Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 1, p. 110-123, 2016
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

# Physiological and biochemical characterization of Cork Oak Seedlings (*Quercus suber*.L) in Tunisia

**Abdessamad Abdessalem[1*], Saidi Ahmed[1], Chkioua Chaker[2], Ksontini Mustapha[1]**

[1]*Laboratory of Physiology and Ecology Forestry, National Institute for Research in Rural Engineering & Forestry INRGREF Tunis, Tunisia*

[2]*Laboratory of Plant Ecology, Faculty of Sciences of Tunis, 2092 El Manar Tunis, Tunisia*

Article published on January 26, 2016

**Key words:** Cork oak, water stress, drought adaptation, physiological markers, biochemical markers.

**Abstract**

The cork oak is native forest species, the most represented in Tunisia and especially to Mogods Kroumirie. This species is considerable regression estimated at 1.22% of the area per year . Natural regeneration of cork oak seedlings and by rejecting strain encounters strong difficulties mainly because of the great mortality among seedlings. The failure of this regeneration is mainly caused by environmental conditions characteristic of the Mediterranean climate (long dry seasons with hot winds and high temperatures) and by predation and anthropogenic factors. Our study is to analyse the physiological and biochemical responses of seedlings of cork oak, subjected to moderate and severe water stress, which was conducted in nurseries in Tunis in twelve populations of cork oak. The various treatments are used to test the tolerance of the cork oak plants to water stress by biochemical markers such as proline, sugars and starch. The accumulation of sugars is characteristic of woody plants in difficult environmental conditions, as in our experimentation proline and starch can be regarded as a biochemical marker of stress to the cork oak.

***Corresponding Author:** Abdessamad Abdessalem ✉ abdou.slouma@yahoo.fr

## Introduction

The world's forests and the Tunisian forests are multiple threats. Several divergent elements involved or may be involved in the evolution of these forests. Like the Mediterranean forests, cork oak Tunisian undergo each year a continual decline; since the beginning of the century, they have lost more ground than they have gained. According to several authors this situation results from the combined action of several historical, socio-economic and natural. Today aged, the cork forests are dying gradually giving way to training degraded with poor natural regeneration or occasionally present.

The genus *Quercus* is typical of such a situation. The oaks are angiosperm belonging to the family Fagaceae. This family is divided into seven genera (Manos and *al*., 2001): The genus *Quercus* comprises several hundred species. According to the authors, several classifications have been proposed on the basis of physiological and biochemical studies.

The study of different physiological and biochemical characteristics that allow plants to survive in some environments and not in others, is an important step in understanding the distribution patterns and abundance of species, a central goal of ecology (Givnish, 1988).

Studies have shown that plants could exhibit phenotypic plasticity (independent of the variability of genomes) for many traits, whether morphological traits, physiological, anatomical or traits related to development and reproduction individuals (Sultan, 2000). Whether the scale of the sheet or of the whole plant, these functional features vary with the size and age of the shafts (Mediavilla and Escudero, 2003).

In Tunisia, the cork oak is native hardwood forest species most represented the Tunisian territory and especially in Kroumirie (Hasnaoui, 1991). Therefore, it is very important to study the physiological and biochemical responses of seedlings at the end of cork oak to enhance the regeneration of cork oak on two complementary approaches and look for stronger and more recent sources to cope to water stress of the environment:

In this work, we seek to increase a corpus of knowledge of physiological and biochemical approach for analyzing tolerance among young oak seedlings cork in water stress, and which are essential for identifying a possible structuring of resistance to drought.

## Materials and methods

*Plant material and study site*

Cork oak seedlings (*Quercus suber* L.) are from acorns harvested from adult subjects areas Hammam Bourguiba [HB], Dar Fatma [DF], Oued Zeen [OZ], Ain Zana [AZ]; Mejen Essef [ME]; Blessed Mtir [BM] Jebel zouza [DZ] (Ain Draham) El Feidja [EF] (Gar Dimaou); Bellif [B] (Nefza); Jebel Khroufa [DK] (Tabarka); Keff El Rand [KR] (El Haouaria) and Hammam Jdidi [HJ] (Hammamet) (Table 1).

The choice of sites was performed according to geographical distribution, the bio-climate and relief. The tests were carried out in controlled conditions in the nursery of INRGREF in Ariana on a semi-arid bioclimate higher. Young plants are located in polybags of 12 cm in diameter and 20 cm in depth filled with a substrate (mixture containing 1/3 sand and 2/3 of cork oak humus) and whose composition physico chemical is given by (Table 2).

*Methods*

Our study focused on the following parameters:

*Physiological parameters*

The osmotic adjustment $\Delta\psi s100$: (AO): The osmotic adjustment is determined indirectly from the pressure volume curve; it is calculated from the difference between the osmotic potential at full turgor ($\psi 100\pi$) of stressed and the controls.

The osmotic adjustment = $\psi\pi 100$ (stressed) - $\psi\pi 100$ (controls).

*Biochemical parameters*

*Determination of soluble carbohydrates*

The extraction was performed according to the described by technique of Albouchi, 1977 (inspired by methods developed by Mc Cready *et al.,* 1950 and Staub, 1963. One hundred mg of dry powder plant (stem, leaf or root) were mixed with 10 ml of 80% ethanol. Is heated homogenate stirring in a water bath at 70 ° C for 30 minutes. After cooling and centrifugation for 10 minutes at 6000 revolutions / min, the soluble sugars contained in the supernatant were assayed for anthrone 0.2% (200 mg anthrone dissolved in 100 ml concentrated $H_2 SO_4$) in the presence of ethanol 80%.

The control consisted of a solution containing 2.5 ml of anthrone and 1.25 ml of 80% ethanol. The reference range was established with pure glucose, diluted in distilled water. The OD was read with a spectrophotometer (type Perkin - Elmer 295 E) at the wavelength of 640 nm. Soluble sugars contents are evaluated with reference to a line of predetermined calibration by the equation Y = 0.1468 OD - 0.0034 for translating the optical densities levels of soluble sugars, expressed in mg / g dry matter.

*Determination of Starch*

The starch extraction was inspired by methods developed by Mc Cready *et al.,* 1950 and Staub, 1963 described by Albouchi ,1997. The previous base (assay of soluble carbohydrates) containing starch is taken up in 80% ethanol to warm for 5 min and then centrifuged at 6000 r / min.

