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GENOME WIDE ANALYSIS OF RECURRENT PARENT GENOME RECOVERY IN DIFFERENT BACKCROSS POPULATIONS FOR BLAST RESISTANCE THROUGH SSR MARKERS IN RICE

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Abstract

Marker-assisted backcross breeding technique has been widely applied for incorporation of blast resistance genes into rice varieties. The monitoring of resistance genes along with recovery of recurrent parent is an essential aspect for reducing the donor genome content in backcross population. Current research was conducted to determine the recovery of recurrent parent in each backcross population of rice. Malaysian high yielding but blast susceptible rice variety MR219 was taken as recurrent parent while PongsuSeribu 2 was donor parent. Microsatellite markers commonly called as Simple sequence repeat markers were used to estimate recovery of recurrent parent genome i.e. MR219 in early generation of backcross population. A total of 300 microsatellite markerswere randomly applied on 12 rice chromosomes. 72 SSR markers found distinct and clear polymorphic between the parent PongsuSeribu 2 and MR219. These 72 polymorphic markers were utilized to analyze the recovery of plants in further subsequent generations. The background recovery ranged from 73-93.9% and 79-96.3% in BC_1F_1 and BC_2F_1 generation. The proportion of recurrent parent genome increased and donor genome content decreases after every backcross generation. The recovery of recurrent parent genome content ranged from 94-97.1% in the selected improved advance lines of BC_2F_2 generation. In the advance lines, the average percent for the proportion of recurrent parent genome was 96.16%. Marker-assisted backcrossing efficiently accelerated the recurrent parent genome recovery within few backcrosses, reduced the backcross generation and saved plenty of time to intogress the genes against blast disease. The present results will be helpful for rice breeders for selecting true blast resistant lines along with maximum resemblance with recurrent parent.

Key words: Marker-assisted backcross breeding, Recurrent parent, Blast resistance, SSR, MR219.

Introduction

Plant diseases are one of the main factor which limits rice production. Among the diseases, rice blast caused by fungus *Magnaportheoryzae*is of main attention due to wide spread throughout the world (Sere *et al.,* 2007). About 80 rice growing countries have reported that every year, 10 million tons of rice products loss because of blast disease (Wen & GAO, 2012). The harvested grains of rice affectby blast and ultimately reduce the actual yield. The research conducted on blast has proven that most effective, eco-friendly and the minimum cost input way is the development of blast resistant rice cultivar. Rice is not only the major edible source of food for most of the population of Malaysia, but also a basic source of income and employment of the farmers. MR219 is the most preferable rice variety of the farmers of Malaysia and cultivated on wider scale (Fasahat *et al.,* 2012). Currently MR219 variety is susceptible to blast because of emerging of new pathotypes of *Magnaportheoryzae* and environmental changes. The wide adaptability of MR219 cultivar by local farmers has been declined.

Recent advances in the field of biotechnology have provided more precise and efficient tools for selection on the basis of markers for desire traits instead of phenotype. Among the marker-assisted selection strategy, marker-assisted backcrossing is the most reliable, rapid and effective method for incorporation of blast resistance genes or quantitative trait loci (QTL) into advance rice varieties (Ashraf & Foolad, 2013). Application of conventional breeding has been minimized due to the development of advance genotypes within limited period of time with the utilization of marker-assisted backcross breeding (Septiningsih *et al.,* 2009). So far, various varieties of hybrid rice having greater magnitude of resistance towards blast and bacterial blight disease have been introduced (Basavaraj *et al.,* 2010; Pervaiz *et al.,* 2010). Marker-assisted selection for target genes never influence by the environmental factor and also the reliability of transference of target genes are high (Chen *et al.,* 2005). The selection of target genes is based on the closely linked molecular markers.

The effectiveness of marker-assisted backcross breeding relay upon different factors such as precise selection of locus where gene of interest is present, no. of backcross for recovering most of the recurrent parent characters and reduction of linkage drag (Frisch & Melchinger, 2005). During monitoring the target locus, recovery of the recurrent parent genome is also essential. If the recurrent parent genome is not recovered it is probably to increase the number of backcrosses until the recurrent parent genome is not completely recovered (Miah *et al.,* 2015). The recovery of recurrent parent genome is possible within three generation in marker-

assisted backcross breeding (Tanksley *et al.,* 1989). The combination of foreground and background selection in marker-assisted backcross breeding program ensures the reliability of selecting of the plants with desirable allele, along with maximum recurrent parent genome recovery (Singh *et al.,* 2013). The main aim of background selection is to completely recover the genome of recurrent parent and also to know that how much genome have been recovered in each backcross generation. In the present study recovery of recurrent parent MR219 was calculated in newly developed blast resistant lines by crossing MR219 and PongsuSeribu 2.

Material and Methods

Plant material, leaf sample collection and crossing techniques: Malaysian rice varieties MR219 and PongsuSeribu 2 were used in this breeding program. Leaf samples were collected from young 21 days old healthy plants of every backcross generation for the extraction of genomic DNA. The collected leaf samples were immediately transferred to the ice box and finally the samples were stored in the -80°C freezer.

