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Full Length Research Paper

Influence of salt stress on growth, pigments, soluble sugars and ion accumulation in three pistachio cultivars

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In this study, three pistachio cultivars were treated with different concentration of salt solution to provide a reference for the cultivation of salt tolerant rootstocks. The results revealed that increasing the salinity stress resulted in decreasing growth performances of the plants in the three pistachio cultivars. This decrease was not significant in salinity of 100 mM but was significant in other salinities compared to control. In all salinity levels and control conditions, Akbari (Ak) cultivar exhibited larger growth performances of the plants in comparison with the other two cultivars; this state is more sensible in salinities of 200 and 300 mM. Ak cultivar assumed significantly higher pigment amount than the other two cultivars in high salinities. Impact of salinity on reduction of chlorophyll amount was significant in all cultivars starting from salinity of 200 mM. Moderate and high salinity led to an increase in amount of total soluble sugars in Ak cultivar while no significant contrast was achieved between the other two cultivars compared to control conditions. The impacts of different salinities on Na and Cl were nearly similar and the amounts of these elements increased with exceeding salinity stress in different cultivars compared to control condition. The high stress level resulted in remarkable enhancement of Cl and Na amounts in Ouhadi (Ou) and Kalehghouchi (Ka) cultivars. However, the low and moderate salinities increased the concentration of potassium in AK cultivar compared to the other two cultivars. Therefore, higher plant growth and photosynthetic pigments, lower Na and Cl content, greater accumulation of soluble sugars and K could explain the greater salt-tolerance of AK compared to that of Ka and Ou.

Key words: Pistachio cultivars, salinity, growth, ion accumulation.

INTRODUCTION

Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern (Jaleel et al., 2007b). Over 800 million hectares of land worldwide is affected by salinity (Munns, 2005), comprising nearly 7% of the worlds total land area. Irrigation systems are particularly prone to salinization, with nearly one third of irrigated land being severely affected. Every year, more and more land becomes non-productive due to salt accumulation. Iran, the second largest country in the soil salinity which is considered a serious agricultural problem, accounting for at least 20% of the Middle East and has 165 million hectares irrigated lands (Munns and Tester, 2008). Approximately, 90% of the country is classified as arid and semi-arid region, most of which suffers from low rainfall, high evaporation-transpiration, salinization, shortage of fresh water, soil erosion, excessive heat and desertification. In Iran, the pistachio nut tree (*Pistacia vera* L.) is an important and valuable crop which is usually grown under saline conditions (Sheibani, 1994). Land salinization is a major limiting factor for conventional crop productivity in the

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Abbreviations: Ak, Akbari; **Ou,** Ouhadi; **Ka,** Kalehghouchi; **mM,** milimolar.

country (Cheraghi, 2004). Salinity in soil or water is one of the most damaging abiotic stress factors limiting crop (Debez et al., 2006). High concentration of salts in the soils immediately imposes on plants the osmotic stress effect due to lower soil water potential leading to retardation of water uptake. When exposed for longer period, salinity entails ionic stress when plants absorb and accumulate toxic level of Na^+ and Cl^- in the cytoplasm. Salinity also induced secondary stresses such as nutritional imbalance and oxidative stress (Zhu, 2002). For worldwide crop production, the detrimental effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors (Levit, 1980; Ashraf and Harris, 2004). All of these agents cause adverse pleiotropic effects on plant growth and development at physiological and biochemical levels (Munns, 2002) and the molecular level (Tester and Davenport, 2003).

To survive in hyper-saline soil, plants have evolved complex mechanisms that contribute to the adaptation to osmotic and ionic stresses caused by high salinity. These mechanisms include osmotic adjustment that is usually established by intake of inorganic ions as well as accumulation of compatible solute (Osmoprotectants). Inorganic ions are sequestered in the vacuole, while organic solutes are compartmentalized in the cytoplasm to balance the low osmotic potential in the vacuole (Rontein et al., 2002). Furthermore, plants have to employ a wide range of biochemical and molecular mechanisms including alteration of photosynthetic pathway, change in membrane structure, stimulation of phytohormones and induction of antioxidative enzymes (Parida and Das, 2005; Mudgal et al., 2010). Difference in salt tolerance exists not only among variant genera and species, but also within the different organs of the same species (Bankar and Ranjbar, 2010). Comparing the response of cultivars of one species to salinity provides a convenient and useful tool for unveiling the fundamental mechanisms involved in salt tolerance.

