academicJournals

Vol. 14(12), pp. 1020-1028, 25 March, 2015
DOI: 10.5897/AJB2014.14377
Article Number: 9FBF79251696
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of water deficit on Argan tree seedlings (*Argania spinosa* L. Skeels): Morphological and physiological aspect

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Received 17 December, 2014; Accepted 17 March, 2015

The Argan tree, *Argania spinosa* L., Skeels, is an endemic species in North-West Africa perfectly adapted to aridity and drought. It is in this context that we studied the physiological impact of water deficit on the Argan tree seedlings for eight weeks at a field capacity of 30%. The obtained results reveal that the stressed seedlings manifested by the strategy of the root elongation from the second week, the roots reached 31 cm compared with 15 cm of the control. However, the seedlings showed severe dehydration of 41% in leaves and 45% in roots. Besides, the content of chlorophyll pigments has relatively decreased from the second week, a slight yellowing and leaf drop was observed. The seedlings have accumulated proteins in a very significant way in leaves (from 25 to 107 mg.g^{-1} by fresh weight) and (from 23 to 90 mg.g^{-1} by fresh weight) in roots. Proline was also accumulated; the content was 4 and 2 µg.g^{-1} by fresh weight respectively in leaves and roots compared with 1.3 and 1.1 µg.g^{-1} ^{1} by fresh weight in control respectively. The accumulation of the protein and proline is higher in leaves than in roots. The content of malondialdehyde was higher in leaves than in roots. This increase is significantly related to the prolongation of the stress period from the second week. The enzymatic activity of peroxidase is in relative increase according to the duration of the water stress applied in both leaves and roots. We deduce from these results that Argan seedlings possess the characteristic of xeropyhte that tolerate aridity.

Key words: *Argania spinosa*, drought stress, proline, chlorophylls, proteins.

INTRODUCTION

The Argan tree, (*Argania spinosa* L. Skeels), is a tropical species from the Sapotaceae family which grows in North-West Africa. Its current natural area is limited in Morocco and in the Algerian southwest in Tindouf city

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(Nouaim, 2005). This species plays an important major ecological and socio-economical roles; the oil obtained from this plant is very rich in unsaturated fatty acid (Charrouf and Guillaume, 1998). The Argan tree is considered as being the least demanding tree of rainfall (Msanda et al., 2005). It is a xerophytic species which can survive at less than 120 mm of precipitation a year, as it can survive for several successive years of drought (Ferradous et al., 1996; Zahidi et al., 2013a). It is also a thermophilous species which is able to stand at very high temperatures reaching 50°C. Roots play an essential role in the survival of the Argan tree in dry climate. It has swiveling roots able to reach great depth to scoop out water (Mokhtari, 2002). Also, its physiological characteristic allows this tree to avoid water stress as its capacity of defoliation or partial closing of stomata (Faouzi, 2006; Berka and Aïd, 2009); no stomata are observed at adaxial leaf surface characterizing Argan as a hypostomatous species (Bani-Aameur and Zahidi, 2005). This tree loses its foliage only in case of a hard drought; dry conditions were spread over a longer period, trees are completely defoliated (Zahidi et al., 2013b) otherwise, the Atlantic ocean offers an important atmospheric humidity which allows the Argan tree to trap fresh air (Msanda et al., 2005). Tindouf city is an arid area where the average annual rainfall is recorded to 33.7 mm. This region has an average temperature of 22.90°C and the heat reaches its maximum in July to August until 52°C. The coincidence of the maximum heat with a period of rainfall deficiency causes a marked water stress in this region.

