Full Length Research Paper

The physiological and behavioural responses of argan seedlings (*Argania spinosa* (L.) Skeels) to water stress in the semi-arid Western Algeria

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This paper presents the behavior, morpho-physiological and biochemical responses of argan seedlings to three water regimes at 30, 60 and 100% of field capacity. Based on the results of the growth parameters, we use the best result obtained from height growth. The increase in the number of leaves and thorns is obtained in seedlings subjected to water stress levels of about 60 and 100% against the application of a severe water stress of 30 and 0%. This 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. Regarding the water parameters, we noted a gradual decline in the relative water, the fresh and dry weight of leaves, stems and roots contents, with minimum values in the most severe stress (30 and 0%). Regarding the biochemical parameters, a decline in the relative water content is followed by a significant accumulation of soluble sugars and protein. Regarding the physiological parameters, stomata density intensifies level maintained at low water contents where the number of stomata per unit area behaves greatly compared to that of the hydrated treatments. Sweating is greatly reduced by increased water deficit; it is even more pronounced in the most severe treatment (30 and 0%).

Key words: *Argania spinosa*, germination, morphological, physiological, growth parameters, water, physiological water stress, biochemical water stress.

INTRODUCTION

The argan tree (*Argania spinosa* (L.) Skeels) is found only in large areas in Southern Morocco and the south-west of Algeria. It is the northernmost representative of a family in this region that hardly accepts tropical

Abbreviations: RWC, Relative water content; **DWR,** dry weight of the root; **DWA,** dry weight of the aerial part.

representatives (Lewalle, 1991). In Algeria, the argan tree is seen on the margins of Northwest Tindouf. It occupies the Wadi beds; tree density increases gradually toward the Atlantic Ocean, showing the tree of monotypic genus. It is the only survivor of tropical flora outside its natural range. It is considered an endemic tree (Otmani, 1995). The argan tree is considered as xerophytic and thermo-philic species that adapts to high periods of prolonged drought and the drying effects of wind. This adaptation of the argan faculty is not related to the fact that this tree saves water, but its ability to draw water from great depths (Mokhtari, 2002). Also in the same periods of drought, the growth of certain branches of the tree decreases (El aboudi et al., 1991). According to Boudy (1950), more than a forest region is dry (arid and semi-arid floor), and the density of its older stock is reduced, because the roots need a large living space to draw soil water. The ecology of the argan tree is closely related to climatic factors; it is considered to be the least demanding tree species in rainfall (Boudy, 1952). Nevertheless, it needs a hydrometric degree air, where it can live only above a certain temperature in the moist coast (Victor, 1917). According to Nouaim and Chaussoud (1993), argan installs inward only weakly beyond 150 kilometer of the Atlantic Ocean, thus justifying that humidity seems to be a key parameter for this ecology species. In this context, Wattier (1917) and Emberger (1939) and Boudy (1950) argue that the altitudinal limit of the argan tree is the isotherm (m = 3.8°C) and a remarkably high thermal support of the order of 50°C. Similarly, Emberger (1924) reported that cold is the determinant of the geographical distribution of the argan tree. Plants generally require optimal environmental conditions for normal growth, but they are often subject to extreme factors of water potential, temperature and salinity, generating different types of stress (Hopkins, 1999; Bouaouina et al., 2000). However, water stress has a special place because of its frequency, and the place that water plays in metabolic processes (Turner, 1990; Bálo et al., 1995). Due to its role in photosynthesis, transport and accumulation, as well as multiplication and cell enlargement, water has a critical role in the growth and development of plants (Rascio et al., 1990; Blake et al., 1991; Medrano et al., 1992; Cabeza et al., 1995). Damage caused by water stress results from drying protoplasmic; the departure of water causes an increase in the concentration of solutes. If the volume of protoplasm decreases, membrane integrity and protein is also affected by the drying, this causes metabolic disorder (Hopkins, 1999). Water deficit can also reduce turgor pressure of the plant. This loss of turgor state may cause the cessation of cell elongation in particular (Gate, 1995). The aim of this work is to determine argan seedlings' morpho- physiological parameters for assessing the degree of tolerance of the species to drought conditions (water stress). This section focuses on water parameters (fresh and dry weight of leaves, stems, roots, and the relative water content), physiological parameters (stomata density and transpiration), and biochemical parameters through an analysis of foliar soluble sugars and protein.

MATERIALS AND METHODS

Argan seedlings (nr = 200) were obtained after germination tests were performed in the laboratory. The seedlings were taken to a greenhouse at the University of Mascara and controlled under light and temperature conditions. The night and daytime temperatures are maintained respectively (15 and 28°C) and photoperiod is 12 h day. Seedlings are irrigated regularly every 10 days and divided into 4 groups:

Lot 1: Witness (T) was maintained at 100% of field capacity.
Lot 2: Seedlings were maintained at 0% of field capacity.
Lot 3: Seedlings were maintained at 60% of field capacity.
Lot 4: Seedlings were maintained at 30% of field capacity.

