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Biochemical and Physiological Changes in *Artemisia herba-alba* Plants under Water Stress Conditions

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Abstract: Container experiments were carried out in order to underline the main effects of water stress on physiological and biochemical behaviour of *Artemisia herba–alba Asso.* After germination, seedlings were well-watered until the age of 4 months when they were subjected to a limiting water supply (70 % and 40 % of container capacity, CC). This first treatment (T1) lasted 36 days and was followed by a re-watering treatment of 40 days period (T2). Several parameters known as indicators of plant status were monitored after drought treatment and subsequent re-watering. It was specially, about biomass production, plant water content, chlorophyll, sugars and proline contents and osmotic potential. Water drought led to a significant decrease of biomass accumulation, water content and chlorophyll content under both 70% and 40 % CC. However, the intensity of water stress showed a positive correlation with sugars and proline accumulation. Well watered plants showed the higher biomass production but the lowest organic components concentration unlike treated plants which showed the contrary evolution of these parameters. Drought stress, again, had a significant impact on the osmotic potential of plants, thereby it showed a significant decrease ($p < 0.05$) when irrigation was reduced to 70% CC or 40% CC. The stop of stress and the re-watering allowed the progressive return to the initial values and helped in the recovery of plants. Altogether, these data indicate that *A.herba–alba* has high aptitude permitting adaptation to unkind environmental conditions of the natural habitat.

Key words: *Artemisia herba-alba* (Asso.) · Water stress · Re-watering · Biomass production · Water content · Chlorophyll content · Sugars content · Proline content and osmotic potential

INTRODUCTION

Arid and semi arid ecosystems represent 60% of globe surface [1] and they are submitted to the combined effects of ground and climate constraints that affect plants properties. Water deficit is frequently the principal restrictive factor for yield production under these conditions [2]. It creates a stress state that affects nearly all the plant growth [3]. But then, plant response to water deficit depends on its acclimatization abilities and the level of climate and soil drought [4].

Water stress is known to induce a significant decrease of the total dry matter biomass [5] and it provokes also a progressive stopping of the growth of young organs cell [6]. On the contrary, organic compounds showed a significant increase under both 70% and 40% FC. In fact, Bohnert and Jensen, [7] indicated that proline accumulation is a means of osmotic adjustment and carbon storage. This accumulation can be the result of synthesis stimulation [8,9], inhibition of proline oxidation [10,11] and/or alteration of proteins synthesis [10]. Soluble sugars synthesis submit also an increase under drought conditions [12,13]. These substances can react as osmoprotectants and carbon source to keep the plants survive during water stress periods [14].

In these circumstances, selecting a drought-resistant species is a promising alternative to overcome drought-induced damages and to benefit to a large extent from the agriculture estate. For this selection, the adaptive behaviour of plants should be tested and their performance should be evaluated either at morphological, anatomical, cellular or molecular levels. Current study, dealing with *Artemisia herba-alba,* was attempted to evaluate changes in the physiological and biochemical aspects of plants under water limited conditions.

The selection of this species was chosen out of its various interests. *Artemisia herba-alba* is a medicinal

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and aromatic dwarf shrub that grows wild in arid areas of the Mediterranean basin [15]. It has a considerable fodder value and it is rich in essential oils with antiseptic, vermifuge and antispasmodic characteristic [16]. Moreover, its leaves are used to treat bronchitis and diarrhoea [17] and its roots are used against convulsions [18]. This species is recommended for neurological troubles and two flavonoids of its extracts have in vitro GABAA-benzodiazepine receptor activity [19]. Again, *Artemisia herba-alba* is characterised, on the one hand by the seasonal dimorphism of its foliage which allows it to reduce the transpiration surfaces and consequently to reduce the loss of water [20,21] and on the other hand by its root system which is dense at surface and allows it to take advantage of little rains [22].

Our objectives were to examine the main changes in physiological and biochemical aspects of drought treated plants in order to consider traits that may be associated with a greater ability to resist water stress.

