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MARKER-ASSISTED BACKCROSSING (MAB) FOR RICE SUBMEGENCE TOLERANCE IN MEKONG DELTA

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ABSTRACT

Submergence is a recurring problem in rainfed lowland rice of Mekong Delta, Vietnam. Developing rice cultivars with submergence tolerance and acceptable traits to farmers is a feasible approach to address this problem. The study objective aims at developing new submergence tolerance varieties from OM1490/IR64-Sub1. Marker-assisted backcrossing of the cross was implemented and their performance was evaluated to determine the effect of Sub1 in different genetic backgrounds. All lines from OM1490/IR64-Sub1 introgression had a significantly higher survival rate than the original parents. The band corresponds to an allele from susceptible parent OM1490 and tolerant one IR64-Sub1 as 240 bp and 230 bp bands, respectively, at the locus RM23805. New lines from OM 1490/IR 64 -Sub1 provided a substantial enhancement in the level of tolerance of all the lines varieties. OM 1490/ IR64-Sub1 provided a substantial enhancement in the level of tolerance of all the lines. Only 10 lines (namely 10, 11, 12, 13, 14, 15, 16, 17, 18, and 19) carried 90-99% survival under field submergence condition. Marker RM23805 showed a positive signal in breeding lines.

Key words. abiotic stress, marker-assisted backcrossing, hybrid, IR64-Sub1, *Oryza sativa*, recombinant, submergence tolerance.

INTRODUCTION

The development of rice varieties with high yield potential, stability of performance through building in resistance to diseases and insects, tolerance to adverse environments, and satisfactory grain quality are the major goals of most breeding programs in Vietnam. Flooding of croplands is a frequent natural disaster in many regions of the Mekong delta. The flooding of root systems and partial to complete submergence of aerial organs can dramatically reduce crop productivity. Plant submergence attributable to complete flooding restricts the diffusion of oxygen and carbon dioxide by 104-fold, which has a dramatic impact on biochemical activities, such as aerobic respiration and photosynthesis (Armstrong and Drew 2002). Development of submergence tolerance genotypes is generally considered as the most effective entry point for improving productivity under submergence affected by typhoon and flash flood. It is also the cheapest option for farmers. In Mekong delta, some varieties as OM4900, IR64-Sub 1, Swarna-Sub 1, Br11-Sub 1, IR82355-5-2-3, IR84194-9 and IR66876-11NDR-1-1-1-1 were developed that can yield 4-5 ton ha^{-1} under water depth of 0.8-1m during 20- 25 days (Lang et al. 2010). The cloning of the major QTL Sub1 has provided an excellent opportunity not only to gain a better understanding of the molecular mechanisms and unravel the pathways underlying submergence tolerance, but also to design gene-based or tightly linked markers for more precise genotyping. Submergence tolerance is controlled by a single major quantitative trait locus (QTL) on chromosome 9, along with a number of minor QTLs (Xu and Mackill 1996; Nandi et al. 1997; Toojinda et al. 2003). All these studies have used the traditional genotype FR13A, which is one of the most submergence-tolerant donor varieties. The major QTL, named *Sub1*, with a LOD score of 36 and an R^2 value of 69% (Xu and Mackill 1996), provides tolerance to complete submergence for up to 2 weeks. The fine-mapping of *Sub1* employed 2950

F_2 segregating individuals. More recently, this gene has been successfully introgressed through marker-assisted backcrossing (MAB) into a popular high-yielding variety from Swarna genotype (India), within a 2-year time frame (Neeraja et al. 2007). Although the region had a low recombination rate, *Sub1* was delineated to a genomic region of approx. 0.06 cM (Xu et al. 2000). Previous studies have reported the development of submergence tolerant varieties by introducing the *Sub1* locus using marker-aided selection (Siangliw et al. 2003; Toojinda et al. 2005). However, these efforts did not take advantage of the benefits of marker-aided selection in precisely and rapidly transferring with the gene was verified. Using BC_2F_2 segregating data for submergence SSR analysis, it showed that the gene is linked to a single copy DNA clone, RM23805, on chromosome 9 at a distance of 0.06 cM (Xu et al. 2000). Thereby, providing an opportunity to initiate marker-aided selection should be addressed. In the present article, we report the development of a PCR based DNA marker based on the **RM23805** clone. These results prove the usefulness of marker-assisted backcross selection, as a complementary tool for conventional breeding.

