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## Evaluation of salt tolerance in almond [*Prunus dulcis* (L.) Batsch] rootstocks

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In this study, four interspecific *Prunus* rootstocks ('HS314', 'HS312', 'HS302' and 'GF677') and the Iranian almond cultivar 'Sahand' were subjected to four different salinity levels (1.5, 3, 6 and 9 dSm<sup>-1</sup>) to determine the effects of salt level on growth parameters and chemical compositions. The results obtained indicate that increased salinity level had significant negative effects on leaf chlorophyll content, leaf area, dry and fresh weight of root and shoot. In addition, increasing the salinity level in general caused an increase in leaf proline concentration; however, the different genotypes were significantly different in response to the salinity level. According to these findings, proline content increase in 'Sahand' cultivar was lower than those of the other studied genotypes were. The majority of the plant's responses to the high salinity levels (6 and 9 dSm<sup>-1</sup>) were significant with no deleterious effects observed on plant growth triggered by lower salt concentrations of 1.5 and 3 dSm<sup>-1</sup>. A significant decrease in total chlorophyll and chlorophyll b content was also found at the high salinity levels but no significant change in chlorophyll a was evident. The potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>), calcium (Ca<sup>2+</sup>), sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ion concentrations of the leaves and roots were significantly different among the studied genotypes due to their exposure to different salinity levels. The concentration of Mg<sup>2+</sup>, Cl<sup>-</sup> and Na<sup>+</sup> as well as the Na<sup>+</sup>/K<sup>+</sup> ratio in the leaves of all the genotypes were increased by the salinity stress, whereas it had no significant effect on the Ca<sup>2+</sup> and K<sup>+</sup> concentrations as well as the Na<sup>+</sup>/Ca<sup>2+</sup> ratio. The result obtained in this study suggest that 'HS314' and 'GF677' interspecific hybrids may represent novel sources of salinity tolerance.

**Key words:** Breeding, interspecific hybridization, proline, salinity.

### INTRODUCTION

Throughout the world, million hectares of land are too saline to produce economic crop yield and much land is becoming non-productive each year due to salt accumulation (Munns, 1993; Tanji, 1990). Salinity problems are usually confined to arid and semiarid regions where rainfall is not sufficient to leach salts from the plant root zone (Epstein and Rains, 1987). The land area

negatively affected by salinization in arid and semiarid regions of south Asia is estimated to be about 42 million hectares (FAO, 1994). More specifically in Iran, 55% of the currently arable land are saline and according to the FAO estimation, the salt-affected land in Iran by low to moderate and high salinity are about 25.5 and 8.5 million hectares, respectively (Aliasgarzad et al., 2005). Therefore, salinity or in other word, salt accumulation can be a threat to plant growth in nearly every arable area of Iran.

Sodium chloride is the dominant salt in saline soils but other ions such as calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>) and

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sulphate ( $\text{SO}_4^{2-}$ ) are also important (Grattan and Grieve, 1999). The term salinity refers to the total concentration of the main dissolved inorganic ions, that is, sodium  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , bicarbonate ( $\text{HCO}_3^-$ ),  $\text{SO}_4^{2-}$  and chloride ( $\text{Cl}^-$ ) in ground-water, channel waters and drainage waters (Epstein and Rains, 1987). We can find different ions combination in saline soils. For example, in some of them, the predominant anion is basically  $\text{SO}_4^{2-}$  (not  $\text{Cl}^-$ ) and the  $\text{Mg}^{2+}$  concentration may exceed those of  $\text{Ca}^{2+}$  by large factors. The concentration of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  combined may exceed that of  $\text{Na}^+$  (Epstein and Rains, 1987). However, presence of  $\text{Ca}^{2+}$  even in lower levels could markedly affect plant response to salinity (Bolat et al., 2006; Jafarzadeh and Aliasgarzad, 2007).

