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# Morphophysiological and anatomical responses of *Quercus suber* L. seedlings under drought stress conditions

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**Morphophysiological and anatomical responses of *Quercus suber* L. seedlings under drought stress conditions**

**Abstract:** This study was performed to investigate the effect of drought stress on behavior of cork oak seedlings (*Quercus suber* L.) from five different eco-geographical zones in Algeria (Tlemcen, Oum Tboul, Jijel, Dradra, Tizi Ouzou). 28-month-old seedlingswere submitted to two water regimes: watered regularly three times a week (controlled seedlings) and drought-induced conditions (stressed seedlings) by withholding watering for 21 days during summer 2022. Drought stress noticeably inhibited the growth traits of *Quercus suber* seedlings which showed significant decrease in shoot height, collar diameter, and relative growth in height and diameter of stems decreased in stressed seedlings in Oum Tboul, Tlemcen and Tizi Ouzou provenances compared to control ones, also a decrease in leaf number, fresh and dry masses, and the relative water content in all provenances, whereas a significant increase in length of the main root, root to shoot ratio, as well as a decrease in the total chlorophyll and carotenoids contents and significant increase in proline content especially in Tizi Ouzou and Tlemcen provenances under drought stress conditions. Moreover, the anatomical study suggested root-anatomical adaptations against drought,the vessel diameters were much larger in the well-watered plants as compared to the water-deficit plants.

**Key words:** cork oak,ecogeographical zones, drought stress, root vessel diameter.

**Morfofiziološki in anatomski odzivi sadik hrasta plutovca (*Quercus suber* L.) v razmerah sušnega stresa**

**Izvleček:** Raziskava je bila izvedena za preučevanje vpliva sušnega stresa na sadike hrasta plutovca (*Quercus suber* L.) iz petih različnih ekološko-geografskih območij v Alžiriji (Tlemcen, Oum Tboul, Jijel, Dradra, Tizi Ouzou). 28-mesečne sadike so bile podvržene dvema vodnima režimoma: redno zalivanje trikrat na teden (nadzorovane sadike) in sušnim razmeram (sadike v stresu), z zadrževanjem zalivanja za 21 dni, poleti 2022. Sušni stres je opazno zaviral rastne lastnosti sadik plutovca, ki so imele znatno krajše poganke, manjši premer koreničnika, manjšo relativno višino in manjši premer debel pri proveniencah Oum Tboul, Tlemcen in Tizi Ouzou rastočih v stresnih razmerah v primerjavi s kontrolnimi. Zmanjšalo se je število listov, kot tudi sveža in suha masa ter relativna vsebnost vode v vseh proveniencah, medtem ko se je znatno povečala dolžina glavne korenine, razmerje med korenino in poganjkom. Zmanjšala se je skupna vsebnost klorofila in karotenoidov, znatno se je povečala vsebnost prolina, zlasti v proveniencah Tizi Ouzou in Tlemcen v razmerahsušnega stresa. Poleg tega so anatomske raziskave ugotovile anatomske prilagoditve korenin proti suši, kjer so bili premeri trahej veliko večji pri dobro zalivanih rastlinah v primerjavi z rastlinami s pomanjkanjem vode.

**Ključne besede:** hrast plutovec, ekogeografske cone, sušni stres, premer koreninskih trahej.

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## 1 INTRODUCTION

The Mediterranean area is considered to be a hotspot for climate change for which the models predict an increase in the temperature and a pronounced decrease in the precipitation, corresponding to an increase in frequency, intensity and drought period, especially during the warm season (Gauqueluin et al, 2018). Consequently, this region will undergo high drought stress conditions and reduced vegetation production. Cork oak (*Quercus suber* L.) is a sclerophyllous evergreen Mediterranean tree of high socioeconomic value in its natural range area (Camilo Alves et al., 2020); it covers approximately 2.1 million ha, distributed in areas with mild climate conditions characterized by acidic, often nutrient-poor soils, where it is usually managed for cork production (Pereira, 2007).

In Algeria, 4/5 of cork oak distribution area is located in the North East, from Tizi Ouzou to the Tunisian border, but in the West, the populations are scattered as small islands (Bouhraoua, 2015). It provides multiple ecological services, such as enhancing biodiversity, promoting carbon sequestration, protecting soils from erosion and supplying economic (cork, wood, timber) and cultural services (Aronson et al, 2009).

In recent years the death rate of cork oak trees in Algeria increased owing to biotic stresses caused by insects' attacks and fungi which are induced by drought stress (Sánchez-Cuesta et al, 2021). Although several reforestation programs have been undertaken, the success rate is very low and unsatisfactory(Bouhraoua, 2015).

The impact of drought on oak growth was addressed in numerous studies concluding that drought severely reduces oak species growth (Oliveira et al, 2016), but cork oak is very resilient and its growth recovers rapidly at the end of drought conditions (Leite et al, 2018), because it responds to the typical Mediterranean summer drought by changes in several traits, such as leaf osmotic adjustments, isohydric behavior, uptake of deep water sources, reduction in root xylem vessel diameter, which make it a drought-avoiding species(Otiento et al, 2006).

Moreover, understanding the survival performance of cork oak plants in the first years of growth is very interesting;and research on mechanisms that allow plants to survive during a prolonged drought period would help to select more drought-tolerant genotypes by identifying morphological and physiological traits associated with drought resistance (Manavalan et al. 2009).

The objective of this study was to investigate the effect of drought stress on cork oak seedlings in nursery conditions, we compared the morphophysiological and anatomical parameters between five cork oak provenances to better understand the behaviour of plants from different origins for their early selection in afforestation programs.

