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Research Article

Physicochemical and Antioxidant Responses of St. John's Wort (*Hypericum perforatum* L.) under Drought Stress

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ABSTRACT

This study investigated the effects of drought stress on the physiological and biochemical responses of the medicinal and aromatic plant *Hypericum perforatum* (St. John's Wort). Changes were determined in leaf length, relative water content (RWC), osmotic potential, chlorophyll fluorescence (Fv/Fm), lipid peroxidation (TBARS), hydrogen peroxide (H_2O_2), and proline content as well as in the antioxidant system enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), and glutathione reductase (GR). These responses were examined in relation to the tolerance of drought stress in *H. perforatum.* Ninety-day-old seedlings were subjected to drought for three weeks. The physiological parameters of leaf length, RWC, Fv/Fm, and osmotic potential were reduced under drought. The H_2O_2, TBARS, and proline levels were increased significantly under drought stress. Moreover, the proline content increase was greatly pronounced (25.9-fold) compared to the control groups. The high accumulation of proline may have resulted from the 83.8% leaf RWC still remaining under drought stress. On the other hand, the SOD, CAT, and GR enzyme activities were enhanced, whereas the POX and APX activities were reduced. The results indicate that improved tolerance to drought stress in *H. perforatum* plants may be accomplished through increased capacity of the antioxidative defense system.

Keywords: *Antioxidant defense, drought stress, Hypericum perforatum, St. John's wort*

Kuraklık Stresi Altında Sarı Kantaronun (*Hypericum perforatum* L.) Fizikokimyasal ve Antioksidan Tepkileri

ÖZET

Bu çalışmada, kuraklık stresinin *Hypericum perforatum*'daki (St. John's Wort) fizyolojik ve biyokimyasal tepkileri üzerine olan etkileri araştırılmıştır. Kuraklık stresine karşı toleransla ilişkili olarak bu tıbbi ve aromatik bitkide yaprak uzunluğu, bağıl su içeriği (RWC), ozmotik potansiyel, klorofil floresan (Fv/Fm), lipid peroksidasyonu (TBARS), hidrojen peroksit (H2O2), prolin içeriği ve antioksidan sistemdeki (süperoksit dismutaz (SOD), katalaz (CAT), peroksidaz (POX), askorbat peroksidaz (APX) ve glutatyon redüktaz (GR) enzim aktiviteleri) değişimler belirlenmiştir. Doksan günlük fidanlar 3 hafta süreyle kuraklığa maruz bırakılmıştır. Kuraklık altında fizyolojik parametrelerden uzunluk, RWC, Fv/Fm ve ozmotik potansiyel azalmıştır. Kuraklık stresi altında H_2O_2, TBARS ve prolin seviyeleri önemli ölçüde artmıştır; ancak kontrol grupları ile kıyaslandığında bu artış prolin içeriğinde (25.9 kat) daha belirgindir. Yüksek prolin birikimi, yapraktaki RWC'nin kuraklık stresi altında hala %83,8 olarak kalmasının bir sonucu olabilir. Diğer taraftan, SOD, CAT ve GR enzim aktiviteleri artarken, POX ve APX

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aktiviteleri azalmıştır. Sonuçlar, *H. perforatum* bitkisinde kuraklık stresine karşı geliştirilmiş toleransın, artan antioksidatif savunma sistemi kapasitesi ile başarılabileceğini göstermektedir.

Anahtar Kelimeler: *Antioksidan savunma, Kuraklık stresi, Hypericum perforatum, Sarı kantaron*

I. INTRODUCTION

St. John's Wort (*Hypericum perforatum* L.), which is a member of Hypericaceae family, is a herbaceous perennial plant widely distributed throughout the world [1]. It has been used as a medicinal plant for centuries [2] because of its significant levels of biologically active agents, specifically phenolic compounds including phenolics (e.g., caffeic and chlorogenic acids), naphthodianthrones (e.g., hypericin, pseudohypericin, and phlorodianthrones), phloroglucinols (e.g., hyperforin), and flavonoids (e.g., quercetin, hyperoside, quercitrin, isoquercitrin, rutin, and procyanidins) [3-5]. This species is important for medicinal uses because of its anti-inflammatory, antidepressant, analgesic, antimalarial, diuretic, sedative, and vulnerary effects [6]. In spite of its medicinal importance, the response of *Hypericum perforatum* to undesirable environmental conditions such as drought, salinity, heat, and heavy metals has been insufficiently reported in the literature.