To determine the field capacity of the pot, we weighed pots containing 5 kg of dry substrate used in the experiment (W1 = 5 kg of dry soil). Then we proceeded to irrigate seedlings to saturation, while covering the pots with black plastic to prevent evaporation of water. Thereafter, we soaked the pots for 48 h. Finally, we reweighed the W2 pots (weight saturation). So, the difference between W1 and W2 represents the amount of water that the soil was able to keep at 100% of field capacity. On the other hand, due to the evolution of the biomass of seedlings over time, the amount of water returned to each pot and irrigation frequency was adjusted every 10 days for 6 months (Figure 1).

Measured parameters

Water parameters

1. Measurement of fresh and dry weight of leaves, stems, and roots. The dry root (DWR) Weight: It is obtained by passing in an oven at a temperature of 60°C for 48 h (Belkhodja and Sotani, 1992). Air dry weight: The dry weight of the aerial part (DWA) of stems and leaves is evaluated by passing in the oven at a temperature of 60°C for 48 h.
2. Measurements of parameters (DWR and DWA) involved the ratio: DWR/DWA (dry weight of the root/dry weight of the aerial part).
3. Measurement of relative water content (RWC).

Thus, several parameters are used to quantify the water status of plants subjected to water deficit. We include the percentage of moisture, relative water content, and the percentage of imbibitions. So, despite the difficulties inherent to the operations of weighing the plant studied and changes in fresh and dry weight, we chose to study the relative water content. It expresses the amount of water present in % of the saturated amount measured and allows a physiological assessment of water status of the plant as well as the water potential. The formula below shows the estimate of this parameter:

$$RWC = (FW - DW) . 100 / (W\ Sat - DW)$$

FW = Fresh weight; W Sat = weight of saturation; DW = dry weight
1. The fresh weight is determined by weighing the sheet immediately after collection.

Figure 1. A, B and C. Germination tests in laboratoty; D. Argan seedling ; E. Acclimatization of arganseedlings ; F. seedlings argan in a greenhouse.

2. The weight of water saturation is obtained by placing the sheet distilled water for 24 h.
3. The dry weight is obtained by placing the sheet in the oven at 80°C for 24 h.

Physiological parameters

Measurement of stomata density

Stomatal density is obtained by the method of Dohman et al. (1991) and used by Lemeu (1999 and 2000). Principle: The leaf prints are seen by spreading a few drops of nail polish on both epidermis (upper and lower) of the sheet. After drying, the footprints are taken by a transparent adhesive tape, which is adhered to a blade in order to count the number of stomata (mm^2) by using microscope equipped with ocular micrometer calibrated and supporting a phototube ZEISS brand. Finally, measurements are made on the number of stomata per unit area on the two types of leaf epidermis.

Measurement of transpiration

The middle of the leaf sample (perspiration is maximum) is weighed (fresh weight of the leaf = P1). The same sample is used to calculate the leaf surface; whereas in our case, the sheet is treated as an ellipse. The following formula allows us to calculate the leaf surface (Boudersa, 1998),

$$S = 3.14. (A. b / 4)$$

With: - a: This is the length of the leaf

- B: This is the maximum width of the leaf
Then the sheet is left in the open air for one (01) h, and then the weight loss after water is evaluated (Wb).

Therefore, the calculation of sweating is achieved by the following formula:

$$Swt = (Wa - Wb). S^{-2}. T^{-1}.$$

Swt: Sweating (mg H_2O. area $^{-2}$. time $^{-1}$); Wa: fresh leaf weight; Wb: weight loss after water; S^{-2}: unit leaf area (cm^2); T^{-1}: Unit of time (second) s.

Biochemistry parameters

Protein determination

The method used is that of Troll and Lindsley (1955), simplified and developed by Dreier and Goring (1974). Principle: It is to take 100 mg of plant material (1/3 middle of the sheet), and then add 2 ml of 40% methanol. The whole was heated to 85°C in a water bath for 60 min. After cooling, the following extracts were added:

1. 1 ml of acetic acid (CH_3COOH).
2. 25 mg of ninhydrin ($C_6H_6O_4$).
3. 1 ml of a mixture containing 120 ml of distilled water, 300 ml of acetic acid, and 80 ml of phosphoric acid (H_3PO_4, d = 1.7).

The mixture is boiled for 30 min, then the solution turns red. After cooling, 5 ml of toluene was added to the solution and then shaken, at two separate phases (upper and lower phases). After removal of the lower phase, the upper phase is recovered and dried by the addition of a spatula of sodium sulfate anhydrous Na_2SO_4. Finally, we determine the optical density (528 nm). The values are converted to protein content from the standard curve, whose relationship is as follows:

$$Y = 0.1043 x.$$

Figure 2. Determination of soluble sugars.

