INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 5, p. 96-106, 2018
http://www.innspub.net

RESEARCH PAPER

OPEN ACCESS

Response of some Tunisian populations of cork oak (*Quercus suber.* L) to salt stress

Khaoula Chihi*, **Refka Zouaoui, Youssef Ammari, Mejda Abassi**

Laboratory of Forest Ecology, National Research Institute of Rural engineering, Water and Forests (INRGREF), Ariana, Tunisia

Article published November 15, 2018

Key words: Cork oak, Salinity, Ecophysiological, Proline, Soluble sugars.

Abstract

Cork oak is a forest specie with significant ecological and economic benefits. However, it is threatened with extinction due to its progressive degradation due mainly to the difficulty of semi-regeneration and the sensitivity to current environmental conditions. In this context, we are interested in studying the impact of salinity on cork oak behavior. Three cork oak (*Quercus suber*) populations were subjected to three treatments during 6 months: the control, irrigated with tap water and two salt treatments with 3 and 6 g / l of NaCl. The rates of relative growth in height, collar diameter and in terms of biomass production were monitored during the treatments. In order to see oak's response to salt stress, a measurement of baseline water potential and volume pressure curve parameters followed by biochemical analyzes of proline and soluble sugars were performed at the end of the treatment period. The results showed a reduction in relative height, diameter and biomass, varying according to populations and salt doses. However, these disturbances were less pronounced in the plants of the Hammam Bourguiba population, demonstrating their better tolerance to salt doses applied compared to the populations of Kaf Errand and Bellif. In addition, biochemical analyzes helped to explain the significant osmoregulatory capacity in the population of Hammam Bourguiba by large accumulation of proline and soluble sugars, unlike Bellif and Kaf Errand who have depleted rates of soluble sugars and their sensitivities to the salt stress.

***Corresponding Author:** Khaoula Chihi ✉ chihikhaoula@yahoo.fr

Introduction

Cork oak (*Quercus suber* L.) is a native and leafy species in the Tunisian forest (Ennajeh *et al.,* 2013). It has very important ecological and socio-economical values. It is located in the north-west of the country at the Tuniso-Algerian border (Kroumirie and Mogods) which correspond to humid and sub-humid bioclimate zones (Yessad, 2000; Stiti *et al.,* 2014). It stretches between sea level up to 1200 meters altitude with a rainfall ranging from 700 to 1500 mm per year. The boundaries of this area coincide almost exactly with the Numidian sandstone formations. With more than 90 000 ha (Selmi, 2006), cork oak forests are the most widespread stands in Tunisia and are all of natural origin (Mejbri, 2005; Ben Jamâa and Nouiui, 2018). The evolution of the suberie in this region is worrying, as we went from a cork oak surface that was estimated at 140 000 ha in 1920 (Débierre, 1927) to an area of 70 000 ha in 2000, according to the results of the second national forest inventory (CNT-DGF-DGRST, 2005 : Stiti *et al.,* 2014). The causes of the degradation of Tunisian cork forests are multiple and growing, both social, economic and management (Palahi, 2004; Scarascia-Mugnozza *et al.,* 2000). On the other hand, different stresses, biotic (insect attack) and abiotic (water, light, salt and so on) (Nsibi, 2006) will still threaten the health and dynamics of cork forests in the coming years as a result of new issues related to climate change (global warming). In addition, the decline associated mainly with human activities (poorly done demasclage) induces several damages to the forest. Also, pasture is predominant in the cork forests, it outweighs other factors of degradation and deferred grazing is not respected in the majority of cases. Cork oak encounters enormous difficulties to recover naturally by seeds and stem rejection. The situation is further compounded in recent years by the climatic conditions characterized by longer periods of drought and sudden and intense rainfall. The future of the forest of cork oak is seriously threatened. In addition, salinity has become a serious threat to agricultural productivity (Cha-um *et al.,* 2006). It affects 20% of cultivated areas and 7% of the total area in the world (Zhu, 2002). These surfaces are steadily increasing due to uncontrolled irrigation and clearing (FAO, 2006). Due to climate variation , temperatures are expected to rise at least 2-4 °C, precipitation will decrease, with an overall decrease in the availability of water due to evapotranspiration, especially during the summer, and salinity will reach wider surfaces. Given recent issues of salinity, several studies are trying to understand the different forms of response, resistance mechanisms and adaptations developed by plants cope with this stress, but much less is known about the salt sensitivity of tree species especially cork oak. It is important to understand the physiological responses of forest species in particular cork oak to salinity in order to predict changes in some physiological and morphological processes. The objective of this investigation was to evaluate the effects of salt stress on morphological, eco-physiological and biochemical behavior of three populations of cork oak.

