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Effect of Foliar Boron and Proline Applications on Some Biochemical Properties in Soybean (*Glycine max* L.)

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ABSTRACT
This study was conducted to determine the regulatory effects of proline (0, 10 and 20 ppm) against boron (0, 2.5, 5, and 10 ppm) toxicity on some biochemical properties of soybean (*Glycine max* L.) plants. In the research, the levels of lipid peroxidation (MDA), chlorophyll a, chlorophyll b, total chlorophyll, total antioxidant activity, total phenolic content, and total flavonoid content were investigated in soybean after application. As a result of the study, it was determined that boron applications in soybean plants increased MDA, chlorophyll a, anthocyanin, phenolic values, and chlorophyll b, total chlorophyll, but total flavonoid content first increased and then decreased (the highest values are usually obtained at a boron dose of 5 ppm (B2)). There was a continuous decrease in carotenoid values compared to the control group. Proline applications had a positive and regulatory effect on total phenolic and flavonoid contents against boron toxicity. The data obtained show that boron applications significantly increase antioxidant capacity, total phenolic and flavonoid content, and chlorophyll levels in the plant, with the most balanced and high results obtained especially at a boron (B2) dose of 5 ppm. Proline applications, on the other hand, suppressed biochemical parameters at high doses, and it was determined that control-to-low doses (P0–P10) produced more positive effects in combination with boron.

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Yapraktan Bor ve Prolin Uygulamalarının Soya Fasulyesinde (Glycine max L.) Bor Toksisitesine Karşı Bazı Biyokimyasal Özellikler Üzerine Etkisi

ÖZET
Bu çalışma, soya fasulyesi (*Glycine max* L.) bitkisinin bazı biyokimyasal özellikleri üzerine bor (0, 2.5, 5 ve 10 ppm) toksisitesine karşı prolinin (0, 10 ve 20 ppm) düzenleyici etkilerini belirlemek amacıyla yürütülmüştür. Araştırmada, uygulamalar sonrasında soya fasulyesinde Lipid Peroksidasyon Seviyeleri (MDA), klorofil a, klorofil b, toplam klorofil, karotenoid, toplam antioksidan aktivite, toplam fenolik madde miktarı ve toplam flavonoid miktarı özellikleri araştırılmıştır. Çalışma sonucunda, soya bitkisinde bor uygulamalarının MDA, klorofil a, antosiyanin ve toplam fenolik içerik değerlerini artırdığı, klorofil b, toplam klorofil ve toplam flavonoid içeriklerinin ise önce artıp sonra azaltığı (en yüksek değerlerin genellikle 5 ppm bor dozunda (B2) elde edildiği) belirlenmiştir. Çalışmada karotenoid değerlerinde kontrol grubuna göre sürekli bir azalma olmuştur. Bu çalışmada prolin uygulamalarının bor toksisitesine karşı toplam fenolik ve flavonoid içerikleri üzerinde olumlu ve düzenleyici bir etkiye sahip olduğu belirlenmiştir. Elde edilen veriler, bor uygulamalarının bitkide antioksidan kapasite, toplam fenolik ve flavonoid içerik ile klorofil düzeylerini anlamlı biçimde artırdığını, en dengeli ve yüksek sonuçların özellikle 5 ppm bor (B2) dozunda elde edildiğini göstermektedir. Prolin uygulamaları

Tarla Bitkileri

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Anahtar Kelimeler
Bor
Fenolik
Klorofil
Prolin
Soya

ise yüksek dozlarda biyokimyasal parametreleri baskılamış, kontrol–düşük dozların (P0–P10) bor ile birlikte daha olumlu etkiler oluşturduğu belirlenmiştir.

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INTRODUCTION

Soybean (*Glycine max* L.) is native to China and the Korean Peninsula, and it became a staple food in Asia approximately,5 000 years ago, highlighting its cultural and economic significance in the region. Despite its introduction to Europe in the early 17th century, desired yields were not achieved due to climatic factors. The nutritional value of soybeans, particularly their high protein content and other nutrients, supports its use as livestock feed, further enhancing their importance (Mert and İlker, 2016).

Soybeans hold important utility in human and animal nutrition due to their composition, which includes 36-40% protein, 18-24% oil (including Omega-3 and similar fatty acids), vitamins B1, B2, K, and E, elements such as Zn, Fe, and Ca, 27% carbohydrates, and 18% minerals. Its derivatives find application in various products such as infant foods, bakery products, confectionery products, and hypoallergenic dairy products. With its seeds containing 18-26% oil, soybeans have also become a major oilseed crop. The meal remaining after oil extraction is suitable for silage, enhancing its value as animal feed (Kolsarıcı et al., 2005).

Boron is an essential micronutrient for plants, but it is considered one of the elements at highest risk of toxicity due to the narrow boundary between optimal and toxic levels. In plants, boron plays important roles in sugar transport, cell wall synthesis and lignification, carbohydrate and RNA metabolism, respiration, indole-3-acetic acid (IAA) metabolism, phenol metabolism, and the structural and functional properties of biological membranes (Lukaszewski & Blevins, 1996). However, high concentrations of boron lead to accumulation, especially in leaf tissue, causing damage to the cell wall structure, impairment of membrane integrity, and disruption of metabolic processes. The main symptoms of boron toxicity include chlorosis, necrosis, decreased photosynthetic activity, and increased oxidative stress. It has been reported that increased boron levels promote the production of reactive oxygen species (ROS) and increase lipid peroxidation (Nable et al., 1997; Reid et al., 2004). These physiological disruptions result in growth retardation and yield losses in plants; toxic effects become more pronounced, especially in arid and semi-arid regions and saline soil conditions, due to increased boron accumulation (Keren & Bingham, 1985; Demirtaş, 2005).

Plants develop various defense mechanisms against abiotic stress conditions, such as boron toxicity

, salt and drought, the most important of which is proline accumulation. Under water-restricted conditions, plants reduce the rate of photosynthesis and CO_2 emissions by closing their stomata, while simultaneously increasing proline and antioxidant enzyme activities (Kutlu, 2010; Sepanlo et al., 2014; Küçükkılıç et al., 2026). Proline increases stress tolerance through its multifaceted functions, including maintaining osmotic balance, preserving intracellular water potential, stabilizing protein and membrane structures, and limiting the harmful effects of free radicals (Ashraf & Foolad, 2007; Szabados & Savouré, 2010). Proline's long-term effect is reported to be an important biochemical defense mechanism protecting plants under abiotic stress conditions (Ercan, 2008). Stewart and Rothwell (1983) also reported that proline is a protective compound for plants against stress factors such as drought. In this context, proline accumulation observed under boron toxicity conditions is considered an adaptive and remedial response aimed at limiting the physiological and biochemical damage caused by boron (Hayat et al., 2012).