Woody plants are usually relatively salt-tolerant during the seed germination stage but much more sensitive during the young seedling stage and progressively more tolerant with increasing age through the reproductive stage (Najafian et al., 2008). Temperate fruit trees are generally rated and sensitive to soluble salts and particularly sensitive to chloride, and irrigation with saline water may significantly reduce their yields (Grattan and Grieve, 1999; Najafian et al., 2008). Also, most of the stone fruit trees and almond are sensitive to salt stresses and their productivity gradually reduces at salt concentrations above  $1.5 \text{ dSm}^{-1}$  and down to 50% of normal yield at the salt concentration of  $4 \text{ dSm}^{-1}$  (Maas and Hoffman, 1977; Ottman et al., 1988; Hassan and El-Azayem, 1990). The reduction in growth and yield is related in part to the total concentration of soluble salts and osmotic potential of the soil solution. Tree crops are also susceptible to specific ion toxicities resulting from the excessive uptake of  $\text{Cl}^-$  and  $\text{Na}^+$  (Aliasgarzad et al., 2005; Rahmani et al., 2003). The first symptoms of ion toxicity are usually those caused by excessive  $\text{Cl}^-$  concentrations in the leaves. Whereas, sodium tends to be retained in the roots, trunk and branches, so its concentration in the leaves remains relatively low for several years (Picchioni et al., 1991; Boland et al., 1997b).

In most glycophytic plants such as trees, the degree of salinity tolerance depends on the roots' ability to exclude or retain potentially toxic ions. Therefore, the role of the rootstock is crucial in determining the tree's performance under saline conditions (Grattan and Grieve, 1999). Previous studies have shown differences in salt tolerance between citrus, grape and pistachio rootstock (Ranjbar et al., 2005; Storey and Walker, 1999; Zekri and Parsons, 1992). Some studies have also shown variations in salt tolerance and boron sensitivity between *Prunus* rootstocks. This genetic diversity, therefore, makes some rootstocks potentially more suitable for cultivation in saline soil (Massai et al., 1998). Direct utilization of interspecific hybrids as rootstock in *Prunus* species has been reported and strongly recommended by several authors (El-Motaium and Brown, 1994; Noitsakis et al., 1997). Therefore, exploiting interspecific hybrids is one of

the most promising ways to improve new clonal rootstocks in *Prunus* species and one of the most important characteristic that should be taken into consideration when selecting new rootstock for fruit trees is their tolerance to salinity and drought.

Since 1998, a breeding programme has been initiated at Sahand Horticultural Research Station, Tabriz, Iran in order to improve stone fruit and almond rootstocks through hybridization between peach  $\times$  almond, apricot  $\times$  prune, almond  $\times$  prune, apricot  $\times$  plum followed by the selection of the candidate interspecific hybrids (for example: salt-tolerant). In the course of this project, several interspecific hybrids have been selected as promising genotypes based on some individual characteristics (Dejampour et al., 2006). To further complete the project, the objective of the present study was to evaluate the tolerance of the newly developed hybrids to the salinity stress using different mixed-salt solutions.

## MATERIALS AND METHODS

The experiment was carried out with 300 seedlings of four interspecific hybrids including 'HS314', 'HS312' and 'GF677' almond [*Prunus dulcis* (Miller) D. A. Webb, syn. *Prunus amygdalus* Batsch.]  $\times$  peach [*Prunus persica* (L.) Batsch], and 'HS302' apricot (*Prunus armeniaca* L.)  $\times$  plum (*Prunus cerasifera* L.). Almond cultivar 'Sahand' was assayed as control. Seedlings were grown in plastic pots arranged in factorial randomized complete-block design by five genotypes, four salinity levels and three replicate, and maintained in a greenhouse with natural sunlight and temperature estimated at 30 to 35°C and 25 to 30°C in day and night, respectively.

