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Full Length Article

Introgression of *Pi-kh* Resistance Gene into a Malaysian Cultivar, MR264 using Marker-Assisted Backcrossing (MABC)

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Abstract

Magnophorthe oryzae is one of the most destructive disease that affected rice productivity. Developing new breeding strategies for durable blast resistant varieties is essential for crop protection. In this study, we aimed to introgress a blast resistance gene using local resistant genotypes, Pongsu Seribu 2 into MR264, a high-yielding rice variety that is blast susceptible thru Marker-Assisted Backcrossing (MABC) approach. Results demonstrated that, resistance gene (*Pi-kh*) was successfully incorporated into MR264-rice variety. Two foreground markers, RM206 and RM5961 were identified tightly linked to the target gene which located at chromosome 11. Both markers were utilized at each backcrossed generation to identify plants possessing heterozygous alleles for the target gene. A total of 82 background markers were used to evaluate recovery of recurrent parent genome in each backcrossed generation. The percentage of recovery of parent genome in BC_1F_1 and BC_2F_1 were 79.1% and 89.7% indicated a higher percentage compared to conventional method. The expected number of resistant and susceptible plants in segregation ratio for a backcrossed model fitted with 1:1 with (p>0.05) was not significantly different form the number of observed plants. This research finding will be helpful guidance of application of MABC in developing a blast resistant variety with highest recovery of RP genome with reducing number of generation.

Keywords: Background selection; Blast; Foreground marker; Resistance gene; Rice

Introduction

Plant disease had been one of the most important restraints to grain production. Rice blast caused by *Magnophorthe oryzae* is among serious rice disease, which induced significantly economic loss each year. In Peninsular Malaysia, a large number of lands; 640, 700 ha were devoted to cultivated rice. Unfortunately, the rice production has not met the national demand and Malaysia are the biggest rice importer countries in Asia (FAO, 2013). Due to this concern, more research and technology advancement should be made to help the poor farmers who totally depend on the rice as their staple food. Disease management by using fungicide has been normally practiced however, fungicides had adverse effect on health problem and quality in addition of not environmental friendly. Breeding for the host-plant resistance is an economical strategy and viable option to alleviate this malady in an eco-friendly manner (Latif *et al.,* 2011; Ragimekula *et al.,* 2013). Generally, there are two group of blast resistance in rice: complete and incomplete (field or partial) resistance (Yunoki *et al.*, 1970; Fukuoka and Okuno 2001; Zenbayashi *et al.,* 2002). In general, field resistance is durable; hence the use of field resistance is one of the most promising measures for blast control (Zenbayashi *et al.*, 2002; Ashkani *et al*., 2011).

Breeding for the host-resistance requires the crossing between identified resistant plants with agronomically acceptable but tolerant (moderately resistant to moderately susceptible) plants. A conventional backcrossing program involved the production of varieties that are similar to the susceptible parent but incorporated with required resistance gene. Regardless of, this conventional breeding takes more years to produce resistant variety and in some instances; the pathogen has already evolved a variant that is not recognized by the improved cultivar, leading to susceptibility. Breeders had developed a number of resistant varieties with genetic resistance to possibly

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overcome pathogen virulences, however insistent and diligent effort in disease management are required for instable pathogenic races of fungus (Chen *et al.*, 2003).

Advances in modern molecular technique make it possible to use markers to introgress several R-genes into a single cultivar from various sources during a crossing program. Marker-assisted selection together with DNA markers closely linked to the genotype of the target gene had an advantages to improves the efficiency of conventional plant breeding by facilitating the screening progeny without extensive disease testing and reliance on laborious methods (Chen *et al.*, 2005).Currently, Marker assisted Backcrossing (MABC) had been applied to incorporate genes controlling a desirable trait, while inheriting the characteristic of recurrent parents (Hospital, 2003). An ideal genotype developed through MABC had 3 essential benefits; (1) improve the phenotypical selection of target allele with effect (2) improve the recombinant selection by minimizing the linkage drag (3) improve the background selection with maximum recurrent parent genome (Collard and Mackill, 2008). In addition, as compared to MABC, development of completely new variety requires much time with conventional breeding (Mackill, 2006). Tanksley and Nelson (1996) were the first researcher applied the MABC to successfully introgress QTLs from unadapted germplasm to an elite breeding line. To date, many new varieties have emerged from this breeding program by introgressing the agriculturaly important genes (Liu *et al.*, 2002). Using this approach, Chen has succeeded in the introgression of *xa21* into hybrid restorer line, Minghui 63, and recently Vikas *et al.* (2012) had successfully pyramid blast resistance gene *Piz5* and *Pi54* into an elite Basmati line. Therefore, the objective in this study was to introgress the blast resistance gene into local Malaysian variety, MR264 by applying marker-assisted backcrossing breeding program and thereby enhancing the background selection.