Material and methods

Plant materials

Experiments were conducted in nursery at the National Institute of Research on Rural Engineering, Water and Forests (Tunis, Tunisia), in a semi-arid to mild winter bioclimatic zone at N36°50', E10°14', 3 m of altitude, 475 mm annual average rainfall, 2952 hours/year sunlight and 7.2 - 34.8°C of monthly average temperature. The study was carried out on seedlings of Oak cork (*Quercus suber)*, of two years and are coming from three different sites: Hammam Bourguiba (among humid climatic area), Bellif (humid climatic area) and Kaf Errand (subhumid) (Fig.1 and Table 1). Seedlings of Oak cork (*Quercus suber*) known to show variability in their tolerance to salinity, were grown in semi-controlled conditions in plastic pots containing an inert substrate (coarse sand). They were placed under a shade house with 50% of permeability to avoid the negative effects of excessive sunlight. The experimental design was a split-plot with four replications. Populations were assigned to the main plot and water regime (control and salt stress) to the split plots. The latter consisted of 12 plants per plot and the total number of plant was evaluated to 144.

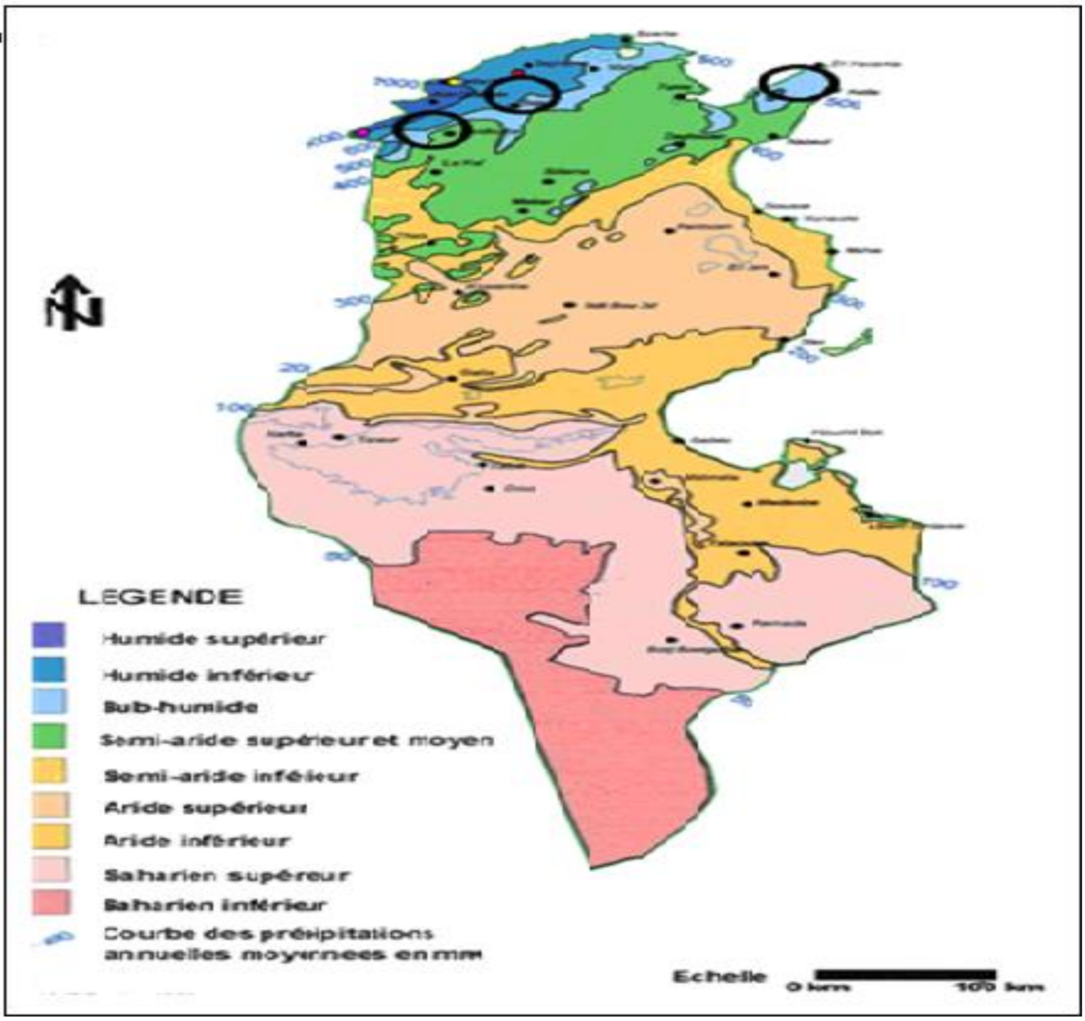

Fig. 1. Map showing the locations of collection sites of Cork oak (*Quercus suber*) in Tunisia.

Salt treatments

The salt stress experiment was carried out during 6 months. The plants were divided into three treatments: control and two salt treatments (3 and 6g/l NaCl). The control plants and the salt treated ones were irrigated with half strength Hoagland solution (Hoagland and Arnon, 1950).

