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Research Article

Physiological and biochemical responses at leaf and root levels in two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration

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Abstract

To set-up afforestation and reforestation projects in arid regions southern Tunisia, several indigenous and exotic forest species were used among them are *Acacia* spp. However, the success of these projects remains highly sceptical because of the intensified aridity during the last decade. To overcome this issue, the selection of genotypes resistant to severe drought is crucial as first step. For this reason, the aim of the present study is to compare tolerance capacity to severe drought between two *Acacia* species (*A. cyclops* and *A. salicina*) and evaluate efficacy of their biochemical responses at leaf and root levels. Combined physiological and biochemical approaches were adopted. Two-years-old plants of two *Acacia* species (*A. cyclops* and *A. salicina*), frequently used in forestation projects in arid regions southern Tunisia, were subjected to severe water stress by withholding watering during 60 days. At regular intervals, water relations and net photosynthetic rate (P_n) were measured. In addition, the biochemical response was characterized by quantifying one sugar alcohol (arabitol) and three cyclitols (myo-inositol, pinitol and quercitol) in leaves and roots. Our results revealed that *A. cyclops* was more tolerant to severe drought than *A. salicina*. The turgor of its leaf tissues and its P_n were less affected. The superiority of *A. cyclops* to tolerate severe water stress might be attributed to greater efficiency of its biochemical defense mechanisms compared to *A. salicina*. Comparison of biochemical profiles between species exhibited some differences depending on the organs and the species. For development and survival under severe drought conditions, *A. cyclops* accomplished efficient osmoregualation and osmoprotection mechanisms by massive accumulation of specific polyols distinctly in leaves and roots. Indeed, compared to *A. salicina*, *A. cyclops* accumulated higher amount of arabitol, myo-inositol and quercitol in roots, but pinitol in leaves. So, contents of these polyols might be used as promising criteria for the selection of drought-tolerant *Acacia* species.

Keywords: *Acacia* sp.; arabitol; cyclitols; drought tolerance; forestation; photosynthesis; water relations

Introduction

The water deficit was the major factor causing ligneous mortality over the past century (Allen *et al.*, 2010; McDowell, 2011). Its enhanced aridity degree and lead to desertification phenomenon. As a

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Mediterranean country, Tunisia was highly affected by aridity. Desertification, deforestation and anthropogenic activities affected the vegetal cover over semi-arid and arid regions southern Tunisia (Le Houérou, 1959; Floret and Pontanier, 1982). 25% of the Tunisian territories are affected by desertification (DGF, 2010; Fetoui *et al.*, 2015). Face to this ecological threat, the protection of fragile arid zones and their rehabilitation are mandatory. The success of any afforestation or reforestation project required co-existence of several key factors among them are the use of suitable plant species. These species should be principally drought-resistant. Many autochthonous or exotic species can be used. The xerophytic plants of *Acacia* had a great ecological value to restore aridity-affected zones (Maslin and McDonald, 2004; Yang *et al.*, 2009). There are almost 1 380 species of *Acacia* found throughout the world. They are distributed as around 1000 species in Australia, 144 species in Africa, 89 species in Asia, and about 185 species in North and South America (Lorenzo *et al.*, 2010). These nitrogen-fixing leguminous have high ability to adapt to various climates, and so to be suitable plants for afforestation of new areas and rehabilitation of degraded areas (Noumi *et al.*, 2011). They are greatly tolerant to water deficit and exhibit fast-growing characteristic (Oba *et al.*, 2001; Aref *et al.*, 2003; Yelenik *et al.*, 2004). In southern arid of Tunisia, a strategic rehabilitation program was launched since 1988. It was based on the introduction of many species of *Acacia* like *Acacia cyclops* and *Acacia salicina* (Zaafouri, 1993). The principal goal of this program was to prevent erosion by soil stabilization (Genin, 2006; Ouessar *et al.*, 2009).

Acacia species adopt various water-use strategies and mechanisms allowing them to sustain water shortage and to maintain cell turgor (Aref and El-Juhany, 1999). They involved an immediate response by reducing stomatal aperture which cope with water deficit conditions (Warren *et al.*, 2011; Kebbas *et al.*, 2015). However, the decline of stomatal conductance reduced photosynthetic assimilation rate in many *Acacia* species (Lassouane *et al.*, 2013; Kebbas *et al.*, 2015). Several in-vivo studies demonstrated that dehydration caused damages to their PSII reaction centres (Yu and Ong, 2002; Kebbas *et al.*, 2015).

