Response of Badami-Zarand Pistachio Rootstock to Salinity Stress

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Abstract

Salinity is one of the most important problems that restrict cultivation of crops in arid and semi-arid regions. Pistachio (*Pistacia vera* L.) is one of the most important trees that grow in these restricts. In this greenhouse study, the effects of salinity on growth of pistachio rootstock Badami evaluated. Seedlings were grown in plastic bags containing perlite. Salinity was imposed, by adding salts Nacl and $Cacl_2$ to the pots to obtain concentration of 0, 100, 200 mM Nacl and $Cacl_2$. 150 days after salt treatment, pistachio seedlings were harvested and length of shoots and roots, number of leaves and fresh and dry weight of shoot and root were measured. Photosynthesis and transpiration rates were measured before harvesting the plants. Also Chlorophyll fluorescence of plants measured. Salinity decreased shoots and to some extent root growth and number of leaves per plant in comparison with the control. Salt treatments reduced length of shoot and fresh and dry weight of shoots, but these values were more in roots for $Cacl_2$ in comparison with control. Photosynthesis rate reduced in response to salinity but for Nacl treatments was more than the other. Transpiration rates and chlorophyll fluorescence did not show any significant differences with control. Data obtained in present study emphasized that growth Badami rootstock is affected by salinity and Nacl more than $Cacl_2$ reduced growth of this rootstock. The aim of this study was to evaluate response of Badami rootstock to different level of salinity.

Key words: pistachio, salt treatments, Badami rootstock, reducing growth.

Introduction

Salinity is a widespread problem especially in arid and semiarid regions. Some studies indicate that 20-50 % of all irrigated croplands are affected by high salt concentration, resulting in considerable economic losses (Flowers, 1999). There are two main negative effects of high salt concentration that influence plant growth and development. Water deficit and ion toxicity associated with them, will excessive Cl and Na, leading to a Ca and K deficiency and to other nutrient imbalances (Marschner, 1995).

Temperate fruit trees are generally rated as sensitive to soluble salts (Mass and Hoffman, 1997; Mass, 1986) and particularly sensitive to chloride (Bernstein, 1980) and irrigation with saline water may significantly reduce yield (Shannon *et al.*, 1994).

Salt stress has been reported to cause an inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Boyer, 1982; Pal *et al.*, 2004). Salinity and its effects on biomass production have been considered by numerous authors (McKell, 1994; Ungar, 1996; Khan *et al.*, 2000 a, b; Mehari *et al.*, 2005).

Pistachio *(Pistacia vera* L.) is one of the most important commercial trees grown in Iran, Turkey, and recently in the USA. Pistachio is considered a potential crop for many arid and semi-arid regions. Pistachio plants are known to be tolerant to salts (Sepaskhah and Maftoun, 1981; Behboudian *et al.*, 1986a; Picchioni and Miyamota, 1990; Ferguson *et al.*, 2002).

Najmabadi (1969) also stated that pistachio can grow on lands that are too saline for other crops; however Parsa and Karimian (1975) have shown that salt adversely affects the aerial and root growth of *P. vera.* Adverse effects of salinity on growth, photosynthetic rates, and morphological changes in the leaves of pistachio have been shown (Behboudian *et al.*, 1986a; Picchioni and Miyamota, 1990; Munns *et al.*, 2002; Ranjbar *et al.*, 2002).

According to Zohary (1973), Iran is a country with a lot of saline soils. In this country, the areas with saline and alkaline soils are expanding especially in arid and semiarid regions. Twelve and a half percent (12.5%) of the total area of the country has been covered with the saline soils through a number of processes (Alkhani and Ghorbani, 1992). Nevertheless, pistachio are planted on sodic soils and irrigated with low quality, saline water in Iran. These conditions result in reduction of pistachio yields over recent years (Karimi *et al.*, 2009).

The aim of present study was to evaluate the vegetative characteristics (Leaf number, shoot height, root length and fresh and dry weight of shoot and root) and ecophysiological characteristics (photosynthesis and transpiration rates and chlorophyll fluorescence) as responses of *P. vera* Badami to salinity.

