

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *RHUS TRIPARTITA* (UCRIA) GRANDE UNDER WATER STRESS

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Abstract

The present study belongs to the program of biodiversity conservation inside BouHedma national Park. Among the plant species in this park, *Rhus tripartita* (Ucria) Grande, Anacardiaceae, has several interests. Drought tolerance of *R. tripartita* seedlings subject to different levels of water stress: T (100% FC; 50% FC (S1); 25% FC (S2) and severe (S3) (No watering) based on photosynthetic gas exchange, chlorophyll content and biochemical analysis (soluble sugars and proline) was examined. A highly significant decrease was recorded in photosynthetic gas exchanges, in particular the stomatal conductance which has been diminished to zero with the intensity of water stress (S3). Similarly, leaf transpiration and photosynthetic assimilation are highly affected. These changes were accompanied by a reduction in chlorophyll pigment content (Chl and total chlorophyll content) and stabilization of Chb content. In contrary to that, the water stress induced an accumulation of organic solutes (soluble sugars and proline) in leaves and roots of young plants.

Key words: Photosynthetic activity, Stomatal conductance, *Rhustripartita*, Organic solutes, Water stress.

Introduction

Water and food security are closely associated. Nearly 800 million peoples are hungry in the world and many of them live in areas that lack water (Anon, 2002). Moreover, many countries already see their water resources becoming scarce and many others experiencing severe shortages in some of their regions. The number of regions in the world suffering from a shortage of fresh water is constantly increasing and users are fighting for access to water (Radhouane, 2009). Tunisia is among the threatened countries by water shortage problem (especially in quality). Faced with this problem, it is obliged to save water resources by focusing on determining the real water needs of different cultures. The application of deficient irrigation or the use of brackish water may be accompanied by morphological transformations (Baldy, 1973; Albouchi, 1997; Garg *et al.*, 2002) and physiological changes (Monneveux, 1997) and may induce changes in the biochemical metabolism of the plant (Zhang *et al.*, 1999). In addition, tolerance to various stresses depends on species, varieties and even ecotypes (Ullaha *et al.*, 2008; Li *et al.*, 2008).

The plant species of the Mediterranean regions, which are characterized by a dry summer season with high temperature and irradiance, can acclimate to these constraints by escape, avoidance and tolerance (Colmer *et al.*, 2006). Plants may be subjected to environmental stresses that adversely affect their growth, metabolism and yield (Lawlor, 2002). Indeed, the plants tolerance degree to the environmental stress varies greatly not only between species, but also in different varieties of the same species. Also, genotypic differences in drought tolerance could be attributed to the ability of plants to grow (Turtola *et al.*, 2006). A large number of environmental stresses, such as water, salinity and

thermal may limit crop growth and productivity by imposing osmotic stress to plants (Navarro *et al.*, 2007). Photosynthesis is considered among the physiological processes affected by biotic and abiotic stresses (Farquhar *et al.*, 1989). Indeed, water shortage and salinity are limiting factors in stomatal conductance as well as the photosynthetic capacity (Greenway & Munns, 1980; Cornic & Briantais, 1991; Lawler, 1995; Tezara *et al.*, 1999). The reduction of photosynthesis due to water deficiency is due to both stomatal and non-stomatal limitation (Parida *et al.*, 2003; Flexas *et al.*, 2004; Ennahli *et al.*, 2005). Water stress can affect one or more steps in the photosynthetic process: CO₂ diffusion through stomata and intercellular spaces, electron transport, photophosphorylations and carboxylation reactions as such (Brestic *et al.*, 1995; Massacci *et al.*, 1996). Indeed, some species of arid regions suffer from this alarming situation of drought among which *Rhus tripartita*. The latter is threatened with extinction and is very rare in the young seedling stage. Note that this species has multiple interests such as pastoral, food (tea infusion and consumption as a treat of these drupes), medicinal. It must therefore protect and better value this plant heritage and especially see its requirements and responses among constraint. In order to know the photosynthetic capacity of this plant under water stress, we started a physiological test on young plants of *R. tripartita* in order to rationalize the water demand and to optimize the efficiency of its use.

