Identification of some drought resistance mechanisms in four pistachio seedling rootstocks under water stress

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Abstract

This research was conducted to identify the drought resistance mechanisms of four pistachio seedling rootstocks. Irrigation treatments were 100% ET_c (control), 65% ET_c (medium stress), and 30% ET_c (severe stress), which were applied on 4-month-old seedlings for 75 days. Evaluated rootstocks were *Pistacia vera* 'Badami-e-Zarand', 'Ghazvini', 'Sarakhs', and *P. mutica*. Physiological parameters such as gas exchanges, water relations, photochemical efficiency of photosystem II (F_v/F_m ratio) and carbon isotope discrimination (Δ), biochemical parameters such as contents of proline, carbohydrate, and chlorophyll, and growth parameters were investigated. Generally, water stress decreased CO_2 assimilation (A), stomatal conductivity (g_s), leaf water potential (Ψ_w), relative water content (RWC), F_v/F_m ratio, carbon isotope [illegible] ...lorophyll content, and biomass, while contents of proline and [illegible] ...increased. Based on the results, *P. mutica* and *P. vera* 'Badami-e-[illegible] ...photosynthesis and higher RWC under water stress. The higher [illegible] ...espite its lower leaf water potential, can be attributed to better [illegible] ...The highest F_v/F_m ratio and chlorophyll content under water stress [illegible] ...in *P. mutica*. A smaller reduction in growth parameters also was [illegible] ...ca and *P. vera* 'Badami-e-Zarand' under water stress conditions. According to the mentioned reasons, it can be concluded that the rootstocks *P. mutica* and *P. vera* 'Badami-e-Zarand were the most drought tolerant rootstocks. Carbon isotope discrimination and proline accumulation were not suitable indexes for the screening of drought-tolerant rootstocks.

Keywords: pistachio, seedling rootstocks, water stress, carbon isotope discrimination

INTRODUCTION

Pistachio is one of the most important parts of Iran's non-oil exports. Currently, there are over 316,000 ha of pistachio orchards in Iran (FAOSTAT, 2014). Most of the Iranian pistachio orchards (more than 70%) are located in Kerman province and the rest are in the provinces of Yazd, Qazvin, Semnan, Sistan and Baluchestan, Fars, Markazi, and Isfahan. According to reports, Iran's pistachio yield (1300 kg ha^{-1}) is lower than the performance of the main countries producing pistachios in the world (FAOSTAT, 2014). Drought stress is one of the most important reasons for low pistachio yield in Iran. Water stress as one of the main factors limiting plant growth and development affects physiological and biochemical processes in plants and reduces performance, product quality, and finally causes plant death (Jones, 1997). Pistachio trees are xerophyte and drought-resistant plants but it does not mean that these trees require little water to produce optimal, but they can produce moderate yield with very low water (Goldhamer, 1995) and the quantity and quality of pistachio trees due to drought stress significantly is reduced (Goldhamer and Beede, 2004). Identification of

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drought-tolerant rootstocks is an important goal of plant breeding. Evaluation of the parameters gas exchanges, photosynthesis, chlorophyll fluorescence, status of water, changes of proline, total soluble sugars, and carbon isotope discrimination have great importance to find mechanisms of drought tolerance and screening and selection of cultivars and rootstocks. These screening methods and examinations in controlled environments can complement the field assessments (Stoddard et al., 2006). This study aimed to investigate the effects of drought stress on the mechanisms of drought tolerance in pistachio seedlings rootstocks and to identify the best rootstocks in terms of drought tolerance and water use efficiency.