This study hypothesizes that exogenous proline application will mitigate boron toxicity in soybeans by reducing oxidative damage (as measured by MDA) induced by high-dose boron, protecting photosynthetic pigments (chlorophyll and carotenoids), and strengthening the antioxidant defense system (total antioxidant activity, phenolics, and flavonoids).

MATERIAL and METHOD

This study was conducted in a fully controlled growth chamber at the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University (Van YYU). The experiment was conducted with the "Yeşilsoy" variety, which was obtained from the Çukurova Agricultural Research Institute. The main reason for choosing a silage soybean variety (Yeşilsoy) (Vurarak, 2024) in this study is its high biomass production potential and well-developed

vegetative parts. Since boron toxicity and the physiological responses developed against it primarily occur in leaf and stem tissues, forage varieties with strong vegetative growth constitute a more suitable model for evaluating stress conditions.

This trial was carried out according to the randomized plots experimental design in a factorial arrangement with 4 replications. The study was carried out in a mixture of peat + perlite + soil (1:1:2) using 500 cc pots, under conditions of 65% relative humidity, an 8/16-hour dark/light photoperiod, and a temperature of 25°C. The seeds were germinated in 40 x 20 cm pots in a perlite medium. The seeds were planted in the vials on January 15. After the real leaves emerged (V2), they were transferred to pots on February 1, 2024. A few days later, the Hoagland nutrient solution was applied to supply the nutritional needs of the seedlings (Hoagland and Arnon, 1950). In this study, the doses of boron (0, 2.5, 5, and 10 ppm) and proline (0, 10, and 20 ppm) were applied. Boron, while contributing to growth and development in trace amounts in plants, is toxic at high concentrations. In this study, boron toxicity was induced through foliar application. Although boron toxicity mostly occurs in soil-borne forms under natural conditions and in agricultural production areas, foliar application is a controlled stress model that allows for the rapid and controlled demonstration of the physiological and toxic effects of boron on plant tissue (Fernández, & Brown, 2013; Wimmer & Eichert, 2013; Yolci et al., 2022a). In this study, different doses (0, 2.5, 5, and 10 ppm) of boric acid (H_2BO_3) were used as the boron source. Proline application to plants started when plants had V6-V10 stage (12th day) (Demiralay et. al., 2017). Boron applications were started to plants in pots via foliar application on the 16th day. Proline application was terminated on day 19. Boron applications continued until the 23rd day. In this study, a total of 7 boron and proline applications were made day after day. Proline was continued alongside boron concentration applications. Physiological problems were observed in plants approximately 1 week later on 30th day. This study was terminated by harvesting for analyses (33rd day, 06.03.2024).

Vegetative development stages in soybeans (Fehr and Caviness, 1977); VE: Germination and emergence, VC: Cotyledon stage, V1: Formation of the first true leaf, V2: Formation of the second true leaf, V3-V5: 4-6 node stage, V6-Vn: 7 or more node stage. Plants were harvested after stress symptoms (Chlorosis at the leaf tips and edges, followed by necrosis) appeared. The following analyses were conducted on the collected plant material:

Lipid Peroxidation Levels (MDA): A 0.5 g leaf sample was homogenized in 10 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15.000 g for 5 minutes. From the supernatant, 1 mL was taken and mixed with 4 mL of 0.5% thiobarbituric acid (TBA) dissolved in 20% TCA. The mixture was incubated in a 95°C water bath for 30 minutes, rapidly cooled in an ice bath, and centrifuged at 10.000 g for 10 minutes. Absorbance readings of the supernatant were taken at 532 nm and 600 nm. The MDA content was calculated in nmol g^{-1} fresh weight (FW) according to the method of Güneri Bağcı (2010).

Chlorophyll and Carotenoid Concentrations: Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid concentrations were determined based on the formulas proposed by Lichtenthaler and Wellburn (1983),

- Total chlorophyll (µg/g FW) = (27.8 x A652) (1)
- Chlorophyll a (Chl a; µg/g FW) = (11.75 x A662) - (2.350 x A645) (2)
- Chlorophyll b (Chl b; µg/g FW) = (18.61 x A645) - (3.960 x A662) (3)
- Carotenoid (Car; µg/g FW) = [(1000 x A470) - (2.270 x Chl a) - (81.4 x Chl b)] / 227 (4)

Where:

- FW: Fresh weight
- A662: Absorbance at 662 nm
- A652: Absorbance at 652 nm
- A645: Absorbance at 645 nm
- A470: Absorbance at 470 nm

Total Phenolic Content: The total phenolic content in samples was measured using a modified Folin-Ciocalteu spectrophotometric method described by Obanda and Owuor (1997).

Total Flavonoid Content: Total flavonoid content was determined using the method developed by Quettier-Deleu et al. (2000). In this method, 2 ml of extract was mixed with 2 ml of 2% $AlCl_3$ solution and incubated at room temperature in darkness for 60 minutes. The prepared samples were measured at 415 nm using a spectrophotometer. The flavonoid content was calculated in mg QE $100g^{-1}$ using a standard quercetin (QE) calibration curve.

Total Antioxidant Activity Analysis: Total Antioxidant activity was based on the FRAP method (Benzie and Strain, 1996), and then the absorbance value was read at 593 nm, and the antioxidant activity values were recorded as Trolox equivalents (TE).

The data obtained in the study were subjected to variance analysis according to the factorial order in the randomized plots experimental design with the help of Costat (version 6.34) package program and the averages

were made according to the LSD multiple comparison test.

RESULTS and DISCUSSION

At the end of the study, the effects of boron (B) and boron × proline (B × P) applications on MDA content were found to be statistically significant ($p<0.01$). However, the effect of proline doses on MDA content was not statistically significant. The highest MDA content obtained from boron applications was recorded at 20.85 nmol/g FW with B3, whereas the lowest value was determined in the control dose at 17.72 nm/go $^{-1}$FW (Figure 1). In the boron × proline interactions, the highest MDA content was recorded at 22.03 nmol g^{-1} FW in the B3 × P0 treatment, while the lowest value was measured at 17.94 nmol g^{-1} FW in the B2 × P0 treatment (Table 1).

MDA is a crucial parameter for assessing stress-induced cellular damage in plants (Jain et al., 2001). Studies on boron have highlighted that, while it is an essential element for plants, excessive doses induce stress, leading to yield and quality losses (Brown et al., 2002). However, in most scientific studies, the distinction between the optimal beneficial dose and toxic levels is relatively narrow, making it challenging to determine the precise effect on plants (Sakal & Singh, 1985). Based on our findings, the observed fluctuations appear to be influenced by plant species and varieties, as well as the interactive effects of boron × proline applications. While proline suppressed MDA at low-to-medium boron doses (B2) (lowest value at B2xP0), proline application at high boron doses (B3) further increased MDA (together with P0). This was identified as an important point showing that the effect of proline is dose-dependent and that proline alone may not provide sufficient protection under high stress.

