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Marker-assisted selection in back cross progenies for transfer of bacterial leaf blight resistance genes into a popular lowland rice cultivar

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ABSTRACT

Bacterial blight (BB) disease caused by Xanthomonas oryza*e pv.* Oryzae *(*Xoo*) is a wide spread disease of rice and causes significant yield losses. BB is predominant in lowland and waterlogged ecology. Many rice cultivars have been pyramided with resistant-gene combinations such as* xa5, xa13 *and* Xa21 *with durable resistance to BB. In this study, the target genes* Xa21, xa13 *and* xa5 *were selected in backcross progenies of Jalmagna and Swarna BB pyramid line using the markers pTA248, RG136 and xa5S,R (multiplex) during foreground selection. In foreground screening of* BC_1F_1 *progenies, fifteen plants were obtained with three BB resistant genes (*xa5, xa13 and Xa21*). The parents were screened with 236 rice microsatellite markers covering all the chromosomes to be used for background recovery of recurrent genome in the backcross progenies. Out of these markers, 120 were observed to be polymorphic for the two parents. Among the polymorphic markers, 60 best distinguishing markers were selected for background selection in the backcross progenies. As high as 85% recurrent genome content was observed in a foreground screened* BC_1F_1 *plant in background selection of the backcross progenies.*

Key words: *Low land rice, Bacterial leaf blight, marker assisted selection, BB resistance gene pyramiding*

Rice serves as a major carbohydrate source for nearly half of the world's population. In India, rice production area is around 43 million hectares. Rice accounts for 42% of food grain production and 55% of cereal production in India. With continuous increase in population, the food grain requirement is also increasing. India has to produce 135-140 million tons of rice by 2030. Many biotic and abiotic factors are limiting the rice production. Bacterial blight (BB) disease caused by *Xanthomonas oryza*e pv. *Oryzae* (*Xoo*) is a wide spread disease of rice causing significant losses. In some areas of Asia, it can reduce crop yield by 50% (Khush et al., 1989) or even up to 80% (Singh et al., 1977). Lowland and deep water ecology is a challenging ecology to increase rice productivity. BB is more common in this ecology reducing the yield to a greater extent. To counter the fast evolving pathogen and have eco-friendly management of BB, employment of cultivars carrying multiple resistance genes is an important approach. Till date 40 BB resistant genes are identified (Kim et al 2015). Out of these, eleven genes are recessive (*xa5*, *-xa5(t)*, *xa8*, *xa13*, *xa15*, *xa19*, *xa20*, *xa24*, *xa28*, *xa31* and *xa32*) and rest are dominant. Out of these genes, nine resistant-genes *Xa*1, *Xa*3/*Xa*26, *xa*5, *Xa*10, *xa*13, *Xa*21, *Xa*25, *Xa*27 and *Xa*38 have been cloned successfully following map-based cloning strategy (Han *et al*., 2014; Bhasin, *et al*., 2012; Chu *et al.*, 2006; Singh *et al*., 2001). Now-a-days, marker-assisted selection (MAS) provides more advantage to detect the transferred resistance genes during the breeding program precisely and easily using molecular markers. Molecular markers can increase the efficiency of backcrossing by allowing selection of genotypes with the maximum percentage of recurrent parent genome and minimizing linkage drag (Hospital, 2005). Based on marker-assisted selection, many rice cultivars have been pyramided with R-genes such as *xa5, xa13* and *Xa21* (Singh et al. 2001), *Xa4, xa5* and *Xa21* (Jung, et al. 2013). Jalmagna is a popular variety with good yield under waterlogged condition but

susceptible to BB. The present study targets introgression of three BB resistance genes into Jalmagna background and development of BB resistant popular rice cultivar for deep water ecology by using marker assisted backcross strategy.