### Experimental design

The experiment consisted of four salinity concentrations ( $1.5, 3, 6$  and  $9 \text{ dSm}^{-1}$ ) prepared by mixing different salt [magnesium sulfate ( $\text{MgSO}_4$ ), sodium chloride ( $\text{NaCl}$ ), sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) and calcium chloride ( $\text{CaCl}_2$ )] at different proportions of 12.8, 11.1, 10.2, 20.7 (w/v), respectively. The plough layer (0 to 30 cm) samples were collected from Sahand Station in East-Azerbaijan Research Center for Agriculture. The initial physico-chemical properties of the soil were as follows: pH 7.74; EC  $1.5 \text{ dSm}^{-1}$ ; 0.75% organic carbon; 6% clay; 10.1% silt; 86% sand; 262 mg/kg K; 14.8 mg/kg P; 3.75% carbonate calcium equivalent (CCE). In order to avoid salt shock, plants were acclimated to stress by using lower salt levels ( $1.5$  to  $3 \text{ dSm}^{-1}$ ) for one week and then exposed to each treatment for two weeks. The pots were irrigated using distilled water. The soil moisture was maintained at 60 to 80% of field capacity during the experiment (150 days). In order to ensure optimum vegetative growth of seedling, nitrogen and phosphorus in the form of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) and potassium di-hydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) were applied uniformly to all pots at the rate of  $40 \text{ mg}^{-1}$ .

### Evaluation of salt tolerance

After 150 days of planting, seedlings were cut from the root systems approximately 1 cm above the soil surface and the roots were washed gently free of soil. The leaf area was measured using leaf area-meter (LI-COR, model Li-1300, California, USA) and leaf proline content was determined as described by Bates et al. (1973). Chlorophyll a, b and total chlorophyll were also determined by

**Table 1.** The effects of salinity on vegetative traits [leaf area, leaf number, plant height, fresh weight of stem (FWS), fresh weight of root (FWR), total fresh weight (TFW), percentage of total dry weight (TDW%), dry weight of stem (DWS), dry weight of root (DWR), total dry weight (TDW), DWS/DWR, proline content, chlorophyll a, b, total concentrations and chlorophyll index] of interspecific hybrids and almond ('Sahand' Cv.).

Salinity (dSm <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Leaf number	Plant height (cm)	FWS (g/pl)	FWR (g/pl)	TFW (g/plant)	TDW (%)	DWS (g/plant)	DWR (g/pl)	TDW (g/plant)	DWS/DWR	Proline (μmol/g)	Chl. a (mg/g)	Chl. b (mg/g)	Chl.T. (mg/g)	Chl. index
1.5	526.38 <sup>a*</sup>	43.53 <sup>a</sup>	61.28 <sup>a</sup>	11.60 <sup>a</sup>	7.10 <sup>a</sup>	18.70 <sup>a</sup>	41.18 <sup>ab</sup>	4.83 <sup>a</sup>	2.83 <sup>a</sup>	7.67 <sup>a</sup>	1.92 <sup>b</sup>	28.56 <sup>d</sup>	0.006 <sup>a</sup>	0.130 <sup>a</sup>	0.028 <sup>ab</sup>	43.37 <sup>a</sup>
3	521.14 <sup>a</sup>	43.29 <sup>a</sup>	60.35 <sup>a</sup>	11.12 <sup>a</sup>	5.80 <sup>ab</sup>	16.92 <sup>ab</sup>	42.50 <sup>a</sup>	4.87 <sup>a</sup>	2.37 <sup>ab</sup>	7.67 <sup>a</sup>	2.43 <sup>a</sup>	57.66 <sup>c</sup>	0.006 <sup>a</sup>	0.137 <sup>a</sup>	0.30 <sup>a</sup>	42.35 <sup>ab</sup>
6	439.56 <sup>b</sup>	41.32 <sup>a</sup>	56.12 <sup>a</sup>	9.55 <sup>b</sup>	5.55 <sup>ab</sup>	15.11 <sup>b</sup>	40.26 <sup>ab</sup>	3.98 <sup>b</sup>	2.08 <sup>b</sup>	6.07 <sup>ab</sup>	2.18 <sup>a</sup>	79.92 <sup>b</sup>	0.006 <sup>a</sup>	0.120 <sup>ab</sup>	0.027 <sup>ab</sup>	40.80 <sup>bc</sup>
9	466.39 <sup>b</sup>	38.58 <sup>b</sup>	52.00 <sup>b</sup>	7.61 <sup>c</sup>	4.34 <sup>b</sup>	11.96 <sup>c</sup>	37.31 <sup>b</sup>	2.97 <sup>c</sup>	1.43 <sup>c</sup>	4.40 <sup>c</sup>	2.66 <sup>a</sup>	101.75 <sup>a</sup>	0.009 <sup>a</sup>	0.113 <sup>b</sup>	0.026 <sup>b</sup>	39.26 <sup>c</sup>

\*Values with different letters are significantly different at the  $p < 0.01$  level. Means values at different salinity levels. (Different letters indicate.....ANOVA;  $P < 0.05$ ).