Determination of soluble sugars

The total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are determined by the method of Dubois et al., 1956. Principle: It is to take 100 mg of plant material (1/3 of the middle sheet) in test tubes, add 3 ml of 80% ethanol to extract sugars and then leave in room temperature for 48 hours. At the time of the assay, the tubes are placed in an oven at 80 ° C to evaporate the alcohol in each tube; 20 ml of distilled extract (sample solution) water is added. In clean glass tubes, we put 2 ml of the sample solution, 1 ml of 5% phenol (phenol is dissolved in distilled water), and adds up quickly 5 ml of 96% concentrated sulfuric acid (d = 1.86), while avoiding pouring acid on the tube walls. We obtained an orange yellow solution on the surface; it passes through a vortex to homogenize the color of the solution. The tubes were left for 10 min and placed in water for 10 to 20 min at a temperature of 30°C.
Note: The color of the reaction is stable for several hours. The values obtained are reported in the standard range, Y = 4.3918 x - 0.1946 (Figure 2).

Statistical analysis

All tests were repeated three times on measures of water, biochemical and morpho- physiological parameters. The results presented as histograms usually mean values. They were produced by Excel. In the case of paintings, the results were subjected to statistical analysis (analysis of variance, ANOVA / MANOVA) with the help of STATISTICA software.

RESULTS

Observations and measurements of water physiological and biochemical parameters

Because the physiological measurements are made at the end of the period of water stress for different treatments, we perform statistical analysis of morphological, physiological and biochemical parameters under the effect of water stress at the same time (after 9 months).
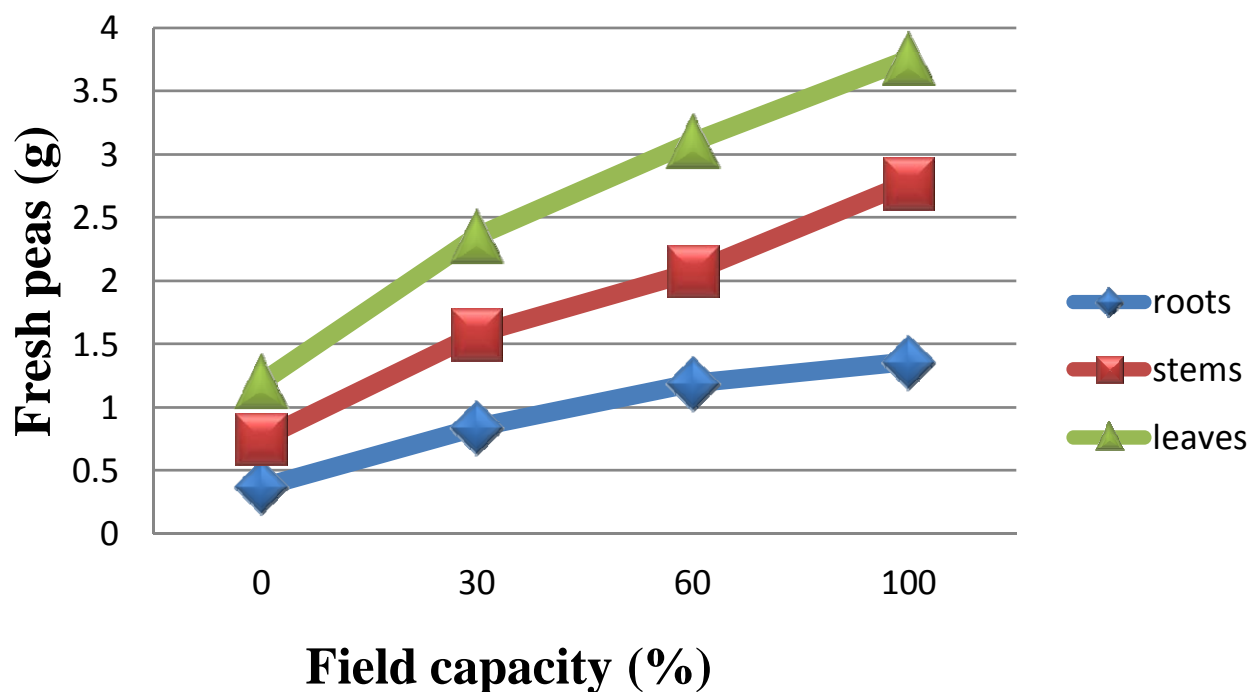
Water Parameters

Effect of water stress on fresh and dry weight of leaves, stems, and roots

The decrease in fresh and dry weight of leaves, stems and roots is due to the level of water stress.–Indeed, in the argan seedlings, processed at 0% and 30% of the

Table 1. The standard deviation and the average fresh weight of leaves, stems, and roots of argan seedlings.

Stress (%)	The average		
	leaves	stems	roots
0	0.37 ±0.09	0.75 ±0.15	1.20 ±0.14
30	0.84 ±0.11	1.58 ±0.16	2.35 ±0.14
60	1.19 ±0.14	2.07 ±0.14	3.10 ±0.20
Witnesses (100%)	1.36 ±0.58	2.77 ±0.19	3.75 ±0.12

Figure 3 . Change in fresh weight of leaves, stems, and roots of seedlings argan, aged 9 months and subject to different levels of water stress. Base of formula

field capacity, we observed lower values of fresh weight of leaves, stems, and roots, respectively in the following order: 0%: 0.37 g, 0.75 g and 1.2 g; 30%: 0.84 g, 1.58 g and 2.35 g (Table 1, Figure 3).

