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Ecophysiological characteristics of two pistachio species (*Pistacia khinjuk* and *Pistacia mutica*) in response to salinity

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SUMMARY – The changes caused by NaCl and CaCl₂ induced salinity on several ecophysiological characteristics were measured in two pistachio species (*Pistacia khinjuk* and *P. mutica*). Both pistachio species are growing with almond, oak and other forest trees in different areas of Iran and they have been selected as resistant rootstocks for producing edible pistachio trees. Salinity was imposed, by adding salt (NaCl and CaCl₂) to the nutrient solution to obtain conductivity values of (EC_w) 6, 12 and 19 dS/m respectively. Salinity decreased leaf water potential (ψ), leaf osmotic potential (ψ_s), net photosynthesis (P_n), transpiration rate (E) and chlorophyll content in comparison with the control. Non-photochemical quenching (Q_n) increased by increasing salinity and was higher in *P. khinjuk* than in *P. mutica*. At all of the salinity levels studied, *P. mutica* showed fewer changes than *P. khinjuk*. Finally we found that *P. mutica* is more tolerant to salinity than *P. khinjuk*.

Key words: Pistachio, photosynthesis, transpiration, water status, chlorophyll content.

RESUME – "Caractéristiques écophysiologicals de deux espèces de pistachiers (*Pistacia khinjuk* et *Pistacia mutica*) en réponse à la salinité". Les changements causés par NaCl et CaCl₂ ont induit la salinité concernant plusieurs caractéristiques écophysiologicals qui ont été mesurées chez deux espèces de pistachiers (*Pistacia khinjuk* et *Pistacia mutica*). Ces deux espèces de pistachiers sont cultivées avec des amandiers, des chênes et autres arbres forestiers dans différentes zones d'Iran et elles ont été sélectionnées comme porte-greffes résistants pour des arbres produisant des pistaches consommables. La salinité a été imposée en ajoutant du sel (NaCl et CaCl₂) à la solution de nutriments pour obtenir des valeurs de conductivité (EC_w) de 6, 12 et 19 dS/m respectivement. La salinité a fait baisser le potentiel hydrique des feuilles (ψ), le potentiel osmotique des feuilles (ψ_s), la photosynthèse nette (P_n), le taux de transpiration (E) et la teneur en chlorophylle en comparaison avec le témoin. Le quenching non photochimique (Q_n) a augmenté en élevant la salinité et était plus élevé chez *P. khinjuk* que chez *P. mutica*. A tous les niveaux de salinité étudiés, *P. mutica* a montré moins de changements que *P. khinjuk*. Finalement nous avons trouvé que *P. mutica* était plus tolérante à la salinité que *P. khinjuk*.

Mots-clés : Pistachier, photosynthèse, transpiration, état hydrique, teneur en chlorophylle.

Introduction

Mastic and khonjok pistachio trees (*Pistacia mutica* and *P. khinjuk*) are growing together with almond, oak and other forest trees in alpine areas, foothills and at different altitudes between 600-3000 m in Iran. These species may form forest stands or can be found in solitary stands (Zohary, 1952; Sheibani, 1995). According to Mobayen and Tregubove (1970), the areas where these species occur have rainfall between 100-600 mm per year.

Mastic pistachio (*Pistacia mutica*) has been selected as a stock resistant to root-knot nematodes for edible pistachio tree (*P. vera*) in many parts of Iran (Sheibani, 1987). Khonjok pistachio (*P. khinjuk*) is another important and well-known species in pistachio nut production as it is also used as a rootstock (Dahab *et al.*, 1975).

Salinity is an important problem to crop production in many parts of the world, especially in irrigated fields of arid and semiarid regions. Saline soils contain sufficient soluble salts to suppress plant growth through a series of interacting factors such as osmotic potential effect, ion toxicity and antagonism, which induce nutrient imbalances (Sepaskhah and Maftoun, 1988; Neumann, 1997). 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 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).