The Argan tree is maintained in Tindouf due to natural conditions remaining favorable in a residual ecosystem resistant but vulnerable in view of the water deficit. The nursery of Touiref Bouaâm belonging to the forest conservation of Tindouf (105 km to the north) offers some advantages in order to regenerate the Argan tree. The water deficit of the environment constitutes a constraint to circumvent in the development of the Argan tree by plantation that requires the mobilization of water resources. Under the conditions of water deficit, plants develop morpho-functional and physiological strategies to improve water absorption (Larcher, 1995). Many plants adapted to arid areas control only very little of their water loss through respiration, but they have a very major rooting able to extract water from the ground (Rivlland, 2003). The tolerance to the water constraint is a strategy that allows the plant to provide its physiological functions in spite of the degradation of its water state. It is the result of complex physical, biochemical and molecular mechanisms. The expression of various genes and the accumulation of various osmolytes coupled with an effective antioxidant system are often the principal mechanisms of tolerance to water deficit (Tardieu, 2005). Consequently, the environmental stresses including the water stress, is the appearance of an oxidative stress, that is, the accumulation of reactive oxygen species (ROS) which damages the cellular structures (Appel and Hirt, 2004). Under the optimal conditions, the leaves are equipped with enzymes such as: catalase and peroxidase and other antioxidant metabolites sufficient to face ROS.

It is for this aim that we have studied the effect of water stress on the Argan seedlings and their behavior (morphological and physiological) opposite this constraint. So, through the analysis of some stress markers such as proline or malondialdehyde that allow us to estimate the degree of damage caused by the water deficit, also the content of protein and peroxidase activity would evaluate the seedlings tolerance. We have also targeted to define the degree of the seedlings' resistance to the water deficit. Finally, *A. spinosa* needs to be reforested in the Algerian forest in order to ensure sustainable development of our forest, and this requires controlling plant growth's conditions especially at young stage in an arid environmental.

MATERIALS AND METHODS

Plant material and growth conditions

The Argan seedlings are obtained from the germination of kernels collected from Tindouf city to the north (27° 39' 15'' N latitude and 8° 9' 6'' W longitude and 450 m altitude) after soaking for 48 h in plain water (Meslem et al., 2009). The seedlings development continues under greenhouse up to one year in polyethylene bags of 15 and 8 cm of diameter containing original soil. Watering was done every week with tap water.

Water deficit experiment

Five batches of 15 seedlings (one year old each) are put under a water deficit in different durations of 2, 4, 6 and 8 weeks at 30% of field capacity (F.C). The control seedlings are watered every week. The field capacity was determined according to Côme and Corbineau (1998); we dried the soil completely in an oven. Later we weighed 1 kg that we put in a perforated cylinder on both sides, and then in a tank containing 1 L of tap water for 48 h. The water will rise surface by capillary phenomena. Finally, we measured the amount of water absorbed by the soil and this value corresponds to 100% of field capacity. At the end of the treatment, triplicate samples of leaves and roots are sorted out, washed with fresh distilled water and conserved at -80°C for further physiological analyses. The follow-up of growth was carried out throughout the water stress. The measurements of the stems' length were taken each week. However, the roots' length was measured at the end of the treatment.

Determination of relative water content (RWC)

The RWC was determined according to the method of Heller et al. (1998); the RWC was calculated as follows:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

FW = fresh weight; TW = Turgescent weight; DW = dry weight.

Chlorophyll determination

50 mg of fresh leaves were homogenized in 2 ml of ice-cold acetone 80°, then centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was used for the determination of chlorophyll pigments by spectrophotometer (6715 UV/Visible- JENWAY) at 645 and 663 nm. The chlorophyll (a) and (b) concentration were quantified as mg. gr^{-1} FW according to Arnon (1949) formulae:

Total chlorophyll = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a = 12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b = 22.9 (A_{645}) - 4.68 (A_{663})

Estimation of soluble total proteins

Fresh biomass was homogenized in 0.06 M phosphate-buffer (pH 7.8) with PVP (polyvinylpyrrolidone) and then centrifuged. Proteins concentration was determined as described by Bradford (1976) method; 2 ml of Bradford reagent was added to 100 µl of the supernatant or distilled water for the standard.