The supernatant was discarded. The previous step is repeated (4 times) until the reaction of the supernatant anthrone or negative. The final pellet is taken up in 1.5 ml of 35% perchloric acid and placed in an ice bath for 15 min. Centrifuged and remove the supernatant (S1). The pellet is réhydrolysed cold for 30 minutes. Centrifuged and the supernatant is recovered (S2). S1 and S2 are combined and the whole 5 ml with distilled water. The dosage of starch was carried anthrone in the presence of perchloric acid at 21%. The calibration range of the starch has been established with pure glucose, diluted with 21% perchloric acid. The OD was read with a spectrophotometer (type Perkin - Elmer 295 E). The readings are plotted on a calibration curve relating the absorbance at 640 nm for the pure glucose concentration, which can translate the optical densities starch content, expressed as mg / g of dry matter.

*Determination of proline*

The method used is that of Troll and Lindsley (1955), streamlined and developed by Dreier and Goring (1974). The assay is performed on the leaves, stems or roots. A vegetable powder sample of 100 mg of dry matter is attacked by 5 ml of methanol at 40%. The mixture is heated in a water bath at 80 ° C for 30 minutes. After cooling 1 ml is removed from the extract, to which is added 2 ml of acetic acid, 25 mg of ninhydrin and 2 ml of a mixture containing (300 ml of acetic acid + 80 ml of absolute orthophosphoric acid + 120 ml of distilled water).

The mixture is heated in a water bath at 100 ° C for 30 minutes. The solution gradually turns red. After cooling, 5 ml of toluene are added to the solution. After shaking several times, the upper phase was recovered, to which a spatula anhydrous $Na_2SO_4$ was added. Reading is performed by the spectrophotometer at a wavelength of 528 nm. The values obtained are plotted on a standard curve and are expressed in mg / g of dry matter. The standard solution is prepared with different concentrations of proline.

*Static Analysis*

The performed statistical analysis is to compare the variables studied; it is produced with the SPSS18 software. The measures have been an analysis of the variance by a factor depending on the case by the Fisher F test to verify the equality of the means of hypothesis risk threshold of 5%. The graphical outputs were achieved with the Excel 2007 software (Table 3).

**Results**

*Physiological characterization of different populations of cork oak*

*Osmotic adjustment*

The values obtained from the cork oak seedlings are of the order of 0.04; 0.17; 0.39; 0.28; 0.66; 0.10; 0.51; 0.22; -0.01; 0.2; 0.44 and 0.10 MPa, respectively, for the populations of Oued Zeen; Keff El Rand; Hammam Jdidi; Dar Fatma; Jebel zouza; Jebel Khroufa; Ain Zana; Mejen Essef; Hammam Bourguiba; El Feidja; Béni Mtir and Bellif at the end of the first cycle. They are 0.02; 0.10; 0.11; 0.16; 0.38; 0.57; 0.44; 0.67; 0.06; 0.04; 0,02et 0.03 MPa at the end of the second cycle (Fig.1).

Statistical analysis showed a highly significant difference between all populations except between people group AB (Ain Zana; Hammam Jdidi, Béni Mtir, Dar Fatma El Keff Rand, El Feidja and Mejen Essef) and group B (Bellif , Djebel Khroufa, Oued Zeen and Hammam Bourguiba) during the cycle 1in 5% threshold.

**Tabe 1.** Characteristics geographical, bioclimatic and relief of different populations of oak cork (*Quercus suber* L) in Tunisia.

| Site | Abbreviation | Area | Geographical distribution | Bioclimat (bioclimatic stage) | Relief |
|---|---|---|---|---|---|
| El Feidja | EF | Ghardimaou | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Beni M'ttir | BM | Ain Drahem | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Hammam Bourguiba | HB | Ain Drahem | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Dar Fatma | DF | Ain Drahem | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Oued Zena | OZ | Ain Drahem | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Mejen Essef | ME | Ain Drahem | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Aîn Zana | AZ | Bouselm | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Djebel Zouza | DjZ | Nefza | Western North (Kroumirie) | Wet superior at moderate winter | Montagne |
| Keff El Rand | KR | Haouaria | North Est (Cap Bon) | Sub-wet superior at hot winter | Montagne |
| Hammam Jdidi | HJ | Hammamet | North Est (Cap Bon) | Semi-arid lower than hot winter | Montagne |
| Djebel Khroufa | DjK | Tabarka | Western North (Mogods) | Wet inferior at hot winter | Chaine tellienne |
| Bellif | B | Tabarka | Western North (Mogods) | Wet inferior at hot winter | Chaine tellienne |

Statistical analysis showed a highly significant difference between all populations except between the CD group populations (Dar Fatma El Keff Rand, Hammam Bourguiba, Bellif, Oued Zeen and El Feidja) during cycle 2 to 5% threshold (Table 4).

*Biochemical characterization of different populations of cork oak*

*Changes in sugar content*

The values of sugar content in cork oak seedlings are of the order of 2664.50; 1196.50; 2194.74; 1871.78; 1769.02; 1832.64; 2023.48; 2444.30; 2155.60; 1132.89; 3065.76 and 3780.18 mmol / g of dry matter for stressed plants against 2145.81; 1279.69; 3036.40; 3775.29; 1201.40; 2229; 927.37; 1421.60; 1402.02; 1529.25; 1568.40 and 2571.53 mmol / g of dry matter for control plants respectively in populations of Bellif; Dar Fatma; Jebel zouza; Béni Mtir; Hammam Bourguiba; El Feidja; Jebel Khroufa; Oued Zeen; Keff El Rand; Hammam Jdidi; Ain Zana and Mejen Essef

At the end of two cycles of water stress, (Fig.2).

The statistical analysis shows no significant difference among all populations of cork oak (Hammam Bourguiba, Ain Zana; Hammam Jdidi; Bellif El Feidja, Dar Fatma, Oued Zeen, Mejen Essef; Béni Mtir, Djebel zouza, Djebel Khroufa and Keff El Rand) for the control plants and a highly significant difference between all populations except among populations of Dar Fatma; Hammam Jdidi and between populations of Hammam Bourguiba; El Feidja; Béni Mtir; Jebel Khroufa; Keff El Rand; Jebel zouza; Oued Zeen; Bellif and Ain Zana for stressed plants at the 5% level (Table 4).

