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Selection of *Sub1* Locus for Submergence-Tolerant Introgression in a Backcrossing of South Sumatra Rice based on SSR Markers

(Pemilihan Lokus *Sub1* untuk Introgresi Ketahanan Penenggelaman dalam Kacukan Balik Padi Sumatera Selatan berasaskan Penanda SSR)

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ABSTRACT

The development of a new submergence-tolerant variety is one ideal solution to reduce submergence stress impact caused by the unpredictable occurrence of flooding in the South Sumatra riparian wetland rice cultivation area. The *Sub1* gene for submergence tolerance was introgressed into South Sumatra riparian wetland rice from the donor parent FR13A using marker-assisted backcrossing. This study involves a backcrossing between Pelita Rampak and BC_1F_1 Pelita Rampak, FR13A-derived *Sub1* breeding lines. The main objective of this study was to introgress the *Sub1* gene in BC_2F_1 using marker-assisted selection (MAS). The introgression of the *Sub1* gene in the backcrossed lines was confirmed by the tightly linked markers RM219 and RM23915. The segregation ratio of RM219 was a good fit to the expected 1:1 Mendelian single-gene model (DF = 1.0, $p \leq 0.05$). In the background study, out of 237 SSR markers unlinked to the target loci, 84 were found to be polymorphic between the two parents and were used for background selection among the selected progeny. Recurrent parent genome recovery in the backcrossed lines ranged from 57.1% to 72.6%. Improvements in the tiller number, percentage of filled grain, productive tiller number and percentage of tiller number were found on these backcrossed lines. The five best backcrossed lines were selected based on SSR markers, submergence tolerance, phenotypic study and agronomic performance.

Keywords: Backcrossing; rice; SSR markers; *Sub1* gene; submergence

ABSTRAK

Pembangunan varieti baru yang toleran terhadap penenggelaman adalah salah satu penyelesaian yang ideal untuk mengurangkan kesan tekanan banjir yang tidak dapat dijangkakan di kawasan penanaman padi di lembah Sumatera Selatan. Gen *Sub1* untuk toleransi penenggelaman telah diintrogresikan kepada padi lembah Sumatera Selatan daripada induk penderma FR13A menggunakan Kacukan Balik Berbantukan Penanda. Kajian ini melibatkan kacukan balik antara Pelita Rampak dan BC_1F_1 Pelita Rampak, titisan biak baka *Sub1* yang berasal daripada FR13A. Objektif utama kajian ini adalah untuk memasukkan gen *Sub1* kepada BC_2F_1 menggunakan Pemilihan Berbantukan Penanda (MAS). Introgresi gen *Sub1* dalam titisan kacukan balik telah disahkan oleh penanda yang berkait rapat iaitu RM219 dan RM23915. Nisbah segregasi RM219 adalah bersesuaian dengan model gen tunggal Mendelian 1:1 yang dijangkakan (DF = 1.0, $p \leq 0.05$). Dalam kajian latar belakang, daripada 237 penanda SSR yang tidak berkaitan dengan lokasi sasaran, 84 didapati polimorfik antara dua induk dan digunakan untuk pemilihan latar belakang antara progeni terpilih. Pemulihan genom induk yang berulang dalam titisan kacukan balik berada antara julat 57.1-72.6%.

Peningkatan bilangan tiler, peratusan butiran terisi, bilangan tiler produktif dan peratusan bilangan tiler dapat dilihat pada titisan kacukan balik. Lima titisan kacukan balik terbaik telah dipilih berdasarkan penanda SSR, toleransi penenggelaman, kajian fenotip dan prestasi agronomi.

Kata kunci: Gen *Sub1*; kacukan balik; padi; penanda SSR; penenggelaman

Introduction

South Sumatra has 2.98 million hectares of riparian wetlands that can be used for rice cultivation (BPS 2015). Submergence stress caused by the unpredictable occurrence of flooding during the rainy season is one of the most critical constraints for rice production in the South Sumatra riparian wetlands (Irmawati et al. 2015; Lakitan et al. 2018). Septiningsih et al. (2009) estimated that about 20 million hectares of rice cultivation areas suffered crop failures caused by this constraint. Over the year, various abiotic stress and climate change factors have caused total rice production to decrease. Wassmann et al. (2009) estimated that yield and crop failure caused by this constraint will increase as a result of the impacts of climate change.

The development of a submergence-tolerant variety is one ideal effort to reduce the impacts of this constraint. In rice, submergence tolerance was mainly regulated by the quantitative trait locus (QTL) *SUB1* in chromosome 9, which can improve the plant survival rate for 10-14 days in complete submergence (Joho et al. 2008; Nandi et al. 1997; Xu & Mackill 1996; Xu et al. 2000, 2006). Several developed *Sub1* gene varieties (i.e. Swarna-*Sub1* and Samba Mahsuri) were introgressed by the QTL *SUB1*, which can be adapted in unfavorable agroecosystems (Neeraja et al. 2007). In another study, Indonesian submergence-tolerant rice varieties were developed, such as Ciherang-*Sub1* and PSB Rc18-*Sub1* (Septiningsih et al. 2014).

