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COMPARATIVE DROUGHT RESPONSES OF *QUERCUS SUBER* SEEDLINGS OF THREE ALGERIAN PROVENANCES UNDER GREENHOUSE CONDITIONS

Henia DAOUDI[1]*, Arezki DERRIDJ[1], Lynda HANNACHI[1] & Jean Philippe MÉVY[2].

[1] Laboratoire d'Écophysiologie Végétale, Département de Biologie, Faculté des Sciences Biologiques et des Sciences Agronomiques. Université Mouloud Mammeri. Tizi-Ouzou. Algeria. E-mails: hedaoudi@yahoo.fr ; aderridj@yahoo.fr ; lyndahannachi@yahoo.fr

[2] Aix Marseille Université, Avignon Université, CNRS, IRD, IMBE, Marseille, France - Équipe Diversité et Fonctionnement : des Molécules aux Écosystèmes. 3 Place Victor Hugo. 13003 Marseille cedex 3 France. E-mail: jean-philippe.mevy@univ-amu.fr

* Corresponding author.

RÉSUMÉ.— *Réponses comparatives à la sécheresse en condition de serre des plantules de* Quercus suber *de trois provenances algériennes*.— Le Chêne-liège est une espèce typiquement méditerranéenne présentant un intérêt économique et écologique. L'existence de mécanismes de tolérance à la sécheresse estivale méditerranéenne chez cette espèce a été démontrée par de nombreuses études réalisées, essentiellement en Europe. Cependant, ces dernières années, d'autres études ont montré que les mécanismes de tolérance diffèrent entre les provenances. Le comportement des provenances algériennes de Chêne-liège a été le sujet de très peu d'études. L'Algérie, étant un pays plus aride que les pays européens, ses provenances pourraient montrer des différences de comportement par rapport aux provenances européennes. L'objectif de ce travail est l'étude de la réponse à une sécheresse estivale des plantules originaires de trois provenances algériennes caractérisées par des étages bioclimatiques différents : Azazga (subhumide), Jijel (humide) et M'Sila (semi-aride). Un arrêt d'arrosage a été appliqué aux plantules, cultivées de façon homogène en serre, pendant 10 semaines, de fin-juin à mi-septembre, pour simuler la sécheresse estivale méditerranéenne. Le statut hydrique et des paramètres morphologiques et biochimiques ont été évalués chez les plantules arrosées (témoins) et non arrosées (stressées) et les taux de survie des plantules non arrosées ont été déterminés pour les trois provenances. Les résultats ont révélé des différences de comportement entre les trois provenances dans les deux conditions d'arrosage et de non arrosage. Dans les conditions d'arrosage, les plantules originaires de M'Sila ont montré la teneur relative en eau la plus élevée ainsi que la meilleure croissance. L'arrêt d'arrosage, ayant provoqué une diminution significative des teneurs en eau du sol, a provoqué une réduction de la teneur relative en eau et de la croissance (hauteur et diamètre de la tige) des plantules originaires de Jijel et d'Azazga alors que seul le diamètre des tiges était réduit chez les plantules originaire de M'Sila. Les feuilles des plantules originaires du site le plus aride, M'Sila, ont montré des modifications morphologiques et physiologiques considérées comme étant des stratégies d'adaptation à la sécheresse : une faible surface foliaire spécifique, une diminution des teneurs en Chlorophylle a, pour éviter une absorption excessive de l'énergie lumineuse, une diminution des teneurs en amidon, une accumulation des protéines, des sucres et de la proline afin de permettre un ajustement osmotique et une augmentation du $\delta^{13}C$ pour une meilleure utilisation de l'eau. Dans les conditions de non arrosage, les plantules d'Azazga ont montré un comportement intermédiaire entre les plantules originaires de Jijel et de M'Sila. Cependant, contre toute attente, les plantules de M'Sila ont montré le plus faible taux de survie, le meilleur taux de survie étant enregistré pour les plantules d'Azazga. Les écotypes étudiés dans ce travail montrent donc des traits fonctionnels différents pouvant être liés aux conditions environnementales des provenances. Malgré le faible taux de survie des provenances du milieu le plus aride on peut penser qu'elles constituent de bons candidats, sur le long terme, en matière de reboisement dans le contexte du changement climatique global.

SUMMARY.— Cork oak (*Quercus suber* L.) is one of the most representative Mediterranean forest species that is well studied in Europe and recognized as drought tolerant. While many studies showed that differences exist in drought tolerance mechanisms among provenances, few reports exist on drought responses of Algerian *Q. suber* seedlings. The present study investigates summer drought behaviour of seedlings originating from three Algerian provenances: from humid (Jijel), sub humid (Azazga) and semi-arid (M'Sila) Mediterranean areas. The summer conditions were simulated by stopping irrigation of the seedlings grown homogenously in greenhouse during 10 weeks from last June to mid-September. Water status, morphological and biochemical parameters were evaluated in watered (control) and non-watered seedlings and survival rate of non-watered seedlings was determined for the three provenances. The results showed differences between seedlings behaviour of the three provenances in watered and non-watered conditions. In watered conditions, M'Sila seedlings showed the highest Relative Water Content and the highest growth. In non-watered conditions, the reduction of soil water content had negative effect on the Relative Water Content and growth (height and shoot diameter) of the seedlings of Jijel and Azazga provenances. Only the stem basal diameter was reduced in the seedlings of M'Sila provenance. Leaves of M'Sila non irrigated seedlings, originating from the drier site (semi-arid), showed morphological and physiological modifications that are known as drought adaptive strategy: low Specific Leaf Area, decrease in Chlorophyll a contents thus avoiding excessive absorption of light energy, a decrease in starch content, an increased accumulation of proteins, sugars and proline. An increase in leaves $\delta^{13}C$ was also obtained suggesting a more efficient water use. Azazga seedlings showed an intermediate behaviour between M'Sila and Jijel seedlings in drought conditions. However, contrary to expectation, survival rate was lowest for M'Sila and highest for Azazga seedlings. The ecotypes studied in this work exhibited different functional traits related to the environmental conditions of the original provenance. Despite the low survival rate of provenances from the most arid environment, they are thought to be suitable candidates in long-term for reforestation in the context of global climate change.