**Table 2.** Soil texture culture.

| | | | | | |
|---|---|---|---|---|---|
| Argile (en%) | 10% | pH | 7.5 | Sable grossier | 48% |
| Limons fins | 3% | Calcaire total | 2% | Azote total | 560 ppm |
| Limons grossiers | 5% | $P_2O_5$ | 15 ppm | Matière organique | 1.8% |
| Sable fin | 32% | $K_2O$ | 70 ppm | | |

*Changes in proline content*

The values obtained for proline content in young cork oak plants are of the order of 6.32; 2.72; 3.60; 3.32; 3.28; 2.50; 4.73; 5.07; 7.44; 2.72; 4.16 and 4.10 mmol / mg of dry matter for stressed plants against 1.71; 4.34; 6.57; 3.61; 0.94; 7.29; 1.27; 2.16; 3.95; 4.40; 3.73 and 2.40 mmol / mg of dry matter for the control plants respectively in populations of Bellif; Dar Fatma; Jebel zouza; Béni Mtir; Hammam Bourguiba; El Feidja; Jebel Khroufa; Oued Zeen; Keff El Rand; Hammam Jdidi; Ain Zana and Mejen Essef at the end of two cycles of water stress (Fig. 3).

Statistical analysis showed a highly significant difference between all populations except between populations Hammam Bourguiba; Jebel Khroufa and Bellif; between populations of Beni Mtir; El Ain Zana and Keff Rand; between populations of Dar Fatma; Hammam Jdidi and between populations Jebel zouza and El Feidja to control plants and a highly significant difference between all populations except between populations Keff El Rand; El Feidja; Dar Fatma; Hammam Jdidi; Hammam Bourguiba; Béni Mtir and Djebel zouza and between populations of Mejen Essef; Ain Zana; Djebel Khroufa and Oued Zeen for stressed plants at the 5% level (Table 4).

**Table 3.** Result of Duncan test (0.05) is Conducted biochemical characters of different populations of oak cork (*Quercus suber* L) in Tunisia: B: Bellif, DF: Dar Fatma KR: Keff Rand, DZ: Jebel zouza, BM Blessed Mtir, HJ: Hammam Jdidi, HB: Hammam Bourguiba, DjK: Jebel Khroufa, OZ: Oued Zen EF: El Feidja, AZ Ain Zana, ME: Mejen Essef.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sucrose soluble | | | | | | | | | | | |
| B | DF | KR | DZ | BM | HJ | HB | DjK | OZ | EF | AZ | ME |
| 5.493 | 5.840 | 5.424 | 5.820 | 5.257 | 5.632 | 6.100 | 5.436 | 5.105 | 6.000 | 6.508 | 5.759 |
| Proline | | | | | | | | | | | |
| B | DF | KR | DZ | BM | HJ | HB | DjK | OZ | EF | AZ | ME |
| 3.072 | 3.265 | 3.148 | 3.102 | 2.709 | 3.094 | 3.092 | 3.082 | 2.817 | 3.362 | 3.412 | 3.211 |
| Amidon | | | | | | | | | | | |
| B | DF | KR | DZ | BM | HJ | HB | DjK | OZ | EF | AZ | ME |
| 1.803 | 1.817 | 1.741 | 1.897 | 1.967 | 1.841 | 2.013 | 1.781 | 1.833 | 1.801 | 1.967 | 1.823 |

*Changes in starch content*

The values obtained from the starch content in cork oak seedlings are of the order of 0.36; 0.53; 0.16; 0.16; 0.24; 0.15; 0.95; 0.25; 0.66; 0.21; 0.26 and 0.42 mg / g of dry matter for stressed plants against 0.24; 0.62; 0.25; 0.34; 0.23; 0.92; 0.68; 0.23; 0.20; 0.33;

0.17 and 0.22 mg / g of dry matter, respectively to the control plants in populations of Bellif; Dar Fatma; Jebel zouza; Béni Mtir; Hammam Bourguiba; El Feidja; Jebel Khroufa; Oued Zeen; Keff El Rand; Hammam Jdidi; Ain Zana and Mejen Essef at the end of two cycles of water stress (Fig.4).

Statistical analysis showed a highly significant difference between all populations except between populations Ain Zana; Keff El Rand; Mejen Essef; Oued Zeen; Hammam Bourguiba; Bellif and Djebel zouza and between populations of Hammam Jdidi and Béni Mtir to control plants and a highly significant difference between all populations except among the peoples of El Feidja; Jebel zouza and Béni Mtir and between populations of Hammam Jdidi; Hammam Bourguiba; Oued Zeen; Ain Zana and Bellif for stressed plants at the 5% level (Table 4).

**Table 4.** One way analysis of variance of (ANOVA) applied quantitative parameters referring to biochemical characters.

| Morphological traits | Mean square | F.observed | P |
|---|---|---|---|
| Sucres solubles | 1,535 | 4,395 ** | 0,000 |
| proline | 0,400 | 3,008 * | 0,002 |
| amidon | 0,072 | 2,125 NS | 0,024 |

NS: Non Significant,*: Significant, **: High Significant.

**Discussion**

Osmotic adjustment is considered an important criterion determining the ability of osmo- regulation it allows the maintenance of turgor compatible with physiological activities in water limiting conditions. Our results show that osmotic adjustment increases dramatically at the end of the second cycle in populations Méjen Essef (0.22 to 0.67 MPa); Dj Khroufa (0.10 to 0.57 MPa) and Hammam Bourguiba (0.01 to 0.06 MPa) which explains that these populations have a high capacity to osmo-regulation allowing tissues to develop a higher turgor and strategy of resistance to water stress. Similarly, for Keff El Rand populations (from 0.17 to 0.10 MPa); Oued Zeen (0.04 to 0.02 MPa); Bellif (0.10 to 0.03 MPa); Ain Zana (0.51 to 0.44 MPa); but this is limited in time. A greater decrease in the population of Hammam Jdidi (0.39 to 0.11 MPa); Dar Fatma (0.28 to 0.16 MPa); Jebel zouza (0.66 to 0.38 MPa); El Feidja (0.20 to 0.04 MPa) and Béni Mtir (0.44 to 0.02 MPa), at the end of the 2nd cycle, which indicates that the population therefore exhibit sensitivity face to the constraint water.

