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# Effects of Drought and Salinity Stress on Antioxidant Enzymes and Yield Parameters of Laurel Plant (*Laurus nobilis* L.)

Kuraklık ve Tuzluluk Stresinin Defne Bitkisinin (*Laurus nobilis L.*) Antioksidan Enzimler ve Verim Parametreleri Üzerine Etkileri

**Mutlu Yalçın[1], Deniz Ekinci[2], Hakan Arslan[3]**

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**Abstract:** Plants are exposed to various environmental stressors throughout their life cycle, including cold, drought, high temperature, salt, and heavy metals. These environmental variables, known as abiotic stressors, lead to oxidative stress and promote the formation of reactive and dangerous reactive oxygen species in plants. In this study, laurel plants were exposed to two different abiotic stress conditions (salinity (10 dS $m^{-1}$), drought). Under both stress conditions, chlorophyll content, stomatal conductance and antioxidant enzyme activities Glutathione S-transferase (GST), glutathione reductase (GR), guaiacol peroxidase (GPx), ascorbate peroxidase (APx) were determined. Chlorophyll content was observed to decrease by 58.53% and 40.31% for drought and salinity treatments, respectively, compared to the control treatment. In addition, stomatal conductance was reduced by 52.75% and 35.15% for drought and salinity treatments, respectively. These results indicate that chlorophyll content and stomatal conductance of laurel plants were more affected by drought stress than salinity. The activity of all antioxidant enzymes decreased in both drought and salinity stress. GR and GPx were significantly reduced by 49.29% and 74.51%, respectively, in drought treatment compared to the control group. In addition, GST and APx activity decreased by 22.01% and 6.26%, respectively, in salinity stress compared to the control group. According to the data obtained, GR and GPx enzyme activities in laurel plants were more affected by drought stress, while GST and APx enzyme activities decreased more significantly under salinity stress.
**Keywords:** *Laurus nobilis L.*, antioxidant, enzyme activity, stress factor, chlorophyll

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**Öz:** Bitkiler yaşam döngüleri boyunca soğuk, kuraklık, yüksek sıcaklık, tuz ve ağır metaller dahil olmak üzere çeşitli çevresel stres faktörlerine maruz kalırlar. Abiyotik stresler olarak bilinen bu çevresel değişkenler oksidatif strese yol açmakta ve bitkilerde reaktif ve tehlikeli reaktif oksijen türlerinin oluşumunu teşvik etmektedir. Bu çalışmada, Defne bitkisi 2 farklı abiyotik stres koşuluna (tuzluluk (10 dS $m^{-1}$), kuraklık) maruz bırakılmıştır. Her iki stres koşulunda da klorofil içeriği, stoma iletkenliği ve antioksidan enzim aktiviteleri Glutatyon S-transferaz (GST), glutatyon redüktaz (GR), guaiakol peroksidaz (GPx), askorbat peroksidaz (APx) belirlenmiştir. Klorofil içeriğinin kontrol uygulamasına kıyasla kuraklık ve tuzluluk uygulamaları için sırasıyla %58.53 ve %40.31 oranında azaldığı gözlenmiştir. Buna ek olarak, stoma iletkenliği kuraklık ve tuzluluk uygulamaları için sırasıyla %52.75 ve %35.15 oranında azalmıştır. Bu sonuçlar, defne bitkilerinin klorofil içeriği ve stoma iletkenliğinin kuraklık stresinden tuzluluğa göre daha fazla etkilendiğini göstermektedir. Tüm antioksidan enzimlerin aktivitesi hem kuraklık hem de tuzluluk stresinde azalmıştır. GR ve GPx, kontrol grubuna kıyasla kuraklık uygulamasında sırasıyla %49.29 ve %74.51 oranında önemli ölçüde azalmıştır. Ayrıca, GST ve APx aktivitesi tuzluluk stresinde kontrol grubuna kıyasla sırasıyla %22.01 ve %6.26 oranında azalmıştır. Elde edilen verilere göre, defne bitkisinde GR ve GPx enzim aktiviteleri kuraklık stresinden daha fazla etkilenirken, GST ve APx enzim aktiviteleri tuzluluk stresi altında daha önemli ölçüde azalmıştır.
**Anahtar Kelimeler:** *Laurus nobilis L.*, antioksidan, enzim aktivitesi, stres faktörü, klorofil

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[1] Mutlu Yalçın, Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, mutluyalcin.26@gmail.com

[2] Prof. Dr. Deniz Ekinci, Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, deniz.ekinci@omu.edu.tr (Corresponding author)

[3] Prof. Dr. Hakan Arslan, Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, hakan.arslan@omu.edu.tr

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## INTRODUCTION

Climate change is predicted to become more visible due to global warming caused by excessive industrialization to meet human demands, and rising carbon emissions generated by the world's rapid population growth. Climate change will cause unfavorable changes in the precipitation regime, as well as the use of low-quality water in agricultural production and the inability of some regions to irrigate agricultural lands (Zandalinas et al., 2018). Salt and drought stress are the two major abiotic stress factors that limit plant morphological and physiological development and degrade yield and quality. The rise in salt-containing soils produced using saline waters, drainage water, wastewater in agricultural irrigation, and drought due to climate change plays a significant part in the loss of productive farm areas (Machado and Serralheiro, 2017).