Cork oak (*Quercus suber* L., Fagaceae), is a species, widely distributed among Mediterranean forest trees (Quézel & Médail, 2003) of great ecological (carbone sequestration, soil protection, hydrological cycle regulation) and economic (production of cork) importance (Pausas *et al.*, 2009). It is an evergreen and sclerophyllous species growing from the sea level up to 700 m in altitude between 13 and 16°C and is strictly calcifuge colonizing siliceous soils (Quézel & Médail, 2003). Cork oak stands in Algeria (227 000 ha) constitute the second largest stand after the Aleppo pine. Moreover, Algerian stands represent 14 % of the world cork oak forests (FAO 2013). Nearly 4/5 of the cork oak areas are located essentially in the north-east, from Tizi-Ouzou to the Tunisian border but, in the western part of Algeria the stands are scattered in the form of small islands (Bouhraoua, 2015).

Q. suber response to drought conditions which characterize summer in Mediterranean areas has been well studied, essentially in Europe (Faria *et al.*, 1999; Nardini *et al.*, 1999; Nardini & Tyree, 1999; Kurze-Besson *et al.*, 2006; Otieno *et al.*, 2006; Pardos *et al.*, 2006; Kwak *et al.*, 2011). Cork oak is well known as drought tolerant species (Nardini *et al.*, 1999; Nardini & Tyree, 1999). There are many mechanisms by which it resists to drought periods: deep rooting, osmotic adjustment (Otieno *et al.*, 2006; Pardos *et al.*, 2006; Kwak *et al.*, 2011) and anti-oxidant system (Faria *et al.*, 1999). Ecophysiological investigations demonstrated that *Q. suber* is well adapted to summer conditions because it maintains a favourable ratio between water loss and uptake during the dry period. Maintaining a favourable water status in tissues, with a high relative water content (RWC) during summer drought, is ensured by deep roots and/or osmotic adjustment through accumulation of molecules such as proteins, sugars and proline (Otieno *et al.*, 2006; Pardos *et al.*, 2006; Oufir *et al.*, 2009; Kwak *et al.*, 2011). A decrease in shoots and leaves biomass by reduced growth and root drop is also noted (Ksontini *et al.*, 1998; Kurze-Besson *et al.*, 2006). A reduction of specific leaf area (SLA) was also observed (Ramirez-Valiente *et al.*, 2010). The presence and the nature of the cork in which cell walls are suberin impregnated, contributes to protection against fire and also resistance to tissues desiccation (Pausas *et al.*, 2009). Under drought conditions, an increase of leaves $\delta^{13}C$ contents reflects a better water use efficiency (WUE) in *Q. suber* seedlings (Gouveia & Freitas, 2009).

Although the cork oak seems well adapted to the dry conditions of the Mediterranean climate, the mechanisms involved in this adaptation are still scare (Almeida *et al.*, 2013). Also, the natural regeneration is low and poorly understood (Gonzalez-Rodriguez *et al.*, 2010) because *Q. suber* is particularly more sensitive to drought in the early stages of development (Aranda *et al.*, 2005) due to the quasi-absence of cork (Pereira *et al.*, 2009). In Algeria, facing the decline of the cork oak forest due to many factors like fires (the most important factor), grazing and diseases, many reforestation operations have been undertaken (the cork oak occupies the first place of the reforested species with 24 % of the wooded areas). However, the success rates of these reforestations are often low and unsatisfactory; the survival rate of plants decreased from 80-90 % to 20-50 % after the summer season (Messaoudène *et al.*, 2011; Bouhraoua, 2015). This seedlings recruitment limitation probably will be amplified with the predicted global changes in Mediterranean region (Caldeira *et al.*, 2014). Hence, climate change is expected to lead to longer dry spells, higher evaporative demand

and more intense droughts in the coming decades in several regions of the world, including the Mediterranean basin (IPCC, 2007).

Since cork oak has a large distribution area with large variation in environmental conditions [areas with a mean annual rainfall of 400–1500 mm and a mean annual temperature of 13–20°C (Díaz-Fernández *et al.*, 1995)], a large differentiation is expected among populations for significant adaptive traits such as the ability to tolerate extended periods of drought (Varela *et al.*, 2014).

Many studies showed a high level of differentiation among the populations of *Q. suber* species. Differences among cork oak plants originated from different populations in phenotypic traits were mainly due to divergent selection imposed by temperature and rainfall variation and to neutral evolutionary processes such as founder effect or genetic drift (Ramirez-Valiente *et al.*, 2010). Genetic diversity parameters determined for different geographic areas of the entire *Q. suber* range, showed variation where paleogeography, hybridization, adaptation, fragmentation, and human impact play an important role in the evolutionary history of this species (Simeone *et al.*, 2010). Ennajah *et al.* (2013) showed a high phenotypic variability among and within Moroccan cork oak tree populations which was significantly correlated with rainfall; large differences between populations from highest and coldest sites as well as those of lowest and warm sites were detected and adaptive responses specific to some populations were founded. Some differences among populations have been observed concerning their ability for adaptation and production under drought conditions (Gandour *et al.*, 2007).

Hence, a better understanding of the effects of drought on plants originating from different provenances is essential for early selection of provenances for afforestation.

Based on the concept of plant-climate-coevolution, our working hypothesis was that differences exist in drought tolerance mechanisms among provenances: provenances native to dry regions would have more capability to acclimate to drought conditions than provenances originated from a more temperate climate region. Thus, ecophysiological comparisons may prove useful for the choice of *Q. suber* provenances for afforestation.

Few reports exist on drought responses of Algerian *Q. suber* seedlings (Acherar *et al.*, 1991; Daoudi *et al.*, 2016). The present study investigates summer drought effects on seedlings originating from three Algerian provenances. The summer conditions were simulated by stopping irrigation of the seedlings grown in greenhouse during two months, July and August. Then, different parameters were evaluated: water status of the seedlings (RWC), morphological traits (height and thickness growths and specific leaf area) and physiological parameters (Chlorophylls, Carotenoids, proteins, sugars, starch, $\delta^{13}C$, % C and % N) in leaves.