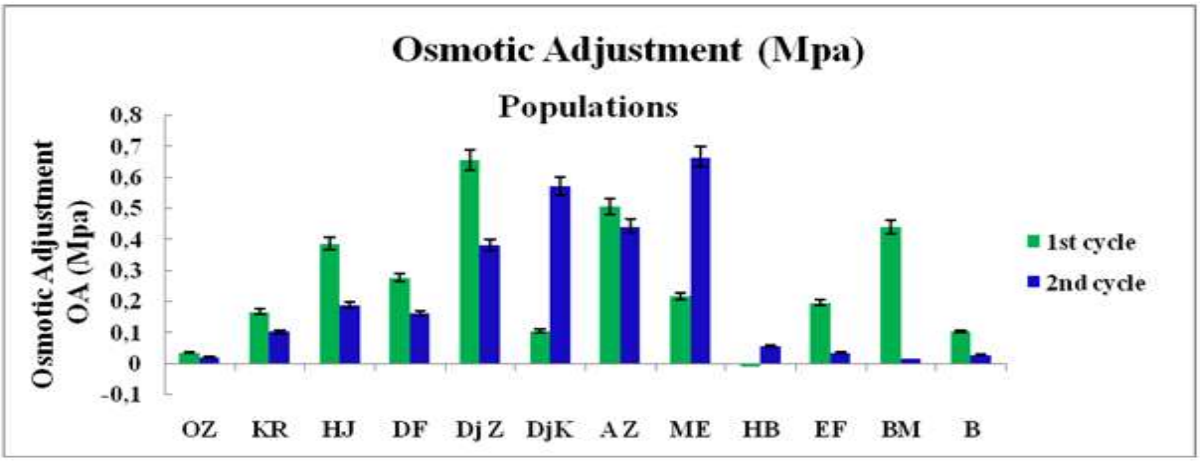


**Fig. 1.** Osmotic adjustment (MPa) of different populations of cork oak (*Quercus suber*.L) in Tunisia during the 1st cycle and the 2nd cycle of water stress.

Osmotic adjustment allows the maintenance of turgor compatible with the physiological activities by limiting water conditions (Turner *et al.*, 1980) These values are higher than those recorded for the Kermes oak that has an osmotic adjustment relative 9.83% (Ksontini, 1996). Similar results were found by Hireche (2006) for two varieties Dessica and Moapa.

*Analysis of the change of sugar content*

The values of sugar content obtained are greater in stressed plants compared to control plants for populations Bellif, Hammam Bourguiba, Jebel Khroufa, Oued Zeen; Keff El Rand; Ain Zana and Mejen Essef at the end of two stress cycles, the highest values are recorded in populations of Mejen Essef (3780.18 mmol / g of dry matter) and Ain Zana (3065.76 mmol / g of dry matter) to a lesser degree among populations Bellif (2664.50 mmol / g of dry matter), Oued Zeen (2444.30 mmol / g of dry matter), Jebel Khroufa (2023.48 mmol / g of dry matter) and El Keff Rand (2155 60 mmol / g of dry matter) and low among the population of Hammam Bourguiba (1769.02 mmol / g of dry matter) (Fig.2). Carbohydrates have a fundamental role in the life of plants, are the primary products of photosynthesis and compounds from which are synthesized lipids and proteins. These are indicators of degrees of stress, because of its significant increase in the severity; metabolic sugars (glucose, galactose, sucrose and fructose) provide resistance to various stresses.

The sugars play a predominant role in the life of the plant, they are produced by photosynthesis. Transported to the deep tissue and channeled, breathing is converted to compounds of reserves (lipids, sucrose, starch) which are optionally degraded, regulation of metabolic process depends on the concentration of sugars (Loretti *et al.,*2001). Soluble sugars in combination with other organic solutes (proteins, carbohydrates, organic acids (malate), amino acid) are involved in the process of osmoregulation (Kinet *et al.,*1998).

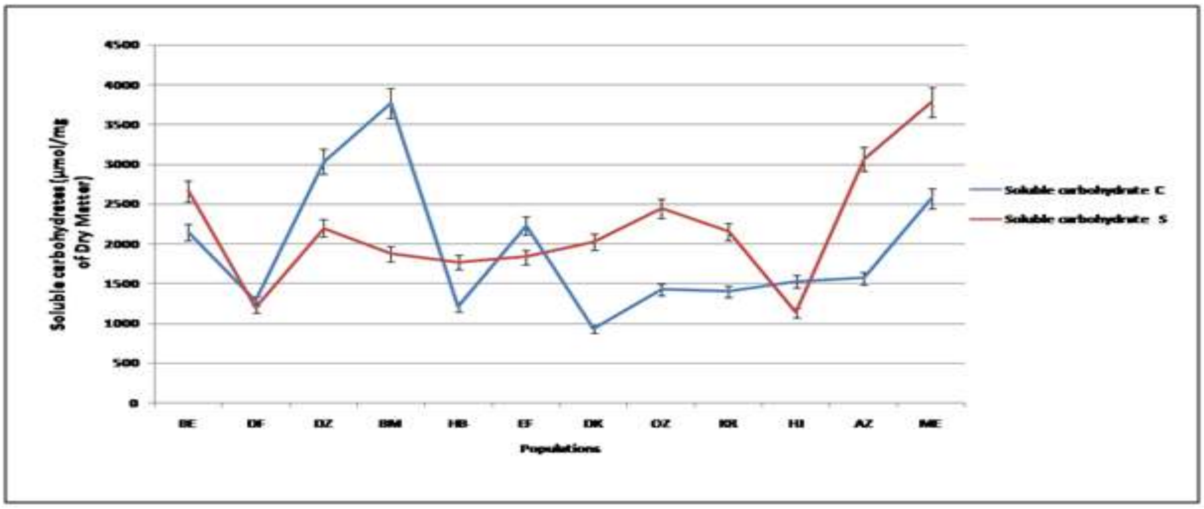


**Fig. 2.** The sugar content (μmol/mg MD) of leaves young's controlled (T) and stressed (S) plants of different populations of cork oak (*Quercus suber*.L) in Tunisia after two water stress cycles separated by rehydration.

Sucrose and monosaccharides, osmotic play a role in lowering the osmotic potential, and consequently in the osmotic adjustment in different plants and give them a stress tolerant life life (Bezzela, 2005).

The accumulation of soluble sugars in warm climatic conditions has been reported by several authors. Indeed, Barlow and *a.,* 1976 show that the presence of soluble sugars during periods of heat and drought protect the thylakoids of irreversible alteration of membranes and exert a favorable effect on the protoplasmic drought resistance. Similarly, Sauter and Kloth, 1987 in Decourteix, 2005 reported that in poplar, the sucrose content of xylémien parenchyma is very strong in August during the deposition of starch, low to the end of fall and maximum in January. The amounts of fructose and glucose remain constant throughout the year. Maltose, when to him is the most abundant sugar from August to November, but its content is negligible in January.

