

*Full Length Research Paper*

# Evaluation of rice genotypes to salt stress in different growth stages via phenotypic and random amplified polymorphic DNA (RAPD) marker assisted selection

Shahrbano Mirdar Mansuri\*, Nadali Babaeian Jelodar and Nadali Bagheri

Department of Agronomy and Plant Breeding, Sari Agricultural Sciences and Natural Resources University (SANRU), Iran.

Accepted 17 October, 2011

Soil salinity represents an increasing threat to rice production. The success of salt tolerance breeding programs employing traditional screening and selection has been limited in the past decades. 40 rice genotypes were screened in saline soil of electrical conductivity (EC) of 4, 8 and 12 ds/m in vegetative growth stage in 2009. Tolerant genotypes were tested in young seedling stage in hydroponic system and then reproductive stage in 2010. Results show that vegetative growth was less affected by salt stress comparison to reproductive stage. Na and Na-K ratio in tolerant genotypes were lower than susceptible genotypes in salt condition in young seedling stage. Shastak-mohammadi, Hasani, Trom-Danesh, Line109 and Line75 were more tolerant to salt stress for the evaluated traits in reproductive stage. Molecular analysis showed that UBC-251 and UBC-244 displayed variation in the banding pattern of individual rice genotypes. Results of molecular analysis confirmed evaluation of phenotypic analysis.

**Key words:** *Oryza sativa*, salt stress, different growth stage, random amplified polymorphic DNA (RAPD) markers.

## INTRODUCTION

Rice is one of the most widely cultivated crops which provide food for one-half of the world's population (Zeng et al., 2004). The soil salinity of reclaimed paddy fields is one of the important stresses which limit rice growth and yield in Asia and Africa (Lee et al., 2007). There are 380 million ha of saline soils on the earth's land surface which are widely distributed in arid and semi-arid areas and seasonally dry coastal areas, where they severely affect the agricultural production of many countries (Xie et al., 2000). Rice is sensitive to salinity particularly at the seedling stage. Screening/breeding of rice varieties for tolerance to salinity have been carried out for over three decades and various screening methodologies are used to screen out tolerant varieties (Surekha et al., 2008). Although salinity affects all stages of growth and development of rice, salinity at the reproductive stage

depresses grain yield much more than salinity at the vegetative stages, therefore, screening for tolerance at reproductive stages has been considered to be more useful (Surekha et al., 2008). The use of physiological characters as selection criteria in salt tolerance breeding requires the identification of the contribution of each individual character to salt tolerance (Sabouri et al., 2007). Plant growth, plant height or shoot biomass, were reported to have dilution effects on sodium accumulation in leaves of rice. Panicle weight, tiller numbers per plant and harvest index are important agronomic characters for the prediction of final yield in rice. These yield components are severely affected by salinity (Zeng et al., 2004). Breeding for salinity tolerance in rice requires variable screening techniques and application of molecular marker technology (Gregorio et al., 2002). In recent years, DNA polymorphism assays and molecular marker have been used for genetic mapping and for marker assisted selection (MAS). The mapping of salt tolerant genes in rice would greatly facilitate breeding for

\*Corresponding author. E-mail: [mirdar-mansuri@yahoo.com](mailto:mirdar-mansuri@yahoo.com).

salt tolerance. In rice, random amplified polymorphic DNA (RAPD) markers have proven useful for efficiently tagging genes (Xie et al., 2000). The objective of this study was to screen salinity tolerance between new source of breeding material and Iranian landrace genotypes based morphological and ionic traits and RAPD marker at different growth stages.

## MATERIALS AND METHODS

### Plant material

A population of 40 genotypes (20 Iranian landraces, 19 improved rice cultivars and 2 foreign genotypes) with Pokkali (tolerant check) and IR29 (susceptible check) were tested in salt stress (0, 6, 8 and 12 ds m<sup>-1</sup>) in vegetative stage in controlled condition in greenhouse in 2009 (Table 2). IRR standard protocol (Gregorio et al., 1997) was used to evaluate salt tolerance of rice germplasms. Finally, 15 genotypes were selected as salt tolerant with Tarom-Jelodar, Tarom-Milad and Tarom-Danesh and these genotypes were evaluated in other stage.

### Evaluation of rice genotypes at seedling stage

At seedling stage, germinated seeds were sown in hydroponic system with tap water in a plastic box (32x24x18 cm). One seed per hole, on a Styrofoam sheet with 100 holes attached a nylon net bottom. The sheet was floated on the distilled water for four days, on the nutrient solution (Yoshida et al., 1976) for four days and persalinized to 0.3% NaCl for four days. Then, seedlings were transferred to 0.7% NaCl for four weeks until the second to third leaf stages (Lee et al., 2007). The nutrient solution was renewed once every week, pH was maintained at 5.0 daily and mean temperature was 31/25°C. The salinity symptom in genotypes was scored according to a modified standard evaluation system (SES) for rice (Table 1). Then, root and shoot length, root and shoot dry weight, Na and K percent were measured.

### Evaluation of rice genotypes at reproductive stage

At the reproductive stage, seedlings were sown in perforated pots which were served as a water tank (Gregorio et al., 1997). In flowering stage, tap water was replaced with salinized water (6 and 12 d/sm). Finally, yield, yield components and biomass were measured. Salinized and non-salinized setups with three replications were maintained at vegetative, seedling and reproductive stages. Analysis of variance (ANOVA) was done using SPSS to compute genotypes (G), environmental effects (E) and G×E interactions across the two environments.

### Molecular analysis with RAPD marker

Modified CTAB was used to extract DNA from leaf samples of genotypes. Two primers, UBC251 (5'-CTTGACGGG-3') and UBC244 (5'-CAGCCAACCG-3') were chosen for the study (Xie et al., 2000). 30 µl polymerase chain reaction (PCR) reaction contained 15 ng DNA samples, 1.25 units Taq DNA polymerase, 0.2 µM primer, 1.7 mM MgCl<sub>2</sub>, 0.17 mM dNTPs and PCR buffer. PCR profile was maintained as initial denaturation at 94°C for 4 min, then the reaction was subjected to 45 cycles of 94°C for 1 min, 35.8°C for 1 min, 72°C for 2 min with a final elongation step of 4 min at 72°C. Amplification products were resolved by electrophoresis on a

1.5% agarose gel with ethidium bromide in TBE buffer and visualized under UV illumination.

