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Effects of osmotic drought stress induced by a combination of NaCl and polyethylene glycol on leaf water status, photosynthetic gas exchange, and water use efficiency of *Pistacia khinjuk* and *P. mutica*

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Abstract

Leaf water potential, leaf osmotic potential, chlorophyll *a* and *b* contents, stomatal conductance, net photosynthetic rate, and water use efficiency were determined in two pistachio species (*Pistacia khinjuk* L. and *P. mutica* L.) grown under osmotic drought stress induced by a combination of NaCl and polyethylene glycol 6000. A decrease in values for all mentioned variables was observed as the osmotic potential of the nutrient solution (Ψ_s) decreased. The osmotic adjustment ($\Delta\Psi_\pi$) of the species increased by decreasing Ψ_s. Thus *P. khinjuk* had a higher osmotic drought stress tolerance than *P. mutica*.

Additional key words: chlorophyll content; leaf osmotic potential; net photosynthetic rate; osmotic adjustment; stomatal conductance.

Introduction

Pistachio (*Pistacia vera*) is a major orchard crop in Iran, Turkey, and recently the USA. Crop reliance on seedling rootstocks has improved pistachio tree growth and nut production (Picchioni and Miyamoto 1990, Abrishami 1995). Khinjuk and mastic species (*P. khinjuk* L. and *P. mutica* L.) are used as drought resistant rootstocks (Sheibani 1995).

High temperature, high irradiance, low vapour pressure, and lack of precipitation in most months characterise arid and semi-arid ecosystems (Kardavani 1990). In these ecosystems, soils are mostly saline because of large and rapid evaporation of subsoil water (Ashraf and O'Leary 1996). Under these conditions, tree crops such as pistachio often suffer from drought combined with salinity stress. The first eco-physiological reaction of the plants, in such hostile environments, is a reduction in photosynthetic activity, which has been reported by numerous researchers such as Deidda *et al.* (1990), Stewart and Bernier (1995), and Sean *et al.* (1998). Leidi *et al.* (1993) reported that transpiration rate (E) and stomatal conductance (g_s) in cotton were decreased as osmotic potential of soil decreased.

Some studies have been carried out on the effects of drought and salt stress on some ecophysiological parameters of pistachio tree (Behboudian *et al.* 1986, Rieger 1995, Ranjbarfordoei *et al.* 1999, 2000), but stresses were usually induced separately. Less is known about the effects of a combined drought and salt stress on ecophysiological behaviour of pistachio. Polyethylene glycol (PEG) solutions are often used to induce drought stress in higher plants (Nepomuceno *et al.* 1998). PEG with molecular mass of 6000 is a non-ionic, water-soluble polymer, which is not expected to penetrate intact plant tissues rapidly (Chazen *et al.* 1995).

The first objective of the present study was to evaluate the effects of osmotic drought stress, induced by a combination of salt (NaCl) and PEG 6000, on some ecophysiological parameters of two pistachio species (*P. khinjuk* and *P. mutica*), leaf water (Ψ_l) and osmotic (Ψ_π) potentials, osmotic adjustment ($\Delta\Psi_\pi$), chlorophyll content (Chl *a* and Chl *b*), g_s, and net photosynthetic rate (P_N). The second objective was to compare both species with respect to their osmotic stress tolerance.

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Abbreviations: Chl – chlorophyll; E – transpiration rate; FM – fresh mass; g_s – stomatal conductance; P_N – net photosynthetic rate; ODS – osmotic drought stress; PAR – photosynthetically active radiation; PEG – polyethylene glycol; WUE – water use efficiency (P_N/E); Ψ_l – leaf water potential; Ψ_p – leaf pressure potential; Ψ_π – leaf osmotic potential; $\Delta\Psi_\pi$ – osmotic adjustment; Ψ_s – osmotic potential of the nutrient solution.