MATERIALS AND METHODS

Plant materials

Two indica BC_2F_2 populations were developed from OM1490/IR64-Sub1 and OMCS2000/IR64-Sub1. IR64-Sub1 has been selected by breeders as donor for submergence. OM 1490 and OMCS 2000 are improved high yielding varieties developed by CLRRI with short duration as 90-95 days. One hundred twenty five BC_2F_2 seeds were produced in OM1490/IR64-Sub1. About 1,000 BC_2F_2 seeds in OMCS2000/IR64-Sub1 were planted in the field. One panicle per plant was harvested and two seeds per panicle were selected for BC_2F_3 generation.

Assessment of submergence.

F_1 heterozygous plants for *Sub1* were derived from crosses OM1490/IR64-Sub1 and OMCS2000/ IR64-Sub1. About 100 F_2 plants were used for submergence screening including the parents and some checks. To determine the level of submergence tolerance of OM1490/IR64-Sub1 and OMCS 2000/IR64-Sub1 recombinants and F_2 hybrids, a randomized complete block design (RCBD) was used in two independent trials for both experiments, with two or three replications used per trial. About 30 plants were used in each replication. Submergence tests and expression studies for both recombinants and F_2 progenies were conducted as described by Xu et al. (2006). BC_2F_2 lines and parents were soaked until germination. They were sown into plastic trays. Ten seeds from each BC_2F_2 and the parents were completely submerged at 14–25 days old seedlings with susceptible check IR42. When the susceptible check exhibited 50% damage, usually after about 14 days of complete submergence, trays were de-submerged and the survival of plants and recovers was scored after 10–21 days.

Submergence evaluation under field condition, 2010 wet season at CLRRI

The experimental design was a randomized complete block design with five replications. Transplanting at each plot (2 x 10 m size), was done with 15x15 cm spacing. Plot sites were located outside the commercial rice production areas of the respective states. The following variables were measured at the plant height (measured from soil line to tip of flag leaf), date of first and 50% tillering, and last heading (heading defined as date of panicle emergence from the boot). Five panicles per plant and five plants per plot were harvested at physiological maturity. After collection, panicles were carefully transported to a laboratory and rapped ten times against a plastic bucket. The percentage of seeds remaining on the panicle was used to give a seed. Total seed weight, 1,000-grain weight, and total seed number were also determined. Panicles were stripped by hand into a bucket, all seed returned to the sample envelope and allowed to dry for 3 days at 50°C. The seed were then passed through a small seed lot thresher to separate the blank florets from the seed. Both empty florets and total seed weights were obtained. A sample of 1000 seeds was weighed and the total number of seeds was calculated.

DNA extraction

In most cases, 14-d-old seedlings were submerged

and de-submerged when the susceptible check showed a substantial level of damage (50 %). DNA was extracted from young leaf tissues following a standard protocol (Lang 2002). DNA suitable for PCR analysis was prepared using a simplified miniscale procedure. A piece of young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was ground using a polished glass rod in a well of a Spot Test Plate (Thomas Scientific) after adding 400 µl of extraction buffer (50 mM Tris-HCl pH 8.0, 25mM EDTA, 300mM NaCl and 1% SDS). Grinding was done until the buffer turned green, which is an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of the extraction buffer was added and mixed into the well by pipetting. Amount 400 µl of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized using 400 µl of chloroform. The aqueous supernatant was transferred to a new 1.5 ml tube and DNA precipitated using absolute ethanol. DNA was air-dried and resuspended in 50 µl of TE buffer (10mM Tris-HCl pH 8.0; 1mM EDTA pH 8.0). An aliquot of 1 µl is sufficient for PCR analysis. The remaining DNA was stored in -20^{o}C for any later use.