MATERIALS AND METHODS

The donor parent CRMAS 2232-85 contained three BB resistance genes *xa5, xa13* and *Xa21* in the background of mega variety Swarna. The donor parent was developed at National Rice Research Institute (NRRI), Cuttack, India (Pradhan et al. 2015). The recurrent parent was Jalmagna, a highly popular variety of deepwater ecosystem of India but highly susceptible to bacterial blight disease. Jalmagna was hybridized with CRMAS 2232-85 and F_1 plants were backcrossed with recipient parent Jalmagna to produce BC_1F_1. Selection based on foreground and background for identification of lines that were similar to the recurrent parent possessing the three BB resistance genes.

Mini scale DNA isolation for PCR analysis was carried out as per Dellaporta et al. (1983). The PCR reaction mixture contained 50ng 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 (Table 1). The list of rice microsatellite markers used for parent polymorphism study for background selection (Table 2). The PCR products of STS marker RG 136 were digested with restriction enzymes *Hinf*I as per manufacturer's instructions. The PCR products and the DNA fragments produced by restriction digestions were separated by gel electrophoresis and gel images were analyzed on gel documentation system (SynGene).

RESULTS AND DISCUSSION

Swarna pyramided line (CRMAS 2232-85) contained three BB resistance genes (*xa5, xa13 and Xa21*) where as Jalmagna is a deepwater rice variety but highly susceptible to bacterial leaf blight. The parent polymorphism study can differentiate the donor and recipient parent at molecular level showing the presence/absence of particular genes or alleles. The parent polymorphism survey was carried out for the donor (CRMAS 2232–85) and recurrent parent (Jalmagna) for the three target genes *Xa21, xa13* and *xa5* using the markers pTA248, RG136 and xa5S,R (multiplex) for their use in foreground selection in the marker assisted backcross program (Table 1). The parents were polymorphic with respect to these three genes. This is because of absence of the resistance genes in the recurrent parent, Jalmagna. In addition, the parents were screened with 236 rice microsatellite markers (Table 2) covering all the chromosomes to be used for background recovery of recurrent genome in the backcross progenies. Out of these markers, 120 were polymorphic for the two parents (Table 3). Among the polymorphic markers, clearly distinguishing best 60 markers were selected for background selection in the backcross progenies. A representative electrophoregram of parent polymorphism survey for background selection is depicted in Figure 1.

Table 1. Markers used for foreground selection of three bacterial blight resistance genes in marker-assisted backcross breeding

Resistance gene	Chromosome number	Marker	Primer sequences used for gene detection Forward(5'-3')	Reverse(5'-3')	Expected size (bp)	Band type	Reference
xa5	5	xa5S (Multiplex)	GTCTGGAATTT GCTCGCGTTCG	TGGTAAAGTAGATA CCTTATCAAACTGGA	410bp, 310bp, 180bp	STS	Sundaram *et al.*,2011
		xa5SR/R (Multiplex)	AGCTCGCCATTC AAGTTCTTGAG	TGACTTGGTTCT CCAAGGCTT			
xa13	8	RG136	TCCCAGAAAG CTACTACAGC	GCAGACTCCAG TTTGACTTC	530bp, 490bp	STS	Huang *et al.*,1997
Xa21	11	pTA248	AGACGCGGAAGG GTGGTTCCCGGA	AGACGCGGTAA TCGAAGATGAAA	1000bp	STS	Huang *et al.*,1997