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Materials and methods

Plants: Seeds of *Pistacia khinjuk* L. and *P. mutica* L. were obtained from the Natural Resources Research Institute of Charmahal-Bakhtiary, Iran. The seed outer shells were scarified by sulphuric acid following Mahmood (1979). Seeds were then sown in pots containing sand and peat. Seedlings were grown in the greenhouse at 27±5 °C, relative humidity of 65±10 %, and normal irradiance for 21 months. Thereafter, plants were transplanted to vermiculite with minimal root disturbance. The plants were simultaneously irrigated using a circulating system consisting of a water pump, gutter, and reservoir containing the nutrient solutions. Treatments were made on both pistachio species and four levels of osmotic drought stress induced by a combination of PEG 6000 and NaCl. Both these osmogenic agents contributed to the same amount to the osmotic potential of the nutrient solution. Osmotic stress levels were chosen with respect to the climatic and pedologic conditions of the areas to which the species are native (Dewan and Famouri 1964). Similar studies have been carried out by Nepomuceno *et al.* (1998). A preliminary experiment to compare PEG 6000 and PEG 10000 showed that the former one had no side effects on pistachio plants. In order to prevent adverse effect of PEG 6000 on oxygen availability to the plant roots (Plaut and Federman 1985), the nutrient solutions were continuously aerated. The concentration of PEG 6000 [g kg^{-1}(water)] for each level of drought was determined using the equation of Burlyn and Merrill (1973).

Osmotic potential of the nutrient solution (Ψ_s) was decreased by –0.14 MPa per day until final potential was reached. The final osmotic potential for each osmotic drought stress (ODS) treatment was –0.1 MPa (control), –0.42, –0.81, and –1.40 MPa; these values were maintained for three weeks. The plants were fertilised by adding a modified (50 %) Hoagland nutrient solution (see Picchioni and Miyamoto 1990). The volume and electrical conductivity (EC) of each treatment solution in the container were controlled daily and kept constant with the addition of tap water. A total of 32 pistachio plants were used in this experiment.

Methods: Leaf water and osmotic potentials [MPa] were measured, three weeks after reaching the final stress levels, on the fourth leaf from the top of *P. khinjuk* and *P. mutica*. From each plant (stressed and non-stressed), two leaf disks (0.25 cm^2) were punched early in the morning. Leaf water potential was directly determined using a Peltier thermocouple psychrometer (*Wescor*, Utah, USA) on one of the two leaf disks. The second leaf disk was wrapped in aluminium foil and frozen (–18 °C) in order to collapse the cell wall and the semi-permeable membranes. The leaf osmotic potential was then determined after thawing these leaf disks, also using the same thermocouple psychrometer.

At the end of the greenhouse experiments, a fully extended leaf from each plant was collected. These leaves were wrapped in aluminium foil to avoid degradation of pigments by light. Half-gram samples were taken from the collected leaves. These samples were then pulverised with liquid nitrogen. Subsequently, 0.25 g of each sample was extracted by 80 % acetone and put in the freezer at –5 °C for 24 h.

Pigment wavelengths were determined using a spectrophotometer (*Uvikon 930*, *Kontron Instruments*, UK). The amounts of Chl *a* and *b* [g kg^{-1}(FM)] were calculated according to Alan (1994). P_N [$\mu mol(CO_2)$ m^{-2} s^{-1}], *E* [$mmol(H_2O)$ m^{-2} s^{-1}], and g_s [$mmol(H_2O)$ m^{-2} s^{-1}] were measured on the same leaves as the leaf water potential using a CO_2 and H_2O gas exchange apparatus (differential infrared analysers *ADC 225 MK3*, *ADC*, Hoddesdon, England) at an irradiance of 500±20 μmol(PAR) m^{-2} s^{-1}, 60 % relative humidity, and 390-425 $\mu mol(CO_2)$ mol^{-1}. The above-mentioned parameters were calculated following Allen *et al.* (1994).