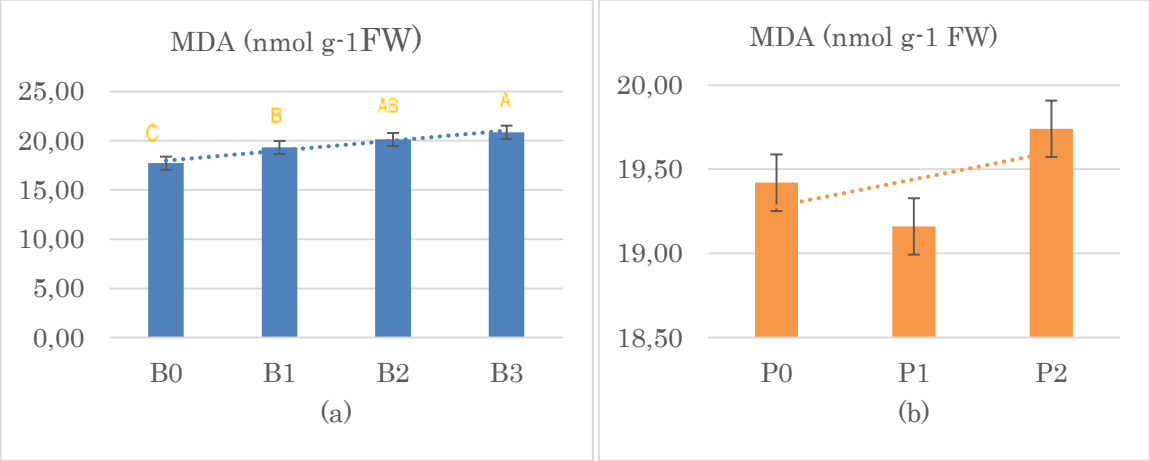

Figure 1. Effects of boron applications (a), and proline applications (b) on MDA content.
Şekil 1. Bor uygulamalarının (a) ve prolin uygulamalarının (b) MDA içeriği üzerindeki etkileri.

As shown in Table 1, the effects of boron (B) and boron × proline (B × P) applications on chlorophyll a content were found to be statistically significant ($p<0.01$). Among the boron treatments, the highest chlorophyll a content was recorded at 15.75 and 15.56 µg g^{-1} FW in the B2 and B3 doses, respectively, while the lowest value was measured at 12.08 µg g^{-1} FW in the control treatment (Figure 2).

In the boron × proline interactions, the highest chlorophyll a content was recorded at 17.19 µg g^{-1} FW in the B3 × P20 treatment, whereas the lowest value was determined at 10.95 µg g^{-1} FW in the B0 × P0 treatment (Table 1). In a similar study, it was reported that boron applications not changed chlorophyll a content in wheat cultivars (Doğru & Bildiren, 2020).

The effect of boron and boron × proline applications on chlorophyll b content in soybean was found to be statistically significant ($p<0.01$). The highest chlorophyll b content was recorded at 10.67 µg g^{-1} fw with the B2 application, while the lowest value was observed at 5.53 µg g^{-1} fw in the control treatment (Figure 3). In terms of boron × proline applications, the highest chlorophyll b content was obtained at 16.43 µg g^{-1} fw with the B2 × P20 treatment, whereas the lowest value was recorded at 4.95 µg g^{-1} fw with the B0 × P0 treatment (Table 1). Our findings indicate that while the effect of proline applications on chlorophyll b content was not statistically significant, chlorophyll a content initially increased with increasing boron doses, followed by a decline due to the onset of toxicity. Supporting our results, a study on German chamomile reported that different boron doses (0, 5, 10, and 20 ppm) initially led to an increase in chlorophyll b levels, followed by a decrease (Yolci et al., 2022b). Similarly, another study on rice found an inverse relationship between increasing boron doses and the levels of chlorophyll a, chlorophyll b, and total chlorophyll content (Riaz et al., 2021). These results suggest that the toxic effect of boron on plants varies not only with plant species and cultivars but also with other environmental factors.

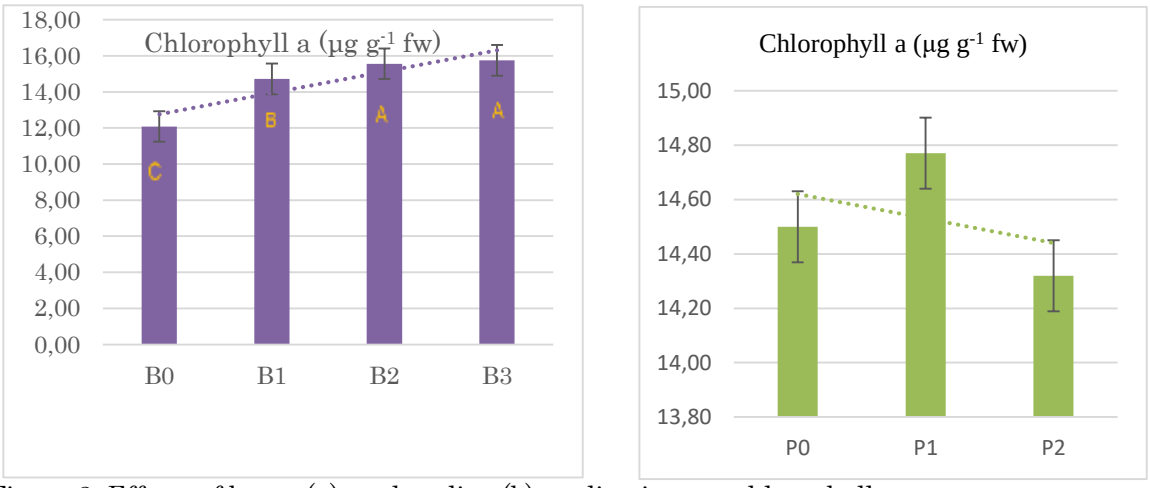

Figure 2. Effects of boron (a), and proline (b) applications on chlorophyll a
Şekil 2. Bor (a) ve prolin (b) uygulamalarının klorofil a üzerindeki etkileri