Materials and Methods

Plant Materials and Breeding Strategy

Local Malaysian variety with high yielding, MR264 was used as recurrent parent, while Pongsu Seribu 2 carrying resistance gene was used as donor parent. F_1 seeds were developed from the normal hybridization between MR264 and Pongsu Seribu 2. Four selected F_1 plant was then backcrossed with MR264 to produce BC_1F_1 seeds. Selected plant carrying resistance gene with highest background recovery and maximum phenotypic similarity to the recurrent parent were backcrossed with MR264 to generate BC_2F_1 seeds. Foreground and background selection were carried out to select the elite plant from each backcross generation.

Molecular Marker Analysis

A total of 325 SSR marker distributed across 12-chromosome of rice were screened for parental polymorphism. Information of SSR primer were extracted from Gramene website (http://www.gramene.org) and Table 1 demonstrated the position of polymorphic marker. For background selection, at least four markers per chromosome were used. Six foreground markers namely RM168, RM148, RM413, RM206, RM5961 and RM101 was found to be related to *Pi*-gene and used to evaluate F_1 and each backcross generation.

Genomic DNA Extraction

Youngest leaves of three weeks seedling were cut apart with sterilized scissors priory washed with 70% ethanol to discard any source of foreign DNA or spore. 5 mL of extraction buffer were added to 2 gram of chopped leaf samples prior to 2 min ground in Tissue Lyser. Dried pellet was re-suspended with 1xTE buffer after precipitated with 70% ethanol. Quality of DNA was checked with 1.5% agarose gel electrophoresis at 90 V for 30 min and the concentration of DNA was measured by Nano-Drop spectrophotometry.

PCR Amplification

PCR amplification was performed following a program in 30 cycles of denaturation at 94°C for 1 min, annealing for 1 minute at 55°Cand polymerization at 72°C for 2 min followed by rapid cooling to 4°C prior to analysis. The amplification products were analyzed by electrophoresis in 3.0% agarose in 1xTBE at 80 V for 80 min and visualized using Molecular Imager® (GelDocTM XR, Bio-Rad).

Statistical Analysis

The marker banding pattern was scored with reference to two parents. For foreground selection, the band similar with Pongsu Seribu 2 was scored as "B", while band having same level with MR264 was scored as "A". For background selection, marker data was analyzed using Graphical Genotyper (GGT 2.0) software (ref). "A", "B" and "H" was scored for homozygous recipient allele; homozygous donor allele and heterozygous allele and the percentage of each allele were calculated. Chi-square analysis for goodness of fit to 1: 1 ratioswas calculated by using the formula, $Chi^2 = (O-E)^2/E$, where O is the observed value, and E is the expected value.

Results

Parental Polymorphism

Out of 325 SSR markers only 82 SSR markers distributed across 12-rice chromosome were demonstrated polymorphism between both parents (Table 1) and used as a

Table 1: List of polymorphic marker used in this study

Chromosome position	Polymorphic SSR markers
1	RM462, RM495, RM1, RM1167, RM151, RM5359, RM259, RM140, RM128
2	RM109, RM250, RM166, RM208, RM138
3	RM231, RM546, RM338, RM487, RM16, RM168, RM85, RM148
4	RM142, RM273, RM317, RM255, RM303
5	RM249, RM169, RM146, RM161, RM143
6	RM133, RM469, RM225, RM111, RM136, RM8225, RM3, RM541, RM454, RM162
7	RM413,RM336 , RM234, RM172
8	RM462, RM152, RM310, RM339, RM149
9	RM285 , RM3912, RM296, RM242, RM245, RM205, RM8303
10	RM244, RM239, RM311, RM269, RM147, RM333
11	RM332, RM5961, RM206, RM1233, RM224, RM144
12	RM19, RM247, RM179, RM101, RM511, RM519, RM155, RM309

Table 2: Information of Foreground marker used in this study

SSR markers	Primer sequences (5'-3')		Chr.	Repeat motif	Expected product size (bp)
	F: Forward primer	R: reverse primer			
RM206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	11	(CT)21	130-212
RM5961	GTATGCTCCTCCTCACCTGC	ACATGCGACGTGATGTGAAC	11	(CAG)8	129

Table 3: Proportion of susceptible and resistant genotypes using 2 foreground marker in BC_1F_1 and BC_2F_1 generation

Marker	Generation	Observed ratio		Expected ratio	X^2 value	p-value
		Resistant (Rr)	Susceptible(rr)			
RM206	BC_1F_1	73	63	1:1	0.735	0.39
	BC_2F_1	52	123		28.01	0.00
RM5961	BC_1F_1	59	77		2.38	0.12
	BC_2F_1	82	88		0.571	0.45

*at 0.05 significant level

background markers. All background markers were used to evaluate the RG percentage in each backcrossed generation, BC_1F_1 and BC_2F_1. All foreground markers showed a clear polymorphism in parental line. The ratio of polymorphic markers on parental analysis is 25.2%.

