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IMPROVING SUBMERGENCE TOLERANCE OF VIETNAMESE RICE CULTIVAR BY MOLECULAR BREEDING

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A B S T R A C T

Submergence stress has caused by climate change is the major hindrance to enhancing rice production of Vietnam. In this study, we have evaluated the levels of submergence tolerance ability of the imported rice cultivars under the 4 different field trials. Among these, IR64-*Sub1* exhibits the highest submergence tolerance with stable and high yield, and was used as a donor plant, while Bacthom 7, an elite Vietnamese rice cultivar was used as the recipient plant. In molecular markers study, we have used closely linkage markers with *Sub1*, flanking markers *Sub1*, and unlinked marker to *Sub1* for the foreground, recombinant and background selections in the backcrossing generations between the donor and the recipient plants. In BC_3F_1 generation, the individual plant number 116 has carried QTL/*Sub1* and retained the highest genetic background of the recipient parent up to 98.6%. The newly improved rice line may be useful for growing in the flooding areas of Vietnam to cope with the climate change.

Keywords: Marker-assisted backcrossing (MABC), Introgression, Submergence tolerance, Simple sequence repeat (SSR).

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cash crop in Vietnam, and plays a key role to enhance economy in this country. Rice plant is cultivated on 82% of the arable land in two main fertile deltas with 18% of rice produced in Red River delta in the north and 52% in Cuulong delta in the south, respectively. Vietnam is currently leading a biggest rice exporter with over 30 million tons annually, accounts for 50 percent of the world rice trade (IRRI 2013).

Submergence stress is considered as a major challenge for rice production in South and Southeast Asia, causing annual losses of over one billion US dollars (Xu *et al.,* 2006; Khanh *et al.,* 2013). In Vietnam, the erratic floods experienced in rainfed and flood-affected areas are usually caused by heavy rainfall, overflow in the vicinity of rivers, canals and tidal movement as in the coastal areas. Moreover, flooding areas are expected to increase substantially due to consequence of climate change as polar ice-caps melt causes sea-level rise, the uneven distribution of rains and the predicted increases in frequencies and intensities of flooding caused by extreme weather events (Coumou and Rahmstorf, 2012). Specifically, over 10.000 hectares of rice were inundated by floods in 2011, which caused severe economic losses (MARD 2011). Vast rice growing areas (approximately 40.000 km^2) will be disappeared if sea level rise is by 1m (Khanh *et al.,* 2013).

Previous researches have shown that submergence tolerance is managed by a single major quantitative trait locus (QTL) on the chromosome 9, along with a number of minor QTLs. The major QTL/*Sub1*, with a LOD score of 36 and an R^2 value of 69 %, provides tolerance to complete submergence for up to 2 weeks. The fine-mapping of *Sub1* employed 2950 F_2 segregating individuals and, although the region had a low recombination rate, *Sub1* was delineated to a genomic region of approx. 0.06 cM (Xu *et al.,* 2000). Sequencing the *Sub1* region in an FR13A-derived line revealed the presence of 3 genes encoding putative ethylene responsive factors (ERF), *Sub1A*, *Sub1B* and *Sub1C* were subsequently identified as the major determinant of submergence tolerance (Xu *et al.,* 2006). On the other

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hand, the improved rice cultivars (carrying *Sub1* gene) can withstand over 2 weeks of complete submergence (Ismail *et al.,* 2012).

The basis of a marker-assisted backcrossing (MABC) strategy is to transfer a specific allele at the target locus from a donor line to a recipient line while selecting against donor introgressions across the rest of the genome. The use of molecular markers, which permits the genetic dissection of the progeny at each generation, increases the speed of the selection process, thus increasing genetic gain per unit time (Hospital 2003). The main advantages of MABC are: (1) efficient foreground selection for the target locus, (2) efficient background selection for the recurrent parent genome, (3) minimization of linkage drag surrounding the locus being introgressed, and (4) rapid breeding of new genotypes with favorable traits. The effectiveness of MABC depends on the availability of closely linked markers and/or flanking markers for the target locus, the size of the population, the number of backcrosses and the position and number of markers for background selection (Frisch *et al.,* 1999; Frisch and Melchinger 2005).

Development of the improved rice varieties with tolerance of submergence stress is an imperative task to limit extent of damage caused by the flooding problem in this country. Hence, the objectives of this study were to evaluate the levels of submergence tolerance ability of the imported rice cultivars, then to apply MABC breeding strategy to introgress QTL/*Sub1* into an elite Vietnamese rice variety Bacthom 7.

MATERIALS AND METHODS

Plant Materials, SSR Markers and Crossing Scheme: Parental rice varieties (total 8 varieties) carrying QTL/*Sub1* were imported from International Rice Research Institute (IRRI) and considered to use as the donor plants. IR42 (without QTL/*Sub1*) was used as a sensitive check cultivar. Three Vietnamese elite rice cultivars (Khangdan 18, Bacthom 7 and OM6976 were used as the recipient plants shown in Table 1. The use of 53 SSR polymorphism markers and their information for parental lines were shown in Table 2.

Table 1. List of rice cultivars used in breeding program

Sr.	Name of variety	*Sub 1*present	Origin
1	Swarna-*Sub1*	+	IRRI
2	IR64-*Sub1*	+	IRRI
3	Samba Mahsuri –*Sub1*	+	IRRI
4	TDK1-*Sub1*	+	IRRI
5	IR49830-7	+	IRRI
6	BR11-*Sub1*	+	IRRI
7	PSB Rc68	+	IRRI
8	INPARA3	+	IRRI
9	IR42	-	IRRI
10	Khang dan 18	-	FCRI
11	Bac thom 7	-	FCRI
12	OM6976	-	CLRRI

(+) Sub1 present; (-) non- Sub1 present, IRRI : Rice genotypes used at IRRI, Philippines.
FCRI: Rice genotypes used at Food Crops Research Institute, Vietnam : CLRRI: Rice genotypes used at Cuu Long Delta Rice Research Institute, Vietnam.