Backcross breeding was used for crossing between recurrent parent (MR219) and donor parent (PongsuSeribu 2). The crossing was carried out until BC_2F_1 generation by following selfing and BC_2F_2 seed were produced. Six F_1 plants which showed heterozygous band were selected and backcrossed for the production of BC_1F_1 plants. Foreground selection and background selection were carried out. The plants showing the presence of blast resistance gene along with maximum recovery of recurrent parent were selected and backcrossed again to produce BC_2F_1 plants. Foreground and background selection were done in every back cross generations to select the best plants.

Molecular markers analysis: SSR marker tightly linked with *Pi* genes were selected and used for foreground selection. Both parents MR219 and PongsuSeribu 2 were subjected for polymorphic survey by using 11 foreground markers (RM495, RM5, RM208, RM168, RM251, RM413, RM340, RM5961, RM229, RM206 and RM101). Background selection was carried out by screening randomly 300 SSR markers for polymorphism between the parental line of recurrent parent MR219 and resistant donor PongsuSeribu 2 covering all rice chromosomes. Primer sequences of these markers were downloaded from publicly available database (www.gramene.org). For recovery of recurrent parent minimum 4-5 polymorphic SSR markers were selected.

Protocol for DNA extraction, PCR analysis and Gel electrophoresis: For DNA extraction, CTAB method as described by Doyle & Doyle (1990) was applied with minor modification in protocol. The DNA concentration was measured by using nano-drop spectrophotometer (ND1000 spectrophotometer). After measuring the DNA concentration the samples were diluted with 1×TAE (10 MmTris-HCl, Ph 8.0, 1 mM, EDTA, pH8.0) to adjust the concentration at 70 ng/µl. The samples were stored at -80°C.

The PCR amplification was run through protocol described by McCouch *et al.,* (2002). Each PCR reaction volume was 15 µl containing DNA template (70 Nano gram), forward primer (1.0 micro lit), reverse primer (1.0 micro lit), green master mix (7.4 micro lit) and water (4.6 micro lit). The standard PCR amplification protocol using touch down PCR was followed. The protocol consist of 94°C for 3 min followed by 10 cycles of 94°C for 30 s, 62°C for 1 min (decreasing 1°C per cycle), and 72°C for 30 s, and 30 cycles of 94°C for 30 s, 52°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min by rapid cooling to 4°C prior to analysis.

Preparation of gel was carried out by dissolving 3.0% metaphor^{TM}agarose (Lonza) gel in 1×TBE buffer, however, for staining Midori green (1 µl) was also added. The voltage for running gel was 80 for 1 hour and 20 minutes and amplified production was visualized in Molecular imager.

Data analysis

The banding patterns obtained after amplification with the primers were calculated by comparison to both parents. For foreground selection, if the band showing similarity with the level of donor variety was marked as 'R' (resistant) means expressing homozygous allele for resistant parent for specific SSR marker. Similarly, if the band showing same level as MR219 it was consider as 'S' (susceptible). The molecular weight of the different alleles was calculated with the Alpha Ease Fc5.0 software. The estimation of recurrent parent as background selection was calculated through Graphical Genotype (GGT 2.0) software (Van Berloo, 2008). The homozygous recipient allele was scored as "A", the homozygous donor allele was scored as "B" and heterozygous allele was scored as "H". The data wasanalysed through computer software GGT-2.0 program. The percentage of marker homozygous for recipient parent (%A) and the parent donor allele, including heterozygous plant (%B) was also calculated.

Results

Parental survey for markers polymorphism: All eleven tightly linked SSR markers with blast resistance genes (RM495, RM5, RM208, RM168, RM251, RM413, RM340, RM5961, RM229, RM206 and RM101) produced clear distinct polymorphism between the donor (PongsuSeribu 2) and recipient (MR219). For background survey, 72 random markers covering all twelve rice chromosome showed clear polymorphism between the MR219 and PongsuSeribu 2. Among them, 11 markers within *Pi* gene locus and 61 covering other loci were detected. The details about these markers including primer sequence, chromosomal locations and product size have been described in Table 1. Meanwhile, the %age of polymorphic markers on parental polymorphism was 24.1%. Further, these markers were utilized in BC_1F_1 and BC_2F_1 generation to detect the target gene along with recovery of recurrent parent. The position of polymorphic markers between two parents was identified on each chromosome of rice.

Table 1. Detail of seventy two SSR polymorphic markers between MR219×Pongsu Seribu 2.

