Variation of relative water content, water use efficiency and stomatal density during drought stress and subsequent recovery in pistachio cultivars (*Pistacia vera* L.)

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Abstract

Pistachio species belong to the *Anacardiaceae* family. *Pistacia vera* L., cultivated pistachio, is the most important species in the genus. Pistachio is a major orchard crop in Iran, whereby 'Akbari', 'Kaleghochi' and 'Ohadi' are very common cultivars. Pistachio has a high resistance to soil drought conditions but differences exist between cultivars. This greenhouse study was conducted to evaluate the effects of an osmotic drought stress (Ψ_s=-0.75 and Ψ_s=-1.5 MPa) on relative water content (RWC), water use efficiency (WUE) and stomatal characteristics in the cultivars mentioned above. Results reveal that the different osmotic drought stress levels applied significantly lowered RWC compared to a well-watered control. At the same time, the treatments significantly increased WUE compared to the control. WUE varied in different cultivars but differences were not significant. There were no significant differences between the different drought stress levels as for their influence on stomatal length, width and numbers, but cultivars showed different stomatal characteristics between them: 'Ohadi' and 'Kaleghochi' had widest stomata, whereas 'Akbari' had most stomata. In conclusion, drought stress treatments lowered RWC and leaf water potential. Therefore, under osmotic stress conditions, pistachio cultivars showed drought tolerance mechanism to cope with water deficiency. Also the results of this study show that drought stress treatments increased WUE. However, further research in field condition and under more severe drought treatments is needed to confirm this survey's research.

Keywords: water relationship, leaf water potential, ecophysiology, arid zone agriculture

INTRODUCTION

Pistacia vera is a subtropical nut. It is a major horticulture crop in Iran with more than 60% of its production exported every year (Sedaghat, 2010). Pistachio is usually recognized to have a good capacity to resist drought stress because of its deep root system and waxy leaf structure (Panahi et al., 2002). It has been claimed that the performance of pistachio trees under drought stress surpasses that of all other fruit tree species (Spiegel-Roy et al., 1977). Pistachio has a high resistance to drought and salinity conditions of both soil and water. Most of Iran's pistachio orchards are situated in the drier and warmer regions of the country (Panahi et al., 2002).

Plant responses and the plant's overall development status to drought stress depend on timing, speed, severity and length of the drought event (Anjum et al., 2011). Impairing effects of drought stress are more important than any other environmental factor (Shao et al., 2009). Stomatal closure is one of the first active responses to drought stress which results in a declined rate of photosynthesis (Anjum et al., 2011).

Drought stress treatments have shown to increase intrinsic water use efficiency (WUE) in *Ziziphus rotundifolia* Lamk (Arndt et al., 2001). In pistachio, the value of relative water content (RWC) and WUE vary with different fruit development stages and different cultivars. RWC of 'Ohadi' is highest followed by 'Kalehghochi', 'Akbari', 'Haratii', 'Ahmad-Aghaii' and 'Rezaii Zoudras' cultivars, respectively. Also, higher WUE relates to 'Akbari', followed by 'Ohadi', 'Kalehghochi', 'Ahmad-Aghaii', 'Haratii', and 'Rezaii Zoudras' cultivars, respectively (Sajjadinia et al., 2010). In wild almond (*Prunus dulcis*), intrinsic WUE increases with increasing drought stress (Rouhi et al., 2007). In the same experiment, WUE – which was defined as the ratio of net photosynthesis over stomatal conductance – did not show any significant differences in value, neither between the various levels of stress applied nor between the two wild pistachios (*P. mutica* and *P. khinjuk*) species tested (Ranjbarfordoei et al., 2001).

Environmental stresses such as drought stress have most effects on leaf traits (Dong and Zhang, 2000). Studies of the epidermal cells in leaves of four pistachio rootstocks under drought stress show that effect of irrigation on stomatal width was significant, while rootstock and interaction of irrigation and rootstock had no significant effects on stomatal width. The highest stomatal density and the lowest stomatal length and width were obtained with severest drought stress (Arzani et al., 2013).

The objective of this investigation was to evaluate the effects of osmotic drought stress on water relationship parameters and stomatal features of three pistachio cultivars towards selection of drought-tolerant genotypes. These investigations should ultimately lead to better informed more recommendations for development of new pistachio orchards.

MATERIALS AND METHODS

This study was carried out in a greenhouse of the Bioscience Engineering Faculty, Ghent University (51°3'N, 3°42'E). Certified seeds of three pistachio cultivars, *Pistacia vera* L. 'Akbari', 'Kaleghochi' and 'Ohadi' were obtained from the Iranian Pistachio Research Institute (30°39'N, 55°94'E), Rafsanjan, Iran. Seeds of these pistachio cultivars were first soaked in water for 12 h and then pre-treated in a fungicide (captan, a broad-spectrum fungicide) solution for 20 min to eliminate fungal contamination (Panahi et al., 2002).