To avoid or tolerate dehydration, plants involved various defence mechanisms acting at different levels. These mechanisms are generally synergic and complementary converging to enhance plant resistance to water deficit. The biochemical response, generally allowing to physiological adaptations, is among the main defence tools against drought (Albouchi *et al.*, 1997).This response is performed by the coordination of series of specific biochemical mechanisms in roots as well as in leaves. Indeed, the osmotic adjustment acts in plants under drought conditions and it can allow to a dehydration avoidance strategy (Turner *et al.*, 1986; Warren *et al.*, 2011). In *Acacia* plants, the adjustment of internal osmotic potential is possible thanks to the active accumulation of organic solutes (Liu *et al.*, 2008; Chen *et al.*, 2011; Kebbas *et al.*, 2015). However, a genetic variability in net solute accumulation under water stress has been reported between many *Acacia* species suggesting its use as criteria for selecting droughts resistant genotypes (Chaves *et al.*, 2003; Warren *et al.*, 2011). The accumulation of soluble sugars and sugar alcohols is a common metabolic response in many *Acacia* species to alleviate water stress (Otieno *et al.*, 2005; Chen *et al.*, 2011). Cyclitols are a group of sugar alcohols that occur with appreciable concentrations in a wide range of *Acacia* species (Liu *et al.*, 2008; Warren *et al.*, 2011). They accumulate osmotically to significant concentrations in leaves of a range of Australian tree species under water stress condition, suggesting their osmoregulatory function (Merchant and Adams, 2005; Adams *et al.*, 2005). In addition, these polyols have further 'osmoprotective' and antioxidant roles in plant tissues (Orthen and Popp, 2000). Several polyols belong to the cyclitols pool, such as pinnitol, myo-inositol, quercitol and arabitol (Nguyen and Lamant, 1988). Pinitol is the major cyclitol accumulated in mature leaves of many *Acacia* species subjected to water deficit (Rontein *et al.*, 2002; Griffin *et al.*, 2004).

Although, there are some studies investigate cyclitols accumulation in leaves of *Acacia* trees, but no study has interested to quantification of these metabolites in roots under drought conditions. Determining profile of cyclitols in roots and leaves is crucial to highlight biochemical response at whole plant scale in severely water-stressed *Acacia* species. Considering the frequent use and the multiple benefits of *Acacia* species in forestation projects in arid regions of Tunisia, it is essential to study their biochemical defence mechanisms against drought. In fact, the main objectives of the present study are to compare capacity of tolerance to severe drought of two

Acacia species and to highlight biochemical profiles in their roots and leaves on term of sugar alcohol and cyclitols. In addition, we investigated the possibility to find use cyclitols as reliable criteria to select drought-tolerant genotypes of *Acacia*. To achieve these objectives, two *Acacia* species (*A. salicina* and *A. cyclops*), frequently planted during forestation projects in arid regions southern Tunisia, were used. During severe water-stress period, water relation parameters and net photosynthetic rate (P_n) were measured, as well as arabitol, pinitol, myo-inositol and quercitol were quantified in roots and leaves.

Materials and Methods

Plant material and treatments

Two-year-old rooted cuttings of *Acacia salicina* (Lindl.) and *Acacia cyclops* (A. Cunn. and G. Don) comparable in size (70-80 cm) were used in this study. They were provided by the 'Nursery of Zerkine' (Gabes, Tunisia). For each *Acacia* species, 15 plants were individually transplanted into 17-L pots filled with a mixture of sandy soil (12% coarse sand, 22% middle sand, 51% fine sand and 15% silt). They were watered to field capacity every day during one month after their transplantation. Following this initial period of acclimation, pots were covered with plastic film and aluminum foil to reduce evaporation from the soil surface and to minimize solar heating. Then, plants of both *Acacia* species were subjected to water stress by watering-off during 60 days. The experiment was conducted outdoor in the campus of the Faculty of Science of Gabes (Southern Tunisia: 33°50'N, 10°5'E) during September-November 2015. It was dry period and no-rain detected. At regular intervals, three plants from each *Acacia* species were randomly selected, and their water status and gas exchanges parameters were measured. After that, their leaves and roots were separated, dipped in liquid nitrogen and powdered then stored in a freezer (−30 °C) for biochemical analysis.

Plant water relations

The plant water status was characterized by measuring predawn leaf water potential (Ψ_{pd}), osmotic potentials (Ψ_s) and turgor potential (Ψ_p). Ψ_{pd} was measured early morning before sun-rise on three leaves by plant using Scholander pressure chamber (Model 1000, PMS Instrument Company, Albany, OR, USA) (Scholander *et al.*, 1965).

