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Screening of Rice Genotypes for Salinity Tolerance at Seedling Stage Through SSR Marker

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ABSTRACT

Twenty-two rice lines were used to evaluate salt tolerance at seedling stage. Salinity screening was conducted following IRRI standard protocol and salinized (EC14dS/m) with culture solution at vegetative stage. Initial and final scoring for visualsalt injury using the IRRI Standard Evaluation System (SES) was done 21 days after salinization (DAS). Based on 1-9 scale scoring, eight rice genotypes (Binadhan 8,Binadhan 10, FL478, RC217, RC221, RC222, RC225) were found as salt tolerant, five genotypes (RC191, RC192, RC193, RC251, RC249) were identified as moderately tolerant, the rest genotypes were susceptible and the Binadhan 7 was highly susceptible at seedling stage. In seedling stage, Binadhan 8, Binadhan 10, FL478, RC217, RC221, RC222 and RC225 were identified as tolerant while RC227, RC229, Binadhan 12, Joli Aman, Binadhan 11, BRRI dhan29, Pajam and BRRI dhan39 were found as susceptible. Three selected SSR markers viz. RM7075, RM10701 and RM11504 were used to screen the germplasm for salt tolerance. The highest genetic diversity (0.8967) was observed in loci RM7075 and the lowest gene diversity (0.5537) was found in loci RM10701 with a mean diversity of 0.7231. This SSR markers offer a potential, simple, rapid and reliable method in marker assisted breeding and a great impact on identifying salt tolerant rice genotypes.

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Introduction

Rice is the most important cereal crop among the low- and middle-income countries of the world and developing countries contribute 96.24 % of the total world rice production (Anonymous, 2012). In 2025, 21% increase of rice production will be needed over the production of 2000 (Bhuiyan *et al*., 2002). Rice is a salt sensitive crop species for which soil salinity is a major factor restricting yield throughout substantial areas of Africa and South and Southeastern Asia (Walia *et al.,* 2005). At present, salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increase rice production worldwide (Gregorio, 1997). In Bangladesh, saline soil covers about 2.8 million hectors of land (BBS, 2009). The total saline area forms one third of the 9 million hectares of total national cultivated area of Bangladesh (ABSPII, 2006). The existing modern varieties are not adapted to this ecosystem because of their lack in salt tolerance. But now it becomes essential for us to adapt modern varieties under saline environment. Rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage (IRRI, 1997). So development of salt tolerant rice varieties has become a demand for this country to feed the bursting population. Among various strategies to overcome this problem, the possibility of selection and breeding for enhanced salinity tolerance in crop species has received considerable attention as it is an economic and efficient alternative (Ashraf *et al*., 2008; Ashraf, 2009). Screening under controlled condition has the benefit of reduced environment effects and the hydroponic system is free of difficulties associated with soil related stress factors. The conventional method of plant selection for salt tolerance is not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance (Gregorio, 1997). This hinders the development of an accurate, rapid and reliable screening technique. However, DNA markers seem to be the best candidates for efficient evaluation and selection of plant material.

Molecular markers are the molecules that could be used to trace a desired gene(s) in examined genotypes. The technology provides a powerful tool in the assessment of genetic relationships within and among species in which differences among accessions can be revealed at the DNA level (Mishra *et al.,* 2002). SSRs (Simple Sequence Repeat) or microsatellite markers have been proved to be ideal for making genetic maps (Islam, 2004), assisting selection (Bhuiyan, 2005) and studying genetic diversity in germplasms. SSR markers are playing an important role to identify gene for salt tolerance that can be helpful for plant breeders to develop new cultivars. They have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application (Cho et al., 2000) and also been useful in integrating genetic, physical, and sequence-based maps of rice, and provide breeders and geneticists with efficient tool to link phenotypic and genotypic variations. The molecular characterization information as well as genetic diversity analysis could be helpful for planning of rice breeding program to improve grain quality, yield quality and specially for minimizing stress such as salinity, cold, flood etc. tolerant genotype development. The objective of this study is to screen rice germplasm under saline and non-saline conditions at both vegetative stages; and to determine genetic diversity and identify salt tolerant rice genotypes using microsatellite markers.

