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AYŞE DELİGÖZ

ESRA BAYAR

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Drought stress responses of seedlings of two oak species (*Quercus cerris* and *Quercus robur*)

Ayşe DELİGÖZ*, Esra BAYAR

Faculty of Forestry, Süleyman Demirel University, Isparta, Turkey

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Abstract: Effects of drought stress on growth, predawn xylem water potential (Ψ_{pd}), osmotic solutes (soluble sugar and proline), and stomatal conductance were assessed in two oak (*Quercus cerris* L. and *Quercus robur* L.) seedlings. Seedlings of both species were subjected to three drought treatments with the following irrigation intervals: well-watered (control: irrigation every 2–3 days), moderate drought stress (irrigation every 15 days), and severe drought stress (irrigation every 30 days). Drought-stressed seedlings of *Q. cerris* and *Q. robur* had more negative predawn xylem water potential than their well-watered seedlings. In *Q. cerris*, root collar diameter and root dry weights were negatively influenced by drought, while height, shoot dry weight, and root:shoot ratios were unaffected. Height and shoot dry weight of drought-stressed *Q. robur* were decreased, while root:shoot ratio increased. Drought caused significant decreases in stomatal conductance of both species. *Q. cerris* seedlings sustained higher stomatal conductance compared with *Q. robur*. Proline and soluble sugar increased in response to drought stress. *Q. robur* had a higher proline accumulation than *Q. cerris*. These findings suggested that the *Q. cerris* and *Q. robur* seedlings showed a drought stress adaptive mechanism either by restricting their growth or increasing root:shoot ratio or by decreasing water loss (reduced stomatal conductance) and accumulating of osmotic solutes.

Key words: Growth, osmotic adjustment, proline, *Quercus*, stomatal conductance, water potential

1. Introduction

Global warming and climate change are among the most prominent threats to the world. It is expected that there will be several regional and global effects of global climate change, especially on agriculture, forests, freshwater reserves, sea level, energy, human health, biodiversity (Doğan and Tüzer, 2011), and forest trees (Provost et al., 2013). Therefore, understanding the adaptation responses of tree species is quite important for plant production and sustainable forests.

The plant drought resistant mechanism has been identified on a morphological, physiological, and molecular basis. Several strategies of management have been suggested for coping with drought stress (Farooq et al., 2009). Plants rely on genetically changeable mechanisms of drought-avoidance and drought-tolerance for coping with periods of drought (Chaves et al., 2002). Morphological adaptations in leaves, stems, and roots exhibit drought avoidance and tolerance. Physiological adaptations include the control of stomatal conductance, leaf water potential, osmotic adjustment, and photosynthetic carbon fixation (Dickson and Tomlinson 1996). Water stress has decreased stomatal conductance and photosynthesis in quite a few species (Peguero-Pina et al., 2009). As stomatal conductance decreases, plant water loss also decreases (Raftoyannis and

Radoglou, 2002) and photosynthesis efficiency declines. This decrease affects plant growth and development due to the fact that carbohydrate molecules and energy, which are used in plant growth and development, are produced by photosynthesis (Öztürk, 2015). Essentially, drought tolerance is a tool for maintaining the turgor pressure and it is related to the elasticity of the cell wall and osmotic adjustment (Xu et al., 2010). Osmotic adjustment seems to be one of the critical stages for a plant's adaptation to drought. It is because of that, osmotic adjustments sustain the metabolic activity of the tissue and help for regrowth after rewetting. For osmotic adjustment, proline and water-soluble sugar are the most important compatible solutes in plants (Chaves et al., 2003).

Oaks are considered tolerant to drought and heat, and this feature is beneficial in warmer and drier climates (Arend et al., 2012). Success of oaks on drought-prone sites is related to diversity of oaks in their morphological and physiological characteristics (Abram, 1990; Arend et al., 2011). Tolerance to drought may differ between oak species and their origins (Dickson and Tomlinson, 1996). The forest ecosystem in Turkey contains significant diversity with regard to oak species. *Quercus robur* L. and *Quercus cerris* L. are quite important species of forest trees. According to several recent studies, adaptation of oak species to drought

* Correspondence: aysedeligoz@sdu.edu.tr

has been reported (Dickson and Tomlinson, 1996; Popović et al., 2010; Öztürk, 2013). On the other hand, adaptation to abiotic stresses varies according to family, genus, and even species (Franco et al., 2006). Furthermore, the response of drought and re-watering in oak species and its mechanism is relatively insufficient. Understanding the morphological and physiological response of plants to water scarcity is important for successful establishments of plantations in semiarid and arid areas. In the present study, morphological traits, predawn xylem water potential, total soluble sugar, proline, and stomatal conductance in two oak species (*Q. cerris* and *Q. robur*) under drought stress conditions were researched. The main objectives of this study were to investigate physiological and morphological response in the two species against drought stress and their availability for reforestation under dry conditions.

