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ORIGINAL PAPER

Drought effects on growth, biochemical changes and leaf gas exchange in laurel (*Laurus nobilis* L.)

Christine Ishimwe, Ayşe Deligöz*

Isparta University of Applied Sciences, Faculty of Forestry, Department of Forest Engineering, Çünür, TR-32260 Isparta Merkez, Turkey

Abstract
Drought is one of the abiotic factors that negatively affect the productivity of laurel (*Laurus nobilis* L.). Increasing water scarcity in arid and semi-arid regions is pushing the physiological limits of woody species, necessitating strong resistance to drought. Studies should be conducted on the effects of drought stress not only on forest trees but also some medicinal and ornamental species, and ways to increase their resistance should be explored. This study was carried out to determine the effect of drought stress on seedling growth and some physiological and biochemical properties in laurel. The seedlings were subjected to drought stress for about 4 months with three different irrigation regimes: watering once every 2–3 days (control), every 15 days, and every 30 days. Morphological properties (root collar diameter, height, dry weight, stem: root ratio, and number of leaves), physiological properties (midday xylem water potential and photosynthesis properties), and biochemical properties (photosynthetic pigments, total carbohydrate, and proline contents) were determined. Results have shown that drought stress has a negative effect on root collar diameter, seedling height, and dry weight. Likewise, leaf number and midday xylem water potential decreased due to increased drought stress. Furthermore, the net photosynthesis rate, stomatal conductance, and transpiration rate of the least irrigated (30th day) seedlings were quite low compared to those of control seedlings. The differences in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were also found to be statistically significant. Total carbohydrate and proline contents showed the highest values for the least irrigated seedlings and the lowest values for controlled irrigation seedlings. Our results reflect the improved tolerance capacity of laurel to increased drought stress.

Key words: midday stem water potential; laurel; morphology; photosynthesis; stomatal conductance; water stress

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1. Introduction

Each plant has an environment to which it is best adapted and its own specific environmental demands. Plants perceive the effects of environmental factors outside of the appropriate values as stress to maintain their lives and create appropriate physiological responses in their metabolism (Korkmaz & Durmaz 2017). Trees can be resistant to stress and recover when the stress is gone. However, if the stress lasts for a long time, increases in severity or if resistance cannot be achieved, the vitality of the tree may begin to decrease, turn into an irreversible damage and cause the death of the tree (Akça et al. 2008; Hussain et al. 2008).

Stress in the tree can be caused by a number of biotic or abiotic factors, and drought is the most important cause of drying in trees with 26% of all evaluated factors worldwide (Kalefetoğlu & Ekmekçi 2005). The gradual decrease in water resources due to climate change and intense population growth seems to pose a serious threat to the world's life in the coming years. As drought is directly related to water scarcity, its impact on trees is serious and causes stress. The effects of drought have been reported for a large number of tree species, resulting on considerable changes many morphological, physiological, biochemical and molecular processes (Ni & Pallardy 1991; Atta et al. 2012; Deligöz & Bayar 2018, 2021; Jafarnia et al.2018; Jalili et al. 2023; Sun et al. 2023). Drought stress reduces plant height, leaf area, stem diameter and plant biomass. Under drought conditions, shoot and root growth may be stunted, leaf curl, mottling, scorching and chlorosis, pest attack.

*Corresponding author. Ayşe Deligöz, e-mail: aysedeligoz@isparta.edu.tr
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In addition, a decrease in cell membrane integrity and tree defense ability may eventually lead to difficulties in survival (Nonami 1998; Kaya et al. 2006; Hussain et al. 2008). In the case of drought stress, photosynthesis is impaired and food storage is also affected. Drought reduces the availability, uptake, transfer and metabolism of nutrients in plants (Farooq et al. 2009). Drought stress reduces the relative water content, turgor pressure, leaf water potential, stomatal conductance and transpiration rate in plants (Rhizopoulou & Mitrakos 1990; Farooq et al. 2009). Through osmotic adjustment as water stress increases, trees attempt to accumulate solutes, including sugars, proline, and other amino acids in response to decreasing water potential (Moradi 2016). Accumulation of proline and soluble sugars in leaves is characterized as the plant's response to environmental stresses, especially drought stress (Sakamoto & Murata 2002; Shehab et al. 2010). Proline is an important cell solute, as at high concentrations it can protect plants from environmental stresses through its contribution to cellular osmotic regulation, detoxification of reactive oxygen species, maintenance of membrane integrity, and stabilization of enzymes or proteins (Toscano et al. 2016). Proline and soluble sugar content are high in water-deficient plants in response to drought stress, as proline and carbohydrates facilitate water uptake and retention by acting as cytoplasmic osmolytes. This helps the plant minimize the damage caused by lack of water (Jafarnia et al. 2018).