The objective of this investigation was to evaluate the effects of salt stress on growth, soluble sugars and ion accumulation of three pistachio cultivars, to provide a reference for the cultivation of salt-tolerant trees.

MATERIALS AND METHODS

Three types of pistachio seeds (*Pistacia vera* L. Cultivars *Akbari*, *Ouhadi* and *Kalehghouchi*) were used in this study. Seeds were collected from the Anar area, Kerman province, which is one of the main pistachio growing regions in Iran with saline condition. The trial was conducted at Biology Department, Islamic Azad University, Damghan Branch, Damghan, Iran during 2011. The seeds were surface-sterilized for 10 min in 10% H_2O_2, washed three times with tap water to remove any trace of chemicals that could impair seed germination and were placed on sterile vermiculite at 18 to 25°C to germinate. After 21 days of germination, seedlings were sown in plastic post containing sandy soil (pH 7.6, 3.8% silt, 14.6% clay, 81.6% sand, and electrical conductivity (EC_e) = 0.98 ds/m). Seedlings were transplanted in 20 ×15 cm plastic pots containing a mixture of salinity clay: sand (1:5 v/v) (four seedlings/pot).

Each pot contained 3 seedlings which were sufficiently irrigated with urban water every 4 days. All pots were placed in a greenhouse condition with mean relative humidity 45 to 60%, day / night temperature 18 to 30°C and photoperiod from 14 to 16 h (photo-synthetically active radiation of approximately of 430 to 460 mol/ms).

Seedlings were grown under these conditions for 4 weeks before initiation of NaCl treatments and salinity treatments were 0, 100, 200, and 300 mM of NaCl. The soil was salinized in step-wise manner to avoid subjecting plants to an osmotic shock. After 55 days of salt treatment, the plants were harvested and separated into shoots and roots. After complete washing with distilled deionized water, fresh masses of shoots and roots and the other morphological parameters such as shoot height, number of branches and leaves were measured in fresh samples. Total leaf area was calculated with an AM-200 leaf-area meter. Total chlorophyll and cartenoid were extracted and estimated from fresh leaves according to the standard method of Arnon (1949). Chlorophyll was extracted in 80% (v/v) aceton from 1 g of fresh leaf sample in the dark at room temperature. Absorbance was measured at 663 and 645 ηm in a UV/VIS spectrophotometer. Chlorophyll concentration was calculated using the equation:

$$Chl = 0.0202 \times A_{645} + 0.00802 \times A_{663}$$

Cartenoid concentration was calculated using the equation:

$$Car = (A_{436} \times V) / 196 \times b)$$

Where V is volume of dilution, b is the length of cell (1 cm). The value 196 is coefficients of specific absorption. The remaining samples were then over-dried at 80°C for 48 h so as to record dry masses. A flame photometer was used for Na^+ and K^+ determination. Phosphorus was analyzed colorimetrically (Boltz and Lueck, 1958) and Cl content was determined by atomic absorption.

Fresh roots and leaves were collected for the determination of total soluble sugars content. Soluble sugar content was determined by 0.1 ml of the alcoholic extractor reacting with 3 ml freshly prepared anthrone (2000 mg anthrone + 100 ml 72% H_2So_4) then was placed in a boiling water bath for 10 min as described by Irigoyen et al. (1992). After cooling, the absorbance was read to be equal to 620 nm.

Statistical analysis

The experiment was arranged in a completely randomized design (CRD) in a two-factor factorial arrangement with 3 replications (n = 3). All parameters were investigated by analysis of variance (ANOVA) using SPSS software. The means were compared by the Tukey's test at a 0.05 confidence level.

RESULTS

Growth performances of the plants were estimated by measuring plant height, leaf area, number of leaves and branches, and total fresh and dry weights. With increase in salinity stress, dry weight of shoots decreases in the three pistachio cultivars. However, this decline was not statistically significantly different in salinity of 100 mM but was significant different in the other salinities compared to the control plant. In all salinity levels and control conditions, Akbari cultivar exhibited larger dry weight

Table 1. Fresh and dry weight (g), number of leaves and branches, leaf area (cm^2) and plant height (cm) of pistachio plants under NaCl stress.