Materials and methods

Plant materials and management

The experiment was performed at the glass house and laboratory, Division of Biotechnology, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202. A total of twenty-two rice genotypes were obtained from IRRI, BRRI and BINA (Table 1). Three markers were used for salt tolerance evaluation of 22 rice genotypes. The banding patterns were identified with known salt tolerant genotypes like Binadhan 8, Binadhan 10 and FL 478. The experimental design was completely randomized design (CRD) with three replications.

Table 1. Identity, source and category of rice genotypes in the study

SL. No.	Genotypes	Category	Origin/Source of Collection
1	RC191*	Salt tolerance + *Sub-1*	IRRI
2	RC192*	Salt tolerance + *Sub-1*	
3	RC193*	Salt tolerance + *Sub-1*	
4	RC217*	Salt tolerance	
5	RC221*	Salt tolerance	
6	RC222*	Salt tolerance	
7	RC225*	Salt tolerance	
8	RC227*	Zn deficiency tolerance	
9	RC229*	Zn deficiency tolerance	
10	RC251*	Salt tolerance + *Sub-I*	
11	RC249*	Salt tolerance + *Sub-I*	
12	BRRI dhan11	Salt tolerance	BRRI
13	Binadhan 7	HYV	
14	Binadhan 8	Salt tolerance	BINA
15	Binadhan 10	Salt tolerance	
16	Binadhan 11	*Sub-I*	
17	Binadhan 12	*Sub-I*	
18	Joli Aman	*Sub-1*	
19	BRRI dhan 29	HYV	BRRI
20	Pajam	HYV	
21	BRRI dhan 39	HYV	
22	FL478	Salt tolerance	IRRI

*=IRRI advanced rice lines and HYV= High yielding varieties

Table 2. 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 3. The sequence and size of the microsatellite markers used for screening salt tolerant rice lines

Markers	Primer sequence (5'-3')		Estimated length (bp)	Repeat motif	Annealing temp. (ºC)
RM 7075	For.	TATGGACTGGAGCAAACCTC	155	(ACAT)	55
	Rev.	GGCACAGCACCAATGTCTC		13	
RM 10701	For.	GAGACACGGCACAATATACAACG	69	(AG) 10	55
	Rev.	TTCTATCTCCGACCTCTTCTCAAGG			
RM 11504	For.	TCGTCTTTGAGCCCACCATATTCG	281	(AAG)18	55
	Rev.	CGCACCAGCACCCTTGTATCC			

Phenotypic study of salinity tolerance at seedling stage

Modified Hydroponic system (Gregorio *et al.,* 1997) was used at the glasshouse to evaluate salt tolerance of the rice genotypes using Peter's solution (Yoshida *et al.,* 1976). The nutrient solution was salinized by adding crude salt to obtain desired EC of 14 dS/m. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI, 1997). Visual rating of salinity tolerance was done according to Table 2. This scoring discriminated the highly tolerant, tolerant, moderately tolerant, susceptible and highly susceptible rice genotypes. Initial and final scoring was done at 15th day and 21st day respectively after salinization.

Genotyping of salinity tolerance rice

Modified Cetyl Trimethyl Ammonium Bromide (CTAB) method was used for DNA extraction for 21-days-old seedling (IRRI, 1997). The quality of the isolated DNA through this procedure was satisfactory for PCR analysis. Three selected primers were used for this study (Table 3). Each PCR reaction carried out with 10.0 µl reactions containing master mix 5.0 µl, 0.5 µl primer forward, 0.5 µl primer reverse 3.0 µl dd H_2O and 1.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94ºC for 5 min, followed by 32 cycles of denaturation at 94ºC for 4 min, annealing at 55ºC for 1 min and polymerization at 72ºC for 1 min; and final extension by 5 min at 72ºC. Then electrophoresis in 2% agarose gel was done after polymorphism in the PCR products and stained in ethidium bromide. Banding patterns were visualized with ultraviolet gel documentation system. The banding patterns of 22 rice germplasm were compared to the band obtained from salt tolerant variety like FL478, Binadhan 8 and Binadhan 10, those were used as salt tolerant genotype in this study.

