

DEVELOPMENT OF RICE LINES (*Oryza sativa* L.) TOLERANT TO SUBMERGENCE VIA *Sub1* GENE INTRODUCTION INTO LANDRACES AND ELITE BREEDING LINES

Nguyen Thi Lang^{1*}, Nguyen Thanh Hoa¹, Pham Thi Thu Ha¹, Nguyen Van Hieu¹,
 Nguyen Ngoc Huong¹, Bui Chi Buu², Russell Reinke^{3,4}, Tran Bao Toan⁵,
 Abdelbagi M. Ismail⁴, Reiner Wassmann⁴

¹Cuu Long Delta Rice Research Institute (CLRRI), Thoi Lai, Can Tho, Viet Nam

²Institute of Agricultural Science for Southern Viet Nam (IAS), Viet Nam

³Temperate Rice Breeder – IRRI & NICS project, Suwon 441-857, Republic of Korea

⁴International Rice Research Institute, DAPO 7777, Metro Manila, Philippines

⁵Biotechnology PCR Company, Can Tho, Viet Nam

Email*: ntlang@hcm.vnn.vn

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ABSTRACT

Development of rice genotypes tolerant to complete submergence in the Mekong Delta was carried out for three continuous years by CLUES Project fund. Eighty five high-yielding cultivars and eighty four progenies from backcrossing population (BC₃F₃) of OM1490/IR64 *Sub1* were used to study the yield components and submergence tolerance. Phenotyping was implemented at three stages: seedling, tillering and heading. Different segregation ratio and phenotypical variation indicated the complex genetic background. All genotypes have been evaluated under both submergence and non-submergence treatments. Correlation coefficient between the survival rate (%) and tiller number /10 hills was positively significant of 0.8880**. Marker-assisted backcrossing (MAB) was exploited on chromosomes 9 to address ten molecular markers (SSRs). Of them, only three polymorphic SSRs really linked to the target QTLs. Marker-assisted selection helped identify elite lines, which would be breeding materials via three molecular markers on the target region viz. RM3269, RM5304 and RM1367 on chromosome 9. Three selected offspring lines from BC₃F₃ population of OM1490/IR64 *Sub1* as 26, 38 and 50 exhibited good adaptation to submergence.

Keywords: chromosome, molecular markers, QTL, submergence tolerance.

Phát triển dòng lúa (*Oryza sativa* L.) chống chịu ngập thông qua khai thác nguồn gen *Sub1* để chuyển vào giống lúa địa phương và dòng cải tiến

TÓM TẮT

Phát triển dòng lúa chống chịu ngập hoàn toàn ở Đồng Bằng Sông Cửu Long đã được thực hiện suốt 3 năm thông qua dự án CLUES. Tám mươi lăm giống lúa cao sản và 84 dòng con lai từ tổ hợp lai hồi giao (BC₃F₃) của cặp lai OM1490/IR64 *Sub1* đã được nghiên cứu về năng suất và tính chống chịu ngập. Đánh giá kiểu hình được thực hiện vào 3 giai đoạn sinh học của cây lúa: giai đoạn mạ, giai đoạn đẻ nhánh và giai đoạn lúa trổ. Kết quả từ sự phân ly khác nhau và biến thiên di truyền cho thấy tình trạng chống chịu ngập có nền tảng di truyền phức tạp. Tất cả các dòng lúa được đánh giá trong 2 điều kiện có ngập và không ngập. Hệ số tương quan giữa mật độ sống sót (%) và số chồi lúa tính trên 10 khóm lúa tương quan thuận rất có ý nghĩa $r = 0.8880^{**}$. Hồi giao nhờ chỉ thị phân tử (MAB) được khai thác thành công trên nhiễm sắc thể số 9 với 10 chỉ thị phân tử SSRs. Trong số đó, chỉ có ba chỉ thị cho kết quả đa hình rõ ràng liên kết với QTL mục tiêu. Thực hiện chọn giống nhờ chỉ thị phân tử giúp tìm ra các dòng lúa tối ưu, thông qua 3 chỉ thị phân tử viz. RM3269, RM5304 và RM1367 trên nhiễm sắc thể 9. Ba dòng triển vọng đã được chọn từ quần thể hồi giao BC₃F₃ của cặp lai OM1490/IR64 *Sub1* là dòng số 26, 38 và 50 chịu ngập tốt thông qua kết quả đánh giá kiểu gen và đánh giá kiểu hình.