Plant height	Leaf area	Number of branches	Number of leaves	Root weight		Shoot weight		Cultivars	Salinity levels (mM)
				Fresh	Dry	Fresh	Dry		
27^{a}	91.04^{a}	7^{a}	43^{a}	3.19^{a}	4.86^{a}	8.2^{a}	5.38^{a}	Ak	
28^{a}	95.4^{a}	7^{a}	46^{a}	1.9^{ce}	3.08^{c}	7.9^{a}	4.9^{a}	Ka	0
27^{a}	93.2^{a}	8^{a}	48^{a}	2.01^{ce}	3.19^{c}	7.4^{a}	5.05^{a}	Ou	
22^{ab}	85.44^{a}	6^{ac}	40^{a}	2.4^{ae}	3.4^{ac}	5.6^{b}	4.7^{a}	Ak	
21^{ab}	87.35^{a}	8^{a}	41^{a}	1.2^{bc}	2.78^{c}	5.4^{b}	3.9^{a}	Ka	100
24^{ab}	86.2^{a}	7^{a}	44^{a}	1.15^{bc}	2.67^{c}	5.2^{b}	3.8^{a}	Ou	
19^{bd}	77.37^{ae}	4^{cd}	29^{c}	1.7^{bce}	2.7^{c}	3.9^{bd}	2.7b^{e}	Ak	
10^{c}	61.30^{de}	3^{d}	21^{c}	0.87^{bd}	2.2^{dc}	2.6^{de}	1.5^{c}	Ka	200
12^{c}	64.35^{de}	3^{d}	24^{c}	0.98^{bd}	2.1^{dc}	2.3^{de}	1.6^{c}	Ou	
17^{d}	65.43^{e}	3^{d}	23^{c}	1.2^{bd}	2.2^{dc}	3.94^{bd}	1.9^{ce}	Ak	
9^{c}	37.3^{c}	2^{d}	10^{d}	1.1^{d}	0.49^{d}	1.58^{e}	0.23^{d}	Ka	300
10^{c}	35.4^{c}	3^{d}	13^{d}	1.05^{d}	0.45^{d}	1.32^{e}	0.19^{d}	Ou	

Within each column, means superscript with different letters are significantly different ($P \leq 0.05$).

compared to the other two cultivars. Moreover, this state was more sensible in salinities of 200 and 300 mM and was statistically significant at $P \leq 0.05$. No significant difference was observed in the dry weight of shoots between the two cultivars, that is, Ka and Ou in all salinity levels and control conditions. Fresh weight was significantly declined in all salinity levels compared to control plant. However, a significant difference in fresh weight was only observed between Ak cultivar and the other two cultivars in the salinity of 300 mM (Table 1). Root weight was higher in Ak cultivar than the other two cultivars under the control and salinity stress conditions. However, this increase was statistically significant only under the control conditions. The results presented in Table 1 also indicated that root weight was decreased with exceeding stress in all pistachio cultivars. This decrease was statistically significant in salinities over 200 mM. No significant contrast was observed between the two cultivars, Ou and Ka regarding the fresh and dry weights of root under the control and different salinity conditions.

No significant difference in number of branches was observed between the control and salinity level of 100 mM among different pistachio cultivars. However, under stress condition at 200 and 300 mM, number of branches showed a significant decrease in all varieties. This decrease was more pronounced in the higher level of salinity and in Ou and Ka cultivars compared to Ak cultivar in different salinities. On the other hand, no significant difference was observed between Ak cultivar and the other two varieties regarding the number of leaves for the control and low or medium salinity conditions. However, number of leaves in Ak cultivar was significantly higher in the high salinity level compared to the other two cultivars (Table 1). It is of interest to mention that the number of leaves was decreased in all the different pistachio cultivars as salinity stress was increased. This decrease was statistically significant only at the medium and higher salinity levels compared to the control plants. Moreover, the reduction leaves number in Ou and Ka cultivars was more intense from moderate salinities onwards (Table 1).

No tangible difference was observed for plant height between the control and the low salinity conditions among different cultivars. However, Ak cultivar had a significant increase in plant height under the moderate and high salinities in comparison with the two other ones. However, the plant height of different pistachio cultivars was shortened with exceeding salinity stress; this reduction was more intense in Ou and Ka cultivars under the moderate and high salinities (Table 1).

