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Research Article

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Marker Assisted Selection for Bacterial Leaf Blight resistance in Segregating Populations of *Karma Mahsuri*

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Abstract

Bacterial leaf blight (BLB) of rice is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), this is a major disease of rice in several countries. The present investigation was undertaken with the objective to develop high yielding rice varieties possessing broad spectrum durable resistance by transferring bacterial leaf blight (BLB) resistant genes *viz*., *xa5 xa13* and *Xa21* from a donor IRBB59 and a popular high yielding rice variety i.e., *Karma Mahsuri*, susceptible to BLB and were selected as parents. The presence of target genes by using gene linked primers *viz*., *xa5R, xa5S, xa13* promoter and Xa21F/R was done. Segregating population was advanced to BC_2F_3 generation and foreground selection was done using gene linked markers. Genetic analysis in BC_2F_3 populations confirmed the presence of genes (*xa5*, *xa13* & *Xa21*) governing BLB resistance in 22 lines.

Keywords

Rice; Bacterial leaf blight; Background selection; Foreground selection; Marker assisted selection; Segregating population

Introduction

Rice is the most important staple food for more than half of the world's population. But rice production is limited by various biotic and abiotic factors; bacterial leaf blight (BLB) being one of the major diseases. Host plant resistance (HPR) has been considered as the most economical and eco friendly strategy for management of biotic stresses. Molecular markers are widely applied in agriculture, and their application in rice improvement has been recently reviewed [1,2,3,4]. Kalaichelvan used 78 SSRs for varietal identification and also assessed the genetic relationship among the elite rice cultivars using morphological and molecular markers. The markers used in the selection must have tight linkage with the target gene in order to have relatively high selection efficiency. MAS has been employed for moving genes from pyramided lines into new plant type [3], as well as into improved varieties grown in India [5]. Development of broad spectrum durable resistance through gene pyramiding or gene stacking for biotic stress resistance can be accelerated through the process of marker assisted selection (MAS) [6].

BLB is caused by the *Xanthomonas oryzae pv. oryzae* and is one of the devastating diseases of rice causing yield loss ranging from 74 to 81% in severe conditions. Till date, 34 BLB genes [7] have been identified in rice and a number of them have been deployed into breeding lines, but disease breakdown has resulted due to significant shift in pathogen race frequency. Such breakdown can be delayed by marker assisted gene pyramiding. The *xa13,* a recessive gene, conferring resistance only in the homozygous state [8]. Perumalsamy et al. [9], introgressed three BLB resistance genes *xa5*, *xa13* and *Xa21* into two high yielding BLB susceptible *indica* rice cultivars, 'ADT43' and 'ASD16' from isoline IRBB60. These pyramided genotypes with two or three resistance genes exhibited high levels of resistance against two predominant *Xanthomonas oryzae* isolates of South India. The broad spectrum BLB resistance gene *Xa21* is expressed in dominant condition and was introgressed from a wild species *O. longistaminata* into *O. sativa* in chromosome 11 through conventional breeding [10]. Basavaraj et al. [5] also used markers *RG 136* and *pTA 248* linked to BLB resistance genes *xa13* and *Xa21*, respectively for foreground selection and improved Pusa 6A by using improved Pusa 6B as donor for *xa13* and *Xa21*.

Thus, the present study was undertaken to develop a high yielding, medium duration rice variety resistant to BLB by introgression of three BLB resistance genes *viz., xa5*, *xa13* and *Xa21* from IRBB59 (donor parent) into the genetic background of *Karma Mahsuri* (recipient parent). The gene linked markers *viz*., *xa5R, xa5S*, *xa13 promotor* and *Xa21* were taken for the study.

Material and Methods

Location of work

The experiment was conducted at the Research cum Instructional Farm of Department of Genetics and Plant Breeding and MAS laboratory, Richhariya Research Laboratory, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), in summer 2016 to generate the phenotypic and genotypic data. The genotypic data was developed using microsatellite (SSR) markers for the transfer of BLB resistant genes *xa5*, *xa13* and *Xa21* in *Karma Mahsuri* variety of rice.