Từ khóa: Chỉ thị phân tử, chống chịu ngập, nhiễm sắc thể, QTL.

1. INTRODUCTION

Most *Oryza sativa* cultivars die within a week of complete submergence - a major constraint to rice production in south and Southeast Asia. A few cultivars, such as FR13A, are highly tolerant and survive up to two weeks of complete submergence owing to a major quantitative trait locus designated Submergence 1 (*Sub1*) near the centromere of chromosome 9. The identification of a cluster of three genes at the *Sub1* locus, encoding putative ethylene response factors. Two of these genes, *Sub1B* and *Sub1C*, are invariably present in the *Sub1* region of all rice accessions analysed. In contrast, the presence of *Sub1A* is variable (Xu *et al.*, 2006).

If rice is submerged under water more than a few days, gradual loss of oxygen leads to withering and death due to the effect on respiration and photosynthesis. Tolerance to excess water includes tolerance to complete submergence and tolerance to gradually rising water levels that stagnate for one or more months. In the latter situation, deepwater rices are suitable where water levels go beyond 50 cm (Mackill 2007). Under these situations, rapid internode elongation is usually needed. A major QTL was mapped on chromosome 12 for early elongation ability in deepwater rice (Tang *et al.* 2005). Rice will lack necessary air and light to carry out the functions of life, thus the growth of rice plants will be limited and the plant will die if it is flooded in water for 4 days (Mackill 2006). The flooding could affect the growth of rice plants at any stage of growth, even for a long time or a short time. Chance of survival is very low when the rice plants were completely submerged in water. Faced with this reality, the long-term solution to help farmers growing rice in the flooded area is to develop submergence tolerant rice varieties with wide adaptability, high yield and stability (Ismail *et al.* 2008).

The application of molecular markers in breeding has allowed us to think of a new rice variety in shape and new biological structure of rice with resistance to pests and diseases, tolerance to acid sulfate soil and flooding with

high productivity (Bui Chi Buu and Nguyen Thi Lang, 2007). In particular, Mekong Delta area in the period 2030-2040 is likely to be flooded and the flood will spread over the area of the Bac Lieu and Ca Mau and the sea level will rise higher (Le Anh Tuan, 2009). Mapping on 12 chromosomes of a backcrossing population was conducted to address 300 molecular markers including 225 ones to express polymorphism that suggested implementing the experiment (Lang, 2013). The strategy of breeding crops, including rice plants, needs to identify a synthetic goal that a variety can tolerate to many adverse factors such as salinity and drought, in which submergence tolerance is also one of the objectives to be considered for timely responses to complex movements of climate change today (Tao, 2010). Starting from one of the above urgent problems, the study aims at evaluating and selecting the promising submergence-tolerant rice lines as an important step to develop new high-yielding rice genotypes adapted to climate change in the Mekong Delta.

2. MATERIALS AND METHODS

2.1. Plant Materials

The experiment consisted of 85 high-yielding rice varieties and 81 progenies in BC₃F₃ of OM1490/IR64 *Sub1* from Department of Genetics and Plant Breeding, CLRRRI. Two donors, Swarna *Sub1* and IR64 *Sub1* and two intolerant genotypes viz. IR42 and OM1490 were used as checks.

2.2. Evaluation of parents and BC₃F₃ progenies for tolerance to complete submergence

The non-submerged control trays were placed a top of the walls around the concrete tanks where the other trays are being submerged completely.

Seeds were first soaked then incubated in a small amount of water for 3 days at 30 °C to germinate in the dark. Seeds were then sown in trays containing soil fertilized with 6 g N, 3g P₂O₅, 3g K₂O per 6L soil and with 60 seedlings

of each cultivar per tray. Seven-day-old seedlings (days after seeding) were submerged in tap water for 14 days in a concrete tank, at the time the intolerant cultivar, Swarna *Sub1* showed visual symptoms of injury. The seedling survival was recorded at 14 days after submergence. Shoot length and root length were measured for non-submerged and submerged condition. Shoot and root elongation rate of tolerant and intolerant genotypes were also compared. The survived seedlings were further observed at flowering and harvesting, especially for important agronomic traits and grain yield.

