RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
47 (4) 448-459, 2015

DEVELOPMENT OF SUBMERGENCE TOLERANT BREEDING LINES FOR VIETNAM

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SUMMARY

Development of rice varieties tolerant to submergence, high-yielding and good quality is essential due to increased flooding in the lowland areas in the Mekong delta, Vietnam. The purpose of this experiment was to develop rice varieties tolerant to submergence on the basis of a combination of two breeding methods by molecular markers, single cross and backcross. Evaluating tolerance of F8 and BC2F4 generation on the basis in the field of flooded and unflooded conditions to select promising lines to meet for farmers applying into production. The *Sub1* gene was introgressed into the new breeding lines. Some high yielding and good submergence tolerant lines were developed (e.g. BC2F4-4-3) however, also many lines failed were not acceptable due to their long duration between 120-130 days or their high rate of unfilled grain. This is a opportunity to improve good rice varieties for condition of breeding submergence rice varieties in Vietnam.

Keywords: backcross lines, combination of agronomic traits, molecular markers, submergence tolerance.

Manuscript received: July 29, 2014; Decision on manuscript: February 3, 2015; Manuscript accepted: July 17, 2015.

Communicating Editor: Bertrand Collard

INTRODUCTION

Vietnam is the second largest exporter of rice in the world. A long-term strategy for increasing Vietnam's exports has been in place, to ensure food security for the country and to prepare for the challenge of climate change. Therefore, rice breeders need new tools and strategies to mitigate the adverse conditions from climate change such as submergence and salinity. These abiotic stresses are great challenges for today's breeders (Buu *et al.*, 2013).

Rice genome sequencing of *indica* and *japonica* genomes and the development of DNA markers have provided breeders with necessary tools for marker-assisted selection. Simple sequence repeat (SSRs) are available for many important genes and QTLs in rice (Mackill *et al.*, 2004). The targets of MAS include yield and agronomic characteristics, cooking quality and nutritional quality, and resistance to abiotic and

biotic stresses. Septiningsih *et al.* (2009) developed a few molecular markers for *Sub1* which have been used to develop varieties with tolerance to submergence. Conventional and modern methods using molecular markers have been used for many breeding applications in rice. In fact Lang *et al.* (2009a, 2009b, 2011) have successfully developed new rice varieties such as OM4900, OM6162, and OM6161 which are tolerant to stagnant flooding. The flooded tolerant varieties can survive for 2 weeks or more when complete flooding, while most of the susceptible varieties died within a week. The best tolerant varieties (e.g. FR13A, Kurkaruppan, Goda Heenati) are from Orissa, India or Sri Lanka, and their tolerance is controlled at the *Sub1* locus on chromosome 9 (Xu *et al.*, 2006; Neeraja *et al*., 2007).

The objective of this study was to investigate the effect of submergence stress at seedling and reproductive stages in development of submergence and tolerance. In this study, we also developed new submergence tolerant breeding lines in order to try to develop new submergence-tolerant varieties, with high yield and good quality.

MATERIALS AND METHODS

Breeding populations were developed 6 different crosses. IR64-Sub1 was the *Sub1* donor. The BC2F4 backcross lines were developed from OM1490/IR64-Sub1//OM1490. OM1490 is high-yielding with broad-spectrum disease resistance. In total, 1,491 progenies were selected from F2, F3, F4, F5, F6, F7, F8 and BC2F4 generations under rainfed lowland conditions at CLRRI. A total of 24 lines with submergence tolerance were advanced and evaluated for yield and yield components using OM1490 as the check.

Method of screening submergence

Parents and breeding lines were flooded in submerged tanks in Cuulong Delta Rice Research Institute to ensure complete submergence for 14 days at seedling stage. After de-submergence, surviving lines were transplanted in fields for observing the recovery of the lines. Plants were transplanted and grown to flowering stage and harvest and data analysis of yield and yield components at CLRRI.

The experimental lines were assessed for yield and yield component traits under both flooded groups and non-flooded. Twenty four fixed F8 and BC2F4 lines were evaluated against resistant check IR64-Sub1 and OM1490 (Table 1) under controlled submergence conditions. Ten-day-old seedlings were transplanted using 1 plant/hill and spacing of 20 x 15 cm in the field. The crops were submerged at 14 d after transplanting for 30 days. After submerged at 14 days removed water and keep around 10 cm water height was maintained until drained during flowering. Fertilizers were applied.