2. Materials and methods

2.1. Plant material, growth conditions, and experimental design

Acorns of *Q. robur* were collected in Balıkesir/Sındırgı (no. 3365 seed collection area, elevation: 734 m) in the Marmara region, while acorns of *Q. cerris* were collected in Isparta/Sütçüler (no. 5516 seed collection area, elevation: 860 m) in the Mediterranean region, in Turkey. The acorns of *Q. robur* and *Q. cerris*, collected in mid-November 2013, were soaked in tap water in plastic boxes; unhealthy seeds were mostly eliminated and then they were stored at cold temperature (4 °C) until the sowing date. Acorns of both oak species were sown in polyethylene bags (12.0 cm diameter × 25.0 cm height) containing a mixture of forest soil and humus (3:1, v/v) in a forest nursery, Eğirdir, Turkey (37°53'N, 30°52'E, altitude 926 m above sea level), in February 2014. In early May, the seedlings of the both oak species for the drought experiment were moved to a greenhouse located in Süleyman Demirel University, Isparta, Turkey. Before the start of the experiment, for maintaining the adaptation of seedlings to the conditions

of greenhouse, the seedlings of the both oak species were watered close to field capacity every 2 or 3 days. In early June, 10 g of nitrogen per m² of soil ammonium sulfate fertilizer was applied. A total of 432 seedlings of each species were divided into three treatment groups using a completely randomized design with three replicates per treatment (total 864; 2 species × 3 drought treatments × 3 replicates × 48 seedlings). Three drought treatments with different irrigation intervals, namely well-watered (control treatment: irrigation every 2–3 days), moderate drought stress (irrigation every 15 days), and severe drought stress (irrigation every 30 days), were assigned. The control seedlings continued to be well watered throughout the experimental period. For the cyclic drought treatments, seedlings of both oak species were re-watered close to field capacity for recovery at the end of every 15 (moderate drought stress) and 30 (severe drought stress) days during the experiment. The drought stress experiment started on 24 June (2014) and ended approximately 3 months later (19 September 2014). The side windows of the greenhouse were almost fully open all day long because of the high air temperature inside the greenhouse. During the experiment, daily air temperature and relative humidity were recorded using a data logger (model ebro; EBI 20-TH) inside the greenhouse (Figure 1). The monthly mean air temperature and relative humidity during the experimental period varied between 21 and 26 °C, and between 46% and 64%, respectively.

2.2. Predawn xylem water potential and soil water content

Predawn xylem water potential (Ψ_{pd}) was determined using a pressure chamber instrument (PMS Instruments, Corvallis, OR, USA) following standard techniques (Scholander et al., 1965). Predawn measurements were made on six shoots samples excised from the root collar level per treatment for each oak species between 0400 and 0630. Ψ_{pd} was measured at approximately 2-week intervals during the experiment. Measurements of volumetric water content (VWC) at a depth of 0–20 cm of growing medium

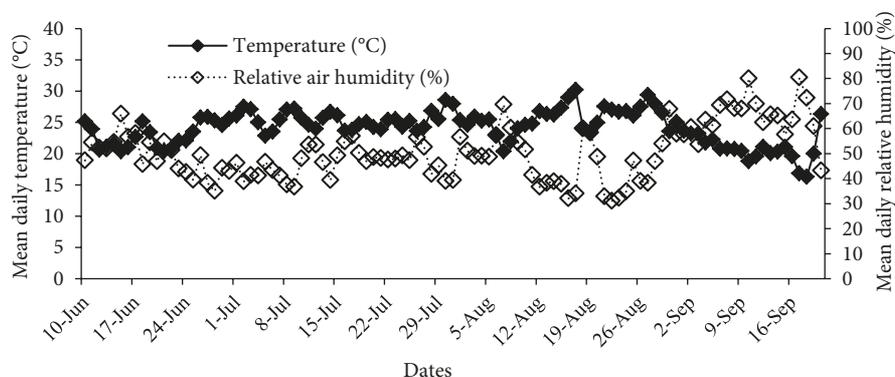


Figure 1. The mean daily relative humidity and temperature changes in the greenhouse during the experiment.

were taken subsequent to the measurements of Ψ_{pd} on predawn measurement days. A Fieldscout TDR 300 soil moisture meter (Spectrum Technologies, Inc., Plainfield, IL, USA) was used to measure the VWC of medium.

2.3. Stomatal conductance

Twelve seedlings (4 seedling \times 3 replications) per treatment for each oak species were chosen for the measurements of stomatal conductance at the beginning of the experiment. Measurements of stomatal conductance were made on leaves of the same seedlings during the experiment between 1130 and 1430 on predawn measurement days. Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) was measured on the well exposed to direct sunlight leaves using a diffusion steady-state porometer (Decagon Devices Leaf Porometer SC-1).

2.4. Proline and total soluble sugars

Proline and total soluble sugars were determined using all leaves of 6 seedlings per treatment for each oak species. All leaves from each seedling were sampled and ground after being dried at 65 °C for 48 h. Proline analysis was conducted according to Bates et al. (1973). About 0.5 g of dry leaves was homogenized with 10 mL of 3% sulfosalicylic acid. Next 2 mL of the supernatant was taken and 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were added to it. The reaction mixture was incubated in a boiling water bath for 1 h and the reaction was finished in an ice bath. Then 4 mL of toluene were added to it and the absorbance was measured with a spectrophotometer at 520 nm. The content of proline was

expressed in units of $\mu\text{mol/g DW}$. Total soluble sugars were determined according to Dubois et al. (1956). Next 100 mg of dry leaves was homogenized by adding 10 mL of 80% ethanol. The supernatant obtained was separated into another test tube and treated with 5% phenol solution and sulfuric acid. Total soluble sugar was calculated by creating a standard curve using standard glucose and was expressed as mg g^{-1} dry weight by reading absorbance at 490 nm.