The NaCl concentration started from 3g/l NaCl and increased stepwise by weekly 1g/l NaCl steps until reaching 6g/l NaCl. The salinity in the soil was monitored periodically by measuring the electrical conductivity in the drain solution by a WTW Cellox 325.

Morphological measurements

The measurements were applied of height and collar diameter of the cork oak seedlings were been taken once every two weeks.

The biomass estimation was performed by weighing the fresh and dry matter of cork oak seedlings. Initial weights were performed on 4 plants / population. The plants collected were divided into stems, leaves and roots. The fresh matter was determined immediately after harvest using a precision balance. The dry matter of aerial and root parts was determined by weighing after drying in an oven at 65 ° C for a period of time to have a constant dry weight.

To better appreciate the effect of salinity on plant growth the relative growth rate (RGR) in height, root collar diameter and biomass were calculated. This parameter is used to evaluate the effectiveness of growth expressed by the ability of plants to produce biomass (Kozlowski *et al.*, 1991).

It is defined by the relationship:

RGRn = (Ln Wn – Ln Wn-1)/(Tn – Tn-1) where Wn and Wn-1 represents the height, stem diameter and biomass after a period of stress, Tn and Tn-1, respectively.

Relative electrolyte leakage

Relative electrolyte leakage was determined in leafs of oak seedlings to study the effect of salinity on membrane integrity. We used the technique as described by Lutts *et al.,* (2004).

Leaves were immersed in distilled water for 2h at 25°C and the electrical conductivity was determined (C1). Then, Samples were autoclaved at 120°C for 20 min and the conductivity was again measured (C2) after equilibration at 25°C.

The relative electrolyte leakage (REL) was calculated as follows: REL (%) = (C1/C2)*100.

Pressure-volume (P-V) curves

P-V curves were determined using the Scholander pressure-chamber technique (Scholander and Hammel, 1965). The P-V curves of each leaf were obtained by expressing the relationship between the relative water content (RWC) values and the reciprocals of the water potentials measured (-1/Ψw).

Osmotic potential at full turgor (Ψ_π^{100}) was estimated via linear regression of data in the straight-line region of the P-V curves.

Osmotic potential at the turgor loss point (Ψ_π^{0}) was derived from the RWC and -1/ Ψw coordinates respectively of the first point in the straight-line region of the P-V curves (Patakas and Notsakis 1999).

The osmotic adjustment (OA) was defined as the difference in Ψ_π^{100} between the stressed and the control plants, respectively: OA = (Ψ_π^{100}) stressed - (Ψ_π^{100}) control.

Volumetric modulus elasticity (ε) was calculated as the slope of the relationship between turgor pressure and RWC (Patakas and Notakis, 1999):

$\varepsilon_{max} = (\Psi_\pi^{100} - \Psi_\pi^{0}) \times (1 - AWC) / (1 - RWC_o)$.

Relative water content (RWC) was determined on three to four fully expanded leaves. Leaves were excised before dawn, weighed freshly (FW) and placed in vials to rehydrate in the dark for 20 h. Preliminary experiments had indicated that full rehydration was complete after 18 h. The next morning, leaf turgid weight (TW) was measured and then leaves were dried at 80 °C for 48 h and dry weight (DW) was determined. The RWC was calculated from the equation:

RWC =100[(FW –DW)/(TW–DW)].

Osmolytes contents

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

Statistical analysis

The data were analyzed using appropriate procedures of the XLSTAT (version 2012). Analysis of variance (ANOVA) was performed with the statistical program SPSS (version 16), involving two levels of classification (salinity and population) with interactions. A Duncan's multiple range test was carried out to determine if significant ($P < 0.05$) difference occurred between populations and treatments.

Results

Morphological behavior

To better evaluate the effect of salinity on growth, we studied morphological values of the populations of cork oak.

The height and collar diameter were significantly reduced by the NaCl treatments compared to the control, a variability between populations was showed (Table 2).

The most important decreases on height growth are shown under 6g/l of NaCl by all the populations. By comparison to control, the population of Bellif was more inhibited on height growth (-83%), Kaf Errand (-79%), and Hammam Bourguiba (-61%) under 6g/l of NaCl. Collar diameter was less affected for the population of HB (-24%), compared to Kaf Errand (31%) and Hammam Bourguiba (59%) under 6g/l of NaCl.

Table 1. Origin and geographic position of Cork oak (*Quercus suber*) sites.

Site	Area	Governorate	Bioclimat area	Altitude (m)
Hammam Bourguiba (HB)	Ain drahem	Jendouba	Wet superior at moderate winter	570 m
Bellif (B)	Tabarka	Jendouba	Wet inferior at hot winter	160 m
Kaf Errand (KR)	Haouaria	Nabeul	Superior at hot winter	320m

The relative growth rate in shoot and root biomass (RGRd) of Hammam Bourguiba wasn't reduced significantly by salt stress (Table2). Whereas, there were a significant decreases within the populations of Bellif and Kaf Erand in the presence of 3 and 6 g/l of NaCl compared to the control. Shoot biomass was reduced respectively under 3 and 6 g/l of NaCl by 11.2% and 70% for Kaf Erand and 44% and 84% for Bellif. While, root biomass was reduced by 22% and 63% for Kaf Erand 42% and 61% for Bellif.

