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Variation in biochemical characteristics, water status, stomata features, leaf carbon isotope composition and its relationship to water use efficiency in pistachio (*Pistacia vera* L.) cultivars under drought stress condition

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Highlights

- Sucrose is an important osmolyte in pistachio submitted to osmotic drought stress.
- RWC is not strongly affected by the osmotic stress up to $\Psi s=-1.5$ MPa thought cultivar differences exist.
- WUE increases in pistachio cultivars under osmotic stress especially for $\Psi s=-1.5$ MPa.
- Leaf carbon isotope composition of pistachio cultivars were not affected by a 2-week stress period.
- Leaf nitrogen isotope composition decreases under a 2-week stress regardless off the cultivar.

Abstract

Pistachio (*P. vera* L., Anacardiaceae) is cultivated in regions where soil water deficits and salinity conditions are higher than usual. Despite adult pistachio trees having been documented as being drought tolerant; there is only a limited understanding of the physiological mechanisms pistachio cultivars use to survive drought. We therefore, carried out a greenhouse experiment to evaluate the effects of three osmotic drought stress treatments including; control conditions (−0.1 MPa), moderate (−0.75 MPa) and severe drought (−1.5 MPa) stress, using PEG 6000 for 14 days with a subsequent two weeks recovery period. Carbohydrate contents, relative water content, water use efficiency, stomatal characteristics, and nitrogen and carbon isotope composition were evaluated in three Iranian pistachio cultivars, i.e. Akbari, Kaleghochi and Ohadi.

Results revealed that the drought stress treatments induced osmotic adjustment by carbohydrate accumulation. Both drought stress treatments increased soluble carbohydrate and starch contents of the leaves. Relative water content was only affected by drought stress in Kaleghochi. Stomatal density and morphology varied with pistachio cultivars but was hardly affected by the stress treatments.

Drought stress significantly increased the overall mean of water use efficiency (intrinsic and instantaneous WUE). There were no significant differences between the leaf carbon isotope compositions of all pistachio cultivars under stress. This indicates that this relation may not be used to determine pistachio cultivars with appropriate WUE via leaf carbon isotope composition within the time frame of the experiment. Leaf nitrogen isotope composition decreased under drought stress regardless off the cultivar.

Introduction

The pistachio is a subtropical nut crop. It is a major horticultural crop in Iran, with an annual production of around 472,000 tons of pistachio nuts (FAO, 2012), more than 60% of the total produce is exported (Sedaghat, 2010). Most of Iran's pistachio orchards are established in the drier and warmer regions of the country (Panahi et al., 2002) and this affects the productivity of the trees.

Plant responses to drought stress depend on timing, speed, severity and length of the drought event (Anjum et al., 2011, Chaves et al., 2002, Praba et al., 2009). Stomatal closure is one of the first responses to drought stress (Anyia and Herzog, 2004, Arndt et al., 2001). Stomatal characteristics, such as frequency and dimensions, are greatly affected by the species but also by environmental factors (Dong and Zhang, 2000, Munir et al., 2011). In pistachio rootstocks, the highest stomatal density and the lowest stomatal length and width were obtained under severe drought stress (Arzani et al., 2013) and this adaptation allows a better control of the leaf water content. Another common mechanism to maintain the turgor at low water availability is osmotic adjustment. Drought stress has been shown to promote the accumulation of soluble sugars and proline in both the leaves and roots of mycorrhizal and non-mycorrhizal pistachio plants, compared to those under well-watered conditions (Abbaspour et al., 2012a).

