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Araştırma Makalesi / Research Article

Antioxidative Responses of Sage to PEG-induced Drought Stress

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Abstract

The current study was conducted to find out the effect of two polyethylene glycol (PEG) induced drought treatments (-0.4 and -0.8 MPa) on sage (*Salvia officinalis* L.). Twenty five-day old cuttings were exposed to drought treatments for seven days. Membrane damage and water loss were gradually increased with the severity of drought. While reductions in membrane integrity and water content showed that sage was affected by drought, even increased flavonoid and antioxidant enzyme activities could not alleviate this effect. H_2O_2 content of leaves increased with decreasing water potential conditions, indication an oxidative stress caused by drought. Elevated levels of SOD and POD activities indicated that the enzymes were involved in scavenging of H_2O_2. Sage was successfully increased the tolerance to withstand to drought.

Keywords: Antioxidant enzymes, *Salvia officinalis* L., Water deficit.

Adaçayının PEG ile İndüklenen Kuraklık Stresine Antioksidatif Yanıtları

Öz

Bu çalışma, polietilen glikol (PEG) ile indüklenen iki kuraklık uygulamasının (-0.4 ve -0.8 MPa) adaçayı (*Salvia officinalis* L.) bitkisi üzerindeki etkisini belirlemek amacıyla yapılmıştır. Yirmi beş günlük çelikler yedi gün boyunca kuraklık uygulamalarına maruz bırakılmıştır. Zar hasarı ve su kaybı, kuraklığın şiddeti ile kademeli olarak artmıştır. Zar bütünlüğündeki ve su içeriğindeki azalmalar adaçayının kuraklıktan etkilendiğini gösterirken, artan flavonoid ve antioksidan enzim aktiviteleri bile bu etkiyi azaltamamıştır. Su potansiyeli koşullarının azalmasıyla yaprakların H_2O_2 içeriğinin artması, kuraklığın neden olduğu oksidatif stresin göstergesidir. Yüksek seviyelerdeki SOD ve POD aktiviteleri, enzimlerin H_2O_2'nin uzaklaştırılmasında rol oynadığını göstermiştir. Adaçayı, kuraklığa dayanma toleransını başarıyla artırmıştır.

Anahtar Kelimeler: Antioksidant enzimler, *Salvia officinalis* L., Su kıtlığı.

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1. Introduction

Abiotic stresses as salinity, heat, drought, chilling and freezing affect adversely the several physiological processes and growth of plants (Basu et al., 2010). Global climate changes often threaten the availability of water in the world, and this causes plants to be exposed to drought stress. The plant's responses to drought stress are highly complex process which includes multiple mechanisms on different severity levels (Basal et al., 2020). Exposure to water deficit induces various biochemical and physiological changes in photosynthesis, transpiration, ion and nutrient uptake, photosynthetic pigment contents, and membrane transport and permeability (Çiçek et al., 2015; Kaya et al., 2016; Arslan et al., 2020; Pekcan et al., 2021). The plant's survival from drought depends on their ability to sense stimuli and initiate various biochemical and physiological changes (Basu et al., 2010). The primary response to drought is an alteration in photosynthesis that includes the stomatal closure and reduction the activity of photosynthetic enzymes and as well as reduction of ATP synthesis (Çulha Erdal et al., 2021). Moreover, drought triggers oxidative stress by causing the synthesis of reactive oxygen species (ROS) to exceed the detoxification capacity of the plant. Excess levels of ROS such as hydroxyl radical ($OH^{\bullet}$), superoxide radical ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) production lead to oxidative injury in cells, with consequent photoinhibition, damage of structures of cell, premature senescence and growth reduction of plants (Hajihashemi and Sofo, 2018). In plant cells, a highly efficient antioxidant defense system exists for ROS detoxification as non-enzymatic antioxidants, such as glutathione, ascorbate and flavonoids, and antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT). The SOD produces the conversion of $O_2^{\bullet -}$ to H_2O_2 and O_2; H_2O_2 is then cleared by POD, CAT and APX.

