



## Morphological and Biochemical Responses of Common Bean (*Phaseolus vulgaris* L.) to PEG-Induced Drought Stress and Salicylic Acid Application

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### ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is a major food source and an important legume worldwide, yet its production is significantly limited by abiotic stress factors, such as drought. This study investigated the effects of Polyethylene Glycol (PEG)-induced drought stress and salicylic acid (SA) application on the morphological and biochemical responses of this plant. To simulate drought conditions, plants were exposed to PEG6000 at osmotic potentials of  $-0.351$  MPa and  $-0.75$  MPa, and treated with 1 mM SA. Drought stress significantly reduced growth parameters. Notably, the most severe stress level ( $-0.75$  MPa PEG) decreased seedling height by approximately 52% and relative water content (RWC) by 59% compared to the control group. Biochemically, this stress led to a 51% reduction in chlorophyll a content, while significantly increasing the levels of MDA and  $H_2O_2$ , the main indicators of oxidative stress, by 33% and 27%, respectively. In contrast, the SA application, both alone and in combination with PEG, induced drought stress, resulting in significant improvements in growth and chlorophyll content. For instance, the 1 mM SA application alleviated the reduction in seedling height by 30% and the loss in chlorophyll a by 40%. This protective effect was accompanied by a noticeable decrease in MDA and  $H_2O_2$  levels. These findings demonstrate that SA effectively mitigates the adverse effects of drought stress on common bean, primarily by activating antioxidant defense mechanisms to manage oxidative stress. SA can thus be utilized as a promising tool to enhance drought resistance in plants.

### Plant Physiology

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### Keywords

*Phaseolus vulgaris* L.

Salicylic acid

Drought stress

Polyethylene glycol (PEG)

Oxidative stress

## Fasulyenin (*Phaseolus vulgaris* L.) PEG Kaynaklı Kuraklık Stresine ve Salisilik Asit Uygulamasına Morfolojik ve Biyokimyasal Tepkileri

### ÖZET

Fasulye (*Phaseolus vulgaris* L.), dünya çapında temel bir besin kaynağı ve önemli bir baklagil olmasına rağmen, üretimi kuraklık gibi abiyotik stres faktörleri tarafından önemli ölçüde sınırlandırılmaktadır. Bu çalışma, polietilen glikol (PEG) kaynaklı kuraklık stresinin ve salisilik asit (SA) uygulamasının bu bitkinin morfolojik ve biyokimyasal tepkileri üzerindeki etkilerini incelemiştir. Kuraklık koşullarını simüle etmek için bitkiler,  $-0.351$  MPa ve  $-0.75$  MPa ozmotik potansiyellerdeki PEG6000'e maruz bırakılmış ve 1 mM SA ile muamele edilmiştir. Kuraklık stresi, büyüme parametrelerini önemli ölçüde azaltmıştır; özellikle en şiddetli stres seviyesi olan  $-0.75$  MPa PEG uygulaması, fide boyunu kontrol grubuna göre yaklaşık %52 ve nispi su içeriğini (RWC) %59 oranında düşürmüştür. Biyokimyasal olarak, bu stres klorofil a içeriğinde %51 azalmaya yol açarken, oksidatif stresin ana göstergeleri olan MDA ve  $H_2O_2$  seviyelerini sırasıyla %33 ve %27 oranında artırmıştır. Buna karşılık, SA uygulaması, hem tek başına hem de PEG kaynaklı kuraklık stresi ile kombinasyon halinde, büyüme ve klorofil içeriğinde önemli iyileşmelere neden olmuştur. Örneğin, 1 mM SA uygulaması, fide boyundaki kaybı %30 ve klorofil a kaybını %40 oranında telafi etmiştir. Bu koruyucu etki, MDA ve  $H_2O_2$  seviyelerinde belirgin bir azalmayla gözlenmiştir. Bu bulgular, SA'nın özellikle antioksidan savunma

### Bitki Fizyolojisi

### Araştırma Makalesi

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### Anahtar Kelimeler

*Phaseolus vulgaris* L.

Salisilik asit

Kuraklık stresi

Polietilen glikol (PEG)

Oksidatif stres

mekanizmalarını aktive ederek oksidatif stresi yönettiğini ve kuraklık stresinin fasulye üzerindeki olumsuz etkilerini etkili bir şekilde hafiflettiğini göstermektedir. SA, bitkilerde kuraklık direncini artırmak için umut verici bir araç olarak kullanılabilir.

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## INTRODUCTION

Drought, one of the abiotic stress factors, is considered a devastating environmental stressor (Gholami & Zahedi, 2019). Due to global warming and climate change, it is predicted that drought frequency and severity will increase in many regions of the world (Pokhrel et al., 2021). Drought is one of the most critical global factors that significantly reduces the yield of all major field and horticultural crops (Sirisuntornlak et al., 2019). Drought stress adversely affects plant growth and development by disrupting cell division, cell expansion, and various physiological and morphological processes, as well as their complex interactions. It leads to oxidative stress by triggering excessive production of reactive oxygen species (ROS), such as singlet oxygen ( $^1O_2$ ), superoxide anion radicals ( $O_2^{\bullet-}$ ), hydroxyl radicals ( $\cdot OH$ ), and hydrogen peroxide ( $H_2O_2$ ). Oxidative stress impairs cellular structures and functions by promoting membrane lipid peroxidation and the degradation of proteins and nucleic acids (Farooq et al., 2009), ultimately resulting in decreased photosynthetic efficiency, loss of membrane stability, cellular damage, and even cell death (Demidchik, 2015; Zhu, 2016).

Plants respond to stress stimuli rapidly upon exposure by initiating physiological and biochemical changes through signal transduction to ensure survival and continued growth (Sohag et al., 2020). They develop complex tolerance mechanisms such as the activation of antioxidant production, stimulation of primary and secondary metabolites, gene regulation, and execution of metabolic responses (Kumar et al., 2021; Zahedi et al., 2021). However, the resistance mechanisms that plants employ in response to stress may not be sufficient under prolonged drought conditions. Therefore, several strategies have been developed to mitigate the impacts of drought stress. One such strategy is the breeding of drought-tolerant genotypes to prevent reductions in crop yield. Nevertheless, this approach has proven inadequate in fully addressing stress-related challenges, prompting scientists to explore alternative solutions to enhance plant survival under adverse environmental conditions. In recent years, plant growth regulators (PGRs) have been reported to play an integrative role in modulating plant responses under drought stress (Ahammed et al., 2020). Among these, salicylic acid (SA), a phenolic phytohormone known to alleviate the adverse effects of abiotic stresses, has gained attention (Maruri López et al., 2019; Kaya et al., 2020). Due to its established role in modulating oxidative stress responses and enhancing antioxidant defense systems, key mechanisms for drought tolerance, SA was specifically selected for this study. Its ability to regulate ROS accumulation and improve membrane stability makes it a promising candidate for mitigating drought damage in common bean. Furthermore, SA has been reported to regulate not only plant growth but also various physiological and biochemical traits (Fardus et al., 2018; Sohag et al., 2020). It improves seedling growth by reducing malondialdehyde (MDA) and hydrogen peroxide ( $H_2O_2$ ) content (Farooq et al., 2010). SA also mitigates drought-induced inhibition of seed germination (Sohag et al., 2020). Similarly, application of SA under drought conditions supports plant growth by enhancing stomatal conductance (Khan et al., 2015).

Common bean (*Phaseolus vulgaris* L.) is an annual legume crop widely cultivated in subtropical and temperate regions worldwide, and it is extensively consumed due to its high content of protein, vitamins, carbohydrates, dietary fiber, folic acid, and minerals (Mitchell et al., 2009; Ganesan & Xu, 2017; Ibrahim et al., 2021). It is one of the major bean species grown globally, particularly in arid and semi-arid regions where water scarcity is a frequent issue (Losa et al., 2022). With the progression of climate change and increasing atmospheric dryness, drought stress is projected to have an even greater impact on *P. vulgaris* production (Mohammed & Feleke, 2022). To achieve optimal yield in common bean, irrigation is required during all growth stages (Sezen et al., 2008). This necessity arises from the plant's sensitivity to fluctuations in soil moisture, as water deficiency leads to a significant reduction in seed and pod yield (Hinkossa et al., 2013; Darkwa et al., 2016). The primary reason for this sensitivity is the shallow root system characteristic of the species (Durigon et al., 2019).

Although existing studies have examined the effects of drought and SA individually, the interaction between different osmotic stress levels and SA on the morphological and biochemical responses of common bean remains insufficiently investigated. Filling this gap is particularly crucial because the common bean is one of the most widely consumed legumes globally, serving as a vital source of protein, fiber, and minerals for millions of people.

Moreover, climate change projections indicate that the frequency and severity of drought will increase, especially in arid and semi-arid regions, where common bean production is already at high risk. Therefore, this study hypothesizes that exogenous salicylic acid application can mitigate the negative effects of PEG-induced drought stress on common bean by enhancing antioxidant defense mechanisms and improving plant growth performance. To test this hypothesis, the effects of two different osmotic stress levels induced by PEG6000 ( $-0.351$  MPa and  $-0.75$  MPa), alone and in combination with  $1$  mM SA, were evaluated on morphological characteristics and biochemical parameters. The findings are expected to provide new perspectives on drought tolerance mechanisms in common bean and offer a practical strategy to enhance the resilience of this crop under water-limited conditions.

