human needs. More than 50% of the World's population depends on rice as the basic food material [1]. Rice is called the "monocot model species" [2]. Though, more than 36 fungal, 6 bacterial and 21 viral diseases of rice were found, but among them Bacterial Leaf Blight (BLB) disease is very harmful in nature [3,4]. The causative agent of BLB disease is *Xanthomonas oryzae* pv. *oryzae* which is transmitted through seed. As the symptoms of BLB disease, "Seedling wilt-Kresek" and "bacterial ooze of the cut ends symptom" can be observed. In both tropical and temperate regions, especially in the irrigated and rainfed lowland regions, rice plants are affected by BLB [5]. Approximately 50% yield loss happened in the world [6] and up to 81.3% yield loss happened in India as the effects of BLB [7,8].

Due to availability of the sequencing information on rice, the proper position of genes or Quantitative trait locus (QTL)s controlling resistant to biotic stress like BLB disease can be identified. Screening of the genotype using the BLB resistant molecular markers which are gene based or tightly linked is more effective than the screening based on morphological parameters [9]. About 40 genes resistant to BLB disease have been recognized [10], among which *Xa4*, *xa5*, *Xa7*, *xa13*, and *Xa21* are considered as the major resistant genes [11]. Among the BLB resistant genes, 20 genes [Xa1, Xa2, Xa3, Xa4, Xa6, Xa7, Xa9, Xa10,

Xa11, Xa12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22, Xa23(t), Xa25(t), Xa27(t) and Xa29(t)] were identified as dominant genes and 9 genes [xa5, xa8, xa13, xa15, xa19, xa20, xa24 (t), xa26(t) and xa28(t)] were identified as recessive genes. Availability of Simple Sequence Repeats (SSR) markers for *Xa4*, *xa5*, *xa13*, and *Xa21* were already reported [12]. The present study performed the screening of BLB resistant rice genotypes among 27 rice genotypes using three Simple Sequence Repeats (SSR) markers tagged with BLB resistant genes. These will help the breeders to select the BLB resistant genotypes.

2. MATERIALS AND METHODS

The seeds of 27 rice genotypes (Table 1) were collected from the Department of Genetic and Plant Breeding, RKMVERI, Narendrapur and the experiments were carried out at RKMVERI, Narendrapur in 2019. Seeds were kept for germination in BOD incubator (YOMA, Mfg by Indian INSTRUMENTS MANUFACTURING CO., Kolkata) at 25°C. The germinated seeds were placed in small pots where soil and cocopeat (1:1 ratio) were added and the plants were grown in the green house. From the three leaves stage, the DNA was isolated using CTAB (Cetyl trimethyl ammonium bromide) method [13], separated in 0.8% agarose gel, visualized and quantified using Gel documentation system (BIO-RAD) and Nanodrop (Thermo Scientific) respectively. The DNA was amplified using a 25 µl reaction mixture in a Polymerase chain reaction (PCR) tube containing 2.5 µl 10X Taq buffer, 0.2 µl each Forward and Reverse primer, 2 µl $MgCl_2$ (25 mM), 2 µl dNTPs, 0.2 µl Taq pol (3U/ µl) and 200 ng isolated rice genomic DNA on a Thermocycler machine (Eppendorf). The program of the thermocycler was- 5 minutes preheating at 95°C, 35 cycles of denaturation at 95°C for 30 seconds, 30 seconds primer annealing at specific temperature for particular primer pair (Annealing temperature of primer-1:MP1, Primer-2:RM13 and Primer-3:RM264 were 56.3°C, 56.6°C and 58.5°C respectively), 1 minute primer extension at 72°C and 5 minutes final extension at 72°C. The used SSR primers are Primer-1: MP1 [Gene name- *Xa4*, Chromosome No: 11, (Forward primer sequence- 5′-ATCGATCGATCTTCACGAGG-3′), (Reverse primer sequence- 5′-TCGTATAAAAGGCATTCGGG-3′)], Primer-2: RM13 [Gene name- *xa5*, Chromosome No: 5, (Forward primer sequence- 5′-TCCAACATGGCAAGAGAGAG-3′), (Reverse primer sequence- 5′-GGTGGCATTCGATTCCAG-3′)] and Primer-3: RM264 [Gene name- *xa13*, Chromosome No: 8, (Forward primer sequence- 5′-GTTGCGTCCTACTGCTACTTC-3′), (Reverse primer sequence- 5′-GATCCGTGTCGATGATTAGC-3′)] [14]. After separating the amplified product on 2% agarose gel, it was observed using Gel documentation system (BIO-RAD) and the band size was estimated based on the size of 50bp DNA ladder.