Research on *Sub1* gene introgression into susceptible rice varieties was widely performed via conventional breeding combined with marker-assisted selection (MAS) (Xu et al. 2006). The development of submergence-tolerant varieties through MAS was more effective to introgress the *Sub1* gene into susceptible varieties while retaining the desirable recipient parent genome (Acquaah 2007; Ahmed et al. 2016; Collard et al. 2005; Neeraja et al. 2007; Septiningsih et al. 2014). MAS was useful to select other specific alleles/genes, such as genes for salinity tolerance (Linh et al. 2012) and blast resistance (Hasan et al. 2016; Miah et al. 2015). Xu et al. (2006) reported that the enhancement of submergence tolerance for susceptible varieties was significant and did not reflect any negative effects on agronomical performance for yield and grain in normal conditions. The combination of MAS in plant breeding increases the quality and consistency of the introgression alleles from the donor parents to the recipients (Collard & Mackill 2008).

Mackill (2006) reported that submergence-tolerant varieties were not extensively cultivated by farmers given the lack of yield and short stature. Therefore, to increase total production and sustainability, one should develop high-yielding varieties of farmers' preference as subjects for submergence tolerance improvement. The study provides a selection of BC_2F_1 generation by SSR markers associated with the submergence tolerance gene. The main objective is to select a BC_2F_1-*Sub1* variety based on SSR markers correlated with the *Sub1* gene.

Materials and Methods

Plant materials and backcrossing scheme

The recurrent parent was Pelita Rampak, a South Sumatra rice variety - high yielding, with a good cooked taste, susceptible to submergence. BC_1F_1 Pelita Rampak-*Sub1* (FR13A × Pelita Rampak), a backcrossed generation of the FR13A-derived *Sub1* breeding line (Gusmiatun et al. 2015), was used as the donor parent. In this study, single backcrossing was performed in Pelita Rampak × BC_1F_1 Pelita Rampak to obtain BC_2F_1 generation. A total of 40 BC_2F_1 plants were genotyped by tightly linked SSR markers to the *Sub1* gene. Next, a background study was performed to find an individual with high similarity to the recurrent parent. The selected plants were self-pollinated to obtain BC_2F_2 seeds. The molecular analysis was laid out in the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and Laboratory of Plant Physiology, Indonesia and Departement of Agriculture, Universitas Sriwijaya, Indonesia.

DNA EXTRACTION AND QUANTIFICATION

DNA was isolated using a protocol as described by Dellaporta et al. (1983). The procedure involved grinding 5-10 young leaves of 2-week-old plants in liquid nitrogen, which was then transferred in 800 µL of a CTAB extraction buffer (100 mM Tris-CL, pH 8.0, 25 mM EDTA, pH 8.0, 1.25 M NaCl, and 30 µl β-mercaptoethanol) and incubated in 65 °C for 15 min (gently shaken at 5 min intervals). A total of 800 µL of chloroform-isoamyl alcohol was added. After being centrifuged at 12,000 rpm for 15 min, 400 µL of a supernatant was transferred into a new sterilized 1.5 mL micro tube (Eppendorf, Germany). The supernatant was mixed with 40 µL of NaoAc and 800 µL of ice-cold absolute ethanol before being centrifuged at 12,000 rpm for 10 min, and the supernatant was then discarded. The DNA pellets were extracted and rinsed with 1 mL of 70% ethanol and centrifuged at 12,000 rpm for 5 min. The pellets were then air-dried for 12 h and dissolved in 10 µL of ddH_20, O, and 2 µL of RNAse was added to each DNA pellet to remove the RNA. The DNA sample was kept at -20 °C.

DNA quantification was performed using the NanoDrop spectrophotometer (ND1000), followed by electrophoresis using a 1X TAE buffer at 65 V for 30 min on 1% agarose gel stained by 1 µL of GelRed. The gel product was visualized using a UV transluminator, and ethidium bromide was used for staining. The DNA concentration was adjusted to 10 ng.

DNA AMPLIFICATION

A polymerase chain reaction (PCR) was carried out in a single 96-well Bio-Rad system (MJ Research Inc., USA). The total volume of single-locus PCRs was 20 µL, containing 2 µL of a DNA template, 2 µL of a dNTP mix, 1 µL of a forward primer, 1 µL of a reverse primer of the SSR marker, 0.2 µL of the Taq polymerase enzyme, 2 µL of a polymerase buffer, and 10.8 µL of free ion water (Miliq-water). Amplification was carried out with the following conditions: pre-denaturation at 94 °C for 5 min and 34 cycles of 45 s at 94 °C, 45 s of annealing at 52 °C, a 1 min extension of 72 °C, and the last extension at 72 °C for 45 s.

