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Original Article

Physiological, oxidative, and antioxidative responses of *Quercus vulcanica* Boiss. and *Quercus aucheri* Jaub. & Spach. under drought stress conditions

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ABSTRACT

Background and Aims: Drought stress is one of the most common global factors of abiotic stress affecting plant growth and productivity world-wide. The present study aims to assess the effects of 1, 2, and 4 weeks of drought stress on the two endemic tree species, *Quercus vulcanica* and *Q. aucheri*

Methods: After applying drought stress conditions, the study determines the physiological parameters, markers of oxidative damage, and levels of antioxidant enzymes in the leaves and stems of 6-month-old oak seedlings.

Results: The dry and fresh weights were observed to decrease by at least 11.44%, as well as the chlorophyll levels by at least 14%, in both the *Q. vulcanica* and *Q. aucheri* that were subjected to 1, 2, and 4 weeks of drought stress. The carotenoid, proline, and anthocyanin levels also increased in the leaves of both Quercus species; however, the amount of ascorbic acid, reduced glutathione (GSH), and total soluble protein decreased in the leaves and stems of both *Quercus* species. As oxidative stress markers, the levels of hydrogen peroxide (H_2O_2) and lipid peroxidation were seen to elevate by at least 1.21 fold under drought stress conditions. This revealed some of the alterations in the activities of antioxidant enzymes in the leaves and stems of both *Q. aucheri* and *Q. vulcanica*.

Conclusion: In conclusion, this study revealed increasing drought stress to have a substantial impact on the physiological parameters and oxidative and antioxidant responses in *Q. vulcanica* and *Q. aucheri*. This will contribute to understanding how oak species respond to drought stress and their adaptation strategies.

Keywords: *Q. vulcanica, Q. aucheri*, drought stress, oxidative stress, antioxidant enzyme system

INTRODUCTION

Due to increased industrial activity, the combustion of fossil fuels, the release of hazardous waste materials into the biosphere, and the release of greenhouse gases into the atmosphere as technology advances, global warming and droughts have become important environmental concerns. Plants cannot avoid stress in nature due to being in fixed locations and being exposed to various stress factors throughout their lifespan (Rao et al., 2006). Drought causes various changes in plants in terms of their ecological, morphological, physiological, biochemical, and molecular aspects (Lichtenthaler, 1996; Rao et al., 2006). These might result in different outcomes, such as inhibited growth, turgor loss, decreased pigment and protein content, and reductions in the quantity and quality of their yield. Additionally, drought increases the formation of free radicals and reactive oxygen species (ROS) that damage photosynthetic pigments, membrane lipids, proteins, and nucleic acids and also affects antioxidant enzyme systems (Egert & Tevini, 2002; Mittler, 2006; Noctor et al., 2018; Suzuki & Mittler, 2006; Yordanov et al., 2000). A balance exists between free radical formation and antioxidant defense systems in organisms. The excessive formation of free radicals and insufficient antioxidant defense due to various environmental factors or metabolic activities cause oxidative damage to the structure and functions of plant cells (Noctor et al., 2018; Suzuki & Mittler, 2006).

Drought causes negative effects on plants, and as a result, plants develop an adaptive response to stressful conditions. Therefore, studies on identifying plant species with high drought resistance or tolerance have become increasingly important.

Oaks are widely distributed trees in the Mediterranean region and can cope with a variety of environmental stressors due to

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the remarkable flexibility of their phenotypic and physiological features (Cotrozzi et al., 2016). The literature contains some studies on the negative effects of various drought stress conditions on some *Quercus* species, including *Q. ilex* (Echevarría-Zomeño et al., 2009; Cotrozzi, Pellegrini, et al., 2017; Simova-Stoilova et al., 2018); *Q. ilex, Q. cerris, Q. pubescens* (Cotrozzi et al., 2016), *Q. cerris* (Cotrozzi, Remorini, et al., 2017); *Q. infectoria* and *Q. libani* (Ghanbary et al., 2020); *Q. cerris* and *Q. pubescens* (Landi et al., 2019); *Q. ilex, Q. pubescens,* and *Q. robur* (Pellegrini et al., 2019); Q. lusitanica (Santamarina et al., 2022); and Q. serrata, *Q. serrata, Q. acutissima*, and *Q. variabilis* (Xiong et al., 2022). The aim of the study is to investigate drought stress on two endemic oak species (*Q. vulcanica* and *Q. aucheri*) in Türkiye by evaluating their physiological and biochemical responses. This approach will enable the investigation of the potential negative effects and adaptive responses in these species, ensuring the determination of resistant species. The research involves subjecting oak saplings to controlled drought conditions and comparing them with well-watered control groups. Thus, the study comparatively examines the effects of 1, 2, and 4 weeks of drought stress on the physiological and biochemical parameters (fresh weight, dry weight, chlorophyll, and carotenoid content, proline and anthocyanin content), oxidative damage (MDA, H_2O_2), and antioxidant systems (ascorbic acid, GSH, oxidized glutathione, superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase) in the leaves and stems of *Q. vulcanica* and *Q. aucheri*. This study will contribute to understanding the mechanisms underlying drought stress and determining the effects of drought stress on the biological diversity of endemic oaks.

MATERIALS AND METHODS

Collection, cultivation and preparation of plant materials for the experiment

The study was able to collect 100 seeds in autumn from different parts of 10 oak trees for each of the studied species in their natural distribution area. The seeds for the *Kasnak Meşesi* [Kotschy oak (Fagaceae)], or *Q. vulcanica* Boiss. & Heldr. ex, were collected from the Yukarıgökdere village of the Eğirdir municipality in Isparta province and for the Boz-Pırnal oak (Fagaceae), or *Q. aucheri* Jaub & Spach., from the city of Muğla in Muğla province. The seeds were then moved to a laboratory and stored at room temperature until the analysis. The seeds were identified and confirmed by Prof. Dr. Osman Erol and Asist. Prof. Dr. Erdal Üzen. The collected seeds were carefully cleaned of debris in the laboratory before being subjected to vernalization at +4°C for approximately one month. After vernalization, the seeds were soaked in a 10% sodium hypochlorite solution for 25 minutes and washed with distilled water three to four times for surface sterilization. The seeds were placed in petri dishes containing filter paper soaked with distilled water and left to germinate in a growth chamber at 25°C. To determine the germination rate of the seeds, they were placed in petri dishes with a diameter of 15 cm in an air-conditioned cabinet and placed in an oven at 25°C. Trials were carried out, with a germination rate of 90% being determined for both species.