Sage (*Salvia officinalis* L.) is an aromatic perennial subshrub native to the Mediterranean region and belongs to the Lamiaceae family. It is a popular herb which has been used in various food preparations since ancient times. Moreover, dried leaves are used as food flavoring as well as raw materials in medicine, perfumery and food industry (Bettaieb et al., 2009). *Salvia officinalis* L. has been the subject of studies mostly focusing on secondary metabolites, essential oils, fatty acids and phenolic compounds and abiotic stress responses of these metabolites in the literature (Lu and Foo et al., 2001; Hendawy and Khalid, 2005; Bettaieb et al., 2009; Taarit et al., 2010; Soltanbeigi et al., 2021). However, there are few reports regarding the responses of enzymatic antioxidative pathway of sage leaves to drought stress. Due to the complexity of drought stress metabolism and adaptation mechanisms, it is necessary to improve our current knowledge with each changing conditions and plants. Therefore, the aim of this study was to clarify the drought stress responses induced by different polyethylene glycol (PEG) concentrations in sage plant on oxidative damage and the effects of the antioxidant defense system involved in the tolerance mechanism. The enhancement of H_2O_2, membrane damage, changes in the amount of flavonoids and water loss, and the enzymatic antioxidant activities such as SOD, POD and CAT, were compared in sage leaves exposed to two different (-0.4 MPa and -0.8 MPa) PEG treatments.

2. Materials and Methods

2.1. Plant materials and treatments

Salvia officinalis L. plants were propagated by cutting mother plants obtained from a plant nursery. Stems (10 cm) that contain 2 nodes and 4 opposite leaves were cultivated in 1 L pods filled with distilled water until adventive roots emerged for 15 days in a growth cabin at 25 ± 1°C, 40 ± 5% humidness, 16 h photoperiod and 250 μmol m^{-2} s^{-1} light intensity. The rooted cuttings continued to grow in half strength Hoagland's solution for 10 days (Hoagland and Arnon, 1950). At the 25th day of the growth, cuttings were transferred to sterile hydroponic cultures for drought treatments, containing two different PEG-6000 concentrations (-0.4 MPa and -0.8 MPa) for 7 days. Control plants were continued to grow at half strength Hoagland's solution. The control and drought treatment solutions are renewed every 2 days.

2.2. Relative leakage ratio (RLR), relative water content (RWC), flavonoid content and H_2O_2 content

Leaf discs (R=0.5 cm) in distilled water were measured at 280 nm for 24 hours before and after incubation in liquid nitrogen for 20 minutes (Redmann et al., 1986). RLR was calculated according to the formula ($A_{280}/A_{280'}$). The water content of the leaves was calculated and evaluated as a percentage of RWC with the formula:

$$RWC\ (\%) = [(FW - DW)/(SW - DW)] \times 100 \quad (1)$$

FW, DW and SW are referred as the fresh weight, dry weight and water-saturated weight, respectively (Farrant, 2000). The flavonoid content of sage was done by the method of Mirecki and Teramura (1984), was estimated from the absorbance at 300 nm of acidified methanol leaf extracts and calculated as the percentage of corresponding controls (C). The content of H_2O_2 was made according to Esterbauer and Cheeseman (1990) and calculated using the standard curve.

2.3. Antioxidant enzyme activities

Leaves from each application were weighed 0.5 g to determine enzyme activities. It was triturated with liquid nitrogen and kept in the specific buffer for each different enzyme extraction studied. The protein concentrations of each extract were found (Bradford, 1976). The powdered sample was homogenized in Tris–HCl buffer (9 mM and pH 6.8) and glycerol (13.6 %). SOD (EC 1.15.1.1) activity was tested using the method of Beyer and Fridovich (1987). One SOD unit is expressed as the enzyme quantity that causes a 50% reduction in NBT reduction inhibited by SOD. POD (EC 1.11.1.7) activity was determined as guaiacol oxidation (ε = 26.6 mM cm^{-1}) with H_2O_2 at 470 nm (Pütter, 1974). One unit of peroxidase activity was described as nmol H_2O_2 dissociated per min per mg of protein. CAT (EC 1.11.1.6) activity was calculated by the decay of H_2O_2 measured at 240 nm (Chance and Maehly, 1955). CAT activity [nmol H_2O_2 mg^{-1} (protein) min^{-1}] was assessed from the reaction's beginning rate applying the extinction coefficient (ε) of H_2O_2 (ε = 40 mM cm^{-1} at 240 nm).

2.4. Statistical analysis

The experiment was performed in randomized design with three replicates for each application. ANOVA and the least significant difference (LSD) ($P < 0.05$) were used to analyze the differences in variables between applications. All the analyses were conducted by the *SPSS v. 20.0*, Inc, Chicago, IL, USA.