The maximum fresh weight of leaves, stems, and roots was found in seedlings treated with 60% and 100% of field capacity, in the following order: 60%: 1.19 g, 2.07 g and 3.1 g; 100 %: 1.36 g, 2.77 g and 3.75 g. Concerning the dry weight, leaves, stems and roots of argan and control seedlings treated at 30% of field capacity had low values, in the following order: 0.125, 0.290 and 0.27 , and 0.19, 0.58 and 0.59 g. And the highest values are observed in seedlings treated at 60 and 100% of field capacity, in the order of 0.275, 0.7 and 1.18, and 0.39, 0.87 and 2.18 g. The results show that the aerial part is more sensitive to the effect of water stress than the root. The reduction in vegetative growth observed in seedlings can be explained by the increased osmotic pressure of

the medium, which prevents the absorption of water by the root system and consequently causes a reduction in the growth of the vegetative apparatus. Similar effects have been seen on the vegetative growth in the seedlings of argan (Table 2, Figure 4).
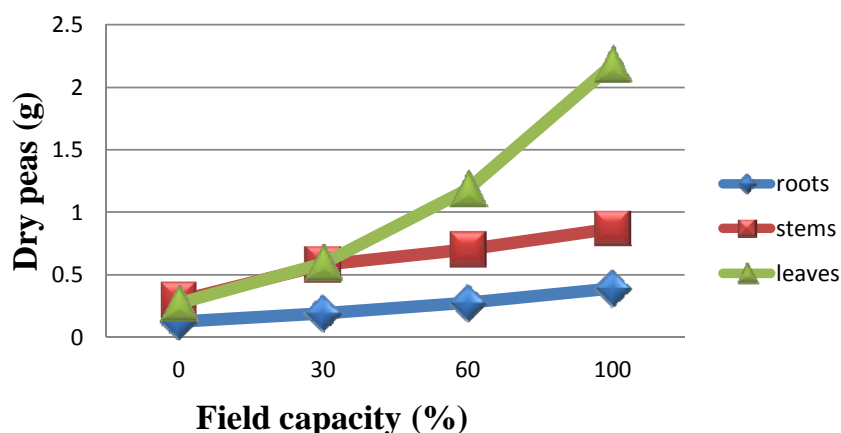
Effect of water stress on the DWR/ADW ratio

It is reported that root dry weight and air dry weight depend mainly on water supply. The DWR/ADW ratio has changed by 1.55, 2.52, 4.07 and 4.6 in argan seedlings witness at 30%, 60% and 100% of field capacity. Lack of water caused a decrease in the DWR / ADW ratio between the two groups; at the same time the seedling root system is well developed and the air dry weight values are still low in relation to the dry weight root.

Indeed, the lowest values of DWR and ADW are

Table 2. The standard deviation and the average dry weight of leaves, stems, and roots of argan seedlings.

Stress (%)	Average		
	leaves	stems	roots
0	0.125 ±0.026	0.29 ±0.099	0.27 ±0.067
30	0.190 ±0.039	0.58 ±0.122	0.59 ±0.137
60	0.275 ±0.075	0.70 ±0.081	1.18 ±0.252
Witnesses (100%)	0.39 ±0.196	0.87 ±0.200	2.18 ±0.122

Figure 4. Change in dry weight of leaves, stems, and roots of seedlings argan, aged 9 months and subject to different levels of water stress.

Table 3. The standard deviation and the mean DWR / ADW ratio argan seedlings.

Stress (%)	The average
0	1.55 ±0.11
30	2.52 ±0.071
60	4.07 ±0.17
Witnesses (100%)	4.60 ±0.25

Table 4. The standard deviation and the mean of the relative water content of argan seedlings.

Stress (%)	The average (%)
0 %	59.89 ±8.17
30 %	75.55 ±6.90
60 %	87.60 ±3.29
Witnesses (100%)	95.45 ±1.51

recorded in the control seedlings with respective values of 0.35 and 0.24 g. While the mean values of the DWR and ADW are recorded in seedlings at 30 and 60% of field capacity, with 0.86 and 1.55 and 0.34 and 0.39 g

values, respectively. The maximum values of DWR and ADW are identified in seedlings at 100% of field capacity, with respective values of 2.24 and 0.47 g. The results show that the ratio of dry root weight and dry weight of air is negatively correlated with the intensity of water stress applied. This correlation is driven by the values of the DWR/ADW which remain lower in the most stressed argan seedlings (Table 3, Figure 5).

Effect of water stress on the variation of the relative water content (RWC)

The analysis of the results shows that the relative water content is greatly influenced by the water regime. The relative water content decreases as the intensity of the stress is high. The highest in water tenure is noted in the lot with 60% of field capacity. It has a maximum value of about 100% (control) of field capacity, ranging between 87.60 and 95.45%.