Drought stress causes plant transcriptional reprogramming, followed by physiological responses such as antioxidant generation, osmolyte synthesis, and stomatal closure (Ding et al., 2013). It has been established that reactive oxygen species (ROS), which begin to accumulate in plants under drought stress, are the product of metabolism, which plays a role in the plant's signal transmission process (Cabello et al., 2014). Excessive plant accumulation of these reactive oxygen species can cause cell death through chain reactions such as lipid peroxidation and DNA damage. Non-enzymatic antioxidants in plants protect non-photosynthetic zones from reactive oxygen species, whereas enzymatic antioxidants mitigate their effects by reducing reactive oxygen species (Osakabe et al., 2014).

Salt stress is the most significant abiotic stressor, an imbalance in edaphic conditions and devastating impacts on plant growth and physiological functioning (Shahbaz and Ashraf, 2013). Salt stress leads to the degradation of physiological and chemical components after creating osmotic and ion stress in the plant. Plants are also subjected to oxidative stress at the subcellular level. These are the primary causes of long-term plant damage caused by salt stress (Acosta-Motos et al., 2015). As a result of the osmotic stress generated by salt stress, the amount of water in the plant decreases. Such a case is known as a physiological drought. As a result of physiological droughty, cell growth slows and eventually stops. Although plants require $Na^+$ and $Cl^-$ ions, increased quantities of $Na^+$ and $Cl^-$ ions in ion stress caused by osmotic stress compete with nutrients such as $K^+$ and $Ca^{2+}$, resulting in a nutritional deficiency (Hu and Schmidhalter, 2005). High concentrations of these ions in leaves can damage cell activity and eventually cause programmed cell death. Furthermore, increased $Na^+$ and $Cl^-$ ion levels inhibit protein synthesis, plant lipid metabolism, and enzyme performance. Also, the presence of such ions in high concentrations may promote the creation of reactive oxygen species, producing severe oxidative stress in plants. The body has defensive systems known as antioxidants to avoid creating free radicals due to numerous reactions and structural damage. By responding swiftly to the radicals generated, these defense mechanisms avoid oxidation and peroxidation (Dündar and Aslan, 1999). Antioxidants form an electron target and affix free radicals to it (Aydemir and Sarı, 2009). The basic roles of antioxidants are to detoxify excess free radicals protect cells from the destructive effects of radicals, and contribute to the prevention of many diseases (Pham-Huy et al., 2008). They help cells operate properly and maintain their integrity. The damaging effects of free radicals do not impact the organism as long as the equilibrium between free radicals and antioxidants is maintained. With various components, oxygen metabolized in metabolism leads to the creation of active oxygens. Active oxygens degrade the structure of lipids, carbohydrates, and proteins (Önenç and Açıkgöz, 2005). Endogenous and exogenous antioxidants are the two types of antioxidants that neutralize free radicals and maintain the oxidant/antioxidant balance to protect the organism (Gupta et al., 2014). In addition, endogenous antioxidants are divided into two groups as enzymatic and nonenzymatic. Plants have developed several defense mechanisms, as well as signaling actions, to regulate both the formation and the removal of ROS to avoid oxidative damage (Hasanuzzaman et al., 2018). The antioxidant defense system efficiently scavenges excess ROS by the coordinated action of different enzymes that include glutathione reductase (GR), Glutathione S transferase (GST), ascorbate peroxidase (APX), Guaiacol peroxidase (GPX) as well as by the involvement of multiple nonenzymatic reactions (Soares et al., 2019; Al Mahmud et al., 2018).

The Laurel plant is a plant that falls into the category of forest products and the laurel plant, which has a wide distribution area in Turkey, is encountered in Antalya, Mersin, Hatay, İzmir, Muğla, and Bursa, especially on the Black Sea coasts. *Laurus nobilis L.* is an aromatic and medicinal plant belonging to the Lauraceae family, which comprises approximately 2500-3500 species (Dobroslavic et al., 2022). It is an evergreen shrub native to the Mediterranean region and is also known by a number of other names, including sweet laurel, Grecian laurel, true laurel and simply laurel (Anzano et al., 2022). Turkey is the primary producer of L. nobilis, exporting it to 64 countries (Anzano et al., 2022). Approximately 97% of the global total production is sourced from Turkey. In addition to its aromatic properties, *L. nobilis* has been esteemed for millennia for its purifying attributes. *L. nobilis* is widely cultivated in many parts of the world and is primarily used as a culinary herb. The different body parts and essential oil (EO) of L. nobilis have been demonstrated to possess a multitude of intriguing properties with potential applications in a diverse range of fields, including agriculture, medicine, food, and the pharmaceutical industry. The leaves are frequently employed as a pungent, aromatic seasoning for soups, fish, meats, stews, puddings, vinegar, and beverages. Due to its antimicrobial and insecticidal properties, laurel is utilized as a food preservative in the food industry (Sırıken et al., 2018). Additionally, the cosmetics industry incorporates L. nobilis EO into creams, perfumes, and soaps (Ordoudi et al., 2022).