## RESULTS

In the first experiment, salt stress reduced growth of rice cultivars. Effect of salinity, genotypes and their interaction were significant on all measured traits (Table 3). In final vegetative growth stage, most of the genotypes died in EC 8 and 12 ds m<sup>-1</sup>. 15 rice genotypes representing a range of tolerance to salt response were selected for the study (Table 2). Result in this step showed that the tolerant genotypes belonged to traditional land races (tall) compared to other genotypes (medium and dwarf).

### Salinity effect on seedling stage

Result show that tolerant rice genotypes in the first experiment were tolerant in seedling stage too, expect Gerdeh cultivar that was identified as a susceptible cultivar. Scoring was performed on day 7 after salinization, when four categories of tolerance can be visually distinguished (Table 4). Symptoms of salt stress included a reduction in growth, whitish leaf tip, leaf rolling, drying of leaves and reduction of height seedling in salinized condition (Figure 1). Root and shoot dry weight and biomass were reduced in rice seedling in salinity stress (Figure 2). Salinity treatment increased Na<sup>+</sup> concentration in all cultivars whereas tolerant cultivars maintained lower amount of Na<sup>+</sup> and Na<sup>+</sup>: K<sup>+</sup> ratio; tolerant genotypes was lower than susceptible genotypes in salt condition (Figure 3). Correlation analysis results showed that significant correlation was detected between biomass and height; this result shows that tall cultivar produced more biomass in the salt condition. Significant correlations were detected between height of seedling and root dry weight and biomass (Table 5). Tolerant cultivars had high biomass and low Na:K ratio. Factor analysis showed that biomass, Na/K ratio and root lengths explained most of the variation. The first component was most influenced by Na: K ratio and biomass; therefore the result shows that Na: K ratio and biomass are important traits in salt tolerance whereas, Na/K ratio (-0.84%) and biomass (0.80%) had negative and positive effect on the first factor respectively.

Observation showed that biomass of tolerant cultivars was more than biomass of susceptible cultivar; therefore this trait can be used for identification of tolerant genotypes of rice in salt stress. Root dry weight (0.81%) and biomass (0.52%) explained most of the variation in the second factor respectively. Therefore, the second factor showed that increase in root dry weight and biomass had positive effect on rice salinity tolerance. The third factor was influenced by root length (Table 6). Genotypes were grouped by wards cluster analysis method and three groups including tolerant, semi

**Table 1.** Modified standard evaluation system of visual salt injury at seedling stage.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leave rolled	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plant dying	Susceptible
9	Almost all plant dead or dying	Highly susceptible

**Table 2.** Response of genotypes to salinity stress in vegetative growth.

Genotype	Country of origin	Classification of germplasm	Salinity tolerance	Genotype	Country of origin	Classification of germplasm	Salinity tolerance
MTM2	Iran	Breeding line	S	Hsani	Iran	Cultivar	T
Ghasrodashti	Iran	Cultivar	S	F <sub>114</sub>	Iran	Breeding line	S
Shafagh	Iran	Cultivar	S	126 (IRRI2-2x deylamani)	Iran	Breeding line	S
Mosa-Tarom	Iran	Cultivar	S	109 (Sang- taromxHassani)	Iran	Breeding line	T
Domsiyah	Iran	Cultivar	T	83 (IRRI2-2xSang-Tarom)	Iran	Breeding line	S
Khazar	Iran	Cultivar	S	76 (IR58025A / R <sub>2</sub> //Sepidrood)	Iran	Breeding line	S
IR28	Philippines	Cultivar	S	75 (Shastakmohammadix SangTarom)	Iran	Breeding line	T
Gerdeh	Iran	Cultivar	T	41 (IRRI2-2 xShastakmohammadi)	Iran	Breeding line	S
Dasht	Iran	Cultivar	S	39 (IR58025A / R <sub>9</sub> // xSepidrood)	Iran	Breeding line	S
Sepidrood	Iran	Cultivar	S	23 (IRRI2-2 xHassni)	Iran	Breeding line	S
Nemat	Iran	Cultivar	S	19 (IR58025A / R <sub>2</sub> //x Sang-Tarom)	Iran	Breeding line	S
Neda	Iran	Cultivar	S	Line 17 (Sahelx Sang-Tarom)	Iran	Breeding line	S
IR229	Philippines	Cultivar	T	Line 10 (IRRI2-2x Shastakmohammadi) // Sepidrood	Iran	Breeding line	S
Amol-3	Iran	Cultivar	S	Line 7 (IRRI2-2xHassani)	Iran	Breeding line	S
Tarom-Mahali	Iran	Cultivar	T	Line 5 (IR58025A// IR68061-27-3-2-2-3R)//Neda	Iran	Breeding line	S
Anbarboo	Iran	Cultivar	T	Line 3 (Sang-TaromxDeylamani)	Iran	Breeding line	S
Sahel	Iran	Cultivar	S	Tarom-Danesh	Iran	Cultivar	T
Fajr	Iran	Cultivar	S	Tarom-Jelodar	Iran	Cultivar	T
Noksiyah	Iran	Cultivar	T	Tarom-Milad	Iran	Cultivar	T

**Table 2.** Contd

Sang-Tarom	Iran	Cultivar	S	IR29	Philippines	Cultivar	S
Shastak-mohammadi	Iran	Cultivar	T	Pokkali	India	Cultivar	T
Deylamani	Iran	Cultivar	T				

\*Genotypes were classified into two categories: S, salt sensitive and T, salt tolerant.