Material and Methods

The experiment was conducted during the spring and summer of 2009 at the research greenhouse of the pistachio research center, Rafsanjan, Iran. First seeds of pistachio rootstock (*Pistacia vera* L.)(Badami) were sterilized with 0.1% sodium hypochlorite for 4 hours and 10 min and rinsing repeatedly with distilled water. After sterilization seeds were planted in pots (25 per 30 cm) containing sterile perlite with the diameter less than 0.5mm. After 30 days, when seeds was germinated Hoagland nutrient solution (0.5-strength) and treatments have been added to pots. Salt treatments were 0 (distilled water), 100, 200 mM of Nacl and $Cacl_2$ obtained by adding Nacl and $Cacl_2$ separately to distilled water. The experiment was conducted in randomized complete design with three replications in each two salinity levels. Pots were maintained under controlled condition at temperature of 25/16 Celsius day/night and relative humidity of 45% and without additional artificial lightning. On 150 days after treatments, seedlings were harvested from pots and plants were divided into root and shoot. Number of leaves per shoot, height of shoot and root and shoot and root fresh and dry weights were recorded. Photosynthesis and transpiration rates were measured by using an apparatus model (LCA4, ADC, Bioscientific LTD, UK) and chlorophyll fluorescence was measured by (Opti Sciences Inc ADC, UK) apparatus. The analysis of variance (ANOVA) was done using Duncan's method.

Results

Effect of salinity on leaf number of pistachio

Analysis of variance indicated that the levels of 200 mM $Cacl_2$ and 100 mM Nacl decreased leaf number in compared with control but this was not significant. 200 mM Nacl showed minimum number of leaves and 100 mM $Cacl_2$ did not show any significant difference with control as is showed in figure 1.

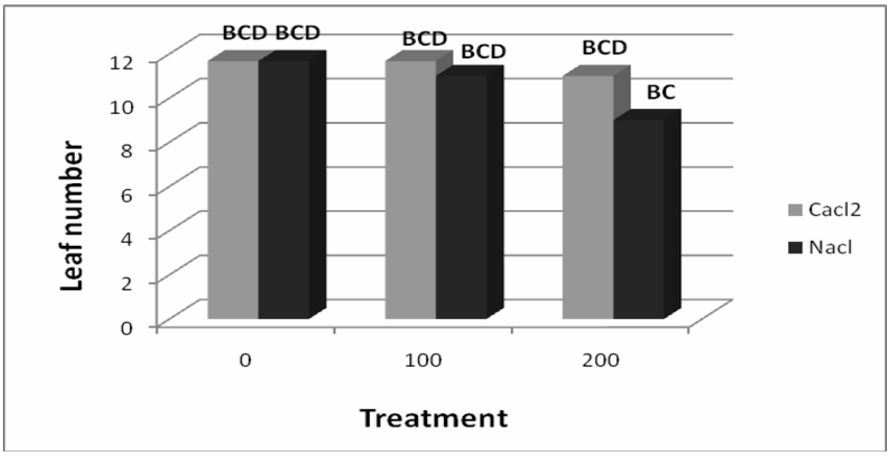

Figure 1: effect of salinity on leaf number of pistachio

Effect of salinity on shoot height of pistachio

Data show that maximum height of shoot observed in control and minimum of that was in 200 mM Nacl. The other treatments were 100 mM $Cacl_2$, 200 mM $Cacl_2$ and 100 mM Nacl for purposes of decrease in shoot height. Completely Nacl treatments more than $Cacl_2$ treatments decreased shoot height. Figure 2 showed the effect of salinity on shoot height of pistachio.

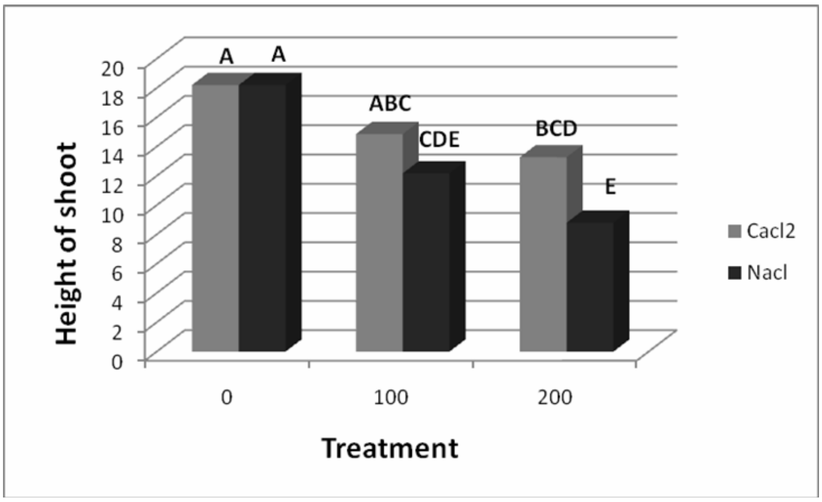

Figure 2: effect of salinity on shoot height of pistachio

Effect of salinity on root length of pistachio

Among all of treatments $Cacl_2$ showed maximum root length. This difference for 100 mM $Cacl_2$ was more than 200 mM. Completely Nacl treatments indicated minimum root length in comparison with the other treatments as it showed in figure 3.

