



DETERMINATION OF RECURRENT PARENT GENOME IN RICE (*Oryza sativa* L.) THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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Received 20th April, 2026; Accepted 20th June, 2026

ABSTRACT: Marker-assisted backcrossing (MABC) strategy was used to introgress brown planthopper (BPH) resistant genes/quantitative trait loci (QTLs) from Rathu Heenati rice variety into the genome of Putra-2. A bi-parental mating design was used to develop F₁ plants and subsequent backcross generations. The primary objective of this research was to develop high yielding and BPH resistant rice variety for cultivation in Malaysia, and also to determine the percentage recurrent parent genome (RPG) recovery of Putra-2 rice variety in BC₁F₁, BC₂F₁ and BC₂F₂ generations. Foreground and background selections were conducted at each generation of backcrossing in order to select progenies having desired genes/QTLs and maximum percentage RPG recovery. In respect of this, out of 316 simple sequence repeats (SSRs) markers screened for polymorphism between the two parents, 79 were found polymorphic, and these markers were used to screen the plants in BC₁F₁, BC₂F₁ and BC₂F₂ generations. The RPG recovery analysis ranged from 80.3 to 84.9% with a mean of 82.1% and from 93.0 to 95.2% with an average of 94.1%, in BC₁F₁ and BC₂F₁ generations, respectively. In the BC₂F₂ selected improved lines, the RPG analysis ranged from 96.4 to 97.2% with an average of 96.4%. Unlike conventional backcrossing, the utilisation of MABC accelerated RPG recovery and reduced the number of backcross generations required to incorporate BPH resistance genes/QTLs into genetic background of Putra-2 rice variety. Thus, marker-assisted selection (MAS) saves energy and time, cost effective, more efficient and reliable in the selection processes compared to traditional breeding methods.

Keywords: RPG recovery, Rice, MABC, BPH resistance genes/QTLs, foreground selection, background selection

INTRODUCTION

Rice is the main source of energy for majority of the population world-wide (Khush, 2005; Ashkani *et al.*, 2012). Due to continuous population growth, there is a need to increase the global rice production by more than 40% in order to meet the demand of the world

population by the year 2030 (Khush, 2005). Yield of rice has greatly enhanced after green revolution, however, the expected yield capability of improved cultivars is not attained at the farmers' field condition. This predicament is attributed to biotic and abiotic factors that restrict rice production. processes (Korinsak *et al.*, 2016). Among the most important rice pests, BPH is one of the major devastating Asian rice insect pests that cause yield loss immensely to rice farmers (Kumari *et al.*, 2010; Haliru *et al.*, 2020). BPH management measures have been employed

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such as pesticide use and biological control, however, host plant resistance is the majorly preferred control strategy because it is economically viable and environmentally friendly (Song *et al.*, 2002; Jena *et al.*, 2006). The use of resistant varieties will assist to reduce pesticide usage and maintain balance in the ecological system by conserving natural enemies (Gurr *et al.*, 2011).

Current advances in molecular biology techniques have resulted to identification of more robust screening and selection methods to replace old ones in the development of new crop variety (Ashraf and Foolad, 2013). Successes have been recorded in utilisation of MAS for transfer and incorporation of BPH resistant genes to improve resistance to BPH susceptible rice cultivars (Suh *et al.*, 2011; Wang *et al.*, 2019). DNA markers are increasingly taking an important component in MAS programmes for detecting target genes. The MAS outcomes are regarded with higher precision due to their selective reliability and efficiency, which are devoid of environmental factors (Koide *et al.*, 2010; Ragimekula *et al.*, 2013). These factors ensure the development of an ideal line within limited time possible and reduce challenges to develop new line relative to conventional breeding programme (Miah *et al.*, 2015). The main idea of backcrossing is to retain the desired gene and reduce the donor parent genome to the barest minimum as quickly as possible. With each cycle of backcrossing, the mean genome of donor parent is decreasing while that of recipient parent is enhancing in the backcrossed progenies (Lewis and Kernodle, 2009). Marker-assisted foreground selection is the selection of progenies possessing the marker allele of the donor parent. On the other hand, marker-assisted background selection refers to the selection of progenies having highest RPG recovery in MABC programme. These strategies ensure that only progenies with target alleles that acquire highest RPG recovery are advanced to the subsequent generations of backcrossing (Singh *et al.*, 2012). The key objectives of this investigation were: (i) to introgress BPH resistant genes/QTLs from Rathu Heenati into Putra-2 rice variety and (ii) to determine the percentage recovery of puta-2 rice variety through MABC breeding scheme.

MATERIALS AND METHODS

Genetic materials and breeding scheme

The experiment was conducted at Ladang 10 Teaching and Research Farm and Laboratory of

Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security (ITAfOS), University Putra Malaysia (UPM) (Latitude; 2° 59' 59" N and Longitude; 101° 42' 45" E), Selangor, Malaysia. Putra-2 is a Malaysian high yielding and submergence tolerant rice cultivar (Ahmed *et al.*, 2016), but susceptible to BPH, it was used as recurrent parent. Rathu Heenati is a Sri Lankan traditional rice cultivar with wide-spectrum of resistance to BPH biotypes (Jena and Kim, 2010). Bi-parental mating design was used to develop the F₁ hybrids and subsequent backcross generations. The breeding procedure for this experiment was illustrated (Fig. 1). Five progenies that were positive (heterozygous) to the two linked markers (RM544 and RM545) were used for backcross population development. The Five F₁ progenies were then backcrossed to the recurrent parent to produce first filial generation of backcrossing (BC₁F₁). Thereafter, foreground screening for BPH resistant genes/QTLs was carried-out in BC₁F₁ population using the two linked markers. The progenies that were positive to the linked markers and recorded maximum RPG recovery were backcrossed to the recurrent parent to generate BC₂F₁ population. Foreground, background and phenotypic screenings were done at each generation of backcrossing to select the desired progenies having maximum recovery of RPG with phenotypic resemblance to Putra-2.

