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Incorporation of BLB Resistance Genes into Basmati Rice Variety CSR-30 (*Oryza sativa* L.) through Marker Assisted Selection

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A B S T R A C T

In Asia, rice is the most important, widely consumed staple food for a large part of the world's human population. Bacterial leaf blight (BB), caused by the bacterium, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the major disease affecting Basmati rice production in India. CSR-30 is a very popular high yielding, salt tolerant Basmati variety widely grown in Haryana, India but highly susceptible to BB. In the present study, therefore, we successfully introgressed two BB resistance genes (*Xa21* and *xa13*) from BB resistant donor variety Pusa Basmati-1460 into the BB susceptible variety CSR-30 through marker assisted selection (MAS). Hundred and fourteen selected BC_1F_1 pyramided genotypes were tested for BB resistance at maximum tillering stage against *Xoo* strain prevalent in Kaul, Haryana. The double resistance genes pyramided genotypes imparted enhanced resistance as expressed by reduced mean lesion length in comparison to genotypes with individual genes. A total of 111 simple sequence repeat (SSR) markers out of 428 representing whole of the genome were found polymorphic between parental genotypes, CSR-30 and Pusa Basmati-1460. Background analysis using 111 polymorphic SSR markers revealed that recurrent parent genome (RPG) recovery ranged up to 83% among twenty five BC_1F_1 two-gene pyramided genotypes. Based on BB reaction, agronomic evaluation, grain quality and percentage recovery of RPG, four genotypes viz., P-C-70, P-C-123, P-C-142 and P-C-149 were found promising. These promising genotypes possessing yield traits similar or at par with the recurrent Basmati parent CSR-30 were identified and advanced to BC_2F_1 generation. The experimental results of this work demonstrate the effectiveness of MAS for successful introgression of the two BB resistance genes from Pusa Basmati-1460 into CSR-30.

Introduction

Rice is the seed of the monocot plant *Oryza sativa* (Asian rice). Rice, the staple cereal food crop for more than half of the world's population is grown in a wide range of climatic conditions and is threatened by several biotic and abiotic stresses. India is the second largest producer of rice after China according to the Agricultural and Processed

Food Products Export Development Authority (APEDA). It has been estimated that a 40% increase in rice production by 2030 will be needed to meet the predicted demand of the growing world population (Khush, 2005). BB disease is one of the most devastating diseases effecting Rice production in India. BB is caused by the, *Xanthomonas oryzae pv. oryzae* and causes severe yield losses upto 80% depending on the stage of the crop, cultivar susceptibility and the environmental conditions (Singh *et al.*, 1977; Noh *et al.*, 2007). The development of resistant varieties carrying resistance genes have been considered to be the most effective and economical way to control the BB disease (Sundaram *et al.*, 2008; Rajpurohit *et al.*, 2010; Dokku *et al.*, 2013). To date, at least 38 BB resistance genes [27 dominant (*Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, X12, Xa14, Xa16, Xa17, Xa18, Xa21, Xa22* (t), *Xa23, Xa25 (t), Xa26, Xa27, Xa29, Xa30, Xa30 (t), Xa31 (t), Xa32 (t), Xa34, Xa35 (t), Xa36 (t), Xa38*) and 11 recessive (*xa5, xa5(t), xa8, xa13, xa15, xa19, xa20, xa24, xa28 (t)xa31* and *xa32)*] conferring host resistance against various strains of *Xoo* have been identified (Bhasin*et al.*, 2012; Natrajkumar *et al.*, 2012). A number of these resistance genes have been tagged by closely linked molecular markers (Sonti, 1998; Rao *et al.*, 2002).