The PCR products were fused with loading dye and analyzed via electrophoresis on 8% polyacrylamide through mini vertical polyacrylamide gels for manual genotyping at high throughput (CBS Scientific Co. Inc., CA, USA). A DNA ladder (100 bp) was used as a marker to measure the amplicon size. The gels were stained with a silver staining solution, NaOH, and formaldehyde, and images were taken using a camera.

FOREGROUND SELECTION AND RECOMBINANT SELECTION

Three SSR markers tightly linked to *Sub1* were genotyped between the two parents (Ahmed et al. 2016; Iftekharuddaula et al. 2011; Mojulat et al. 2017; Neeraja et al. 2007; Septiningsih et al. 2009; Xu & Mackill 1996). RM219 was used for foreground selection, and RM23915 was used for recombinant selection (Table 1). RM23915 was polymorphic between the two parents in a previous study by Hasmeda et al. (2017).

TABLE 1. List of foreground and recombinant SSR markers

Primer	Primer sequence (5'-3')		Repeat motif
	F: Forward Primer	R: Reverse Primer	
RM219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	(CT)17
RM464A	AACGGGCACCTTCTGTCTTC	TGGAAGACCTGATGGTTT CC	(CT) 27
RM23915	GAGGATCCTTACCATCAAAC TTCG	CCAAGAACCTGCATTCTTCAAGG	(AC)15

PARENTAL POLYMORPHISM AND BACKGROUND SELECTION

A total of 237 SSR markers (http://www.gramene.org) unlinked to the target loci distributed in all 12 chromosomes of the rice genome were used for a parental polymorphism survey between the recurrent parent, Pelita Rampak, and FR13A. Out of these, 84 polymorphic markers were used for the background selection of backcrossed progeny.

PHENOTYPIC SELECTION, SUBMERGENCE SCREENING, AND AGRONOMICAL STUDY

Phenotypic selection was carried out at a greenhouse of the Faculty of Agriculture, Universitas Sriwijaya. The selected BC_2F_1 plants and recurrent parent were evaluated at the vegetative stage following the protocol of Iftekharuddaula et al. (2011) and IRRI (2013). The selected plants were ranked based on their phenotypic score and compared to the recurrent parent. The plants with the highest rank of similarity scores to the recurrent parent were selected.

Submergence tolerance screening of the selected lines was done at the submergence experimental pond in the Faculty of Agriculture, Universitas Sriwijaya, following standard protocols described by Xu et al. (2000). The selected lines and their parents were grown in trays, and FR13A was used as a check variety. The 14-day-old seedlings were submerged for 14 days. The survival and recovery rates were recorded 6 days and 30 days after the plants were de-submerged following standard protocol (IRRI 2013).

Agronomic evaluation of selected lines was laid out in at a greenhouse (non-submerged condition) of the Faculty of Agriculture, Universitas Sriwijaya with 6 replications for selected each line and analysed by Analysis of Variance (ANOVA) followed by Honest Significant Difference (HSD) calculated by SAS software.

DATA ANALYSIS

The marker data was scored based on the presence or absence of amplicons between the two parents. The homozygous recipient allele, homozygous donor allele, and heterozygous allele were scored as 'A', 'B', and 'H', respectively. This data was analyzed using the Graphical Genotyper (Van Berloo 2008). Popgene software (Yeh et al. 1999) was used to analyze the Mendelian segregation ratio chi-square (χ^2). The chi-square formula is $\chi^2 = (O-E)2/E$, where O is the observed value and E is the expected value. The phenotypic data was done by using SPSS 23 and agronomic evaluation of selected lines was laid out with 6 replications and analysed by ANOVA followed by HSD test calculated by SAS software.

RESULTS AND DISCUSSION

PARENTAL POLYMORPHISM SURVEY USING SSR MARKERS

The main objective of backcrossing is to introgress one or more genes of concerned character from the donor parent into the recipient parent while maintaining the recipient parent genome (Hasan et al. 2016). Polymorphic SSR markers were essential for the background study as markers to decline unfavorable genes from the donor parent genome in the backcrossed recombinant lines (Frisch & Melchinger 2005; Hasan et al. 2016; Hospital 2001; Neeraja et al. 2007). To screen SSR markers that will be used for the background study, 237 SSR primers unlinked to the target loci were genotyped between FR13A and Pelita Rampak; only 84 primers (35.443%) were polymorphic and will be used for background selection (Figure 1, Table 2). Basavaraj et al. (2010) accounted for 17.47% of the SSR polymorphic markers between PRR78 and Pusa 1460. In another study, Cuc et al. (2012) reported 12.6% polymorphism between Vietnam elite rice and IR64. Khanh (2013) reported 15.1% polymorphism between Bac Thom 7 and IR64. Finally, Mojulat et al. (2017) reported 21.11% of the SSR polymorphic markers between MR263 and Swarna-*Sub1*.