Many researchers have conducted numerous studies on the influence of essential oils produced by the laurel plant and the antioxidant capacity of the laurel plant when the literature is evaluated (Ramos et al., 2012; Santoyo et al., 2006). In light of this, the response and yield characteristics of the antioxidant enzyme system in laurel plants under stress conditions were not examined. The effects of drought and irrigation water salinity which have significant negative effects on plants were investigated in this study, as were the activities of Glutathione S-transferase (E.C. 2.5.1.18 GST), Guaiacol Peroxidase (E.C. 1.11.1.17 GPx), Ascorbate Peroxidase (E.C. 1.11.1.11 APx) and Glutathione Reductase (E.C. 1.8.1.7 GR) members of the antioxidant mechanism in the laurel plant, as well as chlorophyll content and stomatal conductivity which are photosynthetic parameters were studied.

## MATERIAL AND METHOD

### *Experiment Area and Growing Conditions*

This study was conducted at the Ondokuz Mayıs University Faculty of Agriculture, Research area, in a 120 $m^2$ greenhouse under rain-shelter conditions between November 2019 and January 2020. The temperature and relative humidity of the environment were monitored using an electronic data logger (KISTOCK KMO Data logger). During the experiment, the temperature ranged between 12 and 17 $^0$C, while the relative humidity ranged between 68.7% and 81.4%.

This study used pots with a depth of 31 cm, bottom and top diameters of 36 cm and 38 cm, respectively, for seedling planting. Then, the air-dried soils were sieved with the help of a sieve with a pore size 4 mm, and 32 kg of sieved soil was added to the pots. The experimental soil texture was loamy, which has 52.3% sand, 9.4% clay, and 38.3% silt. The pH of the soil was 8.28, and the EC (dS $m^{-1}$) was 0.63.

### *Experiment Design and Irrigation*

The study was conducted in a randomized design with 1 factor and 3 replications. For this, applications were made in the experiment as the control group (0.38 dS $m^{-1}$), the salinity group (10 dS $m^{-1}$), and the drought group (no water). Thus, a total of 9 plastic pots were used in the experiment. One-year-old laurel seedlings were planted on the first day of November and irrigated twice a week with tap water. Saline water and drought applications were started 3 weeks after planting the seedlings. NaCl and $CaCl_2$ salts were used to prepare saline water. The field capacity of each pot was determined before beginning of the experiment. The pots were saturated with tap water, and then the soil surface was covered with plastic to prevent evaporation. After drainage was completed, each pot was weighed and recognized as the respective pot's field capacity weight (WFC). Equation 1 (Kiremit and Arslan, 2018; Ünlükara et al., 2010) was used to calculate the amount of irrigation water supplied to each pot based on their treatment. Each pot was weighed before irrigation. Irrigation was only applied to the control group (0.38 dS $m^{-1}$) and the

salt stress group (10 dS $m^{-1}$). Irrigation water was not used to the plants in the drought group from the beginning of the experiment until harvest.

$$IW = \frac{\frac{W_{FC} - W_{\rho}}{\rho_W}}{1 - LF} \quad (1)$$

Where; IW; irrigation water amount (L), WFC; Field capacity value of the pot (kg), LF; Leaching fraction, Wϱ; Weight of the pot before irrigation (kg), ϱw; the volume weight of water (1 kg $L^{-1}$). Furthermore, the amount of drainage water was measured after each irrigation.

***Stomatal Conductance and Chlorophyll Content***

A SPAD meter (SPAD-502 Plus) was used to determine the chlorophyll content of the leaves of laurel plants by taking measurements at three different points on the fully developed upper leaves of the plants and calculating an average value. In addition, the same procedures were carried out with a portable porometer (Delta-T AP4) to determine the stomatal conductance (mmol $m^{-2}$ $s^{-1}$) of the plants.

***Preparation of Extracts***

Leaf samples were taken 3, 6 and 9 weeks after the first stress treatments for enzyme analysis. The leaf samples were weighed on a precision balance and the total weight was adjusted to approximately 1 g. The samples were cut into small pieces, placed in a mortar and mechanically crushed using liquid nitrogen until they were pulverized. The pulverized samples were placed in a 50 ml tube and 5 ml of 0.5 mM EDTA, 0.1 mM PVP and 100 mM $KH_2PO_4$ (pH 7.7) crushing buffer were added. Centrifugation was carried out at 15,000 x G and +4 ºC for 20 minutes. At the end of centrifugation, the supernatant was filtered using a Pasteur pipette and filter paper and the homogenate was transferred to another tube.

***Protein Determination***

The protein content of the extracts was determined according to the Bradford method (Bradford, 1976). This method is based on the binding of the dye Coomassie Brilliant Blue G-250 to proteins in different concentrations, resulting in a blue solution with varying color intensity. In order to carry out protein determination using this method, a standart curve must be created. For this purpose, 1, 2, 4, 6, 10 and 16 µl of bovine serum albumin (BSA) solution containing 1 mg protein per 1 ml was added to the tubes. After the volumes of all tubes were made up to 100 µl with distilled water, 900 µl of 1x Bradford solution was added and the final volume was made up to 1 ml. The tubes were mixed by vortexing and incubated for 10 minutes. At the end of this time, instantaneous measurements were performed at 595 nm in a spectrophotometer and the results were converted to a standard curve. The values were read photometrically at 595 nm by mixing 96 µl distilled water, 900 µl Coomassie Brilliant Blue G-250 and 4 µl supernatant samples in a quartz cuvette. The protein content was determined using a standard graph based on the values obtained from each sample.

