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SCREENING OF SALINITY TOLERANCE OF TWENTY RICE GENOTYPES AT THE SEEDLING STAGE THROUGH HYDROPONIC AND SSR MARKER

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ABSTRACT

Salinity is one of the major abiotic stresses which severely affect the production of crops across the world. In this experiment, we examined 20 rice genotypes of diverse origins and sources including few salt tolerant varieties (Binadhan-8, Binadhan-10, Pokkali and FL478) as check. The main objective of this study was to determine salt tolerance at seedling stage and to evaluate genetic variation using SSR markers. IRRI standard protocol was applied to screen out salinity among those varieties, at the glasshouse of Bangladesh Institute of Nuclear Agriculture, BAU campus, Mymensingh-2202. Shoot length, root length and total dry matter were recorded at 6dS/m, 8dS/m, 10dS/m and 12 dS/m salt stress levels. According to the morphological and molecular survey of 20 rice genotypes at the seedling stage it was evident that, Binadhan-8, Binadhan-10, Pokkali, FL478, IR64, IR4630, FR13A and Sadamota identified as salt tolerant whereas THDB, Moulata, MV-20, CPD-23, CPD-29, Pot-18, Pot-27 and Dudkalam those were found as susceptible, BRRI dhan67, Binadhan-17 and Binadhan-21 those were traced as highly susceptible. The highest Nei's genetic distance value 1.0 was found in Moulata vs Sadamota and the lowest value 0.08 was observed in Binadhan-21 vs IR64. It will be used in future breeding program to develop a saline tolerant variety of rice.

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INTRODUCTION

Rice (*O. sativa*) is the staple food of an estimated 3.5 billion people throughout the world (IRRI, 2013). Nowadays, rice is produced in every continent of the world except the Antarctica. Thousands of rice cultivars are cultivated across 100 countries. But worldwide, different biotic and abiotic stresses (drought, flood, salinity) are responsible for the stagnant production of rice (Shelly *et al.,* 2016). Salinity is one of the major hindrances to increase rice production worldwide. One-fifth of the irrigated arable lands are recorded to be severely affected by high salinity throughout the world (Negrao*et al.,* 2011). Bangladesh has a considerable amount of coastal areas that are affected to salinity and occupies 30% of net cultivable land (Mamun*et al.*, 2019). If salinity increases with time it is estimated that there will be reduction in production by 10% by 2050 (IPCC, 2007). Salinity in soils is characterized by the excess presence of sodium ions along with dominant anions like chlorine and sulfate which combine together to increased electrical conductivity. (Ali *et al.,* 2013). The effects of salinity on different parameters like morphological, physiological and biochemical traits have been studied in rice which showed reduced tillering, spikelet filling, florets per panicle, 1000 grain weight, grain yield, harvest index, shoot and root dry matter and potassium uptake and increased leaf and root Na+, Cl- (Mohammadi-Nejad *et al.,* 2008; 2010; De Leon *et al.,* 2015; Morales *et al.,* 2012). Although the genetics of salt tolerance is useful for the researchers to develop salt tolerant varieties, there are some problems those are required to be assessed. The main reason is that salinity is not uniformly distributed throughout a given area and thus it is difficult to recognize tolerant variety added that there are some variations which are found among species and some among cultivars within species (Arzani, 2008; Ashraf and Foolad, 2013).

Hydrophonic system is perfectly compatible with screening as it has less stress effect on plant. Hydroponics is one of the subsets of hydro culture, which is a method of growing plants without soil by using mineral nutrient solutions in a water solvent. Terrestrial plants may be grown with only their roots exposed to the mineral solution, or the roots may be supported by an inert medium, such as perlite or gravel.Assessment of genetic diversity and identification of superior genotypes are important as well as necessary for any crop improvement program (Bhuiyan, 2005). Various means, biometrical and biochemical analysis have studied genetic diversity in rice. In general, molecular markers have been proved to be very useful for crop improvement and crop evaluation in many species, a rapid technically simple SSR analysis and inexpensive PCR based assay which requires only small quantity of DNA (Litt and Lutty, 1989). Through PCR different alleles at a locus can be detected by using conserved DNA sequence flanking SSR as primers. SSR have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application (Choi *et al.,* 2011).

MATERIALS AND METHODS

The experiment was carried out at the glass house and laboratory, Division of Biotechnology, Bangladesh Institute of Nuclear Agriculture (BINA), BAU campus, Mymensingh-2202, during the period from February 2019 to June 29, 2019. Experimental materials used in the study were collected from BINA. Modified hydroponic system (Gregorio *et al.,* 1997) was used at the glasshouse to evaluate salt tolerance of the 20 rice genotypes using Peter's solution (Yoshida *et al.,* 1976). To prepare the nutrient solution 1.0 gm. Peter fertilizer and 200 mg ferrous sulphate were mixed together with 1 liter of distilled water. The PH was adjusted to 5.1 by pH meter using 1N HCL and 1N NaOH when necessary. For salinization, crude salt was dissolved with nutrient solution to reach the desired salinity level. The salinization levels were EC at 6 dS/m, 8 dS/m, 10 dS/m and 12 dS/m. The salinity level was measured through EC using the EC meter. Then the old solution was replaced with the new one in every 8-day and the pH was maintained at 5.1 daily. Salt stresses were applied at 7^{th} days old seedling. After two or three days of salinization, salt stress symptoms were obvious. The genotypes were evaluated for their tolerance to salinity under sustained water bath (hydroponic condition) using IRRI standard protocol (Gregorio *et al.,* 1997). This scoring separated the tolerant, moderately tolerant and susceptible and highly susceptible rice genotypes. Initial and final scoring was done at 14^{th} day and 21^{st} day respectively after salinization.

