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Original Research Article

Towards Developing Bacterial Leaf Blight (BB) Resistance Rice Varieties of Eastern Uttar Pradesh by Using Marker Assisted Selection

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A B S T R A C T

Rice (*Oryza sative* L.) is the most important staple food crop however; diseases are among the most important limiting factors in rice production. Bacterial leaf blight (BLB) of rice, caused by *Xanthomonas oryzae* pv *oryzae* (Xoo) causes loss up to 50% depending on stage, weather, location and variety. So far, in the present study an attempt was conducted to pyramid the two BLB resistant genes *Xa 13* and *Xa 21* from the Improved Pusa Basmati. These two gene(s) was transferred into Swarna, Swarna sub1, NDR 97, Sambha Mahsuri and Pusa Basmati 1. F_1 plants were obtained from the cross of Improved Pusa Basmati 1 (donor) with Swarna, Swarna sub 1, Sambha Mahsuri, NDR 97 and Pusa Basmati 1 (recipient). Further true F_{1s} plants were selected by gene specific primers Xa 13 and *Xa* 21 of the successful crosses of Improved Pusa Basmati 1, NDR 97 ^{X} Improved Pusa Basmati 1 and Swarna 1^X Improved Pusa Basmati 1. Artificial inoculation was also performed among the F_{1s} plants and their respective parents. Different true F_1s of different parents crossed by Improved Pusa Basmati 1 have been preceded for marker assisted back cross breeding programme.

Keywords

Oryza sativa, Bacterial leaf blight, Marker assisted breeding

Introduction

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major diseases infected rice (*Oryza sativa* L.) in the world. In few areas of Asia it can reduce crop yield by up to 50% (Khush and Ogawa, 1989). Rice (*Oryza sativa* L.) Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* Ishiyama (*Xoo*) is one of the most important and destructive diseases affecting rice production worldwide (Adhikari ***et al.,***1995). Ali ***et al.,*** (2009) reported that Bacterial blight of rice (*Xanthomonas oryzae* pv. *oryzae*) significantly reduces the yield and quality of rice all over the world. Screening of 15 Pakistani rice genotypes revealed Kashmir Basmati as a highly resistant genotype and showed ~75% resistance to all the tested strains/isolates, only YR6W14D3 infect the genotype but the severity was not divesting. IR-6, Basmati-370, JP-5 and KSK-370 were ~50% resistant to all the tested strains, while the remaining genotypes were susceptible to all the strains/isolates of *Xanthomonas oryzae* pv. *oryzae*.

Gene pyramiding using conventional breeding alone is difficult to achieve because of linkage with some under sirable

traits that is very difficult to break even, after repeated back crossings. Causse *et al.*, (1994) stated that *Xa 5* resistance gene is not near any previously described resistance genes, while several other BLB resistance genes show clear evidence of clustering. The genes *Xa 3, Xa 4, Xa 10* and *Xa 21* are all located on the short arm of chromosome 11, along with several genes for resistance to rice blast, while *Xa 1* and *Xa 2* are located together on chromosome 4. Basavaraj *et al.*, (2009) reported that marker aided foreground selection for genes *Xa 13* and *Xa 21* was carried out using a CAPS marker RG136 and STS marker PtA 248 respectively. Forty six STMS markers polymorphic between Pusa 6B and Pusa 1460 providing genome wide coverage were used for background selection for recovering recurrent parent genome. In each backcross generation based on background selection, a single plant positive for genes *Xa 13* and *Xa 21* was selected for further backcrossing/selfing.

The present study was taken for the pyramiding of two bacterial resistance genes required for ensuring control of BLB. These two BLB resistant genes *Xa 13* and *Xa 21* lying in Improved Pusa Basmati 1 is transferred into Swarna, Swarna *sub*1, NDR 97, Sambha Mahsuri and Pusa Basmati 1.

Materials and Methods

Plant material

The experimental materials comprised of six rice cultivars along with one resistance as donar Improved Pusa Basmati1 (Pusa 1460) developed through MAS Bacterial blight resistance genes *xa13* and *Xa21* incorporated (Joseph et al. 2004) and five rice cultivars as susceptible used as recurrent parent (Swarna, Swarna *sub*1, NDR97, Sambha Mahsuri and Pusa Basmati1). Twenty one days old single seedlings were transplanted with spacing 20 × 15 cm apart in 3.0 × 1.5 m plot size in randomized block design with three replications. The recommended packages of practices were followed to raise a healthy crop.

