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Pyramiding of bacterial blight resistance genes in rice variety Jyothi (Ptb 39) through marker assisted selection

Kabade Pramod Gorakhanath[1]*, Rose Mary Francies[1] and Patil Namrata Devidas[2]

[1]Kerala Agricultural University, Vellanikara, Thrissur, Kerala, India

[2]Vasantrao Naik Marathwada Krishi Vidyapeeth, Krishinagar, Parbhani, Maharashtra, India

**Corresponding author e-mail: pramodkabade007@gmail.com*

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ABSTRACT

As in other rice growing locales around the world, in Kerala too, various climatic, edaphic, biological, physical, physiological and socio-economic variables impact the area, production, and productivity of the rice. Bacterial blight (BB), an important biotic stress caused by Xanthomonas oryzae pv. oryzae (Xoo) assumes a huge role in deciding rice profitability in Kerala. PTB 39 (Jyothi) and Mo 16 (Uma) are both elite rice varieties of Kerala, but extremely susceptible to the bacterial blight. Host-plant resistance is advocated as the most effective breeding strategy to combat the BB in contrast to the use of hazardous plant protection chemicals. Breeders have attempted to introgress disease resistance genes (R-genes) into rice cultivars to impart BB resistance. Marker-assisted selection (MAS) enables pyramiding multiple R-genes along with rapid background recovery of the recurrent parent, while maintaining the exquisite quality characteristics of rice.Considering the impact of the disease on food security and sustainability, efforts to introgress three R-genes (xa5, xa13 and Xa21) into the variety Jyothi from donor parent Improved Samba Mahsuri (ISM) through MAS were made. Foreground selection of the BC_2F_4 individuals was done using the Sequence Tagged Sites (STS) as well as functional markers. Foreground selection of the BC_2F_4 individuals was done using the xa5 gene linked STS marker RG556. Restriction digestion of the PCR product of the STS marker with Dra1 restriction enzyme, resulted in production of alleles of size 238bp and 438bp in all the BC_2F_4 individuals including the parents, indicating the presence of R-gene xa5. Amplification of DNA of the individuals with the functional marker xa5SR further confirmed the presence of R-gene xa5 in the parents as well as in the 51 BC_2F_4 individuals. Foreground selection with STS marker pTA 248 to detect the presence of Xa21 gene revealed that in BC_2F_4 Plant No. 9 and plant no. 21 amplicon of size 992 bp, as found in the donor parent ISM was present. And background selection was done by using rice microsatellite (RM) simple sequence repeats (SSR) markers.

Key words: *R-genes (xa5, xa13 and Xa21), bacterial blight (BB), marker-assisted selection (MAS), foreground selection, background selection, SSR (Simple sequence repeats)*

INTRODUCTION

Rice (*Oryza sativa* L.) with chromosome number, 2n= 24 and genome size of approximately 400 to 430 Mb, belongs to Poaceae family (Arumuganathan and Earle, 1991). Over 90 per cent of world's rice is grown and consumed in Asia and approximately 60 to 70 per cent of the caloric requisite of the Asian population is gained from rice. Owing to this, rice has become a paramount element of food security (Nanda and Agarwal, 2006). As found elsewhere, in Kerala too, the production and demand scenario of rice is not balanced. Hence, guaranteeing food security in future, demands a quantum jump in production and productivity. This increase in crop productivity is to be achieved in face of challenges from the diminishing rice cropped area owing to urbanization and unscientific land conversions, labor and water shortages and lowered profitability as well as a constant threat from biotic and abiotic stress factors. The humid environment prevailing in Kerala favors occurrence of both insects and pathogens throughout the rice cropping period. Among the various

