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ASSESSMENT OF PHENOTYPIC AND GENOTYPIC DIVERSITY IN RICE FOR SALINITY TOLERANCE AT REPRODUCTIVE STAGE

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Abstract

Soil salinity has turned out to be an important global concern, which affects tenable rice production in many agricultural countries like Bangladesh. A cross was made between Binadhan-7 with FL-478 for developing salt tolerant advance lines to assess phenotypic and genotypic diversity in rice for salinity tolerance. Twenty advance rice lines (Binadhan-7/FL-478) along with check varieties were screened for salinity tolerance at reproductive stage using sea site saline water having electrical conductivity (EC) 10 dS m^{-1}. Among the twenty rice lines, seven lines were tolerant, three were moderately tolerant, six were susceptible, four were highly susceptible on the basis of phenotypic evaluation. Seven simple sequence repeat (SSR) markers linked with salt tolerance quantitative trait loci were used for investigation of salt tolerant rice lines. The result revealed that an average number of 6.286 alleles per locus were detected, with polymorphism information content (PIC) values ranging from 0.672 (RM490) to 0.838 (RM562). The highest gene diversity value (0.812) was found in RM562 and the lowest (0.684) was in RM490. A dendrogram constructed from the genetic distance of the genotypes produced four distinct clusters of twenty rice genotypes. Considering both phenotypic and genotypic observation, seven genotypes *viz.*, Binadhan-10, FL-478, SL-51, SL-56, SL-77, EFSD-59 and IZSD-45 were identified as salt-tolerant; on the other hand, EFSD-21, SL-28, SL-32, SL-10, BRRI dhan28, and Binadhan-7 were identified as salt-susceptible. The identified salt-tolerant rice genotypes could be used in the improvement of rice breeding programs.

Keywords: Rice, Salinity Tolerance, Phenotypic and Genotypic Diversity, Reproductive Stage

Introduction

Rice (*Oryza sativa* L.) is the staple food of an estimated 3.5 billion people worldwide (Amagliani *et al.*, 2016). It is also the most extensively cultivated cereal crop and a vital food for about 156 million people in Bangladesh (Shelley *et al.*, 2016) which

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inevitably meets most of the nutritional demand of the majority of its people. Rice production in the world has become stagnant due to different biotic and abiotic causes. Abiotic stresses as like floods, drought, cold and saline excesses in precipitation increasingly limit food, fiber, and forest production worldwide (Collard *et al.,* 2008). Among them salinity is a major abiotic stress which bring negative effect in crop production worldwide (Abdallah *et al.,* 2016). Bangladesh is not beyond the change. Southern part of Bangladesh is facing this salinity problem which affects the rice crop production in those areas. Soil salinity affects 1.06 million hectares of arable land of Bangladesh (SRDI, 2010). Khulna, Satkhira, Bagerhat, Barguna, Patuakhali, Noakhali, Chittagong and some other districs are the coastal areas of Bangladesh where crop yields are drastically decreases due to salinity problem.

In Bangladesh, existing released varieties for salt tolerance named BRRI dhan40, BRRI dhan41, BRRI dhan47, BRRI dhan53, BRRI dhan54, BRRI dhan55, BRRI dhan61, BRRI dhan67, BRRI dhan73, Binadhan-8 and Binadhan-10 within the last ten years, which are not up to the mark in term of grain quality and tolerance as well. However, none of them are fine grained variety which is a good option for the farmers in the coastal regions. So, for the farmer's choice, development of salt tolerant short duration and fine grained rice variety is a prime need in the coastal region. That's why a cross was made between Binadhan-7 with FL-478 to introgress salt tolerant genes into a potential popular fine grained early rice variety.

Soil salinity affects all stages of growth and development of rice plant, but salinity at reproductive stage declines grain yield much more than salinity at the vegetative stages. (Singh *et al.,* 2021). Therefore, screening for salt tolerance at reproductive stages has been considered to be more useful. In order to manage rice productivity in the field and to develop salt-tolerant rice varieties, tolerance during the reproductive stage of the crop is essential (Ahmadizadeh *et al.,* 2016; Hussain *et al.,* 2017). Breeding for salinity tolerance in rice requires suitable screening techniques and appropriate molecular marker technology (Gregorio *et al.,* 2002).

Genetic diversity is a pre-requisite for any crop improvement program since it facilitates the development of improved recombinants. For assessing changes in genetic diversity over time and space molecular marker based genetic diversity analysis has potential process (Duvick, 1984). In order to create segregating progenies with maximum genetic variability for further selection and introgression of desirable genes from diverse germplasm into the available genetic base, genetic diversity analysis is used to estimate and establish genetic relationships in germplasm collections. It also identifies diverse parental combinations (Thompson *et al.,* 1998; Islam *et al.,* 2012). Many crop species including rice for which SSR markers have been developed. These markers are becoming more popular because of their technical simplicity, the small amount of starting DNA required, relatively

low cost, the level of allelic diversity and high power of resolution (Panaud *et al.*, 1995 and McCouch *et al.*, 1997). For an efficient selection of salt-tolerant genotypes both at the seedling and the reproductive stages, SSR markers can be widely used. The current study set out to assess the diversity of twenty rice genotypes using phenotypic and molecular markers.