**Table 2.** The effects of salinity on vegetative traits [leaf area, leaf number, plant height, fresh weight of stem (FWS), fresh weight of root (FWR), total fresh weight (TFW), percentage of total dry weight (TDW%), dry weight of stem (DWS), dry weight of root (DWR), total dry weight (TDW), DWS/DWR, proline content, chlorophyll a, b, total concentrations and chlorophyll index] of interspecific hybrids and almond ('Sahand' Cv.). Means values of the different genotypes.

Genotype	Leaf area (cm <sup>2</sup> )	Leaf number	Plant height (cm)	FWS (g/pl)	FWR (g/pl)	TFW (g/plant)	TDW (%)	DWS (g/plant)	DWR (g/pl)	TDW (g/plant)	DWS/DWR	Proline (μmol/g)	Chl.a (mg/g)	Chl. b (mg/g)	Chl.T. (mg/g)	Chl. index
'HS314'	670.07 <sup>a*</sup>	49.15 <sup>a</sup>	61.25 <sup>a</sup>	10.6 <sup>ab</sup>	5.92 <sup>a</sup>	16.59 <sup>ab</sup>	40.58 <sup>ab</sup>	4.46 <sup>ab</sup>	2.28 <sup>a</sup>	6.75 <sup>ab</sup>	2.08 <sup>ab</sup>	71.86 <sup>a</sup>	0.005 <sup>ab</sup>	0.92 <sup>c</sup>	0.021 <sup>b</sup>	43.96 <sup>b</sup>
'HS312'	517.61 <sup>b</sup>	40.95 <sup>b</sup>	60.23 <sup>a</sup>	8.93 <sup>c</sup>	6.31 <sup>a</sup>	15.24 <sup>b</sup>	40.59 <sup>ab</sup>	3.93 <sup>c</sup>	2.19 <sup>a</sup>	6.30 <sup>b</sup>	2.12 <sup>ab</sup>	72.82 <sup>a</sup>	0.007 <sup>a</sup>	0.142 <sup>b</sup>	0.031 <sup>a</sup>	43.93 <sup>b</sup>
'HS302'	263.51 <sup>c</sup>	38.09 <sup>c</sup>	46.51 <sup>b</sup>	9.00 <sup>c</sup>	4.26 <sup>b</sup>	13.27 <sup>b</sup>	36.33 <sup>b</sup>	3.64 <sup>c</sup>	1.15 <sup>b</sup>	4.80 <sup>c</sup>	3.43 <sup>a</sup>	73.35 <sup>a</sup>	0.002 <sup>b</sup>	0.034 <sup>c</sup>	0.009 <sup>c</sup>	26.24 <sup>c</sup>
'GF677'	478.42 <sup>b</sup>	42.40 <sup>b</sup>	58.73 <sup>a</sup>	11.68 <sup>a</sup>	5.59 <sup>a</sup>	17.28 <sup>a</sup>	42.30 <sup>a</sup>	4.66 <sup>a</sup>	2.69 <sup>a</sup>	7.35 <sup>a</sup>	1.97 <sup>b</sup>	63.53 <sup>b</sup>	0.009 <sup>a</sup>	0.198 <sup>a</sup>	0.040 <sup>a</sup>	48.09 <sup>a</sup>
'Sahand'	512.25 <sup>b</sup>	39.05 <sup>c</sup>	60.48 <sup>a</sup>	9.58 <sup>bc</sup>	6.41 <sup>a</sup>	15.99 <sup>ab</sup>	41.75 <sup>a</sup>	4.12 <sup>bc</sup>	2.58 <sup>a</sup>	6.71 <sup>ab</sup>	1.77 <sup>b</sup>	53.30 <sup>c</sup>	0.007 <sup>a</sup>	0.195 <sup>b</sup>	0.037 <sup>a</sup>	45.02 <sup>ab</sup>

