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The Physiological and Biochemical Effects of Drought Stress on Sheep Sorrel (*Rumex acetosella* L.)

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Sheep sorrel (*Rumex acetosella* L.) is an herbaceous plant belonging to the Polygonaceae family, known for its sour taste. Its leaves are consumed as food and valued in the health sector for their medicinal properties, giving the plant significant ethnobotanical importance. This study aimed to investigate the effects of drought stress on water availability, membrane stability, biomass, and the antioxidant defense system in sheep sorrel plants. The relative growth rate, water content, osmotic potential, lipid peroxidation levels, hydrogen peroxide concentration, and activities of antioxidant defense enzymes—including superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR)—were measured. Drought stress caused decreases in relative growth rate, water content, and osmotic potential in sheep sorrel plants. Additionally, significant increases in lipid peroxidation and hydrogen peroxide levels were observed. Conversely, chlorophyll fluorescence levels were maintained under drought stress, and simultaneous increases in CAT, APX, POX, GR, and SOD enzyme activities indicate an effective antioxidant defense response in the plant.

KEYWORDS
sheep sorrel, *Rumex acetosella*, drought stress, antioxidant defense

Kuzukulağı (*Rumex acetosella* L.) Bitkisinde Kuraklık Stresinin Fizyolojik ve Biyokimyasal Etkileri

Kuzukulağı (*Rumex acetosella* L.), Polygonaceae familyasına ait, ekşi tadı ile bilinen otsu bir bitkidir. Yaprakları gıda olarak tüketilir ve tıbbi özellikleri nedeniyle sağlık sektöründe değerlidir; bu da bitkiye önemli bir etnobotanik önem kazandırır. Bu çalışma, kuraklık stresinin kuzukulağı bitkilerinde su mevcudiyeti, membran stabilitesi, biyokütle ve antioksidan savunma sistemi üzerindeki etkilerini araştırmayı amaçlamıştır. Nisbi büyüme oranı, su içeriği, ozmotik potansiyel, lipid peroksidasyon seviyeleri, hidrojen peroksit konsantrasyonu ve süperoksit dismutaz (SOD), peroksidaz (POX), katalaz (CAT), askorbat peroksidaz (APX) ve glutatyon redüktaz (GR) dahil olmak üzere antioksidan savunma sistemi enzimlerinin aktiviteleri ölçülmüştür. Kuraklık stresi, kuzukulağı bitkilerinde nisbi büyüme oranı, su içeriği ve ozmotik potansiyelde azalmaya neden olmuştur. Ayrıca, lipid peroksidasyon ve hidrojen peroksit seviyelerinde önemli artışlar gözlenmiştir. Diğer taraftan, klorofil floresan seviyeleri kuraklık stresi altında korunurken, CAT, APX, POX, GR ve SOD enzim aktivitelerinde eşzamanlı artışlar, bitkide etkili bir antioksidan savunma tepkisi olduğunu göstermektedir.

ANAHTAR KELİMELER
kuzukulağı, *Rumex acetosella*, kuraklık stresi, antioksidan savunma

1. INTRODUCTION

Climate change and increasing environmental pollution threaten the sustainability of water resources, limiting water availability and promoting water scarcity. Water scarcity has become a problem affecting approximately 40% of the world's population, and it is predicted that rising temperatures by 2030 could further exacerbate water problems, particularly in arid and semi-arid areas (Elakhdar et al., 2022). Agricultural production and plant material applications in landscaping are among the most negatively affected by this situation. The ability of plants to provide high yield and quality throughout all stages of their development depends on the continuity of optimal environmental conditions, and disruptions here trigger stress events in plants (Morgil et al., 2019). Defined as water scarcity resulting from temporary imbalances in moisture levels in a region, drought is an abiotic stress factor that seriously affects plant development (Haghaninia et al., 2023).