The germinated seeds were transferred into pots and first grown individually in a mixture of sand (sieved and washed) and perlite (1:1 v/v) in a climate chamber under optimal conditions (Ozden & Baycu, 2004). The pot experiment was carried out in the phytotron (14 h light and 10 h dark period; light intensity = 7000 lux; temperature = 22°C ± 2°; humidity = 48% ± 2%), and the tree seedlings were watered every other day with a modified Ingestad nutrient solution (Ingestad, 1970; pH 5.8) for 4 months until they had developed 8–10 pinnate leaves. The tree seedlings were then carefully removed from their containers and transplanted into individual pots. Six plants were grown in black polypropylene vessels of 10 L volume under the same phytotron conditions for another 2 months, with the nutrient solutions being changed every other day. Later, the 6-month-old tree seedlings were divided into six experimental groups that were subjected to a drought stress for 1, 2 and 4 weeks alongside their corresponding control groups. Each treatment was comprised of six seedlings divided into three replicated culture vessels. The control groups maintained their regular development by receiving water and the Ingestad nutrient solution every other day. For the drought stress groups, the Ingestad nutrient solution was applied once a week. After the treatments, the oak seedlings were harvested, and some of leaves and stems were stored at -20°C until the day of the physiological and biochemical analyses; some of these leaves and stems were also used fresh for the physiological assessments.

Measurement of fresh and dry weights

The study measured the fresh and dry weights of the leaves and stem samples from the control and 1, 2, and 4 weeks of drought stress-treated oak seedlings (Horwitz, 1970). The relative water content (RWC) was then calculated as a percentage using Equation 1 (Dhanda & Sethi, 1998).

RWC (%)
= [(Fresh weight (fw) (g) - Dry weight (g)) / fw (g)] x 100 (1)

Measurement of chlorophyll and carotenoid contents

To measure the chlorophyll and carotenoid contents, fresh leaves weighing 2 g were taken, cut into small pieces, and homogenized in ice-cold acetone (100%, v/v) and $CaCO_3$ using an Ultra-Turrax (Janke & Kunkel, Germany). The leaf homogenates were then centrifuged at 3,000 g for 10 min. (Heraeus Labofuge 400 R, Germany) before collecting the supernatant. The absorbance of the supernatant was measured at 662 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for all carotenoids (Jenway 6105 UV-Vis Spectrophotometer, Great Britain). The results were calculated using Equations 2,

3, and 4 (Lichtenthaler & Wellburn, 1983) and expressed as mg/g FW for chlorophylls a and b and for all the carotenoids.

Chlorophyll-a = (11.75 x A662 - 2.35 x A645) x 10/mg fw (2)

Chlorophyll-b = (18.61 x A645 - 3.96 x A662) x 10/mg fw (3)

Total carotenoids
= [(1000 x A470 – 2.27 x Chl-a – 81.4 x Chl-b) /227] x 10/mg fw (4)

Measurement of the contents of proline and anthocyanins

The proline content in the leaves of the oak seedlings was determined according to the method described by Bates et al. (1973) using 200 mg of fresh weight (fw) samples and calculated based on the absorbance values at 520 nm and expressed as µmol/g fw. The anthocyanins content in the leaves of the oak seedlings was determined according to the method described by Mancinelli (1990). Briefly, 500 mg of fw samples were extracted in acidified methanol (1%, v/v) and kept at +4°C for 2 days before being centrifuged at 5,000 g. The supernatant was collected, and the absorbance was measured at 530 nm and 657 nm; the anthocyanins content is calculated using the Equation 5 and expressed as (A_{530}-0.33xA_{657})/g fw.

Anthocyanins content = A_{530} - 0.33 x A_{657} (5)

Measurement of hydrogen peroxide (H_2O_2) content and lipid peroxidation products

The H_2O_2 content in the leaves and stems of the oak seedlings was determined according to the method described by Velikova et al. (2000) using 500 mg of leaves and stem samples. The H_2O_2 content was calculated based on the standard curve prepared using H_2O_2 standard solutions and is expressed as µmol/g fw.

The malondialdehyde (MDA) content, which is an indicator of lipid peroxidation, was determined in the leaves and stems of the oak seedlings in accordance with the method described by Heath and Packer (1968). The principle of the method relies on the reaction of MDA with thiobarbituric acid (TBA) under acidic and high-temperature conditions. The MDA content was calculated using Equation 6 and is expressed as nmol/g fw.

MDA (nmol/g fw) = [(A_{532}-A_{600} / 155 mM^{-1} cm^{-1}) / fw (mg)] x 106 (6)

Measurement of glutathione and ascorbic acid contents

The contents of the reduced glutathione (GSH), oxidized glutathione (GSSG), and total glutathione (GSH + GSSG) in the leaves and stems of the oak seedlings was determined according to the method described by Gossett et al. (1994) using 500 mg of fw samples. The GSH + GSSG and GSSG contents were calculated using the standard curve prepared using GSH standard solutions. The GSH content was calculated by subtracting the GSSG content from the GSH + GSSG content. The results are expressed as nmol/g fw.

The ascorbic acid content in the leaves and stems of the oak seedlings was determined according to the method described by Gossett et al. (1994) using 500 mg of fw samples and calculated based on the standard curve prepared using standard solutions. The value is expressed as µmol/g fw.

Measurement of antioxidant enzymes

For the extraction of the antioxidant enzymes, 200 mg of leaves and stem samples were mixed with Triton X-100 (2%, v/v), ascorbate (5 mM), and 400 mg of polyvinylpolypyrrolidone in a KH2PO4/K2HPO4 buffer (10 mM, pH 7.8) for 1 minute and incubated on ice for 30 minutes. The homogenate was then centrifuged at 48,400 g at 4°C (Schwanz et al., 1996). The supernatant was collected and used to determine the total soluble protein and antioxidant enzyme activities.

The protein content in the leaves and stems of the oak seedlings was determined using the method described by Bradford (1976) and calculated using a standard curve prepared with bovine serum albumin (BSA) standard solutions and expressed as mg/g fw.

