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Screening of rice genotypes for submergence stress at seedling stage

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Abstract: Submergence tolerant rice varieties are essential for rainfed low land rice ecosystems which can obstruct the sudden rise of water level. Appropriate screening procedures are significant to select donor parents, having *Sub1* genes for the development of submergence tolerance, high yielding rice varieties. The morphological screening was performed with 14 rice lines along with 4 check varieties at glass house of Bangladesh Institute of Nuclear Agriculture (BINA) for submergence tolerance in rice. To Evaluate the performance of rice genotypes under submergence condition, genotypes were completely submerged under 40 cm water for 24 days. Data was recorded at 7 days after sowing (DAS), 14 DAS and 24 DAS. The progenies RC-193, BRRI dhan51 and Binadhan-11 performed maximum (99%) survival rate while these three genotypes secured 3 (tolerant) of submergence survival score (SES: 1-9) with the lower elongation rates viz. 13%, 46% and 49% respectively. RC-193 shown same survival rate with BRRI dhan51 and Binadhan-11. Even it performed lower elongation rates compared to these two well-known submergence tolerant rice varieties. So based on this evaluation, the line RC-193 was identified as submergence tolerant line and the line will be used as donor and crossed with recurrent parent to develop submergence tolerant rice variety.

Key words: Screening, genotypes, stress, seedling stage, submergence and rice.

Introduction

Submergence stress adversely affects poor farmers living on 15 million ha of rice-growing areas in the rainfed lowlands in south and south-east Asia (Steptiningsih *et al.*, 2009). Fortunately, local rice landraces cherished by farmers include accessions adapted to extremes in water availability, including tolerance to progressive flooding or rapid submergence. But traditional varieties adapted to the submergence prone environments are low yielding due to their low tillering ability, long droopy leaves, susceptibility to lodging and poor grain quality (Iftekharuddaula *et al.*, 2011). However, these submergence tolerant landraces produce less than 2 ton/ha where yields of advanced semi-dwarf varieties are 6–8 ton/ha. Unfortunately, the popular "mega varieties" grown in large areas of Asia are sensitive to complete submergence and usually die within 7 days of complete inundation and their yield can be severely reduced because of high mortality, lower tillering, reduced panicle size and high sterility (Ismail *et al.*, 2008 and Sing *et al.*, 2009).

Submergence occurred by monsoon flood, flash flood and tidal flood Submergence occurs when a large proportion of the pore spaces in the soil are occupied by water which limits the diffusion of oxygen and gas exchange between the soil, plants and atmosphere and resulted in decreased growth of roots and their functioning, thus negatively affecting the plant growth and survival (Pradhan *et al.*, 2013).

Therefore, the great urgency task for rice breeders is to research and improve the rice varieties that can resist to adverse harsh conditions like submergence. Overall objective of the research work was designed to identify the perfect donor with *Sub1* gene for marker assisted selection (MAS) to improve rice genotypes for low laying areas.

Materials and Methods

The experiments in this study were conducted at the glasshouse with ambient temperature and normal light conditions at the glasshouse and experimental field at Plant Breeding Division in Bangladesh Institute of Nuclear Agriculture (BINA), BAU campus, Mymensingh-2202. Fourteen pedigree advance rice lines were imported from IRRI (Linh *et al.*, 2013), checks varieties from BINA and BRRI were used in this study (Table 1).

Table 1. List of rice genotypes used in the screening

Sl. No.	Genotypes	Type and Source
1	RC-227	Advanced line, IRRI
2	RC-229	Advanced line, IRRI
3	RC-225	Advanced line, IRRI
4	RC-217	Advanced line, IRRI
5	RC-222	Advanced line, IRRI
6	RC-291	Advanced line, IRRI
7	RC-248	Advanced line, IRRI
8	RC-251	Advanced line, IRRI
9	RC-249	Advanced line, IRRI
10	RC-250	Advanced line, IRRI
11	RC-252	Advanced line, IRRI
12	RC-253	Advanced line, IRRI
13	RC-292	Advanced line, IRRI
14	RC-193	Advanced line, IRRI
15	Binadhan-6	High yielding variety, BINA
16	Binadhan-11	Submergence tolerant variety, BINA
17	Binadhan-12	Submergence tolerant variety, BINA
18	BRRI dhan51	Submergence tolerant variety, BRRI

Here, Genotypes 1-14: Advanced lines; Genotype 15-18: Check varieties.