Drought is the greatest disastrous event affecting agriculture, as 83% of all damage and loss caused by drought is absorbed by agriculture [7]. Most physiological processes such as photosynthesis, respiration, and uptake of mineral nutrients are affected by drought, and this inhibiting of plant productivity causes crop losses [8,9]. Drought stress leads to the overproduction of highly reactive and toxic reactive oxygen species (ROS) in plants, which damage carbohydrates, proteins, lipids, and DNA [10]. Singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), super oxide (O^{2-}), and hydroxyl ($OH^{\bullet}$) are known as ROS and are produced in the chloroplast, mitochondria, peroxisomes, and other cellular compartments [11-13]. To surmount the overproduction of these reactive substances, plants have acquired dedicated pathways to protect themselves from ROS toxicity [13,14]. The most important tolerance mechanism for scavenging ROS is the plant antioxidant defense system, which contains enzymatic antioxidants, which include ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), peroxidase (POX), and superoxide dismutase (SOD), as well as non-enzymatic antioxidants (ascorbic acid, glutathione, carotenoids, tocopherols and flavonoids) [13,15].

The ability of plants to control drought stress depends on their tolerance mechanisms [16]. Therefore, revealing the ROS scavenging and detoxifying capacity in plants is important, especially for those used in the pharmaceutical, cosmetics, or food sectors. In the past few years, the drought tolerance has been recorded for many plants, such as *Oriza sativa* [17], *Triticum vulgare* [18], *Salvia officinalis* [19], *Helianthus annus* [20], *Hordeum vulgare* [9], *Glycine max* [16], and many others. From this point of view, although St. John's Wort is of medicinal and aromatic importance, virtually no studies have revealed its drought tolerance regarding ROS formation and detoxification in terms of its antioxidant defense system. Most previous studies have focused on the biologically active substances in *H. perforatum*. Therefore, we aimed to examine the tolerance potential of *H. perforatum* when subjected to drought, based on changes in the values of leaf length as a growth parameter, leaf relative water content (RWC), chlorophyll fluorescence (Fv/Fm), osmotic potential, proline level, lipid peroxidation (TBARS), hydrogen peroxide (H_2O_2) content and antioxidant enzyme activities.

II. MATERIAL AND METHODS

A. PLANT MATERIAL AND GROWTH CONDITIONS

Greenhouse experiments were conducted using St. John's Wort (*Hypericum perforatum* L.) plants. Before planting, seeds were sterilized with sodium hypochlorite (5%) and then rinsed with water. They were sown in polyethylene pots filled with peat moss, perlite, and sand at a ratio of 1:1:1. The seedlings were grown at 27 ℃ (day) and 22 °C (night) and at a relative humidity of 70% in a controlled greenhouse. After 90 days, two experimental groups (control and drought) were randomly formed. The drought period lasted three weeks. Leaving the plants without water was considered as a drought treatment. Before harvesting, the plants were photographed and the lengths of the leaves were measured with a standard ruler. Mature leaves were harvested after 21 days, frozen at -196 °C, and then stored at -80 °C for further analyses.

B. METHODS

B. 1. Leaf Relative Water Content

Leaf relative water content (RWC) was determined according to Smart and Bingham [21]. The fresh mass of the harvested leaves was determined and the turgid mass was found by floating them on dI-H_2O. The leaves were then dried at 70 °C for 72 h and calculations were carried out using the formula: RWC (%) = [(Fresh mass-Dry mass) / (Turgid mass-Dry mass)] × 100.

B. 2. Chlorophyll Fluorescence

The chlorophyll fluorescence quenching, serving as the quantum efficiency of the photosystem II, was measured with a chlorophyll fluorimeter (Plant Efficiency Analyzer, Hansatech, UK) at room temperature. Prior to the measurement, the leaves were dark-adapted for 20 min. The PSII photochemistry was measured as the Fv/Fm ratio.