Determination of proline

300 mg of fresh biomass were homogenized in 10 ml of 3% sulfosalicilic acid, then centrifuged at 3000 rpm for 10 min. 2 ml of supernatant was added to 2 ml of solution contains: 1.5 g Ninhydrine, 30 ml of acetic acid and 20 ml of phosphoric acid; then heated at 80°C for 45 min. The mixture was cooled down in an ice-bath. Afterwards 5 ml of toluene was added (Monneveux and Nemmar, 1986). The proline concentration was calculated by absorbance at 520 nm using the molar extinction coefficient of 387.1 $mg.gr^{-1}$ and expressed as $\mu g.gr^{-1}$ FW according to Beer-Lambert's Law:

$$A_\lambda = -\log \frac{I}{I_0} = \varepsilon_\lambda \cdot \ell \cdot C.$$

I/I_0 is the transmittance of the solution; A is the absorbance or optical density at a wavelength λ; ε is the molar extinction coefficient; ℓ is the optical path length in the crossing solution, it corresponds to the thickness of the cuvette used and C is the molar concentration of the solution.

Estimation of lipid peroxidation (malondialdehyde content)

Lipid peroxidation was estimated by determining the malondialdehyde (MDA) contents in the leaves and roots according to the method of Rajinder et al. (1981). 100 mg of samples was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 *g* for 5 min at 4°C. Aliquot of 0.3 ml of supernatant was mixed with 1.2 ml of 0.5% thiobarbituric acid (TBA) prepared in TCA 20%, then incubated at 95°C for 30 min and cooled down in an ice-bath. The samples were centrifuged at 10 000 × *g* for 10 min at 25°C. The supernatant absorbance was then measured at 532 nm. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the molar extinction coefficient of 155 mM^{-1} cm^{-1} expressed in µM. g^{-1} of FW according to Beer-Lambert's Law.

The assay of peroxidase

The leaves and roots were homogenized in cold (4°C) 50 mM phosphate buffer (pH 6.0) (4 ml/g of biomass). The homogenate was rapidly centrifuged at 11 000 *g* for 15 min at 4°C. The supernatant was taken as the source of peroxidase, and the determination of the activity was performed immediately (Jackson and Ricardo, 1992). Peroxidase activity was determined in triplicate by measurement of the absorbance at 470 nm of tetraguaiacol (ε = 6.65 × 103 M^{-1} cm^{-1}) according to Beer-Lambert's Law. We used 1.5 ml of 1% guaiacol and 0.4 ml of 0.1 M hydrogen peroxide in 50 mM phosphate-buffer (pH 6.0) at 35°C and the reaction was started by adding 0.1 ml of peroxidase solution (Reuveni et al., 1992; Khan and Robinson, 1994).

Analysis of data

All data were analyzed in three replications and the obtained data are evaluated statistically using Student's t-test, and least significant difference (LSD) was calculated at $P < 0.05$ and signaled faces by letters (abc), knowing that; $P > 0.05$ not significant, $P < 0.05$ significant difference (a), $P < 0.01$ very significant difference (ab) and $P < 0.001$ highly significant (abc).

RESULTS AND DISCUSSION

The follow-up of growth (length of stems and roots)

Deficit water did not influence significantly the growth of the stems. In contrast, the root length increased significantly as the duration of the water deficit was prolonged. The difference was very significant from the fourth week; 15 and 31 cm were recorded between the control and the 8-week stressed seedlings respectively (Figure 1).

Relative water content (RWC)

The relative water content in the roots and the leaves decreased gradually depending on the prolongation of the water deficit duration. From the fourth week, RWC decreased significantly from 85 to 41% in the leaves and from 88 to 45% in the roots (Table 1).

