Pertanika J. Trop. Agric. Sci. **32 (2)**: 247 - 259 (2009)

ISSN: 1511-3701

Seed Germination and Proline Accumulation in Rice (*Oryza sativa* L.) as Affected by Salt Concentrations

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ABSTRACT

Plant cells accumulate praline as an osmoprotectant to conserve osmotic stability and prevent damage. However, the accumulation of proline may not be associated with tolerance to salinity. This study considered the influences of NaCl and Na_2SO_4 compositions, at different concentrations, on seed germination and accumulation of proline in eleven rice genotypes. Rice seeds were grown in petri dishes in the laboratory and treated with distilled water as a control and NaCl and Na_2SO_4 (1:1 molar concentration ratio) at 2.5, 5.0, 7.5 and 10 dS m^{-1} electrical conductivity for 14 days. The mean germination time (MGT) was positively affected by the increase in the concentration of salt. Conversely, there was a negative relationship between germination index (GI) and salt concentration. Shirodi, Fajr, and Shafag can be classified into salt tolerant group, while Tarom-e-Hashemi was identified as salt susceptible, based on MGT and GI. Slight changes were recorded within dry weight and water content of seedlings at different salt levels. The maximum accumulation of proline was observed at 5 dS m^{-1} salt concentration. No relationship was established between the accumulation of proline and the growth parameters.

Keywords: Rice genotypes, seed germination, proline accumulation, salt stress, salt composition

INTRODUCTION

Salinity is an agro-environmental problem limiting plant growth and development in most of the coastal, arid, and semi-arid regions of the world (Ashraf and Khan, 1994). Two major effects have been identified as the probable causes of high salt toxicity in crop plants, i.e. the osmotic and the ionic effects. Germination percentage is not affected by salt stress, but at the same concentration of salinity, significant differences in plant height and dry weight have been reported (Ahmad *et al.*, 2000). The biochemical response of plant cells to osmotic stress is the synthesis of special organic solutes (osmolytes) which accumulate at high cytoplasm concentrations (Serrano and Gaxiola, 1994). The cells of plant accumulate proline as an osmoprotectant to conserve the osmotic stability and prevent damages. Plants culture, under salt stress, shows a high accumulation of proline (Delauney and Verma*,* 1993; Roosens *et al.,* 1999). The understanding of the role of proline accumulation in salt-tolerant rice, under salt stress, is still unclear (Moons *et al.,* 1995;

Received: 4 September 2008
Accepted: 24 March 2009

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Lutts *et al.,* 1999). Salt treatment significantly increased the proline content of rice, but this also appeared to be a reaction to stress damage and was not associated with salt tolerance, because the contents of proline were higher in the more sensitive cultivars (Hoai *et al*., 2003). There is a general acceptance that under salt stress, many plants tend to accumulate proline as a defense mechanism against osmotic challenge by acting as a compatible solute (Liu and van Staden, 2000; Ghoulam *et al.,* 2002). It seems that rice accumulates proline as a symptom of injury, rather than an indicator of salt tolerance (Gracia *et al*., 1997).

Rice is rated as an especially salt-sensitive crop (Maas and Hoffman, 1977; Shannon *et al.*, 1998). The response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity (Flowers and Yeo, 1981; Heenan *et al.*, 1988; Lutts *et al.*, 1995). In particular, salinity causes a significant reduction in the growth of seedling, very soon after planting. As the duration of salinity stress increases, there is a significant reduction in the growth of seedling (Zeng and Shannon, 2000).

The major cations in salt-affected soils are Sodium (Na^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), while the major anions are Chloride (Cl^-) and Sulphate ($SO4^{2-}$). Many studies carried out on studying salinity have utilized sodium chloride (NaCl) alone, or a combination of NaCl and calcium chloride ($CaCl_2$) salts. Since the soil solution is a mixture of solutes, the rice responses to the mixture of NaCl and $Na_2SO_{4,}$ at different salt concentrations, would be investigated to approach a saline soil solution. Therefore, the objective of the present study were: (i) to determine the influences of both NaCl and Na_2SO_4 concentrations and the composition on seed germination and proline accumulation in eleven rice genotypes, and (ii) to examine the relationships between the content of proline and the physiological characteristics of the rice seedlings.