2.3. DNA extraction and PCR analysis

Genomic DNA was prepared by using DNA extraction procedure with Mini Scale method modified by Lang (2002). The genomic DNA from both of the 84 BC₃F₄ lines and the parents were subjected to PCR amplification. PCR amplification was performed in 10 mM tris-HCl (pH = 8.3), 50m M MgCl₂, 1 unit DNA Taq Polymerase, 4 nmole of dNTP, 10 pmole of primer, with 30 ng of genomic DNA per 25 µl using a thermal cycler 9600 (Perkin-Elmer). The PCR reactions were denatured at 95°C for 4

min, followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minute. The final extension was at 72°C for 5 min. After PCR 13 µl of loading buffer (98% formamide, 10mm EDTA, 0.025% bromophenol blue, 0.025 % xylene cyanol) were added. Polymorphisms in the PCR products were detected by ethidium bromide staining after electrophoresis on 3 % agarose gel.

3. RESULTS

3.1. Survival rate of BC₃F₃ lines (OM1490/IR64 *Sub1*) under complete submergence

The survival rate (%) of BC₃F₃ lines of OM1490/IR64 *Sub1* under complete submergence in comparison with non-submergence (Fig. 1) are presented in Table 1. Survival rate differed significantly among genotypes. Of 85-treated rice genotypes, 23 genotypes had high survival rate (10-70%) after 14-day complete submergence treatment. The died plants showed drooped leaf sheath and minor tillers and the new tillers, the main stems and roots were partly rotten.



Figure 1. BC₃F₃ lines of OM1490 / IR64 *Sub1* before and 14 days after complete submergence

Table 1. The survival rate (%) of BC₃F₃ progenies in OM1490/IR64 *Sub1* before and 14 days after complete submergence

No.	Designation	Normal condition	Complete submergence
1	1	100a	40c
2	4	100a	30d
3	6	100a	20e
4	7	100a	10f
5	8	100a	20e
6	9	100a	40c
7	12	100a	10f
8	14	100a	10f
9	25	100a	20e
10	26	100a	70a
11	28	100a	30d
12	29	100a	10f
13	30	100a	20e
14	38	100a	50b
15	39	100a	10f
16	41	100a	20e
17	42	100a	20e
18	44	100a	20e
19	49	100a	40c
20	50	100a	70a
21	54	100a	10f
22	67	100a	20e
23	79	100a	10f
24	IR64 <i>Sub1</i>	100a	50b
25	OM1490	100a	30d
26	Swarna	100a	0g
CV (%)		-	7.35
F test		ns	**

Note: Values in the same column followed by the same letter are not significantly different at 5% probability level using DMRT; **: significantly different at 1% level of probability, ns: not significant.

3.2. Plant height of the BC₃F₃ lines of OM1490/IR64 *Sub1* under complete submergence

The plant height of BC₃F₃ lines of OM1490/IR64 *Sub1* was significantly inhibited under complete submergence (Table 2). The genotypes with greatest plant height after 14 days under fully flooded conditions included 1, 4, 6, 29, 38, and 41 with the plant height of 83.1 cm, 84.2 cm, 101.3 cm, 82.5 cm, 86.3 cm, and 87.5 cm, respectively.

The remaining lines exhibited their average height from 0 to 80.6 cm as compared to check (IR64 *Sub1*) of 68.8 cm

3.3. Tiller number of BC₃F₃ lines of OM1490 / IR64 *Sub1* under complete submergence

Tillering ability, indicative of recovering ability under submergence, was measured on 10 hills basis (Table 3) The linen numbered with 28, 29, 38, 67, and 79 exhibited high number of tillers/10 hills of 53, 51, 53, 50, and 54,

respectively. Lines exhibiting their recoverable ability and good tiller development after 14 days under complete submergence were 18, 19, 20, and 23 tillers/10 hills, as compared to check (IR64 *Sub1*) of 8 tillers/10 hills (table 3).

3.4. Root length (cm) of BC₃F₃ lines of OM1490/IR64 *Sub1* under complete submergence

Under flooded conditions, root elongation occurred in all genotypes of the experiment with great variation among genotypes (Table 4 and Fig. 2). However, root length exhibited higher values among the lines tolerant to submergence than intolerant ones a reported by Lang (2012). The lines numbered with 9, 38, 41 and 79 had highest root length of 18.0, 18.3, 18.3 and 18.2 cm, respectively, higher than IR64 *Sub1* and OM 1490 (17.7 cm).