diseases affecting the rice crop in the state, BB caused by Xoo has been found to be the most obliterating one. Drastic reduction in production and productivity of the popular elite varieties Uma and Jyothi grown in the state has been reported by the recurrent occurrence of bacterial leaf blight. Hence, efficient and effective management of BB disease recurrence in these elite cultivars was the need of the hour. The R gene family which confers resistance to the *Xanthomonas* is reported to be a multigene family with genes located throughout the rice genome at multiple loci. Presently, about 42 resistance R genes have been distinguished which provide host resistance against different strains of *Xoo*. These major genes have been designated in series from *Xa1* to *Xa42* and include 27 dominants and 14 recessive genes (*xa5*, *xa8*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa25*, *xa26b*, *xa28*, *xa31*, *xa32*, *xa33*, and *xa34*) in the series from *Xa1* to *Xa42* have been identified (Vikal and Bhatia, 2017). According to Priyadarisini and Gnanamanickam (1999), rice line NH56 carrying four R genes, (*Xa4* + *xa5* + *xa13* + *Xa21*) was found to be resistant to Kerala isolate of the *Xoo* pathogen. Studies conducted at Regional Agricultural Research Station, Kerala Agricultural University, Pattambi revealed that the R gene combinations *Xa4* + *xa13* + *Xa21*, *xa5* + *xa13* + *Xa21* and *Xa4* + *xa5* + *xa13* + *Xa21* confers broad spectrum resistance to Kerala isolate of Zoo (DRR, 2015). However, breakdown of resistance of cultivars with *Xa4* has been reported earlier by Mew et al. (1992). Introgression of multiple resistance genes into rice genotypes has been accepted as an effective methodology to make certain durable resistance against BB pathogen. Considering the above, an attempt was made to pyramid three bacterial blight resistant genes *viz., xa5* + *xa13* + *Xa21* into elite cultivar Jyothi (PTB 39) at the College of Horticulture, Vellanikkara. The BC_2F_4 population developed from the cross between high yielding rice variety Jyothi (PTB 39) and donor Improved Samba Mahsuri (ISM) formed the basis of the present study.

MATERIALS AND METHODS

Plant material

Fifty-one BC_2F_4 plants derived from the cross: Jyothi (PTB 39) x Improved Samba Mahsuri (ISM), along with parents [Recurrent parent: Jyothi (PTB 39), Donor parent: Improved Samba Mahsuri (ISM)] comprised the study material. The experiment was carried out in the field facility of Department of Seed Science and Technology, Kerala Agricultural University (KAU), Thrissur, between 2015 and 2017. Improved Samba Mahsuri released from Indian Institute of Rice Research, Hyderabad (formerly Directorate of Rice Research (DRR), Hyderabad), is an essentially derived variety (EDV) derived from the popular rice variety Samba Mahsuri. It was developed through MABB programme and contains the three bacterial blight resistance genes *xa5*, *xa13* and *Xa21*. The recurrent parent Jyothi is one of the most widely grown red kernelled rice variety in Kerala.

Genomic DNA and quantification

DNA (deoxy ribonucleic acid) of parents and backcross population (BC_2F_4s) was extracted as per the Modified CTAB method advocated by Dellaporta et al. (1983) was used for the isolation of good quality DNA. Agarose gel (2 per cent) was used to visualize and quantify the isolated DNA samples with the help of a lambda digest ladder. Further confirmation of the quality and quantity of the DNA isolated was analysed using Nanometer (JH Bioinovations, India). The maximum absorbance of nucleic acids and proteins occurs at 260 nm and 280 nm respectively. The purity of DNA was assessed based on the A260/A280 ratio. A ratio of 1.8 to 2.0 indicated pure DNA.

Polymerase chain reaction (PCR)

The good quality DNA isolated from the leaf samples were further diluted to make a concentration of 50 ng/µl and were used for polymorphism study. The PCR amplification was performed in 10 µl total volume reaction mixture, 10x Taq buffer 1.00µl, dNTPs mix 0.50µl, $Mgcl_2$ (25mM) 25µl, Taq DNA polymerase (Genei, Bangalore, India) (1U) 0.40µl, primers (Forward and reverse) 1.00µl each, DNA sample 3.00µl. PCR reaction profile was followed with initial denaturation (hot start) at 95ºC for 3 min, denaturation at 94ºC for 1 min, primer annealing varied as per Tm value of primer for 1 min, primer elongation at 72ºC for 1.20 min. These steps were repeated for 35 cycles then final extension at 72ºC for 10.00 min and cooling at 4ºC for infinity time. PCR product was resolved on agarose gel (2 per cent) was prepared by boiling 5g agarose in 250 ml of

1X TAE buffer. After 5 to 10 min of melting agarose, ethidium bromide was added (0.5µg/ml) and mixed well. The DNA sample (5µl) along with 2µl, 6X loading dye was added to the wells using a micropipette. 100bp molecular weight DNA ladder was loaded in one of the wells as a standard marker for easy detection of the molecular weight of PCR product and interpretation of PCR results.

