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Physiological responses of *Pistacia vera* L. versus *Pistacia atlantica* Desf. to water stress conditions under arid bioclimate in Tunisia

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ABSTRACT

Water stress represents the major factor that affects the growth and development of plants in the arid and semi-arid areas. To improve crop management, the selection of better yielding species under such condition is a principal strategy. In this study, the responses of two pistachio species were studied after water stress followed by re-watering. Indeed, the leaf water potential, relative water content, total chlorophyll content and leaf gas exchanges were assessed during water stress and re-watering. The results showed that, under water stress, *Pistacia atlantica* Desf. maintained water status, leaf gas exchanges and total chlorophyll content stable compared to *Pistacia vera* L., which experienced a great decrease. After rehydration, *P. atlantica* showed fast recovery of stomatal parameters, compared to *P. vera*, suggesting a good tolerance to water stress. The variation of *P. vera* and *P. atlantica* responses to water stress and re-watering suggested the higher adaptation of *P. atlantica* to water stress compared to *P. vera*.

1. Introduction

Water stress is one of the most important environmental factors limiting plant growth and production, especially in arid and semi-arid areas (Gorai et al., 2015). It is known to induce many physiological changes in plants. It has been reported that water stress often reduces leaf water status through a decline in leaf water potential, relative water content. This effect depends largely on plant species and water stress severity (Rahimi et al., 2010; Rostami and Rahemi, 2013; Aref et al., 2013). Consequently, the leaf water status affects photosynthesis through the limitation of the efficiency of the photosystem II (PSII) activity (Fini et al., 2013).

Water stress results in photosynthesis disturbances (Mwanamwenge et al., 1999; Yordanov et al., 2000). The decrease in photosynthetic rate may result from stomatal and biochemical limitations (Wise et al., 1992; Angelopoulos et al., 1996; Flexas and Medrano, 2002; Lawlor and Cornic, 2002; Yordanov et al., 2003). The stomatal limitation of photosynthesis is a primary event (Lefi et al., 2004), which is then followed by the adequate changes of photosynthetic reactions (Zlatev and Yordanov, 2004). Indeed, the plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration (Cornic, 2000; Lawlor, 1995; De Souza et al., 2013). As a consequence, the input and diffusion of CO_2 into the leaf is limited (Flexas et al., 2006). The biochemical limitations of photosynthesis has been attributed to reduced carboxylation efficiency (Jia and Gray, 2003), reduced ribulose-1,5-bisphosphate (RuBP) regeneration (Tezara and Lawlor, 1995), reduced amount of functional Rubisco, ATP synthase (Tezara et al., 1999; Nogués and Baker, 2000), adenosine triphosphate (ATP) synthesis, or to the inhibited functional activity of PSII. Concomitantly, inhibition or damages in the primary photochemical and biochemical processes may occur (Lawlor, 2002). The factor limiting photosynthesis during water stress can vary according to species (Galmés et al., 2007), the degree of induced stress. Therefore, the ability to maintain photosynthesis under water stress is of major importance in water stress tolerance (Li et al., 2011).

Under arid conditions, plants can respond to water stress by morphological and physiological changes with modifications that allow the plant either to avoid the stress or increase its tolerance (Chaieb et al., 1992). These adaptations depend largely on species. Plants have developed various mechanisms to withhold or partially reduce the negative effect of drought (Allakhverdiev and Murata, 2004; Kalaji and Loboda, 2009). Examples include the escape from water stress by fast vegetative growth, dehydration avoidance by maintaining hydration or development of physiological tolerance to water stress (Levitt, 1980; Kozlowski et al., 1991; Jones,

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1992; Larcher, 1995; Valladares et al., 2008; Berger et al., 2010). Therefore, it is extremely important to study the mechanism of plant adaptation to water stress. This is necessary especially in arid and semi-arid environments.

Under water stress, the potentiality to recovery is an important component of plant's response (Chaves et al., 2003; Flexas et al., 2004; Miyashita et al., 2005). It determines plant establishment in the future. Many studies have shown that after re-watering, leaf water potential recovers earlier than the physiological components (Abdallah and Chaieb, 2006; Rahimi et al., 2010; Gorai et al., 2015). In the contrary, stomatal conductance showed the slowest recovery. Although the mechanism is unclear, it has been related to lower leaf water potential (Brodribb and Cochard, 2009) and to the accumulation of abscisic acid (ABA) during water stress, which stands against gs recovery (Davies and Zhang, 1991; Lovisolo et al., 2008). The photosynthesis recovery depends on the rate and degree of photosynthesis decline during water withholding and depending on species (Galmés et al., 2007; Flexas et al., 2009), raising from fast and complete recovery under moderate stress to low or never complete after severe stress (Flexas et al., 2006; Chaves et al., 2009). Chaves et al. (2009) reported that since the studies of Kirschbaum (1987, 1988), photosynthesis recovery following water stress has been rarely studied.