Quantitative traits

The following quantitative traits were measured:

1. Panicle length (cm): length of panicle at maturity measured from the base to the tip of the panicle (from 10 randomly selected primary panicles per accession per replication).
2. Panicles per plant (number): total number of panicles per plant (from 10 randomly selected primary panicles per accession per replication).
3. 1000-grain weight: weight in grams of 1000 well-developed grains at 14% MC (from 5 randomly selected primary panicles per accession per replication).
4. Days to maturity: days from seeding when 80% of the grains are fully ripened on a per replication basis.
5. Filled grains (number): obtained from counts of total number of filled grains per panicle (from 5 randomly selected primary panicles per accession per replication).
6. Unfilled grains (number): obtained from counts of total number of unfilled grains per panicle (from 5 randomly selected primary panicles per accession per replication).

Table 1. The origin of F8 lines and BC2F4 from CLRRI (Lang et al., 2013).

Numbers	Lines	Cross
1	F8 -13-2	IR75499-84-1-B/IR64-Sub1
2	F8-15-1	V3M-167-2-B/IR64-Sub1
3	F8-15-2	V3M-167-2-B/IR64-Sub1
4	F8-22	IR75499-73-1-B/IR64-Sub1
5	F8-23	IR75499-73-1-B/IR64-Sub1
6	F8-28-1	IR65191-3B-2-2-2-2/IR64-Sub1
7	F8-28-2	IR65191-3B-2-2-2-2/IR64-Sub1
8	F8-29-1	BP227D-MR-2-12/IR64-Sub1
9	F8-29-2	BP227D-MR-2-12/IR64-Sub1
10	F8-30-1	OM1490/IR64-Sub1
11	F8-30-2	OM1490/IR64-Sub1
12	F8-30-3	OM1490/IR64-Sub1
13	F8-30-4	OM1490/IR64-Sub1
14	F8-30-5	OM1490/OM1490-Sub1
15	BC2F4-1-1	OM1490/IR64-Sub1//OM1490
16	BC2F4-1-2	OM1490/IR64-Sub1//OM1490
17	BC2F4-4-1	OM1490/IR64-Sub1//OM1490
18	BC2F4-4-1	OM1490/ IR64-Sub1//OM1490
19	BC2F4-4-2	OM1490/ IR64-Sub1//OM1490
20	BC2F4-4-3	OM1490/ IR64-Sub1//OM1490
21	BC 2F4-5-1	OM1490/ IR64-Sub1//OM1490
22	BC2F4-5-2	OM1490/ IR64-Sub1//OM1490
23	BC2F4-5-3	OM1490/ IR64-Sub1//OM1490
24	BC2F4-6-1	OM1490/ IR64Sub1//OM1490
25	IR64-Sub1 (Check)	IRRI
26	OM1490 (Check)	OM606/IR44593-62-1-3-3

7. Yield was obtained harvested plants in each replication. Harvested grains were threshed, cleaned, dried, and weighed for each accession per replication. Moisture content (MC) per plot was determined immediately after weighing using a moisture meter, and yield was adjusted for moisture content.

DNA extraction

The 31 lines/varieties were grown in pots. Maximum protection was employed to ensure healthy and disease-free seedlings. The leaves were collected 2-3 weeks after planting for DNA extraction. Standard molecular grade chemicals and general techniques for preparing stock solutions, buffers, reagents were prepared following according to Sambrook *et al.* (1989). Molecular work was conducted at the Genetics and Plant Breeding Department of the Cuu Long Delta Rice Research Institute, Can Tho, Vietnam. DNA suitable for PCR analysis was prepared using a simplified procedure (McCouch *et al*., 1988). A piece of a young rice leaf (2 cm) was collected and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf was grinded using a polished glass rod in a well of a spot test plate (Thomas Scientific) after adding 400 µl of extraction buffer. Grinding was done until the buffer turned green, an indication of cell breakage and release of chloroplasts and cell contents. Another 400 µl of extraction buffer was added into the well by pipetting. Around 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 was precipitated using absolute ethanol. DNA was air-dried and re-suspended in 50 µl of TE buffer (Lang, 2002).
DNA quality checks used 1% agarose by melting 3 g of agarose in 300 ml of TAE buffer. The mixture was heated in a microwave for 5-6 min and then cooled to around 55-60 °C. This was then poured on a previously prepared

electrophoresis box with combs. Gels were prepared and the combs removed after about 45 min. Seven microliters of DNA sample plus 3 µl of loading buffer (Tris 1 M pH = 8.0, glycerol, EDTA 0.5 M pH = 8.0, xylene cyanol 0.2%, bromophenol blue 0.2%, and distilled water) was run at 70-80 V, 60 mA for 45 min or until the loading buffer dye moved far away from the wells. The gel was then taken out and stained with ethidium bromide, after which it was observed under UV light.