Table 3. Polymorphic markers detected by parent polymorphism study

Chrom#	Total No. of microsatellite markers	No. of polymorphic markers	Name of polymorphic markers	Percentage of polymorphic markers
1	25	11	SSR09, SSR 31, SSR 60 ,SSR 71, RM23, RM48, RM212, RM272, RM575, RM428, RM488	44
2	24	12	SSR11, SSR 14, SSR 44 ,SSR 71 ,SSR 85, RM154, RM211, RM233A, RM263, RM475, RM45, RM530	50
3	21	10	SSR 06, SSR 13, SSR 18, SSR 45, SSR 85, SSR 93, RM16, RM130, RM218, RM203	47.62
4	18	12	SSR 04, SSR 10, SSR 19, SSR 32, SSR 40, RM241, RM307, RM401, RM55, RM471, RM518, RM470.	66.66
5	19	12	SSR 05, SSR 13, SSR 21, SSR 27, SSR 34, SSR 37, SSR 43, SSR 50, SSR 59, RM164, RM592, RM440.	63.16
6	20	10	SSR 21, SSR 31, SSR 54, RM225, RM276, RM340, RM402, RM586, RM589, RM588.	50
7	21	9	SSR 28, SSR 37, SSR 41, SSR 44, RM10, RM336, RM560, RM432, RM346	42.86
8	20	10	SSR 14, SSR 48, RM223, RM241, RM407, RM3395, RM6208, RM22550, RM22506, RM8271	50
9	16	7	SSR 40, SSR 42, RM219, RM242, RM257, RM410, RM3555.	43.75
10	17	9	SSR 03, SSR 06, SSR 11, SSR 25, SSR 30, RM171, RM216, RM333, RM330.	52.94
11	18	11	SSR 3, SSR 4, SSR 11, SSR 27, RM21, RM144, M202, RM206, RM209, RM260, RM287.	61.11
12	17	7	SSR 23, SSR 26, SSR 36, RM17, RM195, RM415, RM23.	41.18
Total	236	120		

The recipient and donor parents were hybridized and 600 F_1 seeds were generated. True hybridity of the F_1 plants was tested by using the markers pTA248, RG136 and xa5S,R for the three target genes as well as two rice microsatellite markers (Figure 2). The F_1 plants possessing alleles from both the parents were carried forward for backcrossing with the recurrent parent Jalmagna to get BC_1F_1 seeds. The marker assisted selection helped in selecting the true hybrids and avoiding the selfed plants. Singh et al. (2001) also reported similar results.

BC_1F_1 plants were tested with primers pTA248, RG136 and xa5S,R closely linked to BLB resistance genes, *Xa21*, *xa13* and *xa5* respectively. Out of 344 BC_1F_1 plants, 108 BC_1F_1 plants showed the presence of *Xa21* resistance gene specific bands (1000bp) while 116 plants showed the presence of *xa13* resistance gene specific bands (490bp and 530bp) (Table 4). One hundred twenty BC_1F_1 plants showed the presence of *xa5* resistance gene specific bands (160bp). Based on the amplification of resistance specific bands, 48 BC_1F_1 plants showed the presence of *Xa21* and *xa13* resistance genes while 41 BC_1F_1 plants showed the presence of *Xa21* and *xa5* resistance genes (Table 5). Fifty one BC_1F_1 plants showed the presence of *xa13* and *xa5* resistance genes. Only fifteen plants were positive for all the three BB resistance genes (*Xa21, xa13* and xa5*)*. Figure 3 shows representative electrophoregram for foreground screening of the BC_1F_1 plants.

These plants possessing the three BB resistance genes were screened with the 60 polymorphic rice microsatellite markers in order to identify the plant having highest recurrent genome content. The detailed result of the background selection has been presented in Table 6. After this background selection, BC_1F_1 plant No.07 showed highest percentage (85%) of recurrent genome. Because of the non-survival of this particular plant, further breeding programme was carried out with plant No.127 having 81.7% of recurrent genome. The advantage of marker assisted selection over the conventional breeding here was that we are able to select the plant with 85% of genome content that can be used for next generation backcrossing. But in conventional breeding the average genome content will be 75% in BC_1F_1 and the plant