Verification of F_1 Generation

Normal crosses between MR264 and Pongsu Seribu 2 were conducted according to Virmani and Sharma, 1993 with some modification. The F_1 plants were verified for hybridity using the tightly linked polymorphic foreground SSR markers. A total of 20 F_1 plants showed heterozygous condition using the foreground markers.

Genotyping BC_1F_1 Generation

Marker-assisted foreground selection: Four selected F_1 plants were backcrossed with MR264 to develop BC_1F_1 plants. Out of 136 BC_1F_1 plants, 23 plants showed positive heterozygous for marker RM206 and RM5961 tightly linked to *Pi*-gene (Fig. 1).Sequence information for both foreground markers were summarized in Table 2. Another four foreground marker demonstrated a negative result indicated that some blast-resistant genes disappeared during backcrossing breeding program. Chi^2 analysis showed marker RM206 and RM 5961 fitted with 1:1 ratio of BC_1F_1 generation (Chi^2 = 0.735 and 2.38), which is non-significant at a probability level of 0.05 (Table 3).

Marker-assisted background selection: Twenty three BC_1F_1 plants were subjected to background analysis using 82 SSR markers. The highest number of polymorphic markers per chromosome was shown in chromosome 6 (10 markers) while chromosome 7 only have 4 markers per chromosome. Table 4 showed the summary of RPG recovery in BC_1F_1 population. The range of RPG in BC_1F_1 generation was from 75% to 92.4% (Fig. 2). Based on the foreground and background selection, six selected BC_1F_1 plants were used to develop BC_2F_1 population (Fig. 3).Plant number 32-E demonstrated the best individual in BC_1F_1 generation with 92.4% of RPG. Chromosome 11 and 12 showed the heterozygous segment and gene donor parent segments (Fig. 4).

Genotyping BC_2F_1Generation

Marker-assisted foreground selection: Marker RM206 and RM5961 identified 30 BC_2F_1 plants out of 175 plants with positive introgression of blast resistance gene (Fig. 5). Table 3 showed the proportional of susceptible and resistant plants in BC_2F_1 using both foreground marker. For single gene model, results demonstrated that BC_2F_1generation fitted with the expected ratio (Chi^2 = 28.01 and 0.571), which is non-significant at a probability level of 0.05.

Marker-assisted background selection: All 30 BC_2F_1 plants were analysed using 82 polymorphic markers. Table 5 showed the summary of RPG recovery in BC_1F_1 population. The RPG value ranged from 80% to 94.6%

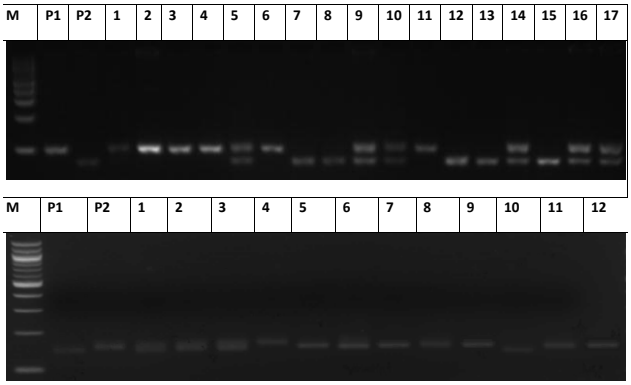

Fig. 1: PCR product for genotyping, with marker RM5961 and RM206 linked to *Pi*-kh resistance gene in BC_1F_1 population derived from MR264 X PongsuSeribu 2. M = 100-bp ladder; P1 = MR264; P2 = PongsuSeribu 2

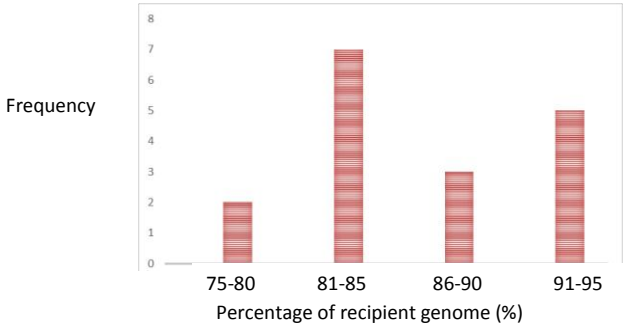

Fig. 2: Frequency distribution of recurrent parent genome recovery (%) in BC_1F_1 population

(Fig. 6). Among plants (32-E-4, 32-E7, 32-E-8, 32-E-9, 32-E-10, 32-E-17, 32-E-24 and 32-E-25), plant number 32-E-8 demonstrated the highest RPG value with 94.6% and lowest H-segments (Fig. 8). Chromosome-wise recipient allele (Fig. 7) shown all chromosome were completely recipient types for plant number 32-E-8 except for chromosome 11 and 12.