Microsatellite analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring. Microsatellite primers were used to survey polymorphism on the samples. These were randomly selected from the 3 microsatellite primer pairs currently available for rice such as RM201; RM105; RM219 (Temnykh *et al*., 2000). The PCR reaction was as follows. Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, with a final extension at 75 °C for 5 min. After amplification, 10 µl of stop solution was added to the PCR product, which was then denatured at 94 °C for 2 min. Eight microliters of each reaction were run on polyacrylamide gel.

Data analysis

Analysis of variance

The agro-morphological data collected were initially analyzed through analysis of variance to verify genetic variation in the traits measured. The few traits with insignificant genetic variation, based on the *F* test, were not considered for further analyses.

Correlation analysis

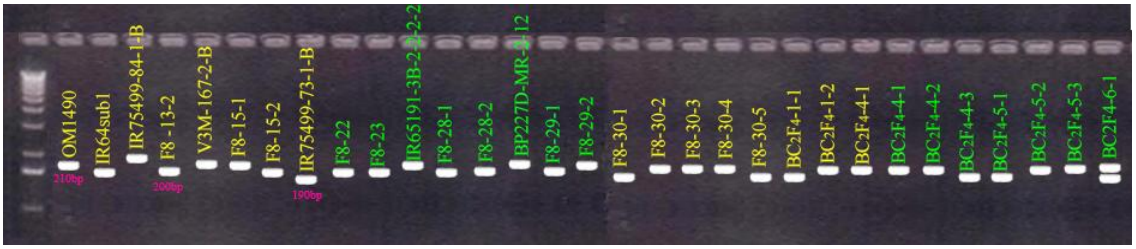
Correlation coefficient (*r*) is a measure of the association between two or more variables. It is a measure of symmetrical association between variables and does not measure the dependence of one variable over other. Correlation among agro-morphological traits was calculated by using SAS software.

RESULTS

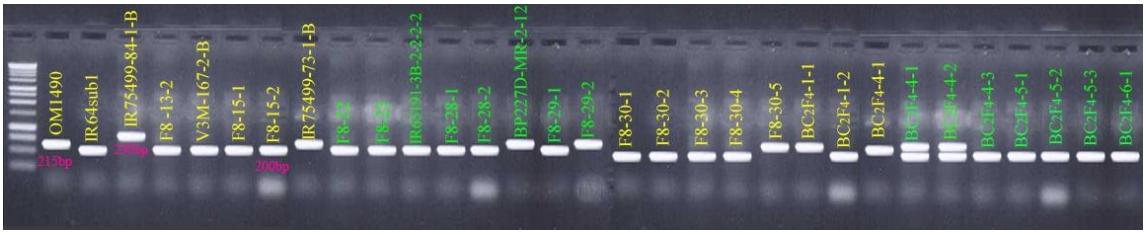
Prior to crossing (hybridization) and line development, there are several applications in which DNA marker data may be useful for breeding, such as cultivar identity, assessment of genetic diversity and parent selection, and confirmation of hybrids (Collard and Mackill, 2008). Screening for Sub1 was based on molecular markers with markers on chromsome 9 based on information of genetic map of Mackill *et al.* (2006). The polymorphism level of the SSR markers was different for each population. The polymorphic markers from IR75499-73-1-B/IR64-Sub1 were RM219, RM8300, RM2383, RM105, SubC173. The polymorphic markers for V3M-167-2-B/IR64-Sub1 were RM201, Sub1c173 and RM219. The polymorphic markers for IR65191-3B-2-2-2-2/IR64-Sub1 were RM219, RM23835, RM105 and RM201. Six markers (RM219, RM8300, RM23835, RM105, Subc173 and RM201) were polymorphic for OM1490/IR64-Sub1 and BP227D-MR-2-12/IR64-Sub1. Some examples of the polymorphisms are shown in Figure 1.

Breeding lines were selected and fixed during 8 continuous seasons in 2011 to 2014 and over the generations selected under submerged conditions until F8 and BC2F4 generations (Lang *et al*., 2013). Evaluation and analysis of yield and yield components was performed after lines were fixed. The results are recorded in Table 2.