2.5. Growth parameters

The seedlings of both oak species were harvested after completing the drought experiment. The height (cm), root collar diameter (mm), shoot and root dry weight (g), and root:shoot ratio (dry weight basis) of 15 seedlings per replicate in each treatment (45 per treatment) for each oak species were determined. Dry weights were determined after oven drying at 105 °C for 24 h.

2.6. Data analysis

SPSS 20.0 was used in the statistical analysis of the data. Statistical analysis was performed using ANOVA. When significant differences occurred in growth, Ψ_{pd} , proline, soluble sugar, and stomatal conductance among treatments, means were separated by Duncan's multiple range tests at $P < 0.05$. SE was calculated and is shown in the figures and table. Significant differences between the two species were determined using Student's t test at $P < 0.05$ for morphological, physiological, and biochemical data. The relationships between the measured parameters were examined using correlation analysis.

Table. Effect of drought stress on the seedling growth of *Quercus cerris* and *Quercus robur*. Data are shown as means \pm SE. Different letters indicate significant differences between treatments ($P < 0.05$). Asterisks show significant difference by Student's t-test ($P < 0.05$) between species

Parameter	P values	Treatments		
		Well-watered	Moderate	Severe
<i>Quercus cerris</i>				
Height (cm)	ns	10.84 \pm 0.54a*	9.57 \pm 0.49a*	11.06 \pm 0.41a*
Root collar diameter (mm)	<0.05	4.43 \pm 0.09b*	4.13 \pm 0.09a*	4.00 \pm 0.11a*
Shoot dry weight (g)	ns	0.95 \pm 0.05a*	0.81 \pm 0.05a*	0.84 \pm 0.05a*
Root dry weight (g)	<0.05	2.50 \pm 0.12b*	2.31 \pm 0.10a*	2.10 \pm 0.10a*
Root:shoot ratio	ns	2.74 \pm 0.11a*	2.99 \pm 0.12a*	2.63 \pm 0.10a ns
<i>Quercus robur</i>				
Height (cm)	<0.05	21.71 \pm 0.90b	17.37 \pm 0.60a	18.22 \pm 0.75a
Root collar diameter (mm)	ns	5.27 \pm 0.12a	5.14 \pm 0.08a	5.16 \pm 0.10a
Shoot dry weight (g)	<0.05	2.15 \pm 0.12b	1.70 \pm 0.09a	1.49 \pm 0.09a
Root dry weight (g)	ns	3.75 \pm 0.26a	3.53 \pm 0.16a	3.50 \pm 0.17a
Root:shoot ratio	<0.05	1.80 \pm 0.10a	2.26 \pm 0.12b	2.49 \pm 0.12b

3. Results

3.1. Soil water content

Volumetric water content was significantly lower in the drought treatments than in the well-watered (control) treatment ($P < 0.001$) during the experiment period. Under well-watered condition, volumetric water content was over 50% in *Q. cerris* and 38% in *Q. robur*. The lowest volumetric water content was 12.4% in *Q. cerris* and 8.4% in *Q. robur* on 24 August. On the same date, volumetric water content under the moderate drought treatment was 19.5% in *Q. cerris* and 16.8% in *Q. robur*, whereas it was 56.9% for *Q. cerris* and 39.4% for *Q. robur* in the well-watered treatment (Figure 2).

3.2. Predawn xylem water potential

During the drought experiment, the well-watered control seedlings of both oak species exhibited very stable water potential values (nearly -0.5 MPa Ψ_{pd}). Under moderate drought stress, Ψ_{pd} was -1.20 MPa in *Q. cerris* seedlings and -1.34 MPa in *Q. robur* seedlings. In the severe drought-stressed seedlings, in the first drought cycle (24 July), Ψ_{pd} was lowered to -2.28 MPa for *Q. cerris* and -3.73 MPa for *Q. robur* (Figure 3). Both oak species showed a rapid recovery after watering following the first drought cycle (24 July), and after 2 weeks (9 August) Ψ_{pd} increased to -0.98 MPa for *Q. cerris* and -1.2 MPa for *Q. robur*. In the second severe drought cycles (24 August), Ψ_{pd} decreased to -2.45 MPa for *Q. cerris* and -2.38 MPa for *Q. robur* (Figure 3). After re-watering following the second severe drought cycles, Ψ_{pd} values recovered to -1.1 MPa (8 September) in both species. Both the well-watered and drought-stressed seedlings of *Q. cerris* and *Q. robur* had nearly the same Ψ_{pd} in almost all periods (Figure 3).

3.3. Proline and total soluble sugars

In the second month of the drought experiment (24 August), the proline content of both oak species was significantly influenced by drought treatments (Figure 4). In *Q. cerris* and *Q. robur*, accumulation of proline was significantly higher in the severe drought treatment than in the other treatments. Proline accumulation in *Q. cerris* increased to 1.51 $\mu\text{mol/g DW}$ and in *Q. robur* to 2.84 $\mu\text{mol/g DW}$. Although there was no difference in proline content between well-watered and moderate stressed seedlings in *Q. cerris*, the lowest proline content in *Q. robur* was determined in well-watered seedlings, i.e. 0.12 $\mu\text{mol/g DW}$.