*Analysis of changes in proline content*

The highest levels of proline are recorded in populations of Keff El Rand (7.44 mmol / g of dry matter and Bellif (6.32 mmol / g of dry matter to a lesser extent in populations Oued Zeen (5, 07 mmol / g of dry matter), Jebel Kroufa (4.74 mmol / g of dry matter), Ain Zana (4.16 mmol / g of dry matter) and Mejen Essef (4.10 mmol / g of dry matter) and lower in the Population Hammam Jdidi and Dar Fatma (2.72 mmol / g of dry matter) and Fl Feidja (2.50 mmol / g of dry matter); Blessed Mtir (3, 32 mol / g of dry matter) and Jebel Zoua (3.60 mmol / g of dry matter). So these populations Mejen Essef and Djebel Kroufa have a capacity for osmoregulation allowing tissues to develop drought resistance strategy. Regarding populations Keff El Rand; Bellif; Oued Zeen and Ain Zana showed tolerance to water stress by an accumulation of proline over time of approximately (6 mmol / g of dry matter) at the end of two cycles of dryness. Both disk populations Hammam Jdidi; Dar Fatma; Fl Feidja; Blessed Mtir and Djebel Zoua have shown vis-à-vis sensitivity to water stress because their proline content decreases over time in the order of (2 mmol / g of dry matter) at the end of two cycles of dryness, Proline is an amino acid essential in plants, it is considered an indicator of stress (Fig.3).

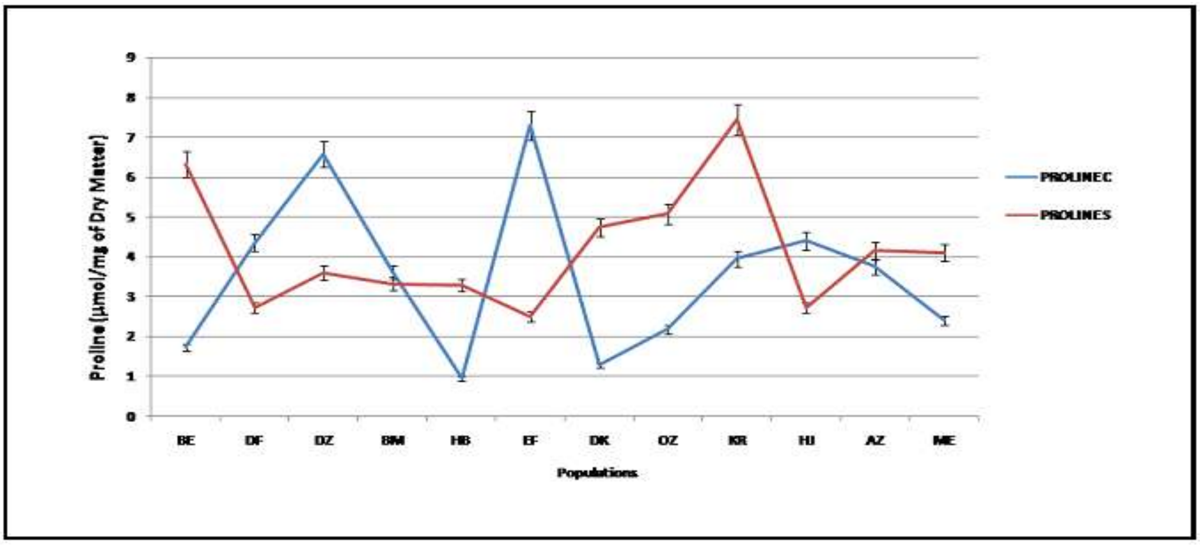


**Fig. 3.** The proline content (µmol/ mg DM) leaves young's controlled (T) and stressed (S) plants of different populations of cork oak (*Quercus suber*.L) in Tunisia after two water stress cycles separated by rehydration.

Proline appears to play an important role in plant response to drought: its rapid accumulation during water stress was demonstrated in many plants, particularly in barley (Lewin *et al.*, 1978) in the Eucalyptus (Chunyang., 2003), in soft wheat (Tan, 1982; Riazi, 1985; Monneveux and Nemmar 1986). It plays a role consisting in osmoprotection and regulation of cytoplasmic pH (Delauney and Verna 1993). It provides a reserve of nitrogen that can be used under stress conditions such as acidity reduction means or residue removal (Delaa, 2003).

The accumulation of proline in the case of stress gives it the role of osmoticum in the cytosol, this amino acid contributes to the osmoprotection in several species, when exposed to osmotic stress (Slama *et al*, 2004).

Belhassen *et al.*, 1995 and Hyun *et al.*, 2003 have shown the important role of proline in maintaining osmotic adjustment, in addition to its protective role (protein stabilization, prevention against denaturation of the enzymes, hydroxyl radicals and protective membranes). Besides, if the accumulation of proline usually corresponds to a damaged event caused by water stress, it is often considered a sign of adaptation to drought. Albouchi *et al.*, 2001 showed that the drought induced accumulation of prolines in phyllodes *Acacia cyanophylla* L. Ksontini (1996) found that the proline content in plants subjected to water stress are higher than in irrigated plants for all species and at the end of two cycles of drought. However, in older plants; the increase of the proline content is shown at the end of the second cycle of drought. It is more important in cork oak of Bellife (0.8mol / GMS) than the Kermes oak Ras Rajel.