Water stress caused by water scarcity disrupts physiological and biochemical functions in plants, leading to a breakdown in metabolic balance that significantly reduces product quality and yield (Esmaielzehi et al., 2024; Jovović et al., 2025). One of the inevitable consequences of drought stress is an increase in the production of reactive oxygen species (ROS) in different cell compartments, namely chloroplasts, peroxisomes, and mitochondria. Under normal conditions, plants continuously produce these substances, as they are by-products of normal cell activity. However, the accumulation of ROS at high concentrations has a toxic effect (Singh et al., 2022); it causes oxidative damage and leads to disruption in photosynthetic events by reducing chlorophyll content (Chowdhury et al., 2021). Lipid peroxidation is triggered, resulting in the disruption of plant cell membrane integrity (Aslam et al., 2022). Plants ensure the detoxification of ROS with the help of an efficient and versatile scavenging system (Cruz de Carvalho, 2008). The main ROS scavenging antioxidants include enzymatic ones such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione peroxidase (GPX), as well as non-enzymatic ones such as ascorbic acid and glutathione (Su et al., 2024). Plant responses to drought stress vary depending on plant species, stress duration and intensity, developmental stage, and cell structure (Ghafar et al., 2021).

Rumex is a genus belonging to the Polygonaceae (Knotweed, Buckwheat) with over 200 species worldwide. It is widely distributed in the flora of Turkey with 28 species and 7 hybrids (Sabuncu et al., 2019; Keskin and Severoğlu, 2023) and is a perennial herbaceous medicinal aromatic plant with a wide distribution in the northern hemisphere, particularly in Europe, Asia and North America (Bello et al., 2019). It occurs naturally in moist rural landscapes and riverbeds in spring; it is also cultivated as a garden plant (Isbilir and Sagiroglu, 2013; Karataş, 2013). Sheep sorrel (*Rumex acetosella*) is a simple plant with green, hairless, lanceolate, alternately arranged leaves (Baig et al., 2011; Ulcay and Şenel, 2020). Its flowers are densely clustered and hermaphroditic (Vasas et al., 2015). The literature reports that the Rumex genus has a high antioxidant content and exhibits anti-inflammatory, anti-tumor, antibacterial, antiviral, and anti-fungal properties (Vasas et al., 2015; Qamar et al., 2018). With its bioactive components and pharmacological properties, sorrel is a medically important species and a plant with ethnobotanical properties that is traditionally used to treat various health problems, such as digestive system diseases and liver disorders, using its above-ground parts and roots (Vasas et al., 2015).

Although several *Rumex* species have been studied under drought, information on *Rumex acetosella* remains limited. Previous studies indicate that *R. acetosella* possesses noteworthy drought tolerance, largely associated with its biomass allocation strategies and physiological plasticity. Zimmerman and Lechowicz (1982) reported that drought resilience in *R. acetosella* is linked more to vegetative resource allocation patterns than to intrinsic physiological distinctions, highlighting the importance of adaptive growth strategies under moisture limitation. Broader research on drought-responsive species further suggests that mechanisms such as improved water-use efficiency,

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regulated gas exchange, and maintenance of leaf water relations contribute to the species' capacity to endure water deficits (Chapman & Augé, 1994). Like other perennial and ornamental plants that adjust metabolic pathways under stress (Tribulato et al., 2019), *R. acetosella* demonstrates both morphological and physiological adjustments that support its survival in arid environments.

Increasing global warming, excessive human population, crop scarcity, environmental degradation and unsustainable agriculture negatively affect the resilience and yield of plants. Therefore, revealing the resilience of plants to abiotic stress conditions will also lead to an understanding of the mechanisms that can positively affect the development and yield of plants under stress. In this context, this study aims to elucidate the effect of drought stress on growth, photochemical processes, and antioxidant mechanisms in sheep sorrel. Furthermore, the findings obtained in this study aim to contribute to the development of strategies to increase the resistance of sheep sorrel to environmental stresses.

2. MATERIAL AND METHODS

2.1. Plant Material

This study was conducted under controlled greenhouse conditions (27/22°C, night/day, 16/8 hours; 70% relative humidity). Sheep sorrel (*Rumex acetosella* L.; Fam: Polygonaceae) seeds were obtained from nature, washed with pure water, and then sterilized with sodium hypochlorite for 15 minutes. Following sterilization, the seeds were sown in pots with a diameter of 16 cm in a substrate consisting of peat, perlite and sand (1:1:1). The plants, cultivated for three months, were divided into two groups; the plants in one group were watered regularly every three days under controlled conditions. The other group of plants was not watered for 3 weeks. The group that was not watered was called the drought stress treatment group. There were 5 pots in each treatment group, with 3 plants in each pot. At the end of the three-week period, leaf lengths were measured, chlorophyll fluorescence was measured, and the plant leaves were harvested. The harvested leaves were immediately frozen with liquid nitrogen and stored at -80°C until analysis.