Artificial Submergence Experiments: The experiments were conducted at the greenhouse of Agricultural Genetics Institute (AGI), Hanoi, Vietnam in the early year of 2010. Submergence tests under greenhouse condition were done in the controlled submergence tank as previously described by Xu *et al. (*2000). Briefly, generations of BC_1F_2, BC_2F_2 and BC_3F_1 and the parents' seeds were soaked in 2 days for germination. Twenty healthy seeds per row were selected to sow into the plastic trays (20 x 15 x 10 cm). After 3 weeks old seedlings, they were completely submerged at 14 to 21 days old under water depth of 1.1 to 1.4 m. Water depth and temperature were controlled and maintained at the same level during submergence test. The susceptible check IR42 cultivar was observed at 6^{th} dates of days of complete submergence. The susceptible check exhibited 70 to 80% damage, trays was de-submerged and the survival of plants and recovers was scored in comparing with control varieties of Khangdan 18; Bacthom 7 and OM6976, respectively.

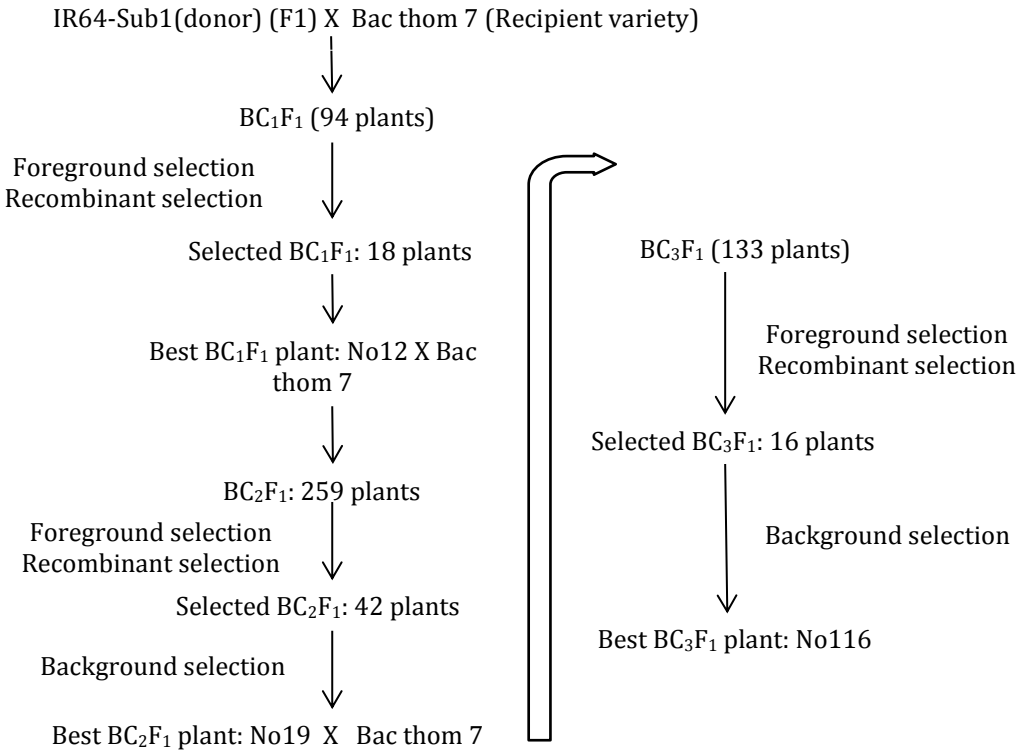
Table 2. Polymorphic data resulted from 378 SSR primer pairs.

Sr.	primer name	Chr	Map positions (Mb)	Repeat Motif
1	RM243	1	7.97	(CT)18
2	RM575	1	8.07	(AG)24
3	RM10713	1	11.23	(AGA)12
4	RM140	1	12.30	(CT)12
5	RM10890	1	14.75	(TATC)5
6	RM10927	1	15.40	(CT)10
7	RM11125	1	20.53	(CT)22
8	RM1349	1	25.07	(AG)23
9	RM7250	1	36.08	(ATCT)6
10	RM5404	2	33.67	(TC)15
11	RM423	2	35.10	(TTC)9
12	RM3202	3	809.95	(TC)12
13	RM5639	3	8.20	(AAG)13
14	S03065*	4	14.50	-
15	RM7097	3	26.87	(AGAT)7
16	RM6329	3	28.80	(CTT)20
17	RM3867	3	31.74	(GA)30
18	RM518	4	2.03	(TC)15
19	RM307	4	11.10	(AT)14(GT)21
20	RM3843	4	31.49	(GA)23
21	S05009*	5	0.80	-
22	RM6317	5	1.52	(CTT)12
23	RM18877	5	23.57	(CTT)25
24	RM510	6	2.83	(GA)15
25	RM585	6	3.20	(TC)45
26	RM527	6	9.86	(GA)17
27	RM508	6	441.61	(AG)17
28	RM20783	7	111.47	(TC)18
29	S07011*	7	2.40	-
30	RM5436	7	9.07	(TC)17
31	RM3753	7	23.66	(GA)17
32	RM18	7	25.65	(GA)4AA(GA)(AG)16
33	RM310	8	5.11	(GT)19
34	RM80	8	24.47	(TCT)25
35	RM447	8	26.54	(CTT)8
36	S08121a*	8	28.20	-
37	S09073*	9	18.80	-
38	S09026b*	9	9.00	-
39	RM24013	9	9.44	(CT)12
40	RM7175	9	16.87	(ATAG)6
41	R9M42*	9	18.90	-
42	RM215	9	21.18	(CT)16
43	RM25022	10	3.58	(TA)45
44	RM25181	10	8.84	(TTC)22
45	RM5806	10	14.48	(AGG)9
46	RM228	10	22.24	(CA)6(GA)36
47	S011055a*	11	10.00	-
48	RM26652	11	15.06	(TTC)9
49	RM21	11	19.10	(GA)18
50	RM206	11	22.01	(CT)21
51	S12055*	12	15.20	-
52	RM28746	12	26.33	(GAA)11
53	RM17	12	27.00	(GA)21

The map position and their information were available at www.gramene.org; * SSR primer was provided by IRRI.