Statistical analysis

Software was used to perform data analysis for non-salinized and salinized condition.

Table 4. Mean performance for plant height, root length, Fresh weight and Dry weight of studied rice lines at seedling stage under non-salinized and salinized condition.

	Plant hieght (cm)		Root length (cm)		Fresh weight (g)		Dry weight (g)	
Genotype	Non-salinized	Salinized (14ds/m)	Non-salinized	Salinized (14ds/m)	Non-salinized	Salinized (14ds/m)	Non-salinized	Salinized (14ds/m)
RC191	48.3 cd	27.3 h	11.67 bc	5.67 b	0.748 d	0.449 d	0.089 bcd	0.075 a
RC192	47.7 de	22.7 kl	11.33 cd	4.33 cd	0.541 g	0.359 f	0.079f	0.048 e
RC193	46.7 f	23.3 jk	12.33 ab	4.67 c	0.547 g	0.353 f	0.079 ef	0.05 e
RC217	48.7 bc	37.7 c	12.67 a	5.67 b	0.745 d	0.45 0 cd	0.094 ab	0.07 ab
RC221	47.3 ef	34.7 e	11.67 bc	6.33 b	0.843 bc	0.429 de	0.086d	0.057 d
RC222	45.3 gh	34.7 e	12.33 ab	7.67 a	0.765 d	0.411 e	0.084de	0.066 bc
RC225	47.7 de	35.7 d	11.33 cd	6.33 b	0.832 bc	0.421 e	0.087 d	0.066 bc
RC 227	44.7 h	22.3 lm	11.33 cd	3.33 e	0.829 c	0.227 h	0.059 h	0.037 f
RC229	45.3gh	23.7 j	12.33 ab	3.67 de	0.772 d	0.226 h	0.05 i	0.027 h
RC251	47.7 de	33.3 f	12.67 a	4.33 cd	0.616 f	0.454 cd	0.067 g	0.035 fg
RC249	47.3 ef	32.3 g	11.67 bc	6.33 b	0.567 g	0.34 f	0.067 g	0.035 fg
BRRI dhn11	47.7 de	32.7 g	11.33 cd	5.67 b	0.768 d	0.413 e	0.087 cd	0.068 abc
Binadhan-7	47.3 ef	32.3 g	10.67 d	4.33 cd	0.772 d	0.413 e	0.056 h	0.079 f
Binadhan-8	49.3 b	39.3 b	12.67 a	4.67 cd	0.942 a	0.535 b	0.093 abc	0.068 bc
Binadhan-10	50.3 a	37.7 c	12.33 ab	6.33 b	0.869 b	0.475 c	0.094 ab	0.063 cd
Binadhan-11	45.3 gh	22.7 kl	10.67 d	4.33 cd	0.741d	0.277 g	0.056 h	0.037 f
Binadhan-12	47.3 ef	20.3 n	12.33 ab	4.67 c	0.765 d	0.27 g	0.055 h	0.029 gh
Joli Aman	45.3 gh	20.3 n	11.33 cd	3.33 e	0.675e	0.257 g	0.064 g	0.039 f
BRRI dhan29	43.7 i	21.7 m	10.67 d	3.67 de	0.738 d	0.217 h	0.066 g	0.035 fg
Pajam	45.7 g	23.3 jk	12.33 ab	4.67 c	0.761 d	0.263 g	0.056 h	0.038 f
BRRI dhan39	43.7 i	25.7 i	9.67 e	3.33 e	0.748 d	0.355 f	0.059 h	0.038 f
FL478	49.3 b	40.3 a	12.33 ab	7.33 a	0.954 a	0.645 a	0.096 a	0.068 bc
%CV	1.23	1.97	4.93	11.48	3.02	4.12	4.43	8.09
level of significance	**	**	**	**	**	**	**	**