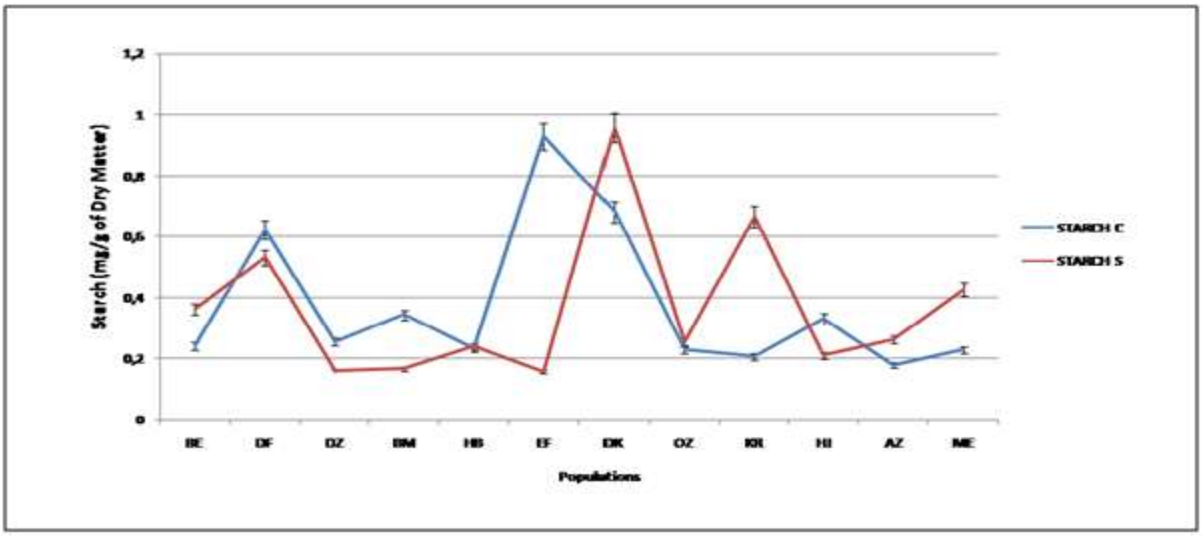


**Fig. 4.** The starch content (mg/g DM) of leaves of young's controlled (T) and stressed (S) plants of different populations of cork oak (*Quercus suber*.L) in Tunisia after two water stress cycles separated by rehydration.

Mefti *et al.*, (1998) found that the water stress applied to *Medicago truncatula* L caused a significant increase in proline accumulation. This accumulation of organic solutes is simply a phenomenon of adaptation to drought, allowing the plant to maintain turgor by lower water potential; it's a form of adjustment of its potential. This type of tolerance allows the plant to ensure its physiological functions normally despite deterioration in its internal water status consequent drought. A value confirms the hypothesis Hinckley, 1982 which suggests that the production of solutes in response to drought is uncommon in trees with oaks (Dreyer *et al.,* 1990, Ksontini, 1996) and Caroubier (Rejeb, 1992).

Examination of the results shows that the seasonal soluble sugar content of Aleppo pine seedlings during the winter season is much higher than in the other seasons. This period is dominated by the frequency of stress where low temperatures ranged between 2 and -4 ° C with a total of 228 hours.

The increase in soluble sugar content in the seed organs during the winter season might be the result of increased degradation of starch during this season. Smith *et al.*, 1979 show that the increase in summer temperatures favors the accumulation of starche retards, its hydrolysis while the low night temperatures during the ripening period accelerate the degradation which is consistent with our results.

The content of Aleppo pine seedlings presents significant accumulations of proline and changes to seasonal temperature variations. While it is relatively low in normal conditions (control). Knu and Chen, 1986 show that the proline content is very low in the leaves and productive members in favorable conditions.

With the exception of the summer season, the proline content is correlated with that of the soluble sugars in other seasons. However during the summer season, the Aleppo pine seedlings accumulate these two metabolites differently when there was a large accumulation of proline and a low concentration of soluble sugars. The simultaneous accumulation or not of these two solutes according to the degree of stress, allows plants to withstand extreme temperatures. The process of concentration of soluble sugars and proline in leaf tissues of the stressed plants is recognized as an adaptation characteristic (Kameli and Losel, 1995). The accumulation of proline is an adaptive strategy triggered by the plant meet the constraints of the environment (Belkhodja, 2000). It accumulates in water stress (Hubac and Viera Da Silva, 1980), salt stress (Lahrer and Stewart, 1980), as well as the effect of low and high temperatures (Chu *et al.*, 1978; Paleg *et al.,* 1981). Indeed, during the spring and summer season, there is a significant accumulation of this amino acid. This increase can be attributed to the development of plants during both seasons and their need for nitrogen compounds, proline, which if necessary, is one of the most easily mobilized compounds. Laplante, 2003 shows that the exposure of plants to frost adaptive triggers the second step in a series of metabolic changes allowing a tolerance to cold.

According to Hare and Cress, 1997, proline helps stabilize within cell structures (membranes and proteins), free radicals, and the cellular redox potential in stress conditions and maintain NADP appropriate ratios + / NADPH compatible with metabolism. It can also act as electron donor (De Ronde *et al.*, 2004).

Regarding the maximum content of soluble sugars stored during the winter period, we can see that the starch is converted into soluble simple carbohydrates during this season, which helps to lower the crystallization temperature by increasing the concentration of solutes (super cooling). The reduction of soluble sugars levels during the summer shows its strong translocation to the growing organs, to be metabolized and used for other purposes; it explains the rapid pace of growth of plants during the summer season (Sakai and Larcher, 1997).

*Analysis of changes in starch content*

The values obtained from the starch content are higher in stressed plants compared to control plants for populations of Bellif; Hammam Bourguiba; Jebel Khroufa; Keff El Rand; Oued Zeen; Ain Zana and Mejen Essef at the end of two stress cycles, the highest values are recorded in populations of Jebel Khroufa (0.95 mg / g of Dry Matter) and Keff Rand El (0.66 mg / g of Dry Matter ) to a lesser degree among populations Bellif (0.36 mg / g of Dry Matter) and Mejen Essef (0.42 mg / g of Dry Matter) and low among the population of Hammam Bourguiba (0.24 mg / g of Dry Matter) , Oued Zeen (0.25 mg / g of Dry Matter) and Ain Zana (0.26 mg / g of Dry Matter) (Fig.4).

Carbohydrates are the major components of the trees. They represent three quarters of their weight (Kramer and Kozlowski, 1991, Alaoui- Sosse *et al.*, 1994). These carbohydrates, starch and sucrose which have been the subject of several studies, starch as the primary sugar stored in the perennial parts of trees and sucrose as the main product of photosynthesis and products of hydrolysis (glucose and fructose). Indeed soluble sugars, even if they represent osmoticums much less powerful, they also participate in them keeps the balance of osmotic force to maintain turgor and cytoplasmic volume as high as possible (Bouzoubaa *et al.*, 2001) and also for preservation’s membrane integrity in the dried organs and a protection protein (Darbyshire, 1974).