Results

Leaf water relations: In both species, leaf water potential decreased as the osmotic potential of the nutrient solution (Ψ_s) decreased. Significant differences were observed at moderate stress and were even more pronounced at severe stress (Fig. 1*A*). In *P. khinjuk*, control-compared decreases of 3, 14, and 32 % were observed for Ψ_s of –0.42, –0.81, and –1.40 MPa, respectively. Accordingly, these values for *P. mutica* were 3, 18, and 38 %, respectively. *P. khinjuk* had invariably a higher Ψ_l than *P. mutica*. A progressive decrease in Ψ_π was also observed with decreasing Ψ_s (Fig. 1*B*). Significant effects of Ψ_s on Ψ_π were observed at –0.81 and –1.40 MPa. No significant difference between Ψ_π of both species was observed. A constant pressure potential (Ψ_p) was observed for each species under control and stress conditions (values not shown). Osmotic adjustment ($\Delta\Psi_\pi$) increased with decreasing Ψ_s (Fig. 2). A higher $\Delta\Psi_\pi$ was observed for *P. khinjuk* compared to *P. mutica*.

Chl content: Exposure of both pistachio species to osmotic drought stress led to a lower Chl *a* content (Fig. 3). Significant differences in this variable for *P. khinjuk*, compared to the control, started at –0.81 MPa (–7.5 %), but were more pronounced at Ψ_s of –1.40 MPa (–11 %). In *P. mutica*, these reductions were 8.2 and 13 %, respectively. Chl *b* content was not significantly affected by decreasing Ψ_s. However, it showed a tendency to decrease by decreasing Ψ_s (Table 1). Analysis of variance showed that Chl *a* content was significantly higher in *P. mutica* than in *P. khinjuk* ($p < 0.05$; $n = 16$), 1.46 *versus* 1.38 g kg^{-1}(FM), while the concentration of Chl *b* was significantly higher in the latter ($p < 0.05$; $n = 16$), 0.57 *versus* 0.85 g kg^{-1}(FM).

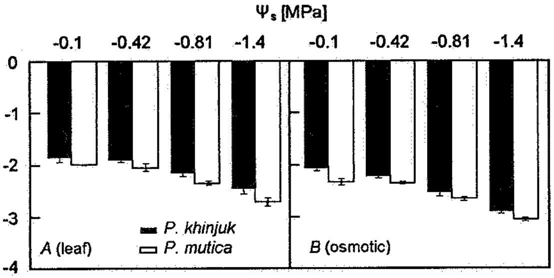

Fig. 1. Changes in (*A*) leaf water potential (Ψ_l) and (*B*) leaf osmotic potential (Ψ_π) in two pistachio species as a function of osmotic potential of the nutrient solution (Ψ_s) induced by a combination of NaCl and PEG 6000. Each bar shows the mean of four observations ± SE.

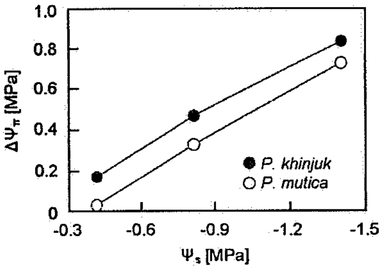

Fig. 2. Changes in leaf osmotic adjustment ($\Delta\Psi_\pi$) of two pistachio species as a function of decreasing osmotic potential of the nutrient (Ψ_s) solution induced by a combination of NaCl and PEG 6000.

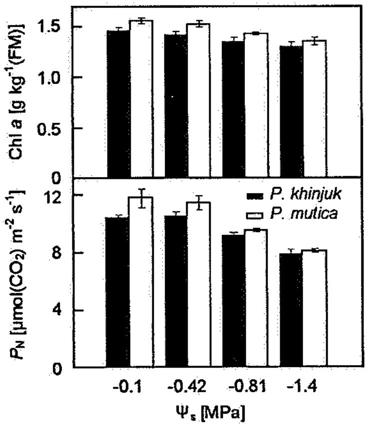

Fig. 3. Chlorophyll (Chl) *a* content (*upper*) and net photosynthetic rate, P_N (*lower*) of two pistachio species as a function of osmotic potential of the nutrient solution (Ψ_s) induced by a combination of NaCl and PEG 6000. Each bar represents a mean of four observations ± SE.