FOREGROUND SELECTION AND RECOMBINANT SELECTION

The performance of MAS as diagnostic markers depends on the availability of closely linked markers and/or flanking markers for the interest gene (Frisch & Melchinger 2005). In this study, RM219 and RM464A, which were previously reported to be tightly associated with the *Sub1* gene on chromosome 9, were genotyped between the two parents. RM219 and RM464A are 3.4 cM and 0.7 cM, respectively, from *SUB1* (Neeraja et al. 2007; Xu et al. 2004). Out of these, only RM219 produced clear polymorphism and was used for foreground selection, the homozygous donor allele in 150 bp and the homozygous recurrent parent in 140 bp.

During foreground selection, 40 plants of BC_2F_1 were genotyped using RM219 (Figure 2). Out of these, 16 plants showed heterozygous alleles (H score), which indicated the introgression of the *Sub1* gene; the rest of the 21 plants showed homozygous recipient alleles (susceptible alleles). In this study, two plants showed fixed donor alleles (score 'B'). The results showed that the RM219 marker fit the 1:1 ratio in the Mendelian single gene model for BC_2F_1 generation for the homozygous recipient allele and heterozygous allele groups, with a non-significant chi-square value of 0.02 at a probability level of 0.05. In earlier studies, the application of RM219 as a diagnostic marker for the development of susceptible rice varieties introgressed by the *Sub1* gene has been carried out (Mojulat et al. 2017; Neeraja et al. 2007; Rathnayake et al. 2013; Siangliw et al. 2003;

TABLE 2. Polymorphic SSR markers between Pelita Rampak and FR13A

No.	Chr.	SSR Marker	Location	No.	Chr.	SSR Marker	Location
1	1	RM576	52.6	43	7	RM481	3.2
2	1	RM84	26.2	44	7	RM5672	44.1
3	1	RM583	58.9	45	7	RM542	49.7
4	1	RM580	68.2	46	7	RM182	61
5	1	RM24	79.1	47	7	RM560	69.2
6	1	RM128	134.8	48	7	RM429	99.9
7	2	RM154	4.8	49	8	RM337	0.5
8	2	RM211	14.4	50	8	RM1959	1.8
9	2	RM233A	16.3	51	8	RM407	3
10	2	RM262	78.4	52	8	RM1235	13.1
11	2	RM110	100.6	53	8	RM1376	25.9
12	3	RM22	13	54	8	RM547	43.7
13	3	RM585	25.1	55	8	RM72	60.9
14	3	RM545	35.3	56	8	RM339	72.2
15	3	RM282	100.6	57	8	RM531	90.3
16	3	RM135	153.7	58	9	RM23679	0.5
17	3	RM570	158.2	59	9	RM434	56.8
18	3	RM448	189.6	60	9	RM410	64.1
19	4	RM537	8.5	61	9	RM257	65.1
20	4	RM2848	16.7	62	9	RM288	69.5
21	4	RM1869	70.6	63	9	RM242	73.6
22	4	RM1388	76.5	64	9	RM108	76.9
23	4	RM273	94.4	65	10	RM330A	2.4
24	4	RM241	106.2	66	10	RM474	3
25	4	RM348	113.2	67	10	RM222	11.3
26	4	RM451	115.5	68	10	RM1375	44.3
27	5	RM153	0.5	69	10	RM1873	51.5
28	5	RM267	33.1	70	10	RM258	70.8
29	5	RM440	76.2	71	10	RM228	94.7
30	5	RM161	96.9	72	11	RM4B	3.4
31	5	RM233B	110	73	11	RM20B	3.8
32	5	RM538	132.7	74	11	RM3717	4.8
33	6	RM540	0	75	11	RM287	64.8
34	6	RM585	25.1	76	11	RM229	77.8
35	6	RM276	33.5	77	11	RM1341	80.3
36	6	RM402	40.3	78	11	RM206	88.7
37	6	RM549	42.7	79	11	RM456C	117
38	6	RM539	45.1	80	12	RM7619	3.8
39	6	RM3431	52.3	81	12	RM4A	5.2
40	6	RM402	52.3	82	12	RM20A	9.7
41	6	RM162	104.8	83	12	RM28195	62.2
42	6	RM1370	110.6	84	12	RM1226	109.2

Toojinda et al. 2005; Xu et al. 2004). The two homozygous donor alleles plants were produced because of accidental backcrossing failure (Figure 2). In plant breeding, normally, backcrossed progenies only produce homozygous susceptible alleles (A) and heterozygous alleles (H); the presence of homozygous donor alleles in backcrossed lines is not expected (Acquaah et al. 2007). The same result was found in a study by Iftekharuddaula et al. (2015) in BC_2F_1 generation from backcrossing between BC_1F_1 and BR11.

Obviously, 16 heterozygotes plants were phenotypically evaluated. Out of these, 9 plants with the highest ranking phenotypic performance close to the recurrent parent were selected. These 9 plants were self-pollinated to obtain BC_2F_2 seeds. In this generation, recombinant selection was done using RM23915. All the selected plants produced fixed donor alleles (resistant alleles) (score 'B') (Figure 3). These 9 lines were selected and subjected to submergence tolerance and agronomical performance.