MATERIAL AND METHODS

Plant Material and Soil: Seeds of Artemisia herba-alba used in this study were collected from the region of Gafsa in February 2004 and conserved, after cleaning, in the seeds bank of the Institute of Arid Regions (IRA) of Medenine. Seeds were germinated in containers at 15°C and irrigated 3 to 4 times a week with distilled water. Following germination, plants were transferred into a growth room in which growth conditions were constantly 13 h light and 11 h dark, a temperature of 19/23 °C, the relative humidity of 40% and irrigation with improved Hogland nutritive solution. A month after germination, plants were transplanted into 1-l pots (one plant per pot) containing a gravel layer below a 2/1 (v/v) soil and sand mixture that was previously treated by chlorhydric acid (0.5 N) during 48 h in order to eliminate micro-organisms and organic wastes.

Drought Treatments: Prior to treatments, plants were regularly irrigated at the field capacity (F C). At the age of 4 months three groups of 24 plants were randomly selected and were submitted to different water conditions: 100 % of the FC, 70 % FC (moderate stress) and 40 % FC (severe stress) respectively, under the similar environmental conditions as described above. Drought stressed plants were watered every 2 days to approximately substitute evapotranspiration, so that the level of water stress remains constant. 36 days of treatment were enough to provoke remarkable changes and therefore 12 plants of each group were harvested. Subsequently, the remaining plants were re-watered to the FC during a 40-days period before being harvested. Control plants were well-watered every day and sampled following the same day intervals as the treated groups.

After harvesting, then vegetable material was freeze dried and dry weights (DW) were determined.

Chlorophyll Content: About 0.1 g of freeze dried leaves was pulverized and added to 5 ml of 80% acetone solution. After 72 h incubation at 4 °C in the dark, chlorophyll a, b and total contents were measured as a function of absorbance at 665 and 649 nm, respectively, using the following equation:

$$\text{chl a } (\mu g/ml) = 11,63\ (A_{665}) - 2,39\ (A_{649}).$$
$$\text{chl b } (\mu g/ml) = 20,11 (A_{649}) - 5,18\ (A_{665}).$$
$$\text{chl total } (\mu g/ml) = 6,45\ (A_{665}) + 17,72\ (A_{649}).$$

Proline and Sugars Contents Measurement: Proline content in 100 mg of freeze dried leaves was extracted and determined according to the method of Troll and Lindsley [23] simplified and pointed by Dreier [24]. Proline content was expressed in $\mu mol.g^{-1}$ MS.

Soluble sugars dose was carried out from 100 mg leaf freeze dried material [25]. Soluble sugars content was expressed in $\mu mol.g^{-1}$ MS.

All the data were measured three times and the mean was used for result analysis and discussion.

Osmotic Potential: For osmotic potential determination, frozen samples were thawed and osmotic potential of the obtained sap was measured using a vapour pressure osmometer (Christ alpha 1-4).

Statistic Analysis: The significance of treatment effects on physiological and biochemical parameters was assessed by analysis of variance using SPSS 12.0 version Models. Comparisons of means between the three treatments, with an equal number of repetitions, were performed by a Duncan test.

RESULTS

Induced drought stress influenced significantly ($p<0.05$) total plants dry weight. The highest dry matter production (1.092 g per plant) was noticed under control conditions and was reduced to about 0.68 and 0.42 g per plant in drought-treated plants under 70%CC and 40%CC, respectively. Untreated plants had again the highest total