\*Values with different letters are significantly different at the  $p < 0.01$  level.

spectrophotometer (Model DR 2000, Hach, Germany) according to the method of Arnon and chlorophyll index was estimated using chlorophyll meter (Spad 502 Minolta, Japan) (Arnon, 1949). In addition, height of plants, fresh and dry weights of shoot and root and total weight of plants were recorded. The concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> in the youngest fully expanded leaves and roots were measured by atomic absorption spectrophotometer (Perkin Elmer model 3110, USA). Also, Cl<sup>-</sup> concentrations and K<sup>+</sup> and Na<sup>+</sup> contents were determined with chloride-meter (Jenway model Pclms, USA) and atomic emission spectrometer (flame photometer, Corning model 410, Germany), respectively. Statistical analysis was carried out using MSTATC and SAS software and means were separated by the Duncan's multiple range test at the 5% probability level.

## RESULTS

Results indicate that high salinity levels (6 and 9 dSm<sup>-1</sup>) caused significant reduction in stem height, leaf number, leaf area, dry and fresh weight of stem and root in all genotypes (Table 1). The effects of salinity levels and genotypes, on all plant growth characteristics, were significant but their interaction was significant only on leaf area index. The effects of salinity treatments on the vegetative traits and plant growth indices were not significant in low salinity levels (1.5 and 3 dSm<sup>-1</sup>). The decline in leaf growth is the earliest response

of plants to salinity (Massai et al.,1998) and according to this study, leaf area of plants were not significantly different between 1.5 and 3 dSm<sup>-1</sup> (Table 1). The results indicate that decrease in plant leaf area (in addition to decline leaves size) is mainly due to a reduction of leaf number and stem height. Unlike low salinity levels, 6 and 9 dSm<sup>-1</sup> significantly reduced fresh and dry weight of leaves, stems and roots. However, growing factors varied between genotypes so that maximum and minimum values were observed in 'GF677' and 'HS302', respectively (Table 2). The interactions between salinity and genotype were

**Table 3.** The effects of salinity on the leaf and root ion concentrations. Means values of the different genotypes.

Genotype	Leaf					Root				
	K (%)	Ca (%)	Mg (%)	Cl (%)	Na (%)	K (%)	Ca (%)	Mg (%)	Cl (%)	Na (%)
'HS314'	2.083 <sup>a*</sup>	1.20 <sup>d</sup>	0.314 <sup>a</sup>	1.97 <sup>a</sup>	1.26 <sup>a</sup>	1.20 <sup>bc</sup>	1.25 <sup>c</sup>	0.31 <sup>b</sup>	2.5 <sup>b</sup>	1.06 <sup>bc</sup>
'HS312'	1.825 <sup>a</sup>	1.31 <sup>ab</sup>	0.310 <sup>ab</sup>	1.25 <sup>c</sup>	0.92 <sup>c</sup>	1.30 <sup>ab</sup>	1.30 <sup>b</sup>	0.30 <sup>bc</sup>	2.5 <sup>b</sup>	1.14 <sup>a</sup>
'HS302'	1.933 <sup>a</sup>	1.35 <sup>a</sup>	0.314 <sup>a</sup>	1.7 <sup>d</sup>	1.07 <sup>bc</sup>	1.35 <sup>ab</sup>	1.35 <sup>a</sup>	0.31 <sup>b</sup>	2.58 <sup>ab</sup>	1.10 <sup>ab</sup>
'GF677'	2.067 <sup>a</sup>	1.27 <sup>bc</sup>	0.304 <sup>b</sup>	1.95 <sup>ab</sup>	1.13 <sup>ab</sup>	1.50 <sup>a</sup>	1.35 <sup>a</sup>	0.32 <sup>ab</sup>	2.62 <sup>a</sup>	0.99 <sup>c</sup>
'Sahand'	1.800 <sup>a</sup>	1.22 <sup>dc</sup>	0.291 <sup>c</sup>	1.87 <sup>b</sup>	1.08 <sup>bc</sup>	1.10 <sup>c</sup>	1.25 <sup>c</sup>	0.34 <sup>a</sup>	2.67 <sup>a</sup>	1.15 <sup>a</sup>

\*Values with different letters are significantly different at the  $p < 0.01$  level.