MATERIALS AND METHODS

Plant Materials

Rice seeds (*Oryza sativa* L., Japonica cultivar-group), from 11 genotypes, were obtained from Iran. These genotypes include Pouya, Shafag, Neda, Kadous, Tabesh, Tarom-e-hashemi, Sahel, Khazar, Shirodi, Fajr and Nemat, which were selected from the widely cultivated cultivars in Iran.

Growth Condition and Salt Treatments

Twenty rice seeds were placed on filter paper-lined petri dish of 9.0 cm diameter. Salt treatments of NaCl and Na_2SO_4 (1:1 molar concentration) were dissolved in distilled water, at 2.5, 5.0, 7.5 and 10 dS m^{-1} electrical conductivity. Distilled water was applied as a control. Twenty ml of this solution was applied to each petri dish. The experiment was conducted in the laboratory, using a completely randomized design (CRD) in 3 replications at room temperature ($27 \pm 2^{\circ}C$), and under dark condition for 14 days. The number of germinated seeds was counted for 7 days (i.e. from the 3rd to 9th day after soaking). Significant differences between the treatments were determined using the Tukey's studentized procedure.

Germination was observed daily according to the recommendation by International Seed Testing Association (ISTA, 1993). The mean germination time (MGT) was calculated according to the equation proposed by Ellis and Roberts (1981), as follows:

$$MGT = \frac{\sum Dn}{\sum n}$$

Where MGT is the mean germination time, n is the number of seeds, which were germinated on day D; D is the number of days counted from the beginning of germination.

Germination index (GI) was calculated as described in the Association of Official Seed Analysis (1983), using the following formulae:

$$GI = \frac{\text{No. of germinated seeds}}{\textit{Days of first count}} + \ldots\ldots + \frac{\text{No. of germinated seeds}}{\textit{Days of final count}}$$

Three seedlings from each replicate were randomly sampled at 14th day after soaking. Root, shoot length, and seedlings fresh weight were measured. Seedlings were dried in a forced-air oven (70˚C) for 72 h and then measured for their dry weights.

Proline was measured as described by Bates *et al*. (1973). Five hundred mg of shoot of seedlings were homogenized in 10 ml of 3% sulphosalicylic acid, and the homogenate was filtered through Whatman No. 2 filter paper. Two ml of the extract was reacted with 2 ml glacial acetic acid and 2 ml acid ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) for 1 h at 100˚C; the reaction was then terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene. The chromophore-containing toluene was collected and the absorbance was read at 520 nm. The amount of proline was determined from a standard curve and presented in µmol g^{-1} fresh weight.

RESULTS AND DISCUSSION

Seed Germination

In the experiment, the imposition of salt stress was found to significantly ($P \leq 0.01$) affect the growth of seedling. The results showed a significant difference in both rice genotypes, and salt treatments. Considerable effect due to salinity, genotype and their interaction was observed for most of the traits evaluated during the seedling stage (Table 1).

The results showed that salinity stress affected rice phenology but to different extents in different genotypes. The germination of rice seeds was significantly ($P \leq 0.01$) influenced by salinity (Table 1). Meanwhile, the imposition of salt stress showed that the MGT was significantly decreased by increasing the salt levels up to 5 dS m^{-1}, as compared to the control, and an upward tendency above 5 dS m^{-1} was recorded (*Fig. 1*). The lowest and the highest MGT were observed for Shirodi and Tarom-e-Hashemi genotypes, respectively (*Fig. 2*).

Germination index (GI) and final germination percentage (FGP) decreased by increasing the salinity level, while the genotypes were found to respond differently to salinity (*Fig. 3*). There

TABLE 1

Analysis of variance of genotype (G), treatment (T) and their interactions (G×T) for the mean germination time (MGT), germination index (GI), final germination percentage (FGP), root length and shoot height, seedling dry matters, water and proline contents

Dependent variables	Independent variables		
	G	T	G × T
MGT (day)	57.0**	95.1**	4.43**
GI	39.0**	64.2**	2.96**
FGP	3.88**	4.78**	1.31ns
Root length (cm)	24.3**	30.24**	1.8**
Shoot height (cm)	11.8**	52.6**	0.95ns
Dry weight	28.4**	9.35**	1.0ns
Water content (%)	5.56**	4.04**	0.18ns
Proline (µmol g^{-1}FW)	13.1**	243.5**	16.0**