B. 3. Osmotic Potential

The osmotic potential of the leaves was detected with a vapor pressure osmometer (Wescor Vapro). First, the leaves were crushed with a glass rod, centrifuged, and measured. The results obtained were then converted to MPa according to Santa-Cruz *et al.* [22].

B. 4. Proline Content

Leaf proline content was quantified according to Bates *et al.* [23]. Fresh leaf tissue (0.5 g) was homogenized in sulphosalycylic acid (3%) and then filtered. Next, 2 mL of the filtered solution were mixed with glacial acetic acid (2 mL) and acid-ninhydrin (2 mL). The mixture was then exposed to 95 °C temperature for 1 h. After cooling, toluene (4 mL) was added and vortexed. The supernatant was used for the readings and recorded at 520 nm.

B. 5. Hydrogen Peroxide Content

Leaf hydrogen peroxide (H_2O_2) content was quantified according to the method described by Liu *et al.* [24]. Fresh leaf samples (0.5 g) were homogenized in 1% trichloroacetic acid (TCA) and the homogenized material was mixed with 1.5 mL of $TiCl_4$ (0.1%). Known concentrations of H_2O_2 were used for preparing a standard curve and the absorbance values were recorded at 410 nm.

B. 6. Lipid Peroxidation

Leaf lipid peroxidation level was quantified according to the method of Madhava Rao and Sresty [25] by measuring the thiobarbituric acid reactive substances (TBARS). Leaf samples (0.5 g) were extracted with TCA (0.1%). After centrifugation, the supernatant was mixed with TCA containing thicbarbutiric acid (20% TCA + 0.5% TBA). The mixture was then exposed to 90 °C temperature for 30 min. Following cooling, the absorbance values were recorded at 532 nm.

B. 7. Crude Plant Extraction and Antioxidant Enzyme Assays

Fresh leaf samples (0.5 g) were homogenized in a pre-cooled mortar in K-phosphate buffer (50 mM; pH 7.0) consisting of ethylenediaminetetraacetic acid (EDTA) and polyvinylpyrrolidone (PVP). For the APX activity assay, ascorbate was added to the buffer. An aliquot was used for enzyme activities and the total protein content of this aliquot was determined by Bradford [26].

Superoxide dismutase (SOD) activity was assayed according to the ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm using the method of Beauchamp and Fridovich [27]. The reaction mixture contained K-phosphate buffer (50 mM; pH 7.0), methionine (13 mM), EDTA (0.1 mM), NBT (0.075 mM), and riboflavin (0.002 mM). One unit of specific enzyme activity for SOD determination was defined as the 50% inhibition of the NBT photoreduction. Total peroxidase (POX) activity was quantified according to Mika and Lüthje [28]. The reaction mixture contained Na-acetate buffer (25 mM; pH 5.0), H_2O_2 (10 mM), and guaiacol (10 mM). The absorbance readings were recorded at 470 nm for 3 min. Total catalase (CAT) activity was estimated according to Aebi [29]. The reaction mixture contained K-phosphate buffer (50 mM; pH 7.0) and H_2O_2 (10 mM). The absorbance readings were recorded at 240 nm for 3 min. Total ascorbate peroxidase (APX) activity was measured according to Nakano and Asada [30]. The reaction mixture contained Na-phosphate buffer (50 mM; pH 7.0), H_2O_2 (5 mM), and ascorbate (250 µM). The absorbance readings were recorded at 290 nm. Total glutathione reductase (GR) activity was assayed according to the method described by Foyer and Halliwell [31]. The reaction mixture contained Tris-HCl buffer (50 mM; pH 7.6), oxidized glutathione (GSSG; 10 mM), and NADPH (5 mM). The absorbance readings were noted at 340 nm for 3 min.

B. 8. Statistical Analysis

A completely randomized design was applied for data analyses and six replications were performed in each experiment. Data was subjected to one-way analysis of variance (ANOVA), and Duncan's post-hoc multiple range test was used to determine significant differences at 5% probability.