Field Experiments: Field experiments were carried out at the 4 different field trials during the years of 2010 and 2011 at the provinces of Thanh Tri, Hanoi, Nam Dinh, and Thai Binh (Red River Delta in the north), and Bac lieu province (Cuulong delta in the south). All the imported rice varieties and 3 Vietnamese elite rice cultivars were grown in a plot (4 x 3 m). The major agronomic traits included days to heading (DTH), plant height (PH), panicles per plant (PP), seeds per spikelet (SPS) and grain yield (GY) were recorded.

Crossing Scheme: Based on the results obtained from greenhouse and field experiments, IR64-*Sub1* was used as a donor plant to follow the marker assisted backcrossing (MABC). Among the Vietnamese elite rice cultivar, Bacthom 7 was selected as a recipient plant because its high quality with aroma smell. To establish the crossing scheme, Bacthom 7 cultivar was crossed with IR64-*Sub1* to obtain F_1 seeds (Fig. 1). F_1 seeds were backcrossed with Bacthom 7 to obtain a large number of BC_1F_1 seeds. In the BC_1F_1 generation, individual plants that were heterozygous at the *Sub1* locus were identified by the markers of the previous report in order to minimize the population size for further screening as the foreground selection (Neeraja *et al.,* 2007). From the individual plants that showed heterozygous for *Sub1*, those that were homozygous for the recipient allele at 2 markers (RM23662 and RM23877) distally flanking the *Sub1* locus (i.e. recombinant) were identified. We termed this as "recombinant selection" (Collard and Mackill 2008). From these recombinant plants, individuals with the fewest number of markers from the donor genome were selected (background selection).



In the second BC generation, the same strategy was followed to select the individual plants with the desired allele combination at the target loci including selection for recombinants between *Sub1* and the nearest proximal markers locus (RM23662 and RM5688) and suitable genomic composition at the non-target loci and crossed with the recipient parent to develop the next generations (BC_2F_1, BC_3F_1). The best plant (BC_3F_1 generation) was selected for further analysis.

Genomic DNA Extraction and PCR Amplification Conditions: A piece of young leaves (1 to 3 cm) was collected at the early morning in the experimental field. They were kept in moist tissue paper in a plastic bag, kept away from sunlight and were frozen immediately in liquid nitrogen on the day of collection. They were used immediately, or frozen at – 20^0C until required. Cetyl trimethyl ammonium bromide (CTAB) method was used for DNA extraction following a standard protocol: A 4.0 g of leaf sample was ground in liquid nitrogen using a mortar and pestle pre-chilled to –20^oC. The pulverized

leaves were quickly transferred to a liquid nitrogen prechilled, 50-mL Falcon tube. The 2% of pre-heated (65°C) CTAB buffer (16 mL) containing 5% v/v β-mercaptoethanol and 2% PVP were quickly added to the tube and stirred with a glass to mix. The tube was incubated at 65°C for 5 min with frequent swirling. An equal volume of chloroform: octanol (24:1) was added and the sample centrifuged for not more than 5 s in a bench-top centrifuge (Biofuge 13, Heraeus) at room temperature to separate the phases. The supernatant was carefully decanted and transferred to a new tube. The above steps, beginning with the addition of chloroform/octanol (24:1) and ending with decanting of supernatant, were repeated twice. The supernatant was precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with the buffer (75% ethanol, 10 mM ammonium acetate, TE). The pellets were air-dried and re-suspended in TE. The dissolved nucleic acids were brought to 2 M NaCl and re-precipitated using 2 volumes of 70% ethanol. The tube was incubated at 65°C for 5 min to dissolve geneomic DNA, and Rnase was then added. The yield of DNA per gram of leaf tissue extracted was measured using a UV-VIS Spectronic Genesys 5 (Milton Roy) spectrophotometer at 260 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA samples from the leaf tissues were digested with *EcoRI* and *HindIII* and electrophoresed on a 0.8% agarose gel.

Statistical Analyses: The measured data from field experiment were analyzed by IRRISTAT 5.0; Statistix 8.0, Excel 2007 programs. The molecular weights of the different alleles were calculated by Alpha Ease Fc 5.0 software. The marker data was analyzed using the software Graphical Geneotyper (Van Berloo 2008). The homozygous recipient allele, homozygous dominant allele and heterozygous allele were scored as "A", "B" and "H". The percent markers homozygous for recipient parent (%A) and the percent recipient alleles including heterozygous plants (%R) were calculated. All treatments of the agronomic traits and artificial submergence analyses were performed in a completely randomized design with at least thrice. Data were analyzed with the use of the Duncan`s multiple-range test ($P<0.05$).

RESULTS

Evaluation on Levels of Submergence Tolerance Ability of the Imported Rice Varieties under the Artificial Conditions: Total 12 rice varieties were evaluated the submergence tolerance ability in 13 to 15 days of complete submergence. The results showed that the Vietnamese elite varieties such as Khangdan 18, Bacthom 7 and OM6976 as well as IR42 (sensitive check) are considered as non-carrying QTL/*Sub1* because they were completely withered and died after 13 to 15 days of complete submergence, while the imported rice varieties such as IR49830-7 and BR11-*Sub1* (carrying QTL/*Sub1*) exhibited the survival ability in 10 to 14 days of complete submergence, and to re-newed growth and development after de-submergence. The survival rate of IR49830-7 and BR11-*Sub1* were 25.4% and 37.2%, respectively (Table 3).