The impact of chloride-sodium on leaf area revealed no considerable difference among different cultivars under the control and low salinity conditions. However, the difference between the three cultivars was intensified with the increase in salinity stress since this difference between Ak cultivar and two other cultivars was significant in high salinity. Ak cultivar showed the maximal leaf area among all varieties although this parameter was reduced in all varieties as stress was increased and it was more noticeable for Ou and Ka cultivars under high salinity (Table 1). The effect of chloride-sodium on the chlorophyll and cartenoid content in leaves is presented in Table (2). These results indicated that no significant difference in cartenoid pigment was observed among different pistachio cultivars

Table 2. Chlorophyll, cartenoides content (mg/g f.w.) and soluble sugars (mg/g d.w.) of three pistachio cultivars as affected by salinity treatment.

Salinity levels (mM)	Cultivar	Total chlorophyll	Cartenoides	Soluble sugars
	Ak	1.2[a]	0.89[a]	22[a]
0	Ka	1.25[a]	0.77[a]	26[a]
	Ou	1.30[a]	0.93[a]	24[a]
	Ak	0.89[ab]	0.78[a]	25[a]
100	Ka	0.85[ab]	0.79[a]	24[a]
	Ou	1.03[ab]	0.81[a]	27[a]
	Ak	0.73[bc]	0.82[a]	33[b]
200	Ka	0.54[cd]	0.71[a]	22[a]
	Ou	0.59[cd]	0.76[a]	24[a]
	Ak	0.75[bc]	0.22[b]	35[b]
300	Ka	0.38[d]	0.27[b]	23[a]
	Ou	0.41[d]	0.31[b]	20[a]

Within each column, means superscript with different letters are significantly different ($P \leq 0.05$).

under the control and different chloride-sodium salinity conditions. However, at salinity of 300 mM, a significant reduction in cartenoid amount was observed in different cultivars. The results indicated chloride-sodium did not induce any remarkable difference in total chlorophyll pigments among different cultivars under the control and low-moderate salinity conditions. However, Ak cultivar showed a significantly higher pigment amount than the other two varieties under high salinities. Moreover, the impact of salinity on the reduction of chlorophyll amount was statistically significant in all varieties starting from salinity of 200 mM (Table 2).

The impact of chloride-sodium salinity on soluble sugar value indicated that low salinity had no considerable impact on dissolved sugars in different cultivars. However, the moderate and high salinity led to an increase in the amount of total soluble sugars in Ak cultivar while no significant difference was achieved between the other two cultivars compared to the control conditions. The difference in soluble sugars between Ak and the other two cultivars was significant in salinities of 200 and 300 mM while no significant difference was observed among the three cultivars under the control and low salinity conditions with regard to soluble sugars (Table 2).

The effect of different concentrations of chloride-sodium on the amounts of Na, Cl, K and N in different cultivars of pistachio shrubs is presented in Table 3. The results indicated that the impacts of different salinities on Na and Cl were nearly similar as the amounts of these two elements were increased with exceeding salinity stress in different cultivars compared to control condition. This increase was statistically significant under all salinity levels in comparison with the control condition. Moreover, no significant difference was observed between the amounts of the respective elements in different cultivars under the control and low-moderate salinity conditions. However, increasing salinity from 200 to 300 resulted in remarkable enhancement of Cl and Na amounts in Ou and Ka varieties. Interestingly, no significant difference was found in the concentration of Na and Cl elements in Ak cultivar due to increasing salinity compared to salinity of 200 mM (Table 3).

Salinity of 100 mM had no significant impact on reduction of potassium level in Ak cultivar but this reduction was significant in the other varieties as compared with the control. The concentration of potassium was significantly higher ($P \leq 0.05$) in Ak cultivar than the other two cultivars under the low and the moderate salinities. However, no significant difference was reported in potassium concentration among different cultivars in control and high salinity conditions (Table 3). The results of nitrogen concentration in different cultivars under the control and salinity conditions revealed insignificant difference under the control and salinity conditions. The concentration of this element decreased in all cultivars as salinity stress increased and the reduction was statistically significant under the moderate and high salinities compared to the control conditions although at low salinity this difference was insignificant (Table 3).

DISCUSSION

In this study, the responses of three different pistachio cultivars to salt stress were compared with regard to plant growth, soluble carbohydrate and ion accumulation. It is well documented that high salinity can inhibit the growth and development of plants and even result in their death (Shi and Wang, 2005; Moghaieb et al., 2004). The current results (Table 1) demonstrated a significant reduction in plant growth with increasing soil salinity. These results are parallel to those reported for different

Table 3. Shoot content of Na and Cl (mol/kg d.m.), K and N (mg/g d.m.) in three pistachio cultivars at different salinity levels.