Table 2. Effects of NaCl treatments on relative growth rate in height, root collar diameter and biomass (shoot and root). Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences.

Populations	Hammam Bourguiba	Bellif	Kaf Errand
RGR_{height} (10^{-3})			
Control	3,1 a	2,4 a	2,8 a
3 g/l	1,6 b	0,6 a	1,5 b
6 g/l	1,2 C	0,4 a	0,6 c
$RGR_{diameter}$ (10^{-3})			
Control	2,5 b	3,2 a	2,6 b
3 g/l	2,4 b	2,0 c	2,1 c
6 g/l	1,9 d	1,3 f	1,8 e
$RGR_{biomass\ shoot}$ (10^{-3})			
Control	15,3 a	10,9 b	14,2 a
3 g/l	14,5 a	6,1 c	12,6 a
6 g/l	13,4 a	1,7 e	4,2 d
$RGR_{biomass\ root}$ (10^{-3})			
Control	3,6 a	3,1 a	5,9 a
3 g/l	3,4 a	1,8 ab	4,7 b
6 g/l	2,6 a	1,2 b	2,2 c

There were significant increases of electrolytes leakage presented by damage percentage under salt treatments for all the populations of oak seedlings (Fig.2a). Under 3g / l NaCl, plants from Bellif showed the highest percentages of damage of about 34% followed by Kaf Errand (22.8%) and lower in

Hammam Bourguiba (6.63%). While, under 6g/l NaCl, damage increased significantly reaching 73.4%, 46.4% and 14.3% respectively in Bellif Kaf Errand and Hammam Bourguiba.

Salinity induced a significant decrease in the water potential in all populations (Fig.2b). The values are around -0.15 MPa in controls, -0.58 MPa under 3g/l and -0.69 MPa under 6 g/l of NaCl. Analysis of variance bi-factors showed a very highly significant treatment effect and a non-significant population effect.

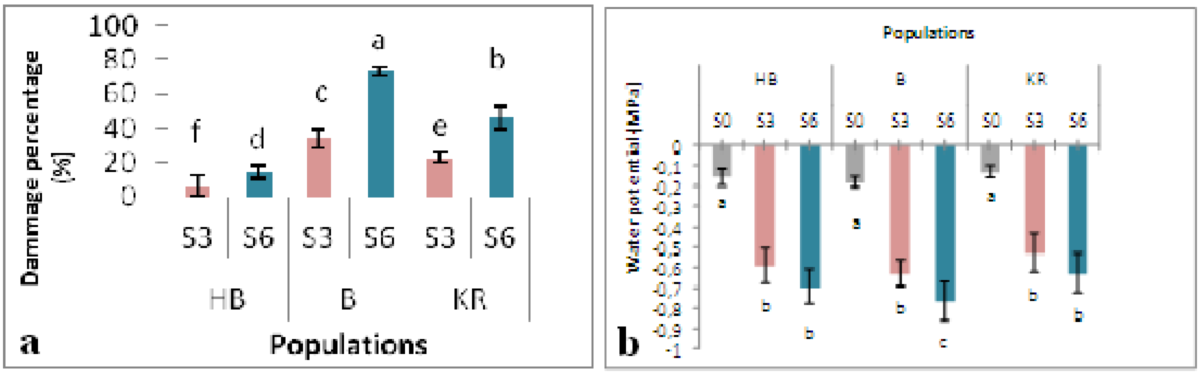

Fig. 2. (a) Damage percentage and (b) Water potential of Cork oak populations (HB, B and KR) grown under different salinity levels (0, 3, 6 g / l NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are LSD 0.05 Different letters indicate significant differences.

Osmolytes accumulation

The effect of salt stress occurs by a hight accumulation of proline (Fig.3a). We noticed a higher significant difference between treatment and populations. Compared to control, the plants from Kaf Errand recorded the largest increases (90%) compared to Hammam Bourguiba (86%) then Bellif (67%) under 6g/l of NaCl. Biochemical analysis of carbohydrates accumulation indicated that levels of soluble carbohydrates decreased under saline conditions in samples of Bellif and Kaf Errand. However, this level increased significantly in samples of Hammam Bourguiba (Fig.3b).