Discussion

Exploitation of local genotypes variety for combating blast disease had been creating an attention among researcher. In Malaysia, Pongsu Seribu 2 is a traditional rice variety that potentially regarded as genetic source for resistance gene (Ashkani *et al.*, 2013). It is hope that the exploration of local resistant genotypes will contribute to the national rice-breeding programmed as well as the livelihood of farmers with the development of new durable resistant varieties. In this study, MR-264 rice variety was selected as a recurrent parent due to the agronomically important characteristic which is high yield and semi-dwarf plant stature. According to Virmani and Kumar (2004), the possession of semi-dwarf plant stature is extremely important for any maintainer parent to be an ideal acceptors.

Table 4: Background and introgressed segment analysis in selected lines of BC_1F_1 population

Selected plant	A (%)	B (%)	H (%)	Total (cM)	H-segments
3-F	92.1	0.8	3.8	1436.3	1
14-A	92.2	0	3.8	1436.3	2
32-B	85.6	1.2	3.8	1436.3	1
32-C	90.9	0	5	1436.3	1
32-E	92.4	0	5	1436.3	2
32-F	84.6	0	7.2	1436.3	2

Table 5: Background and introgressed segment analysis in selected lines of BC_2F_1 population

Selected plant	A (%)	B (%)	H (%)	Total (cM)	H-segments
32-e-4	92	2.4	3.8	1429	1
32-e-7	92.1	1.7	4.3	1429	2
32-e-8	95.4	0.6	3.8	1429	1
32-e-9	92.8	3.4	3.8	1429	1
32-e-10	92.6	2.3	3.8	1429	1
32-e-17	93.4	0.7	3.8	1429	1
32-e-24	94.6	1.6	3.8	1429	1
32-e-25	94.4	1.6	3.8	1429	1

Development of blast resistant variety selection of plant carrying desirable genome can be speed up thru Marker Assisted Selection (MAS) with adventure in molecular marker technology. Microsatellite marker is the most preferable marker for plant breeding application due to well distributed throughout rice genome and hypervariable (Miah *et al.*, 2013). Marker screening is important to represent the population. According to Alam *et al.* (2012) a polymorphic markers is an essential step in breeding program because it can distinguish between two parental genotypes. Narasimhulu *et al.* (2013) stated in his study that selection of primers was best based on repeat number and location on different chromosomes. In this study, the percentage of polymorphic marker was similar with study reported by Linh *et al.* (2012) between BT7 and FL478. Monomorphic primer also were found in this study as also reported in study by Huyen *et al.* (2012) in a study for salt tolerance in rice between (AS996 and IR50404). Monomorphic markers were not valuable for selection process and discarded from marker screening.

MAS offer a wide range of advantages compared to conventional breeding (Collard and Mackill, 2008; Sabina *et al.*, 2010; Jiang, 2013) includes time saving and speed up the selection plant with high reliability. Nevertheless, the success of the application of MAS solely depends upon a few factors, including the correlation between molecular marker and the target gene, number of individuals analyzed, genetic background of the gene to be transferred and the genetic base of the trait. Marker Assisted Backcrossing (MABC) is an application of MAS breeding program that firstly had been introduced by Hospital and Charcosset (1997) and till date many researcher had applied the breeding program to develop many resistant varieties. MABC consist of two selections

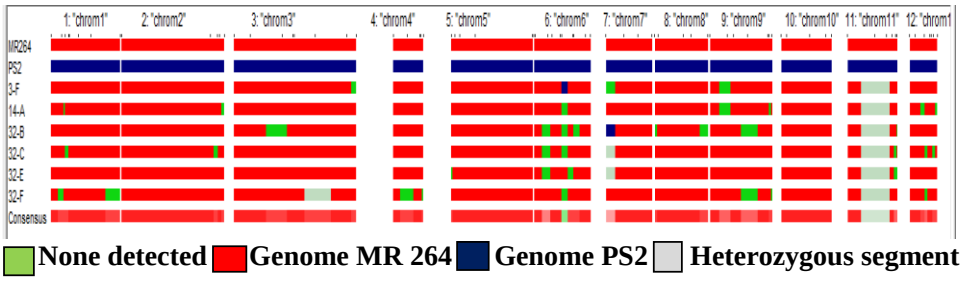

Fig. 3: Chromosome-wise recurrent genome recovery in six selected plants in BC_1F_1 generation