Total soluble sugar content of both oak species showed a strong decrease in the first month of the drought experiment (July 24). However, at this stage of drought, there was no significant difference between the drought treatments of both species (Figure 5). For *Q. cerris*, the total soluble sugar content of severe drought-stressed seedlings was significantly higher than in well-watered and moderate drought-stressed seedlings in the second month of the drought experiment. At the end of the experiment, the total soluble sugar content of severe drought-stressed and well-watered seedlings was significantly higher than that of moderate drought-stressed seedlings, while the difference in the total soluble sugar content between severe drought-stressed and well-watered seedlings was insignificant. In the case of *Q. robur*, in the second month of the drought experiment, significant differences in soluble sugar contents between well-watered and drought-stressed seedlings were determined. The soluble sugar content was higher in drought-stressed seedlings compared to well-

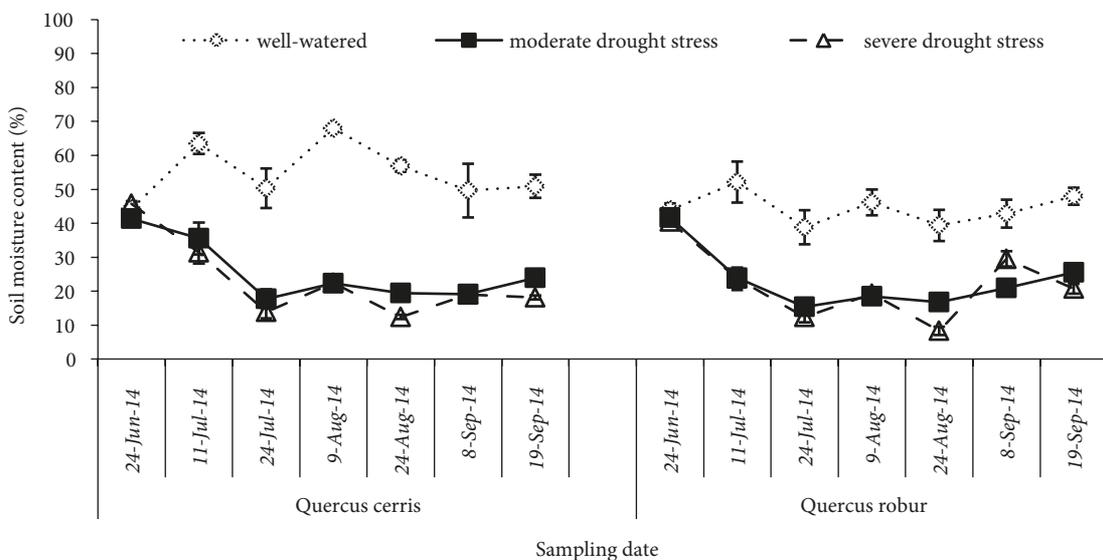


Figure 2. Changes in volumetric water content (soil moisture) during the experimental period. Data are shown as means \pm SE.

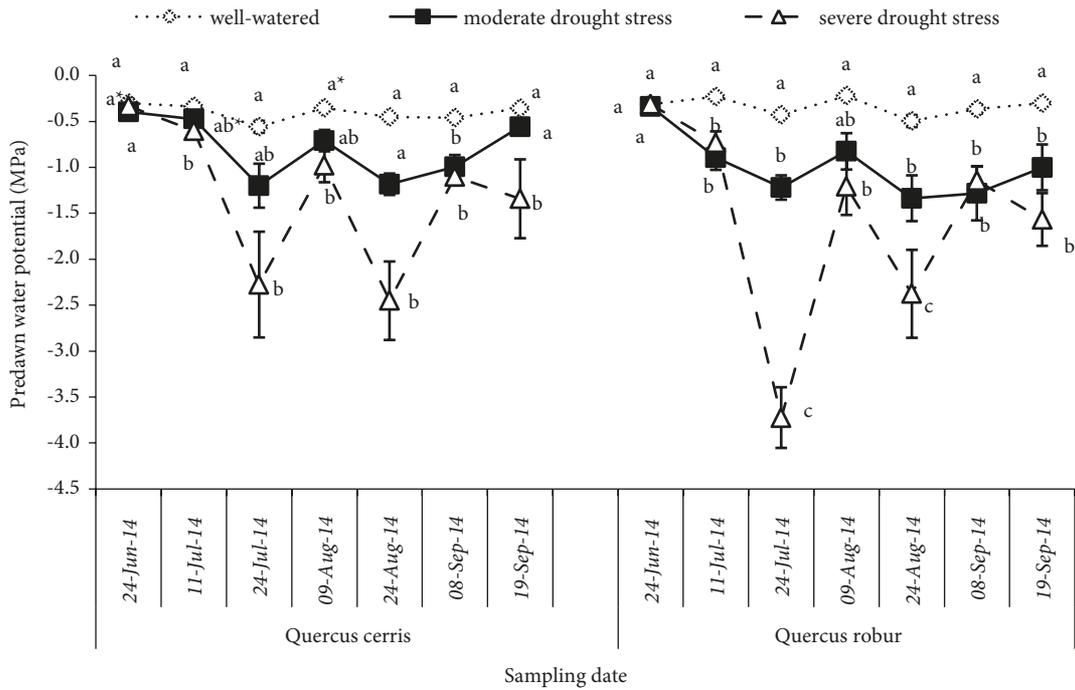


Figure 3. Predawn water potential (Ψ_{pd}) of two oak species growing under different drought stress treatments in greenhouse conditions. Data are shown as means \pm SE. Different letters indicate significant differences between treatments ($P < 0.05$). Asterisks show significant differences by Student's t-test ($P < 0.05$) between species.