Materials and Methods

Seed lot details: Mature seeds of *R. tripartite* were collected during March 2012, harvested from spontaneous adult shrubs located in BouHedma National Park in south of Tunisia (Arid Bioclimate). The study was focused on young plants *R. tripartite* aged of 4 years grown in polythene bags and transplanted into pots of 5kg.

Growth conditions: The study was conducted at semi-controlled conditions (greenhouse in bicarbonate) at the National Institute for Research in Rural Engineering, Waters and Forests of Tunis belonging to superior semi-arid bioclimate with mild winter (36°50'N, 10°14'E, 3m). The seeding was carried out in polyethylene bags filled with a growing substrate by mixing 2/3 sand and 1/3 forest soil. The plants obtained were irrigated regularly for 4 years. Four water regimes were applied during the experiment. A control group (T) was continuously maintained at field capacity (irrigated daily) at basal water potential between -0.18 to -0.25 MPa and 3 lots of plants were subjected to water stress in different level: 50% FC (S1); 25% FC (S2) and severe (S3) (without irrigation) with a base potential between -1.2 and -1.5MPa. The duration of the treatment is 45 days (brackish water). The experimental device used for the study was a "split-plot" with 6 repetitions by water treatment. This study was focused on physiological and biochemical parameters. The seedlings were cut out at the end of the experiment. The leaves and roots were dried in an oven at 40 ° C for 24h to 48 h. The dried material was powdered and kept refrigerated until used.

Effect of water stress on gas exchanges: We conducted various measures gas exchange at fully exposed leaves in full sun (fourth or fifth leaf from the apex of the seedling) using a photosynthesis system (LCpro-SD Photosynthesis System), attached to an infrared gas analyzer (IRGA) and a data logger. The measurements were made during the morning (between 08.00 and 10.00 am). The parameters were: Net photosynthetic rate (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), Transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), Stomatal conductance (gs) ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and the internal concentration of CO_2 [CO_2]_i (PPM).

Determination of the effects of water stress on chemical constituents: Chlorophyll analysis was determined according to Withan *et al.*, (1971) method:

$$\begin{aligned} \text{With: Cha} &= 12,7 * \text{DO}_{663} - 2,69 * \text{DO}_{646} \\ \text{Chb} &= 22,9 * \text{DO}_{646} - 4,68 * \text{DO}_{663} \\ \text{Cha+Chb} &= 7,15 \times \text{DO}_{663} + 18,71 \times \text{DO}_{646} \end{aligned} \quad \text{V / M}$$

Osmolytes contents of proline was determined following the ninhydrin method described by Bates *et al.*, (1973) in leaves and roots, using L-proline as a standard. Proline in the test samples was calculated from a standard curve prepared against L-Proline (5-30 μg , from MERCK KGaA): $y = 0.059x - 0.014$, $R^2 = 0.99$. The content of total soluble carbohydrates in the studied samples (leaves and roots) was determined according to Mc Cready *et al.*, (1950) and Staub (1963), using glucose as a standard. From the standard curve ($y = 0.0095x - 0.0299$, $R^2 = 0.979$), the concentrations of soluble carbohydrates in the test samples were calculated.

Statistical analysis: The experiment was a complete randomized design consisting of four water treatments, and six replicates. The data were analyzed using appropriate procedures of the SPSS software (version 16). Analysis of variance (ANOVA) was performed with the

statistical program Minitab (Minitab Inc.; College Park). A Duncan's multiple range test was carried out to determine if significant ($p < 0.05$) difference occurred between treatments.

Results

Effect of water stress on gas exchanges: The variations of the gas exchanges under water stress are shown in figure 1. The net photosynthetic assimilation (A) measured on the leaves of *R. tripartita* was more important in favorable conditions (T ($6.78 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)). Under water stress, the net photosynthetic rate was decreased significantly in all treatments. The reduction varied from 14% to 89% respectively in S1 and S3 (Fig. 1A). Very significant ($p < 0.000$) variations in stomatal conductance (gs) between treatment were observed. In the control, gs showed the highest value ($0.125 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) compared to the other treatments. gs decreased progressively with stress from 0.04 to $0.01 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ respectively for S1 and S2. The great reduction (100%) was obtained in the non-irrigated plants (S3) since gs was canceled by the closure of stomata (Fig. 1B; Table 1).