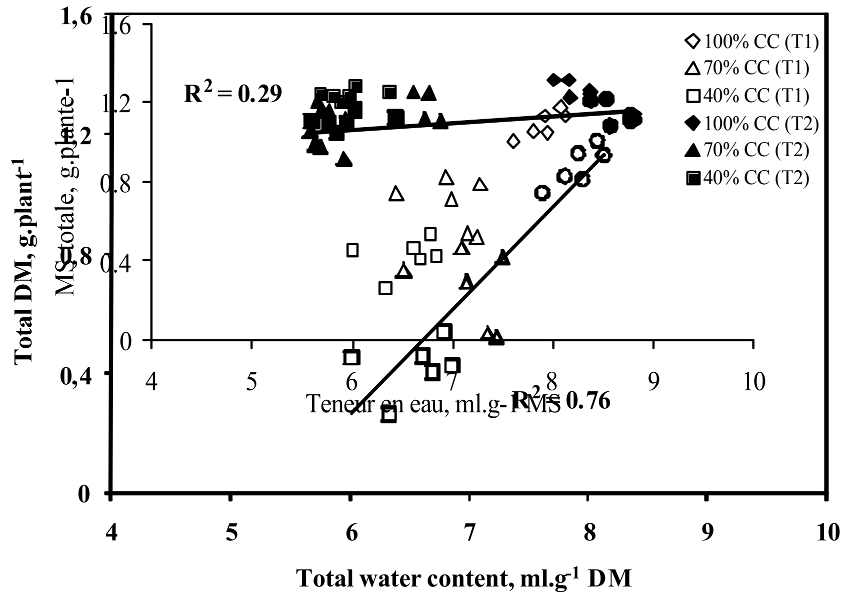


Fig. 1: Correlation between dry matter production and water content after water stress (Light symbols) and re-watering treatments (Dark symbols)

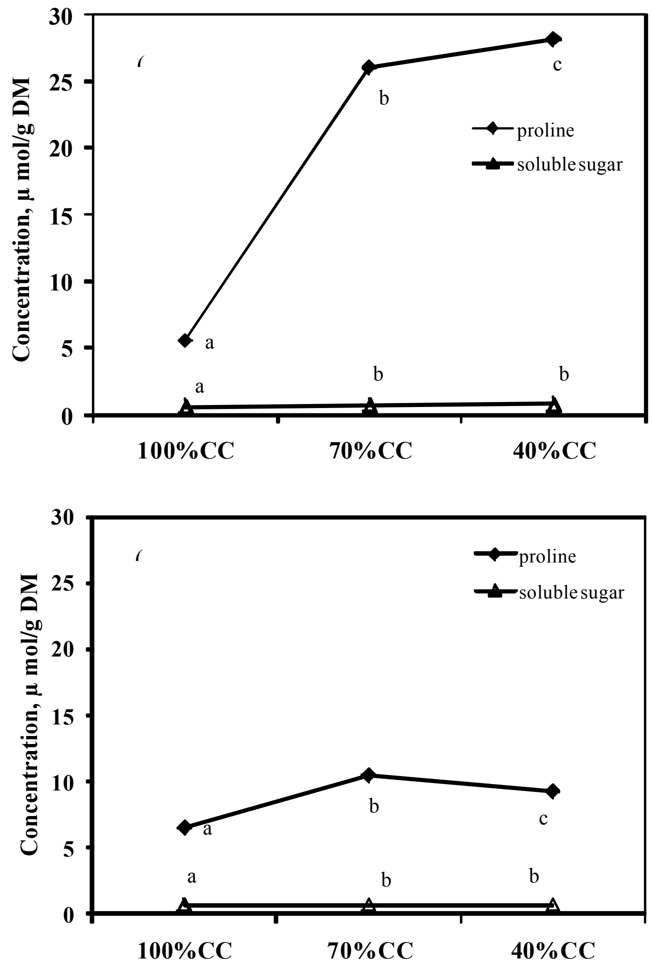


Fig. 2: Proline and soluble sugar content after water stress (a) and re-watering (b) treatment. Aeverage of 3 replicates