Numbers represent F-values at 1% level; ns: not significant; ** $P \leq 0.01$

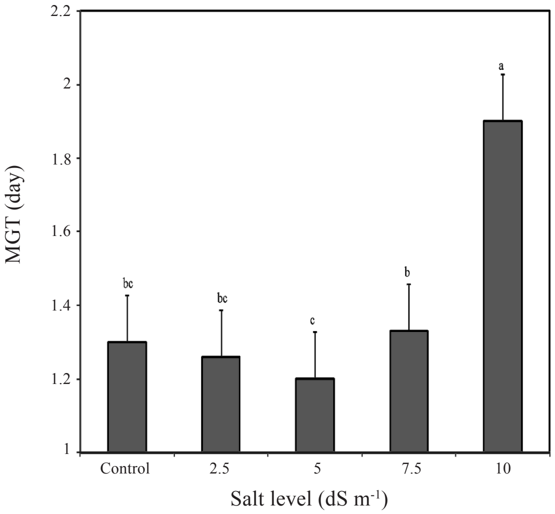

Fig. 1: The mean germination time at five different salt concentrations. Each value is the mean of eleven genotypes, with three replications and vertical bars represents SE

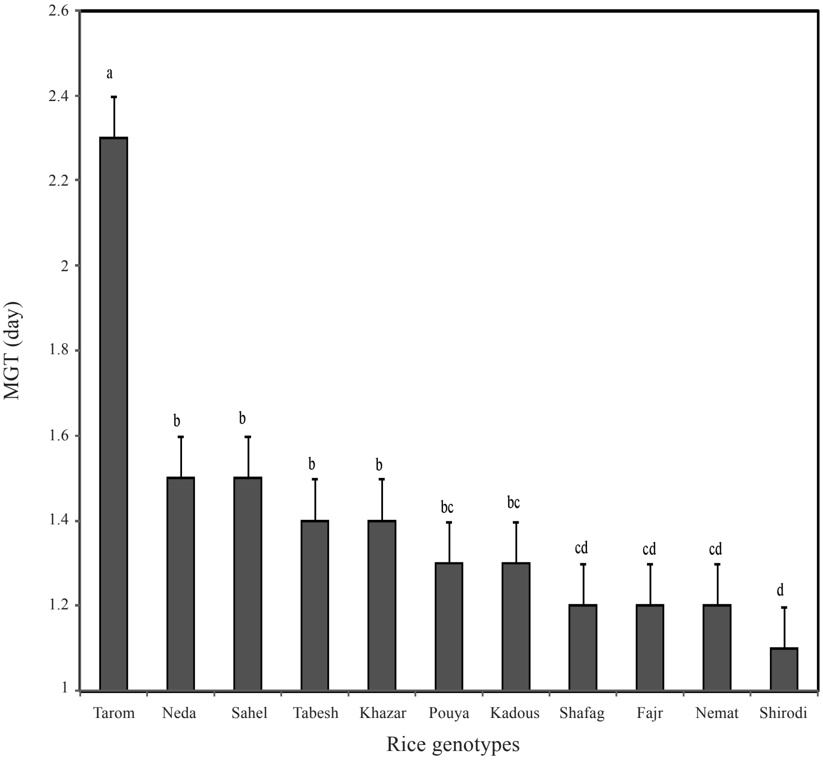

Fig. 2: The effect of salinity on the mean germination time of eleven rice genotypes. Each value is the mean of five salt levels and three replications and vertical bars represents SE

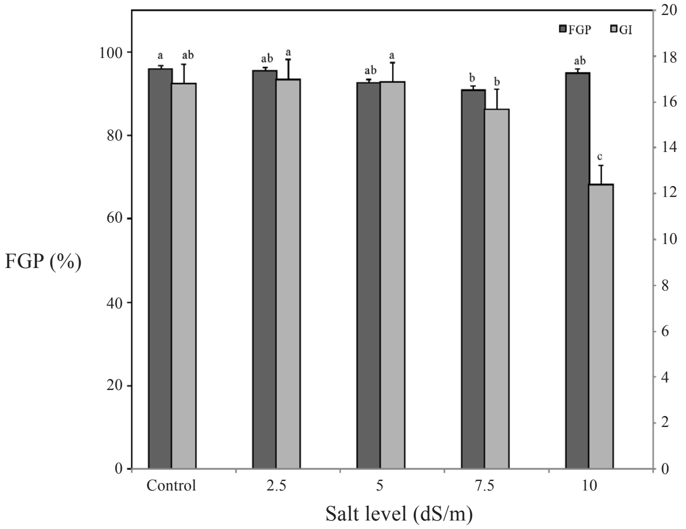

Fig. 3: The effects of salinity on germination index. The value is the mean of eleven genotypes, with three replications and vertical bar represent SE

was no significant interaction between the FGP and the rice genotypes (Table 1). This result was similar to the results obtained by Ahmad *et al*. (2000).