3. Findings and Discussion

Osmotic pressure of the soil solution increases with increasing severity of drought stress often leads to dehydration of cell, wilting of plant and sometimes death (Farooq et al., 2009). The PEG is a non-penetrable and non-toxic osmotic agent that reduces water potential in medium which accurately mimics drought. PEG leads to blockage of water entry and movement pathways, decreased water absorption and drying of the plant (Lawlor, 2010). Therefore, PEG-6000 has been used to promote drought and maintain uniform water potential over an experimental period in laboratory conditions. (Basu et al., 2010). A plant's water status is highly sensitive to water-deficient conditions and determines the plant's responses to stress (Zhang et al., 2018). The primary adverse effect of water deficit is a reduction in water content of plant, accompanied by alterations in molecular, physiological and morphological processes (Hajihashemi and Sofo, 2018). Relative water contents (RWC) of leaves of sage significantly decreased with the reduction of PEG induced water potential, showing that PEG treatments effectively caused drought stress on *Salvia officinalis* L. (Table 1). Drought treatments caused 15%, and 31% reduction of RWC by exposing -0.4 and -0.8 MPa PEG water potential, according to the control respectively. The difference between two different PEG treatments was 19%. The similar negative effect of PEG induced drought on *Stevia rebaudiana* was determined (Hajihashemi and Sofo, 2018). PEG induced drought treatments led to a dramatic increase of RLR that indicates electrolyte leakage from leaf cells of sage for all treatments, especially at -0.8 MPa with 4.2-fold compared to control (Table 1). Excessive ROS production due to drought stress can inhibit plant metabolism and growth. Overproductions of free radicals cause damage to membrane system (Zhang et al., 2018). The gradual increase of RLR results in decreasing water potentials indicated disrupted membrane integrity of sage leaves. Flavonoids, which are plant secondary metabolites, act as antioxidants because of their free radical suppressive activity (Farghaly et al., 2021). Increased content of flavonoid was more pronounced at the severest drought treatment (-0.8 MPa) which was 2.1-fold of control (Table 1).

Table 1. Relative Leakage Ratio (RLR), Relative Water Content (RWC) and Flavonoid content of sage leaves exposed to -0.4 MPa and -0.8 MPa PEG treatment. C represents 32 day old control plants. Means ±SEs, 3 (for RLR) or n= 6 (for RWC). Different letters indicate significant difference at $P < 0.05$ according to LSD 5%.

	Parameters		
Treatments	RLR	RWC	Flavonoid
C	0.009 ± 0^{a}	75 ± 0.3^{a}	100
-0.4 MPa	0.016 ± 0^{b}	64 ± 0.4^{b}	134
-0.8 MPa	0.038 ± 0^{c}	52 ± 0.5^{c}	208
LSD 5%	0.002	2	

Oxidative stress appears as a consequence of unbalanced ROS production in response to environmental stresses such as water deficit (Hajihashemi and Sofo, 2018). H_2O_2 contents of drought treated sage leaves were increased with increasing severity of stress (43 and 100% at -0.4 and -0.8 MPa, respectively) (Figure 1A). H_2O_2, is one of the toxic ROS and increased amount of H_2O_2 determined under drought in several species causes membrane damage (Basu et al., 2010). Likewise, the present study H_2O_2 and RLR results showed that increased contents of these parameters had a correlation between them. H_2O_2 also functions as an intracellular signal by stimulating or deactivating some antioxidant enzymes such as SOD, POD and CAT (Basu et al., 2010). Under both -0.4 and -0.8 MPa stress conditions, SOD activity of sage leaves dramatically increased that increase was 6- and 9.5-fold of control, respectively (Figure 1B). Increasing SOD activity with decreasing water potential might have contributed to the increase in the amount of H_2O_2. Because, SOD is responsible for catalyzing the dismutation of superoxide ion (O_2^-) to hydrogen peroxide and molecular oxygen (O_2) during oxidative metabolism (Turan and Ekmekçi, 2011). POD which scavenges H_2O_2 to H_2O, exhibited statistically significant increase in both -0.4 and -0.8 MPa treatments (Figure 1C). The amount of the increased activity was approximately 4-fold of control at -0.4 MPa, while at severest stress treatment POD activity increased 2.8-fold of control. Higher activity of POD was determined in moderate drought treatment (-0.4 MPa). CAT, the other H_2O_2 scavenge antioxidant enzyme, decreased with PEG treatments (Figure 1D). The decreased values of CAT activity were not statistically significant at -0.4 MPa PEG water potential treatment, while -0.8 MPa treatment resulted in 19% reduction of activity of CAT in sage leaves. Changes in the activities of antioxidant enzymes play an important role in drought stress tolerance.