The data were recorded from the screening at seedling stage in both normal and salinized conditions following SES of IRRI. Root length, shoot length and total dry matter were measured along with reduction rate at different salinity stages. Plants were separately kept into envelops and oven dried at 72°C for a week and weighed. Juvenile, vigorous leaves were collected from 21 days old seedlings for the isolation of genomic DNA using the mini preparation Modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (IRRI, 1988). The leaf samples were cut into 2-3 cm pieces and the sample was grounded. Extraction Buffer (800 µl) and 20% SDS (50 µl) were added. Then the mixture was Vortexed for 20 seconds and incubated for 10 minutes at 65ºC in the hot water bath. 100 µl 5M NaCl was added and inverted gently to suspend the samples evenly. Then 100 µl CTAB was added and mixed well. Again, the mixture was vortexed for 20 seconds and incubated for 10 minutes at 65ºC in the hot water bath. 900µl chloroform mix (Phenol: chloroform: isoamyl alcohol = 25:24:1, v/v) was added and mixed well. The samples were centrifuged at 15000 rpm for 15 minutes. Then the supernatant was transferred into a new eppendorf tube and 600 µl ice-cold isopropanol was added to the supernatant and shacked well. The mixture was again spinned down at 12000 rpm for 15 minutes by centrifuge. The Supernatant was discarded and the pellet was washed with 200 µl 70% ethanol. At last the samples were spinned down again at 15000 rpm for 5 minutes, the ethanol was removed and the pellets were allowed for air-drying for 1 hour. The pellets were then suspended in 30 µl 1X TE buffer. Finally, the DNA samples were stored at -20 °C. To estimate the quantity and quality (in terms of protein and RNA contamination) of isolated genomic DNA, agarose gel electrophoresis and Nanodrop spectrophotometer was used. A set of thirty microsatellite primers developed by several investigators were used in this study. Thirty primers were screened on a sub sample of one randomly chosen individual from twenty rice genotypes to evaluate their suitability for amplifying DNA sequences, which could be accurately scored. Primers were selected on the basis of band resolution intensity, presence of smearing, consistency within individuals and potential for population discrimination. Out of thirty primers, thirteen primers were used for further analysis. The total volume of PCR cocktail for this study was 9 µl per sample. 1 µl genomic DNA was added with 9 µl PCR cocktail and finally, the total volume was 10 µl. The PCR cocktail including DNA was placed in the PCR tubes and run in the DNA thermal cycler. The reaction mixture was preheated at 94˚C for 5 minutes followed by denaturation at 94˚C for 30 seconds and annealing at 55˚C for 1 minute. The polymerization reaction was done at 72˚C for 1 min followed by repeating those cycles for 35 times. At last the amplified products were incubated at 72˚C for 5 minutes. The amplified products were then separated electrophoretically on polyacrylamide gel. After completion of electrophoresis the gel was soaked in ethidium bromide (10 mg/ ml) solution for 12-15 min. After staining, the gel was taken out carefully from the staining tray and placed on high performance ultraviolet light box (UV trans-illuminator) of gel doc for checking the DNA bands. The DNA was observed as band and the records were saved. The pattern of bands obtained after application with the primers was scored with reference of control. The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to a molecular weight size marker with the help of Alpha Ease FC 5.0 software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values were determined using POWER MARKER version 3.23. Allele molecular weight data also used to determine the genetic distance for phylogeny reconstruction.

Table 1. Modified standard evaluation scoring protocol of IRRI

Score	Observation	Tolerance
1	Normal Growth, No leaf symptoms	Highly Tolerant (HT)
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant (T)
5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately Tolerant (MT)
7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible (S)
9	Almost all plants dead or dying	Highly Susceptible (HS)

Table 2. List of markers used in the study

Marker	Primer sequence (5'-3')		Estimated length (bp)	Repeat motif	Annealing temp. (°C)
RM17	For.	TGCCCTGTTATTTTCTTCTCTC	184	$(GA)_{21}$	55
	Rev.	GGTGATCCTTTCCCATTTCA			
RM276	For.	CTCAACGTTGACACCTCGTG	149	$(AG)_8A_3(GA)_{33}$	55
	Rev.	TCCTCCATCGAGCAGTATCA			
RM234	For.	ACAGTATCCAAGGCCCTGG	156	$(CT)_{25}$	55
	Rev.	CACGTGAGACAAAGACGGAG			
RM302	For.	TGCAGGTAGAAATTGAAGC	156	$(GT)_{30}(AT)_8$	55
	Rev.	AGTGGATGTTAGGTGTAACAGG			
RM310	For.	CCAAAACATTTAAAATATCATG	105	$(CT)_{19}$	55
	Rev.	GCTTGTTGGTCATTACCATTC			
RM510	For.	AACCGGATTAGTTTCTCGCC	122	$(GA)_{15}$	55
	Rev.	TGAGGACGACGAGCAGATTC			
RM223	For.	GAGTGAGCTTGGGCTGAAAC	165	$(CT)_{25}$	55
	Rev.	GAGTGAGCTTGGGCTGAAAC			
RM493	For.	TAGCTCCAACAGGATCGACC	211	$(CTT)_9$	55
	Rev.	GTACGTAAACGCGGAAGGTG			
RM435	For.	ATTACGTGCATGTCTGGCTG	166	$(ATG)_7$	55
	Rev.	CGTACCTGACCATGCATCTG			
RM7075	For.	GCGTTGCAGCGGAATTTGTAGG	155	$(ACAT)_{13}$	55
	Rev.	CCCTGCTTCTCTCGTGCAGAG			
RM208	For.	TCTGCAAGCCTTGTCTGATG	173	$(CT)_{17}$	55
	Rev.	TAAGTCGATCATTGTGTGGACC			
RM8094	For.	AAGTTTGTACACATCGTATACA	209	$(AT)_{31}$	55
	Rev.	CGCGACCAGTACTACTACTA			
RM562	For.	CACAACCCACAAACAGCAAG	243	$(AAG)_{13}$	55
	Rev.	CTTCCCCCAAAGTTTTAGCC			

RESULTS

Phenotypic variation of rice genotypes at seedling stage under non-salinized and salinized condition

Among these 20 rice genotypes, according to Standard evaluation score of IRRI, at 6 dS/m, 12 genotypes were tolerant (T) to salt stress whereas 8 genotypes were moderately tolerant (MT). At 8 dS/m, 6 genotypes were tolerant, 10 were moderately tolerant and the rest 4 genotypes were susceptible (S). At 10 dS/m, 5 genotypes were found as tolerant, 7 were moderately tolerant and the rest 8 were susceptible. At 12 dS/m, 3 genotypes were established as salt stress tolerant lines, 5 genotypes were taken as moderately tolerant, 10 were found as susceptible and the rest 2 of them were highly susceptible (HS) to salinity. The SES data are given in Table 3 under different conditions.