Najmabadi (1969) stated pistachio can grow on land too saline for other crops, but Parsa and Karimian (1975) have shown that salt adversely affects the aerial and root growth of *P. vera*. According to Behboudian *et al.* (1986a,b) photosynthesis rates in *P. vera* (with rootstock of *P. atlantica*) were decreased by gradually increasing Cl⁻ from 225 mM to 400 mM. In other species, salinity adversely affects some other aspects of plant metabolism. For instance, photochemical efficiency in *Spondias purpurea* L. (Marler and Mickelbart, 1993); stomatal conductance and photon yield of O₂ evolution (Hill reaction) in cotton (*Gossypium hirsutum* L.) (Enrico and Macro, 1991); net photosynthesis (P_n), stomatal conductance (g_s) and transpiration rate (E) in mulberry (*Morus alba* L.) (Lakshmi *et al.*, 1996) and transpiration rate in bald cypress (*Taxodium distichum* L.) (Javanshir and Ewell, 1992).

The aim of the present study was to evaluate the ecophysiological characteristics (net photosynthesis and transpiration rate, chlorophyll fluorescence, water status and chlorophyll content) responses of *P. khinjuk* and *P. mutica* to salinity and to find which of the two is more tolerant in saline conditions.

Material and methods

The present study was carried out during the spring and summer seasons of 1999 in the greenhouse of the laboratory of Tropical and Subtropical Agronomy and Ethnobotany and within the Laboratory of Plant Ecology located at the campus of the Faculty of Agriculture and Applied Biological Sciences (University of Ghent, Belgium). Seeds of *P. khinjuk* and *P. mutica* were taken from the Natural Resources Researches Institute of Charmahal-Bakhtiary (Zagros), Iran. In order to remove the hard outer shell, seeds are scarified by sulphuric acid, according to the protocol of Sheikh (1979). After scarification, seeds were sown in pots (5 litre) containing sand with organic materials (10% washed sand and 90% peat with the diameter of less than 0.5 mm). Seedlings were grown in the greenhouse under environmental conditions of 26°C (±3°C), RH 65% (±10) and saturated light (without additional artificial lightning). After 21 months (from 10th May 1997 till 10th February 1999), plants were transplanted on 11 February 1999 to new bed material (vermiculite) with the least root disturbance. They were irrigated using a circulating system consisting of a reservoir, pump and gutter (pumping systems had been installed at one side of the gutters and irrigating solution returned spontaneously to the reservoir from the other side). The treatments were made on two pistachio species and four levels of salinity (totally eight treatments). Saline treatments began when the plants were 23 months old. The concentration of salt in irrigating water increased by steps of 20 mM/l (NaCl + CaCl₂, 82% and 18% respectively) every other day until final concentration was reached. The final electrical conductivity for each salinity treatment was as follows: 0.8 dS/m (control), 6 ± 0.4 dS/m, 12 ± 5 dS/m, and 19 ± 5 dS/m. A modified Hogland nutrient solution (0.5-strength) no. 1 with the trace elements at the 0.5-strength concentration was applied. The analysis of variance (ANOVA) was done using Duncan's method for randomised complete block design (RCBD) with pistachio species (*P. khinjuk* and *P. mutica*) as the main plots and salt solutions as sub-plots, replicated four times.

Net photosynthesis and transpiration rates

Measurements were done on the fourth leaf (from the top) of *P. khinjuk* and *P. mutica* using a CO₂ and H₂O gas exchange apparatus (Porometer CQP130, H. Walz GmbH, Effeltrich, Germany) provided with a gas analyser (BINOS 100/4P, Walz), 3 leaf cuvettes (PMK-10, Walz), a measuring gas cooler (MGK1, Walz), a temperature controller (MGE 130, Walz) and humidity compensator (HC130, Walz). Three test plants from each treatment were put in the three cuvettes simultaneously. Net photosynthesis and transpiration rates were synchronously measured at different light intensities.

For calculating maximal photosynthesis rate P_n (max), maximal light conditions (here: 500 mol/m²/s) were used. By use of black plastic, plants in the cuvette were put in complete darkness and dark respiration (R_d) was measured. On the basis of these data (maximum net photosynthesis and dark respiration), the typical light response curve was drawn according to Peat (1970)

method. The maximal slope of the curve was shown the quantum light efficiency (ϕ_c). Light compensation rate (I_c) was determined at the point the net photosynthetic rate is equal to zero. Net photosynthetic rate was calculated for each plant by use of the following equation (see Thornly, 1976) and with respect to data, received from the light response curve.

$$P_n = \frac{P_n(\max) (I - I_c) \phi_c}{P_n(\max) + (I - I_c) \phi_c} \quad [1]$$

where:

P_n = net photosynthesis rate (mol CO₂/m²/s)
 $P_n(\max)$ = maximal net photosynthesis rate (mol CO₂/m²/s)
 I = light intensity (mol photon/m²/s)
 I_c = light compensation point (mol photon/m²/s)
 ϕ_c = quantum efficiency (mol CO₂/ mol photon)
 R_d = dark respiration (mol CO₂/m²/s).