Given that drought stress is a serious threat to the environment, more research is needed on the effects of drought stress and particularly the resilience of plants or trees. This research was carried out to determine the effects of drought stress on laurel (*Laurus nobilis* L.). Laurel (or bay laurel) is an evergreen aromatic shrub or small tree reaching up to height of 18 meters. It is widespread in Mediterranean country such as in Turkey, Algeria, Morocco, Portugal, Spain, Italy, France, Greece and Mexico, and cultivated in other temperate and warm region of the world. It grows well in sunny and well-drained soils, in regions with a mild, cold climate and humid atmospheric conditions near the sea. It is not found in high altitude regions due to frost sensitivity. Laurel grown commercially for its essential oil in Turkey, Italy, Belgium, Algeria, France, Tunisia, Iran, Morocco, Serbia, Greece, Portugal, Centers America, and the Southern United States (Maatallah et al. 2016; Paparella et al. 2022). The leaves are 10 cm long by 3 to 5 cm wide. In essential oils obtained from laurel leaves identified 41 compounds. The fruits are olive-like black berries in 10–15 mm long. Its fruit contains 17–25% fixed oil. Laurel is has not only an aromatic leaves wide used in gastronomy, pharmacy and perfumery industry, but has also been used as an ornamental plant. On the other hand, laurel essential oils have the potential to use in the fight against harmful insect due to their repellent and lethal effects (Sönmez 2023). Laurel has leaf characteristics that enable the plant to withstand the stressful conditions of the Mediterranean summer. It is also resistant to air pollution, up to injury and regenerative ability (Christodoulakis & Fasseas 1990). However, young laurel is susceptible to drought due to insufficiently long root systems. This species can suffer greatly from lack of water in field conditions at an early stage of its development (Rhizopoulou & Mitrakos 1990; Akça et al. 2008). The reason for choosing the laurel species in this research is that it is an important medicinal and ornamental plant, as well as playing a major role in the country economy. We hypothesized that different degrees of drought stress will cause changes in the level of leaf defensive compounds, water relations and morphology. The objective of this study is to elucidate the mechanism of drought stress tolerance by investigating morphology, midday xylem water potential, photosynthesis properties, carbohydrate and proline contents of laurel.

2. Material and methods

2.1. Plant material and study area

As material in this study we used the laurel seeds collected in December 2020 from the seed collection field no. 221 situated at an altitude of 110 m a.s.l. in Yeşilova locality in the western part of the Mediterranean region of Turkey (37°4´N, 28°25´E). The study area has a Mediterranean – type climate characterized by mean annual temperature of 18 °C, with mild, rainy winters and warm summers. Annual precipitation is mean 900 mm for the 1979–2023 climatic periods (Meteoblue 2024). After collection, the laurel seeds were put into cold wet folding process in the cold storage on January 20, 2021. The seeds with the first signs of germination were removed from the cold storage and planted in 11×25 cm polyethylene bags on March 7, 2021. A mixture of peat, vermiculite and soil (1 : 1 : 1) was used as the growing medium. Sowing was carried out under greenhouse conditions in the Research and Application Nursery in Isparta, Turkey. The greenhouse where the research was carried out is unheated and is a simple glass greenhouse model with an area of 60 m^2 with manual ventilation from the side. Irrigation was carried out regularly after planting. Maintenance works such as weeding and misfire were carried out.

2.2. Establishment of the experiment and application of stress

In mid-June of 2021, 270 uniformly sized seedlings were selected to drought stress application. At the beginning of the trial, the mean of root collar diameter and height of the seedlings were 1.65 ±0.03 mm and 6.7 ±0.12 cm respectively. Drought stress was started with the irriga-

tion process applied at approximately field capacity in all seedlings. Afterwards, the three different irrigation intervals were applied: 1/ watering at full capacity once in 2–3 days (control); 2/ watering every 15 days; and 3/ watering every 30 days. At the end of each irrigation interval, all the seedlings were irrigated at approximately field capacity. The experiment was set up in a completely randomized design with 3 different irrigation treatments under greenhouse conditions. Each treatment was conducted in three replications and 30 seedlings per replication. The stress treatments continued for four months. The monthly average temperature and humidity values measured in the greenhouse in June–October 2021, when the experiment was carried out, are given in Fig. 1. During the experiment, soil moisture content, seedling height, root collar diameter, midday xylem water potential, and leaf gas exchanges were all evaluated four times (once every month). At the end of the experiment, all seedlings were harvested and biomass, number of leaves and biochemical analysis (photosynthetic pigments, total carbohydrate content and proline) were assessed. All these samplings were performed just before irrigation.