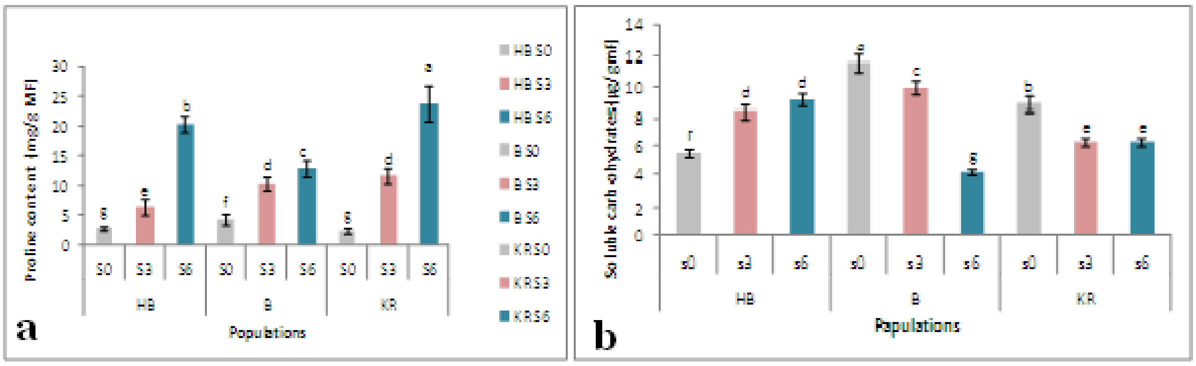

Fig. 3. Solute concentrations in leaves: (a) proline content and (b) soluble carbohydrates content of Cork oak populations (HB, B and KR) grown under different salinity levels (0, 3, 6 g / l NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are LSD0.05 Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences.

Water relations

Osmotique potential at the full trugor (Ψ_{π}^{100}) and at the loss point (Ψ_{π}^{0}) decreased significantly with concentrations of NaCl only for the population of Hammam Bourguiba (Fig.4a and b). In fact, Similar values were observed between control and stressed plants from populations of Bellif and Kaf Errand. Osmotic adjustment values were low among the populations of Bellif and Kaf Errand compared to Hammam Bourguiba which showed moderately higher values with 0.13 and 0.16 MPa, respectively under 3 and 6 g / l NaCl. The relative water content at the turgor loss point (RWC0) in stressed plants of three populations increased significantly with an increase of salinity compared to control (Fig.4c).

The salt stress induced a significant increase in water content at zero turgor RWC_0 and in apoplasmique water content AWC (Fig.4d). Control plants showed the lowest values of AWC 82%. Under 3 and 6 g / l NaCl, AWC have varied from 86 to 89% in the three populations.

The elasticity modulus is directly related to the relationship between the potential at turgor and the relative water content. The values of the modulus of elasticity don't change significantly under salt stress however values were very low (Fig.4f).

Discussion

The application of NaCl induced a significant decrease in relative growth rate on height (RGR_h) and collar diameter (RGR_d), with significant variability between populations.

The population of Hammam Bourguiba seems the most suited by lesser reductions in growth, displaying better growth compared to Kaf Errand and Bellif. These results confirm the best behavior of this population under salt conditions. Such effect has been reported in poplar clones under salt stress (Abassi *et al.,* 2004).

On the other hand, saline treatment didn't affect the relative growth rate in biomass of shoots and roots parts of Hammam Bourguiba's population which reflect a best ability to exploit the nutrient for the production of dry matter and a better tolerance to salt concentrations applied.

In the contrary, there was a depressive action of the salt on the production of shoot and root biomass for the populations of Bellif and Kaf Errand.

Indeed, Epron *et al.* (1999) reported that 3 g / l of NaCl induced 20% of decrease in root biomass of seedlings of *Quercus rubra*. L stressed.

Under environmental stresses plant membranes are subject to changes often associated with increases in permeability and loss of integrity. Thus, membrane injury increased in a condition of high soil salinity in the seedling stage (Farkhondeh *et al.,* 2012).

Water deficit-induced by salinity is the cause of the electrolyte lackage as result of loss integrity of membrane structures of populations of cork oak. Hammam Bourguiba recorded lowest damages indicating greater membrane integrity. The most significant damage are recorded in the population of Bellif.

The accumulation of carbohydrate reserves provides stability of membrane structures during salt treatments. While depletion in reserves may aggravate cellular damage (Bailey- Serres and Voesenek, 2008).

In addition, osmoticum contribute to the protection of the enzyme systems in these plants which allows the continuation of physiological processes.

Various studies have demonstrated such effects of salinity on the deterioration of the integrity of membrane structures of plants (Kaya *et al.,* 2001; Abassi *et al.,* 2010).

Moreover, increasing the intensity of the salt stress caused a significant reduction of water potential resulting strong dehydration of the cork oak leaves. This decline is even more pronounced when the intensity of stress is more important. In fact, plants irrigated with saline water must exert more energy per unit of absorbed water, compared to a plant irrigated with fresh water.

The decrease in leaf osmotic potential at full turgor (Ψ_{π}^{100}) and at the loss point (Ψ_{π}^{0}) in treated plants from Hammam Bourguiba indicated the ability to maintain turgor and to develop osmotic adjustment under saline conditions. While, values was statistically similar to controls for the other populations. The decrease of the full turgor potential can be attributed to a high accumulation of soluble carbohydrates and proline in leaves. some authors suggest that it may be attributed to an accumulation of Na^+ and Cl^- ions (Navarro *et al.,* 2007) or to a decrease in leaf area under salt stress (Medina and Francisco, 1997) or with an increase in water content apoplasmique (Maury *et al.,* 2000).