## 2 MATERIALS AND METHODS

### 2.1 PLANT MATERIAL AND DROUGHT EXPERIMENTS

Acorns were collected from five populations originating from different environments of five elite trees (for each of the five cork oak provenances: Tizi Ouzou, Tlemcen, Dradra, Oum Tboul, Jijel) which come from regions with different geographical distribution and bioclimate (Tab. 1). Then, they were kept in a cold room at a temperature equal to 4 °C for one week, and were sown without any prior treatment, in perforated polyethylene bags 20 cm deep and 7 cm diameter filled with a mixture of equal parts of sand, potting and forest soil having the following characteristics: pH = 5.9, electrical conductivity CE = 0.5 ds. $cm^{-1}$, total carbon: 0.8 %, total nitrogen: 1.6 %, organic matter: 1.37 %, limestone: 0.6 %, the water retention capacity of the soil is 36.64 %.

The pot experiment was conducted in thethe nursery of the National Institute of Forestry Research (Algeria). Seedlings were irrigated daily and maintained at 75 % field capacity of soil under semi controlled conditions in a subhumid bioclimate with warm winter (2°56'59" to 2°59' 08") East longitude, (36°47' 52"at 36°48'30") north latitude, the average annual rainfall is around 475 mm with minimum monthly average temperatures of 7.2 °C and maximum of 34.8 °C.

Twenty eight months old seedlings were planted into plastic pots (642 g), then they were exposed to drought stress by withholding watering for twenty one days during summer 2022 (18 july 2022 to 07 August 2022). Control seedlings were irrigatedregularly three times a week with tap water, seedlings were distributed according to an experimental design in ten completely random blocks with two hierarchical factors / provenance - treatment; they were maintained in homogeneous nursery conditions (34 ± 2°C during the day and 23 ± 2 °C during the night) at 16 h/8h (light).

Growth parameters were harvested for all the control and stressed provenances 10 replicates each, which gives a total of 50 control plants and 50 stressed plants. The plants from each origin have been numbered in order to avoid any errors during measurements.

### 2.2 HEIGHT AND BASAL STEM DIAMETER

Shoot height was recorded along the stem from the

**Table 1:**Ecological and geographical parameters of the five *Quercus suber* populations.

| Provenance | Source | Bioclimate | Average Annual precipitation (mm) | Average Annual temperature (°C) | Latitude and longitude |
|---|---|---|---|---|---|
| Tizi Ouzou (TO) | Yakouren (Sacchardi forest) | Humid and perhumid | 720.1 | 18.5 | N 36° 43'44", E 4° 27' 20" |
| Tlemcen (T) | Ifriforest (Ain Fezza) | Lower semi-arid | 345.2 | 15.5 | N 34° 52' 38", E 1° 14' 7" |
| Jijel (J) | El Aouana forest (Kissir, Aghzar) | Humid | 900 à 1000 | 18 | N 36°47'27.18" E 5°40'0.63" |
| Oum Tboul (OT) | El Kala | Hot Subhumid | 699 | 18.6 | N 36° 52' 49,2" E 8° 34' 08, 8" |
| Dradra (D) | El Kala | Hot Subhumid | 699 | 18.6 | N 36° 53' 44,1" E 8° 37' 06,8" |

soil surface to the tallest living budusing meter rule.Collar diameter was measured at 1cm above the root collar using a branded digital caliper (Vernier Caliper 150× 0.02 mm).

### 2.3 RELATIVE GROWTH RATE (RGR) IN HEIGHT AND COLLAR DIAMETER

RGR in height and diameter were determined by applying the general formula Relative Growth

*Rate (RGR)* = $(M_2 - M_1 / (t_2 - t_1)$ (Beadle (1993).

where $M_1$ is the measurement (height or diameter) at the start time ($t_1$) and $M_2$ is the measurement at the end time ($t_2$).

### 2.4 DETERMINATION OF PLANT BIOMASS

Shoot and root system were separated and the growth parameters were measured. The number of leaves per plant was monitored and recorded once a week. The root system was carefully cleared of the substrate to keep as much root mass as possible. Fresh masses of the aerial and root parts were determined using electronic weighing balance. Dry masses of root, shoot were measured at the final harvest, they were dried in an oven set at 80 °C for 48 hand weighed. In addition, root to shoot dry ratio were determined.

### 2.5 RELATIVE WATER CONTENT (RWC)

RWC of control and stressed plants was measured at the end of the drought period using five plants from each provenance sampled randomly per treatment on mature leaves most exposed to the sun. The fresh mass (FM) was determined immediately after harvest, and then the leaves were left in distilled water for 24 h at 4 °C, to determine the turgor mass (TM). The dry mass (DM) was obtained after drying at 80 °C for 72 h. RWC is calculated by the following formula:

$$RWC\ (\%) = [(FM - DM) / (TM - DM)] * 100.$$

### 2.6 BIOCHEMICAL PARAMETERS

The chlorophyll pigment contents were determined on five seedlings per provenance/treatmentusing 0.1g of leaves the most exposed to the sun collected from five plants chosen randomly per treatment. Optical density was measured at 663 nm for chlorophyll (a) and 645 nm for chlorophyll (b) and 470 nm for carotenoids.

Pigment contents were expressed in mg. $g^{-1}$ FM and calculated according to the formula of Lichtenthaler (1983).Proline analysis was performed on 0.1g of leaf samples according to Rasio et al., (1987). Absorbance values of samples were recorded read as 515 nm wavelength at UV spectrophotometer. A standard curve was received to determine the content of proline (μmol $g^{-1}$ FM) by using pure proline.

### 2.7 ROOT ANATOMICAL STUDY

The experiment was performed on a total of 60 seedlings. The number of analysed root segments was 70. For each plant, three secondary roots were used for the xylem-diameter measurements. Four-centimeter long root segments were cut from each root.