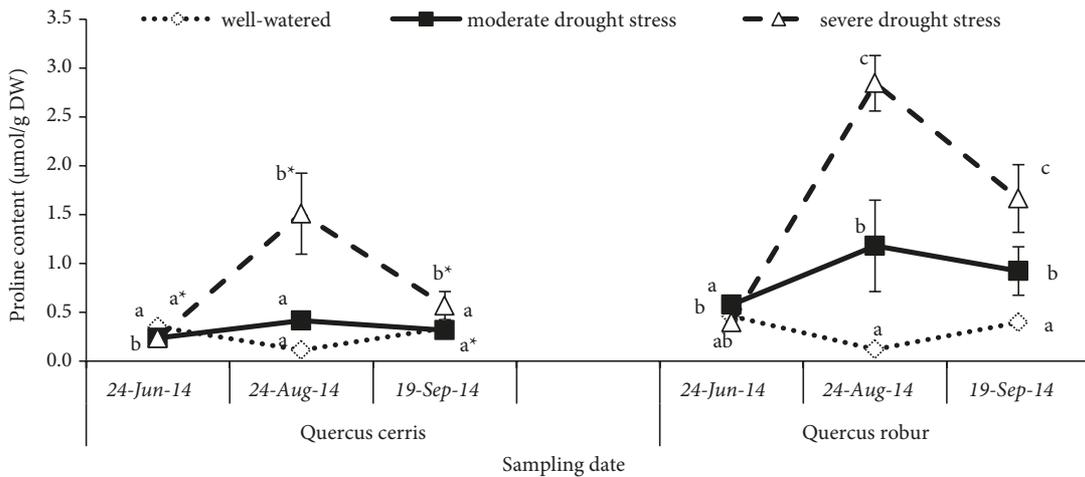


Figure 4. Leaf proline content of two oak species growing under different drought stress treatments in greenhouse conditions. Data are shown as means \pm SE. Different letters indicate significant differences between treatments ($P < 0.05$). Asterisks show significant differences by Student's t-test ($P < 0.05$) between species.

watered seedlings. Differences in soluble sugar content between drought-stressed and well-watered seedlings disappeared at the end of the experiment (Figure 5).

3.4. Stomatal conductance

There were significant differences between well-watered and drought-stressed seedlings in stomatal conductance of *Q. robur* and *Q. cerris* (Figure 6). The stomatal conductance

levels of drought-stressed seedlings of both species were significantly lower than those of well-watered seedlings throughout most of the experimental period ($P < 0.05$). Generally there was no difference between stomatal conductance values of moderate and severe drought-stressed seedlings of both species. A significant difference between species was not determined at the beginning of

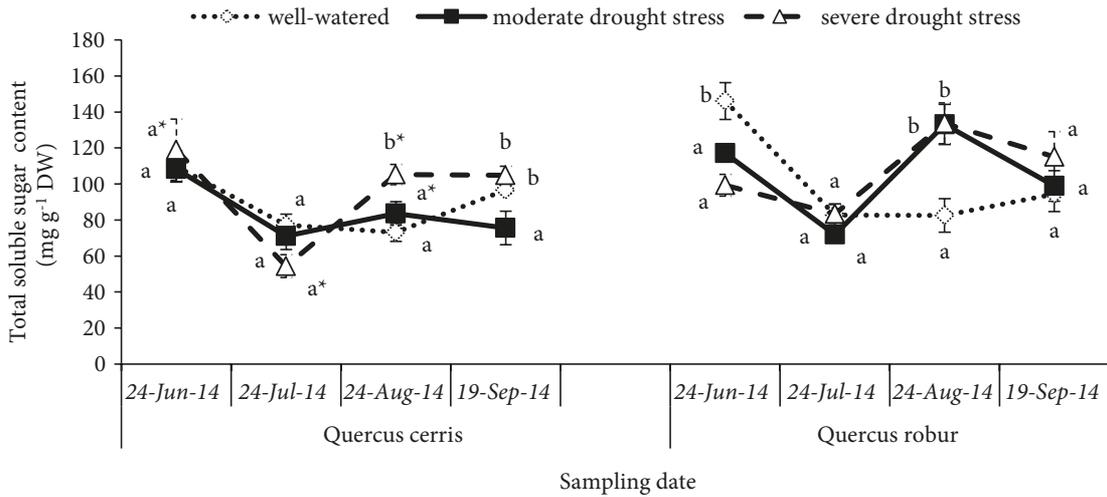


Figure 5. Total soluble sugar contents in leaves of two oak species growing under different drought stress treatments in greenhouse conditions. Data are shown as means \pm SE. Different letters indicate significant differences between treatments ($P < 0.05$). Asterisks show significant differences by Student's t-test ($P < 0.05$) between species.

