

Agronomic Characteristics and Proline Accumulation of Iranian Rice Genotypes at Early Seedling Stage under Sodium Salts Stress

Momayezi, M.R.^{*,1,2}, Zaharah, A.R.², Hanafi, M.M.² & Mohd Razi, I.³

¹ *Faculty of Agriculture, Islamic Azad University, Varamin, Tehran, Iran*

² *Land Resource Management Department, Universiti Putra Malaysia, Serdang, Malaysia*

³ *Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

ABSTRACT

Salt composition can affect rice (*Oryza sativa* L.) growth at germination and early seedling stages. The response of eleven rice genotypes to sodium salt compositions (NaCl and Na₂SO₄ with the ratio of 1:1, 2:1 and 1:2 molar concentrations) and concentrations (2.5, 5.0, 7.5 and 10 dS m⁻¹ salt concentrations) was investigated in the laboratory for 10 days. Effects due to salinity, genotype, and their interaction were observed for most of the measured parameters during the germination and early seedling stages. Mean germination time increased and germination index decreased with increasing salt stress. Measured agronomic characteristics were influenced by salinity stress with the extent differing with salt treatments. The 2:1 molar ratio compared to the other salt compositions showed the greatest effect on rice germination. The results also confirmed that Cl⁻ toxicity effects decreased as SO₄²⁻ increased in the solution. There was a non-significant relationship between water content and proline accumulation. The anion associated with Na⁺ may play a functional role in the responses of rice seedlings and the degree of proline synthesis in stressed plants. According to mean germination time and germination index, Tarom-e-Hashemi and Shirodi can be classified into salt sensitive and salt tolerant groups, respectively.

Keywords: Germination, germination index, mean germination time, proline content, sodium salt composition

INTRODUCTION

Salinity is one of the important abiotic stresses limiting rice productivity. The capacity to tolerate salinity is a key factor in plant productivity. Rice is a species native to swamps and freshwater marshes and its cultivated varieties provide one of the world's most important food crops. In south and south-east Asia, about 100

*Corresponding author : Email: momayeziir@gmail.com

million hectares of land suited to rice production are not utilised because of soil problems such as salinity, alkalinity, strong acidity, or excess organic matter (IRRI 2005). There has been great interest in developing varieties of rice that are resistant to salinity. The physiological basis for salt resistance is not completely understood. Plants which grow in salt affected soils tend to show differences in physiological and biochemical activities from those grown on non-salt affected soils (Lutts *et al.* 1995). Jamil *et al.* (2007) reported that salinity delayed germination and decreased seedling growth.

Growth reduction depends on the period of time over which the plants were grown in saline conditions. No visual differences were observed between control and stressed plants for tolerant and sensitive genotypes by a gradual increase in salinity (Walia *et al.* 2005). Salinity reduced leaf growth more than root growth (Munns, 2002). Studying the physiology of salt tolerance in rice plants may throw light on a number of different aspects of plant stress physiology and may help to develop a unified model of stress tolerance in plants (Orcutt *et al.* 2000).

The commonly characterised biochemical response of plant cells to osmotic stress is the synthesis of special organic solutes (osmolytes) which accumulate at high cytoplasm concentrations (Serrano *et al.* 1994). Plant cells accumulate proline as an osmo-protectant to conserve osmotic stability and to prevent damage. 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 and Ghoulam *et al.* 2002)

Many studies have been carried out to elucidate the physiological responses of rice plants to NaCl salinity stress (Chowdhury *et al.* 1995; and Hoai *et al.* 2003). Gregorio and Senadhira (1993) and Zeng *et al.* (2000 and 2002) reported that NaCl and calcium chloride (CaCl₂) salts affected plant growth. Therefore, the objectives of the present study were: (i) to determine the influence of both NaCl and Na₂SO₄ concentrations and composition on seed germination and proline accumulation in eleven rice genotypes during early growth and (ii) to examine the relationships between proline content and morphological and physiological characteristics of the rice seedlings.

MATERIALS AND METHODS

Growth Condition and Salt Treatments

Rice seeds (*Oryza sativa* L.), from 11 genotypes selected from the widely cultivated cultivars in Iran namely Pouya, Shafag, Neda, Kadous, Tabesh, Tarome-Hashemi, Sahel, Khazar, Shirodi, Fajr and Nemat were used for the present study.

Twenty seeds were placed on a petri dish of 9.0 cm diameter lined with a filter paper. Salt treatments of NaCl and Na₂SO₄ (1:1, 2:1 and 1:2 molar concentrations) were dissolved in distilled water at 2.5, 5.0, 7.5 and 10 dS m⁻¹ electrical conductivity. The salt solutions as well as 20 ml distilled water as a control were applied to 39 different petri dishes. The experiment was conducted

in laboratory using a complete randomized design (CRD) in 3 replications at room temperature ($27\pm 2^\circ\text{C}$) under dark conditions. The number of germinated seeds was counted for 7 days (from 3rd to 9th day after soaking). Ten-day-old rice seedlings were evaluated for root length, shoot height, dry and fresh weight, water content as agronomic characteristics and proline content as a biochemical characteristic. Significant differences between treatments were determined using the Student-Newman-Keuls test.