Analysis of molecular markers

The simple sequence repeats (SSRs) markers (RM544 and RM545) that were reported linked to BPH resistance genes/QTLs (*Bph19* and *Bph13*) (Chen *et al.*, 2006; Shabanimofrad *et al.*, 2015a; Shabanimofrad *et al.*, 2015b) were used for foreground selection. Three hundred and sixteen (316) SSR markers spread across the 12 rice chromosomes were screened for polymorphism between Putra-2 and Rathu Heenati rice varieties for the purpose of background selection. The informations about the SSR markers used such as primer sequences and positions on the chromosomes (data not presented) were sourced from the previous researches (Sun *et al.*, 2005; Chen *et al.*, 2006; Shabanimofrad *et al.*, 2015a; Shabanimofrad *et al.*, 2015b) and the Gramene SSR marker data-base (<http://archive.gramene.org/markers/microsat/>).

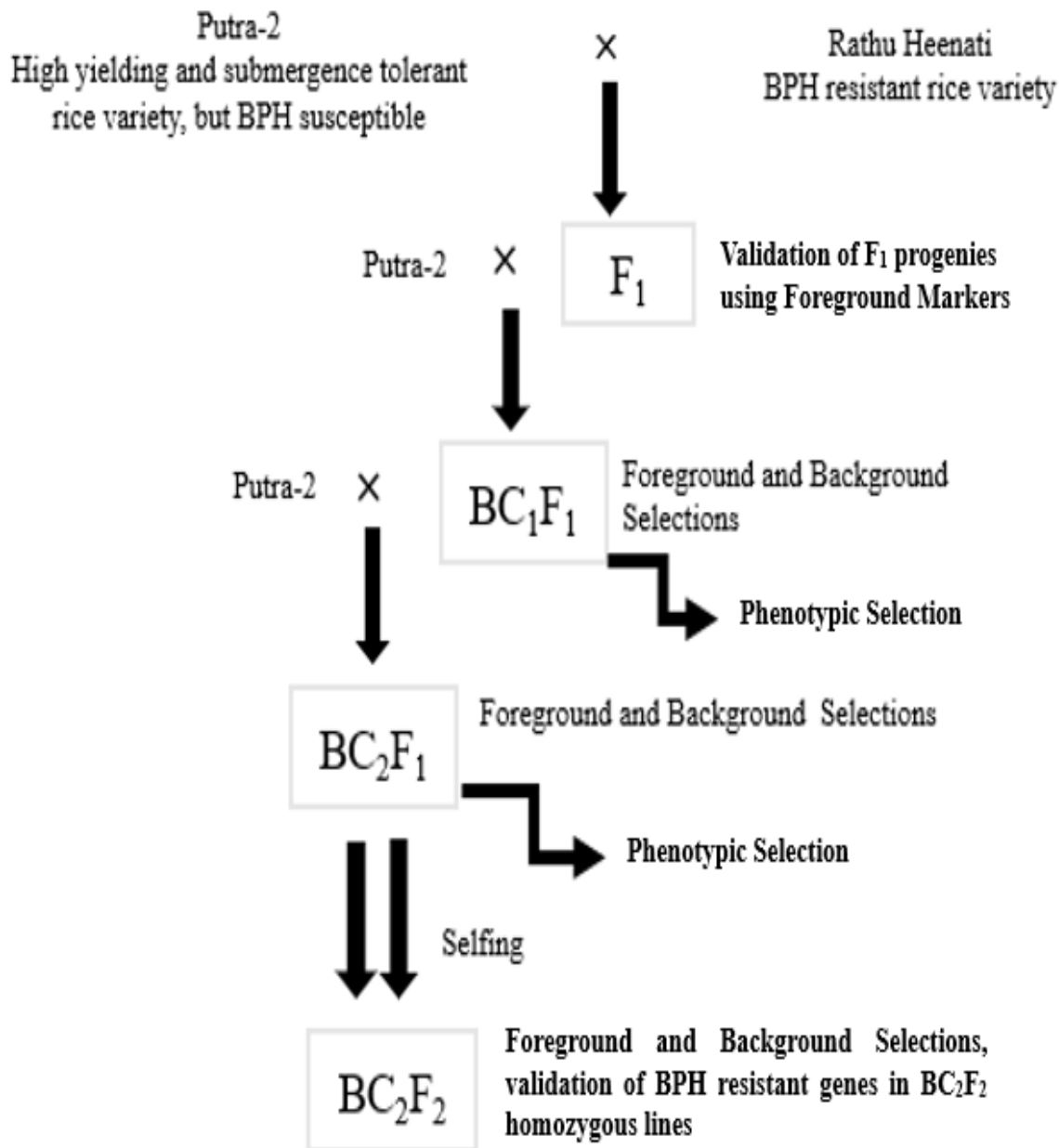


Fig. 1. Marker-assisted backcrossing breeding scheme using bi-parental mating design.