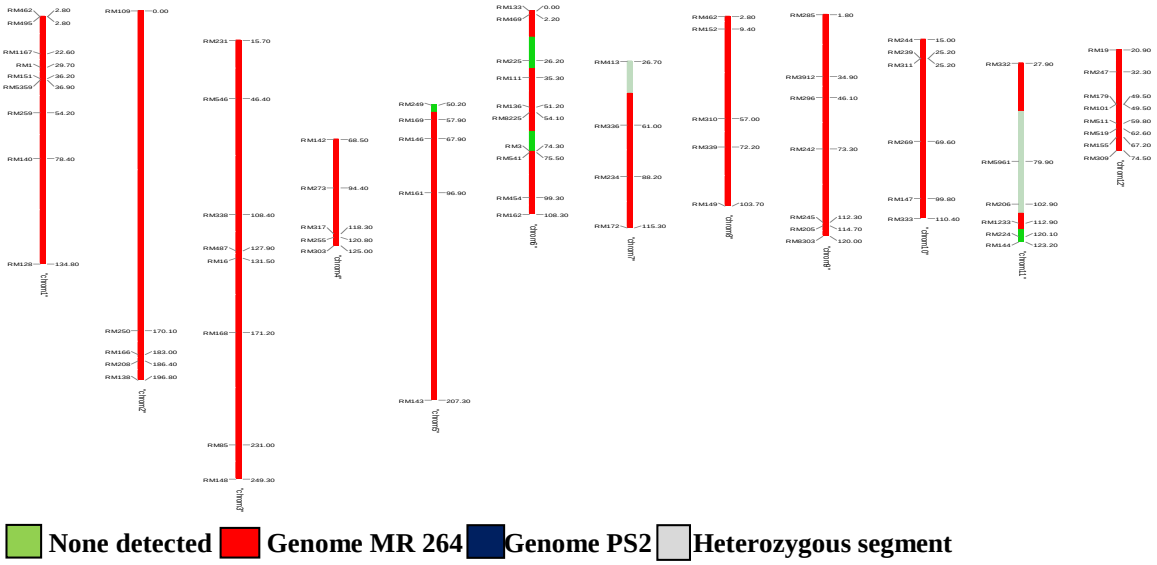

Fig. 4: Chromosome-wise recurrent genome recovery of the best plant 32-E in BC_1F_1 generation

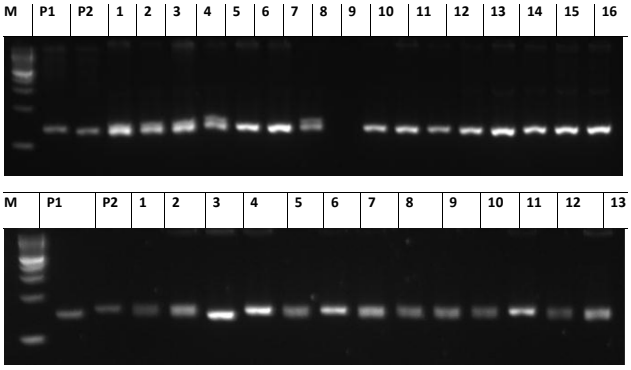

Fig. 5: PCR product for genotyping, with marker RM5961 and RM206 linked to *Pi*-kh resistance gene in BC_2F_1 population derived from MR 264 X PongsuSeribu 2. M = 100-bp ladder; P1 = MR264; P2 = PongsuSeribu 2

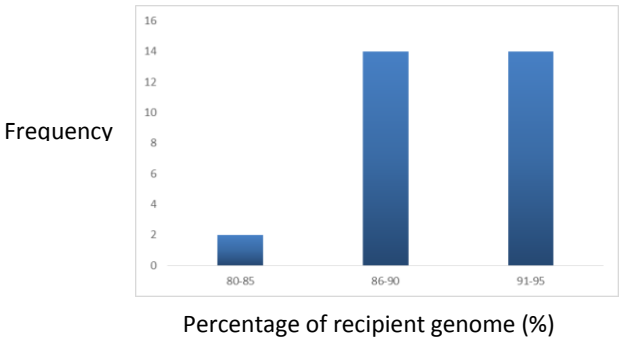

Fig. 6: Frequency distribution of recurrent parent genome recovery (%) in BC_2F_1 population

namely Marker-assisted Foreground (MAF) and Marker-assisted Background (MAB). A successful MABC program depends on the comprehensive primer used in both selections. MAF is mainly focused for the conformation of the target alleles in backcrossed generation or F_1 generation derived from cross between donor and recurrent parent (Allard *et al.*, 1999). According to Collard and Mackill (2008) reliability of marker to predict phenotype will increase by using marker less than 5cM genetic distance. Present study identified two markers, RM206 and RM5961 showed heterozygous plants for blast resistant gene in BC_1F_1. Both markers are linked to blast-resistant *Pi*-