Materials and Methods

Plant materials

Experiments were conducted at the glasshouse and Molecular Plant Breeding Laboratory of Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during October 2020 to June 2021. A total of twenty rice genotypes (BC_2F_7 developed from crossing between Binadhan-7 and FL-478) along with three check varieties (BRRI dhan28, BRRI dhan58 and Binadhan-10) were used in this experiment (Table 1).

Table 1. List of rice genotypes used in the experiment

Sl. No.	Name of the rice lines/variety	Type	Origin
1	SL-10		BINA
2	SL-28		BINA
3	SL-32		BINA
4	SL-39		BINA
5	SL-44		BINA
6	SL-51		BINA
7	SL-56		BINA
8	SL-57	Advanced line	BINA
9	SL-77		BINA
10	EFSD-21		BINA
11	EFSD-58		BINA
12	EFSD-59		BINA
13	IZSD-10		BINA
14	IZSD-45		BINA
15	IZSD-44		BINA
16	FL-478	Salt-tolerant parent	BINA
17	Binadhan-7	Salt-susceptible parent	BINA
18	BRRI dhan28	Susceptible check	BRRI
19	BRRI dhan58	Susceptible check	BRRI
20	Binadhan-10	Tolerant check	BINA

BINA= Bangladesh Institute of Nuclear Agriculture, BRRI = Bangladesh Rice Research Institute

Phenotypic screening

The genotypes were evaluated for their tolerance to salinity using International Rice Research Institute (IRRI) developed standard protocol (Gregorio *et al.*, 1997) at

reproductive stage. The experimental design was completely randomized design (CRD) with three replications. Two conditions were maintained: control (without salt stress) and salinized at EC 10 dS m^{-1}. Rice genotypes were scored based on standard evaluation system (SES) (IRRI, 2002) on flag leaf symptoms at reproductive stage (Table 2).

Table 2. Modified standard evaluation system (SES) of visual salt injury at reproductive stage

Score	Observation (Flag-leaf damage percentage)	Tolerance
1	No flag leaf symptoms	Highly tolerant
3	1-10% damage	Tolerant
5	11-25% damage	Moderately tolerant
7	26-50% damage	Susceptible
9	More than 51% damage	Highly Susceptible

Agronomic parameters and data visualization

Rice yield component data were recorded from the reproductive stage in both control and salinized conditions. Data were recorded on days to first flowering, days to maturity, plant height (cm), number of total tillers $plant^{-1}$**,** number of effective tillers $plant^{-1}$, panicle length (cm)**,** number of filled grains $plant^{-1}$, number of unfilled grains $plant^{-1}$, thousand seed weight (g) and yield (t ha^{-1}). The reduction (%) of plant character was calculated with the following equation:

$$\text{Reduction percentage} = \frac{\text{Traits in normal condition} - \text{Traits in saline condition}}{\text{Traits in normal condition}} \times 100$$

Genotyping of rice genotypes for salinity tolerance through SSR markers

Seven SSR markers were selected for polymorphisms, clear DNA band to use in final polymerase chain reaction (PCR) by amplifying template DNA for this study of the rice genotypes (Table 3). Genomic DNA was extracted from healthy 21-day old leaf samples of the twenty rice genotypes using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method. The extracted DNA was quantified using a Nanodrop and diluted to a final concentration of 50 ng μl^{-1}. A 10 µl PCR reaction mixture containing 2 µl of genomic DNA template, 3.2 µl of PCR master mix, 1 µl of forward primer, 1 µl of reverse primer and 2.8 µl nuclease free water was prepared. PCR program was maintained as initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation for 30 seconds at 94 °C, annealing at (55-60) °C for 45 seconds, extension at 72 °C for 2 minutes and final extension at 72 °C for 7 minutes. PCR amplified products were separated in 8% polyacrylamide gel at 90 V for 1.5 h in 1X TBE buffer and stained with ethidium bromide

(0.5 µg ml^{-1}). DNA banding patterns were visualized using Alpha Imager documentation system. The amplified DNA fragment size was determined by comparing the migration distance of 100-base pair DNA Ladder.

Table 3. Details of the SSR markers used for genotyping

Marker Name	Location on Chromosome	Forward (F) and Reverse (R)	Primer Sequence (5´ – 3´)	Annealing Temperature (°C)	Expected PCR Product Size
RM3412b	1	F	TCATGATGGATCTCTGAGGTG	55	211
		R	GGGAGGATGCACTAATCTTTC		
RM493	1	F	GTACGTAAACGCGGAAGGTGACG	55	211
		R	CGACGTACGAGATGCCGATCC		
RM1287	1	F	GGAAGCATCATGCAATAGCC	55	162
		R	GGCCGTAGTTTTGCTACTGC		
RM490	1	F	ATCTGCACACTGCAAACACC	55	101
		R	AGCAAGCAGTGCTTTCAGAG		
RM10793	1	F	GACTTGCCAACTCCTTCAATTCG	60	123
		R	TCGTCGAGTAGCTTCCCTCTCTACC		
RM562	1	F	CACAACCCACAAACAGCAAG	55	243
		R	CTTCCCCCAAAGTTTTAGCC		
AP3206	1	F	TTCTCATCGCACCATTCTG	55	375
		R	GGAGGAGGAGAGGAAGAAG		

Analysis of SSR markers

Molecular weight for each amplified allele was measured in base pair using AlphaEaseFC 4.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and polymorphism information content (PIC) values were analyzed using PowerMarker 3.25 (Liu and Muse, 2005). The Nei's genetic distance (Nei *et al.*, 1983), coefficient and a dendrogram representing the genetic relationships between genotypes based on the unweighted pair group method with arithmetic mean (UPGMA), which as constructed using the program of MEGA-X software.