The highest and the lowest GI were recorded for Shirodi and Tarom-e-Hashemi genotypes, respectively. Tarom-e-Hashemi had the lowest FGP, while Shirodi and Fajr genotypes demonstrated the highest FGP (*Fig. 4*).

Salinity affects the germination of seeds by creating an external osmotic potential which prevents water uptake. Mechanism of salt tolerance in plant is divided into two categories, namely avoidance and tolerance against salt. In the present study, significant difference in the salt sensitivity was observed at the germination stage of the eleven genotypes tested (*Figs. 2* and *4*). The salt tolerant genotypes showed a faster growth rate under saline conditions (Walia *et al.*, 2005). Meanwhile, the plant growth can decline steadily when external salinity increases. Consequently, the tolerant plants elevate their germination rate to avoid the salinity stress. Both the MGT and GI can be acceptable parameters to be used in evaluating salt tolerance during germination stage because the genotype, which is salt tolerant, has the lowest MGT and the highest GI. Based on the MGT and GI, 11 genotypes can be assigned into three groups, tolerant (with the lowest MGT and the highest GI), semi-tolerant, and sensitive (with the highest MGT and lowest GI). Shirodi, Fajr and Shafag genotypes showed the lowest MGT and the highest GI; therefore, they can be classified into salt tolerant group. Meanwhile, Tarom genotype (with the highest MGT and lowest GI) is categorized into the salt sensitive group (*Figs. 2* and *4*).

Seedlings Growth

Rice seedling growth were significantly ($p \leq 0.01$) influenced by the levels of salt it contained. The growth of rice seedling was evaluated by root measurements, shoot length, and dry matter. The maximum rice seedling growth occurred at 2.5 dS m^{-1}. The data showed that the length of roots of all genotypes was reduced with the increasing salt concentrations. In general, Fajr and Tabesh genotypes had the highest and lowest root lengths, respectively (*Fig. 6*). The results revealed that the length of shoot of the rice seedlings increased at 2.5 dS m^{-1}, and above this concentration, no significant differences were

TABLE 2
The correlation between phenological characteristics and proline content at early seedling stage

	Root length	Shoot height	Dry weight	Water content	Proline content
Root length	0	0.55 **	-0.46 **	0.23 **	-0.13 ns
Shoot height		0	-0.38 **	0.44 **	-0.03 ns
Dry weight			0	-0.25 **	-0.01 ns
Water content				0	-0.04 ns
Proline content					0

Results are r values of eleven rice genotypes, five salt concentrations with three replications
** and *: significant different at 1%, 5% level; ns: not significant

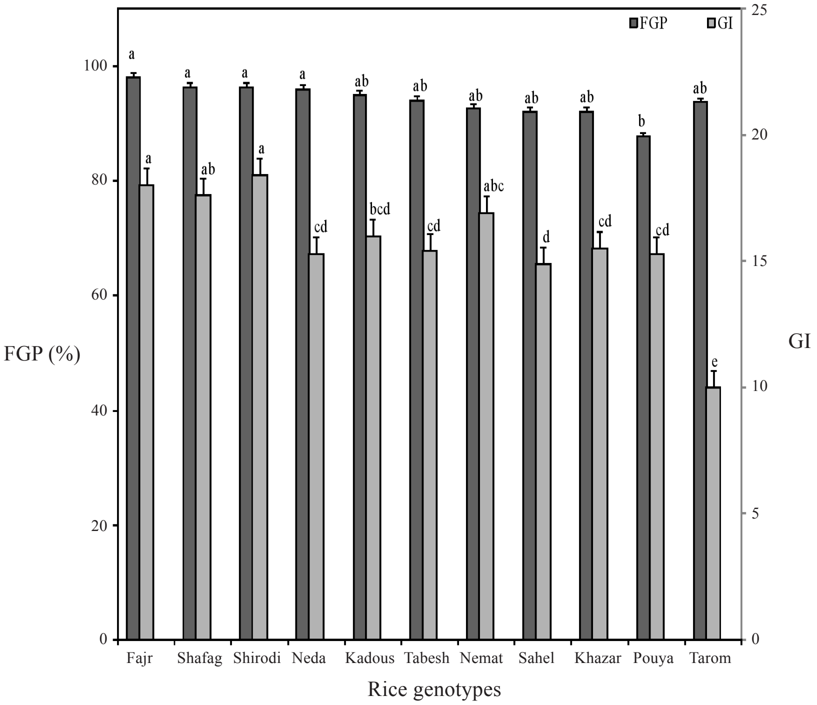

Fig. 4: The effects of salt stress on the germination index of eleven rice genotypes. Each value is the mean of five salt treatments, with three replications and vertical bar represents SE