Table 2. Plant height (cm) among BC₃F₃ lines of OM1490/IR64 *Sub1* 14 days after complete submergence

N ₀	Designation	Plant height (cm)	
		Normal condition	Complete submergence
1	1	54.8a	83.1bcde
2	4	51.3abcd	84.2bcd
3	6	44.8ghi	101.3a
4	7	51.1abcd	78c-h
5	8	49.7a-h	71.5fghi
6	9	47.5c-h	45kl
7	12	44.7ghi	75d-h
8	14	44.3hi	53k
9	25	46.2d-i	70.5ghi
10	26	47.8b-h	74efgh
11	28	53ab	80.6b-f
12	29	47d-h	82.5bcde
13	30	44.8ghi	74.5efgh
14	38	50.3a-f	86.3bc
15	39	47.8b-h	47.5kl
16	41	49.7a-h	87.5b
17	42	50.7a-e	78.8b-g
18	44	49b-h	48.8kl
19	49	47.3c-h	76.2d-h
20	50	45.5e-i	52.7k
21	54	52.7abc	61.5j
22	67	45.5e-i	40.5l
23	79	49.8a-g	42.0l
24	IR64 <i>Sub1</i>	41.7i	68.8hij
25	OM1490	45fghi	63ij
26	Swarna	36.3j	0m
CV (%)		5.8	7.4
F Test		**	**

Note: Values in the same column followed by the same letter are not significantly different at 5% probability level using DMRT; **: significantly different at 1% level of probability

Table 3. Tiller number/10 hills of the BC₃F₃ lines of OM1490/IR64 *Sub1* under normal and complete submergence

N _o	Designation	Tillers/10 hills	
		Normal condition	Complete submergence
1	1	37l	14d
2	4	42h	12e
3	6	40j	9gh
4	7	49e	4lmn
5	8	42h	7ij
6	9	49e	18.3c
7	12	44g	3n
8	14	45f	4lmn
9	25	41i	7ij
10	26	49e	19bc
11	28	53b	11ef
12	29	51c	5kl
13	30	49e	10fg
14	38	53b	20b
15	39	39k	4.3lm
16	41	39k	10fg
17	42	42h	6jk
18	44	44g	6jk
19	49	45f	12e
20	50	42h	23a
21	54	39k	3.7mn
22	67	50d	9gh
23	79	54a	5kl
24	IR64 <i>Sub1</i>	23m	8hi
25	OM1490	21n	5kl
26	Swarna	17o	0o
CV (%)		1.03	6.93
F Test		**	**

Note: Values in the same column followed by the same letter are not significantly different at 5% probability level using DMRT; **: significantly different at 1% level of probability

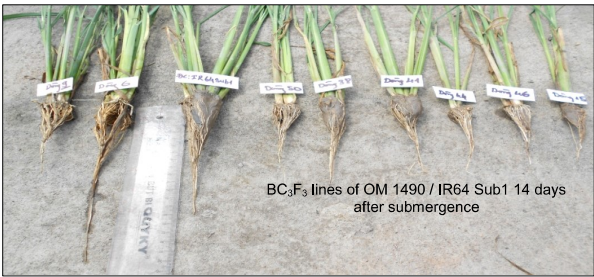


Figure 2. Root length (cm) of BC₃F₃ lines of OM1490/IR64 *Sub1* 14 days after submergence

Table 4. Root length (cm) of the BC₃F₃ lines of OM1490 / IR64 *Sub1* before and 14 days after complete submergence

No	Designation	Root length (cm)	
		Normal condition	After complete submergence
1	1	9efg	14.5abcd
2	4	10.3def	16.1abc
3	6	9.1efg	17.3ab
4	7	10.3def	12.0c-f
5	8	7.5fgh	8.5ef
6	9	8.7efg	18.0a
7	12	7.3fgh	7.9f
8	14	8.2fgh	8.8ef
9	25	8.8efg	9.3def
10	26	7.8fgh	16.5abc
11	28	6.3gh	8.5ef
12	29	6.5gh	10.3def
13	30	5.7h	8.8ef
14	38	12cd	18.3a
15	39	9.8def	10.3def
16	41	8.1fgh	18.3a
17	42	11.5cde	12.6b-f
18	44	12.2cd	13.3a-e
19	49	6.2gh	17.3ab
20	50	6.3gh	16.2abc
21	54	9.7def	10.9def
22	67	16.8a	17.4ab
23	79	17.7a	18.2a
24	IR64 <i>Sub1</i>	15.3ab	17.7ab
25	OM1490	15.7ab	17.7ab
26	Swarna	13.3bc	0.0g
CV (%)		15.4	20.9
F Test		**	**

Note: Values in the same column followed by the same letter are not significantly different at 5% probability level using DMRT; **: significantly different at 1% level of probability

3.5. Correlation between the main traits in the BC₃F₃ lines of OM 1490/IR64 *Sub1* after screening submergence

The correlation among key traits is present in table 5. The survival rate (%) is positively correlated with root length ($r = 0.5725^*$) and highly significant with tiller number /10 hills ($r = 0.8880^{**}$).