This increase in soluble sugars, was remarkable at the leaf level, a deterioration of certain disaccharides (sucrose) where the accumulation of sugars (glucose and fructose) which have shown an increase during the stress period at high temperatures. The carbohydrate reserves are mostly stored long-term in the form of starch. The latter is obtained from the photosynthesis and accumulates during the vegetative phase in perennial tissues and particularly in the parenchyma reserves branches and roots. There are also soluble carbohydrates that can serve as storage form of energy.

The most common remains sucrose that accumulates in large amounts in the vacuoles (Yamaki, 1992). The accumulation of reserves soluble sugars and starch in the roots during the acclimatization, however, is considered a key factor in the level of frost tolerance in lucerne (Bertrand and Paquin, 1991).

## Conclusion

The cork oak (*Quercus suber* L) is a Mediterranean essence of a great ecological and socio-economic interest which ensures a much diversified production, the cork; the wood and the fruits. Cork oaks is characterized by a large polymorphism which it has large individual variations in behavior and botanical characters between individuals and ecotypes.

The accumulation of organic osmolytes is only a phenomenon of adaptation or tolerance water stress, and consequently allows the plant to maintain turgor by adjusting its osmotic potential that is to say; there is an accumulation function of stress and populations which increases according to the intensity of stress. These results allow us to say that this development (In soluble sugar content, in starch and in proline) indicates some metabolic variability.

According to the physiological and biochemical eco polymorphism on this species for different populations in Tunisia, this study allowed us to distinguish between different sources but this work should be completed by a molecular approach based primarily on molecular markers to analyze end genotypic diversity.

## Acknowledgement

This work was supported by grants from the Tunisian «Ministère de l'Enseignement Supérieur et de la Recherche Scientifique».

## References

**Abid H, Selmi K.** 1998. La subéraie tunisienne : Importance et orientation pour une gestion durable, Actes séminaire méditerranéen sur la régénération des forêts de chêne liège, Tabarka Tunisie: 22-24 octobre 1996, Ann INRGREF Suppl.

**Alaoui-Sossé B, Parmentier C, Dizengremel P, Barnola P.** 1994. Rhythmic growth and carbon allocation in *Quercus robur*. I. Starch and sucrose, Plant Physiol. Biochem. **32,** 331 339.

**Albouchi A.** 1997. Effet du préconditionnement à la carence en eau sur certaines manifestations de l'endurcissement à la sécheresse chez *Acacia cyanophylla*. Croissance, comportement hydrique et accumulation active des solutés. Thèse de Doctorat en Sciences Biologiques Uni. Tunis II. Fac. Sci. Tunis. 142.

**Albouchi A, Sebeïb H, Meznic Majid Y, Hédi El Aounid M.** 2001. Influence de la durée d'acclimatation sur l'endurcissement à la sécheresse d'Acacia cyanophylla Lindl. Ann. For. Sci. **58,** 519–528.

**Albouchi A, Ghrir R, El Aouni MH.** 1977. Endurcissement à la sécheresse et accumulation de glucides solubles et d'acides aminés libres dans les phyllodes *d'Acacia cyanophylla* Lindl. Ann.Sci.For., **54,** 155-168.

**Barlow EWR, Boersma L, Young JL.** 1976. Root temperature and soil water potential effects on growth and soluble carbohydrates concentration of corn seedlings. Crop Sci. **16,** 59 62.

**Belhassen E, Monneveux P.** 1995. Adaptation génétique face aux contraintes de sécheresse.Cahier Agriculture **4,** 251-261.

**Belkhodja M.** 2000. Proline response of broad bean (Vicia faba L) under salt stress. Egypt. J. Agric. Res. **78,** 185-195.

**Bertrand A, Paquin R.** 1991. Influence de la température d endurcissement sur la tolérance au gel de la luzerne et sa teneur en sucres, amidon et proline. Can. J. Plant Sci. **71,** 737-747.

**Bouzoubaa Z, El Mourid M, El Gharous M,** 2001. Manuel d analyse chimique et biochimique des plantes. Eds ; INRA Maroc.

**Chu TM , Jusaitis M, Aspinall D, Paleg LG.** 1978. Accumulation of free proline at low temperatures. Physiol Plant, **43,** 254-260.

**Chunyang H, Kaïoyun W.** 2003. Differences in drought microtheca F. meullpopulations. University of Helsinki. Finland. Forest ecology and management **179,** 377-385.

**Darbyshire B.** 1974. The function of the carbohydrate units of three fungal enzymes in their resistance to dehydration. Plant Physiol. **54,** 717-721.

**De Ronde JA, Cress WA, Krüger GHJ, Strasser RJ, Van Staden J.** 2004. Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene, during heat and drought stress. J. Plant. Physiol, **161,** 1211-1224.

**Decourteix M.** 2005. Caractérisations physiologique et moléculaire de transporteurs de saccharose et d hexoses de xylème de noyer (*Juglans regia* L. cv Franquette) : rôles dans les échanges latéraux de sucres pendant la période non-feuillée. Thèse de docteur d université. Université de Blaise Pascal. 88 p.

**Delaa Y.** 2003. Utilisation des végétaux dans l'approche ecotoxycologique, thèse de magister à l'université de Mentouri- Constantine, p 172.

**Delauney AJ, Verma DPS.** 1993. Proline biosynthesis and osmoregulation in plants. *Plant J,* 4, 215-223, European Scientific Journal August edition **9,** 24 ISSN: 1857 – 7881 (Print) e - ISSN 1857- 7431 153.

**Dreier W, Göring M.** 1974. Der Einfluss hoher Salzkonzentration auf verschiedene physiologische Parameters von Maiswurzeln. Wiss. Z. der Humboldt Univ. Berlin, Reihe/Math. Naturwiss. **23,** 641-644.

**Dreyer E, Bousquet F, Ducry M.** 1990. **Use** of pressure volume curves in water relations analysis on woody shoots: influence of rehydration and comparison of four European oak species.Annales des Sciences Forestières **47,** 284-295 p.

**Givnish TJ.** 1988. Adaptation to sun vs. shade: a whole-plant perspective. Australian Journal of Plant Physiology **15,** 63–92.

**Hare PD, Cress WA.** 1997. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul, 21, 79-102,

**Hasnaoui B.** 1991. Régénération naturelle par rejet de souche et par drageonnement d'unesubéraie dans le Nord-Ouest de la Tunisie. XVII (Ecologia Méditerranaea, **79(87),** 16-21 p.

**Hinckley TM, Pezeshki R.** 1982. The stomatal response of red alder and black cottonwood to changing water status. Canadian Journal of Forest Research **12,** 761–771.