The pistachio response to water stress has received little attention in previous studies. There are few works studying the water relations of this species (Behboudian et al., 1986; Gijón et al., 2010). In Tunisia, the pistachio genus include, in addition to *P. vera* which produces edible nuts two species, *Pistacia atlantica* and *Pistacia lentiscus*. In fact, they have other agronomic and ecological interests and are used as rootstocks (Jacquy, 1973), but they are threatened by disappearance. This disappearance is related, on the one hand, to the difficulties of germination of seeds under normal conditions, and, on the other hand, to the spontaneous characters of these species. *Pistacia atlantica* has a great capacity for adaptation to the difficult environmental conditions of the arid and semi-arid regions (Abbaspour et al., 2012). Consequently, it is used as the principal and the best rootstock of *P. vera* (Gijón et al., 2010). These improvements are related to the particular ecophysiological characteristics of *P. atlantica*, which remain unknown.

Therefore, the objectives of this study are (1) to evaluate the response of *P. vera* and *P. atlantica* species to water stress and rewatering, (2) to compare their adaptation to water availability in the soil and their recovery potentiality and (3) to identify the mechanisms of their adaptation to water stress.

2. Materials and methods

2.1. Plant material and water stress treatments

The experimentation was carried from September 2011 to June 2012. *Pistacia vera* L. and *P. atlantica* Desf. seedlings were grown in 11 L plastic pots. The pots were filled with a mix of soil, sand, peat, and gravel in 45:40:10:5% respectively. The soil has a water content of 17%. All plants were watered twice a week. The plants in pots were putted under a plastic cover allowing air movement from two sides of the experimental field but protected from rainfall. The pots were isolated from the soil so that the root system of the plants had no access to rain water.

After 6 months, the seedlings were used for the water stress treatment, which was developed gradually by withholding water. We have not measured the canopy volume of studied species, but according to our observations and experiments, canopy species had a very similar volume, especially in juvenile stage.

The number of repetitions is equal to 12 for each treatment (irrigated, stressed and re-watered treatment). After a period of 40 days, six stressed seedlings were harvested and the six others were re-watered. Measurements were taken during the period March-June 2012. Temperature ranging between 25 and 30 °C.

2.2. Ecophysiological parameters measurements

2.2.1. Leaf water potential (Ψ_L)

Leaf water potential, was measured on youngest and fully expanded leaves with an HR-33T dew point microvoltmeter in combination with a C-52 sample chamber, (Wescor, Inc. USA), by the method of dew point. Five measurements were taken at 11 h during water stress and stress recovery periods.

2.2.2. Relative water content (RWC)

For each treatment, five leaves were cut from different plants at 11 h and directly weighed to determine their fresh weights (FW). Next, the leaves were soaked in distilled water for 24 h at 5 °C. Then, the leaves were weighed again to determine their turgid weights (TW). Finally, the leaves were dried in the drying oven at 85 °C and weighed after 48 h to obtain their dry weights (DW). The relative water content (RWC) was determined at the number of five repetitions using the expression:

$$\mathrm{RWC} = [(\mathrm{FW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW})] \times 100.$$

2.2.3. Analysis of gas exchanges

Gas exchange analysis was carried out on five youngest and fully expanded leaves from different plants, often healthy leaves and at the same physiological age (4th leaf), after the first leaf of emergency), using the LCi portable device of photosynthesis (ADC BioScientific Ltd., USA). This system made it possible to measure the rate of net photosynthesis (A, μmol m^{-2} s^{-1}), the stomatal conductance (gs, mol m^{-2} s^{-1}), the transpiration rate (E, mol m^{-2} s^{-1}), internal CO_2 concentration (Ci, μmol m^{-2} s^{-1}). These measurements were taken at the same Photosynthetic Active Radiation (1000 μmol m^{-2} s^{-1}) at 10 h on sunny days. Instantaneous water uses efficiency (A/E), intrinsic water uses efficiency (A/gs) and mesophyll efficiency (A/Ci) were calculated as fractions of net photosynthesis rate and transpiration rate (A/E), stomatal conductance (A/gs) and internal CO_2 concentration (A/Ci), respectively (Maseyk et al., 2008). The total chlorophyll content was measured on the same leaves used for leaf gas exchanges using the total chlorophyll content meter CCM 200 (Opti-Sciences, USA). Leaf specific weight (LSW = DL/LA), expressed in gcm^{-2}, was continuously estimated on the same leaves that were used for leaf gas exchange assesment for each measurement, where (DL) is the dry mass of the leaf and (LA) is the corresponding leaf area (Baret and Fourty, 1997). The number of repetition is equal to 6.

2.3. Statistical analyses

Data were analyzed using the analysis of variance (ANOVA) procedures according to a factorial model with fixed factors (day of treatment, species, treatment (water stress or rehydration) with the SPSS (Statistical Package for the Social Sciences, SPSS Institute Inc., Cary, NC, USA) base 11.5 software. Means are presented with the standard errors of the mean and significance expressed at $p < 0.05$. Duncan test one ANOVA factor was used to compare means in each date.