Results

Screening based on variation in visual injury of flag leaf

The degree of flag leaf injury and necrosis caused by salinity were ascertained by SES score values on the basis of growth symptoms. Among the 20 rice genotypes, 7 genotypes scored 3 (tolerant), 3 genotypes scored 5 (moderately tolerant), 6 genotypes scored 7 (susceptible), 4 genotypes scored 9 (highly susceptible) (Fig. 1 and Table 4).

Control condition Salinized condition

Fig 1. Performance of rice genotypes under control and salinized setup

Table 4. Performance of twenty rice genotypes under salinized condition (EC: 10 dS m^{-1}) grown in sustained saline water condition at reproductive stage

Sl. No.	Genotypes	SES Score	Tolerance	Sl. No.	Genotypes	SES Score	Tolerance
1	SL- 10	7	S	11	EFSD- 58	7	S
2	SL- 28	9	HS	12	EFSD- 59	3	T
3	SL- 32	9	HS	13	IZSD- 10	7	S
4	SL- 39	5	MT	14	IZSD- 44	3	T
5	SL- 44	5	MT	15	IZSD- 45	7	S
6	SL- 51	3	T	16	FL- 478	3	T
7	SL- 56	3	T	17	Binadhan- 7	7	S
8	SL- 57	5	MT	18	BRRI dhan28	9	HS
9	SL- 77	3	T	19	BRRI dhan58	7	S
10	EFSD- 21	9	HS	20	Binadhan-10	3	T

In 1-9 scale, where 1 = highly tolerant (HT), 3 = tolerant (T), 5 = moderately tolerant (MT), 7 = susceptible (S) and 9 = highly susceptible (HS)

Days to maturity

Due to salt stress, salinized plant mature earlier than the non-salinized plant. Considering the days to maturity, SL-51, SL-10, SL-57, EFSD-58, IZSD-10, IZSD-44 and SL-28 showed higher (>5.0%) reduction. But SL-44, FL-478, BRRI dhan28, Binadhan-10, Binadhan-7, EFSD-21, SL-56, SL-32, EFSD-59, BRRI dhan58, SL-39, IZSD-45 and SL-77 showed lower reduction (<5.0%) (Fig. 2).

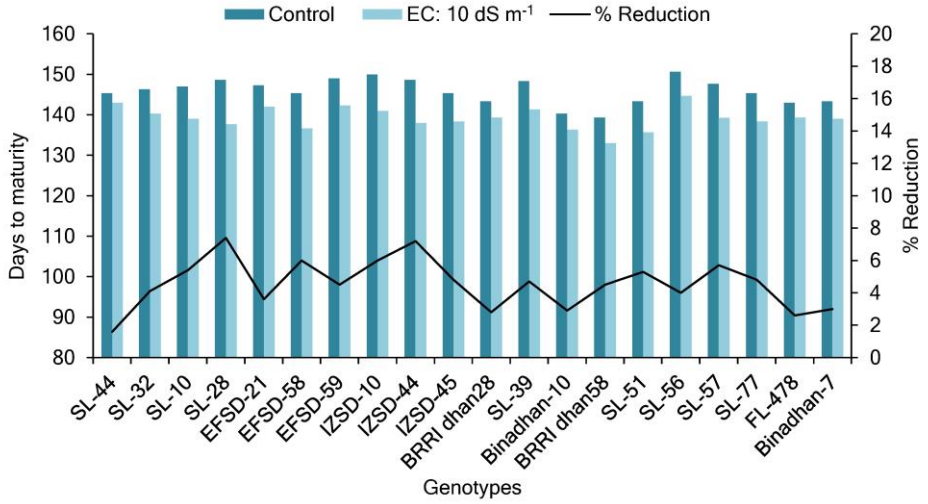

Fig. 2. Days to maturity reduction of rice genotypes under the salinity level at EC 10 dS m^{-1}

Plant height

Plant height reduction percent of different rice genotypes was influenced by salinity. At 10 dS m^{-1} salinity level, EFSD-59, EFSD-58, IZSD-10, FL-478, SL-10, SL-56 and SL-32 showed higher (>5.0 %) reduction. On the other hand, SL-77, SL-57, SL-51, IZSD-44, SL-39, BRRI dhan58 and BRRI dhan28 showed lower reduction (<5.0 %) (Fig. 3).