Germination was observed daily according to recommendations by International Seed Testing Association (ISTA, 1993). Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981). Germination index (GI) was calculated as described in the Association of Official Seed Analysis (1983). In order to evaluate salt tolerance of 11 rice genotypes and investigate the relationship between the measured parameters at germination stage and growth parameters at early seedling stage, the following were also determined: root length, shoot height, dry weight, water content percentage and proline content. Three seedlings from each replicate were randomly sampled and root length, shoot height, and seedlings fresh weight were measured. Root and shoot length were measured with a ruler. Seedlings were dried in a forced-air oven (70°C) for 72 hours and then measured for dry weight.

Proline was measured as described by Bates *et al.* (1973). The amount of proline was determined from a standard curve and presented in $\mu\text{mol g}^{-1}$ fresh weight.

RESULTS AND DISCUSSION

Seed Germination

Seed germination was significantly ($P\leq 0.01$) affected by salinity both in rice genotypes and salt treatments. There was significant interaction between genotype and salt treatment for most of the parameters; however, this interaction was not reflected in the final germination percentage, dry weight and water content percentages.

Germinated rice seeds demonstrated that mean germination time (MGT) was significantly increased by increasing salinity levels up to 10 dS m^{-1} compared to control (Fig. 1). The lowest and the highest MGT were recorded for Shirodi and Tarom-e-Hashemi genotypes, respectively (Fig. 2). Significant ($p\leq 0.01$) effect of salinity was seen on germination index (GI). Germination index decreased with increasing salinity level (Fig. 3) and the genotypes responded differently to salinity stress (Fig. 4). However, different salt compositions did not affect final germination percentage (Fig. 5). The highest and the lowest GI were observed for Shirodi and Tarom-e-Hashemi genotypes.

Significant differences in salt sensitivity were observed at the germination stage of eleven tested rice genotypes. The germination of rice seeds declined steadily when external salinity increased in contrast to the salt tolerant genotypes which attain a faster growth rate under saline conditions (Walia *et al.* 2005).

Consequently, mean germination time and germination index can be reliable parameters for evaluation of salt tolerance during germination stage because the salt tolerant genotype has the lowest MGT and the highest GI. Based on these parameters, eleven genotypes can be assigned to three groups: tolerant (with the lowest MGT and the highest GI), sensitive (with the highest MGT and the lowest GI) and moderate (those that fall outside the first 2 groups) Shirodi, Fajr and Shafag genotypes, with the lowest MGT and the highest GI, were classified into the salt tolerant group. Hence Tarom-e-Hashemi genotype which had the highest MGT and the lowest GI was assigned to the salt sensitive group. Other genotypes were categorised into the moderate group (Figs. 2 and 4).

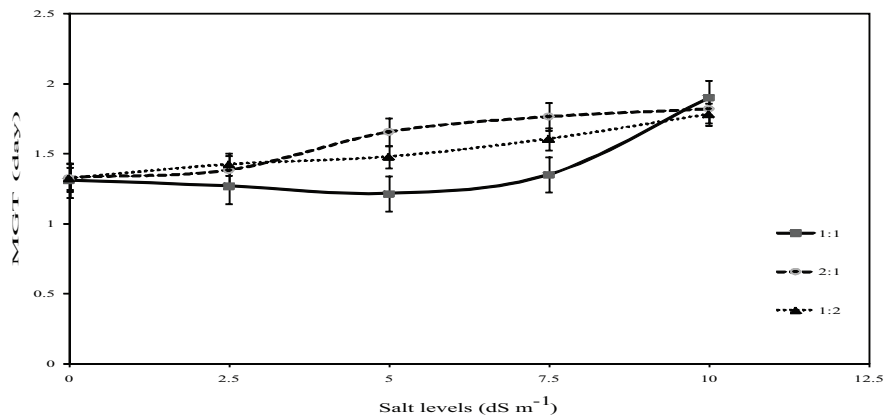


Fig. 1: Mean Germination time (MGT) at three different sodium salt compositions including NaCl:Na₂SO₄ (1:1, 2:1 and 1:2 molar concentrations). Note: Values are means of eleven genotypes with three replications and vertical bars represent SE.

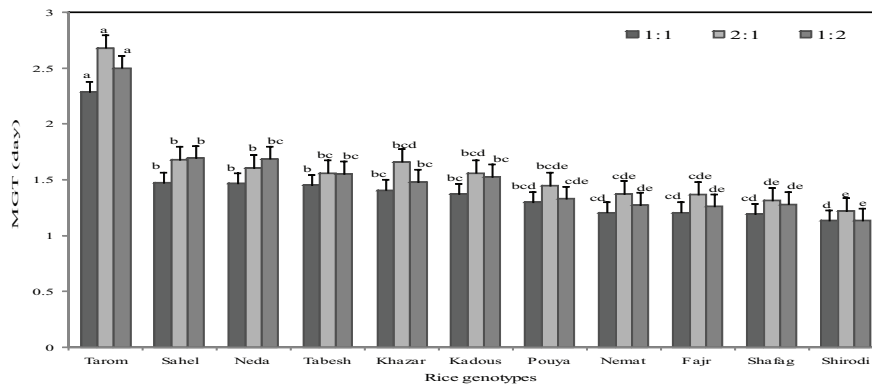


Fig. 2: Effect of salinity on mean germination time (MGT) in different salt compositions including NaCl:Na₂SO₄ (1:1, 2:1 and 1:2 molar concentrations) and different genotypes. Note: Values are means of three replications and vertical bars represent SE.