Table 5. Summary of Nei's (1973)) genetic distance values among studied 22 rice lines for SSR markers

OTU	G1	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G2	G20	G21	G22	G3	G4	G5	G6	G7	G8	G9
G1	0.000																					
G10	1.000	0.000																				
G11	1.000	0.667	0.000																			
G12	1.000	1.000	1.000	0.000																		
G13	1.000	1.000	1.000	0.333	0.000																	
G14	1.000	0.333	0.667	0.667	0.667	0.000																
G15	0.667	1.000	1.000	0.667	0.667	0.667	0.000															
G16	1.000	1.000	0.667	0.667	0.667	0.667	0.667	0.000														
G17	1.000	0.667	1.000	0.667	0.667	0.333	0.667	0.333	0.000													
G18	1.000	1.000	0.667	1.000	1.000	1.000	1.000	0.667	0.667	0.000												
G19	1.000	1.000	0.667	1.000	1.000	1.000	0.667	0.667	0.667	0.333	0.000											
G2	0.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000										
G20	1.000	1.000	1.000	0.667	0.333	0.667	0.667	0.333	0.333	0.667	0.667	1.000	0.000									
G21	1.000	1.000	1.000	0.667	0.333	0.667	0.667	0.333	0.333	0.667	0.667	1.000	0.000	0.000								
G22	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.667	0.667	0.667	1.000	0.667	0.667	0.000							
G3	0.667	1.000	1.000	1.000	1.000	1.000	1.000	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.667	0.000						
G4	1.000	1.000	1.000	0.333	0.333	0.667	0.667	0.667	0.667	1.000	1.000	1.000	0.667	0.667	1.000	0.667	0.000					
G5	1.000	0.667	0.667	0.667	0.667	0.333	0.667	0.667	0.667	1.000	1.000	1.000	0.667	0.667	0.667	1.000	0.667	0.000				
G6	1.000	1.000	1.000	0.667	0.667	0.667	0.333	0.333	0.333	0.667	0.667	1.000	0.333	0.333	0.667	0.667	0.667	0.667	0.000			
G7	1.000	0.667	0.667	0.667	0.667	0.333	0.667	0.667	0.667	1.000	1.000	1.000	0.667	0.667	1.000	1.000	0.667	0.333	0.667	0.000		
G8	1.000	1.000	1.000	0.000	0.333	0.667	0.667	0.667	0.667	1.000	1.000	1.000	0.667	0.667	1.000	1.000	0.333	0.667	0.667	0.667	0.000	
G9	1.000	0.667	1.000	0.333	0.333	0.333	0.667	0.667	0.333	1.000	1.000	1.000	0.667	0.667	1.000	1.000	1.000	0.667	0.667	1.000	1.000	0.333	0.667	0.667	0.667	0.333	0. 000

Here, G1- RC191, G2- RC192, G3- RC193, G4- RC217, G5- RC221, G6- RC222, G7- RC225, G8- RC227, G9- RC229, G10- RC251,G11- RC249, G12- BRRI dhan11, G13 Binadhan 7, G14- Binadhan 8, G15- Binadhan 10, G16- Binadhan 11, G17- Binadhan 12,G18- Joli Aman, G19- BRRI dhan29, G20- Pajam, G21- BRRI dhan39, G22- FL47

Results and Discussion

Phenotypic variation of rice genotypes under non salinized and salinized conditions at the vegetative stage