## MATERIAL and METHODS

### Germination of plants and establishment of experimental groups

In this study, commercially available seeds of common bean (*Phaseolus vulgaris* L.) were used. Prior to sowing, the seeds were surface-sterilized to prevent fungal contamination. The seeds were then sown in seedling trays (outer dimensions:  $51 \times 32$  cm; cell diameter:  $6.5$  cm; depth:  $7$  cm) using peat as the growing medium, with three seeds sown per cell. Following sowing, the trays were transferred to a controlled growth chamber maintained at  $26/22$  °C (day/night),  $50\%$  relative humidity, and a  $16/8$  h light/dark photoperiod. After a 10-day establishment and growth period, the treatment phase was initiated (Figure 1). The experimental groups are detailed in Table 1. The concentrations of PEG, SA, and their combinations were determined based on previous literature (Marcińska et al., 2013). Stock solutions of PEG6000 and SA were prepared separately. Freshly prepared solutions were applied daily as a soil drench at a volume of  $10$  mL per plant. For combined treatments, PEG6000 and SA solutions were applied separately on the same day to ensure equal exposure. The control group received only distilled water. The treatment applications continued for 14 days, and the experiment was terminated on the 24th day after sowing. At the end of the experiment, morphological parameters, including root, shoot, and leaf lengths, fresh and dry weights, number of leaves, relative water content (RWC), and tolerance index were measured. The remaining plant tissues were harvested and stored at  $-80$  °C for biochemical analyses.

Table 1. PEG, SA, and combined PEG+SA treatments

#### Çizelge 1. PEG, SA ve PEG+SA kombinasyon uygulamaları

Treatments
1 Control
2 $-0.351$ MPa PEG (15%, w/v)
3 $-0.75$ MPa PEG (25%, w/v)
4 $1$ mM SA
5 $-0.351$ MPa PEG + $1$ mM SA
6 $-0.75$ MPa PEG + $1$ mM SA



Figure 1. Morphological images of the *P. vulgaris* plant on the 3rd and 10th days after sowing, respectively  
Şekil 1. *P. vulgaris* bitkisinin ekimden sonra sırasıyla 3. ve 10. günlerdeki morfolojik görüntüleri

### Determination of growth parameters

Plant lengths: To measure seedling, root, and stem lengths, three samples were randomly selected from each group,

and the lengths were measured in centimeters (cm) using a ruler.

**Fresh and dry weights:** For the determination of fresh weights of the plant samples, the same three specimens used for length measurements were weighed in grams using a precision balance. The fresh samples were then dried in an oven at 72 °C for 48 hours to obtain the dry weights, which were also measured in grams using a precision balance.

**Number of leaves:** The number of leaves in the plant samples was determined by counting the leaves on the main stem of the same plants used for length and weight measurements.

**Relative water content (%):** Three leaf samples of *P. vulgaris* were randomly selected, and their fresh weights were measured. The samples were then immersed in distilled water at room temperature for 2 hours to determine their turgid weights. Subsequently, the samples were dried in an incubator at 72 °C for 48 hours to obtain their dry weights. The relative water content (RWC) of bean leaves was calculated using the following formula: (Barrs & Weatherley, 1962).

$$\text{RWC (\%)} = [(\text{Fresh weight} - \text{dry weight}) / (\text{Turgid weight} - \text{dry weight})] \times 100$$

**Tolerance index (TI):** The tolerance index (TI) was calculated as the ratio of the root length of seedlings under stress conditions to that of the control group seedlings (Abdul-Baki & Anderson, 1973). As the tolerance index (TI) is calculated relative to the control, the control group is inherently assigned a constant value of 1.00.

### Determination of chlorophyll, MDA, and H<sub>2</sub>O<sub>2</sub> contents

**Chlorophyll a and b contents:** Fresh leaf samples, 0.2 g of *P. vulgaris* were weighed and homogenized in 2 mL of 80% acetone using a mortar and pestle. The homogenate was subsequently centrifuged at 5000 rpm for 10 minutes. Absorbance readings were then taken using a spectrophotometer at 663 nm for chlorophyll a and 645 nm for chlorophyll b. Finally, the concentrations of chlorophyll a and b were calculated using the following formulas (Arnon, 1949).

$$\text{Chlorophyll a mg g}^{-1} \text{ FW} = (12.70 \times A_{663}) - (2.69 \times A_{645})$$

$$\text{Chlorophyll b mg g}^{-1} \text{ FW} = (22.90 \times A_{645}) - (4.68 \times A_{663})$$

**MDA content:** The MDA content, an end product of free radical-induced damage in plants, was determined. Briefly, 0.2 g of fresh *P. Vulgaris* tissue was ground in liquid nitrogen, followed by the addition of 2 mL of 0.1% trichloroacetic acid (TCA). The homogenous mixture was centrifuged at 15,000 rpm for 15 minutes. One milliliter of the resulting supernatant was taken, and 1 mL of 20% TCA and 1 mL of 0.5% thiobarbituric acid (TBA) were added to it. The mixture was incubated in a boiling water bath for 40 minutes, then cooled in an ice bath, and centrifuged again at 10,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm and 600 nm wavelengths, with three biological replicates (Heath & Packer, 1968).

**H<sub>2</sub>O<sub>2</sub> content:** To determine the H<sub>2</sub>O<sub>2</sub> content, which also functions as a signaling molecule in plants, 0.2 g of fresh tissue from *P. vulgaris* was weighed and ground in liquid nitrogen using a mortar and pestle. Then, 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) was added to the sample. The resulting homogenate was centrifuged at 15,000 rpm for 15 minutes. Subsequently, 1 mL of the supernatant was transferred, to which 800 µL of potassium phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>) and 1600 µL of potassium iodide (KI) were added. Absorbance was measured at 390 nm using a spectrophotometer in three biological replicates (Hao et al., 2014).

### Statistical Analysis

All experimental data in the study were obtained in three replicates under the specified treatment conditions. Statistical analyses of the data were performed using SPSS software version 22.0. Differences between the control and PEG/SA treatment groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at a significance level of  $p < 0.05$ . Furthermore, Pearson Correlation Analysis was performed to evaluate the interrelationships among all measured parameters, and the results are presented in a Heatmap with significance determined at  $p < 0.01$ .

## RESULTS and DISCUSSION

### Plant lengths (cm)

The morphological appearances of *P. vulgaris* plants exposed to different concentrations of drought stress and treated with SA after 14 days are presented in Figure 2. When seedling, root, and stem lengths across all treatments were evaluated based on Figure 3, seedling lengths ranged from 19.66±1.33 to 41±2.30 cm, root lengths from 7.16±0.16 to 16.33±1.85 cm, and stem lengths from 4.83±0.33 to 10.33±0.33 cm.



Figure 2. Morphological image of the *P. vulgaris* plant after PEG, SA, and combination treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA

Şekil 2. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası morfolojik görüntüsü 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA

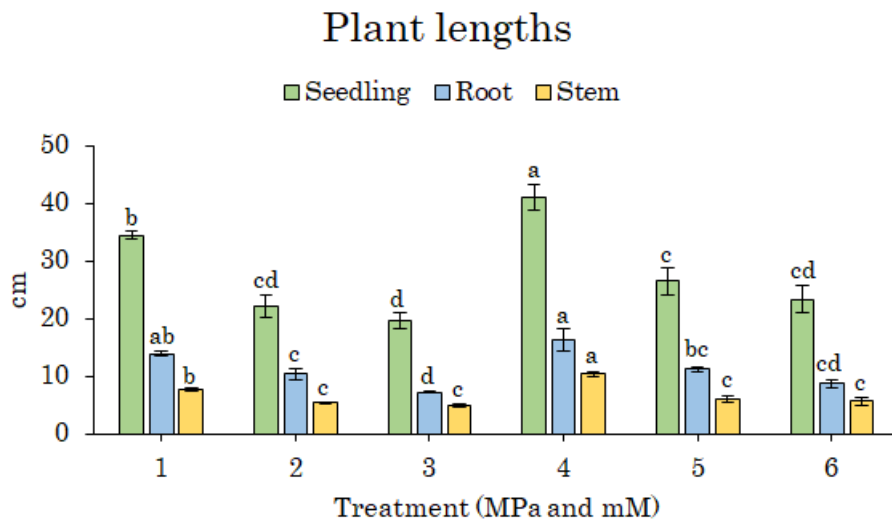


Figure 3. Seedling, root, and stem lengths of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments in seedling length ( $F(5,12)= 17.73, p<0.001$ ), root length ( $F(5,12)= 4.05, p=0.022$ ), and stem length ( $F(5,12)= 9.61, p=0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) (n=3). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 3. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası fide, kök ve gövde uzunlukları 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir fide uzunluğu ( $F(5, 12)= 17.73, p<0.001$ ), kök uzunluğu ( $F(5,12)= 4,05, p=0.021$ ) ve gövde uzunluğu ( $F(5,12)= 9.61, p=0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir (n=3). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