The relative lower water tenure is stored at 0% of field capacity, with 59.89% value. The average value is found in the batch of 30% of capacity field, in the order of 75.55%. The analysis of the relative water content (RWC) describes in a comprehensive manner the water status in response to water stress (Table 4, Figure 6).
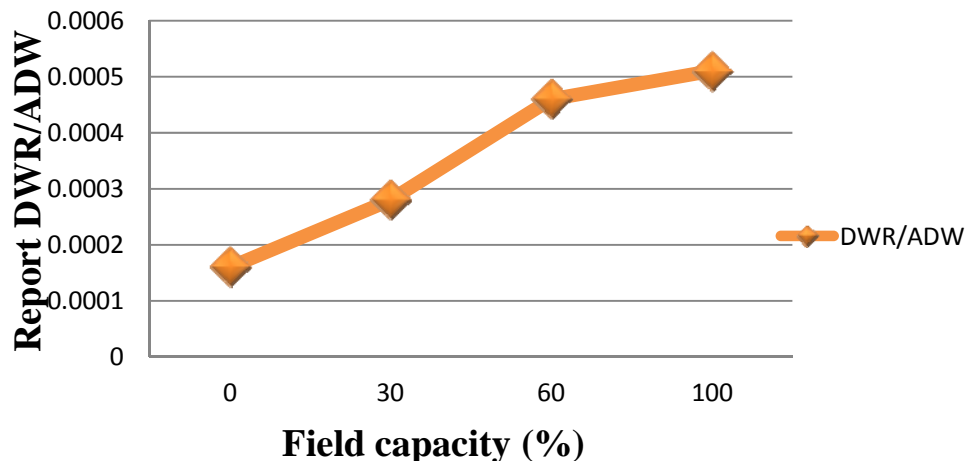
Figure 5. Change DWR / ADW ratio argan seedlings aged 9 months and subject to different levels of water stress.
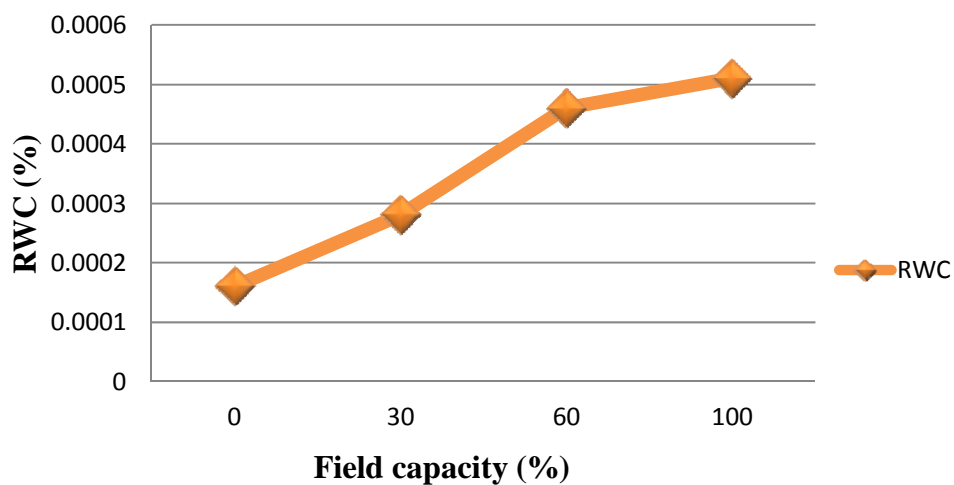

Figure 6. Variation of relative water content of the argan tree seedlings, aged 9 months and subject to different levels of water stress.

Physiological parameters

Stomata density

The density of stomata in the upper and lower epidermis is strongly influenced by the water regime. Thus, we find that increased water deficit causes an increase in stomata density at both upper and lower leaf surfaces of the argan tree (Table 5).

In seedlings of the argan tree with 0% of field capacity, stomata density of the upper and lower epidermis has almost doubled (14.8 s/mm2, 24.5 s/mm2). In seedlings treated with 30 and 60% of field capacity, stomata density of the upper and lower epidermis increased slightly at 12.8 s/mm2, 18.8 s/s/mm2, and 10 s/s/mm2, 15.1 s/mm2. And lower stomata density on the upper and lower

epidermis is recorded in seedlings treated with 100% of field capacity, at 5.7 s/mm^2, 11.7 s/mm^2 (Figure 7 and 8).

Effect of water stress on the variation of sweating

The intensity of sweating is conditioned with the level of water stress. Moreover, we find that the lowest level of perspiration is recorded in seedlings treated with 0% of field capacity and those treated with 30% of field capacity, in the order of 0.000164 mg H2O. $S^{-1}.cm^{-2}$ and 0.000285 mg H2O. $S^{-1}.cm^{-2}$.

On the other hand, maximum sweat is obtained in seedlings treated with 60 and 100% of field capacity, with values of 0.000463 mg $H_2O.S^{-1}.cm^{-2}$ and 0.000517 mg

Table 5. The standard deviation and the mean stomatal density argan seedlings.