P_N, g_s, and water use efficiency (WUE): For both pistachio species osmotic drought stress treatments had a significant inhibitory effect on P_N at Ψ_s –0.81 and –1.40 MPa, while P_N differed not significantly ($p < 0.05$; $n = 16$) between the two species. Compared to the control, *P. khinjuk* revealed less reduction in P_N at Ψ_s of –0.81 and –1.40 MPa (11 and 18 %, respectively) than *P. mutica* (24 and 30 %, respectively) (Fig. 3).

Significant differences were found for g_s with increasing osmotic drought stress. When Ψ_s decreased to –0.81 MPa, g_s dropped significantly in both species, and continued to decrease at Ψ_s of –1.40 MPa (Fig. 4). In this case, *P. khinjuk* with a reduction in g_s of 22 and 41 % at Ψ_s of –0.81 and –1.40 MPa, respectively, showed a higher tolerance against the stress agents than *P. mutica.* The latter species had 27 and 56 % reduction in g_s at the mentioned Ψ_s levels. Analysis of variance revealed also that g_s differed significantly between the species ($p < 0.05$; $n = 16$). For all Ψ_s levels, g_s of *P. mutica* was higher than that of *P. khinjuk* (Fig. 4).

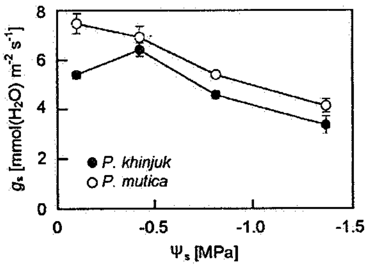

Fig. 4. Stomatal conductance (g_s) of *P. khinjuk* and *P. mutica* as a function of osmotic potential of the nutrient solution (Ψ_s) induced by a combination of NaCl and PEG 6000. Each point represents the mean of four observations ± SE.

Table 1. Effects of decreasing osmotic potential of the nutrient solution, Ψ_s [MPa] on Chl *b* [g kg^{-1}(FM)] and water use efficiency, WUE [μmol(CO$_2$) mmol^{-1}(H$_2$O)] in *P. khinjuk* and *P. mutica* at control (–0.10 MPa) and three levels of osmotic drought stress. Different letters express significantly different results between osmotic drought stress levels in the same species (a, b, c) or between the species with the same osmotic drought stress level (r, s). Means ± SE ($n = 4$).

Species	Treatment	Chl *b*	WUE
P. khinjuk	–0.10	0.95±0.05a/r	22.00±1.23a/r
	–0.42	0.90±0.03a/r	22.43±0.14a/r
	–0.81	0.81±0.01a/r	23.40±3.30a/r
	–1.40	0.73±0.02a/r	20.52±1.17a/r
P. mutica	–0.10	0.64±0.04a/s	20.01±2.23a/s
	–0.42	0.59±0.08a/s	19.80±1.22a/s
	–0.81	0.55±0.01a/s	19.29±1.24a/s
	–1.40	0.50±0.01a/s	18.63±0.67a/s

WUE was calculated as the ratio of P_N [μmol(CO_2) m^{-2} s^{-1}] over E [mmol(H_2O) m^{-2} s^{-1}]. Significant differences in WUE between control and stress treatments were not observed, while the parameter was significantly different between species even when plants experienced drought stress. We found that *P. khinjuk* had a significantly higher WUE than *P. mutica* ($p < 0.05$; $n = 16$) (Table 1).