Microsatellite assay

The used primer is RM23805. PCR amplification was performed in 10mM Tris-HCL(pH 8), 50 mM KCl, 1.5mM $MgCl_2$.1 unit of TAKARA *Taq* 4 nmol dNTP, 10 pmol primer and 50 ng genomic DNA . The PCR reactions were denatured at 95^{o}C for 5 min, followed by 35 cycles of 94^{o}C for 60 seconds, 55^{o}C for 30 seconds and 72^{o}C for 60 Seconds. The final extension was at 72^{o}C for 5 min. After PCR, 13ul of loading buffer (98% formamide, 10 mm EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) were added. Polymorphism in PCR products were detected by ethidium bromide staining after electrophoresis on 5% agarose gels

Microsatellite marker scoring and linkage analysis

The marker was scored for presence or absence of the corresponding bands between two extremes: pool segregant with good submegence and non submegence type along with the parents.

RESULTS

Development of population

OM1490, OMCS2000 used as susceptible parents in cross, IR64-Sub1 lines used as a donor. The F_1 plants were grown and selfed to produce the BC_2F_2 populations for marker-assisted backcross selection (MAB) .

Phenotypic variation of F_2 from OM1490 / IR64-Sub1 and OMCS2000/IR64-Sub1

F_2 progenies derived from the crosses of OM1490/IR64-Sub1 were significantly less tolerant as compared to the tolerant parent IR64-Sub1. In addition, OMCS2000/IR64-Sub1 in F_2 plants was lower tolerated as compared to OM1490/IR64-Sub1 plants. It was also noted that OMCS2000/IR64-Sub 1 breeding lines with a heterozygous *Sub1* locus were less tolerant to submergence stress. The tolerant version of OMCS2000/IR64Sub1 was developed following a similar approach with OM1490/IR64Sub1. Among the 40 F_2 and 125 BC_2F_2 plants that were genotyped, lines # 1-15 was selected (Table 1). This preliminary study suggested that the tolerant allele of *Sub1* should be in both parents in order to maintain the high level of tolerance in hybrids. However, the moderate tolerance of heterozygous hybrids may be beneficial under some circumstances where a combination of moderate submergence tolerance and moderate elongation ability may be desirable as is the case when stagnant longer-term partial flooding is anticipated before or after submergence.

Table 1. Submergence tolerance screening of heterozygotes of two crosses including OM1490 (P1)/IR64-Sub1 (P2) and OMCS2000 (P1)/IR64-Sub1 (P2)

No.	OM1490/IR64 Sub1		OMCS2000/IR64 Sub1	
	Lines	Survival days (%)	Lines	Survival days (%)
1	P1	0.2	P1	0.5
2	P2	79.5	P2	79.5
3	F2-1	74.0	F2-1	62.2
4	F2-12	73.0	F2-7	41.2
5	F2-13	2.0	F2-8	44.2
6	F2-23	3.0	F2-15	85.1
7	F2-45	56.0	F2-18	36.2
8	F2-48	69.0	F2-34	35.7
9	F2-51	68.0	F2-35	32.5
10	F2-63	89.0	F2-40	41.2
11	F2-109	86.0	F2-41	33.2
12	F2-111	56.0	F2-49	31.1
13	F2-145	66.0	F2-62	40.3
14	F2-167	45.0	F2-102	2.6
15	F2-178	5.2	F2-167	0.5

Phenotypic variation of BC_2F_2 from OM1490 / IR64-Sub1

OM1490, IR64 Sub1 and IR42 (control), with 125 BC_2F_2 lines were used evaluated for survival score. This brings a good recombination for submergence reaction in the population. For survival percentage under flooded condition, large differences were noticed in the populations ranges from 0-99%. The evaluation results were exhibited in the table 2. Among the 450 BC_2F_2 lines of the OM1490 x IR64 Sub1; wide variation was observed for survival percentage under flooded condition. The frequency distribution of % survival in flooded reaction among the BC_2F_2 was continuous. This showed a good recombination for plant submegence in the population. Out of 125 BC_2F_2 progenies from the selected ones, plant # 125 was selected based on its possession of the *Sub1* locus and maximum recipient genome, with a similar size of *Sub1* introgression as the first version of OM1490 IR64 Sub 1.