Table 3. Evaluation of submergence tolerance of rice varieties at the controlled condition. unit: *%*

Sr.	Name of variety/ Breeding line	Survival plant (%)		Average	Scoring of survival	Observation
		Spring crop (15 d)	Summer crop (13 d)			
1	Swarna-*Sub1*	71.9	65.8	68.9	7.0	average tolerance
2	IR64-*Sub1*	82.8	70.3	76.5	5.0	good tolerance
3	Samba Mahsuri –*Sub1*	56.2	51.6	53.9	7.0	average tolerance
4	TDK1-*Sub1*	72.4	68.7	70.6	7.0	average tolerance
5	IR49830-7	27.1	23.7	25.4	9.0	non tolerance
6	BR11-*Sub1*	39.8	34.6	37.2	9.0	non tolerance
7	PSB Rc68	56.8	51.1	54.0	7.0	average tolerance
8	INPARA3	50.7	45.6	48.2	7.0-9.0	less tolerance
9	IR42 (sensitive check)	26.7	25.0	25.9	9.0	non tolerance
10	Khang dan 18 (control)	0.0	0.0	0.0	9.0	non tolerance
11	Bac thom 7(control)	0.0	0.0	0.0	9.0	non tolerance
12	OM6976 (control)	0.0	0.0	0.0	9.0	non tolerance
	$CV_{(\%)}$	4.47	3.44			
	LSD (0,05)	1.17	1.91			

Among the imported rice varieties, IR64-*Sub1* revealed the highest survival (76.5%). The lowest variety was INPARA3 by 48.2%. The submergence tolerant varieties showed medium survival proportion including Swarna-*Sub1* (68.9%); Samba Mahsuri-*Sub1* (53.9%), TDK1-*Sub1* (70.6%) and PSBRc68 (54%), respectively. On the other ways, IR64-*Sub1* was the best submergence-tolerant ability with survival score of point 5 in the artificial submergence screening.

Evaluation on the Agronomic Traits and Their Adaptability of the Imported Rice Varieties in the Field Conditions: As aforementioned, the imported rice varieties and the elite Vietnamese rice varieties were evaluated for 5 major agronomic traits in the different field trials as shown in Table 4, 5, 6 and 7. The results showed that all rice cultivars have days to heading from 95 to 158 days. However, there is a slight change in days to heading of those rice varieties in the 4 different field trials. The plant height of the rice varieties ranged from 95 to 163 cm. Similarly, the panicles per plant of and seeds per spikelete of the varieties were about 5.0 to 9.9 plants and 99 to 133 grains. Regarding grain yield, the PSbRc68 showed good adaptation in growth and development at the areas of the Red River Delta.

Table 4. Performance of major agronomic traits of the imported rice varieties in Hanoi field in 2010-2011.

Sr	Cultivar name/ Breeding line	Days to heading (d)		Plant height (cm)	Panicle /plant	Seed/ spikelet (seeds)	Grain yield (ton/ha)
		Summer crop	Spring crop				
1	Swarna-*Sub1*	110	146	95.4 ± 2	6.5 ± 0.5	110 ± 2	3.97
2	IR64-*Sub1*	105	130	98.1 ± 1	6.1 ± 0.5	102 ± 4	4.47
3	Samba Mah –*Sub1*	115	138	106.3 ± 4	7.3 ± 0.5	117 ± 3	3.37
4	TDK1-*Sub1*	110	148	100.8 ± 1	6.3 ± 1	122 ± 3	5.33
5	BR11-*Sub1*	95	145	156.6 ± 6	5.8 ± 1	121 ± 2	4.44
6	PSB Rc68	105	129	119.6 ± 2	6.2 ± 1	129 ± 4	5.92
7	IR49830-7	110	142	119.4 ± 1	5.3 ± 0.5	129 ± 1	3.22
8	INBARA3	130	158	106.4 ± 5	6.5 ± 0.5	133 ± 2	4.42
9	Khang dan18 (control)	110	135	96.5 ± 2	7.5 ± 1	111 ± 5	6.07
10	Bacthom 7 (control)	110	145	107.8 ± 1	7.4 ± 0.5	113 ± 3	5.31

Table 5. Performance of major agronomic traits of of the imported rice varieties in Thai Binh field trials in 2010-2011.

Sr.	Cultivar name/ Breeding line	Days to heading (d)		Plant height (cm)	Panicle /plant	Seed/ spikelet (seeds)	Grain yield (ton/ha)
		Summer crop	Spring crop				
1	Swarna-*Sub1*	110	148	95.7 ± 5	6.4 ± 0.5	108 ± 4	3.77
2	IR64-*Sub1*	106	124	97.9 ± 1	6.2 ± 0.5	99 ± 8	4.02
3	Samba Mah –*Sub1*	116	125	109.7 ± 0.5	6.5 ± 1	119 ± 3	3.23
4	TDK1-*Sub1*	117	155	100.3 ± 2	6.4 ± 1	127 ± 4	5.33
5	BR11-*Sub1*	97	139	160.0 ± 4	5.8 ± 1	123 ± 5	4.44
6	PSB Rc68	107	137	119.4 ± 3	6.4 ± 0.5	130 ± 3	6.06
7	IR49830-7	111	140	119.8 ± 1	5.0 ± 1	129 ± 8	3.47
8	INBARA3	130	154	105.4 ± 5	6.5 ± 1	130 ± 7	4.43
9	Khangdan 18 (control)	110	130	97.4 ± 3	8.0 ± 0.5	110 ± 11	6.42
10	Bacthom 7 (control)	110	140	107.9 ± 1	8.1 ± 0.5	108 ± 4	5.46

Table 7. Performance of major agronomic traits of the imported rice varieties in Bac Lieu field trials in 2010.