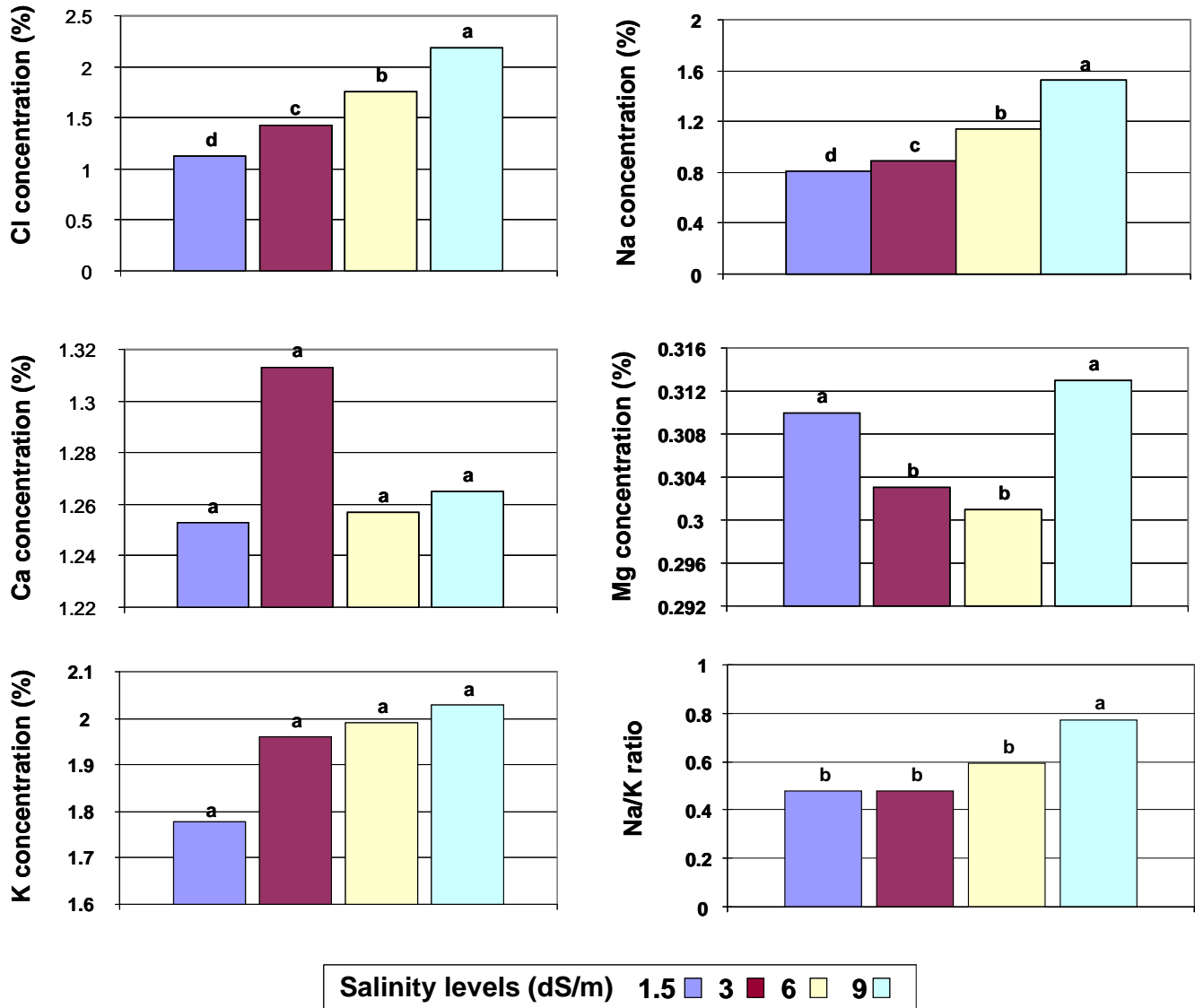
not significant but plant growth of all genotypes decreased at higher salinity levels. Increase in salinity level caused a significant increase in leaf proline contents in all the genotypes. The maximum and minimum amounts of proline were observed in 'HS302', 'HS312' and 'HS314' genotypes and 'Sahand', respectively (Table 2). These results show that the increase in proline content and salt stress tolerance is different between the genotypes. It may also indicate that 'Sahand' is relative salt-tolerance cultivar and it is not necessary to increase the leaf proline for stress responses. It probably means that its tolerance is induced by other factors than proline content. The chlorophyll (b, total and index) contents of leaves were significantly reduced by increase in salinity level but it was not significant in different levels of salinity for chlorophyll a (Table 1). The maximum value reduction of chlorophyll content and index was observed in 'HS302' and 'HS314' genotypes (Table 2). The interactions between salinity and genotype were not significant for chlorophyll contents. In this experiment, leaf chlorophyll content reduced in all genotypes and caused the appearance of chlorosis symptoms. Data concerning leaf ion concentrations in different salinity levels is presented in Table 3. Salinity significantly increased  $Mg^{2+}$ ,  $Cl^-$ ,  $Na^+$  concentrations and  $Na^+/K^+$  ratio in the leaf of all genotypes, whereas no significant effects were observed for  $Ca^{2+}$ ,  $K^+$  concentrations and  $Na^+/Ca^{2+}$  ratio (Table 3 and Figure 1). Also, reduction of  $Mg^{2+}$ ,  $Cl^-$  and  $Na^+$  concentrations were significant ( $p \leq 0.05$ ) among genotypes but it was not significant for  $Ca^{2+}$ ,  $K^+$  concentrations and  $Na^+/K^+$  ratio. Interaction effect of salinity and genotypes on leaf ion concentrations was significant except for  $K^+$  content.