Markers	Chr.	Primer sequence (5'-3')	Repeat motif	Expected size (bp)
RM4959	1	F: GTACAATATTTTTGGTAGGA R: CAACCAGCTTAACTAATTAA	(TA)31	149
RM259	1	F: TGGAGTTTGAGAGGAGGG R: CTTGTTGCATGGTGCCATGT	(CT)17	162
RM1	1	F: GCGAAAACACAATGCAAAAA R: GCGTTGGTTGGACCTGAC	(GA)26	113
RM5	1	F: TGCAACTTCTAGCTGCTCGA R: GCATCCGATCTTGATGGG	(GA)14	113
RM495	1	F: AATCCAAGGTGCAGAGATGG R: CAACGATGACGAACACAACC	(CTG)7	159
RM207	2	F: CCATTCGTGAGAAGATCTGA R: CACCTCATCCTCGTAACGCC	(CT)25	118
RM233	2	F: CCAAATGAACCTACATGTTG R: GCATTGCAGACAGCTATTGA	(CT)20	162
RM208	2	F: TCTGCAAGCCTTGTCTGATG R:TAAGTCGATCATTGTGTGGAC	(CT)17	173
RM250	2	F: GGTTCAAACCAAGCTGATCA R: GATGAAGGCCTTCCACGCAG	(CT)17	153
RM327	2	F:CTACTCCTCTGTCCCTCCTCTC R: CCAGCTAGACACAATCGAGC	(CAT)11(CTT)5	213
RM138	2	F: GTCGAGCTCGTCGTCAGTCGT R: TCGAAAGGCTGTCGCTGTGT	(GT)14	233
RM1106	2	F: CGGAAAGTGAATCGGAGAAC R: GCACCACGCTAAGCTAAACC	(AG)12	194
RM3501	2	F: TCCTAGTGCATCAGCACAGC R: GTCCGTTTCAGCAAGCAAAC	(CT)25	218
RM110	2	F:TCGAAGCCATCCACCAACGAA R:TCCGTACGCCGACGAGGTCGA	(GA)15	156
RM36	3	F: CAACTATGCACCATTGTCGC R: GTACTCCACAAGACCGTACC	(GA)23	192
RM218	3	F: TGGTCAAACCAAGGTCCTTC R: GACATACATTCTACCCCCGG	(TC)24ACT5(GT)11	148
RM251	3	F: GAATGGCAATGGCGCTAG R: ATGCGGTTCAAGATTCGATC	(CT)29	147
RM3131	3	F: CTCTGCACCCTGTTCACATG R: CCCAATGGAATATCAGGTGG	(CA)14	141
RM564	3	F: CATGGCCTTGTGTATGCATC R: ATGCAGAGGATTGGCTTGAG	228	(GT)14
RM252	4	F: TTCGCTGACGTGATAGGTTG R: ATGACTTGATCCCGAGAACG	(CT)19	216
RM7187	4	F: CAGCGAACGTGGTGTCTTC R: CCCACACCAACTTCTCGC	(ATAG)7	157
RM177	4	F:CCCTCTTAGACAGAGGCCAGA R:GTAGCCGAAGATGAGGCCGC	(AG)8	195
RM8212	4	F: CCACCGCACTTGTCTATG R: TCCAATCTCACTCTCGACTC	(GGA)9	182
RM281	4	F: ACCAAGCATCCAGTGACCAG R: GTTCTTCATACAGTCCACATG	(GA)21	138
RM166655	4	F:CCTTGGAAGCTGGAACTTCAC R:GCTCTTAGTTAGATCCCACAC	(CGG)7	138

Table 1. (Cont'd.).

Markers	Chr.	Primer sequence (5'-3')	Repeat motif	Expected size (bp)
RM348	4	F: CCGCTACTAATAGCAGAGAG R: GGAGCTTTGTTCTTGCGAAC	(CAG)7	136
RM413	5	F: GGCGATTCTTGGATGAAGAG R: TCCCCACCAATCTTGTCTTC	(AG)11	79
RM13	5	F: TCCAACATGGCAAGAGAGAG R: GGTGGCATTCGATTCCAG	(GA)6-(GA)16	141
RM5	5	F: TGCAACTTCTAGCTGCTCGA R: GCATCCGATCTTGATGGG	(GT)16TT(GT)4	246
RM164	5	F: TCTTGCCCTCACTGCAGTATC R:GCAGCCCTATGCTACAATTCT	(GT)16TT(GT)4	246
RM26	5	F: GAGTCGACGAGCGGCAGA R: CTGCGAGCGACGGTAACA	(GA)15	112
RM435	6	F: ATTACGTGCATGTCTGGCTG R: CGTACCTGACCATGCATCTG	(ATG)7	166
RM586	6	F: ACCTCGCGTTATTAGGTACC R:GAGATACGCCAACAGATACC	(CT)23	271
RM225	6	F: TGCCCATATGGTCTGGATG R: GAAAGTGGATCAGGAAGGC	(CT)18	140
RM217	6	F: ATCGCAGCAATGCCTCGT R: GGGTGTGAACAAAGACAC	(CT)20	133
RM527	6	F: GGCTCGATCTAGAAAATCCG R: TTGCACAGGTTGCGATAGAG	(GA)17	233
RM3187	6	F: TCCCCACATCGTGTCGTC R: TTTTTCCCCTTCTACCCTCG	(CT)12	142
RM340	6	F:GGTAAATGACAATCCTATGGC R: GACAAATATAGGCAGTGTGC	(CTT)8T3(CTT)14	163
RM30	6	F: GGTTAGGCATCGTCACGG R: GGTTAGGCATCGTCACGG	(AG)9A(GA)12	105
RM1253	7	F: CTGAACTGCCTGAGAACTC R: GACGACTCTCCATGCTCG	(AG)16	175
RM1132	7	F: ATCACCTGAGAAACATCCGG R: CTCCTCCCACGTCAAGGTC	(AG)12	93
RM1209	7	F: CCCAATGATTTGGTACTGCC R: GCCCCAGCTAATTCTTGATG	(AG)14	154
RM1364	7	F: AAGAAATTCAAAACACATGA R: AAAACATCTACTTTGATCCA	(AG)26	158
RM248	7	F: TCCTTGTGAAATCTGGTCCC R: GTAGCCTAGCATGGTGCATG	(CT)25	102
RM118	7	F:CCAATGGAGCCACGGAGAGC R:CACACTCCAGCGACGCCGAG	(GA)8	156
RM544	8	F: TGTGAGCCTGAGCAATAACG R: GAAGCGTGTGATATCGCATG	(TC)9	248
RM342	8	F: CCATCCTCTACTTCAATGAAG R: ACTATGCAGTGGTGTCACCC	(CAT)12	141
RM515	8	F: TAGGACGACCAAAGGGTGAG R: TGGCCTGCTCTCTCTCTCTC	(GA)11	211
RM80	8	F: TTGAAGGCGCTGAAGGAG R: CATCAACCTCGTCTTCACCG	(TCT)25	142

