Effects of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia kharjuk* and *P. mutica*

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Abstract

The effects of drought stress induced by polyethylene glycol, PEG (molecular mass 6000) on some ecophysiological characteristics of two wild pistachio species, Mastic and Khinjuk (*P. mutica* and *P. kharjuk*) selected as rootstocks for production of edible pistachio trees (*P. vera*) in Iran and Turkey, were studied. Net photosynthetic rate (*P*ₙ), stomatal conductance (*gₙ*), chlorophyll (Chl) fluorescence parameters, leaf water potential (*Ψₕ*), leaf osmotic potential (*Ψₒ*), leaf osmotic adjustment (ΔΨₒ), and Chl a and b were measured. All parameters were influenced by increase in concentration of PEG in the nutrient solutions. *P*ₙ, *gₙ*, and Chl a were significantly higher in *P. mutica* than in *P. kharjuk* but, compared to the control treatment, *P. kharjuk* showed a higher resistance to drought stress than *P. mutica*.

Additional key words: chlorophyll content; net photosynthetic rate; osmotic adjustment; stomatal conductance; water use efficiency.

Introduction

Mastic and khinjuk pistachio (*Pistacia mutica* L. and *P. kharjuk* L.) are two wild pistachio species which are growing together with almond, oak, and other forest trees at altitudes between 600-3 000 m (Sheibani 1995). They are distributed in Iran, Turkey, Syria, northern Iraq, and southern Jordan (Zohary 1995). *P. mutica* has been selected as a rootstock for edible pistachio trees (*P. vera*) in many parts of Iran (Sheibani 1987). *P. kharjuk* is another well-known species in pistachio nut production and it is also used as a rootstock (Dahab et al. 1975).

Polyethylene glycols (PEG) with molecular mass of 6000 and above are non-ionic, water-soluble polymers which are not expected to penetrate intact plant tissues rapidly (Chazen et al. 1995). PEG solutions are often used to induce water stress in higher plants (e.g., Leidi 1993, El-Shahaby 1994, Ashraf and O'Leary 1996, Nepomuceno et al. 1998). Dodd and Davies (1996) reported the inhibitory effects of PEG on barley (*Hordeum vulgare* L.) growth through high accumulation of abscisic acid. Johnson et al. (1996) mentioned that PEG-generated osmotic stress (-1.6 to -2.0 MPa) significantly reduced cell turgor pressure in *Closphorpermum mopane* (Fabaceae). Drought stress generated by polyethylene glycol induces significant reduction in *P*ₙ and turgor pressure in white spruce (*Picea glauca*) seedlings (Jiang et al. 1995). Also Zhang and Kirkham (1995) stated that osmotic stress generated by PEG (-0.57 MPa) reduced photosynthetic rate, leaf water potential, osmotic potential and transpiration rate in sunflower (*Helianthus annuus* L.) plants.

The objectives of the present study were to determine if drought stress induced by PEG affects ecophysiological parameters in two pistachio species and if it does, to evaluate the influence of the stress on fluorescence emission, Chl content, *P*ₙ, *gₙ*, and water use efficiency. Also, both species will be compared in relation to their drought tolerance.

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**Abbreviations: Chl - chlorophyll; *E* - transpiration rate; *F₀* - minimal fluorescence yield; *Fₘ* - maximal fluorescence yield; *Fₖ* - variable fluorescence yield; *qₑ* - non-photochemical quenching; *qₑ* - photochemical quenching; *gₙ* - stomatal conductance; *Pₙ* - net photosynthetic rate; PAR - photosynthetically active radiation; PEG - polyethylene glycol; WUE - water use efficiency; *Ψₕ* - leaf water potential; *Ψₒ* - leaf osmotic potential; ΔΨₒ - osmotic adjustment; *Ψᵣ* - osmotic potential of the nutrient solution.

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Materials and methods

Plants: Seeds of *Pistacia khinjuk* and *P. mutica* were obtained from the Natural Resources Research Institute of Charamal-Bakhtiari, 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 65±10 %, and natural irradiance for 21 months. Plants were transplanted on 11 February 1999 to vermiculite with minimal root disturbance. They were simultaneously irrigated using a circulating system consisting of water-pump, gutter, and reservoir containing the nutrient solutions. Treatments made at three levels of drought stress (Ψ_ᵣ = -0.5, -1.0, and -1.6 MPa) induced by PEG 6000 were compared against a control (Ψ_ᵣ = -0.1 MPa). Drought stress levels were chosen with respect to the climatic conditions of the lands where the species are native and similar studies have been carried out, such as that by Nepomuceno et al. (1998). A preliminary experiment to compare PEG 6000 and PEG 10 000 showed that the former had no side effects on pistachio plants. In order to prevent adverse effect of PEG 6000 on oxygen availability to plant roots (Plaut and Federman 1985), the nutrient solutions were continuously aerated. The concentration of PEG 6000 [g kg⁻¹(water)] for each drought stress level was determined using the equation of Burlyn and Merrill (1973).