Number of effective tillers plant^{-1}

Salinity level had significant effect on number of effective tillers plant^{-1}. The maximum number of effective tillers plant^{-1} of all genotypes was found in control condition and the minimum was at 10 dS m^{-1} salinity levels. Due to salinity the effective tillers reduction was varied from 10.2 to 35.8%. At 10 dS m^{-1} salinity levels, other than check variety, the lowest effective tillers reduction percent were found in SL-57, SL-56 and IZSD-10 and the highest was found in BRRI dhan28, SL-44, EFSD-58 and IZSD-45 (Fig. 4). The decrease in number of tillers might be due to the toxic effect of salt on plant growth.

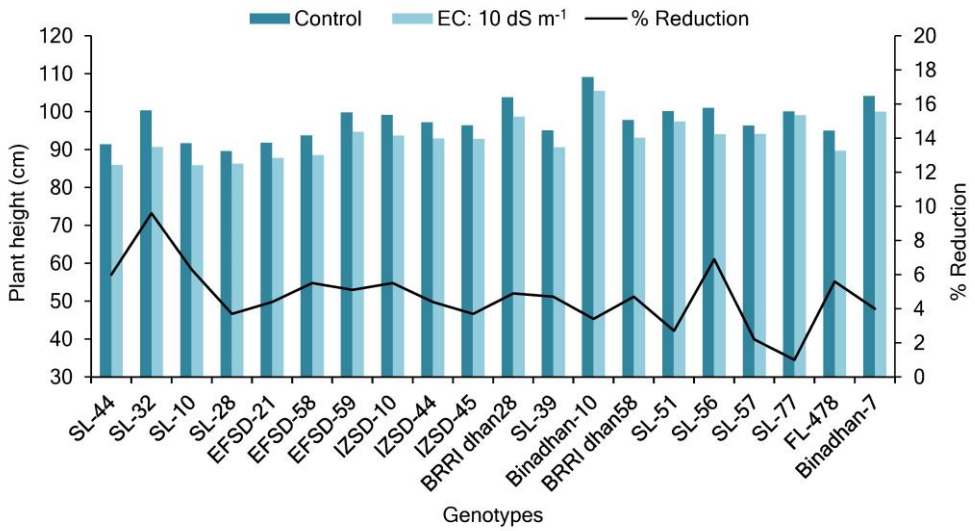

Fig. 3. Plant height reduction (%) of rice genotypes under the salinity level at EC 10 dS m^{-1}.

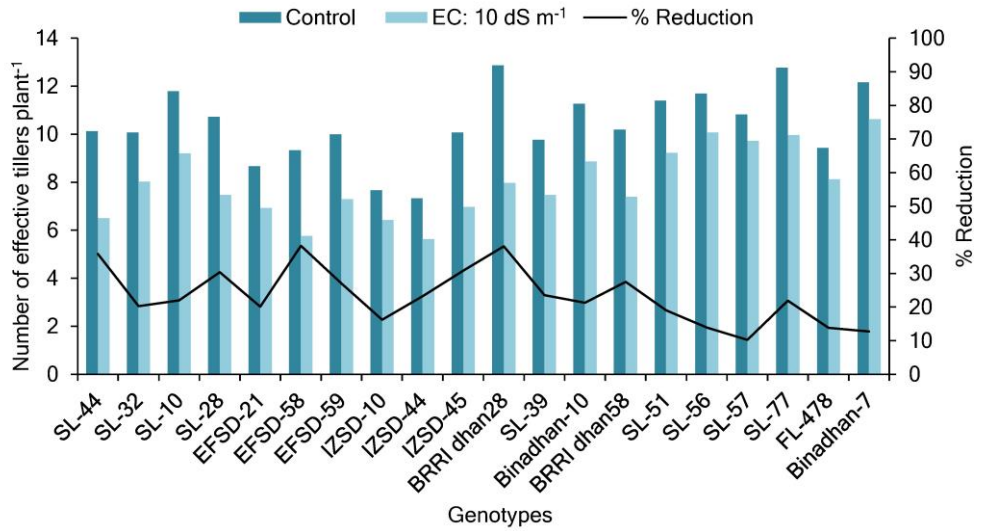

Fig. 4. Number of effective tillers reduction (%) of rice genotypes under the salinity level at EC 10 dS m^{-1}.

Number of filled grain plant^{-1}

The number of filled grain plant^{-1} is the most influential yield component, and most closely correlated with seed yield. Data regarding filled grain plant^{-1} of rice as influenced by different salinity levels. Results showed that the highest number of filled grains plant^{-1} (610.9) was found in Binadhan-7 at control and the lowest (350) was found in SL-39 at 10 dS m^{-1} salinity which has 23.60% decrease in compare with control treatment. At 10 dS m^{-1} salinity, the minimum filled grain reduction was found in SL-51, SL-56 and SL-77 (20.40%, 20.90% and 20.70% respectively) and the maximum (38.30%) was found in SL-10 compared to that of check variety (Fig. 5). Considering no. of filled grains, FL-478, SL-51, Binadhan-10, SL-77, SL-56 and SL-57 showed lower reduction (<2.60%) but SL-39, EFSD-59, EFSD-58, IZSD-45, SL-32, BRRI dhan58 and SL-10 showed higher (>23.60 %) reduction.