3. RESULTS AND DISCUSSION

IRBB 59 was considered as the BLB resistant control variety while the susceptible control variety was IR 24, which were identified earlier [15,16], these two varieties developed from The International Rice Research Institute (IRRI) were used for identification. The MP1 primer is linked with Xa4 gene, RM13 primer is linked with xa5 gene and the primer, RM264 is linked with xa13 gene. The observed band sizes of amplified PCR products of the resistant and susceptible control genotypes were different for individual markers. Bands of 150bp, 160bp and 190bp fragments exhibited for the resistant variety while 140bp, 130bp and 170bp fragments were observed for the susceptible variety with MP1, RM13 and RM264 primers, respectively (Fig. 1). By observing the band sizes, the BLB resistant genotypes among the 27 rice genotypes were identified, which were MTU 1010, IR68144-2B-2-2-3-1-127, ARC-10086, Mali 4, IR 64 and Kalinga-2, these genotypes had all the three resistant genes (*Xa4*, *xa5* and *xa13*) (Table. 1).

Table 1. Genotypic screening for bacterial leaf blight resistance of 27 rice genotypes.*

Sl, No.	Name of the Genotypes	Origin	Genotyping of resistance genes using SSR Markers			Remarks
			MP1	RM13	RM264	
1	IR 24 (Susceptible Control)	IRRI	140bp (Band size)	130bp (Band size)	170bp (Band size)	S
2	IRBB 59 (Resistant Control)	IRRI	150bp (Band size)	160bp (Band size)	190bp (Band size)	R
3	ARC 10086	Assam	+	+	+	R
4	Mali 4	Mali Agritech, Ranaghat	+	+	+	R
5	Kasalath	India	+	+	-	MR/MS
6	MTU 1010	ANGRAU, AP	+	+	+	R
7	Swarna	IRRI collab CRRI	+	+	-	MR/MS
8	Dular	Landrace	0	-	-	S
9	Azucena	Philippines	0	-	0	S
10	Swarna Sub-1	IRRI	0	-	0	S
11	Samba Mahsuri	ANGRAU	0	0	0	S
12	Lemont	Philippines	0	0	0	S

13	Restorer Line-51 (R-51)	Mali Agritech, Ranaghat	0	+	+	MR/MS
14	CN1646-2	Chinsurah, West Bengal	0	+	0	S
15	Sabita	Landrace	+	-	-	S
16	Ratna	India	+	0	0	S
17	Restorer Line (R-71)	Mali Agritech, Ranaghat	+	+	-	MR/MS
18	Patharea	Thane, Maharashtra	+	-	-	S
19	*Indica Javanica* (TC-25-2-1)	Mali Agritech, Ranaghat	+	+	-	MR/MS
20	*Indica Javanica* (TC-26-2-1)	Mali Agritech, Ranaghat	0	-	-	S
21	Nippon bare	Japan	-	-	+	S
22	IR 64	IRRI	+	+	+	R
23	IR 68144-2B-2-2-3-1-127	IRRI	+	+	+	R
24	Kalinga-2	CRRI,Cuttack	+	+	+	R
25	Danaguri	Local landrace, West Bengal	-	-	-	S

26	Zheshan-2	China	+	-	+	MR/MS
27	ARC 100372	Assam	+	-	+	MR/MS

** R = resistant, S = susceptible, + = presence of resistant band, - = presence of susceptible band, 0= no band, MR/MS= moderately resistant / moderately susceptible*