The effects of the treatments on seedling length  $F(5,12)= 17.73, p<0.001$ , root length  $F(5,12)= 4.05, p=0.022$ , and stem length  $F(5,12)= 9.61, p=0.001$  of *P. vulgaris* were statistically significant. Compared to the control group, drought stress induced by PEG treatments -0.351 MPa and -0.75 MPa caused a pronounced reduction in plant lengths, with the lowest values for seedling  $19.66\pm 1.33$  cm, root  $7.16\pm 0.16$  cm, and stem lengths  $4.83\pm 0.33$  cm observed under -0.75 MPa PEG. 1 mM SA treatment significantly promoted plant growth, producing the highest values for all parameters seedling  $41.00\pm 2.30$  cm, root  $16.33\pm 1.85$  cm, and stem lengths  $10.33\pm 0.33$  cm. Seedling

length reached a maximum of 41 cm, while root and stem lengths exceeded those of the control group, highlighting the growth-promoting effect of SA. Evaluation of the PEG+SA combination treatments showed that these combinations partially alleviated the negative effects of PEG, although they were less effective than SA alone. In particular, the  $-0.351$  MPa PEG + 1 mM SA treatment resulted in higher seedling  $26.50 \pm 2.36$  cm, root  $11.17 \pm 0.43$  cm, and stem  $6.00 \pm 0.57$  cm lengths compared to  $-0.351$  MPa PEG alone, demonstrating that SA supports growth even under mild drought conditions (Figure 3). These results demonstrate that drought stress negatively affects seedling, root, and stem lengths, whereas SA application effectively mitigates these reductions. Notably, while Altunlu et al. (2022) reported that PEG6000-induced drought stress reduced seedling and root lengths by 18–22% in two tomato genotypes (M28 and Alyans), our study observed a more pronounced decrease of 25% in *P. vulgaris* under similar stress conditions, indicating the species-specific sensitivity and the potential for SA to alleviate this stress. Similarly, Türkan et al. (2005) reported reductions in seedling, root, and stem lengths in two bean species, but our findings demonstrate that 1 mM SA application can restore these parameters to values exceeding the control, highlighting its growth-promoting effect. In canola (*Brassica napus* L.) and rice (*Oryza sativa* L.), SA also enhanced shoot and root lengths under PEG-induced drought stress, consistent with our observations (Korgaonker & Bhandari, 2023; Tanveer et al., 2023). In line with these findings, Chaghakaboodi et al. (2025) showed that high drought stress 100 mM, significantly reduced shoot length in *Lallemantia iberica* (cv. Sara), whereas 3 mM SA pretreatment enhanced seedling growth under stress. Taken together, these results indicate that applying 1 mM SA effectively supports seedling, root, and stem development under drought stress in *P. vulgaris*, providing a practical approach to improve early growth and establishment. The comparative analysis with literature suggests that the observed improvements may be due to the inherent sensitivity of common bean genotypes to SA-mediated stress alleviation, underlining the importance of genotype-specific responses in SA application strategies.

### Plant fresh weights (g)

When the plant fresh weights across all treatments were evaluated based on Figure 4, root fresh weights ranged from  $0.24 \pm 0.03$  to  $1.16 \pm 0.03$  g, stem fresh weights from  $0.18 \pm 0.01$  to  $0.68 \pm 0.05$  g, and leaf fresh weights from  $0.58 \pm 0.01$  to  $1.82 \pm 0.02$  g. The treatments caused statistically significant differences in root fresh weights  $F(5,12) = 88.59$ ,  $p < 0.001$ , stem fresh weights  $F(5,12) = 91.61$ ,  $p < 0.001$ , and leaf fresh weights  $F(5,12) = 414.2$ ,  $p < 0.001$  of the plants. Among all treatments, 1 mM SA application resulted in the highest fresh weights across all organs: root  $1.16 \pm 0.03$  g, stem  $0.68 \pm 0.05$  g, and leaf  $1.82 \pm 0.02$  g, while PEG treatments had a suppressive effect. Compared to the control group, PEG-induced drought stress ( $-0.351$  MPa and  $-0.75$  MPa) led to a clear reduction in plant fresh weights, with more severe stress resulting in greater reductions. The lowest fresh weights in roots  $0.24 \pm 0.03$  g, stems  $0.18 \pm 0.01$  g, and leaves  $0.58 \pm 0.01$  g were observed under the  $-0.75$  MPa PEG treatment, reflecting the osmotic limitations imposed by PEG, which reduce water uptake and turgor pressure in plant tissues. SA treatment produced a significant increase in all parameters, root, stem, and leaf fresh weights, demonstrating its growth-promoting effect. When evaluating PEG+SA combination treatments, it was observed that SA partially alleviated the negative effects of PEG; however, the improvements were not as substantial as those achieved by SA alone. For instance, the  $-0.75$  MPa PEG + 1 mM SA combination treatment, despite severe stress, showed a notable increase in fresh weight compared to PEG alone, highlighting the protective role of SA, though total fresh weights remained lower than those of the control and SA-only treatments. Overall, these results demonstrate that the SA application effectively mitigates PEG-induced reductions in fresh weight, likely through enhanced antioxidative defense mechanisms and improved water retention. In comparison to previous studies, our results show a higher relative increase in fresh weights under SA treatment than reported in tomato (*Solanum lycopersicum* L.) genotypes under similar PEG stress, where reductions under severe stress were more pronounced (Esan et al., 2018). Similarly, in *Passiflora edulis* Sims, PEG-induced drought reduced fresh weight, but SA application led to a smaller recovery than observed in our study (Qi et al., 2023). In *Impatiens walleriana* L., SA also increased fresh weight under stress, though the effect was moderate compared to *P. vulgaris* (Antonić et al., 2016). Likewise, in banana (*Musa acuminata* cv. 'Berangan', AAA), increasing PEG levels suppressed growth, while SA mitigated this reduction, yet the enhancement remained less than that observed in our results (Bidabadi et al., 2012). These comparisons suggest that the *P. vulgaris* genotype used in this study may exhibit a particularly strong responsiveness to SA treatment. Collectively, these findings indicate that soil application of 1 mM SA could serve as a practical agronomic strategy to improve drought tolerance and sustain biomass production in *P. vulgaris* under water-limited conditions. Future studies could explore the effects of SA across different genotypes and growth stages to optimize its application under field conditions.

## Plant fresh weights

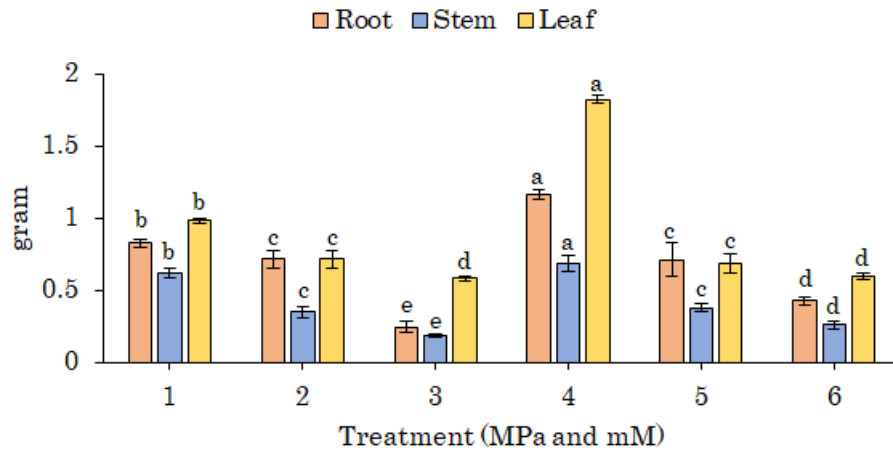


Figure 4. Root, stem, and leaf fresh weights of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. –0.351 MPa PEG, 3. –0.75 MPa PEG, 4. 1 mM SA, 5. –0.351 MPa PEG + 1 mM SA, 6. –0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments root fresh weight ( $F(5,12)= 88.59, p<0.001$ ), stem fresh weight ( $F(5,12)= 91.61, p<0.001$ ), and leaf fresh weight ( $F(5,12)= 414.2, p<0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) (n=3). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 4. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası kök, gövde ve yaprak yaş ağırlıkları 1. Kontrol, 2. –0.351 MPa PEG, 3. –0.75 MPa PEG, 4. 1 mM SA, 5. –0.351 MPa PEG + 1 mM SA, 6. –0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir kök yaş ağırlığı ( $F(5,12)= 88.59, p<0.001$ ), gövde yaş ağırlığı ( $F(5,12)= 91.61, p<0.001$ ) ve yaprak yaş ağırlığı ( $F(5, 12)= 414.2, p<0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir (n=3). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