III. RESULTS AND DISCUSSION

The present study investigated the physiological and biochemical alterations of *Hypericum perforatum* under drought stress. The morphological differences in the *H. perforatum* are shown in Figure 1 and the remarkable reduction in morphology can be seen from this image. Similar differences were also recorded in water stressed *Nicotiana tobaccum* [32]. In addition to morphological changes, cellular changes were determined in the present study. Drought stress (leaving the plants without water for three weeks) significantly reduced leaf RWC and length, the efficiency of photosystem II (Fv/Fm), and osmotic potential (Fig. 2). Compared with the control plants, reductions by 15.4, 21.5, 1.45, and 40.4%, respectively, were recorded in *H. perforatum* leaves. In previous studies, drought stress has been shown to reduce plant growth [16,19,33]. Growth reduction might have been due to the restricted water uptake and reduced water content [34]. Moreover, our investigations showed that drought stress decreased RWC, leaf osmotic potential, and Fv/Fm in *H. perforatum.* The reduction of these parameters also correlated with each other. A possible reason for the reduced Fv/Fm of the drought-stressed leaves may have been the reduction in the plant water status. These results were consistent with those for rice [17], sage [19], and soybean [35] under drought stress.

Figure 1. *Effect of drought stress on Hypericum perforatum. Scale bar, 5 cm.*

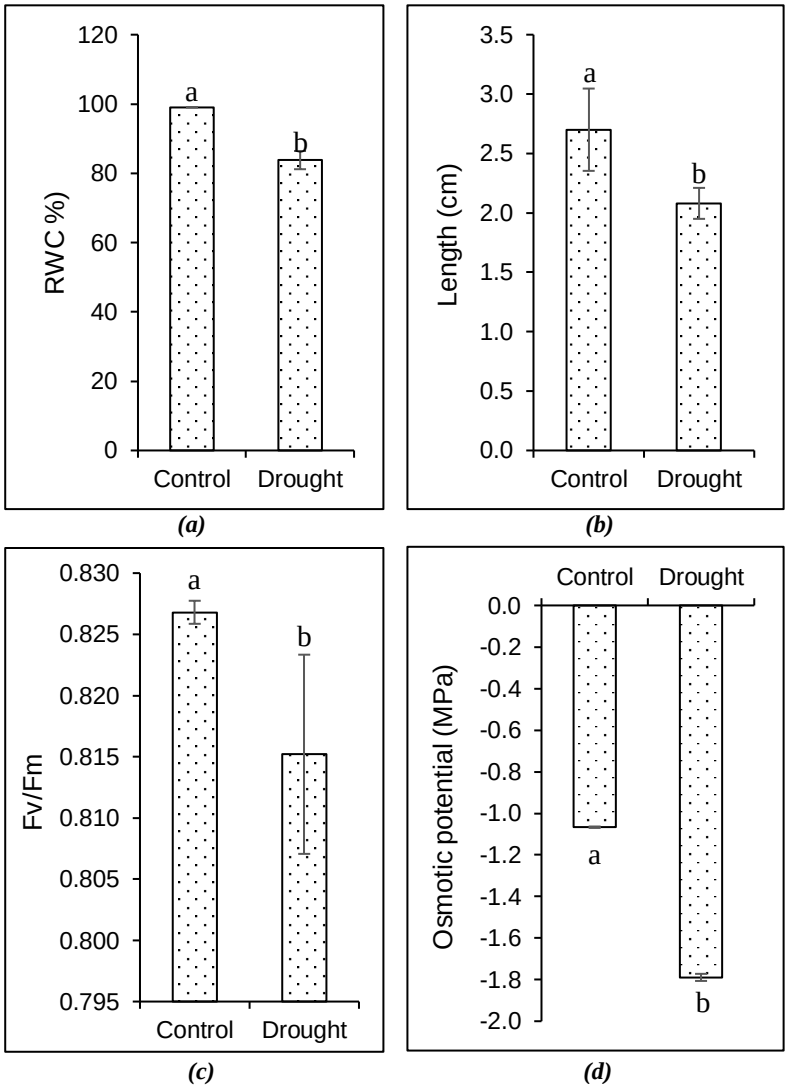

Figure 2. *Leaf relative water content (RWC)* ***(a)****, leaf length* ***(b)****, maximum quantum yield (Fv/Fm)* ***(c)****, and osmotic potential* ***(d)*** *in Hypericum perforatum L. grown under drought stress.*
Mean ± SE; mean values sharing different letters are significantly different at P < 0.05.