Table 1. (Cont'd.).

Markers	Chr.	Primer sequence (5'-3')	Repeat motif	Expected size (bp)
RM284	8	F: ATCTCTGATATCCATCCATCC R: CCTGTACGTTGATCCGAAGC	(GA)8	141
RM3609	9	F: AGGTGCACACATACTGTCGC R: AGGACATGGCATCTTCTTGG	(GA)13	158
RM105	9	F: GTCGTCACCCTCGGAGCCAC R: TGGTCAGGTGGGATCGGGTC	(CCT)6	134
RM242	9	F: GGCCAACGTGTGTATGTCTC R: TATATGCCAAGACGGATGGG	(CT)26	225
RM205	9	F: CTGGTTCTGTATGGAGCAG R: CTGGCCCTTCACGTTTCAGTG	(CT)25	122
RM245	9	F: ATGCCGCCAGTGAATAGC R:CTGAGAATCAATTATCTGGGG	(CT)14	150
RM216	10	F: GCATGGCCGATGGTAAAG R: TGTATAAAACCACACGGCCA	(CT)18	146
RM271	10	F: TCAGATCTACAATTCCATCC R: TCGGTGAGACCTAGAGAGCC	(GA)15	101
RM258	10	F: TGCTGTATGTAGCTCGCACC R: TGGCCTTTAAAGCTGTCGC	(GA)21(GGA)3	148
RM333	10	F:GTACGACTAGAGTGTCACCAA R: GTCTTCGCGATCACTCGC	(TAT)19(CTT)19	191
RM228	10	F: CTGGCCATTAGTCCTTGG R: GCTTGCGGCTCTGCTTAC	(CA)6(GA)36	154
RM167	11	F: GATCCAGGTGAGGAACACGT R:AGTCCGACACGGTGCGTTGTC	(GA)16	128
RM552	11	F: CGCAGTTGTGGATTTCAGTG R: TGCTCAACGTTTGACTGTCC	(TAT)13	195
RM287	11	F: TTCCCTGTTAAGAGAGAAATC R: GTGTATTTGGTGAAAGCAAC	(GA)21	118
RM229	11	F: CACTCACACGAACGACTGAC R: CGCAGGTTCTTGTGAAATGT	(TC)11(CT)5C3(CT)	116
RM5961	11	F: GTATGCTCCTCCTCACCTGC R: ACATGCGACGTGATGTGAAC	(CAG)8	129
RM206	11	F: CCCATGCGTTTAACTATTCT R: CGTTCCATCGATCCGTATGG	(CT)21	147
RM144	11	F: TGCCCTGGCCAAATTTGATCC R:GCTAGAAGATCAGATGGTAG	(ATT)11	237
RM3331	12	F: CCTCCTCCATGAGCTAATGC R: AGGAGGAGCGGATTTCTCTC	(CT)15	129
RM101	12	F:GTGAATGTCAAGTGACTTAGG R:ACACAACAGTCCCTCCCATGC	(CT)37	324
RM1337	12	F: GTGCAATGCTGAGGAGTATC R: CTGAGAATCTGGAGTGCTTG	(AG)21	210
RM260	12	F: ACTCCACTATGACCCAGAG R:GAACAATCCCTTCTACGATCG	(CT)34	111
RM270	12	F: GGCCGTTGGTTCTAAAATC R: TGCGCAGTATCATCGGCGAG	(GA)13	108

Genotyping for recovery of recurrent parent in BC_1F_1 generation: Foreground markers RM495, RM5, RM208, RM168, RM251, RM413, RM340, RM5961, RM229, RM206 and RM101 were used. In F_1 population, RM208 and RM206 produced heterozygous band. The four best F_1 plants carrying the allele linked with these markers were subjected to backcross and 120 BC_1F_1 plants were produced. In BC_1F_1 generation, out of 120 plants 67 plants produced heterozygous band screened with SSR marker RM208 and RM206 closely linked to blast resistance genes (Fig. 1a).