### Plant dry weights (g)

When the plant dry weights across all treatments were evaluated based on Figure 5, root dry weights ranged from  $0.056\pm 0.003$  to  $0.255\pm 0.015$  g, stem dry weights from  $0.032\pm 0.002$  to  $0.096\pm 0.006$  g, and leaf dry weights from  $0.054\pm 0.002$  to  $0.139\pm 0.007$  g. The treatments caused statistically significant differences in the root dry weights,  $F(5,12)= 474.57, p<0.001$ ; stem dry weights,  $F(5,12)= 77.60, p<0.001$ ; and leaf dry weights,  $F(5,12)= 205.36, p<0.001$  of the *P. vulgaris* plants. PEG treatments –0.351 MPa and –0.75 MPa led to a considerable decrease in plant root, stem, and leaf dry weights. The lowest dry weight values were recorded in the root  $0.056\pm 0.003$  g, stem  $0.032\pm 0.002$  g, and leaf  $0.054\pm 0.002$  g under the –0.75 MPa PEG treatment. This result indicates that the osmotic effect of PEG caused severe water loss and biomass reduction in plant tissues. The 1 mM SA application resulted in the highest dry weights across all parameters: root  $0.255\pm 0.015$  g, stem  $0.096\pm 0.006$  g, and leaf  $0.139\pm 0.007$  g, indicating that SA supports plant development and enhances dry matter accumulation even in the absence of stress. The combination treatments (PEG+SA) partially mitigated the negative effects of PEG but did not reach the levels achieved by SA alone. SA appeared to buffer the adverse effects of PEG to some extent. The –0.351 MPa PEG + 1 mM SA treatment demonstrated a better protective effect of SA, whereas the –0.75 MPa PEG + 1 mM SA treatment showed a more limited impact, suggesting that the effectiveness of SA decreases as stress severity increases (Figure 5). Although both combination treatments significantly improved dry weights compared to PEG treatments alone, the SA-only application proved more effective in enhancing biomass accumulation. These findings are consistent with previous studies. For instance, in grass pea (*Lathyrus sativus*) subjected to three different concentrations of PEG (50, 100, and 150 g L<sup>-1</sup>), low PEG concentrations did not affect dry weights, whereas higher concentrations increased them (Piwowarczyk et al., 2014). In another study on barley (*Hordeum vulgare* L.), drought stress significantly reduced leaf dry weights, whereas SA application resulted in a marked increase in these weights (Abdelaal et al., 2020). Likewise, Chishti et al. (2025) reported that drought conditions caused significant reductions in root and shoot dry weights in two radish (*Raphanus sativus* L.) genotypes. Similarly, in a study on two strawberry (*Fragaria  $\times$  ananassa*) genotypes, drought stress decreased root and shoot dry weights compared to the control, but SA application led to increases in both parameters under stress conditions (Ghaderi et al., 2015). These results indicate that soil application of 1 mM SA effectively improves dry matter accumulation in *P. vulgaris* under drought stress, suggesting its potential use to enhance yield stability in water-limited environments.

## Plant dry weights

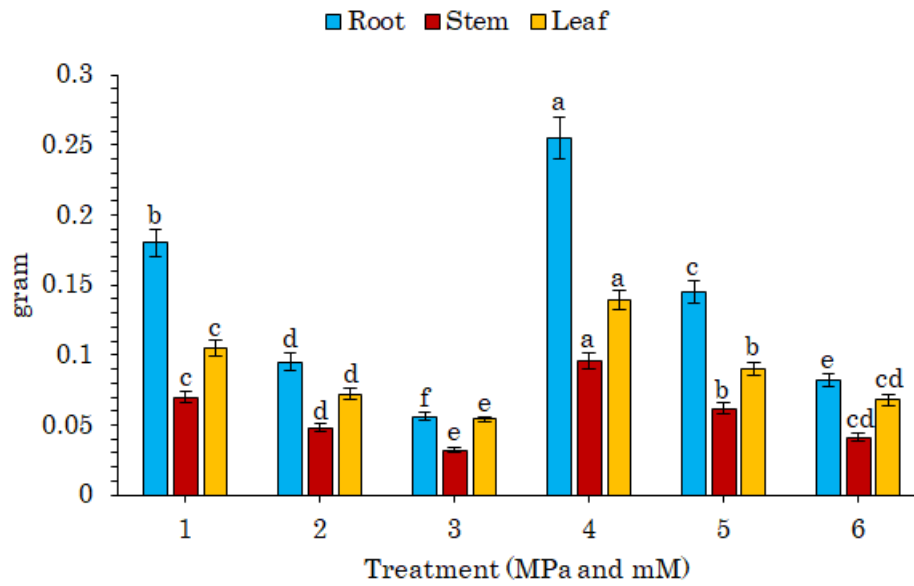


Figure 5. Root, stem, and leaf dry weights of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments root dry weight ( $F(5,12)=474.57, p<0.001$ ), stem dry weight ( $F(5,12)=77.60, p<0.001$ ), and leaf dry weight ( $F(5,12)=205.36, p<0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) (n=3). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 5. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası kök, gövde ve yaprak kuru ağırlıkları 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir kök kuru ağırlığı ( $F(5,12)=474.57, p<0.001$ ), gövde kuru ağırlığı ( $F(5,12)=77.60, p<0.001$ ) ve yaprak kuru ağırlığı ( $F(5,12)=205.36, p<0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir (n=3). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

### Number of leaves (units)

When the number of leaves across all treatments was evaluated based on Figure 6, leaf numbers ranged from  $4.00\pm 1.00$  to  $12.00\pm 2.00$  per plant. The different treatments had statistically significant effects on leaf number in *P. vulgaris*  $F(5,12)=7.686, p=0.002$ . Among the PEG treatments, -0.351 MPa  $6.33\pm 0.66$  slightly limited leaf formation, whereas the -0.75 MPa PEG  $4.00\pm 1.00$  treatment resulted in the lowest number of leaves, indicating that severe drought stress most strongly suppressed leaf development. The control group exhibited the highest number of leaves,  $12.00\pm 2.00$ . Application of 1 mM SA maintained leaf numbers at a comparable level,  $11.66\pm 1.20$ , with no statistically significant difference observed between the SA and control groups. In the combination treatments, -0.351 MPa PEG + 1 mM SA  $8.66\pm 0.88$  demonstrated the promoting effect of SA under mild drought conditions, whereas under -0.75 MPa PEG + 1 mM SA  $5.00\pm 1.15$ , the protective role of SA was limited, reflecting the suppressive impact of severe drought stress (Figure 6). These results are in agreement with previous studies. For instance, drought stress significantly reduced leaf number in pea (*Pisum sativum* L.) cultivars regardless of cultivar differences (Kausar et al., 2023), and Arafa et al. (2021) similarly reported a significant decrease in leaf number under drought stress. In contrast, in broad bean (*Vicia faba* L.), drought stress reduced leaf number, but SA application significantly increased leaf numbers (Abdelaal, 2015). Likewise, in basil (*Ocimum basilicum* L.) and marjoram (*Majorana hortensis*), leaf number increased with rising salicylic acid concentrations, although very high SA doses caused a decline (Gharib & Abed, 2006). Collectively, these findings suggest that soil application of SA can help maintain leaf formation and function in *P. vulgaris* under water-limited conditions, providing a practical strategy to improve drought resilience and yield potential.

### Relative water content (%)

Relative water content (RWC) is a key indicator of the physiological status of plants under stress, reflecting their tissue water retention capacity. As shown in Figure 7, RWC values across all treatments ranged from  $32.61\pm 1.84$  to  $80.10\pm 1.53$ . The treatments had statistically significant effects on leaf RWC in *P. vulgaris*  $F(5,12)=172.68, p<0.001$ . PEG-induced drought stress caused a clear reduction in leaf water content. Under mild stress -0.351 MPa PEG,  $54.92\pm 1.88$ , RWC values were slightly lower than the control  $80.10\pm 1.53$ , whereas severe stress -0.75 MPa

PEG,  $32.61 \pm 1.84$  led to the lowest RWC, indicating substantial loss of cell turgor pressure. Application of 1 mM SA resulted in the highest RWC  $84.12 \pm 1.39$ , significantly exceeding control levels, demonstrating its capacity to enhance water retention, maintain osmotic balance, and reduce water loss. In combination treatments,  $-0.351$  MPa PEG + 1 mM SA  $61.52 \pm 1.61$  significantly alleviated the negative effects of PEG, while  $-0.75$  MPa PEG + 1 mM SA  $37.40 \pm 1.35$  partially restored RWC, although values remained statistically lower than those of SA alone (Figure 7).

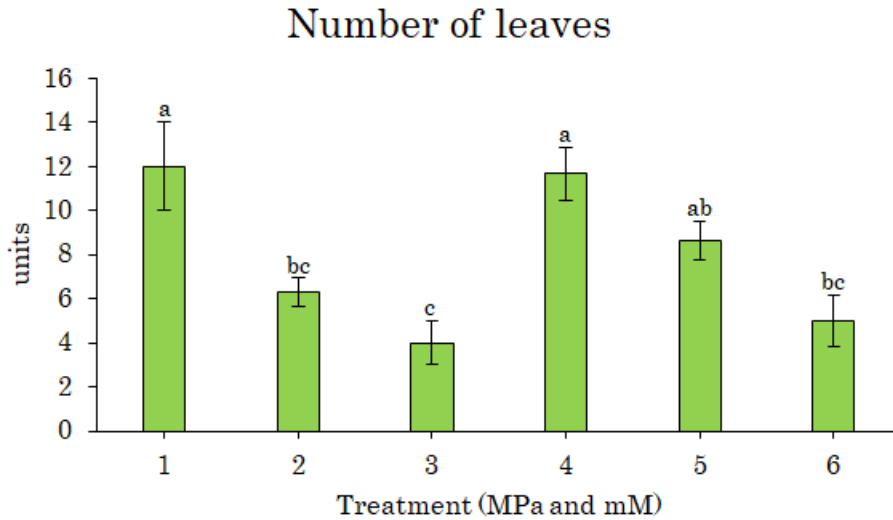


Figure 6. Number of leaves of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4. 1 mM SA, 5.  $-0.351$  MPa PEG + 1 mM SA, 6.  $-0.75$  MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12) = 7.686$ ,  $p=0.002$ ). \*Bars represent mean  $\pm$  standard error (SE) ( $n=3$ ). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 6. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası yaprak sayısı 1. Kontrol, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4. 1 mM SA, 5.  $-0.351$  MPa PEG + 1 mM SA, 6.  $-0.75$  MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12) = 7.686$ ,  $p=0.002$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir ( $n=3$ ). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