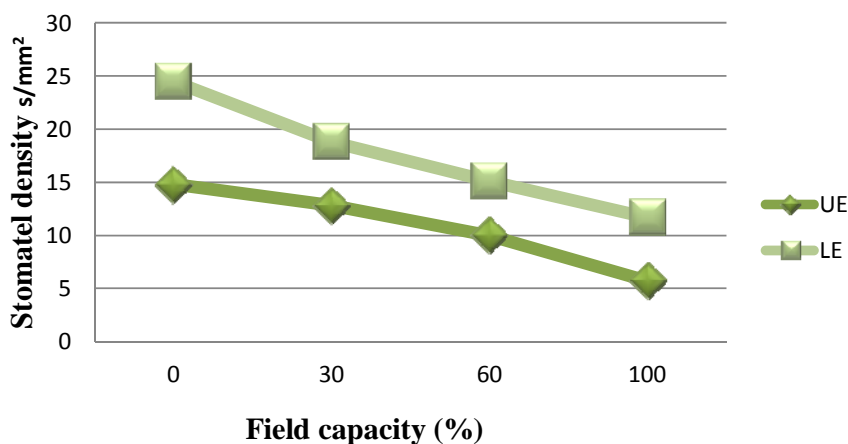| Stress (%)	The average (s/mm^2)	
	lower Epidermis	upper epidermis
0	14.8 ±1.39	24.5 ±2.67
30	12.8 ±1.03	18.8 ±1.68
60	10 ±1.33	15.1 ±1.68
Witnesses (100%)	5.7 ±2.00	11.7 ±1.56

Figure 7. Variation in stomata density of seedlings argan, aged 9 months and subject to different levels of water stress.
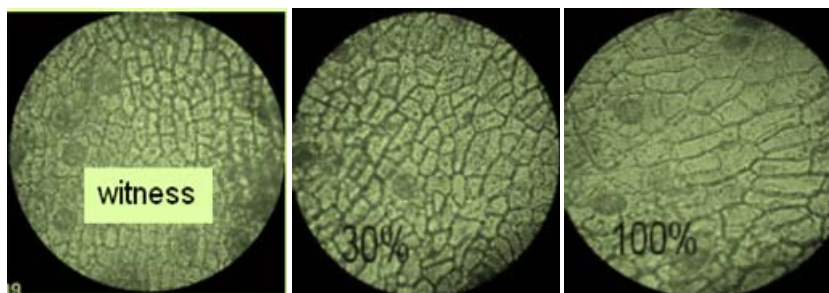

Figure 8. stomatal density (stomata per unit area) of the upper epidermis of the leaves of the argan tree, conducted under different water regimes.

Table 6. The standard deviation and the mean transpiration argan seedlings.

Stress (%)	The average (mg H$_2$O. S^{-1}.cm^{-2})
0	0.00016 ±0.000034
30	0.00028 ±0.000018
60	0.00046 ±0.000020
Witnesses (100%)	0.00051 ±0.000021

$H_2O.S^{-1}.cm^{-2}$. The relationship between water deficit and physiological parameters shows the need for maintaining good sweat (Table 6, Figure 9).

Biochemical parameters

Effect of water stress on the variation in the content of proline

In the results, we note that water stress is causing an increase in proline tenure in leaves of argan seedlings.
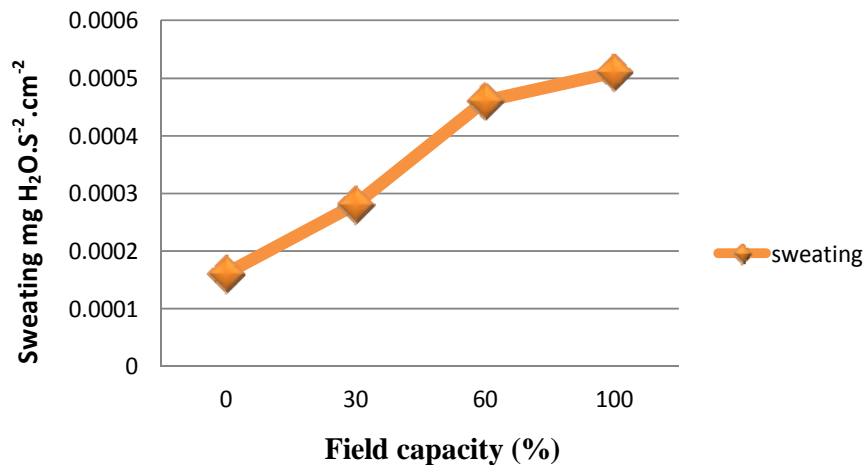
Figure 9. Change sweating argan seedlings, aged 9 months and subject to different levels of water stress.
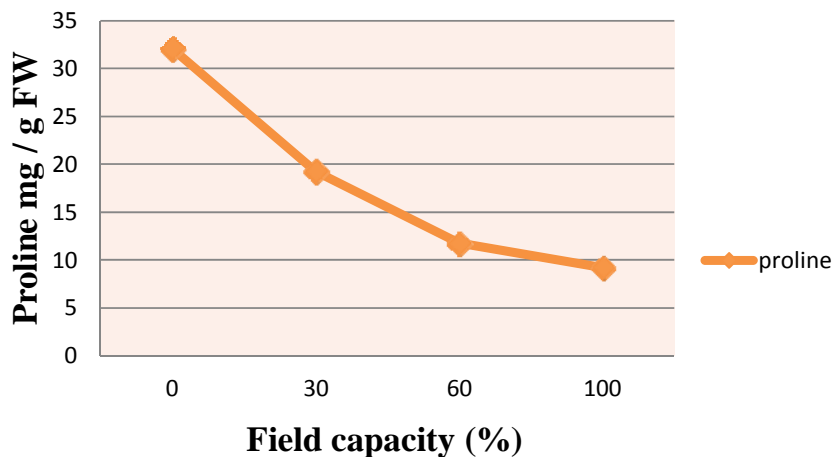