Seedlings after 7 days under 22 cm submergence

Seedlings after 24 days under 40 cm complete submergence

Figure. 1. Screening of genotypes for submergence tolerance followed Standard Evaluation System (SES) (IRRI, 2002)

The arrangement needs rectangular mini-trays with nylon net at bottom of size 30 cm x 25 cm x 5 cm having 30 holes and filled with fertilized soil (Fig. 1). Pregerminated seeds of each fourteen advanced lines along with four check varieties were placed on soil surface of mini-frames (Cuc *et al.*, 2012) (Fig. 1).

The mini-frames were submerged into sustained water bath containing 22 cm tap water. There were two replications. After 7 days, mini-frames with seedlings were transferred and submerged into fiber tank containing 40 cm water (Fig. 1). Standard Evaluation System (SES) score for submergence tolerance in rice (IRRI, 2002) were followed to evaluate the genotypes (Table 2). Data of seedlings was recorded at 7 days after sowing (DAS), 14 DAS and 24 DAS (Azarin *et al.*, 2016 and Cuc *et al.*, 2012).

Table 2. Standard evaluation system of rice for determination of survival score (IRRI, 2002)

Survival (%)	Score	Description	Tolerance
100	1	Minor visible symptom of injury	Highly tolerant
95-99	3	Some visible symptom of injury	Tolerant
75-94	5	Moderate injury	Moderately tolerant
50-74	7	Severe injury	Susceptible
0-49	9	Partial to complete death	Highly susceptible

Stress indices were calculated as follows:

(i) Tolerance score (TS) = Scored on the basis of percent plant survival (Toojinda *et al.*, 2003)

(ii) Percent plant survival (%) = (Total number of survived seedlings/Total number of seedlings before submergence) × 100 (Toojinda *et al.*, 2003)

(iii) % Elongation = {(Plant height before submerge - Plant height after de-submerge)/ Plant height after de-submerge} × 100 (Toojinda *et al.*, 2003).

Results

The performance of the different agro-morphological characters under submerged condition at the early seedling stage could be effectively employed for screening rice genotypes. In present study, multiple rice genotypes including several high yielding varieties from BINA and BRRI and fourteen advanced lines from IRRI were screened for submergence tolerance at early seedling stage. The submergence related traits such as percent plant survival, shoot length and root length were recorded. A similar observation was recorded by Tojinda *et al.*, 2003.

In the evaluation, the survival rate of genotypes (Table 3) was noted in the range of 22-100 percent (Bharathkumar *et al.*, 2015). Regarding submergence tolerance, all the entities survived 100% at 8^{th} day after complete submergence while they showed wider variation after 14 days and 24 days of stressed condition (Fig. 2).

At 15^{th} day of submerged condition, the line RC-250 showed minimum (81%) survival. The lines RC-229, RC-225, RC-217, RC-222, RC-248, RC-251, RC-221, RC-292, RC-193, BRRI dhan51 and Binadhan-11 performed 100% survival rate. The rest genotypes showed 88%-94% survival at 15^{th} day of complete submerged condition. At the 25^{th} day of complete submergence, the maximum (99%) survival rate was recorded in RC-193, BRRI-dhan51 and Binadhan-11 and minimum (22%) survival percentage was observed in the line RC-250 and RC-221. More than 80% survival rate was observed to the genotypes RC-222, RC-291, RC-251, RC-252 and RC-292 while others showed 23%-79% survival rate.