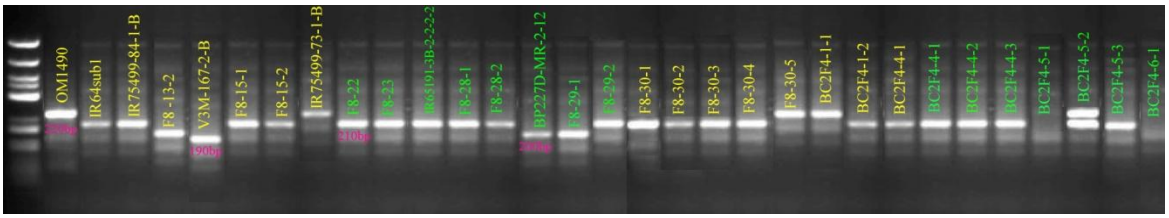
RM 219



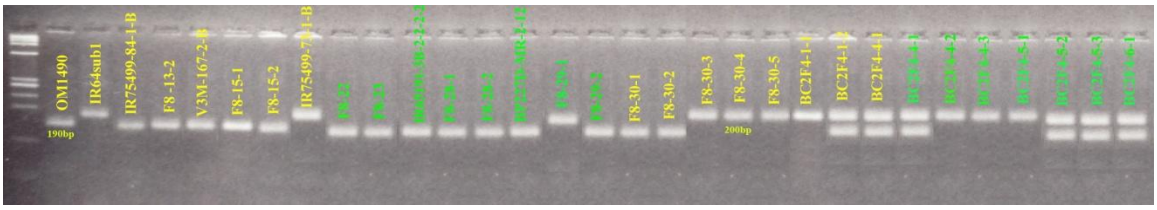
RM 8300



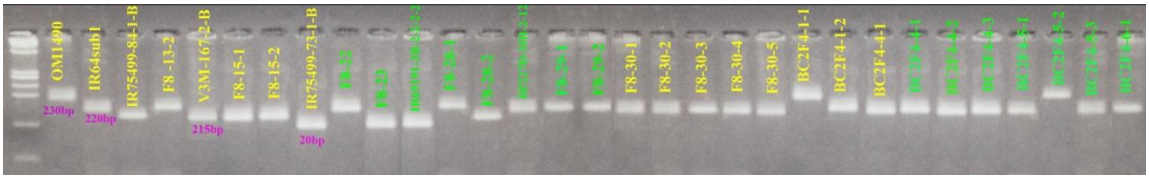
Sub1C137



RM105



RM 23835



RM201

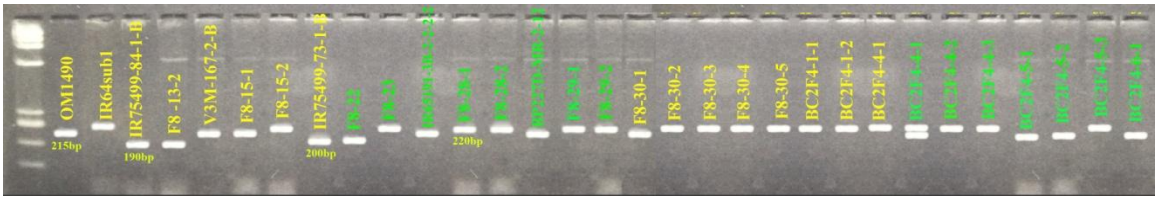


Figure 1. Six different PCR products for individual F8 plants and BC2F4 were loaded with RM219, RM8300, Sub1C137, RM105, RM23835, RM201 indicates primer pairs and microsatellite loci. The number in parenthesis identifies the individual F8 and BC2F4 plant used for PCR analysis.

Table 2. Results of survival rate and plant height of the BC3F3 lines derived from OM1490/IR64-Sub1 before and after the complete submergence.