Table 2. List of markers used for survey of parental polymorphism

Sl. No.	Chrom#	Name of hypervariable markers	Sl. No.	Chrom#	Name of hypervariable markers	Sl. No.	Chrom#	Name of hypervariable markers
1	1	SSR07	80	4	RM241	159	8	RM241
2	1	SSR09	81	4	RM307	160	8	RM337
3	1	SSR13	82	4	RM335	161	8	RM339
4	1	SSR24	83	4	RM401	162	8	RM407
5	1	SSR31	84	4	RM470	163	8	RM433
6	1	SSR41	85	4	RM471	164	8	RM3395
7	1	SSR60	86	4	RM518	165	8	RM6208
8	1	SSR65	87	4	RM537	166	8	RM8271
9	1	SSR71	88	4	RM551	167	8	RM22506
10	1	SSR79	89	5	SSR02	168	8	RM22550
11	1	SSR85	90	5	SSR05	169	9	SSR02
12	1	RM 9	91	5	SSR13	170	9	SSR10
13	1	RM23	92	5	SSR21	171	9	SSR24
14	1	RM48	93	5	SSR27	172	9	SSR39
15	1	RM212	94	5	SSR34	173	9	SSR40
16	1	RM259	95	5	SSR37	174	9	SSR42
17	1	RM272	96	5	SSR43	175	9	SSR45
18	1	RM283	97	5	SSR50	176	9	RM105
19	1	RM428	98	5	SSR59	177	9	RM201
20	1	RM488	99	5	RM26	178	9	RM219
21	1	RM495	100	5	RM164	179	9	RM242
22	1	RM575	101	5	RM233B	180	9	RM257
23	1	RM578	102	5	RM267	181	9	RM285
24	1	RM3453	103	5	RM289	182	9	RM410
25	1	RM10017	104	5	RM440	183	9	RM566
26	2	SSR3	105	5	RM459	184	9	RM3555
27	2	SSR11	106	5	RM480	185	10	SSR03
28	2	SSR14	107	5	RM592	186	10	SSR06
29	2	SSR27	108	6	SSR09	187	10	SSR11
30	2	SSR31	109	6	SSR13	188	10	SSR15
31	2	SSR33	110	6	SSR16	189	10	SSR21
32	2	SSR37	111	6	SSR21	190	10	SSR25
33	2	SSR44	112	6	SSR25	191	10	SSR30
34	2	SSR56	113	6	SSR31	192	10	SSR35
35	2	SSR66	114	6	SSR40	193	10	SSR42
36	2	SSR71	115	6	SSR46	194	10	RM171
37	2	SSR73	116	6	SSR54	195	10	RM216
38	2	SSR74	117	6	SSR63	196	10	RM269
39	2	SSR85	118	6	SSR74	197	10	RM311
40	2	RM6	119	6	RM204	198	10	RM330
41	2	RM154	120	6	RM225	199	10	RM333
42	2	RM211	121	6	RM276	200	10	RM342A
43	2	RM233A	122	6	RM340	201	10	RM484
44	2	RM236	123	6	RM402	202	11	SSR03
45	2	RM263	124	6	RM494	203	11	SSR04
46	2	RM475	125	6	RM586	204	11	SSR11
47	2	RM485	126	6	RM588	205	11	SSR13
48	2	RM526	127	6	RM589	206	11	SSR19
49	2	RM530	128	7	SSR04	207	11	SSR23
50	3	SSR6	129	7	SSR05	208	11	SSR27
51	3	SSR10	130	7	SSR13	209	11	SSR28
52	3	SSR13	131	7	SSR23	210	11	SSR75
53	3	SSR18	132	7	SSR28	211	11	RM21

Sl. No.	Chrom#	Name of hypervariable markers	Sl. No.	Chrom#	Name of hypervariable markers	Sl. No.	Chrom#	Name of hypervariable markers
54	3	SSR21	133	7	SSR37	212	11	RM144
55	3	SSR30	134	7	SSR41	213	11	RM202
56	3	SSR45	135	7	SSR44	214	11	RM206
57	3	SSR64	136	7	RM10	215	11	RM209
58	3	SSR71	137	7	RM11	216	11	RM224
59	3	SSR85	138	7	RM18	217	11	RM260
60	3	SSR93	139	7	RM180	218	11	RM286
61	3	RM16	140	7	RM234	219	11	RM287
62	3	RM130	141	7	RM248	220	12	SSR09
63	3	RM203	142	7	RM336	221	12	SSR10
64	3	RM218	143	7	RM346	222	12	SSR11
65	3	RM422	144	7	RM429	223	12	SSR16
66	3	RM426	145	7	RM432	224	12	SSR18
67	3	RM442	146	7	RM478	225	12	SSR20
68	3	RM514	147	7	RM505	226	12	SSR23
69	3	RM517	148	7	RM560	227	12	SSR26
70	3	RM570	149	8	SSR14	228	12	SSR36
71	4	SSR4	150	8	SSR16	229	12	SSR43
72	4	SSR10	151	8	SSR28	230	12	RM17
73	4	SSR19	152	8	SSR40	231	12	RM19
74	4	SSR21	153	8	SSR41	232	12	RM20
75	4	SSR27	154	8	SSR45	233	12	RM101
76	4	SSR32	155	8	SSR48	234	12	RM117
77	4	SSR38	156	8	RM38	235	12	RM235
78	4	SSR40	157	8	RM210	236	12	RM415
79	4	SSR49	158	8	RM223			