Transpiration rates were measured with the differential infrared H₂O gas analyser set up in the phytotron on the same leaf as used for photosynthesis measurements.

Chlorophyll fluorescence

Chlorophyll fluorescence measurements were done four times on each plant from each treatment, 15 days after initiation of treatments, by use of portable PAM-fluorometer (Pulse Amplitude Modulation) (H. Walz GmbH, Germany) and software DA-2000. Fluorescence parameters were measured on four plants (three same leaf samples as used in phytotron and one more) of each treatment after a dark adaptation period (at least 30 min). Ground fluorescence (F_0), maximum fluorescence (F_m), maximum fluorescence/variable fluorescence (F_m/F_v), photochemical quenching (Q_p) and non-photochemical were recorded on the adaxial surface of the fourth leaf from the top.

Total leaf water potential (ψ) and leaf osmotic potential (ψ_s)

Leaf water and osmotic potentials were measured using the leaves used for gas exchange determinations. Two leaf disks (0.25 cm²) were punched in the morning from each plant. One leaf disk was placed in three (individually) Wescor C-25-SF chambers and allowed to equilibrate for at least 90 min. Water potential was determined using thermocouple psychometric (Wet Bulb) method (see Spanner, 1951). The second disk from each plant was wrapped in aluminium foil and was frozen (-18°C) at least 25 days to collapse cell wall and semi-permeable membranes. The osmotic potential of each disk was determined using the same method used for leaf water potential determination.

Chlorophyll content

The fifth leaf from each plant (the nearest leaf to the leaf which, employed for gas-exchange determination) was collected at the end of greenhouse experiments. The leaves were wrapped in aluminium foil to avoid degradation of pigments by light. The half-gram samples were taken from the leaves. Then they were pulverised with liquid nitrogen. After that 0.25 g of each pulverised sample was extracted by 80% acetone and put in the freezer for 24 hours. Pigments were determined using the Lichtenthaler (1987) method in the Laboratory of Horticulture (Faculty of Agricultural and Applied Biological Sciences, University of Ghent) with spectrophotometer (Uvikon 930). The amount of chlorophyll a (C_a) and chlorophyll b (C_b) were determined according to following equations:

$$C_a = 12.21 A_{663} - 2.81 A_{646} \quad [2]$$

$$C_b = 20.13 A_{646} - 5.03 A_{663} \quad [3]$$

Results

Effects of salinity on photosynthetic rates

According to microscopic observations, both pistachio species are amphistomatous and photosynthetic rates were measured at 500 ± 15 $\text{mol/m}^2/\text{s}$ photon flux density (PAR). Exposure of both pistachio species to salinity led to a significant reduction in leaf P_n at most of the concentrations studied. In *P. khinjuk*, P_n rates increased by increasing salinity up to 6 dS/m and then it decreased at conductivity values of 12 and 19 dS/m. The inhibitory effect of increasing salt concentrations was also seen in *P. mutica*. It was shown a continuous reduction in P_n with increasing salinity. In *P. mutica*, maximum P_n was observed at control (0.8 dS/m) whereas the lowest rates were seen at the highest salinity level (19 dS/m). The effect of salinity levels on P_n in *P. khinjuk* and *P. mutica* are shown in Fig. 1.

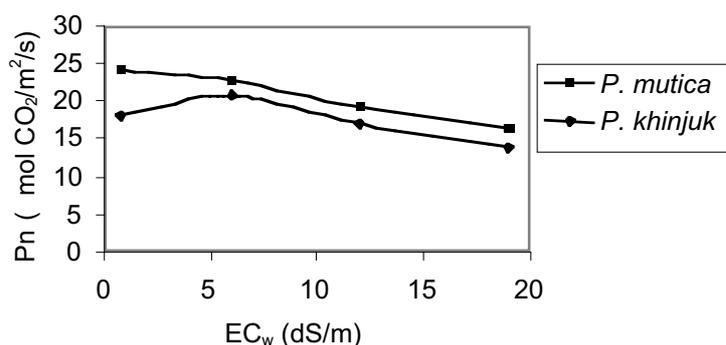


Fig. 1. The effect of increasing salinity on the net photosynthesis rates of *P. khinjuk* and *P. mutica*.