Figure 1. Screening for submegence tolerance at seedling stage in rice from BC_2F_2 population in cross between OM1490 / IR64Sub1

Molecular marker polymorphism of the parents

Electrophoretic analysis of PCR products derived from OM1490 and IR64Sub1 with four primers were screened for DNA polymorphism between two parents. Two polymorphic markers as RM23805, RM8300 were recognized (Figure 2). It revealed that the DNA polymorphism between the parents.

Analysis of the BC_2F_2 population

The phenotype evaluation of submergence and genotype by RM23805 markers in the BC_2F_2 population were conducted. The two extremes of the population were identified for selective genotypes. DNA markers showed polymorphism in parental DNA identified 125 lines. These markers were used to select segregants in BC_2F_2 population. Marker **RM23805** exhibited polymorphism of BC_2F_2 lines of OM1490 / IR64-Sub 1 to distinguish submergence tolerant ones from its allelomorph conferring no submegence tolerance. The bands correspond to an allele from OM1490 (non-tolerant) and IR64 Sub1 (tolerant) were scored at 240 bp and 230 bp sizes, respectively.

In the early applications of MAB for developing submergence-tolerant varieties, the diagnostic marker was used as RM8300. However, in some cases, RM8300 did not distinguish tolerant from non-tolerant varieties in BC_2F_2 populations of OM1490/IR64-Sub1. IR42 was used as a check variety for susceptibility under complete submergence. The heterozygous plants were significantly less tolerant than the homozygous ones for the tolerant allele (Table 2). The results indicate that the *Sub1* locus tolerance is closely associated with the expression level and the dosage of the survival percentage under flooded condition.

Table 2. Estimated phenotype and genotype at locus RM23805 on chromosome 9

Parents and BC_2F_2 lines	Well position no.	Allele		Phenotype
		A 240 bp	B 230 bp	% Survival under flooded condition
OM1490	35		+	0.00
IR64-Sub1	36	+		98.80
OM1490/IR64-Sub1// OM1490	1		+	0.23
	2		+	0.56
	3		+	0.80
	4		+	8.60
	5		+	42.10
	6		+	14.50
	7		+	12.30
	8		+	10.20
	9		+	11.56
	10		+	12.30
	11		+	13.20
	12		+	14.50
	13		+	13.50
	14		+	14.20
	15	+	+	69.35
	16	+	+	43.27
	17	+	+	57.04
	18	+	+	59.08
	19	+	+	32.84
	20	+	+	36.57
	21	+	+	42.60

Parents and BC_2F_2 lines	Well position no.	Allele		Phenotype
		A 240 bp	B 230 bp	% Survival under flooded condition
OM1490/IR64-Sub1// OM1490	22	+	+	55.20
	23	+	+	55.60
	24	+	+	54.20
	25	+	+	32.10
	26	+	+	33.50
	27		+	21.20
	28		+	23.10
	29		+	22.30
	30		+	22.40
	31		+	22.50
	32		+	23.10
	33		+	22.50
	34		+	24.60
	37	+		56.90
	38	+		89.60
	39	+	+	24.30
	40	+	+	55.60
	41	+		77.40
	42	+		77.60
	43	+		77.60
	44		+	13.20
	45	+		56.90
	46	+		85.30
	47	+		85.60
	48	+		45.20
	49	+		41.30
	50	+		44.70
	51	+		44.20
	52	+		44.60
	53	+		44.90
	54	+		45.60
	55	+		48.90
	56		+	0.00
	57	+		14.50
	58	+		16.30
	59	+		85.60
	60	+		89.60
	61	+		88.20
	62	+		88.70
	63		+	0.00
	64	+		22.30
	65	+		28.90
	66	+		27.60
	67	+		24.60
	68	+		85.60
	69	+		87.40