Polymorphic markers on each chromosome ranged from 5 (chromosome 1, 3, 5, 8, 9, 10, 12), 7 on chromosome 4, 6 on chromosome 8, 9 on carrier chromosome 2 containing gene of interest and 7 on carrier chromosome 11 also containing gene of interest. For background recovery analysis, 72 polymorphic markers were usedfor 67-BC_1F_1 plants obtained from foreground selection. In BC_1F_1, recurrent parent genome recovery ranged from 73% to 94% (Table 2). The range of recurrent parent genome recoveries of plants in BC_1F_1 population is shown in Figure 2a. Some of the markers which were utilized for background recovery of plants of BC_1F_1 generation are shown in Figure 2c.

Average recovery of the recurrent parent genome (RPG) of best selected 6 plants was 86.56%. The overall summary of recovery of RPG and a containing heterozygous segment of best selected plants in BC_1F_1 Population is shown in Table 2. The maximum donor segments were present on a genomic regionon chromosome 2 and chromosome 6 due to the linkage drag. The best individual plants with maximum recovery of RPG along with resemblance of the recurrent parent in term of morphological characters was planted no. 5-1. It was found that chromosome 1, 6 and 12 was recovered completely in the improved lines in contrast to other chromosomes. In case of chromosome 7 only one line was not fully recovered from selected best plants (Fig. 3a). The background recovery indicates that the majority of the residual segment was distributed on chromosome 2 and 11.

Genotyping for Recovery of recurrent parent in BC_2F_1generation: In the BC_2F_1 generation, the introgression of target genes were confirmed in 154 plants by using tightly linked markers RM208 and RM206 (Fig. 1b). Eight best progeny having maximum morphological resemblance with MR219 produced from BC_2F_1 generation were selfed to generate BC_2F_2 population. The background selection for recurrent parent genomerecovery of BC_2F_1 generation population was performed by screening 72 polymorphic markers resulting from foreground selection. Some of the polymorphic markers banding pattern in this generation is shown in Figure 1d. In BC2F1 generation, recovery of recurrent parent genome (RPG) ranged from 79.4% to 96.1% (Fig. 2b). Among the recovered plants, eight best plants were selected (5-3-1, 5-3-2, 5-3-4, 5-3-7, 5-3-11, 5-3-17, 5-3-20, 5-3-22) based on the foreground selection, maximum phenotypic resemblance and highest recovery of recurrent parent genome. Furthermore, Table 2 describes the overall summary of recurrent parent genome recovered and heterozygous segments of outstanding eight plants and their average. Figure 3b indicates the recoveries of recipient allele (chromosome wise) of selected outstanding eight plants. Chromosome 1, 6, 8 and 12 was completely recovered among these best eight plants.

Recovery of recurrent parent in BC_2F_2plants: Foreground selection was conducted to select most desirable plants having close resemblance to recurrent parent i.e. MR219 genome background along with homozygous resistant allele. The background selection was carried by constructing the genetic map covering about 1195 cM. However, average distance for each marker was 16.1 from region of whole genome of rice. The percentage of recurrent parent genome screened with 72 polymorphic SSR markers for background selection ranged from 94 to 97.1% in the selected tagged lines of BC_2F_2 population. However, most of the linkage drags (remaining residual segment) of donor genome were observed on chromosome 5, 9 and 10; and other chromosome were completely recovered. The improved lines were further evaluated for yield contributing characters in order to check their performance against their recurrent parent MR219 (Table 3).

Table 2. Estimation of background recovery and introgressed segment carrying target genes in selected advance lines of $BC_1F_1$and BC_2F_1population.

Population	Selected individuals	A (%)	B (%)	H (%)	Total (cM)	H-segment
	5-1	93.9	2.5	3.6	1269.3	3
	5-2	88.2	2.5	9.3	1269.3	6
	5-3	88.7	2.5	8.8	1269.3	6
BC_1F_1	5-4	83.4	2.5	14.1	1269.3	7
	5-5	85.7	2.5	11.8	1269.3	7
	5-6	86.8	2.5	10.7	1269.3	6
	Average	86.56	2.5	9.71	1269.3	5.83
	5-3-1	95.1	2.5	2.3	1266	2
	5-3-2	92.8	2.5	4.6	1266	4
	5-3-4	94	2.5	3.5	1266	3
BC_2F_1	5-3-7	96.3	2.5	1.1	1266	1
	5-3-11	92	2.5	5.5	1266	2
	5-3-17	96	2.5	1.5	1266	2
	5-3-21	95.1	2.5	2.4	1266	2
	5-3-22	95.5	2.5	2.3	1266	2
	Average	94.6	2.5	2.86	1266	2.25