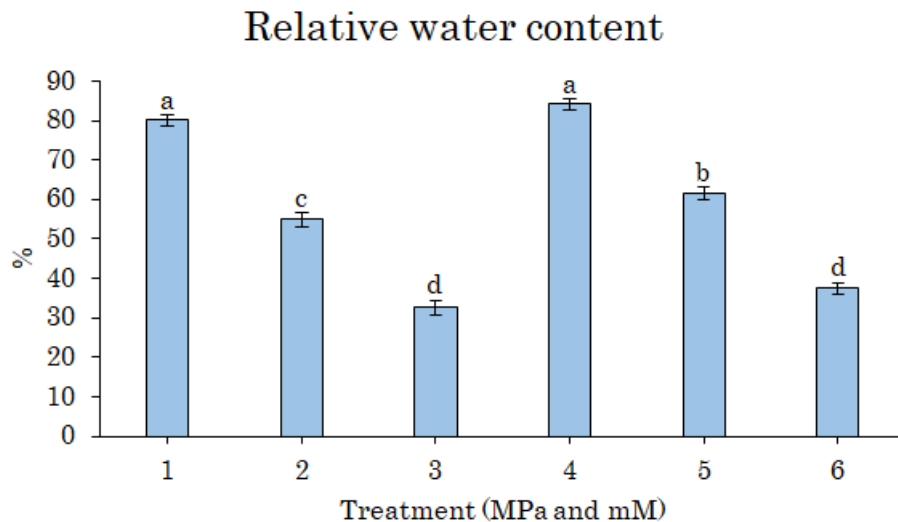


Figure 7. Relative water content in the leaves of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4. 1 mM SA, 5.  $-0.351$  MPa PEG + 1 mM SA, 6.  $-0.75$  MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12) = 172.68$ ,  $p<0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) ( $n=3$ ). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 7. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası yaprakta nispi su içeriği 1. Kontrol, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4. 1 mM SA, 5.  $-0.351$  MPa PEG + 1 mM SA, 6.  $-0.75$  MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12) = 172.68$ ,  $p<0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir ( $n=3$ ). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

These results are consistent with previous studies. For example, Hossain et al. (2024) reported that RWC decreased with increasing PEG-induced drought stress in Tartary buckwheat (*Fagopyrum tataricum* L.). Similarly, Chamani et al. (2025) found that leaf RWC declined under drought stress in *Lawsonia inermis* L., but significantly increased following SA and sodium nitroprusside (SNP) application. Shao et al. (2018) observed that in 20-day-old *Zea mays* L. seedlings, PEG6000-induced drought reduced RWC, while SA pretreatment enhanced water retention. Furthermore, Modara et al. (2025) reported that drought stress decreased RWC by 46.8% in marjoram (*Origanum majorana* L.), whereas SA application improved RWC by 44%. Taken together, these findings indicate that soil application of SA may enhance water retention and physiological stability in *P. vulgaris*, thereby improving tolerance to moderate drought stress.

### Chlorophyll a (mg g<sup>-1</sup> FW)

Chlorophyll a content is a crucial indicator of a plant's photosynthetic capacity and, consequently, its overall health and growth. As shown in Figure 8, chlorophyll a content across all treatments ranged from 1.09±0.11 to 2.59±0.06 mg g<sup>-1</sup> FW. The treatments had statistically significant effects on the chlorophyll a levels of *P. vulgaris*  $F(5,12)=29.61$ ,  $p<0.001$ . Compared to the control group, 2.22±0.05 mg g<sup>-1</sup>, PEG treatments -0.351 MPa 1.72±0.09 and -0.75 MPa 1.09±0.11 mg g<sup>-1</sup> FW led to a reduction in chlorophyll a content. The lowest chlorophyll a level was observed under the -0.75 MPa PEG treatment, 1.09±0.11 mg g<sup>-1</sup> FW, indicating that severe drought stress significantly disrupts chlorophyll a synthesis and thereby compromises photosynthetic efficiency. The highest chlorophyll a content was recorded in the 1 mM SA treatment, 2.59±0.06 mg g<sup>-1</sup> FW, which was statistically higher than all other treatments, including the control. This study demonstrates that SA strongly enhances chlorophyll a biosynthesis or delays its degradation in plants. In the combined treatments, the -0.351 MPa PEG + 1 mM SA application 2.11±0.41 mg g<sup>-1</sup> FW showed a mitigation of the stress-induced reduction in chlorophyll a, highlighting the protective role of SA under moderate drought conditions. However, in the -0.75 MPa PEG + 1 mM SA treatment, 1.23±0.08 mg g<sup>-1</sup> FW, chlorophyll a content did not differ significantly from the -0.75 MPa PEG group, and was significantly lower than in the SA-only group (Figure 8). This indicates that the protective capacity of SA in preserving chlorophyll a under severe drought stress may be limited.

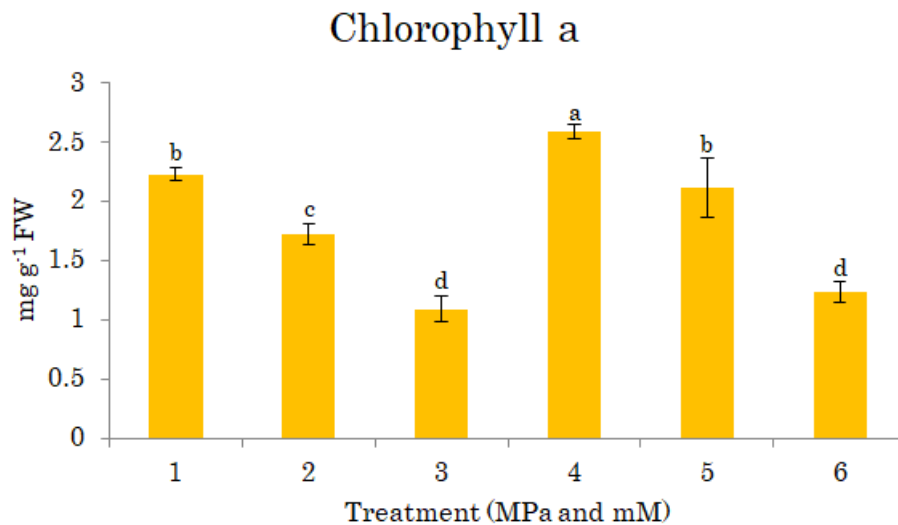


Figure 8. Changes in chlorophyll a content of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12)=29.61$ ,  $p<0.001$ ). \*Bars represent mean ± standard error (SE) (n=3). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 8. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası klorofil a miktarındaki değişiklik 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12)=29.61$ ,  $p<0.001$ ). \*Çubuklar ortalama ± standart hatayı (SE) göstermektedir (n=3). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

### Chlorophyll b (mg g<sup>-1</sup> FW)

Chlorophyll b content, as shown in Figure 9, ranged from 0.62±0.11 to 1.89±0.08 mg g<sup>-1</sup> FW across all treatments, with statistically significant differences  $F(5,12)=116.2, p<0.001$ . PEG-induced drought stress significantly reduced chlorophyll b content under both -0.351 MPa 1.06±0.07 and -0.75 MPa PEG 0.62±0.11 mg g<sup>-1</sup> FW treatments compared to the control 1.37±0.06 mg g<sup>-1</sup> FW. The lowest value was observed under -0.75 MPa PEG, indicating that severe drought stress markedly restricts chlorophyll b synthesis or stability, thereby impairing light-harvesting capacity and overall photosynthetic efficiency. In contrast, 1 mM SA treatment produced the highest chlorophyll b content 1.89±0.08 mg g<sup>-1</sup> FW, significantly higher than all other treatments, including the control. This demonstrates that SA strongly promotes chlorophyll b synthesis or delays its degradation, contributing to enhanced photosynthetic pigment accumulation, consistent with its effect on chlorophyll a. In the PEG + SA combination treatments, -0.351 MPa PEG + 1 mM SA 1.26±0.04 mg g<sup>-1</sup> FW was statistically similar to the control and significantly higher than the corresponding PEG treatment alone, whereas -0.75 MPa PEG + 1 mM SA

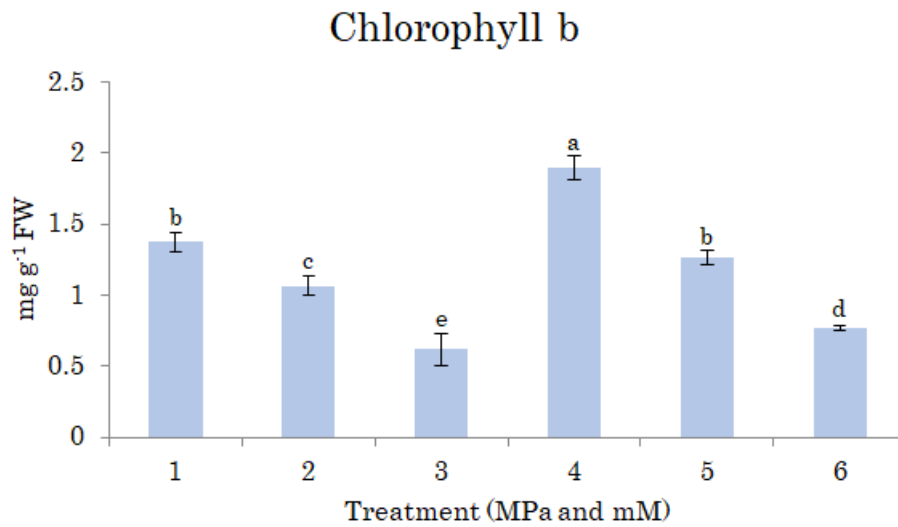


Figure 9. Changes in chlorophyll b content of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12)=116.2, p<0.001$ ). \*Bars represent mean ± standard error (SE) (n=3). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 9. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası klorofil b miktarındaki değişiklik 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12)=116.2, p<0.001$ ). \*Çubuklar ortalama ± standart hatayı (SE) göstermektedir (n=3). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