	Rate of survival (%)		Plant height (cm)		Number of tiller/10 hills		Root length (cm)		
Code	Normal condition	After complete submergence at 14 days	Normal conditions	After complete submergence at 14 days	Normal conditions	After complete submergence af 14 days	Normal conditions	After complete submergence	SES score for submergence tolerance
F8-13-2	100 a	40 c	54.8 a	83.1 bcde	37 l	14 d	9 efg	14.5 abcd	3
F8-15-1	100 a	30 d	51.3 abcd	84.2 bcd	42 h	12 e	10.3 def	16.1 abc	5
F8-15-2	100 a	20 e	44.8 ghi	101.3 a	40 j	9 gh	9.1 efg	17.3 ab	5
F8-22	100 a	10 f	51.1 abcd	78 c-h	49 e	4l mn	10.3 def	12.0 c-f	7
F8-23	100 a	20 e	49.7 a-h	71.5 fghi	42 h	7 ij	7.5 fgh	8.5 ef	7
F8-28-1	100 a	40 c	47.5 c-h	45 kl	49 e	18.3 c	8.7 efg	18.0 a	3
F8-28-2	100 a	10 f	44.7 ghi	75 d-h	44 g	3 n	7.3 fgh	7.9 f	9
F8-29-1	100 a	10 f	44.3 hi	53 k	45 f	4l mn	8.2 fgh	8.8 ef	9
F8-29-2	100 a	20 e	46.2 d-i	70.5 ghi	41 i	7 ij	8.8 efg	9.3 def	7
F8-30-1	100 a	70 a	47.8 b-h	74 efgh	49 e	19 bc	7.8 fgh	16.5 abc	1
F8-30-2	100 a	30 d	53 ab	80.6 b-f	53 b	11 ef	6.3 gh	8.5 ef	5
F8-30-3	100 a	10 f	47 d-h	82.5bcde	51 c	5 kl	6.5 gh	10.3 def	9
F8-30-4	100 a	20 e	44.8 ghi	74.5efgh	49 e	10 fg	5.7 h	8.8 ef	9
F8-30-5	100 a	50 b	50.3 a-f	86.3bc	53 b	20 b	12 cd	18.3 a	3
BC2F4-1-1	100 a	10 f	47.8 b-h	47.5 kl	39 k	4.3 lm	9.8 def	10.3 def	9
BC2F4-1-2	100 a	20 e	49.7 a-h	87.5 b	39 k	10 fg	8.1 fgh	18.3 a	9
BC2F4-4-1	100 a	20 e	50.7 a-e	78.8 b-g	42 h	6 jk	11.5 cde	12.6 b-f	9
BC2F4-4-1	100 a	20 e	49 b-h	48.8 kl	44 g	6 jk	12.2 cd	13.3 a-e	9
BC2F4-4-2	100 a	40 c	47.3 c-h	76.2 d-h	45 f	12 e	6.2 gh	17.3 ab	3
BC2F4-4-3	100 a	70 a	45.5 e-i	52.7 k	42 h	23 a	6.3 gh	16.2 abc	1
BC 2F4-5-1	100 a	10 f	52.7 abc	61.5 j	39 k	3.7 mn	9.7 def	10.9 def	9
BC2F4-5-2	100 a	20 e	45.5 e-i	40.5 l	50 d	9 gh	16.8 a	17.4 ab	7
BC2F4-5-3	100 a	10 f	49.8 a-g	42.0 l	54 a	5 kl	17.7 a	18.2 a	7
BC2F4-6-1	100 a	50 b	41.7 i	68.8 hij	23 m	8 hi	15.3 ab	17.7 ab	3
IR64-Sub1	100 a	50 b	45 fghi	63 ij	21 n	5 kl	15.7 ab	17.7 ab	7
OM1490	100 a	20 e	36.3 j	36 m	17 o	2 o	13.3 bc	4.0 g	7
CV (%)	-	7.35	5.8	7.4	1.03	6.93	15.4	20.9	
F	ns	**	**	**	**	**	**	**	

Note: **: significant at P = 0.01; ns: not significant in statistically significant.

Table 3: Correlation coefficient matrix of a few targets of phenotype after screening submergence of BC3F3 lines in 1490/IR64 OM Sub1 combination.

	Plant height (cm)	Root length (cm)	Number of branches/10 hills	Survival rate (%)
Plant height (cm)	1			
Root length (cm)	0.3508 ns	1		
Number of branches/10 hills	0.2753 ns	0.5697 *	1	
Survival rate (%)	0.2426 ns	0.5725 *	0.8880 **	1

Note: *: significant at P = 0.05; **: significant at P = 0.01; ns: not significant in statistically