**Table 3.** Analysis of variance (ANOVA) for various growths attributes of rice genotypes under salinity.

S.O.V	df	Height	Shoot length	Root length	Tiller number	Shoot dry weight	Root dry weight	Leaf dry weight	Biomass
R	2	1.32ns	0.78ns	0.29ns	0.43*	8682.70 ns	0.25 ns	0.16 ns	0.60 ns
G	39	1219.5**	256.48**	124.57**	21.61**	8750.15**	14.94**	2.45**	51.34**
S	3	6544.6**	1307.57**	51.75**	150.31**	8074.87**	253.91**	30.93**	1267.09**
G×S	117	52.76**	15.61**	9.82**	5.22**	8750.25ns	3.58**	0.86**	10.85**
E	318	1.07	0.54	0.31	0.12	8755.24	8.20	0.24	0.48

\*, \*\*significant at the 0.05 and 0.01 level, respectively; ns, not significant.

**Table 4.** Evaluation system (SES) of the International Rice Research Institute (IRRI) used for evaluation of cultivars for salinity tolerance during young seedling stage.

Score	Genotype
1	Pokkali
3	Tarom-mahali, Deylamani, Hassani, Nok siyah, Line75, IR229, Line109, Domsiyah, Tarom-danesh, Tarom-milad
5	Line3, Anbaboo, Shastak mohammadi, Tarom- jelodar
7	Gerdeh
9	IR29

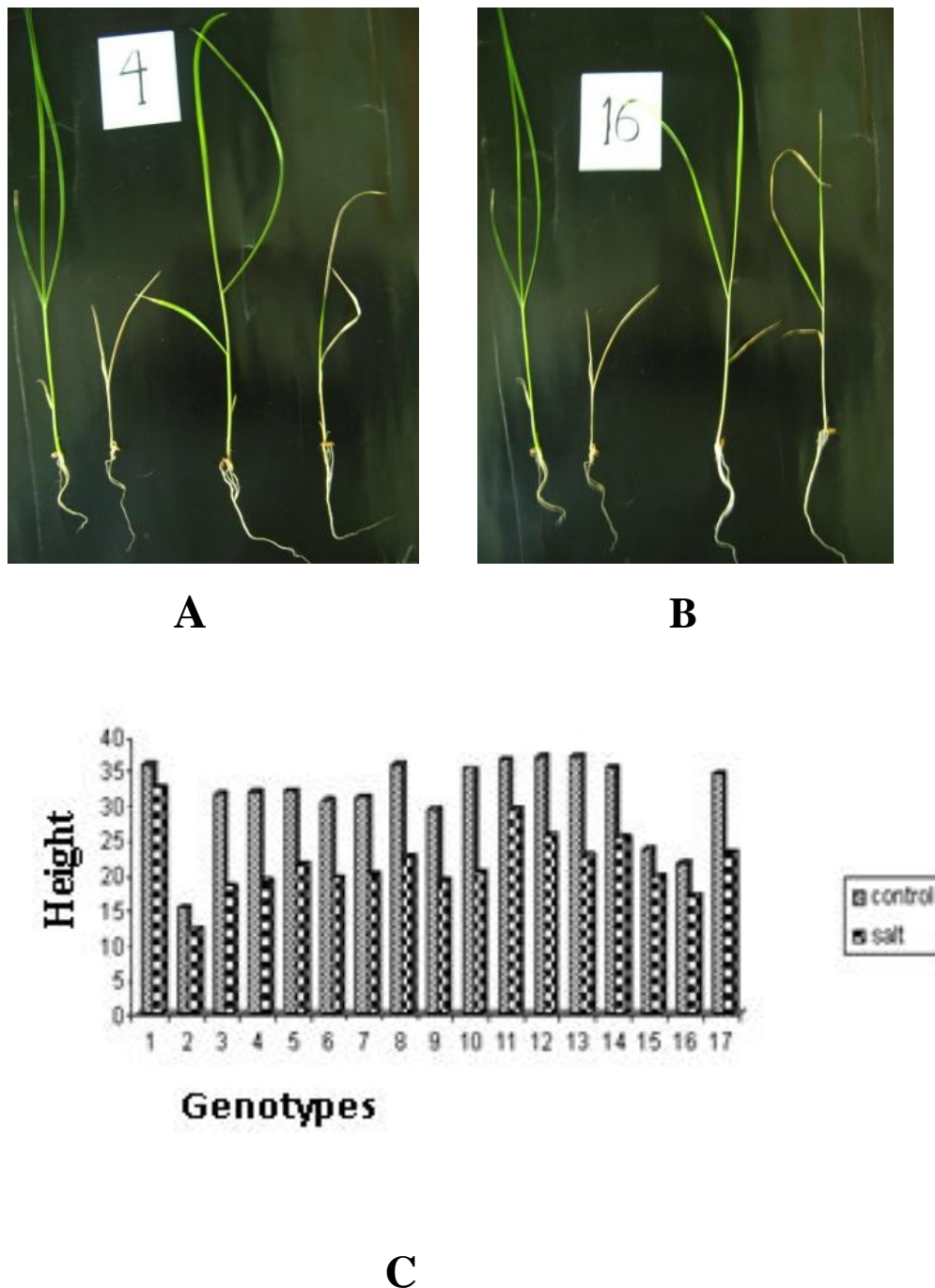
tolerant and sensitive cultivars were obtained (Figure 4).

### Salinity effect on reproductive stage

Results show that rice genotypes were more sensitive at the reproductive stage than at the vegetative and young seedling stages whereas susceptible genotypes were damaged in the first week of salt stress and did not produce panicle. Yield and yield components decreased in all the studied genotypes in salinity stress. Reduction in grain yield under stress over normal was observed in all the genotypes. IR29, Hasani, Neda and domsiyah had maximum grain yield in non stress condition, but Tarom-Danesh, Hasani and Shastak-mohammadi produced more grain yield in 6  $\text{dsm}^{-1}$  whereas IR29, Shafagh and Sahel had 100% reduction in grain yield (Figure 5). In 12  $\text{dsm}^{-1}$ , Hasani, Deylamani, Nona-Bokra, Line109, Noksiyah and Tarom-Mahhali had grain yield but other genotypes could not produce grain yield in salinity stress. Hasani had lower grain yield reduction percent (88.14%) compared to the other genotypes in 12 ds/m. Length of

panicle and number of panicle per plant in genotypes were reduced by salinity stress.