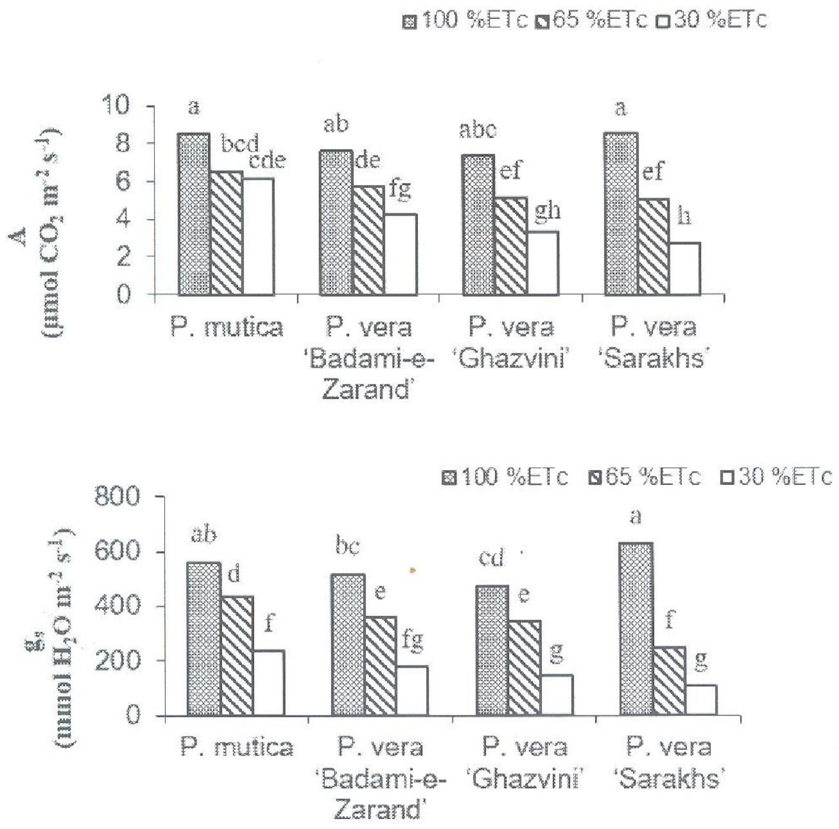
MATERIALS AND METHODS

In this study, we investigated the effect of three irrigation levels (100% ETc, 65% ETc, and 30% ETc) on four pistachio seedling rootstocks. Irrigation treatments applied on 4-month-old seedlings grown in the greenhouse for 75 days. Rootstocks were *Pistacia mutica* and three cultivars of *P. vera* ('Badami', 'Ghazvini', and 'Sarakhs'). During and at the end of the experiment, different physiological parameters such as photosynthesis (A), stomatal conductance (g_s), leaf water potential (Ψ_w), relative water content (RWC), chlorophyll fluorescence (F_v/F_m ratio), and carbon isotope discrimination, biochemical parameters such as contents of proline, total soluble sugars and leaf chlorophyll and finally growth parameters were measured. Photosynthesis (A) and stomatal conductance (g_s) was measured on two fully developed leaves of each plant (replicate) in each pot by photosynthesis meter (LI-6200 Portable, Li-Cor Inc., Lincoln, NE, USA) from 10:00 am to 12:00 pm Measurement of water potential was done by a pressure chamber apparatus in the middle of the day on two leaves of each plant (the youngest developed leaves and taken from the outer branches of the crown) based on the Romero and Botia (2006) method. The leaf relative water content (RWC) was measured according to the method described by Ritchie et al. (1990) on the newest adult leaves of the plant. Measurement of chlorophyll fluorescence was carried out using a plant tensioner device (plant stress meter) made by the Swedish Bio Monitor Company in the middle of the fully developed leaves between 11 and 13 h. The light level and the measurement duration used were adjusted to 400 μmol s^{-1} and 5 s, respectively (O'Neill et al., 2006). Evaluation of carbon isotope discrimination carried out by the method of Farquhar et al. (1989) by a mass spectrometer in the Department of Forest Sciences of the University of British Columbia, Canada. In this method, the content of ^{13}C absorbed in the tissue of plants is measured relative to a fossil standard. Determination of free leaf proline content, total soluble sugars, and leaf chlorophyll content carried out based on Bates et al. (1973), Irigoyen et al. (1992), and Kizhedath and Suneetha (2011), respectively. Also, at the end of the experiment parameters such as roots, shoots and leaves were divided into their parts and the dry weight of them was measured. Data were statistically analyzed by MSTATC software.