Each segmentwas stored in FAA fixative (acetic acid: ethanol: formalin = 1:1:8) for 24 h.Then they were dehydrated by passage through alcohol series baths of increasing degrees (70 °, 95 °, 100 °) and were immersed in 100 % toluene to eliminate traces of ethanol. Then root segments were placed in a mold containing molten paraffin (+1 or 2 °C). thin cross sections (20 μm) were made using a rotary microtome (Leica RM).The sections were deparaffinized in three successive baths of pure toluene for 20 mn each, on the hot plate set at 60 °C. then sections are rehydrated in three baths of absolute alcohol then in 100 ° ethanol + formalin (4 V/ 1 V). The sections were then rinsed under running water. The slides obtained were stained using a double stain, periodic acid shiff (APS) and naphthol blue black. After staining, the sections were immediately dehydrated on a hot plate at 40 °C, then mounted in Canada balsam.

For each cross section, the diameter of the five largest vessels in the metaxylem area were measured. Photographs were taken at x 400 magnification using optical microscope with camera (SMC LAB/QUIMICA).The average of the minimum and maximum diameters (μm) of each of the five vessels from each root segment was calculated to characterize vessel size and image analysis was performed with CANVA online. At least 30 metaxylem vessels were measured per provenance ( for control and stressed plants ).

### 2.8 STATISTICAL ANALYSIS

The data was analyzed with two-way ANOVA; and the means were compared using the multivariate Newman-Keuls test ($p$< 0.05). Drought stress and provenance were used as first and second factors, respectively. The data were expressed on average ± standard deviation of the observations. Significant differences and interaction between factors were calculated at 5 %. The two-way ANOVA was enabling on the data of all the parameters evaluated to compare the effects of drought stress on behavior of all cork oak provenances studied.

## 3 RESULTS

### 3.1 SHOOT HEIGHT DYNAMICS

The fivecork oak provenances had parallel changes in shoot height (Fig. 1). Drought stress did not significantly slow down shoot height growth in all plants.

After seven days, shoot heights of control T,D plants overlapped, butafterwards they differed andreached 57.8 ± 0.015cm and 53.6 ± 0.012cm respectively. The provenances TO, OT and J achievedlower heights compared to T, D provenances;they were 51.3 ± 0.010 cm,47.7±0.013 cm and42.3 ± 0.011 cm, respectively.

Under drought stress conditions, means heights of T and D plants were higher than those of the provenances TO, OT, J to achieve 48.7 ± 0.012 cm and 45.5 ± 0.011 cm, respectively. The plant heights in J and OT provenances started to overlap at the 3rd day of drought stress, and this overlapping continued until the last day of the experiment when theyreached average heights of 40 ± 0.010 cm and 39.5 ± 0.012 cm, respectively,in OT and J provenances. The provenance TO reachedthe lowest average height; i.e. 37.5 ± 0.011 cm(Fig. 1).

### 3.2 COLLAR DIAMETER

In well-watered conditions, the provenance T had the largest collar diameter (6.43 ± 0.011 mm); it was followed by TO, J, OT andD provenances, which reached 6.25 ± 0.012 mm, 6.24 ± 0.010mm, 6.21 ± 0.013 mm, 5.94 ± 0.011 mm, respectively (Fig. 2).Under drought stress, there was no obvious difference in collar diameter among the D, TO and OT plants; it reduced by 6 %, 11 %, 13 %,

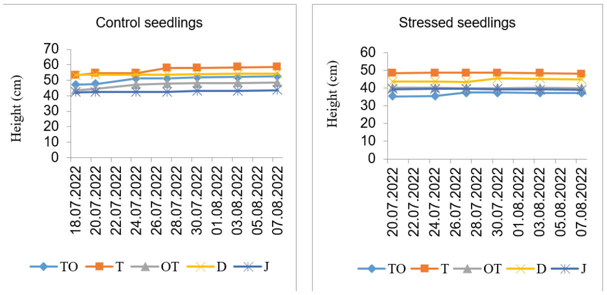


**Figure 1:** Height growth of control and stressed cork oak plants during the experiment. (* significative difference between control and stressed seedlings at 5 %).

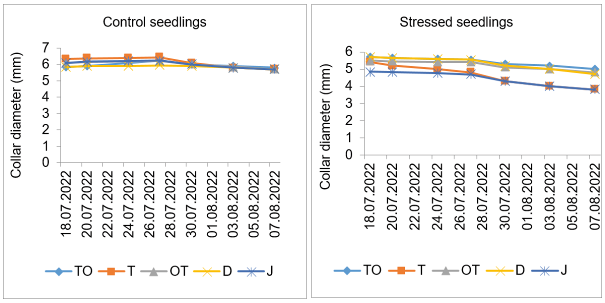


**Figure 2:** Diameter growth of control and stressed cork oak plants during the experiment.(* significative difference between control and stressed seedlings at 5 %).

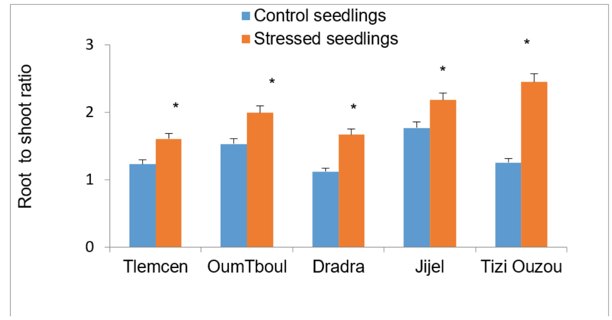


**Figure 4:** Effect of drought stress on root to shoot ratio of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

respectively, whereas; it reduced by 24 %,25 % in J and T plants, respectively.

## 3.3 ROOT LENGTH

As shown in Figure 3, the highest main rootwas recorded in control plants from J provenance (75 ± 0.16 cm). However, average lengths in T, OT, D andTO control plants were 68 ± 0.22 cm, 70 ± 0.65 cm, 60 ± 0.45 cm and 62 ± 0.38 cm,respectively.

In the five provenances, seedlings under water stress had higher root length values than those that were well watered.Hence, a significant increase in root length was noted in all the provenances studied; it was increased by 12 % for T, OT,Jplants,respectively,and 19 %;30 % inD and TO plants respectively (Fig. 3).