Tarom-Milad, Domsiyah, Shastak-mohammadi and Line 3 had maximum panicle length in normal condition. In 6 ds/m, Tarom-Milad had maximum length panicle. Also, Sahel, IR29 and Shafagh cultivars did not produce panicles and maximum damage of salinity stress was obtained in 6 ds/m (Figure 5). Hasani had minimum reduction percent in panicle length (42%) in 12 ds/m over non stress condition compared with other genotypes. Trom-Jelodar, Tarom-Milad and IR29 produced maximum number of panicles per plant in normal condition but IR229 had more panicles in 6  $\text{dsm}^{-1}$  and Line 109 and Shstak-mohammadi produced maximum panicles in 12 ds/m compared to the other genotypes (Figure 5). Salinity stress increased spikelet sterility percent in rice genotypes. IR29, Sahel, Shafagh and Noksiyah had 100% spikelet sterility in 6  $\text{dsm}^{-1}$  and these genotypes were identified as very sensitive to salt stress. Tarom-Mahali, Nona-Bokra and Shastak-mohammadi had the lowest spikelet sterility percentage in this level of salt stress. In 12 ds/m, Shastak-mohammadi (65.45%) and



**Figure 1.** Effect of salinity stress on growth seedling. (A) Left to right respectively, Pokkali (tolerance check), IR29 (sensitive check), Gerdeh in normal condition, and Gerdeh in salt stress. (B) Left to right respectively, Pokkali (tolerance check), IR29 (sensitive check), Tarom-Jelodr in normal condition, and Tarom-Jelodr in salt stress. (C). Reduction of height seedling in salt stress (genotypes number of name like in Figure 3).

Nona Bokra (69%) had minimum spikelet sterility percent over normal condition compared to the other genotypes (Figure 5). Yeo et al. (1990) and Bernastain et al. (1994) reported reduction fertility in rice genotypes in salt stress. In this study, it was observed that grain weight, straw

weight, biomass and harvest index decreased by salinity stress (data not shown) and tolerant genotypes had the lowest reduction for this traits compared to the sensitive genotypes. In the reproductive stage, although most genotypes could not withstand the salinity stress,

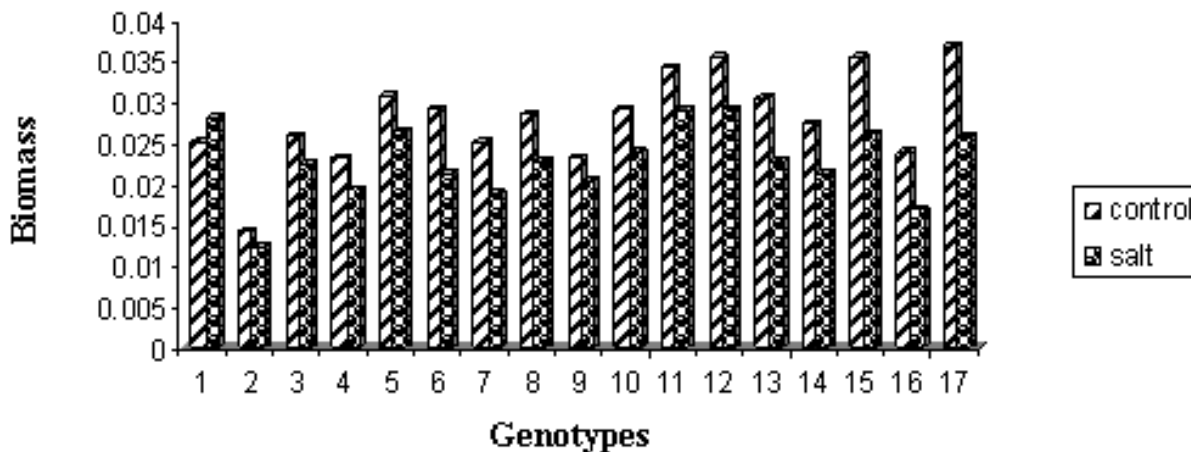


Figure 2. Effect of salinity stress on biomass of rice seedling.

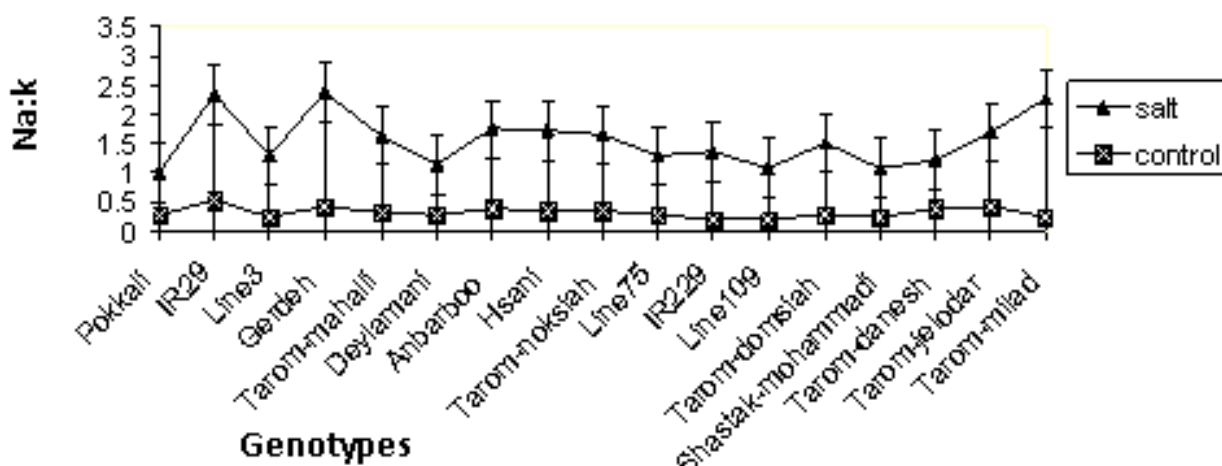


Figure 3. Effect of salt stress on Na:K ratio in rice genotypes in seedling stage.