Genomic DNA extraction

The leaf samples of four weeks old seedlings were excised from the healthy plants for whole genome DNA isolation adopting the cetyl trimethyl-ammonium bromide (CTAB) protocol (Doyle and Doyle, 1990). The quality of DNA was checked by employing nano-drop spectrophotometer (ND 1000). The DNA samples used were within the acceptable range of 1.8-2.0 purity (NDS, 2019). The samples were diluted with 1×TBE buffer (1 Mm EDTA, pH 8.0, 0.05 M boric acid, 0.05 M Tris base) to yield 70

ng/μl composition and stored in -20 °C freezer for future use.

Polymerase chain reaction (PCR)

The total volume of PCR reaction was 15 μl, which included 1 μl of template DNA (70 ng), 1 μM of forward and reverse primer each, 7.5 μl of master mix (2×Power Taq PCR MasterMix, Bioteke Corporation, China) and 4.5 μl of nuclease-free water. The PCR amplification processes for foreground and background markers were accomplished using thermocycler (T100™, Bio-Rad) by using touch-

down PCR protocol as described by Shabanimofrad *et al.* (2015a) as follows: 94 °C for five minutes (mins); followed by 10 cycles of 94 °C for 15 seconds (secs), 62 °C for 15 secs (decreasing 0.5 °C per cycle), then 72 °C for 15 secs, followed by 30 cycles of 94 °C for 15 secs, 52 °C for 15 secs, then 72 °C for 15 secs, and a final extension for 10 mins at 72 °C followed by rapid cooling to 4 °C before gel electrophoresis analysis.

Gel electrophoresis

In case of gel electrophoresis, a 3% w/v Metaphor Agarose gel (Lonza) was formulated with 5 µl staining dye (gel view, Bioteke Corporation, China) to determine amplicons in a 1×TBE buffer (1 Mm EDTA, pH 8.0, 0.05 M boric acid, 0.05 M Tris base). The gel was carried out at 80 V for 1 hour. A 50-base pair (bp) reference ladder (GeneDireX, Inc., Taiwan, ROC) was used to detect the size of alleles of the magnified PCR products. The resolved and magnified PCR products were visualised under ultra-violet light using digital molecular imager® (GelDoc™ XR, Bio-Rad Laboratories Inc., USA).

Phenotypic selection

The phenotypic screening of BC₁F₁, BC₂F₁ and BC₂F₂ populations was carried out after foreground and background selections. The progenies that acquired BPH resistant genes with maximum phenotypic similarity with Putra-2 were selected during vegetative growth and harvesting period based on the following morphological and yield parameters: days to 50% flowering, plant height, number of panicles per hill, days to maturity, grain length, number of tillers per plant, panicle length, grain width, percentage filled grain, percentage unfilled grain length, 1000-grain weight and grain yield. The backcross seeds were derived from progenies with maximum level of phenotypic similarities to the recurrent parent, Putra-2. The same strategy was employed in each generation of backcrossing to choose plants acquiring BPH resistant genes/QTLs with highest resemblance with the recurrent parent.

Statistical analysis

The Chi-square test was computed using Statistical Analysis System (SAS 9.4) to determine whether the observed and expected ratios are similar or distinct among backcross generations. For foreground selection, the homozygous recurrent or recipient

parent (Putra-2) was designated as “A” (Susceptible) while the homozygous donor (Rathu Heenati) was labelled as “B” (Resistant) and the heterozygous individuals were marked as “H” (Segregants) for a specific SSR marker. The Chi-square formula (Snedecor and Cochran, 1967) used was as follows: Chi-square (X^2) = (O – E)²/E, where O refers to number of observed plants, and E number of expected plants. For background selection, on the other hand, the coded SSR marker data generated were subjected to Graphical Geno Types (GGT) software (Van Berloo, 2008) for analysis. The homozygous recurrent parent allele was designated as “A”, the homozygous donor parent allele was labelled as “B” and heterozygous allele was regarded as “H”. The percentage of markers acquiring homozygous recurrent (A %) and donor (B %) parent alleles as well as heterozygous alleles (H %) were computed. Independent t-test (using SAS 9.4 software) was employed to compare the mean difference of agromorphological traits between Putra-2 and BC₂F₂ BPH resistant improved lines.

RESULTS

Molecular marker screening

The two tightly-linked (foreground markers, RM544 and RM545) markers for BPH resistant genes have shown polymorphism between the two parents. Out of 316 SSR markers surveyed (data not presented) between the two parental lines, 79 (25%) markers (including the foreground markers) spanning the 12 rice chromosomes were found polymorphic between the two parent (Table 1).

Confirmation of first filial (F₁) generation plants

A total of 70 F₁ plants were screened to confirm their true hybridity employing foreground markers. The results revealed that out of 70 F₁ plants, 48 progenies acquired the alleles of both parents (heterozygous). Below is the gel picture (Fig. 2) of some progenies after F₁ screening.

Marker genotyping in BC₁F₁ population

Marker-assisted foreground selection

The best five progenies having the alleles of the foreground markers in the F₁ generation were backcrossed to Putra-2, which produced 120 BC₁F₁ progenies. Among the 120 BC₁F₁ progenies screened, 40 plants were found heterozygous or positive to the foreground markers (Fig. 3.).