2.2. Determination of Chlorophyll Fluorescence, Relative Water Content, Osmotic Potential and Relative Growth Rate

To evaluate the photochemical activity of Photosystem II located on the thylakoid membrane, the maximum quantum efficiency (Fv/Fm) was measured. Measurements were performed using a fluorometer after the leaves were dark-adapted. Fv/Fm values were recorded after a 20-minute adaptation period for the treatment groups. Leaf relative water content was determined using the method reported by Smart and Bingham (1974). The leaves were first weighed for wet weight and then soaked in pure water for approximately 12 hours, after which their turgid weight was recorded. For dry weights, the same leaves were dried in an oven (70°C, 72 hours) and substituted in the formula for calculations. Osmotic potential measurement was performed using an extract obtained by crushing sheep sorrel leaves with a glass rod and then centrifuging at 5000 rpm for 5 minutes. The extracts belonging to the application groups were measured and recorded using an osmometer (Santa-Cruz et al., 2002). For the relative growth rate, leaf samples were randomly taken from both groups before the applications began and when the applications ended, dried in an oven (70°C), and their dry weights were determined. Growth rates were calculated by substituting the formula as reported in the study by Hunt and co-workers (2002).

2.3. Determination of Hydrogen Peroxide Content and Lipid Peroxidation

To determine the hydrogen peroxide (H_2O_2) content, 0.5 g of leaves were homogenized with trichloroacetic acid (TCA) and then centrifuged. The resulting extract was treated with 0.1% $TiCl_4$ and then measured using a spectrophotometer at a wavelength of 410 nm to determine the H_2O_2

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content in µmol/g (Liu et al., 2000). To determine the level of lipid peroxidation, 0.5 g of leaves were homogenized with TCA and then centrifuged. The extract obtained after centrifugation was incubated at 90°C for 30 minutes in a solution containing 0.5% thiopental and 20% TCA. After the incubation period, the tubes were allowed to cool to room temperature, and the absorbance was measured at 532 and 600 nm wavelengths (Madhava Rao and Sresty, 2000).

2.4. Determination of Antioxidant Enzyme Activities

To determine the activity levels of enzymes belonging to the antioxidant system, extracts were first obtained from leaf samples. For this purpose, 0.5 g of sample was homogenized with a solution containing 50 mM phosphate buffer (pH: 7.0), 1 mM EDTA, and 1% polyvinylpyrrolidone. For ascorbate peroxidase activity, 2 mM ascorbic acid was additionally added to the medium. Following homogenization, extracts were obtained from the treatment groups by centrifuging at 14,000 g for 30 minutes. The total protein content in the extracts was calculated according to the Bradford (1976) method. The activity of superoxide dismutase (SOD), an antioxidant defence system enzyme, was measured according to Beauchamp and Fridovich (1971), peroxidase activity according to Mika and Lüthje (2003), catalase (CAT) activity according to Aebi (1984), ascorbate peroxidase (APX) activity according to Nakano and Asada (1981), and glutathione reductase (GR) activity according to Foyer and Halliwell (1976). The enzyme activities were expressed as U/mg protein in the measurement results.

2.5. Statistical Analyses

Throughout the study, experiments were conducted using 5 pots, each containing 3 plants. A one-way analysis of variance (ANOVA) was used to analyze the data. Results are expressed as arithmetic mean ± standard error. Differences between means were compared using Duncan's test ($p < 0.05$).

3. RESULTS AND DISCUSSION

The drought condition arising from factors such as the irregular distribution of rainfall, evapotranspiration, and water retention capacity in the rhizosphere is one of the environmental stress factors that most severely limits plant productivity (DeVincentis et al., 2021). This study demonstrated that physiological processes such as growth, photosynthetic efficiency, and water status of sheep sorrel were significantly affected by drought stress and that the antioxidant defense system was activated during this process. The morphological differences in the *Rumex acetosella* are shown in Figure 1 and the remarkable reduction in morphology can be seen from this image. To evaluate the effect of drought stress on the water status of the plant, the relative water content (RWC) of the leaves was measured. The RWC in sheep sorrel plants under drought stress decreased by 20.9% compared to control plants (Figure 2A). Like our study, the decrease in RWC under drought stress has been determined in different studies (Boussadia et al., 2023; Lu et al., 2023; Ghimire et al., 2024; Habibi et al., 2024). Chlorophyll fluorescence data were measured to determine the photochemical efficiency of photosystem II. It was found that drought stress did not cause a statistically significant ($p < 0.05$) difference in the Fv/Fm ratio in sheep sorrel (Figure 2B). The maintenance of photosynthetic activity under drought stress may be a reflection of the plant's effective defense system.