The osmotic potential (Ψ_s) was determined by the method of Nobel (1991). The same leaf used for measuring Ψ_{pd} was used for determining Ψ_s. To obtain cell contents, discs of 0.5 cm diameter obtained from fresh leaves were enclosed in 1 ml Eppendorf tube perforated at its base. The tube was immerged in liquid nitrogen for a few seconds, and then removed and left to thaw for 5 min; three freeze–thaw cycles were performed for each sample. The perforated Eppendorf tube was placed in another larger non-perforated tube which was centrifuged at 8000×g for 15 min at temperature of 4 °C. Cell extracts of leaf discs were collected in the larger tube. Ψ_s of cell extracts was measured using an osmometer (WESCOR, VAPRO model 5600, UT, USA). To express Ψs in MPa, the following equation was used:

$$\Psi s\ (\text{MPa}) = \frac{(\Psi s\ (\text{mosmol/kgH2O}) * 2{,}577433)}{1000} \qquad (1)$$

Turgor potential (Ψ_p) was calculated as the difference between predawn leaf water potential and osmotic potential:

$$\Psi p = \Psi pd - \Psi s \qquad (2)$$

Photosynthesis

The net photosynthetic rate (P_n, μmolm^{-2}/s^{-1}) was measured on mature leaves using the CI- 340 portable photosynthesis system (ADC BioScientific Ltd, Hoddesdon, UK). Measurements were done between 09:30-10:30 am under saturating light conditions at temperatures between 20-30 °C. The measurements were repeated three times for each of three leaves per plant. A total of three plants per *Acacia* species per measurement interval were used. So P_n value at each interval was the mean of 27 measurements.

Polyols

One sugar alcohol, arabitol, and three cyclitols (myo-inositol, pinitol and quercitol) were extracted from frozen-powdered samples of roots and leaves. Sample of 30 mg was mixed with 80% ethanol and heated to 80 °C for 30 min in a thermostatic bath. Then the mixture was centrifuged at 1000×g for 10 min. The supernatant was separated from the pellet before to be stored in 6 ml glass hemolysis tube. On the same pellet sample, a second extraction with 80% ethanol was performed for 15 min and a third one with 50% ethanol. The three supernatants containing the sugar alcohol and cyclitols were purified on a home–mode filtration cartridge on a vacuum filtration system. The filtrate was dried with the Speed Vacuum concentrator (Speed-Vac Plus SC110A, Thermo Savant, Holbrook, NY, USA). The dried sugars were re-solubilised in 1 ml pure water (Classic Labwater, Veolia water, Le Plessis-Robinson, France) in an ultrasonic bath (Bioblock Scientific, Germany) for 15 min. Arabitol, myo-inositol, pinitol and quercitol were assayed from 100 µl of the extract by High Performance Liquid Chromatography (HPLC, 817 BIOSCAN, METROHM, Herisau, Switzerland). The mobile phase, NaOH 0.2 N, is pumped at a flow rate of 0.6 ml min^{-1}. The temperature is adjusted to 37 °C. Arabitol, myo-inositol, pinitol and quercitol were identified and quantified using a calibration curve relative to each one.

Statistical analysis

Acacia plants of both species were arranged as a Completely Randomized Design with three replicates. All values of variables are the means of at least three replicates ±SE. The data were subjected to a statistical analysis of variance using GLM procedure of SAS software (SAS Institute 1999) followed by separation of means by Duncan Post-Hoc test with a level of significance P = 0.05.

Results

Plant water relations

At field capacity, potted plants of *A. salicina* and *A. cyclops* had similar Ψ_{pd} around 0.85 MPa (Figure 1). Water deficit caused a significant decrease in Ψ_{pd} for both *Acacia* species (P=0.0001). However, from day 22 of watering-off, *A. cyclops* plants exhibited slightly more negative Ψ_{pd} than *A. salicina* but this difference was not significant (P=0.65).

The plot Ψ_s as a function of Ψ_{pd} showed that Ψ_s value was about -1.65 MPa in both *Acacia* species when Ψ_{pd} was high and plants were well watered (Figure 2). However, their Ψ_s decreased significantly (P=0.0001) as water stress intensified. *A. cyclops* declined its Ψ_s more acutely than *A. salicina* throughout the studied range of Ψ_{pd} (P=0.039). This behavior indicated that *A. cyclops* accomplished higher accumulation of osmotica than *A. salicina*.