**Hireche Y.** 2006. Réponse de la luzerne (Medicago Sativa L) au stress hydrique et à la profodeur de semis .Université Al HadJ Lakdar Hubac, C., Vieira, Da Silva, J. Indicateurs.

**Hubac C, Viera Da Silva J.** 1980. Indicateurs m6taboliques de contrainte mesologiques. Physiol. Vdg. **18,** 45-53.

**Hyun DY, Lee IS, Kim DS, Lee YI.** 2003. Selection of azetidine-2- carboxylic acid resistant cell lines by in vitro mutagenesis in rice (*Oryza sativa* L). Journal of Plant Biotechnology **5,** 43 - 49.

**Kameli A, Losel DM.** 1995. Contribution of carbohydrates and solutes to osmotic adjustment in wheat leaves under water stress. J. Plant Physiol., **145,** 363–366.

**Kinet JM, Benrebiha F, Bouzid S, Laibacar S, Dutuit P.** 1998. de réseau atriplex : Allier biotechnologies et écologie pour une sécurité alimentaire accrue en régions arides et semis arides. Cahier d 'Agricultures **7,** 505-509.

**Knu, Chen.** 1986. Effect of hight température on proline content in tomato floral buds and leaves. Sci, Hortic. **111 (5)**, 746-750 in chemical abstract.

**Kozlowski TT, Kramer PJ, Pallardy SG.** 1991. The physiological ecology of woody plants. Academic Press, San Diego, Calif.

**Ksontini M.** 1996. Etude ecophysiologique des réponses à la contrainte hydrique du chêne liège (*Quercus suber*) dans le Nord–Tunisie: comparaison avec le chêne Kermès (*Q. coccifera*) et le chêne zeen (*Q.faginea*) Thèse université Paris XII Val De Marne, 157 p.

**Laplante N.** 2003. Paramètres climatiques engendrant le gel des bourgeons chez le bleuet en Corymbe dans la région de Granby (Québec). Université du Québec à Rimouski Travail réalisé dans le cadre du cours Climatologie G2O-250-01, 13-16,

**Lewin LG, Sparrow DHB, Aspinal D.** 1978. Proline accumulation and grought resistance in barley. **23(8),** 12-36.

**Loreti E, De Bellis L, Alpi A, Perata P.** 2001. Why and how do plant cells sense sugars? Ann Bot **88,** 803–812.

**Manos PS, Zhou ZK, Cannon CH.** 2001. Systematics of Fagaceae: phylogenetic tests of reproductive trait evolution. Int. J. Plant Sci. **162(6),** 1361-1379

**McCready RM, Guggolz J, Silviera V. Owens HS.** 1950. Determination of starch and amylose in vegetables. Anal. Chem., **22,** 1156–1158.

**Mediavilla S, Escudero A.** 2003. Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. Forest Ecology and Management **187(2-3),** 281-294.

**Mefti M, Abdelguerfi A, Chebouti A.** 1998. Etude de la tolérance à la sécheresse chez quelques populations de Medicago truncatula L.Gaertn. Science **(5),** 173- 176 p.

**Monneveux P, Nemmar M.** 1986. Contribution à l'étude de la résistance à la sécheresse chez le blé tendre (Triticum aestivum L.) et chez le blé dur (Triticum durum Desf.) : étude de l'accumulation de la proline aucours du cycle de développement. Agronomie **6,** 583-590.

**Paleg LG, Douglas TJ, Van Dali A, Keech DB. 1981.** Proline, betaine and other organic solutes protect enzymes against heat inactivation. Aust. J. Plant Physiol **8,** 107-l 14,

**Rached-Kanouni M, Alatou D, Sakr S.** 2012. Responses of cork oak seedlings to short-term exposures to low temperatures. American Journal of Scientific Research **59,** 28-41,

**Rejeb MN.** 1992. Etude des mécanismes de résistance à la sécheresse du caroubier (ceratonia siliqual L.). Thèse de doctorat d'état es sciences naturelles Fac. Sci. Tunis. 199 p.

**Riazi A, Matruda K, Arslam A.** 1985. Water stress induce changes in concentration of proline and other solutes in growing regions. J. Exp. Bot., **36,** 1716-1725.

**Sakai J, Nohturfft A, Cheng D, Ho YK, Brown MS, Goldstein JL.** 1997. Identification of complexes between the COOH-terminal domains of sterol regulatory element binding proteins (SREBPs) and SREBP cleavage-activating protein (SCAP). J. Biol. Chem. **272,** 20213–20221.

**Sauter JJ, Kloth S.** 1987. Changes in carbohydrates and ultrastructure in xylem ray cells of Populus in response to chilling. Protoplasma **137,** 45-55.

**Slama I, Messedi D, Ghnaya T, Arnould S, Abdelly C.** 2004. Effects of water deficit on growth and proline metabolism in *Sesuvium portulacastrum.* Environmental and Experimental Botany (in press).

**Smith RB, Lougheed EC, Franklin EW, Mc Millan I.** 1979. The starch iodine test for determining stage of maturation in apples. Canadian Journal of Plant Science, **59,** 725-735,

**Staub AM.** 1963. Extraction, identification et dosages des glucides dans les extraits d'organes et les corps bactériens. In : Techniques de laboratoire, tomes 1 et 2, Masson, Paris, 1307-1366.

**Stewart GR, Larher F.** 1980. Accumulation of ammo acids and related compounds in relation to environmental stress In: P. K. Stumpf and E. E. Conn, The Biochemistry of Plants, vol. 5, Amino acids and Derivatives (Ed. by B. j . Milflin), Academic. Press London. **17,** 609-635.

**Sultan SE.** 2000. Phenotypic plasticity for plant development, function, and life history. Trends in Plants Science **5 (12),** 537-542.

**Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF.** 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. **118,** 412–416.

**Troll W, Lindsley JA.** 1955. photometric method for the determination of proline. J. Boil. Chem. 215, 655-660,

**Turner NC, Jones MM.** 1980. Turgor maintenance by osmotic adjustment. A review and evaluation. In: Adaptation of plants to water and high temperature (Turner N.C. and Kramer P.J. eds) 87 and 454 New York John Wiley and Sons.

**Yamaki S.** 1992. Distribution of sorbitol, neutral sugars, free amino acids, malic acid and some hydraulic enzymes in vacuoles of apple cotyledons. Plant Cell Physiol., **23,** 881-889.