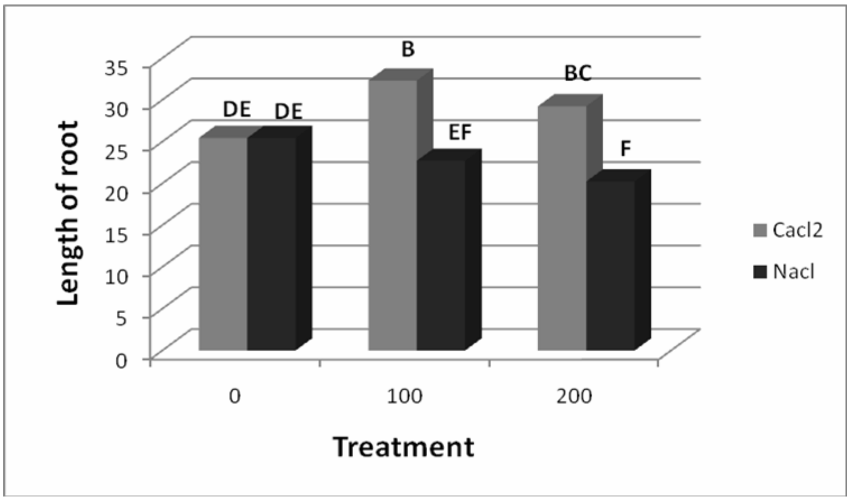

Figure 3: effect of salinity on root length of pistachio

Effect of salinity on fresh and dry weight of shoot and root

In both dry and fresh weight of shoot, control showed maximum values. Level 100 and 200 mM $Cacl_2$ and 100 mM Nacl were in later ranks. 200 mM Nacl showed minimum dry and fresh weights of shoots. Analysis indicated that level 100 mM $Cacl_2$ in both fresh and dry weight of root, showed maximum values than the others. Control and 200 mM $Cacl_2$ were similar and Nacl treatments were in later ranks. The Effect of salinity on fresh and dry weight of shoot and root is presented in table 1.

Table 1.Effect of salinity on fresh and dry weight of shoot and root

Treatment	Fresh weight of shoot	Dry weight of shoot	Fresh weight of root	Dry weight of root
Control	1.523a	1.203a	2.723cd	2.42cd
S_1	0.97c	0.633cd	3.383b	3.147b
S_2	0.94c	0.606d	2.687cd	2.41cd
S_3	0.84c	0.54De	2.35d	2.137d
S_4	0.49d	0.313e	1.56e	1.36f

S_1: 100mM $Cacl_2$, S_2: 200mM $Cacl_2$, S_3: 100mM Nacl, S_4: 200mM Nacl.

Effect of salinity on fluorescence emission

Chlorophyll fluorescence parameters were considered as indicators of damage to light harvesting system in the chloroplasts. Ground fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv) ,variable fluorescence / maximum fluorescence (Fv/ Fm), were recorded on the adaxial surface of the leaves from the top. Analysis of variance indicated that the different saline solutions had no significant effects on none of the chlorophyll fluorescence parameters. These findings are in agreement with Ranjbar et al (1999) on pistachios. Table 2 shows the effect of salinity on fluorescence emission.

Table 2.Effect of salinity on chlorophyll fluorescence.

Treatment	Fo	Fm	Fv	Fv/Fm
Control	179a	788.3ab	609.3ab	0.772ab
S_1	179a	858a	679a	0.791ab
S_2	168a	883.3a	715.3a	0.809a
S_3	179a	782.7ab	603.7ab	0.768ab
S_4	174a	734ab	560ab	0.756ab

S_1: 100mM $Cacl_2$, S_2: 200mM $Cacl_2$, S_3: 100mM Nacl, S_4: 200mM Nacl.

Effect of salinity on photosynthetic rates

According to findings, exposure of pistachios to salinity led to a significant reduction in leaf photosynthesis, but for level 100 mM $Cacl_2$ this reduction was not significant. Control in this case indicated maximum photosynthesis rate in comparison with the other treatments. Figure 4 shows the effect of salinity on photosynthesis rates.