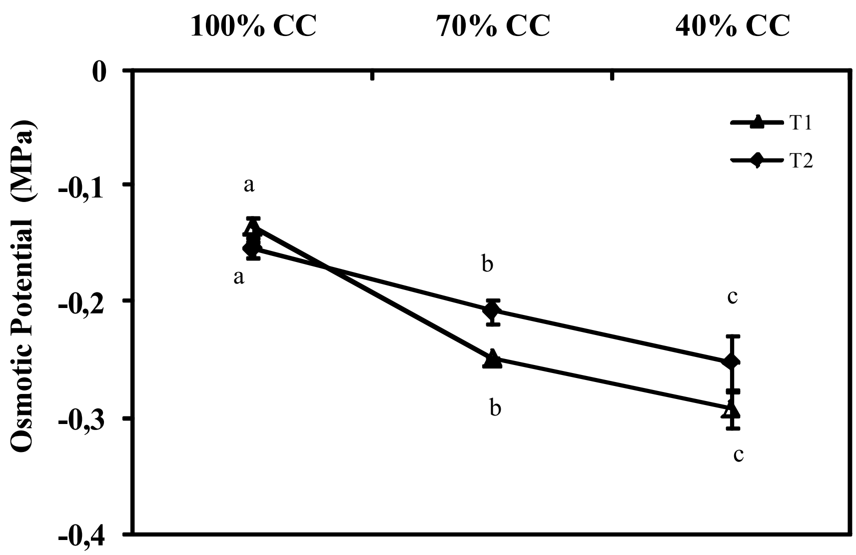


Fig. 3: Osmotic potential of *A.herba-alba* after water stress (T_1) and re-watering