Table 5. Correlation coefficient between evaluated traits.

Trait	Plant height	Root length	Shoot dry weight	Root dry weight	Biomass	Percentage of Na	Percentage of K	Na/ K ratio
Plant height	1.000							
Root length	-0.027	1.000						
Stem dry weight	0.182	0.057	1.000					
Root dry weight	0.356**	-0.013	0.011	1.000				
Biomass	0.742**	-0.090	0.197**	0.708**	1.000			
Percentage of Na	-0.683**	0.015	-0.142	-0.218*	-0.513**	1.000		
Percentage of K	0.403**	-0.079	0.076	-0.062	0.226*	-0.401**	1.000	
Na/ K ratio	-0.669**	0.022	0.146	-0.217*	-0.501*	-0.974**	-0.504**	1.000

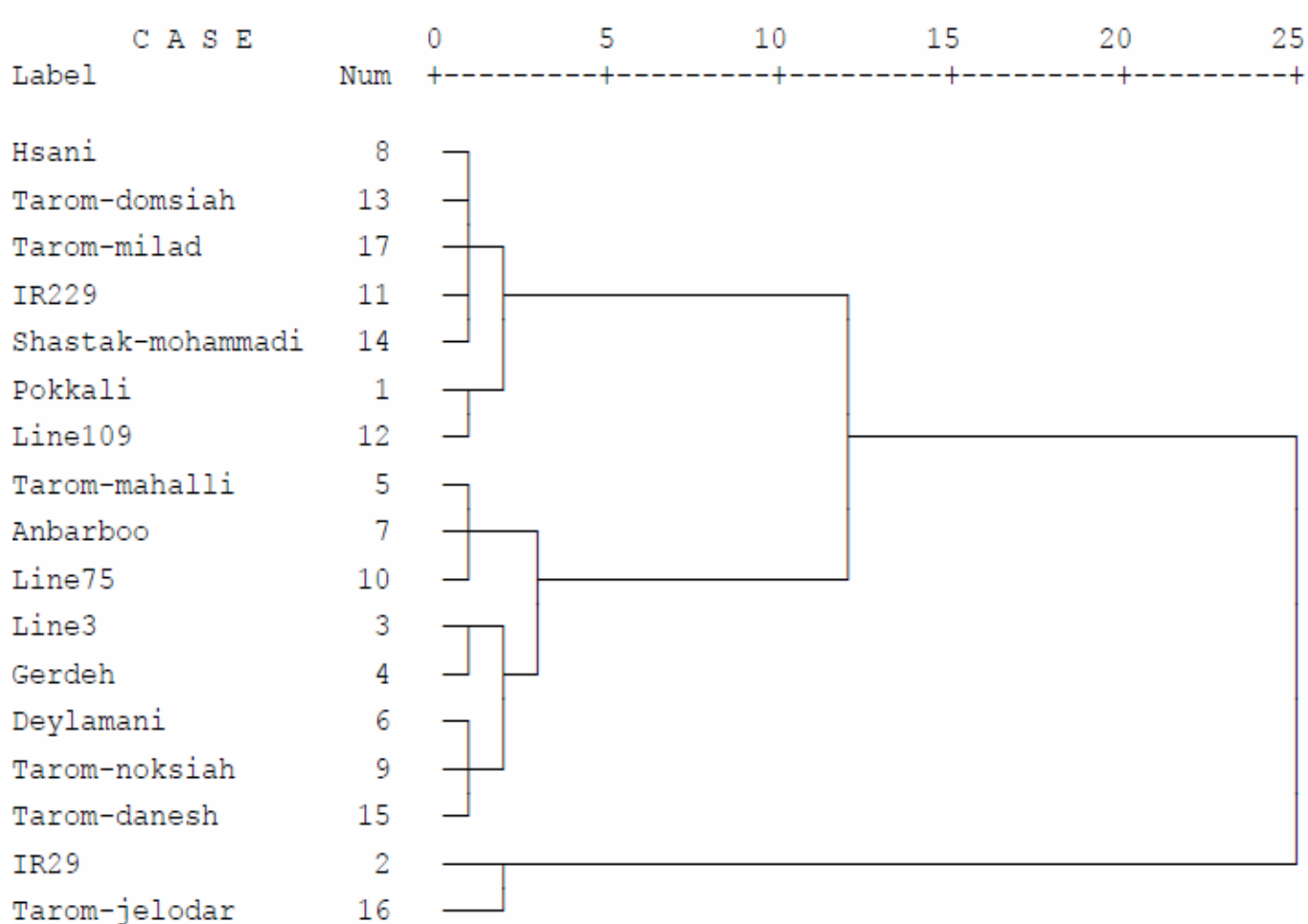
\*, \*\* Correlation is significant at the 0.05, 0.01 level, respectively.