observed (*Fig. 5*). Eight out of the 11 genotypes showed the lowest shoot length, while Fajr genotype was found to have the highest shoot length (*Fig. 6*).

Moreover, the dry weight and percentage of water content in rice seedling genotypes were significantly affected by salinity. There was a slight increase in the dry weight when the

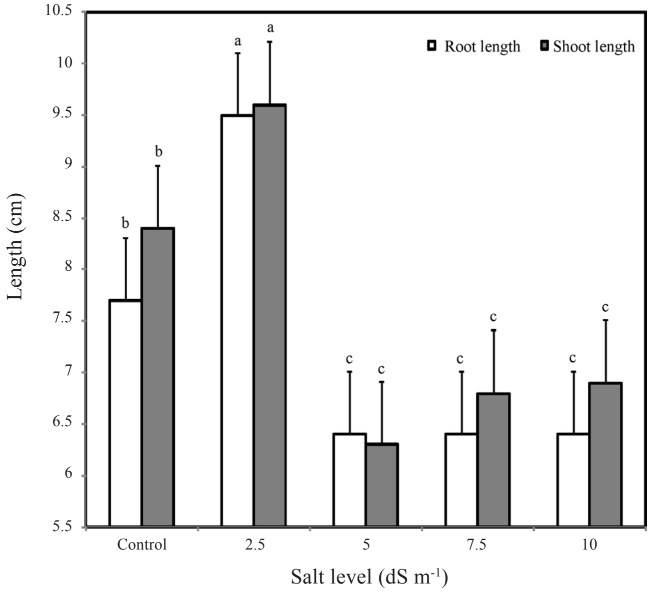

Fig. 5: The lengths of root and shoot at different salt concentrations. Each value is the mean of three replications and vertical bar represents SE

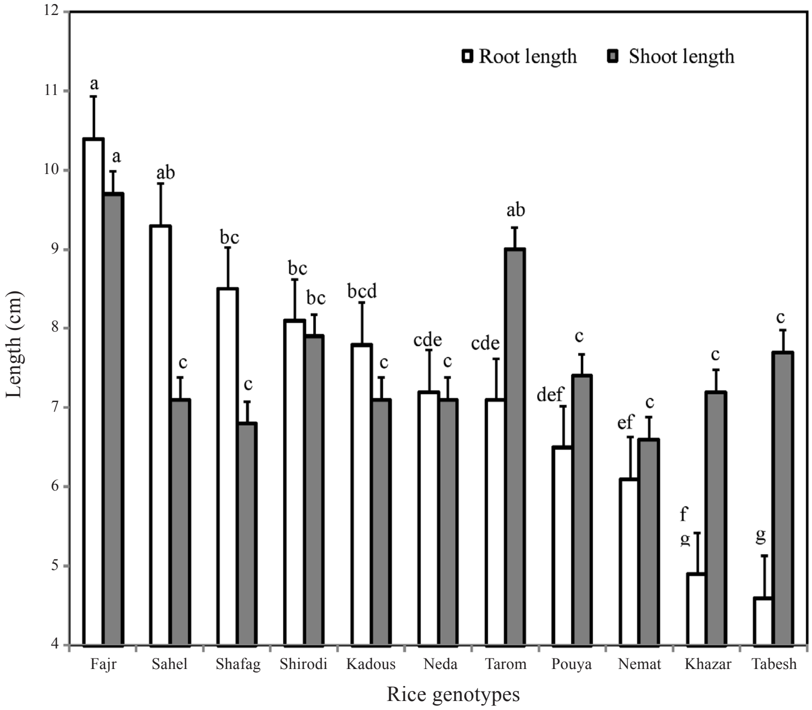

Fig. 6: The root length and shoot height of eleven rice genotypes. Each value is the mean of five salt concentrations, with three replications and vertical bar represents SE