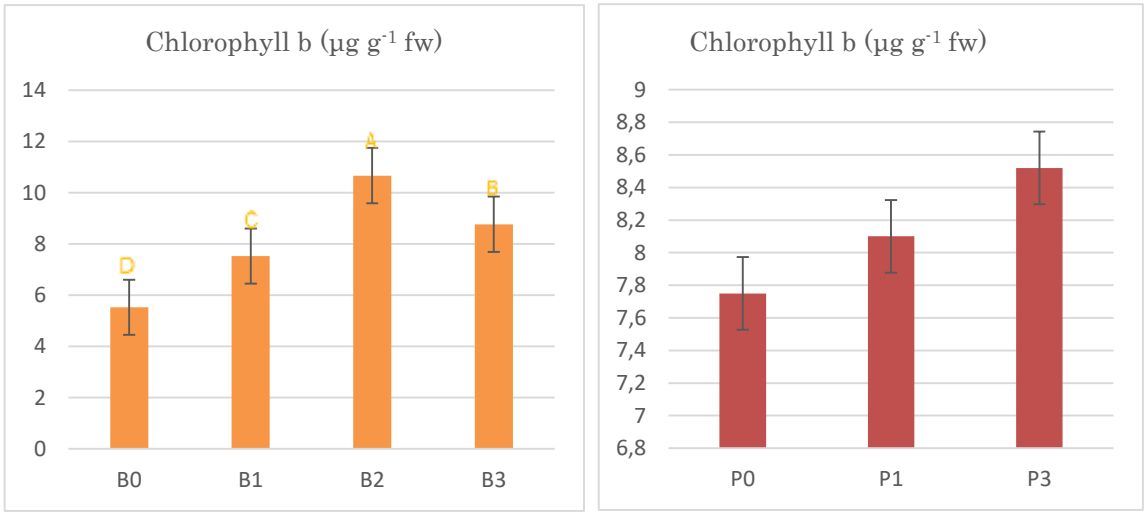

Figure 3. Effects of boron (a), and proline (b) applications on chlorophyll b
Şekil 3. Bor (a) ve prolin (b) uygulamalarının klorofil b üzerindeki etkileri

The effect of boron and boron × proline applications on total chlorophyll content was found to be statistically significant ($p<0.01$). Among the boron dose treatments, the highest total chlorophyll content was recorded at 26.09 µg g^{-1} fw with the B2 dose, whereas the lowest value was measured at 16.28 µg g^{-1} fw in the control treatment (Figure 4). Regarding boron × proline applications, the highest total chlorophyll content was obtained at 33.61 µg g^{-1} fw with the B2 × P20 treatment, while the lowest value was recorded at 15.90 µg g^{-1} fw with the B0 × P0 treatment (Table 1). The observed trend of an initial increase followed by a decrease in chlorophyll a, chlorophyll b, and total chlorophyll content, depending on the boron dose, clearly reveals the bidirectional effect of boron on plant physiology. It appears that at low concentrations, boron stimulates metabolic processes, increasing photosynthetic pigment synthesis, while at high doses it leads to cellular toxicity and accelerates chlorophyll degradation. This can be considered a typical hormesis response, supporting the idea that boron has a stimulatory (hormetic) effect at low doses and an inhibitory effect at high doses. Consistent with our findings, a previous study reported that the effects of boron doses on total chlorophyll and other photosynthetic pigments varied dewithlant variety, environmental conditions, and treatment (Kayıhan et al., 2017). It can be suggested that proline supports chlorophyll biosynthesis or limits chlorophyll degradation by maintaining cellular balance under stress conditions, particularly through mechanisms such as porphyrin ring stabilization and magnesium ion chelation. In this context, this interaction is considered one of the unique contributions of the study and requires further detailed investigation through advanced physiological and biochemical analyses (Phung et al., 2011; Zulfiqar, and Ashraf, 2023; Sarraf et al., 2026).

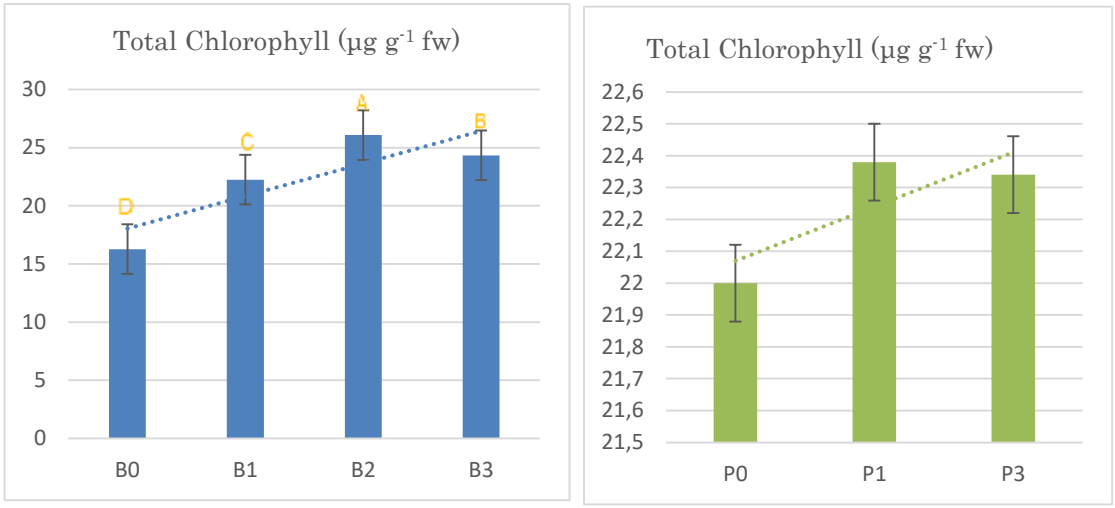

Figure 4. Effects of of boron (a), and proline (b) applications on total chlorophyll
Şekil 4. Bor (a) ve prolin (b) uygulamalarının toplam klorofil üzerindeki etkileri

Table 1. Effects of boron and proline applications on some biochemical properties in soybeans
Çizelge 1. Bor ve prolin uygulamalarının soya fasulyesindeki bazı biyokimyasal özellikler üzerindeki etkileri