treatment (T_2). Aeverage of 3 replicates

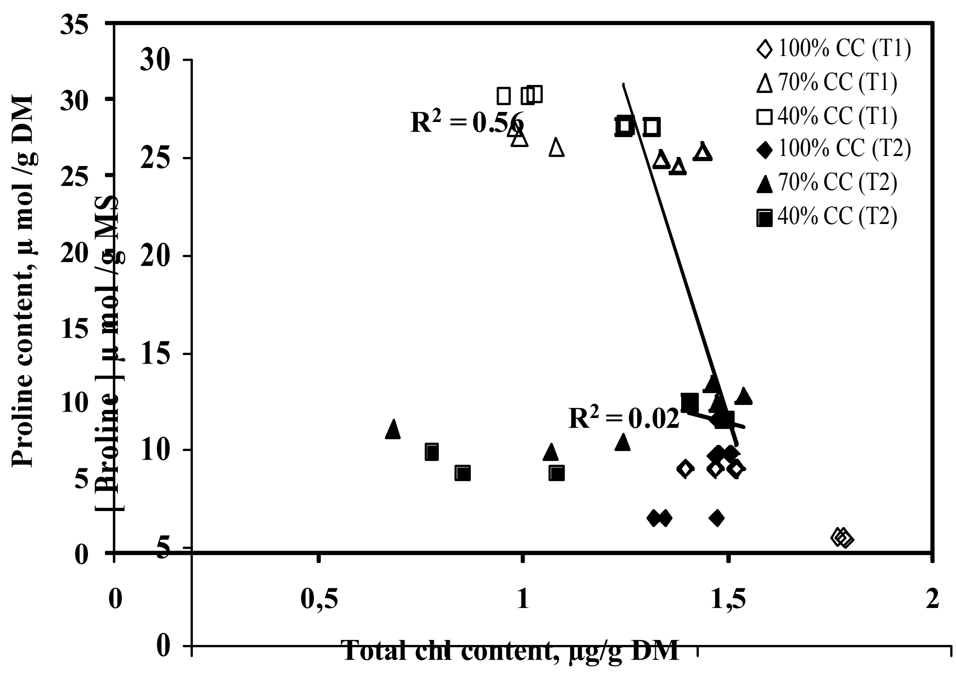
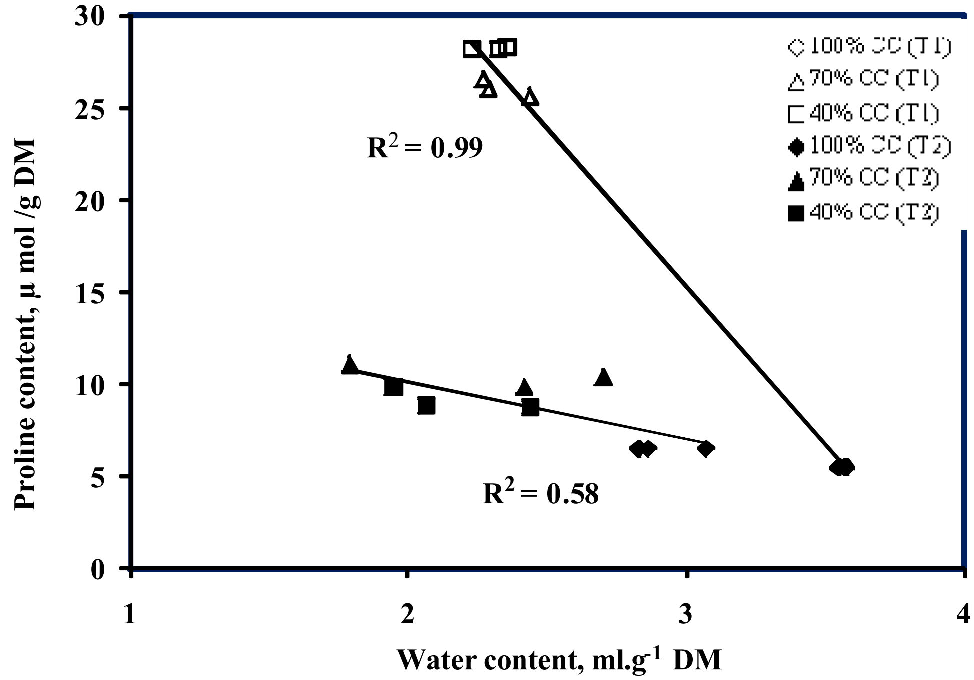