2.3. Data collection

Morphological measurements

During the experiment, seedling height and root collar diameter were measured monthly from mid-June to October 2021. Root collar diameter was determined with a millimetric electronic caliper with an accuracy of 0.01 mm, the seedling height was determined with a precision of 0.5 cm with a ruler. At the end of the experiment (in October), the seedlings were removed from the bags and their roots systems were gently washed to remove the growing medium. Each seedling was divided into roots, stems, and leaves. Number of leaves, stem dry weight, root dry weight and leaf dry weight measurements were carried out on at least 20 seedlings from each treatment. Biomass samples were dried at 65 °C for 48 hours and weighted with an accuracy of 0.001 grams with an electronic balance. Shoot to root ratio were used to evaluate the effect drought stress on seedling morphology. For shoot to root ratio, shoot dry weight was divided by root dry weight.

Soil moisture content and midday xylem water potential

Soil moisture content at 0–20 cm depth was measured volumetrically by a portable TDR using 20 cm rod length (Model FieldScout™ TDR 300, Spectrum Technologies, Inc.) in 15 tube seedlings from each treatment during the experiment. In order to determine the effect of stress treatments on midday xylem water potential, measurements were made with a plant pressure chamber device (PMS model 1000) between 12:00 and 14:00. Midday xylem water potential measurements were made on shoot samples cut from the root collar level of 6 randomly selected seedlings from each treatment.

Leaf gas exchanges

Measurements of net photosynthesis rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}) and transpiration rate (mmol H_2O m^{-2} s^{-1}) were made using portable photosynthesis system LI-6400XT (LICOR, Lincoln, NE, USA) LI-COR brand 6400-02B model and an additional LED light source in June, July, August and October 2021. Photosynthesis measurements were carried out between 09:00 and 11:00 on one fully mature leaf of at least 6 seedlings from each treatment. Before the measurement, the device was calibrated, its photosynthetic active radiation (PAR) was adjusted as 1,000 μmol m^{-2} s^{-1} and the CO_2 concentration was adjusted as 400 μmol s^{-1}. During the measurement, the leaf chamber environment [i.e. temperature (°C); relative humidity (%), vapor pressure deficit (kPa) were similar to the growth conditions in the greenhouse. Instant water use efficiency was calculated using the net photosynthesis rate/ transpiration rate formula (Hatfield & Dold 2019).

Photosynthetic pigments

Two fully developed leaves were collected from each seedling in each treatment (six seedlings per replication) and finely cut with scissors. Approximately, 0.1 g fresh leaf samples were crushed in a mortar with 80% acetone solution. Absorbances were measured at 450, 645 and 663 nm wavelengths in a spectrophotometer against the solvent (acetone) blank. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations were calculated using the following equations (Arnon 1949):

Chlorophyll a: $12.7(A_{663}) - 2.69(A_{645})$ [1]

Chlorophyll b: $22.9(A_{645}) - 4.68(A_{663})$ [2]

Total Chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$ [3]

Carotenoids: $4.07(A_{450}) - [(0.0435 \times \text{Chlorophyll a}) + 0.367 \times \text{Chlorophyll b})]$ [4]

Total carbohydrate content and proline analysis

For total carbohydrate content and proline analysis, samples were dried in oven at 65 °C for 48 hours and then ground in a coffee grinder. Total carbohydrate content in leaf, stem and root samples was measured according to Dubois et al. (1956). Ground samples (0.1 g) of dried leaf, stem and root from each treatment were homogenized in 80% ethanol for 24 hours. Sulphuric acid and phenol were added and mixed. Total carbohydrate content (mg g^{-1} DW) was determined by the phenol sulfuric acid method at a wavelength of 490 nm (Dubois et al. 1956). D-glucose was used to create a calibration curve for carbohydrate analysis.

Proline analysis was carried out according to Bates et al. (1973). Leaf samples (0.1 g) were homogenized with 3% sulfosalicylic acid. The extracts were filtered with filter paper and acid ninhydrin and glacial acetic acid were added. Afterwards, the sample was incubated at 100 °C

for 1 hour, the reaction was terminated in ice and toluene was added. The absorbance values of the samples mixed by vortex were read in the spectrophotometer at 520 nm against toluene control (blind). L-proline was used as a standard and the proline concentration was determined as µmol proline g^{-1} DW.

2.4. Data analysis

Statistical values such as arithmetic mean and standard error of mean of morphological, physiological and biochemical properties determined in laurel seedlings were determined with the help of SPSS 22.0 for Windows package program. Moreover, analysis of variance was applied to determine the effects of drought stress on the measured parameters of laurel saplings. Values with $p < 0.05$ were considered statistically significant in the analyzes and Duncan test was applied to these variables. In order for the analyzes to be reliable, normality control was performed in the necessary data.

3. Results

3.1. Growth characteristics

The changes in root collar diameter and height of seedlings grown under greenhouse conditions (Fig. 1) from the beginning of the drought stress experiment (June) until its completion (October) are given in Table 1 and Fig. 2. While the statistical difference between the three irrigation regimes in terms of root collar diameter and seedling height was insignificant ($p > 0.05$) in the first month of drought stress (July), significant differences emerged in August and October ($p \leq 0.001$) (Table 1).