Foliar transpiration (E) (Fig. 1C) showed similar variations to net photosynthetic assimilation and stomatal conductance. This parameter was $3.18 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ in the control and underwent a sharp and pronounced reduction under water stress. A smaller reduction (29%) was recorded in S1 (50% CC). At the highest stress, E showed the greatest reduction (90%) in S3. Similarly, the reduction of intercellular CO_2 concentration (C_i) was clearly observed at the end of the stress period (Fig. 1D). The most pronounced reduction was obtained (61%) in S2 (25% FC). This parameter varied from (276 ppm), (158.83 ppm), (106.83 ppm) and (152.16 ppm) respectively for T, S1, S2 and S3.

Biochemical response

Effect of water stress on chlorophyll content: Chlorophyll contents (Cha, Chb, Total chlorophyll and Cha / Chb) were done (Fig. 2). In all applied treatments, water stress induced a significant reduction of Cha and the total chlorophyll content as well as the Cha / Chb ratio. This reduction becomes more and more important with stress. Both the pronounced reduction (77%) of Cha was recorded in non-irrigated plants (S3). The both treatments (S1 and S2) underwent reductions of 26 and 56% respectively (Fig. 2A). Similarly, S3 has the greatest reduction for the total chlorophyll content and the Cha / Chb ratio. The analysis of figure 2C showed that the highest total chlorophyll content was obtained in control T ($4.80 \text{ mg.g}^{-1} \text{ DW}$). The water stress gradually decreased the total chlorophyll content for S1 (3.77), S2 (2.62) and S3 ($2.14 \text{ mg.g}^{-1} \text{ DW}$). The highest value of the Cha/Chb ratio was recorded in control T (2.38) while the lowest was obtained in S3 (0.53). The ANOVA analysis depicted the very highly significant effect of water stress on the Cha / Chb ratio (Fig. 2D). There was no significant variation of the Chb under stress (Table 2). So Chb seemed to be insensitive to the applied water treatment (Fig. 2B).