MATERIALS AND METHODS

Cork oak acorns were collected in the end of November 2011 from three Algerian provenances: Jijel (Aghzer forest), M'Sila forest (Oran) and Azazga (Beni Ghobri forest). The forests of the three provenances showed foliar damages attributed to xylophages and phyllophages insect pests (the most important being *Platypus cylindrus*) and cryptogamic diseases (the most important is coal caused by *Hypoxylon mediterraneum*) (Bouchaour-Djabeur, 2013; Rouibah *et al.*, 2011). The decline of Algerian cork oak forests is slow but chronic (Bouhraoua *et al.*, 2010). The characteristics of Jijel, M'Sila and Azazga forests are shown in Tab. I.

Thus, M'Sila is the drier provenance and Jijel is the most humid provenance. The dry period is longer in M'Sila (mid-April to mid-September) and shorter in Jijel (May to September) as shown by the ombrothermic diagrams (Fig. 1).

The acorns of the different provenances were weighed: 2.954 ± 0.155 g, 2.458 ± 0.093 g and 2.195 ± 0.256 g for Jijel, M'Sila and Azazga respectively. After germination at 20°C in Petri dishes, the seedlings with 2 cm root length (mid-April) were transplanted to plastic bags (15 cm diameter and 30 cm deep) filled with substrate consisting of a mixture of loam (2/3) and washed sand (1/3). The loam (N 110-250 mg/l, P2O5 60-140 mg/l, K20 120-280 mg/l) had a fine structure and a pH of 6.2.

All the *Q. Suber* seedlings of the three provenances were grown homogeneously in greenhouse (5 x 3 m dimension), located next to the university of Tizi-Ouzou in Algeria which is characterised by Sub-humid Mediterranean climate (mean annual temperature of 19.2°C, mean annual precipitation of 705 mm). The seedlings were watered regularly (three times a week) until the end of June. Then we constituted three plots of seedlings (60 seedlings/plot). Each plot was constituted by a mixture of 10 seedlings/provenance (Jijel, M'Sila, Azazga)/treatment (watered and none watered): Jijel watered (JW) and non-watered (JNW), M'Sila watered (MW) and non-watered (MNW) and Azazga watered (AW) and non-watered (ANW). Water stress treatment was given by withholding the water supply for 10 weeks, from end of June until mid of September, to simulate Mediterranean summer drought conditions. According to ICCP (2007) dry days are projected to increase markedly in the Mediterranean basin. The greenhouse temperatures varied from 25°C to 38°C during the experiments. Ten weeks after irrigation was stopped (mid-September), plants of JW, JNW, MW, MNW, AW, ANW were harvested for determination of morphological and physiological parameters.

TABLE I

Location, soil characteristics and climatic data of acorns provenances (Jijel, M'Sila and Azazga)

	Jijel	M'Sila	Azazga
Altitude	20 m	350 m	530m
Longitude	36°49'0.259''E	0° 50' 19.7" W	4°22' to 4°27' W
Latitude	5° 44' 56.7''N	35°38' 22.6"N	36°42'to 6°47'N
Annual mean temperature	18	18.1	16.7
Annual mean precipitations	1022 mm/year	397mm/year	944mm/year
Mediterranean Bioclimatic Level	Humid	Semi- arid	Sub-Humid
Substrat	Numidian sandstone	Jurassic schist	Numidian sandstone
Soil Texture (superficial Horizon)	Sandy loam	Clay and silt	Clay loam
Organic Matter	2,65	2,75	4,595
pH	6,525	6,7	5,8
Exposition Dominance	NW	NW	N

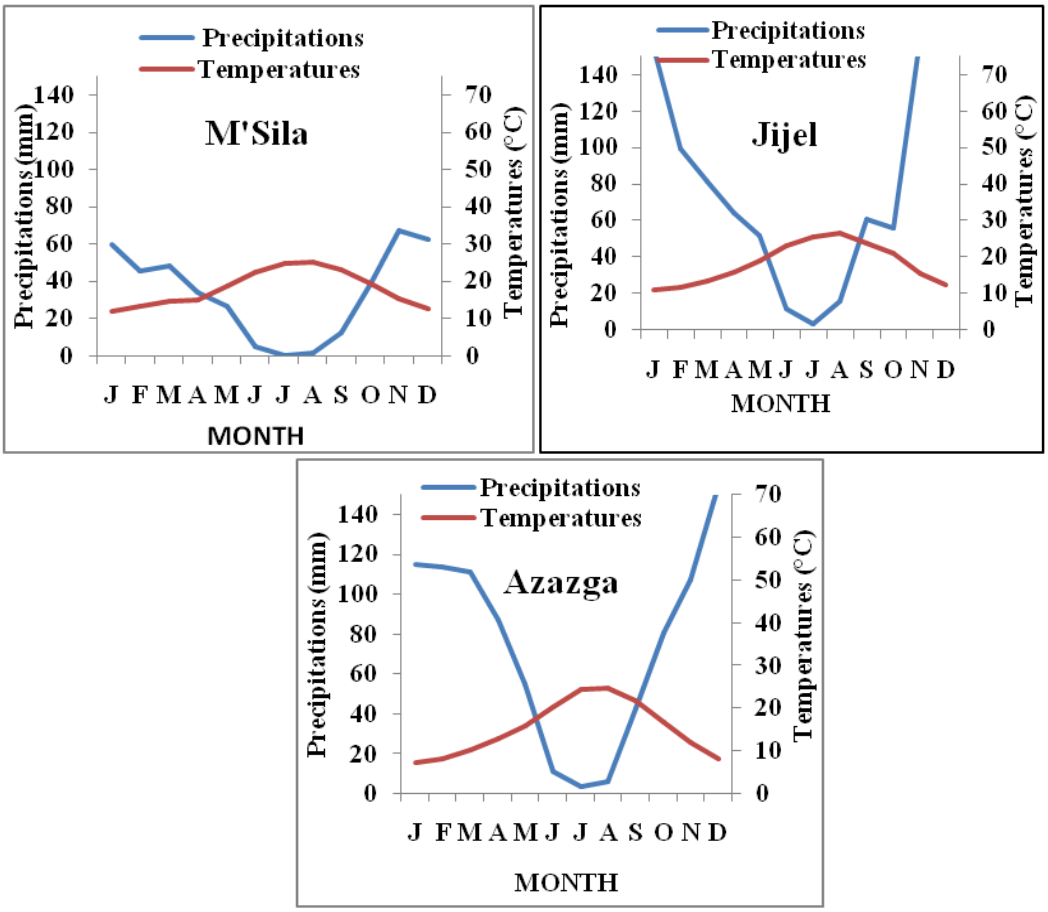

Figure 1.— Ombrothermic diagrams of M'Sila, Jijel and Azaga Forest provenances.