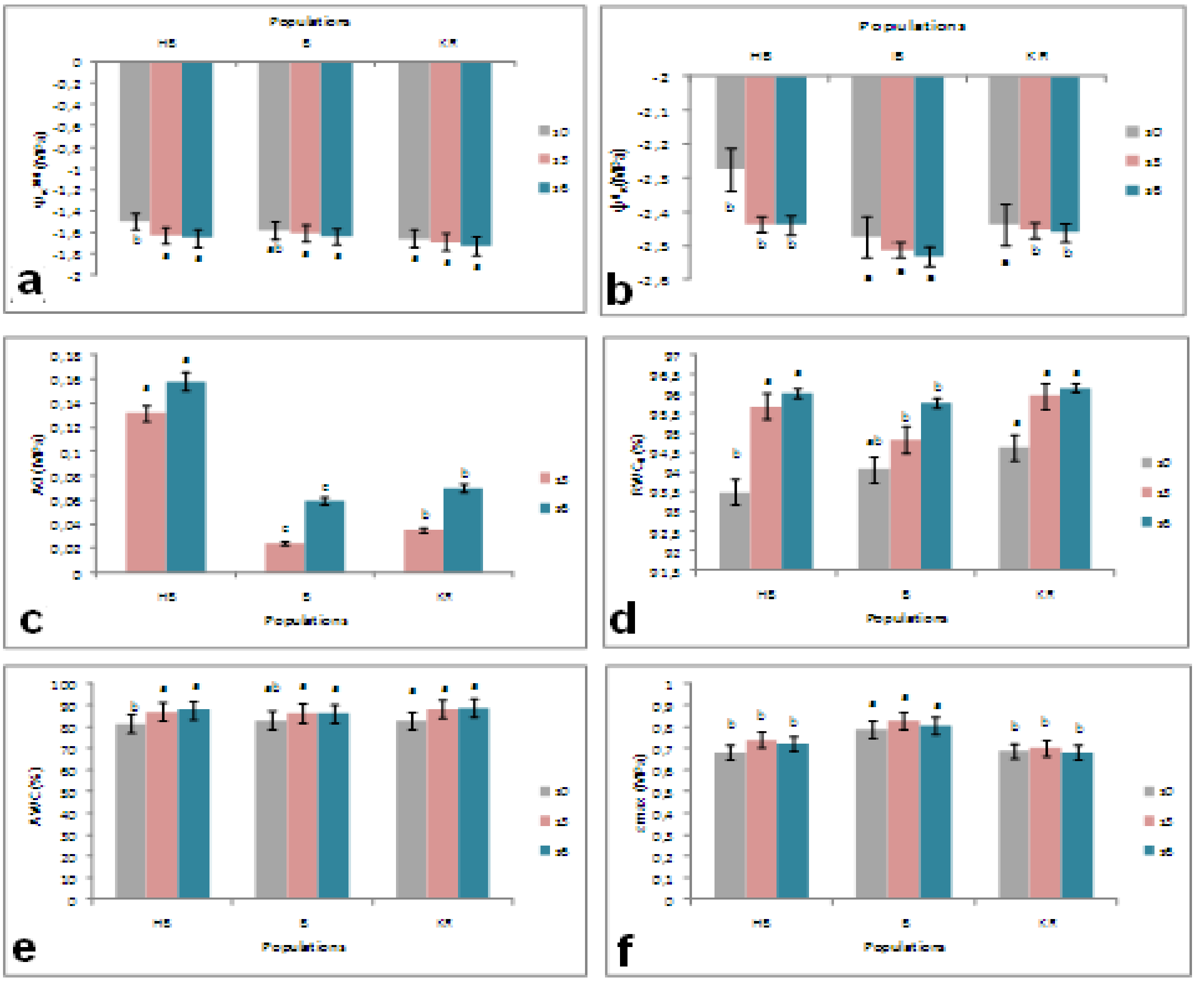

Fig. 4. Water relation parameters derived from P-V curve analysis: (a) the osmotic potential at full trugor (Ψ_π^{100}), (b) the osmotic potential at zero trugor (Ψ_π^0), (c) the osmotic adjustment (OA), (d) the relative water content at the turgor loss point (RWC_0), (e) the apoplastic water content (AWC) and (f) the volumetric modulus elasticity (εmax) of Cork oak populations (HB, B and KR) grown under different salinity levels (0, 3, 6 g / l NaCl) the data are mean values of four replications with three measurements per replicate and vertical bars are LSD0.05 Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences.

These results confirm that the population of Hammam Bourguiba has more developed coping mechanisms to physiological drought compared to Bellif and Kaf Errand.