A= Recurrent, B=Donor, H= Heterozygous, cM= Centimorgan

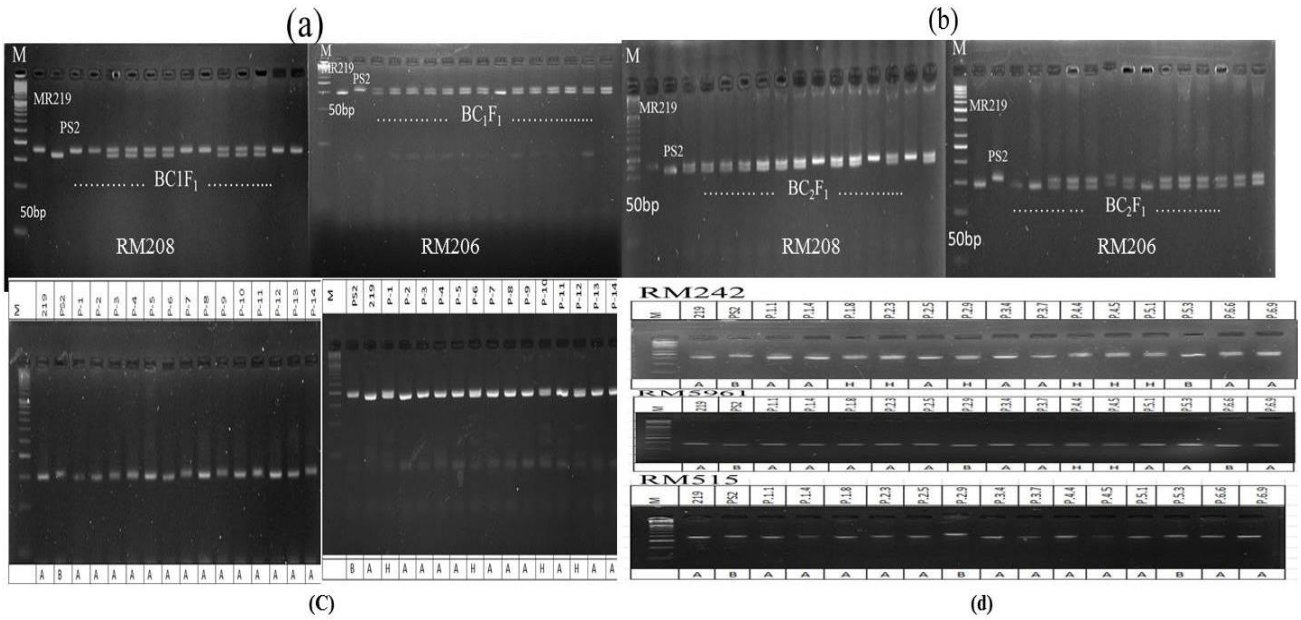

Fig. 1. Screening of resistant and susceptible plants using RM208 and RM206 marker in (a) BC_1F_1 generation (b) BC_2F_1 generation; banding pattern of background marker in (c) BC_1F_1 generation and (d) BC_2F_1 generation.

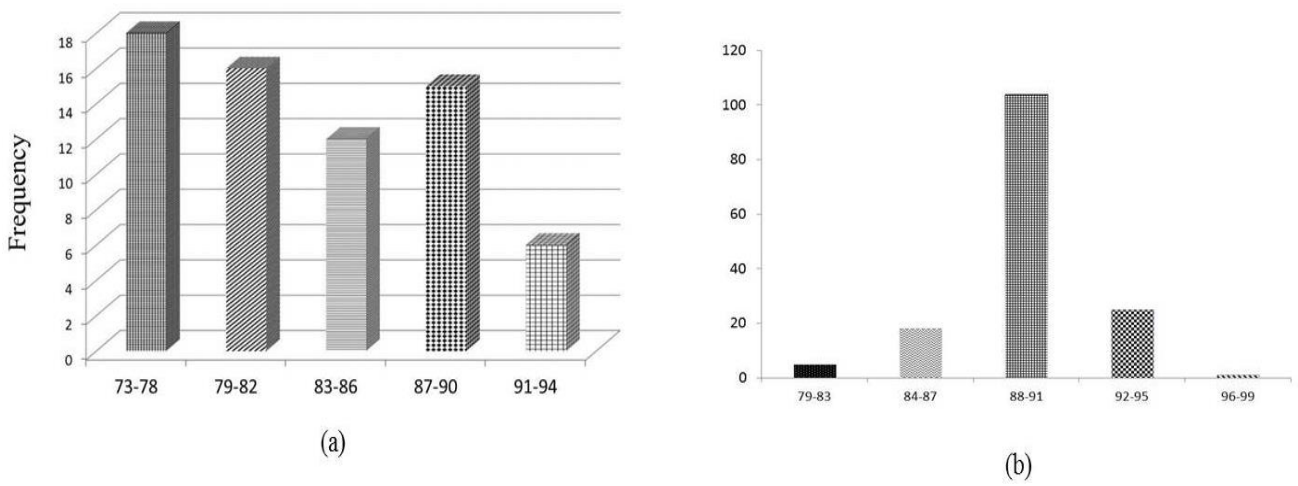

Fig. 2. Estimation of recovered recurrent parent genome in (a) BC_1F_1 and (b) BC_2F_1 generation population produced from cross between MR219 and PongsuSeribu 2.