***Determination of Enzyme Activities***

The leaf samples were taken and stored at -20 °C for enzymatic analyses 3, 6 and 9 weeks after the first application. All spectrophotometric analyses were performed using a spectrophotometer for UV-visible light at 25 °C (Shimadzu UV–1800).

The activity of GR was measured at 340 nm due to the oxidation of NADPH using 200 mM TRIS buffer (pH 7), 2 mM GSSG, 2 mM NADPH and 100 µl supernatant. The spectrophotometric methods were performed at 340 nm for 2 min with the kinetic rate setting (Carlberg and Mannervik, 1975).

The activity of GST, a waveform of the dinitrobenzene-5-glutathione product (DNB-SG) formed using CDNB, was measured using 20 mM TRIS buffer (pH 7.5), 20 mM GSH, 25 mM CDNB and 100 µl supernatant. The increase in absorbance at 340 nm was observed for 2 minutes (Habig et al., 1974).

The activity of the APX enzyme was determined by measuring the rate of ascorbate oxidation using a spectrophotometer. APX activity was measured by observing the decretion in absorbance of ascorbate oxidation at 290 nm for 2 minutes. The mixture of reaction (1 ml) includes 20 mM TRIS buffer (pH 6.0), 1 mM EDTA, 20 mM $H_2O_2$, 2.5 mM L(+)-ascorbic acid (ASA) and enzyme extract (Cakmak et al., 1993).

The activity of the GPX enzyme was detected using the method of Sisecioglu et al., (2010). This method is based on the oxidation of the chromogenic substrate guaiacol by $H_2O_2$ and the observation of the increase in absorbance by the formed colored compound (tetraguaiacol). The reaction solution contained 200 mM $KH_2PO_4$ buffer (pH 6.0), 50 mM $H_2O_2$ and 100 mM guaiacol and the absorbance increase in the supernatant was observed at 470 nm for 2 min.

***Statistical Analysis***

A one-way ANOVA statistically analyzed stomata conductivity and chlorophyll content values, and significant differences among means were separated by using the Duncan test at the %5 probabilty level using SPSS 25.0 statistical software ($p<0.05$). Data are given as mean values ± standard deviation. Each data point is the mean of three independent replicates (n = 3). Each replicate consisted of one plants. The bar graphs were drawn in Microsoft Excel 2019.

## RESULT and DISCUSSION

***The Effect of Salinity and Drought Stresses on Photosynthetic Parameters***

The effect of different stress factors on stomatal conductivity and chlorophyll content in the leaves of the laurel plant is shown in Figures 1 and 2. The main effect of irrigation water salinity and drought stress for stomatal conductance and chlorophyll content was statistically significant ($p< 0.05$). Stomatal conductivity and chlorophyll content decreased in both drought and salinity stress compared to the control group. Stomatal conductance was determined as 176.01 (mmol $m^{-2}$ $s^{-1}$) in the control group, 111.73 (mmol $m^{-2}$ $s^{-1}$) in the salinity group, and 82.68 (mmol $m^{-2}$ $s^{-1}$) in the drought stress group, respectively. The stomatal conductance value decreased by 36.15% in the salinity treatment and 52.75% in the drought treatment. According to these results, drought's effect on the stomatal conductance of laurel plants was greater than that of salinity.

The chlorophyll content in the leaves of the laurel plants was determined as 70.90 CCl in the control group, 43.32 CCl in the salinity stress group, and 29.40 CCl in the drought stress group, respectively (Figure 2). Chlorophyll content value decreased by 40.31% in salinity treatment and 58.53% in drought treatment compared to the control. According to these results, drought's effect on the chlorophyll content of laurel plants was higher than that of salinity.

Salt stress causes $Na^+$ toxicity and ionic imbalance in plant cells, interfering with vital metabolic processes such as protein synthesis, enzymatic reactions, and ribosome function (Alkharabsheh et al., 2021; Mushtaq et al., 2020). Other essential nutrients such as potassium, magnesium, ammonium, nitrate, and phosphate are competed with the high concentration of $Na^+$ salt used (Shabala and Cuin, 2008). Furthermore, salinity-induced osmotic stress impairs the photosynthetic mechanism by decreasing stomatal conductance. As a result of the reduced $CO_2$ input, the rate of photosynthesis decreases (Ouyang et al., 2017). In the study of Ben Ayed et al., in 2018 on the effects of NaCl salts applied in different doses on the growth and mineral parameters of the laurel plant, they reported that there was a decrease in chlorophyll content with the increase in the salinity level applied to the plants (Ben Ayed et al., 2018). The decreased chlorophyll content is accepted as one of the main markers of metabolic problems in plants exposed to drought stress; it is also known that decreases in protein levels and enzyme activity cause the closure of leaf stomata and dehydration (Levitt, 1980).