Screening Salt-tolerant rice genotypes at seedling stage through morpholoigical traits

Rice plant expressed various degrees of responses to salt stress (Table 4). At 6 dS/m, Moulata (47.44%) and BRRI dhan67 (43.37%) showed the highest degrees of reduction on shoot length. On the other hand, Binadhan-20 (5.12%), IR64 (8.16%) and FL478 (8.49%) showed lowest degrees of length reduction at the same condition. Binadhan-20 (5.07%) and Pokkali (8.85%) displayed the lowest level of shoot length reduction opposite to Sadamota (47.0%) and Moulata (43.54%) at 8dS/m. At 10 dS/m, Pot-27 (61.79%) and Moulata (61.65%) showed the highest level of reduction of shoot length, at the same time FL478 (19.70%) and Binadhan-8 (24.52) showed the least amount of reduction in length. At 12dS/m, Dudkalam (66.02%) and Moulata (64.96%) were mostly influenced and were reduced at the highest level in their shoot length; on the contrary, IR4630 (38.11%) and Pokkali (39.57%) were decreased in their length less than other varieties.

Table 3. Salt tolerance scoring of 20 rice genotypes by modified standard evaluation Protocol of IRRI

Sl. No.	Genotype	6dS/m	8dS/m	10 dS/m	12 dS/m	Tolerance
1	Binadhan-8	3	3	3	5	MT
2	Binadhan-10	3	3	3	3	T
3	Binadhan-17	5	5	7	9	HS
4	Binadhan-20	3	5	7	7	S
5	Binadhan-21	3	7	7	9	HS
6	THDB	3	5	7	7	S
7	MV20	5	5	5	7	S
8	Moulata	5	5	7	7	S
9	Sadamota	3	3	5	5	MT
10	Dudkalam	3	5	7	7	S
11	Pot-18	5	5	5	7	S
12	Pot-27	5	5	7	7	S
13	BRRI dhan67	5	5	7	7	S
14	IR4630	5	5	5	5	MT
15	FL478	3	3	3	3	T
16	IR64	3	5	5	5	MT
17	FR13A	3	3	3	5	MT
18	Pokkali	3	3	3	3	T
19	CPD-29	5	5	5	7	S
20	CPD-23	5	5	5	7	S

Here, MT=Moderately Tolerant, T= Tolerant, S=Susceptible and HS=Highly Susceptible

Various levels of changes were observed at the root length when applied to different salt stresses during seedling stage (Figure 1). At 6dS/m, BRRIdhan-67 (42.34%) and IR-4630 (35.92%) showed maximum level of length reduction, on the other hand, Binadhan-21 (1.42%) and Dudkalam (2.11%) showed minimum level of root length reduction but Binadhan-20 exhibited different characteristic by increasing their root length at 0.47% than those of control condition. IR-4630 (27.47%) and Sadamota (21.78%) showed maximum level of reduction in their length, on the opposite, Dudkalam (2.54%) and Binadhan-21 (3.18%) were increased in their length at the lowest amount. Surprisingly all the Binadhan-20, Pot-18, FR13-A, Pokkali, CPD-29 and CPD-23 have increased amount of root length than those of control condition at 8 dS/m. At 10 dS/m, IR-4630 lost its root length by 40.50% which was the highest on the other hand Binadhan-10 lost the length by 3.08%, the lowest in this category. At 12dS/m, Binadhan-10 lost its length by 33.67% and FL-478 lost its length by 2.71%, which were highest and lowest reduction in that category, respectively.

Table 4. Shoot length (cm) and its reduction (%) in 20 rice genotypes upon salinity stress compared to control condition at seedling stage

Genotypes	Control	6 dS/m (cm)	Reduction (%)	8dS/m (cm)	Reduction (%)	10 dS/m (cm)	Reduction (%)	12 dS/m (cm)	Reduction (%)
Binadhan-8	57.81	44.87	22.39	47.56	17.74	43.63	24.52	27.22	52.91
Binadhan-10	64.97	57.63	11.29	53.39	17.82	47.23	27.30	29.83	54.08
Binadhan-17	39.72	30.14	24.11	33.13	16.59	23.38	41.15	18.72	52.87
Binadhan-20	46.00	43.64	5.12	43.67	5.07	30.53	33.62	27.72	39.73
Binadhan-21	48.28	36.50	24.40	35.50	26.47	25.17	47.87	18.80	61.06
THDB	56.44	40.33	28.54	33.44	40.75	28.67	49.21	26.03	53.88
MV-20	45.67	33.88	25.82	36.82	19.37	23.69	48.13	26.01	43.04
Moulata	79.50	41.79	47.44	44.89	43.54	30.49	61.65	27.86	64.96
Sadamota	89.00	56.14	36.92	47.17	47.00	41.36	53.53	37.14	58.26
Dudkalam	87.33	60.37	30.88	50.06	42.68	36.37	58.36	29.68	66.02
Pot-18	73.89	48.72	34.06	47.56	35.64	34.57	53.22	29.34	60.29
Pot-27	72.67	50.36	30.70	50.91	29.94	27.77	61.79	27.28	62.46
BRRI dhan67	58.39	33.07	43.37	37.13	36.40	28.66	50.92	25.94	55.57
IR4630	35.28	30.90	12.41	28.49	19.24	20.07	43.12	21.83	38.11
FL478	69.39	63.50	8.49	62.08	10.54	55.72	19.70	41.89	39.63
IR64	47.39	43.52	8.16	27.22	42.56	25.66	45.86	25.06	47.13
FR13A	68.06	52.17	23.35	46.14	32.20	34.13	49.84	34.94	48.65
Pokkali	80.94	72.72	10.16	73.78	8.85	58.10	28.22	48.91	39.57
CPD-29	70.17	54.33	22.57	47.97	31.64	36.41	48.11	28.48	59.41
CPD-23	77.89	45.22	41.94	47.39	39.16	35.06	54.99	30.41	60.96