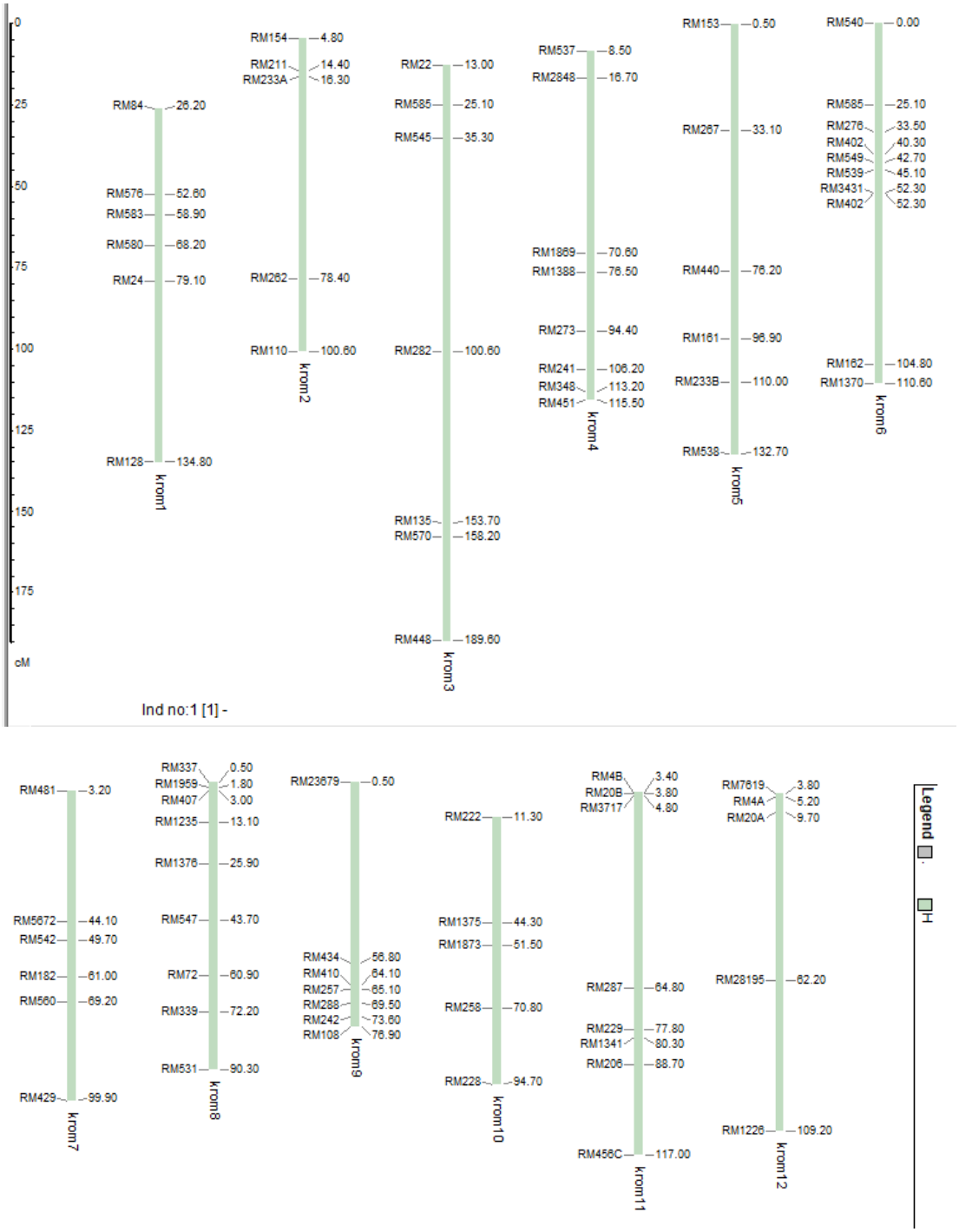

FIGURE 1. Distribution of polymorphic markers in each chromosome between Pelita Rampak and FR13A

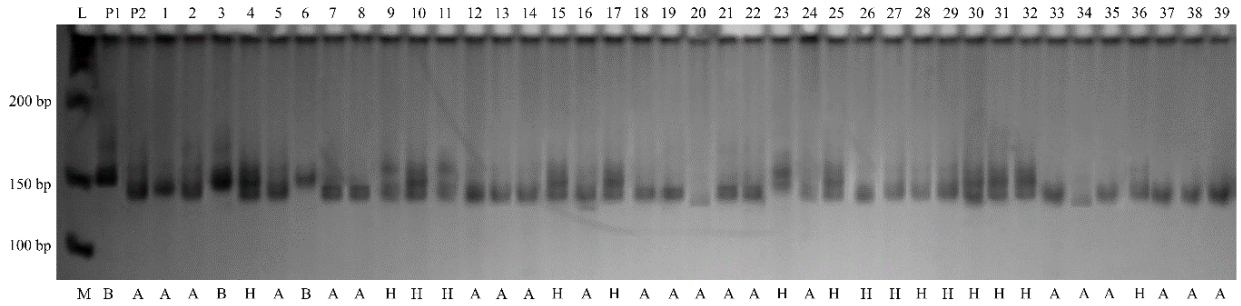

FIGURE 2. Amplification of the BC_2F_1 generation by RM219 marker linked to the *Sub1* gene