3.6. Phenotyping of BC₃F₃ lines of OM1490/IR64 *Sub1* under submergence screening.

The results on phenotyping BC₃F₃ rice lines of OM1490/IR64 *Sub1* after 14 days of complete submergence are presented in Fig. 6. At similarity coefficient of 29.46, BC₃F₃ rice lines were divided into two genetic clusters, A and B.

Table 5. Correlation coefficients among survival rate, plant height, root length and tiller number of the BC₃F₃ lines of OM 1490/IR64 *Sub1*

	Plant height (cm)	Root length (cm)	Tiller number/10 hills	Survival rate (%)
Plant height (cm)	1			
Root length (cm)	0.3508ns	1		
No. of tillers/10 hills	0.2753ns	0.5697*	1	
Survival rate (%)	0.2426ns	0.5725*	0.8880**	1

Note: *, **: significance at 5% and 1% level of probability, répctively; ns: non- significant

Cluster A consists of only variety Swarna (submergence tolerant donor), while cluster B, there includes 23 lines, 1, 4, 6, 7, 8, 9, 12, 14, 25, 26, 28, 29, 30, 38, 39, 41, 42, 44, 49, 50, 54, 67 and 79. These rice lines positioned the cluster with. IR64 *Sub1* and OM1490. Considering the survival rate, this cluster greatly ranged from 10 to 70%, with tolerance score of 7 and 9.

At similarity coefficient of 19.86, the rice lines were divided into two main clusters A and B with subcluster B1 and B2 being the subclusters. Cluster A only included Swarna.

Subcluster B1 included 21 lines as 1, 4, 6, 7, 8, 9, 12, 14, 25, 28, 29, 30, 38, 39, 41, 42, 44, 49, 54, 67 and 79 (including IR64 *Sub1* and OM1490). The rate of the survival of these lines was low ranging from 10 to 40% (average 35%). Subcluster B2 included two lines 26 and 50. This cluster exhibited the highest survival rate of 70%, recovery at score 7.

Accordingly, at similarity coefficient of 16.016, the rice lines of experiment were divided into 4 clusters: A, B1-1, B1-2 and B2 (Fig. 3).

- Cluster A included only Swarna.

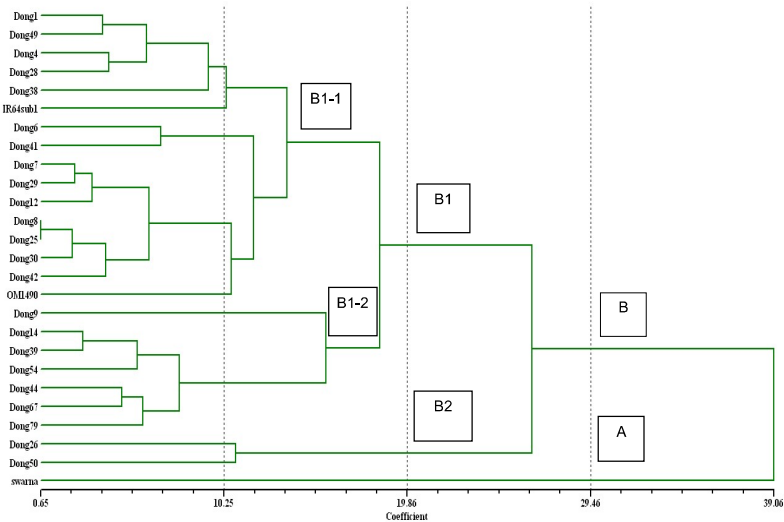


Figure 3. Tree diagram showing the phenotypic relationship between the lines of the BC₃F₃ in OM1490/IR64 *Sub1* after 14-days under complete submergence

- Subcluster B1-1 included 14 lines as 1, 4, 6, 7, 8, 12, 25, 28, 29, 30, 38, 41, 42 and 49 (including IR64 *Sub1* and OM1490) with the survival rate of 10 - 50% and recovery score of 7 and 9.