Effects of salinity on transpiration rate (E)

Analysis of variance did not show any significant difference for transpiration rate at different levels of salinity, but differences were highly significant between both pistachio species. Totally, the rate of transpiration was higher in *P. mutica* than in *P. khinjuk*. The trend of transpiration reduction in *P. mutica* was gentler than in *P. khinjuk*. The highest transpiration rate in *P. mutica* was observed at control level, while the highest rate for *P. khinjuk* was seen at 6 dS/m. The lowest transpiration rates were observed at highest level of salinity for both species. Figure 2 shows the effects of increasing salinity on transpiration rate of *P. khinjuk* and *P. mutica*.

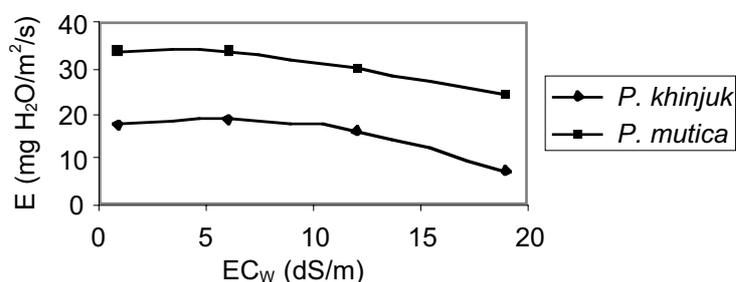


Fig. 2. The effect of increasing salinity on the transpiration rates of *P. khinjuk* and *P. mutica*.

Effects of salinity on fluorescence emission

Chlorophyll fluorescence parameters are considered as indicators of damage to light harvesting system in the chloroplasts. Analysis of variance indicated that the different saline

solutions had no significant effects on none of the chlorophyll fluorescence parameters (changes are insignificant) except on non-photochemical quenching (Q_n). This parameter showed significant differences for both factors of plant species and salinity level. The increasing trend of Q_n in *P. mutica* was gentler than in *P. khinjuk*. Both species showed maximal Q_n at highest level of salinity and the minimum at control level. In *P. mutica*, Q_n changes did not occur until 12 dS/m saline concentration. Figure 3 shows the effect of salinity on Q_n .

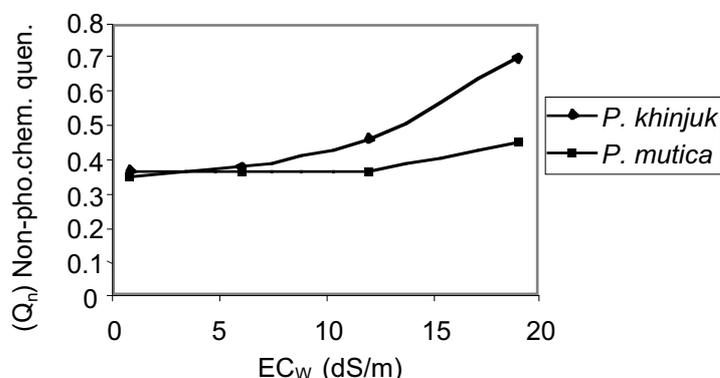


Fig. 3. The effects of different salt concentrations on non photochemical quenching in *P. khinjuk* and *P. Mutica*.

Effect of salinity stress on leaf water and osmotic potentials (ψ and ψ_s)

Analysis of variance showed highly significant changes in leaf water (ψ) and osmotic (ψ_s) potentials in plants subjected to different levels of salinity. In *P. mutica* both, leaf water and osmotic potentials (ψ and ψ_s), exhibited gentle reduction trends. In *P. khinjuk*, increased in leaf water potential was found at 12 dS/m (Fig. 4a). In both species, maximum negative potentials were seen at the highest salinity level (19 dS/m) whereas the lowest rates were observed at control level (0.8 dS/m). Generally, *P. mutica* exhibited more negative leaf water and osmotic potentials than *P. khinjuk*. Figure 4 (a and b) shows the effects of salinity on leaf water and osmotic potentials of both species.