L: ladder; P1: the donor parent; P2: the recurrent parent; 1-39: plant number

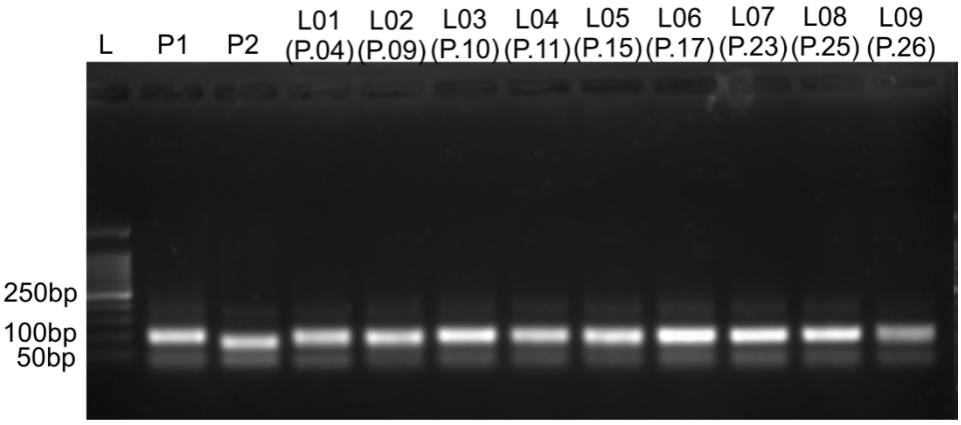

Figure 3. View of gel electrophoresis of BC_2F_2 generation by RM23915 marker linked to the *Sub1* gene

L: ladder; P1: the donor parent; P2: the recurrent parent; L01–L09: plant number

BACKGROUND SELECTION

A total of 84 polymorphic SSR markers unlinked to the target loci were genotyped in 5 lines to select the plants with the highest recurrent parent genome proportion. The application of background markers can accelerate the recovery of the recurrent parent genome (Collard & Mackill 2008). Considering the size of the donor parent, introgression might be affected by submergence tolerance and agronomical performance (Frisch & Melchinger 2005; Hospital 2001). The recurrent parent genome percentages in the 5 selected lines after foreground and recombinant selection ranged from 57.1% to 72.6% (Table 3). The highest recurrent parent genome recovery was 72.6%, found in L06 (PN 17) (Figure 4). Through background selection, we maintained the essential genome of the recurrent parent to retain its desirable traits.

TABLE 3. Background study of selected BC_2F_1 lines

Plant no.	Recurrent parent (A %)	Donor parent (B %)	Heterozygous (H %)
L03 (PN 10)	67.4	22.5	10.1
L04 (PN 11)	57.1	30	12.8
L06 (PN 17)	72.6	15.9	11.5
L07 (PN 23)	70.2	22.6	7.2
L09 (PN 26)	65.8	25.1	9.1