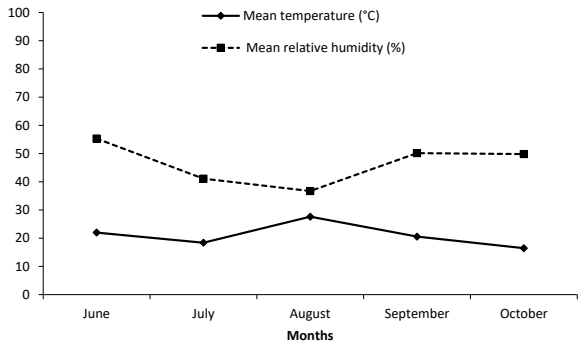


Fig. 1. The average relative humidity and temperature changes in the greenhouse during the experiment in 2021.

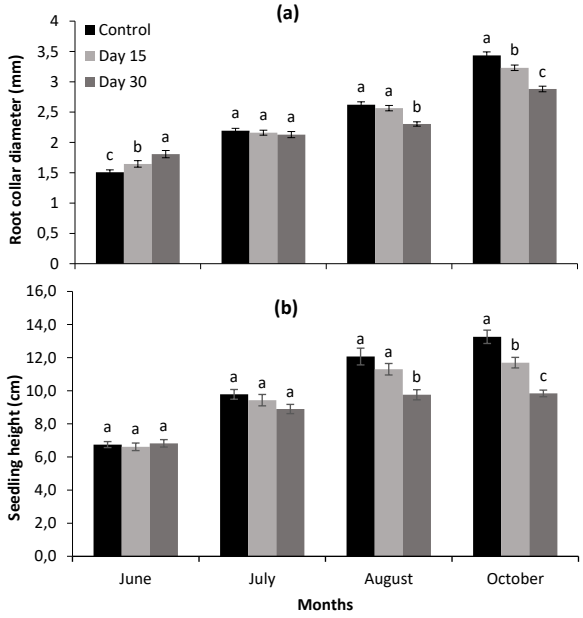


Fig. 2. Root collar diameter (a) and seedling height (b) changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

At the end of the experiment, root collar diameter and seedling height decreased due to increased stress ($p < 0.001$) (Fig. 2).

At the end of the drought stress trial (October), a significant difference was found between the treatments in terms of root, stem and leaf dry weight and stem:root ratio ($p < 0.01$) (Table 1 and Fig. 3–4). The effect of drought stress treatments on the number of leaves was found to be significant ($p < 0.001$). Seedlings irrigated every 30 days had the lowest weight (Fig. 3) and the least number of leaves (Fig. 4b).

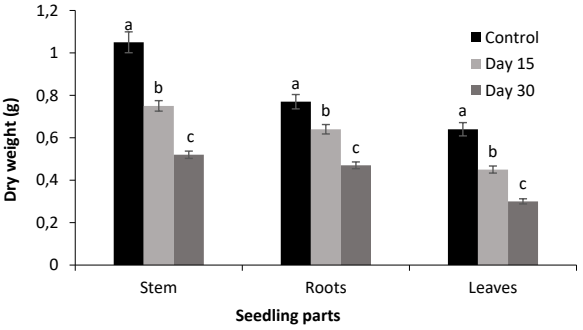


Fig. 3. Dry weight changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 1. Variance analysis (ANOVA) results of morphological, physiological and biochemical traits of the laurel seedlings grown under different drought stress.

Traits	df	F-value			
		June	July	August	October
Root collar diameter (mm)	2	18.05***	0.57^{ns}	13.82***	28.28***
Seedling height (cm)	2	0.22^{ns}	2.05^{ns}	7.44**	21.45***
Stem dry weight (g)	2	—	—	—	51.70***
Root dry weight (g)	2	—	—	—	29.39***
Leaf dry weight (g)	2	—	—	—	50.70***
Shoot:root ratio	2	—	—	—	7.41**
Number of leaves	2	—	—	—	23.61***
Midday xylem water potential (MPa)	2	1.94^{ns}	26.39***	25.49***	59.10***
Net photosynthesis rate (µmol CO_2 m^{-2} s^{-1})	2	1.69^{ns}	34.49***	93.75***	33.47***
Stomatal conductance (mol H_2O m^{-2} s^{-1})	2	19.61***	25.29***	77.27***	31.42***
Transpiration rate (mmol H_2O m^{-2} s^{-1})	2	22.68***	27.27***	85.44***	44.04***
Water use efficiency	2	110.05***	20.50***	11.21***	22.00***
Chlorophyll a (mg g^{-1})	2	—	—	—	27.92***
Chlorophyll b (mg g^{-1})	2	—	—	—	30.81***
Total clorophyll (mg g^{-1})	2	—	—	—	29.65***
Carotenoids (mg g^{-1})	2	—	—	—	67.89***
Proline (µmol g^{-1})	2	—	—	—	28.88***
TCC in stem (mg g^{-1})	2	—	—	—	3.80*
TCC in leaves (mg g^{-1})	2	—	—	—	5.82*
TCC in roots (mg g^{-1})	2	—	—	—	34.20***

Notes: not significant; *, ** and ***: significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. TCC: Total carbohydrate content.