Chlorophyll content

The chlorophyll pigments; total, (a) and (b) are sensitive to water deficit. The total chlorophyll content was 15 $mg.g^{-1}$ FW in the eight weeks. The difference was very significant which is about 66 $mg.g^{-1}$ FW for the control (Table 2). Also, a slight yellowing in leaves was observed from the 6^{th} week.

Total soluble protein content

The protein content increased significantly (Figure 2). This increase was important in the first weeks in the roots. However, from the sixth week, the protein content increased more importantly in the leaves. There was a

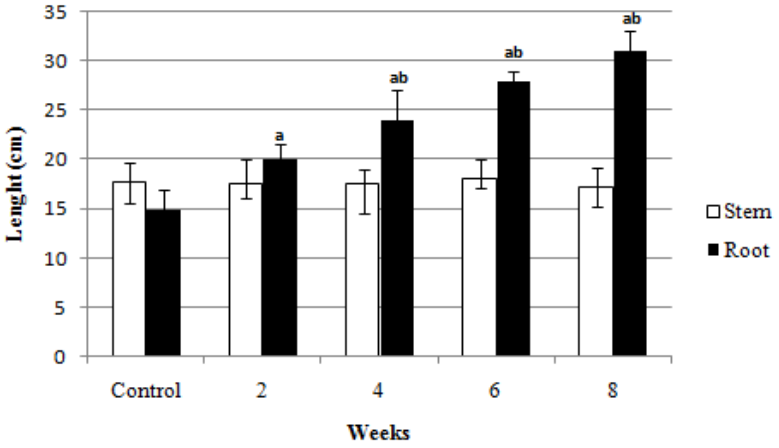

Figure 1. Effect of water deficit on the length of the 8-week stressed seedlings' stems and roots [P>0.05, not significant; P <0.05, significant difference (a), P <0.01 very significant difference (ab), and P<0.001 highly significant difference (abc].

Table 1. Effect of water stress on RWC (P>0.05 not significant, P < 0.05 significant difference (a), P <0.01 very significant difference (ab), and P<0.001highly significant difference (abc).

% RWC	Control	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Leaf	85±2	83±1	70±2^{a}	65±1^{ab}	41±1^{abc}
Root	88±1	85±2	73±1^{ab}	60±1.5^{abc}	45±1^{abc}

Table 2. Effect of water stress on chlorophyllian pigments content [P>0.05 not significant, P <0.05 significant difference (a), P <0.01 very significant difference (ab), and P<0.001highly significant difference (abc)].

Content in mg.g^{-1} FW	Control	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Chl tot	67.2 ± 0.02	66± 0.01	40±0.05^{a}	27.7±0.045^{abc}	15,2± 0.06^{abc}
Chl (a)	61.2±0.3	54.22±0.7	50.88±1.2	38.12±0.35^{abc}	30.24±0.3^{abc}
Chl (b)	91.2±0.15	52.18±0.07^{ab}	47.72±0.57^{ab}	39.4±0,41^{ab}	36.16±0.11^{ab}

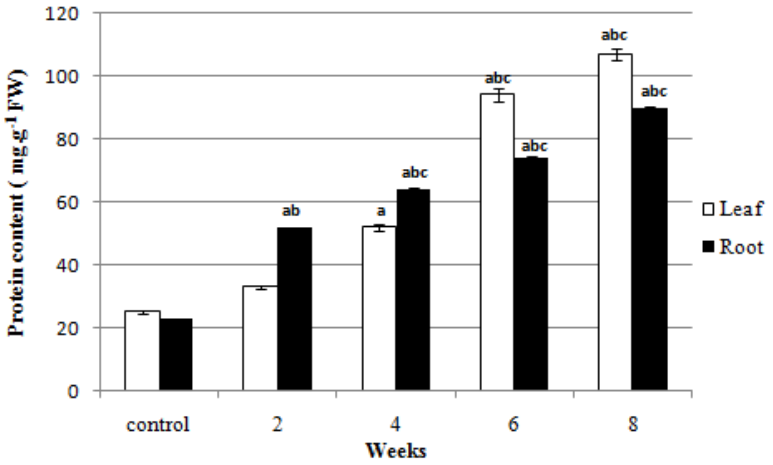

Figure 2. Effect of water deficit on the total soluble protein content [P>0.05 not significant, P <0.05 significant difference (a), P <0.01 very significant difference (ab), and P<0.001highly significant difference (abc)].