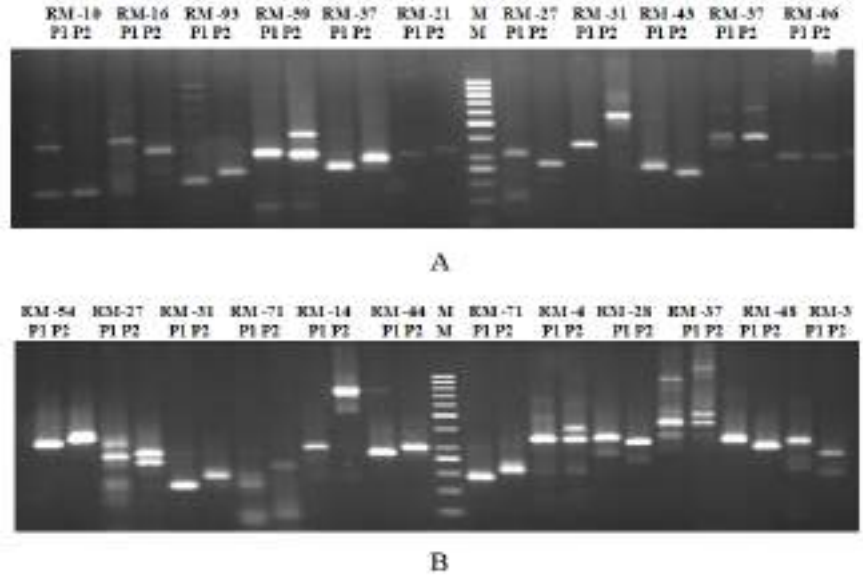

Fig. 1. (A, B) Polymorphism survey of parents CRMAS2232-85 and Jalmagna with rice microsatellite markers
M- 50 bp DNA ladder, P1-CRMAS2232-85, P2-Jalmagna
Numbers on the top of the gel indicate the polymorphic hyper variable rice microsatellite markers

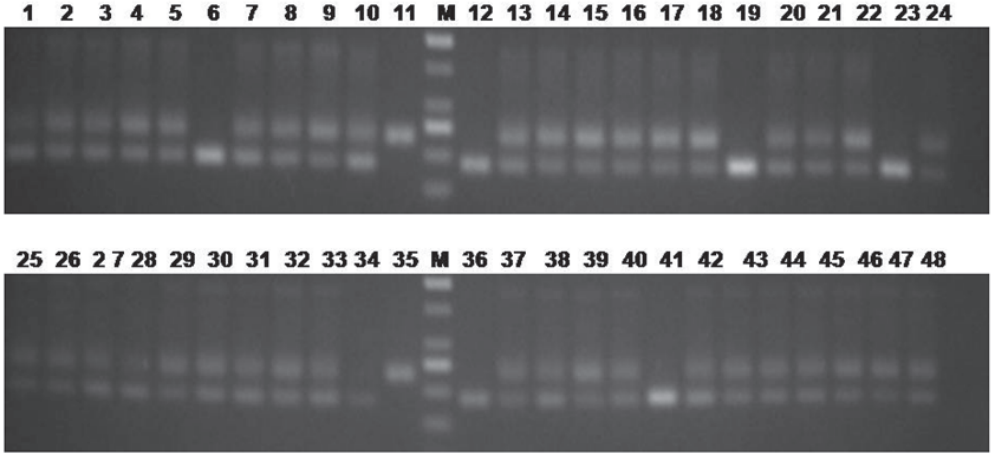

Fig.2. True hybridity test of F_1 progenies using the marker RM16.