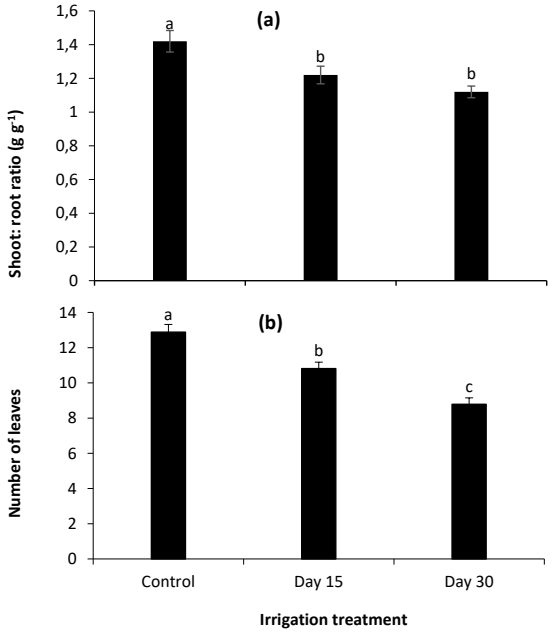


Fig. 4. Shoot : root ratio (a) and number of leaves (b) changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

3.2. Midday xylem water potential and leaf gas exchange

Drought stress was found to be effective on the midday xylem water potential in July, August and October at $p < 0.001$ significance level (Table 1 and Fig. 5). Differences in photosynthetic properties were also found (Fig. 6). A significant difference was found between the three irrigation regimes in terms of net photosynthesis rate, stomatal conductance, transpiration rate and water use efficiency ($p < 0.001$) (Table 1). As drought stress increased, the least irrigated seedlings showed lower values in midday xylem water potential, net photosynthesis rate, stomatal conductance and transpiration rate, while on the other hand, an increase in water use efficiency according to the months was determined (Fig. 5–6).

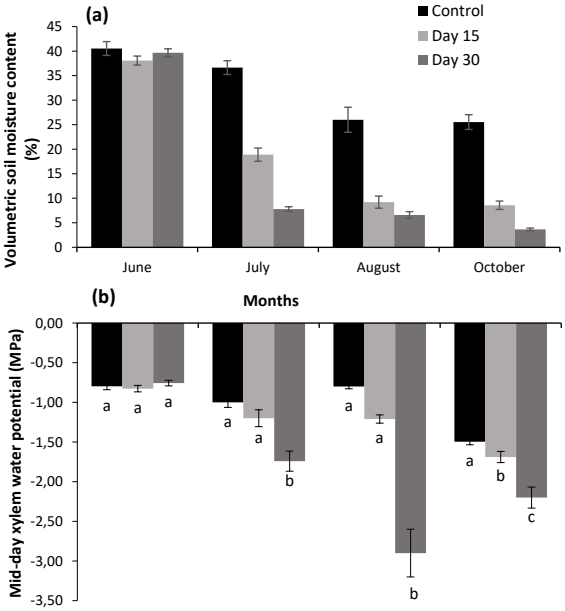


Fig. 5. Volumetric soil moisture content (%) (a) and midday xylem water potential (MPa) (b) changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