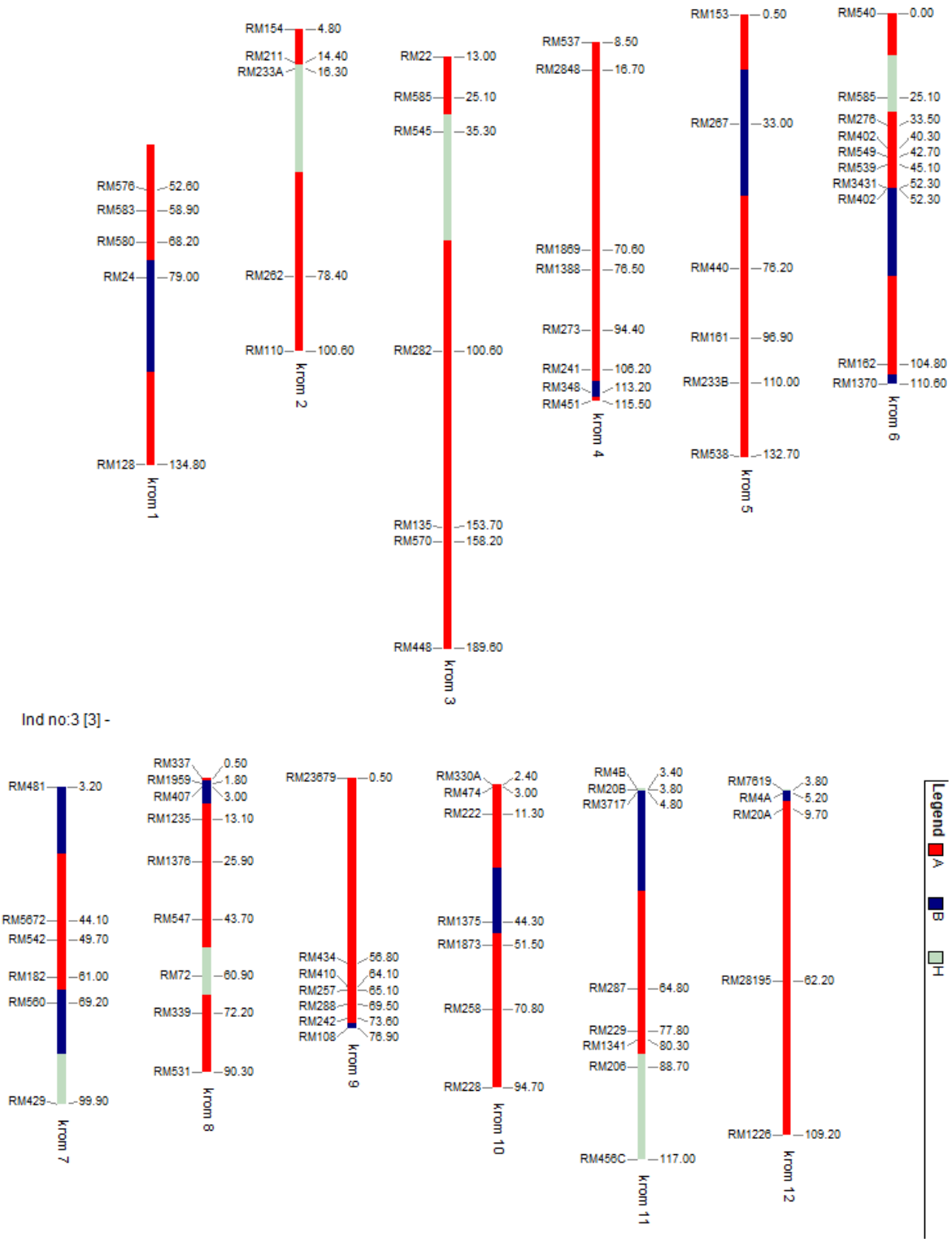

FIGURE 4. Graphical genotype of the plant with the highest BC_2F_2, L06 (PN 17)

SUBMERGENCE SCREENING

The 14-day-old seedlings of the 9 selected lines were submerged for 2 weeks. Only 5 lines showed submergence tolerance. These plants also showed good recovery after de-submergence, with a survival percentage above 70%, while the recurrent parent, Pelita Rampak, showed low submergence tolerance and recovery rates; on the other hand, the resistant checks showed good survival and recovery performance. This result shows improvement in submergence tolerance and recovery performance and confirms the introgression of the *Sub1* gene in backcrossed lines. Fukao and Bailey-Serres (2008) reported, in submergence-tolerant rice, *Sub1* responses to submergence stress by quiescence and high recovery rates by shoot elongation and the development of new leaves soon after de-submergence. Toojinda et al. (2003) found that the inheritance and expression of submergence tolerance at the seedling stage are regulated by physiology and genetic complex. Submergence tolerance is a polygenic trait in which the expression is determined by the results of gene interaction (Mohanty et al. 2000). The rate of submergence was influenced by additive gene action and the environment (Mishra et al. 1996; Mohanty & Khush 1985). The rate of submergence tolerance might be influenced by the donor parent's genome introgression and the *Sub1* gene's introgression in chromosome 9. Positive interaction between these genes will help increase the rate of submergence tolerance (Iftekharuddaula et al. 2015) (Table 4).

TABLE 4. Screening of selected BC_2F_1 lines for submergence tolerance

Variety/Lines	% Survival		Submergence tolerance score
	6 Days after de-submergence	30 Days after de-submergence	
FR13A	100.00	96.67	1
BC_1F_1 Pelita Rampak	90.00	83.33	5
Pelita Rampak	33.33	23.33	9
L03 (PN 10)	76.67	70.00	5
L04 (PN 11)	80.00	73.33	5
L06 (PN 17)	73.33	66.67	5
L07 (PN 23)	80.00	73.33	5
L09 (PN 26)	83.33	73.33	5

Score for tolerance after 6 days: 1, erect dark green leaves, very little elongation, very tolerant, 100% survival rate; 3, erect green leaves, little elongation, tolerant, 95-99% survival rate; 5, droopy, pale green leaves, moderate elongation, moderate, 75-94% survival rate; 7, long, pale green leaves, elongated, few survived, susceptible, 50-74% survival rate; 9, long whitish leaves, elongated, completely dead, very susceptible, 0-49% survival rate