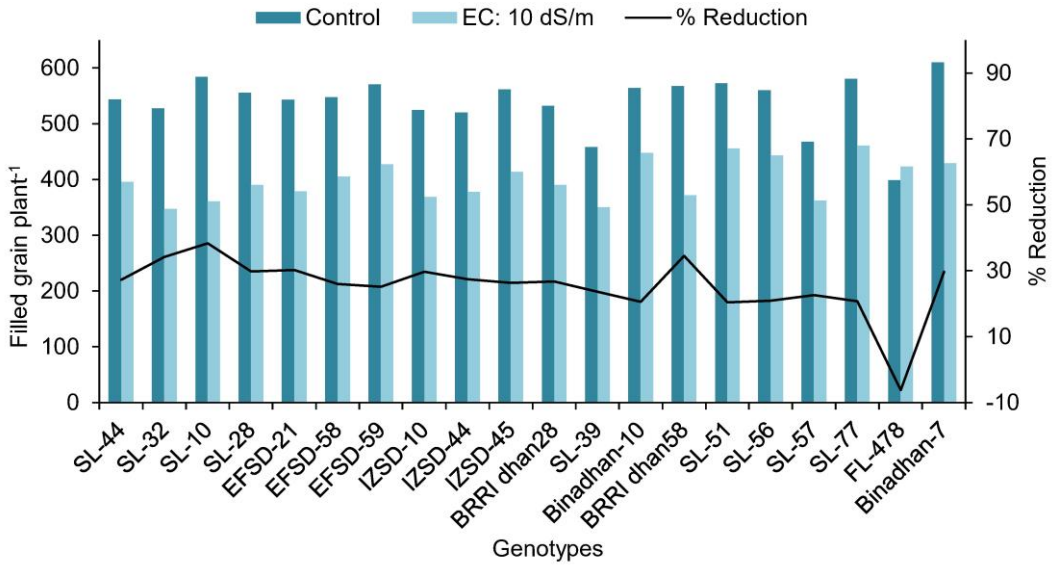

Fig. 5. Number of filled grain reduction (%) of rice genotypes under the salinity level at EC 10 dS m^{-1}.

Grain yield (t ha^{-1})

The results indicated that seed yield of rice was significantly influenced by salinity level. The maximum seed yields of all genotypes were found in control and the minimum were at 10 dS m^{-1} salinity. At control, the maximum seed yield (5.72 t ha^{-1}) was found in

SL-51 while at 10 dS m^{-1} salinity, the maximum seed yield (5.85 t ha^{-1}) was found in SL-77 and the minimum (1.7 t ha^{-1}) was found in SL-32 other than check variety. SL-51 (4.09 t ha^{-1}) and SL-56 (3.56 t ha^{-1}) also showed better seed yield at 10 dS m^{-1} salinity (Fig. 6). Other than the check variety, the minimum seed yield reduction (22.7%) was found in SL-77 and the maximum (57.8%) was found in SL-32 at 10 dS m^{-1} salinity level. SL-51 (28.5%) and SL-56 (33.6%) also showed better result compared to other genotypes. Considering yield (t ha^{-1}), SL-77, SL-51, FL-478, SL-56, Binadhan-10 and EFSD-59 showed reduction less than (<38.5%). On the other hand, SL-44, IZSD-45, IZSD-10, SL-28, BRRI dhan28, Binadhan-7, SL-32 and SL-10 showed reduction more than (>43.5%).

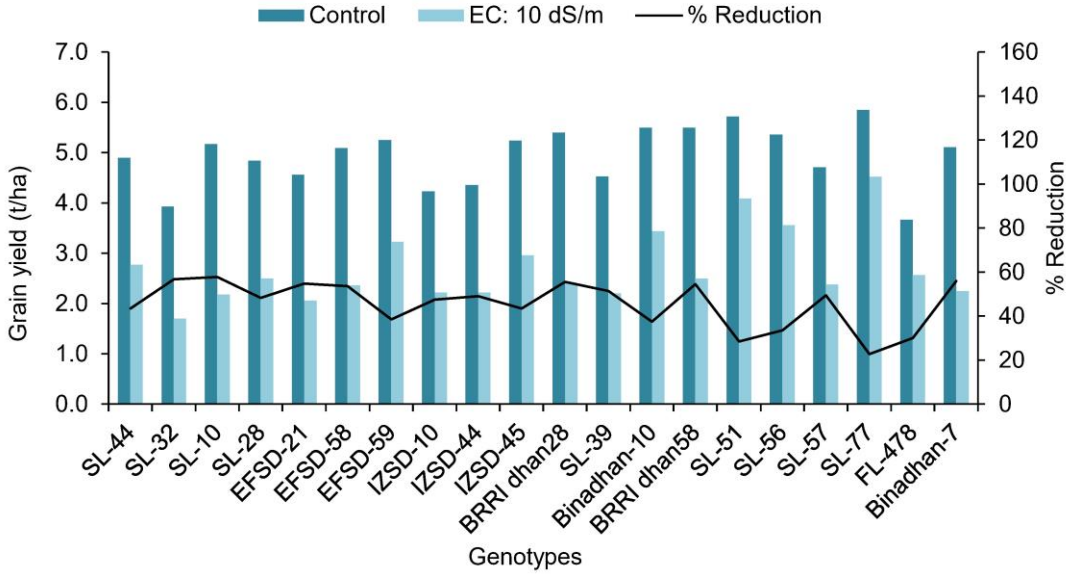

Fig. 6. Grain yield reduction (%) of rice genotypes under the salinity level at EC 10 dS m^{-1}.