Marker assisted breeding overcomes the limitations of conventional breeding by providing an effective solution for resistance breakdown in form of gene pyramiding. The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene (Mundt, 1990). Pyramiding multiple genes into a single variety through conventional breeding is cumbersome, requires much time and material resource. Pronounced dominance and epistatic effect along with linkage drag are major drawbacks associated with conventional breeding approach. Marker assisted breeding employs foreground selection for trait of interest and background selection for assessment of recurrent parent genome (RPG) recovery in the recombinants. Improved rice cultivars with broad spectrum and durable BB resistance have been developed by using the gene pyramiding approach. Pandey *et al.*, (2012) intogressed BB resistance genes (*Xa21* and *xa13*) into Taraori Basmati and Basmati 386 and reported 83.6% recovery at BC_1F_4 generation while selecting for RPG. BB resistance genes *xa13* and *Xa21* have been introgressed into 'Pusa Basmati 1' through MAS coupled with phenotypic selection for agronomic, grain and cooking quality traits (Joseph *et al.*, 2004). In the present study, we employed MAS for introgression of two BB resistance genes viz. *Xa21* and *xa13* into Basmati variety CSR-30. Foreground selection was followed by background selection assisted by SSR markers that efficiently recovered maximum RPG along with the yield traits and minimum introgression of non-targeted genome from the donor into the pyramided genotypes. The success will facilitate future efforts to transfer combinations of BB resistance genes into other preferred rice cultivars.

Materials and Methods

Plant material and pyramiding strategy

The experimental material for the present study consisted of BB susceptible Basmati rice variety CSR-30 as recurrent parent, while Pusa Basmati-1460 having BB resistance genes, *xa13* and *Xa21* was used as donor parent. Crosses were made between CSR-30 and Pusa Basmati-1460 and F_1 plants were selfed to obtain F_2. The positive F_2 plants were backcrossed with CSR-30 and BC_1F_1 generation was obtained. Among the BC_1F_1 population, the foreground selection was performed using polymerase chain reaction

(PCR) based sequence tagged sites (STS) markers linked to the two BB resistance genes *xa13* and *Xa21* followed by BB incidence analysis on artificial inoculation and background analysis. The BC_1F_1 plants having the two BB resistance genes were advanced to BC_2F_1 generation.

Molecular marker analysis and PCR amplification

Mini-scale genomic DNA isolation of the parents and backcross progenies for foreground selection was carried out from 35-day old seedlings using CTAB extraction method of Murray and Thompson (1980) modified by Saghai-Maroof *et al.,* (1984) and Xu *et al.,* (1994). Two STS markers viz. pTA248 and RG136 linked to the BB resistance genes,*Xa21* and *xa13*, respectively, were used to confirm the presence of these resistance genes at F_2 and backcross generation. The pTA248 marker is 0.2 cM from *Xa21* (Ronald *et al.,* 1992) and RG136 marker is 3.8 cM from *xa13* (Zhang *et al.,* 1996) (Table 1). PCR reaction was carried out in 20 µl reaction mixture containing 50ng genomic DNA, 2 units of TaqDNA polymerase, 1X PCR Buffer (10mM Tris HCL, 1.5mM $MgCl_2$), 100µM each of dNTPs and 10 µM of primer. Template DNA was initially denatured at 94ºC for 5 min followed by 30 cycles of PCR amplification with the following conditions: 30 sec denaturation at 94ºC, 1 min annealing at 55ºC and 1 min of primer extension at 72ºC followed by final extension of 72ºC for 10 min. The amplified product of pTA248 was electrophoretically resolved on 1.5 % agarose gel containing 0.5 µg/ml of ethidium bromide in 1.0 X TBE buffer and visualized under UV light. For the amplified products of RG136, 5 µl of PCR product was used for gel electrophoresis tocheck DNA amplification. The remaining PCR product was used for restriction digestion. The reaction mixture used for digestion of PCR product with the respective restriction enzyme consisted of 0.3 µl (10 U/µl) of restriction enzyme *Hinf*1for RG136 amplicons, 2.5 µl of 10 X restriction buffer, 7.2 µl of sterile distilled water and 15 µl PCR product to make a final volume of 25 µl (Perumalsamy *et al.,* 2010). The reaction mixture was incubated for 5 hr at 37°C and the products of restriction digestion were separated by gel electrophoresis (2.0% agarose) and visualized under UV light.