Sr.	Cultivar name/ Breeding line	Days to heading(d)		Plant height (cm)	Panicle /plant	Seed/ spikelet (seeds)	Grain yield (ton/ha)
		Summer-Autumn	Spring crop				
1	IR64-*Sub1*	96	110	85.0 ± 3	6.2 ± 1	58 ± 14	4.47
2	Swarna-*Sub1*	118	140	84.6 ± 1	8.6 ± 0.5	114 ± 37	6.24
3	BR11-*Sub1*	91	115	86.0 ± 2	9.9 ± 0.5	88 ± 14	6.35
4	PSB Rc68	107	115	102.2 ± 4	5.2 ± 1	102 ± 24	4.27
5	OM6976 (control)	105	110	88.1 ± 2	9.3 ± 0.5	124 ± 1	7.06

Table 8. Grain yield of the rice varieties in field trials during 4 consecutive season crops in the different areas.

Sr.	Cultivar name/ Breeding line	Summer crop			Spring crop		
		Grain yield (ton/ha)		(b_i)	Grain yield (ton/ha)		(b_i)
1	Swarna-*Sub1*	3.699	e	0.159	3.934	c	4.000
2	IR64-*Sub1*	4.217	d	1.501	4.550	c	0.021
3	Samba Mah –*Sub1*	3.342	e	0.865	3.393	d	8.277
4	TDK1-*Sub1*	5.456	b	0.965	5.212	b	-2.503
5	BR11-*Sub1*	4.756	c	2.574	4.445	c	-0.077
6	PSBRc68	5.829	a	0.354	6.136	a	0.417
7	IR49830-7	3.670	e	1.441	3.305	d	-0.061
8	INBARA3	4.474	cd	0.942	4.420	c	0.688
9	Khangdan 18 (control)	6.116	a	0.618	6.382	a	-0.960
10	Bacthom 7 (control)	5.251	b	0.582	5.503	b	0.187
	F-test	Ns			Ns		

ns: means with the same letter in a column are not significantly difference at P<0.05

Based on the results of greenhouse and field screenings of the imported rice cultivars performed during 4 consecutive season crops, the PSbRc68 variety demonstrated to well grow and develop with high and stable yield in the different cultivation areas. The yield of IR64-*Sub1* was slighly lower than the local check variety but exhibited the highest submergence ability to compare with the others. It is suggested that this variety may be suitable for field condition of Cuu long delta. Noteworthily that both above rice varieties are not only used as good materials for submergence tolerance breeding programes but also may be developed as the varieties to deal with submergence stress in Vietnamsese deltas areas. To compare with the Khangdan 18 (as the control variety), the average of yield from the field trials during 2010 to 2011 were ranged from 3.3 to 6.1 ton/ha in the summer crop and 3.3 to 6.4 ton/ha in the spring season crop. The obtained results showed the yield of the imported rice were higher than the Bacthom 7. Among the tested cultivars, the PSBRc68 cultivar revealed a good regression values with b_i index at both growing season crops (b_i = 0,417 in spring season crop; b_i = 0,354 in summer season crop). The other cultivars showed high bi regression values and were considered for their adapability at some specific environmental conditions. On the other hand, the evaluated results on the levels of submergence ability and field trials via 4 consecutive season crops (2010 to 2011) exhibited that the PSbRc68 showed stable and high yield with medium submergence ability, whereas the IR64-*Sub1* showed good yield and stable genetic background with the highest submergence tolerance ability. It disclosed that QTL/*Sub1* of IR64-*Sub1* was activated and worked well in the completed submergence condition.

Application of MABC in Rice Breeding of Bacthom 7-Sub1

Foreground and Recombinant Selections: In this study, 11 out of 24 SSR markers showed polymorphism on the chromosome 9 between Bacthom 7 and IR64-*Sub1* varieties. At the initial stages of the experiment, for selection of the *Sub1* locus (foreground), the reported rice microsatellite markers namely SC3 and ART5 which were found to be linked to *Sub1* (Neeraja *et al.,* 2007) and fine mapping of the *Sub1* locus and sequence information (Xu *et al.,* 2006). Only 5 SSR polymorphic markers exhibited linkage with the target QTL/ *Sub1* were identified including RM23662, RM5688, ART5, SC3, and RM23877.

Background Selection: Microsatellite markers showed unlinked to *Sub1* covering in all 12 chromosomes including the *Sub1* carrier chromosome 9 that were polymorphism between the parents, were used for background selection to recover the recipient genome (Table 2). Based on the polymorphism information, initially evenly spaced microsatellite markers were selected per chromosome. To select the best plants from BC_1F_1, BC_2F_1 and BC_3F_1 generations, the total 53 polymorphic SSR distributed on 12 chromosomes were used to screen to select the individual plants with the highest recurrent parent genotype. The microsatellite markers that revealed fixed (homozygous) alleles at non-target loci at one generation were not screened at the next BC generation.

BC_1F_1: Foreground/recombinant selections: 2 SSR

markers were used for foreground and recombinant selections. A total of 41/53 SSR markers were used for genotyping 18 BC_1F_1 plants after foreground and recombinant selections. The individuals with the highest proportion (70-80%) of the recurrent parent genotype occupying 50%, while the proportion of 80% was only 6.2% were observed. Similarly, the individuals with the proportion of (60-65%), (65-70%), (75-80%) of the recurrent parent genotype were 12.5%, 25% and 18.7%, respectively. By using Graphical Geneotypes 2 (GGT2) software, plant No.2 that had the highest recipient alleles up to 80.7%, were identified for the next backcrossing in the next generation of BC_2F_1.