Osmotic potential is known as the potential energy of dissolved substances in a solution that affects water uptake into plant cells, particularly during drought stress, to maintain osmotic balance. This is one of the physiological responses of plants to water stress and plays an important role in retaining intracellular water (Bartlett et al., 2014). In our study, drought treatment caused a 2.8-fold decrease in osmotic potential compared to control plants (Figure 2C). Similarly, drought stress in *Mentha*

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spicata (Araniti et al., 2024) and *Triticum aestivum* (Pandya et al., 2023) plants also reduced osmotic potential by inducing osmolyte synthesis. A decrease in growth was also observed in sheep sorrel under drought conditions. Plants under drought stress showed a 73% decrease in relative growth compared to those without drought treatment (Figure 2D). The restrictive effects of drought on growth are directly proportional to the decrease in the plant's water content. Like the results obtained in our study, drought stress has also reduced growth in rapeseed (Biswas et al., 2019), lentils (Jahantigh and Amiri, 2020) and olives (Boughalleb and Hajlaoui, 2011). Furthermore, drought has been reported to limit growth in other species belonging to the *Rumex* genus (Simova-Stoilova et al., 2011).

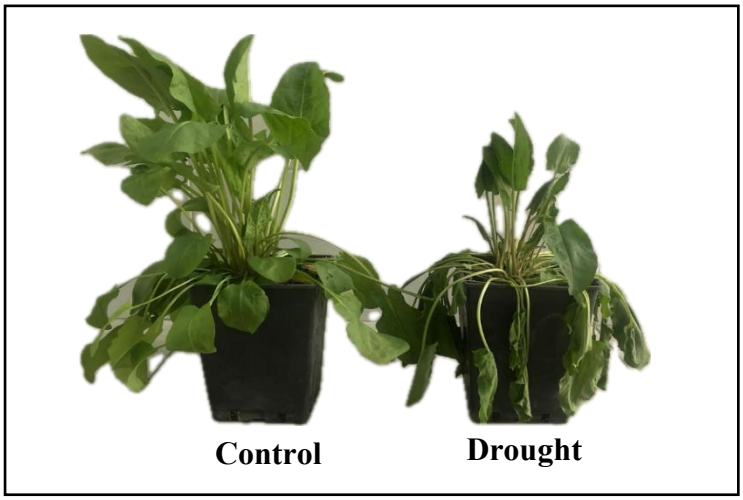


Figure 1. *Effect of drought stress on Rumex acetosella*

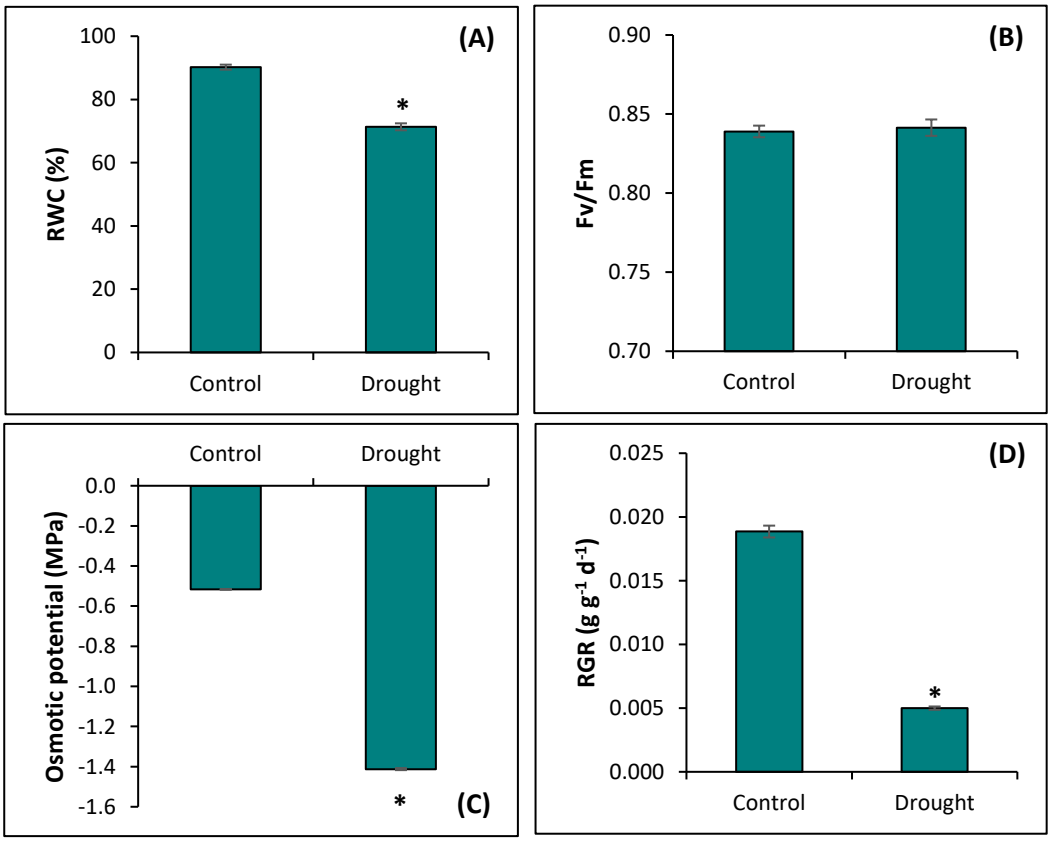


Figure 2. *A: Relative water content (RWC). B: Chlorophyll fluorescence (Fv/Fm). C: Osmotic potential. D: Relative growth rate (RGR). Data represents the mean ± standard deviation. Columns marked with an asterisk indicate a significant difference at the p < 0.05 level.*