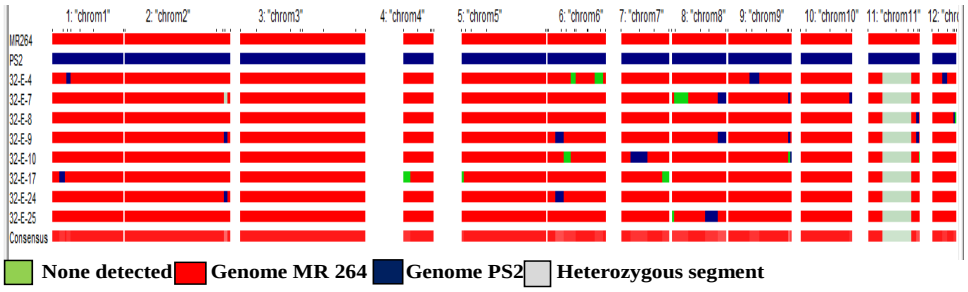

Fig. 7: Chromosome-wise recurrent genome recovery in eight selected plants in BC_2F_1 generation

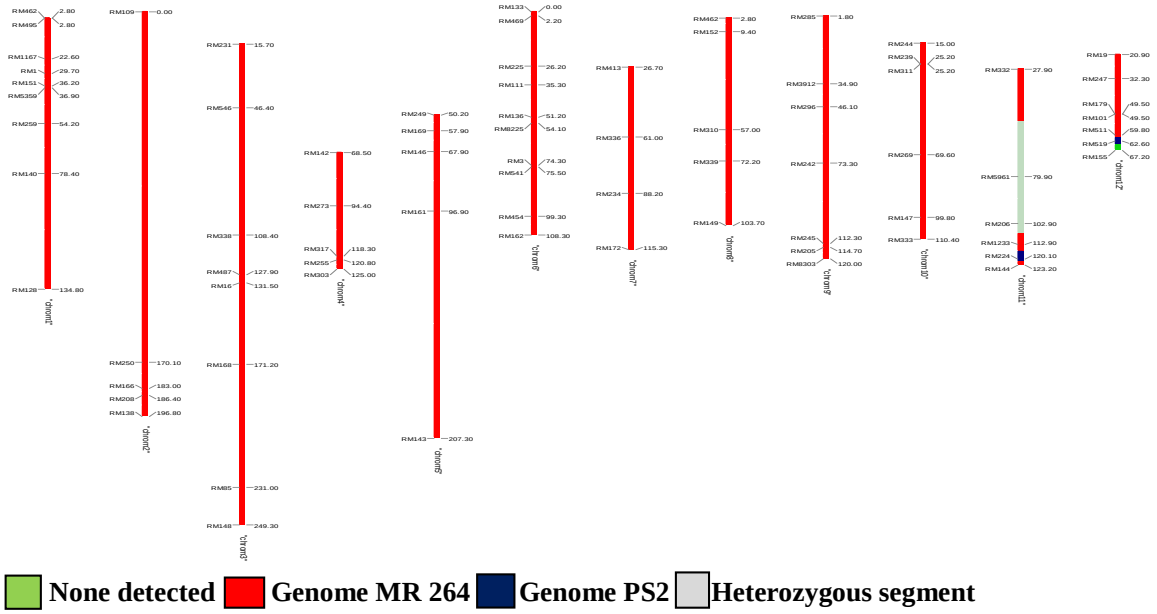

Fig. 8: Chromosome-wise recurrent genome recovery of the best plant 32-E-8 in BC_2F_1 generation

kh gene. The markers RM206 and RM5961, which were found to be tightly linked with blast-resistant gene (putative *Pi-kh*) in this research, were situated on chromosome 11.This research confirmed the study made by Fatah *et al.*(2014) suggesting that Pongsu Seribu 2 contained 23% of *Pi-kh* blast resistance gene in different chromosome location. Similar resistance gene was also incorporated in Tetep variety reported by Sharma *et al.* (2010) and Gupta *et al.* (2012). *Pi-kh* is a resistance gene that has been effectively used to combat a broad-spectrum of races of the rice blast fungus *M. oryzae* (Fjellstrom *et al.*, 2006). In Malaysia, a few studies had been reported incorporating resistance gene using local cultivar including gene *Piz* by Miah *et al.* (2015) using MR219 and Pongsu Seribu 1 as donor parent.

Identification of appropriate background marker in MABC selection is also equally important to speed up the recovery of recurrent genome. Factors such as the extent of saturation of the molecular marker map, the availability of technical resources at a given point of time, and the required levels of line conversion affected the selection response for background analysis. Present study used a higher number of parental polymorphic markers i.e., 82 SSR markers with ~4 polymorphic markers per chromosomefor a better coverage per chromosome in genetic background selection. Basavaraj *et al.*(2010) also successfully reported the same number of polymorphic markers in their studies. In this present study, the percentage of RP genome was higher compared to conventional method. In $BC_1F_1$and BC_2F_1 the average RPG is 79.1% and 89.7%, respectively.