Foreground selection

For confirming the presence of the resistance allele of each gene in the backcross generation, three STS markers RG556, RG136 and pTA248, closely linked to the BB resistance genes *xa5*, *xa13*, and *Xa21*, respectively were used (Table 1). Restriction digestion of the STS markers RG556 with restriction enzyme *Dra1* and marker RG136 with enzyme *Hinf 1*, was done after PCR amplification as advocated by Sundaram et al. (2008). In addition, the functional marker *xa5SR* and *xa13* promoter were also used to confirm the presence of R genes *xa5* and *xa13*, respectively.

Background selection

Fifty rice microsatellite (RM) markers [SSR (Simple Sequence Repeats) markers] (Table 3) reported to be polymorphism between the parental genotypes Jyothi and Improved Samba Mahsuri, were chosen to genotype BC_2F_4 generation, in order to determine the genotypic background of the pyramided lines with recurrent parent Jyothi. PCR products were separated by agarose gel electrophoresis. No restriction digestion of PCR product was done for markers used for background selection.

Analysis of DNA amplification pattern in parents and BC_2F_4s

The banding pattern of PCR bands was observed to be either monomorphic or polymorphic using UVITEC fire reader software (Cambridge, UK). The molecular weight of every PCR bands was measured by comparing with given known reference molecular weight marker ladder. Amplicons of the same size were recorded as monomorphic bands whereas bands of different sizes were recorded as polymorphic. The obtained results of molecular weight were then processed with graphical genotyping tool (GGT) version 2.0 (Van Berloo, 1999) software and used as output (Fig. 4 and Fig. 5).

Statistical analysis

The data generated from genotyping of BC_2F_4 population where analysedwith graphical geno types (GGT) version 2.0 (Van Berloo, 1999) software. It was used for evaluating the genomic contribution of the parent in each recombinant based on the SSR data. The software generates similarity matrix as per Sneath and Sokal (1973) and clusters based on default similarity coefficient and dendrogram were generated. Mini-tab software was used for cluster analysis of parents and selected BC_2F_4s. The proportion of genome of the recipient parent was estimated according to Sundaram et al. (2008) as follows:

$G\ [(X+1/2Y)\times 100]/N$, where N = total number of parental polymorphic markers screened, X = number of markers showing homozygosity for the recipient

Table 1. List of markers used for foreground selection

Gene	Primer name	Primer sequence	Marker distance(cM)	PCR product size (bp)	Reference
xa5	xa5SR F	AGC TCG CCA TTC AAG TTC TTG AG	0.0	410, 310, 180	Petpisit et al. (1977)
	xa5SR R	TGA CTT GGT TCT CCA AGG CTT			
	RG 556 F	ATA CTG TCA CAC ACT TCA CGG	0.1	440, 410	
	RG 556 R	GAA TAT TTC AGT GTG TGC ATC			
xa13	RG 136 F	TCC CAG AAA GCT ACT ACA GC	3.8	530, 490	Sundaram et al.(2008)
	RG 136 R	GCA GAC TCC AGT TTG ACT TC			
	xa13 Pro F	GGC CAT GGC TCA GTG TTT AT	0.7	500	
	xa13 pro-R	GAG CTC CAG CTC TCC AAA TG			
Xa21	pTA 248 F	AGA CGC GGA AGG GTG GTT CCC GGA	0.2	1000	Sundaram et al. (2008)
	pTA 248 R	AGA CGC GGT AAT CGA AAG ATG AAA			

parent alleles, Y = number of markers showing heterozygosity for the parental alleles.

RESULTS AND DISCUSSION

Bacterial blight (BB) in rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a devastating disease affecting rice crop and impacts rice production and productivity world over. Considering the advantage of host-plant resistance over cultural practices or chemical means to check the pathogen, an attempt was made to pyramid three genes conferring resistance to bacterial blight pathogen (*xa5*, *xa13*, and *Xa21*) into Jyothi (PTB 39) to develop durable resistance from improved Samba Mahsuri (ISM) through marker assisted selection. The outcome of the attempt is detailed below.

Foreground selection

Results confirmed that the genomic DNA extracted from the parents and the BC_2F_4 plantswere of good quality (OD260/OD280 of DNA extract: 1.7 to 1.9). Sufficient quantity of good quality total genomic DNA extracted from 51 BC_2F_4 plants, were subjected to foreground selection along with recurrent parent Jyothi and the donor parent ISM using rice microsatellites (Table 1).