Twenty-two rice lines were screened for salinity tolerance by their phenotypic variation at vegetative stage. All genotypes were uniform in colour and height in the non-salinized condition (Figure 1). The phenotypic variation in some rice lines under salinized are given in Table 4. Islam *et al*., (2004) also observed wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) lines using modified SES of IRRI standard protocol. Seedling height was reduced in salinized condition, compared to the seedlings grown in non-salinized conditions (Table 4). According to SES scoring, Binadhan 8, Binadhan 10, FL478, RC217, RC221, RC222, RC225 were identified as tolerant. RC191, RC192, RC193, RC251, RC249 were identified as moderately tolerant, the rest genotypes were susceptible. Lower reduction of seedling height, fresh weight and dry weight was recorded in Binadhan 8, Binadhan 10, FL478, RC217, RC221, RC222, RC225 genotypes. On the other hand, higher reduction of seedling height, fresh weight and dry weight was showed

by RC227, RC229, Binadhan 12, Joli Aman, Binadhan 11, BRRI dhan29, Pajam, BRRI dhan39 genotypes, Tolerant genotypes showed lower growth reduction than susceptible genotypes under salinized conditions (Suplick-Ploense *et al*., 2002). Seedling height, fresh weight, dry weight of susceptible genotypes showed more reduction than tolerant genotypes. Bhowmik 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%. Munns & Tester, (2008) also reported that salinity might directly or indirectly inhibit cell division and enlargement during plant growing period. As a result, leaves and stems of the affected plants appeared stunted.

Figure 1. Non –salinized set up (A) and salinized set up (B) of 15dSm^{-1} for 21 days, respectively

Genotyping of salinity tolerance

Molecular marker helps to identify alleles that are associated with key phenotypic traits (Xu et al., 2004). Identifying molecular markers that are linked to genes controlling salinity tolerance could facilitate selection of rice lines with salinity tolerance having high heritability and expressivity. In this study, 22 lines of rice were analyzed. Three SSR primers such as RM7075, RM10701 and RM11504 were used for molecular screening. Chakravarthi and Naravaneni (2006) also reported that SSR primers had distinct polymorphism in rice while they studied 30 SSR primers on 15 rice genotypes. Amplified microsatellite loci were analyzed for polymorphism using Polyacrylamide Gel Electrophoresis (PAGE) and the result revealed that all the primer pairs detected polymorphism among the rice genotypes analyzed. The microsatellite loci were also polimorphic (5 to 12 alleles per locus with a mean of 7.33 alleles per locus). Microsatellite profiles of 22 rice lines at loci RM7075, RM10701 and RM11504 were shown in the Figure 2A, 2B and 2C, respectively. The bands obtained from other lines were compared to the band obtained from salt tolerant variety like FL478, Binadhan 8 and Binadhan 10, those were used as salt tolerant genotype in this study because it is widely known as salt tolerant. RC217, RC221, RC222, RC225 were identified as tolerant. RC191, RC192, RC193, RC251, RC249 were identified as moderately tolerant and the rest genotypes were susceptible.

Genetic Diversity

The assessment of genetic diversity or similarity is not onlyimportant for rice improvement but also for efficient management and protection of germplasm resources. Rice collected from different origin was analyzed using a highly repeatable PCR based fingerprinting assay known as Simple Sequence Repeat (SSR) or microsatellites markers. These microsatellite DNA markers produce a higher level of DNA polymorphism than other DNA markers with respect to rice lines. 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 Heenan et al. (2000). According to Nei's, (1973), the highest level of gene diversity value (0.8967) was observed in loci RM7075 and the lowest level of gene diversity value (0.5537) was observed in loci RM10701 with a mean diversity of 0.7231 (Table 4.3). 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. RM11504 primer showed a gene diversity value of 0.7190. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity.