Discussion

In this study we investigated the effect of osmotic stress induced by a combination of salt and PEG 6000 on the ecophysiological performance of *P. khinjuk* and *P. mutica*. By decreasing Ψ_s, significant reduction of both Ψ_l and Ψ_π was observed at Ψ_s of –0.81 and –1.40 MPa (Fig. 1). Accumulation of osmolytes (Nepomuceno *et al.* 1998) could cause these alterations. In contrast to osmotic drought stress induced by solely salt (Ranjbarfordoei *et al.* 1999) and PEG 6000 (Ranjbarfordoei *et al.* 2000), no significant difference between the species was observed in both Ψ_l and Ψ_π, for the osmotic drought stress induced by a combination of NaCl and PEG 6000. So the inhibitory effect of osmotic drought stress induced by a combination of salt and PEG 6000 is lower than the separately induced stress. Chazen *et al.* (1995) came to the same conclusion with maize (*Zea mays*). The osmotic adjustment of both studied species increased with increasing Ψ_s (Fig. 2) which can be attributed to the accumulation of osmotically active solutes under osmotic stress conditions (Ashraf and Karim 1991). *Pistacia khinjuk* had the highest $\Delta\Psi_\pi$, which shows larger resistance of this species to drought osmotic stress than *P. mutica*. Similar results were reported by Weng (1993) on *Miscanthus* spp. and by Arnau *et al.* (1997) on barley (*Hordeum vulgare*). With respect to previous studies (Ranjbarfordoei *et al.* 1999, 2000) it can be concluded that *P. khinjuk* has a selective capacity for osmotic adjustment. Namely, when *P. khinjuk* was exposed to drought stress, only induced by PEG 6000, it showed a lower $\Delta\Psi_\pi$ than when it was exposed to salt stress (Ranjbarfordoei *et al.* 1999) or a combination of NaCl and PEG 6000 stress. Thus, $\Delta\Psi_\pi$ of *P. khinjuk* changes with the type and severity of stress.

At the lowest Ψ_l, –2.9 and –3.1 MPa for *P. khinjuk* and *P. mutica*, respectively (Fig. 1), a Ψ_p of approximately 0.38 MPa was maintained. However, in many fruit tree species a decreasing Ψ_l is accompanied with a loss of Ψ_p. For example, Sean *et al.* (1998) showed that ber (*Ziziphus mauritiana*) leaves reached zero Ψ_p at a Ψ_l of –2.3 to –2.5 MPa. Therefore maintaining Ψ_p in the studied species could be an effective mechanism in tolerating drought stress.

We found that decreasing Ψ_s at –0.81 and –1.40 MPa negatively influenced g_s of both pistachio species. This decrease in g_s was synchronous with the decrease in Ψ_l and Ψ_π. The synchronised alterations show that leaf water status could influence stomatal closure and thus g_s (Glenn *et al.* 1993, Lambers *et al.* 1998). These results are consistent with the hypothesis that stomata operate to maintain Ψ_l above a minimum threshold.

Both species showed a relatively high correlation between Chl *a* content and P_N ($r = 0.73$ and 0.82 for *P. khinjuk* and *P. mutica*, respectively). These *r*-values illustrate that the decrease in P_N is largely explained by the decrease in Chl *a*. However, a part of the decrease in P_N can be explained by the reduction in g_s. Thus P_N is controlled by both stomatal and non-stomatal factors. The decrease in Chl *a* at decreasing Ψ_s can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially to salinity and drought stress, which has been reported by several researchers. For instance, Singh and Dubey (1995) stated that salinity stress caused destruction of Chl *a* in rice (*Oryza sativa*) and Jagtap *et al.* (1998) mentioned that drought stress degraded Chl *a* in *Sorghum bicolor*.

WUE in both species did not significantly change at increased stress, which demonstrates that alterations in E and P_N are parallel. *P. khinjuk* had a significantly higher WUE than *P. mutica*. The conservative character of WUE under stress for both pistachio species agrees with the findings on *Atriplex canescens* (Glenn *et al.* 1993) and sunflower (Ashraf and O'Leary 1996) which can indicate that WUE is a constant value for each species.

Despite the high Chl *a* content in *P. mutica*, this species showed a greater reduction of this parameter at increasing osmotic drought stress, compared to *P. khinjuk*. The higher resistance to osmotic drought stress, induced by a combination of NaCl and PEG 6000, of *P. khinjuk* compared to *P. mutica* was evident from the lower reduction in P_N, g_s, Chl *a*, Ψ_l, Ψ_π, and the higher $\Delta\Psi_\pi$ at the various stress levels.

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