Parents and BC_2F_2 lines	Well position no.	Allele		Phenotype
		A 240 bp	B 230 bp	% Survival under flooded condition
OM1490/IR64-Sub1// OM1490	70	+		82.30
	71	+		84.50
	72	+		84.50
	73	+		87.60
	74	+		52.30
	75		+	12.00
	76	+		18.90
	77	+		52.30
	78	+		55.40
	79	+		55.60
	80	+		55.70
	81	+		55.90
	82	+		86.30
	83	+		86.40
	84		+	0.00
	85		+	0.00
	86		+	0.15
	87		+	0.56
	88		+	1.23
	89		+	8.90
	90	+		42.30
	91		+	12.30
	92	+		94.20
	93	+		99.10
	94	+		99.30
	95	+		99.50
	96	+		95.20
	97		+	2.30
	98	+		45.60
	99	+		47.80
	100	+		77.90
	101	+		90.20
	102	+		99.50
	103	+		23.50
	104	+		35.60
	105	+		56.70
	106		+	11.23
	107		+	14.50
	108	+		56.90
	109	+		77.40
	110	+		77.80
	111	+		77.60
	112	+		77.40
	113	+		45.60
	114	+		48.90
	115	+		56.20

Parents and BC_2F_2 lines	Well position no.	Allele		Phenotype
		A 240 bp	B 230 bp	% Survival under flooded condition
OM1490/IR64-Sub1// OM1490	116	+		57.80
	117	+		55.40
	118		+	12.30
	119		+	0.50
	120		+	0.12
	121		+	1.36
	122		+	13.45
	123		+	1.78
	124		+	7.80
	125		+	4.90

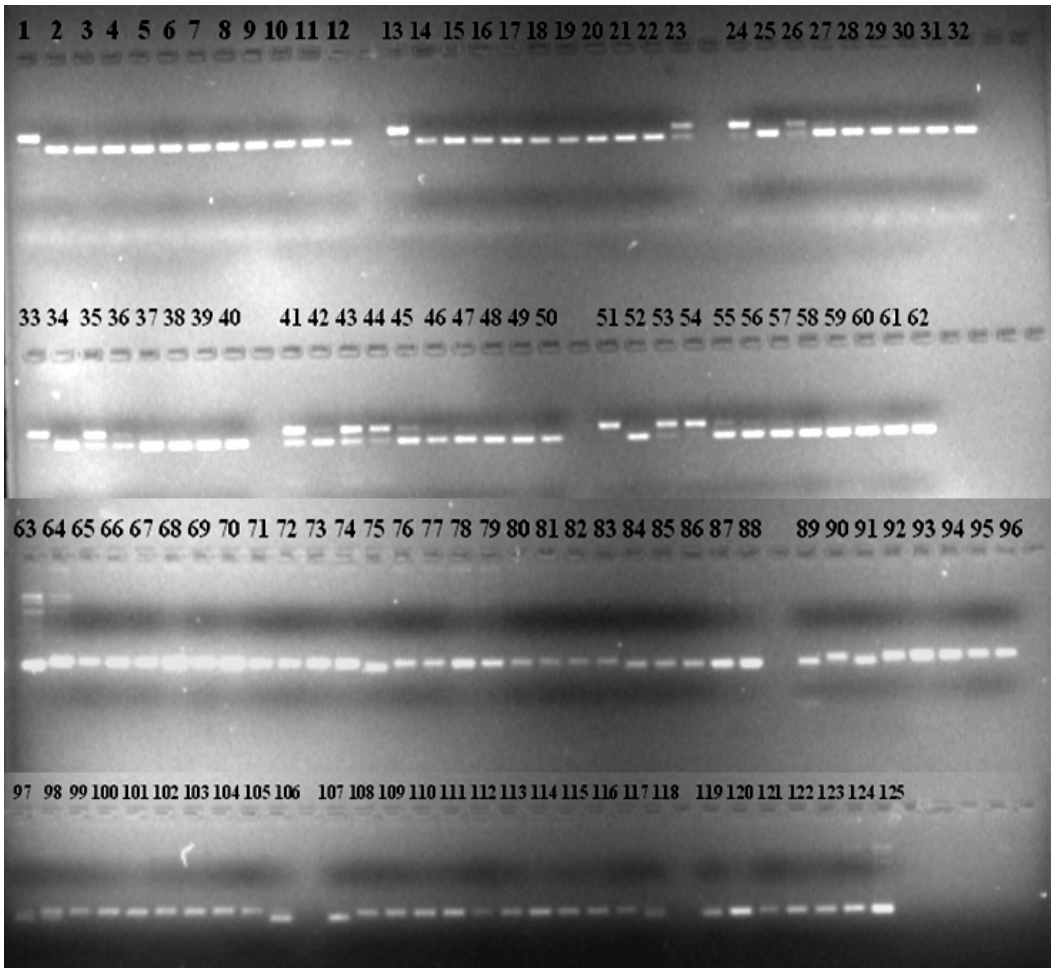

Figure 2. PCR products of BC_2F_2 population from OM1490 / IR64-Sub 1, at locus RM23805 on chromosome 9.