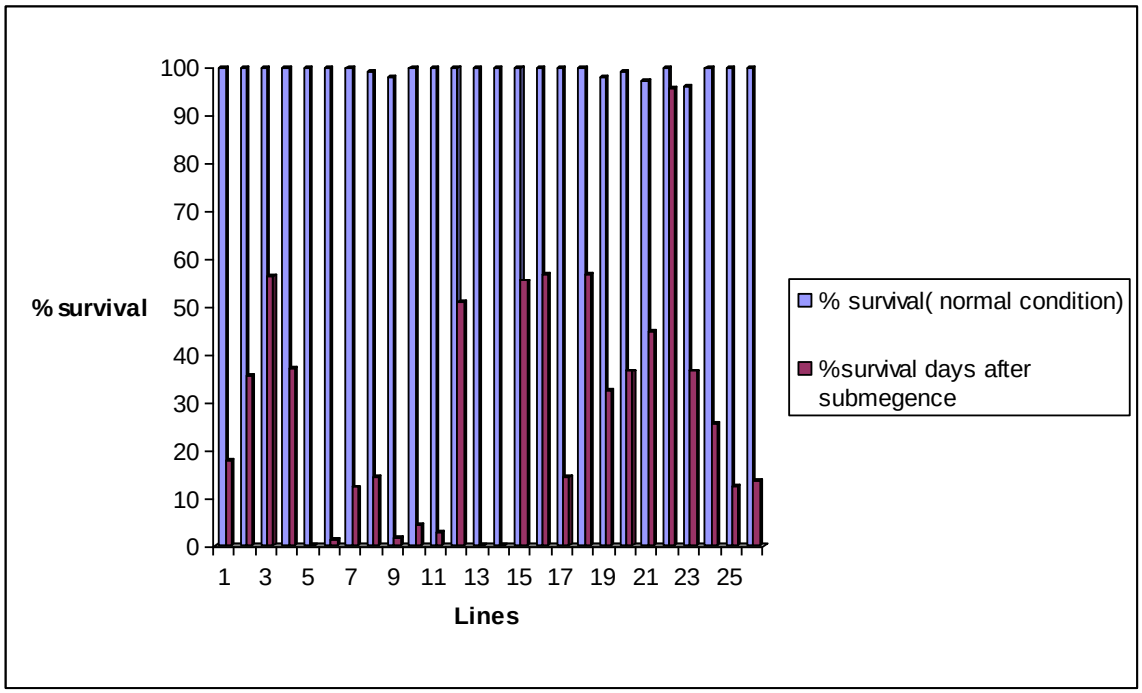


Figure 2. Frequency of percentage survival for tolerance to submergence conditions during reproductive of 26 lines (1-26) from F8, BC2F4 and OM1490, IR64-Sub1

Table 3: Agronomic traits of F8 and BC lines in unflooded and completely submerged conditions (14 days)

		Plant height (cm)		Number of tillers		Filled grains		Unfilled-grain		Yield (g/hill)	
No.	Line name	Normal conditions	After complete submergence	Normal conditions	After complete submergence	Normal conditions	After complete submergence	Normal conditions	After complete submergence	Normal conditions	After complete submergence
P1	OM1490 (Check 1)	117 abc	125 ab	17 ab	12 a	126 g	56 g	9.5 m	12.1 no	42.3 a	10.5 ij
P2	IR64-Sub1 (Check 2)	100 gh	115 c-d	15 cd	11 ab	105 ij	80 f	12.8 l	13.6 mn	18.9 ij	16.5 ef
1	F8-13	105 efg	115 cd	14 de	7 ef	158 cd	85 cde	23.2 e	17.5 k	39.6 b	16.2 f
2	F8-15-1	114 a-d	120 a-d	12 fg	6 f	156d	84 def	21.5f	18.6jk	35.6c	12.5h
3	F8-15-2	115 a-d	0	15 cd	0	140ef	0	16.5ghi	0	40.3b	0
4	F8-22	102 fgh	105 ef	14 de	7 ef	163bcd	100 b	15.4ijk	56.2b	25.6ef	20.1c
5	F8-23	100 gh	105 ef	15 cd	8 de	142ef	104 ab	14.2k	42.1d	12.4n	10.2ij
6	F8-28-1	96 h	100 f	15 cd	10 bc	144 e	89 c	12.3 l	35.2e	13.4mn	7.8k
7	F8-28-2	107 d-g	110.67 de	14 de	11ab	135 f	87 cd	17.5 g	63.5 a	10.6 o	5.6 l
8	F8-29-1	108 c-g	115 cd	14 de	10bc	124 g	56 g	15.8 hij	42.3 d	14.5 lm	8.5 k
9	F8-29-2	115 a-d	117 bcd	13 ef	11ab	123 g	55 g	15.6 hij	44.5 c	12.3 n	10.6 ij
10	F8-30-1	114 a-d	117 bcd	16 bc	9cd	124 g	52 g	16.9 gh	41.2 d	17.8 jk	14.5 g
11	F8-30-2	120 abc	0	14 de	0	100 j	0	27.1 b	0	15.6 l	0
12	F8-30-3	115 a-d	0	11 g	0	112 hi	0	34.2 a	0	19.3 hi	0
13	F8-30-4	116 a-d	120 a-d	14 de	8 de	114 h	100 b	26.1bc	30.2f	20.5 h	18.6 d
14	F8-30-5	117 abc	125 ab	11g	7ef	115 h	56 g	12.8l	20.5i	18.5 ijk	16.5 ef
15	BC2F4-1-1	120 a	120 a-d	15cd	6f	108 hi	56 g	11.9l	15.6l	19.2 i	10.1 j
16	BC2F4-1-2	118 ab	120 a-d	18a	10bc	147 e	104 ab	10.2m	17.5k	26.3e	23.1b
17	BC2F4-4-1	114 a-d	115 cd	12 fg	10 bc	165 bc	34 h	12.9 l	42.1 d	25.6 ef	5.5 l
18	BC2F4-4-1	105 efg	115 cd	14 de	10 bc	145 e	36 h	17.8 g	19.2 ij	24.5 fg	4.2 m
19	BC2F4-4-2	100 gh	128 a	16 bc	11 ab	125 g	100 b	14.5 jk	15.6 l	23.3 g	17.6 de
20	BC2F4-4-3	110 b-f	120 a-d	14 de	12 a	142 ef	106 a	10.2 m	11.9 o	42.5 a	29.6 a
21	BC 2F4-5-1	110 b-f	125 ab	17 ab	10 bc	163 bcd	85 cde	25.7 cd	28.6 f	17.6 k	10.5 ij
22	BC2F4-5-2	112 a-e	124 abc	15 cd	12 a	168 ab	84 def	26.3 bc	26.5 g	19.5 hi	11.5 hi
23	BC2F4-5-3	114 a-d	123 abc	12 fg	10 bc	174 a	86 cde	25.6 cd	26.9 g	42.1 a	18.9 cd
24	BC2F4-6-1	113 a-e	124 abc	14 de	10 bc	112 hi	82 ef	24.5 d	24.8 h	32.3 d	17.2 ef
CV%		4.18	4.72	6.07	8.91	3.11	3.53	4.04	3.98	2.99	6.04
LSD 0.05		7.58	2.07	1.42	1.75	6.93	5.92	1.2	2.41	1.19	1.86