For background analysis, 111 polymorphic SSR markers out of 428 tested were used for the assessment of recovery of RPG in the pyramided genotypes. The computer package NTSYS-PC (Rohlf, 2000) was used for cluster analysis. Graphical genotypes (GGT) Version 2.0 (Van Berloo, 1999) software programme was used for the assessment of the genomic contribution of the parent in the selected genotypes based on SSR marker data

BB incidence

Plants selected on the basis of foreground selection from the BC_1F_1 generation carrying resistance genes (*Xa21* and *xa13*) individually and in combination, along with the control, were inoculated with the *Xoo* isolate prevalent in Kaul, Haryana, using a bacterial suspension of 10^9cells/ml (Kauffman *et al.,* 1973). The plants were clip inoculated at maximum tillering stage. The leaf blades were inoculated by clipping with scissors at 3 cm below the leaf tips. On an average five leaves per plant were inoculated and the disease incidence using 0-5 scale was measured 14 days after inoculation.

Agronomic parameters

Agronomic traits were recorded in the BC_1F_1 pyramided rice genotypes for plant height, effective tillers per plant, panicle length, filled grains per panicle, 1,000 grain weight and

grain yield. The pyramided BC_1F_1 genotypes were raised in during the kharif season in net house at CCSHAU, Hisar and in field at Regional Rice Research Station, Kaul. The two-gene pyramided BC_1F_1 genotypes with maximum RPG were advanced to BC_2F_1 generation.

Results and Discussion

Foreground and Background selection

In F_2 population, the presence of BB resistance genes viz., *Xa21* and *xa13* was determined by using respective linked marker and 6/88 genotypes had both the BB resistance genes. The two-gene pyramided F_2 genotypes were backcrossed with CSR-30 and BC_1F_1 seeds were obtained. In BC_1F_1 generation, plants with single resistance gene and with two resistance genes were identified. In BC_1F_1 generation, 25/150 genotypes were found positive for both the BB resistance genes (Figure 1). Among resistant genotypes, 47 plants had *Xa21*, 42 plants had *xa13* whereas 25 plants were found to have both *xa13* and *Xa21* BB resistance genes. A total of 111 polymorphic SSR markers spanning uniformly across all the rice chromosomes were used for background selection (Figure 2). The similarity indices ranged from 0.15 to 0.84 across all the genotypes. The donor parent (Pusa Basmati-1460) and recurrent parent (CSR-30) had low genetic similarity coefficient and bifurcated at coefficient value of 0.22. The maximum genetic similarity coefficient for the two-gene pyramided BC_1F_1genotypes with respect to recurrent parent CSR-30 was observed in P-C-70 (0.84) followed by P-C-123(0.82), P-C-142 (0.82) and P-C-149 (0.79). UPGMA cluster tree was divided into two groups (Figure 3). The recipient parent CSR-30 and all the BC_1F_1genotypespositive for *Xa21* and *xa13* BB resistance genes fell in one group with two major sub-groups. Sub-group I consisted of recipient parent CSR-30 and four genotypes, P-C-70, P- C-123, P-C-142 and P-C-149 with maximum similarity to CSR-30. Sub-group II consisted of the remaining twenty one genotypes. Among the two-gene pyramided BC_1F_1 genotypes, P-C-70 showed 84% genetic similarity with CSR-30, whereas, P-C-123 and P-C-142 showed 82% genetic similarity with CSR-30 followed by P-C-149 which showed 79% genetic similarity with the same. Among the twenty five two-gene pyramided genotypes, the RPG recovery ranged from 62.7% to 83%. Six and ten SSR markers were found polymorphic on BB carrier chromosomes 8 and 11, respectively (Figure 4). Genotypes, P-C-70, P-C-123, P-C-142 and P-C-149 were found to have least introgression of donor parent genome as conferred by background analysis. These four two-gene pyramided genotypes with RPG above 78.7% were crossed with CSR-30 and BC_2F_1 seeds were harvested.

BB incidence scoring

The pyramided BC_1F_1 lines were evaluated stringently for their resistance to BB in the field and under glass house conditions using the *Xanthomonas oryzae* strain isolated from the BB infected fields of RRS, Kaul, CCSHAU, Hisar. The twenty five two-gene pyramided BC_1F_1 genotypes (mean lesion length of 0.8 cm) were found to be more effective against the virulent *Xoo* strain than the donor parent Pusa Basmati-1460 (mean lesion length of 1.67 cm). On screening for BB resistance, the mean lesion length among pyramided lines varied from 0.8 cm to 4.6 cm. Also the forty seven lines having *Xa21* (mean lesion length of 2.04 cm) gene alone were found to be resistant or moderately resistant to the BB disease. However, lines with *xa13* gene (mean lesion length of 4.6 cm) alone were found to be susceptible to BB disease. The pyramided lines with two gene combination exhibited broader spectrum of