Fig.4. Variation of proline content of AP of *A.herba-alba* plants in relation total chlorophyll content under water stress (T_1) and after re-watering (T2). 3 individual replicates per treatmentent.



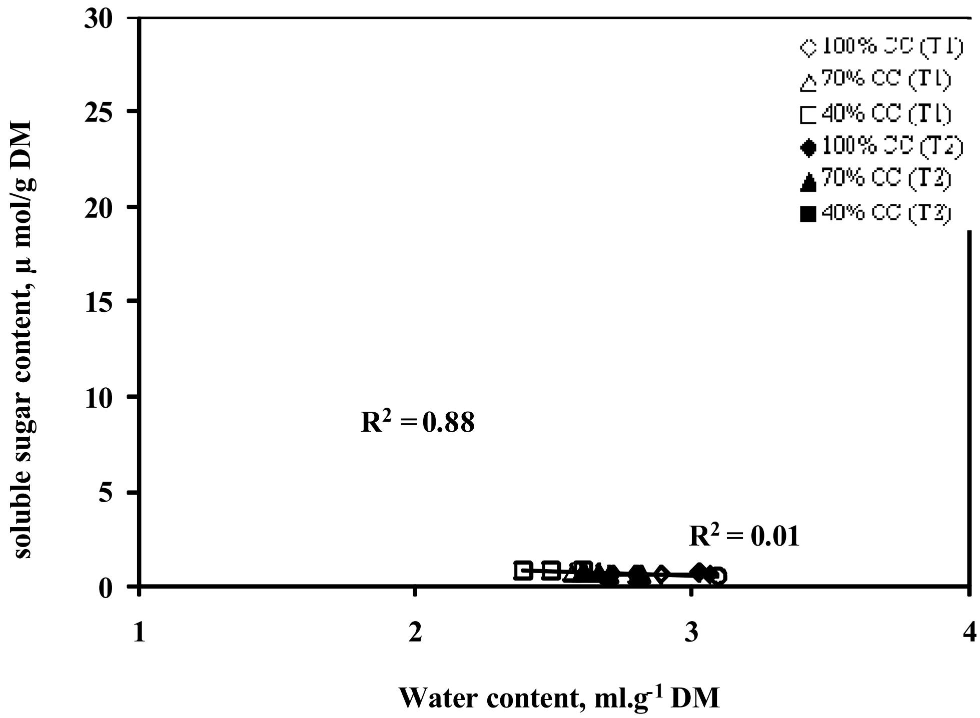


Fig. 5: Variation of proline and soluble sugar contents of AP of *A.herba-alba* plants in relation to water content under water stress (T_1) and after re-watering(T2). 3 individual replicates per treatmentent

Table 1: Chlorophyll a, b and total content of *A. herba-alba* after water stress and re-watering treatment. Average of 3 replicates and SE at 5%

Treatment		chl a (µg /g MS)	chl b (µg/g MS)	Total chl (µg/g MS)
Water stress	100%CC	0.88±0.13a	0.58±0.061a	1.46±0.06a
	70%CC	0.84±0.10b	0.54±0.04b	1.38±0.05b
	40%CC	0.74±0.078c	0.52±0.03c	1.26±0.04c
Re-watering	100%CC	0.89±0.03a	0.59±0.01a	1.48±0.02a
	70%CC	0.89±0.05a	0.59±0.01a	1.48±0.04a
	40%CC	0.91±0.067a	0.54±0.02b	1.45±0.05a

water content with an amount of 6.1 $ml.g^{-1}$ DM. Severe stressed plants showed in contrary a lower water content that was 23% fewer than the witness level. Interestingly, 40 days of re-watering helped the recovery of both total dry weight and total water status. Compared to the control plants, previously severe treated plants showed an increase of dry matter production and water content to 97% and 94%, respectively.

In order to illustrate the between the two parameters, total dry matter produced was expressed in relation to total plant water content.

A high correlation (R^2=0.8) was noticed and revealed that the growth of plants under water deficit conditions was highly dependent on their water status (Fig. 1).

Chlorophyll Content: As shown in Tab.1, leaf chlorophyll content changed significantly ($p<0.05$) after 36 days of water stress treatment. The highest chlorophyll content was found in control plants. The lowest values were observed in treated plants. At 40% CC, the decrease of Chl*a,* Chl*b* and total chl content were about 0,14 µg/g DM; 0,06 µg/g DM and 0,2 µg/g DM, respectively. However the differences decreased further with a return to optimum irrigation.

Proline Content: The proline contents of untreated and treated plants were significantly different ($P = 0.023$). The control plants had lower aerial part proline contents compared with stressed plants. Fig. 2 shows that the amounts of proline in control plants were within a range of 5.54 $\mu mol.g^{-1}$ DM aerial part dry weight. Limiting water supply for 36 days increased that level by 4 and 5 times under 70 and 40% CC, respectively. After 40 days of re-watering, this amount decreased specially for plants that were severely stressed but it didn't reach the control level.

Soluble Sugar Content: Soluble sugar contents were remarkably lower than those of proline (Fig. 2). Water stressed plants showed a higher soluble sugar content by comparaison to control plants. It was about 0.69 and 0.74 $\mu mol.g^{-1}$ DM with an increase of 22 and 35% under 70% and 40% CC, respectively. Soluble sugar content of re-watered plants showed a decrease especially for previously severe stressed plants which reached the control level (0.6 $\mu mol.g^{-1}$ DM).

Osmotic Potential: Osmotic potential was remarkably reduced after the water stress treatment (Fig. 3). It decreased from -0.8 MPa under optimal water conditions to -1.5 MPa and -1.8 MPa under 70%CC and 40%CC, respectively, but it increased further when plants were re-watered for 40 days.

DISCUSSION

The present study aimed at investigating the response of *A. herba-alba* to water deficit stress. Plants were submitted for 36 days to 70 and 40 %CC and the reversibility of water deficit effect was assessed by analysing the behaviour of water-stressed plants, once transferred to 100% CC treatment for 40 days.