Screening of rice genotypes for salinity tolerance through SSR markers

Using seven markers across twenty genotypes, 44 alleles were identified. The loci RM562 and RM1287 had the highest number of alleles (8), whereas the loci RM490 had the lowest number of alleles (4). The average value of the allele was 6.286 (Table 5). The highest genetic diversity was found for RM562 (0.812) and lowest for RM490 (0.684) (Table 5). It was observed that marker detecting the lower number of alleles showed lower gene diversity than those which detected higher number of alleles which revealed higher gene diversity. Major allele is defined as the allele with the highest frequency and also known as most common allele at each locus. On an average 32.10% of the twenty genotypes

shared a common major allele ranging from 20% (RM562) to 40% (RM490 and RM3412b) at each locus (Table 5). PIC value is a reflection of allele diversity and frequency among the varieties that can be evaluated on the basis of its alleles. It varied significantly for all the studied SSR loci. In the present study, the level of polymorphism among the 20 genotypes was evaluated by calculating PIC values for each of the 7 loci. The PIC value ranged from 0.672 (RM490) to 0.838 (RM562) with an average of 0.749 per locus (Table 5).

Table 5. Allele number, allele size, frequency, gene diversity and PIC of twenty rice genotypes for seven SSR markers

Locus name	No. of Allele	Major Allele Frequency	Gene Diversity	PIC
RM493	6	o.35	0.732	0.736
RM490	4	0.4	0.684	0.672
RM10793	6	0.3	0.746	0.753
RM3412b	6	0.4	0.694	0.688
RM1287	8	0.3	0.789	0.811
RM562	8	0.2	0.812	0.838
AP3206	6	0.3	0.741	0.746
Mean	6.286	0.321	0.742	0.749

Banding pattern of twenty rice genotypes using seven SSR markers

Figures of banding patterns of twenty rice genotypes for molecular analysis using seven SSR markers are presented (Fig. 7 - 9). The banding patterns were compared with reference to those of Binadhan-7 and Binadhan-10. According to the phenotypic performance, Binadhan-10 was considered as tolerant and Binadhan-7 was considered as susceptible. The genotypes which gave bands with same position or near with salinity tolerant Binadhan-10 were supposed to be tolerant to salinity and those similar to Binadhan-7 were considered as salt susceptible.

Genetic distance based analysis

The values of pair-wise comparisons of Nei's (Nei *et al.,* 1983) genetic distance between genotypes were computed from combined data for the seven markers, ranged from 0.00 to 1.00 (Table 6). Comparatively higher genetic distance (1.00) was observed between a number of genotypes or genotypes pair. Among them Binadhan-10 vs. Binadhan-7, Binadhan-10 vs. BRRI dhan28, Binadhan-10 vs. FL-478, Binadhan-10 vs. IZSD-44, Binadhan-10 vs. IZSD-45, Binadhan-10 vs. SL-32 and FL-478 vs. SL-51, FL-478 vs. SL-57 were important. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is more dissimilar than a pair with a lower value. The lowest genetic distance (0.29) was found SL-10 vs. SL-28 and SL-28 vs. SL-32 etc.

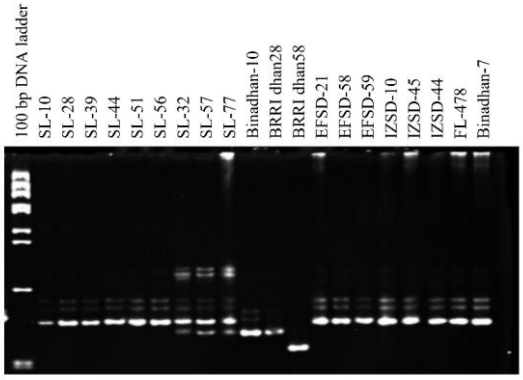

Fig 7: Banding pattern of 20 rice genotypes for SSR marker RM562.

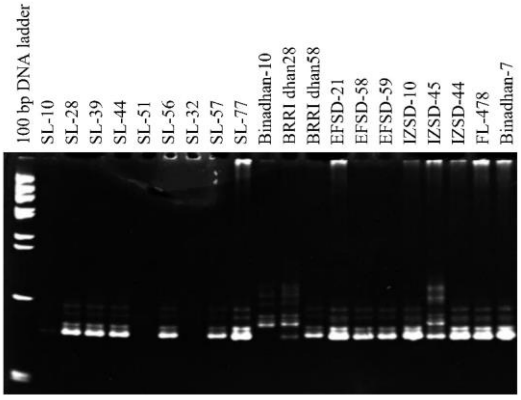

Fig 8: Banding pattern of 20 rice genotypes for SSR marker RM1287.