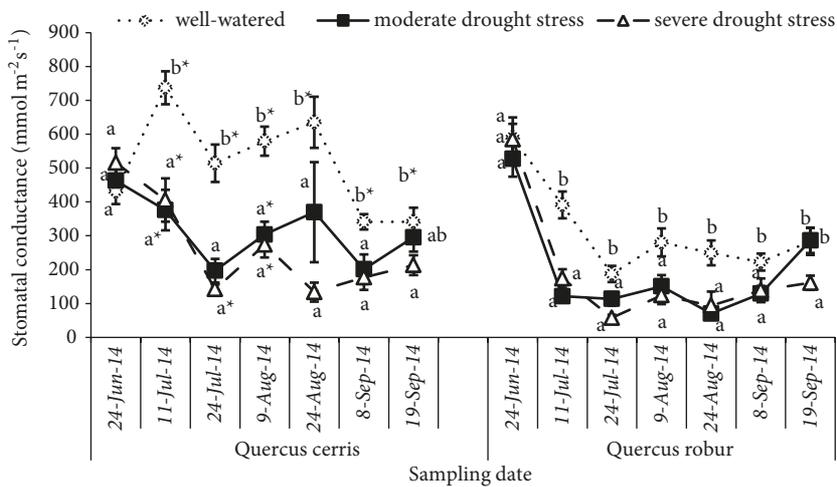


Figure 6. Stomatal conductance of two oak species growing under different drought stress treatments in greenhouse conditions during the experimental period. Data are shown as means \pm SE. Different letters indicate significant differences between treatments ($P < 0.05$). Asterisks show significant differences by Student's t-test ($P < 0.05$) between species.

the experiment (24 June). However, stomatal conductance in well-watered seedlings of *Q. cerris* was significantly higher than that of well-watered seedlings of *Q. robur*. Despite the fact that stomatal conductance of *Q. cerris* was generally higher than that of *Q. robur* during the severe drought stress, this difference was significant only on 11 and 24 July and 9 August (Figure 6).

3.5. Relationships between some parameters

In both species, significant negative relationships between Ψ_{pd} and proline content (*Q. cerris*: $R^2 = 0.87$; *Q. robur*: R^2

$= 0.93$; $P: 0.001$) were determined (Figure 7a). There was a significant positive and linear relationship between the soil moisture content and Ψ_{pd} (*Q. cerris*: $R^2 = 0.55$; *Q. robur*: $R^2 = 0.62$; $P: 0.001$) (Figure 7b). Stomatal conductance and soil moisture content were positively correlated (*Q. cerris*: $R^2 = 0.79$; *Q. robur*: $R^2 = 0.54$; $P: 0.001$) (Figure 7c).

3.6. Plant growth

The well-watered and drought-stressed *Q. robur* seedlings had significantly greater height, root collar diameter, and shoot and root dry weights than *Q. cerris* seedlings, but

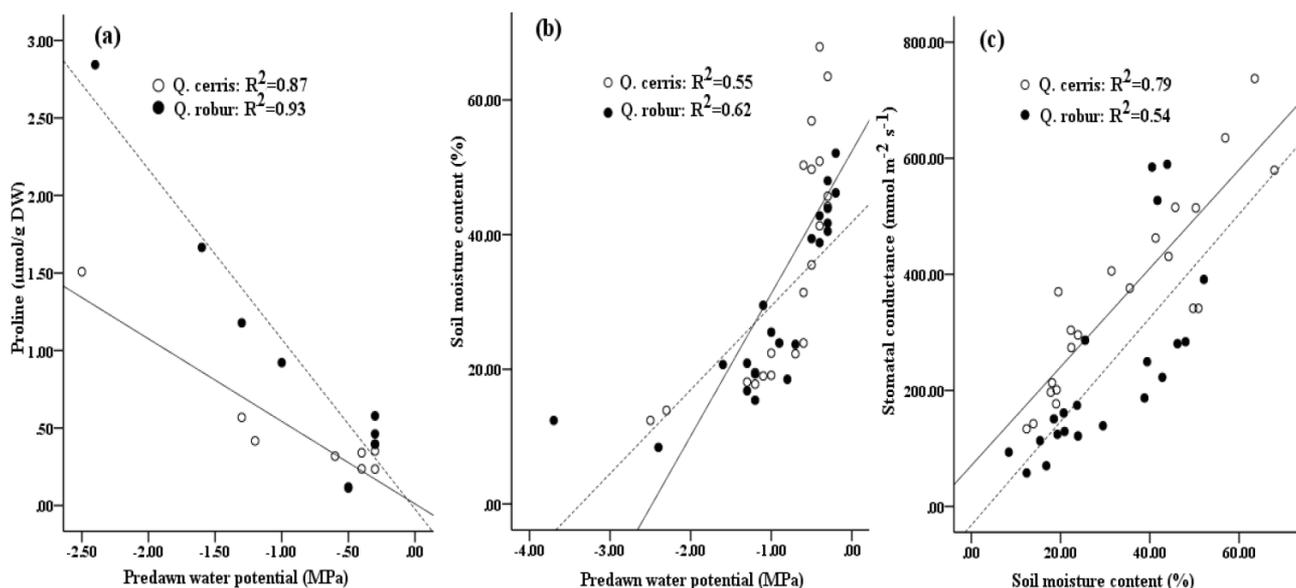


Figure 7. Correlations between predawn water potential (Ψ_{pd}) and proline content (a), soil moisture content (b); between soil moisture content and stomatal conductance (c). The solid lines represent the best fitted linear regressions for *Q. cerris*. The dotted lines represent the best-fitted linear regressions for *Q. robur*.

lower root:shoot dry weight ratios than *Q. cerris* (Table). At the end of the experiment, there was a significant effect of drought stress on root collar diameter and root dry weight of *Q. cerris* seedlings and on the height, shoot dry weight, and root:shoot ratio for *Q. robur*. In *Q. cerris*, well-watered seedlings had greater root collar diameter and root dry weight than drought-stressed seedlings. Root collar diameter and root dry weight of moderate and severe drought-stressed seedlings were also similar. When it was compared to well-watered, drought-stressed *Q. robur* seedlings' height and shoot dry weight were lower, whereas the root:shoot ratio was higher. However, differences between the treatments in terms of height, shoot dry weight, and root:shoot ratio for *Q. cerris* and root collar diameter and root dry weight for *Q. robur* were not significant (Table).