The effectiveness of Xa4, xa5, Xa7, xa13 and Xa21 genes which conferred resistance to the BLB disease in rice, were already reported [12,17,18]. The presence of Xa4, xa5, xa13 and Xa7 BLB resistant genes were previously found for IR 64, IR68144-2B-2-2-3-1-127 and Kalinga-2 [14] which was also confirmed in this research work based on Xa4, xa5 and xa13 genes. In this study, Dular, Azucena, Swarna Sub-1, Samba Mahsuri, Lemont, Danaguri, CN1646-2, Sabita, Ratna, Patharea, *Indica Javanica* (TC-26-2-1) and Nippon bare were classified as susceptible genotypes for exhibiting susceptible result. The other varieties exhibited intermediate result.

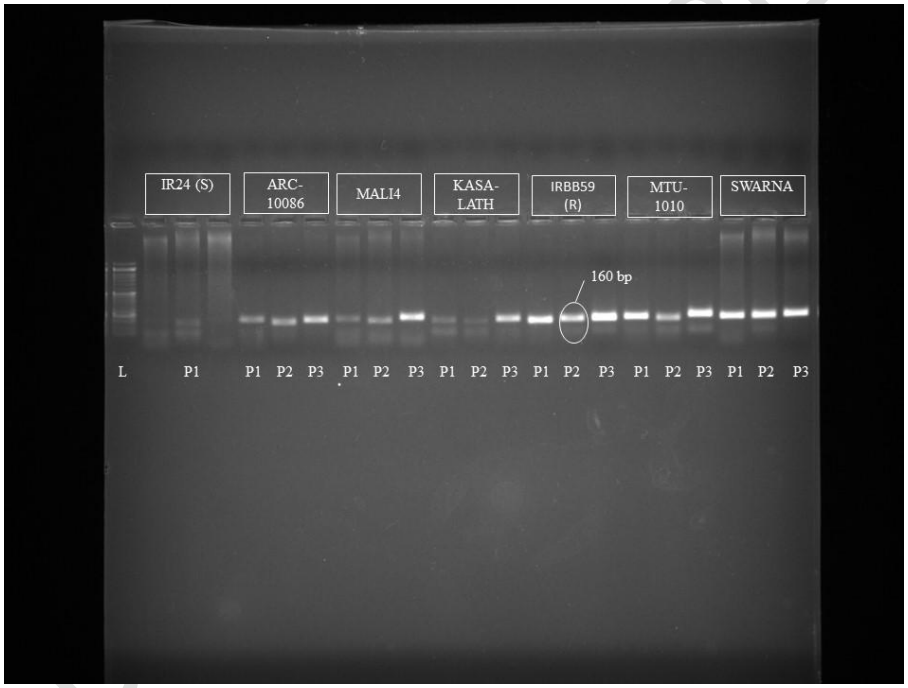

Fig. 1. Electrophoregram of different rice cultivars using three markers (where L= 50bp DNA ladder, P1= Primer-1: MP1, P2= Primer-2: RM13, P3= Primer-3: RM264)

In a previous study, where 34 rice cultivars were used for the identification of Xa4, xa13 and Xa21 genes, it was found that, only xa5 gene was present among 20 cultivars along with the resistant cultivar but xa13 and Xa21 were not present in all of the cultivars [19]. It was also observed that, the rice genotypes where no resistant gene was present, showed high susceptibility in green house condition. If more than one BLB resistant genes were present in the genotypes, then those genotypes became BLB resistant with the inoculums in green house condition. This observation stated that combination of resistant genes through gene

pyramiding will be effective for developing BLB resistant varieties [18]. In this study, presence of all three genes were found in the 6 genotypes and the resistant control genotype, so it can be said that the SSR markers, tagged with the three BLB resistant genes (Xa4, xa5 and xa13), which were used in the present study, are highly effective to identify the BLB resistant genes.

4. CONCLUSION

The presence of the bands of all the three markers tagged with BLB-resistant genes in the classified six rice varieties indicated that these varieties will be useful as donor of the BLB genes in the development of BLB resistant rice varieties in breeding programme.