0.77±0.02 mg g<sup>-1</sup> FW showed partial recovery, exceeding the PEG-only value but remaining below the SA-alone and control levels. These results indicate that SA confers partial protection to chlorophyll b under severe drought stress, although it is insufficient to fully restore levels to those of unstressed plants. When evaluated together, chlorophyll a and b displayed parallel responses PEG stress reduced both pigments, while SA application maintained or enhanced their levels, highlighting that drought adversely affects both the photosystem reaction center chlorophyll a and the light-harvesting complex chlorophyll b. These findings align with previous studies. In *P. vulgaris*, drought stress applied on day 15 reduced chlorophyll a, b, total chlorophyll, and carotenoid contents by over 50% by day 25; however, SA and methyl jasmonate treatment significantly increased pigment levels in stressed plants (Mohi-Ud-Din et al., 2025). Similarly, in *Ocimum basilicum* L., SA or trehalose treatment after 20 days of drought stress enhanced chlorophyll a and b contents relative to untreated stressed plants (Zulfiqar et al., 2021). In *Triticum aestivum*, moderate and severe drought stress significantly decreased chlorophyll b content, whereas SA application alleviated these effects and improved both chlorophyll a and b levels (Munsif et al., 2022). Taken together, these results demonstrate that SA application, particularly under mild to moderate drought stress, effectively maintains or enhances chlorophyll a and b contents, suggesting its potential to sustain photosynthetic capacity and improve crop performance under water-limited conditions.

### Tolerance index

The tolerance index is a dimensionless parameter reflecting a plant's capacity to withstand stress conditions. As shown in Figure 10, tolerance index values across all treatments ranged from 0.51±0.01 to 1.02±0.34, with

statistically significant differences among treatments  $F(5,12)= 16.43, p<0.001$ . PEG-induced drought stress led to reduced tolerance index values, with  $-0.351$  MPa PEG  $0.74\pm0.07$ , indicating that even mild drought imposes noticeable stress. The lowest tolerance index was observed under  $-0.75$  MPa PEG  $0.51\pm0.01$ , demonstrating that severe drought considerably limits plant resilience. Application of  $1$  mM SA resulted in the highest tolerance index  $1.02\pm0.34$ , highlighting its capacity to enhance plant performance and physiological resilience even in the absence of stress. In combination treatments,  $-0.351$  MPa PEG +  $1$  mM SA  $0.80\pm0.05$  significantly improved the tolerance index under mild drought, reflecting the stress-mitigating role of SA. Under severe stress  $-0.75$  MPa PEG +  $1$  mM SA,  $0.62\pm0.08$ , SA slightly improved the tolerance index, yet values remained lower than those with SA alone (Figure 10), indicating a partial protective effect under high stress levels. These results are consistent with previous studies. For example, in *Triticum durum*–*Aegilops tauschii*, PEG-6000-induced osmotic stress decreased the tolerance index in proportion to PEG concentration (Sharma et al., 2022), and similar reductions were reported in wheat genotypes under PEG stress (Bukhari et al., 2021). Conversely, in drought-stressed *Ocimum basilicum*, SA application enhanced the drought tolerance index (Damalas, 2019). Collectively, these findings suggest that soil application of  $1$  mM SA can be adopted as a practical crop management strategy to improve drought resilience and sustain the growth and productivity of *P. vulgaris* under water-limited conditions.

### Tolerance index

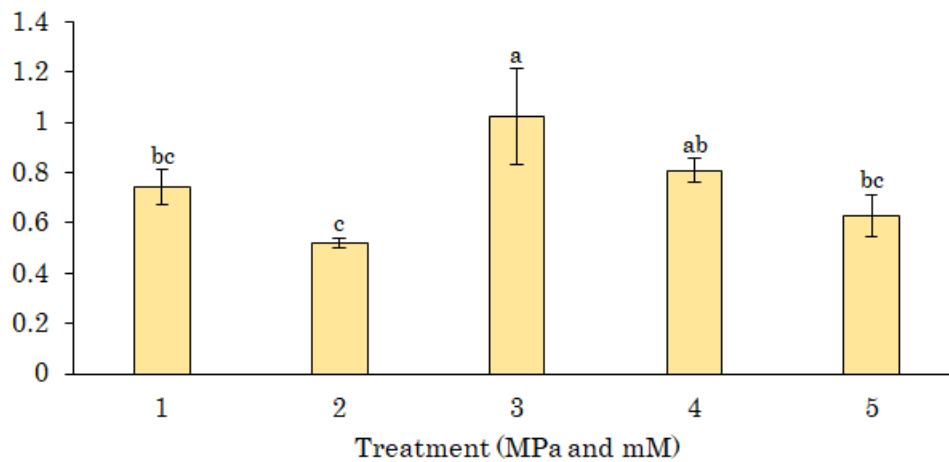


Figure 10. Tolerance index of the *P. vulgaris* plant after PEG, SA, and combined treatments 1.  $-0.351$  MPa PEG, 2.  $-0.75$  MPa PEG, 3.  $1$  mM SA, 4.  $-0.351$  MPa PEG +  $1$  mM SA, 5.  $-0.75$  MPa PEG +  $1$  mM SA. The TI value of the control group is  $1.00$  by definition and therefore not shown in the graph. ANOVA revealed significant differences among the treatments ( $F(5,12)= 16.43, p<0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) ( $n=3$ ). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 10. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası tolerans indeksi 1.  $-0.351$  MPa PEG, 2.  $-0.75$  MPa PEG, 3.  $1$  mM SA, 4.  $-0.351$  MPa PEG +  $1$  mM SA, 5.  $-0.75$  MPa PEG +  $1$  mM SA. Kontrol grubunun TI değeri  $1,00$ 'dir ve bu nedenle grafikte gösterilmemiştir. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12)= 16.43, p<0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir ( $n=3$ ). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

### MDA content ( $\mu\text{mol g}^{-1}$ FW)

Malondialdehyde (MDA), the terminal product of lipid peroxidation in plants, serves as a direct indicator of oxidative damage to cellular membranes induced by environmental stress and is thus a key biomarker for assessing plant tolerance to abiotic stress. As illustrated in Figure 11, MDA content across treatments ranged from  $0.75\pm0.03$  to  $1.50\pm0.16$   $\mu\text{mol g}^{-1}$  FW, with significant differences among treatments  $F(5,12)= 7.59, p=0.002$ . PEG-induced drought stress significantly elevated MDA levels, with  $-0.351$  MPa PEG  $1.32\pm0.09$   $\mu\text{mol g}^{-1}$  FW and  $-0.75$  MPa PEG  $1.50\pm0.16$   $\mu\text{mol g}^{-1}$  FW exceeding the control  $1.13\pm0.04$   $\mu\text{mol g}^{-1}$  FW. The highest MDA accumulation was observed under  $-0.75$  MPa PEG, highlighting that severe drought stress triggers excessive ROS accumulation, thereby amplifying lipid peroxidation and compromising membrane integrity. Conversely,  $1$  mM SA treatment resulted in the lowest MDA content,  $0.75\pm0.03$   $\mu\text{mol g}^{-1}$  FW, significantly lower than both PEG treatments, demonstrating SA's potent antioxidative role in mitigating ROS accumulation and protecting cellular membranes. In PEG + SA combination treatments,  $-0.351$  MPa PEG +  $1$  mM SA  $1.17\pm0.11$   $\mu\text{mol g}^{-1}$  FW significantly reduced MDA compared to PEG alone, illustrating that SA effectively alleviates oxidative stress under mild drought conditions. However, under severe stress  $-0.75$  MPa PEG +  $1$  mM SA,  $1.36\pm0.04$   $\mu\text{mol g}^{-1}$  FW, MDA levels were

not significantly lower than PEG-only treatment, though they remained higher than SA-alone, indicating that SA's protective capacity is limited under extreme drought. These findings are consistent with prior studies drought stress increased MDA by 26–39% in common bean cultivars 'Dorsa and Shekofa (Dolatkhah Dashtmian et al., 2023), and elevated MDA levels were observed in roots and leaves of black goji after 28 days of drought (Guo et al., 2018). Similarly, in mung bean, MDA content rose with increasing PEG-induced stress (Ajmal et al., 2023). damage, thereby limiting lipid peroxidation (Taghizadeh Tabari et al., 2022). Furthermore, SA enzymatically removes ROS that trigger membrane lipid peroxidation. In addition, numerous studies have demonstrated that SA enhances the activity and expression of major antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), which together form the core enzymatic ROS-scavenging system. These enzymes work synergistically to convert superoxide radicals and hydrogen peroxide into non-toxic molecules, thereby preventing the initiation of lipid peroxidation (Hasanuzzaman et al., 2020; Sedaghat et al., 2022; Ali et al., 2023). The activation of these enzymes under SA treatment has been reported in drought-stressed wheat, maize, and bean plants, leading to reduced MDA accumulation and enhanced membrane stability (Islam et al., 2023; Zafar et al., 2023). This suggests that the lowered MDA levels observed in this study are closely associated with SA-induced upregulation of antioxidant defense enzymes that suppress ROS-driven lipid degradation. Collectively, these results demonstrate that SA provides a robust physiological mechanism to protect cellular membranes and enhance stress tolerance in crop plants, particularly under mild to moderate drought conditions.