resistance than parental lines or lines with a single gene. The results indicated that the genes in combinations were more effective against the pathogen strain than a single resistance gene. The combination of different resistance genes thus provide more durable resistance (Jeung *et al.*, 2006; Kim *et al.*, 2009; Suh *et al.*, 2009; Baliyan *et al.*, 2016). This indicates that there is some kind of quantitative complementation with the presence of multiple resistance genes having an additive effect on the overall level of resistance. Accumulating major genes for resistance in an elite genotype by conventional breeding is laborious, time consuming and very difficult when two or more of the resistance genes are pyramided into an elite cultivar. The higher lesion lengths observed in some two gene combinations could be the result of recombination between marker locus and the target gene. This is more likely for *xa13* since the linked marker RG136 is 3.7 cM away from the resistant gene *xa13*.

Yield and its attributes

Data was recorded for yield and agro-morphological traits of the pyramided BC_1F_1genotypes and the parental genotypes. Significant variation was observed among the pyramided lines and parental rice genotypes for plant height, tillers per plant, panicle length, filled grains/panicle, and grain yield per plant. Most of the two-gene pyramided BC_1F_1 genotypes were similar or superior to the recurrent parent CSR-30 with respect to the yield and its components. In the field conditions, for the pyramided BC_1F_1 genotypes, plant height ranged from 122 cm-159 cm, whereas the plant height of recurrent parent CSR-30 was 154 cm. All the pyramided genotypes except P-C-129 were found dwarf than the recurrent parent. The pyramided BC_1F_1 genotypes in field exhibited 1000 grain weight greater than the recurrent parent CSR-30 (23.1 g). The genotypes with maximum recovery of RPG, P-C-70 (74.12 g/plant) and P-C-149 (81.23 g/plant), showed yield/plant higher than the recurrent parent CSR-30 (73.89 g/ plant) (Table 2). In the net house, similar trend was observed, where, plant height of the pyramided BC_1F_1 genotypes ranged from 87.2 cm-126 cm, the plant height of CSR-30 was recorded 143 cm. All the pyramided genotypes were found dwarf in comparison to the recurrent parent. The pyramided BC_1F_1 genotypes except P-C-117 exhibited 1000 grain weight greater than the recurrent parent CSR-30 (21.2 g). The genotypes with maximum recovery of RPG, P-C-142 (39.66 g/plant), showed yield/plant higher than the recurrent parent CSR-30 (36.78 g/ plant) whereas, genotype P-C-123 (35.62 g/plant) exhibited yield/plant almost similar to CSR-30 (Table 4).

Grain and cooking quality traits

A total of twenty five bacterial leaf blight (BB) resistance genes (xa13 and Xa21) pyramided BC_1F_1 genotypes from a cross between CSR-30 and Pusa Basmati-1460 were evaluated for grain and cooking quality traits. Quality parameters measured were, kernel dimensions before and after cooking, aroma, alkali spread value (ASV) and amylose content (AC).

Correlation studies were made on data to find out association between grain quality traits (Table 3). Correlation coefficient analysis of the two-gene pyramided BC_1F_1genotypes revealed that kernel length/breadth ratio before cooking exhibited positive correlation with kernel length before cooking (0.741, p=0.01) whereas it showed significant negative correlation with kernel breadth before cooking (-0.632, p=0.01). Kernel length/breadth ratio after cooking showed significant negative correlation (-0.851, p=0.01) with kernel breadth after cooking.

These findings were in agreement with other studies (Danbaba N *et al.,* 2011;Mathure S *et al.,* 2011, Mehta *et al.,* 2020). Amylose percent exhibited a significant negative correlation (-0.537, p=0.01) with ASV. Elongation ratio revealed significant negative correlation (-0.873, p=0.01) with kernel length before cooking and positive correlation with kernel length after cooking (0.767, p=0.01), which is in consistent with the findings of other similar studies (Chakraborty *et al.,* 2009).