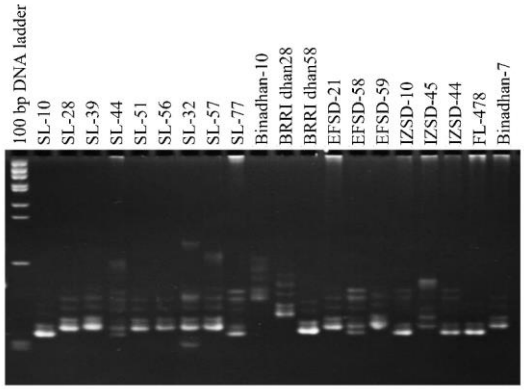

Fig 9: Banding pattern of 20 rice genotypes for SSR marker RM3412b

Table 6: Pair-wise comparisons of Nei's genetic distance for twenty rice genotypes

Genotypes	**Binadhan-10**	**Binadhan-7**	**BRRI dhan28**	**BRRI dhan58**	**EFSD-21**	**EFSD-58**	**EFSD-59**	**FL-478**	**IZSD-10**	**IZSD-44**	**IZSD-45**	**SL-10**	**SL-28**	**SL-32**	**SL-39**	**SL-44**	**SL-51**	**SL-56**	**SL-57**
Binadhan-7	1																		
BRRI dhan28	1	1																	
BRRI dhan58	0.57	1	0.86																
EFSD-21	0.86	0.86	1	1															
EFSD-58	0.71	0.86	0.86	1	0.71														
EFSD-59	0.86	0.86	0.86	1	0.57	0.71													
FL-478	1	0.71	0.86	1	1	1	0.57												
IZSD-10	0.71	0.71	1	0.86	1	0.71	0.71	0.86											
IZSD-44	1	0.86	0.43	1	1	0.71	0.86	0.71	0.71										
IZSD-45	1	0.71	1	1	0.86	0.86	0.57	0.57	0.86	1									
SL-10	0.86	0.71	0.86	1	0.86	0.57	0.86	0.71	0.71	0.57	0.71								
SL-28	0.86	1	0.86	1	0.57	0.71	1	0.86	0.86	0.57	1	0.29							
SL-32	1	1	0.71	0.86	0.86	0.86	1	0.86	0.86	0.57	1	0.43	0.29						
SL-39	0.86	1	0.57	0.86	1	1	0.86	0.71	0.86	0.57	1	0.86	0.86	0.71					
SL-44	1	0.86	1	1	0.43	0.86	0.57	0.86	0.86	0.71	0.86	0.86	0.71	0.57	0.86				
SL-51	0.43	0.71	0.86	0.57	0.71	0.86	1	1	1	0.86	1	0.71	0.71	0.86	1	1			
SL-56	0.71	0.71	1	0.86	1	0.86	0.86	0.71	0.86	0.86	0.71	0.71	0.86	0.86	0.71	0.86	0.71		
SL-57	0.57	0.86	0.86	0.71	0.71	0.86	1	1	0.71	1	1	0.86	0.86	1	0.86	1	0.57	0.86	
SL-77	0.71	0.86	0.86	1	0.86	0.71	0.57	0.43	0.71	0.86	0.71	0.71	0.86	1	0.86	1	0.86	0.86	0.86

Cluster analysis

The UPGMA cluster analysis led to the grouping of the twenty genotypes in four major clusters (Fig. 10). Except for BRRI dhan58, SL-57 was moderately tolerant and SL-51, Binadhan-10 were tolerant but BRRI dhan58 was found susceptible in SES scoring. Cluster II was the biggest group which contained seven genotypes. All of them are tolerant in SES score. Cluster III was divided into two sub clusters and consists of SL-39 which were moderately tolerant whereas BRRI dhan28, IZSD-44, EFSD-21, SL-44, EFSD-58, SL-32, SL-10 and SL-28 were found susceptible. The dendrogram revealed that the genotypes that found genetically similar type were clustered together.