Shastak-mohammadi, Hasani, Tarom-Danesh, Line 109 and Line 75 showed more tolerance to salt stress compared to the other genotypes. Correlation analysis

showed that grain yield had very significant correlation with panicles per plant in 6 and 12 dsm<sup>-1</sup>. Harvest index had negative and very significant correlation with spikelet

**Table 6.** Component matrix with three principle component in rice genotypes.

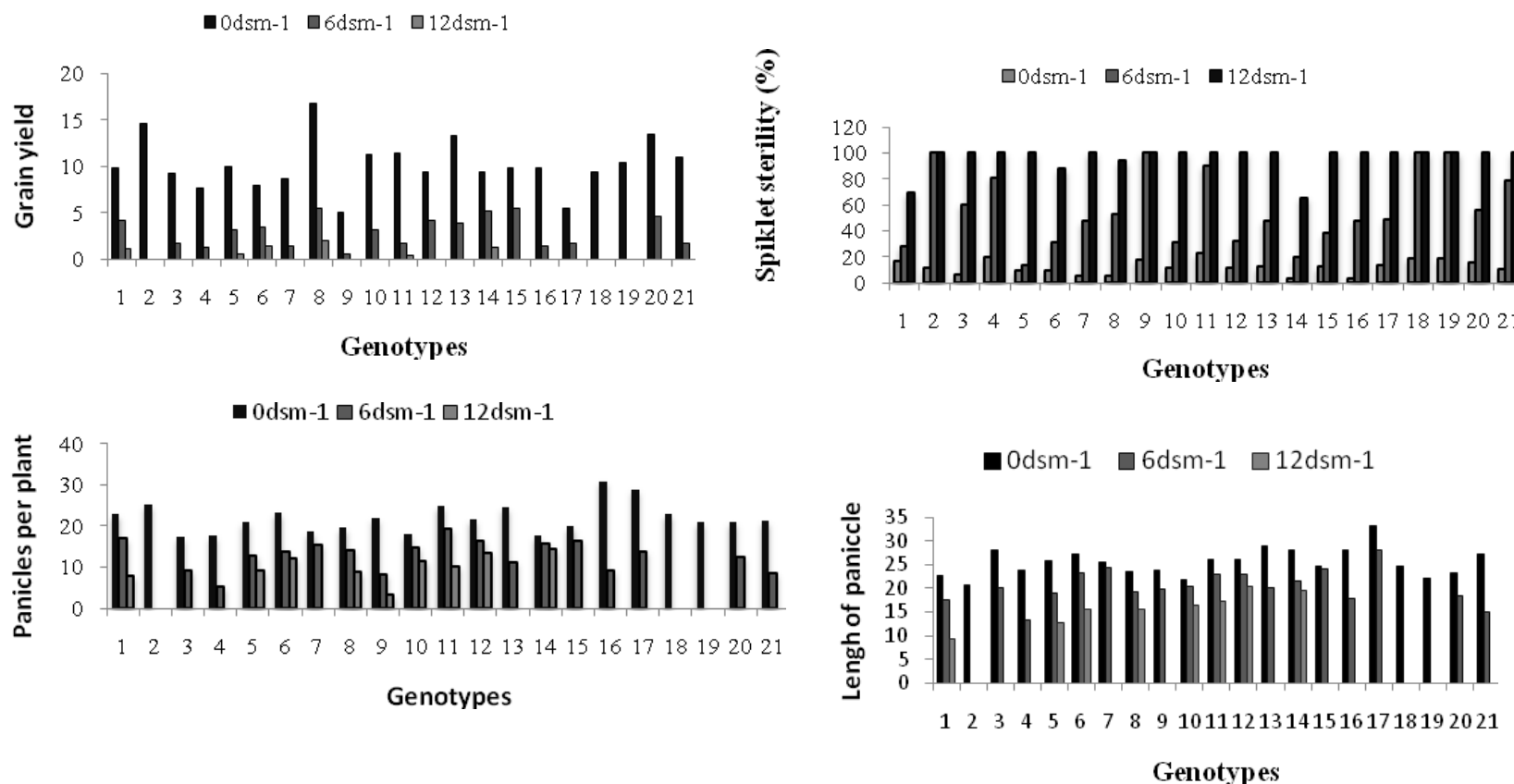
Trait	Factor		
	1	2	3
Plant height	0.777	0.154	0.318
Root length	-0.248	-0.234	0.866
Stem dry weight	0.641	-0.282	0.333
Root dry weight	0.444	0.814	0.031
Biomass	0.806	0.529	0.089
Percentage of Na	-0.828	0.285	0.135
Percentage of K	0.458	-0.515	-0.129
Na/ K ratio	-0.849	0.369	0.189
variance%	44.23	19.78	13.02
Cumulative variance	44.23	64.02	77.04

**Figure 4.** Genotype grouping by Ward's cluster analysis method for evaluated traits.

sterility in both salinity levels. Also, length of panicles had negative and significant correlation with spikelet sterility. Correlation between biomass with panicles per plant, straw weight and grain weight were significant in 6 and 12  $\text{dsm}^{-1}$  (Table 7).

#### **RAPD polymorphism of rice genotypes differing in salt tolerance**

Results of molecular analysis showed that UBC-251 and UBC-244 displayed variation in the banding pattern of



**Figure 5.** Effect of soil salinity (ds/m) on reproductive growth of rice genotypes.

individual rice genotypes. UBC-251 generated a fragment of about 1100 bp in Pokkali, Nona-Bokra, Deylamani, Tarom-Danesh, Tarom-Milad, Shastak-mohammadi, Hasani, Line109, Neda, Line 3 and Anbarboo whereas IR29, Gerdeh, Tarom-Jelodar, Nemat, Domsiyah, Noksiyah, Shafagh, IR229, Sahel and Tarom-Mahali did not produce same banding in 1100 bp for UBC-251 primer (Figure 6). Primer UBC-244 produced a prominent diagnostic fragment of about 800 bp.

IR29, Gerdeh, Tarom-Jelodar, Shafagh, Nemat, Noksiyah and Sahel did not produce specific fragment for UBC-244 whereas other genotypes showed one band in 800 bp (Figure 6).