Increasing levels of H_2O_2 (a ROS member) lead to a concomitant increase in the levels of TBARS (a lipid peroxidation marker) and this situation causes oxidative damage to membranes, proteins, and RNA and DNA molecules [36]. In our study, in the *H. perforatum* leaves, significant increases were found in both H_2O_2 and TBARS content (by 60.4 and 96.3%, respectively) when compared to the control plants (Fig. 3a and b). Similarly, drought stress-induced H_2O_2 and lipid peroxidation increases have been detected in plants such as rice [17], *Oudneya africana* [37], wheat [38], and soybean [16]. Hence, an increase in the levels of H_2O_2 and TBARS could be a good indicator of oxidative stress in plant cells caused by the reduction in RWC as well as a consequence of cellular damage and cell death. On the other hand, proline, one of the most compatible osmolytes accumulating under drought stress, was also measured in this study. Proline acts as a non-toxic osmolyte and mitigates drought stress in plants by quenching ROS and maintaining the stabilization of cell membranes [39]. In our study, there was a decline in RWC and a significant increase in proline content. Drought stress increased proline levels 25.9-fold in *H. perforatum* leaves compared to the control plants (Fig. 3c). Moreover, the leaf osmotic potential of *H. perforatum* detected was -1.8 MPa under drought stress. The reason the water potential did not significantly decrease may have been the excessive increase in proline. In relation to this, with its elevating proline content, *H. perforatum* sustained its leaf water status better than many plants under drought stress. These results are consistent with previous studies [38,40].

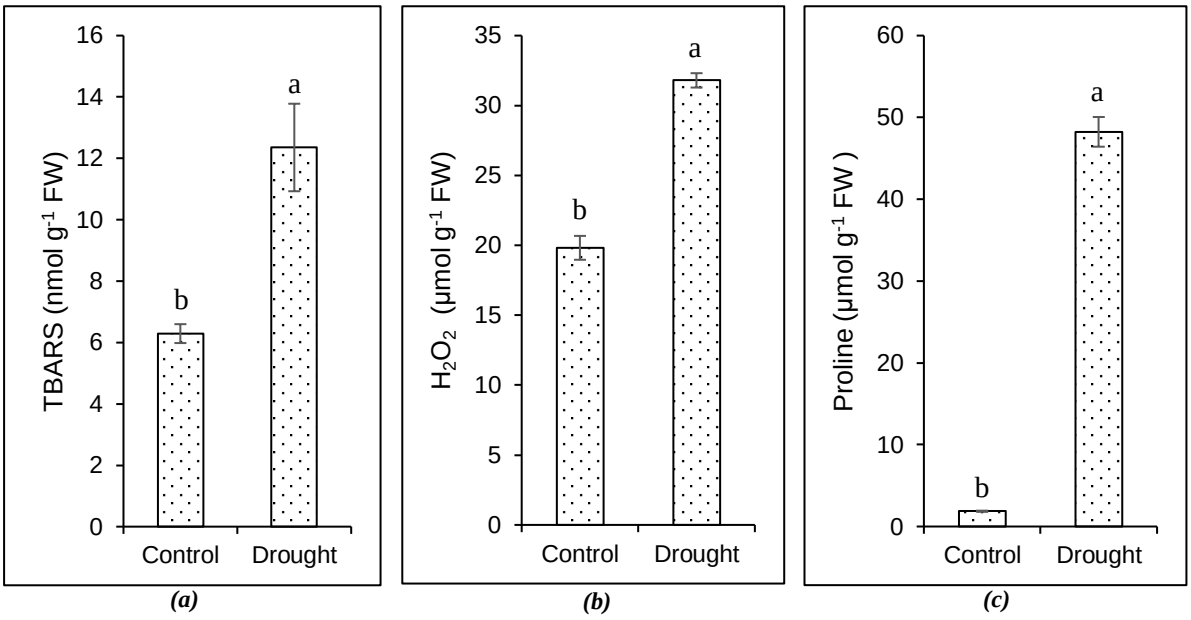

Figure 3. *Lipid peroxidation (TBARS)* ***(a)****, hydrogen peroxide (H_2O_2* ***(b)****, and proline* ***(c)*** *contents in Hypericum perforatum L. grown under drought stress.*
Mean ± SE; mean values sharing different letters are significantly different at $P < 0.05$.

