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RICE BREEDING LINES DEVELOPED WITH HIGHLY EFFICIENT SUBMERGENCE TOLERANCE THROUGH ADVANCED SINGLE SEED DESCENT METHOD FOR SEMI-LOWLAND AND -DEEP LOWLAND AREAS

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Abstract- In the present study, sixty-three breeding lines of rice cultivars adapted for semi-lowland and semi-deep lowland areas were developed for submergence tolerance through the single seed descent (SSD) method. In submergence evaluation of these lines, we have selected many breeding lines associated with highly submergence tolerance. For instance, some breeding lines, CR 2589-8-1-1-1, CR 2590-9-1-1-2 and CR 2530-B-1-5-1-1 accounted for 100 percent survival rate in the evaluation. Besides, many breeding lines have showed survival rate more than 90 percent. In PCR level, we confirmed the presence of Sub1 locus in the submergence tolerant lines. Moreover, these selected lines were more efficient than donor and tolerant checks also. Thus developed breeding lines with high efficiency for submergence tolerance at field level could be complementary for rice farmers who cultivate rice in semi-lowland and semi-deep lowland areas of eastern region of India.

Keywords- Advanced single seed descent method, Sub1 locus, IYT1, Sub1BC2, breeding lines, donor and submergence stress

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Introduction

Even though rice (*Oryza sativa* L.) is a typical-water plant, it is very sensitive to submerged condition. Because of rice plants are not well adapted to sudden and total water inundation for several days. Therefore submergence stress affects severely the rice production in the rainfed lowland areas. To overcome this problem, recently more sustainable and permanent solution has been identified with a major quantitative trait locus (QTL) for submergence tolerance in rice landrace from eastern region of India [1]. This QTL named as Submergence tolerance 1 (Sub1) helps the rice plants to survive for 10-14 days under submerged condition and renews its growth when the water subsides. This locus has been mapped on chromosome 9 and cloned and presence of three genes encoding putative ethylene responsive factors (ERF) (Sub1A, Sub1B and Sub1C) has been identified within this locus. Among them, *Sub1*A gene has been confirmed as major determinant of submergence tolerance in Sub1 locus [1, 2]. Using Sub1 locus, development of submergence tolerance in rice high-yield rice cultivars is being done in different parts of the world since it is the most promising solution to develop high-yielding local rice varieties for submergence tolerance. For example, many mega-rice varieties have been developed for submergence tolerance by introgression of Sub1 locus through marker-assisted backcrossing (MABC) [3-5]. Very recently also, high yielding rice cultivars have been improved for submergence tolerance in flash-flood lowland areas of Bangladesh [6] and in eastern Indian [7]. As rural poverty and food insecurity are especially persistent in rainfed and flood-prone rice production areas in Asia and Africa, the flooding is considered a major challenge for rice production in these areas since the modern rice varieties are sensitive to complete submergence or long-duration stagnant floods to depths of over 30 cm. In India, about 16.1 million ha of rainfed lowland rice are grown each year, of which 5.2 million ha are periodically affected by submergence [8]. In this study, a total of 63 breeding lines developed for submergence tolerance from high yielding rice cultivars (Savitri, Gayatri, Sarala, Varshadhan and Durga) by introgression of *Sub1* locus for rain-fed lowland and semi deep lowland areas in eastern India were evaluated for their efficiency in submergence tolerance.

Materials and Method

Plant Materials

In this study, a total of 63 breeding lines developed for submergence tolerance from high yielding rice cultivars adapted to semi-lowland (Savitri, Gayatri, Sarala) and semi-deep lowland ecosystem (Varshadhan and Durga) by introgression of Sub1 locus using different donor lines (Swarna-Sub1, Samba mahsuri-Sub1, IR64-Sub1 and IR 49830-7) [5] as well as same cultivar or different cultivar by single-way or two-way cross in CRRI, Odisha (20.5°N, 86°E, and 23.5 meters above mean sea level) and advanced up to F_6 generation through single seed descent (SSD) method [9] were used

[Table-1].