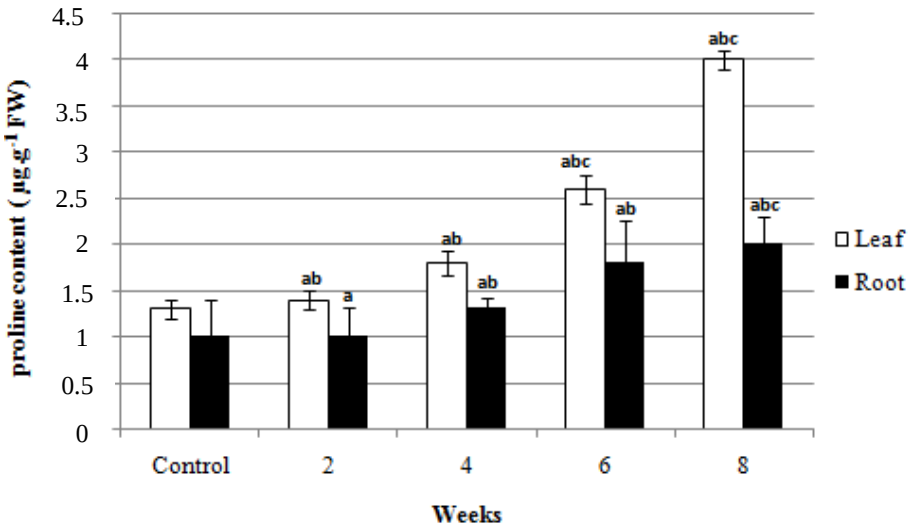

Figure 3. Effect of water deficit on proline content [P>0.05 not significant, P <0.05 significant difference (a), P <0.01 very significant difference (ab), and P<0.001highly significant difference (abc)].

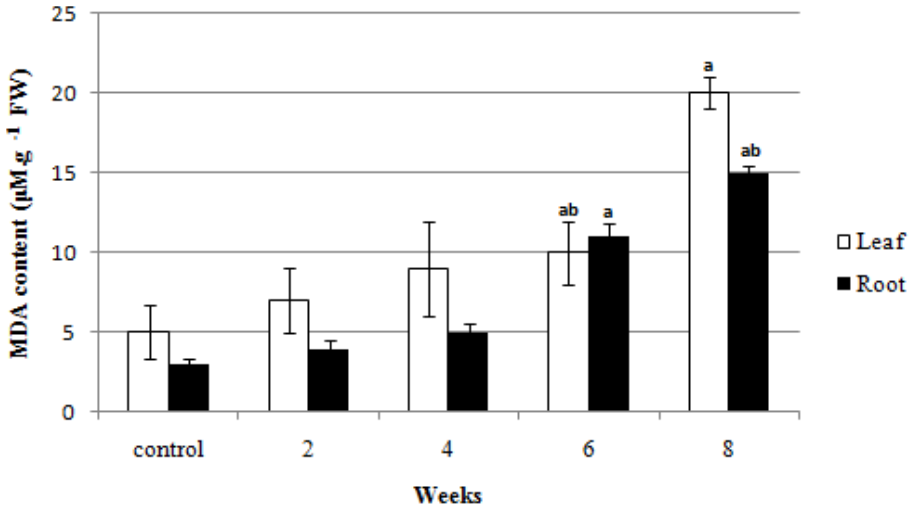

Figure 4. Effect of water deficit on the MDA content [P>0.05 not significant, P <0.05 significant difference (a), P < 0.01 very significant difference (ab), and P<0.001 highly significant difference (abc)].

positive correlation between the duration of the water deficit and the accumulation of total protein.