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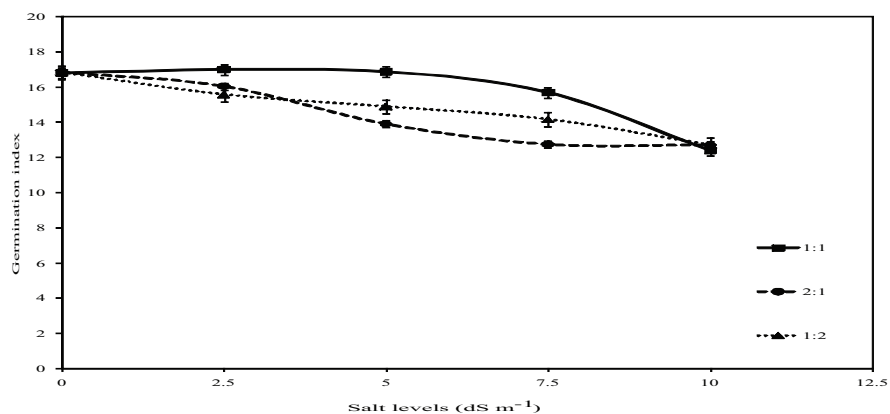


Fig. 3: Effect of salinity on germination index. Note: Values are means of eleven genotypes with three replications and vertical bars represent SE.

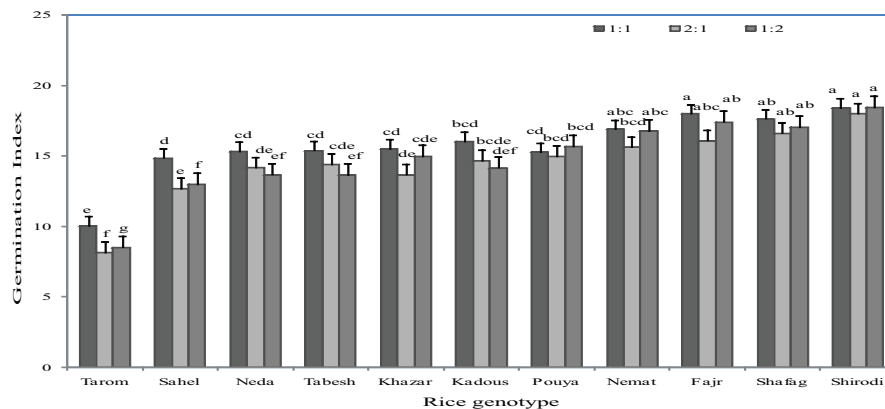


Fig. 4: Effect of salinity on germination index of eleven rice genotypes in different sodium salt compositions. Note: Values are means of three replications and vertical bars represent SE.

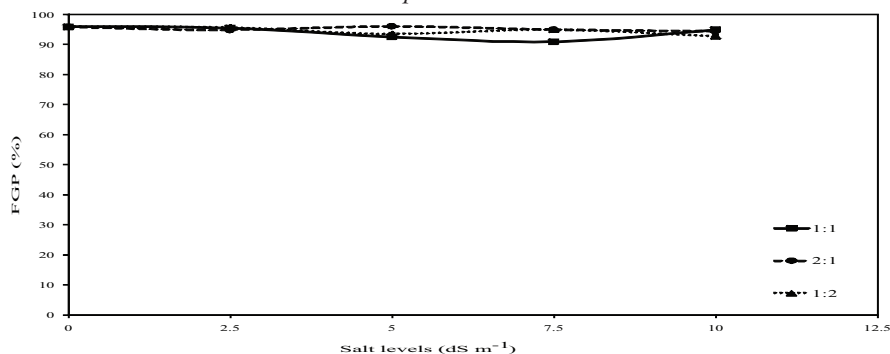


Fig. 5: Final germination percentage (FGP) at different salt levels and salt compositions. Note: Values are mean of eleven genotypes with three replications and vertical bars represent SE.

Early Seedling Growth

Rice seedling growth were significantly ($P \leq 0.01$) influenced by salt levels. Root length of rice seedlings was reduced by increasing the levels of salt concentration (Fig. 6). These finding are consistent with those of Jamil *et al.* (2007) and Rodríguez *et al.* (1997). Maximum root length occurred at 2.5 dS m⁻¹ when salt compositions were applied at 1:1 and 2:1 molar ratio; whereas exposure of the seedlings to 1:2 molar concentrations resulted in the highest root length at 5 dS m⁻¹. Besides, a relation was observed between variations in root length and sodium salt composition. The root length of seedlings slightly increased as sulphate and chloride concentration changed (Fig. 7). Root length increased up to 2.5 dS m⁻¹ for 1:1 and 2:1 molar ratios and thereafter decreased up to 10 dS m⁻¹. For 1:2 salt composition, there was an upward tendency to 5.0 dS m⁻¹, decreasing as salinity concentration increased. In general, Fajr and Tabesh genotypes had the longest and the shortest root lengths, respectively (Fig. 7).