The superoxide dismutase (SOD) enzyme activity in the leaves and stems of oak seedlings was determined using the method described by Beyer and Fridovich (1987). The measurement of SOD activity is based on the reduction of nitro blue tetrazolium chloride (NBT) by O_2 radicals under light. The amount of enzyme that causes a 50% inhibition in the reduction of NBT was considered one unit, with SOD activity being expressed as U/g fw. The catalase (CAT) enzyme activity in the leaves and stems of oak seedlings was determined using the method described by Aebi (1984). The principle of the method is based on the enzymatic decomposition of the H_2O_2 substrate by CAT, which is monitored at 240 nm. The CAT activity was calculated using a standard curve prepared with H_2O_2 standard solutions and expressed as U/g fw.

The ascorbate peroxidase (APX) enzyme activity in the leaves and stems of oak seedlings was determined using the method described by Nakano and Asada (1987). The APX activity was calculated using the molar extinction coefficient of 2.8 $mM^{-1}cm^{-1}$ at 290 nm and expressed as U/g fw. The guaiacol peroxidase (GuPX) enzyme activity in the leaves and stems of the oak seedlings was determined using the method described by Cakmak (1994) and expressed as U/g fw. The glutathione reductase (GR) enzyme activity in the leaves and stems of the oak seedlings was determined using the method described by Foyer and Halliwell (1976) and expressed as U/g fw.

Statistical analysis

All values in the obtained data were calculated as a mean ± standard deviation (*M* ± *SD*). The statistical evaluation of the

data was performed using the software package SPSS 14.0 for Windows and applying the one-way analysis of variance analysis (ANOVA) with post-hoc test and least significant difference (LSD) test. A p-value of less than 0.05 or 0.001 was considered statistically significant.

RESULTS

The effect of drought stress on the fresh and dry weight of oak seedlings

The fresh and dry weight of the *Q. vulcanica* and *Q. aucheri* leaves and stems significantly decreased in the groups that had been subjected to drought stress for 1, 2, and 4 weeks compared to the control group, indicating a significant decrease of at least 11.44% ($p < 0.001$), decreasing more with longer durations of drought stress (Figures 1a & 1b). The RWC in the leaves and stems of the *Q. vulcanica* seedlings was observed to significantly decrease by at least 3.77% ($p < 0.001$) after 1, 2, and 4 weeks of drought stress compared to the control group (Figure 1c). However, the decrease in RWC in the leaves of the *Q. aucheri* seedlings was not significant, while a significant decrease of at least 5.29% ($p < 0.001$) was observed in their stems after 2 and 4 weeks of drought stress (Figure 1c).

The effect of drought stress on the chlorophyll and total carotenoid contents

In the groups subjected to 1, 2, and 4 weeks of drought stress, a significant decrease of at least 14% ($p < 0.05$) was observed in the content of chlorophyll a in the leaves of the *Q. vulcanica* seedlings and of at least 49.17% ($p < 0.001$) in the leaves of the *Q. aucheri* seedlings compared to the control group (Figure 2a). The chlorophyll b content showed a non-significant decrease of 5.53% in the 1-week drought stress group, while significant decreases of at least 29.35% ($p < 0.05$) were observed in the 2- and 4- week groups for *Q. vulcanica* and of at least 46.68% ($p < 0.001$) for *Q. aucheri* in all drought stress groups (Figure 2b). The total chlorophyll content showed a significant decrease of 11.85% in the 1-week drought stress group and a significant decrease of at least 32.85% ($p < 0.05$) was observed in the 2- and 4-week groups for *Q. vulcanica*, while significant decreases of at least 34.63% ($p < 0.001$) were observed for *Q. aucheri* in all drought stress groups (Figure 2c). With regard to the total carotenoid content, increases of 3.7 fold ($p < 0.001$) and 1.52 fold ($p < 0.001$) were observed in all the drought stress groups for *Q. vulcanica* and *Q. aucheri*, respectively (Figure 2d).

The effect of drought stress on the proline, anthocyanins, and total soluble protein contents

The groups subjected to 1, 2, and 4 weeks of drought stress showed a significant 1.17-fold increase in the proline content in the leaves for *Q. vulcanica* ($p < 0.05$) and 2.11-fold for *Q.*

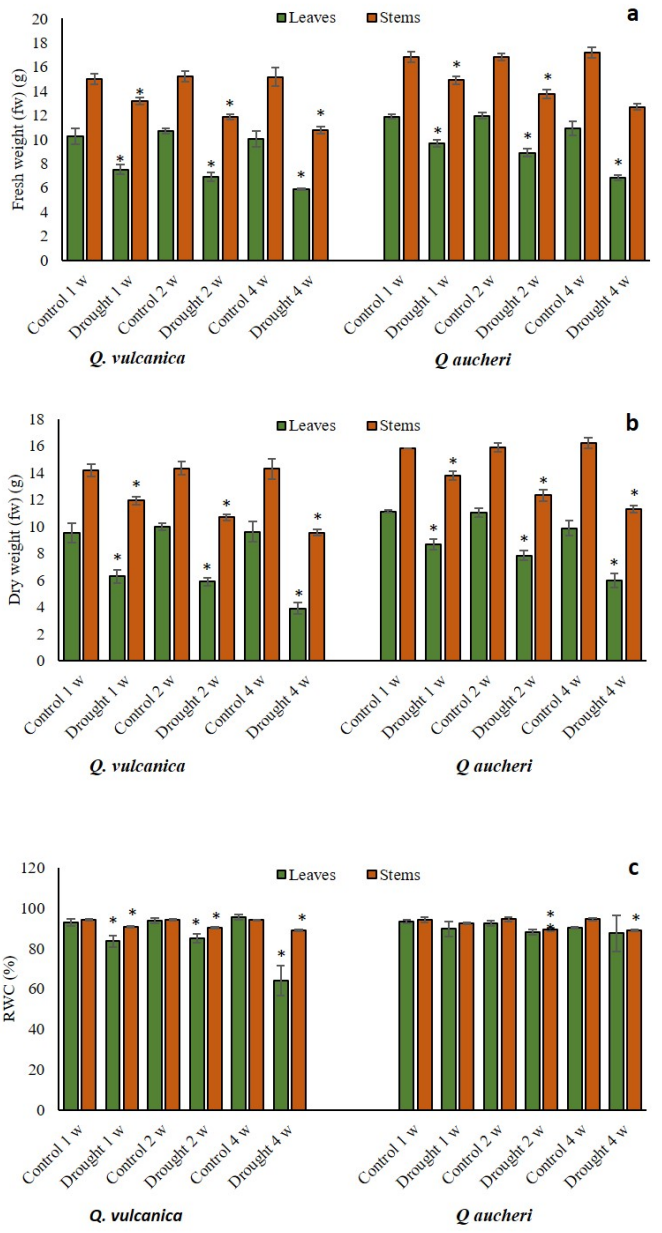

Figure 1. The effects of 1, 2, and 4 weeks of drought stress on (a) fresh weight, (b) dry weight, and (c) relative water content in the leaves and stems of *Q. vulcanica* and *Q.aucheri.* Data are presented as $M \pm SD$ (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by $*p < 0.05$ and $**p < 0.001$).