RESULTS AND DISCUSSION

Effect of drought stress on gas exchanges

Drought stress significantly reduced photosynthesis and stomatal conductance of all rootstocks. As shown in Figure 1, in all irrigation treatments, the highest and the lowest photosynthesis rates belonged to *P. mutica*. and 'Sarakhs', respectively. Under severe water stress condition *P. mutica*, 'Badami', 'Ghazvini', and 'Sarakhs' showed the highest to the lowest rate of photosynthesis, respectively. The lowest values of stomatal conductance in severe water stress conditions also were observed in the rootstock 'Sarakhs', while this rootstock under full irrigation conditions had higher stomatal conductance than other rootstocks. The *P. mutica* showed higher stomatal conductance rates in comparison to others under water stress conditions (Figure 1). In medium stress conditions, reduction of photosynthesis is primarily due to the closure of the stomata, but in the case of severe water stress, both stomatal constraint and increase of mesophilic resistance and the effect on the membrane of the thylakoid and reduction of chlorophyll content reduce photosynthesis (Burce, 1991). A significant decrease in stomatal conductance (g_s) and closure of stomata in very early stages of soil drying has been observed even before significant reduction of the leaf water potential by the chemical-derived signals from the root and leaf (Jefferies and Mackerron, 1989).



igation treatments on photosynthesis (A) and stomatal conductance (g_s)
ı seedlings.

ress on water relations

Based on the results, water stress reduced leaf water potential and relative water content of the leaf. The minimum leaf water potential was obtained in *P. mutica* under severe water stress. There was no significant difference between the other rootstocks (Figure 2). In the case of RWC, the rootstocks *P. mutica* and 'Badami' showed higher RWC under water stress conditions. The high RWC despite low water potential in *P. mutica* under water stress may be due to higher stomatal conductance and mechanisms such as osmotic adjustment or root ability for water uptake (high hydraulic conductivity). Therefore, the physiological activity of this rootstock is preserved despite water loss due to water loss. It is reported that the genotypes that can maintain more water without closing their stomata are more suitable for dry areas (Blum et al., 1981).

Effects of drought stress on chlorophyll a fluorescence

Water stress reduced F_v/F_m of studied plants. The largest contents of F_v/F_m which is a good indicator for efficiency of photosystem II belonged to the *P. mutica* that followed by the rootstocks 'Badami', 'Ghazvini', and 'Sarakhs'. The difference between 'Badami', 'Ghazvini', and 'Sarakhs' was not significant. Based on the results, the maximum value of F_v/F_m under severe water stress was related to *P. mutica* (Figure 3). It can be said that *P. mutica* can withstand water stress conditions better than other rootstocks. Water stress damages the center of photosystem II and reduces the in-use efficiency of the photon by the photosystem and resulted in the release of energy as fluorescence and heat. Thus the efficiency of photosystem II decreases (Mohammad et al., 1996). It has been reported that there is a high correlation between photosynthesis efficiency and F_v/F_m and genotypes with higher F_v/F_m have higher photosynthetic efficiency (Bongi and Loreto, 1989).