Maximum and minimum root  $Na^+$  concentration was recorded in 'Sahand' (1.15%) and 'GF677' (0.99%), respectively, whereas it was reverse for  $Ca^{2+}$  concentration (Table 3).

Leaf  $Na^+$  and  $Cl^-$  concentrations in 'GF677' and 'HS314' genotypes were higher than in 'HS312' and 'HS302' in high level of salinity.  $Na^+$  was accumulated significantly only in the leaves of 'HS314' genotype; however, in high level of salinity, vegetative growth, plant biomass and tolerance of this genotype were better than others.

## DISCUSSION

Woody trees have been shown to be more susceptible to sodium and chloride toxicities (Boland et al., 1997b). Almond, apricot, plum and peach are all rated as sensitive to salinity (Maas and Hoffman, 1977). According to the research and considering growth measures, proline and ions concentrations as salt tolerance indices, it can be concluded that genotypes 'GF677', 'HS314' and 'Sahand' Cv. have relatively higher salt tolerance. Mechanism of salinity tolerance in 'Sahand' Cv. may be related to slow  $Na^+$  movement from roots to leaves like many woody species that rely on  $Na^+$  exclusion from leaves. In this way, several mechanisms such as  $Na^+$  accumulation in woods are involved in reduced uptake (Boland et al., 1997a; Maas et al., 1977). For this reason, it seems logical that leaf proline content of 'Sahand' Cv. was lower than other genotypes at different salinity levels. Mechanism leading to salt tolerance in 'GF677' and 'HS314' (peach × almond hybrids) probably are related to increased levels of leaf proline and enhanced threshold of the genotypes. Maximum reduction in chlorophyll content and chlorophyll index was observed in HS302 and HS314 genotypes. This may be due to chlorophyll degradation, reduced chlorophyll synthesis and stability of thylakoid membrane. In addition, it may be associated with the increased activity of chlorophyll degrading enzyme, chlorophyllase (Gunes et al., 2007). It should be noted that pot experiments may not accurately show the differences between the genotypes and therefore, will not allow us to recommend the more stable rootstocks in field under salinity conditions. But these results clearly indicate that under saline conditions, 'GF677' and 'HS314' accumulated more  $Na^+$  in leaves than 'HS312' and 'Sahand'. Therefore, 'GF677' and 'HS314' genotypes are able to keep osmotic adjustment and maintain adequate conditions for growth under saline environment than the sensitive ones (Boland et al., 1997b; Massai et al., 1998). This tolerance may result from their relative growth vigour due to heterosis phenomena that is genetically controlled. Generally, in saline soils,  $Na^+$  ions compete with  $K^+$  for uptake across the plasma membrane of plant cells. This can result in low  $K^+/Na^+$  ratio that reduce plant growth and eventually