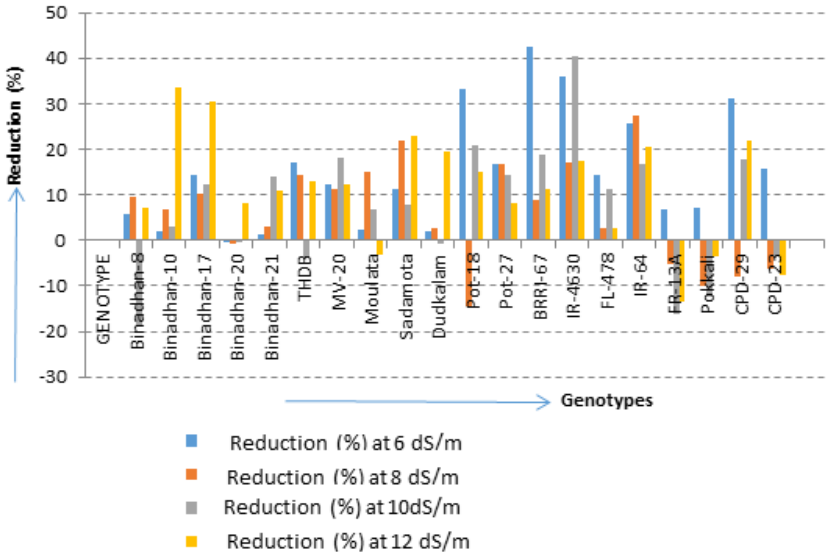

Figure 1. Graphical representation of reduction (%) of root length

In terms of total dry matter, Moulata (66.37%) was reduced to the highest level and Pokkali (0.68%) was reduced to the lowest level. FL478 (4.59%) showed increased amount of total dry matter at 6 dS/m (Table 5). At 8 dS/m, Moulata (59.45%) and IR-64 (59.45%) expressed the highest level of reduction and Binadhan-21 (5.66%) had the least amount of reduction in their total dry matter. At the same time Binadhan-20, FL-478 and Pokkali were found to have increased total dry matter. Moulata lost its total dry matter at about 85.64%, on the other hand, FL478 was reduced to its TDM at only 1.88%, at 10 dS/m. In the final treatment at 12 dS/m, Moulata (86.90%) was decreased to the highest level, at the same time; FL478 (44.05%) was reduced to its TDM at the lowest amount.

Table 5. Dry weight (gm) and its reduction (%) in 20 rice genotypes upon salinity stress compared to control condition at seedling stage

Genotypes	Control (gm)	6 dS/m (gm)	Reduction (%)	8 dS/m (gm)	Reduction (%)	10 dS/m (gm)	Reduction (%)	12 dS/m (gm)	Reduction (%)
Binadhan-8	0.40	0.26	34.26	0.35	11.98	0.34	14.21	0.12	68.80
Binadhan-10	0.44	0.36	18.25	0.40	10.00	0.35	21.00	0.14	68.93
Binadhan-17	0.16	0.11	30.14	0.13	17.81	0.06	65.21	0.04	78.36
Binadhan-20	0.23	0.21	9.71	0.30	-29.13	0.14	38.35	0.11	52.43
Binadhan-21	0.24	0.21	11.32	0.22	5.66	0.08	66.04	0.03	85.80
THDB	0.32	0.20	37.72	0.18	44.64	0.13	58.13	0.11	65.40
MV-20	0.21	0.14	32.11	0.19	9.47	0.09	57.89	0.10	50.53
Moulata	0.44	0.15	66.37	0.18	59.45	0.06	85.64	0.06	86.90
Sadamota	0.58	0.27	53.90	0.26	56.19	0.22	63.05	0.14	76.00
Dudkalam	0.51	0.29	41.89	0.26	48.90	0.14	73.03	0.07	86.40
+Pot-18	0.41	0.24	41.62	0.26	36.49	0.12	71.84	0.12	71.89
Pot-27	0.41	0.28	33.24	0.27	35.92	0.08	80.16	0.07	83.65
BRRI dhan67	0.22	0.08	61.60	0.12	44.85	0.07	65.98	0.05	76.49
IR4630	0.14	0.13	7.03	0.12	15.63	0.05	65.86	0.08	44.14
FL478	0.53	0.56	-4.59	0.63	-18.79	0.52	1.88	0.30	44.05
IR64	0.24	0.22	6.91	0.10	59.45	0.10	59.45	0.07	71.61
FR13A	0.41	0.29	28.49	0.27	32.60	0.17	59.18	0.17	57.26
Pokkali	0.49	0.48	0.68	0.55	-12.56	0.36	26.48	0.22	55.25
CPD-29	0.31	0.28	9.71	0.21	33.45	0.14	56.22	0.08	73.02
CPD-23	0.40	0.22	44.44	0.23	43.61	0.13	67.50	0.08	80.33

According to the morphological study, it is obvious that, THDB and Moulata, MV-20, CPD-23, CPD-29, Pot-18, Pot-27 and Dudkalam those were found as susceptible; BRRI dhan67, Binadhan-17 and Binadhan-21 those were identified as highly susceptible whereas Pokkali, Binadhan-8, Binadhan-10 and FL478, which were known as tolerant. IR64, IR4630 and FR13A acted as moderate tolerant line.

Screening Salt-tolerant rice genotypes at seedling stage through SSR markers

In this SSR marker based DNA fingerprinting technique, 20 rice genotypes were analyzed using 13 loci. Amplified microsatellite loci were analyzed to find out polymorphism. All 13 microsatellite loci were polymorphic and had 4 alleles (mean) per locus. The bands obtained, were compared to the band of salt tolerant variety like Pokkali, FL478, Binadhan-10. The results of the banding patterns obtained from the study are presented (Figure 2-9).