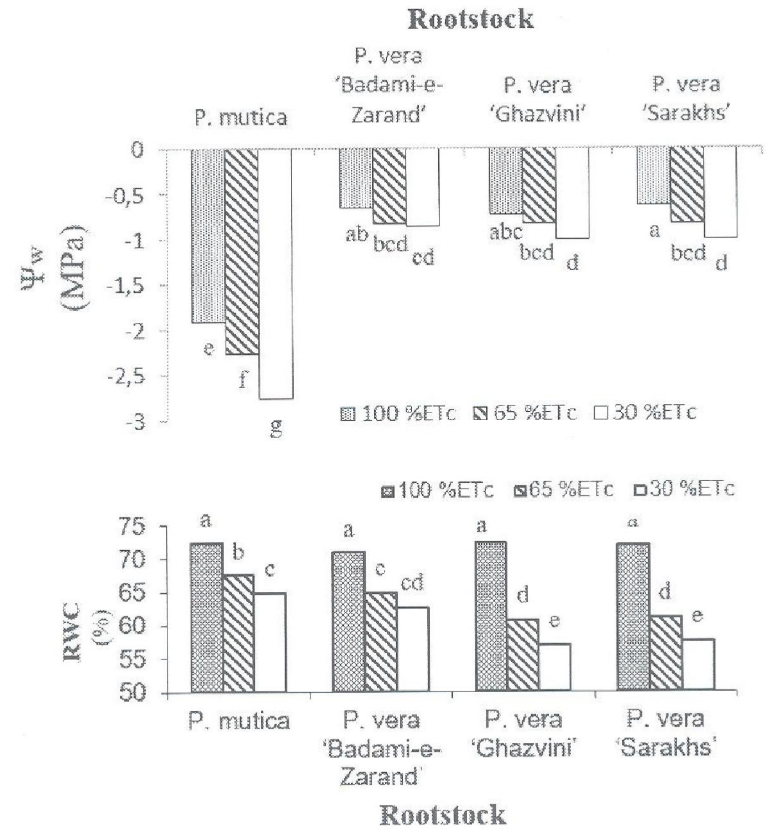


Figure 2. Effect of different water treatments on leaf water potential (Ψ_w) and relative water content (RWC) of pistachio seedlings.

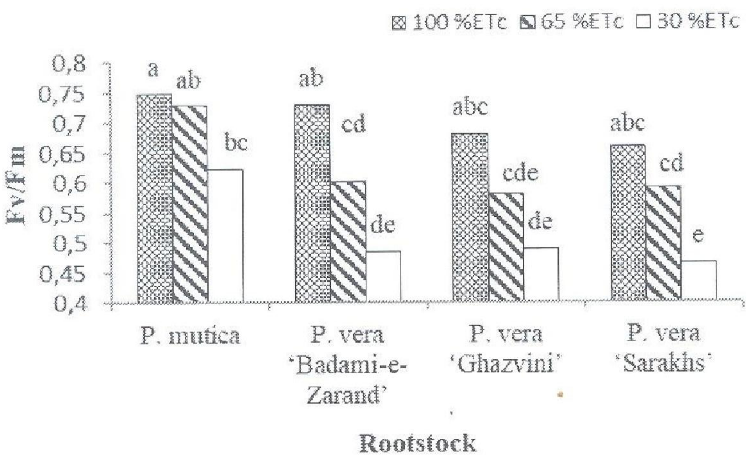


Figure 3. Effect of different water treatments on efficiency of photosystem II of pistachio seedlings.

Effects of drought stress on leaf carbon isotope discrimination

Water stress reduced carbon isotope discrimination (Δ) and under severe water stress, the lowest carbon isotope discrimination was observed in the rootstock 'Sarakhs' (29.44‰) (Figure 4). The greatest discrimination under severe water stress also belonged to Badami (30.89‰). According to Farquhar et al. (1989), a reduction in the amount of carbon isotope discrimination in stress conditions is due to effects of stress on the activity of plant photosynthesis, change in rubisco enzyme activity, and a decrease in stomatal conductance. With a decrease in stomatal conductance, the CO_2 concentration of intercellular (Ci) decreases, and the amount of Δ decreases. While when Ci is high (high stomatal conductance), more differentiation and more diagnosis against carbon 13 is done. So when Δ is high, the plant lost more water and reduces water use efficiency as a result of this operation. The lower stomatal conductance of 'Sarakhs' under drought stress can be a factor in the low level of isotope discrimination (Δ) in this rootstock.