All seeds were sown in 4-L pots containing sand and organic material in June 2011 (one seed/plant pot^{-1}). Plant management was done according to good agricultural practices (Panahi et al., 2002) during the first year. In March 2012, seedlings (27 plants for each cultivar) were transplanted to 5-L pots filled with vermiculite following (Ranjbarfordoei et al., 2002). Transplanted 1-year-old seedlings were grown in a controlled glasshouse environment in a hydroponic system using standard Hoagland solution (Picchioni et al., 1991) for fertigation. Temperature and relative humidity (day/night) in the glasshouse ranged between 27.1/21.7°C and 49.4/71% RH, respectively, while mean light intensity was 212.4 μmol m^{-2} s^{-1} PAR for the duration of the experiment.

In May 2012, drought treatments were initiated. They consisted of a control (Ψ_s=-0.10 MPa), and two drought stress levels (Ψ_s=-0.75 MPa, Ψ_s=-1.5 MPa) using PEG 6000 as osmotic agent. Drought stress levels were maintained for two weeks, then all solutions were replaced by the control treatment (-0.10 MPa), and this level was maintained for two recovery weeks.

Relative leaf water content (RWC)

Relative leaf water content (RWC) was determined on fully expanded leaves. From each plant, one leaf at the end of the second drought stress and recovery weeks, respectively, were clipped and immediately transferred to the laboratory in the icebox. There we determined fresh leaf weight (FW) by digital balance (Mettler Toledo PB602-L, Laboratory & Weighing Technologies, CH-8606 Greifensee, Switzerland; with precision ±0.1 mg). In order to obtain turgid weight (TW), leaves were floated in distilled water inside a closed petri dish and kept in a fridge for 24 h. Subsequently, we gently wiped the water from the leaf surface with tissue paper and measured turgid weight. Afterward, leaf samples were placed in an electric oven (L031, Jouan laboratory oven, UK) at 85°C for 48 h in order to obtain leaf dry weight (DW). Values of FW, TW and DW were then used to calculate RWC, using (Ritchie et al., 1990): RWC (%)=[(FW-DW)/(TW-DW)]×100%, where FW, DW and TW are fresh, dry and turgid weight (g), respectively.

Water use efficiency (WUE)

Gas exchange measurements were done on the 8th leaf (from the top) using a CO_2 and H_2O infrared gas analyzer (Licor-6400, LI-COR Inc., Lincoln, USA). Net photosynthetic rate (P_n) was measured after 14 days of drought stress and after 14 days of recovery, respectively. All measurements were made under standard environmental conditions (light intensity of 1500 µmol m^{-2} s^{-1}; CO_2 concentration = 400 µmol CO_2 mol^{-1}; leaf temperature (T_l) = 26.9°C; leaf vapour pressure deficit (VPD) = 1.1-2.2 kPa) between 10 am and 3 pm. Besides measuring net photosynthetic rate, the gas exchange instrument also provides data about the stomatal conductance for water vapour (g_s in mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, in µmol CO_2 mol^{-1} air) and leaf transpiration rate (E in mmol (H_2O) m^{-2} s^{-1}). Following Ashraf (2002) and Mediavilla et al. (2002), P_n/E ratio was taken as an estimate of water use efficiency.

Stomatal density

In this study, a fully expanded leaf of each plant from each treatment was used for measurement of stomatal density. Imprints of epidermal cells and leaf stomata were taken from the 8th leaf from the top by using colourless nail polish. A thin layer of polish was applied with a small brush onto the abaxial side of the leaf and was allowed to dry, after which the resulting print of the leaf surface can be removed with transparent tape and put on a microscope slide (Elagöz et al., 2006).

Replicas were analysed at a magnification of 10×40 using a bright field microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan). To facilitate stomatal counting, the image from the microscope was transferred to a TV screen by means of a video camera (JVC TK - 860 E). The number of stomata was directly counted on the TV screen and converted to stomatal density (number of stomata per mm^2). For each treatment, stomata of 81 fields were counted (3 replications × 9 plants × 3 counts per area) according to the method of Zaid and Hughes (1995). Stomata density and stomata dimension (length and width) were determined by light microscope and scanning electron microscope (SEM). 12 stomata per plant were measured directly in the snapshot on the screen.

Statistical analysis

Treatments and cultivars were arranged in a randomized complete block design (RCBD) with three blocks and three replicates per experimental unit. A two-way analysis of variance was used to test for drought treatment differences and cultivar effects. Means were compared by a Tukey's test. All analyses were performed in SPSS 20 (IBM Corporation, USA).