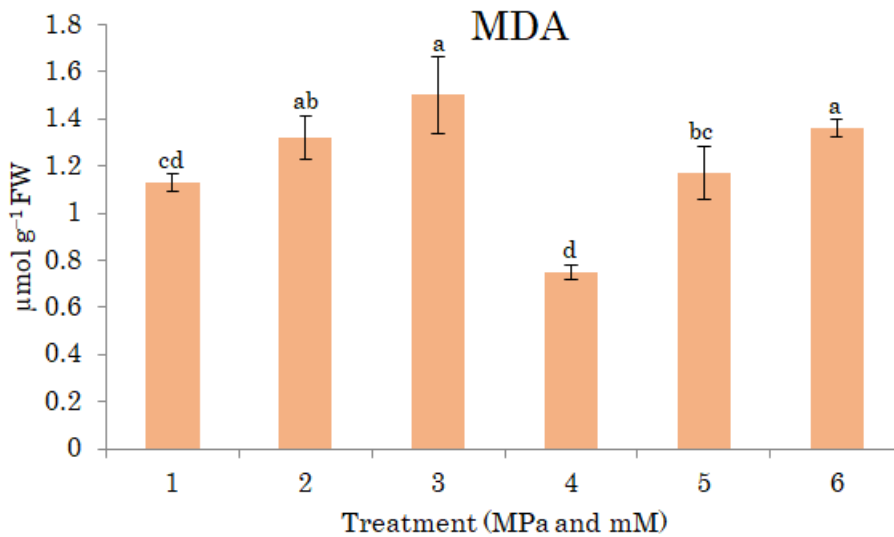


Figure 11. Lipid peroxidation content of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12)= 7.59, p=0.002$ ). \*Bars represent mean  $\pm$  standard error (SE) ( $n=3$ ). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 11. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası lipid peroksidasyon içeriği 1. Kontrol, 2. -0.351 MPa PEG, 3. -0.75 MPa PEG, 4. 1 mM SA, 5. -0.351 MPa PEG + 1 mM SA, 6. -0.75 MPa PEG + 1 mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12)= 7.59, p=0.002$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir ( $n=3$ ). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

### H<sub>2</sub>O<sub>2</sub> content (µmol g<sup>-1</sup> FW)

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), one of the major ROS, plays a dual role in plants, acting as both a signaling molecule under normal conditions and as a marker of oxidative stress when accumulated excessively. As shown in Figure 12, H<sub>2</sub>O<sub>2</sub> content in *P. vulgaris* leaves ranged from 0.47 $\pm$ 0.02 to 0.66 $\pm$ 0.01 µmol g<sup>-1</sup> FW across all treatments, displaying a pattern similar to MDA accumulation. Statistical analysis confirmed that the treatments significantly influenced H<sub>2</sub>O<sub>2</sub> levels,  $F(5,12)= 28.24, p<0.001$ . The most pronounced H<sub>2</sub>O<sub>2</sub> accumulation, 0.66 $\pm$ 0.01 µmol g<sup>-1</sup> FW, was detected under the -0.75 MPa PEG treatment, which was statistically higher than all other treatments. This dramatic increase reflects the severe oxidative stress caused by excessive ROS generation under strong drought conditions, ultimately leading to lipid peroxidation and membrane destabilization. The -0.75 MPa PEG + 1 mM SA treatment exhibited slightly lower H<sub>2</sub>O<sub>2</sub> levels 0.62 $\pm$ 0.04 µmol g<sup>-1</sup> FW compared to PEG alone, but remained significantly higher than the SA-only treatment, suggesting that SA provides only partial protection against oxidative damage under severe stress. In contrast, the lowest H<sub>2</sub>O<sub>2</sub> level, 0.47 $\pm$ 0.02 µmol g<sup>-1</sup> FW, was recorded in the 1 mM SA treatment, which was significantly lower than both the control and all PEG-stressed groups. This

sharp decline clearly demonstrates SA's potent ability to activate the antioxidant defense machinery, effectively suppress ROS accumulation, and maintain cellular redox homeostasis. Under mild drought stress  $-0.351$  MPa PEG,  $H_2O_2$  content increased to  $0.57 \pm 0.009 \mu\text{mol g}^{-1}$  FW compared with the control  $0.52 \pm 0.01 \mu\text{mol g}^{-1}$  FW, whereas the addition of SA  $-0.351$  MPa PEG +  $1$  mM SA significantly reduced it to  $0.53 \pm 0.003 \mu\text{mol g}^{-1}$  FW. This highlights that the SA application under mild drought is highly effective in suppressing oxidative stress, whereas its protective effect becomes less pronounced as stress intensity increases. Overall, the parallel trends observed for  $H_2O_2$  and MDA strongly indicate that PEG-induced drought triggers oxidative damage by stimulating ROS overproduction, while SA acts as an efficient antioxidant modulator that mitigates this response. However, the efficiency of SA is stress-intensity-dependent, being most effective under moderate drought but less so under extreme stress levels. These findings are in strong agreement with previous reports. Zulfiqar et al. (2021) observed that salicylic acid significantly reduced  $H_2O_2$  accumulation in drought-stressed sweet basil plants. Similarly, Urmi et al. (2023) demonstrated that SA application suppressed drought-induced increases in  $H_2O_2$  in rice, while Islam et al. (2023) reported comparable effects in barley genotypes. Likewise, Akin and Kaya (2024) found that SA treatment substantially reduced  $H_2O_2$  levels in drought-stressed wheat plants, further confirming its antioxidant role. Mechanistically, the observed decrease in  $H_2O_2$  content under SA treatment provides clear biochemical evidence of enhanced ROS scavenging capacity. SA is known to upregulate the expression and activity of key antioxidant enzymes such as catalase (CAT) and peroxidases (POD), which catalyze the conversion of  $H_2O_2$  into water and oxygen (Anjum et al., 2016; Ghafari et al., 2018; Abdelaal et al., 2020). This enzymatic upregulation enables plants to efficiently detoxify the ROS generated during drought stress. Furthermore, SA-mediated regulation of  $H_2O_2$  involves signaling pathways such as NPR1 (Nonexpressor of Pathogenesis-Related genes1) and downstream MAPK (Mitogen-Activated Protein Kinase) cascades. NPR1 functions as the main receptor transmitting SA signals inside the cell and plays a key role in oxidative stress responses. Its activation triggers MAPK cascades that regulate the expression of genes controlling  $H_2O_2$  production, thereby enhancing plant resistance to oxidative stress (Jayakannan et al., 2015). Additionally, SA modulates  $H_2O_2$  generation through interaction with ABA (Abscisic Acid)-dependent signaling pathways, coordinating stress responses such as stomatal closure and water loss (Zhao et al., 2025).

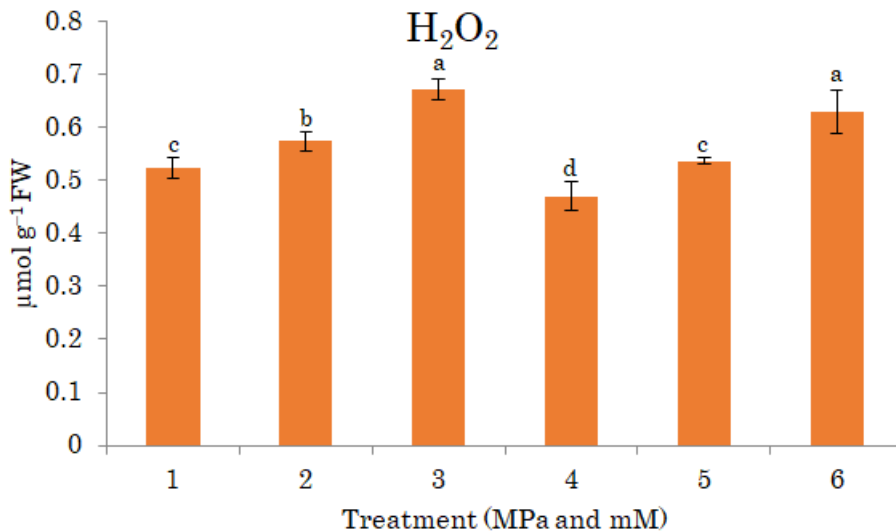


Figure12.  $H_2O_2$  content of the *P. vulgaris* plant after PEG, SA, and combined treatments 1. Control, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4.  $1$  mM SA, 5.  $-0.351$  MPa PEG +  $1$  mM SA, 6.  $-0.75$  MPa PEG +  $1$  mM SA. ANOVA revealed significant differences among the treatments ( $F(5,12)= 28.24, p<0.001$ ). \*Bars represent mean  $\pm$  standard error (SE) ( $n=3$ ). Different letters indicate statistically significant differences at  $p<0.05$