Osmotic potential of the nutrient solution was decreased by -0.15 MPa per day until final Ψᵣ was reached. The final Ψᵣ for each drought treatment was kept for three weeks. The plants were fertilised by adding a modified (50 %) Hoagland nutrient solution (see Picchioni and Miyamoto 1990). The volume of each treatment solution in the container was maintained daily with the addition of tap water. A total of 32 pistachio plants were used in this experiment.

Methods: Leaf water and osmotic potentials were measured three weeks after reaching the final stress treatments, on the fourth leaf from the top of plants. From each plant (stressed and non-stressed plants) two leaf disks (0.25 cm²) were punched early in the morning. Leaf water potential was directly determined using the thermocouple psychrometer (Spanner 1951) on one of the two leaf disks. The second group of leaf disks was wrapped in aluminium foil and was 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 thermocouple psychrometer.

Chl fluorescence parameters were measured four times within the experimental period (weeks 1, 2, 3, and 4 after reaching the final stress levels), using a portable PAM-fluorometer (*H. Walz*, Effeltrich, Germany) and *DA-2000* software. Fluorescence parameters were measured on four plants of each treatment after a dark adaptation of 30 min. Minimal fluorescence (F₀), maximal fluorescence (Fₘ), the ratio of variable fluorescence to maximal fluorescence (Fᵥ/Fₘ), photochemical quenching (qₑ), and non-photochemical quenching (qₑ) were recorded on the adaxial surface of the fourth leaf from the top.

At the end of the greenhouse experiments a fully extended leaf from each plant was collected. The leaves were wrapped in aluminium foil to avoid degradation of pigments by light. Half-gram samples were taken from the collected leaves. They 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. Pigments were determined according to Lichtenthaler (1987) by means of spectrophotometer analysis (*Unikon 930*).

F₀, E, and g, were measured according to Samson et al. (1997) on the same leaves as the leaf water potential using a CO₂ and H₂O gas exchange apparatus (*ADC 225 MK3*) at an irradiance of 500±20 μmol(PAR) m⁻² s⁻¹, 60 % relative humidity, 23 °C, and 390-425 μmol(CO₂) mol⁻¹.

Results

Leaf water potential (Ψᵣ) and leaf osmotic potential (Ψₑ): Both Ψᵣ and Ψₑ of the two species were significantly affected by Ψᵣ, indicating that PEG indeed induced drought stress on the plants. Overall, *P. mutica* showed more negative Ψᵣ and Ψₑ than *P. khinjuk*. No significant difference was found in Ψᵣ and Ψₑ at Ψᵣ higher than -0.5 MPa. Significant deviation from the control started at Ψᵣ = -1.0 MPa and increasingly continued at Ψᵣ = -1.6 MPa (Fig. 1A, B). The leaf pressure potential (Ψₑ) was maintained almost constant (0.35 and 0.43 MPa for *P. khinjuk* and *P. mutica*, respectively). The degree of osmotic adjustment (ΔΨₑ), calculated as the difference of Ψₑ between the stressed and control plants, for all stress levels increased by more negative Ψᵣ.

Chl fluorescence and Chl content: Exposure of pistachio plants to drought stress led to a significant effect in non-photochemical quenching (qₑ) as well as Chl a and b contents (Table 1). The study indicates an increasing and a decreasing trend for qₑ and Chl content, respec-
tively. Non-photochemical quenching showed a significant augmentation (1.21 times the control) only at \( \Psi_s = -1.6 \text{ MPa} \). The value of \( q_n \) in *P. klinjuk* was lower than that of *P. mutica* for all stress levels (Table 1). Other Chl fluorescence parameters were not affected.

Significant decrease in Chl a content started at \( \Psi_s = -1.0 \text{ MPa} \) and continued with further decrease of \( \Psi_s \) down to -1.6 MPa while a significant reduction in Chl b was only observed at \( \Psi_s \) of -1.6 MPa. *P. mutica* was characterised with higher Chl a concentration and *P. klinjuk* revealed higher Chl b content. The trend of reduction in Chl a and b in both species was parallel, which was reflected in the nearly constant Chl a/b ratio (Table 1). Analysis of variance confirmed a significant difference of Chl a/b between the species, with the higher value for *P. mutica*.