**Figure 1.** Mean ion concentrations in leaves of the five genotypes assayed at different salinity levels. Values with the same letter are not statistically different according to Duncan's multiple range test at the 5% probability level.

become toxic (Munns, 1993). It seems that the rate of decrease in  $K^+/Na^+$  ratio had inverse relationship with the rate of rootstocks resistance. Many attempts have been made to study the effects of salt stress on plant growth using NaCl. Saline soils under natural conditions consist of some cations and anions which affect the growth of plants in different ways. For example, chloride salts are more toxic than sulfate salts and calcium ions reduce the deleterious effects of sodium ion in saline conditions (Aliasgarzad et al., 2005; Boland et al., 1997a; Rieger, 2001). Threshold salinity levels for fruit tree crops are generally based on vegetative growth of plants. According to report by Maas and Hoffman, (1977), tolerance threshold of almond, apricot and plum is about  $1.5 \text{ dSm}^{-1}$ . As expected, in this research, some growth related characteristics were the highest by increasing

salinity level up to  $3 \text{ dSm}^{-1}$  in all genotypes, especially 'HS314' and 'GF677'. Also, the results indicate that most of physiological, biochemical and morphological characteristics did not show significant reduction at the salinity of  $>3 \text{ dSm}^{-1}$ . Similar results were reported in two bitter almonds (Najafian et al., 2008). The reason is explained as follows: Salt mixture-induced salinity enhances salinity threshold of *Prunus* species from  $1.5$  up to  $3 \text{ dSm}^{-1}$  due to the presence of  $Ca^{2+}$ ,  $Mg^{2+}$  and  $SO_4^{2-}$  ions in saline solution (Bolat et al., 2006). Such results have been reported for alfalfa (Soltanpour et al., 1999). Although, NaCl is the dominant salt in saline soil of Iran, but as mentioned above, other cations and anions are present at low concentrations which affect salt tolerance of plants (Aliasgarzad et al., 2005; Tabatabaei, 2006). Using soil instead of perlite or other inert substrates and irrigation

only with pure water instead of nutrient solution may alter root morphology and its functions. This may lead to an increased plant tolerance to salt stress (Zekri and Parsons, 1992; Gunes et al., 2007). Moreover, using salt mixtures salinity and soil make situations very similar to the natural conditions. So with this method, the results will be applicable for field condition. Salt tolerance and vegetative growth rate may change in long-term salinization in field condition, because some studies have shown that salts accumulate in the wood for several years (even in low salinity level), become toxic and trees gradually decline (Boland et al., 1997a; Catlin et al., 1993; Rengel, 1992; Zekri et al., 1992). It has been shown that the woody tissue serves as a sink and when the storage capacity is exceeded, Na<sup>+</sup> or Cl<sup>-</sup> rapidly moves into the leaves (Boland et al., 1997a; Massai et al., 1998). Therefore, further studies should also investigate the effects of salinity on promising and new rootstocks in field condition for long term.

The result show that 'HS314' and 'GF677' genotypes were able to tolerate the high concentrations of mixture salinity than other genotypes and it seems that salinity threshold of the genotypes may be more than 1.5 dSm<sup>-1</sup> under field conditions. Moreover, using mixed-salt solutions and soil in the experiments make situations very similar to the natural field conditions. Therefore, with this method, determination of salinity threshold of plants could be more reliable.

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