- Subcluster B1-2 included 7 lines as 9, 14, 39, 44, 54, 67 and 79. The lines exhibited low ratio of survival ratio of score 9.

- Subcluster B2 included 2 lines as 26 and 50. The rice lines of this group obtained the survival ratio with the highest value of 70%.

3.7. Genotyping of the BC₃F₃ lines using SSRs

3.7.1. PCR products by SSR markers

To transfer the tolerant *Sub1* allele into the mega varieties, a MAB strategy was followed, with closely flanking markers used for recombinant selection to reduce the target introgression size and background markers used to select for recurrent parent alleles (Collard and Mackill, 2008).

Three microsatellite markers, viz. RM3269, RM5304, and RM1367 were used. Amplified products from the primers were observed on agarose gel of 3%. Based on the difference between the alleles expressed in the bands on the gel, we could determine the differences among lines.

PCR products by RM3269.

RM3269 was used as a polymorphic marker with its products of 210-220 bp (Fig. 4).

The sequence of primer RM3269 :

RM3269 F 5' GCATTGCTCACTCACACTC - 3'

RM3269 R 5' CAATGGCGCCTCTCATGTC - 3'

- Name of the corresponding lines in the wells on the gel.

1-Line 1	9-Line 25	17-Line 42
2-Line 4	10-Line 26	18-Line 44
3-Line 6	11-Line 28	19-Line 49
4-Line 7	12-Line 29	20-Line 50
5-Line 8	13-Line 30	21-Line 54
6-Line 9	14-Line 38	22-Line 67
7-Line 12	15-Line 39	23-Line 79
8-Line 14	16-Line 41	24-Line 44-2'

The BC₃F₃ lines exhibited high polymorphism (Figure 4) with OM1490 and OMCS2000 alleles of 210 bp and IR64 *Sub1* allele of 220bp. PCR products reached 100% of the recorded lines, of which, the varieties and lines expressed relatively clear polymorphism in tolerance to submergence. RM3269 could be used to assist rice breeding program and selecting submergence tolerant variety (Lang, 2011).

In BC₃F₃ populations, 8 lines in the position numbered with 1, 2, 6, 10, 11, 14, 19 and 20, corresponding to lines of 1, 4, 9, 26, 28, 38, 49 and 50, respectively, showed their bands at the same position with the control., IR64 *Sub1* (220bp).

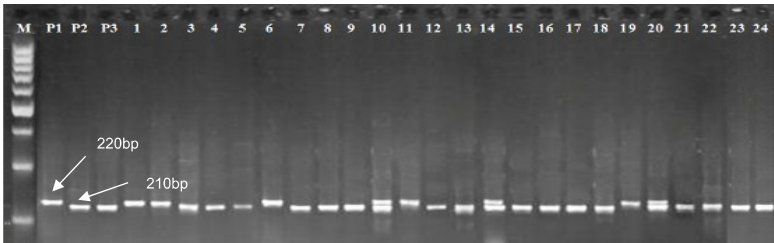


Figure 4. PCR product of the BC₃F₃ lines of OM1490/IR64 *Sub1* with RM3269 primer

Note: M: Standard marker (Φ 174); P1: IR64 *Sub1*; P2: OM1490; P3: OMCS2000; 1-23: lines of OM1490/IR64*Sub1* combinations; 24: lines of OMCS2000/IR64*Sub1*

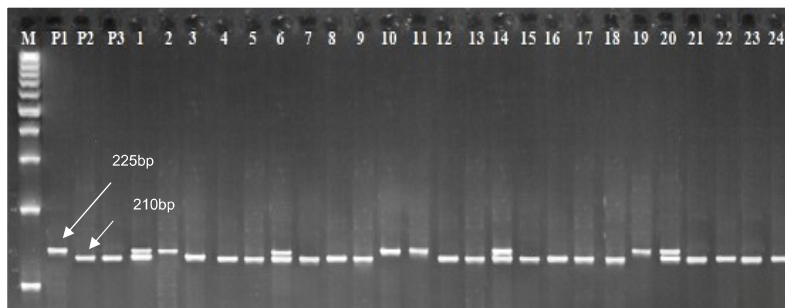


Figure 9. PCR product at the locus of RM5304 in population BC₃F₃ of OM1490/IR64 *Sub1* on agarose gel of 3%

Note: M: Standard marker (Φ 174); P1: IR64 *Sub1*; P2: OM1490; P3: OMCS2000; 1-23: lines of OM1490/IR64*Sub1* combination; 24: lines of OMCS2000/IR64*Sub1* combination.