The significant difference in Ψ_s between the two studied *Acacia* species despite the no-significant difference between their Ψ_{pd} may explain the significant difference between their leaf Ψ_p (Figure 3). Water deficit affected Ψ_p more acutely in *A. salicina* than in *A. cyclops* ($p < 0.0001$). Indeed, *A. salicina* loses cell turgor (Ψ_p=0 MPa) starting from Ψ_{pd} of -1.86 MPa, but *A. cyclops* maintained positive turgor ($0<\Psi_p$) until Ψ_{pd} of -4.16 MPa. This finding may affirm the superiority in resistance to water stress of *A. cyclops* compared to *A. salicina* throughout the studied water stress range.

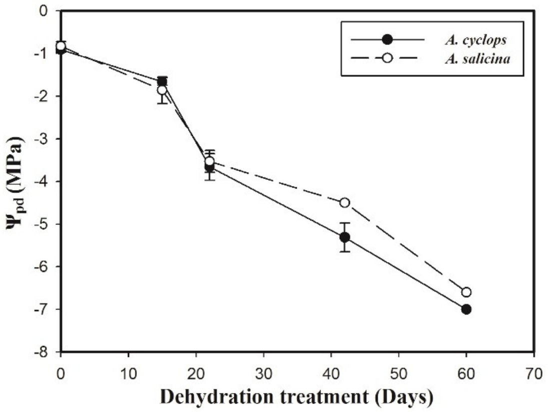

Figure 1. Evolution of predawn leaf water potential (Ψ_{pd}) as a function of dehydration treatment time (days) in two *Acacia* species (*A. cyclops* and *A. salicina*)

Each point is the average of 3 replicates and the vertical bars indicate SE.

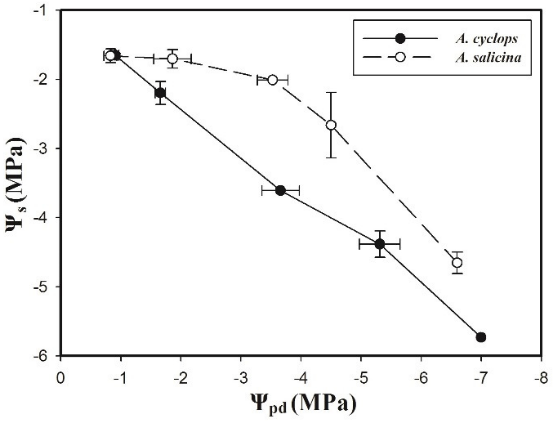

Figure 2. Variation of osmotic potential (Ψ_s) as a function of predawn leaf water potential (Ψpd) in two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period

Each point is the average of 3 replicates and the vertical bars indicate SE.

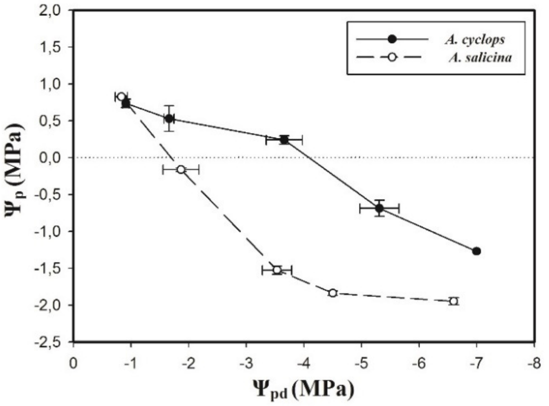

Figure 3. Variation of turgor potential (Ψ_p) as a function of predawn leaf water potential (Ψ_{pd}) in two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period

Each point is the average of 3 replicates and the vertical bars indicate SE.

Photosynthesis

Evolution of net photosynthetic rate (P_n) as function of plant water status (Ψ_{pd}) showed inter-specific difference (Figure 4). Under well watering conditions, *A. cyclops* plants accomplished higher P_n (17.91 µmol m^{-2} s^{-1}) than *A. salicina* plants (13.56 µmol m^{-2} s^{-1}). Water deficit affected significantly P_n in both studied *Acacia* ($p < 0.0001$). However, for the same leaf hydration (Ψ_{pd}) *A. cyclops* always exhibited higher P_n than *A. salicina*. So, the significant difference in P_n between studied *Acacia* species ($P=0.05$) throughout the Ψ_{pd} range testify the superiority of *A. cyclops* compared to *A. salicina* in resistance to drought.