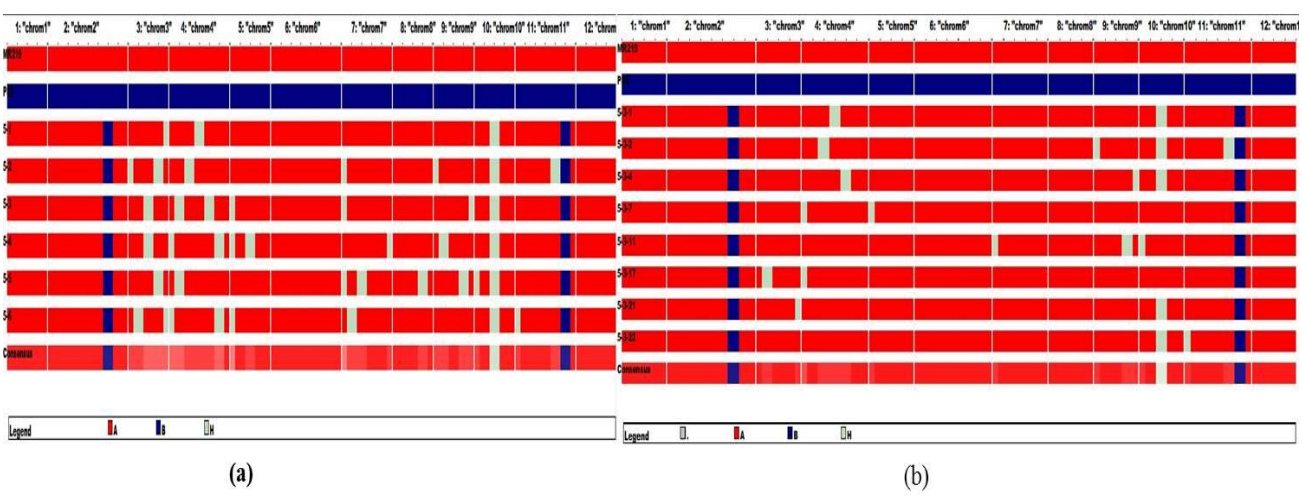

Fig. 3. Chromosome-wise recovery of recurrent parent genome in (a) BC_1F_1 and BC_2F_1 generation. Blue color indicate regions homozygous for PongsuSeribu 2, red color indicate homozygous region for MR219 and green color indicate the residual segments.

Table 3. Performance of yield-contributing traits in developed and recurrent parent population.

Characters	BC_2F_2 population	Recurrent parent
No. of filled grains/panicle	155.8 ± 0.89	154.1 ± 1.35
Grain length	9.77 ± 0.02	9.63 ± 0.04
Grain width (mm)	2.07 ± 0.03	1.94 ± 0.02
Yield/ plant (gm)	43.26 ± 0.22	42.06 ± 0.28
1000 grain weight (gm)	26.66 ± 0.25	25.86 ± 0.23
Total grain/panicle (no)	172.5 ± 0.79	166.9 ± 1.46
Panicle length (cm)	26.4 ± 0.25	25.2 ± 0.20
Total tiller/Hill (no)	18.46 ± 0.30	17.00 ± 0.30
Plant height	96.40 ± 0.23	95.33 ± 0.31

Significance at 5% level with independent t-test

Discussion

The success of the marker assisted backcross breeding totally depends upon the polymorphic markers which differentiate the parental genotype. However there would be a great acceleration of the recurrent parent genome if numbers of polymorphic markers per chromosomes increase (Vishwakarma *et al.,* 2014). Microsatellite markers have strong potential to improve the selection of better plants with desirable characters in marker-assisted selection program comparatively to conventional breeding. In the present study, the percentage of polymorphic markers was 24.1% throughout the genome covering all 12 rice chromosomes. Similar results was found by Linh *et al.,* (2012) while screening 447 markers between the parents BT7 and FL478 for salinity trait and found 89 (18.7%) markers polymorphic. Huyen *et al.,* (2012) also found 12.6% SSR markers polymorphic between the parents AS996 and FL478. Basavaraj *et al.,* (2010) found 54 markers (17.49%) polymorphic STMS markers distributed throughout the genome while studying the parental population of PRR78 and Pusa1460. Similarly Khanh *et al.,* (2013) screened 384 SSR markers and found 58 markers (15.1%) polymorphic between the parents Bac Thom and IR64. Miah *et al.,* (2015) screened 375 SSR markers between the population of PongsuSeribu 1 and MR219 and found 70 polymorphic SSR markers. The present results of our research and results of different researcher indicate that there would be more recovery of the recurrent parent if the numbers of markers per chromosomes are greater. The selection on the basis of markers provides authentic results and good idea to identify the best progeny for further backcrossing (Ahmed *et al.,* 2016). Ribaut *et al.,* (2002) mention that there are many factors responsible for selection of non-carrier chromosome for background analysis, including saturation of molecular map, technical resource availability and required level of line conversion.