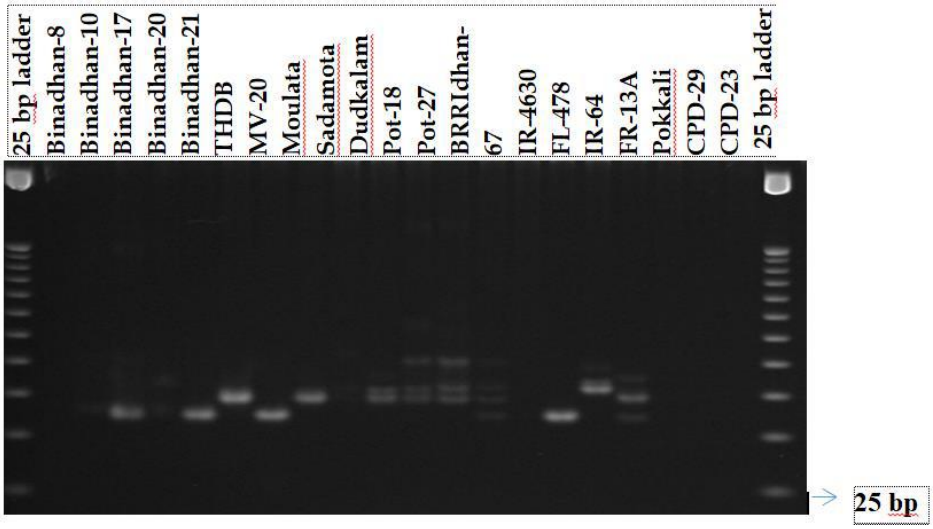

Figure 2. Microsatellite profiles of 20 rice genotypes at locus RM310

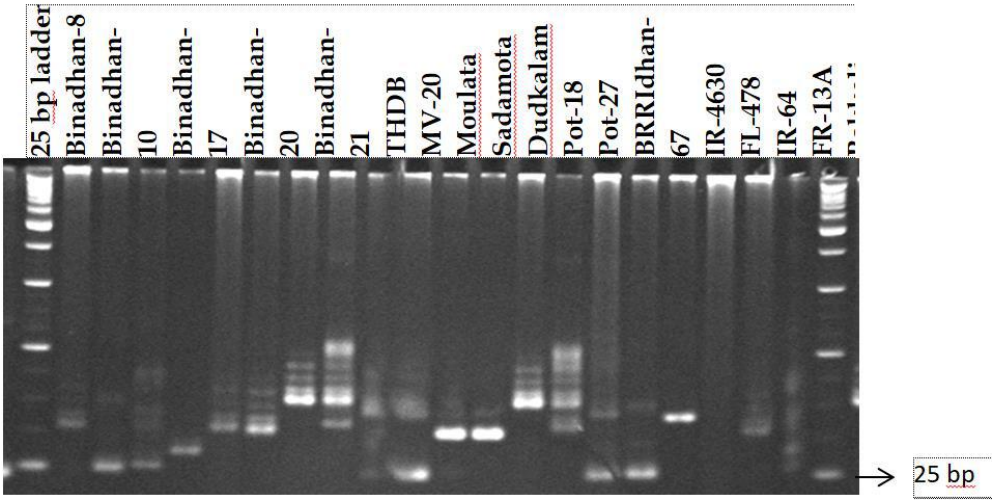

Figure 3. Microsatellite profiles of 20 rice genotypes at locus RM302

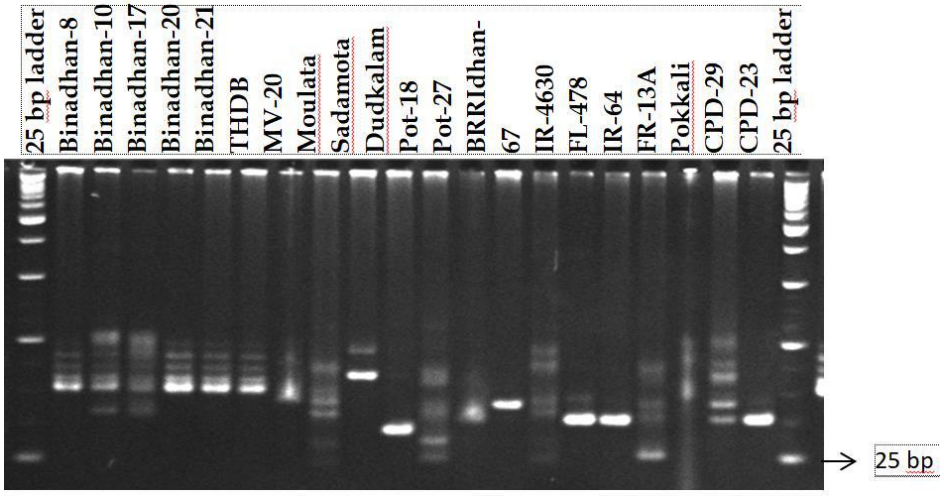

Figure 4. Microsatellite profiles of 20 rice genotypes at locus RM223

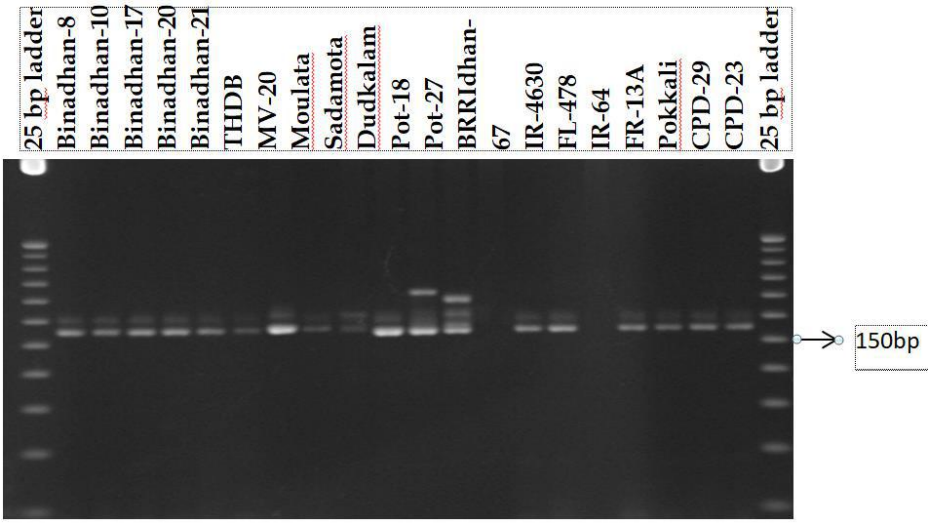

Figure 5. Microsatellite profiles of 20 rice genotypes at locus RM435

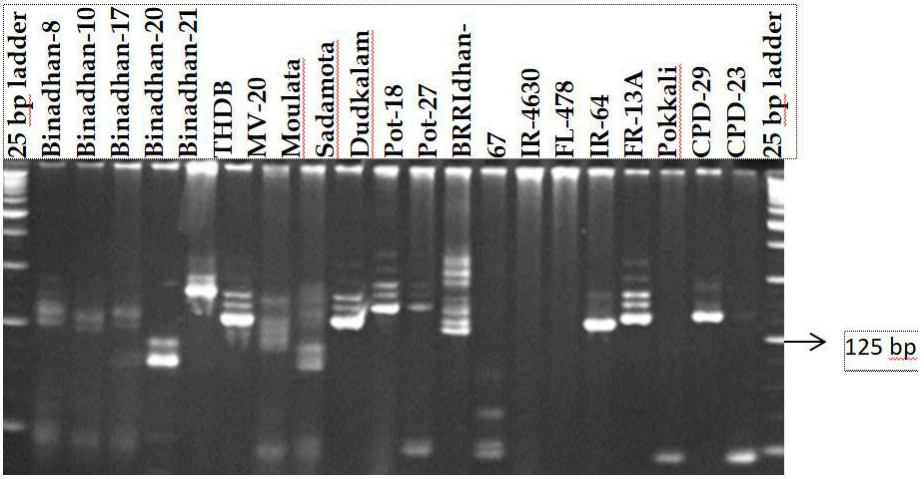

Figure 6. Microsatellite profiles of 20 rice genotypes at locus RM234

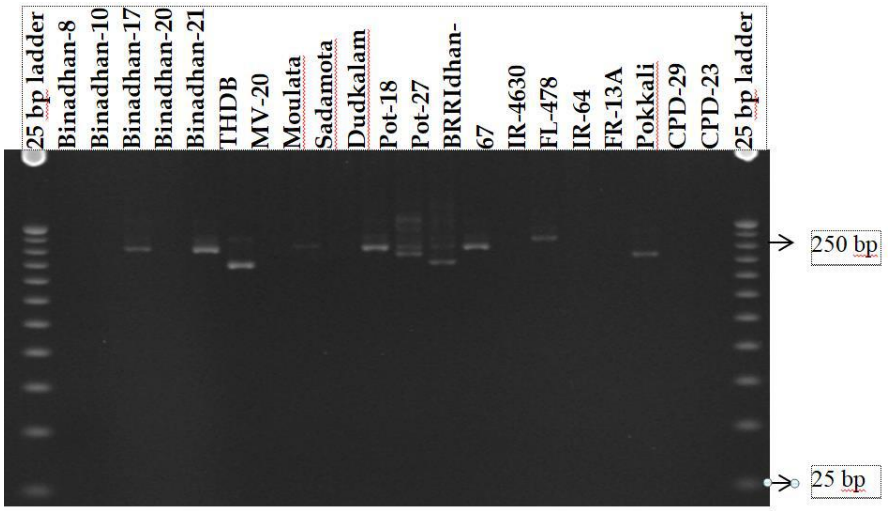

Figure 7. Microsatellite profiles of 20 rice genotypes at locus RM562

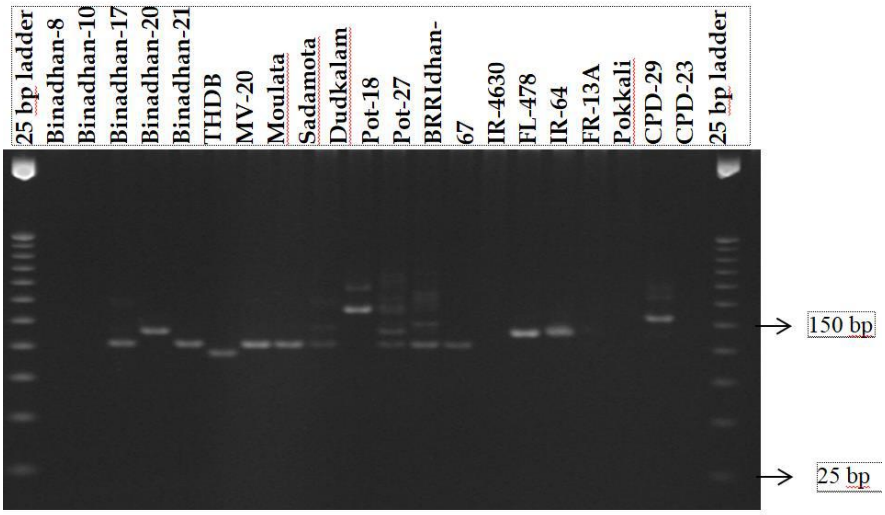

Figure 8. Microsatellite profiles of 20 rice genotypes at locus RM7075

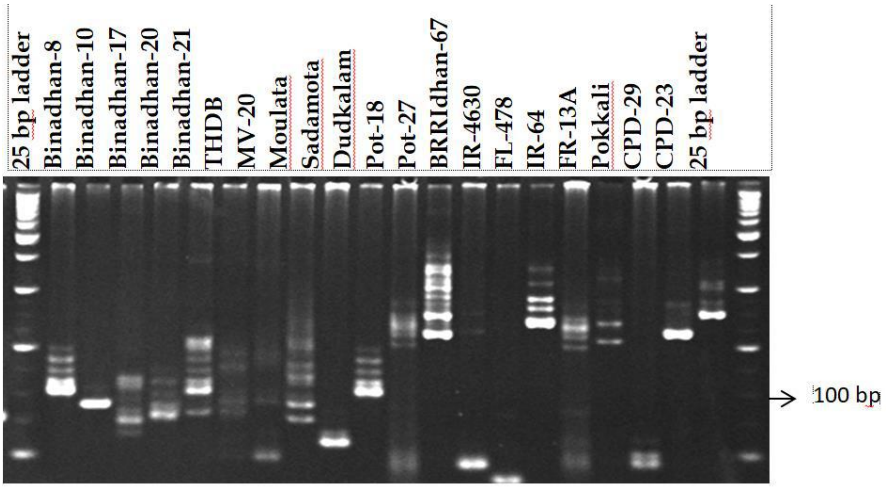

Figure 9. Microsatellite profiles of 20 rice genotypes at locus RM276

Table 6. Summary of genetic distance values among 20 rice genotypes using 13 SSR markers

Gen.*	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20
P1	0.00	0.69	0.54	0.69	0.15	0.77	0.85	0.69	0.92	0.31	0.77	0.69	0.77	0.69	0.85	0.23	0.54	0.69	0.77	0.77
P2		0.00	0.46	0.46	0.62	0.23	0.77	0.77	0.62	0.54	0.62	0.69	0.77	0.46	0.62	0.62	0.85	0.85	0.62	0.38
P3			0.00	0.54	0.54	0.46	0.62	0.62	0.85	0.46	0.54	0.54	0.54	0.62	0.62	0.54	0.69	0.77	0.38	0.54