The analysed data show that in terms of shoot height, rice seedlings responded differently to different salt mixtures and levels. There was a significant ($P \leq 0.01$) increase in the shoot height at 2.5 dS m⁻¹ compared to the control (Fig. 8). This process continued up to 5 dS m⁻¹ while salinity composition was at 2:1 molar concentration. However, this significant increase in shoot height was not observed when the salt composition was changed to 1:1 or 1:2 molar ratios. The shoot height decreased when salinity was increased above 2.5 dS m⁻¹ when the rice seedlings were treated by 1:1 and 1:2 molar concentrations. However, there was a downward tendency in shoot height when salt composition reached 1:1 and 1:2 ratios. Conversely, rice seedlings tended to increase their shoot height when they were exposed to 2:1 salt ratio (Fig. 8). The data revealed that Fajr and Shafag genotypes had the tallest and shortest shoot heights, respectively (Fig. 9).

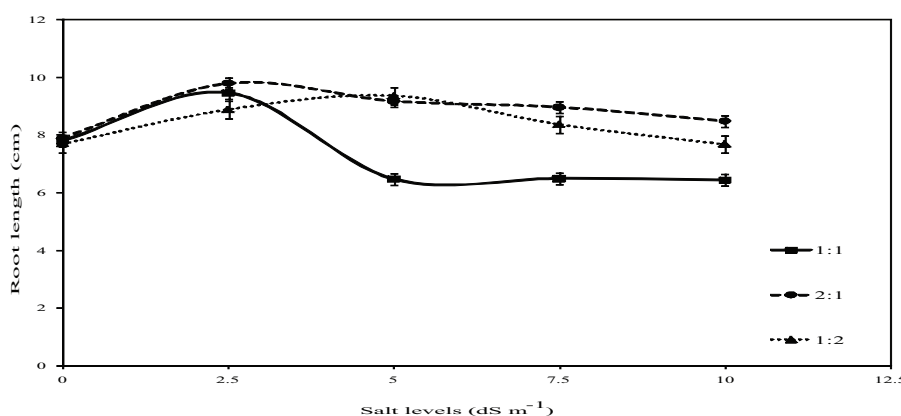


Fig. 6: Effect of salinity on rice seedling root length. Note: Values are means of eleven genotypes with three replications and vertical bars represent SE.

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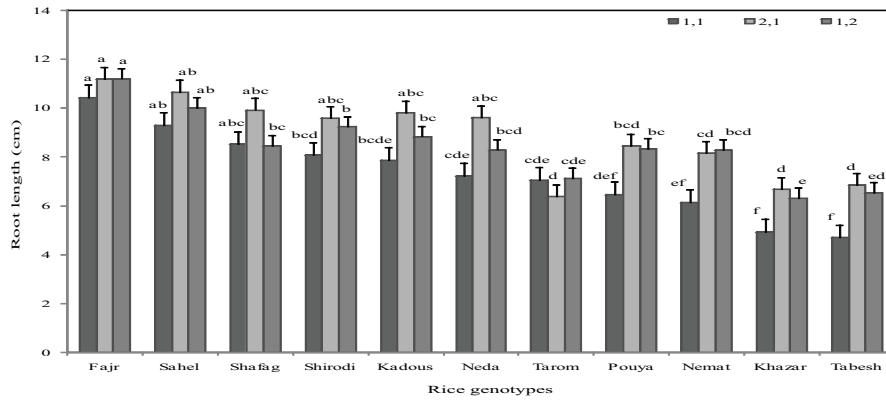


Fig. 7: Effect of salinity on root length of eleven rice genotypes in different sodium salt compositions. Note: Values are means of three replications and vertical bars represent SE.

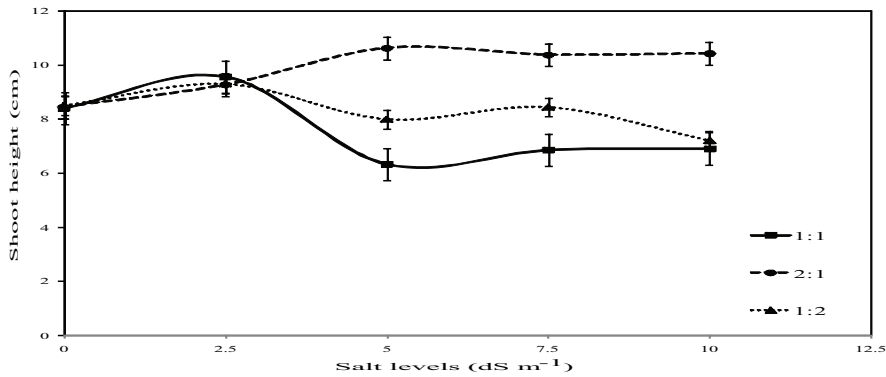


Fig. 8: Effect of different salt mixtures on rice seedling shoots length. Note: Values are means of eleven genotypes with three replications and vertical bar represent SE.

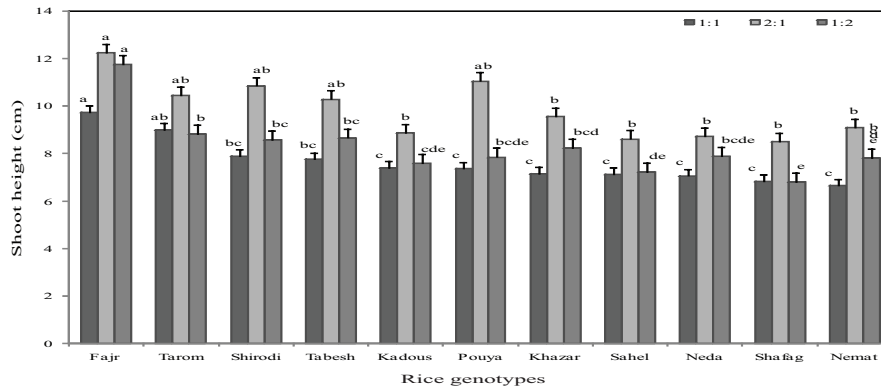


Fig. 9: Effect of salinity on shoot length of eleven rice genotypes in different sodium salt compositions. Note: Values are means of three replications and vertical bars represent SE.