Table 1: Polymorphic SSR markers used in the experiment

Chromosomal location	Name of polymorphic markers	No. of polymorphic markers
1	RM9, RM431, RM10000, RM488, RM1067, RM1003, RM495, RM312	8
2	RM154, RM262, RM5529, RM3501, RM1358, RM109, RM525, RM87, RM324, RM475	9
3	RM218, RM232, RM1002, RM3134, RM5474, RM8204, RM545	7
4	RM177, RM5511, RM8213, RM5953, RM317	5
5	RM163, RM169, RM421, RM267, RM3322, RM87	6
6	RM3827, RM19311, RM585, RM7488, RM528	5
7	RM1279, RM1306, RM336, RM234, RM21975 RM501	6
8	RM407, RM310, RM3395, RM6925, RM6208, RM264, RM544	7
9	RM205, RM105, RM23869, RM23865, RM24382, RM3909, RM219, RM23805, Sub1C173, RM8300	10
10	RM228, RM1125, RM311, RM216	4
11	RM206, RM441, RM552, RM224	4
12	RM247, RM3726, RM313, RM519, RM5479, RM235, RM17, RM3331	8
Total		79

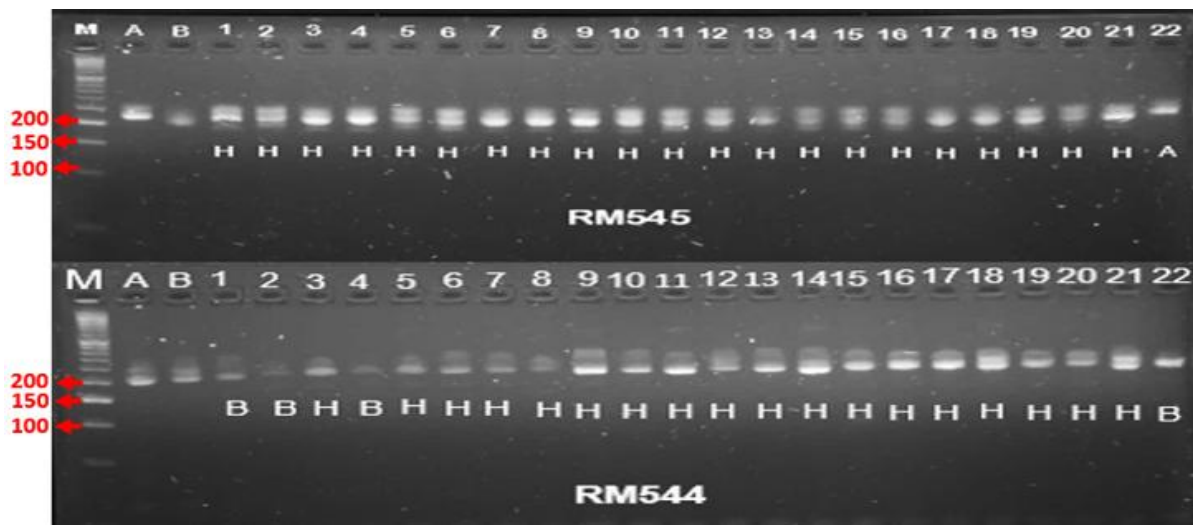


Fig. 2. Validation of F_1 plants using foreground markers, RM544 and RM545. A= Putra-2, B= Rathu Heenati, H= Heterozygous individuals and M= 50-bp ladder. Only 22 individuals shown.

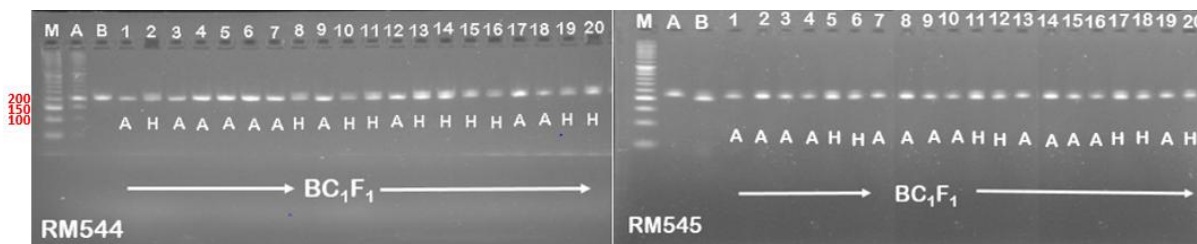


Fig. 3. Foreground selection using linked markers, RM544 and RM545 in BC_1F_1 generation. A= Putra-2, B= Rathu Heenati, H= Heterozygous individuals and M= 50-bp ladder. Only 20 individuals shown.

The fraction of resistant and sensitive progenies in BC₁F₁ population utilising foreground markers were presented in Table 2. The two linked markers conformed to goodness of fit test-cross proportion of

1:1 in BC₁F₁ population, which showed non-significance probability at 5% level using Chi-square analysis.

Table 2. Fraction of resistant and susceptible plants in BC₁F₁ population using foreground markers

Foreground marker(s)	Chr. no.	Marker analysis		Total number of BC ₁ F ₁ plants	Chi-square (X ²) test (1:1)	Probability (P<0.05)
		Aa	aa			
RM544	8	64	56	120	0.53	0.47
RM545	3	57	63	120	0.30	0.58

Degree of freedom (DF) = 1; X² (0.05, 1) = 3.84; Aa = Segregant; aa = Susceptible, Chr. no. = Chromosome number

Marker-assisted background selection

In BC₁F₁ population, 79 polymorphic SSR markers were applied for background selection. A total of 40 BC₁F₁ progenies that possessed the alleles of foreground markers were subjected to background selection using GGT 2.0 software (Van Berloo, 2008). Figure 4 shows some background markers genotyping in BC₁F₁ population. The recovery of recipient parent genome ranged from 80.3 to 84.9 % in BC₁F₁ population (Table 3).

Mean recovery of the recipient parent genome (RPG) of chosen four BC₁F₁ progenies was 82.3% (Table 3). The section of BPH resistant genes/QTLs of the donor parent were positioned on chromosomes 3 and 8 based on foreground selection. Chromosomes 1, 3, 9 and 12 were almost regained in all the selected BC₁F₁ lines (Fig. 5), in contrast to remaining chromosomes. Due to foreground and background screening, four BC₁F₁ progenies were utilised for development of BC₂F₁ generations.