Plant materials

The material under study included two parents' *viz., Karma Mahsuri* and IRBB59 and 92 BC_2F_3 lines derived from the cross between these two parents (*i.e., Karma Mahsuri* and IRBB59). Initially, the cross (*Karma Mahsuri* x IRBB59) was attempted in *Kharif* season 2011, later on it was advanced and during the research work *i.e.,* summer 2016 it was in BC_2F_3 generation.

Phenotypic screening

The pyramided lines were evaluated for their reaction to BLB under field conditions using Dhamtari isolates of *Xoo* which is very prominent in Chhattisgarh. Forty-day old plants were clip inoculated with bacterial suspensions of 10^9 cfu/ml at the maximum tillering stage. Lesion lengths were measured fifteen days inoculation based on SES. This set along with both parents was used for molecular studies.

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Molecular analysis

DNA was isolated from young leaves of 2-week-old plants using CTAB method. The DNA samples were quantified by using Nano Drop Spectroscopy (*NANODROP* 2000) and were then diluted with Sigma (sterilized) water to make the final concentration to 50 ηg/µl. PCR was performed in 20 µl reaction mixture containing 2 µl of template DNA (50 ηg/µl), 2 µl of 10x PCR buffer, 1 µl of 1.0 mM dNTPs, 0.5 µl of 5 ρmol forward and reverse primers each, 0.5 µl of 1 U/µl *Taq* polymerase and 13.5 µl of nanopure water each. After initial denaturation for 5 minutes at 95°C, each cycle comprised denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 2 minutes and finally final extension at 72°C for 10 minutes at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analysed by electrophoresis on 5% polyacrylamide gel. The gels were stained with Ethidium bromide (10 µl/ 100 ml of double distilled water) and were documented using BIORAD Gel Doc XR$^+$ (Figure 1).

Foreground selection

Selection for the specific trait under consideration is known as foreground selection; here for the bacterial leaf blight resistant three genes *viz., xa5*, *xa13* and *Xa21* from IRBB59 were selected. These particular genes were located on chromosome 5, 8 and 11 respectively. For foreground selection, linked markers were used [3]. Four primer *xa5S, xa5R, xa13 prom and Xa21 F/R* exhibited parental polymorphism and were subsequently used to generate the genotyping data (Table 1).

Background selection

Selection for the background of the recipient parent is known as background selection. For this purpose, only polymorphic markers which were not linked to the concerned resistance genes *viz., xa5*, *xa13* and *Xa21* and distributed well throughout the genome were used [3]. So, a total of 72 markers were used from the panel of highly polymorphic markers distributed throughout the genome of rice. Out of these 72 primers, 42 were monomorphic and 22 primers exhibited polymorphism. Those exhibiting polymorphism were subsequently used for background selection (Table 2).

Result and Discussion

Selection of parents

The reason of selecting *Karma Mahsuri*, for transferring the BLB resistant genes *xa5*, *xa13* and *Xa21* is that it is a high yielding *indica* rice variety possesses resistance to gall midge biotype *Gm1, gm4 and Gm5* and tolerant to leaf blast and brown spot. Being early maturing, it is suitable for double cropping in whole Chhattisgarh. IRBB59 (IRRI), taken as resistant parent as it possesses BLB resistant genes *xa5*, *xa13* and *Xa21.*

Phenotypic screening

A total of 92 lines were screened out on the basis of similarity in plant type to that of the recipient parent (*Karma Mahsuri*). This *set al*ong with both the parents were used for further genotypic screening (Foreground selection).

With changing scenario, new technologies must be developed to accelerate breeding by improving genotyping and phenotyping methods. The combination of reliable phenotyping and MAS has been particularly important in transferring desirable alleles by simple backcrossing into elite germplasm. Integrated skillful phenotypic screening reduces the cost of experiment and also helps in proper handling of the material under study. Here in present context, the basis of screening was the selection of all the lines similar to the recipient parent in plant type, i.e., having maximum of recipient parent background. This reduced the cost of foreground selection by reducing the initial segregating population to 92 individuals only.

Foreground selection

These 4 polymorphic (Table 1) SSR markers showing polymorphism were further used for PCR amplification with all 92 phenotypically screened BC_2F_3 lines along with the parents using standardized PCR protocol. The gel pictures of different SSR primers used for foreground selection are presented in Figure 2A,B and C. It is important to note that in foreground selection, we select for donor parent type allele. MAB is an effective and efficient strategy in crop improvement as it speeds up and simplifies the selection process especially for complex traits. Several researchers successfully pyramided genes for multiple disease resistance to provide a broader spectrum of resistance than those conferred by a single QTL/gene [8].