Dry matter weight increased when salinity stress was raised to 7.5 dS m⁻¹ (Fig. 10). But above 7.5 dS m⁻¹ when salt compositions were 2:1 and 1:2 molar ratios, there was a decrease in dry matter weight. However, a slight increase was observed after 7.5 dS m⁻¹ when salt composition was 1:1 molar composition (Fig. 10). Tabesh and Fajr genotypes had the highest and the lowest dry matter weight (Fig. 11). The percentage of water content was influenced by salt stress. A downward tendency in water content percentage was observed above 2.5 dS m⁻¹, when the sodium salts ratio was changed to 1:2 and 2:1 molar compositions. As the duration of salinity stress increased, a significant increase in water content occurred at 2.5 dS m⁻¹; thereafter, a decrease was observed (Fig. 12). Fajr and Shafag genotypes demonstrated the highest and lowest water content respectively (Fig. 13). Generally, root length and water content significantly decreased by raising salt stress particularly at 7.5 dS m⁻¹. A similar conclusion was made by Akbar *et al.* (1974) and Basra *et al.* (2006). Shoot height and dry weight were observed to increase up to 7.5 dS m⁻¹ but when salt concentration exceeded 7.5 dS m⁻¹, seedling growth was inhibited. These parameters were similarly affected by different salt compositions. Seedling growth was acutely restrained by salt stress at 10 dS m⁻¹ because osmotic pressure of medium was increased by high salt concentration. As the seedlings could not absorb water, survival in this hard condition became difficult. Similar results have been documented by Kim *et al.* (2005).

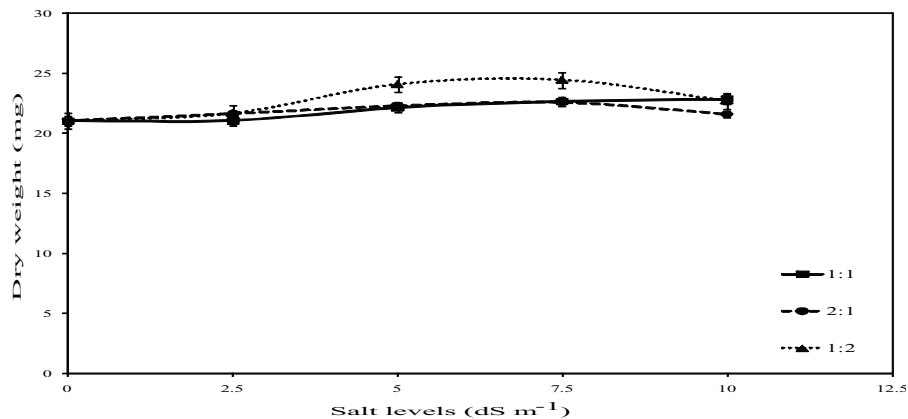


Fig. 10: Dry weight at different salt compositions and concentrations
 Note: Values are means of eleven genotypes with three replications and vertical bars represent SE.

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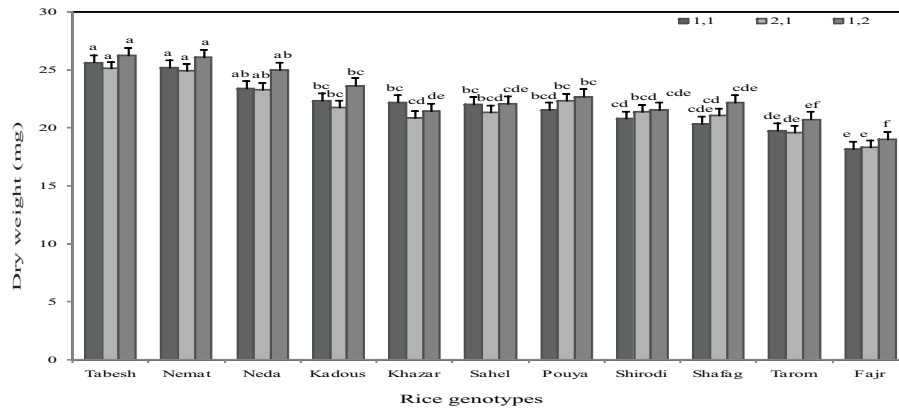


Fig. 11: Dry weight of eleven rice genotypes at different salt compositions

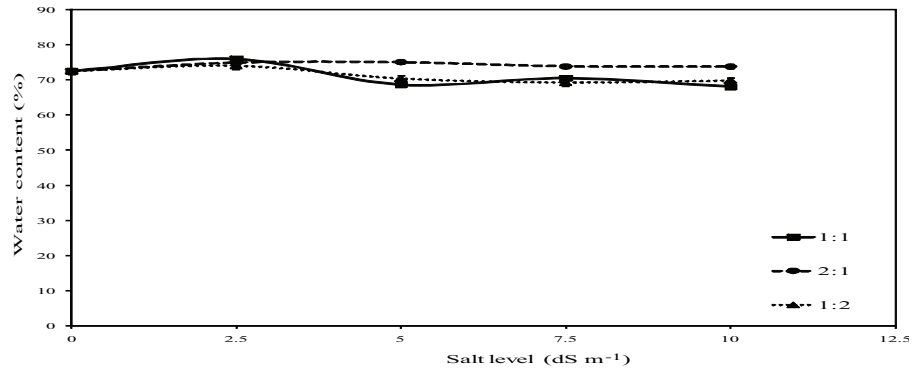


Fig. 12: Water content percentage at different salt compositions and concentrations