Şekil 12. *P. vulgaris* bitkisinin PEG, SA ve kombinasyon uygulamaları sonrası  $H_2O_2$  içeriği 1. Kontrol, 2.  $-0.351$  MPa PEG, 3.  $-0.75$  MPa PEG, 4.  $1$  mM SA, 5.  $-0.351$  MPa PEG +  $1$  mM SA, 6.  $-0.75$  MPa PEG +  $1$  mM SA. ANOVA, uygulamalar arasında anlamlı farklılıklar olduğunu göstermektedir ( $F(5,12)= 28.24, p<0.001$ ). \*Çubuklar ortalama  $\pm$  standart hatayı (SE) göstermektedir ( $n=3$ ). Aynı sütunda farklı harflerle gösterilen ortalamalar  $p<0.05$  düzeyinde istatistiksel olarak anlamlı farklılık göstermektedir

Collectively, these mechanisms strengthen plant defenses against oxidative stress and improve drought tolerance. Taken together, these results demonstrate that SA confers a robust antioxidative defense by reducing  $H_2O_2$  accumulation, stabilizing cellular structures, and maintaining metabolic balance under stress conditions. The pronounced decrease in  $H_2O_2$  levels, particularly in the SA-alone and mild-stress + SA groups, highlights the crucial role of SA in protecting plant cells from oxidative injury. Therefore, the SA application represents an

effective physiological strategy to enhance drought tolerance in crops by sustaining redox homeostasis and limiting oxidative membrane damage.

### Correlation analysis

In order to statistically substantiate the internal mechanisms and physiological interrelationships among the studied parameters, a Pearson correlation analysis was conducted on key stress markers and growth indicators. The analysis revealed robust statistical connections that strongly support our experimental findings. Specifically, a highly significant positive correlation was observed between MDA and H<sub>2</sub>O<sub>2</sub> levels ( $r= 0.829^{**}$ ), statistically confirming that the accumulation of ROS directly drives lipid peroxidation under stress conditions. From a physiological standpoint, the strong negative correlation of RWC with both MDA ( $r= -0.747^{**}$ ) and H<sub>2</sub>O<sub>2</sub> ( $r= -0.917^{**}$ ) highlights the critical role of water deficit in exacerbating oxidative stress and cellular damage. Regarding the photosynthetic apparatus, the very strong positive correlations observed between RWC and both Chlorophyll a ( $r= 0.937^{**}$ ) and Chlorophyll b ( $r= 0.944^{**}$ ) suggest that maintaining cellular turgor is a primary determinant of pigment stability. Conversely, the potent negative relationship of H<sub>2</sub>O<sub>2</sub> with Chlorophyll a ( $r= -0.941^{**}$ ) and Chlorophyll b ( $r= -0.919^{**}$ ) demonstrates that oxidative stress induces severe pigment degradation, proving a direct destructive effect on the plant's photosynthetic capacity (Fig 13).

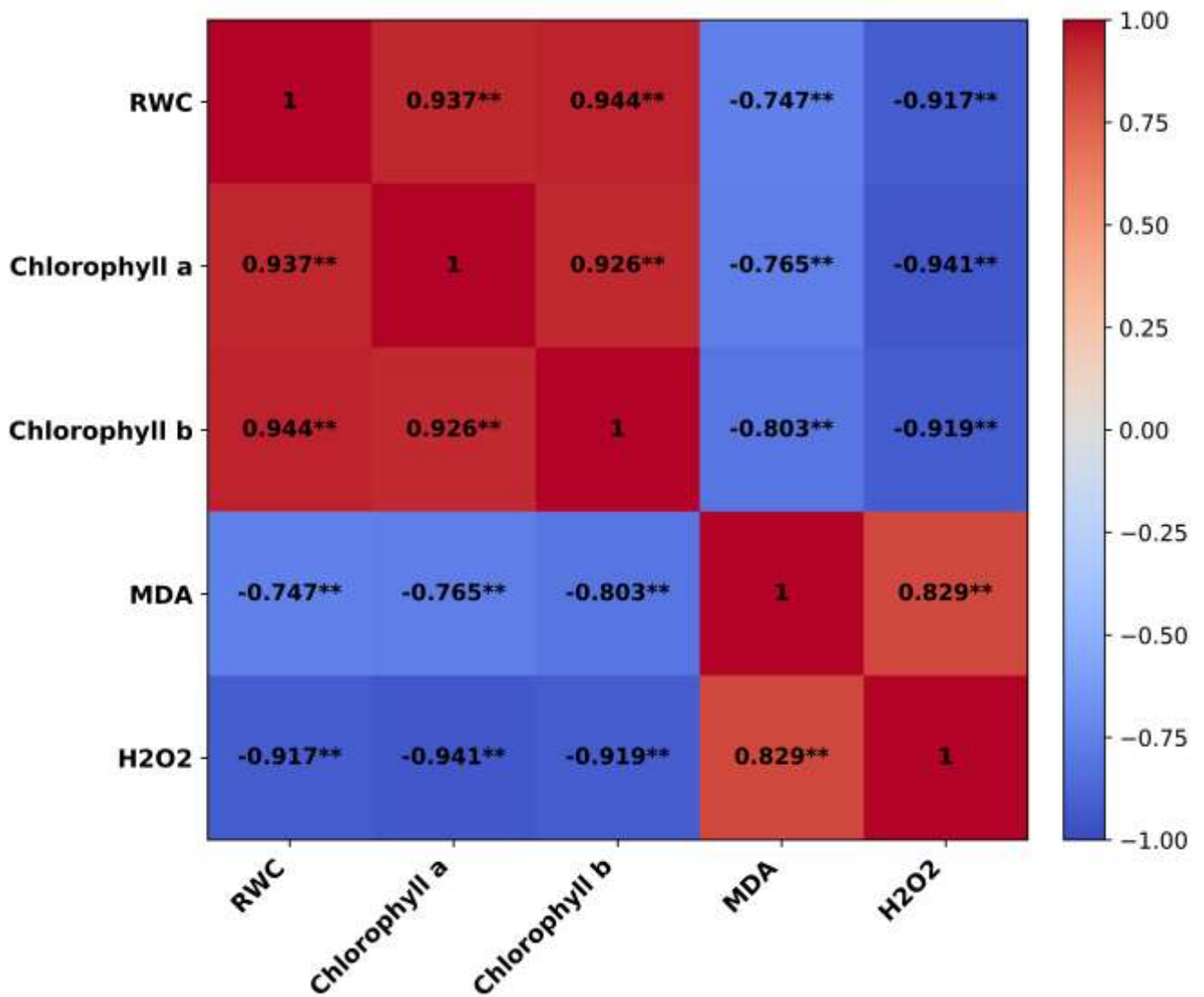


Figure 13. Pearson Correlation Matrix (heatmap) among MDA, H<sub>2</sub>O<sub>2</sub>, RWC, chlorophyll a and chlorophyll b parameters. \*Values within cells indicate the correlation coefficient (r) and statistical significance level. Red shades indicate positive correlation, and blue shades indicate negative correlation. \*\*Correlation is statistically significant at the  $p<0.01$  level

Şekil 13. MDA, H<sub>2</sub>O<sub>2</sub>, RWC, klorofil a ve klorofil b parametreleri arasındaki Pearson Korelasyon Matrisi (ısı haritası). \*Hücre içindeki değerler korelasyon katsayısını (r) ve istatistiksel önem düzeyini belirtmektedir. Kırmızı tonlar pozitif korelasyonu, mavi tonlar ise negatif korelasyonu ifade eder. \*\*Korelasyon,  $p<0.01$  düzeyinde istatistiksel olarak anlamlıdır

## CONCLUSION

This study demonstrates that drought stress severely compromises the physiological and biochemical integrity of common bean (*P. vulgaris* L.), while the application of SA offers a potent mitigation strategy. PEG-induced drought stress led to significant reductions in key morphological parameters including seedling height, root and shoot lengths, biomass accumulation (fresh and dry weights), and leaf number as well as physiological markers such as RWC. At the biochemical level, drought stress induced a sharp decline in chlorophyll a and b pigments while triggering severe oxidative stress, evidenced by significantly elevated MDA and H<sub>2</sub>O<sub>2</sub> levels. Notably, the surge in H<sub>2</sub>O<sub>2</sub> under severe PEG stress reflects the overproduction of ROS, leading to substantial cellular damage. Conversely, the exogenous application of 1 mM SA, both alone and in combination with drought stress, proved effective in alleviating these adverse effects. SA treatment improved growth metrics, preserved photosynthetic performance by maintaining higher chlorophyll contents, and mitigated oxidative damage by significantly lowering ROS accumulation H<sub>2</sub>O<sub>2</sub> and membrane lipid peroxidation MDA. It is noteworthy that while SA provided robust protection under mild drought conditions, its efficacy was partially limited under severe stress, suggesting a stress-intensity-dependent response. Consequently, the soil application of 1 mM SA is recommended as a practical and cost-effective agronomic approach to enhance drought resilience and minimize yield losses in *P. vulgaris* cultivation. Future research should validate these findings under field conditions and further elucidate the molecular mechanisms, specifically examining the influence of SA on antioxidant enzyme kinetics, osmolyte accumulation, and stress-responsive gene expression profiles across different growth stages.

### Contribution rate statement summary of researchers

The authors declare that they have contributed equally to the article.

### Conflict of interest statement

The authors declare that there is no conflict of interest among them.

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