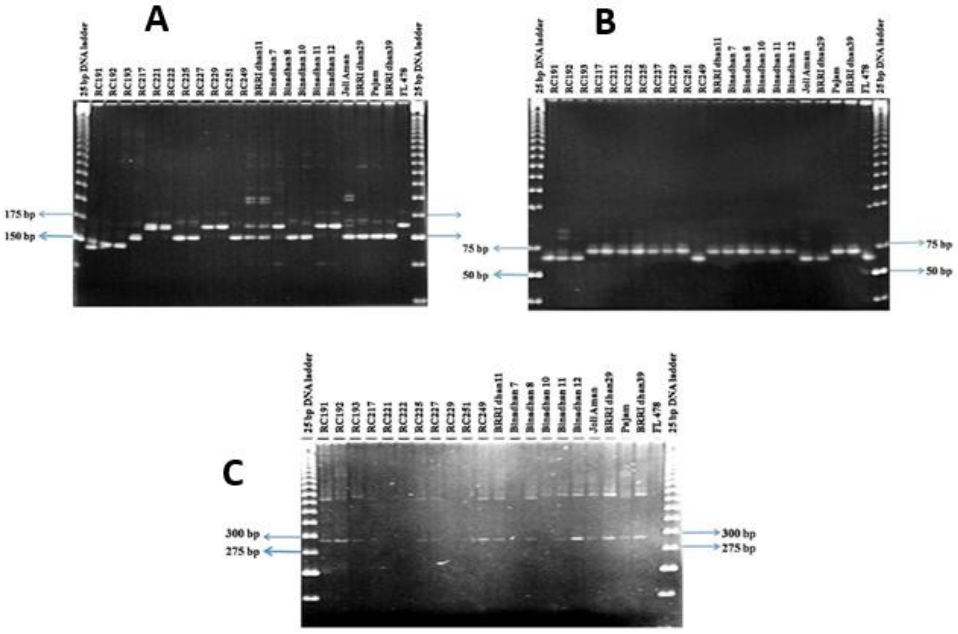

Figure 2. Microsatellite profiles of 22 rice lines at loci RM7075 (2A), RM10701 (2B) and RM11504 (2C)

Genetic diversity analysis

The values of pair-wise comparisons of Nei's (1973) genetic distance between 22 rice lines was computed from combined data sets for the three primers ranging from 1.00 to 0.33 (Supplementary Table 1). Comparatively higher genetic distance genetic (1.00) was observed between RC191 and RC251, RC191 and RC249, RC191 and BRRI dhan11, RC191 and Binadhan 7, RC191 and Binadhan 8. Binadhan 8 is a salt tolerant landrace variety and RC191 is an advanced line, both are developed by IRRI. The lowest genetic distance (0.33) was revealed between Pajam and Binadhan7, Pajam and Binadhan 11, Pajam and Binadhan 12. The smaller number of pair-wise differences (high genetic similarity value) among some rice lines were likely due to their genetically relatedness. On the other hand, large number of pair-wise differences (low genetic similarity value) was observed among those rice lines developed from genetically distant parental. According to the relationship of lower genetic distance among the varieties most of them were tolerant. Table 8: Summary of Nei's (1973) genetic distance values among studied 22 rice lines for SSR marker.

Genetic similarity analysis using UPGMA

Dendrogram based on Nei's (1973) genetic distance using Unweighted Pair Group Method of Arithmetic Means (UPGMA) indicated differentiation of the 22 rice lines (by 3 markers). All 22 rice lines could be easily distinguished. The UPGMA cluster analysisled to the grouping of the 22 lines in five major clusters (Figure 3). RC222 and Binadhan 10 formed cluster-1. Observed similarity value between RC222 and Binadhan 10 was 0.333 and genetic distance value was 0.667. According to SES scoring Binadhan 10 and RC222 rice lines of cluster 1 is highly salt tolerent atvegetative and reproduvtive stage. On the other hand, cluster 2 was formed by BRRI dhan11 and FL478. Both of them were tolerant under salt stress at different growth stage. Whereas, cluster 3 showed three sub clusters (Sub cluster 3a, Sub cluster 3b and Sub cluster 3c). Sub cluster 3a had two lines RC251 and Binadhan 8. The similarity value between RC251 and Binadhan 8 was 0.333. In Sub cluster 3b had two varieties RC221 and RC225. Similarity value between RC221 and RC225 was 0.333. The remaining group (Sub cluster 3c) had only one variety, which is RC249. All of them were found tolerantby SES scoring and agronomic performance. While, cluster 4 was formed two sub clusters (Sub cluster 4a, Sub cluster 4b). Sub cluster 4a had five lines RC192, RC193, Joli Aman, RC229 and RC217. The similarity two lines RC217 and RC229 was 1.000. It should be noted that RC217 was tolerant along with RC192, RC193, Joli Aman but RC229 was susceptible by SES scoring. It was observed that RC192 and RC193 were in same position in cluster whereas, similarity was 0.667 and they are moderately tolerant and cluster 5 showed only three lines which are BRRI dhan29, RC227 and Binadhan 7. RC227 was found as moderately tolerant whereas BRRI dhan29 are whilst and susceptible in SES scoring except Binadhan 7. In SES Scoring Binadhan 7 was found highly Susceptible. They had similarity value of 1.000. Based on above result, line which showed the maximum tolerant and moderately tolerant under salt stress at different stage and they grouped I same cluster due to lower genetic distance.