Drought stress induction generally results in higher accumulation of ROS. Over-production of these species leads to damage in cellular redox homeostasis and promotes oxidative damage, which can also affect redox signaling and the regulation of cell response [41]. In the present study, over-production of H_2O_2, one of the main ROS members, was detected (Fig. 3b). Scavenging of H_2O_2 is vital for maintenance of the cellular redox state under drought conditions. As a result, the antioxidant defense system is activated and high antioxidant enzyme activities cause better ROS scavenging capacity in plant genotypes. The SOD plays a significant role in ROS-mediated oxidative defense and converts $O_2^{\cdot-}$ to more stable H_2O_2 [42]. This H_2O_2 can diffuse among different compartments such as cytosol, peroxisomes, and mitochondria [15,43]. The CAT and APX, as the main scavengers of H_2O_2 in plants, break down H_2O_2 into water and oxygen [15]. Moreover, APX and GR are crucial enzymes involved in the ascorbate-glutathione (AsA-GSH) cycles [10]. In the literature, there is little information about the antioxidant defense system of *H. perforatum* plants under drought stress.

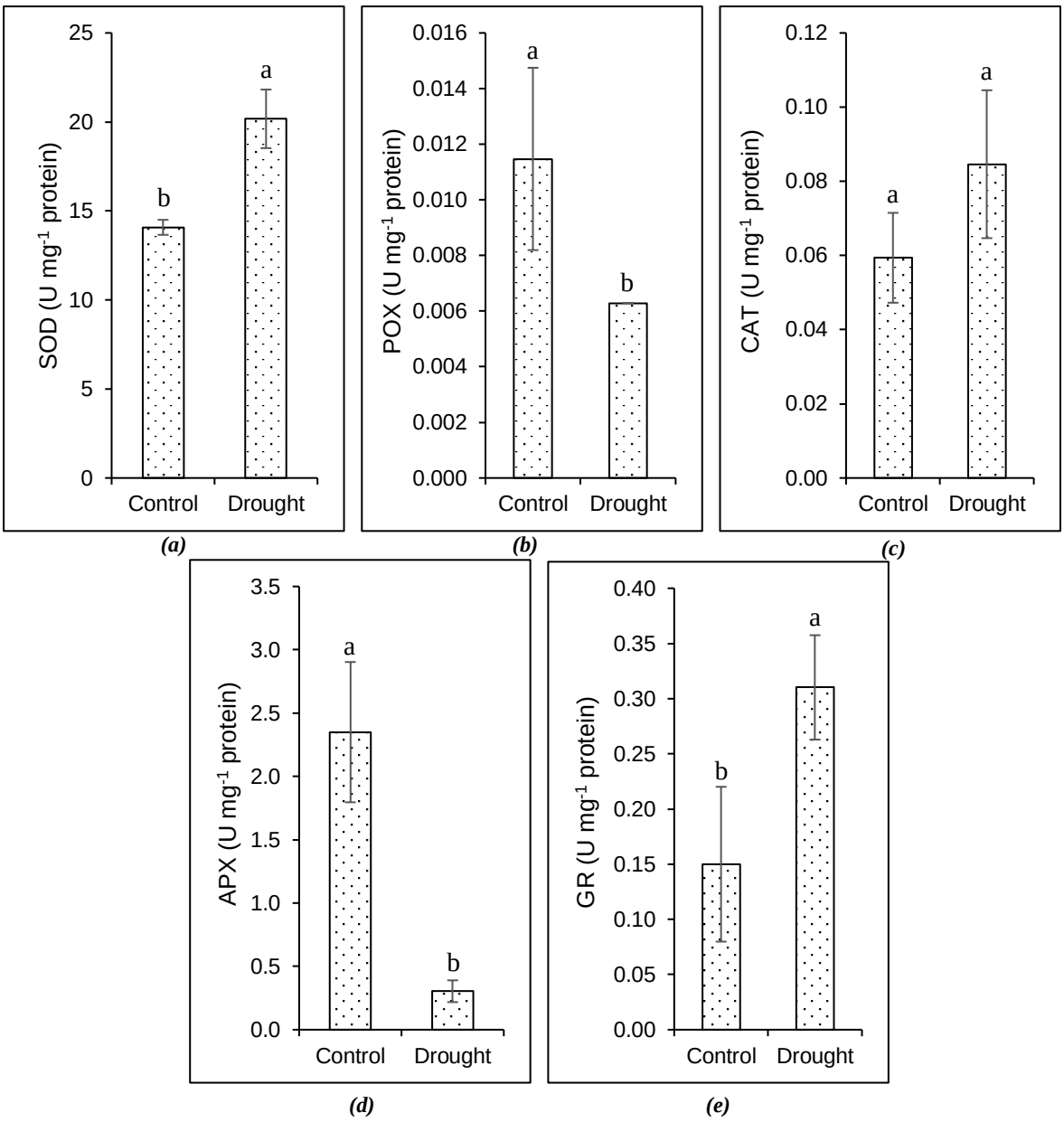

Figure 4. *Activities of superoxide dismutase (SOD)* ***(a)****, peroxidase (POX)* ***(b)****, catalase (CAT)* ***(c)****, ascorbate peroxidase (APX)* ***(d)****, and glutathione reductase (GR)* ***(e)*** *in Hypericum perforatum L. grown under drought stress.*

Mean ± SE; mean values sharing different letters are significantly different at P < 0.05.

In the current research, APX, CAT, GR, POX, and SOD activity levels in *H. perforatum* displayed changes under drought conditions. The SOD, CAT, and GR activities were enhanced by 43.3%, 44.1% and 2.1-fold, whereas the POX and APX activities decreased by 45.5% and 87.1%, respectively, compared to the control plants (Fig. 4). Similar observations for SOD, CAT, and GR increases were reported for tomato [44], chrysanthemum [45], *Oudneya africana* [37], wheat [38], soybean [16], and pepper [46] under