Table.1 Molecular markers used for marker-assisted selection of BB resistance genes

Molecular marker	R Genes for BB	Chromosome	primer sequences (5'-3')	Reference
pTA248 (0.2cM)	*Xa21*	5	F:5'AGACGCGGAAGGGTGGTTCCCGGA3' R:5'AGACCGGTAATCGAAAGATGAAAA3'	Yoshimura *et al.* (1995)
RG136 (3.8cM)	*xa13*	8	F:5'TCCCAGAAAGCTACTACAGC3' R:5'GCAGACTCCAGTTTGACTTC3'	Zhang *et al.* (1996)

Table.2 Agronomic traits and % RPG recovery in the two-gene pyramided BC_1F_1 genotypes grown in the field

S.No.	Plant no.	PH (cm)	NT	1000 GW (g)	PL (cm)	FG/P	Awns	Y/P (g)	RPG%
1	PB-1460	136	27	28.6	23.16±0.23	69.91±0.18	long	63.68	0
2	CSR-30	154	37	23.1	28.24±0.13	79.37±0.29	small	73.89	100
3	P-C-4	150	29	25.4	27.30±0.37	80.00±1.58	small	72.8	69.1
4	P-C-19	148	19	28.9	29.73±0.09	98.74±0.54	small	41.33	78.4
5	P-C-23	122	22	25.7	26.00±0.25	102.50±1.69	long	64.23	73.5
6	P-C-30	153	29	27.9	28.85±0.23	87.00±0.84	small	68.57	68.8
7	P-C-37	136	32	26.7	28.05±0.17	100.00±0.56	small	78.67	67.1
8	P-C-46	142	37	28.9	30.00±0.51	81.00±1.12	small	81.16	69
9	P-C-59	147	30	27.1	28.13±0.05	94.00±1.33	long	75.94	62.7
10	P-C-63	127	24	24.0	27.50±0.37	78.50±0.23	small	70.79	66.2
11	P-C-70*	139	26	25.3	23.83±0.43	93.6±0.59	small	74.12	83
12	P-C-81	134	18	26.6	30.58±0.27	120.00±0.61	absent	40.96	69
13	P-C-99	125	24	23.7	23.20±0.13	83.25±0.27	long	63.78	66.5
14	P-C-100	143	24	25.6	25.38±0.49	71.00±0.66	small	69.15	80.5
15	P-C-111	142	31	26.0	23.88±0.42	70.00±0.74	long	77.83	65.8
16	P-C-129	159	39	24.0	27.00±0.16	67.75±0.61	absent	83.71	75.1
17	P-C-134	148	28	31.8	26.63±0.14	60.00±2.87	long	72.59	77.6
18	P-C-149*	152	38	27.7	23.72±0.43	74.50±1.27	small	81.23	78.7

PH plant height, *NT* no. of tillers, *1000 GW* 1000 grain weight, *PL* panicle length, *Y/P* yield per plant, *RPG(%)* recurrent parent genome (%)

* indicates two-gene pyramided genotypes with maximum RPG

Table.3 Correlation coefficients analysis for grain quality traits of parental genotypes and two-gene pyramided BC_1F_1 genotypes

Variables	KLBC (mm)	KBBC (mm)	KBC L/B	KLAC (mm)	KBAC (mm)	KAC L/B	ER	ASV	AC%
KLBC(mm)	1.000								
KBBC(mm)	0.043	1.000							
KBC L/B	0.741**	-0.632**	1.000						
KLAC(mm)	-0.365	-0.031	-0.249	1.000					
KBAC(mm)	0.013	-0.053	0.043	0.197	1.000				
KAC L/B	-0.237	0.034	-0.197	0.332	-0.851**	1.000			
ER	-0.873**	-0.042	-0.640**	0.767**	0.109	0.320	1.000		
ASV	-0.007	-0.552**	0.361	-0.122	0.046	-0.099	-0.080	1.000	
AC%	0.207	0.325	-0.041	-0.031	-0.220	0.188	-0.154	-0.537**	1.000

*Significant at 5%, **Significant at 1% level

KLBC-Kernel length before cooking, *KBBC*-Kernel breadth before cooking, *KBC-L/B*-Kernel length to breadth ratio before cooking, *KLAC*-Kernel length after cooking, *KBAC*-kernel breadth after cooking, *KAC-L/B*-Kernel length to breadth ratio after cooking, *ER*-Elongation ratio, *ASV*-Alkali spread value, *AC%*-Amylose content%