Submergence Evaluation and Morphological Characterization

Twenty-one day old seedlings of Sixty-three breeding lines, donor lines, tolerant check (FR13A and Karkuruppan), intolerant checks (IR42 and IR64) and rice varieties (Gayatri, Sarala, Savitri, Varshadhan and Durga) were transplanted in two rows (each rice line) in submergence tank, CRRI, Cuttack during Kharif season-2014. Each row consisted of 20 seedlings with 15 x 10 cm gap. Seedlings were allowed 10-days to establish and necessary fertilizers were applied to seedlings. Then, seedlings were submerged and the water level was maintained at the level of 95cm height for 14-days. Following the completion of stress period, seedlings were de-submerged and allowed them 10-days to regenerate. Following this, survival rate of (re-generated) seedlings was calculated in percentage [5]. For morphological characterization, the mean value of 5 plants of each line was recorded for plant height and tiller number during flowering stage.

DNA Isolation and PCR Screening

A crude DNA preparation suitable for PCR screening was prepared using a simplified miniscale procedure [10]. A single piece of healthy young leaf was harvested and placed in a labeled 1.5 ml centrifuge tube in ice. The leaf sample was macerated using thick glass rod after adding 400 µl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The leaf was grounded until the buffer turned into green colour. After grinding, another 400 µl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 g in micro centrifuge for 10min. Nearly 400 µl of lysate was extracted with 400 µl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol. The contents were centrifuged for 3 min at full speed and the supernatants were discarded. The pellet was washed with 70% ethanol. The DNA was air dried and re-suspended in 50 µl of TE buffer (10mMTris-HCl, pH 8.0, 1mM EDTA, pH 8.0). One ml of aliquot was used for PCR analysis and the remaining solution was stored at -20ºC for any further use. PCR amplification was performed using gene-specific markers IYT1 and IYT3 (in promoter region of Sub1A gene), Sub1BC$_2$ (in between *Sub1B* and *Sub1C* gene) and Sub1C173 (exon of *Sub1C* gene). Scoring was done based on presence and absence of allele [5].

Results

Submergence Evaluation

In the evaluation, the survival rate of 63 breeding lines was noted in the range of 65-100 percent. In donor lines, the maximum survival rate was recorded in IR64-Sub1 (89.2%) and minimum rate was in IR49830-7 (64.2%). In tolerant checks, FR13 had higher survival rate (96.7%) than Karkuruppan (74.6%). In intolerant checks, the maximum survival rate was 3.3 percent in IR64 and the lower rate was zero percent in IR42. Among local rice cultivars, the higher survival rate was recorded in Durga (20%) as well as zero percent rate in Gayatri, Sarala, and Savitri (0%). In morphological characterization during flowering stage, the mean value of 5 plants of each line was recorded in the range of 83.3-151.6 cm for plant height and 4.3-20.0 for tiller number [Table-1].

Table 1- Details of developed breeding lines, their parental combinations, plant survival rate, mean value of 5 plants for plant height and tiller number and presence and absence of gene-specific alleles of *Sub1* locus

Designation No./Cultivars	Parental combination	Phenotypic evaluation	Mean value		Gene-specific markers			
		Survival rate (%)	plant height (cm)	tiller number	1	2	3	4
CR2582-7-1-1-1	Varshadhan/Swarna-Sub1	65	144	8.6	+	+	+	+
CR2582-7-1-1-1	“	75	144	7.3	+	+	+	+
CR2582-7-2-1-1	“	60	125.3	5.3	+	+	+	+
CR2582-7-2-1-2	“	65	119.3	6.3	+	+	+	+
CR3015-3-1-1-1	Durga/IR64-Sub1	65	115	9.3	+	+	+	+
CR3015-4-1-1-1	“	85	106.6	8	+	+	+	+
CR2586-4-1-1-1	Durga/Samba Masuri-Sub1	60	88	14.3	+	+	-	-
CR2587-2-1-1-1	Durga/Swarna-Sub1	95	96.6	11	+	+	-	-
CR2587-3-1-1-1	“	95	101.3	10	+	+	+	-
CR3011-10-1-1-1	Savitri/IR64-Sub1	95	105	11.6	+	+	+	+
CR2585-7-1-1-1	Gayatri/Samba Mahsuri-Sub1	90	120	6	+	+	+	+
CR3013-7-1-1-1	Sarala/IR64-Sub1	80	89	20	+	+	+	+
CR3013-16-1-2-1	“	90	126.3	11.6	+	+	+	+
CR3013-18-1-1-1	“	85	151.6	8.6	+	+	+	+
CR2589-7-1-1-1-1	Gayatri/EC516602//Swarna-Sub1	75	94.3	8	+	+	H	-
CR2589-7-1-1-1-2	“	75	151.6	9	+	+	H	-
CR2589-8-1-1-1	“	100	118.3	9.3	+	+	+	-
CR2590-1-1-1-1	Gayatri/Varshadhan//IR49830-7	95	118.6	8.6	+	+	+	-
CR2590-1-1-1-2	“	65	125.6	11.3	+	+	H	-
CR2590-9-1-1-1	“	90	120	7.6	+	+	+	-
CR2590-9-1-1-2	“	100	109.6	10	+	+	+	-
CR2590-10-1-1-1	“	95	104.3	15.3	+	+	-	-
CR2590-10-1-1-2	“	95	108.6	12.6	+	+	-	+
CR2590-10-1-1-3	“	85	114	8	+	+	-	+
CR2590-11-1-1-1	“	90	113.3	6.3	+	+	+	+
CR2590-11-1-1-2	“	95	98.3	7.6	+	+	+	+
CR2590-12-1-1-1	“	60	104.6	10	+	+	-	-
CR2590-12-1-1-2	“	75	142.6	8	+	+	-	-