aucheri ($p < 0.001$) compared to the control group (Figure 3a). The groups subjected to 1, 2, and 4 weeks of drought stress saw the anthocyanins contents in the leaves of the *Q. vulcanica* and *Q. aucheri* seedlings to significantly increase compared to the control group at respective rates of at least 12.1 fold and 4.9 fold ($p < 0.001$) and to correlate with the applied duration of drought stress (Figure 3b). The total soluble protein content increased by 1.6 fold ($p < 0.001$) under one week of drought stress while decreasing by 17.42% under two weeks and 19.61% under four weeks of drought stress ($p < 0.05$) in the leaves

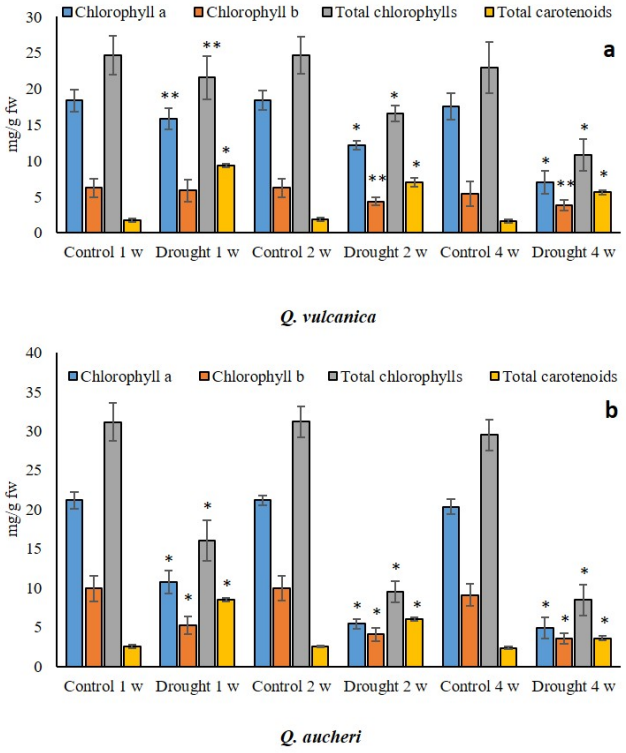

Figure 2. The effects of 1, 2, and 4 weeks of drought stress on the chlorophyll and total carotenoid levels in the leaves of (a) *Q. vulcanica* and (b) *Q.aucheri.* Data are presented as *M* ± *SD* (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by **p* < 0.05 and ***p* < 0.001).

of the *Q. vulcanica* seedling (Figure 3c). In the *Q. aucheri*'s leaves, a decrease of at least 27.23% ($p < 0.001$) in total protein content was observed for all drought stress groups. However, both Quercus species exhibited at least a 1.58-fold increase ($p < 0.001$) in total protein content in their stems for all drought stress groups (Figure 3c).

The effect of drought stress on the oxidative stress markers

As shown in Figure 4a, H_2O_2 content was observed to significantly increase in the 1-, 2-, and 4-week drought stress groups at least 1.4-fold ($p < 0.001$) for the *Q. vulcanica* leaves, at least 2.3-fold ($p < 0.001$) for the leaves, at least 1.41-fold ($p < 0.05$) for the *Q. vulcanica* stems and at least 2.2-fold ($p < 0.001$) for the *Q. aucheri* stems compared to the control group, increasing in correlation to the duration of the applied drought stress. The MDA content showed varying responses in the leaves of *Q. vulcanica* for the drought stress durations compared to the control group (Figure 4b), with the 1-week group resulting in a notable decrease (54.64%, $p < 0.001$), the 2-week group showing minimal change, and the 4-week group leading to a significant 2.38-fold increase ($p < 0.001$) in MDA content, indicating a significant increase in oxidative stress under prolonged (i.e., 4-week) drought conditions. Meanwhile, MDA levels had at least a 1.21-fold increase in the stems of *Q. vulcanica* under all drought stress groups ($p < 0.05$). As for *Q. aucheri*, the MDA content showed a 1.39-fold increase in the leaves ($p < 0.001$) and 1.21-fold increase in the stems ($p < 0.05$) in response to drought stress durations compared to the control group (Figure 4b).