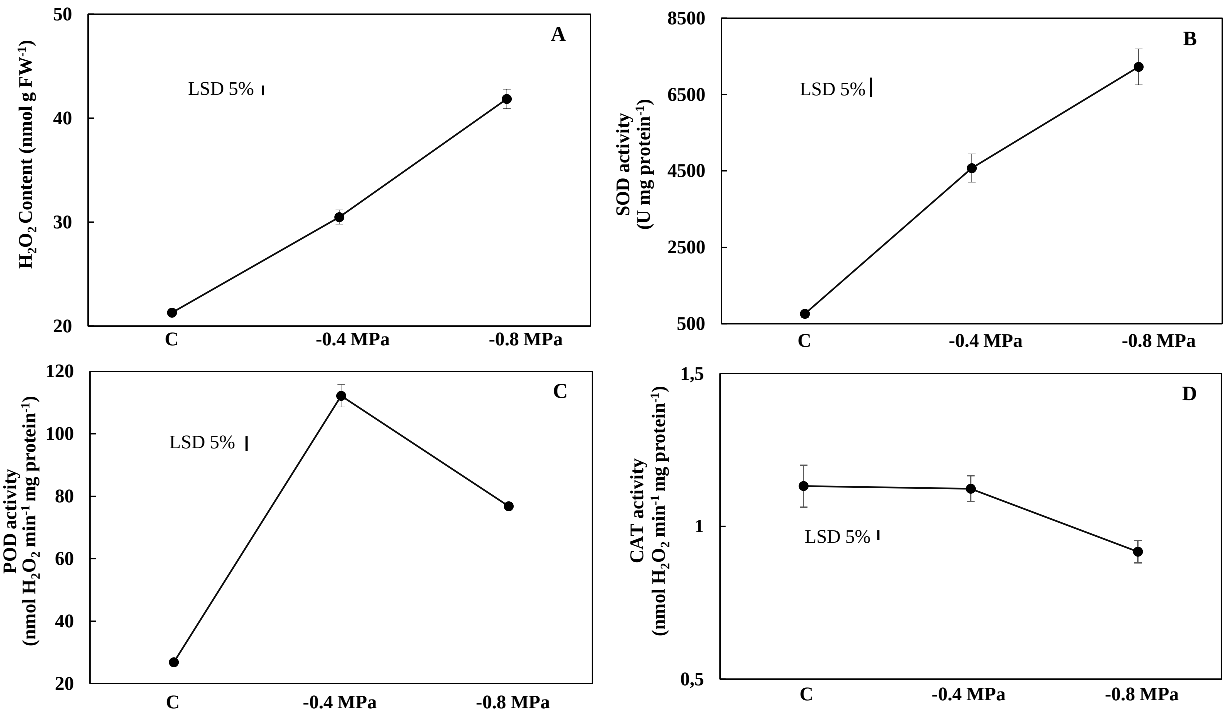

Figure 1. H_2O_2 content (A) and Antioxidant enzyme activities of sage exposed to different PEG induced drought: B, superoxide dismutase SOD, C peroxidase POD, D catalase CAT. C represents 32 day old control plants. The error bars represent the standard error (±SE) for three replicates.

4. Conclusions and Recommendations

In conclusion, the current study showed that the applied PEG treatments of sage lead deteriorating effect on physiological mechanisms of sage. Decreased water potential values may be an indication that treated drought stress triggers defense mechanisms and thus tolerance mechanisms in the sage. Accordingly, the correlation of increased H_2O_2 with RLR, the indicator of membrane damage, indicates an increase in ROS production. Even though there is an increase in antioxidant enzyme activities and flavonoid, sage leaves which were subjected to drought could not eliminate ROS. While high SOD and POD activities were a result of functional antioxidative defense mechanism, decreased activity of CAT indicated that it was not effective in this defense mechanism.

Increased flavonoid content and SOD and POD activity may play a role in withstanding the damaging effects of drought on sage. As discussed in this study, a more detailed comparison of physiological responses in future studies may be useful to elucidate the mechanism of drought stress tolerance of sage in the future.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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