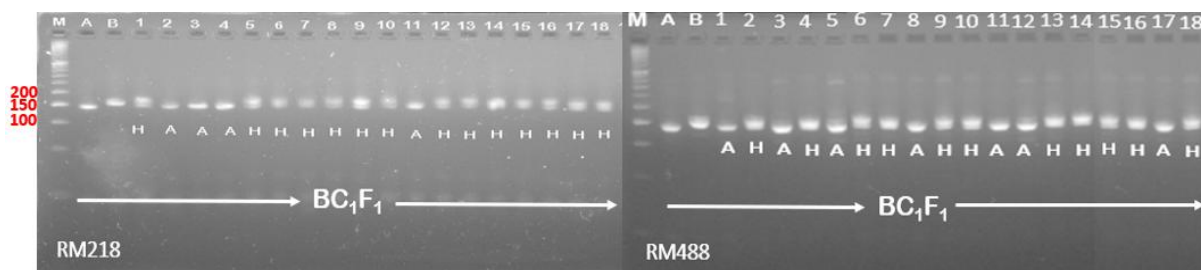


Fig. 4. Background selection using background markers, RM218 and RM488 in BC₁F₁ generation. A= Putra-2, B= Rathu Heenati, H= Heterozygous individuals and M= 50-bp ladder. Only 18 individuals shown.

Table 3: Percentage recovery of recurrent parent genome and introgressed sections analysis of the selected plants in BC₁F₁ generation

Selected BC ₁ F ₁ plants	A (%)	B (%)	H (%)	Total genetic map (cM)	H-segments
BC ₁ -1	80.3	8.7	11.0	1441.6	6.0
BC ₁ -3	81.7	7.7	10.6	1441.6	8.0
BC ₁ -7	84.9	6.9	8.3	1441.6	7.0
BC ₁ -19	82.1	10.2	7.7	1441.6	6.0
Average	82.3	8.4	9.4	1441.6	6.8

A=Putra-2, B=Rathu Heenati, H=Heterozygous, cM=centi Morgan

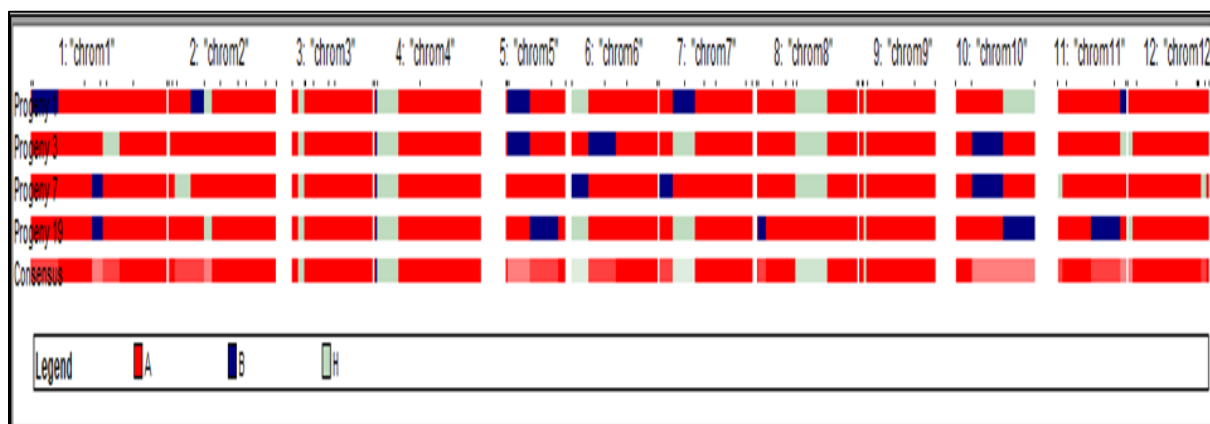


Fig. 5. Chromosome-wise analysis of recurrent parent genome recovery of the four chosen progenies in BC₁F₁ population using GGT software. A=Putra-2 (Red), B=Rathu Heenati (Dark blue) and H=Heterozygous segment (Grey)

Marker genotyping in BC₂F₁ population Marker-assisted foreground selection

Out of 140 BC₂F₁ progenies screened, 30 were found positive to the two foreground markers (Fig. 6). The fraction of resistant and susceptible individual plants in BC₂F₁ population using the linked markers were

presented in Table 4. The results conformed to the expected testcross proportion of 1:1 using Chi-square test of association at 5% level of probability. Five positive plants possessing the BPH resistant alleles and acquiring maximum genome of Putra-2 were self-pollinated to yield BC₂F₂ populations.

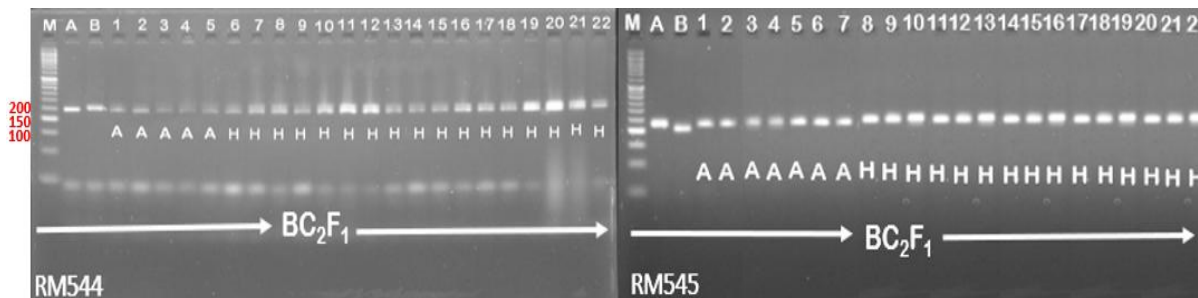


Fig. 6. Foreground selection using linked markers, RM544 and RM545 in BC₂F₁ generation. A= Putra-2, B= Rathu Heenati, H= Heterozygous individuals and M= 50-bp ladder. Only 22 individuals shown.