**DISCUSSION**

Evaluation of salinity tolerance in rice is often difficult and needs long time but tolerance to

salinity of rice is different in growth stages, thus breeding and screening for salinity tolerance in rice require a rapid and reliable technique. Our study done for the three stages of rice growth showed that salinity tolerance of rice genotypes was same in the vegetative growth, seedling growth and reproductive stages but salinity affected more the yield and yield components at the reproductive stage, rather than at the previous stages as reported by Blouch et al. (2003). Thus,



**Table 7.** Correlation coefficient between evaluated traits.

Trait	Lengths of panicle		Panicles per plant		Spikelet sterility		Grain weight		Straw weight		Biomass		Harvest index	
	6 ds/m	12 ds/m	6 ds/m	12 ds/m	6 ds/m	12 ds/m	6 ds/m	12 ds/m	6 ds/m	12 ds/m	6 ds/m	12 ds/m	6 ds/m	12 ds/m
Lengths of panicles	1	1												
Panicles per plant	0.767**	0.833**	1	1										
Spikelet sterility	0.609**	-0.459*	-0.531*	-0.360	1	1								
Grain weight	-0.020	0.057	0.684**	0.653**	-0.363	-0.188	1	1						
Straw weight	0.041	0.172	0.025	0.013	-0.122	-0.034	0.526*	0.503*	1	1				
Biomass	0.025	0.729**	0.436*	0.042	-0.217	-0.084	0.747**	0.711**	0.958**	0.965**	1	1		
Harvest index	0.358	0.411	0.420	0.449*	-0.615**	-0.719**	0.473*	0.367	-0.344	-0.327	-0.110	-0.156	1	1

\*, \*\* Correlation is significant at the 0.05, 0.01 level, respectively.

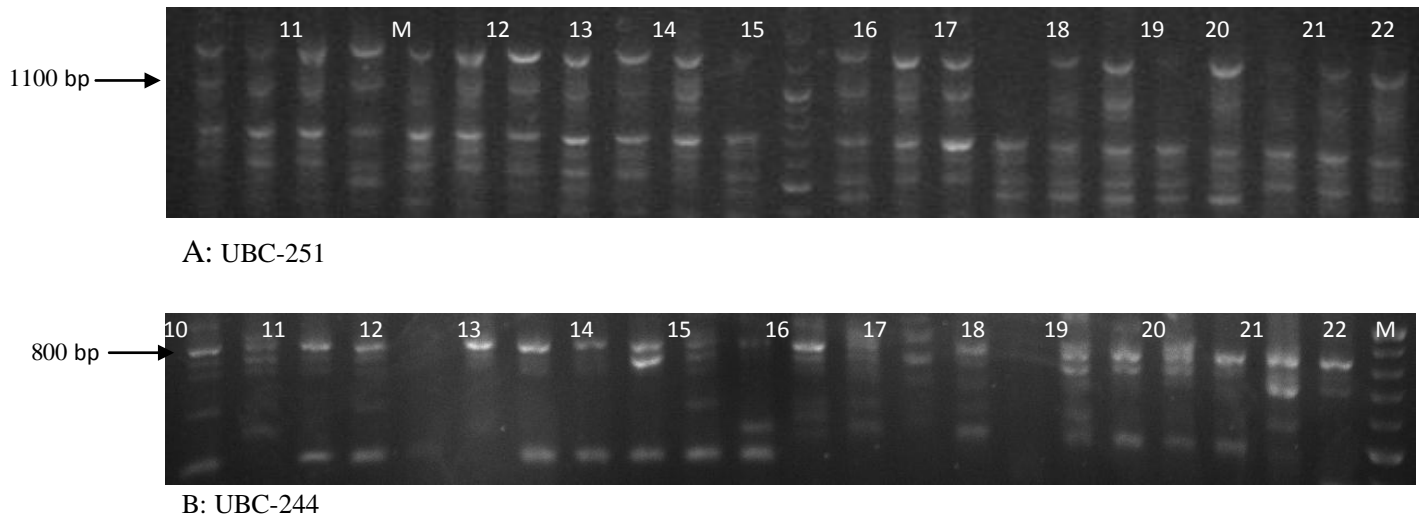
in the areas where rice was cultivated in the direct system, the screening done was more rapid and better than that in the seedling stage. Evaluation for salt tolerance in natural saline condition is difficult due to stress heterogeneity, the presence of other soil stress and influence of climatic factors, therefore hydroponic system and control condition are the best ways for screening salt tolerance. Bhowmik et al. (2007) confirmed that salt stress changed morphological traits of rice such as reduction of root, shoot length and tiller number that lead to reduction in biomass. In this study, growth of all genotypes was decreased by salinity stress in all stages, but reduction of growth in tolerant genotypes was lower than that in susceptible genotypes. Results show that most tolerant and semi tolerant genotypes had tall height. Also, tall height cultivars had low Na/K ratio in salinity stress. Walia et al (2005) reported that dwarf rice in lower growth young seedling stage were damaged by Na<sup>+</sup> accumulation in salt stress. Shastak-mohammadi, Hasani, Line 109, Line 75 are tall cultivars that show high tolerant to

salt stress. Thus, height of rice genotypes has important role in screening for salinity tolerance and genotypes with minimum height reduction were more tolerant to salinity than the other genotypes.

In this research, Na/K of the studied rice genotypes increased in salt condition. Shastak-mohammadi, Line 109, Deylamani, Tarom-Danesh, Line 75, Line 3 and IR229 had no significant difference compared with Pokkali (Tolerance check) for Na/K trait, perhaps tolerance mechanisms in these genotypes were avoidance that means these had low Na<sup>+</sup> decrease concentration inter cell because Na<sup>+</sup> absorption were minimum for these genotypes. Munns et al. (2008) reported that susceptible genotypes have more Na<sup>+</sup> concentration in cells than tolerant genotypes thus, Na<sup>+</sup> accumulation resulted in reduction in the growth of susceptible genotypes. Results of mean comparison showed that the variation between genotypes for K absorption was less than that for Na<sup>+</sup> absorption; therefore, it pointed out that the genotypes had

low Na<sup>+</sup> absorption until the variant in K absorption neutralized the negative effects of Na in the cells. Also, Domsiyah, Noksiyah, Tarom-Mahali, Hasani and Anbarboo had low Na/K ratio. Correlation between biomass and Na/K ratio ( $r = -0.501$ ), K % ( $r = 0.513$ ) and Na ( $r = 0.226$ ) showed that rice genotypes had more biomass and thus, had minimum Na<sup>+</sup> absorption and minimum Na/K ratio too. Negative correlation between K and Na/K ratio show that genotypes compete for absorption of K and Na in the cell. K is an important cofactor for most enzyme whereas Na<sup>+</sup> is a high ion in salt condition thus plant requires K; shortage of K in susceptible genotypes are more than that in tolerant genotypes (Schachtman et al., 1992).