STS marker RG 556 is reported to be tightly linked to R gene *xa5* at a distance of 0.1cM. The PCR analysis of the genomic DNA of the 51 BC_2F_4 and the two parents with the marker did not produce any polymorphism even after restriction digestion of the PCR product with *Dra1* restriction enzyme. In all the 51 BC_2F_4 and the parents, alleles of size 238 bp and 438 bp were present, indicating that all the BC_2F_4 individuals and the parents carried the R gene *xa5* (Fig. 1 and Table 2). On resolving the DNA of BC_2F_4s and parents on agarose gels, the functional marker *xa5* SR proved to be monomorphic as amplicon of size 190 bp was present in all the BC_2F_4 individuals and parents Jyothi and ISM. This further confirmed the presence of *xa5* gene in both the parents as well as in all the BC_2F_4s studied (Fig. 1 and Table 2). Studies by Bharathkumar et al. (2008) revealed that resistance in rice cultivars with single BB resistance gene breaks down in the field whereas a R gene pyramid is more durable. This substantiates the susceptibility reaction of parent Jyothi in spite of occurrence of the R gene xa5. Higher level of resistance to the *Xoo* pathogen, than would be expected from the sum of the parental levels has been reported in multiple BB resistance gene pyramided lines compared to those with single resistance gene (Yoshimura et al., 1996; Huang et al., 1997; Sundaram et al., 2008; Nayak et al., 2015; Pradhan et al., 2015).

Restriction digestion of PCR products of STS marker RG 136 with Hinf1 enzyme had produced homozygous alleles for the gene *xa13* in all BC_2F_4 individuals as found in the susceptible parent. Hence, it was evident that none of the BC_2F_4 plants possessed the R gene *xa13*. Similarly, the 280bp amplicon associated with the resistant allele found in donor parent ISM was absent in the BC_2F_4 individuals when amplified with functional marker *xa13* promoter. This further confirmed that the 51 BC_2F_4 individuals screened were not introgressed with the R-gene *xa13* (Fig. 2 and Table 2). The results thus pointed that all the 51 BC_2F_4s possessed only a single R gene (*xa5*).

The STS marker pTA 248 reported to be tightly linked to dominant R gene *Xa21* is located at a distance of 0.2cM from *Xa21* (Dokku et al., 2013; Nayak et al.,2015; Pradhan et al., 2015). Hence, it has been widely used for precise and early detection of genotypes carrying the R gene *Xa21*. Out of the 51 BC_2F_4

Table 2. Segregation of molecular markers in selected BC_2F_4s and parents (Foreground selection)

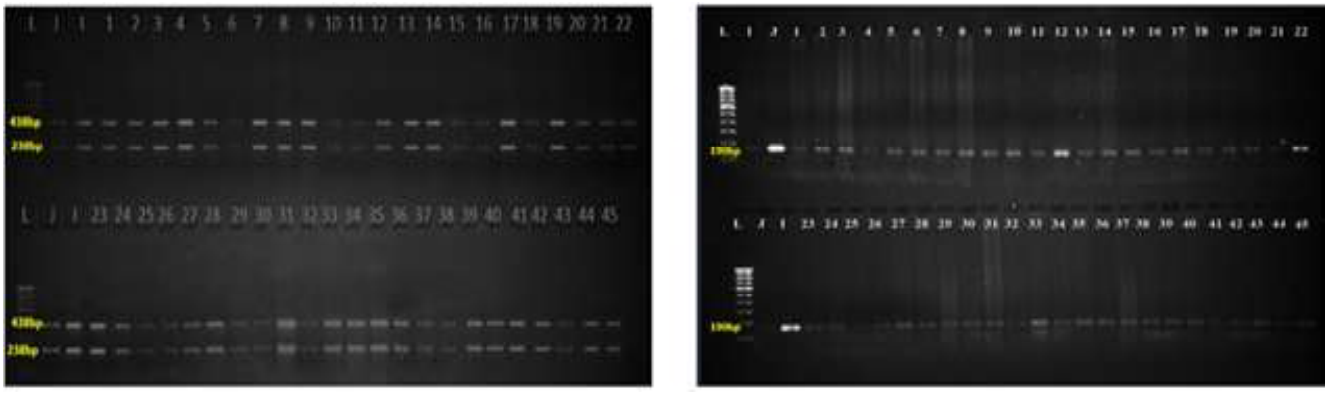
Sl. No.	Markers	Nature of amplification	Number of amplification	Size of amplicon (bp) Polymorphic		
				Recurrent parent (Jyothi)	R-gene introgressed BC_2F_4 plants	Donor parent (ISM)
Markers employed in the foreground selection						
1	*xa5* SR	Monomorphic	1		190	
2	RG 556	Monomorphic	2		238, 438	
3	*xa13* promoter	Polymorphic	2	280		428
4	RG 136	Polymorphic	2	945		635
5	pTA 248	Polymorphic	2	678	992	992