Table 4: Fraction of resistant and susceptible plants in BC₂F₁ population using foreground markers

Foreground marker(s)	Chr. no.	Marker analysis		Total number of BC ₂ F ₁ plants	Chi-square test (1:1)	Probability (P<0.05)
		Aa	aa			
RM544	8	75	65	140	0.71	0.40
RM545	3	72	68	140	0.11	0.74

DF = 1; X² (0.05, 1) = 3.84, Aa= Segregant, aa= Susceptible, Chr. no. = Chromosome number

Marker-assisted background selection

Seventy-nine polymorphic SSR markers were employed for background selection in BC₂F₁ population. The recovery of recipient parent genome

in selected BC₂F₁ population ranged from 93.0 to 95.2% (Table 5). Five best progenies were chosen based on foreground selection, phenotypic similarity with Putra-2 and background data in the present

population. The mean RPG recovery of the chosen progenies was 94.1% (Table 5). Chromosome-wise analysis of RPG recoveries of the chosen five individuals were displayed in Figure 7. In these plants, the recurrent parent alleles were fully restored in chromosomes 1, 2, 4, 5, 6, 9, and 10. However, the target or carrier chromosomes (3 and 8) are of donor origin.

Marker genotyping in BC₂F₂ population

Marker-assisted foreground selection

Foreground screening was conducted to choose individuals with phenotypic resemblance to Putra-2, and at the same instance acquiring homozygous BPH resistant genes/QTLs using the linked markers, RM544 and RM545. These markers segregation fractions (Table 6) were not significantly different at 5% probability level, and thus fit to Mendelian segregation proportion of 1:2:1 for a one-gene model.

Table 5. Percentage recovery of recurrent parent genome and heterozygous segments of selected plants in BC₂F₁ generation

Selected BC ₂ F ₁ plants	A (%)	B (%)	H (%)	Total genetic map (cM)	H-segments
BC ₂ -P ₁₉ -P ₁	95.2	1.2	3.6	1441.6	2
BC ₂ -P ₁₉ -P ₆	93.0	3.3	3.7	1441.6	3
BC ₂ -P ₇ -P ₉	94.9	1.5	3.6	1441.6	2
BC ₂ -P ₃ -P ₁₃	93.5	2.2	4.3	1441.6	4
BC ₂ -P ₁ -P ₁₅	94.1	2.1	3.8	1441.6	3
Average	94.1	2.1	3.8	1441.6	2.8

A=Putra-2, B=Rathu Heenati, H=Heterozygous, cM=Centi Morgan

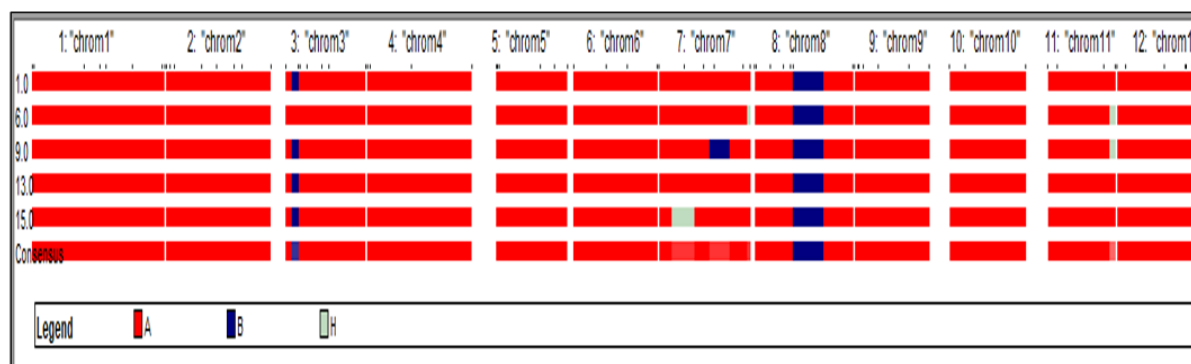


Fig. 7. Chromosome-wise analysis of recurrent parent genome recovery of the five chosen progenies in BC₂F₁ population. A=Putra-2 (Red), B=Rathu Heenati (Dark blue) and H=Heterozygous segment (Grey).

Table 6: Chi-square analysis for fraction of resistant and susceptible plants in BC₂F₂ population to test the expected ratio (1:2:1) of marker segregation for a single-gene model

Foreground marker	Chr. no.	Marker analysis			Total number of BC ₂ F ₂ plants	Chi-square test (1:2:1)	Probability (P<0.05)
		AA	Aa	aa			
RM544	8	47	87	46	180	0.21	0.90
RM545	3	49	89	42	180	0.57	0.75

DF= 2; X² (0.05, 2) = 5.99, AA = Resistant, Aa = Segregant, aa = Susceptible, Chr. no. = Chromosome number

3.5.2 Marker-assisted background selection

In BC₂F₂ population, the background analysis was achieved using the 79 polymorphic SSR markers. The average distance per marker was 18.25 cM and a genetic map of over 1441.6 cM of the RPG examined. A total of 20 plants fixed with donor parent alleles using foreground markers were used for background selection and subsequently, 10 best BC₂F₂ homozygous lines were selected based on their phenotypic resemblance with Putra-2 rice variety. Among the chosen BC₂F₂ plants, the RPG enhanced from 95.7 to 97.2%, with a mean of 96.4%. The mean ratio of the Putra-2 in the selected advance lines was 96.4% (Table 7).