RESULTS

Relative water content (RWC)

RWC of leaves decreased with drought stress applied compared to their corresponding controls during both drought and recovery stages. Under recovery conditions, RWC was higher than under drought condition in both well-watered and drought-exposed plants (Figure 1A). Statistical analysis showed that there were no differences for RWC values among pistachio cultivars in drought and recovery stages (Figure 1B).

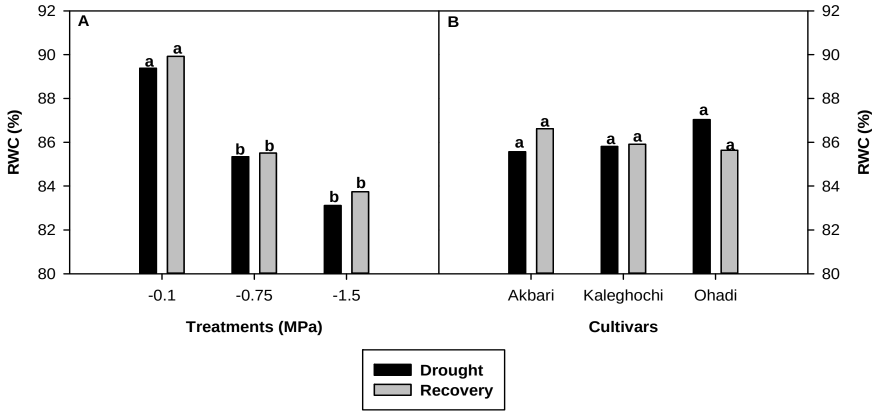

Figure 1. Changes in RWC in different treatments (A) and pistachio cultivars (B) during drought and subsequent recovery stages.

Water use efficiency (WUE)

In the drought stage, both drought stress levels increased water use efficiency in all evaluated cultivars and differences were significant compared to their control. During recovery, WUE values were higher than the previous stress stage. During recovery, there was a significant difference between highest those measured during drought stress level compared to control (Figure 2A). Increase percentages in WUE were 25 and 62.1% under the most severe drought stress level compared to control during drought and recovery stages, respectively.

There were no significant differences between the pistachio cultivars in both drought and recovery stages (Figure 2B), even though WUE values were higher during recovery compared to those obtained during the drought stage.

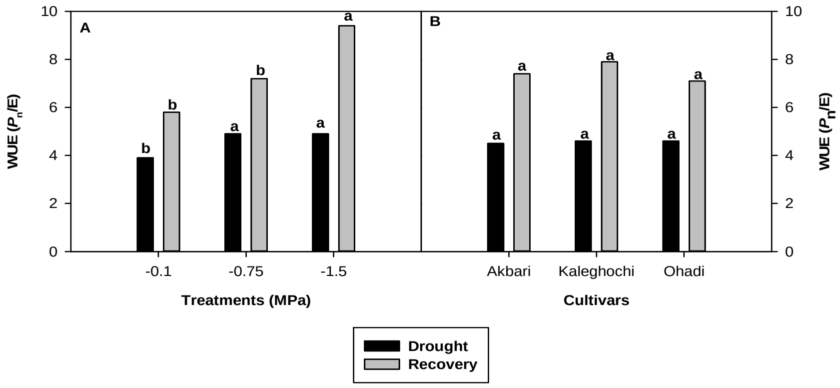

Figure 2. Changes in WUE in different treatments (A) and pistachio cultivars (B) during drought and subsequent recovery stages.

Stomatal width

Drought stress treatments had no significant effects on stomatal length (data not shown) and width values compared to control dimensions (Figure 3). There were significant differences in stomatal width between the pistachio cultivars (Figure 3). 'Akbari' had the lowest value whereas 'Ohadi' had the highest value for stomatal width in this experiment. Differences between these two cultivars were significant.

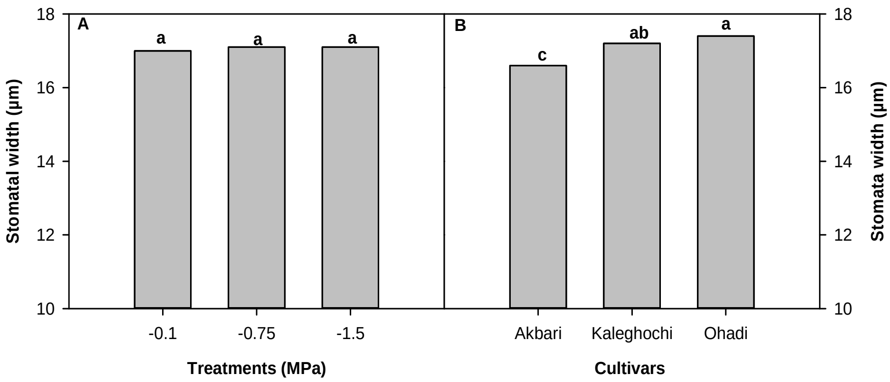

Figure 3. Changes in stomatal width in different treatments (A) and in different pistachio cultivars (B).