P4				0.00	0.62	0.46	0.77	0.77	0.62	0.54	0.54	0.62	0.69	0.54	0.54	0.69	0.77	0.77	0.62	0.54
P5					0.00	0.69	0.92	0.77	0.77	0.15	0.69	0.77	0.69	0.69	0.85	**0.08**	0.62	0.77	0.77	0.69
P6						0.00	0.77	0.77	0.69	0.62	0.54	0.69	0.62	0.54	0.69	0.69	0.85	0.85	0.69	0.54
P7							0.00	0.31	0.92	0.85	0.46	0.23	0.85	0.85	0.77	0.85	0.85	0.92	0.46	0.85
P8								0.00	**1.00**	0.85	0.46	0.23	0.77	0.85	0.85	0.69	0.92	0.85	0.38	0.85
P9									0.00	0.69	0.77	0.92	0.69	0.62	0.92	0.77	0.62	0.54	0.92	0.54
P10										0.00	0.62	0.69	0.69	0.62	0.85	0.23	0.62	0.69	0.69	0.62
P11											0.00	0.23	0.77	0.69	0.85	0.77	0.69	0.69	0.46	0.62
P12												0.00	0.77	0.77	0.85	0.85	0.77	0.77	0.38	0.77
P13													0.00	0.77	0.62	0.69	0.77	0.85	0.69	0.69
P14														0.00	0.54	0.69	0.77	0.77	0.77	0.46
P15															0.00	0.85	0.85	0.92	0.77	0.62
P16																0.00	0.69	0.85	0.69	0.69
P17																	0.00	0.23	0.69	0.69
P18																		0.00	0.77	0.62
P19																			0.00	0.77
P20																				0.00

Here, P1=Binadhan-8, P2=Binadhan-10, P3=Binadhan-17, P4=Binadhan-20, P5=Binadhan-21, P6=THDB, P7=MV-20, P8=Moulata, P9=Sadamota, P10=Dudkalam, P11=Pot-18, P12=Pot-27, P13=BRRI-67,P14=IR-4630, P15=FL-478, P16=IR-64,P17=FR-13A, P18=Pokkali, P19=CPD-29, P20=CPD-23.