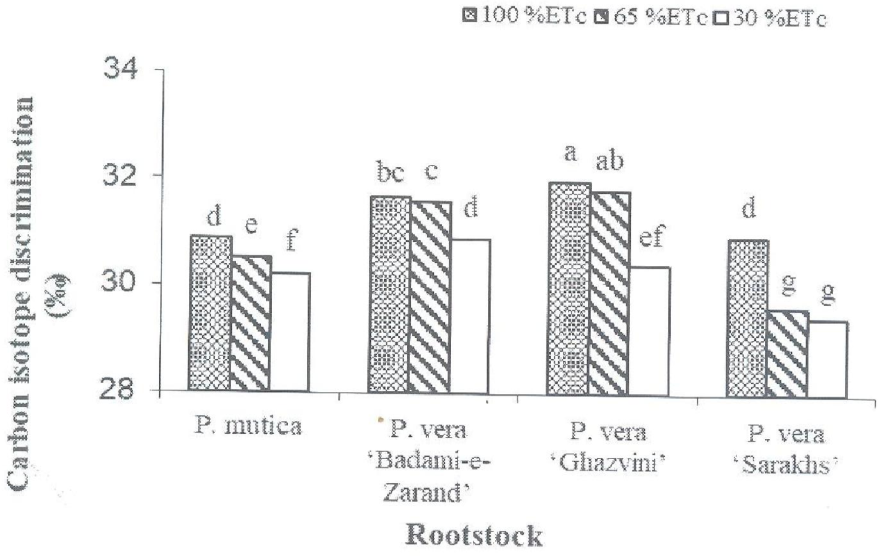


Figure 4. Effect of different water treatments on carbon isotope discrimination of pistachio seedlings.

ress on the accumulation of proline and soluble sugars

ion showed that in all the rootstocks, increasing intensity of water stress l proline and soluble sugars. The results showed that the largest proline vater stress was obtained in the rootstock 'Sarakhs' and then 'Ghazvini' tly different from other rootstocks (Figure 5). While the highest le carbohydrates under water stress conditions was obliged to *P. mutica*. 'Sarakhs' also had less accumulation of carbohydrates under water stress (Figure 5). More accumulation of soluble salts or osmotic adjustment is one of the special mechanisms of cellular defense against water loss. In this situation, the cell can maintain its enzymatic activity (Levitt, 1980).

Effects of drought stress on chlorophyll content of leaves

The largest and the least contents of total chlorophyll were observed in control and severe water stress treatment. The rootstock 'Sarakhs' under severe water stress conditions had the least chlorophyll content in the leaves and other rootstocks showed no significant difference (Figure 6). Reduction in the amount of chlorophyll under water stress can be due to chlorophyll degradation or prevent the synthesis of chlorophyll and can reduce net photosynthesis. Chlorophyll degradation caused by drought may be due to the production of oxygen free radicals in cells that cause oxidation and decomposition of pigments (Schütz and Fangmeier, 2001).

The effect of drought on growth parameters

Growth parameters of *P. mutica* and 'Badami' were less affected by drought. Results showed that the shoot and leaf dry weight of *P. mutica* were less affected by drought stress in comparison to other rootstocks. In the case of root dry weight also the rootstock 'Badami' was less affected by drought and was followed by *P. mutica*, 'Ghazvini', and 'Sarakhs', respectively (Figure 7). There are several reasons for the reduction of growth under water stress including the reduction of photosynthesis, turgor, RWC, leaf area, and consumption of more ATP for the hydrolysis of osmotic compounds and synthesis of hormones such as ABA (Chartzoulakis et al., 1993; Dubey, 1997).