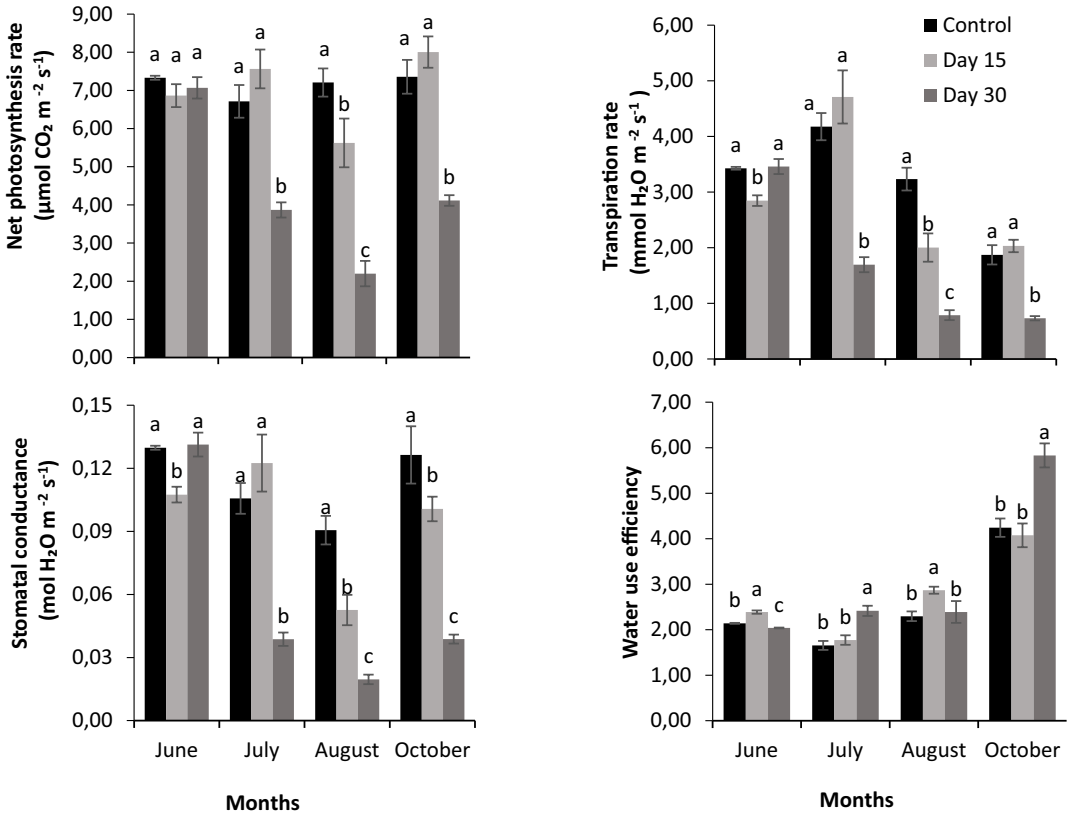


Fig. 6. Photosynthetic characteristics changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

3.3. Photosynthetic pigments, proline and total carbohydrate content

As a result of the analysis of variance for photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), the difference between different irrigation groups was found significant ($p < 0.001$) (Table 1). As drought stress increased, an increase in photosynthetic pigments was detected (Fig. 7). According to the results of the proline analysis, a difference was determined at the $p < 0.001$ significance level of the treatments (Table 1). It was determined that while the seedlings exposed to drought stress had high proline values, control seedlings had the lowest (Fig. 8). It was determined that the applied irrigation regimes had significant effects on the total carbohydrate content ($p < 0.05$) (Table 1). It was observed that in the stem and root samples the highest values were obtained under the irrigation process applied every 30 days, while in the leaf samples the highest values were obtained within the irrigation regime applied every 15 days (Fig. 9).

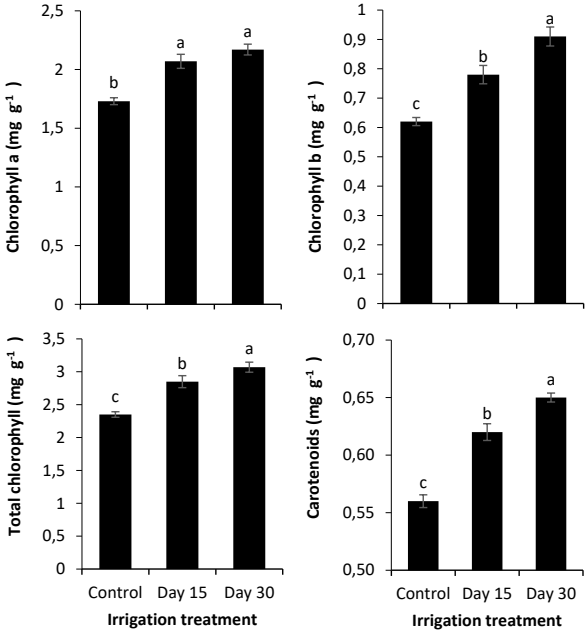


Fig. 7. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

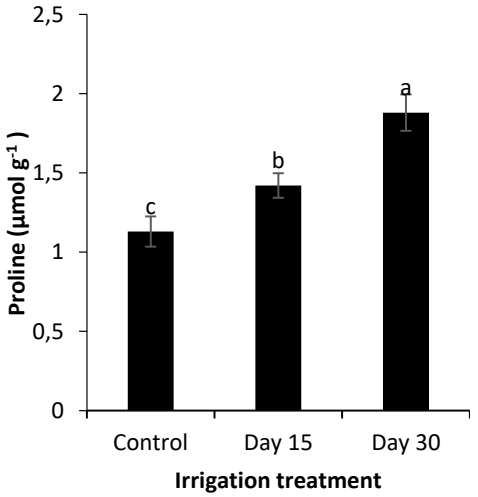


Fig. 8. Proline content changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

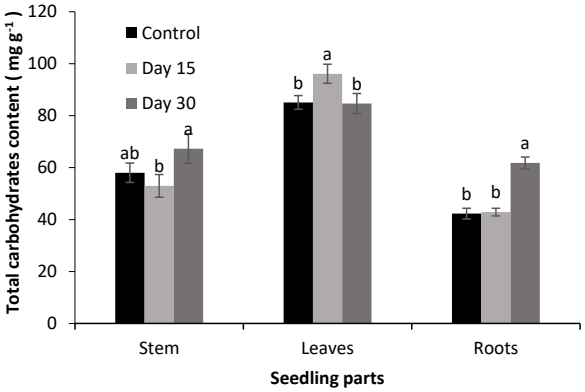


Fig. 9. Total carbohydrate content changes of laurel seedlings grown under different drought stress treatments under greenhouse conditions. Data are shown as mean ± SE. Different letters indicate significant differences between treatments ($p < 0.05$).