Gen.*= Genotypes used in this study.

The pair wise comparison values of Nei's (1973) genetic distance among 20 rice genotypes were calculated from combined data sets for 13 loci. The value ranged from 0.08 to 1.0 (Table 4.6). The highest Nei's genetic distance value 1.0 was found in Moulata vs Sadamota. The lowest genetic distance value 0.08 was observed in Binadhan-21 vs IR64. The lower value of pair wise differences among rice genotypes was likely due to their genetic relatedness. On the other hand, higher value of pair-wise difference was observed among those rice lines developed from genetically distal parental.

Dendogram based on Nei's (1973) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated differentiation of the 20 rice genotypes by 13 markers. All the 20 rice genotypes could be easily distinguished. The UPGMA cluster analysis led to the grouping of 20 rice genotypes in three major clusters at 40% cut off (Figure 7).

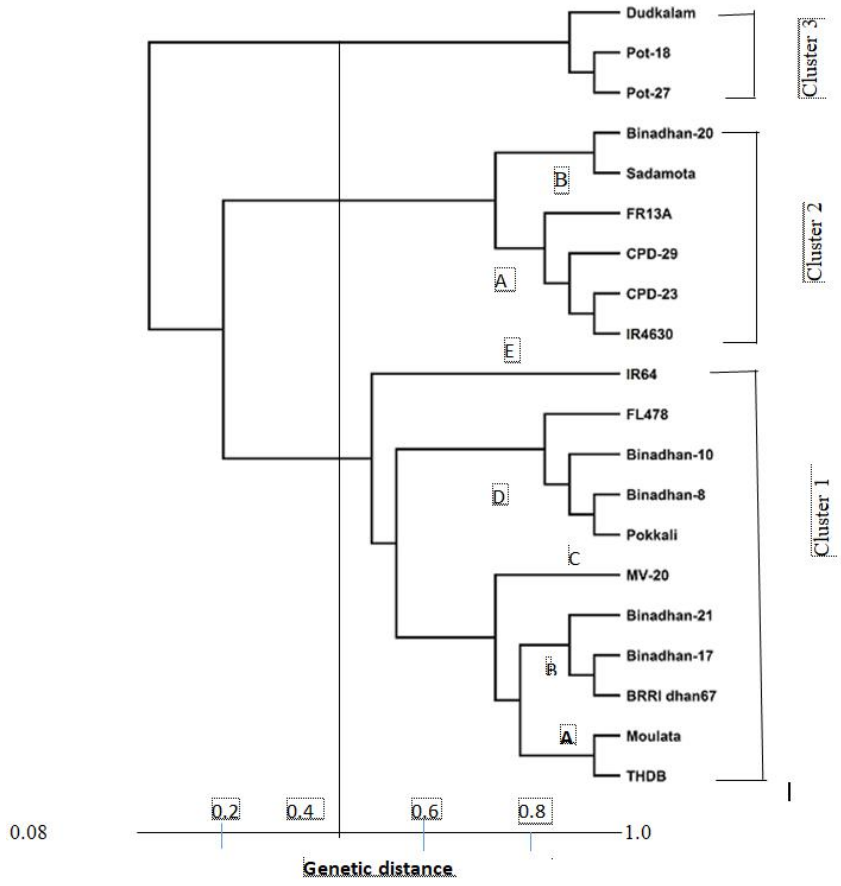

Figure 10. UPGMA Dendogram based on Nei's Genetic Distance according to SSR analysis

Cluster-1 showed 5 subcluster (1A, 1B, 1C, 1D and 1E), Sub cluster 1A comprised of 2 genotypes, THDB and Moulata, those were found as susceptible in morphological characterization , 1B contains BRRI dhan67, Binadhan-17 and Binadhan-21, those were identified as highly susceptible, Cluster 1C has a single genotype, MV-20, which was susceptible, Cluster 1D comprises of Pokkali, Binadhan-8, Binadhan-10 and FL478, which were known tolerant in morphological analysis. Cluster 1E had a single genotype IR64 which was found as moderate tolerant line in earlier analysis. Cluster -2 consisted of 6 genotypes having two sub clusters (2A and 2B). In 2A, IR4630, CPD-23, CPD-29, FR13A were clustered. Among those, CPD-23 and CPD-29 were selected as susceptible in phenotypic analysis on the other hand IR4630 and FR13A was found as moderately tolerant. In Cluster-3, 3 genotypes namely, Pot-18, Pot-27 and Dudkalam, clustered together and they all were grouped in susceptible category in morphological analysis. Based on the above result, it can be concluded that the genotypes showing diverse ranges of salt injury upon salinity stress condition during morphological characterization, tend to cluster together in the dendogram with some exceptions.

DISCUSSIONS

Screening Salt-tolerant rice genotypes at seedling stage through morpholoigical traits

The findings in our study that the rice roots and shoots exhibited a significant reduction in their length, fresh weight and dry weight were consistent with Amirjani, (2010) who reported that salt stress level decreased as the salt concentration increased in rice. Silveira *et al.,* (2009) reported that plant height of tolerant lines of rice were reduced by 19% under salt stress (EC 12 dS/m), whereas those of susceptible lines were reduced by 46%. Due to variation of genotype the change also varied in different extent. Our study also complied with Mansour, (2005) who reported that salt stress suppresses the growth of leaves in the plants resulting in complete cessation of growth and development. Rai *et al.,* (2003) found that salt stress significantly reduced the total dry matter of rice cultivars which was similar to our findings. Ali *et al.,* (2004) observed that the salt stress of 50mM NaCl caused a significant decrease in both fresh weight and dry weight. These results were also compatible with the findings of Nicolas *et al.,* (2012) and weon *et al.,* (2003).