Boron Doses	Proline Doses	MDA (nmol g-1FW)	Chlorophyll a (µg g-1fw)	Chlorophyll b (µg g-1fw)	TotalChlorophyll (µg g-1fw)
	P0 (Control)	19.35±0.56 bc	10.95±0.32 g	4.95±0.14 f	15.90±0.46 fg
Control (B0)	P10	14.71±0.42 d	11.72±0.34 fg	6.46±0.19 e	16.18±0.50 ef
	P20	19.10±0.55 bc	13.57±0.39 de	5.19±0.15 e	16.76±0.48 f
B0 average		**17.72±0.80 C**	**12.08±0.43 C**	**5.53±0.25 D**	**16.28 ±0.29 D**
	P0 (Control)	19.35±0.56 abc	16.19±0.47 ab	8.87±0.26 cd	25.06±0.72 bc
2.5 ppm (B1)	P10	20.52±0.59 b	15.10±0.44 bc	7.44±0.21 de	22.54±0.65 cd
	P20	18.06±0.52 c	12.88±0.37 ef	6.28±0.18 e	19.16±0.55 ef
B1 average		**19.31±0.45 B**	**14.72±0.53 B**	**7.53±0.39 C**	**22.25±0.91 C**
	P0 (Control)	17.94±0.52 d	16.21±0.45 ab	7.97±0.23 d	21.60±0.62 de
5 ppm (B2)	P10	21.16±0.61 ab	16.85±0.49 ab	7.61±0.22 de	23.04±0.67 cd
	P20	21.29±0.63 ab	13.64±0.39 de	16.43±0.47 a	33.61±0.97 a
B2 average		**20.13±0.62 AB**	**15.56±0.62 A**	**10.67±1.45 A**	**26.09±1.93 A**
	P0 (Control)	22.03±0.64 a	14.63±0.42 cd	9.20±0.27 c	25.41±0.73 bc
10 ppm (B3)	P10	20.26±0.59 b	15.43±0.45 bc	10.9±0.31 b	27.76±0.80 b
	P20	20.52±0.57 b	17.19±0.50 a	6.20±0.18 e	19.84±0.57 ef
B3 average		**20.85±0.41 A**	**15.75±0.48 A**	**8.77±0.70 B**	**24.34±1.23 B**
	P0 (kontrol)	19.42± 0.51	14.50± 0.67	7.75±0.51	22.00± 1.18
Proline average	P10	19.16±0.8	14.77±0.60	8.10±0.53	22.38± 1.27
	P20	19.74± 0.45	14.32± 0.54	8.52± 1.39	22.34± 2.01
Coefficient of Variation (%)		3.93	5.25	11.39	7.18
Bor(B)		**	**	**	**
Prolin (P)		ns	ns	ns	ns
B x P		**	**	**	**

B0: Boron Control, B0: 2.5 ppm Boron, B1: 5 ppm Boron, B3: 10 ppm Boron, P0: Proline Control, P10: 10 ppm Proline, P20: 20 ppm Proline, *Significant at P<0.05 level, **Significant at P<0.01 level.

The effects of boron and boron × proline applications on carotenoid content were found to be statistically significant. However, the effect of proline doses on carotenoid content was determined to be statistically insignificant (Table 2). The highest carotenoid content (3.11 µg g-1 fw) was observed in B0 average, while the lowest (1.98 µg g-1 fw) was observed in the B3 average (Fig. 5 and Table 2) with the 10 ppm dose, whereas the lowest value was measured at 1.98 µg g-1 fw in the control treatment (Figure 5). Regarding boron × proline applications, the highest carotenoid content was obtained at 3.34 µg g-1 fw with the B0 × P0 treatment, while the lowest value was recorded at 0.19 µg g-1 fw with the P10× P20 application (Table 2). Similar to total chlorophyll, carotenoids also function within the same mechanism under stress conditions and exhibit similar behaviors (Doğru & Bildiren, 2020). As non-enzymatic components of the antioxidant system, carotenoids play a protective role in plant tissues and organs under stress

conditions by preventing both singlet oxygen and triplet chlorophyll formation (Trebst, 2003). In this study, it is hypothesized that the reduction in carotenoid content serves to maintain chlorophyll content. Despite decreasing carotenoid levels, the rapid consumption of these pigments under oxidative stress, which scavenge reactive oxygen species, may temporarily protect chlorophyll molecules from photo-oxidative damage. It is also thought that boron toxicity may directly suppress carotenoid synthesis or increase its consumption. The extreme value obtained may also have resulted from tissue loss due to excessive toxicity.

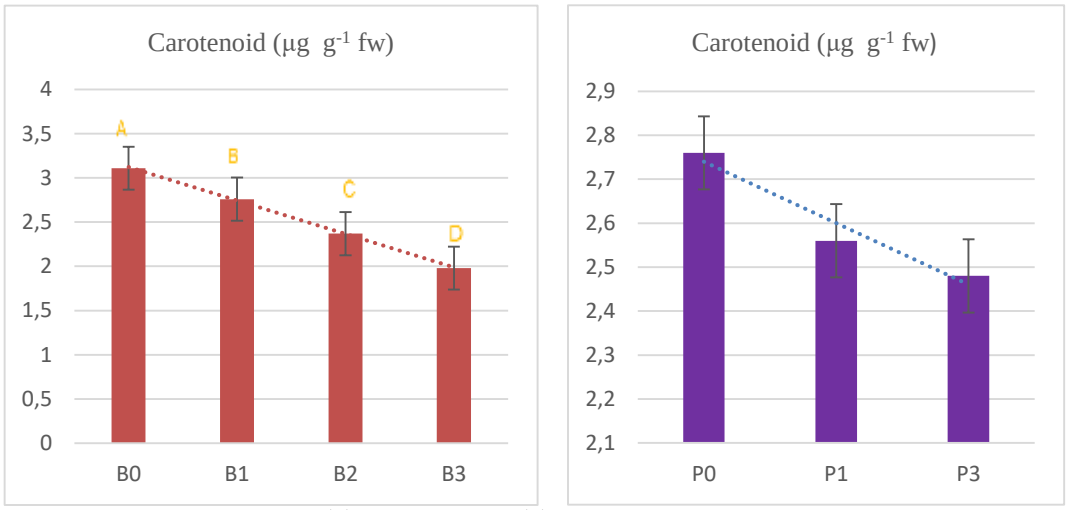

Figure 5. Effects of boron (a), and proline (b) applications on carotenoid
Şekil 5. Bor (a) ve prolin (b) uygulamalarının karotenoid üzerindeki etkileri

The effect of boron and boron x proline applications on total antioxidant activity was found to be statistically significant ($p<0.01$). The effect of proline doses on total antioxidant activity, however, was found to be insignificant. Among the boron dose applications, the highest total antioxidant activity value was 16.74 µmol TE g^{-1}, observed with the B3 dose, while the lowest value was 13.98 µmol TE g^{-1}, observed with the B0 dose (Figure 6). Following the B x P applications, the highest total antioxidant activity value was 17.42 µmol TE g^{-1} with the B3 x P10 combination, while the lowest value was 12.66 µmol TE g^{-1} with the B0 x P20 combination (Table 2). According to our findings, increasing boron doses increased total antioxidant activity compared to the control. Inal (2015) reported that boron applications in beans resulted in total antioxidant activity values ranging from 59.37 to 70.11 mmol kg^{-1} DM. Karadağ (2018) evaluated the antioxidant activity of *Onobrychis argyrea* subsp. *isaurica* (Fabaceae) and found that the activity varied between 1.63 and 2.11 mmol TE g^{-1} extract in different extracts. Proline is reported to be an important stress-immune component supporting antioxidant defense in plants. Proline accumulates in plants under various abiotic stresses and has been shown to help scavenge reactive oxygen species (ROS), thereby supporting antioxidant capacity. Proline did not have a significant independent effect on total antioxidant activity. This suggests that proline may be effective not alone but in combination with other factors (e.g., in combination with boron or under specific stress conditions). The literature reports that proline modulates the antioxidant system, but this effect varies depending on the application dose and type of stress; the positive effect of proline on antioxidant defense is generally evident under stress conditions or when interacting with other metabolic processes (Hayat et al., 2012).