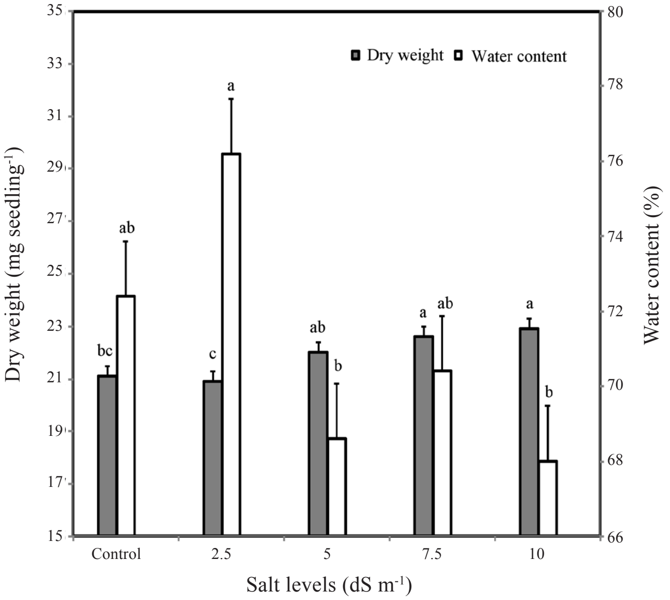

Fig. 7: The percentage of the dry weight and water content at different salt concentrations

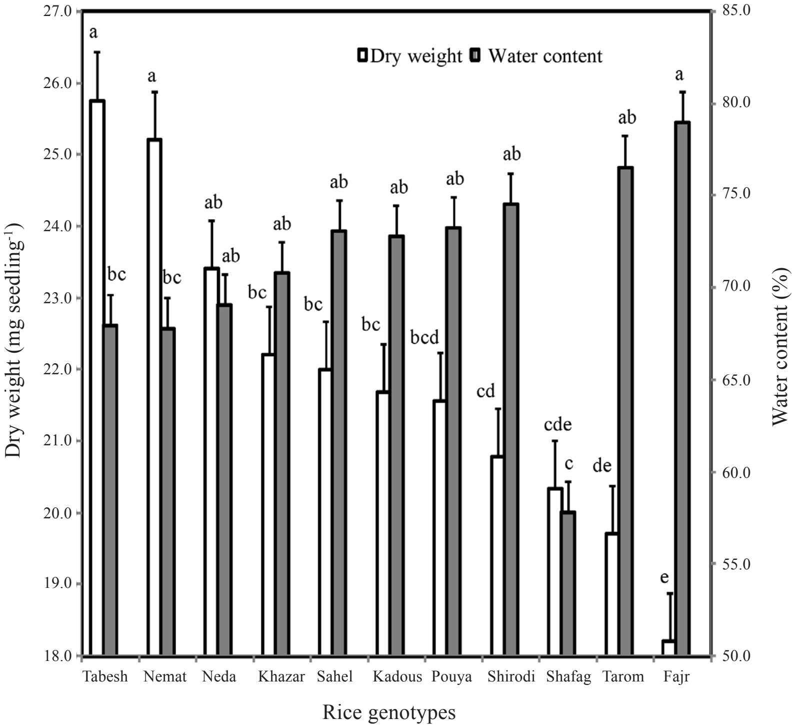

Fig. 8: Dry weight and water content of eleven rice genotypes at seedling stage

salinity stress increased. As the concentration of salinity increased, a significant increase in the water content occurred at lower salt level, thereafter, a decrease was observed (*Fig. 7*). Tabesh and Fajr genotypes demonstrated the highest and the lowest dry weights, respectively. In addition, they also showed the lowest and the highest water contents, respectively (*Fig. 8*). The decrease in the dry weight is accompanied by the increase in the water content in seedling after the salt treatment. The results proved that there was a negative relation between the dry weight and the water content under salt stress (Table 3).

The comparison of the data derived from germination and seedling stages revealed that Fajr genotype, which was found with the longest root, the highest shoot and water content, can be classified into the salt tolerance group at its seedling stage. It seems that Fajr genotype is salt tolerant, not only at germination stage but also at the seedling stage and it could adapt well to salinity condition. However, Tarom-e-Hashemi is salt sensitive at germination stage and it could tolerate at seedling stage.