MABC program applied in this study demonstrated that a few individual plants in both generation showed a fully recovery for recurrent genome. This had been observed using the graphical genotypes concept that was first introduced by Van Berloo (2008). This also were observed in a study made by Prigge *et al.* (2008), who mentioned that mean RPG recovery in both generation (BC_1 and BC_2) was highest by using Swarna and Samba Mahsuri variety.

Conclusion

It can be concluded that MABC successfully introgressed *Pi-kh* gene into backcrossed generation with highest RPG

value. This molecular breeding approach also speeds up the development of backcrossed generation and facilitates the selection process through application of molecular marker.

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References

Alam, M.S., M. Salim, M. Moniruzzaman, J.A. Rashid, M.M. Islam, 2012. Marker-assisted Foreground Selection for Identification of Salt Tolerant Rice Genotypes. *Agriculture*, 10: 1–8

Allard, R., 1999. *Principles of Plant Breeding*, 2th edition. Wiley and Sons, New York, USA

Ashkani S., M.Y. Rafii, M. Sariah, A.S.N. Akmar., Rusli I., H.AbdulRahim, M.A. Latif, 2011. Analysis of simple sequence repeats markers linked with blast disease resistance genes in segregating population of rice (*Oryza sativa* L.) *Genet. Mol. Res*. 10: 1345–1355

Ashkani, S., M.Y. Rafii, H.A. Rahim and M.A. Latif, 2013. Mapping of the quantitative trait locus (QTL) conferring partial resistance to rice leaf blast disease. *Biotechnol. Lett.*, 35: 799–810

Basavaraj, S.H, V.K.Singh, A. Singh, D. Anand, S. Yadav, R.K.D. Ellur, S. Singh, M. Gopala Krishnan, T. Nagarajan, K.V. Mohapatra and A.K. Prabhu, 2010. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid.*Mol. Breed.*, 26: 293–305

Chen, X.W., S.G. Liy, M.H.Y. Li, K.D. Zhou and L.H. Zhu, 2003. Marker-assisted selection and pyramiding for three blast resistance genes, *Pi-d(t)1, Pi-b, Pi-ta2*, in rice, Shengwu Gongcheng Xuebao. *Chin. J. Biotechnol*., 20: 708–714

Chen, Z.W., H.Z. Guan, W.R. Wu, Y.C. Zhou and Q.D. Han, 2005. The screening of molecular markers closely linked to rice blast resistant gene *Pi*-1 and their application, Fujian Nonglin Daxue Xuebao. *J. Fujian Agric. For. Univ*., 34: 74–77

Collard, B.C.Y. and D.J. Mackill, 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B*., 363: 557–572

FAO (Food and Agriculture Organization), 2013. *Rice Market Monitor*. Trade and Markets Division, Food and Agriculture Organization of the United Nations, July 2013 Volume XVI - Issue No. 3

Fatah, T., M.Y. Rafii, H.A. Rahim, S. Meon, M. Azhar and M.A. Latif, 2014. Cloning and analysis of QTL linked to blast disease resistance in Malaysian rice variety PongsuSeribu 2. *Int. J. Agric. Biol.*, 16: 395–400

Fjellstrom, R., A.M. McClung and A.R. Shank, 2006. SSR markers closely linked to the *Piz* locus are useful for selection of blast resistance in a broad array of rice germplasm. *Mol. Breed.*, 17: 149–157

Fukuoka,S. andK.Okuno,2001. QTL analysis and mapping of *Pi-21*, a recessive gene for field resistance to rice blast in Japanese upland rice. *Theor. Appl. Genet*., 103: 185–190

Gupta, S.K., A.K. Rai and S.S. Kanwar, 2012. The single functional blast resistance gene *Pi-54* activates a complex defence mechanism in rice. *J. Exp. Bot.*, 63: 757–772

Hospital, F., 2003. *Marker-Assisted Breeding: Plant Molecular Breeding*, 2th edition. Blackwell Publishing and CRC Press, Oxford and Boca Raton, Florida, USA

Hospital, F. and A. Charcosset, 1997. Marker-assisted introgression of quantitative trait loci. *Genetics*, 147: 1469–1485

Huyen, L.T.N., L.M. Cuc, A.M. Ismail and L.H. Ham, 2012. Introgression the salinity tolerance QTLs *saltol* into AS996, the elite rice variety of Vietnam. *Amer. J. Plant Sci*., 3: 981–987