As shown in Table 2, the effects of boron, proline, and boron × proline applications on phenolic content were found to be statistically significant ($p<0.01$). Among the boron dose treatments, the highest phenolic content was recorded at 127.40 and 126.93 mg GAE g^{-1} with the B2 and B3 doses, respectively, whereas the lowest value was measured at 120.39 mg GAE g^{-1} in the B0 treatment (Figure 7). Regarding proline dose applications, the highest phenolic content was obtained at 131.49 mg GAE g^{-1} with the P0 dose, while the lowest value was recorded at 118.09 mg GAE g^{-1} with the P20 dose. Boron is an essential micronutrient that influences multiple metabolic pathways in plants, including cell wall integrity and secondary metabolism, and changes in boron status have been shown to alter phenolic compound concentrations, with boron deficiency often leading to increased phenolics as a stress response and shifts in metabolic partitioning (In contrast, proline is a stress-responsive amino acid that accumulates in plants under abiotic stress and contributes to osmotic adjustment, redox balance, and antioxidant defense, and exogenous proline application has been demonstrated to elevate total phenolic acids and flavonoids in stressed plants such as celery under salt stress, indicating that proline can influence phenolic metabolism indirectly through enhancement of antioxidant systems (Camacho-Cristóbal et al., 2004; Gao et al., 2023; Zhang

et al., 2023).

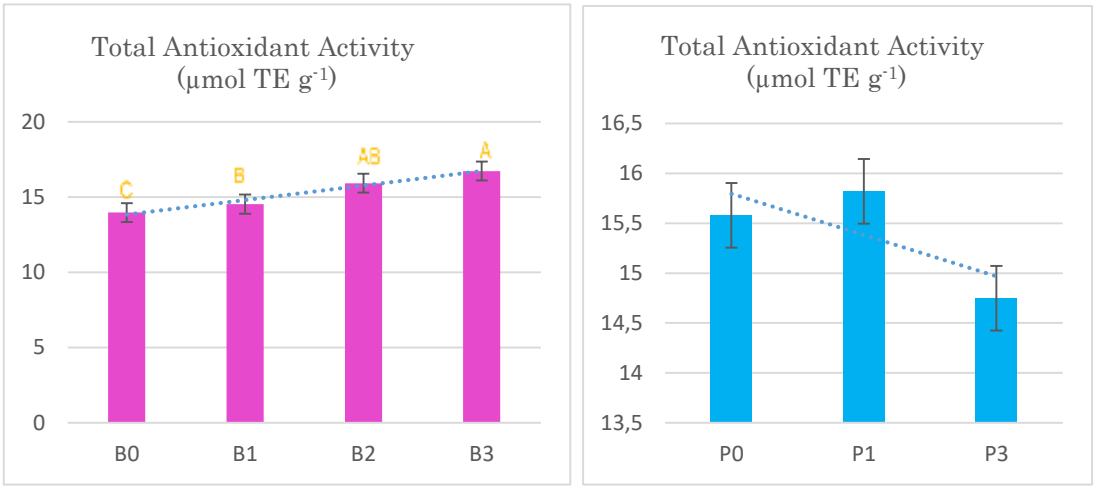

Figure 6. Effects of boron applications (a), and proline (b) applications on total antioxidant activity
Şekil 6. Bor (a) ve prolin (b) uygulamalarının toplam antioksidan aktivite üzerindeki etkileri

Among the boron × proline applications, the highest phenolic content was recorded at 140.14 mg GAE/g with the B3 × P10 treatment, while the lowest value was observed at 107.29 mg GAE g^{-1} with the B1 × P20 treatment. In plants, phenolic acids (or phenolcarboxylic acids) are small-molecule compounds, often volatile, that include flavonoids (Schoeter et al., 2000). In this study, increasing boron doses corresponded to an increase in phenolic content.

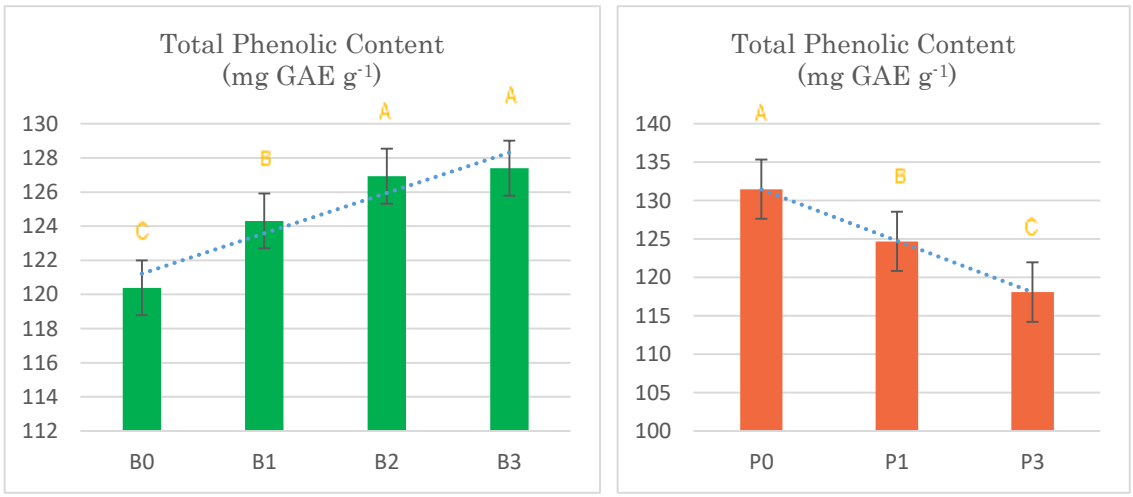

Figure 7. Effects of boron (a), and proline (b) applications on total phenolic
Şekil 7. Bor (a) ve prolin (b) uygulamalarının toplam fenolik üzerindeki etkileri