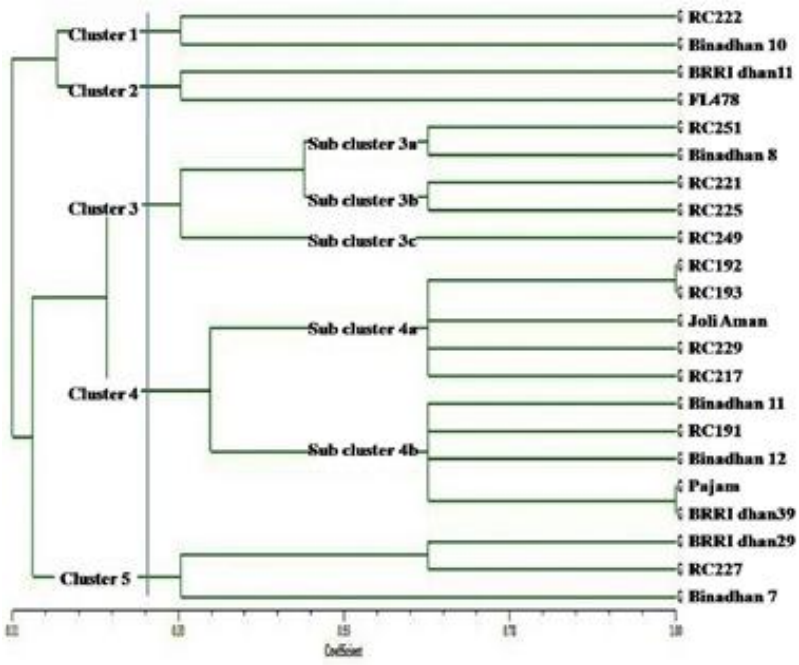

Figure 3. UPGMA dendrogram based on Nei's (1973) genetic distance summarizing the data on differentiation among 22 rice lines according to SSR analysis. Arrow line indicates the scale of genetic distance (0.11-1.00)

Conclusion

Twenty two rice lines were used for salinity screening and morphological performance at vegetative stage and molecular characterization for salt tolerance using SSR markers. According to the modified SES system 1-9 scale, among 22 lines eight lines were identified as salinity tolerant; five genotypes were identified as moderately tolerant. One line was identified as highly susceptible and eight genotypes were susceptible. Tolerant lines also showed higher number of plant height, fresh weight and dry weight than the susceptible genotypes. Salt tolerance of the lines was tested with 3 markers viz. RM7075, RM10701 and RM11504. From the genetic analysis, Binadhan 10, RC217, RC221, RC222, BRRI dhan11, RC225 were salt tolerant compared to FL478 and Binadhan 8. Binahan 8, Binadhan 10, FL478, RC217, RC221, RC222, RC225 could be utilized to develop salt tolerant rice varieties with all desirable characters using marker-assisted backcrossing. Marker-assisted selection (MAS) has great potentialities and impact on identifying salt tolerant rice lines. Thus, this technique can be used to identify traditional more land races from saline prone region of Bangladesh.

Competing interest

To developed salt tolerant rice variety in Bangladesh.

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