Table 3. Segregation of molecular markers in selected BC_2F_4s and parents (Background selection).

Sl. No.	Markers	Nature of amplification	Number of amplicon	Size of amplicon (bp)						
				Recurrent parent(Jyothi)	Plant No.5	Plant No.9	Plant No.21	Plant No.25	Plant No.27	Donor parent (ISM)
1	RM1	Polymorphic	2	107	*	*	*	*	*	132
2	RM6340	Polymorphic	2	154	*	***	**	*	*	148
3	RM10871	Polymorphic	2	190	*	*	*	*	*	232
4	RM11069	Polymorphic	2	210	*	*	*	*	*	280
5	RM11313	Polymorphic	2	350	*	***	*	*	*	303
6	RM11342	Polymorphic	2	130	*	*	*	*	*	125
7	RM12038	Polymorphic	2	310	*	*	*	***	***	321
8	RM482	Polymorphic	2	487	*	*	***	*	*	469
9	RM3340	Polymorphic	2	150	*	***	*	*	*	146
10	RM12941	Polymorphic	2	180	**	*	*	***	***	167
11	RM11390	Polymorphic	2	210	*	*	*	*	*	180
12	RM16	Polymorphic	2	187	*	*	*	*	*	230
13	RM49	Polymorphic	2	179	**	**	**	***	*	192
14	RM85	Polymorphic	2	98	*	***	*	*	***	130
15	RM251	Polymorphic	2	147	*	***	*	*	*	123
16	RM15583	Polymorphic	2	140	*	*	*	*	*	122
17	RM307	Polymorphic	2	198	*	*	*	*	*	220
18	RM5586	Polymorphic	2	110	*	***	***	*	*	105
19	RM6089	Polymorphic	2	174	*	***	*	*	*	154
20	RM6679	Polymorphic	2	110	*	***	***	*	*	106
21	RM17377	Polymorphic	2	290	*	***	***	***	***	285
22	RM18225	Polymorphic	2	409	*	***	*	*	*	429
23	RM18919	Polymorphic	2	485	*	***	*	***	*	460
24	RM19218	Polymorphic	2	172	*	*	*	***	*	160
25	RM217	Polymorphic	2	187	**	*	*	***	***	170
26	RM214	Polymorphic	2	132	*	*	*	*	*	121
27	RM248	Polymorphic	2	360	*	*	*	*	*	309
28	RM295	Polymorphic	2	210	***	*	*	***	*	200
29	RM20833	Polymorphic	2	198	***	*	*	*	*	172
30	RM21345	Polymorphic	2	218	*	**	**	***	*	215
31	RM72	Polymorphic	2	179	**	**	*	*	*	164
32	RM5545	Polymorphic	2	130	*	*	*	*	*	125
33	RM5556	Polymorphic	2	146	*	*	*	***	*	103
34	RM23087	Polymorphic	2	410	*	*	*	***	*	392
35	RM107	Polymorphic	2	129	*	*	*	***	*	98
36	RM205	Polymorphic	2	127	*	**	**	*	*	110
37	RM524	Polymorphic	2	190	*	***	*	*	*	170
38	RM23998	Polymorphic	2	279	*	***	***	*	*	260
39	RM304	Polymorphic	2	120	*	*	*	***	***	125
40	RM7545	Polymorphic	2	234	*	*	*	***	*	203
41	RM202	Polymorphic	2	141	*	***	***	*	*	165
42	RM224	Polymorphic	2	200	*	***	***	*	*	179
43	RM26213	Polymorphic	2	309	*	*	*	***	*	299
44	RM26868	Polymorphic	2	170	*	*	*	*	*	175
45	RM17	Polymorphic	2	459	*	*	***	*	*	472
46	RM19	Polymorphic	2	208	*	***	***	*	*	206
47	RM247	Polymorphic	2	198	*	***	***	*	*	175
48	RM260	Polymorphic	2	94	*	***	*	*	*	82
49	RM28277	Polymorphic	2	240	*	***	***	**	**	209
50	RM308	Polymorphic	2	149	*	*	*	***	*	141