SUBSTRATE WATER CONTENTS MEASUREMENT

Substrate water contents (SWC) was determined as described by Mathieu & Pieltain (2003). Substrate samples of 1 g were taken from the plastic bags of the six lots at a depth of 10 cm (10 repetitions/provenance/treatment) and dried at 105°C for 72 h and then water content was calculated using the formula:

$$SWC=\frac{FW-DW}{FW} \text{ X } 100$$

where FW and DW are the fresh and dry weight, respectively.

RELATIVE WATER CONTENTS (RWC) MEASUREMENT

Relative water content (RWC) was measured in three young leaves of five plants per provenance/treatment using Nardini *et al.* (2000) method. Leaves were detached and immediately weighed to get their fresh weight (FW). Then, the leaves were restored with distilled water to near full turgor by immersing their petioles in water, covering the leaf blade with plastic film and leaving them in the dark for 12 h. Leaves were then reweighed to obtain their turgid weight (TW) and put into oven at 70°C for 72 h to obtain their dry weight (DW). Finally, RWC was calculated as:

$$RWC=\frac{FW-DW}{TW-DW} \text{ X } 100$$

MORPHOLOGICAL PARAMETERS MEASUREMENTS

To evaluate seedlings growth in both conditions, watered and non-watered, different morphological parameters of 10 plants per provenance/treatment were measured: stem height, basal stem diameter and shoots (stems and leaves), roots (total roots washed with distilled water) and leaves (with petioles) biomass were determined after drying at 75°C during 72 h. Then the root to shoot ratio was estimated.

The specific leaf area (SLA) was determined on three mature leaves per plant and ten plants per provenance/treatment as the ratio of leaf area (determined with AM350 Portable Leaf Area Meter) to individual leaves dry mass (DM), measured after oven-drying at 70°C to a constant weight (Faria *et al.*, 1999).

PHYSIOLOGICAL PARAMETERS MEASUREMENTS:

Pigments, sugar, starch, proteins, proline, %C, %N and $\delta^{13}C$ contents were determined on five seedlings per provenance/treatment.

After extraction in 80 % acetone in dark, the photosynthetic pigments absorbances were determined spectrophotometrically and their contents were calculated as proposed by Lichtenthaler & Buschmann (2001):

Chlorophyll a as: Chla = 12.25 × A663 - 2.79 × A647

Chlorophyll b as: Chlb = 21.50 × A647 - 5.10 × A663

Total chlorophylls as: ChlT = 7.15 × A663 - 18.71 × A647

Total carotenoids as: CarT = 1000 × A470 - 1.82 × Chla - 85.02 × Chlb

After the extraction of the soluble sugars from fresh leaves in ethanol (70 %), the solid fraction was used for starch analysis. Starch was incubated in HCl (1.1 % v/v) for 30 min at 95°C for hydrolysis into simple sugar. Then, soluble sugars and starch concentrations were determined colorimetrically at 625 nm with anthrone reagent following Cerning-Berorard (1975) method. Glucose was used as standard for both soluble sugars and starch.

Soluble proteins were extracted from fresh leaves in distilled water and then quantified spectrophotometrically at 595 nm after colorimetric reaction with Bioard reagent following Bradford (1976) method. BSA was used as standard.

Proline content was quantified by the ninhydrin-colorimetric method at 515 nm after extraction in methanol (70 %) as described by Monneveux & Nemmar (1986). Proline was used as standard.

Foliage samples for analysis of $\delta^{13}C$, %C and %N were dried at 70ºC for 72 h and ground to a fine powder. The abundance in combusted samples were performed using a mass spectrometer (Finnigan, Delta-S, Bremen, Germany) at UMR-CNRS7266 LIENSS with a precision of 0.1 ‰. We calculated $\delta^{13}C$ (‰) with respect to the PDB Pee Dee Belemnite standard:

$$\delta^{13}C=\frac{Rsample}{Rstandard-1} \text{ X } 1000$$

where *Rsample* and *Rstandard* are the $_{13}C/_{12}C$ ratios in a sample and the standard (Pee Dee Belemnite) respectively (Warren & Adams, 2000).

STATISTICAL ANALYSIS

Statistical analysis was performed using STATISTICA software (Version 7.1; Stat Soft Inc.). The differences among the plots (JW, JNW, JW, JNW, AW and ANW) for all recorded data were compared by the one-way analysis of variance (ANOVA) where the conditions of normality and equality of variances are checked followed by LSD test. Otherwise, a Kruskal-Wallis test was achieved. The significant level for all the tests was $P < 0.05$.

RESULTS

This study investigated the response to drought of *Q. suber* seedlings from three Algerian provenances. Results showed some morphological and physiological differences between humid (Jijel), semi-arid (M'Sila) and sub-humid (Azazga) provenances in watered and non-watered conditions.

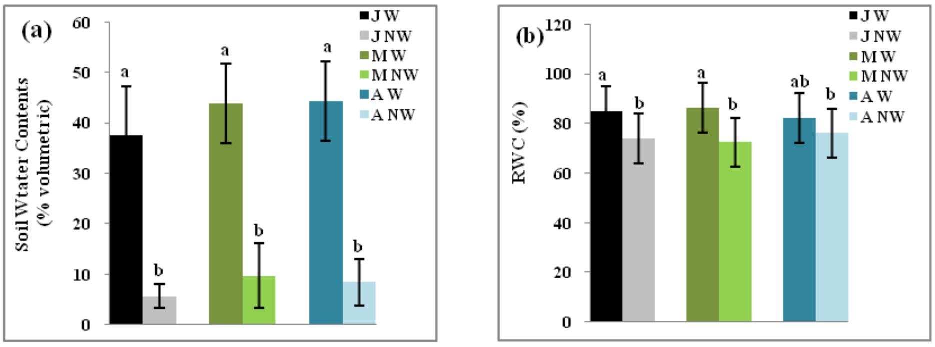

Figure 2.— Water status measurements (a) soil water contents and (b) relative water contents (RWC) in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0.05$). Means ± SE.