The effects of boron, proline, and boron × proline applications on flavonoid content in soybean were found to be statistically significant ($p<0.01$). The highest flavonoid content was recorded at 93.32 mg QE/100 g with the B3 application, whereas the lowest value was measured at 52.94 mg QE $100g^{-1}$ in the control treatment. The effect of proline dose applications on flavonoid content initially increased and then declined. Accordingly, the lowest value was observed with the P10 dose (62.15 mg QE $100g^{-1}$), while the highest value (79.73 mg QE $100g^{-1}$) was recorded in the control treatment (Figure 8). Regarding boron × proline applications, the highest flavonoid content was obtained at 112.72 mg QE $100g^{-1}$ with the B2 × P0 treatment, whereas the lowest value was recorded at 45.14 mg QE $100g^{-1}$ with the B0 × P0 treatment (Table 2). It is well established that under biotic and abiotic stress conditions, antioxidants such as flavonoids become activated and contribute to plant protection (Shah & Smith, 2020). It is known that proline can function not only as a protective osmolyte but also as a metabolic and signaling regulatory molecule under certain stress conditions. In this context, the reduction of the intensity of stress signals by improving cellular redox balance through exogenous proline application and consequently suppressing the

phenylpropanoid pathway can be considered a possible mechanism that may explain the observed decrease in flavonoid biosynthesis (Zhang, & Becker, 2015; Alvarez et al., 2022; Zhang et al., 2022).

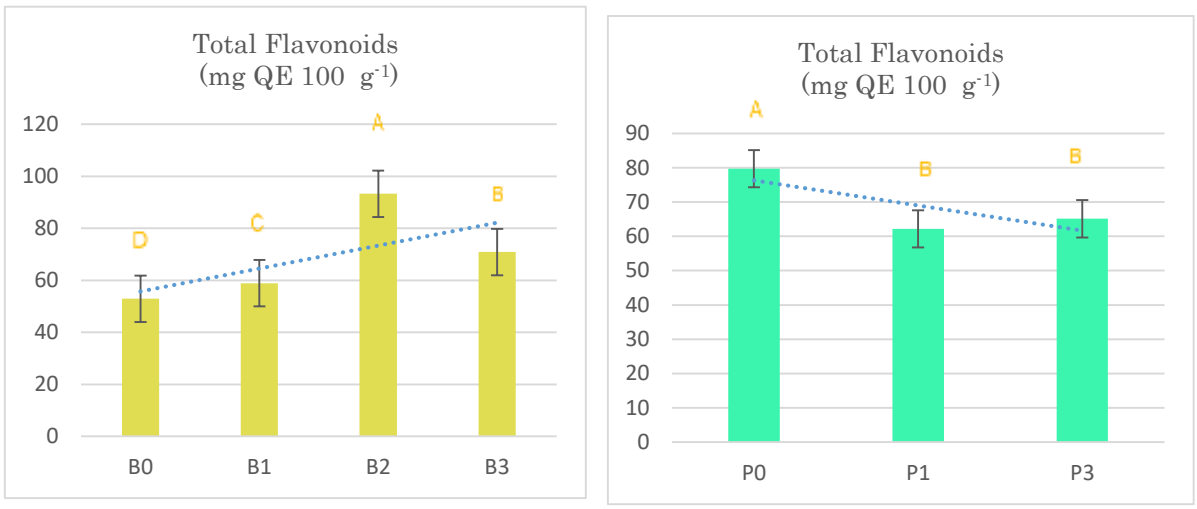

Figure 8. Effects of boron applications (a), and proline (b) applications on total flavonoids
Şekil 8. Bor (a) ve prolin (b) uygulamalarının toplam flavonoid üzerindeki etkileri

Table 2. Effects of boron and proline applications on some biochemical properties in soybeans
Çizelge 2. Bor ve prolin uygulamalarının soya fasulyesindeki bazı biyokimyasal özellikler üzerindeki etkileri

Bor Doses	Proline Doses	Carotenoid (µg g^{-1} fw)	Total Antioxidant Activity (µmol TE g^{-1})	Total Phenolic (mg GAE g^{-1})	Total Flavonoid (mg QE 100 g^{-1})
	P0 (Control)	3.34±0.10 a	15.85±0.46 c	120.52±3.48 d	45.14±1.30 g
Kontrol (B0)	P10	2.90±0.08 bc	13.43±0.39 de	122.29±3.55 cd	64.91±1.87 cd
	P20	3.10±0.11 ab	12.66±0.33 e	118.36±3.42 d	48.77±1.41 f
B0 average		**3.11±0.08 A**	**13.98±0.52 C**	**120.39±1.83 C**	**52.94±3.14 D**
	P0 (Control)	2.37±0.07 e	15.03±0.45cd	151.21±4.37 b	64.45±1.86 cd
2.5 ppm (B1)	P10	2.86±0.09 c	15.08±0.40cd	114.43±3.30 de	59.11±1.71 de
	P20	3.05±0.10 b	13.52±0.39de	107.29±3.10 e	53.11±1.53 f
B1 average		**2.76±0.11 B**	**14.54±0,33 B**	**124.31±7.04 B**	**58.89±1.95 C**
	P0 (Control)	2.32±0.05 e	15.69±0.45 c	126.57±4.65 cd	112.72±3.25 a
5 ppm (B2)	P10	1.75±0.08 f	17.35±0.50 ab	121.93±3.52 cd	70.14±2.02 c
	P20	3.03±0.09 b	14.77±0.43 d	132.28±3.82 c	97.10±2.80 b
B2 average		**2.37±1.83 C**	**15.93±0.44 AB**	**126.93±3.27 A**	**93.32±6.37 A**
	P0 (Control)	3.02±0.07 b	15.77±0.46 c	127.64±3.68 cd	96.64±2.79 b
10 ppm (B3)	P10	2.73±0.12 d	17.42±0.58 a	140.14±4.05 a	54.45±1.57 ef
	P20	0.19±0.11 g	17.05±070 b	114.43±3.30 de	61.52±1.78 d
B3 average		**1.98±1.28 D**	**16.74±0.35 A**	**127.40±4.14 A**	**70.87±6.61 B**
	P0 (Control)	2.76± 0.13	15.58± 0.22	131.49±3.89 A	79.73±8.05 A
Proline average	P10	2.56± 0.15	15.82± 0.54	124.69±3.24 B	62.15±1.95 B
	P20	2.48±0.38	14.75± 0.53	118.09±3.11 C	65.12±5.0 B
Coefficient of Variation (%)		3.07	5.38	4.92	4.98
Bor(B)		**	**	**	**
Prolin (P)		ns	ns	**	**
B x P		**	**	**	**

B0: Boron Control, B0: 2.5 ppm Boron, B1: 5 ppm Boron, B3: 10 ppm Boron, P0: Proline Control, P10: 10 ppm Proline, P20: 20 ppm Proline, *Significant at P<0.05 level, **Significant at P<0.01 level.