Jiang, G.L., 2013. *Molecular Markers and Marker-Assisted Breeding in Plants*. http://dx.doi.org/10.5772/52583

Latif M.A., M.A.Badsha,,M.I.Tajul, M.S.Kabir, M.Y.Rafii, M.A.T.Mia, 2011. Identification of genotypes against blast, bacterial leaf blight, sheath blight and tungro and efficacy of seed treating fungicides against blast disease of rice. *Sci. Res. Essays* 6: 2804–2811

Linh, L.H., T.H. Linh, T.D. Xuan, L.H. Ham, A.M. Ismail and T.D. Khanh, 2012. Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *Int. J. Plant Genomics*, 2012. Article ID 949038

Liu, G., G. Lu, L. Zeng and G.L. Wang, 2002. Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t)*, are physically linked on rice chromosome 6. *Mol. Genet. Genomics*, 267: 472–480

Narasimhulu, R.N. Naidu, P. Shanthi, G. Govardhan, K.R. Rupesh and R.K. Hariprasad, 2013. Marker-assisted selection in disease resistance breeding. *J. Plant. Breed. Genet.*, 1: 90–109

Mackill, D.J., 2006. *Breeding for Resistance to Abiotic Stresses in Rice: the Value of Quantitative Trait Loci*. Blackwell Publisher, New Jersey, USA

Miah, G., M.Y. Rafii, M.R. Ismail, A.B. Puteh, H.A. Rahim, R. Asfaliza and M.A. Latif, 2013. Blast resistance in rice: a review of conventional breeding to molecular approaches. *Mol. Biol. Rep.*, 40: 2369–2388

Miah, G., M.Y. Rafii, M.R. Ismail, A.B. Puteh, H.A. Rahim and M.A. Latif, 2015.Recurrent parent genome recovery analysis in a marker-assisted backcrossing program of rice (*Oryza sativa* L.). *C. R. Biol*., 338: 83–94

Prigge, V., H.P. Maurer, D.J. Mackill, A.E. Melchinger and M. Frisch, 2008.Comparison of the observed with the simulated distributions of the parental genome contribution in two marker-assisted backcross programs in rice. *Theor. Appl. Genet.*, 116: 739–744

Ragimekula, N., N.N. Varadarajula, S.P. Mallapuram, G. Gangimeni, R.K. Reddy and H.R. Kondreddy, 2013. Marker Assisted selection in disease resistance breeding. *J. Plant Breed. Genet.*, 1: 90–109

Sabina, A., M.A. Shafiq, A. Wani, K.A. Bhat, S. Chalkoo, M.R. Mir and A. Shabir, 2010. Marker assisted selection in rice. *J. Phytol.*, 2: 66–81

Sharma, T.R., A.K. Rai, S.K. Gupta and N.K. Singh, 2010. Broadspectrum blast resistance gene *Pi-kh* cloned from the rice line Tetep designated as *Pi54*. *J. Plant Biochem. Biotechnol.*, 19: 87–89

Sundaram, R.M., M.R. Vishnupriya, G.S. Laha, Shobha N. Rani, P. Srinivas Rao, S.M. Balachandaran, G. Ashok Reddy, N.P. Sarma and R.V. Shonti, 2009. Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety. *Biotechnol. J*., 4: 400–407

Tanksley, S.D. and C.J. Nelson, 1996. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadaptedgermplasm into elite breeding lines. *Theor. Appl. Genet.*, 92: 191–203

Van Berloo, R., 2008. GGT 2.0: versatile software for visualization and analysis of genetic data. *J. Hered*., 99: 232–236

Vikas, K.S., S. Atul, S.P. Singh, K.E. Ranjith, C. Vikas, S. Sarkel, S. Devinder, S. Gopala, M.N. Krishnan, K.K. Vinod, U.D. Singh, R. Rathore, S.K. Prashanthi, P.K. Agrawal, J.C. Bhatt, T. Mohapatra, K.V. Prabhu and A.K. Singh, 2012. Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line through marker assisted backcross breeding. *Field Crops Res.*, 128: 8–16

Virmani, S.S. and I. Kumar, 2004. Development and use of hybrid rice technology to increase rice productivity in the tropics. *Int. Rice Res. Notes*, 29: 10–20

Virmani, S.S. and H.L. Sharma, 1993. *Manual for Hybrid Rice Seed Production*. IRRI, Manila, Philippines

Yunoki, T., A. Ezuka, H. Sakurai, H. Shinoda and K. Toriyama, 1970. Studies on the varietal resistance to rice blast III. Testing methods for field resistance on young seedlings grown in greenhouse. *Bull. Chugoku. Nat. Agric. Expl. Stn*., 6: 1–19

Zenbayashi, K., T. Ashizawa, T. Tani andS. Koizumi, 2002. Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. *Theor. Appl. Genet.*, 104: 547–552

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