## 3.4 ROOT TO SHOOT RATIO

In control plants, root to shoot ratio was lower than that of stressed plants. It was 1.23 ± 0.01,1.53 ± 0.012,1.11 ± 0.010, 1.77± 0.012 and1.25 ± 0.013,in T, OT, D, J,TO provenances,respectively. In dry conditions, root to shoot ratio was observed to increase significantly by 23 %, 24 %, 33 %, 19 % and 49 % in T, OT, D, J, TO provenances,respectively (Fig.4).

## 3.5 LEAF NUMBER

In control cork oak plants, the average leaf number was 50, 40, 48, 45 and 42, respectivelyin T, OT, D, J and TO provenances.Drought stress caused a decrease in leaf number, compared to the control seedlings, provenances OT, J, TOsignificantly reduced by 30 %, 24 %, 23 % respectively.

## 3.6 RELATIVE GROWTH RATE (RGR) IN HEIGHT AND DIAMETER

The variation in RGR in height for all control to stressed plants ranged from 4.5 to 0.56 mm, 4.25 to

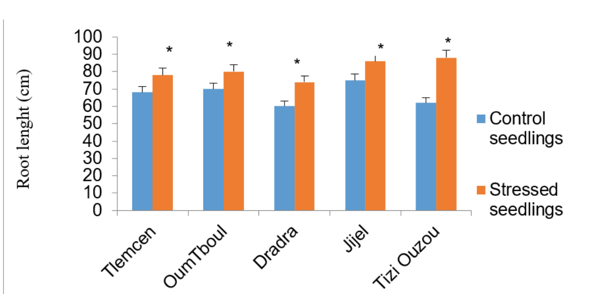


**Figure 3:**Effect of drought stress on the length of the main root of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

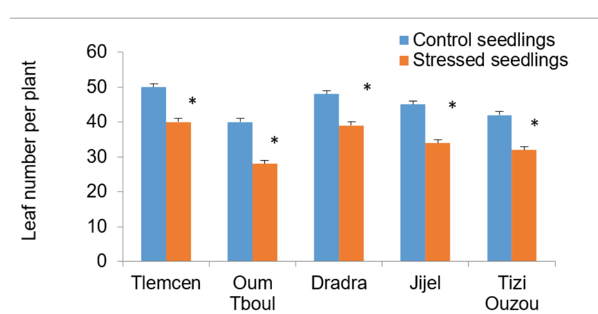


**Figure5:** Effect of drought stress on leaf number of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

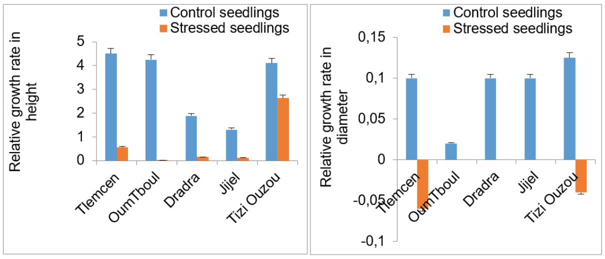


**Figure 6:** Effect of drought stress on relative growth rate in height and diameter of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

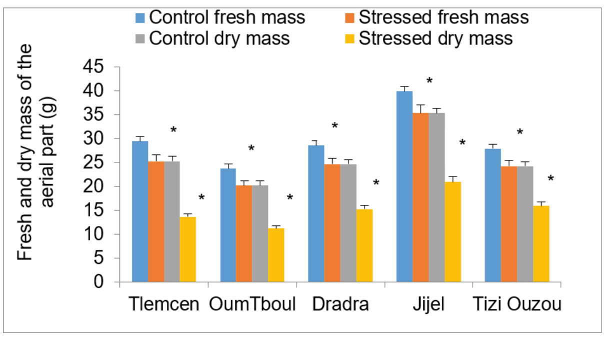


**Figure 7:** Effect of drought stress on fresh and dry mass of the aerial parts of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

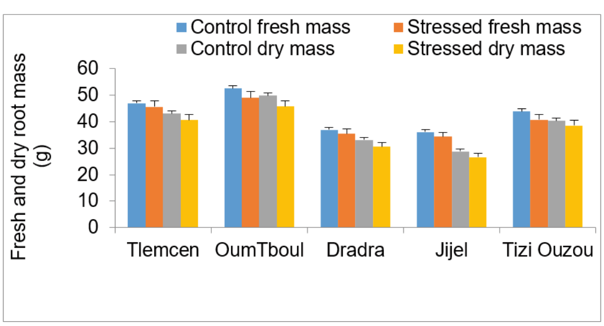


**Figure 8:** Effect of drought stress on the fresh and dry roots masses of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

0.02 mm, 1.88 to 0.15 mm, 1.3 to 0.12 mm and 4.1 to 2.63 mm in T, OT, D, J and TO, respectively.

Moreover, RGR in diameter was also slowed down under drought stress conditions; growth was absent in OT, D and J provenances. The same observations were recorded for T and TO provenances, we noted a decrease in RGR, with negative values of -0.06mm and-0.04mm,respectively, for T and TO provenances, which reflected a reduction in diametric growth under drought conditions (Fig.6).

## 3.7 FRESH AND DRY MASS OF AERIAL PARTS

The average fresh mass of the aerial parts in control plants were: 29.4 ± 0.01 g; 23.7 ± 0.01 g; 28.6 ± 0.03 g; 39.9 ± 0.02 g; 27.9± 0.01 g respectively in T, OT, D, J and-TO provenances. The J provenance had the highest mean fresh mass.

The biomass of aerial part was affected by drought stress, the average fresh mass of the aerial parts in the five provenances declined by 14 %; 15 %, 13 %; 12 %; 13 % respectively in T, OT, D, J andTO plants. Otherwise, dry mass decreased significantly by 46 %, 44 %, 38 %, 40 %, 34 %in T, OT, D, J, TO respectively compared to that of the control seedlings (Fig.7).