Note: numbers followed by the same letter are not statistically difference at the 5% level

Evaluating results on the survival rate (%) of the BC2F4 lines from OM1490/IR64-Sub1//OM1490 before and after completely flooding were recorded in Table 2. Results showed that the ability of lines to survive under conditions of complete submergence for 14 days, with the survival rate ranging from 10-70% (Table 2). Observing dead plants, we found that leaf sheath was dead, and the the main stem and the roots were rotten.

Results in Table 2 shows, the rice lines when grown in normal conditions had a 100% survival rate. The lines with the highest survival rate (%) were: F8-30-1, BC2F4-4-3 with an average survival rate of 70% (with tolerance level was 3) and line BC2F4-6-1, with a survival rate of 50% (with a tolerance of 5). The remaining lines had a survival rate that was lower than the control variety IR64-Sub1 (50%) ranging from 10-40%, including OM1490 which had a survival rate of 30%.

The submerged rice plants had a faster plant height growth due to elongation of internodes. Because, plant height growth is a combination of increasing the length of the internodes, leaf sheath and leaf blade and the elongation only the increase of length of the internodes. Results are shown in Table 1.

The results show that plant height differed between two different conditions. The selection for short plant is very important for high yield and easier machine havesting. The farmers in the Mekong area of Vietnam need varieties with high plant around 100-115 cm. If the lines are too high plants can lodge and affect yield.

The plant height of plants after being submerged was more than the plant height in normal conditions, except for Sub1 lines. This occurs because susceptible lines will grow rapidly to rise up out of the water, but die if the depth is too deep, or when the flood water recedes.

The tallest lines in normal conditions were F8-13-2, F8-15-1, F8-22, F8-30-2, BC 2F4-5-1, which were 54.8 cm, 51.3 cm, 51.1 cm, 53 cm, 52.7 cm. The remaining lines had an average height ranging from 36.3 to 50.7 cm, in which the control IR64-Sub1 was 41.7 cm.

The tallest lines after 14 days of complete submergence were F8-13-21, F8-15-1, F8-15-2, F8-30-5, F8-30-3 and BC2F4-1-2 which were 83.1 cm, 84.2 cm, 101.3 cm, 82.5 cm, 86.3 cm, and 87.5 cm, respectively. The remaining lines had an average height range from 0 to 80.6 cm, IR64-Sub1 was 68.8 cm. Fluctuations between the lines in the two environments were quite small with CV% < 10% was recorded as 5.8 and 7.4.

Tillering ability of variety depends on the genetic characteristics of the variety, in addition to farming practices and environmental conditions. When stable water levels are maintained (e.g. 10 - 15 cm), the tillering ability of rice will be strong but tillering will be affected when water levels are higher than this.
Tillering ability of the experimental lines before submergence and after complete submergence was assessed through evaluation of number of tillers of 10 hills of each line. Results are reported in Table 2.

Results showed the recovery ability and good growth of the lines are approximately 1 week after transplantation. Speed of tillering begins 14th days before submergence. The lines F8-30-2, F8-30-3, BC2F4-5-3 had the highest number of tillers: 53, 53, 54 tillers/10 hills, respectively.

According to the results in Table 3, some of the lines had good recovery and tiller growth 14 days complete submergence. The best lines were: F8-30-1, F8-30-5, BC2F4-4-3 which had 19, 20, 23 tillers/10 hills, respectively, and were higher than the control variety IR64-Sub1 (8 tillers/hill).

During the two periods before and after complete submergence, rice lines with tillers/10 hills gradually reduced. This can be explained that, in conditions of prolonged submergence the new-born branches and main stem rot. Analysis of the number of tillers/10 hills after screening for submergence were statistically significant at level 1%.