The main interest of these osmotic changes is to maintain turgor which provides the physiological activity of plants. Similar effects have been reported in *Arbutus unedo* (Navarro *et al.,* 2007).

Osmotic adjustment is generally induced by accumulation of solutes in the vacuole of tolerant plants. Only the population of Hammam Bourguina presented moderate OA compared to other populations. This reflects a capacity of osmoregulation which enables its tissues turgor and develops a higher resistance to osmotic stress.

The increasing of RWC_0 reflects sensitivity to dehydration under the effect of salinity, besides, the elevation of the apoplast water content at zero turgor is responsible to maintain turgor in limited water conditions. Salinity induced a significant increase in the water content of the apoplast (AWC). Populations of Kaf Errand and Hammam Bourguiba showed higher values of AWC compared to Bellif. These results are similar to those found by Haddad (2002) on *Eucalyptus camaldulensis* and *Eucalyptus microtheca*, and those found by Mguis *et al.* (2011) on some wheat plants treated by salinity gradient.

The results of modulus elasticity showed that the populations behaved significantly in the same way as 0, 3 and 6 g/l of NaCl. Indeed, Margio *et al.* (2000) have shown that a high modulus of elasticity is disadvantageous for foliar cells because they plasmolys easily during dehydration. The increase of cell elasticity (low ε_{max}) associated with high concentration of solutes, is an adaptation process, insofar as the combination of these two factors provides water absorption and a turgor maintenance over a wide range of water potential (Suarez *et al.,* 1998).

Salinity induced significant accumulation of proline in leaves of the three populations studied. Whereas, the rate of soluble sugars was increased for the population of Hammam Bourguiba plants, however, it was decreased belong Bellif and Kaf Errand samples. This is related to the osmotic adjustment recorded and the best behavior of this population to salt stress. It seems to be the result from combined effect of proline and soluble sugars. This accumulation of organic solutes is simply a phenomenon of adaptation to salinity, allowing the plant to ensure its normal physiological functions despite deterioration in its internal water status.

In fact, Hyun *et al.* (2003) showed the important role of proline in maintaining osmotic adjustment in addition to its protective role. A decrease in leaf soluble sugars content was mentioned in citrus plants under salt stress (Arbona *et al.,* 2005).

Conclusion

The results obtained in this study reflect a variable behavior depending on the population and the amount of salt applied. The population of Hammam Bourguiba showed an average salinity adaptation, by small reductions in growth, and an average of osmoregulation capacity resulting from an accumulation of proline and soluble sugars. The populations of Bellif and Kaf Errand showed sensitivity to salinity, by strong growth reductions, their inability to osmoregulation despite the accumulation of proline and a decrease in levels of soluble sugars.

Acknowledgments

We wish to thank the National Institute for Research in Agricultural Engineering, Water and Forestry (INRGREF, Tunisia) for funding this project.

References

Abassi M, Albouchi A, Béjaoui Z, Sellami D. 2010. Incidence de la salinité sur la croissance de jeunes plants de peuplier blanc (*Populus alba* L.). Dans: Actes des Journées Scientifiques de l'INRGREF: Valorisation Agricole des Eaux Salées, des Eaux Usées Traitées et des Boues Résiduaires, Hammamet 9-10 novembre 2009. Annales de l'INRGREF, Numéro spécial **14**, 29-38.

Abassi M, Albouchi A, Ben Mansoura A, Béjaoui Z, Rejeb MN, Mougou A. 2004. Tolérance de divers clones de peuplier à la salinité. Annales de l'INRGREF **6,** 17-34.

Arbona V, Iglesias DJ, Jacas J, Primo-Millo E, Talon M, Gomez-Cadenas A. 2005. Hydrogel substrate amendment alleviates drought effects on young citrus plants. Plant and Soil **270**, 73-82.

Bailey-Serres J, Voesenek LACJ. 2008. Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biology **59,** 313-339.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water- stress studies. Plant and Soil **39,** 205-207.

Ben Jamâa ML, Nouiui M. 2018. Le liège et le vin en Tunisie, Actes du colloque de VIVEXPO et de l'IML, Institut de liege 1-9.

Centre National de Telédétection (Cnt)-Direction Générale Des Forêts (DGF)-Direction Générale de la Recherche Scientifique (DGRS). 2005. Résultats du deuxième inventaire forestier et pastoral national. Gouvernorats de Jendouba, Béja et Bizerte. Tunisie p 129.

Cha-um S, Supaibulwatana K, Kirdmanee C. 2006. Water relation, photosynthetic ability and growth of Thai Jasmine rice (*Oryza sativa* L. ssp. Indica Cv. KDML 105) to salt stress by application of exogenous glycinebetaine and choline. J Agron. Crop Sci. **192,** 25-36.

Débierre F. 1927. Le chêne-liège en Tunisie. Imprimerie Centrale-Tunis, 60 p.