Figure 10. Change in proline accumulation in leaves of argan tree seedlings, aged 9 months and subject to different levels of water stress.

Table 7. The standard deviation and the average proline argan seedlings.

Stress (%)	The average (mg/g PF)
0	0.05 ±0.0013
30	0.037 ±0.0022
60	0.025 ±0.0016
Witnesses (100%)	0.013 ±0.0012

Indeed, in control seedlings and those treated with 30% of field capacity, the contents of proline are more marked, in the order of 0.0505 mg/g FW and 0.0379 mg/g FW.

The average values of proline levels are identified in argan seedlings treated at 60% of the field capacity, of about 0.0251 mg/g FW. And the lowest levels of proline are stored in the control seedlings treated with 100% of the field capacity of about 0.0132 mg/g FW (Table 7, Figure 10).

Effect of water stress on the accumulation of soluble sugars

The levels of applied water stress cause significant increase in the levels of soluble sugar. Indeed, when the level of water stress becomes severe, the argan tree seedlings record very high levels of soluble sugar. So the highest level of soluble sugar is stored in the argan tree seedlings with 0% of field capacity and those treated with 30% of field capacity, with values of 32.28 mg/g FW and

Table 8. The standard deviation and the mean of soluble sugars argan seedlings.

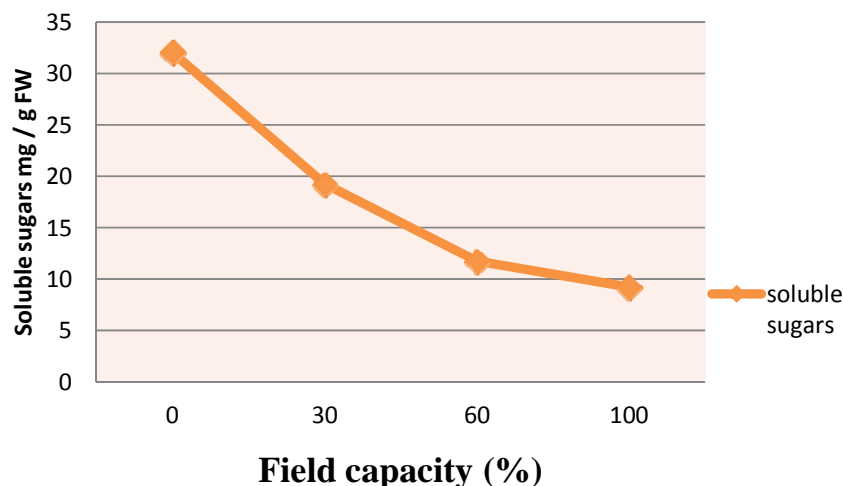| Stress (%)	The average (mg/g PF)
0	32.12 ±0.49
30	19.27 ±0.337
60	11.74 ±0.26
Witnesses (100%)	9.21 ±0.43

Figure 11. rate of accumulation of soluble sugars in the leaves of the argan tree seedlings, aged 9 months and subject to different levels of water stress.

19.34 mg/g FW. The contents of soluble sugar readings are the lowest in seedlings treated with 100% of field capacity (9.348 mg/g FW).In seedlings treated with 50% of field capacity, we find that the levels of soluble sugar are average, about 11.60 mg/g FW. The effect of water deficit on biochemical parameters is used to highlight a highly significant effect of water deficit on the accumulation of leaf protein (Table 8, Figure 11).

DISCUSSION

The effect of drought is also expressed by a progressive and rapid slowdown in growth as the primary water deficit reduces turgor and consequently the expansive power of the leaves. Results linking water deficit and water parameters show that the effect of water stress on root growth, leaf and stem is very remarkable, corresponding to a statistically significant difference noted between the different treatments. It is also shown that the aerial part is more sensitive to the effect of water stress than the root. The reduction of vegetative growth observed in seedlings argan can be explained by the increase of the osmotic pressure of the medium, which prevents the absorption of water by the root system and thus leads to a reduction in