The irrigation cessation after ten weeks (mid-September), significantly reduced the SWC of the three provenances (Fig. 2a). In watered conditions, the seedlings of M'Sila provenances showed a better water status with a higher RWC (86.40 %) compare to Jijel (84.96 %) and Azazga (82.08 %) provenances but the reduction of the SWC decreased the RWC of the seedlings of the three provenances (Fig. 2b). The RWC of non-irrigated plants of M'Sila decreased from 86.40 to 72.41 %. Azazga showed the lowest decrease of the RWC (from 82.08 to 76.09 %).

The reduction of SWC had also negative effect on the seedlings growth of Jijel and Azazga provenances by reducing slightly their stem height while the seedlings of M'Sila provenance were not affected; their stem height were similar in watered and non-watered conditions (Fig. 3a).

In watered conditions, the diameter growth of the seedlings was higher for Jijel provenance and lower for Azazga provenance. The lack of irrigation reduced the stem basal diameter of the seedlings of the three provenances. However, the stem basal diameter of non-watered seedlings of Jijel provenance were similar to those of the watered seedlings of M'Sila provenance and the stem basal diameter of non-watered seedlings of this provenance were similar to those of watered seedlings of Azazga provenance (Fig. 3b).

The leaf biomass and the roots/shoots ratios did not significantly differ between the three provenances and drought conditions did not affect this parameter (Fig. 3c, d).

The SLA did not differ significantly between the three provenances in irrigated conditions. The seedlings growing under conditions of water deficiency showed leaves with low SLA in the three provenances. The M'Sila seedlings showed the lowest value of the SLA (Fig. 3e).

Significant differences in Chla+b concentrations were observed among the three provenances. Higher values of Chla+b were observed in the leaves of Jijel provenance seedlings and the lowest values were noted for Azazga seedlings. The reduction of the SWC decreased the total leaves chlorophyll contents only in M'Sila provenance (Fig. 4a). The concentration of chlorophyll a was higher in Jijel and M'Sila seedlings compared to Azazga seedlings but drought conditions reduced chlorophyll a contents in Jijel and M'Sila provenances while for Azazga seedlings this biochemical parameter increased (Fig. 4b). Leaves contents of chlorophyll b were higher in Jijel and M'Sila than in Azazga seedlings. Under drought conditions, the leaves of M'Sila and Jijel seedlings showed an increase in chlorophyll b concentration while Azazga seedlings were not affected (Fig. 4c). Chlorophyll a/ chlorophyll b ratios did not significantly differ between the three provenances in watered and non-watered conditions (Fig. 4d). Carotenoids concentrations in leaves were higher in Jijel and M'Sila than in Azazga watered seedlings and in non-watered seedlings they significantly decreased in M'Sila and Azazga seedlings (Fig. 4e).

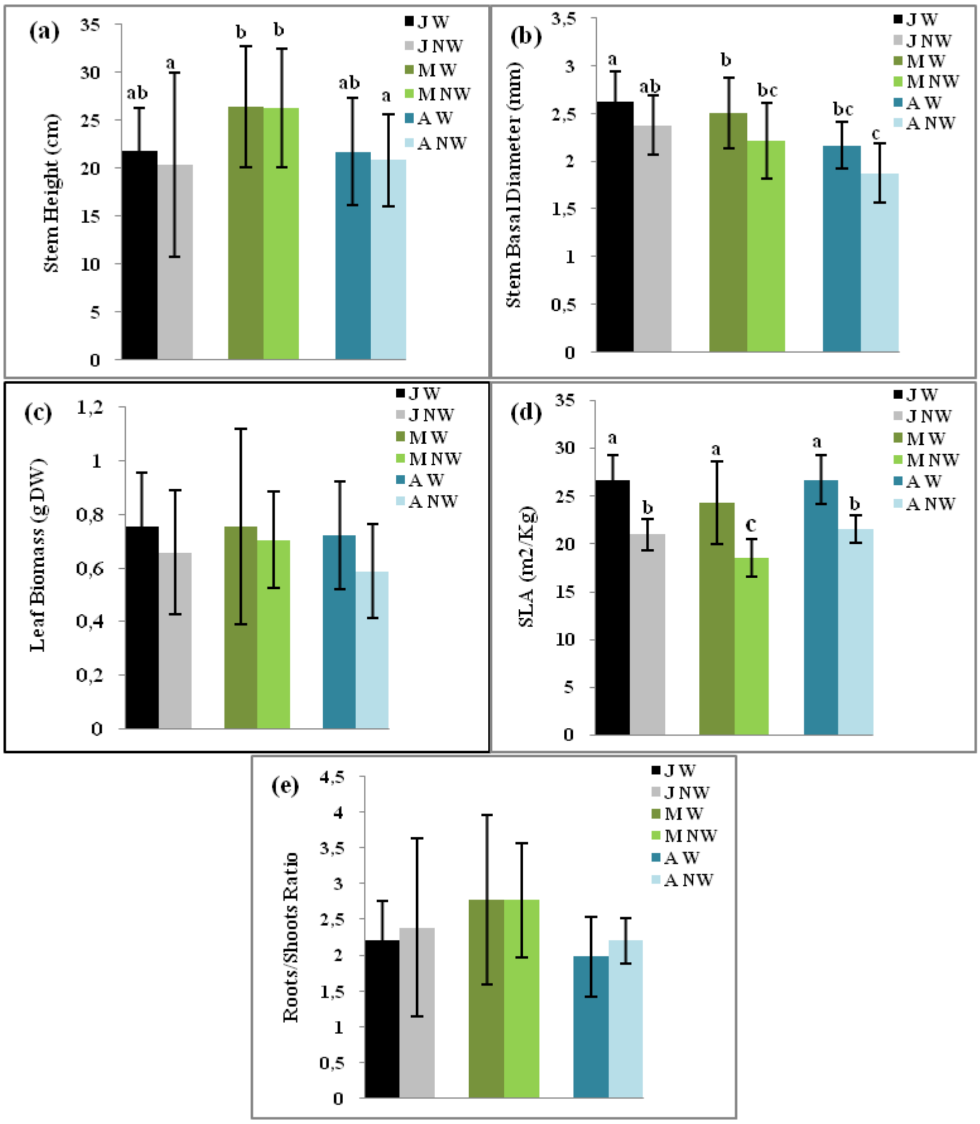

Figure 3.— Morphological parameters measurements (a) shoot height, (b) stem basal diameter, (c) leaf biomass, (e) SLA and (d) roots/shoots ratio in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0,05$). Means ± SE.