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Lipid peroxidation disrupts the integrity of cell and organelle membranes in plants, thereby weakening their selectivity (Rawat et al., 2021). Therefore, lipid peroxidation is an important indicator in determining drought-induced damage. In our study, drought treatment increased lipid peroxidation in sheep sorrel leaves by 2.7 times compared to untreated plants (Figure 3A). This drought-induced condition is related to damage to the membrane structure. Our findings are consistent with those of many studies showing that drought stress increases lipid peroxidation (Wei et al., 2013; Chen et al., 2017; Kulundžić et al., 2022; Meenakshi et al., 2022; Sadak et al., 2022; Guzel Deger et al., 2025). Lipid peroxidation has also been detected under drought stress in another species belonging to the *Rumex* genus, and increased electrolyte leakage as a result of membrane damage has been reported (Gilgen and Feller, 2014).

Drought stress increases the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, singlet oxygen, ozone, and organic peroxides, leading to oxidative damage in leaf cells and disruption of cellular integrity (Černý et al., 2018). Hydrogen peroxide (H_2O_2) is one of the most stable ROS, easily transported between cells and playing an important role in signal transduction (Hasanuzzaman et al., 2017). In our study, the amount of H_2O_2 in the leaves of sheep sorrel increased by 98% under drought stress compared to control plants (Figure 3B). Similarly, studies conducted on wheat (Günay et al., 2022) and tomato (Teker Yıldız and Akı, 2025) have also reported that drought stress increases H_2O_2 levels.