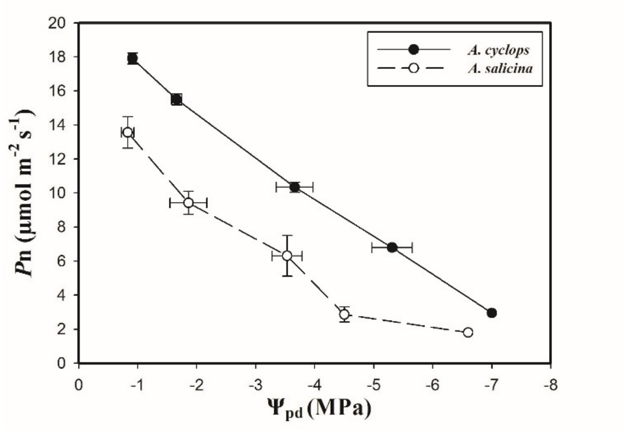

Figure 4. Variation of net photosynthetic rate (P_n, µmol m^{-2} s^{-1}) as a function of predawn leaf water potential (Ψ_{pd}) in two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period Each point is the average of 27 measurements and the vertical bars indicate SE.

Contents of arabitol and cyclitols in roots and leaves

Arabitol and three cyclitols (myo-inositol, pinitol and quercitol) accumulated distinctly in roots and leaves of water-stressed *Acacia* plants depending to plant species and metabolite type. Indeed, both studied *Acacia* species didn't accumulate arabitol in their leaves during drought treatment (Figure 5). However, arabitol content increased greatly in roots of '*A. cyclops*' according to drought stress intensity, but the content of this metabolite was very weak in roots of *A. salicina*. Concerning cyclitols, myo-inositol content increased strongly according to drought stress intensity in leaves of *A. cyclops* and slightly in leaves of *A. salicina* within the range 0.5-2.5 mg g^{-1} DW (Figure 6). In roots, *A. salicina* didn't involve myo-inositol in response to water scarcity, but *A. cyclops* accumulated high quantity of this cyclitol (7-19 mg g^{-1} DW) according to drought intensity. At the most severe water stress level, in *A. cyclops*, myo-inositol content in roots was eight times than that in leaves.

In overall, both *Acacia* species used pinitol in response to drought specifically at leaf level (Figure 7). Pinitol content in leaves increased according to Ψ_{pd} but more strongly in *A. cyclops* than in *A. salicina*. In the most stressed plants, pinitol content was four times than that in well-watered plants for *A. cyclops*, but just two times for *A. salicina*. In addition, water stress induced accumulation of pinitol in roots of both *Acacia* species. Content of this cyclitol was similar in roots of the two studied *Acacia* species until Ψ_{pd} of -3.3 MPa. Whereas further dehydration ($\Psi_{pd} < -3.3$ MPa) enhanced pinitol content more in roots of *A. salicina* than *A. cyclops*.

Concerning the third quantified cyclitol, quercitol, a significant difference existed in accumulation of this metabolite between leaves and roots depending to species (Figure 8). *A. salicina* didn't used quercitol in their roots and leaves in response to drought. However, in *A. cyclops*, no quercitol was detected in leaves, but it was acutely accumulated in its roots. In this species, quercitol content in roots of water-stressed plants was seven-times than that in roots of well-watered plants. In addition, quercitol content (37.08 mg g^{-1} DW) in roots of water-stressed *A. cyclops* was the highest compared to contents of all quantified metabolites in this study.

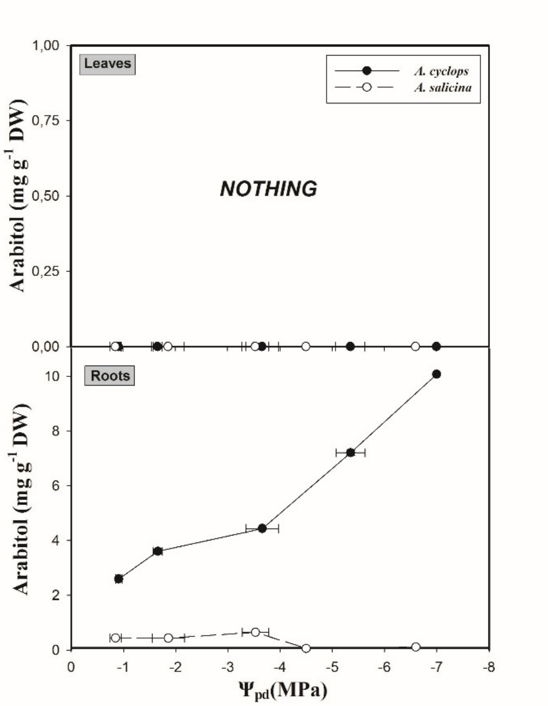

Figure 5. Variation of arabitol content as a function of predawn leaf water potential (Ψ_{pd}) in leaves and roots of two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period
Each point is the average of 3 replicates and the vertical bars indicate SE.