Proline Content

The content of proline in rice seedlings was differently altered with the increase in salt concentration. There was only a slight increase in the content of proline at 2.5 dS m^{-1} as compared to the control. Generally, rice seedlings tend to accumulate proline at 5 dS m^{-1} and a downward trend beyond 5 dS m^{-1} was observed (*Fig. 9*). The considerable decrease in the content of proline at 10 dS m^{-1} might be due to the inhibition of the seedling growth by salt stress (Table 3). The highest and the lowest amounts of proline were found in Shafag and Fajr genotypes, respectively (*Fig. 10*).

In addition, proline also plays a role as an osmoprotectant to adjust osmotic stability; it was expected that the accumulation of proline was associated with the percentage of water content, as compared to the control especially at 10 dS m^{-1}. However, no significant correlation among the physiological parameters and proline content was found. It seemed that salt stress provoked rice genotypes to different extents. The accumulation of proline in Shafag genotype

TABLE 3
The relationship between proline content and shoot length

Rice Genotype	Proline (µmol g^{-1}FW)			Water Content (%)		
	Control	EC=10	Change%	Control	EC=10	Change%
Fajr	[#]0.58a	0.23b	-62.1	79.3a	75.3a	-5.0
Kadous	0.59a	0.02b	-96.6	73.6a	67.9a	-7.7
Khazar	0.75a	0.16b	-78.7	75.6a	68.1b	-9.9
Neda	0.53a	0.14b	-73.6	70.6a	65.3a	-7.5
Nemat	0.45a	0.01b	-98.7	69.8a	62.9a	-9.9
Pouya	0.67a	0.02b	-97.0	74.3a	68.8b	-7.4
Sahel	0.45a	0.01b	-98.7	73.4a	72.1a	-1.8
Shafag	0.69a	0.05b	-92.7	57.7a	57.7a	0
Shirodi	0.63a	0.01b	-98.4	75.0a	72.0a	-4.0
Tabesh	0.65a	0.02b	-96.9	70.5a	66.7a	-5.4
Tarom	0.60a	0.09b	-85.0	77.0a	71.7a	-6.9

Results are the mean values of three replications. The values with the same letter in a row are not significantly different

$$\text{Change\%} = \left[\left(\text{EC}_{10} - \text{Control}\right)/\left(\text{Control}\right)\right] \times 100$$

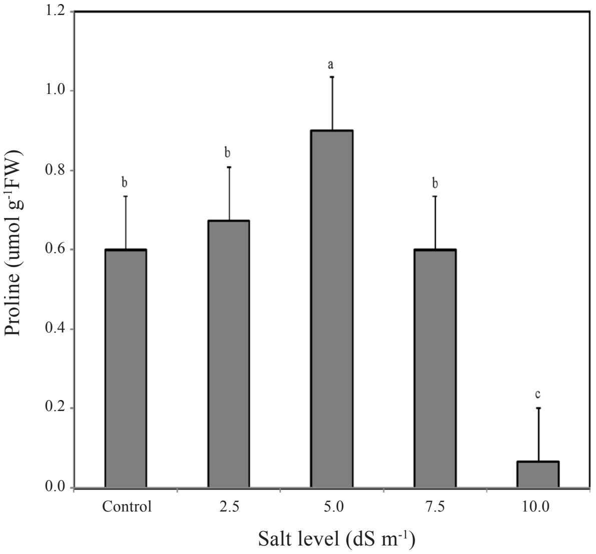

Fig. 9: The comparison of the proline content of rice seedlings at different salt concentrations