BC_2F_1: Foreground and recombinant selections: BC_2F_1 lines were characterized with 2 markers for foreground selection (SC3, RM23877 and 2 newly-designed flanking markers recombinant selection was RM23662 and RM5688). A total of 259 individual plants carrying QTL/gene *Sub1* (BC2F1) were identified by using ART5 and RM23877 markers. The result has shown that using ART5 and RM23877 markers identified total of 42 individual plants carrying QTL/ *Sub1* and further used for background selection.

For background selection: total 46/53 SSR markers which evenly distributed in the 12 chromosomes were used to screen in BC_2F_1 generation of Bacthom 7/IR64-*Sub1* to select individual plants with the highest recurrent parent genotype. The results showed the highest proportion of the recurrent parent genotype ranging 75-80% (19 plants) and 80-85% (12 plants), equivalent to 45.2% and 28.5%, respectively. The individuals with the proportion of 85-90% (3 plants) of the recurrent parent genotype account 7.1%, only. By using Graphical Geneotypes 2 (GGT2) software, 2 plants No.14 and No.19 had the highest recipient alleles up to 86.4% and 89.8%, respectively but only plant No.19 was used to develop for the next generation.

BC_3F_1: The selected plant No.19 in BC_2F_1 generation with 89.8% genetic background of recipient plant was further developed BC_3F_1 generation. Total of 133 plants were screened by the same previous procedures with the ART5 và RM23877 markers to identify the individual plants carrying target QTL/gene *Sub1*. By applying the marker/primer ART5, total of 33 individual plants carrying QTL/gene *Sub1* were identified and used to develop for the next generation, consisting of plant No. 1, 3, 5, 7, 8, 9, 13, 16, 17, 18, 19, 22, 23, 30, 31, 38, 39, 40, 41, 111, 113, 114, 115, 116, 121, 122, 123, 125, 127, 128, 130, 131, 132 (Figure 2). By using the primer RM23877, total of 72 individual plants carrying QTL/*Sub1* were identified and used for the next generation, including plant No. 9, 13, 14, 15, 36, 38, 39, 40, 43, 44, 46, 47, 48, 49, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 76, 77, 78, 79, 82, 84, 85, 86, 87, 88, 89, 90, 93, 94, 102, 107, 108, 109, 111, 112, 113, 114, 115, 116, 117, 118, 120, 121, 122, 123, 124, 126, 127, 131, 132, 133 (Figure 3). By the same aforementioned procedure in combination of markers RM23877 and ATR5 for screening heterozygous plants, total of 16 individual plants carrying QTL/gene *Sub1* were identified and used to develop for the next generation, consisting of plant No. 9, 13, 38, 39, 40, 111, 113, 114, 115, 116, 121, 122, 123, 127, 131, and 132.

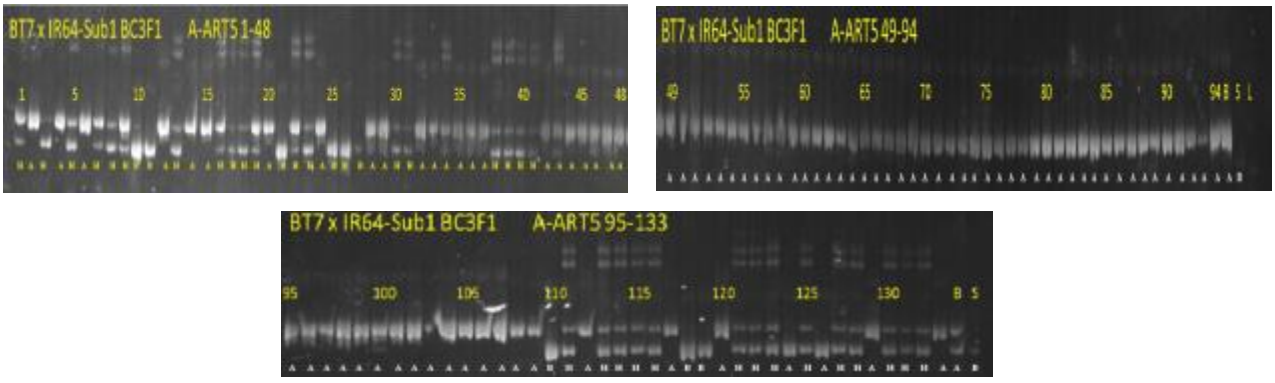


Figure 3. Screening individuals on crossed BC3F1(Bacthom 7/IR64-*Sub1*) using ART5. 1-133: BC3F1 in dividuals, A: Bacthom 7; B: IR64-Sub1; H: heterozygous.

In order to select the genetic background of Bacthom 7 from 16 individual plants in BC_3F_1 generation, 49/53 SSR markers polymorphic distributed in the 12 chromosomes were used. The result displayed that 2 individual plants have retained the genetic background (80 to 85%) of Bacthom 7, 5 plants were arranged 85 to

90%, 6 plants were 90 to 95%, and 3 plants had genetic background from 95 to 100% (Figure 4), especially individual plant No.116 had the highest genetic background 98,6% (Figure 5).