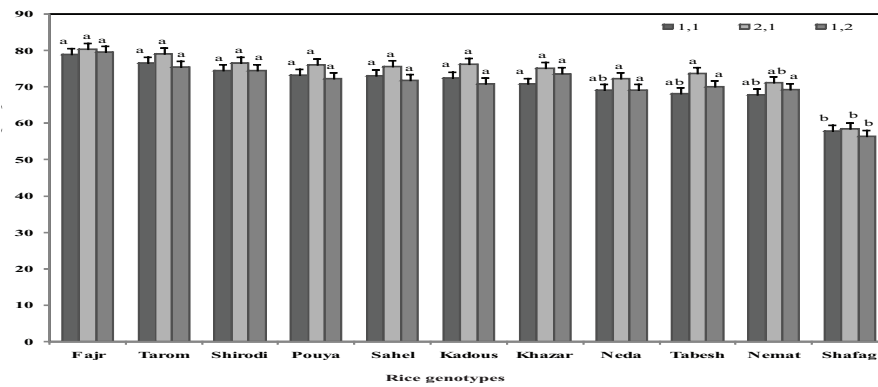


Fig. 13: Water content percentage of eleven rice genotypes at different salt stress and salt compositions

Fajr and shirodi genotypes with low MGT and high GI at germination stage were clustered into the salt tolerant group. These genotypes also demonstrated long root length, high shoot height, low dry weight and high water content percentage at early seedling stage. It is seen that this group was able to adapt to the limiting conditions because it had the lowest dry weight and the highest water content percentage (Table 1). Consequently, these genotypes were better able to take up water from the media compared to other genotypes.

Tarom Hashemi genotype, which was classified as salt sensitive at germination stage, had long root length and dry weight, high water content percentage and shoot height; consequently it was clustered into salt tolerant group at early seedling stage. It appears that these genotypes are more sensitive at germination stage than at early seedling stage (Tables 1 and 2).

However, Khazar genotypes which were categorised into moderate group at germination stage, had short root length, medium shoot height, high dry weight and low water content; therefore it was assayed into the salt sensitive group indicating that they were more sensitive at seedling stage than at germination stage (Tables 1 and 2).

The accumulation of proline was significantly ($P \leq 0.01$) related to salt levels and sodium salt compositions (Fig. 14). With the exception of 2:1 salt composition, there was a slight increase in proline content at 2.5 dS m⁻¹ compared to control, at salt composition of 1:1 and 1:2 molar concentrations. The rice seedlings tended to accumulate proline up to 5 dS m⁻¹ when the applied sodium salt composition was at 1:1 and 2:1 molar concentrations. Thereafter, the amount of proline within seedlings was reduced by salinity up to 7.5 dS m⁻¹ at 1:1 and 2:1 salt compositions. The considerable decrease at 10 dS m⁻¹ (1:1 salt composition) might be due to inhibition of seedling growth by salinity (Fig. 14). Shafag and Tabesh genotypes accumulated the maximum amount of proline while, Fajr and Sahel genotypes had the minimum amount of proline in their shoot tissue (Fig. 15).

The different salt combinations diversely affected growth parameters. Dry weights increased irrespective of salt composition. As shown in Table 3, the comparison of two rice salt tolerant and salt sensitive groups revealed that the response of rice seedlings varied in different salt compositions. At germination stage, salt concentration in the medium appears to be more important for emergence than salt composition because the osmotic pressure of medium is dominated by salt concentration. A diverse relationship between water and proline content was observed in salt tolerant and salt sensitive groups, for example, Fajr genotype classified as salt tolerant showed an upward tendency in water content as proline decreased at 1:1 molar ratio but this relation was not observed in other genotypes. It was also noted that the decrease in water content was accompanied by an increase in proline content in sensitive genotypes. There is a likelihood that the anion associated with Na⁺ plays an important role in salt tolerance.

TABLE 1
Effects of different salt compositions on characteristics of salt tolerant (Shirodi and Fajr) and salt sensitive (Khazar and Taron-e-Hashemi) rice genotypes