Table 1- Continue..

Designation No./Cultivars	Parental combination	Phenotypic evaluation	Mean value		Gene-specific markers			
		Survival rate (%)	plant height (cm)	tiller number	1	2	3	4
CR2591-2-1-1-1	Gayatri/Varshadhan/Swarna-Sub1	80	116.3	9	+	+	-	-
CR2591-2-1-1-2	“	100	106	11	+	+	+	+
CR2594-1-1-1-1	Savitri/IR49830-7//CR2232-71	75	129	5.6	+	+	+	+
CR2594-1-1-1-2	“	95	123	15	+	+	+	+
CR2594-2-1-1-1	“	80	102	8.3	+	+	+	+
CR2594-5-1-1-1	“	90	102.6	15.3	+	+	+	+
CR2594-7-1-1-2	“	65	117	11.3	+	+	+	+
CR2594-4-7-1-1-1	“	90	142	13.3	+	+	+	+
CR2595-4-1-1-2	Gayatri/IR49830-7//CR2232-85	65	88.6	5.3	+	+	+	+
CR2595-11-1-1-2	“	80	108.3	12.6	+	+	+	+
CR2530-B-1-1-1-1	Savitri//Savitri/IR49830-7	85	115.6	14.6	+	+	+	+
CR2530-B-2-1-1-1	“	95	112.3	12	+	+	+	+
CR2530-B-3-1-1-1	“	65	94.6	4.3	+	+	-	-
CR2530-B-1-3-1-1	“	90	111.6	7.3	+	+	+	+
CR2530-B-1-5-1-1	“	100	112	8.3	+	+	+	+
CR2530-B-2-1-1-1	“	90	124	6.6	+	+	+	+
CR2530-B-2-2-1-1	“	95	116	8.6	+	+	+	+
CR2530-B-2-3-1-1	“	90	112.3	12	+	+	+	+
CR2530-B-3-1-1-1	“	60	117.3	7.6	+	+	+	+
CR2530-B-3-2-1-1	“	75	117	5.3	+	+	-	-
CR2530-B-3-3-1-1	“	90	114.3	7	+	+	+	+
CR2530-B-3-5-1-1	“	70	107.3	10.3	+	+	-	+
CR2530-B-4-2-2-1	“	90	112.6	7	+	+	+	+
CR2530-B-5-1-1-1	“	75	107	8.3	+	+	+	+
CR2530-B-5-2-1-1	“	80	114	13.3	+	+	+	-
CR2530-B-5-4-1-1	“	75	106.6	11.6	+	+	+	+
CR2530-B-5-6-1-1	“	60	114.6	8.3	+	+	H	+
CR2531-B-3-1-1-1	Gayatri//Gayatri/IR49830-7	90	127.3	7	+	+	+	-
CR2532-B-1-1-1-1	Sarala//Sarala/IR49830-7	90	114.3	9	+	+	+	-
CR2532-B-1-1-2-1	“	90	118	9	+	+	+	-
CR2532-B-2-1-1-2	“	85	113.3	4.6	+	+	+	+
CR2532-B-6-2-1-1	“	60	124.6	7	+	+	+	+
CR2508-B-B-1-1-1-1	Savitri/IR49830-7	95	118	8.3	+	+	+	-
CR2508-B-B-4-1-1-1	“	80	117.3	12	+	+	+	-
CR2530-B-B-4-1-1-1	Gayatri//Gayatri/IR49830-7	70	89	10.6	+	+	+	+
Swarna-*Sub1*	Donor	70	83.3	10.6	+	+	+	-
Samba Mahsrui-*Sub1*	Donor	88.3	95.3	10.6	+	+	+	-
IR64-*Sub1*	Donor	89.2	88.3	9	+	+	+	+
IR 49830-7	Donor	76	89	10	+	+	+	+
FR13A	Tolerant check	96.7	144	9.6	+	+	+	+
Karkuruppan	Tolerant check	74.2	147	10	+	+	+	+
IR64	Intolerant check	3.3	-	-	-	-	-	-
IR42	Intolerant check	0	-	-	-	-	-	-
Gayatri	Intolerant	0	-	-	-	-	-	-
Sarala	Intolerant	0	-	-	-	-	-	-
Savitri	Intolerant	0	-	-	-	-	-	-
Durga	Intolerant	10	-	-	-	-	-	-
Varshadhan	Intolerant	5	-	-	-	-	-	-