## 3.8 FRESH AND DRY ROOT MASS

The fresh root mass in control plants varied among provenances. In fact, the OT provenance maintained the highest fresh mass (52.5 ± 0.12 g), it was followed by T and TO provenances with respective average fresh masses of 46.9 ± 0.11 g and 43.8 ± 0.10 g,and finally, the lowest fresh masses of 36.92 ± 0.13g and 36.1 ± 0.12g,respectively, were detected in provenances D and J.

The effect of drought stress resulted in a reduction in fresh roots mass for all provenances. Indeed, results indicated that reduction in fresh root mass wasnot significant for all origins. Average fresh masses of 45.5±0.11 g, 48.9±0.13 g,35.4±0.12 g, 34.28±0.10 g and 40.69± 0.11 g were noted in T, OT, D, J and TO provenances, respectively. Likewise, the dry roots mass of the control plants OT provenance showed the highest root dry mass; it was followed by T, TO, D and J provenances. Moreover, in stressed plants, no significant reduction was noted in root dry mass for all provenances. Average dry masses were: 45.6 ± 0.13 g,40.6 ± 0.13 g,30.5 ± 0.10 g, 26.6 ± 0.11 g and 38.5 ± 0.12 g, respectively, for OT,T,D,J and TO provenances (Fig. 8).

## 3.9 DRY MATTER DISTRIBUTION INDEX (IR)

The index ratio of aerial dry matter to root dry matter (IR) enabled us to highlight the relationship between the aerial and the root parts. As shown in Figure 9 this ratio was significantly higher in stressed plants compared to control plants. Indeed, in stressed plants, the OT provenance recorded the highest ratio (4.04 ± 0.01); it was fol-

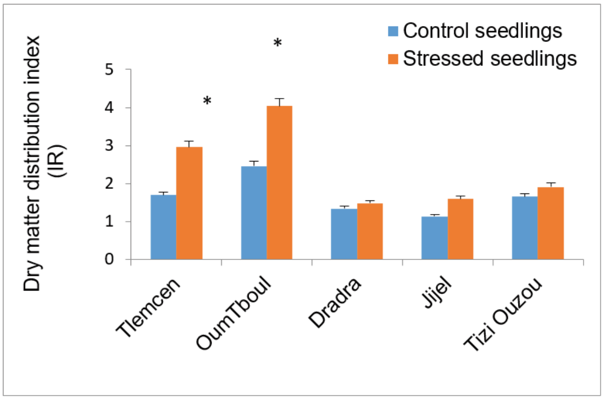


**Figure 9:** Effect of drought stress on dry matter distribution index of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

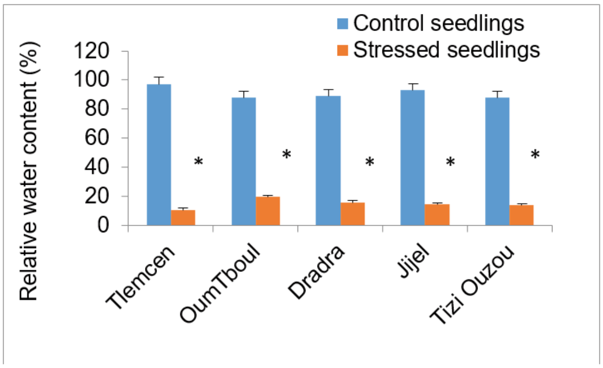


**Figure 10:** Effect of drought stress on the relative water content of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

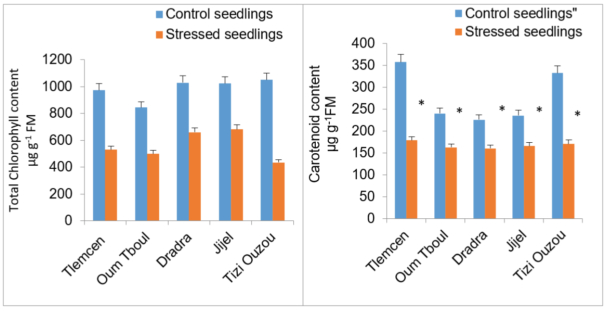


**Figure 11:** Effect of drought stress on total chlorophyll and carotenoids content in leaves of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

lowed by T, TO, J and D provenances with respective average ratios of 2.69± 0.01,1.65±0.03,1.34 ± 0.02 and1.13 ± 0.01(Fig. 9).

## 3.10 RELATIVE WATER CONTENT (RWC)

For the five provenances, seedlings under water stress had lower RWC values than those that were controls. RWC reduced drastically by 89 %, 78 %, 82 %, 85 % and84 %,in T, OT, D, J and TO provenances respectively, (Fig. 10).

## 3.11 TOTAL CHLOROPHYLL AND CAROTENOID CONTENTS

The difference in chlorophyll content was significant between the control and stressed plants. In terms of the decline relative to the controls one, that of the J, D, OT, T decreased significantly by 33 %. 36 %. 40 % and 45 % and that of the TO decreased more (59 %) under drought stress.

Similarly, the carotenoid content of cork oak plants from the five provenances tended to decrease in general. Numerically, D, J and OT provenances showed lower reductions in carotenoid contents, which were 29 %,29.5 % and 32 %, respectively, compared to TO and T provenances, which noted respectively significant reductions of 48 % and 50 % (Fig. 11).

## 3.12 PROLINE CONTENT

Under drought stress, the proline content increased slightly in stressed plants(40 %, 28 %, 23 %, 22 %, 24 % )in T, OT, D, J, TO respectively compared to that of the control plants. (Fig. 12).