Rice genotype	Salt composition ratio	GI	MGT	Root length	Shoot height	Dry weight	Water content
			(day)	(cm)	(mg)	(%)	
Shirodi	1:1	18.4±0.3a [#]	1.13±0.02a	8.1±0.6a	7.9±0.4b	20.8±0.4a	74.5±0.9a
	2:1	18.0±0.5ab	1.22±0.06a	9.6±0.5a	8.5±0.3b	21.4±0.4a	76.6±0.7a
	1:2	18.4±0.2a	1.13±0.02a	9.2±0.4a	11.4±0.5a	21.6±0.3a	74.5±0.5a
Fajr	1:1	18.0±0.4a	1.21±0.04b	10.4±0.6a	9.7±0.5b	18.2±0.4a	78.9±0.7a
	2:1	16.1±0.7b	1.37±0.08a	11.2±0.4a	11.2±0.6a	18.3±0.4a	80.3±0.8a
	1:2	17.4±0.4a	1.26±0.05ab	11.2±0.4a	12.7±0.4a	19.0±0.3a	79.5±0.6a
Khazar	1:1	15.5±0.7a	1.41±0.08bc	4.9±0.3c	7.1±0.6b	22.2±0.5a	70.8±1.8a
	2:1	13.7±0.8a	1.66±0.09a	6.3±0.3ab	8.2±0.4ab	20.9±0.5a	75.1±0.9a
	1:2	15.0±0.6a	1.48±0.07ab	6.6±0.4a	9.5±0.4a	21.4±0.5a	73.6±0.7a
Taron-e-Hashemi	1:1	8.9±0.5a	2.3±0.2bc	6.8±0.4a	9.4±0.5a	23.6±0.5ab	76.4±1.2ab
	2:1	8.3±0.7b	2.8±0.2a	7.1±0.4a	9.4±0.6a	25.0±0.5a	75.0±1.1b
	1:2	8.0±0.8b	2.6±0.1ab	6.0±0.6a	8.8±0.5a	20.5±0.5b	79.0±1.3a

[#] Results are mean values of three replications ± SE.

Means with the same letter in the column are not significantly different in each rice genotype

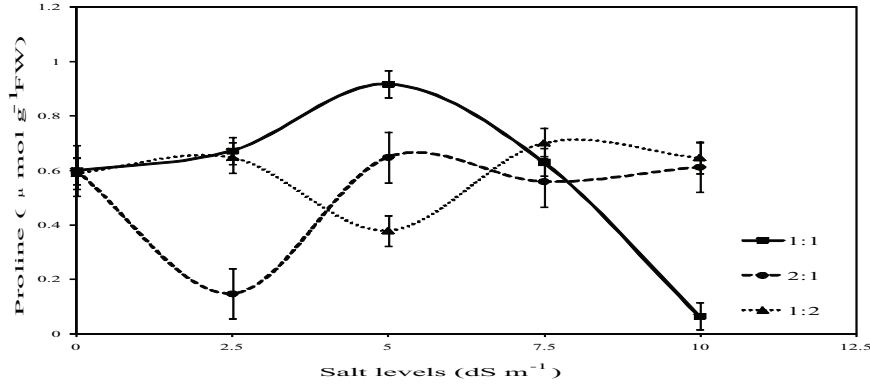


Fig. 14: Differing effects of salt levels with different compositions on proline content of rice seedlings

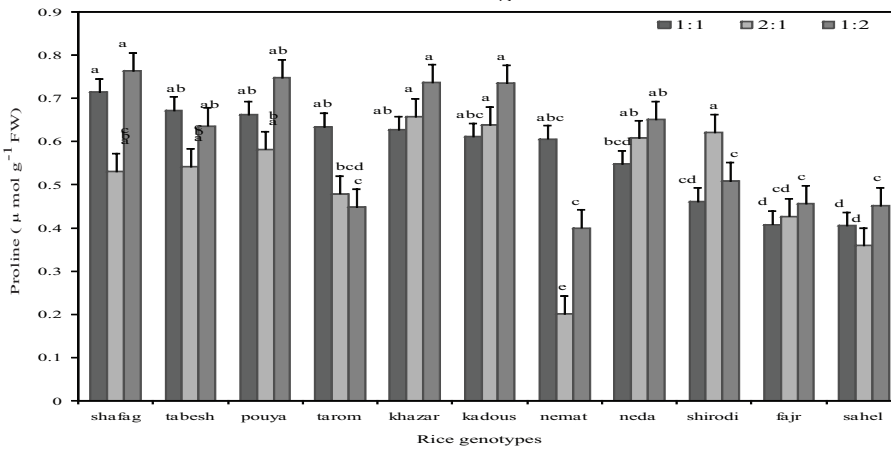


Fig. 15: Salinity effects on proline accumulation in different rice genotypes

Proline accumulation in plants is one of the most commonly reported modifications, and cytoplasmic accumulation of proline is thought to be involved in osmotic adjustment of stressed tissues. Therefore, proline content as a main compatible solute of rice was measured. Salt tolerant cultivated rice accumulated less free-proline than salt sensitive ones (Lutts *et al.* 1996). Nakamura *et al.* (2002) revealed that there was a positive correlation between free proline content and osmotic potential in salt tolerant rice species. Proline participates in decreasing osmotic potential. But in this study, it was observed that there was no correlation between the amount of proline and water content percentage, at least in these rice genotypes ($r = -0.11$) (data not shown). It is feasible that proline, with other free amino acids, is involved in ameliorating salt tolerance. These results support those of Hoai *et al.* (2003), where it was found that free amino acids were influenced by salt concentration. The lowest and the highest reduction of proline were at

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1:1 and 1:2 molar salt ratios, respectively (Table 2). Proline content increases when the anion ratio (chloride to sulphate) decreases (Fig. 14). This demonstrates that chloride had a more toxic effect on rice seeds than sulphate at germination stage. A similar effect was recorded in most measured agronomic characteristics. This suggests that there is a difference in plants species response to salt stress and the degree of proline synthesis in stressed plant can be controlled by Na⁺ concentration and anion associated with Na⁺ in solution (Cl⁻ and SO₄²⁻).