Ennajeh A, Azri W, Khaldi A, Nasr Z, Selmi H, Khouja M. 2013. Variabilité génétique du Chêne liège (*Quercus suber* L.) en Tunisie. Bilan d'un essai comparatif multisites de plantations de provenances diverses, Geo-Eco-Trop **37(2),** 191-200.

Epron D, Toussaint M, Badot PM. 1999. Effects of sodium chloride salinity on root growth and respiration in oak seedlings. Ann. Forest.Sci. **56,** 41-47.

FAO (Organisation des Nations Unies pour l'alimentation et l'Agriculture). 2006. Conférence électronique sur la salinization: Extension de la salinisation et stratégie de prévention et réhabilitation. Projet CISEAU: Centre d'information sur l'Eau Agricole et ses Usagers.

Farkhondeh R, Nabizadeh E, Jalilnezhad N. 2012. Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. International Journal of Agricultural Science **2**, 385-392.

Haddad I. 2002. Statut hydrique dans l'adaptation à la sécheresse de 5 espèces d'Eucalyptus le plus utilisées dans le reboisement en Tunisie. Université de Tunis El Manar. 62.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plant without soil. California Agriculture Experiment Station Circulation, University of California Berkley Press, CA. USA, p 347.

Hyun PS, Simm J, Ritzema H. 2009. Development of tidal areas: Some principles and issues towards sustainability, Irrig. Drain., **58(S1),** 52-59.
https://doi.org/10.1002/ird.474

Kaya C, Higgs D, Kirnak H. 2001. The effect of high salinity (NaCl) and supplementary phosphorus and potassium on physiology and nutrition development of spinach. Bulg. J. Plant Physiol., **27(3-4),** 47-59.

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

Lutts S, Almansouri M, Kinet JM. 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Sci. **167**, 9-18.

Margio G, Peltier JP, Girel J, Pautou G. 2000. Success in the demographic expansion of *Fraxinus excelesior* L. Tree. **15**, 1-13.

Maury P, Breger M, Mojayed F, Planchon C. 2000. Leaf water caracteritics and drought acclimatation in sun flower genotypes. Plant and Soil., **223**, 153-160.

Mc Cready RM, Guggolz J, Silviera V, Owes HS. 1950. Determination of starch ad amylase in vegetables. Application to peas. Annal. Chem **22,** 1156-1158.

Medina E, Francisco M. 1997. Osmolality and d13C of leaf tissues of mangrove species from environments of contrasting rainfall and salinity. Estuar Coast Shelf Sci. **45,** 337-344.

Mejbri N. 2005. Histoire de l'exploitation forestière en Kroumirie durant la période coloniale. Mémoire de Mast ère. Univ. De Manouba (Tunis), p 131.

Mguis K, Albouchi A, khadhri A, Abassi M, Yakoubi-Tej M, Mahjoub A, Ouerghi Z, Ben Brahim N. 2011. Adjustments in leaf water relations of wild wheat relative Aegilops geniculata Rooth. and wheat (*Triticum durum* Desf.) plants grown in a salinity gradient. Australian Journal of Crop Science **5(10).**

Navarro A, Banon E, Sanchez-Blanco. 2007. Effects of sodium chloride on water potential components, hydraulic conductivity, gaz exchange and leaf ultrastructure of Arbutus unedo plants. Plant Science **172,** 473-480.

Nsibi R, Souayah N, Khouja LM, Khaldi A, Bouzid S. 2006. Impacts des facteurs biotiques et abiotiques sur la dégradation de suberaie tunisienne. Geo-Eco-Trop **30(1),** 25-34.

Palahi M. 2004. New tools and methods for Mediterranean forest management and planning. Tempus IMG. Centre Tecnologic Forestal de Catalunya.

Patakas A, Noitsakis B. 1999. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. J Plant Physiol **154,** 767-740.

Scarascia-Mugnozza G, Oswald H, Piussi P, Radoglou K. 2000. Forests of Mediterranean region: gaps in knowledge and research needs. For. Ecol. Manage **132,** 97-109.

Selmi K. 2006. Utilisation des données et résultats de l'inventaire forestier national pour la gestion des Forêts de chêne-liège en Tunisie. Annales de l'INRGREF, N° Spècial **9(1),** 21-30.

Staub AM. 1963. Extraction, identification et dosage des glucides dans les extraits d'organes et les corps bactériens In: Technique de laboratoire, Tome 1 et 2 (Masson Compagnies, Eds.) Paris. 1307-1366.

Stiti B, Piazzetta R, Khaldi A. 2014. Régénération de la subéraie tunisienne: état des lieux, contraintes et avancées techniques, Forêt méditerranéenne t. **35(2),** 151-160.

Suarez N, Sobra MA, Medina E. 1998. Salinity on the leaf water relations components and ion accumulation patterns in *Avicennia germinans* (L.) seedlings. Oecologia **114**, 299-304.

Yessad SA. 2000. Le chêne-liège et le chêne dans les pays du méditerrané occidental. Edition ASBL foret Wallonne, p 190.

Zhu JK. 2002. Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biology **53,** 247-273.