1-IYT1; 2-IYT3; 3-*Sub1*BC$_2$; 4-*Sub1*C173

‘+’ and ‘-’ indicate presence and absence of tolerant alleles, respectively.

‘H’ -indicates heterozygous condition of alleles.

PCR

In PCR screening, positive alleles were documented for IYT1, IYT3 and Sub1BC$_2$ in rice lines which showed survival rate > 60% as well as positive or negative allele for Sub1C173 marker. Also, there were positive alleles in some intolerant lines for Sub1C gene but not for markers linked with Sub1A gene.

Discussion

As reported earlier, flash flooding and submergence are widespread in south-east Asia, Bangladesh and northeastern India, and affect at least 22 million hectares (16% of world rice lands) including 15 million hectares of potential short-duration flash floods in rainfed lowlands and 5 million hectares of deepwater rice [11]. According to Sarker et a l. [12], approximately 10 million hectares of rice lands is affected by flash floods and complete submergence in eastern India alone and rice yields in the rainfed lowlands of this region are low, averaging 2.4 t/ha. In this study, we identified rice breeding lines having highly efficient submergence tolerance amidst variations of survival rate. This variation in the survival rate is associated with the smaller rate of starch depletion during submergence and maintenance of higher level of starch in tolerant cultivars when compared to intolerant lines [1]. Moreover, crossing rice variety with different donor lines has showed variations in the survival rate in some cases. For instance, breeding lines obtained by crossing Durga with IR64-Sub1 or Samba Mahsuri-Sub1 or Swarna-Sub1 had maximum survival rate at 85 or 60 or 95 percent, respectively. Moreover, breeding lines developed by two-way cross using different variety or same variety along with donor line have accounted for highest survival rate (100%) i.e. CR2590-9-1-1-1 (Gayatri/Varshadha//IR49830-7), CR2591-2-1-1-2 (Gayatri/Varshadhan/Swarna-Sub1), CR2589-8-1-1-1 (Gayatri/EC516602//Swarana-Sub1) and CR2530-B-1-5-1-1 (Savitri//Savitri/IR49830-7) when compared with breeding line by one-way cross i.e. CR25827-1-1-1 (Varshadhan/Swarna-Sub1) (up to 75%). At the same time, breeding lines developed by one-way cross have also showed higher survival rate (up to 95%) like CR2587-2-1-1-1 (Durga/Swarna-Sub1) and CR3011-10-1-1-1-1 (Savitri/IR64-Sub1).