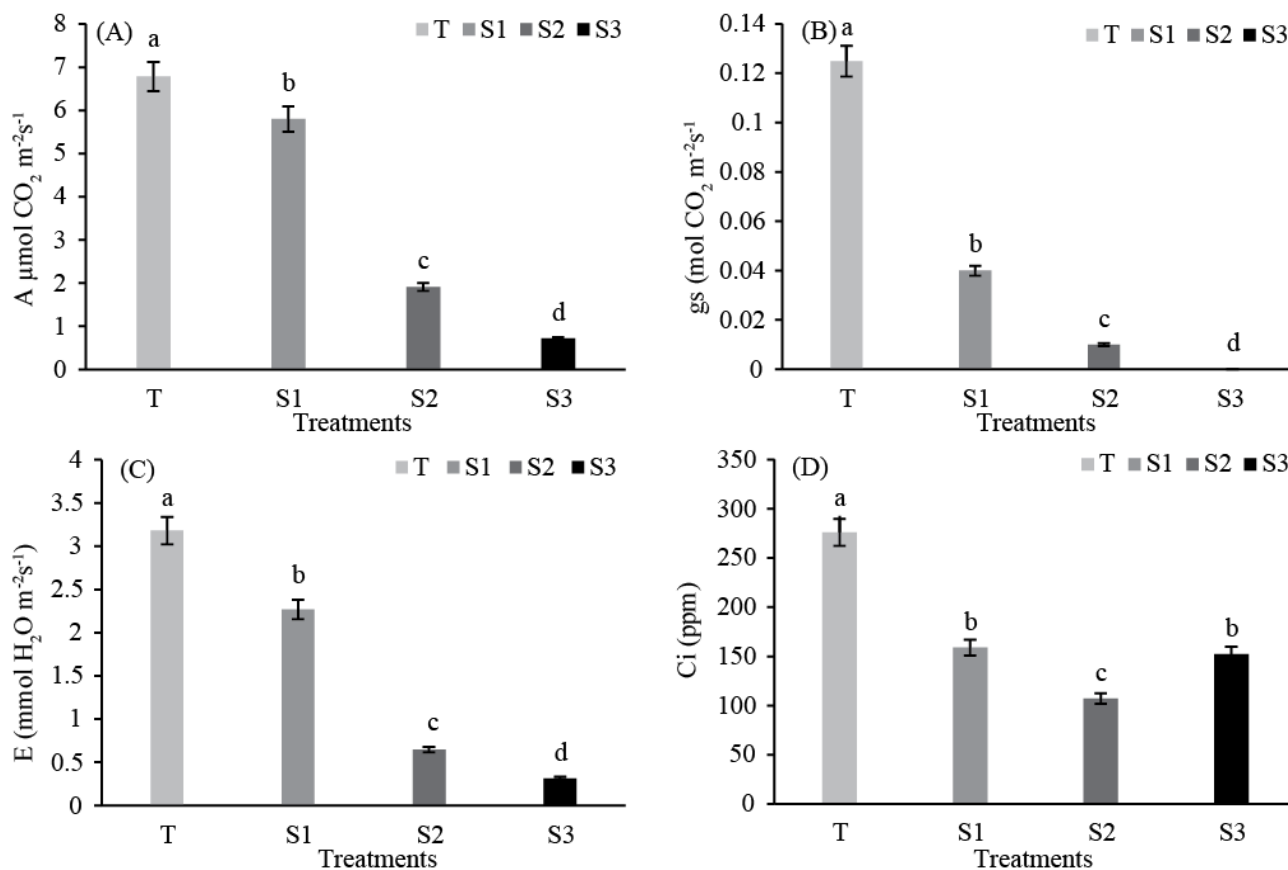


Fig. 1. Variation of net photosynthetic rate: A (A), Stomatal conductance: gs (B), transpiration rate: E (C) and internal concentration of CO₂: Ci (D) for seedlings of *Rhus tripartita* under water stress (Means with same letters are not significantly different by Duncan's test ($p < 0.05$)).