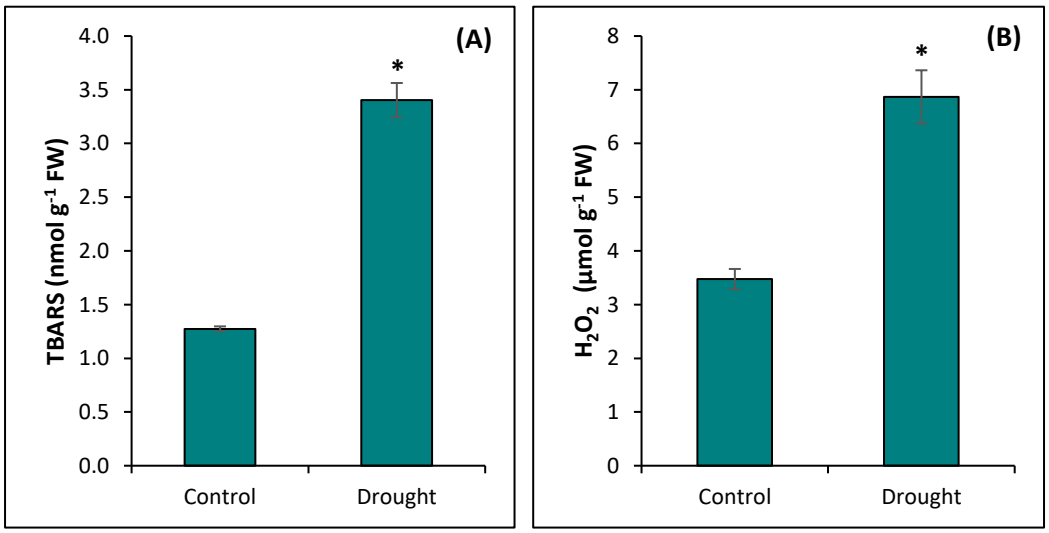


Figure 3. *A: Lipid peroxidation (TBARS). B: Hydrogen peroxide (H_2O_2). Data represents the mean ± standard deviation. Columns marked with an asterisk indicate a significant difference at $p < 0.05$.*

Among abiotic stress types, drought stress disrupts critical physiological processes such as photosynthesis and respiration, leading to excessive ROS accumulation and causing secondary stresses such as oxidative stress (Lv et al., 2023; Qiao et al., 2024). Plants activate complex antioxidant defense systems consisting of enzymatic and non-enzymatic mechanisms to combat oxidative damage (Huang et al., 2019; Mohagheghian et al., 2025). In our study, the antioxidant defense system enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), peroxidase (POX), and glutathione peroxidase (GPX) were measured in the leaves of the sheep sorrel plant. These defense system enzymes work together to neutralize ROS by maintaining it at non-toxic signaling levels and to protect cellular components from oxidative stress damage in adverse environments (Farooq et al., 2009; Sun et al., 2020; Hasanuzzaman et al., 2020). SOD functions as a primary defense enzyme and effectively reduces the precursor of toxic hydroxyl radical formation via Haber-Weiss/Fenton reactions by catalyzing the dismutation of the highly reactive superoxide radical into less reactive hydrogen peroxide and molecular oxygen (Tyagi et al., 2019). Downstream antioxidant enzymes such as CAT, APX, and POX are also required for the subsequent clearance and detoxification of H_2O_2 produced by SOD (Rajput et al., 2021; Rao et al., 2025). It is

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important to note that the overall cellular H_2O_2 pool is not solely dependent on SOD activity, as H_2O_2 is continuously produced as a by-product of normal metabolism and is actively generated in various cellular compartments (ul Islam et al., 2023). Major H_2O_2 production sites unrelated to SOD include glycolate oxidase activity during photorespiration in peroxisomes, *β*-oxidation of fatty acids in glycoxisomes and peroxisomes, and NADPH oxidase enzymes localized in the plasma membrane in the apoplast, which produce superoxide that is rapidly converted to H_2O_2. (Hasanuzzaman et al., 2020; Rajput et al., 2021).

To determine the effect of drought stress on the antioxidant defense system, SOD, POX, CAT, APX and GR enzyme activities were measured in sheep sorrel, and the changes occurring under stress conditions were determined. In our study, CAT, APX, POX, GR, and SOD enzyme activities in sheep sorrel plants under drought stress increased by 83.3%, 66.7%, 5.7-fold, 3.6-fold, and 6.9-fold, respectively, compared to the control group (Figure 4A-E).