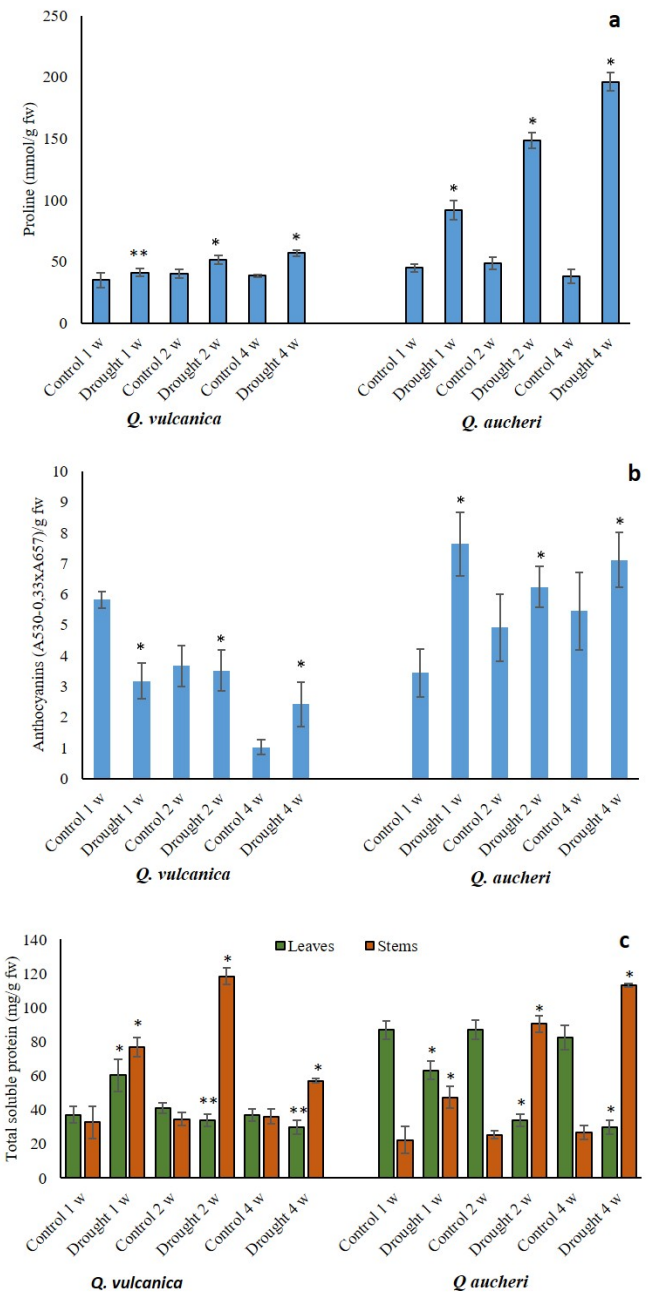

Figure 3. The effects of 1, 2, and 4 weeks of drought stress on the levels of (a) proline, (b) anthocyanins, and (c) total soluble protein in the leaves of *Q. vulcanica* and *Q.aucheri*. Data are presented as *M* ± *SD* (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by **p* < 0.05 and ***p* < 0.001).

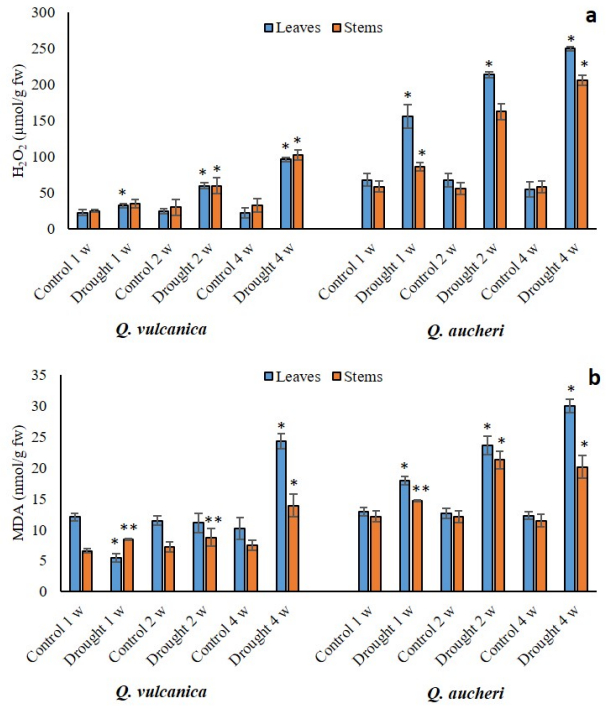

Figure 4. The effects of 1, 2, and 4 weeks of drought stress on (a) H_2O_2 and (b) MDA content in the leaves and stems of Q. vulcanica and Q.aucheri. Data are presented as M ± SD (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by $*p < 0.05$ and $**p < 0.001$).

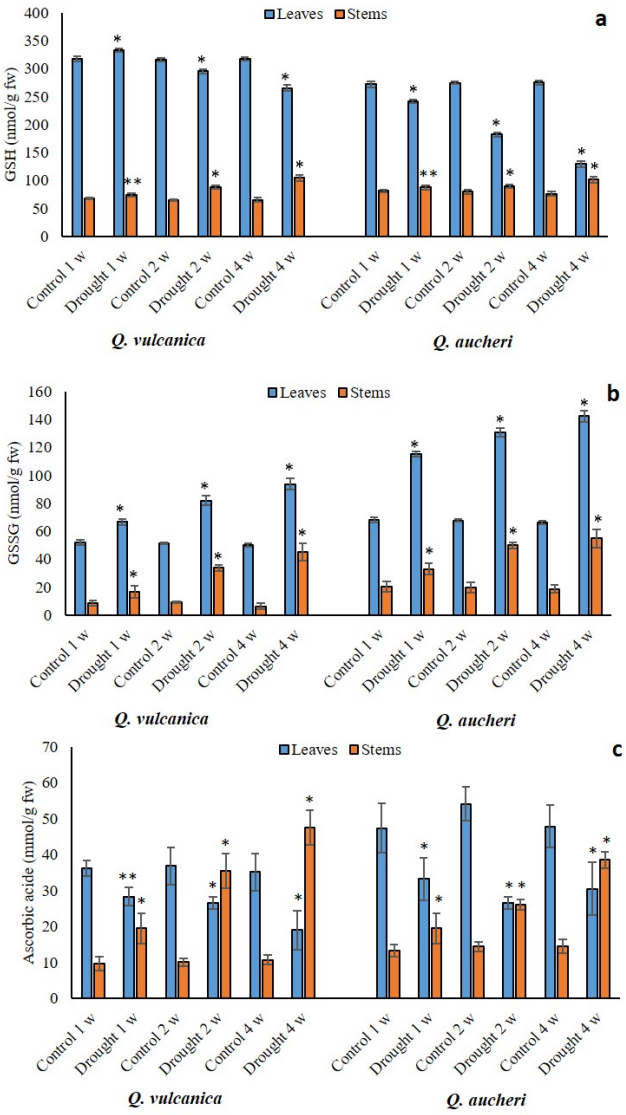

Figure 5. The effects of 1, 2, and 4 weeks of drought stress on the (a) GSH and (b) GSSG and (c) ascorbic acid content levels in the leaves and stems of *Q. vulcanica* and *Q.aucheri*. Data are presented as *M ± SD* (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by $*p < 0.05$ and $**p < 0.001$).

The effect of drought stress on the contents of GSH and GSSG and ascorbic acid

GSH content in the leaves was observed to significantly decrease by at least 6.75% in the 2-week and 4-week drought stress groups for *Q. vulcanica* ($p < 0.001$) and by at least 11.13% in all drought stress groups for *Q. aucheri* ($p < 0.001$), while GSH content increased at least 1.083 fold ($p < 0.05$) in the stems of both oak seedling types for all drought stress groups (Figure 5a).

The GSSG content was observed to significantly increase in the leaves of *Q. vulcanica* by at least 1.28 fold ($p < 0.001$), in the leaves of *Q. aucheri* by at least 1.69 fold ($p < 0.001$), in the stems of *Q. vulcanica* by at least 1.94 fold ($p < 0.001$), and in the stems of *Q. aucheri* by at least 1.67 fold ($p < 0.001$) for the 1-, 2-, and 4-week drought stress groups in line with the applied duration of drought stress (Figure 5b). In accordance with these results, alterations in the GSH+GSSG content were observed in both oak species.