The proline content

Water deficit induced an accumulation of proline in leaves and roots (Figure 3); we recorded an increase from 1.3 to 4 µg.gr^{-1} FW in leaves and from 1 to 2 µg.gr^{-1} FW in roots relative to the control, respectively. This accumulation is significantly higher in leaves ($p< 0.001$) compared to roots for the eight weeks. But, in control seedlings MDA content was also higher in leaves; indeed the increase in MDA was higher in roots (x5) than in leaves (x4) with respect to controls.

The content of MDA (lipid peroxidation)

The lipid peroxidation was significantly higher in leaves than in roots compared to control; from 5 to 20 µM.g^{-1} FW and from 3 to 15 µM.g^{-1} FW (Figure 4). The amount of MDA accumulation has manifested significantly from the sixth week of stress ($p < 0.05$).

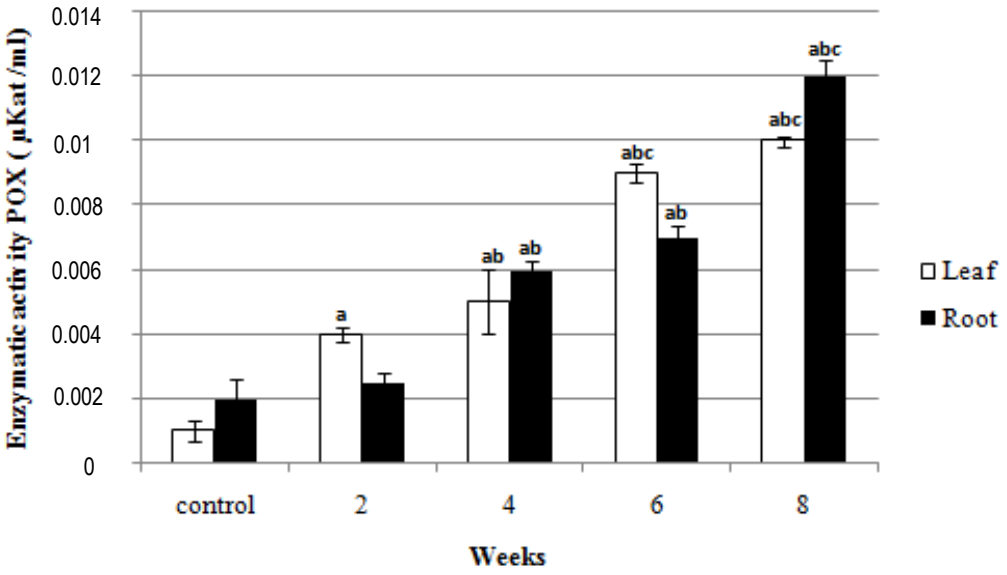

Figure 5. Effect of water deficit on the enzymatic activity of peroxidases [P > 0.05 not significant, P < 0.05 significant difference (a), P < 0.01 very significant difference (ab), and P < 0.001 highly significant difference (abc)].

Total enzymatic activity of peroxidase

The enzymatic activity of peroxidases increased progressively according to the stress duration in the leaves and the roots as well (Figure 5).

DISCUSSION

The water deficit occurs by the restriction of the availability of soil water. The degree of adaptation of the plants to the lack of water varies according to the species (Parker and Pallardy, 1985). Moreover, drought resistance is the result of complex physiological, biochemical and molecular mechanisms; at the biochemical level, there is the intervention of various osmolytes that help to maintain osmotic balance in the cell in the terms of dehydration (Wang et al., 2004). Indeed, tolerance to drought remains poorly described for the Argan tree. The mechanisms adopted by this species to withstand drought are still largely unknown (Zahidi et al., 2013a). Currently, it is proven that the Argan adult tree is drought resistant, survives in its area for many years (Emberger, 1925). We report here, responses of the Argan seedling to draught. The water deficit applied to Argan seedlings induced proteins accumulation in leaves and roots. This accumulation was from 25 to 107 $mg.gr^{-1}$ by F.W in leaves and from 23 to 90 mg^{-1} by F.W in roots after eight weeks compared to control, respectively. This accumulation of protein has been demonstrated also among abiotic stress in other species; Zerrad et al. (2008) reported an increase in protein content in wheat under water stress conditions and a stimulation of a new protein synthesis which ensures a protection of the cellular membranes (David and Grongnet, 2001).