Stomatal density

There were no significant differences in stomatal number between drought stress treatments (Figure 4A). Numbers of stomata in different pistachio cultivars varied whereby 'Akbari' had the highest number among the cultivars tested. The stomatal number was lowest in 'Ohadi' cultivar (Figure 4B). There was an inverse relationship between stomatal length and width, and stomatal numbers (data not shown).

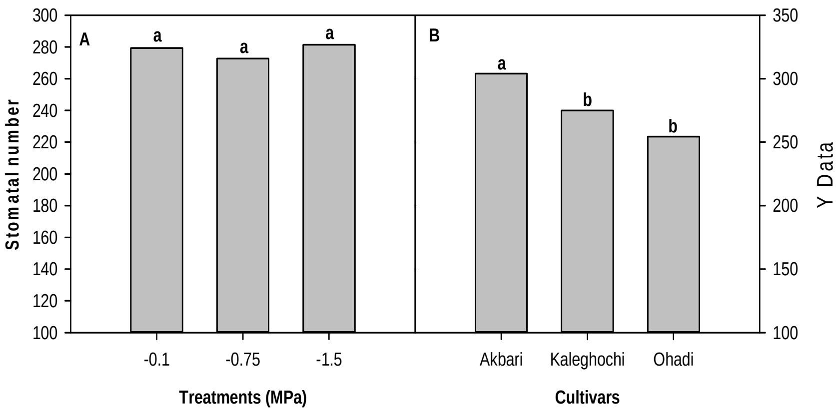

Figure 4. Changes in stomatal number in different treatments (A) and in different pistachio cultivars (B).

DISCUSSION

The findings of this experiment show that applying osmotic drought stress treatments caused a decrease in RWC in leaves of pistachio cultivars in drought conditions (Figure 1A), confirming that stress causes dehydration in leaf cells (Anjum et al., 2011). These results are in agreement with previous reports on pistachio seedlings that indicated RWC was lowered by drought stress treatments (Habibi and Hajiboland, 2013; Panahi, 2009) and by an increase in salinity (Benhassaini et al., 2012; Karimi et al., 2009; Panahi, 2009).

Instantaneous WUE (P_n/E) increased with decreasing osmotic potential (Figure 1C, D), as both P_n and E decreased with increasing drought stress (data not shown). These results are consistent with the report of Behboudian et al. (1986) on pistachio, Arndt et al. (2001) on *Ziziphus rotundifolia*, Rouhi et al. (2007) on almond rootstocks but are in disagreement with Ranjbarfordoei et al. (2001) on *P. mutica* and *P. khinjuk* (wild pistachio rootstocks).

Since all the cultivars used in this experiment are related to the same species (*P. vera*), we can conclude that this disagreement may be related to the fact the latter research focused on different pistachio species.

The increase of WUE by decreasing osmotic potential could be an illustration of the presence of a stomatal resistance factor (Behboudian et al., 1986). Regarding the inverse effects of drought stress treatments on photosynthesis rates in this experiment (data not shown), stomatal factors may have played a more important role than internal physiological factors because of the increase of WUE by decreasing osmotic potential (Behboudian et al., 1986). Based on these WUE data, we estimate that water use efficiency rates are lower in the drought stress stage compared to the subsequent recovery phase, and higher in drought stress levels compared to their corresponding control values.

Environmental stresses such as drought primarily affect leaf traits. Stomatal density values for the above-evaluated cultivars were lower than those reported for a number of pistachio rootstocks (Ranjbarfordoei et al., 2002) and cultivars (Arzani et al., 2013). These differences can be explained by the different cultivars and rootstocks (Munir et al., 2011).

Drought stress levels had no significant effects on stomatal length and width for the pistachio cultivars examined. These results are in contrast with the result of Arzani et al. (2013) who reported the highest stomatal density and the lowest stomatal length and width were obtained with the most severe drought stress.

The lower stomatal density in leaves of 'Ohadi' can be attributed to an anatomical adaptation at leaf level to reduce transpiration. Also, results of this study confirmed prior finding, that there is a negative correlation between stomatal density and stomatal width (Arzani et al., 2013). A negative relationship between the stomatal length and width with stomatal numbers suggests that stomata have an adaptation mechanism to cope with excessive drought stress by a negative linkage between the stomata characteristics. Possibly these cultivars require higher drought stress intensities for the stomatal characteristic to change.

CONCLUSIONS

Findings of the present study show that drought stress treatments lowered the RWC and leaf water potential. Therefore, under osmotic stress conditions pistachio cultivars apparently developed drought tolerance mechanisms to cope with water deficiency. Also, the results of this study show that drought stress treatments increased WUE. However, further research in field condition and also more severe drought treatments are needed to confirm this survey's research.

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