The ascorbic acid content in the leaves of *Q. vulcanica* showed a significant decrease of at least 21.63% ($p < 0.05$), while the stems exhibited an increase of at least 2-fold ($p < 0.001$). Accordingly, the ascorbic acid content in the leaves of *Q. aucheri* showed a significant decrease of at least 29.91% ($p < 0.001$), while the stems exhibited an increase of at least1.47-fold ($p < 0.001$). These changes were observed to increase in line with the applied duration of drought stress (Figure 5c).

The effect of drought stress on antioxidant enzyme activity in oak seedlings' leaves and stems

The antioxidant enzyme activity is shown in Figures 6a-6e. The SOD activities were observed to significantly increase in the respective *Q. vulcanica* and *Q. aucheri* leaves by at least 8.6 fold ($p < 0.001$) or 1.6 fold ($p < 0.001$), and in the respective *Q. vulcanica* and *Q. aucheri* stems by at least 8.2 fold ($p < 0.001$) or 2.5 fold ($p < 0.001$). CAT activities were observed to significantly decrease in the respective *Q. vulcanica* and *Q.*

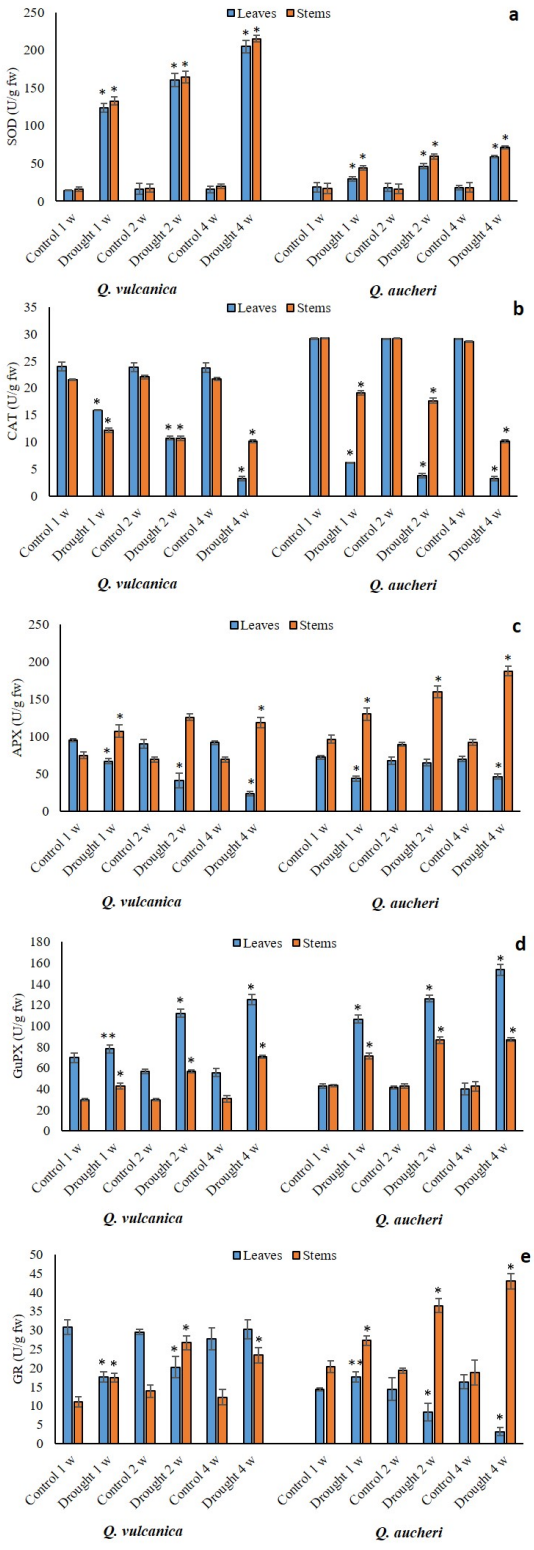

Figure 6. The effects of 1, 2, and 4 weeks of drought stress on the activities of (a) SOD, (b) CAT, (c) APX, (d) GuPx, and (e) GR content levels and (c) ascorbic acid levels in the leaves and stems of *Q. vulcanica* and *Q.aucheri*. Data are presented as mean ± SD. (Statistical analysis was performed using the ANOVA + LSD post hoc test. Statistically significant changes are indicated by $*p < 0.05$ and $**p < 0.001$).

aucheri leaves by at least 33.74% ($p < 0.001$) or 78.85% ($p < 0.001$), and in the respective *Q. vulcanica* and *Q. aucheri* stems by at least 43.58% ($p < 0.001$) or 34.7% ($p < 0.001$). APX activities were observed to significantly decrease by at least 29.7% ($p < 0.001$) in the leaves of *Q. vulcanica* and *Q. aucheri* (excluding the 2-week group), while increased at least 1.35-fold ($p < 0.001$) in the stems of both oak species. GuPx activity was observed to significantly increase in the leaves and stems of *Q. vulcanica* by at least 1.12 fold ($p < 0.05$) and of *Q. aucheri* by at least 1.65 fold ($p < 0.001$). Some alteration occurred regarding GR activity in the leaves of both oak species' seedlings, decreasing in particular in the 4-week drought stress group by at least 80.9% ($p < 0.001$) for *Q. aucheri*, while increasing significantly by at least 1.34-fold ($p < 0.001$) in the stems of both oak species' seedlings.

DISCUSSION

Drought stress is a significant environmental factor that can have profound effects on plant growth and survival. Among the various plant species, oaks (*Quercus* spp.) are particularly important due to their ecological and economic value. Understanding the impact of drought stress on oak species is crucial for effective conservation and management strategies. Therefore, this study has aimed to investigate the effects of drought stress on the physiological, oxidative and antioxidant parameters of two oak species.