Breeding aspect

In this programe of research crosses was made between Swarna (recipient) to Improved Pusa Basmati 1 (donor), Swarna *sub* 1 (recipient) to Improved Pusa Basmati 1 (donor), Sambha mahsuri (recipient) to Improved Pusa Basmati 1 (donor), NDR 97 (recipient) to Improved Pusa Basmati 1 (donor) and Pusa Basmati 1 (recipient) to Improved Pusa Basmati 1 (donor), keeping in mind the fact that donor having *Xa 13* and *Xa 21* BLB resistant genes, where as Swarna, Swarna *sub* 1, Sambha Mahsuri and NDR 97 are commonly grown high yielding varieties of eastern U.P. and Pusa Basmati 1 is a susceptible with BLB. This work was done in Kharif season 2009.

Pathogenicity screening for bacterial blight (BB)

The F_1 and all the parents lines with appropriate checks were screened for bacterial blight for their disease resistance. For bacterial blight screening PB1 used as susceptible check and Improved Pusa Basmati 1 (Pusa 1460) used as resistant check. A virulent isolate of the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (Xoo) collected from Indian Agricultural Research Institute New Delhi, India, was used to screen F_1s progenies of along with susceptible and resistant checks and other parents for BB resistance under both glasshouse and field conditions. The culture of *Xanthomonas oryzae* pv. *oryzae* subcultured on peptone sucrose agar (PSA) medium (Distilled water: 1 L, Sucrose: 20g,

Peptone: 5g, K_2HPO_4: 0.5g, $MgSO_4.7H_2O$: 0.25g and Agar: 15g) and maintained it at pH 7.2–7.4 (Fahy and Persley 1983). For pathogenicity test, clipping method was used for inoculation in the rice plants with *Xanthomonas oryzae* pv. *oryzae*. The Xoo strains were cultured and stored as described by Laha et al. (2009). The rice plants were clip inoculated with a bacterial suspension of 108-9 cfu/ml at maximum tillering stage (45–55 days after transplanting) through the methodology of Kauffman et al. (1973). Following inoculation, the plants were observed after every 24 hours time interval to note the appearance of disease symptoms. On the basis of lesion length these cultivars were categorized by using standard IRRI (2013) procedure as resistant 1-5 cm, moderately resistant 510 cm, moderately susceptible 10-15 cm and susceptible >15 cm. The average lesion length at 15 days after inoculation (DAI) was recorded to identify the degree of pathogenecity on 0–4 rating scale, Standard Evaluation System IRRI (2013).

Phenotypic studies

Data were recorded on five plants from each family for agronomic merits namely plant height(cm), panicle length(cm), panicle bearing tillers plant^{-1}, spikelets panicle^{-1}, grains panicle^{-1}, 1000-seed weight(g) and grain yield plant^{-1}(g).

DNA isolation

The young leaves were collected from three week of transplanted cultivar .Leaves sample of F_{1s} and their parents were collected from the field. The total genomic DNA was isolated by CTAB (Cetyl trimethylammonium bromide) method given by Murray and Thompson (1980) and DNA quality was checked by electrophoresis in 0.8% agarose gel.

PCR amplification

It was essentially done as described by William et al. (1990), Welsh and McClelland (1990). The PCR reaction mixture contained 50 ng templates DNA, 5 pico mole of each of the primers, 200 µM dNTPs, 1 X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, and 0.01 mg/ml gelatin) and 0.6 unit of Taq DNA polymerase in a volume of 20 µl and amplification of target sequences were as per earlier reports. The RAPD primers OPA16 and OPA11 (Table 1) were used for the amplification purpose with the following programme: initial incubation for 5 min. at 94°C, followed by 40 cycles, each consisting of denaturation step of 1 min. at 94°C, followed by an annealing step of 1 min. at 35°C and an extension of 1 min. at 72°C. The *xa13* and *xa21* (table 1) primer pair were amplified as following programme: initial incubation for 5 min. at 94°C, followed by 35 cycles, each consisting of denaturation step of 1 min. at 94°C, followed by an annealing step of 1 min. at 55°C and an extension of 2 min. at 72°C. The amplification reaction was concluded by a final extension at 72°C for 10 min. and then the temperature was decreased to 4°C until the reaction mixture was removed. The amplified products were resolved on the 1.5 % agarose gel and analyzed in the presence of ethidium bromide.

Results and Discussion

Improved Pusa Basmati 1 which is improved version of Pusa Basmati 1, a Basmati rice variety developed through MAS by pyramiding genes *Xa 13* and *Xa 21* in the genetic background of first semi-dwarf Basmati rice variety Pusa Basmati 1 (Gopalakrishnan *et al.*, 2008), was used as the donor parent for genes *Xa 13* and *Xa 21*.