Plant physiological processes are directly or indirectly affected by inadequate water availability. Photosynthesis is directly affected in plants that are subjected to drought stress (Li et al., 2018; Wang et al., 2019). Drought stress reduces plant morphological and physiological characteristics, photosynthesis, leaf water potential and stomatal conductance (Bhusal et al., 2019). The two main factors that cause plants to close their stomata during drought are hydraulic signals (leaf water potential, cell turgor) and chemical

signals (e.g. abscisic acid). Abscisic acid (ABA), synthesized in roots and transported to guard cells by transpiration runoff, binds to the hypothetical ABA receptor in guard cells and causes stomatal closure under drought stress conditions (Teiz and Zeiger, 1998). Drought stress can cause excessive ROS production in plants (Abdelaal et al., 2021). Overproduction of ROS in plants due to stress causes protein denaturation, lipid peroxidation, DNA damage, carbohydrate oxidation, pigment degradation and impairment of enzymatic activity (Hasanuzzaman et al., 2019). Drought-induced stomatal closure reduces the plant's ability to use sunlight. Similarly, drought stress disrupts plant nutrient homeostasis and photosynthesis (Razi and Muneer, 2021). Under drought stress, plant cells lose turgor due to lack of water, which inhibits plant growth (Nardini, 2022).

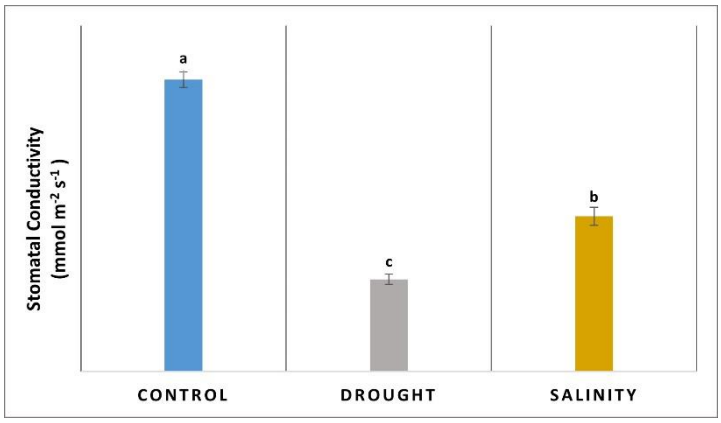


**Figure 1.** Graphs of stomatal conductivity in laurel plants under stress conditions. Means ± SD. (n=3) means denoted by different letters indicate a significant difference among the treatments at a $p < 0.05$ level according to Duncan's test.
*Şekil 1. Stres koşulları altında defne bitkilerinde stoma iletkenliği grafikleri. Ortalamalar ± SD. (n=3) farklı harflerle gösterilen ortalamalar, Duncan testine göre p < 0.05 düzeyinde uygulamalar arasında anlamlı bir fark olduğunu göstermektedir.*

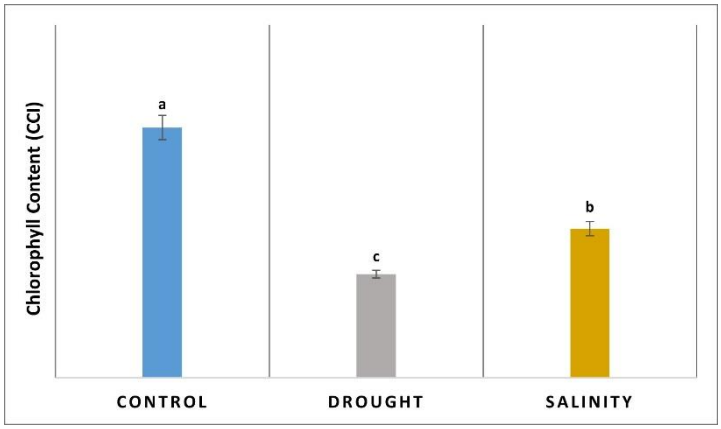


**Figure 2.** Graphs of chlorophyll content in laurel plants under stress conditions. Means ± SD. (n=3) means denoted by different letters indicate a significant difference among the treatments at a $p < 0.05$ level according to Duncan's test.
*Şekil 2. Stres koşulları altında defne bitkilerinde klorofil içeriği grafikleri. Ortalamalar ± SD. (n=3) farklı harflerle gösterilen ortalamalar, Duncan testine göre p < 0.05 düzeyinde uygulamalar arasında anlamlı bir fark olduğunu göstermektedir.*

### *Antioxidant Enzymes*

The results of the effect of salinity and drought stress on the activities of antioxidant enzymes in laurel plants for three periods are shown in Figure 3. The main effect of irrigation water salinity and drought stress for GR, GST, GPX and APX specific activity was statistically significant ($p< 0.05$). The average specific activity (three measurements) of GR enzyme was determined to be 0.71 (EU $mg^{-1}$) in the control group, 0.57 (EU $mg^{-1}$) in the salinity group, and 0.36 (EU $mg^{-1}$) in the drought group. The activity of the enzyme was reduced by 19.71% in the salinity group and 49.29% in the drought group compared to the control.