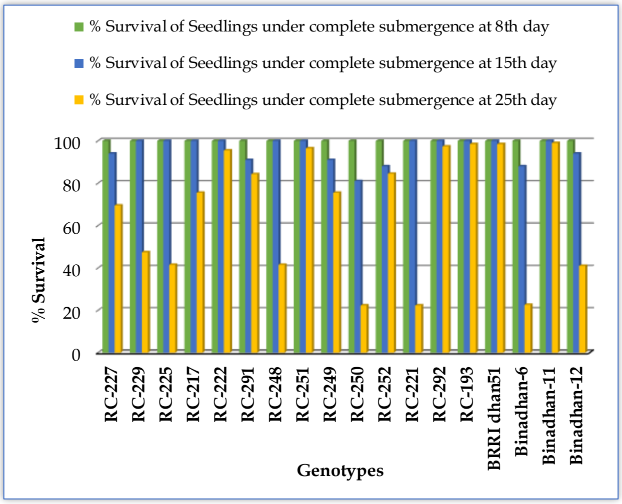

Figure 2. Survival percentage of genotypes under complete submergence for 24 days

Table 3: Survival percentage (%) of Genotypes for Submergence Tolerance

Genotypes	% Survival of Seedlings under Complete Submergence		
	At 8^{th} day	At 15^{th} day	At 25^{th} day
RC-227	100	94	69.5
RC-229	100	100	47.4
RC-225	100	100	41.5
RC-217	100	100	75.5
RC-222	100	100	95.5
RC-291	100	91	84.3
RC-248	100	100	41.5
RC-251	100	100	96.5
RC-249	100	91	75.5
RC-250	100	81	22.3
RC-252	100	88	84.5
RC-221	100	100	22.3
RC-292	100	100	97.4
RC-193	100	100	98.5
BRRI-51	100	100	98.5
Binadhan-6	100	88	22.6
Binadhan-11	100	100	99.0
Binadhan-12	100	94	41.0

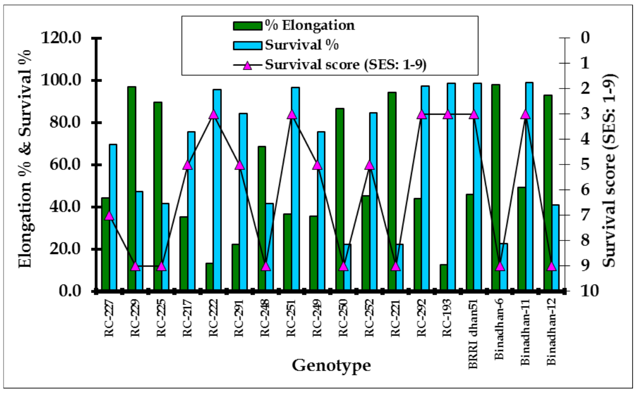

Figure 3. Performance of rice genotypes with regard to elongation (%), survival (%) and survival Score (1-9) under submerged condition

Genotypes were compared on the basis of elongation (%), survival (%) and submergence survival score (1-9) (Table 4). Highest survival rates were observed in the genotypes RC-193 (98.5%), BRRI dhan51 (98.5%) and Binadhan-11 (99%) with the lower elongation rates viz. 13%, 46% and 49%, respectively (Fig. 3).

On the other hand, these three genotypes secured 3 (tolerant) of submergence survival score (SES: 1-9). The genotypes RC-222, RC-291, RC-251, RC-252 and RC-292 showed more than 80% survival rate while their elongation rates were 13%, 22%, 37%, 45% and 44%, respectively. Among the tested lines RC-222, RC-251 and RC-292 were submergence tolerant (3) and the lines RC-291 and RC-252 were scored 5 (Moderately tolerant). Rest of the genotypes performed <80% survival rates and the elongation percentage range from 35-97%. Among the line, RC-217 was moderately tolerant (5) while all the rest were susceptible (7) to highly susceptible (9) genotypes (Fig.3).

Table 4. Survival percentage (%) of % Elongation and SES of Genotypes for Submergence Tolerance