Drought conditions induced an accumulation of soluble sugars and proteins only in leaves of M'Sila and Azazga seedlings (Fig. 5a, b); especially in M'Sila seedlings for sugars, this increase was approximately 1.5-fold in non-watered compared to watered conditions (Fig. 5a). The starch content showed an important decrease in non-watered seedlings of the three provenances, the reduction was 2.7, 2.1 and 1.3-fold in Jijel, M'Sila and Azazga provenances, respectively (Fig.

5c).The drought conditions also induced an increase in leaves proline content in the seedlings originating from M'Sila and Azazga provenances (Fig. 5d).

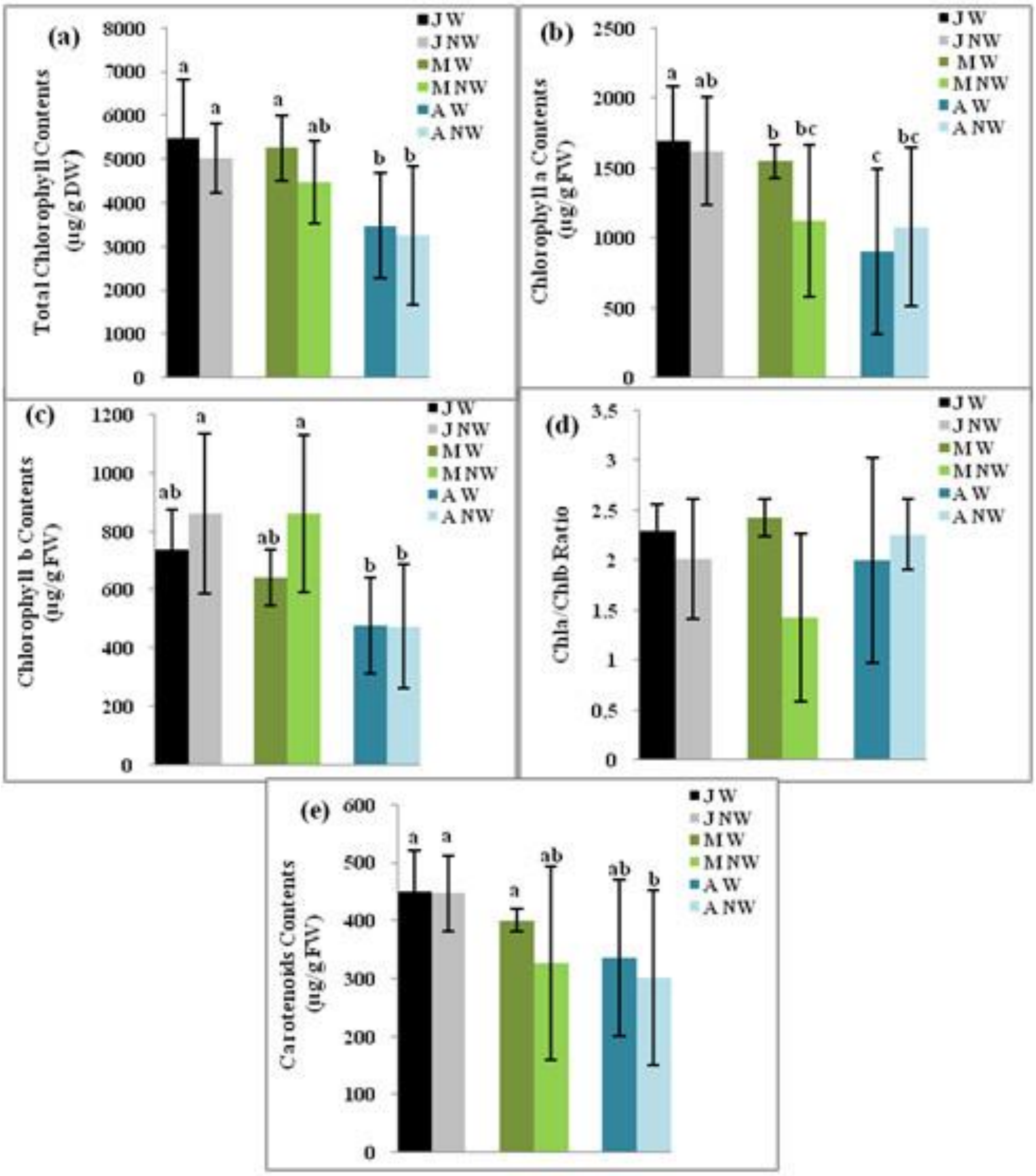

Figure 4.— Pigments leaves contents (a) Total Chlorophyll, (b) Chlorophyll a, (c) Chlorophyll b, (e) Carotenoids in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0,05$). Means ± SE.

The three provenances studied showed differences in leaves $\delta^{13}C$. In watered conditions, the lower value was obtained in Azazga seedlings provenance (-31.72 ‰). The reduction of the SWC enhanced significantly the $\delta^{13}C$ leaves content in Azazga and M'Sila provenances. The higher value of δ^{13}C (30.54 ‰) was recorded in M'Sila non-watered seedlings (Fig. 6a). The δ^{13}C from Jijel seedlings was not affected by drought.