In the present study five varieties of rice *viz.* Swarna, Swarna *sub* 1, Sambha Mahsuri, NDR 97, Pusa Basmati 1 were crossed as female with the donor Improved Pusa Basmati 1 as male parent and the F_1s obtained. With the aim to check the disease resistance in different rice varieties or the gene transfer in F_1or not. The phenotypic observation of F_1 was recorded (Table 2 and 3). Disease reaction was scored 21 days after inoculation. Plants with an average lesion length of up to 6 cm were considered as resistant and those with lesion length above 6 cm were scored as susceptible.

The similar work was also done by Kauffman *et al.,* (1973). In which plants in early as well as in advanced generations were inoculated with the bacterial suspension at a density of10^9 cells/ml at maximum tillering stage using the most virulent 'Kaul' isolate of *Xoo* multiplied from single scored culture and maintained in the Division of plant pathology, IARI, New Delhi. Five young leaves in each plant were inoculated through the clip inoculation method and disease reaction was scored 21 days after inoculation. The isolation of DNA was done as primary requirement to carry out RAPD and BLB specific marker *Xa 21*. This similar work was also done by Basavaraj *et al.,* (2010). In which Pusa RH10, the widely cultivated superfine grain aromatic rice hybrid, and its parental line Pusa 6B and PRR78 are susceptible to bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae.* Pusa 1460, a Basmati rice variety, was utilized as the donor for introgressing BB resistance *genes Xa* 13 and *Xa 21* into Pusa 6B and PRR 78 using a marker assisted selection. The markers RG136 and pTA248 linked to BB resistance genes *Xa 13* and *Xa 21* respectively were used for foreground selection.

With the aim to identify the F_1 the two primers were used i.e. OPA 11 and OPA 16. OPA 11 amplified good and reproducible DNA fragments of both parent and F_1 and showed intact bands. Huang *et al.,* (1997) reported that a CAPS marker, RG 136 (3.7 cM from *Xa 13)* and a STS marker pTA248 (a gene sequence-based marker for *Xa 21*), were used for foreground selection of genes *Xa 13* and *Xa 21*, respectively. Out of these five F_1 developed using improved parental lines, based on phenotypic observations and bacterial blight reactions to promising combinations Swarna and NDR 97 were identified with high level of resistance to BLB while Swarna *sub* 1, Sambha Mahsuri and Pusa Basmati 1 were identified as susceptible to BLB.

Figure.1 Foreground selection at F_1 generation using 'R' gene linked PCR based markers by using *Xa21* linked marker pTA248

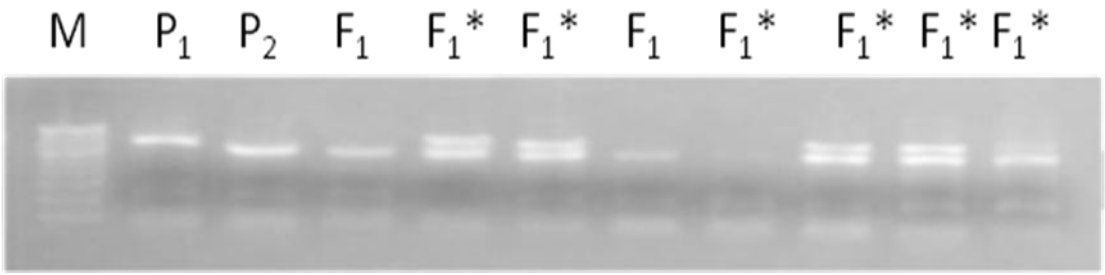

Table.1 RAPD and gene specific primers used in the study

S. No.	Gene	Primers	Chromosome	Sequence	References
1	-	OPA 16	-	AGCCAGCGAA	-
2	-	OPA 11	-	CAATCGCCGT	-
3	Xa13	Xa13 promoter specific marker	**8**	F-5`TCCCAGAAAGCTACTACAGC3` R-5`GCAGACTCCAGTTTGACTTC3`	Huang et al. (1997)
4	pTA248	*Xa 21*	11	F-5`AGACGCGGAAGGGTGGTTCCCGCA3` R-5`AGCGCGGTGTAATCGAAAGATGAAA3`	Huang et al. (1997)

Table.2 Mean performance of phenotypic observation of parents of different rice varieties