Screening Salt-tolerant rice genotypes at seedling stage through SSR markers

Molecular marker helps to identify alleles that are associated with key phenotypic traits (Islam *et al.,* 2007). In our study, all markers generated polymorphic banding patterns where a total of 53 alleles were detected across the genotypes with an average of 4 alleles per locus which was compatible with the previous work of Ali *et al.,* (2004). The number of alleles detected is one of the selection categories in assessing a marker's usefulness in diversity analyses since a higher allele count per locus means that the marker is able to discriminate genotypes that might be indistinguishable to other markers (Khanam *et al.,* 2018). In our study 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. This result is consistent with previous work done by Singh *et al.*, (2008).

CONCLUSION

Binadhan-8, Binadhan-10, Pokkali, FL478, IR64, IR4630, FR13A and Sadamota can be used to develop salt stress tolerant varieties. Observation on the reproductive stage of selected rice genotypes can be performed to assess their performance. Field trial of the selected genotypes may be examined to evaluate their production.

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CONFLICT OF INTEREST

There are no conflicts of research for this study.

REFERENCES

1. Amirjani M, 2010. Effect of Salinity Stress on Growth, Mineral Composition, Proline Content, Antioxidant Enzymes of Soybean. American Journal of Plant Physiology, 5(6): 350-360
2. Ali S, Gautam RK, Mahajan R, Krishnamurthy SL, Sharma SK, Singh RK and Ismail AM, 2013. Stress indices and selectable traits in Saltol QTL introgressed rice genotypes for reproductive stage tolerance to sodicity and salinity stresses. Field Crops Research, 154: 65-73.
3. Arzani A, 2008. Improving salinity tolerance in crop plants: A Biotechnological View. In Vitro Cellular and Developmental Biology. Plant, 44 (5): 373-383.
4. Ashraf M and Foolad M, 2013. Crop breeding for salt tolerance in the era of molecular markers and marker assisted selection. Plant Breeding, 132: 10-20.

5. Bhuiyan MAR, 20005. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. MS Thesis. Dept. of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, pp. 96.
6. Choi WY, Lee KS, Ko JC, Choi SY, Choi DY and Kim TS, 2003: Critical concentration of saline water for rice cultivation on a reclaimed coastal soil in Korea. Korea Rep. International Rice Research, 28(1): 54-55.
7. Leon TB, Linscombe S, Gregorio G and Subudhi PK, 2015. Genetic variation in southern USA rice genotypes for seedling salinity tolerance. Plant science, 6: 1-3.
8. Gregorio GB, Senadhira D and Mendoza RT, 1997. Screening rice for salinity tolerance. IRRI discussion paper series, 22. IRRI, Manila.
9. IPCC, 2007. Climate change in Asia too alarming to contemplate. Intergovernmental panel on climate change. pp.85
10. IRRI, 2013. World rice statistics. Los Banos, Laguna, Philippines. pp.125.
11. Litt M and Lutly JA, 1989. A hyper variable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. The American Journal of Human Genetics, 44: 397-401.
12. Mamun A, Sarker P and Noor MMA, 2019. Influence of irrigation and gypsum on wheat cultivation in saline soil. Research in Agriculture, Livestock and Fisheries, 6 (1): 1-10
13. Mohammadi-Nejad G, Arzani A, Rezai AM, Singh RK and Gregorio GB, 2008: Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the Saltol QTL. African Journal of Biotechnology, 7: 730-736.
14. Mohammadi-Nejad G, Singh RK, Arzani A, Rezaiec AM, Sabourid H and Gregorio GB, 2010. Evaluation of Salinity Tolerance in Rice Genotypes. International Journal of Plant Production, 4 (3): 199-208.
15. Morales SG, Tellez LIT, Merino FCG, Caldana C, Victoria DE and Cabrera BEH, 2012. Growth photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. Acta Scientiarum Agronomy, 34(3): 317-324.
16. Negrao S, Courtois B, Ahmadi N, Abreu I, Saibo N and Oliveira MM, 2011. Recent updates on salinity stress in rice: from physiological to molecular responses. Critical Reviews in Plant Sciences, 30: 329-377.
17. Shelly IJ, Takahashi-Nosaka M, Kano-Nakata M, Haque M and Inukai Y, 2016. Rice cultivation in Bangladesh: present scenario, problems, and prospects. Journal of International Cooperation for Agricultural Development, 14: 20-29.
18. Yoshida S, Forno DA, Cock JH and Gomez KA, 1976. Laboratory manual for physiological studies of rice. IRRI, Las Banos, Laguna, pp. 83.
19. Mansour M.M.F, 2005. Nitrogen containing compounds and adaptation of plants to salinity stress. Biological Plantrum, 43: 491-500.
20. Silveira JA, Rde IM, Viegas DA, Rocha AC, Moreira and Oliveira JT, 2009. Proline accumulation and glutamine synthetase activity are increased by salt-induced proteolysis in cashew leaves. Journal of Plant Physiology. 160: 115-123.
21. Rai SP, Luthra R and Kumar S, 2003. Salt-tolerant mutants in glycophytic salinity response (GRS) genes in *Catharanthusroseus*. Theoritical& Applied Genetics, 106: 221-230.
22. Ali Y, Aslam Z, Ashraf MY and Tahir GR, 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. International Journal of Environmental Science & Technology, 1(3): 221-225
23. Nicolas ME, Munns R, Samarakoon AB and Gifford RM, 2012. Elevated CO_2 improves the growth of wheat under salinity. Australian. Journal of Plant Physiology, 20: 349-360.
24. Weon YC, Kyu SL, Jong CK, Song, YC and Don HC, 2003. Critical saline concentration of soil and water for rice cultivation on a reclaimed saline soil. Korean Journal of Crop Science, 48(3): 238-242.
25. Islam M.Z, Baset MA, Islam MR and Akter A, 2007. Effect of different saline levels on growth and yield attributes of mutant rice. Journal of Soil Nature, 1(2): 18-22.
26. Khanam TN, Akhtar MA, Halim F and Hossain M, 2018. Effect of irrigation salinity on the growth and yield of two Aus rice cultivars of Bangladesh.Jahangirnagar University Journal of Biological Science, 7(2): 1-12.
27. Singh SK, Kakani VG, Brand D, Baldwin B and Reddy KR, 2008. Assessment of cold and heat tolerance of winter-grown canola (Brassica napus L.) cultivars by pollen-based parameters. Journal of Agronomy and Crop Science, 194: 225–236.