From all the lines similar to the recipient parent, some are expected to contain the intact portion of the desired genes, due to crossing over events. We have selected those lines which have the desired genes for resistance. So, from 92 BC_2F_3 lines, 22 lines were having three resistant genes *xa5*, *xa13 and* Xa21 in the intact form and thus can be further used for background selection.

Background selection

On the basis of data generated by foreground selection, 22 lines confirmed the presence of the genes *xa5*, *xa13* and Xa21; and were further selected on the basis of yield and yield attributing traits for background selection. The gel pictures of different SSR primers used for background selection are presented in Figure 3. Here in background selection, we select for recipient parent type allele.

The individuals showed proportion of background similar (recipient parent) this is presented in Table 3. This was calculated by using the following formula [3]:

$$\%\ of\ background\ \text{similiar to recepient parent [Karma Mahsuri]} = \frac{Number\ of\ markers\ showing\ bands\ similar\ to\ recipient\ parent\ [Recipient\ parent\ type\ allele]}{Total\ number\ of\ polymorphic\ markers} * 100$$

On the basis of 22 polymorphic markers we have found that the proportion of background is similar to recipient parent in various individual. Based on a general conclusion; that a few suitably placed markers provide adequate coverage of the genome in breeding programs [11,12]. Based on this concept, the markers for background selection in the present study were selected from the panel of highly

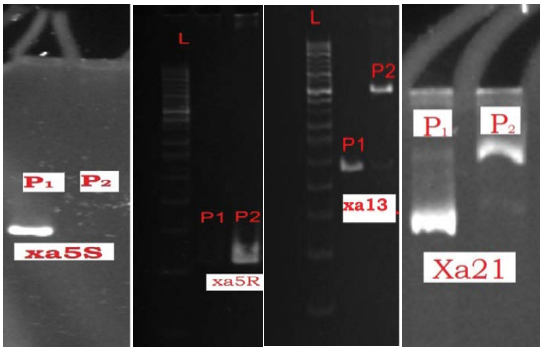

Figure 1: Parental polymorphism for xa5S, xa5R, xa13 and Xa21.

Table 1: List of bacterial leaf blight resistance genes and sequence of primers.

S. No.	Resistance gene	Marker	Chromosome	Primer sequence
1	**xa5**	xa5S	5	**F:** GTCTGGAATTTGCTCGCGTTCG **R:** TGGTAAAGTAGATACCTTATCAAACTGGA
2	**xa5**	xa5R	5	**F:** AGCTCGCCATTCAAGTTCTTGAG **R:** TGACTTGGTTCTCCAAGGCTT
3	**xa13**	xa13prom	8	**F:** GGCCATGGCTCAGTGTTTAT **R:** GAGCTCCAGCTCTCCAAATG
4	**Xa21**	Xa21 F/R	12	**F:**TCCAACATGGCAAGAGAGAG **R:**GGTGGCATTCGATTCCAG

Table 2: List of 22 polymorphic SSR markers used for background selection of *Karma Mahsuri* in the $BC_2F_3F_3$ population.

S. No.	SSR primers	Chromosome	Position [in cM]
1	RM1	1	29.7
2	RM106	2	123.2
3	RM574	5	41.0
4	RM162	6	108.3
5	RM510	6	20.8
6	RM586	6	7.4
7	RM588	6	1.4
8	RM118	7	96.9
9	RM25	8	52.2
10	RM22565	8	0.90
11	RM105	9	32.1
12	RM219	9	11.7
13	RM242	9	73.3
14	RM278	9	77.5
15	RM171	10	73.0
16	RM222	10	11.3
17	RM244	10	15.0
18	RM144	11	123.2
19	RM1233	11	112.9
20	RM17	12	109.1
21	RM511	12	59.8
22	RM1261	12	61.6