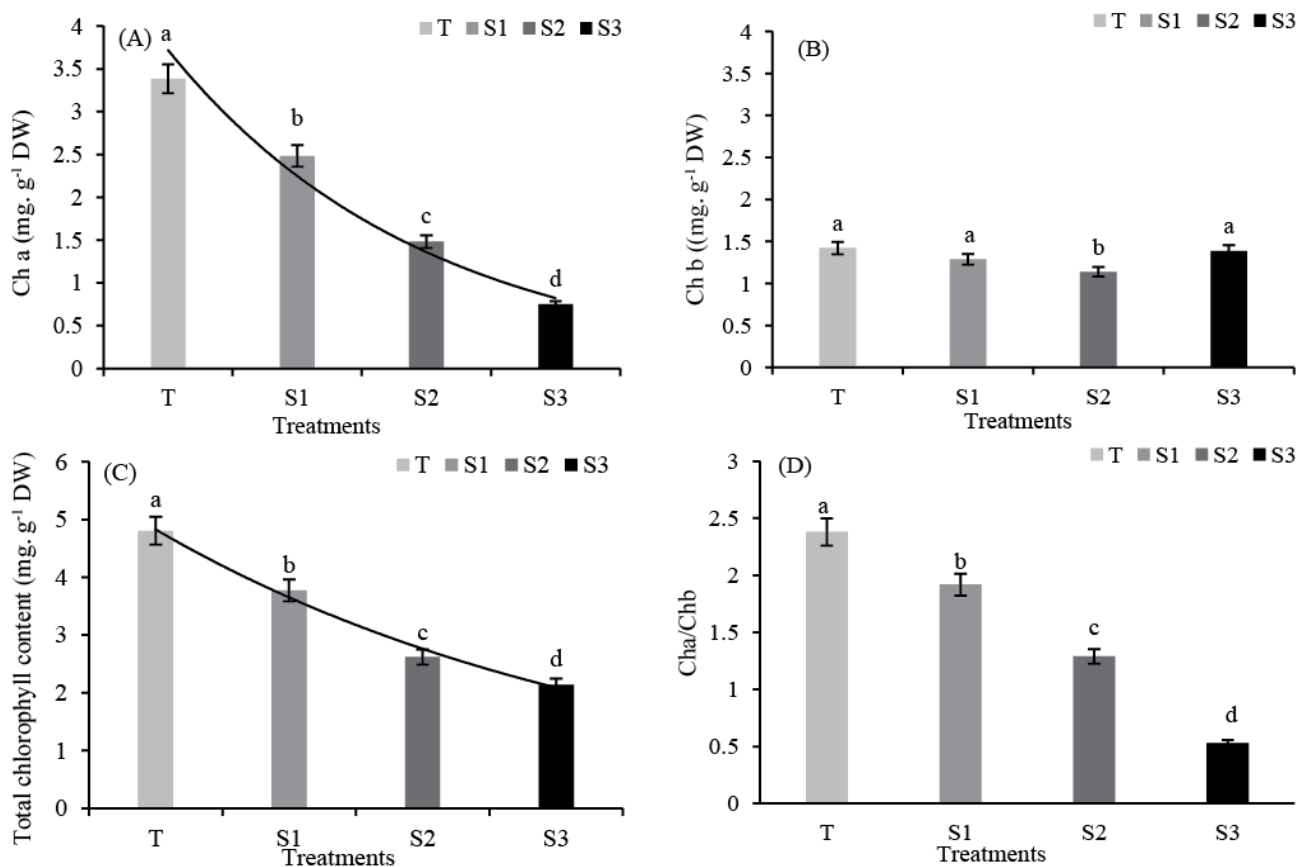


Fig. 2. Variation of chlorophyll content "Ch a"; "Ch b"; total chlorophyll content and (Ch a / Ch b) in seedlings of *Rhus tripartita* under water stress (Means with same letters are not significantly different by Duncan's test ($p < 0.05$)).

Table 1. Analysis of variance of photosynthetic parameters (net photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs) and internal concentration of CO₂ (Ci) of young seedlings of *Rhus tripartita* under water stress.

Parameters		ANOVA				
		Sum of squares	df	Mean square	F	Sig.
Ci	Between groups	93725.458	3	31241.819	12.978	0.000***
	Within groups	48146.500	20	2407.325		
	Total	141871.958	23			
E	Between groups	33.044	3	11.015	2.404E3	0.000***
	Within groups	0.092	20	0.005		
	Total	33.135	23			
gs	Between groups	0.058	3	0.019	198.333	0.000***
	Within groups	0.002	20	0.000		
	Total	0.060	23			
A	Between groups	156.175	3	52.058	203.325	0.000***
	Within groups	5.121	20	0.256		
	Total	161.295	23			

Table 2. ANOVA of the biochemical parameters of *Rhus tripartita* seedlings under water stress (F significance and homogeneous group average)

Parameters		ANOVA				
		Sum of squares	df	Mean square	F	Sig.
Cha	Between groups	15.977	3	5.326	19.322	0.000***
	Within groups	3.308	12	0.276		
	Total	19.284	15			
Chb	Between groups	0.192	3	0.064	0.222	0.879NS
	Within groups	3.465	12	0.289		
	Total	3.658	15			
Cha+Chb	Between groups	15.952	3	5.317	23.921	0.000***
	Within groups	2.668	12	0.222		
	Total	18.620	15			
Cha/Chb	Between groups	15.977	3	5.326	19.322	0.000***
	Within groups	3.308	12	0.276		
	Total	19.284	15			