Performance of agro-morphological traits in the improved BC₂F₂ BPH resistant lines

In order to ascertain the successful RPG recovery in the newly improved BC₂F₂ lines, the performance of the traits of agronomic importance of the developed lines and Putra-2 were compared using two samples independent t-test (Table 8). The results revealed no significant difference ($P < 0.05$) between Putra-2 and

improved BC₂F₂ lines for all the agronomic traits examined.

DISCUSSION

Screening of parental lines for polymorphism using molecular markers is very critical for embarking on any MAS breeding programme. Markers that do not differentiate between the parental lines lack relevance in selection processes, and therefore cannot be used. Polymorphic markers were applied in each stage of selection to screen F₁, BC₁F₁, BC₂F₁ and BC₂F₂ plant populations. The percentage of marker polymorphism in the present study (25%) is in congruence with Usman *et al.* (2018), who conducted parental survey with 252 SSR markers and reported that 68 (26%) markers were polymorphic between Kulai and AVPP0702 chilli varieties. In another investigation, Linh *et al.* (2012) surveyed 477 SSR markers between parents BT7 and FL478, and detected 89 polymorphic markers, which represent 18.7% polymorphism between the two parents

Table 7: Background and introgressed segments analysis in chosen BC₂F₂ populations

Selected BC ₂ F ₂ lines	A (%)	B (%)	H (%)	Total genetic map (cM)	H-segments
BC ₁ -P ₁ -P ₁₅ -P ₆	96.5	3.0	0.6	1441.6	1
BC ₁ -P ₁ -P ₁₅ -P ₁₃	96.4	3.6	0.0	1441.6	0
BC ₁ -P ₁ -P ₁₅ -P ₁₆	95.9	3.6	0.6	1441.6	1
BC ₁ -P ₃ -P ₁₃ -P ₉	95.9	3.6	0.6	1441.6	1
BC ₁ -P ₃ -P ₁₃ -P ₁₉	97.2	2.8	0.0	1441.6	0
BC ₁ -P ₇ -P ₉ -P ₁	96.4	3.6	0.0	1441.6	0
BC ₁ -P ₇ -P ₉ -P ₁₁	96.4	2.6	0.0	1441.6	0
BC ₁ -P ₇ -P ₉ -P ₁₇	96.9	3.0	0.2	1441.6	1
BC ₁ -P ₁₉ -P ₁ -P ₃	95.7	3.6	0.7	1441.6	1
BC ₁ -P ₁₉ -P ₆ -P ₁₀	97.0	3.0	0.0	1441.6	0
Average	96.4	3.3	0.3	1441.6	0.5

A= Recurrent parent, B= Donor parent, H= Heterozygous, cM= centi Morgan

The results of foreground selection in BC₁F₁ population conform to expected Chi-square test-cross proportion of 1:1 for a one-gene model, thus showing an association between the linked markers and BPH resistance genes/QTLs. Iftekharudaula (2008) screened BC₁F₁ population of rice for submergence tolerance, and revealed that resistance and

susceptibility fractions correspond to 1:1 test-cross ratio, indicating a Mendelian single-gene model. In addition, Mondal *et al.* (2013) reported a Mendelian single-gene inheritance model with segregation proportions for resistance and susceptibility of 1:1, in screening rice BC₁F₁ population for salt tolerance. It is now clear that the target alleles can be successfully

incorporated through marker-assisted foreground selection. This further shows that the two foreground markers are tightly-linked to the target genes/QTLs

on chromosomes 3 and 8 (Chen *et al.*, 2006; Shabanimofrad *et al.*, 2015b).

Table 8: Mean comparison of agro-morphological traits between Putra-2 and improved BC₂F₂ lines

Traits	Putra-2 (Mean $\pm$ SD)	BC ₂ F ₂ (Mean $\pm$ SD)	t-values	P-values
Days to 50% flowering	80.00 $\pm$ 1.73	80.00 $\pm$ 3.00	0.00	1.00 ^{ns}
Days to maturity	105.33 $\pm$ 1.53	105.00 $\pm$ 1.73	0.25	0.82 ^{ns}
Plant height (cm)	80.90 $\pm$ 1.15	80.23 $\pm$ 1.10	0.72	0.51 ^{ns}
Number of tillers per plant	9.67 $\pm$ 1.15	10.00 $\pm$ 0.00	-0.50	0.64 ^{ns}
Number of panicles per hill	9.00 $\pm$ 1.00	9.67 $\pm$ 0.58	-1.00	0.37 ^{ns}
Panicle length (cm)	27.57 $\pm$ 1.95	27.03 $\pm$ 0.40	0.46	0.67 ^{ns}
Grain length (mm)	9.85 $\pm$ 0.45	9.65 $\pm$ 0.08	0.77	0.49 ^{ns}
Grain width (mm)	2.22 $\pm$ 0.06	2.20 $\pm$ 0.11	0.18	0.86 ^{ns}
Percentage filled grain (%)	93.15 $\pm$ 2.77	93.66 $\pm$ 2.37	-0.24	0.82 ^{ns}
Percentage unfilled grain (%)	7.15 $\pm$ 1.92	6.63 $\pm$ 1.91	0.33	0.77 ^{ns}
1000-grain weight (g)	23.19 $\pm$ 1.56	24.30 $\pm$ 1.56	-0.87	0.43 ^{ns}
Grain yield (t/ha)	6.80 $\pm$ 0.59	6.82 $\pm$ 0.59	-0.04	0.97 ^{ns}