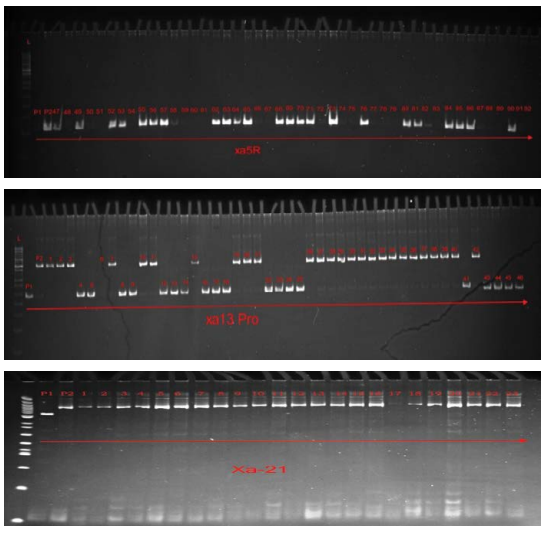

Figure 2A, B and C: Gel picture of BC_2F_3 lines along with both parents *Karma Mahsuri* (P_1 recipient) and IRBB59 (P_2 donor) used for foreground selection. A: Amplification shows line having xa5 resistant gene for bacterial leaf blight. B: Bands like P1 shows susceptible line and like P2 shows resistant line for xa13. C: Lines showing Xa21 resistant gene along with parents.

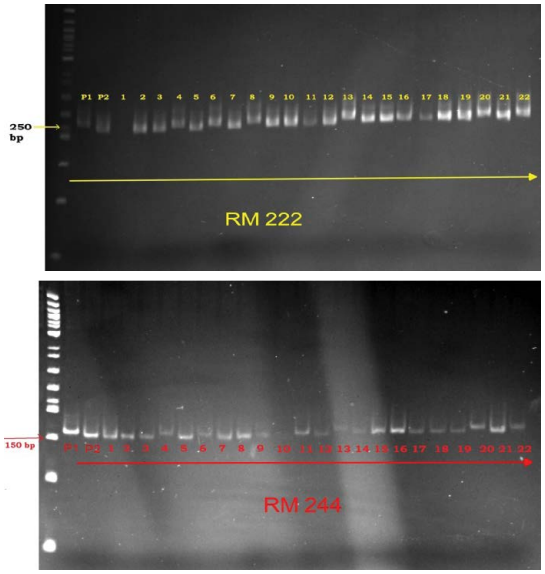

Figure 3A and 3B: Gel picture of 22 BC_2F_3 lines along with both parents *Karma Mahsuri* (P_1 recipient) and IRBB59 (P_2 donor) used for background selection. 3A: RM 222 P1, P2 and 22 lines for for background selection. 3B: RM 244 P1, P2 and 22 lines for for background selection.

polymorphic SSR markers which are usually used for diversity assessment (gramene.org). By using markers for background selection, there was a great acceleration of recipient genome recovery in the study conducted by Neeraja et al. (2007) and several other cases including this study.

Frisch and Melchinger [13] concluded that the effectiveness of marker assisted breeding depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population as well as the number of backcrosses, and the position and number of background markers. However, in another study conducted by Muthurajan and Balasubramaniam [9] indicated that the selection of the recipient and donor parents was more crucial. MAB with a stepwise screening technique was applied to select genotypes with desirable genes/QTLs by reducing a number of selected individuals in each step [4,14].

Major outcome

The outcome of the experiment was that, out of 92 lines 60 were found resistant for Xa21, 55 were resistance for *xa13* and 38 lines were found resistant for *xa5* individually. In twenty two lines have all the three genes *viz*., Xa21, *xa13 and xa5* were present which shows highly resistant in field also. Twenty five lines shows the combination of two resistant genes *viz. Xa21, and xa13* with the presence of one susceptible gene *Xa5* whereas fifteen lines show combination of resistant genes

Xa21 and *xa5* along with the presence of *Xa13* susceptible gene [15]. Four line shows combination of two recessive resistant genes *viz*., *xa13, xa5* along with one susceptible recessive xa21gene, five lines have only one resistant gene *xa5* with the combination of two susceptible genes xa21 and *Xa13*. Ten lines shows resistant for *xa13* with combination of two susceptible genes xa21 and Xa5. These facts suggest that besides carrying forward the entire lines; one can get the expected result by merely carrying these finally selected 2-4 lines as demonstrated in this study, which reflects how effective MAS can be in accelerating crop improvement process [16].

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