According to these results, drought's effect on GR enzyme activity in laurel plants was higher than salinity. The results clearly showed that there were differences between the groups according to the activity readings made three weeks after in GR enzyme. The results of the first measurements showed that the specific activities decreased by 32.71% in the salt stress group and 46.72% in the drought group compared to the control group. Besides, the specific activity values in the drought group decreased by 20.83% more than the salinity group. In the measurements made 6 weeks later, the salt and drought groups showed a lower decrease compared to the third week decreasing by 7.27% in the salt group and 65.45% in the drought group compared to the control group. Moreover, the specific activity in the drought group decreased by 62.74% more than the salt stress group. The data obtained nine weeks after the application revealed a 5.76% reduction in the specific activities of the plants in the salt stress group in comparison to the control group. In the drought group, this rate was determined to be 33.53%. Also, the specific activity in the drought group plants decreased by 32.65% more than the salinity group. GR, a flavoenzyme with a disulfide group, is active in the AsA-GSH cycle and converts oxidized glutathione (GSSG) into its reduced form (GSH) (De Vega et al., 2003). GR thus regulates the cellular GSH/GSSG ratio and provides a GSH source for the enzymes glutathione peroxidase, which removes hydrogen peroxide, and dehydroascorbate reductase, which reduces oxidized ascorbate. In order to see the effects of hydrogen sulphide on common bean plant under salt stress, T1 (irrigated with water as control); T2 (50 µM NaHS); T3 (100 µM NaHS); T4 (irrigated with 75 mM NaCl); T5 (50 µM NaHS + 75 mM NaCl); T6 (100 µM NaHS + 75 mM NaCl); T7 (irrigated with 150 mM NaCl); T8 (50 µM NaHS + 150 mM NaCl); and T9 (100 µM NaHS + 150 mM NaCl). To evaluate the effect of H2S in modulating salt stress, the activities of antioxidant enzymes (CAT, POX, SOD, APX, GR, NR) in the leaves of common bean plants were measured. Compared to control plants, CAT, POX, SOD, APX, GR and NR activities were found to increase significantly with increasing NaCl concentrations. CAT (65.3%), POX (43.4%), SOD (134.8%), APX (140.4%), GR (43.4%) and NR (20.5%) activities were higher in plants irrigated with high concentrations of salt stress (150 mM) compared to non-stressed plants (Dawood et al., 2022). In a study conducted to investigate the effects of salt stress on antioxidant enzyme activity and lipid peroxidation in the above- and below-ground parts of two maize genotypes BR5033 (salt tolerant) and BR5011 (salt sensitive) grown under control and salt stress conditions (solution containing 100 mM NaCl), it was reported that GR activity increased in a time-dependent manner in control and salt-stressed plants of BR5033 and BR5011 genotypes. It was found that GR activity was higher in the leaves of plants under salt stress than in the control group, and that GR activity was less affected by salinity in the roots of BR5033, but decreased by about 30% in the roots of BR5011 after 15 days of salt application (de Azevedo et al., 2006). In a study on mustard plants, 100 and 200 mM NaCl concentrations increased GR activity by about 20 % and 25 % on day 90 of treatment (Ahmad et al., 2017). Increasing salt concentrations significantly increased GR activity in different kiwifruit genotypes compared to control plants (Abid et al., 2020). The GR activity of rice plants exposed to three different NaCl concentrations increased approximately 1.5 times (Wutipraditkul et al., 2015). In this study, GR activity decreased under irrigation water salinity and drought stress. GR, an enzymatic flavoprotein antioxidant involved in removing $H_2O_2$ in the AsA-GSH cycle, could not contribute to the fight against salinity-induced oxidative stress in the aerial parts of laurel plants.

For GST, the average specific activity was determined as 3.61 (EU $mg^{-1}$) in the control group, 2.82 (EU $mg^{-1}$) in the salinity group, and 2.86 (EU $mg^{-1}$) in the drought group. Specific activity values for GST enzyme decreased by 22.01% in salinity treatment and 20.90% in drought treatment compared to the control. When the activity of the GST was analyzed periodically, the specific activity value decreased by 2% in the salinity stress treatment compared to the control group in the first period including the 3 weeks after the application, while no change was observed in the drought stress treatment. In the second period, the specific activity value was reduced by 43.65% in the salinity stress group and by 33.74% in the drought stress group compared to the control. In the third period, while these rates were 34.76% in the salinity group, the specific activity value in the drought group decreased by 44.92%. According to these results, salinity stress had the highest effect on the specific activity of GST in the second period, at 43.65%. In drought stress, this effect was 44.92% in the third period. It was determined that the effects of salinity and drought stress on GST activity in laurel plants varied periodically. GST, which generally utilizes the reduced form of glutathione (GSH), is involved in the detoxification of xenobiotics and toxic lipid

peroxides, redox signaling through the reduction of dehydroascorbate, maintenance of reducing pools such as AsA, α-tocopherol, and anthocyanins, primary metabolism, and biochemical reactions of secondary products such as flavonoids (Dixon et al., 2011). In Brassica juncea plants under salt stress, GST activity increased by about 47 % in all 30, 60 and 90-day treatments (Ahmad et al., 2017). In another study with mustard, it was observed that the GST activity of $NaCl^-$ stressed plants increased by more than 88% (Ahmad et al., 2018). In a study investigating the role of antioxidant defence system and glyoxalase system in tomato plants under salt stress and recovery period, an experiment was established by treating 15-day-old tomato plants (Solanum lycopersicum L. cv. Pusa Ruby) grown hydroponically with 150 and 250 mM NaCl for 4 days. According to the enzyme activity results of the plant samples, APX activity increased significantly with salinity (250 mM NaCl) compared to the unstressed control condition, but this activity decreased after the recovery period. GR activity was slightly decreased under salt exposure compared to the unstressed condition, but increased after the recovery period. The thiol-dependent enzymes, GPX and GST, showed increased activity in response to NaCl stress compared to control, but both activities decreased after the recovery period (Parvin et al., 2019).