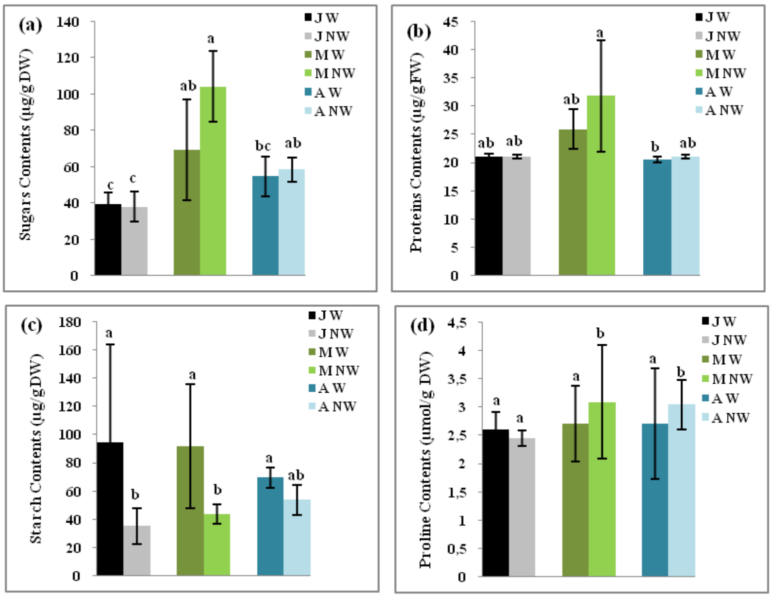

Figure 5.— Biochemical parameters measurements (a) solubles sugars, (b) starch contents, (c) solubles proteins contents and (d) proline contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0,05$). Means ± SE.

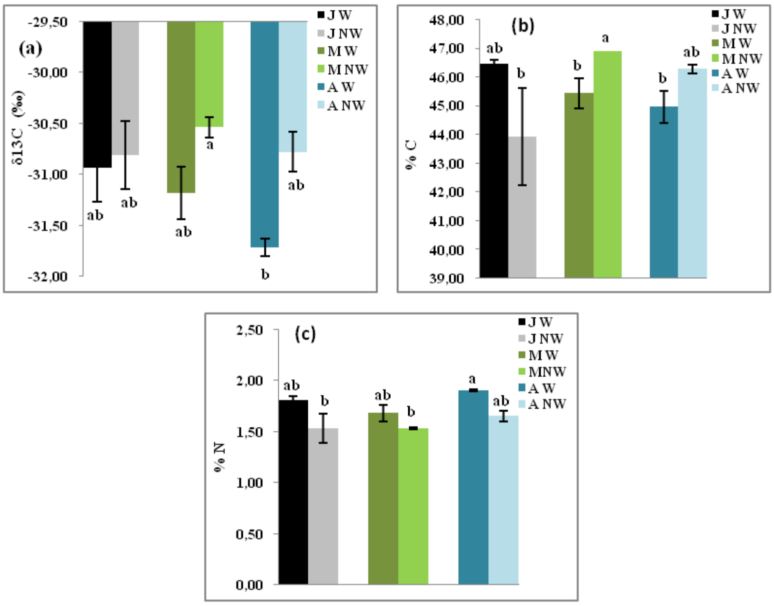

Figure 6.— δ^{13}C, C and N measurements (a) δ^{13}C, (b) %C and (c) %N leaves contents in *Quercus suber* seedlings from humid (J: Jijel), semi-arid (M: M'Sila) and sub-humid (A: Azazga) provenances in watered (W) and non-watered (NW) conditions. ($P < 0,05$). Means ± SE.

In watered seedlings, the carbon rate was higher for Jijel provenance. The drought conditions reduced the level of %C in Jijel and enhanced it in M'Sila and Azazga provenances (Fig. 6b).

The nitrogen rate in watered seedlings did not differ between Jijel and M'Sila provenances and Azazga provenances showed the highest value. The reduction of the SWC induced a decrease of %N in the provenances studied (Fig. 6c).

Mortality rate showed a significant difference among provenances (*P* = 0.01). It was 17.64 %, 29.41 % and 35.29 % for Azazga, Jijel and M'Sila provenances respectively. M'Sila seedlings showed the lower survival rate (Fig. 7).

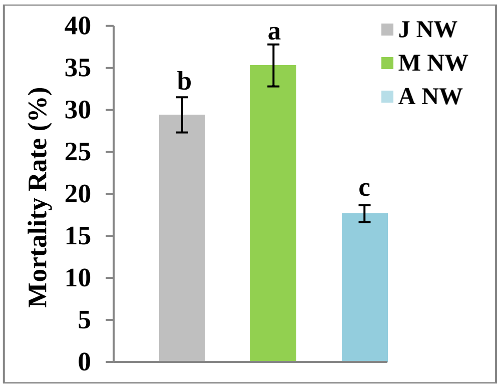

Figure 7.— Survival rates of *Q.suber* Jijel, M'Sila and Azazga seedlings provenances in drought conditions (P < 0.05). Means ± SE.

DISCUSSION

This study showed differences between seedlings of the three provenances: Jijel (humid), M'Sila (semi-arid) and Azazga (sub-humid) elevated homogeneously in greenhouse watered and non-watered conditions.

In watered treatments of all the morphological traits measured, only height, acorn weight and diameter growth exhibited significant population divergence. Previous studies have shown large differences between populations originating from different sites for morphological parameters. Total height was the most discriminating variable between 26 populations originating from six Mediterranean countries (Portugal, Spain, Tunisia, Morocco, Italia and Algeria). In our study, samples of the semi-arid area (M'Sila) exhibited the highest height growth while, in others report, seedlings originating from high temperature sites displayed the lowest growth traits (Gandour *et al.*, 2007). This discrepancy may be explained by the local environmental conditions and/or seeds mass. Among the three provenances investigated, the lightest acorns were from the sub-humid population (Azazga). Seed mass represents the reserves available for growth in the first stages of plant establishment and variation in seed mass is an important trait which may have consequences for growth and survival of seedlings (González-Rodríguez *et al.*, 2011). Our results are in accordance with several authors who reported that differences among seedlings provenances may be related to the climate of the seedling sources (Gandour *et al.*, 2007; Ramirez-Valiente *et al.*, 2010; Ennejah *et al.*, 2013) and to the seed mass (Quero *et al.*, 2007).

Drought conditions also induced various morphological and physiological responses in the three provenances studied. This is in agreement with Aranda *et al.* (2007), who have shown that *Q. suber* is more sensitive to drought in the early stages of development. In particular, the reduction of diameter growth and the SLA recorded from all the seedlings and the reduction of shoot growth are in agreement with previously obtained data (Ksontini *et al.*, 1998; Ramirez-Valiente *et al.*, 2010;

Daoudi *et al.*, 2016). Interruption of shoot growth in the dry summer is a significant adaptation trait (Kurze-Besson *et al.*, 2006). SLA has often been observed to be reduced under drought conditions. In dry conditions, low SLA allows a more conservative water use maintaining photosynthetic activity and carbon gain over a longer period of time (Dudley, 1996).