4. Discussion

Throughout the experiment period, the drought stress caused significant decreases in soil moisture content and consequently the predawn xylem water potentials of *Q. cerris* and *Q. robur* seedlings were lower in the drought treatments than in the well-watered treatment. In the first severe drought cycle (24 July), soil moisture content was lowered by 13.9% in *Q. cerris* and 12.4% in *Q. robur*, and Ψ_{pd} was lowered to -2.28 MPa for *Q. cerris* and -3.73 MPa for *Q. robur*. While the differences between species in Ψ_{pd} were not significant, the lower values of *Q. robur* may be related to the sizes of seedlings. Both the well-watered and drought-stressed *Q. robur* seedlings had taller, larger

diameter, and heavier shoots than *Q. cerris* while the root:shoot ratio was lower than in *Q. cerris*. As a matter of fact, water condition of a plant is controlled by relative rates of water absorption and loss. Moreover, it is also related to the plant's ability to maintain and adjust adequate water status (Chavarría and Santos, 2012). Therefore, the greater seedling size of *Q. robur* than *Q. cerris* seedlings may have used the most water per seedling (Struve et al., 2009).

A predawn water potential of -2.0 MPa is not likely to result in death of a seedling, but through the day normal stomatal activities may be blocked, photosynthesis will gradually decrease, and seedling growth may stop (Lopushinsky, 1990). In the present study, although watering following every drought cycle increased Ψ_{pd} , repeated drought stress caused a reduction in plant growth. Repeated drought stress decreased the root collar diameter and root dry weight in *Q. cerris*, but it did not affect seedling height. In *Q. robur*, while seedling height and shoot dry weight were significantly reduced due to drought stress, the reductions in root collar diameter and root dry weight were not significant. Similarly, earlier results from a study of 11 species including *Q. robur* and *Q. pyrenaica* Willd. revealed that growth was decreased in drought-treated seedlings compared with the controls (Valladares and Sánchez-Gómez, 2006). In the case of *Pistacia lentiscus*, the growth of shoots, transpiration, and stomatal conductance of seedlings decreased under drought conditions (Vasques et al., 2016). Fort et al. (1997) noted that drought stress was ineffective on root collar diameter in *Q. robur*. Water stress decreased the

leaf water potential, shoot height, and diameter in *Acacia ehrenbergiana* Hayne and *Acacia tortilis* (Forssk.) Haynes subsp. *raddiana* seedlings (Atta et al., 2012).

Under well-watered condition, *Q. cerris* had higher root:shoot ratio than *Q. robur*. While drought stress was statistically ineffective on root:shoot ratio for *Q. cerris*, it increased the root:shoot ratio in *Q. robur*. It was also noted that, in *Fagus sylvatica* L. seedlings, root:shoot ratio increased with increasing drought stress (Zang et al., 2014). Many studies have shown that there is an increase in root:shoot dry weight under water stress (Xu et al., 2010; Vasques et al., 2013). The increase in root:shoot ratio was noted as an avoidance mechanism of plants, for providing maximum water absorption under drought condition (Chaves et al., 2003). It was also reported that *Q. robur* is very sensitive to moisture stress (Dickson and Tomlinson, 1996). Probably *Q. robur* develops an avoidance mechanism against drought by increasing the root:shoot ratio.

Proline has an important biological role in response to stress (Liang et al., 2013). Many plant species accumulate proline in response to drought stress (Bhaskara et al., 2015; Deligoz and Gur, 2015). Proline accumulation can influence stress tolerance in various ways (Szabados and Savoure, 2010). Generally, osmolite accumulation in plant cells results in a decrease in cell osmotic potential and therefore in maintenance water absorption and cell turgor pressure, helping physiological processes such as stomatal opening, photosynthesis, and growth under dry conditions (Blum, 1996). Moreover, accumulation of sugar and proline in plant tissues can protect cell membranes and it can increase the tolerance against loss of water (Shvaleva et al., 2005). In the present study, repeated drought stress caused increased proline contents in both oak species. Especially under severe drought stress, accumulation of proline was higher. The accumulation of proline significantly increased with increasing drought stress in *Q. variabilis* Bl. seedlings (Wu et al., 2013). Drought stress increased proline content in three Mediterranean oak species (*Q. ilex*, *Q. pubescens*, and *Q. cerris*) (Cotrozzi et al., 2016). Deligoz et al. (2016) reported that proline accumulation is one of the responses of *Cedrus libani* seedlings to repeated drought stress. The level of proline accumulation is changeable from species to species (Verbruggen and Hermans, 2008). In the present study, in the second severe drought cycles (24 August), proline content increased from 0.24 $\mu\text{mol/g DW}$ to 1.51 $\mu\text{mol/g DW}$ in *Q. cerris*, while it increased from 0.40 $\mu\text{mol/g DW}$ to 2.84 $\mu\text{mol/g DW}$ in *Q. robur*. However, *Q. robur* had a higher proline accumulation than *Q. cerris*. High solute concentrations can contribute to a greater capacity for turgor maintenance (Ranney et al., 1991). In both species, a strong negative correlation was detected between Ψ_{pd} and proline. Watering following the drought

stress cycles increased both soil moisture content and Ψ_{pd} ; it also lowered the stress level of seedlings, and therefore the proline level also decreased at the end of the drought experiment (19 September). Both oak species accumulated proline in the case of drought stress; it seems likely that they developed their osmotic adjustment and they have shown tolerance to drought.