Table 4. BC1F1 plants having single BLB resistance gene

Sl. No	Gene	No. of BC1F1	Plant No.
1	*Xa21*	108	1,6,7,9,13,14,16,19, 21, 24, 26,28, 32,38,40,42,44,47,53,59,60,61,62, 66,69,70,75,78, 80,85,91,93,95,105,111,112, 114,115,116,117, 119,125,127,128,129, 132,140,143,145, 150, 152, 158,163,170, 175,177,1 82,186,187,193, 194,196,198,199,200,201,203, 206, 207, 208, 210,212,214,216,218,219,221, 222, 226, 229, 230,231, 233,236,239,240,242, 244, 248, 252, 254, 255,256, 258, 260, 263,264,266,273, 277, 286, 291, 293, 300, 302, 304, 307, 319.
2	*Xa13*	116	1,5,6,7,9,12,13,14,17,18,22,24,28,29,30, 32,33,36, 37,40,41,45, 46,47,48,51,52, 53,54,56, 57, 58, 60,66,68,69,70,71,72,79, 81,84,93, 100,113,114,115, 121,124,127,128,130,137,147, 153,158, 163,164 ,166,167,168,170,174,175,180,181,1 82,186,187, 188,190, 191,193, 195, 201, 203, 207, 208,211,212,213,214,218,224,225,226,227, 228,229,230,233,235,237, 240,241,242,246,247,248,250,252, 253,257, 262, 263, 264,269,271, 273,275,282, 294, 302, 309, 313, 317.
3	*Xa5*	120	2,3,6,7,8,9,10,11,12,14,15,16,20, 25, 27, 28,29,30,31,33,36,37,38,41,46,48 ,51,54,55,58, 62, 64, 65,67,70,71,76,7 7,78,79,80,81,82,91,95,101,106,110,111,112,113,115,1 17,118,123, 124, 125, 127,130, 133,134,136,138,147,159,160,161,175,183 ,184,189,190,193, 197,199, 200, 205,207,210,211,213,221,222,223,224,225,226,227,232,234,236,237,239, 240,243, 244, 245, 246, 247,250,251,252,253,257,258,259,260,263, 266,268,271,272,273,282, 289,293, 299,307,312,317.

Table 5. BC_1F_1 plants having BLB resistance genes combination

Gene combination	No. of BC_1F_1	Plants No.
Xa21-xa13	48	1,6,7,13,14, 17, 24,28,32,40,47,53, 60,66,69,70,113,114,115,117,127,128,158, 163,170,175,182,186, 187,193, 201,203,207, 208,212,214,218,226,230,233,240,242,248,252,263,264,273,302.
Xa21-xa5	41	6,7,14, 16, 28,38,62,70,80,85,91,95, 111,112,115,117,125,127, 175,193, 199,200, 207, 210,221,222, 226, 236,237,239,240,244,252,256,258,260,263,266,273,293,307.
Xa13-xa5	51	6,7,9,12,14, 28, 29,30,33,36,37,41,46,48,51,54, 58,70, 71,79,81,115,124,127,130, 134,147,175, 182, 190,193, 207, 211,213,224,225,226,227,237,240,246,247,250,252,253,257,263,271,273,282,317.
Xa21-xa13-xa5	15	6, 7, 14, 28, 70,115, 127, 175, 193, 207, 226, 240, 252, 263, 273

Table 6. Background selection of the BC1F1 plants having three BB resistance genes