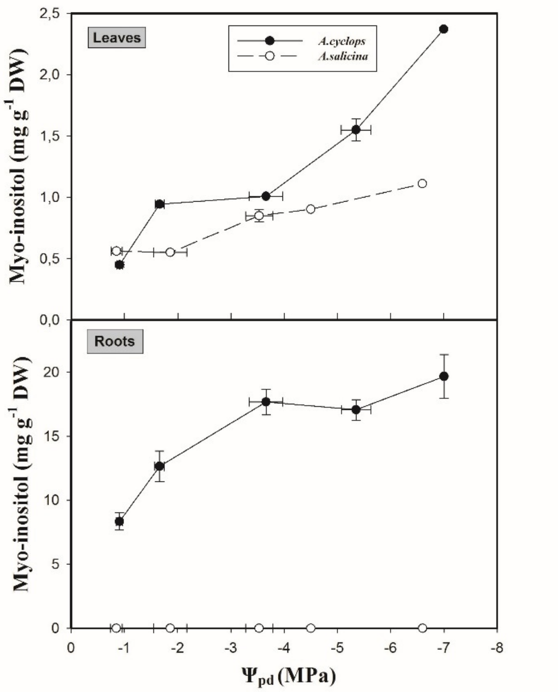

Figure 6. Variation of myo-inositol content as a function of predawn leaf water potential (Ψ_{pd}) in leaves and roots of two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period
Each point is the average of 3 replicates and the vertical bars indicate SE.

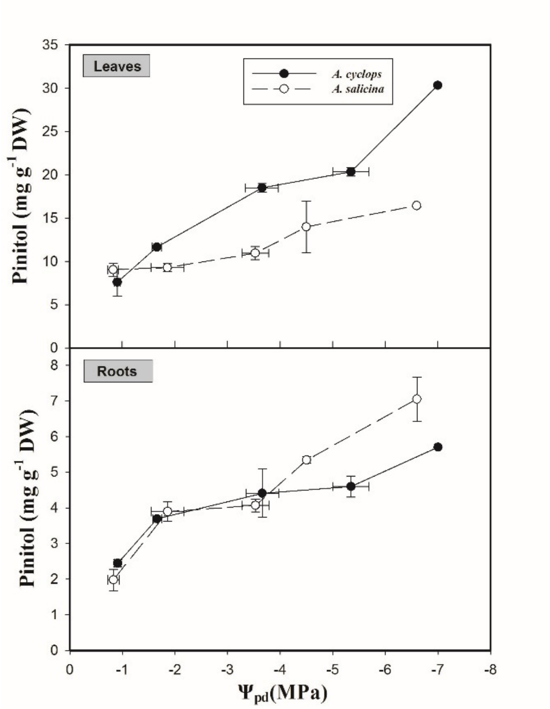

Figure 7. Variation of pinitol content as a function of predawn leaf water potential (Ψ_{pd}) in leaves and roots of two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period
Each point is the average of 3 replicates and the vertical bars indicate SE.

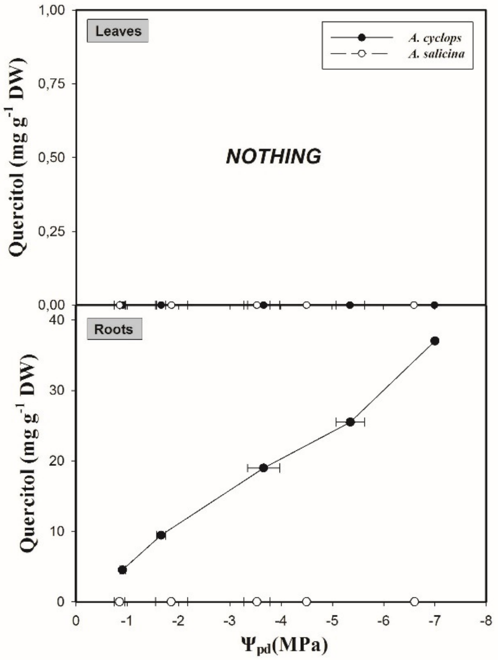

Figure 8. Variation of quercitol content as a function of predawn leaf water potential (Ψ_{pd}) in leaves and roots of two *Acacia* species (*A. cyclops* and *A. salicina*) subjected to dehydration treatment period
Each point is the average of 3 replicates and the vertical bars indicate SE.