CONSENT

Not applicable

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol. 2005;59(1):1-6.

2. Coudert Y, Périn C, Courtois B, Khong NG, Gantet P. Genetic control of root development in rice, the model cereal. Trends Plant Sci. 2010;15(4):219-226.

3. Shekhar S, Sinha D, Kumari A. An Overview of Bacterial Leaf Blight Disease of Rice and Different Strategies for its Management. Int. J. Curr. Microbiol. App. Sci. 2020;9(4):2250-2265.

4. Khan MA., Naeem M, Iqbal M. Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. Eur J Plant Pathol. 2014;139:27–37.

5. Disease- and pest- resistant rice. Accessed on 10 January 2022.

https://www.irri.org/disease-and-pest-resistant-rice

6. Kulkarni S, Jahagirdar S. Evaluation of new molecules in the management of bacterial blight of paddy in India. Internat J Plant Protec. 2011;4(2):289-291.

7. Prasad D, Singh R, Deep S. In-vitro and In-vivo Efficacy of Antibacterial Compounds against *Xanthomonas oryzae* pv. *oryzae*, A Cause of Bacterial Leaf Blight of Rice. Int. J. Curr. Microbiol. App. Sci. 2018;7(5):2960-2969.

8. Swati, Kumar A, Roy SP, Kumari P. Studies on efficacy of different chemicals treatments against Bacterial leaf blight of rice in Bihar. The Biobrio. 2015;2(1-2):56-61.

9. Sinha S, Kumar A, Satyendra, Kumar M, Singh SP, Singh PK. Screening of rice genotypes for abiotic and biotic stresses using molecular markers. J. Pharmacogn. Phytochem. 2018;7(2):2111-2115.

10. Kim SM, Suh JP, Qin Y, Noh TH, Reinke RF, Jena KK. Identification and fine-mapping of a new resistance gene, Xa40, conferring resistance to bacterial blight races in rice (*Oryza sativa* L.). Theor Appl Genet. 2015;128:1933-1943.

11. Singh AK, Dharmraj E, Nayak R, Singh PK, Singh NK. Identification of bacterial leaf blight resistance genes in wild rice of eastern India. Turk. J. Bot. 2015;39(6):1060-1066.

12. Shah BH, Xiaohua D, Liexian Z, Talukdar A, Zemin Z, Ruizhen Z, Guiquan Z. Pyramiding four bacterial blight resistance genes into rice cultivars in south China. Mol Plant Breed, 2006;4(4):493-499.

13. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 1987;19:11-15.

14. Majumder K, Mondal SI, Mallick R, Dasgupta T. Identification of BLB resistant genes in some rice varieties for development of high yielding bacterial leaf blight tolerant types. J. Environ. Biol. 2020;41(1):85-91.

15. Salgotra RK, Gupta BB, Millwood RJ, Balasubramaniam M, Stewart CN Jr. Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). Euphytica, 2012;187(3):313-323.

16. Panwar BS, Trivedi R, Ravikiran R, Ram C, Narayanan S. Molecular Marker-based Screening for Bacterial Leaf Blight Resistance Genes in Landraces and Cultivars of Rice in Gujarat. Indian J. Plant Genet. Resour., 2018;31(1):51-56.

17. Sabar M, Bibi T, Farooq HU, Haider Z, Naseem I, Mahmood A, Akhter M. Molecular screening of rice (*Oryza sativa* L.) germplasm for Xa4, xa5 and Xa21 bacterial leaf blight (BLB) resistant genes using linked marker approach. Afr. J. Biotechnol., 2016;15(41):2317-2324.

18. Acharya A, Adhikari NR, Amgain RB, Poudel A, Yadav R, Poudyal K. Identification of rice genotypes resistant to bacterial leaf blight disease using SSR markers. J. Inst. Agric. Anim. Sci., 2018;35(1):113-120.

19. Singh AK, Nayak R, Singh PK. Identification of bacterial leaf blight resistance genes in rice (*Oryza sativa* L.). Int. J. Sci. Nature, 2015;6(2):283-287.