Water stressed plants showed a significant decrease in dry matter production similarly to the results noticed by Inman-Bamber and Smith [26], Asch *et al*. [27] and Singh and Singh [5].

The response of *A. herba-alba* to water stress deficit was also characterised by a significant decrease of chlorophyll content under both 70 and 40%CC. This was also noticed by Tahri *et al*. [28] and Scheirs et De Bruyn [29] and was considered as a means to reduce photooxidation threat [30,31]. Similar results were obtained by Thalooth *et al*. [30] who noticed that the decrease of water supply diminish the chlorophyll (a and b) and carotenoids content in the leaves of *Vigna radiata* at any stage of plant developpment. The same study explained this decrease by the decrease of magnesium that is one of the principal chlorophyll constituents. It was also revealed that this can be attributed to the high level of chlorophyll degradation more than to its biosynthesis limitation under water stress conditions [32].

On the other hand, a marked increase was found in proline and soluble sugar accumulation by *A. herba-alba* under water deficit stress (Fig. 3). At the end of treatment, stressed plants accumulated highly soluble sugar content as was noticed by Mohsenzadeh et *al*. [33]. Again, treated plants showed four to five-fold more proline than controls and similar results were revealed with *Pringlea Antiscorbutica* [34] and *solanum tuberosum* [35]. In stress tolerance of plants, the soluble compounds containing sugars, proline, soluble proteins and phenolics have an important role [36]. Proline accumulation was considered as an indicator of plant tolerance to drought conditions [35]. It improves water absorption [1] and reduces water loss [32] and may serve as a means of osmotic adjustment and storing carbon and nitrogen when stress leads to slower growth [7].

Soluble sugar content noticed in the current study was lower than that detected in other similar studies. This difference could be explained by the limited period of water stress treatment considered in our experiments. In Fact, Mohsenzadeh *et al*. [33] noticed that soluble sugar accumulation is highly dependent on the period of the applicated water stress. Therefore, it's estimated that *A. herba-alba* could accumulate more sugar under a long natural drought period.

Re-watered plants showed a decrease in aroline and soluble sugar content similarly to the result of Mohsenzadeh *et al.* [33] with *Aeluropus lagopoides.*

The decrease in proline concentration noticed in this study is associated to a decrease in chlorophyll content (Fig. 4) as was observed by Tahri *et al.* [28] with *Triticum durum* submitted to an osmotic stress. This inversal correlation was considered as a result of a competition between these two metabolits for their precursor, glutamate [28].

To appreciate the effect of water stress on organic solutes accumulation, proline and solubles sugars were expressed in relation to water content (Fig. 5).

The current study showed also that proline synthesis under water stress is correlated to a decrease in water status (**Fig. 0**). This correlation is comparable to that described by Mohsenzadeh *et al*. [33] who noticed that these solutes production rised when the relative water content (RWC) was reduced.

Previously stressed plants of *A. herba-alba* showed an increase of water status and a decrease in proline content after re-watering as was described with *Aeluropus lagopoides* [33].

The measurment of plants osmotic potential in this study showed a significant increase of this parameter under water stress conditions. Drunasky et Struve [37] found the same results with *Quercus macrocarpa* and concluded that that species had a big resistance and tolerance to water deficit conditions, which can be so deduced for *A. herba-alba*.

The osmotic potential measured in our experiment was very high and couldn't be explained by proline and sugars lonely. Other compounds could be implicated in this osmotic adjustment such as potassium which was considered by Hong Bo *et al*. [2] as an osmotic regulator.

Results obtained by Ourcival [20], which revealed that *A. herba-alba* didn't have a high osmotic adjustment didn't disapprove our results if we consider the differences in developpment stages. In Fact, Zid et Grignion [38] noticed that water stress resistance is highly dependant on plant growth stage and couldn't be neither defined nor absolutely predicted, but must be considered as a relative parameter.

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