AGRONOMICAL PERFORMANCE

The agronomical performance of the 5 selected lines was observed in the non-submerged condition in the greenhouse of the Faculty of Agriculture, Universitas Sriwijaya. Significant variation was found among all the characters (Table 5). The result generally shows that the agronomical performances of all the selected backcrossed lines were close to that of the recurrent parent. However, the highest tiller number, percentage of filled grain, productive tiller number, and percentage of tiller number values were found in the backcrossed lines. In the MABC program, the level of donor parent introgression influenced agronomical performance; if the introgression of the donor genome consists of desirable trait, it might increase agronomic performance and vice versa (Frisch & Melchinger 2005; Hospital 2001; Iftekharuddaula et al. 2015).

TABLE 5. Agronomical evaluation of the selected backcrossed submergence-tolerant lines

Variety/ Lines	Days of maturation (#)	Plant height (cm)	Tiller number (#)	Productive tiller (#)	Flag leaf length (cm)	Panicle length (cm)	Grain/ panicle (#)	Filled grain/ panicle (#)	Percentage of filled grain (%)	Weight of grain/ panicle (g)	Grain length (cm)	Grain width (cm)	1,000-grain weight (g)	Yield (ha)
Pelita Rampak	124.83 ± 0.41	156.83 ± 5.64	24.67 ± 1.51	22.00 ± 2.00	35.89 ± 10.45	25.60 ± 2.74	196.56 ± 10.23	148.83 ± 8.66	75.78 ± 3.78	2.86 ± 0.05	1.01 ± 2.45	0.28 ± 0.01	27.25 ± 0.34	6.23 ± 0.58
BC_1F_1 Pelita Rampak	131.17 ± 1.60	181.83 ± 16.63	27.33 ± 1.75	22.50 ± 0.55	43.16 ± 4.85	25.14 ± 2.33	168.17 ± 13.42	127.22 ± 2.66	75.99 ± 5.22	2.60 ± 0.02	1.03 ± 0.02	0.29 ± 0.01	25.57 ± 0.81	5.68 ± 0.38
L03 (PN 10)	130.33 ± 0.52	141.23 ± 4.95	25.83 ± 1.72	23.00 ± 1.41	39.76 ± 5.72	23.07 ± 0.88	156.67 ± 2.95	126.39 ± 1.00	80.69 ± 1.15	2.59 ± 0.01	0.97 ± 0.02	0.28 ± 0.01	25.98 ± 0.13	6.14 ± 0.34
L04 (PN 11)	130.00 ± 0.00	130.50 ± 5.39	27.50 ± 2.17	22.83 ± 1.47	42.38 ± 5.30	22.65 ± 0.58	155.22 ± 2.96	125.67 ± 0.82	80.98 ± 1.64	2.59 ± 0.02	0.96 ± 0.03	0.27 ± 0.01	26.40 ± 0.13	6.11±0.47
L06 (PN 17)	130.00 ± 0.00	158.27 ± 4.06	25.00 ± 1.26	21.67 ± 1.21	38.84 ± 1.74	22.17 ± 0.72	156.89 ± 1.60	124.95 ± 2.03	79.65 ± 1.53	2.65 ± 0.29	1.06 ± 0.03	0.27 ± 0.01	26.60 ± 0.24	5.99 ± 0.95
L07 (PN 23)	130.17 ± 0.41	142.17 ± 7.96	27.50 ± 1.38	22.83 ± 2.32	35.28 ± 0.91	22.33 ± 0.46	156.50 ± 0.84	125.61 ± 1.02	80.27 ± 0.66	2.57 ± 0.04	1.06 ± 0.02	0.27 ± 0.00	25.35 ± 1.22	6.08 ± 0.57
L09 (PN 26)	130.00 ± 0.00	153.30 ± 3.13	25.50 ± 1.05	22.50 ± 1.05	35.90 ± 2.90	21.17 ± 0.36	154.84 ± 2.72	126.72 ± 3.38	81.87 ± 2.67	2.59 ± 0.02	1.05 ± 0.01	0.27 ± 0.01	25.96 ± 0.11	6.10 ± 0.41
HSD (0.05)	58.45	25.25	3.61	0.63	2.10	7.41	31.25	31.16	4.51	4.74	21.57	5.37	7.46	0.60

CONCLUSION

In this parental polymorphism study, out of 237 SSR markers, only 84 showed polymorphism and were used for the background study. The selection of *Sub1* gene introgression was done using foreground marker RM219 and recombinant marker RM23915. Out of 40 plants, 16 plants were heterozygous, as amplified by RM219. Nine plants were selected based on phenotypic selection and recombinant selection. Out of these, 5 lines were selected based on their submergence performance and agronomical performance. These lines were evaluated using background markers. The recurrent parent genome recovery of these selected lines ranged from 57.1% to 72.6%. The highest recurrent parent genome recovery was observed in L06 (PN 17).

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