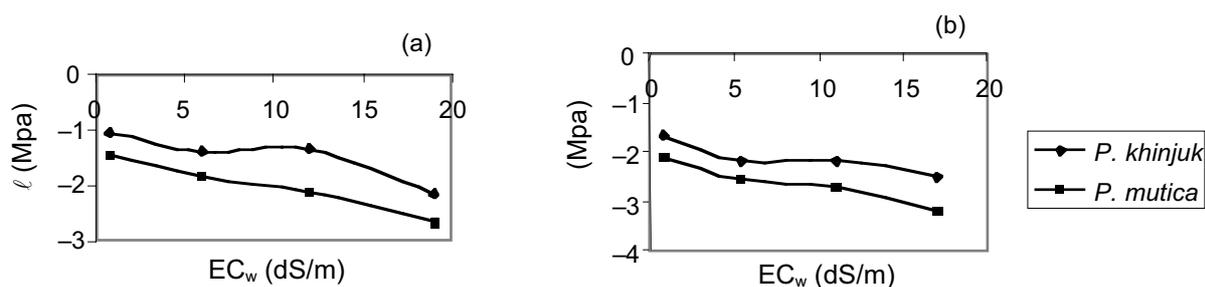


Fig. 4. Effect of increasing salt concentration on leaf water potential (a) and leaf osmotic potential (b) in *P. khinjuk* and *P. mutica*.

Effects of increasing salinity on chlorophyll content

Analysis of variance showed significant reduction of chlorophyll a (C_a) content with salinity, whereas it was not significant for the species. C_a quantity was higher in *P. mutica* than in *P. khinjuk*. Both species showed maximum and minimum C_a rates at control and 19 dS/m levels respectively (Fig. 5a). The value of total chlorophyll ($C_a + C_b$) was significantly decreased with salinity in both species. This value was higher in *P. khinjuk* up to 12 dS/m. The trend of reduction was more gradual in *P. mutica* than *P. khinjuk*. The maximum values of ($C_a + C_b$) were observed at

control and 6 dS/m levels for *P. mutica* and *P. khinjuk*, respectively (Fig. 5b). The mean C_a/C_b ratios were not significant at different salinity levels, but were different with species. The trend of reduction in C_a/C_b was more gradual in both species. Generally, the C_a/C_b ratio was higher in *P. mutica* than *P. khinjuk* (Fig. 5c).

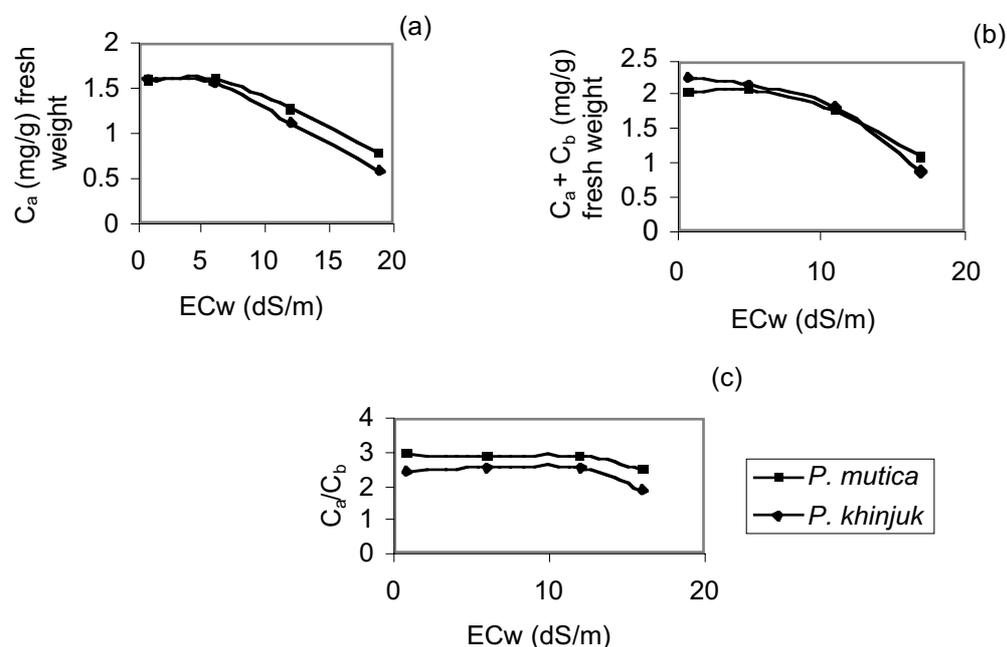


Fig. 5. The effects of different salinity levels on C_a (5a), total chlorophyll content ($C_a + C_b$) (5b) and C_a/C_b ratio (5c).