ORG NO	6	7	14	28	70	115	127	175	193	207	226	240	252	263	273		
Primers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	cr	jal
hyv1-31	JJ	JJ	JJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYv1-71	JJ	JJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
HYV1-60	JJ	JJ	JJ	CJ	JJ	CJ	JJ	CJ	JJ	JJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYV2-14	CJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYV1-30	JJ	JJ	CJ	JJ	CJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
HYV5-27	JJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	CC	JJ
HYV5-43	CJ	JJ	CJ	CJ	JJ	CJ	JJ	CJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	CC	JJ
HYV6-54	CJ	CJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
HYV5-59	JJ	CJ	CJ	JJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM336	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
RM488	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM530	CJ	JJ	CJ	JJ	CJ	CJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
HYV3-85	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
HYV4-19	CJ	JJ	CJ	JJ	JJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	CC	JJ
HYV4-32	CJ	CJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	CC	JJ
HYV11-11	JJ	JJ	CJ	JJ	CJ	JJ	CJ	CJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
HYV7-28	JJ	JJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	CJ	JJ	JJ	JJ	CC	JJ
HYV3-93	JJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYV11-13	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
RM16	JJ	JJ	JJ	JJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
RM307	JJ	JJ	CJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM19	CJ	CJ	CJ	JJ	JJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	CC	JJ
RM144	JJ	CJ	CJ	CJ	CJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CC	JJ
RM330	JJ	CJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	CJ	CJ	JJ	CJ	JJ	CJ	CC	JJ
RM263	CJ	JJ	JJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
RM276	JJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	JJ	CC	JJ
RM401	JJ	JJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
RM333	JJ	JJ	JJ	JJ	CJ	JJ	JJ	CJ	CJ	CJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
RM415	JJ	JJ	CJ	CJ	JJ	CJ	JJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	CC	JJ
RM428	CJ	CJ	CJ	CJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
RM219	CJ	JJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CC	JJ
HYV12-18	CJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYV11-3	JJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
HYV8-14	JJ	CJ	CJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	CJ	JJ	CJ	JJ	CJ	CC	JJ
HYV7-37	CJ	JJ	CJ	CJ	CJ	JJ	CJ	CJ	JJ	JJ	CJ	JJ	CJ	JJ	CJ	CC	JJ
HYV8-48	CJ	JJ	JJ	CJ	JJ	CJ	JJ	JJ	JJ	JJ	JJ	CJ	CJ	CJ	JJ	CC	JJ
HYV5-37	CJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	CJ	CJ	CC	JJ
RM218	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
RM410	JJ	CJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	JJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM3555	JJ	JJ	CJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
RM340	CJ	CJ	JJ	CJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	CJ	JJ	CJ	CC	JJ

RG NO	6	7	14	28	70	115	127	175	193	207	226	240	252	263	273		
Primers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	cr	jal
RM592	CJ	JJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	JJ	CJ	CJ	CJ	JJ	CJ	CC	JJ
RM588	JJ	CJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
RM223	CJ	JJ	JJ	CJ	JJ	JJ	CJ	JJ	JJ	CJ	CJ	JJ	JJ	CJ	CJ	CC	JJ
RM241	CJ	JJ	CJ	CJ	CJ	CJ	JJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CC	JJ
RM171	JJ	JJ	JJ	CJ	CJ	JJ	JJ	CJ	CJ	CJ	JJ	JJ	JJ	JJ	JJ	CC	JJ
RM180	JJ	CJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	CC	JJ
RM427	CJ	JJ	CJ	CJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
HYV2-18	JJ	JJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
HYV3-13	JJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
HYV5-43	CJ	CJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	JJ	CC	JJ
HYV9-42	JJ	JJ	CJ	JJ	CJ	JJ	JJ	JJ	JJ	JJ	CJ	CJ	CJ	JJ	CJ	CC	JJ
HYV10-30	JJ	JJ	JJ	JJ	CJ	JJ	JJ	JJ	JJ	JJ	CJ	JJ	CJ	JJ	CJ	CC	JJ
HYV12-26	JJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
RM287	JJ	CJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM235	CJ	JJ	CJ	JJ	CJ	CJ	CJ	JJ	CJ	JJ	CJ	CJ	JJ	JJ	CJ	CC	JJ
RM21	CJ	JJ	CJ	CJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	JJ	CJ	CJ	CJ	CC	JJ
RM216	JJ	CJ	CJ	JJ	JJ	JJ	JJ	CJ	CJ	JJ	JJ	JJ	CJ	CJ	JJ	CC	JJ
RM444	CJ	JJ	CJ	CJ	JJ	CJ	CJ	CJ	JJ	JJ	CJ	JJ	JJ	JJ	CJ	CC	JJ
RM475	JJ	JJ	CJ	JJ	CJ	JJ	JJ	CJ	JJ	CJ	CJ	CJ	CJ	CJ	CJ	CC	JJ
	94	102	77	92	92	93	98	86	94	88	78	95	86	95	78		
PERCENTAGE	78.3	85	64.2	76.7	76.7	77.5	81.7	71.7	78.3	73.3	65	79.2	71.7	79.2	65		
AVERAGE	75.60																