4. Discussion

Drought stress is one of the most common environmental limitations affecting the growth and productivity of plants (Kalefetoğlu & Ekmekçi 2005). Water shortage causes loss of turgor, impaired mitosis and reduced cell elongation and growth, which affects overall plant growth. As the water potential decreases, the plant decreases in height, diameter and number of leaves, resulting in decreased photosynthesis and dry weight accumulation. One change in plant functions leads to another and may cause irreversible damage to the plant (Jaleel et al. 2009; Anjum et al. 2017).

Root collar diameter and seedling height were affected by drought stress applied with different irrigation regimes. In August the root collar diameter of the control group laurel seedlings irrigated regularly and the seedlings irrigated every 15 days were found to be thicker than the seedlings irrigated every 30 days. At the end of the drought stress trial, it was determined that the diameter of the root collar decreased as the stress level increased. Almost similar results were obtained in terms of seedling height. Although the seedling height was similar in all treatments in June and July, in August and October the shortest seedlings were found in 30 days of irrigation group. The decrease in growth may be due to some reduction in leaf biomass (leaf number and weight) in drought-stressed seedlings. Lower net photosynthesis rate in 30 days of irrigation group contributed to their declining growth. The negative effect of drought stress on root collar diameter and seedling height has also been determined by many researchers in different tree and shrub species (Atta et al. 2012; Ravanbakhsh et al. 2023). It has been determined that as the stress level increases in some oak species, the diameter of the root collar and the height of the seedling decrease (Deligöz & Bayar 2018). It was observed that the increase in plant height slowed down as water stress increased in *Prunus mahaleb* L. rootstocks (Özyurt & Akça 2017). As drought stress increased in *Ostrya carpinifolia* Scop. populations, decreases in diameter, height and biomass of seedlings occured (Yılmaz et al. 2022). It has been reported that the decrease in plant height after drought stress is due to decreased cell expansion, increased leaf fall, and impaired mitosis (Yang et al. 2021). In this study, it was determined that the number of leaves decreased as the irrigation interval increased in August and October. Plants try to reduce the number of leaves by shedding their leaves in the face of water loss. In addition, they also differed significantly in their stem : root ratio and dry weight. Decreased shoot : root ratio under drought stress can be considered as a drought avoidance strategy (Kou et al. 2022). Root, stem and leaf weight were adversely affected by drought stress. Likewise, Pasha et al. (2019), got similar results in a study and reported a decrease in stem length, stem diameter, number of leaves, root diameter, stem and root fresh and dry weights of laurel seedlings following water restriction. These findings are in line with those of many other studies on drought stress.

In recent years, it was determined that long-term drought stress changed the morphological, physiological and biochemical properties of 11 tree species (Bhusal et al. 2021). Likewise, the same results were found for eucalyptus species exposed to drought conditions. Drought stress significantly affected anatomical, physiological and biochemical features (Saadaoui et al. 2017). In response to water deficiency, leaf water potential, stomatal conductance, photosynthesis, transpiration, chlorophyll, proline, carbohydrate content etc. the change in properties has been reported in many studies for many years. Leaf water potential is considered a reliable parameter to measure the plant's response to

water stress (Siddique et al. 2000). Due to the increase in the applied irrigation interval, drought stress caused a decrease in the midday xylem water potential in laurel seedlings. In another study, Acacia species responded in the same way. It has been reported that drought stress applied at 100%, 50% and 25% field capacity in *Acacia ehrenbergiana* Hayne and *Acacia tortilis* (Forssk.) Hayne seedlings causes significant decreases in leaf water potential (Atta et al. 2012). Likewise, in a study conducted by Maatallah et al. (2010) in two different laurel origins, it was determined that the predawn leaf water potential was negatively affected by water stress.