In both species, total soluble sugar content, which was quite high at the beginning of the drought experiment, declined in the first month (24 July) of the drought experiment. This decrease was probably related to seedling growth, which is in agreement with Kulac et al. (2012). In the first month of the drought experiment (24 July), drought stress did not significantly affect total soluble sugar content. However, in the second month of the drought experiment (24 August), close to the end of the growth period, total soluble sugar content increased in drought-stressed seedlings of both species. The severe drought stressed seedlings of *Q. cerris* and *Q. robur* had higher total soluble sugar content than well-watered seedlings. In *Q. robur*, the total soluble sugar content differences between drought-stressed and well-watered seedlings disappeared with stress at the end of the drought experiment. An increase or decrease in accumulation of sugars depends on the intensity of stress and the role of sugar signal is not fully understood in these processes (Akıncı and Lösel, 2012). Indeed, accumulation of soluble sugars and proline under drought conditions has been associated with tolerance to drought in many plant species (Liu et al., 2011). The results of the present study suggest that both species showed tolerance to drought stress by increasing the total soluble sugar content, which largely contributed to osmotic adjustment.

Stomatal conductance of *Q. robur* and *Q. cerris* seedlings was significantly decreased in drought-stressed seedlings. However, there was not a significant difference between stomatal conductance of severe and moderate drought-stressed seedlings for both oak species. *Q. cerris* and *Q. robur* seedlings continue to exhibit some stomatal conductance at low water potential (Fotelli et al., 2000). Water stress significantly decreased stomatal conductance of *Quercus robur* L. and *Carpinus betulus* L. grown at a nonirrigated site (Stojnić et al., 2016). Similar data have been reported by Peguero-Pina et al. (2009) for *Q. coccifera* L., *Q. ilex* subsp. *ballota* (Desf.) Samp., and *Q. suber* L. by Vasques et al. (2016) for *P. lentiscus*, and by Fotelli et al. (2000) for *Q. frainetto* Ten., *Q. pubescens* Willd., *Q. macrolepis* Kotschy, and *Q. ilex* L. Plants grown under drought conditions tend to have lower stomatal conductance, thus helping water conservation and maintenance of an adequate leaf water status, but at the same time reducing leaf internal CO_2 concentration and photosynthesis (Chaves et al., 2002). This study indicates

that lowering of the stomatal conductance in order to reduce the water loss of both species in drought stress may be an indication of tolerance mechanism for adapting to drought stress. In addition, despite the fact that the two species had similar water potentials during the experiment, *Q. cerris* always had higher stomatal conductance than *Q. robur*. These differences can be explained by reducing stomatal conductance of *Q. robur* that copes with drought stress. In both species, the reduction in stomatal conductance during drought stress is closely related to a decrease in Ψ_{pd} and soil moisture. A similar conclusion has also been noted for *Acacia confusa* (Liang and Zhang, 1999). Stomatal conductance correlated positively with soil moisture content in *Q. cerris*. Parallel decrease in stomatal conductance and leaf water potential reveals cautious water usage, which is a characteristic response in Mediterranean species to daily and seasonal water scarcity (Manes et al., 2006).

In conclusion, seedling establishment is a critical part of the life cycle. Survival rates of seedlings under drought conditions will decrease proportionally to drought intensity and duration. Therefore, appropriate silvicultural measures are required to be applied in forest practice to enable seedlings to survive this critical life stage. One silvicultural solution to avoid this problem could be the use of more drought-adapted tree species or provenances. In this study,

Q. cerris and *Q. robur* seedlings showed differential growth responses to drought. Drought stress decreased growth and stomatal conductance, but increased proline and total soluble sugar in both species. In addition, root:shoot ratio increased during drought stress in *Q. robur* seedlings. The decrease in growth observed in this study can be regarded as a morphological adaptation of plants to drought stress via lowering water consumption and reducing transpiration. Both oak species showed drought avoidance mechanisms (increasing the ratio of root:shoot or decreasing growth) and drought tolerance mechanisms (control of stomatal conductance and osmotic adjustment). Similarly, water potential values of *Q. robur* followed by low stomatal conductance might indicate that this species is more sensitive to drought than *Q. cerris*. Under drought stress conditions, increased root:shoot ratio, sugar and proline accumulation, and the osmotic adjustment appear to be important adaptations for establishment and regeneration in *Q. robur*. These mechanisms might be very important for both oak seedlings to overcome drought stress in a dryer and warmer climate. The stage in which tree species are most vulnerable to drought and death is the seedling stage. Overall, the results of this study will provide critical information for assessing ongoing afforestation programs and optimizing the design of future ones.

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