The foreground selection of the target gene is feasible and economical with the use of SSR markers. Genetic analysis with molecular marker identified many markers located in the *Pi-b* gene region out of them RM208 is tightly linked marker with *Pi-b* gene (Miyamoto *et al.,* 1996; Monna *et al.,* 1997). Wang *et al.,* (1999) cloned the *Pi-b* gene and dissect the tightly linked SSR marker RM208 located on chromosome 2. Similarly Sharma *et al.,* (2005) cloned the *Pi-kh* gene and found that RM206 is tightly linked SSR marker and located on chromosome 11. Both of these markers provide great accuracy to introgress*Pi-b* and *Pi-kh* gene into blast susceptible rice cultivars with the marker-assisted selection (Tanweer *et al.,* 2015). The BC_1F_1 generation plants segregated into (1:1) for resistant versus susceptible with Chi square value 1.633. Mondal *et al.,* (2013) also found the same results while introgressing the salinity trait into recurrent parent Binadhan-7 from donor parent FL-478. The present results support the idea that phenotypically plants segregating into expected ratio reduce the selection pressure for the next generation of backcrossing.

Once the F_1 is backcrossed and BC_1 generation is produced, desirable plant with maximum recovery of recurrent parent can be selected. If the multiple plants showing the desirable condition, selection among those plants should carry out on the basis of analysis of the other marker present either on the carrier or no carrier chromosome for selection of the most desirable one for producing BC_2 generation (Frisch *et al.,* 1999). If individual lacking the target allele in BC_1 means that backcrossing failed in BC_1 generation. Different researcher has indicated in their research that less no. of individuals are required to obtain at least one individual with the target allele in BC_1 generation for producing the BC_2 (Hospital & Charcosset, 1997).

The background recovery for BC_1F_1 generation ranged from 73% to 94%, which is more or less similar to the results found by Cuc *et al.,* (2012) who also found the recovery range from 80.00% to 89.01%. The analysis of background recovery indicates that most of the residual segments were distributed over the chromosome 2 and 11. The present results also supported by the finding of Prigge *et al.,* (2008) who described that mean recovery of the recurrent parent genome in BC_1F_1 was (83% in the case of Swarna and 85% in the case of Samba Mashuri) generated.

The recovery of the recurrent parent in BC_2F_1 generation ranged from 79.4% to 96.1%. Our results are in agreement with the finding of Prigge *et al.,* (2008) who also found 97% in the case of Swarna and 95% in Samba Mashuri in BC_2F_1 generation. Ours results were also in correlation with the finding of Singh *et al.,* (2013) who found recovery of recurrent parent genome 91.6%, while pyramiding the blast resistant genes *Piz-5* and *Pi54* into an elite Basmati rice restorer line PRR78 using marker-assisted backcrossing approach. Khanh *et al.,* (2013) also

found 89.9% genetic background recovery in BC_2F_1 generation. The estimation of recurrent parent allele in each backcross generation made it possible to select the best genotype in each generation. Thus, increase the chance of selection of best plants carrying target allele along with maximum similarity with the elite recurrent line. The present finding strongly evident that analysis of the recurrent parent genome in each backcross greatly facilitates the reduction of linkage drag mainly spread throughout the genome carried by donor parent. Background selection is an important step for quick recovery of RPG within few backcrosses. In the present study the recovery of RPG was way much faster as compared to conventional breeding. The evenly spaced markers at least (two to four markers per chromosome of 100 cM) provide wide coverage of whole genome for background selection (Visscher, 1996). In our finding at least 5 markers per chromosome found polymorphic were evenly spaced and the recoveries of the recurrent parent genome within few backcrosses were greater. Visscher *et al.,* (1996) also demonstrates that marker distributed at the middle of the chromosome will be more informative than located near the end of chromosome. Along with marker distribution the number of target genes to be introgressed, the saturated dense molecular map, backcrossing steps, and applied breeding strategy is also should be considered for success of marker-assisted backcross breeding.

The highest recovery of the recurrent parent genome can widely be achieved with the utilization of background markers and proper phenotypic selection. The current approach not only reduces the time, but greatly enhances the breeding for desirable trait with minimum cost input and labors. The application of marker-assisted selection could be very effective if the number of polymorphic markers include in this study is more, but with the 24.1% polymorphic SSR markers between the parental line of PongsuSeribu 2 and MR219, marker-assisted selection for background recovery was still adequate. The combination of background markers with a strong phenotypic selection will rapidly increase the selection of the best plants within the minimum number of backcross generations.

Conclusion

The monitoring of the recurrent parent background along with the target loci in marker-assisted backcross breeding program is an important step toward developing of superior line. If the target loci is completely transferred, butthe recurrent parent background is not fully recovered, it will also produce linkage drag, in results require more number of backcross generations. The improve line carrying the blast resistance gene in the background of MR219 were completely advance in grain quality and yield contributing characters than a recurrent parent with additional blast resistance trait. The improved lines will be also useful for donor of blast resistance genes in future blast resistance breeding program. The present finding strongly support that Marker assisted backcross breeding has potential to recover the genetic background of recurrent parents in less number of backcross repeats.

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