Besides, EL-Tayeb and Ahmed (2007) reported that a tolerant broad bean to drought reveals an increase in proteins that protects the wall against damage, plus the synthesis of 13 new polypeptides. This accumulation is probably due to a protein synthesis resistance to abiotic stress, or to an activation of antioxidant enzymes. The growth of the stem during the eight weeks of stress remains indifferent compared to control. We noted also that root's elongation increased progressively as the duration of water deficit, the difference is very clear; 15 and 31cm, respectively, are recorded between the control and seedlings stress after eight weeks. These results complement those found before by Zahidi et al. (2013a) about Argan seedling, it was reported that drought stress decreased seedlings height, basal diameter, leaf number, leaf area, biomass production and water content.

Generally, any implied stress affects the growth in plants, the application of a metallic copper stress inhibits the root's elongation in radish (Sun et al., 2010) and a reduction in the amount of water in young sunflower seedlings (Jouili and El Ferjani, 2003). In spite of that, our seedlings were able to grow but at a low rate and started shedding leaves. However, Zahidi et al. (2013a) reported also that when water stress is high, leaf fall was more intense, but after lower or moderate water deficit, leaf drop was less important in humid seasons. Also, Benouf et al. (2014) reported that subjection Argan seedlings to several water stress led to reduction in the height of the stem, the number of leaves, and radial growth of biomass, and increased the length of the root portion. In seedlings of *Adenanthera pavonina*, Paliwal and Kannan

(1999) showed that water deficit causes a reduction of the stem height. In many species, root system in acquiring water has long been recognized as crucial to cope with drought conditions (Kashiwagi et al., 2006). Additionally, photosynthesis and growth (biomass production) are the primary processes to be affected by drought.

Our results agree with the finding in other species, so, in order to diminish consumption and increase absorption of water, plants in dry conditions often decrease their growth rate and biomass production, and contribute more biomass to roots (Wu et al., 2008). Our results show a remarkable reduction in the water content in roots and leaves; the RWC decreased progressively according to the duration of the water deficit. After eight weeks, we noted a decrease in the RWC from 85 to 41% in leaves and 88 to 45% in roots compared to the control. These results agree with those found by Berka and Aïd (2009) about the Argan tree subjected to several field capacity and by Fahmi et al. (2011) at different sampling arid areas. Several studies have showed the effect of the water stress on the RWC on other species; Zerrad et al. (2008) and Tahi (2008) about tomato and Kasraoui et al. (2004) about the Olive tree. The leaves of the Argan seedlings subjected to a water deficit present a gradual decrease in the content of chlorophyll pigments from the fourth week, and a yellowing of the leaves was also observed. This reflects the degradation of chlorophyll pigments which influences the photosynthetic capacity thereafter (M'Hamdi et al., 2009). It was reported that an oxidative stress causes the degradation of the total chlorophyll in Argan tree (Fahmi et al., 2011), in wheat (Tahri et al., 1998) and in sunflower (Ahmed et al., 2013). Also Majumdar et al. (1991) reported that chlorophyll is degraded following an activation of chlorophyllase. It was suggested that the existence of a probable connection between the synthesis of chlorophyll and proline, there is competition on the common precursor, which is the glutamate (Tahri et al., 1998).