Genotypes	Before submergence (7 day old seedlings)			After 18 days complete submergence (25 day old seedlings)			% Elongation	Survival %	Survival score (SES: 1-9)
	Shoot Length (cm)	Root Length (cm)	Plant height (cm)	Shoot Length (cm)	Root Length (cm)	Plant height (cm)			
	Mean	Mean	Mean	Mean	Mean	Mean			
RC-227	14.4	5.7	20.1	24.5	11.5	36.0	79	69.5	7
RC-229	13.1	4.8	17.9	24.8	10.5	35.3	97	47.4	9
RC-225	16.8	5.4	22.2	29.5	12.5	42.0	89	41.5	9
RC-217	12.6	6.1	18.8	25.1	10.8	35.8	91	75.5	5
RC-222	17.0	5.0	22.0	15.7	9.3	25.0	13	95.5	3
RC-291	17.1	6.7	23.7	21.0	8.0	29.0	22	84.3	1
RC-248	11.7	6.7	18.4	23.1	8.0	31.1	69	41.5	9
RC-251	16.3	6.1	22.4	22.9	7.8	30.6	37	96.5	3
RC-249	18.2	5.3	23.5	23.7	8.3	31.9	36	75.5	3
RC-250	10.9	6.7	17.7	23.5	9.5	33.0	87	22.3	9
RC-252	18.7	6.3	25.0	27.3	9.0	36.3	45	84.5	3
RC-221	14.4	5.9	20.3	28.1	11.3	39.4	94	22.3	9
RC-292	19.3	6.3	25.5	28.0	8.8	36.7	44	97.4	1
RC-193	18.8	5.8	24.6	17.3	10.5	27.8	13	98.5	3
BRRI dhan51	17.5	6.7	24.2	28.4	7.0	35.4	46	98.5	3
Binadhan-6	11.9	5.0	16.9	24.9	8.5	33.4	98	22.6	9
Binadhan-11	13.3	6.7	20.0	22.4	7.5	29.9	49	99.0	3
Binadhan-12	12.5	7.1	19.6	25.1	12.8	37.8	93	41.0	9

Discussion

The ideal response to flooding is submergence tolerance (survival under water) together with some elongating ability (Mackill *et al.*, 2010; Bailey-Serres and Voesenek, 2010). To identify novel sources of submergence tolerant donor parents for breeding program, artificial screening was a faster and easy program. In current study, fourteen advanced lines from IRRI together with four high yielding varieties from BINA and BRRI screened for submergence tolerance at early seedling stage.

In the evaluation, all the genotypes were survived (100%) at 8th day after complete submergence and showed 80%-100% survival at 15th day after submerged condition. Bharathkumar *et al.,* (2015) evaluated some progenies under submerged condition and observed that the survival rate of 63 genotypes ranged of 65-100%. At the final scoring at 25th day of complete submergence stress elongation (%), survival (%) and submergence survival score (1-9) were compared. The progenies RC-193, BRRI dhan51 and Binadhan-11 gave maximum (99%) survival rate while these three genotypes secured 3 (tolerant) of submergence survival score (SES: 1-9) with the lower elongation rates viz. 13%, 46% and 49% respectively. The major physiological trait involved in submergence tolerance is maintenance of minimum elongation growth during submergence. Addition of GA increased elongation and reduced survival of even submergence tolerant lines. Plant height did not increase much in *Sub1* introgressed cultivars, resulted significantly lower elongation compared to other genotypes (Sarkar and Bhattacharjee, 2011). A few studies presented that survival is positively associated with limited stem elongation. Lang *et al.,* (2013) observed that under submerged conditions, shoot elongation occurred in all genotypes but to a lesser extent in the tolerant genotypes than in the sensitive genotypes. Tolerant genotypes showed suppressing of shoot elongation may be for energy conservation under floodwater. Development of oxidative stress is the common response to hypoxia and anoxia in plant tissues. The possibility to grow under submergence condition (hypoxia and anoxia) is provided by the locus *Sub1A*, encodes by ethylene-response factor transcription (Xu *et al.,* 2006; Mickelbart *et al.,* 2015).

For the screening, the progenies were completely submerged for 25 days under 40 cm water. The progenies RC-193, BRRI dhan51 and Binadhan-11 performed maximum (99%) survival rate while these three genotypes secured 3 (tolerant) of submergence survival score (SES: 1-9) with the lower elongation rates viz. 13%, 46% and 49% respectively. BRRI dhan51 and Binadhan-11 are the popular submergence tolerant rice varieties. RC-193 shown same survival rate with BRRI dhan51 and Binadhan-11. Even it performed lower elongation rates compared to these two well-known submergence tolerant rice varieties. So based on this selected lines the line RC-193 was identified as submergence tolerant and this advanced lines would be used as donors and crossed with recurrent parents to develop submergence tolerant variety.

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