In morphological characterization also we found the variations in terms of plant height and tiller number among breeding lines, but there was no association in survival rate. Because breeding lines which having more plant height showed both higher and lower survival percentage. For instance, CR2589-7-1-1-1-1 line had more plant height (151.6cm) but less survival rate (75%) when compared to CR2590-10-1-1-1-1(104.3cm) accounted for 100 percent survival rate. However, in most of the lines with less than 120cm plant height, the higher survival rate was recorded in the range of 80-100 percent i.e. CR2589-8-1-1-1-1 (100%) with 118.3cm, CR2590-9-1-1-2 (100%) with 109.6, CR2530-B-1-5-1-1 (100%) with 112 cm and CR2591-2-1-1-2 (100%) with 106 cm plant height. In case of tiller number, breeding line (CR2585-7-1-1-1) having more tiller number (20) has accounted for higher survival rate (90%) as well as breeding line (CR2591-2-1-1-2) having less tiller number (5.6) for highest survival rate (100%). Meanwhile, very recently, the application of fertilizer followed by de-submergence has increased tiller number and yield than unfertilized rice plants [13]. However, in this study, we found the variation in the survival rate and plant growth among breeding lines significantly at higher and lower level. Generally, Sub1 locus do not affect characters of recipient parents after introgression but increases yield by increasing tiller numbers [14]. Supportively, some rice lines which showed 100 percent survival rate had more plant height and tiller number in this study. Even though, we found variations in survival rate, plant height and tiller number. Perhaps, these variations might have associated with the phenomenon of phenotypic plasticity in which organisms (particularly monocots) can adjust their phenotype in response to changing environmental condition. This phenomenon is controlled by rice plasticity 1 (RPL1) gene which encodes a nuclear protein of unknown function which alters the overall DNA methylation and histone modifications and affects plant response to several phyto hormones such as brassinosteroid, gibberellin and cytokinins. It matched with the rice genotype having Sub1 locus in which the plant growth is controlled through inhibition of gibberellic (GA) synthesis by the accumulation of the GA-signaling suppressors Slender Rice-1 (SLR1) and SLR1 Like 1 (SLRL1) during submergence stress and it leads to increase survival rate after stress [15]. But, in case of non-Sub1 lines under stress condition, GA synthesis occurs and promotes plant growth which ultimately leads to decrease the survival rate significantly due to lack of sufficient food source to survive [1]. Thus the variations in survival rate of rice plants under submergence condition is associated with the phyto hormone level which accounts for higher and lower survival rate [2]. According to recent report, RPL1 gene enhances variations in plant height and tiller number of rice and its reduced function provides a larger range of phenotypes for natural and/or artificial selection, both in evolution and in plant breeding [16].

In PCR screening, the results of IYT1, IYT3 and Sub1BC$_2$ markers matched with survival rate of rice lines with > 60% rather than that of Sub1C173. And also, it was found that the results obtained for Sub1BC$_2$ marker coincided with that of IYT1 and IYT3 markers. It indicates that Sub1BC$_2$ marker which linked with SUB1B gene also associated with Sub1A gene and helped to identify the tolerant plants. As reported earlier, Sub1B gene is more closely related to Sub1A than Sub1C due to the high amino acid sequence similarity at the N-terminus shared by Sub1A and Sub1B, but not Sub1C [1]. Therefore, Sub1BC$_2$ marker will be helpful in the selection of tolerant and heterozygous plants in submergence breeding program and also it accounts for heterozygous alleles.

Addition to this, following the submergence stress, all donors and breeding lines of semi-lowland rice varieties completed their life-cycle through seed setting successfully except semi-deep rice lines (Varshadhan and Durga). In which, there was no seed setting even though they attained reproductive stage due to commencement of cold season. This indicates that the long duration rice lines are not able to complete their life cycle because of delayed growth due to submergence stress when compared to short or medium duration varieties like, Swarna, Gayatri, Sarala and Savitri. Very recently, it has been reported that urea foliar spray after desubmergence significantly enhances the photosynthesis and narrows down the flowering time which led to higher grain yield and productivity in medium duration rice varieties such as IR 64-Sub1 and Swarna-Sub1 [13] but it is not for long duration varieties. Context to this, it is needed to concentrate on cold tolerance also in the long duration varieties in flood proven areas. Moreover, tall plants like Varshadhan and Durga can tolerate under submergence as well as stagnant condition when compared to short plants since they are not able to elongate if water depth remains at or above the canopy level for longer due to the Sub1-mediated suppression of elongation [5].

In conclusion, we selected many breeding lines which accounted for highest survival rate for each rice variety in this task. These breeding lines showed higher efficiency in submergence tolerance than donors and tolerant checks (FR13A and Karkuruppan). It is most important selection of highly efficient rice genotypes for cultivation in flood prone areas. Thus, a significant progress is achieved to develope rice lines (for semi-deep lowland and semi-lowland areas) with account of a higher level tolerance for submergence stress in this study. Generally improved rice varieties have not been adopted by the farmers in submergence prone areas because of the sensitivity of rice and the prevalence of the stress. So, submergence tolerance has been an important breeding objective for rainfed lowland areas of Asia. Therefore, this work with local high-yielding rice varieties developed for submergence tolerance will be very good compliment to Indian farmers facing the economic losses by submergence, particularly in coastal region of eastern India.

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Conflicts of Interest: None declared.

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