Our data reveal an accumulation of proline much more in leaves than in roots. That was relative to the stress duration. It is a stress indicator that constitutes a source of nitrogen that can be used by the plant at the stress period (Stewart and Lee, 1974; Tal and Rosenthal, 1979) and a regulator of cytoplasmic pH (Pesci and Beffagna, 1984). It has even been recommended by several authors as an early screening test for the tolerance to water deficit (Singh et al., 1972). Our results show the conformity with the observations obtained by Berka and Aïd (2009) and Benouf et al. (2014) who reported that Argan seedlings overcome deficit water by osmotic adjustment resulting from a rapid and significant increase of active soluble sugars and a rapid accumulation of proline in leaves at several field capacity.

Tahri et al. (1998) and Chaib et al. (2010) reported that the PEG induced an accumulation of proline in wheat and a decrease in the activity of glutamine synthetase. The reactive oxygen species at high levels can react with unsaturated acids that cause the peroxidation of the membrane essential lipids (Scandalois, 1993). The content of MDA is often considered as an indicator of tissue damage (Ding et al., 2004). Our results demonstrate that MDA was accumulated in Argan seedlings and correlated with the stress duration. These results are consistent with the observations of Tahi (2008) who reported an accumulation of MDA in tomato and radish (Sun et al., 2010). Also, Gülen et al. (2008) reported that low temperatures induced a rise of MDA in strawberry. The peroxidase is an enzyme involved in the mechanism of detoxification. The enzymatic activity was considered as an indicator of resistance to oxidative stress (Castillo, 1987). Argan seedlings submitted under water deficit showed a high peroxidase activity in leaves and roots compared to the control. At the beginning of treatment, the enzymatic activity increased significantly in the leaves. From the second week, the phenomenon was reversed, and the activity becomes more important in roots to reach its maximum at the eighth week. Many studies show that enzymes as superoxide dismutase, ascorbate peroxidase, catalase and peroxidase especially are accumulated during the water stress (Zhang and Kirkham, 1994). The capacity of the antioxidant system is crucial to maintain the integrity of the photosynthetic system (Reddy et al., 2004). In plants, the peroxydase is known by a wide distribution of iso-enzymes, which differ by their physiological role (Maciel et al., 2007).

Also, EL-Tayeb and Ahmed (2007) have shown that a variety of tolerant broad bean to water stress had a high peroxydase activity compared to other sensitive. Thus, Gülen et al. (2008), reported the appearance of new isoforms of peroxydase in Strawberry seedlings subjected to low temperature. The detoxifying enzymes can be used as important bio-markers in response to environmental stresses (Sun et al., 2010). Indeed, the response of the Argan seedlings through the enzymatic reaction reflects its great capacity to cope with water deficit.

Finally, an important factor must be looking for which is the genotype and its effect; it was be conducted by Ait Aabd et al. (2010) in order to identify promising wild Argan trees, who found that some provenances exhibited high performance yield and appeared to be the best adapted to drought conditions, contrary to others. So, it would be interesting to select tolerant genotypes. Same observations was made by Zahidi et al. (2013b) who suggested that some genotypes are resistant to dry conditions and will be useful for selecting plus trees which are essential for management and conservation practices of genetic resources in Argan forest.

Conclusion

This study demonstrates that Argan tree seedlings have

developed metabolic strategies to cope with the effect of water deficit and to minimize damage. This study has produced interesting results on the mechanisms of resistance of the Argan seedlings against a water deficit for two months, which can be useful in the applied aspect, that is, at a young stage; we can irrigate the Argan seedlings on field at least every six weeks. It would be also interesting to look further into this study by the prolongation of the duration of the stress and to follow other parameters for the successful regeneration and reforestation of this species in the Algerian forests (Tindouf) which is on the way of defacement.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

We express our thanks and gratitude to the forest sector of Tindouf city for providing seeds of the Argan tree, for their supporting for sampling and also for the documentation provided.

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