1 IR64-Sub1; 2 OM1490; 1 and 125 BC_2F_2 progenies

Breeding for submergence tolerance in rice

Under field condition of CLRRI in 2009 and 2010 wet seasons; complete submergence occurred at 20 days after transplanting. Evaluation of breeding lines was conducted with IR42 (S check). Agronomical traits were obtained as growth duration, plant height, panicles/hill, fertility. Early growth duration genotypes (90-110 days), semi dwarf (90-110 cm), medium number of panicles/hill (7-15 panicles), high number of grains/panicle (77-199 grains) were predominantly selected for further testing. Recovering ability after 20-25 day submergence under 0.8-1.0 m of water depth was observed (Table 3)

Table 3. Yield and yield components of rice genotypes tested in BC_2F_3 at CLRRI

No.	Designation	Panicles/ plants (no.)	Filled grains/ Panicle (no.)	Unfilled grains/Panicle (no.)	1,000-grain weight *(g)*	Yield (g/10 plants)	(%) survival under flooded condition
1	OM1490	18 c	198 d	12.0 l	26.7 cde	75 m	5.2 v
2	IR64-Sub1	16 de	148 n	14.2 j	26.8 cd	56 t	78.8 i
3	Line 1	17 cd	77 x	14.2 j	27.6 b	99 g	45.8 mn
4	Line 2	16 de	169 i	14.5 ij	26.0 g	95 i	45.6 n
5	Line 3	15 ef	210 c	16.8 f	26.8 cd	93 j	47.8 k
6	Line 4	18 c	230 a	16.9 f	27.0 c	94 i	42.3 s
7	Line 5	12 g	120 t	20.3 b	26.5 def	96 h	45.9 m
8	Line 6	13 fg	145 o	18.9 c	26.8 cd	95 i	46.8 l
9	Line 7	14 ef	187 f	17.5 e	26.9 c	99 g	44.8 p
10	Line 8	15 ef	176 h	16.8 f	26.4 ef	153 a	44.5 r
11	Line 9	12 g	189 e	18.9 c	26.8 cd	124 c	68.9 j
12	Line 10	10 h	163 j	18.4 d	26.8 cd	114 e	95.3 e
13	Line 11	12 g	152 m	17.5 e	26.5 def	121 d	92.4 g
14	Line 12	13 fg	148 n	16.8 f	26.7 cde	112 f	95.8 d
15	Line13	14 ef	199 d	20.3 b	26.5 def	49 u	97.5 c
16	Line 14	12 g	185 g	21.3 a	26.4 ef	86 k	99.8 a
17	Line 15	14 ef	175 h	20.1 b	26.5 def	112 f	99.5 a
18	Line 16	15 ef	126 r	12.3 kl	26.3 f	135 b	99.8 a
19	Line 17	15 ef	142 p	10.2 n	26.8 cd	99 g	91.2 h
20	Line 18	9 h	158 k	10.5 n	26.8 cd	86 k	99.3 b
21	Line 19	9 h	135 q	15.4 g	26.9 c	68 o	99.7 a
22	Line 20	7 i	147 n	14.2 j	28.7 a	67 o	45.2 o
23	Line 21	4 j	156 l	11.2 m	24.2 h	65 p	0.0 y
24	Line 22	13 fg	123 s	11 m	26.8 cd	64 q	0.0 y
25	Line 23	15 ef	145 o	14.3 ij	26.4 ef	62 s	0.0 y
26	Line 24	16 de	163 j	12.6 k	26.5 def	63 r	12.3 u
27	Line 25	18 c	221 b	14.7 hi	26.4 ef	69 n	2.3 x
28	Line 26	22 b	110 w	18.0 d	26.7 cde	68 o	2.8 w
29	Line 27	23 ab	112 v	17.0 f	26.8 cd	85 k	0.2 y
30	Line 28	24 a	114 u	15.0 gh	27 c	84 l	22.3 t
31	IR42 (check)	13 fg	112 v	10.5 n	26.8 cd	65 p	77.5 i