Table.4 Agronomic traits and % RPG recovery in the two-gene pyramided BC_1F_1 genotypes grown in the net house

S.No.	Plant no.	PH (cm)	NT	1000 GW (g)	PL (cm)	FG/P	Awns	Y/P (g)	RPG%
1	PB-1460	122	12	27.6	31.00±0.46	102.36±1.02	long	31.45	0
2	CSR-30	143	16	21.2	31.12±0.57	89.3±1.20	small	36.78	100
3	P-C-89	119	12	24.2	22.87±0.30	67.25±0.93	small	33.13	66
4	P-C-90	126	18	23.2	24.45±0.44	79.50±0.47	small	22.88	70.1
5	P-C-96	112	16	22.5	20.37±0.42	65.50±0.55	long	40.18	68.2
6	P-C-106	91	12	21.6	24.40±0.53	54.75±0.56	absent	30.25	67
7	P-C-117	87.2	11	17.2	17.15±0.43	32.00±1.39	absent	29.32	62.7
8	P-C-122	95	11	22.1	23.39±0.31	47.54±0.16	small	33.14	68
9	P-C-123*	120	15	24.2	21.54±0.57	68.24±0.76	small	35.62	79.7
10	P-C-126	98.5	10	21.4	19.35±0.38	43.00±0.13	absent	31.65	74.5
11	P-C-142*	102	9	27.0	22.45±0.47	78.32±0.61	small	39.66	79.6

PH plant height, *NT* no. of tillers, *1000 GW* 1000 grain weight, *PL* panicle length, *Y/P* yield per plant, *RPG(%)* recurrent parent genome (%)

* indicates two-gene pyramided genotypes with maximum RPG

Fig.1 Marker assisted selection in BC_1F_1 genotypes from cross CSR-30 x Pusa Basmati-1460 for (A, B) *Xa21* using pTA248 (C, D) *xa13* using RG136

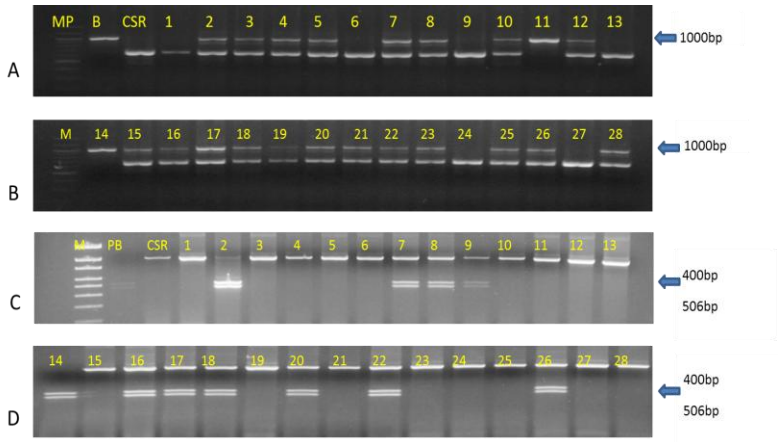


Fig.2 Background selection of the pyramided BC_1F_1 genotypes from cross CSR-30 x Pusa Basmati-1460 using polymorphic SSR markers (a) RM-1221 (b) RM1134

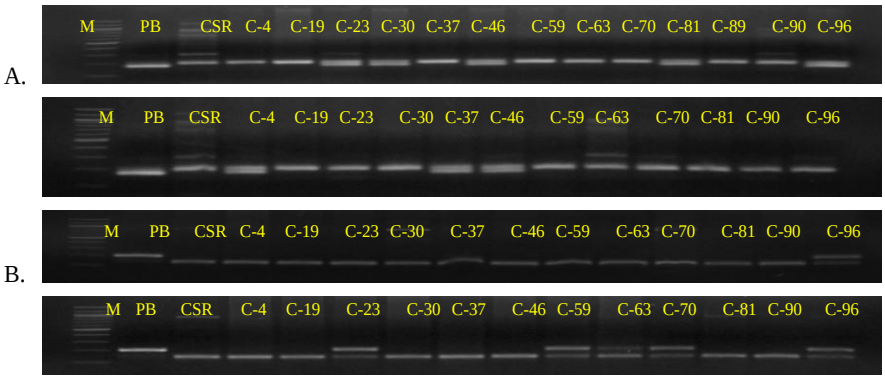


Fig.3 Dendrogram (NTSYS-pc, UPGMA) showing genetic similarity among the BC_1F_1 two gene-pyramided genotypes and parental rice genotypes based on SSR diversity data at 428 loci.