drought stress. Another antioxidant enzyme measured in this study was POX, which is also responsible for detoxifying H_2O_2 in chloroplasts and cytosols. The POX displayed a reduction under these conditions (Fig. 4b). Like POX activity, APX activity was also reduced in *H. perforatum* leaves under drought treatment (Fig. 4d). Similarly reduced activities of POX and APX under drought stress have been reported [38]. The increased accumulation of H_2O_2 and TBARS might have been caused by this decrease in POX and APX activities in this species. This suggests that the activity of CAT may be more important than POX and APX activities in scavenging H_2O_2 in drought-stressed *H. perforatum*.

On the other hand, it can also be suggested that the duration time of the drought application in the present study might have been insufficient for the *H. perforatum* and therefore, APX activity might play a significant role in scavenging H_2O_2 for this species during long-term stress.

IV. CONCLUSION

Overall, in the present study, drought stress adversely affected the physicochemical processes, leading to a decline in plant growth of St. John's Wort (*Hypericum perforatum*), a medicinal and aromatic plant. Leaf relative water content, leaf length, maximum quantum yield of Photosystem II, and osmotic potential decreased, whereas leaf water level was maintained at a high level (83.8%). Moreover, lipid peroxidation and H_2O_2 were both induced by drought. Proline content increased with the reduction of osmotic potential in the leaves. The antioxidant defense enzyme activities, especially those of SOD, CAT, and GR, were suppressed under drought stress. The higher levels of antioxidant enzyme activities in spite of increased levels of H_2O_2 and TBARS might have resulted from the powerful defense of this medicinal plant against drought stress. In future studies, for better regulation of the *H. perforatum* antioxidant defense system, the involvement of signal molecules and the stress duration might be considered.

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