Fresh and dry weights for the leaves and stems of *Q. vulcanica* and *Q. aucheri* seedlings is seen to decrease significantly as the applied duration of drought stress increases due to increased water loss and protein degradation (Perales-Vela et al., 2007). Accordingly, RWC also decreased in the leaves and stems of the two oak species under drought stress. Decreased water content has been detected in the leaves of *Q. pubescens* during drought (Gallé & Feller, 2007). Water content has also been seen to decrease after 14 days of drought stress in the leaves of *Q. ilex* (Echevarría-Zomeño et al., 2009) and in the leaves of *Q. ilex, Q. cerris,* and *Q. pubescens* (Cotrozzi et al., 2016), as well as in the shoots of *Q. lusitanica* after 22 weeks (Santamarina et al., 2022). In Cotrozzi, Pellegrini et al.'s (2017) study, a 15-day drought stress period showed no changes in RWC in *Q. ilex*. Landi et al. (2019) showed RWC to decrease in the leaves of *Q. cerris* and *Q. pubescens* (10% and 6%, respectively). The leaf water potential of *Q. infectoria* and *Q. libani* changed significantly in response to 1 month of drought stress (Ghanbary et al., 2020). The RWC and water potential of the leaves of *Q. fabri, Q. serrata, Q. acutissima,* and *Q. variabilis* decreased under continued drought stress (Xiong et al., 2022). As shown, water content in plant tissues decreases as a result of drought stress. This decrease in water quantity causes a decrease in turgor pressure, which is an indicator of water stress. The interaction between plants and drought stress results in an increase in solute concentrations within the cell. Additional im-

pacts of drought stress include reduced cell growth, decreased cell wall and protein synthesis, stomatal closure that reduces CO_2 absorption and respiration, and an increase in osmolytes, including proline and carbohydrates (Öpik & Rolfe, 2005).

Drought alters photosynthetic pigments and impairs photosynthesis due to disruptions in chloroplast structure, decreased chlorophyll production, and stomatal closure that prevents CO_2 from entering the plant. Determining chlorophyll content is one of the best methods used to determine the effects of environmental stress factors such as drought, low and high temperatures, soil and air pollution, and radiation on plants (Baycu et al., 2006). In the present study, the chlorophyll content in the leaves of both *Quercus* species considerably reduced as the length of drought stress increased. Consistent with this study, one study on young *Q. pubescens* trees demonstrated a decrease in chlorophyll content of leaves due to drought stress affecting photosynthetic performance (Gallé & Feller, 2007). Chlorophyll a (fluorescence of photosystem II) decreased in *Q. ilex* after a 15-day drought stress period (Cotrozzi, Pellegrini, et al., 2017). Landi et al (2019) showed chlorophyll a to decrease in the leaves of *Q. cerris* and *Q. pubescens* (by 3% and 37%, respectively). The decrease in chlorophyll content during drought can occur as a result of membrane damage due to oxidative stress (Alonso et al., 2001).

Carotenoids are pigments found in plants that protect the plant against oxidative damage. Carotenoids are weakly bound to proteins within the cell, protecting the pigment from reactions such as oxidation, degradation, and isomerization (Çinar, 2004). Carotenoids are highly efficient at scavenging singlet oxygen and can directly react with hydroxyl radicals, peroxyl radicals, and alkoxyl radicals, thus preventing lipid peroxidation chain reactions (Burton & Ingold, 1984). The current study has observed the carotenoid content in the leaves of *Q. vulcanica* and *Q. aucheri* seedlings to increase significantly compared to the control group, which indicates carotenoid content to protect the plant from damage. Furthermore, the lower carotenoid content in the 2- and 4-week drought stress groups compared to the 1-week stress group indicates that damage is more severe in the 2- and 4-week drought stress periods for both Quercus species. According to Pellegrini et al.'s (2019) results, total carotenoid content increased in severe drought conditions in *Q. ilex*, while slightly decreased in moderate drought for *Q. pubescens*; as for *Q. robur*, a significant decrease was shown under both moderate and severe drought conditions.

The accumulation of free proline, a nitrogenous osmoprotectant in plants, is a response to stress. This study observed the proline content in the leaves of both *Quercus* species to increase significantly under drought stress. Consistent with this, a study on the leaves of a 5-year-old *Q. robur*. clone showed an approximately 70% increase in proline content after 8 days of drought stress (Oufir et al., 2009). Proline content is seen to have increased under drought stress conditions in the leaves of *Q. ilex, Q. cerris,* and *Q. pubescens* (Cotrozzi et al., 2016); in *Q. ilex* (Cotrozzi, Pellegrini, et al., 2017); in *Q. cerris* (Cotrozzi, Remorini, et al., 2017); in *Q. cerris* and *Q. pubescens* (Landi et al., 2019); in *Q. infectoria* (Ghanbary et al., 2020); and in *Q. fabri, Q. serrata, Q. acutissima* and *Q. variabilis* (Xiong et al., 2022), with no change being observed in the proline content in *Q. libani* (Ghanbary et al., 2020). The proline content in the current study increased higher in *Q. aucheri* (2.05-5.15 fold) than in *Q. vulcanica* (1.17-1.48 fold). This shows that proline accumulation in plants can vary from species to species (Kocheva & Georgiev, 2008). Thomas et al. (2002) demonstrated the response of drought tolerance in oak species in Central Europe to be associated with an increase in endogenous nitrogenous osmoprotectants.

Anthocyanins are phenolic compounds found in all parts of higher plants and through their antioxidant properties protect plants against the harmful effects of ROS generated under various abiotic and biotic stresses. This study found the anthocyanin levels in the leaves of *Q. vulcanica* and *Q. aucheri* seedlings to increase significantly depending on the applied duration of drought stress. Thus, the plant increased its anthocyanin content to protect itself against drought stress. The content of free phenolics increased in the roots of *Q. ilex* under 9 days of limited water (Simova-Stoilova et al., 2018) and in the leaves of *Q. libani* (Ghanbary et al., 2020). Pellegrini et al. (2019) showed no change to occur in total phenols in the leaves of *Q. ilex, Q. pubescens,* and *Q. robur*, which they suggested was due to *Q. ilex* having a superior ability to counteract oxidative conditions. The total soluble protein content is considered an important indicator in plants for determining the physiological status of cells under stress conditions. The current study found the total protein content of *Q. vulcanica* and *Q. aucheri* under drought stress to decrease in the leaves while increasing in the stems. Xiong et al. (2022) reported that total soluble protein content increased under drought stress conditions in the leaves of *Q. serrata, Q. acutissima*, and *Q. variabilis*. Some variations have been observed in total soluble protein content in oak species, which suggests drought stress may have different effects on protein metabolism and synthesis in plants.