***: Very significant difference; NS: Non-significant difference

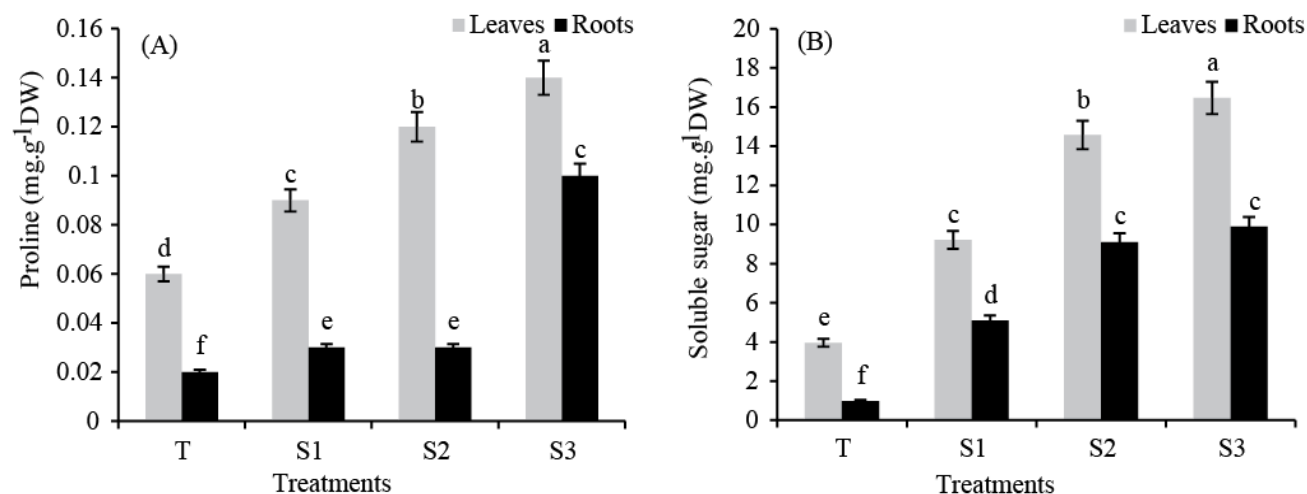


Fig. 3. Variation in proline content (A) and soluble sugar content (B) under water stress. Data are means \pm SD of 4 replications. Means with same letters are not significantly different by Duncan's test ($p < 0.05$).

Effect of water stress on proline content: The water stress induced a highly significant increase in proline content both in the leaves and roots of the *R. tripartita* seedlings relative to their respective controls (Fig. 3A). In leaves, the highest value (0.14 mg.g⁻¹ DW) was recorded in S3 and the lowest was obtained in the control plants (0.06 mg.g⁻¹ DW). In roots, proline content showed a significant increase with the intensity of stress. The highest content was recorded in S3 (0.10) compared to the control (0.02 mg.g⁻¹ DW). According to the DUNCAN

test, four homogeneous groups were found for leaves and three groups for roots. The treatment effect was highly significant for the two plant organs.

Effect of water stress on soluble sugars content: The variation of soluble sugar content in leaves and roots of seedlings of *R. tripartita* is shown in Figure 3B. Water stress induced an increase of soluble sugars (SS) in leaves and roots. Seedlings recorded very high sugar content in leaves under severe treatments (S3) and 25% FC (S2),

with 16.47 and 14.58 mg.g⁻¹ DW respectively. On the other hand, the lowest values were observed for control plants (3.95) and S1 (9.21) mg.g⁻¹ DW. A very highly significant effect of stress under different water treatments. Similarly, for the SS content in roots, we found a significant increase with the intensity of stress. The highest content was recorded in S3 (9.88) compared to the control (0.98 mg.g⁻¹ DW). Always the leaves are most loaded with soluble sugars.

Discussion

The results showed that water stress induced a reduction of gas exchange parameters: A, E, gs and internal CO₂ concentration. These results were in agreement with those reported by Rodriguez-Gamir *et al.*, (2010 & 2011), Hutton & Loveys (2011) and De Campos *et al.*, (2011). This fall in stomatal conductance is a consequence of stomatal closure. It should be noted that stomatal conductance has been used in several studies to detect the effects of water deficit on the functioning of the photosynthetic system (Beniken *et al.*, 2013).