Key: * Recurrent parent allele **Heterozygous allele ***Donor parent allele

individuals scored with the STS marker pTA 248, two individuals *i.e.,* Plant No. 9 and Plant No. 21 were found to possess alleles (855 bp) similar to the donor parent ISM. These were also found to be homozygous



xa5 linked STS marker RG556 *xa5* linked functional marker *xa5* SR

Fig 1. Foreground selection of BC_2F_4s using *xa5* linked STS marker RG556 and functional marker *xa5SR* (L: Ladder, J: yothi, I: Improved Samba Mahsuri,1-45: BC_2F_4 plants).

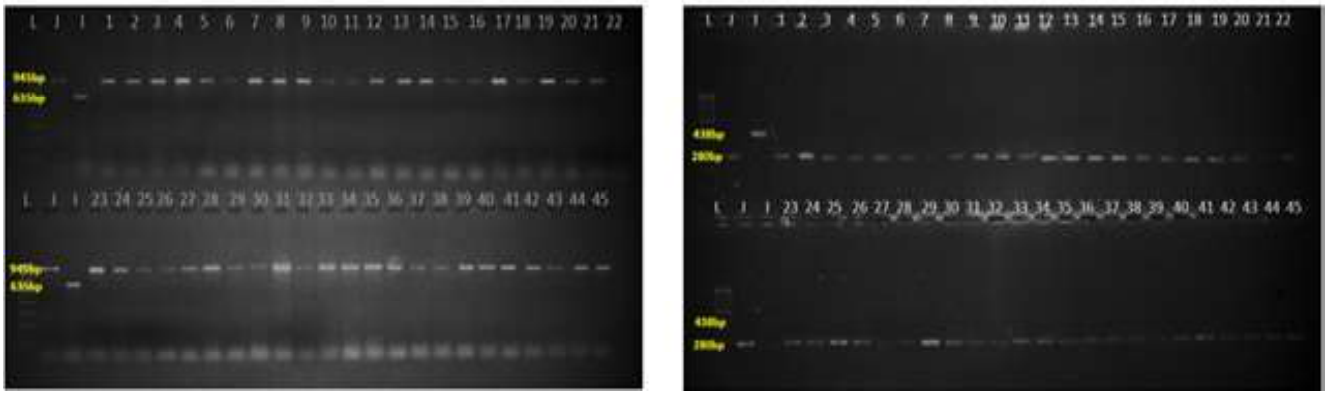
with the donor parent allele (Fig. 3 and Table 2).

To summarise, the foreground selection of the 51 BC_2F_4 individuals revealed that two BC_2F_4 plants (Plant No. 9 and Plant No. 21) were introgressed with the two R-genes *xa5* and *Xa21* (Table 2). Absence or no recovery of 3-R gene pyramids, recovery of 2-R gene pyramids with various combinations of R genes and occurrence of single R gene introgressions, are a common occurrence in a backcross programme owing to gene segregation and independent assortment. Arunakumari et al. (2016) in their attempt to pyramid two major bacterial blight (BB) resistance genes (*Xa21* and *xa13*) and a major gene for blast resistance (*Pi54*) into an Indian rice variety MTU1010 through marker-assisted backcross breeding found that, out of a total of 293 BC_1F_1 plants generated, only 55 were identified to be positive for *Xa21*, 68 were positive for *xa13* and 8 were double positive for both *Xa21* and *xa13*.

The study also pointed out the presence of R gene *xa5* in both parents (Jyothi and ISM) as well as in the BC_2F_4s. The alleles of the R-genes in each of the two 2-R gene pyramids (*xa5* + *Xa21*) thus obtained were also found to be in the homozygous state as in the donor parent and therefore expected to show no segregation in further selfed generation. It is also expected to exhibit a higher degree of resistance to the BB pathogen than the remaining single gene introgressions.