TABLE 2
Variation percentage of measured parameters in rice genotypes under different salt compositions at 10 dS m⁻¹ compared to control

Rice genotypes	Salt composition rate	GI ¹	MGT ²	Root length	Shoot height	Dry weight	Water content	Proline
		(day)	(day)	cm	cm	(mg)	(%)	($\mu\text{mol g}^{-1}\text{FW}$)
Shirodi	1:1	4.2	-6.5	-38.3	-13.0	5.9	-3.9	-98.3
	2:1	43.1	-25.8	-14.2	-14.9	9.4	-4.4	35.3
	1:2	7.4	-9.7	8.7	43.8	11.5	2.0	0.2
Fajr	1:1	31.7	-13.1	-16.2	-11.8	20.6	-5.0	-60.7
	2:1	68.3	-30.4	-7.3	-1.2	18.4	-0.2	-0.2
	1:2	44.1	-19.5	-6.0	2.5	12.8	-2.3	-5.8
Khazar	1:1	58.3	-34.5	3.2	-31.7	14.4	-9.9	-77.5
	2:1	44.7	-33.5	30.8	-25.2	5.7	-6.0	19.4
	1:2	38.2	-29.4	24.7	-4.9	4.2	-4.1	42.5
Tarom Hashemi	1:1	43.4	-39.5	-5.0	12.1	2.3	-3.0	-44.3
	2:1	64.1	-45.6	21.7	5.8	0.6	-0.6	-23.7
	1:2	22.9	-20.8	-19.1	-6.0	4.2	-4.4	-37.0

¹ Germination index; ² mean germination time

Values are percentage of change in observations at EC 10 dS m⁻¹ compared with controls Value are means (n=9).

TABLE 3
Effect of different salt concentrations and compositions on two rice salt tolerant and sensitive genotypes
^I Mean germination time; ^{II} germination index; ^{III} Water content percentage; ^{IV} proline content ($\mu\text{mol.g}^{-1}\text{FW}$); * Means with the same

Salt treatment	Rice genotypes									
	Fajr					Tarom Hashemi				
	MG ^I	GI ^{II}	WC ^{III}	Pro. ^{IV}	Pro.	MG ^I	GI	WC	Pro.	Pro.
Control	1.0 cd*	19.7 a	79.8 ab	0.6 ab	0.6 bc	2.1 f	10.6 a	80 bcd	0.6 bc	0.6 bc
2.5 dS m ⁻¹	1.2 abcd	17.8 abc	82.2 a	0.1 e	83.7 a	1.9 f	11.6 a	73 cd	1.4 a	1.4 a
5 dS m ⁻¹	1.1 cd	18.8 ab	79.9 ab	0.5 abc	77.3 abcd	1.8 f	12.3 a	72.1 d	0.6 bc	0.6 bc
7.5 dS m ⁻¹	1.3 abcd	16.6 abcde	78 ab	0.7 a	72.1 d	2.1 ef	9.8 ab	77.3 abcd	0.5 bc	0.5 bc
10 dS m ⁻¹	1.3 abcd	17.1 abcd	75.3 b	0.2 cde	72.1 d	3.5 a	5.9 d	72.1 d	0.1 d	0.1 d
2.5 dS m ⁻¹	1.0 d	18.8 ab	82.8 a	0.1 de	79.4 abc	2.4 cdef	9.5 abc	79.4 abc	0.1 d	0.1 d
5 dS m ⁻¹	1.5 abc	14.9 cde	75.5 b	0.5 abc	75.1 bcd	2.8 bcde	7.2 bcd	75.1 bcd	0.7 b	0.7 b
7.5 dS m ⁻¹	1.6 ab	13.3 e	80.9 ab	0.5 ab	72.1 d	2.8 abcd	7.4 bcd	72.1 d	0.5 bc	0.5 bc
10 dS m ⁻¹	1.7 a	13.7 de	79.1 ab	0.4 abc	73.4 cd	3.3 ab	6.0 d	73.4 cd	0.6 bc	0.6 bc
2.5 dS m ⁻¹	1.2 cd	18.3 abc	82.6 a	0.5 abc	79.5 abc	2.2 def	9.3 abc	79.5 abc	0.4 bcd	0.4 bcd
5 dS m ⁻¹	1.2 bcd	17.1 abcd	80.3 ab	0.3 bcde	81.3 ab	2.3 def	9.5 abc	81.3 ab	0.3 cd	0.3 cd
7.5 dS m ⁻¹	1.4 abc	16.1 abcde	82 a	0.4 abcd	77 abcd	2.9 abcd	6.5 cd	77 abcd	0.5 bc	0.5 bc
10 dS m ⁻¹	1.4 abc	15.8 bcde	77.5 ab	0.5 ab	80.2 ab	3.0 abc	6.6 cd	80.2 ab	0.5 bc	0.5 bc

letter in the column are not significantly different.

CONCLUSION

There was no relationship between growth parameters of different rice genotypes and MGT and GI. The salt tolerant and salt sensitive groups showed no significant relationship between proline production and salt tolerance as the proline content also increased in salt tolerant genotypes. Our results confirm that rice responds differently to salt stress as the salt composition is changed. Since the soil solution consists of a mixture of solutes, application of different salt compositions can shed light on the response of plants to salt in natural conditions.

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