Discussion

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, stomatal conductance or the mesophyll capacity to CO_2 assimilation (Farquhar and Sharkey, 1982). It has been demonstrated that both Na and Cl can reduce the mesophyll capacity to assimilate CO_2 (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). Our data show that the addition of salts ($NaCl + CaCl_2$) to the nutrient solution reduced P_n and transpiration (E) rates in both pistachio species studied here. This effect was highest in *P. khinjuk* and lowest in *P. mutica*. Lower leaf water and osmotic potentials in latter species may explain relatively better maintenance of P_n under increased salinity in *P. mutica*. These results are in agreement with those reported by Behboudian *et al.* (1986b) on nut pistachio (*P. vera* L. cv. kerman), Janvanshir and Ewell (1992) on bald cypress (*Taxodium distichum*), Lakshmi *et al.* (1996) on two cultivars of mulberry (*Morus alba* cvs S30 and K2), Romero-Aranda and Syvertsen (1996), on grapefruit (*Citrus paradisi* Macf.) and Valencia orange (*Citrus sinensis* L.), Wang *et al.* (1997) on salt bush (*Atriplex prostrata*) and Chaudhuri and Chaudhuri (1998) on two jute species (*Corchorus capsularis* and *C. olitorius*). The relatively high salt tolerance of *P. mutica* was evident from a smaller reduction in P_n and transpiration rates than *P. khinjuk*.

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. Also the duration, during which plant, are exposed to stress, affects the type of damage (osmotic effect or ion accumulation). For instance, Rana and Annie (1986) found that short-term effect of NaCl on leaf elongation in wheat arise from osmotic effects. 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.

Salinity in the nutrient solution decreased total (ℓ) and osmotic () potentials in both pistachio species. But the better was found with *P. mutica* and so that it would seem this species is more salt tolerant than *P. khinjuk*, because osmotic adjustment in *P. mutica* is better than in *P. khinjuk*. Our findings on both pistachio species are in agreement with findings by Behboudian *et al.* (1986b) on *P. vera*, Ranjith and Meinzer (1997) on sugarcane, Fernandez-Ballester *et al.* (1998) on sour orange (*Citrus aurantium* L.) who mentioned that osmotic potential of leaves decreased with increasing salt concentration in nutrient solution.

When there is 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. For instance Lichtenthaler (1988) mentioned, stress decreases variable fluorescence of dark-adapted leaves, increases non-photochemical quenching (Q_n). Also, he stated the height of ground fluorescence (F_0) depends on chlorophyll content of the leaf. 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).

Results of the present study show reduction in C_a and total chlorophyll ($C_a + C_b$) with salt-stressed pistachio species. The positively reduction of these characteristics was greater in *P. khinjuk* than in *P. mutica* and it attributed to C_a , which is more sensitive to salinity than C_b (Reddy and Vora, 1986). The reduction in chlorophyll concentration of salinized plants could be also attributed to the increased activity of chlorophyll degrading enzyme chlorophyllase (Rao and Rao, 1981). Our results are in agreement with findings by Joshi and Nimbalkar (1984) on *Cajanus cajan*, and Singh and Dubey (1995) on rice (*Oriza sativa*). The C_a/C_b ratios were relatively stable at all levels of salt concentration in *P. mutica* whereas this characteristic showed fluctuations in *P. khinjuk*. Increasing of non-photochemical quenching (Q_n) in *P. khinjuk* was higher than in *P. mutica* at all salinity levels and it similar with findings of Lichtenthaler (1988) on spruce needles (*Picea omorika*). With respect to the results which were discussed in this study, we found that *P. mutica* is more tolerant than *P. khinjuk* for salinized environments.

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