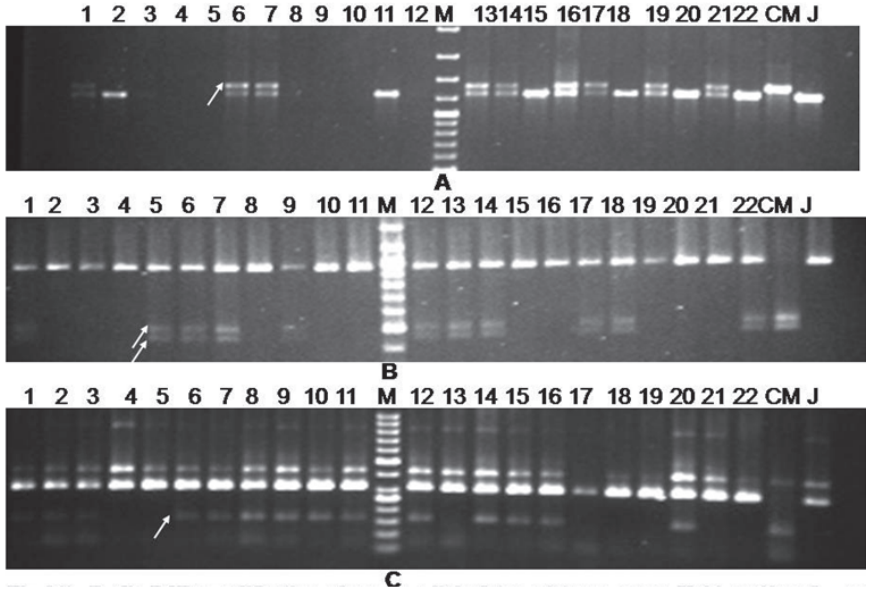

Fig 3. (A, B, C). PCR amplification of markers linked to resistance genes, *Xa21, xa13* and *xa5* using primers A) pAT248, B) RG136 and C) xa5S and xa5R/R, respectively. Lanes on the top of the gel shows the BC_1F_1 plant no. CM- CRMAS 2232-85, J-Jalmagna, M-Molecular weight marker. Arrow indicates tolerant band.

selected might possess even less genome content than the average where there may necessity of more number of backcrossing in order to attain required recurrent genome content. Many earlier successful studies on marker assisted backcrossing in rice were reported with less number of generations (Singh *et al.* 2001; Shanti *et al.,* 2001; Bharatkumar *et al.,* 2008; Sundaram *et al.,* 2008; Rajpurohit *et al.,* 2011; Dokku et al., 2013; Suh *et al.,* 2013; Jung *et al.,* 2013).

Marker-assisted backcrossing using functional markers reduce the risk of false selection in recombination between the molecular marker and the gene of interest. We were successful in identifying superior recombinations for three BB resistance genes (*Xa21, xa13 and xa5*) in BC_1F_1 generation. Using background selection, breeding generation can be reduced due to selection of high recurrent genome content plant in early generation. Further backcrossing programme with the gene introgression lines will lead to development of a BB resistant cultivar in Jalmagna background that can be more popular in lowland ecology than the recurrent parent.

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