In biological systems, H_2O_2 forms as a result of enzymatic and non-enzymatic dismutation of superoxide radicals and plays a significant role in free radical biochemistry (Halliwell & Gutteridge, 2015; Karpinski et al., 1999). The present study observed the H_2O_2 content in the leaves and stems of *Q. vulcanica* and *Q. aucheri* seedlings to increase significantly based on the applied duration of drought stress. As a result, the onset of oxidative damage, which causes lipid peroxidation, becomes inevitable in plants under drought stress conditions. Lipid peroxidation is one of the most important mechanisms causing oxidative damage. Therefore, measuring the content of MDA is considered an indicator of cellular breakdown. This study observed high MDA contents in the leaves and stems of both Quercus species, especially in those under 4 weeks drought

stress. Accordingly, H2O2 and MDA contents were seen to increase in the leaves of *Q. ilex, Q. pubescens* and *Q. robur* (Pellegrini et al., 2019), while no change occurred regarding H_2O_2 content in the leaves of *Q. ilex* (Cotrozzi, Pellegrini, et al., 2017). MDA levels have been seen to increase in the leaves of *Q. ilex, Q. cerris, Q. pubescens* (Cotrozzi et al., 2016), and *Q. cerris* (Cotrozzi, Remorini, et al., 2017); in the cotyledons of *Q. ilex* (Simova-Stoilova et al., 2018); and in the leaves of *Q. serrata, Q. acutissima*, and *Q. variabilis* (Xiong et al., 2022) under drought stress. H_2O_2 levels have been seen to increase in the leaves of *Q. pubescens* (Landi et al., 2019) under drought stress. In the present study, the decrease in MDA content in the leaves of *Q. vulcanica* under 1- and 2-weeks of drought stress can be explained by antioxidant components inhibiting lipid peroxidation. In addition, the increase in MDA content in the 4-week drought stress group may be associated with an increase in lipid peroxidation, resulting in depletion of antioxidant capacity based on the duration of drought stress.

Ascorbic acid, which is found in all plant parts and helps protect plant cells from ROS and other electrophilic compounds, plays an important role in the Halliwell-Asada cycle as the electron donor and substrate of the APX enzyme. In addition, ascorbic acid also has important functions in various biochemical processes such as growth promotion, cell division, photosynthesis, and electron transport (Noctor & Foyer, 1998). The current study observed the ascorbic acid content in the leaves of both *Quercus* species to decrease based on the applied duration of drought stress but to increase in the stems, resulting in the leaves being more sensitive than stems in terms of ascorbic acid content under drought stress. Interestingly, Pellegrini et al. (2019) showed total ascorbic acid to increase in the leaves of *Q. robur* but to decrease in *Q. pubescens*. Water limitation caused a slight decrease in total ascorbic acid while increasing the percentage of oxidized ascorbate in root tips on day 3 in *Q. ilex* (Simova-Stoilova et al., 2018). This might suggest that changes in ascorbic acid content could be an important variable in the different parts of plant species. GSH is one of the mechanisms that protect cell DNA, lipoproteins in the cell membrane, and enzymes from ROS and other electrophilic compounds. GSH acts as a substrate for GPX (Glutathione Peroxidase) and dehydroascorbate reductase enzymes. In the Halliwell-Asada cycle, GSH is oxidized to ascorbate and reduced again by GR with the help of NADPH (nicotinamide adenine dinucleotide phosphate) during the regeneration of ascorbic acid (Creissen et al., 1994). The present study observed the GSH content to decrease in the leaves of both *Quercus* species but to increase in the stems. In the leaves of drought-tolerant plants acclimated to severe drought stress, the level of GSH and the GSH:GSSG ratio show a smaller decrease compared to non-acclimated drought-tolerant plants (Khanna-Chopra & Selote, 2007). The decrease in GSH content in the leaves of both *Quercus* species under drought stress in the current study might be attributable to increased oxidative damage, which also results in consistently high levels of GSSG. No change has been observed in the GSH levels in the leaves of *Q. ilex, Q. pubescens*, and *Q. robur* (Pellegrini et al., 2019). GSH levels were seen to increase in the roots of *Q. ilex* on day 6 after having decreased on day 3 without any changes to the GSH:GSSG ration (Simova-Stoilova et al., 2018).

Enzymatic antioxidants such as SOD, CAT, GR, and peroxidases play an important role in the elimination of free radicals and the prevention of oxidative damage at the cellular level (Halliwell & Gutteridge, 2015). According to the current study's results, SOD activity increased in the leaves and stems of both Quercus species, which supports a response to oxidative damage under drought stress. This response is also confirmed by the higher H_2O_2 level found in this study. The CAT enzyme activity in the leaves and stems of *Q. vulcanica* and *Q. aucheri* was observed to decrease significantly under drought stress. APX enzyme activity was observed to decrease in the leaves of *Q. vulcanica* and *Q. aucheri* but to increase in the stems. The increased amount of H_2O_2 can be concluded to deplete the CAT and APX enzyme activity in the leaves. In addition, GuPx enzyme activities in the leaves of *Q. vulcanica* and *Q. aucheri* increased significantly. Some alterations in GR activity were also shown to have occurred; this is thought to be relevant to maintaining the balance of the GSH:GSSG ratio in the cell. Xiong et al. (2022) showed the activities of peroxidase, SOD, and CAT to increase in the leaves of *Q. serrata, Q. serrata, Q. acutissima*, and *Q. variabilis* when under drought stress. According to Simova-Stoilova et al. (2018) the activities of CAT, SOD, and peroxidase in the roots and cotyledons had different profiles in *Q. ilex*, with the roots having high peroxidase and low CAT activity and cotyledones having the opposite effect. Ghanbary et al. (2020) showed drought stress to alter the antioxidant system in *Q. libani* and *Q. infectoria* seedlings. Similar to the current study. other studies have revealed different prevailing metabolic processes to appear in leaves and stems during drought stress and the need for distinct enzymes to deal with the generated ROS.

CONCLUSION

As a current significant environmental issue, drought has been exacerbated globally due to climate change and has varying impacts on countries including Türkiye. This is the first comparative study on the effects of drought stress and tolerance mechanisms on the endemic species of *Q. vulcanica* and *Q. aucheri* in Türkiye. This study has revealed that increasing drought stress significantly affects the physiological and biochemical parameters in *Q. vulcanica* and *Q. aucheri*, and this will contribute to understanding how oak species respond to drought stress and their adaptive strategies. By comparing *Q. vulcanica* and *Q. aucheri* based on the tolerance levels under drought stress, this study is able to suggest that *Q. aucheri* may be more drought tolerant than *Q. vulcanica*. This study is be-

lieved to be able to play a special role in the preservation and continuity of Türkiye's natural plant diversity.

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