SD = Standard deviation, NS = not significant at 5% probability level

In BC₂F₁ plants, the proportion of resistant and susceptible plants using the foreground markers follow similar trend. In otherwords, the two foreground markers exhibited a good to fit test-cross proportion of 1:1 for a one-gene model, and thus revealing that there is association between target trait and the foreground markers. Other previous findings (Tanweer *et al.*, 2015a; Usman, 2018) had earlier reported similar results. It is now evident that the desired genes/QTLs were successfully incorporated into Putra-2 through marker-assisted foreground selection. This means that RM545 and RM544 co-segregate with BPH resistant loci on chromosomes 3 and 8, respectively (Chen *et al.*, 2006; Shabanimofrad *et al.*, 2015b). Also, in BC₂F₂ generation, the two foreground markers showed the same trend (as in BC₁F₁ and BC₂F₁), with each marker observed individual plant proportions conforming to the expected Mendelian segregation ratio of 1:2:1 using Chi-square test at $P < 0.05$. This implies that BPH resistance in the present population is administered by a single major gene (Liu *et al.*, 2014; Shabanimofrad *et al.*, 2015a; Shabanimofrad *et al.*, 2015b). Furthermore, many previous investigations (Balakrishna and Satyanarayana, 2013; Kiran *et al.*, 2014; Shabanimofrad *et al.*, 2015a; Tanweer *et al.*, 2015b; Deen *et al.*, 2017; Rather and Deo, 2018)

indicated that insect pest resistance including BPH, is regulated by a single major or minor gene(s), moderate as well as horizontal (polygenic) resistance based on the cultivars or genotypes

For the background selection in BC₁F₁ population, the RPG recovery extends from 80.3 to 84.9% with an average of 82.39%. These results supported the finding of Miah *et al.* (2015), who revealed RPG recovery in a range of 75.40 to 91.3% in BC₁F₁ generation screened for blast resistance, with an average of 87.3% in the selected lines. Hospital (2003) made a comparison between MABC and traditional backcrossing, and reported a maximum RPG recovery of 79% in BC₁F₁ population for MABC programme. In BC₂F₁ population, the RPG recovery extends from 93.0 to 95.2% with a mean of 94.1%. This coincided with Neeraja *et al.* (2007), who reported RPG recovery of 87.2 to 94.2% in submergence tolerant BC₂F₁ rice population. In another finding, Ahmed *et al.* (2016) reported a maximum RPG recovery of 95.4% in BC₂F₁ rice population screened for submergence tolerance. Majority of the chromosomes were almost or fully recovered in the chosen BC₂F₁ individuals, and consequently, this provides a good means for easier and efficient selection. On the basis of this, the chosen plants were used to develop BC₂F₂ population.

Among the BC₂F₂ advanced lines, the RPG enhances from 95.7 to 97.2%, with a mean of 96.4%. The mean RPG of the advanced BC₂F₂ selected lines was higher relative to the theory based mean RPG (87.5%) (Hospital *et al.*, 2003), after two generations of repeated backcrossing and one cycle of selfing. Similar observations were made by Basavarajet *et al.* (2010) and Tanweer *et al.* (2015b) in their studies.

Introgression of desirable alleles and recovery of the recurrent parent genome is usually validated by phenotyping the backcross generation individually. Individuals that acquired the desirable gene(s) and resemble the recurrent parent are selected after phenotyping, and vice-versa. The results of independent t-test indicated that the growth, yield and yield related traits performance of the Putra-2 and the selected BC₂F₂ lines are statistically similar. This means that there is no variation in terms of performance between BC₂F₂ improved lines and Putra-2. In otherwords, the genome of Putra-2 was recovered in BC₂F₂ plants through MABC. Good phenotypic performance is a feature of a good genotype especially if the allele or genes have a major effect on physical characteristics, and the error value is less on the phenotype (Ye and Smith, 2008). Phenotypic selection along with background selection have usually been adopted to identify superior individuals (Singh *et al.*, 2011).

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In conclusion, it is clear that MABC and phenotypic screening were successfully applied to regain the genome of Putra-2 in the improved BC₂F₂ lines having BPH resistance genes/QTLs. The phenotypic characteristics of the improved lines in terms of yield and other traits of agronomic importance are similar to recipient parent. Unlike conventional backcrossing, the utilisation of MABC expedited RPG recovery, reduce the time desired for introgression of BPH resistance genes/QTLs, and decrease the number of backcross generations needed to attain maximum RPG recovery. The improved lines will be potential sources of BPH resistance gene in subsequent rice breeding programmes. Finally, it can be inferred that MABC is a good breeding strategy to incorporate and monitor desired gene(s) and also regain the RPG in rice breeding programme.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors are grateful to the Ministry of Higher Education, Malaysia, for providing fund to conduct this research through the Higher Institutions Centre of Excellence (HiCoE Vot No. 6369105).

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HOW TO CITE THIS ARTICLE:

Haliru, B. S., Rafii, M. Y., Mazlan, N. and Ramlee, S. I. (2026) Determination of recurrent parent genome in rice (*oryza sativa* l.) through marker-assisted backcross breeding. *Nigeria Journal Of Plant Breeding* (<https://pbanjournal.org/>), 3(2), 155-180. ISSN: 2814-3531