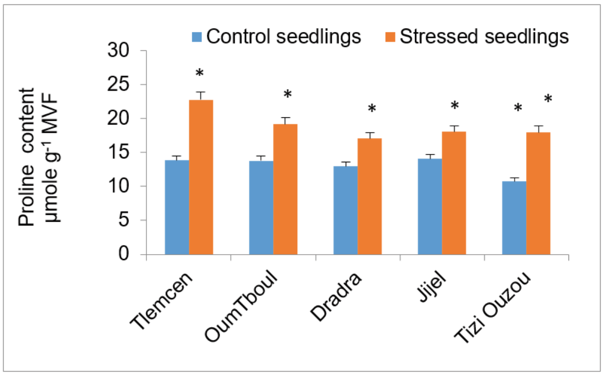


**Figure 12:** Effect of drought stress on proline content in the leaves of cork oak plants. (* significative difference between control and stressed seedlings at 5 %).

3.13 ROOT ANATOMY

The average vessel diameter for all control plants was slightly larger (98 µm ±2.3 µm; 90± 1.8 µm, 88± 3.4 µm) in T, OT,D, respectively.whereas TO, J dispalyed the narrowest vessels (82 ± 1.8 µm, 80 ± 2.5 µm respectively).

The water deficit induced decrease inroot xylem vessel diameter. Significant differences were recorded in lateral roots of all stressed seedlings. For the provenances TO, J, the vessel diameter were shifted towards lower diameter when compared to the other three ones. We registred 40± 1.5 µm and 42 ± 2.3 µmrespectively for TO and J. Whereas they decreased to 58± 1.7 µm, 53± 2.1 µm, 50± 1.9 µmrespectively in T, D, OT provenances. (Fig. 13)

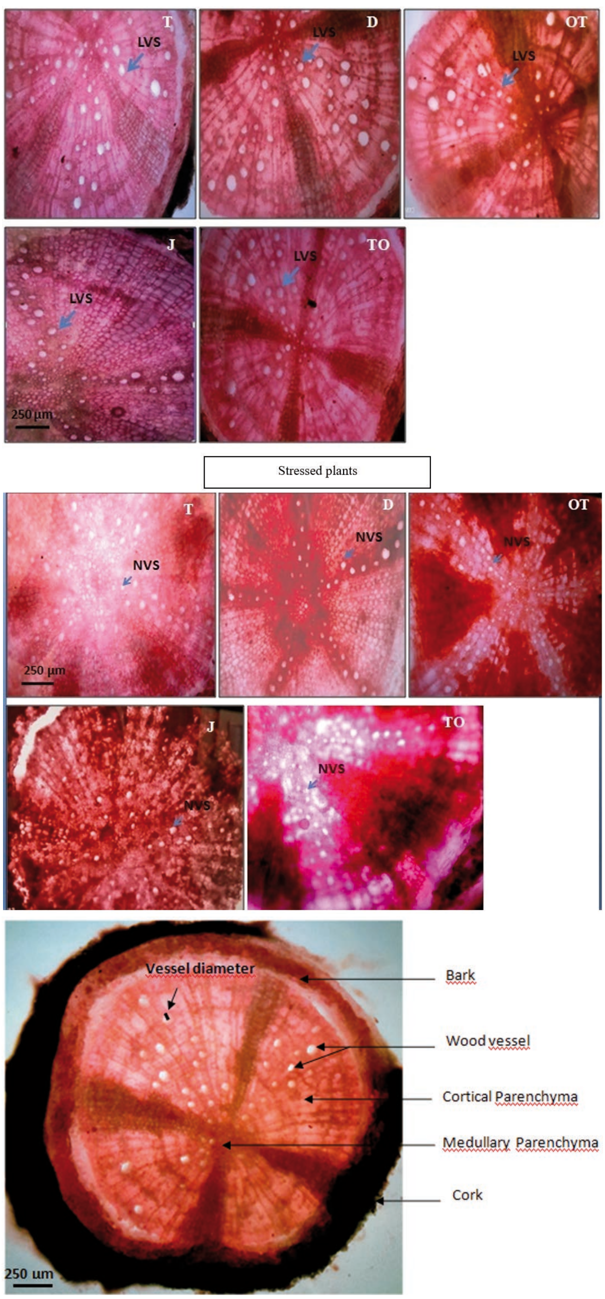


**Figure 13:** Photomicrographs of root cross sections showing vessel diameter in plants grown under control and drought conditions observed under an optic microscope (400x.).

## 4 DISCUSSION

Cork oak plants from five provenances did not have the same response to dehydration by stopping watering.

Results of this study showed no significant increase in shoot height for T and D provenances, but significant decrease in collar diameter in J and T provenances was registred, also significant increase in root to shoot ratio in TO and J provenances. This is in agreement with Aranda et al. (2007), who showed that *Q. suber* is more sensitive to drought in the early stages of development.

In particular, the reduction of growth diameter of seedlings and the reduction of shoot growth are in agreement with previously obtained data (Kurze-Besson et al., 2006. Daoudi et al., 2016; Ennejah et al., 2014).The collar diameter is an important morphological trait that can best predict the performance of plants after planting. Plants with a large crown diameter generally have well-developed lateral roots. This parameter can explain up to 97 % of the observed variation in total plant mass (Lamhamedi et al., 2007).

Also the increase in root to shoot ratio is known as an avoidance mechanism of plants, for providing maximum water absorption under drought conditions (Chaves et al., 2003). It was also noted in *Fagus sylvatica* L seedlings that root to shoot ratio increased with increasing drought stress (Zang et al., 2014).

According to Aziadekey et al.(2014), this response to water drought is complex and depends on the stage of plant growth, severity, and stress duration. Growth of the aboveground part of young plants varied depending on the provenance.