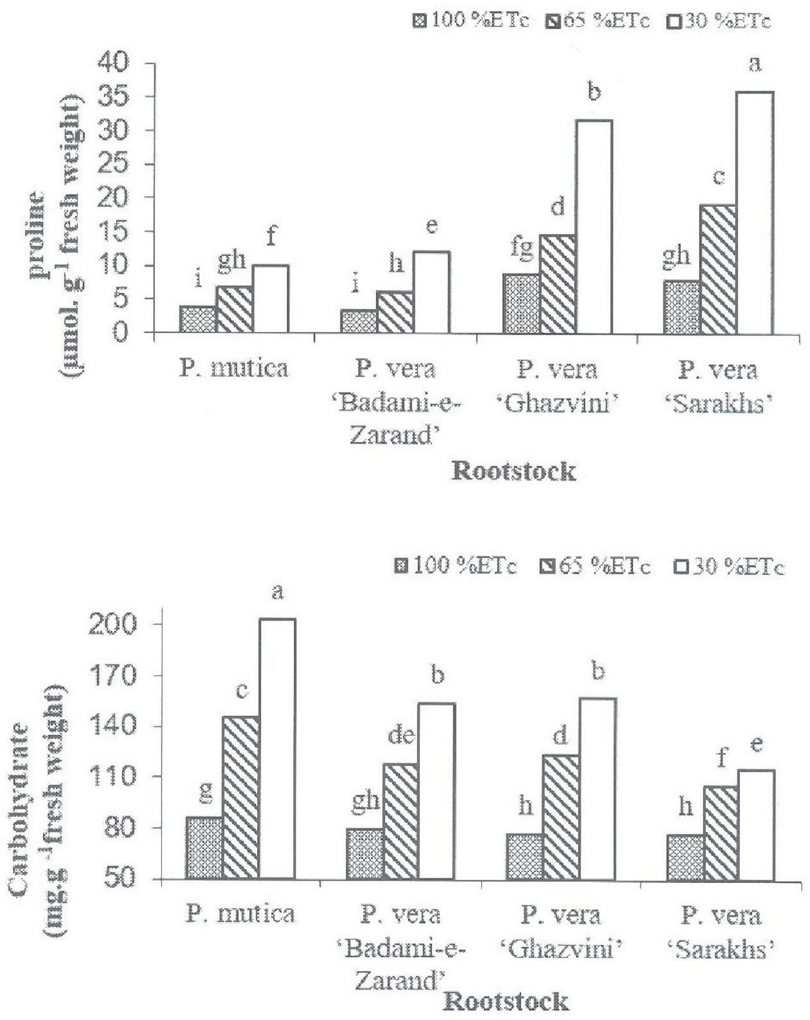


Figure 5. Effect of different water treatments on the accumulation of proline and soluble sugars.

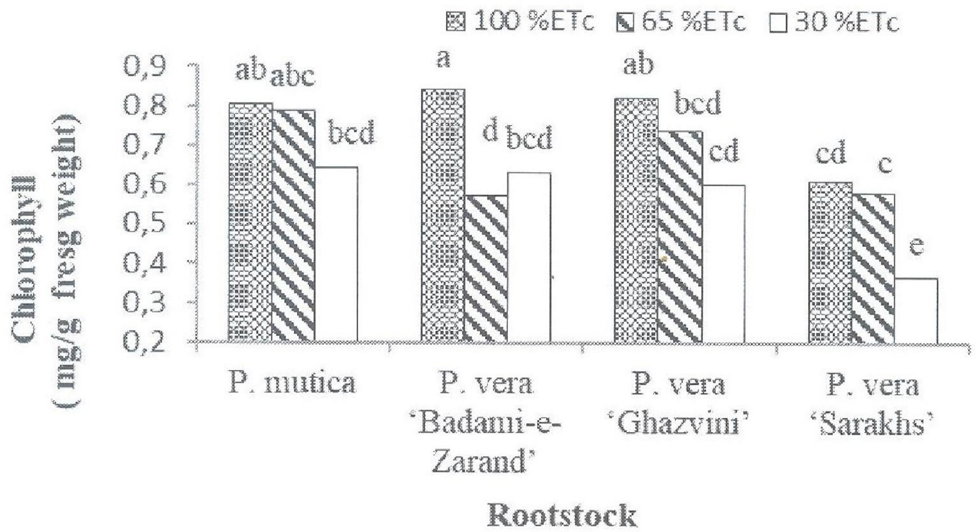


Figure 6. Effect of different water treatments on contents of leaves chlorophyll of pistachio rootstocks.