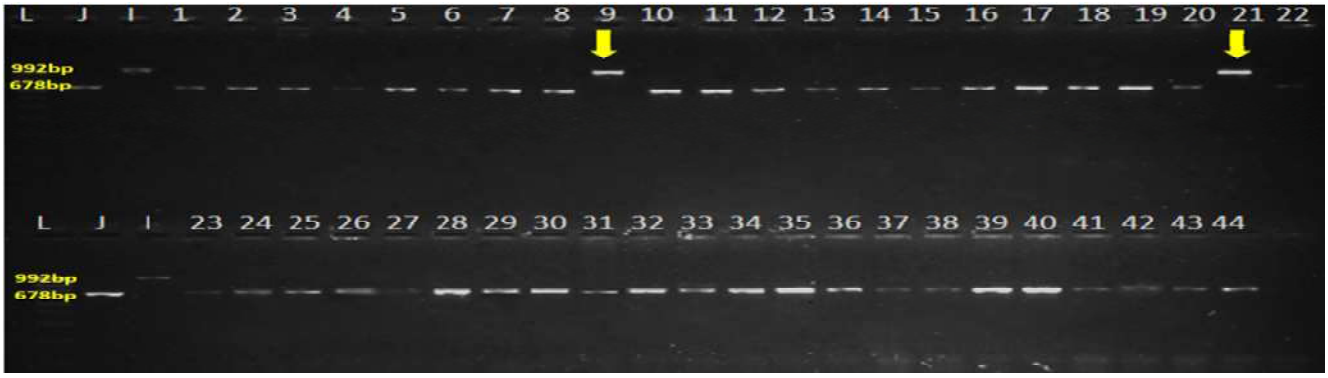
Background selection

According to Joseph et al. (2004), background selection in the backcross generation would greatly enhance the efficiency of marker assisted backcross breeding and



xa13 linked STS marker RG136 *xa13* linked functional marker *xa13 promoter*

Fig. 2. Foreground selection of BC_2F_4s using *xa13* linked STS marker RG136 and functional marker *xa13* promoter (L: Ladder, J: Jyothi, I: Improved Samba Mahsuri, 1-45: BC_2F_4 plants).



Foreground selection BC_2F_4s using *xa21* linked STS marker pTA248

Fig. 3. Foreground selection of BC_2F_4s using *Xa21* linked STS marker pTA248 (L: Ladder, J: Jyothi, I: Improved Samba Mahsuri, 1-44: BC_2F_4 plants).

help release a cultivar with increased BB resistance. The background profiling of the two 2-R gene introgressed BC_2F_4 plants (Plant No. 9 and Plant No. 21) was done along with the donor parent ISM and the recurrent parent Jyothi

Results (Table 3) revealed that the banding pattern in all the BC_2F_4s individuals (Plant No. 5, Plant No. 9, Plant No. 21, Plant No. 25, and Plant No. 27) were similar to recurrent parent on analysis with twelve RM markers (RM1, RM10871, RM11069, RM11342, RM11390, RM15583, RM16, RM19255, RM19514, RM19793, RM20416, RM214, RM248, RM26868, RM307 and RM439). This indicated that the BC_2F_4s possessed the same allele as in recurrent parent Jyothi at these marker loci.

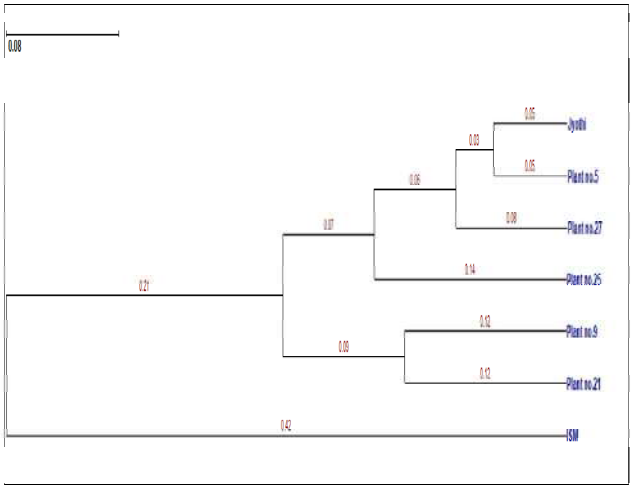


Fig. 4. Clustering of parents and selected BC_2F_4S based molecular data.