PCR products by RM5304

This marker has size of 210 bp-225 bp with DNA template to be established with a special primer fragments.

RM5304 primer sequence:

RM5304 F 5' CAGCCATCTCTCTCTCTG - 3'

RM5304 R 5' GATAGCAGGAAGAGGCGTTG - 3'

- Name of the lines corresponding to the wells on the gel

1-Line 1	9-Line 25	17-Line 42
2-Line 4	10-Line 26	18-Line 44
3-Line 6	11-Line 28	19-Line 49
4-Line 7	12-Line 29	20-Line 50
5-Line 8	13-Line 30	21-Line 54
6-Line 9	14-Line 38	22-Line 67
7-Line 12	15-Line 39	23-Line 79
8-Line 14	16-Line 41	24-Line 44-2'

Eight genotypes carried submergence tolerance genes including 1, 2, 6, 10, 11, 14, 19 and 20, corresponding to lines 1, 4, 9, 26, 28, 38, 49 and 50, respectively.

One allele exhibited at the band of 225 bp carrying tolerance gene of IR64 *Sub1* (P1) and lanes 1, 2, 6, 10, 11, 14, 19, and 20, corresponding to lines 1, 4, 9, 26, 28, 38, 49 and 50, respectively.

One allele exhibited at the band of 210 bp carrying intolerance of OM1490 (P2),

OMCS2000 (P3) and lanes 3, 4, 5, 7, 8, 9, 12, 13, 15, 16, 17, 18, 21, 22, 23 and 24 corresponding to lines 6, 7, 8, 12, 14, 25, 29, 30, 39, 41, 42, 44, 54, 67, 79 and 44, respectively.

In particular, four lines 1, 9, 38 and 50 exhibited two bands (225 bp and 210bp) due to their heterozygous condition.

RM5304 marker associated with submergence tolerance trait exhibited 33.3% and 66.7% band of tolerance and intolerance response, respectively.

PCR products of RM1367

RM1367 has size of 230 bp - 250 bp and was used as DNA template to establish a special primer fragment.

The sequence of RM1367:

RM1367 F 5' GTGTGTACGTAGGATCGGAG - 3'

RM1367 R 5' TGCTACTCTAGCTGCTACC - 3'

- Name of the lines corresponding to the wells in the gel

1-Line 1	9-Line 25	17-Line 42
2-Line 4	10-Line 26	18-Line 44
3-Line 6	11-Line 28	19-Line 49
4-Line 7	12-Line 29	20-Line 50
5-Line 8	13-Line 30	21-Line 54
6-Line 9	14-Line 38	22-Line 67
7-Line 12	15-Line 39	23-Line 79
8-Line 14	16-Line 41	24-Line 44-2

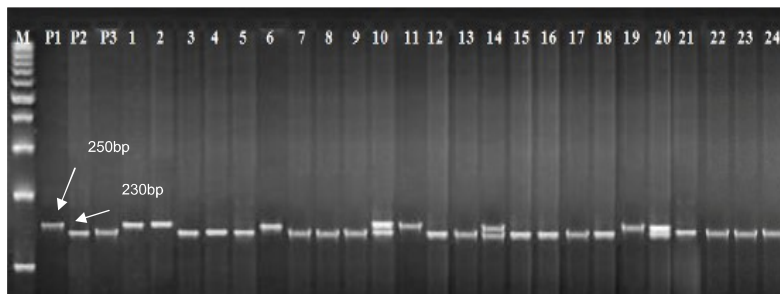


Figure 10. PCR product at the locus RM1367 in the population BC₃F₃ of two backcrosses OM1490/IR64 *Sub1* on 3% agarose gel

Note: M: standard marker (Φ 174); P1: IR64 *Sub1*; P2: OM1490; P3: IR42; 1-23: lines of OM1490/IR64*Sub1*

Hybrid lines carrying the submergence tolerance included lanes 1, 2, 6, 10, 11, 14, 19 and 20, corresponding to lines of 1, 4, 9, 26, 28, 38, 49 and 50, respectively.

Two heterozygous lines at the lanes 10 and 14 carrying both alleles of 250 bp and 230 bp, corresponding to the lines 26 and 38, respectively.

3.2.3 Predictive accuracy between phenotyping and genotyping

The predictive accuracy between phenotyping and genotyping using 3 primers RM3269, RM5304 and RM13670, is presented in table 6.