Many studies had reported that when water stress was experienced by plants, the first short-term response was to reduce stomatal conductance to avoid water loss through sweating and in the medium term by increase in Root growth to maximize water absorption (Kramer & Boyer, 1995). In addition, these results agreed with those obtained in *Ziziphus lotus* (Zouaoui *et al.*, 2013). According to Beniken *et al.*, (2013) and Rodriguez-Gamir *et al.*, (2010), the decrease in stomatal conductance causes a reduction in transpiration and, consequently, a decrease in photosynthesis. It should also be noted that the chlorophyll a content is affected in a highly significant manner under prolonged water stress. It decreased progressively compared to the control especially for S2 (1.48 mg.g⁻¹DW) and S3 (0.75 mg.g⁻¹DW). More severe stress (S3), decreased again the content of the pigment. This decrease is also verified by the work of Fahmi *et al.*, (2011), which show that during a water stress the "Ch a" content is higher in *Arganiaspinosa* (L.) Skeels in the region of Essaouira (Semi-arid) (1.99 ± 0, 75 mg.g⁻¹DW) compared to the dry region of Guelmim (arid) (1.5 ± 0.33 mg.g⁻¹ DW). So, the chlorophyll content decreased with increasing gradient of aridity. On the contrary, the variation of the Chb content is not significant. Our results were in agreement with those of Fahmi *et al.*, (2011) which showed that the "Ch b" content did not reveal a significant variation in *A. spinosa* between the Essaouira and Guelmim regions. In addition, a decrease in "Ch a" and "Ch b" was found in West Indian lime citrus (Shirley M. Norman *et al.*, 1990), although other species of citrus showed an increase in the "Ch b" content under the effect of water stress.

These results are consistent with those obtained in *Vignaradiata* (Thalooth *et al.*, 2006). In arid regions, plant species develop different coping strategies such as the increase in antioxidant concentrations (carotenoids, Ascorbat) following the degradation of chlorophyll pigments (Streb *et al.*, 1997). In water deficiency, photosynthetic activity of the leaf is affected and chlorophyll concentration decreases with increasing water stress (Fahmi *et al.*, 2011). These results were consistent

with those of Garcia-Plazaola *et al.*, (2000), which showed a decrease in total foliar chlorophylls. The total chlorophyll content (Ch a + Ch b) was decreased progressively in stressed plants S1, S2 and S3. Our results confirm those of Guerfel *et al.*, (2009) which indicate the decrease of (Ch a + Ch b) for two varieties of olive (*Olea europaea* L.) Chemlali and Che'toui in two different water regimes (Irrigated and Stressed). For the irrigated Chemlali variety, the total chlorophyll content was in order of (11.6 ± 1.5b mg.g⁻¹ DW), whereas for the stressed variety, the content was (8.2 ± 1.3a mg g⁻¹ DW). These results agree Hireche (2006). It has been shown that 25% of the field capacity (FC) leads to a total chlorophyll reduction of 2.95% compared to the control (100%) in the Dessica and Moapa varieties of *Medicago sativa* L. Amoumen & Benhebreche (2013) found that when a plant was under stress, the level of chlorophyll decreased, affecting the color of leaves and slowing its growth activities. The reduction in the total chlorophyll content of the vitron wheat variety observed in "Cha", "Ch b" probably results from the synergy of several factors: reduced stomatal opening that limited water loss through evapotranspiration and by increasing the resistance.