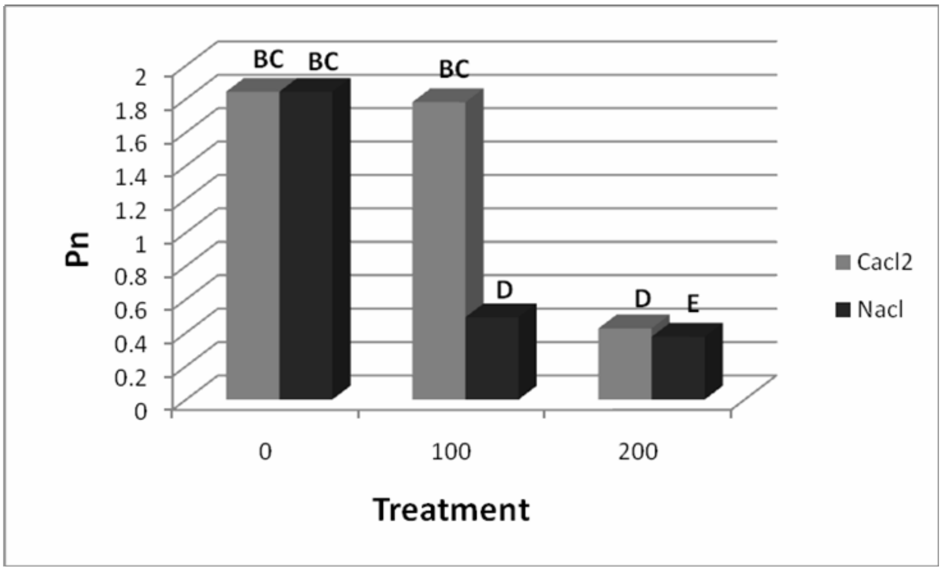

Figure 4: Effect of salinity on photosynthesis rate.

Effect of salinity on transpiration rate (E)

Analysis of variance did not show any significant differences for transpiration rate at different levels of salinity. The highest transpiration rate was observed in 100 mm $Cacl_2$.transpiration rates were measured with the differential infrared H_2O gas analyzer set up in the phytoron on the same leaf as used for photosynthesis measurements. Figure 5 shows the effect of salinity on transpiration rate.

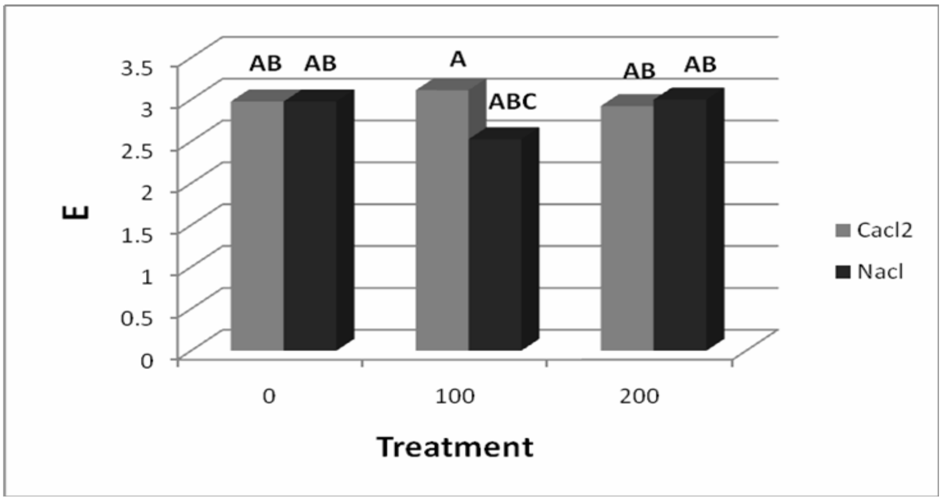

Figure 5: Effect of salinity on transpiration rate of pistachio

Discussion

Salinity normally decreases vegetative growth of plants such as pistachios. It has been claimed that the growth of pistachio trees under stress surpasses that of all other fruit trees species (Spigel-Roy *et al.*, 1997).

Our data showed that the addition of salts (Nacl + $Cacl_2$) to the nutrient solution reduced fresh and dry weight of shoots, length of stem and leaf number but length of root for $Cacl_2$ treatments was more than control and Nacl treatments. Fresh and dry weight of roots in 100 mM $Cacl_2$ were considered revising, but control and 200 mM $Cacl_2$ showed similar values. Nacl treatments again showed minimum values in this case. These results are in agreement with findings by Karimi *et al.*, (2009) on pistachios and Najafian *et al.*, (2008) on bitter almond.

Exposure to salt may affect plant metabolism by an osmotic effect, causing a water deficit, or by a specific ion effect (depends on the types of salt and species), causing excessive ion accumulation. The reduction in tissue masses when salinity level increased, as well as visible-injury observations, suggests that the severe saline environment caused not only growth reduction but also chlorosis, defoliation and other toxicity symptoms. (Sepaskhah and Maftoun, 1981, 1988; Tvallali *et al.*, 2008).