![Graph](image1.png)

**Fig. 1.** Leaf water potential (\( \Psi_l \)) (A), leaf osmotic potential (\( \Psi_s \)) (B), net photosynthetic rate (\( P_n \)) (C), and stomatal conductance (\( g_s \)) (D) in *P. klinjuk* and *P. mutica* at control (-0.1 MPa) and three levels of drought stress (-0.5, -1.0 and -1.6 MPa) induced by PEG 6000 (\( \Psi_s \)). Each bar represents the mean of four observations ± SE.

![Graph](image2.png)

**Fig. 2.** Osmotic adjustment (\( \Delta \Psi_s \)) at different drought stress (\( \Psi_s \) = -0.5, -1.0, and -1.6 MPa) (A), and (B) the relation between chlorophyll a (Chl a) content and net photosynthetic rate (\( P_n \)) in *P. klinjuk* and *P. mutica*. Regression models for *P. klinjuk* and *P. mutica* are \( P_n = 6.18 \text{ (Chl a) } - 0.40 \) and \( P_n = 4.87 \text{ (Chl a) } + 2.16 \), respectively.

**Table 1.** Chlorophyll a and b contents [g kg\(^{-1}\) (f.m.)], non-photochemical quenching (\( q_n \)), and water use efficiency, WUE [nmol(CO\(_2\)) mol\(^{-1}\)(H\(_2\)O)] in *P. klinjuk* and *P. mutica* at control (-0.1 MPa) and three drought stress levels (-0.5, -1.0, and -1.6 MPa) induced by PEG 6000. Means ± SE (n = 4). Different letters express significantly different results between drought stress levels (\( \Psi_s \)) in the same species (a, b, c) or between species with same drought stress level (r, s).

<table>
<thead>
<tr>
<th>Species</th>
<th>( \Psi_s ) [-MPa]</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Chl a/b</th>
<th>( q_n )</th>
<th>WUE</th>
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<tr>
<td><em>P. klinjuk</em></td>
<td>0.1</td>
<td>1.57±0.03a/r</td>
<td>0.75±0.04a/r</td>
<td>2.10±0.16a/r</td>
<td>0.248±0.010a/r</td>
<td>18.80±1.35a/r</td>
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<td></td>
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<td>1.58±0.03a/r</td>
<td>0.75±0.02a/r</td>
<td>2.10±0.03a/r</td>
<td>0.250±0.000a/r</td>
<td>19.66±1.27a/r</td>
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<td></td>
<td>1.0</td>
<td>1.37±0.01b/r</td>
<td>0.69±0.01b/r</td>
<td>2.10±0.06b/r</td>
<td>0.264±0.010a/r</td>
<td>18.34±0.59b/r</td>
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<tr>
<td></td>
<td>1.6</td>
<td>1.22±0.02c/rs</td>
<td>0.61±0.05b/r</td>
<td>2.00±0.14a/r</td>
<td>0.303±0.000b/r</td>
<td>18.01±0.99b/r</td>
</tr>
<tr>
<td><em>P. mutica</em></td>
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<td>1.73±0.03a/s</td>
<td>0.69±0.00a/s</td>
<td>2.50±0.07b/s</td>
<td>0.259±0.010a/r</td>
<td>18.01±0.26a/b</td>
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<td>0.66±0.03a/s</td>
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<tr>
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<tr>
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<td>2.47±0.08b/s</td>
<td>0.312±0.010b/r</td>
<td>18.31±0.47a/R</td>
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</table>

\( P_n \), \( g_s \), and water use efficiency (WUE): \( P_n \) and \( g_s \) decreased with decreasing \( \Psi_s \) for both species (Fig. 1C,D). Relative to the control plants, both species showed a similar decrease of 42 and 75% for \( P_n \) and \( g_s \), respectively, when \( \Psi_s \) was -1.6 MPa. Stomatal conductance differed significantly at \( \Psi_s = -1.0 \) and -1.6 MPa, while \( P_n \) was only significantly different at \( \Psi_s = -1.6 \) MPa. The performances of \( P_n \) and \( g_s \) in *P. mutica* were significantly superior to that of *P. klinjuk* either in drought-stressed and control plants. *P. mutica* showed higher stomatal frequency and larger stomata (values not shown), which might account for the higher \( g_s \) compared to *P. klinjuk*. WUE was calculated as the ratio of \( P_n \) over \( E \). Analysis of variance did not show any significant difference in value of WUE, neither between the various levels of stress nor between the two species (Table 1).
Discussion

By decreasing the $\Psi_s$, both $\Psi_f$ and $\Psi_n$ decreased and this could be caused by an accumulation of osmolytes at cellular level (Nepomuceno et al. 1998). Higher values of $\Psi_f$ and $\Psi_n$ were found in P. khinjuk compared to P. mutica which illustrates that the former species tolerates a higher drought stress.