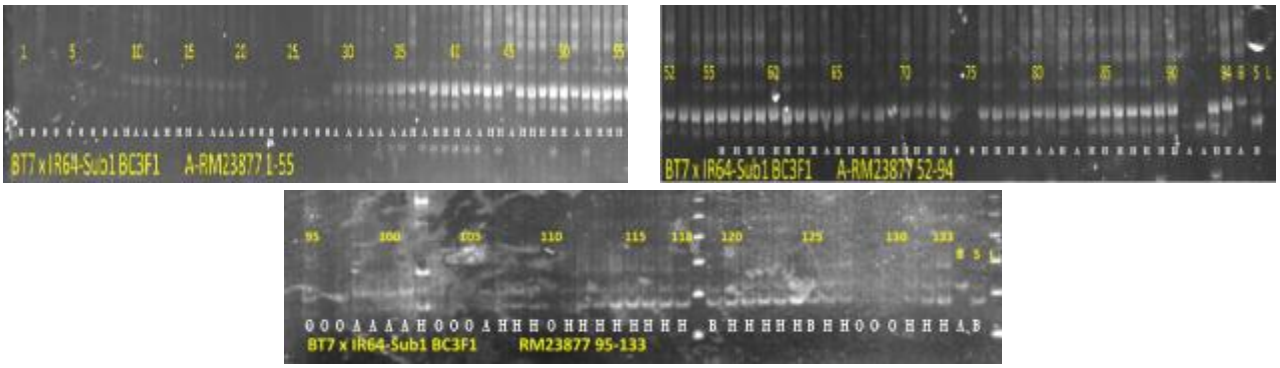


Figure 4. Screening individuals on crossed BC3F1(Bacthom 7/IR64-*Sub1*) using RM23877.
1-133: BC3F1 in dividuals, A: Bacthom 7; B: IR64-Sub1; H: heterozygous

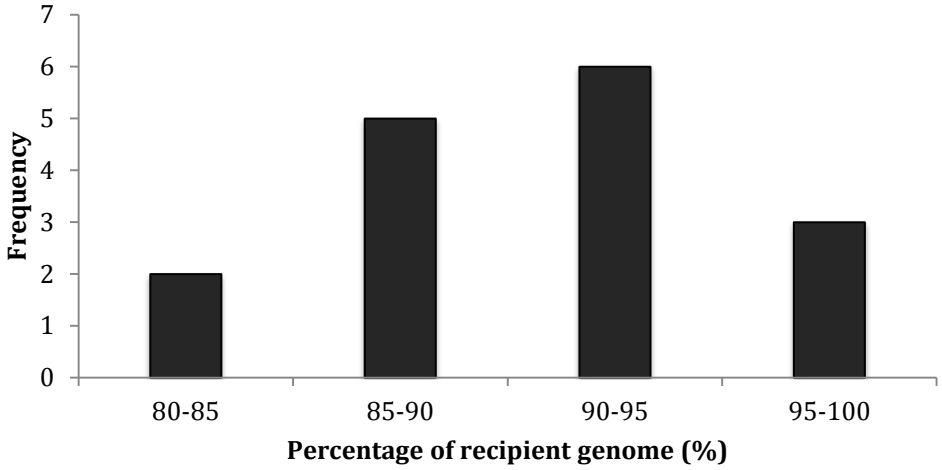


Figure 5. Percentage of recipience genome (%) in the BC3F1 population derived from the cross between Bac thom 7 and IR64-*Sub1*
Vertical axis: individual plants ; Horizontal axis: Percentage of recipience genome (%)

DISCUSSION

Submergence stress has frequently encountered in rice crop, is a widespread curb rice production in Southeast Asia where is mainly irrigated and high-rainfall environment, causing regularly adverse effects over 20 million ha of rice in the tropics (Mackill *et al.,* 2012). Climate change is causing adverse effects on rice production, which is the most important crop in Vietnam, and its production is mostly confined to the most vulnerable coastal deltas (Linh *et al.,* 2012; Khanh *et al.,* 2013). In the fact that any decline in rice production in two major coastal deltas of Vietnam will have considerable negative impact on local food security as well as on rice availability for international trade because Vietnam is now being the biggest rice exporter in the world. Hence, it is imperative to develop submergence tolerance rice variety with high yield potential and grain quality using modern tools of biotechnology. The MABC strategy is an effective means of using QTLs with large effects in rice breeding program. SSR markers were used in this study because of its predominant use in mapping and introgressing agronomically important QTLs into the elite rice variety by MABC method. One of most important pre-requisites before launching MABC is to precisely select and evaluate the parental plants (donor and recipient plants). Therefore, total 8 imported rice varieties (carrying QTL/*Sub1*) included a sensitive check IR24 and 3 Vietnamese elite cultivars were assessed and monitored the ability of submergence tolerance in the controlled conditions. Simultaneously, the agronomic traits of those rice cultivars were evaluated their adaptability in the 4 different field trials.

There is no significant difference between one variety which was grown at the same season crop of the different field trials (Table 8). It implies that the imported rice cultivars exhibited stable inheritance in the major agronomic traits. The b_i index was used in this study to evaluate the yield stability, and agronomical traits of the imported rice cultivars at the different growing season crops and different field trials. If the absolute value of the b_i index was $b_i \sim 1$ in the experiments, exhibited that the rice cultivars were stable yield. Based on the obtained results, IR64-*Sub1* has shown the highest submergence tolerance ability and selected as the donor plant. Similarly, among the imported rice cultivars, 2 rice varieties PSbRc68 and IR64-*Sub1* exhibits stable and potential yield during 4 consecutive growing season crops in both areas of Red River and Cuu Long deltas.

In this study, we selected IR64-*Sub1* as the donor plants because it shows higher submergence tolerance ability and stable yield than other imported rice cultivars. The results on evaluation of submergence tolerance ability and field screening were re-confirmed to ensure the adaptation of the final success of MABC breeding strategy. Moreover, PSbRc68 variety showed a high and stable yield during 4 consecutive season crops which are equally and higher than the controlled varieties Khangdan 18 and Bacthom 7 in the same experimental conditions. It is suggested to further develop this variety as a good potential submergence tolerance material in Vietnam. This is the first report to re-confirm submergence tolerance ability and screen the growth adaptability of the imported QTL/*Sub1* rice varieties at the region where would transfer QTL/*Sub1* into the local elite rice cultivar. Along with selection of the donor plant, Bacthom 7 was selected and used as the recipient material because Bacthom 7 is an elite Vietnamese rice cultivar that was first introduced in Vietnam in 1992 and has widely grown in large areas due to its stable yield, aromatic and good quality rice, and always gives high cost for milled rice.