In the reproductive stage, a few genotypes could produce grain yield in salinity condition. High or low grain yield of the studied genotypes was related with yield components and different behaviors of rice genotypes to salt stress. Thus, screening for salinity tolerance do not affect the grain yield alone; it is required that yield



**Figure 6.** Monomorphic bands among salt tolerant and susceptible varieties with primer UBC-251 and UBC-244. M, DNA marker.

components should be measured too. The results confirm the reports of the effect of salt stress on spikelet sterility. Secondly, we report trends of adverse effects of salinity stress on spikelet length and spikelet number. Shastak-mohammadi, Hasani, Trom-Danesh, Line 109 and Line75 were more tolerant to salt stress for the evaluated traits in the reproductive stage. These genotypes are early and semi early maturity genotypes whereas salt stress has more effect at the end of the agricultural season because temperature was high, thus early maturity and semi early maturity genotypes produced more grain yield. Zeng and Shannon (2000) reported that the time of salt stress on plant is more effective than growth stage in salinity stress.

In our study, although tolerance of genotypes was withstood in 6 and 12 ds/m, beyond which significant yield reduction were observed, it seems that threshold tolerance of the studied rice genotypes is lower than 6 and 12 ds/m in general. Result of the molecular analysis confirmed the evaluation of the phenotypic IR229 of Domsiyah and Tarom-Mahali which produced a fragment band for UBC-244, but did not for UBC-251 primer. Molecular evaluation of inbred species, such as rice, is often based on an accession or variety of homogeneous. When genetic identity is based on phenotypic evaluation at the whole plant level, this assumption is often an acceptable approximation to reality. However, evidence of within-cultivar heterogeneity has been documented in rice cultivars (McCouch et al., 1988; Virk et al., 1995; Olufowote et al., 1997).

Sensitive genotypes did not produce band for UBC-244 whereas Gerdeh and Noksiyah were identified as tolerant cultivar in screening, which had no band in 800 bp for UBC-244 primer. Gerdeh, Noksiyah, Domsiyah, IR229 and Tarom-Mahali was identified as tolerant to salinity whereas it did not produce band in 1100 bp and Neda (sensitive tolerance) had band in 1100 bp for UBC-251

primer. This observation might result in inter-varietal variation that may be induced by out crossing or rare cases and spontaneous mutation, as reported by Ko et al. (1994) and Olufowote et al. (1997). Other reason may be that RAPD marker is random. This research points out the possibility to use beneficial potentials of rice genotypes identified as tolerant to salinity. However, the crosses identified between the sensitive and tolerance genotypes can be used in breeding rice to salt tolerance.

## REFERENCES

- Baloch WA, Soomro AM, Javed MA, Bughio HR, Alam SM, Bughio MS, Mohammad T, Mastio NN (2003). Induction of salt tolerance on rice through mutation breeding. *Asian J. Plant Sci.* 2(3): 273-276.
- Bhowmik Sk, Islam MM, Emon RM, Begum SN, Siddika A, Sultana S (2007). Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. *Pak. J. Biol. Sci.* 10(24): 4449-4454.
- Gregorio GB, Senadhira D, Mendoza RD (1997). Screening Rice for Salinity tolerance. IRRI. Philippine. p. 22.
- Gregorio GB, Senadhira D, Mendoza RD, Manigbbas NL, Roxas JP, Guerta CQ (2002). Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crop Res.* 76: 91-101.
- Ko H, Cowan DC, Henry RJ, Graham GC, Blakeney AB, Lewin LG (1994). Random amplified polymorphic DNA analysis of Australian rice (*Oryza sativa* L.) varieties. *Euphytica*, 80: 179-189.
- Lee SY, Ahn J H, Cha YS, Yun DW, Lee MC, Ko JC, Lee KS, Fun MY (2007). Mapping QTLs related to salinity tolerance of rice at the young seedling stage. *Plant Breed.* 126: 43-46.
- McCouch SR, Doerge RW (1988). QTL mapping in rice. *Trends Genet.* 11:482-487.
- Munns R, Tester M (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59: 651-681.
- Olufowote JO, Xu YB, Chen XL, Park WD, Beachell HM, Dilday RH, Goto M, McCouch SR (1997). Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genomic*, 40: 370-378.
- Sabouri H, Rezaei A, Moumeni A, Kavousi M (2007). Investigation of genetic diversity of Iranian rice genotypes under salinity condition: Multivariate analysis. 4<sup>th</sup> International Temperate Rice Conference.
- Schachtman D, Munns R (1992). Sodium accumulation in leaves of Triticum species that differ in salt tolerance. *Australian Journal of Plant Physiology.* 19: 331-340.

- Surekha R, Mishra PB, Gupta SR, Rathore A (2008). Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant Breed.* 127: 256-261.
- Virk PS, Newbury HJ, Jackson MT, Ford-Lloyd BV (1995). The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theor. Appl. Genet.* 90: 1041-1055.
- Walia H, Wilson C, COndamine P, Liu X, Ismail AM, Zeng L, Wanamaker SI, Mandal J, Xu J, Cui X, Close TJ (2005). Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiol.* 84: 61-66.
- Xie HJ, Zapata-Arias FJ, Shen M, Afza R (2000). salinity tolerant performance and genetic diversity of four rice varieties. *Euphytica*, 116: 105-110.
- Yoshida S, Forno DA, Cock JH, Gomez KA (1976). Laboratory manual for physiological studies of rice. IRRI, Los Babos, Philippines.
- Zeng L, Kwon TR, Xuan L, Wilson C, Grieve CM, Gregorio GB (2004). Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. *Plant. Sci.* 166(2004): 1275-1258.
- Zeng L, Shannon MC (2000). Salinity effect on seedling growth and yield components of Rice. *Crop Sci.* 40: 996-1003.