Short Research Article

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

Comment [u1]: I will like to suggest a modified topic as this '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 varieties including two control varieties using three Simple Sequence Repeats markers tagged with Bacterial leaf blight genes which are *Xa4, xa5* and *xa13*. As a result, six varieties ~~naming~~, 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 of all of the three markers. 12 varieties were considered as susceptible and the other 7 varieties showed moderate resistance or susceptible results. The identified resistant genotypes can be utilized as a donor for developing Bacterial leaf blight ~~tolerant~~ resistant rice varieties in ~~future~~ breeding programme.

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

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1. INTRODUCTION

Rice is the most popular cereal crop because of its extensive consumption as a food crop for human needs. Approximately 60% of the World's population depends on rice ~~for using~~ as the basic food material [1]. Rice is ~~also~~ called ~~as~~ the "monocot model species" [2]. Though, around 21 viral and 6 bacterial 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 [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 ~~of~~ on rice, the proper position of genes or Quantitative trait locus (QTL)s controlling resistant to abiotic 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]. 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 varieties among 27 rice varieties using three Simple Sequence Repeats (SSR) markers tagged with BLB resistant genes. These will help the breeders to select the BLB resistant varieties.

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2. MATERIALS AND METHODS

The seeds of 27 rice varieties (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. 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, 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- ATCGATCGATCTTCACGAGG), (Reverse primer sequence- TCGTATAAAAGGCATTCGGG)], Primer-2: RM13 [Gene name- *xa5*, Chromosome No: 5, (Forward primer sequence- TCCAACATGGCAAGAGAGAG), (Reverse primer sequence- GGTGGCATTCGATTCCAG)] and Primer-3: RM264 [Gene name- *xa13*, Chromosome No: 8, (Forward primer sequence- GTTGCGTCCTACTGCTACTTC), (Reverse primer sequence- GATCCGTGTCGATGATTAGC)] [14]. After separating the amplified product on 2% agarose gel, it was observed on Gel documentation system (BIO-RAD) and the band size was estimated based on the size of 50bp DNA ladder.

Comment [u6]: Please, recast. Did the author plant the seeds before harvesting leaves for DNA isolation? If yes, how and where?
Screenhouse or field?

Comment [u7]: Show the annealing temmperature for each primer.

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3. RESULTS AND DISCUSSION

IRBB 59 was considered as the BLB resistant control variety ~~whereas~~ while the susceptible control variety ~~control~~ was IR 24, which were also described [15.16], these two varieties developed from IRRI were used for identification. The MP1 primer is linked with Xa4 gene, RM13 primer is linked with xa5 gene and the primer ~~named~~, RM264 is linked with xa13 gene. 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 RM256 primers, respectively (Fig. 1). ~~For the susceptible variety, 140bp, 130bp and 170bp fragments were observed using MP1, RM13 and RM256 primers respectively.~~ By observing the band sizes, the BLB resistant varieties among the 27 rice varieties were identified, which were MTU 1010, IR68144-2B-2-2-3-1-127, ARC-10086, Mali 4, IR 64 and Kalinga-2, these varieties ~~were consisting~~had all ~~of~~ the three resistant genes (*Xa4*, *xa5* and *xa13*) (Table. 1).

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Comment [u10]: This is the first mention of this abbreviation, you need to explain it.

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Tables should be explanatory enough to be understandable without any text reference. Double spacing should be maintained throughout the table, including table headings and footnotes. Table headings should be placed above the table. Footnotes should be placed below the table with superscript lowercase letters. Sample table format is given below.

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

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Sl, No.	Name of the	Origin	Genotyping of resistance genes using SSR Primer	Remarks

	Varieties		MP1	RM13	RM264	
1	IR 24 (Susceptible Control)	IRRI	140bp	130bp	170bp	S
2	IRBB 59 (Resistant Control)	IRRI	150bp	160bp	190bp	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	0	+	0	S

		Bengal				
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 bands, 0= no bands, MR/MS= moderately resistant / moderately susceptible*

The presence of Xa4, xa5, xa13 and xa21 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 ~~are~~ were classified as susceptible varieties due to having susceptible reactions. The other varieties exhibited intermediate result.

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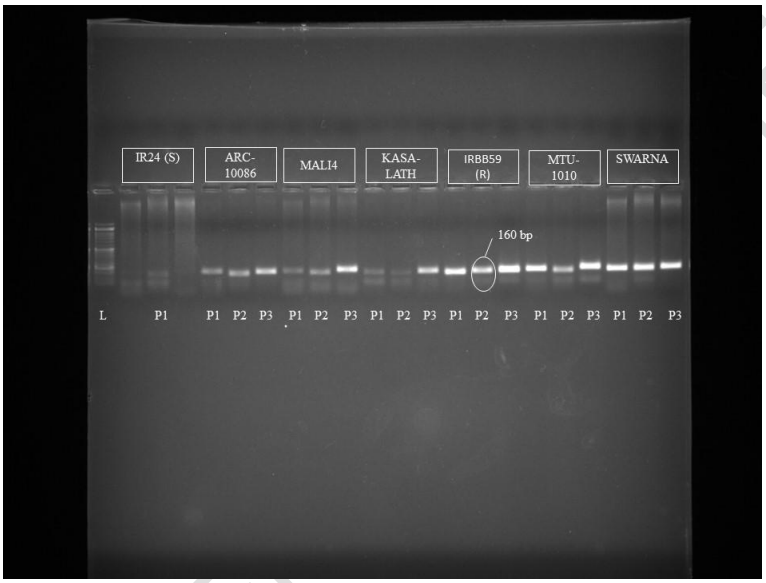

Fig. 1. Electrophoregram of different rice cultivars using three markers (where L= 50bp DNA ladder, P1= Primer-1: MP1, P2= Primer-2: RM13, P3= Primer-3: RM264)

4. CONCLUSION

The ~~appearance~~ presence of the bands of all the three markers tagged with BLB-~~-~~resistan~~ce~~t genes in the classified six rice varieties indicate~~d~~s ~~that~~ these varieties will be useful as donor of the BLB genes in the develop~~ing~~ment of BLB resistant rice varieties ~~with the help of advance~~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.

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Comment [u14]: Please, do proper pagination. E.g 219-226

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

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ABBREVIATIONS

BLB- Bacterial Leaf Blight
Bp- Base pair
CTAB- Cetyl trimethyl ammonium bromide
dNTPs- Deoxynucleotide triphosphates
$MgCl_2$- Magnesium chloride
SSR- Simple Sequence Repeat
QTL- Quantitative trait locus

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