DISCUSSION

Submergence tolerance has been an important breeding objective for more than three decades (HilleRisLambers and Vergara 1982; Mackill 1986; Mohanty and Chaudhary 1986), especially in term of climate change in Mekong Delta. Breeders at IRRI made crosses between the tolerant donor FR13A and high-yielding varieties such as IR48 and IR36 in the mid- to late-1970s. Submergence tolerant lines with high yield potential were obtained in the early 1990s (Mackill et al. 1993). However, these tolerant prototypes were never widely adopted by farmers since they were inferior in grain quality or in other traits needed for local adaptation. On the other hand, mega varieties that possessed most traits desired by farmers but were submergence intolerant began to spread widely in both irrigated and rainfed lowland areas of south and south-east Asia (Mackill et al. 2006). To ensure the adoption of the final breeding product by the farmers, an approach of converting these mega varieties by MAB was undertaken. The objective was to convert these varieties to submergence-tolerant types while retaining the desirable traits of the original parents through a precise MAB approach (Collard and Mackill 2008). Molecular marker-assisted backcrossing selection (MAB) is an approach that has been developed to avoid the problems connected with conventional plant breeding changing the selection criteria from selection of phenotypes towards selection of genes, either directly or indirectly. Molecular markers are clearly not environmentally regulated. They are unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth. With the availability of an array of molecular markers and genetic maps, usefulness of a given molecular marker is dependent from its capability in revealing polymorphisms in the nucleotide sequence. Allowing microsetellite it should, therefore, be possible to exploit this information to trace the flow of genes or quantitative trait loci of interest in rice. It would make prediction about crossing and selection that will increase the efficiency of varietal improvement. In addition, microsatellite marker analysis can be automated. This feature is attractive for marker assisted selection program. This study reports the localization of RM23805 on chromosome 9, which can serve as a starting point for the positional cloning RM23805. For this purpose and for fine mapping, saturation of the genetic map by increasing the amount of markers at the chromosomal region of interest is in progress. Only one marker showed a positive signal in breeding lines (OM1490/IR64-Sub1). Thus, selection for progeny with the gene of interest is not always possible based on submergence in rice, and molecular marker linked to the gene of interest is required. Compared to the phenotype, MAS for IR64-Sub1 can improve the cost effectiveness and significantly speed up the introgression of submergence to rice.

CONCLUSION

OM1490/IR64-Sub1 provided a substantial enhancement in the level of tolerance of all the lines. With 30 lines, it is confirmed as the primary contributor to tolerance in the field; only 10 lines (number 10, 11, 12, 13, 14, 15, 16, 17, 18, 19) exhibited their recovery ability with survival percentage of 90-99% under field condition. Marker RM23805 showed a positive signal in breeding lines (OM1490/IR64-Sub1). Compared to the phenotype, the marker- assisted selection can improve the cost effectiveness and significantly speed up the introgression of submergence gene to rice .

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Chọn tạo giống lúa chịu ngập nhờ chỉ thị phân tử

Úng ngập là vấn đề lớn của thay dổi khí hậu tại ĐBSCL. Phát triển giống lúa chống chịu ngập hoàn toàn trong vòng 15-20 ngày, khả năng hồi phục nhanh là mục tiêu của nghiên cứu. Các dòng triển vọng trong quần thể phân ly của tổ hợp lai OM1490/IR64-Sub1 đã được phát hiện nhờ chỉ thị phân tử RM23850 liên kết chặt chẽ với gen *Sub-1* định vị trên nhiễm sắc thể số 9. Đa hình được ghi nhận với kích thước phân tử của băng giống nhiễm OM1490 và băng giống chống chịu IR64-Sub1 là 240 bp và 230 bp, theo thứ tự. Có 10 dòng (được đánh số 10, 11, 12, 13, 14, 15, 16, 17, 18, và 19) có tỷ lệ sống sót cao (90-99%) được ghi nhận.