An increasing trend of $\Delta\Psi_n$ with decreasing $\Psi_s$ of the nutrient solution was observed (Fig. 2A). This behaviour has been described as a mechanism developed for the plant to survive in dry conditions (Herrade et al. 1998), and can be attributed to the active accumulation of solutes in the cell sap or through passive solute accumulation resulting from reduced cell volume (Morgan 1984). P. mutica exhibited a higher $\Delta\Psi_n$ (0.62) than P. khinjuk (0.41), which reflects the ecological adaptations to the land where they are native. The former species is native in semi-arid to arid area while the latter one is native in arid area (Abrahimi 1995), and the period of dry season in the regions where P. mutica is native is shorter than in the areas where P. khinjuk is distributed. Wong et al. (1985) stated osmotic adjustment might delay stomatal closure. Therefore the lower $\Delta\Psi_n$ in P. khinjuk than in P. mutica could be effective in stomatal closure, thus reducing transpirational water losses which is important to survive in arid environments.

At $\Psi_f$ of -3 MPa, a $\Psi_p$ of approximately 0.4 MPa was maintained. In many fruit tree species, a decreasing $\Psi_f$ is accompanied with the loss of $\Psi_p$. For example, Sean et al. (1998) showed that ber (Ziziphus mauritiana Lamk.) leaves reached zero $\Psi_p$ at a $\Psi_f$ of -2.3 to -2.5 MPa. Therefore maintaining $\Psi_p$ in the species studied here could be an effective tool in tolerating drought stress.

Non-stomatal restrictions on $CO_2$ assimilation under drought stress can be assessed through measuring Chl fluorescence parameters. Non-photochemical quenching ($q_n$), which is related to the energy state of the chloroplast, shows that the energetic state of this organelle increased as a consequence of drought stress. The other fluorescence parameters such as $q_p$ show that the redox state of photosystem 2 was not affected by drought stress. Our results for $q_n$ are consistent with the findings of, e.g., Lichtenthaler and Rinderle (1988) and Eastman and Camm (1995) on spruce needles. Nevertheless, fluorescence parameters change also during vegetation season (for P. lentiscus see Karavatas and Manetas 1999).

Imposition of drought caused a reduction in Chl content in both pistachio species. The reduction in Chl a content was higher than in Chl b, which is mainly due to sensitivity of the former parameter to environmental stress, especially to salt and drought stress (Reddy and Vora 1986). Our findings agree with the results of Sayed (1996) on Zygophyllum qatarnase and Luquez et al. (1997) on soybean (Glycine max).

Photosynthetic performance in P. mutica is superior to that of P. khinjuk in unstressed and stressed conditions, and this could be related to the difference in Chl a content between the species (Luquez et al. 1997) (Fig. 2B). With respect to the effect of drought treatments, the control-compared values of $P_N$ show that P. mutica is more affected by stress compared to P. khinjuk. Both stomatal and mesophyll limitations could reduce $P_N$ in both species. The decrease in $g_s$ at decreasing $\Psi_s$ (Fig. 1D) and the accompanied decrease in $P_N$ ($r = 0.61$) (Fig. 1C) illustrates the stomatal limitations. The maintenance of WUE with decreasing $\Psi_s$, in both species, could be an illustration of the presence of mesophyll resistance (Behboudian et al. 1986). Also the reduction in Chl content (especially Chl a) and alteration in energy state of the chloroplast (as increasing $q_n$) were non-stomatal limitations, which reduce $P_N$. Our results with $P_N$ and $g_s$ (Fig. 1C,D) are in agreement with the findings of Goldhamer et al. (1984) on Pistacia vera and Loreto et al. (1993) on Quercus ilex and Pistacia lentiscus.

In conclusion, drought stress induced by PEG-6000 promoted significant differences in stressed plants versus control plants in their water relations and gas exchange. Both pistachio species have the capacity to maintain a relatively high $P_N$ with decreasing $\Psi_s$ down to at least -1.0 MPa. P. khinjuk has a relatively greater drought resistance than P. mutica, and it is relatively more suited for arid environments.

References

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