Discussion

Like other xerophytic species, *Acacia* has the ability to defy water deficit and survive under conditions with limited water supply and high evaporative demands (Ramoliya and Pandey 2002; Abbas *et al.*, 2016). It displays a wide range of genetic traits involving biochemical and physiological adaptations (Otieno *et al.*, 2005; Gimeno *et al.*, 2010; Warren *et al.*, 2011). The present study aimed to assess the physiological behaviour of two *Acacia* species (*A. salicina*, *A. cyclops*) subjected to water deficit and to compare their biochemical response at leaf and root levels. Results showed differences depending to species, organ and biochemical metabolites involved in drought defence mechanisms.

Water deprivation had significant effects on plant water relations in the two *Acacia* species. Ψ_{pd} is a primary indicator of the degree of plant's stress under drought conditions (McCutchan and Shackel, 1992). Water deficit significantly affected Ψ_{pd} of *A. salicina* and *A. cyclops*. Indeed, the restriction of water supply for 60 days dropped Ψ_{pd} to -7 MPa and -6.6 MPa in A. *cyclops* and *A. salicina*, respectively. This is generally a common impact for drought on most ligneous species as *Acacia* tree (Otieno *et al.*, 2005; Donoso *et al.*, 2011; El Atta *et al.*, 2012), *Quercus pubescens* (Galmés *et al.*, 2007), *Quercus suber* (Aranda *et al.*, 2007) as well *Eucalyptus globulus* (Guarnaschelli *et al.*, 2003). However, in our study, from day 22 of watering-off, *A. cyclops* plants exhibited slightly more negative Ψ_{pd} than *A. salicina* but this difference was not significant. In fact, in plant tissue, water potential is principally composed by two elements: osmotic potential (Ψ_s) and turgor potential (Ψ_p). By examining Ψ_{pd} components, we remarked that *A. cyclops* exhibited higher Ψ_p than *A. salicina* associated with more negative Ψ_s in the former species than in the later one. This variability in physiological behaviour may prove difference in biochemical response testifying that *A. cyclops* accomplished stronger osmoregulation than *A. salicina*. Many studies demonstrated that decreased Ψ_s is a common response within the genus of *Acacia* under drought stress (Donoso *et al.*, 2011; El Atta *et al.*, 2012). In *A. saligna* and *A. radianna*, water stress led to more negative Ψ_s and increased accumulation of osmotically active solutes in leaves (Nativ *et al.*, 1999; Kebbas *et al.*, 2015).

In our study, *A. cyclops* plants maintained positive cell turgor ($0<\Psi_p$) until Ψ_{pd} of -4.16 MPa. On contrary, *A. salicina* lost cell turgor (Ψ_p=0 MPa) starting from Ψ_{pd} of -1.86 MPa. Positive plant cell turgor is prerequisite factor for plant growth and survival (Jaleel *et al.*, 2009). In fact, photosynthetic activity is the primary physiological processes greatly affected during water stress episode (Chaves, 1991; Sapeta *et al.*, 2013). Our results showed that water deficit caused P_n decrease in both *Acacia*, but intra-specific difference existed. Indeed, *A. cyclops* had higher photosynthetic activity than *A. salicina* through whole the studied water status range. Wujeska-Klause *et al.* (2015) noticed that P_n decreased in *A. aneura* subjected to dehydration cycle. Similar behaviour was reported in *A. mangium* (Novriyanti *et al.*, 2012). The greater photosynthetic activity of *A. cyclops* compared to *A. salicina* might be related to efficiency of its defence mechanisms against drought. These mechanisms involved those avoiding dehydration by improving water supply at root level and so well hydration of leaf mesohyll as well as those preserving structural and functional integrities of membranes and macromolecules of photosynthetic machinery. One of the key tools of drought tolerance in higher plants is osmotic adjustment leading to osmoregulation and osmoprotection mechanisms. This biochemical response might be accomplished by accumulation of numerous osmotic compounds including inorganic ions and organic solutes (Wu and Xia, 2006; Jabeen and Ahmad, 2012). Sugar alcohols and cyclitols were among these solutes. They play important roles in cell functioning because they are involved in signal transduction, cell wall formation, osmoregulation and anti-oxidation response (Merchant *et al.*, 2006; Donahue *et al.*, 2010). Because their hydroxyl groups, these polyols may mimic the structure of water and maintain structural integrity of membranes and macromolecules (Schobert, 1977). In *Acacia* from dry areas, sugar alcohols and cyclitols (myo-inositol, quercitol, pinitol) dominated leaf and root metabolites profiles (Griffin *et al.*, 2004; Liu *et al.*, 2008; Warren *et al.*, 2011). Results of our study revealed significant differences in accumulation of these metabolites depending to species and organs (leaf and root) in drought-stressed *Acacia* plants.