However, there was no consistency in banding pattern among the selected BC_2F_4s on genotyping with the remaining 34 RM markers. BC_2F_4 Plant No.9 followed by Plant No. 21 possessed higher number of donor alleles than the other three BC_2F_4 individuals screened. In addition, these plants along with Plant No. 5 also registered higher number (4 nos.) of heterozygous loci.Such variations may be attributed to the segregation and independent assortment of alleles in the early backcross generations. These plants could be expected to segregate for the alleles in subsequent generation.

Rice microsatellites have been successfully used to estimate the recovery of recurrent parent genome during background selection. The expected recovery of background of recurrent parent in BC2F4 generation is 87.50 per cent (Meksem et al., 2009).

In the present study, based on the segregation of the 50 markers, the recurrent parent genome contribution (Fig. 4 and 5) among the selected BC_2F_4 plants were estimated. Among the five BC_2F_4 individuals, the recovery of recurrent parent genome was found to be highest in plant no. 5 (92.20 %) followed by plant no. 27 (91.60%) while it was lower than the expected recovery in Plant No. 25 (76.40%), plant no. 21 (64.40%) and Plant No.9 (58.80%). The graphical representation of the results of genotyping of the BC_2F_4 plants done using the GGT software (Fig. 4 and 5) also confirmed the above findings.

The dendrogram generated out of the marker data resulted in two clusters, one with the five selected BC_2F_4 individuals along with the recurrent parent Jyothi.

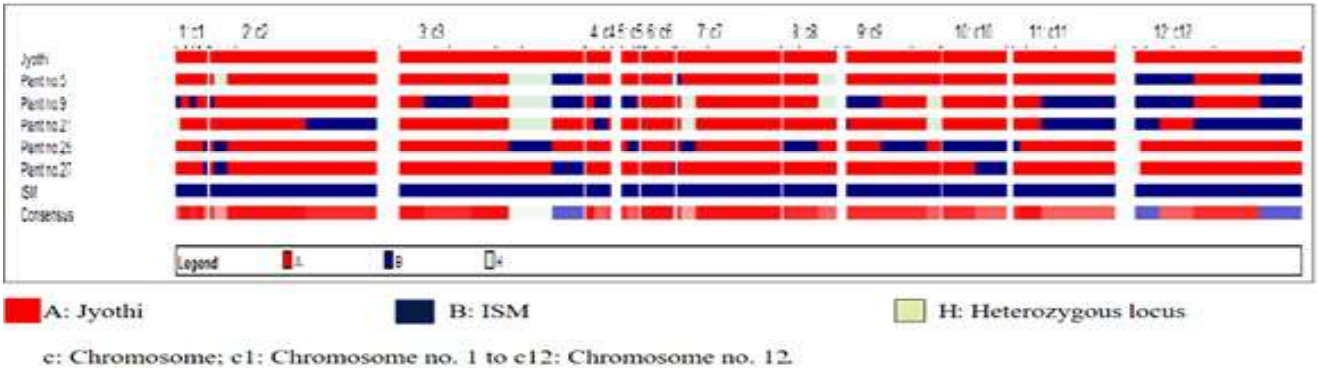


Fig. 5. Recovery of recurrent parent genome in selected BC_2F_4s

However, plant no. 9 and plant no. 21 were clustered into a sub-cluster of the cluster I containing Jyothi, indicating that they were less similar to the recurrent parent when compared to the other three BC_2F_4 individuals studied. This indicated that, the R-gene introgressed plants were genetically more similar to the donor parent ISM owing to larger linkage drag with the introgressed R-genes

CONCLUSION

The low per cent recovery of recurrent parent genome may also be due to the extremely lower number of rice microsatellite markers used for background screening. In the present study, only a small fraction of the marker loci has been covered (50 RM markers) and this might be the reason for the low recovery obtained. The results of foreground selection thus pointed out that, among the 51 BC_2F_4s studied, two individuals (plant no. 9 and plant no. 21) were the only 2-R gene pyramids. All the other individuals possessed only a single R gene (*xa5*). Based on background selection, the R-gene pyramids (plant no. 9 and plant no. 21) grouped into a separate sub-cluster farthest from recurrent parent Jyothi indicating that they were less similar to the recurrent parent when compared to the other three BC_2F_4 individuals studied. Conversely, this indicated that, the R gene introgressed plants were genetically more similar to the donor parent ISM. The Morphological results also further indicated that the selection based on genotypic data is reproducing at phenotypic level.

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