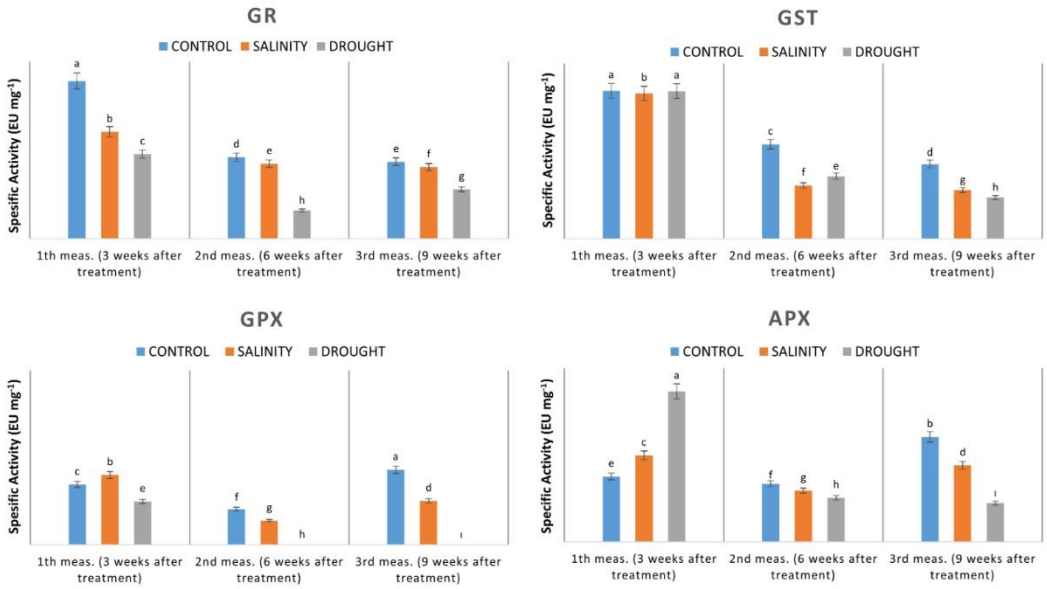


**Figure 3.** Activity results of GPx APx GST, GR antioxidant enzymes. Means ± SD. (n=3) means denoted by different letters indicate a significant difference among the treatments at a $p < 0.05$ level according to Duncan's test.
*Şekil 3. GPx APx GST, GR antioksidan enzimlerinin aktivite sonuçları. Ortalamalar ± SD. (n=3) farklı harflerle gösterilen ortalamalar, Duncan testine göre $p < 0.05$ düzeyinde uygulamalar arasında anlamlı bir fark olduğunu göstermektedir.*

The average specific activity of GPX enzyme in laurel plants in the treatments was 29.43 (EU $mg^{-1}$) in the control group, 23.73 (EU $mg^{-1}$) in the salinity group, and 7.5 (EU $mg^{-1}$) in the drought group. The specific activity decreased by 19.36% in the salinity treatment compared to the control, while this rate diminished by 74.51% in the drought treatment. According to the results of these measurements, drought's effect on GPX enzyme activity in laurel plants was significantly higher than salinity. The results of the first measurement of GPX enzyme showed that the specific activity increased by 15.84% in the salt stress condition compared to the control group and decreased by 28.09% in the drought group. In addition, the specific activity in the drought group plants decreased by 37.93% more than the salinity stress-treated plants. After 6 weeks, it decreased by 32.30% in the salt stress group compared to the control. Specific activities decreased by 99.40% in the drought stress compared to the control group. In addition, specific activity decreased by 99.11% more in the drought group than in the salt stress group. According to the data obtained $9^{th}$ weeks, the specific activity decreased by 41.47% in the salt stress group compared to the control group, and this rate was determined as 99.89% in the drought group. GPX is primarily involved in the

removal of hydrogen peroxide by oxidizing aromatic electron donors such as guaiacol and pyrogallol (Schuller et al., 1996). In a study on salt stress in two maize genotypes, it was reported that APX activity was significantly increased in the leaves of both genotypes as a result of salt stress. However, it was unaffected in the roots of BR5033 (salt tolerant), while it decreased slightly, albeit significantly, in the roots of BR5011 (salt sensitive). In BR5033 plants under salt stress, APX and GPX activities in leaves increased by about 137% and 58%, respectively, after the tenth day of stress compared to control plants. Salinity also increased APX and GPX activities in the leaves of BR5011, but these increases were lower than those observed in BR5033. On the other hand, salt stress decreased APX and GPX activities in the roots of BR5011 by about 29% and 55%, respectively, while these enzyme activities were not affected in the roots of BR5033 (de Azevedo et al., 2006). POD activity increased by about 60% in Brassica chinensis plants exposed to NaCl stress (Ren et al., 2020). In a study conducted on two types of clover plants, specifically Xinmu No. 1 (stress-tolerant) and Northstar (stress-sensitive), it was observed that peroxidase (GPX) activity was higher in the roots than in the shoots for both varieties under normal conditions. However, upon treatment with 200 mM NaCl, a significant reduction (approximately 50%) in GPX activity was noted in the roots of Northstar when compared to control conditions. In contrast, Xinmu No. 1 exhibited a GPX activity level that was approximately 1.96 times higher than that of Northstar, indicating only a marginal decrease (Wang et al., 2009).