Midday xylem water potential can be considered an indicator of daily maximum water stress because it is measured at midday when the temperature and irradiance and vapor pressure deficit reached the highest value. The decrease in vapor pressure in the sub-stomatal space in the leaves due to negative water potential has an important role in the leaf gas exchange (Vesala et al. 2017). The rate of photosynthesis is determined by changes in the rate of CO_2 diffusion into the leaves through stomata and photosynthetic activity in mesophyll cells. Drought has a negative effect on the photosynthetic apparatus, membrane and enzymes (David et al. 2007). In laurel seedlings, drought stress was affected net photosynthesis rate, transpiration, stomatal conductance and water use efficiency. During the dry period, a decrease in the net photosynthesis rate, stomatal conductivity and transpiration rate in August and an increase in water use efficiency according to the months affiliated with the increased drought stress were determined. Similarly, a decrease in net photosynthesis rate and stomatal conductance was observed in stressful conditions (summer drought in July) compared to non-stressed conditions (spring – May) on *Laurus nobilis* L. and *Quercus ilex* L. leaves collected at different seasons of the year (Arena et al. 2008). For *Quercus alba* L. and *Q. stellata* Wangenh., net photosynthesis and stomatal conductance decreased in arid conditions compared to well-watered conditions (Ni & Pallardy 1991). Although the decrease in photosynthesis caused by water stress can be attributed to the stomatal closure, CO_2 uptake may be limited by non-stomatal limitations, especially when water stress is intense and continues for long time (Varonea et al. 2012). In this study, additional parameters are needed to determine the stomatal and non-stomatal limitations to photosynthesis.

Under drought conditions, stomatal conductance in leaves of laurel decreased along with transpiration rate and photosynthesis rate. Photosynthesis and transpiration are directly affected by changes in stomatal conductance. Increase water use efficiency the least irrigated (30^{th} day) seedlings may be associated with slow decrease in photosynthesis rate along with a rapid decrease in stomatal conductance and transpiration rate. This may indicate that laurel may improve its ability to adapt to drought stress by increasing the water use efficiency (Wu et al. 2022). Net photosynthesis rate, transpiration rate, a decrease in stomatal conductance, and an increase in water use efficiency were reported in *Populus przewalskii* Maxim. cuttings under two different irrigation regime conditions (well-watered, stressed) (Yin et al. 2006). Likewise, there was an increase in water use efficiency in response to water stress in *Acer negundo* L. and *Malus baccata* [L.] Borkh. species (Ranney et al. 1990).

Another reliable indicator of drought stress in plants is the change in chlorophyll content. Since chlorophyll pigments play a major role in photosynthesis, they show the response of plants to habitats, weather conditions and anthropogenic conditions, thus the vitality of plants and their resistance to stressful conditions (Zielewicz et al. 2020). A common issue under drought stress is decreases in chlorophyll content (Atta et al. 2012; Wu et al. 2013; Maatallah et al. 2016; Yavas & Unay 2016; Tariq et al. 2018; Guo et al. 2019). However, Pirzad et al. (2011) reported increased chlorophyll accumulation. When the results of this study were evaluated, significant differences were determined in terms of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid between well-watered seedlings and those under drought stress, and an increase in the determined photosynthetic pigments was detected. An increase in chlorophyll content was also found in eucalyptus under water stress conditions (Michelozzi et al. 1995). High chlorophyll values in dry conditions indicate the severity of stress on plants and reduced leaf area (Yavas & Unay 2016). Increase in chlorophyll content helps the plants to cope with changes in water status of plants for some extent. In this study, increased chlorophyll content under drought stress may mean that laurel seedlings tolerate drought stress by keeping higher chlorophyll concentration due to dehydration.

In this study, high proline values were determined in the most stressed seedlings. Similar results were obtained in the drought stress experiment applied to *Ostrya* populations. It was determined that there was an increase of 14% of the proline amount in *Ostrya* seedlings subjected to severe drought (Yılmaz et al. 2022). Similarly, similar findings were obtained for *Swietenia macrophylla* King species. Proline content was higher in water-stressed plants. It is suggested that this is due to the osmotic adaptation that occurs in plants exposed to drought (Yvens et al. 2009). In this study, increases in proline content at the end of experiment seem to be in line with decreases midday xylem water potential. The least irrigated (30^{th} day) seedlings had higher soluble sugar in roots and proline content than the control seedlings. The increase in soluble sugar concentration may be contribute to enhanced desiccation tolerance and allows metabolic activity to be maintained (Naser et al. 2010). The drought tolerance mechanism of laurel may be related to the accumulation of osmoprotectants such as proline and soluble sugars. Osmolytes regulate the opening of guard cells that influence inflow of CO_2 for photosynthesis. An increase in soluble sugar and proline content was determined in

coniferous species *Pinus sylvestris* L. and *Pinus massoniana* Lamb. under drought stress conditions (Sudachkova et al. 2002; Shao et al. 2022).

5. Conclusions

Drought stress negatively affected the growth of laurel seedlings. As drought stress increased, seedling height, root collar diameter and number of leaves decreased. Seedlings exposed to intense drought stress showed decreased midday xylem water potential, net photosynthesis rate, stomatal conductivity and transpiration rate. However, as a result of laurel's tolerance to drought stress, photosynthetic pigments, total soluble sugar and proline accumulation increased. Results shows that when exposed to drought, laurel finds a sustainable use of water and strategies that allow it to tolerate the negative effects of drought stress. Laurel is known as a drought tolerant species. However, such studies should be followed by other studies to find ways to increase drought resistance.

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