The polyol arabitol accumulated significantly only in roots of water-stressed *A. cyclops* plants. Our finding was in the same line with Shvaleva *et al.* (2006) showing a great accumulation of arabitol in roots of *Fagus sylvatica* under water stress conditions. Under moderate water stress, *A. cyclops* accumulated slightly arabitol in its roots, but when the stress become severe, arabitol content increased acutely. This trend in arabitol accumulation indicated the involvement of the solute in osmorgulation but more strongly in osmoprotection at root level in *A. cyclops*. In addition to arabitol, the biochemical response against drought of both studied *Acacia* species involved cyclitols distinctly in their leaves and roots. Drought-stressed plants of *A. cyclops* accumulated pinitol and myo-inositol in their leaves as well as in their roots according to stress intensity. Pinitol was highly accumulated in other water-stressed species of *Acacia* genera like *A. crassicarpa* (Xu *et al.*, 2007), *A. auriculiformis* (Liu *et al.*, 2008) and *A. cyanophylla* (Albouchi *et al.*, 1997). In addition, our findings about *Acacia* were similar to those obtained in Eucalyptuses showing an accumulation of myo-inositol in the leaves of four Eucalyptus species (*E. regnans*, *E. arenace*, *E. socialis* and *E. delegatensis*) under water stress condition (Warren *et al.*, 2011). However, contents of pinitol and myo-inositol in *A. cyclops* plants were higher than those in water-stressed plants of *A. salicina*. Pinitol accumulation exhibited inter-organ difference. Indeed, in both species pinitol content in leaves was around five times more than that in roots. This may suggest the importance of pinitol in the osmoprotection of leaf tissue under dehydration-stress. Myo-inositol was totally absent in *A. salicina* roots. In *A. cyclops* it was 10 times higher in roots than in leaves. So, in *A. cyclops* roots, myo-inositol might be highly involved in the osmoregulation mechanism. Increasing concentrations of osmotically active solutes may have greater significance in roots because it is a major facilitation mechanism for water uptake from drying soils (Chaves *et al.*, 2003). All results concerning pinitol and myo-inositol accumulation in both studied *Acacia* species suggested that *A. cyclops* accomplished stronger osmoregulation and osmoprotection reactions than *A. salicina* at leaf level and specifically in roots.

Accumulation of the cyclitol quercitol in the two studied water-stressed *Acacia* species showed distinctive result compared to all quantified metabolites. It was the most accumulated solute under severe drought intensity. Its content was 37 mg g^{-1} DW in roots of *A. cyclops*. Previews study demonstrated a great accumulation of quercitol and myo-inositol in roots of *Eucalyptus globulus* clones under water stress conditions (Adams *et al.*, 2005). Also Merchant *et al.* (2006) demonstrated that only Eucalyptuses from xeric environment highly accumulated quercitol in their roots. In our study, quercitol might be the major contributor to osmotic adjustment in roots of *A. cyclops*.

Conclusions

In conclusion, the physiological and biochemical properties at leaf and root level in plants of two *Acacia* species were affected by water availability. *A. cyclops* appeared more tolerant to drought than *A. salicina*. It maintained higher cell turgor and greater photosynthetic activity than *A. salicina*. The superiority in tolerance to severe drought of *A. cyclops* might be attributed to its efficient biochemical response at leaf and root levels. It accomplished stronger osmoregulation and osmoprotection mechanisms than *A. salicina* by great accumulation of polyols: arabitol, myo-inositol and quercitol in roots, and pinitol in leaves. So, accumulation of these polyols might be used as promising criteria for the selection of drought-resistant *Acacia* species suitable to conduct forestation projects in arid regions of Tunisia. However, to confirm this finding, further studies using more *Acacia* species are required.

Authors' Contributions

The manuscript was written through contributions of all authors. Conceived and designed the experiments: ME, SS and HK. Performed the experiments: SS and ME. Analysed the data: SS, ME and HK. Wrote the paper: SS, ME and HK.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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