S. No.	Varieties	Plant height (cm)	Panicle length (cm)	Panicle bearing tillers plant^{-1}	Spikelets Panicle^{-1}	1000-Seed weight (g)	Grains Yield Plant^{-1} (g)
1	Swarna	93.30	24.86	10.67	192.33	24.50	20.20
2	Swarna sub 1	95.20	26.30	15.33	204.00	23.33	21.70
3	Sambha Mahsuri	98.50	25.73	11.33	177.67	19.00	24.73
4	NDR 97	90.90	22.33	9.33	162.67	25.66	22.86
5	Pusa Basmati 1	111.80	26.36	8.33	11367	27.33	14.23
6	Improved Pusa Basmati 1	115.76	26.36	9.33	109.33	24.00	15.26
C.D		9.70	N/A	4.17	16.30	2.47	3.62
SE(m)		3.04	1.62	1.30	5.10	0.77	1.13
SE(d)		4.30	2.28	1.85	7.22	1.09	1.60
C.V		5.22	11.05	21.10	5.53	5.59	9.90

Table.3 Mean performance of phenotypic observation of F_{1s} of different rice varieties

S. No.	Variety	Plant height (cm)	Panicle length (cm)	Panicle bearing tillers plant^{-1}	Spikelets Panicle^{-1}	1000-Seed weight (g)	Grains Yield Plant^{-1} (g)
1	Swarna × Improved Pusa Basmati 1	97.03	23.70	9.67	192.33	20.33	22.23
2	Swarna *sub* 1 × Improved Pusa Basmati 1	96.93	23.66	9.33	181.00	23.67	24.60
3	Sambha Mahsuri × Improved Pusa Basmati 1	102.03	21.83	6.67	157.33	20.00	17.06
4	NDR 97 × Improved Pusa Basmati 1	94.03	21.93	5.00	145.33	18.67	18.16
5	Pusa Basmati 1 × Improved Pusa Basmati 1	108.13	21.70	6.00	133.66	19.67	14.33
C.D		5.45	N/A	N/A	9.29	1.93	3.27
SE(m)		1.64	1.58	1.07	2.80	0.58	0.98
SE(d)		2.32	2.23	1.51	3.96	0.82	1.39
C.V		2.86	12.15	25.26	3.00	4.92	8.87

Table.4 Reaction of the different parents and F1s obtained by crossing over with improved Pusa Basmati 1 to bacterial blight

Genotypes	Disease reaction (lesion length)	Disease Score	Genotypes	Disease reaction (average of 10 plants)	Disease Score
Swarna	6.67	S	Swarna × Improved Pusa Basmati 1	2.97	MR
Swarna sub 1	6.76	S	Swarna *sub* 1 × Improved Pusa Basmati 1	4.83	MS
Sambha Mahsuri	6.86	S	Sambha Mahsuri × Improved Pusa Basmati 1	4.97	MS
NDR 97	6.86	S	NDR 97 × Improved Pusa Basmati 1	3.00	MR
Pusa Basmati 1	8.83	S	Pusa Basmati 1 × Improved Pusa Basmati 1	5.00	MS
Improved Pusa Basmati 1	1.40	R			
C.D	0.314		C.D	0.304	
SE(m)	0.098		SE(m)	0.092	
SE(d)	0.139		SE(d)	0.130	
C.V	2.73		C.V	3.83	

In the present, the F_{1s} progenies were selected with the help of marker linked with the xa13 and xa21 (Figure 1) using marker-assisted foreground selection for gene(s)/QTLs of interest along with stringent phenotypic selection as described earlier (Singh *et al.*, 2011). The parents and F_{1s} progenies obtained by the crossing with the Improved Pusa Basmati 1 were screened for resistance to BB, 'Improved Pusa Basmati 1' as checks using standard procedures (Table 4). In contrast, the donar parent 'Improved Pusa Basmati 1' was resistant to BB (lesion length of 1.40 cm) and other reciepent parents were found to be highly susceptible to moderate resistance under artificial inoculation conditions ranged from 6.67 cm Swarna to 8.83 cm Pusa Basmati 1. All five F_{1s} progenies were found to moderate to susceptible reactions by artificial inoculation and 10 best F_{1s} were selected which showed resistant to BB and the lesion lengths ranged from 2.97 cm in Swarna to 5.00 cm in Pusa Basmati1, in comparison with 1.40 cm in Improved Pusa Basmati 1.

The disease reaction for BB resistance in some of the F_1s progenies showed greater or lesser resistance than the donar parent Improved Pusa Basmati '. Such type has been also reported due to complementarity of genes from the donor parent Tetep, which have been reported to carry other BB-resistant genes, namely *Xa1, Xa2, Xa12* and *Xa16* (Nelson *et al.*, 1994; *et al.*, 1998).

In conclusion, foreground selection coupled with stringent phenotypic selection identified true F_{1s} plants for *xa13* and *Xa21*, which were advanced to back crossing with their respective recurrent parents to proceed marker assisted back cross breeding for the development of important rice lines against bacterial blight resistance.

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