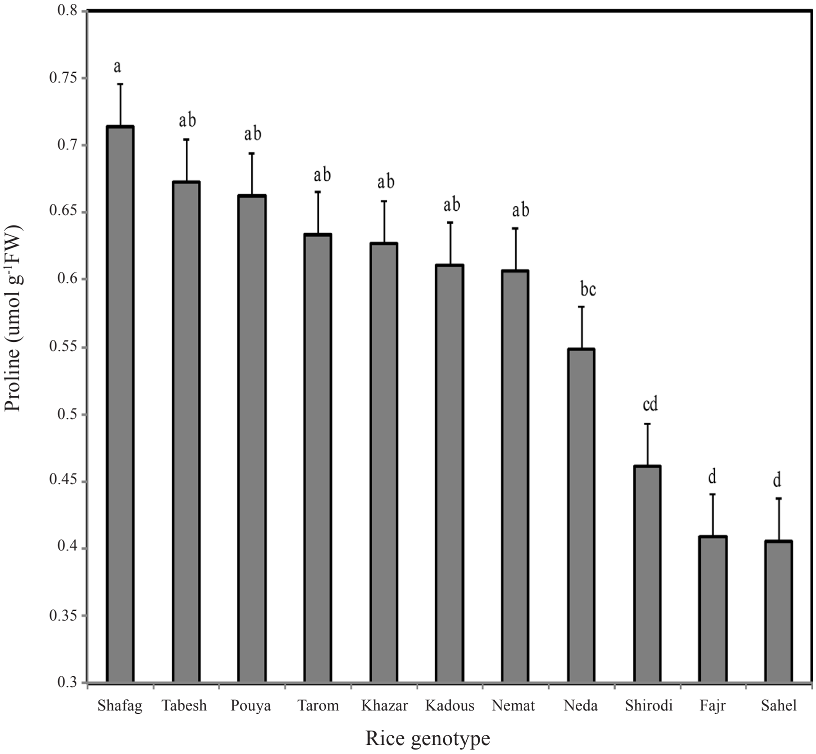

Fig. 10: The accumulation of proline in eleven rice genotypes. Each value is the mean of five salt concentrations, with three replications and vertical bar represents SE

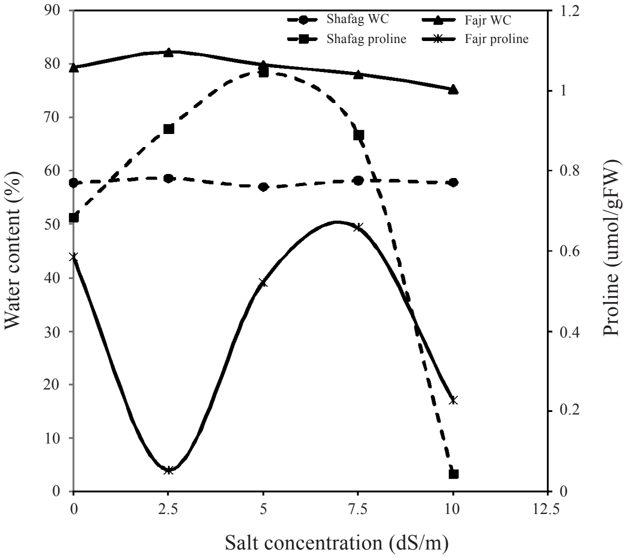

Fig. 11: The effect of salt levels on the contents of water and proline in Shafag genotype as salt susceptible and Fajr genotype as salt tolerant at the early seedling stage

as a salt susceptible followed a regular pattern, as compared to Fajr genotype as a salt tolerant. Fajr genotype also tended to accumulate proline less than Shafag genotype (*Fig. 11*). This result was similar to the ones obtained by Luttus *et al.* (1996). Therefore, the content of proline did not play a functional role to enhance water uptake by rice seedlings, at least in these rice genotypes (Tables 2 and 3). This conclusion is in agreement with the previous report by Khedr *et al.* (2003).

Salt stress can affect the synthesis of organic compatible solutes such as proline, betaine and soluble sugars (Phap *et al.*, 2006). The accumulation of proline has been reported in many plants under salt stress. However, there are reports which indicate that salt tolerant cultivated rice accumulates less free-proline than salt sensitive ones (Lutts *et al.,* 1996). Jain *et al.* (2001) suggested that salt stressed plants accumulate increased amount of low-molecular weight water-soluble metabolites, like proline and betaines, in their cells, possibly for osmotic adjustment. However, it is not clear whether the accumulated proline is involved in ameliorating salt tolerance because the accumulation was the highest in more sensitive genotypes (*Fig. 10*). The result suggested that the increase in proline content might not be associated with salinity tolerance, but rather with the extent of damage encountered by salt stress, as shown by the greater increase in the content of proline in the susceptible genotypes. This conclusion is confirmed by Hoai *et al.* (2003).

CONCLUSIONS

Increasing salt levels had detrimental effects on germination percentage, root and shoot length, water content and seed germination index of the rice genotypes tested. In particular, Fajr and Shirodi genotypes as salt tolerant group and Tarom-e-Hashemi genotype as salt susceptible were classified based on their MGT and GI. However, the relationship between the growth parameters and MGT and GI was not found. The salt tolerant and salt sensitive groups showed that there was no significant relationship between proline and salt tolerant, at least in these rice genotypes, because proline content also increased in salt tolerant.

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