Salinity level (mM)	Cultivar	Na	Cl	K
0	Ak	0.054[a]	0.301[a]	236[a]
	Ka	0.061[a]	0.298[a]	218[a]
	Ou	0.056[a]	0.291[a]	240[a]
100	Ak	0.258[b]	0.898[b]	195[a]
	Ka	0.241[b]	0.842[b]	141[b]
	Ou	0.269[b]	0.901[b]	150[b]
200	Ak	0.284[b]	0.810[b]	140[b]
	Ka	0.345[b]	0.911[b]	85[c]
	Ou	0.301[b]	0.943[b]	79[c]
300	Ak	0.315[b]	0.894[b]	120[bc]
	Ka	0.637[c]	1.987[c]	71[c]
	Ou	0.748[c]	1.785[c]	84[c]

Within each column, means superscript with different letters are significantly different ($P \leq 0.05$).

plants such as carthamus (Abbaspour, 2010), pistachio (Bankar and Ranjbar, 2010) and wheat (Khan et al., 2006). In order to improve salt tolerance in pistachio plants, inter-cultivar variation in pistachio varieties should be examined to select the promising lines-genotypes. Differences in growth of pistachio cultivars in response to salt stress observed in the present study might have occurred due to variation in a number of biochemical or physiological traits that are associated with the processes related to the mechanism of salt tolerance such as photosynthetic pigments, nutrient homeostasis and accumulation at compatible solute. The comparison of different cultivars under investigation showed that the growth of Ou and Ka varieties were significantly inhibited and these cultivars cannot survive in 300 mM NaCl. However, Ak cultivar can grow well at the same degree of salinity.

In the present study, the photosynthetic pigment content of pistachio plants was decreased under salt stress. Photosynthetic pigments play a key role in maintaining photosynthetic capacity of most plants (Dubey, 2005). The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (levit, 1980). It is also attributed to the interference of salt ion with the de novo synthesis of proteins and the structural component of chlorophyll rather than the breakdown of chlorophyll (Jaleel et al., 2007a). Moreover, the changes of pigments content under salt stress are used as parameter for selection of tolerant and sensitive cultivars in plants (Eryilmaz, 2007).

The three tested cultivars showed differences in the accumulation of soluble sugar with increasing salinity. The content of soluble sugars in Ou and Ka did not changed; while, the respective value was increased in Ak at medium and high salinities. Thus, it is proposed that the accumulation of soluble sugars might be important to cytoplasmic osmotic of Ak and the destructive effects of salinity are commonly thought to be a result of low water potential and ion toxicity (Parida and Das, 2005).

Na^+ is the main poisonous ion in salinized soil and plants growing in saline conditions generally compartmentalize Na^+ into vacuoles resulting in Na^+ toxicity in the cytosol. It is essential for maintaining a number of enzymatic processes (James et al., 2006; Zhu, 2003; Munus, 2002), and the Na^+/K^+ ratio is an important index representing the salt-tolerance ability of a plant (Parida and Das, 2005). The current results indicated that the increasing levels of NaCl induced a progressive absorption of Na and Cl. Such pattern of accumulation of the toxic ion has earlier been reported in a number of plant species referred as “salt accumulators” (Turan et al., 2010). Accumulation of Cl in the tissue is disruptive to membrane uptake mechanisms (Yousif et al., 1972). The reduction in potassium in shoots as result of salt stress (Table 3) has been observed previously, interpreted as resulting from competition between this ion and Na (Karmoker et al., 2008; Turan et al., 2010). According to Cordovilla et al. (1995), NaCl decreased N concentration in the shoot tissues and the salinity has a negative impact on the nitrogen acquisition and utilization (Lewis, 1986). Similar negative effect of chloride-sodium on NO^-_3 was reported by other authors (Wehrman and Handel, 1984; Turan et al., 2010).

By comparing the accumulation traits of the three pistachio cultivars, it is obvious that under the same degree of salinity, especially in high salinity, Na^+, Cl^- and Na^+/K^+ were much lower in Ak than those of Ou and Ka cultivars which was suggestive of greater capacity of Ak in controlling Na^+. Tolerance of salt stress is greater in Ak cultivar than that in Ou and Ka cultivars and the difference might be due to different salt-tolerance mechanisms. Higher plant growth and photosynthetic pigments, lower Na and Cl content, greater accumulation of soluble sugars and K could explain the greater salt-tolerance of Ak compared to that of Ou and Ka.

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