the growth of the vegetative apparatus. Similar effects have been seen on the vegetative growth in seedlings of argan. The same results were also obtained by the same species (Harrouni, 1995); and according to Thakur and Rai (1982), water deficit causes a delay in plant growth. It results in a reduction in the height and diameter of the nod, a shortening of the internodes and a decrease in the number of leaves and leaf surface (Aspinall, 1986). With a decrease in root growth depending on the degree of exploitation of the soil by the root system, the argan seedlings tend to reduce the length of the roots to overcome the effect of water stress caused. The results show that the ratio of dry root weight and dry weight of air is negatively correlated with the intensity of water stress applied. This correlation is driven by the values of the PSR / PSA which are still lower in the most stressed seedlings of argan. This indicates that the water deficit did not affect similarly the two parts of the plant. The increase in the ratio could be explained by an increase in root growth mostly affected by lack of water than air. The preferential development of the root system to the detriment of the aviation system is considered by many authors as a criterion for drought resistance; making better use of available water more accessible (Bensalem, 1993; Bchini et al., 2002). The analysis of the relative

water content (TRE) is used to describe in a comprehensive way the water status in response to water stress and to assess the ability to achieve a good osmoregulation and maintain cell turgor (Eljaafari, 2000).

The water status of the plant, expressed by the relative water content was sensitive to changes in water available in the soil. Indeed, water deficit causes a regression of the values of relative water content in which these findings are confirmed by the net negative and significant relationship revealed between water deficit and relative water content. During our experiments, the results obtained indicate that the application of severe water deficit negatively affects and significantly reduces the relative water content of the tissue. The greater the intensity of water stress, the more the water content is lowered in the most severe water stress (0 and 30%). This reduction is due to dehydration, leading to a loss of water in the cells. The relative water content is high compared to control seedlings subjected to water stress levels at 60 and 100%. This is probably due to active osmoregulation. Similar work was done by Morgan (1984) Bennaceur (1994) and Nouri (2002) on different types of plants subjected to water stress of different intensities.

The relationship between water deficit and physiological parameters shows the need for maintaining good sweat, and consequently maintaining stomatal coverage during water deficit directly involved in the level of the relative water content (Denden and Leneur, 1999; Nouri, 2002). This indirectly conditions the use of water effectively for photosynthesis (Erchidi et al., 2000). According to Slama et al. (2005), the reduction of water loss by stomatal closure is a means by which plants adapt to drought. Thus, our results show a strong positive relationship between water stress and stomatal density at both upper and lower epidermis of the leaves. According to Slama (2002), the increase in the number of stomata per unit area could be a factor in resistance to water stress if it is accompanied by a good physiological activity.

The increase in stomatal density can increase the net CO_2 assimilation and reduce water loss. Indeed, a large number of stomata can cause stomata small size and fast closing (Slama et al., 2005). Passive cell dehydration of argan seedlings induces turgor loss at the cellular level. To overcome this, plants try to limit water loss, which results in decreased sweating due to the closure of the stomata (Yakhlef, 2001; Nouri, 2002). The results show a very highly significant positive correlation between transpiration and relative water content. Estimated through water loss by excised leaf transpiration is greatly reduced by the accentuation of the water deficit. This decrease was more marked in the most severe treatment. Similarly, these data are confirmed through the work of Clark et al. (1989, 1991), which indicates that sweating is a key parameter variation of the water status of the plant and affects productivity. The effect of water

deficit on biochemical parameters is used to highlight a highly significant effect of water deficit on the accumulation of leaf proline. Similar results were encountered in different forest species such as *Fraxinus excelsior* and *Quercus petrea* (Ladefoged, 1963), *Quescus coccifera* (Losch et al., 1982) and *Argania spinosa* (Peltier et al., 1992). However, Claussen (2005), working on tomato under salt and drought stress, suggested that the accumulation of proline could be due to an induction/activation of the enzyme involved in the biosynthesis of proline or lowering of glutamate in its oxidation and improved proteins. One of the causes of the accumulation of proline is membrane proteolysis which could accumulate proline following a disturbance in the metabolism of proteins (Bezzala, 2005). Clifford et al. (1998), in their work, show an accumulation of proline and soluble sugars (hexoses , glucose). This is in agreement with our results that a very highly significant positive correlation exists between soluble sugars and proline. This suggests the latter is extremely sensitive to the synthesis of proline at the reduction of NADP + and enhances cellular data, stating that the synthesis of proline is a means for regulating cellular redox potential "contribution in energy adjustment" (Bellinger et al., 1987; Rai, 2002). Furthermore, it can be inferred that soluble sugars (sucrose and glucose) are effectors of the accumulation of proline. Mastrangelo et al. (2000) and Nouri (2002) suggest that the ability to accumulate in these genotypes osmoticums (sugars and proline) is used as the basis for selecting drought tolerant genotypes. The levels of soluble sugars sheets show a very highly significant negative correlation between the relative water content and accumulation of soluble sugars (glucose). Therefore, an increase in soluble sugars was seen in many woody plants such as Eucalyptus and microtheca (Chunyang, 1998; Pesoli et al., 2003). According to these authors, the increase in soluble sugars is attributed to degradation of starch due to the rapid conversion of sucrose and inhibition of the synthesis of starch reserves.

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