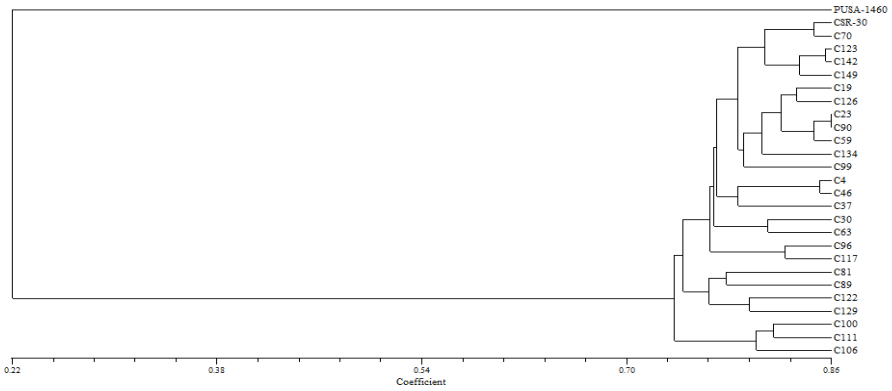
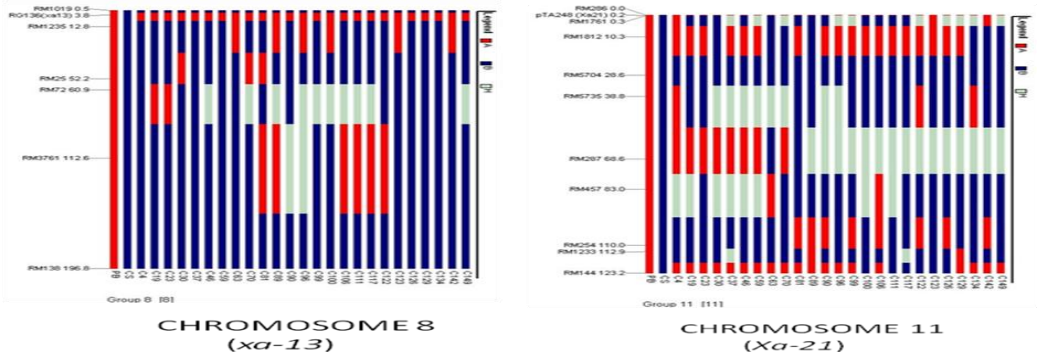


Fig.4 Demonstration of donor parent and recurrent parent genome on chromosome 8 and 11(having BLB resistance genes *xa13* and *Xa21*) in the twenty five, two-gene pyramided BC_1F_1 genotypes



Several BB resistance genes have been identified and characterized in non-aromatic rice and incorporated and pyramided through MAS to develop resistant cultivars (Perumalsamy *et al.,* 2010; Rajpurohit *et al.,* 2010). Gene pyramiding through MAS aims to assemble desirable genes from multiple parents into a single genotype. Tightly linked DNA markers have been developed for several BB resistance genes. Using the gene pyramiding approach, improved rice cultivars with broad spectrum BB resistance have been developed by combining multiple resistance genes (Huang *et al.,* 1997; Sanchez *et al.,* 2000; Singh *et al.,* 2001; Joseph *et al.,* 2004; Perez *et al.,* 2008; Sundaram *et al.,* 2008; Rajpurohit *et al.,* 2010; Dokku *et al.,* 2013; Suh *et al.,* 2013, Baliyan *et al.,* 2018).