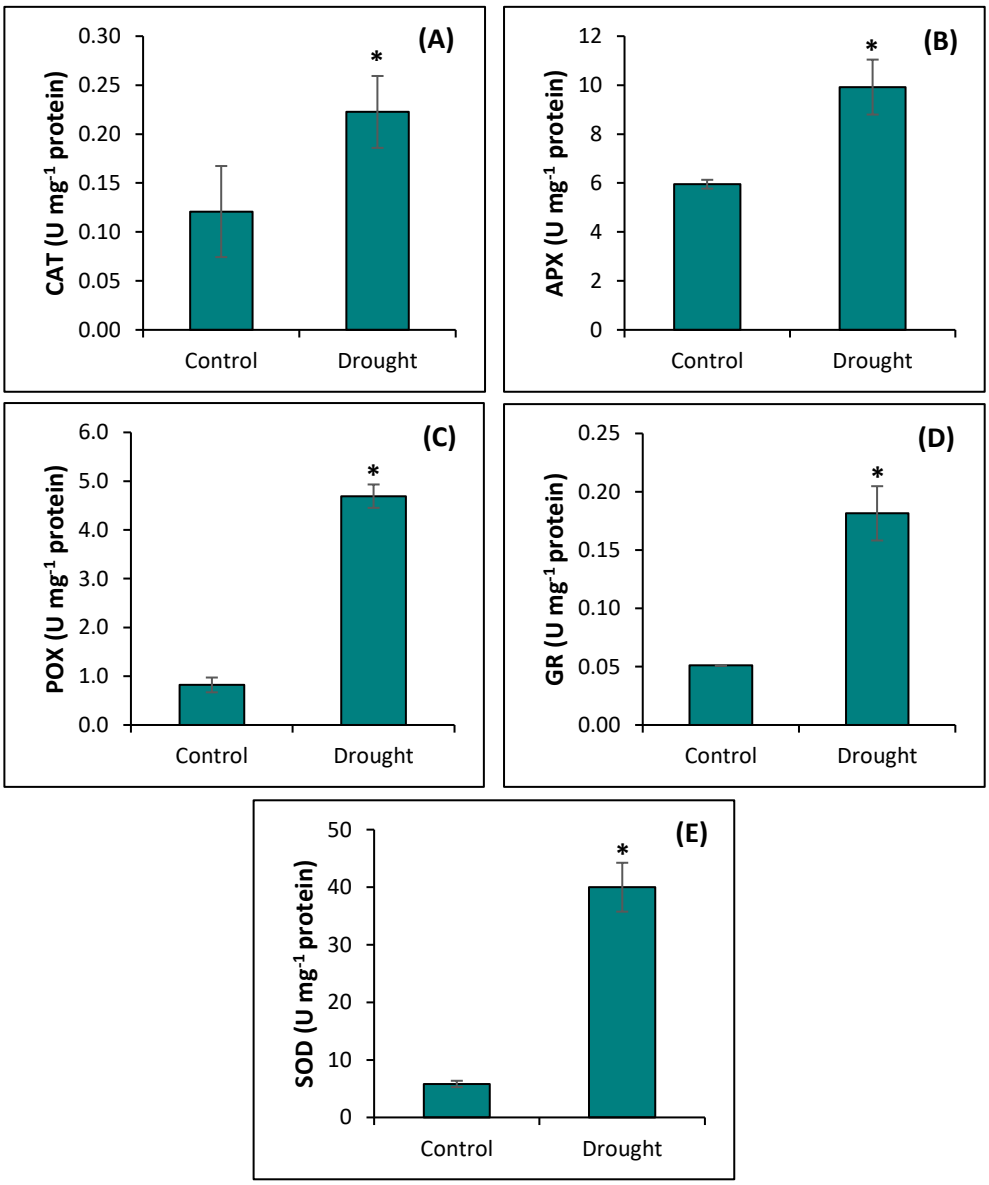


Figure 4. *Enzyme activities in Rumex acetosella. A: Catalase (CAT). B: Ascorbate peroxidase (APX). C: Peroxidase (POX). D: Glutathione reductase (GR). E: Superoxide dismutase (SOD). Data represent mean ± standard deviation. Columns marked with an asterisk indicate a difference at the $p < 0.05$ level.*

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4. CONCLUSION

This study investigated the physiological and biochemical changes occurring in *Rumex acetosella* under drought stress. Drought caused a significant decrease in relative water content, osmotic potential, and relative growth rate in the sheep sorrel plant. Furthermore, it was observed that drought stress did not cause changes in chlorophyll fluorescence but led to an increase in lipid peroxidation levels and hydrogen peroxide amounts. In addition, significant increases were detected in the activities of antioxidant defense system enzymes, namely catalase, ascorbate peroxidase, peroxidase, glutathione reductase, and superoxide dismutase. Under arid conditions, *Rumex acetosella*, which stands out with its strong antioxidant defense system, can be considered an important plant for medicinal aromatic plant cultivation and ethnobotanical uses due to its potential to adapt to other harsh environmental conditions. Research on drought tolerance may contribute to a better understanding of the plant's stress responses and the development of more resilient species.

Author Declarations and CRediT Roles

There is no conflict of interest. No financial support has been received. Ethical committee approval is not required. Generative artificial intelligence tools have not been used. It has not been previously presented or published. The data are presented within the article.

Conceptualization: SŞ, HT; Data curation: SŞ, HT; Formal analysis: SŞ; Investigation: SŞ, HT; Methodology: SŞ, HT; Resources: SŞ, HT; Software: SŞ, HT; Supervision: HT; Validation: HT; Visualization: SŞ, HT; Writing – original draft: SŞ, HT; Writing – review & editing: HT.

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