The results of the Ch a / Ch b ratio under different water treatments show that the highest value was recorded in the control while the lowest was obtained in S3. These results were consistent with those of Hireche (2006), who found a reduction of the Ch a / Ch b ratio in plants subjected to 50% and 25% FC. Our study also agrees with Nana *et al.*, (2009). Under water deficit, the Ch a / Ch b ratio was decreased in the five okra varieties. The Ch a / Ch b ratio is considered a good indicator of the tolerance threshold (Nana *et al.*, 2010). The conservation of water in cells requires an accumulation of solutes (proline, glycine betaine, soluble sugars). Therefore an increase in the content of certain solutes, in fact is a parameter of adaptation to water stress (Hireche, 2006). In particular, soluble sugars protect the membranes against dehydration. They contribute largely to the lowering the osmotic potential in the plants (Amoumen & Benhebreche, 2013). Our results show an increase in the soluble sugar content under the applied water treatments. Severe stress (S3) had the highest leaf content (16.47) compared to roots (9.88 mg.g⁻¹ DW). The accumulation of soluble sugars in cells is adapted by plants to resist to the environment stresses (Mouellef, 2010; Hireche, 2006). This increase was confirmed by Amoumen & Benhebreche (2013), who found a strong accumulation of SS content at 25% FC, for two varieties (vitron and carioca) of Wheat (*Triticum durum* Desf). Therefore, an increase in soluble sugars was observed in a good number of plants (wheat, bean) under water stress (Bezzala, 2005) and in many woody species such as *Eucalyptus microtheca* (Chunyang, 1998) and *Quercus ilex* (Pesoli *et al.*, 2003). Different soluble sugars may be present in well-hydrated tissues, but sucrose is preferentially accumulated in dehydrated tissues in dehydration (Amoumen&Benhebreche, 2013). The accumulation of soluble sugars may be due to an increase in hydrolysis of starch (Bouchelaghem, 2012). The application of water stress caused an increase in the foliar proline content (from 0.06 in T to 0.14 mg.g⁻¹ in S3). Since the increase of this element is one of the adaptive strategies

frequently observed in plants to limit the effects of water stress. It is linked to cytoplasmic osmoregulation (Hireche, 2006). The results obtained were consistent with those of Houasliet *et al.*, (2013) who showed that water stress caused an increase in proline content in all genotypes of *Cicerarietinum*. Similarly, for the work of Hireche (2006), which showed an increase in the proline content for the Dessica variety of 21.05 $\mu\text{g} / 100 \text{ mg}$ in S1 (50% FC) to 111.84 in S2 (Dry at 25% FC) and an accumulation of the latter in the Moapa variety from 42.22 for S1 to 131.12 $\mu\text{g} / 100 \text{ mg}$ for S2. He also found an important capacity of osmoregulation which is due to the accumulation of proline. In addition, Moulineau (1993) showed that when relative water content (RWC) decreased, proline accumulation and alanine disappearance were observed. During the water stress, proline becomes a major element for lower RWC for the two populations of MIL (HKP and IC 30). The accumulation of proline is more pronounced for the HKP population (4 times). According to the study by Nana (2010), an increase in the foliar proline content was observed in *Abelmoschus esculentus* subjected to a water restriction at the vegetative and flowering stages. In addition, accumulation of proline content was recorded in roots (from 0.02 for T to 0.10 $\text{mg}\cdot\text{g}^{-1}$ for S3.) depending on the intensity of stress. Our results were in agreement with those of Zerrad *et al.*, (2008), which showed a change in the amount of proline in roots of both durum wheat varieties according to the duration of water stress. The increase in proline content is positively correlated with the degree of water stress. In absence of water stress, the proline level in both varieties remains very low and almost steady for the roots.

Conclusion

Water stress has led a pronounced reduction in the gas exchange parameters. The net photosynthetic assimilation (A), foliar transpiration (T), stomatal conductance (gs) and intercellular concentration of CO_2 (C_i) had undergone similar variations. The control plants recorded the highest values. On the other hand, the severe stress S3 showed the lowest values except for C_i . These changes were accompanied by an increase in organic solutes (soluble sugars and proline) and an accentuated decrease in total chlorophyll. According to the previous results, this species is tolerant to conditions of water stress at some level (level of experimentation) and it can be recommended for planting and use in Tunisia, especially in industry (tanning of skins), feeding (infusion of tea for leaves and treats for drupes) and especially the medicinal aspect.

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