The reduction in shoot dry weight was attributed to lower leaf number and development of smaller leaves with increasing salinity of the growth medium. Ion toxic effects of salts are attributed to excess accumulation of certain ions in plant tissues and to nutritional imbalances caused by such ions. It was shown that damage to pistachio plants has been mainly attributed to excessive accumulation of Cland Na+ in the leaves (Sepaskhah and Maftoun, 1981, 1988; Tvallali *et al.*, 2008).

Also the duration, during which plant, are exposed to stress, affects the type of damage (osmotic effect or ion accumulation). (Ranjbar *et al.*, 1999). For instance, Rana and Annie (1986) found that short-term effect of Nacl on leaf elongation in wheat arise from osmotic effects. Salt treatment decreased the photosynthetic rate, and subsequently the whole plant biomass. These suggested that the impairment of plant growth by salt stress mainly resulted from the suppression of photosynthetic activity. Current data showing that leaves and stem growth depressed more than root growth by salt stress (Karimi *et al.*, 2009).

Salinity normally decreases plant photosynthetic rates. Exceptions are the halophytes, in which photosynthetic rates do not always decrease and may even be increased with increasing conductivity of the soil solution (Gale, 1975). Photosynthetic activity can be interfered within two ways: by modifying either, stomata conductance or the mesophyll capacity to CO2 assimilation (Farquhar and Sharkey, 1982). It has been demonstrated that both Na and Cl can reduce the mesophyll capacity to assimilate CO2 (Behboudian *et al*., 1986a, b). It has been claimed that the growth of pistachio trees under stress surpasses that of all other fruit trees species (Spiegel-Roy *et al.*, 1997).

In present study, salinity decreased photosynthesis rates and this reduction was more for Nacl treatment in comparison with the other treatments. Level of 100mM $Cacl_2$ did not show any significant difference with control.

Our data show that the addition of salts (Nacl + CaCl2) to the nutrient solution reduced Pn when there was stress on the process of photosynthesis, one can detect this, with the change(s) in chlorophyll fluorescence emission and/or chlorophyll content. In most plants there are relationships (direct/inverted) between chlorophyll emission parameters and chlorophyll content. Soil salinity is one of the major environmental stresses. It can lead to destruction of the fine structure of chloroplasts, instability of pigment-protein complexes (Lapina and Popov, 1984) and decreases in chlorophyll content (Kingsbury *et al.*, 1984; Downton and Millhouse, 1985; Reddy and Vora, 1986).

Transpiration is the evaporation of water from the aerial parts of plants, especially leaves but also stems flowers and roots. Leaf transpiration occurs through stomata which are found on the undersides of leaves, and can be thought of as a necessary "cost" associated with the opening of stomata to allow the diffusion of carbon dioxide gas from the air for photosynthesis. Transpiration also cools plants and enables mass flow of mineral nutrients from roots to shoots (Bilger *et al.*, 1984).

The rate of transpiration is directly related to the degree of stomatal opening, and to the evaporative demand of the atmosphere surrounding the leaf. Deficiency of elements can influence stomata opening, and thus transpiration rate (Fracheboud, 1999). The amount of transpiration by a plant depends on the number and size of leaves, leaf areas, and plant's roots. Transpiration rate of plants can be measured by a

number of techniques, including potometers, lysimeters, porometers, and heat balance sap flow gauges (Demmig *et al.*, 1996).

Transpiration rates in this study did not show any significant difference with control but salinity decreased it to some extent. This reduction can be attributing by low number of leaves and low sizes of roots and leaves in plants affecting by salt treatments. (Figure 5).

In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiological studies (Maxwell and Johnson, 2000). No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. Chlorophyll fluorescence allows us to study the different functional levels of photosynthesis indirectly (Bron *et al.*, 2004). Photosynthesis is often reduced in plants experiencing adverse conditions, such as temperature, nutrient deficiency and attack by pathogens (Godedheer, 1964).

Our data show that the addition of salts to the nutrient solution did not reduce Chlorophyll fluorescence namely salt stress did not affect chlorophyll fluorescence parameters. These findings were in agreement were Ranjbar et al, (1999) on pistachios.

Levitt (1980) has mentioned salt stress, exposes plant to physiological drought stress (osmotic dehydration) which is, analogous to freeze and evaporative dehydration's. There also is another similarity between the injurious effects of the osmotic to evaporative dehydration, and that is increasing of leaf RNase activity. In conclusion, the findings of this study showed that salt stress negatively impact pistachio growth and photosynthesis.

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