Progeny lines had variability towards donor variety of IR64*Sub1* carrying submergence tolerance when tested for survival after 14 days of complete submergence. Of 84 initial BC₃F₃ lines, only 8 hybrid lines of OM1490/IR64 *Sub1* exhibited their submergence tolerance including 1, 4, 9, 26, 28, 38, 49 and 50. With phenotyping, only 3 lines were recorded with survival percentage over 50%, i.e lines 26, 38 and 50.

Three molecular markers RM3269, RM5304 and RM1367 could detect submergence tolerance accurately (100%) and high reliability (Table 7).

4. DISCUSSION

The study opened new direction for integration of conventional and molecular breeding methods for improvement of Vietnamese rice. Molecular marker has been proved as a powerful tool in rice breeding program. The application of marker-assisted selection (MAS) in rice breeding is emphasized. Submergence tolerant cultivars can survive 2 weeks or more under complete submergence, whereas most cultivars die within a week. The most tolerant cultivars (e.g. FR13A, Kurkaruppan, Goda Heenati) are originated from Orissa, India, or Sri Lanka, and their tolerance is controlled at the *Sub1* locus on chromosome 9 (Xu *et al.* 2006).

After extraction, total DNA was analyzed by PCR with specific primers located at target regions of submergence genes. RM3269, RM5304, and RM1367 linked to genes located on chromosome 9.

Phenotypic evaluation and testing of submergence tolerant genotypes recorded the lines with good submergence tolerance and carrying both submergence genes. Backcross lines 26, 38 and 50 had high percentage of survival (50%).

The evaluation of molecular markers showed that the volatility of molecular markers depended on polymorphisms of SSRs.

Table 6. Survival rate (%) as compared to three primers in BC₃F₃ population of OM1490 / IR64 *Sub1*

Variety/line	% survival under submerged condition	Alleles					
		RM3269		RM5304		RM1367	
		A (220bp) flooding	B (210bp) Unflooding	A (225bp) Flooding	B (210bp) Unflooding	A (250bp) Flooding	B (230bp) Unflooding
IR64 <i>Sub1</i>	50	+		+		+	
OM1490	30		+		+		+
IR42	0		+		+		+
Line 1	40	+		+	+	+	
Line 4	30	+		+		+	
Line 6	20		+		+		+
Line 7	10		+		+		+
Line 8	20		+		+		+
Line 9	40	+		+	+	+	
Line 12	10		+		+		+
Line 14	10		+		+		+
Line 25	20		+		+		+
Line 26	70	+	+	+		+	+
Line 28	30	+		+		+	
Line 29	10		+		+		+
Line 30	20		+		+		+
Line 38	50	+	+	+	+	+	+
Line 39	10		+		+		+
Line 41	20		+		+		+
Line 42	20		+		+		+
Line 44	20		+		+		+
Line 49	40	+		+		+	
Line 50	70	+	+	+	+	+	
Line 54	10		+		+		+
Line 67	20		+		+		+
Line 79	10		+		+		+
Line 44'	10		+		+		+

Note: Line 1 line to line 79 represents offspring lines of BC₃F₃ of OM1490/IR64 *Sub1* combination and line 44' is offspring line of BC₃F₃ of OMCS2000/IR64 *Sub1* combination.

Table 7. Genotyping and phenotyping accuracy on the BC₃F₃ population of OM1490 / IR64 *Sub1*

	Number of individuals	Tolerant	Intolerant	Heterzygous	Tolerance estimate (%)
Submergence phenotype	24	3	21	-	
RM3269	24	5	16	3	100
RM5304	24	4	16	4	100
RM1367	24	6	16	2	100

5. CONCLUSIONS

- Phenotyping 84 BC₃F₃ lines from OM1490/IR64-*Sub1* 14 days after complete submergence indicated that survival rate, root length and tillering ability are key traits.
- Eight selected offspring lines from BC₃F₃ population of OM1490/IR64 *Sub1* exhibited good adaptation to submergence. Of them three lines of 26, 38 and 50 were selected.
- The polymorphic and codominant SSRs, RM3269, RM5304, and RM1367 were useful for detecting the target genes in the BC₃F₃ population and help distinguish submergence tolerant from intolerant genotypes.
- Both phenotyping and genotyping pinpointed three lines of 26, 38 and 50, which are considered the most promising breeding materials.

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