Water use efficiency (WUE) is widely used to evaluate the plant adaptation to limited water supply (Araus et al., 2002, Moghaddam et al., 2013). WUE may be estimated as the ratio between net photosynthesis (Pn) and transpiration (E), which is known as instantaneous water use efficiency (physiological index) (Mediavilla et al., 2002, Polley, 2002), as the ratio between Pn and stomatal conductance (gs), which is known as intrinsic water use efficiency (physiological index) (Boyer, 1996, Pascual et al., 2013) and as the ratio of dry matter accumulation over time to the amount of water transpired which is identified as biomass/yield water use efficiency (agronomic index). Physiological WUE indices are widely used in comparative studies involving plant responses to changes in water supply and demand (Cabrera-Bosquet et al., 2009, Condon et al., 2004) and provide information about the physiological performance in response to short-term changes in the plant water status (Centritto et al., 2002). In pistachio, WUE varies with different fruit growth stages and cultivars (Sajjadinia et al., 2010). It was shown that intrinsic WUE values increased in wild almonds (*Prunus dulcis*) (Rouhi et al., 2007) and *Ziziphus rotundifolia* Lamk. (Rhamnaceae) (Arndt et al., 2001) by increasing drought stress. In contrast, WUE was not affected under drought in two wild pistachio species (*P. mutica* and *P. khinjuk*) (Ranjbarfordoei et al., 2000).

A decreasing stomatal conductance results in increasing WUE and declining leaf intercellular CO_2 (Ci) and consequently decreasing carbon isotope discrimination (Δ). Therefore, there should be a negative relation between WUE and Δ due to the independent relation between Ci and Δ or WUE (Farquhar et al., 1989, Farquhar and Sharkey, 1982, Saugier et al., 2012). In breeding programs, the variation in the CO_2 assimilation to stomatal conductance or water transpiration ratio can be exploited by indirect selection for WUE via Δ (Moghaddam et al., 2013). Hokmabadi et al. (2005) reported that carbon isotope discrimination (Δ) decreased with increasing salinity in the leaves, stems and roots of pistachio (*P. vera*) seedlings though there were no significant differences in carbon isotope discrimination between three *P. vera* (Sarakhs, Badami-zarand, and Ghazvini) rootstocks under the same salinity treatments. Yet, carbon isotope discrimination values can vary in different plant species. Alternatively the leaf carbon isotope composition ($\boldsymbol{\delta}^{13}$c) can be measured and this parameter is positively linked to instantaneous WUE and intrinsic WUE in C_3 plants (Farquhar et al., 1989) especially in wheat (Cabrera-Bosquet et al., 2009), and grapevine (de Souza et al., 2005). Carbon isotope ratios of *Pistacia lentiscus*, *Quercus ilex* and *Phillyrea argustifoli* showed similar $\boldsymbol{\delta}^{13}$c values, while two deciduous oak species, *Q. pubescens* and *Q. cerris*, had lower δ^{13}c values in a Mediterranean ecosystem (Valentini et al., 1992).

Decreasing water availability will also reduce the N availability and use and this could be assessed by nitrogen discrimination (δ^{15}N). In durum wheat root δ^{15}N increased under drought stress while the same stress treatments significantly decreased shoot δ^{15}N (Yousfi et al., 2012). In broccoli, it was found that high salinity significantly decreased nitrogen isotope discrimination and increased carbon isotope discrimination in leaf dry matter (Del Amor and Cuadra-Crespo, 2011). Carbon and nitrogen isotope ratios constitute a tool that is widely used to detect environmental effects especially in forests and herbaceous plants and to link these effects to water stress, WUE and N availability (Pascual et al., 2013). However, to our knowledge, uses of C and N isotope ratios to assess drought stress in fruit trees are very limited.

There is a wide genetic variation of more than 150 edible pistachio (*P. vera*) cultivars in Iran (Esmaeilpour et al., 2010, Esmaeilpour and Khezri, 2006, Sheibani, 1995). They have originated from natural pistachio forests in the northeast part of the country and are grown in different environmental conditions. Although pistachio is well-known for its drought tolerance (Abbaspour et al., 2012b, Bagheri et al., 2011, Esmaeilpour et al., 2015, Fardooei, 2001, Habibi and Hajiboland, 2013, Khoyerdi et al., 2016, Panahi, 2009, Tajabadipour et al., 2006), limited evidence is available on the underlying mechanisms of drought tolerance associated with the plant's water relations, and especially carbon and nitrogen isotope composition.