Our results supported those of Elfeel and Al-Namo (2011), who reported that drought significantly reduced growth traits in *Acacia tortilis* (Forssk.) Haynesub sp. *raddiana*(Savi) Brenan, *Salvadora persica* L. and *Leptadenia pyrotechnica*(Forssk.) Decne.;they showed that drought stress enhanced adaptive traits of seedlings resulting in better survival. Hence, drought treatment significantly reduced growth traits in the three species and increased root to shoot ratio and survival.In addition, results of Xiao et al.(2008) on *Populus cathayana*Rehder demonstratedthe adaptive responses of seedlings subjected to water stress which depend on drought intensity and poplar genotype (population).

Otherwise, significant increase in the length of main root in all stressed cork oak plants was recorded. In holm oak, the best survival rate is due to deep roots because the difference of length root system associated with water availability can be implicated on seedlings survive in field conditions (Mancilla-Leytón et al., 2016). Sustained growth of the root system is thought to be a factor in tolerance to water stress (Kang et al., 2022).Under mild drought, plants can improve their tolerance to drought by increasing the length of the main roots and the number of lateral roots and root hairs (Salazar-Henao, Vélez-Bermúdez & Schmidt, 2016).

A deep root system is the favourite characteristic of plant, because it allows to a better use of water reserves that are often found deep down (Babé et al., 2012). *Q. suber* is particularly more sensitive to drought in the early stages of development because of the absence of cork (Pereira et al., 2009).

The decrease in growth can be regarded as a morphological adaptation of plants to drought stress via lowering water consumption and reducing transpiration. Both oak species (*Q. robur*L. and *Q. cerris*L.) showed drought avoidance mechanisms (increasing the root to shoot ratio or decreasing growth) ( Deligoz et Bayar, 2018).

In cork oak species the drought-avoiding strategy should help it to alleviate the negative impacts of severe water shortages on radial growth and reduce the risk of hydraulic failure through controlling stomatal conductance, tissue hydration, xylem embolism and root access to deep water ( Vaz et al., 2010).Our results noted significant reduction in leaf number for all stressed plants;this parameter is a good indicator of water and mineral nutrition, and the low rate of leaf regeneration performs the nutriments use efficiency with avoiding a supplementary carbon and water demand (Casper et al., 2001).The water stress reduced the growth in height and leaf production and increased leaf loss, which is a typical response to drought (Tenopala et al., 2012). Also, Misson et al. (2011) demontrated that the production of new leaves may be reduced increasing leaf retention and longevity under water loss. Nonetheless, drought conditions can increase leaf fall because of xylem cavitation ( Misson et al., 2011), by accumulating abscisic acid and ethylene hormones inducing senescence and abscission ( Zhang et al., 2014),Indeed, the estimation of leaves number was a good indicator of the plant assimilative capacities and its biomass production (Pena-Rojas et al., 2005).

Moreover, there was a significant reduction in fresh and dry masses of the above-ground in all cork oak provenances. Previous studies showed intra-specific variability between different origins of Tunisian cork oak in response to summer drought (Ben Fradj, 2016), intraspecific variability is an adaptation way to different climatic conditions(Zine El Abidine et al., 2016a).

For all provenances, seedlings under water stress had lower RGR values than those that were well watered.RGR is a prominent indicator of plant strategy with respect to productivity as related to environmental stress and disturbance regimes.It is the amount of growth per day and plants under water stress which tend to arrest their growth rate for metabolic efficiency and economy .In addition, Abdelbasit et al. (2012) reported that water stress causes significant variation on seedlings relative growth rate (stem length, leaf number, root and total plant biomass) in tree provenances of *Acacia tortilis*of Sudan.Previous studies have shown significant difference in growth and morphological traits of cork oak population (Gandour et al., 2007) as well as physiological traits mainly related to drought tolerance (Ramírez-Valiente et al., 2014c).

Significant differences were registred between RWC of control and those of stressed plants for all provenances. RWC is generally used to estimate the plant water status under drought stress, the evolution of this parameter accounts not only for variations in the water content into the tissues, but also for changes in their ability to incorporate water in drought conditions. RWC is an indicator of the ability to maintain a given state of hydration or to withstand some tissue dehydration (Arndt et al., 2015).

Leaves pigment contents of the five provenances were not similarly affected by drought. Hence, T, TO, OT seedlings showed a significant decrease in total chlorophylls and carotenoids contents.It has been shown that drought stress caused chlorophyll decomposition and chlorophyll content decreases, leading to changes in photosynthetic function (Jafari, Hashemi Garmdareh & Azadegan, 2019).This reduction is due to the destruction of cellular thylakoid membranes: a loss of cellular compartmentalization can inhibit the development of major metabolic functions (Cornic & Ghashghaie, 1991). Chloroplasts are the first organelles to be degraded (loss of chloroplast membrane, chloroplast distortion) with chlorophyll degradation (Jafari, Hashemi Garmdareh & Azadegan, 2019).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.

Additionally, Ghouil et al. (2003) demonstrated the tolerance of cork oakto high temperatures.We hypothesis that leaf pigment contents at cork oak juvenile stage of seedlings is more sensitive to drought stress than adult cork oak trees.Other studies reported that water stress reduce the tissue concentrations of chlorophylls and carotenoids (Xiao et al., 2008, Tang et al., 2021), primarily with the production of reactive oxygen species in the thylakoids (Chakhchar et al., 2017)

Moreover, an increase in proline content was recorded in all cork oak stressed plants. Stimulating the synthesis of organic solutes is an adaptation mechanism to drought stress developed by several species of the genus *Quercus* in order to preserve the structural integrity of their membranes and maintain their cellular turgor (Deligoz et al., 2016). However, our results showed in both T and OT provenances a higher accumulation of proline indicating dehydration state requiring higher osmoregulation (Deligoz & Gur, 2015). It can protect cell membranes and increase tolerance to water loss (Shvaleva et al., 2005). Studies undertaken on the genus *Quercus* showed that water stress increased proline content in three Mediterranean oak species (*Q. ilex* L., *Q. pubescens* Willd. and *Q. cerris*) (Cotrozzi et al., 2016).Similar results were also found in *Quercus vulcanica* Boiss.& Heldr. Ex Kotschy and *Quercus aucheri* Jaub. & Spach(Ozden 2009) seedlings.