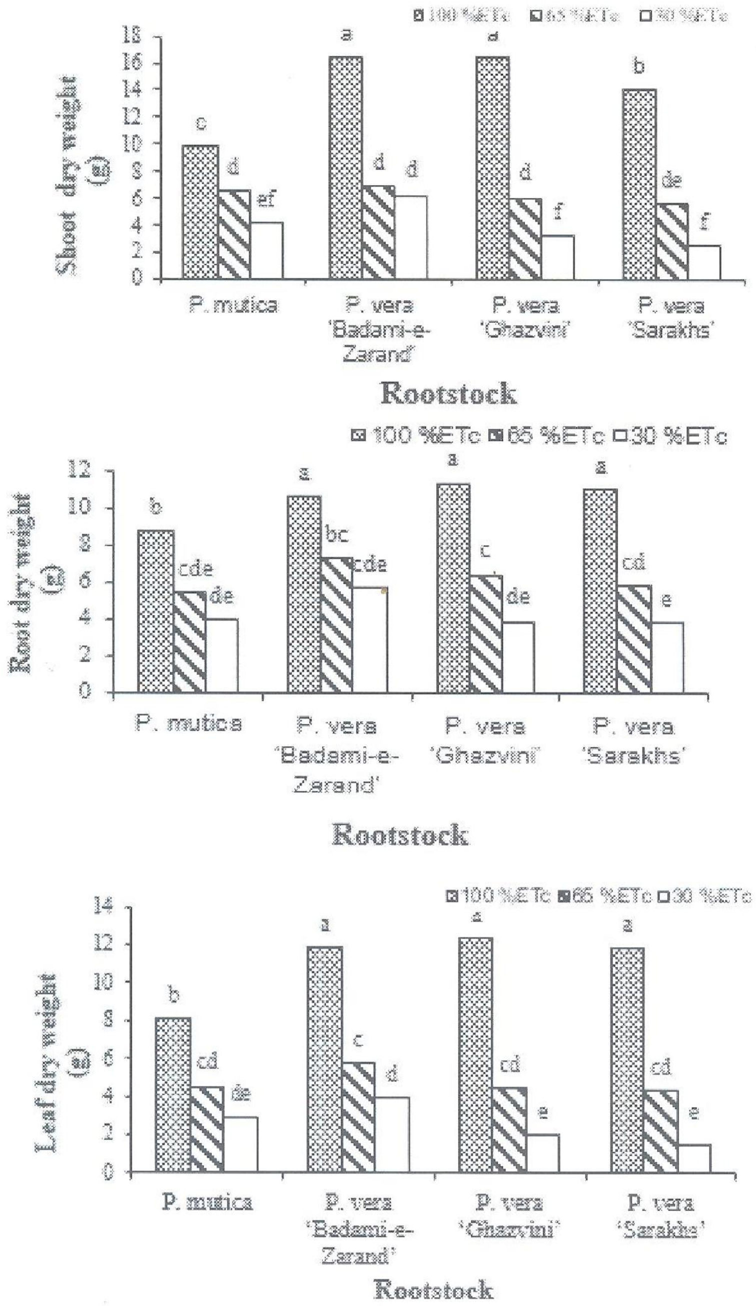


Figure 7. Effect of different water treatments on dry weight of shoot, root and leaves of pistachio rootstocks.

CONCLUSIONS

Based on the results, it can be said that the rootstocks *P. mutica* and *P. vera* 'Badami-e-Zarand' had higher drought tolerance than other rootstocks. Because in both the parameters photosynthesis, RWC, F_v/F_m ratio, and chlorophyll content were higher, and on the other hand their growth parameters were less affected by drought. In severe water stress conditions, the lowest carbon isotope discrimination was observed in the rootstock 'Sarakhs'. Due to the lack of correlation between the carbon isotope discrimination and the drought resistance of the studied rootstocks, it can be said that this parameter is not an appropriate index for selecting resistant drought genotypes.

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