Results of evaluating root length (cm) of the BC2F4 lines (derived from OM1490/IR64-Sub1) before and after complete submergence were presented in Table 2. The results showed that under submerged conditions, elongation of roots occurred in all experimental lines but the root length was higher for the lines with tolerance under waterlogged conditions compared to susceptible lines (Lang *et al.*,

2013). The results presented in Table 2 showed that the lines F8-30-5, BC2F4-1-2 and BC2F4-5-3 had the highest root length (18.3, 18.3, 18.2 cm, respectively), which was higher than the control varieties IR64-Sub1 and OM1490 with root length was recorded as 17.7 cm.

Root length of the experimental lines after submergence screening indicated that there was no great variation of root length in the experimental lines. Analysis of root length of the lines after screening submergence was statistically significant at the level of 1%. Results evaluating the correlation between several target traits after screening submergence in BC3F3 lines derived from the OM 1490/IR64-Sub1 cross are shown in Table 3.

The results showed that the survival rate (%) correlated with root length (r = 0.5725). This increase in root length and their function during completely submerged conditions is almost certainly beneficial and different from the increase in plant height.

The correlation between survival rate (%) and number of tillers/10 hills was high (r = 0.8880; $P < 0.01$). The good tillering ability of rice plants during submerged conditions and good recovery and growth after submergence appears to be an important determinant of survival.

The lines were screened at seedling stage after surviving submergence stress and were screened at flowering stage. Breeding lines were tested under two treatments: normal conditions and submergence for 14 days. The range of survival rates (%) in the BC2F4 lines of derived from OM1490/IR64-Sub1 are shown in Figure 2.

Some lines still segregated, and a few lines were fully dead (F8-15-2, F8-30-2, F8-30-3). Results indicated that most of the lines ranged in height between 96 to 120 cm. Some lines had high tillering ability (e.g. BC2F4-5-1, BC2F4-4-2, F8-30-1, F8-30-1-BC2F4 5-2, F8-15-2, F8-23, and F8-28-1).

Some lines have a high number of filled grains/panicle (>150), including: BC2F4-5-3-5-2 BC2F4, BC2F4-4-1, BC2F4-5-1, and F8-22). All lines had a low rate of unfilled-grain. Considering yield components, many lines had high yield potential, with an average of weight (g/hill) of 30 to 40 g (e.g. BC2F4-4-3, BC2F4-5-3, F8-15-2, F8-15-1-BC2F4 6-1). However these lines need to be tested in plots to get an accurate measure of yield potential.

Analysis of filled grains/panicle ranged from 52 to 106 grains; the best lines the lines were F8-30-5, BC2F4-4-3, F8-23, and F8-22.
Only 1 line BC2F4-4-3 had a high survival rate in completely submerged conditions accounting for 95% survival at reproductive stage.

DISCUSSION

New breeding lines consisting of F8 and BC-derived lines were screening for submergence tolerance using phenotyping and molecular markers. For self-pollinated crops, an important aim may be to fix alleles in their homozygous state as early as possible. For example, in bulk and single-seed descent breeding methods, screening is may be performed at the F8 generations when most loci are homozygous. Using co-dominant DNA markers, it is possible to fix specific alleles in their homozygous state as early as the F2 generation. However, this may require large population sizes; thus, in practical terms, a small number of loci may be fixed at each generation (Koebner and Summers, 2003). An alternative strategy is to ‘enrich’ rather than fix alleles—by selecting homozygotes and heterozygotes for a target locus—within a population in order to reduce the size of the breeding populations required (Bonnett *et al*. 2005).

In conclusion, new breeding lines were developed after several generations of selection after submergence stress and agronomic and yield-component traits (Table 3). Grain yield in rice is a complex trait determined by its three component traits: number of panicles, number of grains per panicle, and grain weight (Yongzhong *et al*., 2010). After thorough evaluation, only 2 lines BC2F4-1-2 and BC2F4-4-3 - had high yield and a growth duration of less than 100 days. The number of grains per panicle is usually highly proportional to the spikelet number. To understand number of grains per panicle, it is essential to understand the basic biological processes of panicle development, as well as the differentiation of meristems into spikelets at submergence in rice. From an

agronomic perspective, the number of spikelets per panicle can be attributed to two components: the duration of panicle differentiation and the rate of spikelet differentiation (Huang *et al*., 2006).

ACKNOWLEDGEMENTS

The authors are grateful to International Rice Research Institute (IRRI) and Australian Centre for International Agricultural Research (ACIAR) for funding this research on rice. Appreciation is expressed for CLRRI provided for the project, and to our colleagues in Genetics and Plant Breeding Division for their support and valuable suggestions.

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