The significant difference in diameter growth between Jijel, M'Sila and Azazga cork oaks, facing to water deficit were previously shown for Morocco populations; the early stages of oak population's seedlings development are affected differently by changes in soil water reserves and temperatures (Ennejah *et al.*, 2013, 2014).

These intra-specific differences of traits appeared to be linked to interspecific differences in seed size and its confounding effect (Sanchez-Gomez *et al.*, 2007).

Water stress decreased the ratio of shoot biomass / root biomass of *Q. suber* seedlings (Ksontini *et al.*, 1998). The similar leaves biomass and root to shoot ratio traits in well-watered and water stressed plants may be due to a similar degree of osmotic adjustment in root and leaf cells (Sobrado & Turner, 1986). The reduction of starch and the accumulation of soluble sugars, soluble proteins and proline in M'Sila and Azazga seedlings suggest the occurrence of an osmotic adjustment at leaf level which decreases leaf osmotic potential; this mechanism enhances seedlings potential to extract water from the drying soil by increasing the soil-plant water potential gradient (Kurz- Besson *et al.*, 2014). In Jijel provenance, osmotic adjustment can be accomplished by the accumulation of other metabolites. So, the compounds involved in osmotic adjustment differ widely among plant species and perhaps among populations (Patakas *et al.*, 2002).

Leaves pigment contents of the three provenances were not similarly affected by drought. Only M'Sila seedlings showed a decrease in total chlorophylls, chlorophyll a, and carotenoids contents while Azazga seedlings showed a decrease only in carotenoids contents. Jijel seedlings were not affected for total chlorophylls and carotenoids contents. Generally, *Q. suber* water stressed seedlings showed a decrease of total chlorophylls with an increase in chlorophyll b and carotenoids to cope with oxidative stress and a decrease of chlorophyll a to avoid excessive absorption of light energy (Faria *et al.*, 1999). Daoudi *et al.* (2016) did not find any difference in total chlorophyll contents of seedlings originating from Azazga provenance and Vaz *et al.* (2010) reported that summer dry conditions did not affect chlorophyll concentrations of *Q. suber* trees. Contrary to expectation, seedlings of M'Sila provenance which showed the better length growth had the lower chlorophyll contents. A negative correlation between chlorophyll contents and growth was previously recorded by Ramirez-Valiente *et al.*(2010); plants exhibiting lower leaf chlorophyll content had larger annual shoot growth.

Important variations of leaf $\delta^{13}C$ have been observed for many species across their distribution range like *Q. suber* (Gouveia & Freitas, 2009). The decrease of $\delta^{13}C$ leaves content in M'Sila and Azazga seedlings were similar to the results of Gouveia & Fortas (2009) who reported that trees subjected to greater water stress showed an increase of carbon isotope discrimination. This is due to the fact that water supply affects the stomatal conductance and photosynthesis of plants, which changes $_{13}C/_{12}C$ ratios in the synthesized carbohydrates (Du *et al.*, 2015). The reduction of %N in leaves of the three provenances was previously recorded by Daoudi *et al.* (2016) but this was not observed in Tunisian samples by Kwak *et al.* (2011).

Summer drought is the main cause of seedling mortality in Mediterranean-climate areas. The significant difference in mortality rate under dry conditions between these Algerian provenances was not found by Gandour *et al.* (2007) for 26 provenances studied from six countries. However, differences in sapling survival across *Q. suber* provenances were recorded and positively related to the height of planted seedlings, and seedling size was closely related to acorn size, which was bigger in populations from warm and drier locations (Ramírez-Valiente *et al.*, 2009) in contrary to our results where M'Sila showed the better height but the lowest survival rate. Navarro *et al.* (2006) concluded that positive relationships between survival and seedling size were three times more frequent than the cases showing negative relationships. Variation in seed mass is an important trait which may have consequence for growth and survival of seedlings because seed mass represents the reserves available for growth in the first stage of plant establishment (González-Rodríguez *et al.*, 2011). An increase in seedling size can result in higher transpiration, which increases plant vulnerability to drought on the short-term and this is the main argument for using small seedlings in dry sites (Villar-Salvador *et al.*, 2012).

CONCLUSION

In our experiments water stress did not act alone but was associated with high temperatures and high light stresses. Therefore, seedlings response to drought involves adjustment to stresses associated to drought.

This study showed differences in watered and non-watered conditions for morphological and physiological traits of three Algerian provenances (Jijel, M'Sila and Azazga) originating from humid, sub-humid and semi-arid areas at seedling stage. In terms of survival rate and water status, Azazga provenance seems to be the better provenance adapted to summer drought conditions of the Mediterranean area while survival rate of M'Sila provenance which is located in semi-arid climate was more affected by drought. However, the growth of the seedlings was less affected for M'Sila than Azazga provenances. Indeed specimens from the site of M'Sila (semi-arid) when cultivated in water privation conditions, showed morphological and physiological changes that could be related to drought tolerance. They exhibited the lowest SLA, which means a reduction of the leaf evaporative surface. A decrease of chlorophyll a contents was also noticed suggesting a potential reduction of the number of PSII for an efficient regulation of solar radiation absorbed in excess. A substantial enhancement of the water use efficiency (WUE) was obtained by $\delta^{13}C$ measurements. While starch content decreases, soluble sugars and proline increase, probably as potential compatible osmolytes and electron donor in response to drought. The intense metabolic activity related to plant tolerance to drought seems to be related to an increased accumulation and/or synthesis of total soluble proteins.

These results showed probably the existence of an inter-populations variability that may be linked to the specificity of plant-climate-coevolution. Hence, semi-arid sites of *Q. suber* may be considered as potential germplasm banks for reforestation in response to global climate change. However, further studies are needed to deepen the physiological knowledge of *Q. suber* seedlings. The photosynthesis machinery should be explored both in terms of gas exchange and quantum yield as well as field measurements.

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