The pyramided BC_1F_1 lines were evaluated stringently for their resistance to BB in the field and under glass house conditions using the *Xanthomonas oryzae* strain isolated from the BB infected fields of RRS, Kaul, CCSHAU, Hisar. The twenty five two-gene pyramided BC_1F_1 plants derived in this study from the cross CSR-30 x Pusa Basmati-1460, were found to be more effective against the virulent *Xoo* strain than the donor parent Pusa Basmati-1460. The pyramided lines with two gene combination had a higher level of resistance and broader spectrum of resistance than parental lines or lines with a single gene. The results indicated that the genes in combinations were more effective against the pathogen strain than a single resistance gene. This indicates that there is some kind of quantitative complementation with the presence of multiple resistance genes having an additive effect on the overall level of resistance. The marker RG 136 for *xa13* is 3.7 cM away from the resistant gene and hence there could be recombination between marker locus and the target gene. This may account for higher lesion lengths observed in some two gene combinations.

Molecular marker assisted background analysis of pyramided genotypes is useful in determining the relative contribution of the parents genome. Molecular marker analysis with SSR gives quick evaluation of the genetic background of the pyramided genotypes. A total of 111 out of 428 SSR markers produced polymorphism between the parental genotypes Pusa Basmati-1460 and CSR-30. The polymorphic primers were used to find out the recovery of RPG in the pyramided BC_1F_1 genotypes having two BB resistance genes *Xa21* and *xa13*. The percentage recovery of RPG in the pyramided BC_1F_1genotypes ranged from 62.7% to 83% as revealed by the global statistics using the software Graphical Geno Types (GGT)

version 2.0. Theoretically with single backcross average background recovery should be 75%. In the present study, higher RPG recovery was achieved, contrary to the theoretical expected value of approximately 75% at the BC_1F_1 generation. Reduced background recovery in some lines is largely due to linkage drag of the donor genotype on the carrier chromosomes around two target genes *Xa21* and *xa13*. Gopalakrishnan *et al.*, (2008) carried out MAS for the two resistance genes inBC_1F_1, BC_1F_2 and BC_1F_3 generations. On background analysis using 252 polymorphic AFLP markers they detected 80.4 to 86.7% recurrent parent alleles in BC_1F_3 selections. The results obtained employing AFLP markers and phenotypic selection (Joseph *et al.*, 2004) suggests addition of one round of background selection in BC_1F_1 may greatly increase the efficiency of the program. This approach used in the study ensured the realization of the major objective for generation of pyramided genotypes with enhanced resistance to BB.

Most of the two-gene pyramided BC_1F_1 genotypes were similar or superior to the recurrent parent CSR-30 with respect to the agronomic traits. A considerable reduction in the plant height was observed among pyramided BC_1F_1 genotypes than the recurrent parent CSR-30 and had 1000 grain weight greater than the recurrent parent. In field conditions, the genotypes with maximum recovery of RPG, P-C-70 and P-C-149, showed yield/plant higher than the recurrent parent CSR-30. Similarly in net house, the genotypes with maximum recovery of RPG, P-C-142, showed yield/plant higher than the recurrent parent CSR-30 whereas, genotype P-C-123 exhibited yield/plant almost similar to CSR-30. Therefore, all of the selected pyramided BC_1F_1 genotypes were found to have the agronomic traits similar to that of recurrent parent.

In conclusion the pyramided lines selected from cross CSR-30 x Pusa Basmati-1460 showed the agronomic features similar or at par with the recurrent parent, CSR-30. Based on agronomic evaluation, BB reaction and percentage recovery of RPG, grain quality characteristics, four genotypes viz. P-C-70, P-C-123, P-C-142 and P-C-149were found promising.

Rice is highly susceptible to BB disease for which a number of effective genes have been tagged and cloned. More resistance genes need to be identified and pyramid together into the elite cultivars to ensure the durability of BB resistance. Breakdown of resistance by new or unrecognized pathogen races is a major drawback. However, this can be prevented by pyramiding number of resistance genes into single cultivar. In this study, genes conferring broad spectrum resistance were successfully introgressed through MAS. The pyramided genotypes performing better or equivalent to CSR-30 in grain yield in addition to resistance against BB should be further evaluated at different locations. The pyramided genotypes obtained in our study can be used as genetic resources for BB resistance in breeding programs that will help to achieve better disease management. Marker assisted selection thus can be successfully utilized to transfer combinations of BB resistance genes into other preferred rice cultivars without compromising yield traits.

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