Roots have evolutionarily become the first organ that senses the changes in soil moisture and adapts to them at morphological, anatomical scales (Amtmann et al., 2022).Superficial roots of *Q. suber* have been shown to have different behaviour during the dry summer, i.e. mainly responding to soil water (shallow connected roots) or groundwater (deep connected roots), depending on their relative positioning to sinkers (Nadezhdina et al., 2008).

Our results showed that among the anatomical features, there were decreases in vessel diameter of the root. As compared to control plants, a narrowing xylem vessel diameter observed in roots of cork oak seedlings grown under drought stress for the five provenancesstudied.

Vessel xylem diameter is affecetd by environmental conditions and genetic control(Uga et al., 2008). Also, the response of xylem hydraulic transport to drought depends on xylem anatomy, and xylem anatomical plasticity during water deficit also varies among different organs and different drought intensities (Köcher etal., 2012). The xylem anatomical structure of oak saplings is influenced during its development by external factors such as soil drought and air warming (Fonti et al., 2013 b).

Most drought-tolerant crops opt for numerous small xylem vessels (Strock et al., 2020) which can have big consequences for plant survival under drought (Levionnois et al., 2020). But, the diameter, and number of xylem vessels changed according to the drought stress intensity (Konijnendijk & Randrup, 2002). Other studies found that drought stress reduced the average root diameter and the root vessel diameter (Wang et al., 2018). Martinez-Vilalta et al.(2002) showed that water deficit reduced diameter of conductive elements, increased hydraulic resistance and consequently facilitated the sap circulationof nine Mediterranean woody species: *Quercus ilex*; *Acer monspessulanum* L.; *Arbutus unedo* L.; *Sorbus torminalis* (L.) Crantz; *Cistus laurifolius* L.; *Cistus albidus* L.; *Ilex aquifolium* L.; *Phillyrea latifolia* L.; *Juniperus oxycedrus* L..

Similar anatomical responses were also found in the roots of *Astragalus gombiformis* subjected to water stress (Boughalleb et al, 2014). Species living in environments where water is available might only episodically have larger xylem vessels and larger diameter roots to maximize water uptake when it is available. However, large vessels may also be more prone to cavitation and embolism during water stress. The lower sensitivity of vessel diameter to hydrological alterations has already been reported by Oladi et al. (2014) in oriental beech (*Fagus orientalis* Lipsky) and by Schume et al. (2004) in hybrid poplar (*Populus deltoides* W.Bartram ex Marshall*x Populus nigraL.)*.Whereas,in *Olea europaea*L.large xylem vessels increased root conductivity during drought stress, allowing deep rooting and extended water acquisition (Torres et al, 2013).

Several authors successfully revealed a clear signal in vessel traits of ring-porous species mainly linked to the water availability (Campelo et al., 2010; Gea Izquierdo et al., 2012). The xylem anatomy is highly heterogeneous at the interspecific level but also within a species or even a single tree (Schuldt et al., 2013).The formation of smaller vessel elements suggests that *Eucalyptus grandis*W.Hill exhibit a strategic response to improve xylem hydraulic safety and to enable survival under drought periods (Keret et al., 2023).

The structure and properties of the root cortical tissue, the diameter, and the number of xylem vessels changed according to the degree of drought stress (Konijnendijk & Randrup, 2002).Some studies have also found that drought stress reduced the average root diameter and the diameter of the root vessel (Wang et al., 2018; Strock et al., 2020); which can have big consequences for plant survival under drought ( Levionnois et al., 2020).

Also it has been demonstrated in several oak species, such as *Q.cerris* L., *Q.pubescens* Willd. (Rita et al., 2016), *Q.ilex* L.(Zalloni et al., 2019) that adaptive responses to the loss of hydraulic conductivity result from the adjustments in the vessel anatomy of diffuse-porous wood that is required to adapt to new environmental requirements (von Arx et al., 2013).

Thangthong et al. (2019) suggested that reduction in root vessel sectional area of peanut species in response to water-deficit stress was due to a diminished plant growth, and this was the main factor affecting hydraulic conductance.

Other studies suggested that roots with few narrow metaxylem vessels reduced capacity for axial transport and therefore require a larger water potential gradient between the soil and atmosphere to transport an equivalent volume of water as roots with many wider xylem vessels. This anatomical trait improved drought tolerance to root phenotypes (Klein et al., 2020).

## 5 CONCLUSION

This study showed differences in control and stressed cork oak seedlings for morphological , physiological and root anatomical traits of five Algerian provenances (Jijel, Tlemcen, Oum Tboul; Tizi Ouzou, Dradra). Different strategies were observed among seedlings submitted to drought conditions, such as decrease incollar diameter,leaf number, fresh and dry mass, RGR in growth, alsoreduction in RWC, total chlorophyll and carotenoids contents, and increase in proline content related to osmoregulation.

The morphological variability between the five provenances is most likely linked to phenotypic characters, which mainly depend on genetic and environmental factors in these plants native to different regions of Algeria.

The behavior ofplants submitted to drought stress highlighted the intraspecific variability presented in cork oak species. Then, the plasticity occurred in root plants by reducing vessel diameterisone of several strategies adopted by cork oak plants to tolerate drought stress. Indeed, the knowledge obtained from this study will help guide foresters to identify the most suitable seed sources to use in reforestation practices and thus contribute to cork oak stands that are more adapted to drought and more productive.

The use of provenances with greater genetic potential in terms of growth and root architecture would contribute to improving the survival rate and their growth in reforestation sites. It would be interesting to carry outadditionalresearch in natural conditions and over long periods to better understand the behavior of cork oak plants from other provenances, in order to harvest their acclimation capacity and determine their resistance thresholds.

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**Data vailability statement**

All original data are included in manuscript

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