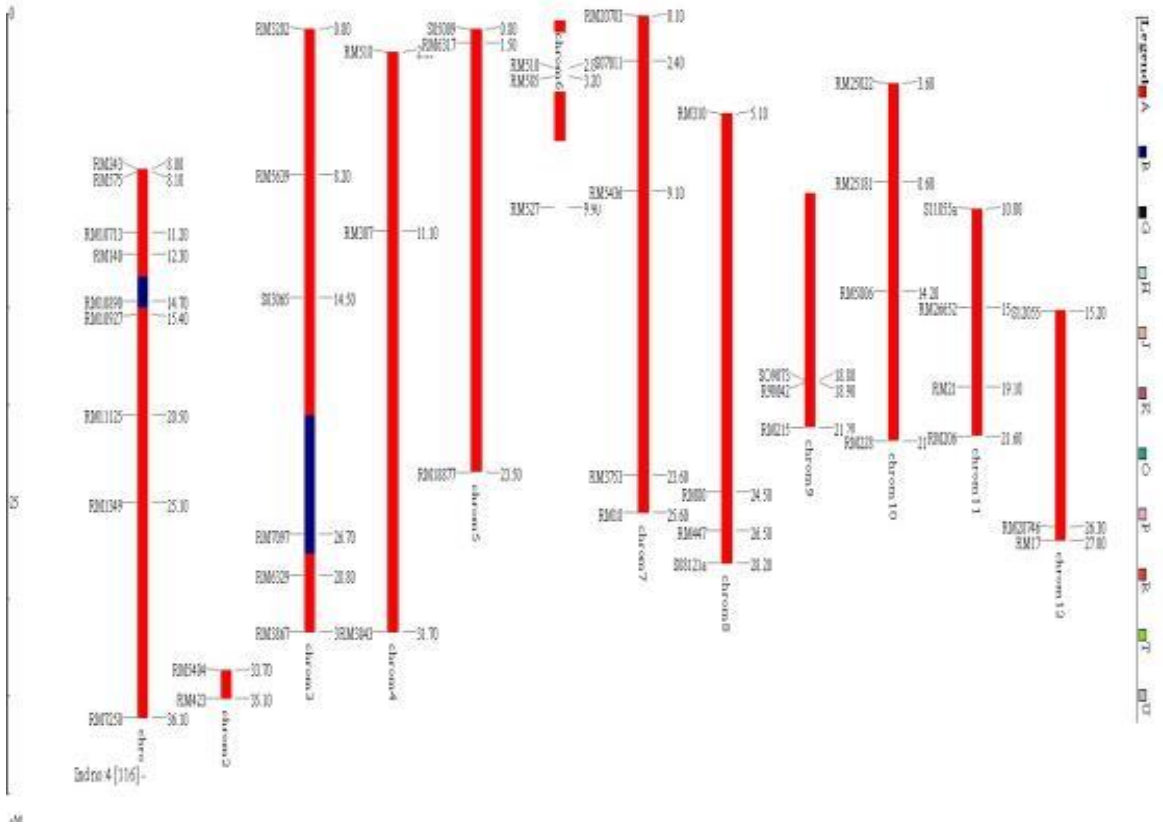


Figure 6. Graphical genotypes of plant number 116.

Graphical representation of genertic mapping base on the software Graphical Geneotyper (GGT 2.0). Red portions of the bars are IR64-Sub1 genetics and blue regions are Bacthom 7 genetics. Polymophic markers between Bacthom 7 and IR64-Sub1 are labeled on the left and position on the right of the chromosomes.

A major QTL/*Sub1* explaining about 70% of phenotypic variation in submergence tolerance has been fine mapped on the chromosome 9 in the strong submergence tolerant cultivar FR12A (Xu and Mackill 1996; Xu *et al.,* 2000). Moreover, *Sub1* locus was found to consist of 3 ethylene response-like transcription factors designated *Sub1*A, *Sub1*B and *Sub1*C (Xu *et al.,* 2006). Exploiting molecular breeding has just initiated in some recent years in Vietnam. Lang *et al. (*2011) and Cuc *et al. (*2012) reported to successfully applied MABC to improve submergence tolerance of OM1490 and AS996 rice varieties which are widely cultivated in the areas of Cuu Long delta. This is the first report to introgress QTL/*Sub1* into Bacthom 7, an elite high quality, largely grown in the Red River Delta in the north via MABC program. Initially, the *Sub1* locus was monitored by markers which displayed to be closely linked with the gene (Neeraja *et al.,* 2007). Applying the 5 SSR markers RM23662, RM5688, ART5, SC3, and RM23877 linked to *Sub1* and flanking RM23662 and RM23877 markers as the previous introduced by Collard and Mackill (2008), ensured efficient foreground and recombinant selections. In the backcross generations, the foreground and recombinant selections have done similar with the aforementioned steps. We have used total 53 polymorphic markers distributed on the 12 chromosome for background selection in order to select the best individual plants obtained the highest genetic background from the BC generations. The percentage of retaining the recurrent parent genotype has directly proportional to the number of BC with the highest recurrent genome recovery up to 98.6% of the individual plant No116 in BC_3F_1.

CONCLUSIONS

IR64-*Sub1* variety was selected as the donor plant because of its widely adaptable in the natural conditions of field test in some delta areas of Vietnam, with stable yield (b_i<1). Especially, IR64-*Sub1* was the highest submergence tolerance ability among the other donors test. We have successfully improved a submergence tolerance of Bacthom 7 cultivar via applying marker assisted backcross, which controlled by a major *Sub1* QTL. The recovery of the current parent genome and selection by MABC could reduce time and field work. It may be useful to grow the improved lines in the submergence delta areas of Vietnam.

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