The objectives of this investigation were: (1) to evaluate the effects of osmotic drought stress on RWC, carbohydrate content and stomatal characteristics of three pistachio cultivars; (2) to determine the effect of drought stress on WUE, δ^{13}C and δ^{15}N under drought stress conditions; (3) to study the relationship between WUE and δ^{13}C; (4) to assess the use of carbone isotope composition in pistachio trees as an indicator of water use efficiency (WUE) under drought stress.

Section snippets

Plant material and experimental set-up

This study was carried out in a greenhouse at the Faculty of Bioscience Engineering, Ghent University (51°3′N, 3°42′ E). Certified seeds of three pistachio cultivars, *Pistacia vera* L. i.e. Akbari, Kaleghochi and Ohadi were obtained from the Iranian Pistachio Research Institute, Rafsanjan, Iran (30°39′N, 55°94′E). Seeds of these pistachio cultivars were first soaked in water for 12 h and then treated for 20 min with 0.01% captan, a broad-spectrum fungicide (Panahi et al., 2002).

All seeds were sown …

Changes in carbohydrate concentrations

Sucrose content significantly accumulated under severe drought stress in all three cultivars (Table 1). Fructose significantly increased in Kaleghochi for the highest drought stress level. Also, starch accumulated in all three cultivars at both drought stress levels (−0.75 and −1.5 MPa). When compared to their corresponding control plants, these changes were significant in Ohadi and non-significant in Akbari and Kaleghochi cultivars. No significant effect on glucose levels was observed (Table 1).…

Discussion

In this study, we investigated the effect of osmotic drought stress induced by PEG on leaf water status as assessed by RWC, carbohydrates and stomatal characteristics and on WUE, nitrogen and carbon isotope composition of three important pistachio cultivars, i.e., *P. vera* Akbari, Kaleghochi and Ohadi.

RWC content is a good measure of plant water status, in terms of monitoring the physiological consequences of a cellular water deficit (Anjum et al., 2011, Blum, 2005, Farooq et al., 2009). Our…

Conclusion

Under osmotic stress conditions, pistachio cultivars developed an active drought tolerance mechanism to cope with water deficiency. The present study has shown that drought stress treatments increased soluble sugars and starch levels in our pistachio cultivars, although relative water content was only moderately affected. WUE increased with the higher content of osmotically active molecules, i.e. soluble sugars and this increase was still present after a recovery of two weeks. The observed…

Acknowledgements

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> …Although continuous monitoring of photosynthetic carbon fixation and stomatal conductance is limited in the field (Ghrab et al., 2013; Klein et al., 2013), the time-integrated WUEi can be inferred using stable carbon isotope ratios (δ13C) of plant tissues, because photosynthetic carbon fixation and stomatal conductance is correlated with the ratio of intercellular to ambient CO_2 partial pressures (Ci/Ca) in C3 plants, which reflects the balance between stomatal conductance and the photosynthetic capacity (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984). Thus, leaf δ13C values can be used as a proxy for WUEi of C3 plants based on a positive correlation between δ13C values and WUEi (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984; Boyer, 1996; Dawson et al., 2002; Craven et al., 2013; Pascual et al., 2013; Esmaeilpour et al., 2016). Moreover, foliar δ13C values can be used to evaluate plant response to water stress within and across species and ecosystems and provide information about the physiological performance in response to changes in the plant water status (Centritto et al., 2002; Hasselquist et al., 2010; Esmaeilpour et al., 2016).…

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> …Pistachio production is concentrated in a few countries and the main producers are Iran, United States and Turkey (FAO, 2019). Numerous cultivars are grown in pistachio producing countries, which significantly vary for growth, physiology, resource utilization, nutrient accumulation, yield, quality and tolerance to adverse environmental conditions (Benhassaini et al., 2012; Ghrab et al., 2012; Kebour et al., 2012; Hajiboland et al., 2014; Esmaeilpour et al., 2016; Mehdi-Tounsi et al., 2017; Rahneshan et al., 2018a, 2018b). Pistachio trees comprise of two parts: the rootstock providing root system and the scion that produces the commercial crop.…

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