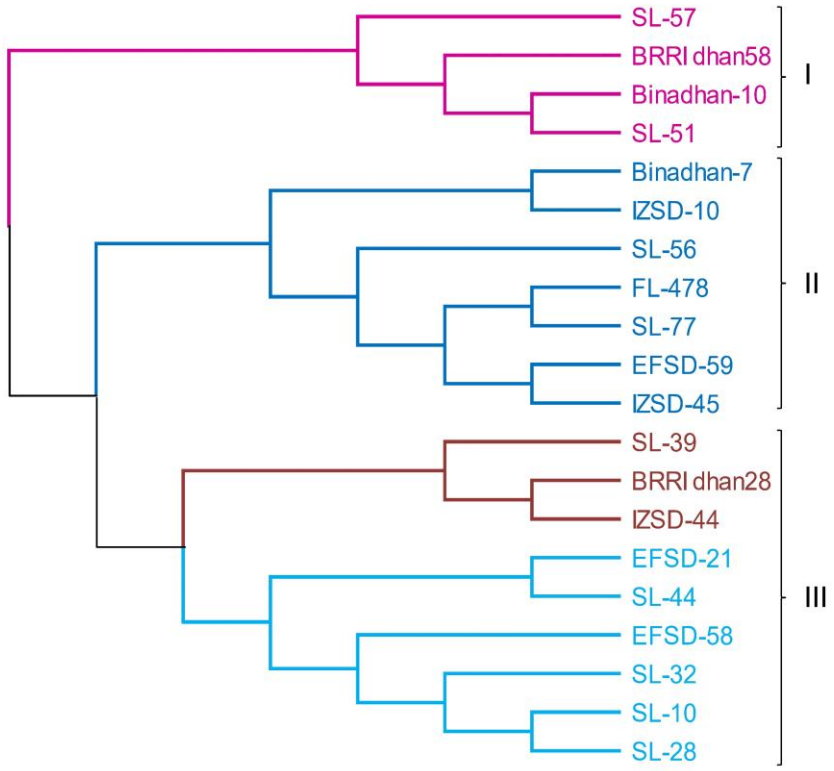

Fig. 10. A dendrogram showing the genetic relationships between twenty rice genotypes based on the alleles detected by seven SSR markers.

Discussion

The genotypes of the salt-tolerant lines showed relatively better growth and lower symptoms than susceptible genotypes after salinization (Bhuiyan, 2005) (Fig. 1). Plant height is the most significant morpho-physiological characteristic, which also determines shoot yield and overall biomass production. Salt stress might inhibit cell division or cell enlargement so that plant height was reduced. It has been reported that plant height decreased progressively with increase in salinity levels. Therefore, the reduction might be occurred due to salt stress during growth and development (Rad *et al.,* 2011). These results indicate that high salinity decreased filled grains plant^{-1} of rice. This is because of loss of biomass production was lower in tolerant genotypes which increased the assimilation and ultimately produced the higher number of grains. Therefore, increased salinity resulted in increased total number of empty grains plant^{-1} and finally it decreases yield. Increased number of empty grains might be a result of assimilate shortage during grain filling, brought about by early leaf senescence caused in this case by salinity (Aref and Ebrahimi-Rad, 2012). Grain yield production was also reduced due to salt stress. The same result was reported by Asch *et al.,* 1998 where eighty rice cultivars were used. This result suggests that the salt tolerant cultivars are different from susceptible in up taking salt and yield production. Rice has been reported as being salt-sensitive at reproductive stage (Moradi and Ismail, 2007), leading to a reduction in productivity of more than 50% when exposed to 6.65 dS m^{-1} salinity (Fig. 6) (Chaum and Kirdmanee, 2010).

The results of SSR markers also consistent with previous work done by Heenan *et al.*, (1988), who observed that the gene diversity at each SSR locus was significantly correlated with the number of alleles detected, number of repeat motif and with the allele size range. Nearly similar observation was found by Dhar *et al.,* (2012), where they got that average number of allele per locus was 10, with a range of 8 (RM152) to as many as 12 (RM7075 and RM10701) among the 26 rice germplasms by using 6 SSR markers. It also observed that the highest level of gene diversity value (0.899) was in loci RM10701 and the lowest level of gene diversity value (0.774) was observed in loci RM152 with a mean diversity of 0.854, the frequency of the most common allele at each locus ranged from 15.38% (RM10701) to 37.51% (RM152) with a mean frequency of 24.15. PIC values showed a significant, positive correlation with the number of alleles and allele size range for SSR markers evaluated in this study. The allele size range and the number of alleles were themselves also highly correlated. PIC values ranged from a low of 0.746 (RM152) to a high of 0.891 (RM10701) and averaged 0.857 was observed by Dhar *et al.,* (2012). Mohammadi-Nejad *et al.* (2010) also found that PIC value varied from 0.56 to 0.88, the highest value belonged to RM8094, while RM8095 showed the lowest PIC value (0.56). The SSR marker RM8094 was found to be superior for analysis of genetic diversity among the markers in the region. Genotypic pair indicating that they are genetically much closer among the genotype tested. Hence SSR marker based molecular fingerprinting could serve as a potential basis for the identification of genetically distance genotypes as well as sorting

of morphologically closer genotypes. The cluster analysis based on pair-wise comparison of Nei's genetic distance agreed with the allelic diversity observed among Basmati and Non-basmati long grain *indica* rice varieties (Chakravarthi and Naravaneni, 2006; Siwach *et al*. 2004; Ren *et al*., 2003).

Conclusion

Salinity is major constraint to cereal production worldwide and has become huge challenge for agriculture and food security in developing countries. In fighting against this problem development of salt-tolerant rice line is realized as the most promising, less resource consuming, economically viable and socially acceptable approach. Salinity tolerance of rice could noticeably be enhanced if superior alleles for all useful mechanisms are combined into popular rice varieties using molecular technique. Considering both phenotypic and genotypic observation, seven genotypes viz. Binadhan-10, FL- 478, SL-51, SL-56, SL-77, EFSD-59 and IZSD-45 were identified as salt-tolerant and on the other hand, EFSD-21, SL-28, SL-32, SL-10, BRRI dhan28, and Binadhan-7 were identified as salt-susceptible. The markers (RM490, RM493, RM562, RM1287, AP3206, RM3412b and RM10793) were used in marker-assisted selection, and identified salt-tolerant rice genotypes could be used in the improvement of rice breeding program.

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