The mean specific activity of the APX was 8.78 (EU $mg^{-1}$) in control group, 8.23 (EU $mg^{-1}$) in the salinity stress group, and 8.96 (EU $mg^{-1}$) in the drought stress group. The specific activity value for the APX enzyme decreased by 6% in the salinity treatment compared to the control, while this rate increased by 2% in the drought treatment. In addition, the rate of decrease in the first period of the application was determined as 40.82% in salinity stress compared to the control group, while it was determined as 70.58% in the drought stress group. According to these measurements, drought's effect on APX activity in the laurel plant was significantly higher than salinity in the first period. According to the results of the first measurement of APX enzyme, the specific activity increased by 32.22% in the plant groups exposed to salt stress compared to the control group and decreased by 129.97% in the drought group. In addition, the specific activity in the drought group plants increased by 73.92% compared to the salinity group. After 6 weeks, it reduced by 11.80% in the salt stress group compared to the control group. Specific activities in the drought stress group decreased by 23.76% compared to the control group. Moreover, the specific activity in the drought group plants decreased by 13.55% more than salt stress. The measurements taken $9^{th}$ weeks showed that the specific activity decreased by 27.22% in the salt-stressed plants compared to the control group, and this rate was 63.20% in the drought group. As one of the main components of the AsA-GSH cycle, ascorbate peroxidase, which plays one of the main roles in controlling intracellular ROS levels, is considered one of the most important enzymes in protecting cells from oxidative stress (Kumar et al., 2018). Antioxidant activity and stress tolerance were studied in tobacco plants exposed to NaCl stress; it was found that APX activity in the leaves decreased by about 29% as a result of NaCl treatment compared to the control (Che et al., 2022). In Lolium perenne, NaCl stress caused a decrease in APX activity (Wu et al., 2017). In rice seedlings, NaCl stress caused a significant decrease in APX activity in the aerial part (Mekawy et al., 2018). In maize plants grown with different NaCl concentrations, it was found that APX activity decreased significantly as a result of salt stress (Gong et al., 2011), and it was concluded that the oxidative attack caused by the applied stress may have significantly weakened the plants antioxidant defense system. The results of this study are consistent with the results reported by Nalina et al. (2021) showing that GR and APX activities in Tea (*Camellia sinensis* (L) O. Kuntze) decreased under drought stress in the two plant genotypes they used in the study. In the present study, the salinity of irrigation water led to an increase in APX activity in the aerial parts of laurel plants. In view of the results, it can be said that the APX enzyme played one of the major roles in the elimination of hydrogen peroxide under salt stress conditions and is one of the important catalysts of the AsA-GSH cycle. Salinity can disturb the balance between antioxidant enzymes and reactive free radicals, and oxidative stress that occurs in parallel with the increase in salinity can weaken the antioxidant system by producing deleterious effects (Foyer and Noctor, 2003). The study clearly demonstrates that drought stress has a significant impact on Medicago sativa L. The results indicate that when treated with 200 mM NaCl, APX activity in shoots and roots of alfalfa plants of the two cultivars

used increased significantly. These findings provide strong evidence for the impact of drought stress on alfalfa plants and highlight the importance of stress-tolerant cultivars in mitigating the effects of drought. It is noteworthy that Xinmu No. 1, which is stress-tolerant, exhibited higher APX activity in shoots and roots compared to Northstar, which is stress-sensitive (1.59-fold and 1.48-fold increase, respectively) (Wang et al., 2009). In the present study, the salinity of the irrigation water and the drought stress to which the laurel plants were subjected caused a decrease in APX activity. Given these results, it can be said that the APX enzyme does not play a significant role in the eliminating hydrogen peroxide under salinity and drought conditions and is not one of the critical catalysts of the AsA-GSH cycle.

## CONCLUSION

In this study, the effects of salinity and drought stresses on photosynthetic parameters and antioxidant enzyme activities in laurel (*Laurus nobilis L.*) plants were investigated. Overall, present findings showed that salinity and drought stress had a negative effect on chlorophyll content, stomatal conductance and antioxidant enzyme activities in laurel plants. Drought stress inhibited photosynthesis more than salt stress due to decreased chlorophyll content and stomatal conductance. Antioxidant enzyme activity levels were less tolerant to drought stress compared to salinity stress. Considering the increase in saline and drought areas due to global warming and changes in precipitation regime, it will be possible to better determine the potential value of the laurel plant in terms of the producing of high-yielding and economically important plants. Detailed determination of the effects of salinity and drought stress on laurel may be a new research source for breeders and plant scientists by helping to develop genetically modified resistant plants against these stress factors.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest concerning this article's research, authorship, and/or publication.

## DECLARATION OF AUTHOR CONTRIBUTION

D.E. and H.A. designed the study; M.Y carried out the experiments; M.Y., D.E. and H.A. analyzed the data, wrote and revised the manuscript. All authors read and approved the final manuscript.

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