CONCLUSION

This study revealed that foliar application of boron exhibits a dose-dependent toxic effect on soybeans, and the plant responds to this toxicity by activating its antioxidant defense system (phenolics, flavonoids, antioxidant activity). The most important finding of the study is that exogenous proline application has a dose- and boron concentration-dependent regulatory role on these defense mechanisms. Proline can be activated through different

mechanisms, particularly by increasing chlorophyll b and total chlorophyll content (B2xP20) under moderate boron stress, and by promoting phenolic compound accumulation (B3xP10) under high boron stress. The molecular mechanisms (gene expression, enzyme activities) underlying the dose-dependent dual effect of proline observed in this study (protective at low doses, suppressive at high doses) need to be investigated. Testing the findings under field conditions and across different soybean varieties will yield more reliable data for practical agricultural applications. Long-term studies should be conducted to examine the effects of combined proline and boron application on yield and yield components. The effectiveness of different proline application methods (seed priming, soil application) should be compared with that of foliar application.

Contribution Rate Statement Summary of Researchers

Conceptualization: EO, RT, MT; Data curation: EO, RT, MT; Formal analysis: EO, RT, MT, ES, LN; Investigation: EO, RT, MT, ES, LN; Methodology: EO, RT, MT; Resources: RT, MT, ES, LN; Software: RT, MT, LN, ES; Supervision: RT, EO, MT; Validation: RT, EO, MT; Visualization: ES, LN; Writing - original draft: EO, RT, MT; Writing - review and editing: EO, RT, MT. All authors read and approved the final manuscript.

Conflict of Interest

The authors of the article declare that they have no conflict of interest.

REFERENCES

Alvarez, M. E., Savouré, A., & Szabados, L. (2022). Proline metabolism as regulatory hub. *Trends in Plant Science*, *27*(1), 39-55.

Ashraf, M. F. M. R., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, *59*(2), 206-216.

Benzie I.E.F., & Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry, 239*(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, V., & Romheld, V. (2002). Boron in plant biology. *Plant biology, 4*(02), 205-223. Doi: 10.1055/s-2002-25740

Camacho-Cristóbal, J.J., Lunar, L., Lafont, F., Baumert, A., González-Fontes, A. (2004). Boron deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine conjugates in tobacco leaves. *J Plant Physiol*, *161*(7), 879-81. doi: 10.1016/j.jplph.2003.12.003.

Demiralay, M., Altuntaş, C., Sezgin, A., Terzi, R., & Kadioğlu, A. (2017). Application of proline to root medium is more effective for amelioration of photosynthetic damages as compared to foliar spraying or seed soaking in maize seedlings under short-term drought. *Turkish Journal of Biology, 41*(4), 649-660.

Demirtaş, A. (2005). Bitkide bor ve etkileri. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 36* (2), 217-225.

Doğru, A., & Bildiren, Ş. (2020). Physiological and biochemical changes in wheat cultivars under salt stress as affected by foliar boron application. *Journal of Boron, 5*(2), 100 – 107. https://doi.org/10.30728/boron.654920

Ercan, O. (2008). *Effect of drought and salt stress on antioksidant defense system and physiology of lentil (Lens culinaris M.) seedlings (Publication No. 177563).* [MSc Thesis, Middle East Technical University Institute of Science Department of Biology]. Databases of National Thesis Center of the Council of Higher Education.].

Fehr, W.R., & Caviness. C.E. (1977). Stages of soybean development. Iowa Agric. Home Econ. Exp. Stn., Iowa Coop. Ext. Serv. Spec. Rep. 80.

Fernández, V., & Brown, P. H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science*, *4*, 289.

Gao Y, Zhang J, Wang C, Han K, Hu L, Niu T, Yang Y, Chang Y, Xie J. (2023). Exogenous Proline Enhances Systemic Defense against Salt Stress in Celery by Regulating Photosystem, Phenolic Compounds, and Antioxidant System. *Plants (Basel)*, *12*(4), 928. doi: 10.3390/plants12040928.

Güneri Bagci, E. (2010). *Nohut (Cicer arietinum L.) çeşitlerinde kuraklığa bağlı oksidatif stresin fizyolojik ve biyokimyasal parametrelerle belirlenmesi. (Publication No. 297575).* [Yüksek Lisans Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü Toprak anabilim Dalı]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signal Behav*, *7*(11), 1456-66. doi: 10.4161/psb.21949.

Hoagland, D.R., Arnold, D.I., 1950. The water culturemethod of growing plants without soil. Circular.California *Agricultural Experiment Station*, 347, 1-6.

İnal, A. (2015). Yapraktan bor ve indol asetik asit (İAA) uygulamalarının fasulye (*Phaseolus vulgaris* L.) bitkisinin gelişimi ve kimi fizyolojik özelliklerine etkisi. Ankara Üniversitesi Bilimsel Araştirma Projesi Sonuç Raporu, Proje Numarası 14L0447001. https://dspace.ankara.edu.tr/server/api/core/bitstreams/4875ce01-0c97-4afb-b250-b865a1e9f536/content

Jain M., Mathur G., Koul S.,& Sarin N. B. (2001). Ameliorative effects of proline on salt stressed lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L.). *Plant Cell Reports, 20*, 463-468. Doi: 10.1007/s002990100353

Karadağ, F. (2018). *Evolution of Antioxidant Activity of Onobrychis argyrea subsp. isaurica (Fabaceae) (Publication No. 493822).* [Master's thesis, Necmettin Erbakan University Institute of Science Department of Molecular Biology and Genetics]. Databases of National Thesis Center of the Council of Higher Education.

Kayıhan, C., Öz, M.T., Eyidoğan, F., Yücel, M., & Öktem, H.A. (2017). Physiological, biochemical, and transcriptomic responses to boron toxicity in leaf and root tissues of contrasting wheat cultivars. *Plant Molecular Biology Reporter, 35*(1), 97– 109.

Kolsarıcı, Ö., Gür, A., Başalma, D., Kaya, D., & İşler, N., (2005). Yağlı tohumlu bitkiler üretimi. TMMOB Ziraat Müh. Odası Türkiye Ziraat Müh. VI. Teknik Kongresi (3-7 Ocak 2005), Ankara. 409-429.

Keren, R., & Bingham, F.T., (1985). *Boron in water. Sois and Plants.* In Adv. In Soil Sci. (Fd. By B.A. Stewart) Vol.l, 229-276.

Küçükkılıç, B., Canavar, Ö., & Gören, H.K. (2026). Salt Stress Effects Physiology Properties, Malondialdehyde and Proline Accumulation in Relation To Osmotic Adjustment Of Soybean [Glycine Max (L) Merrill]. Kahramanmaraş *Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *29*(1), 79-97. https://doi.org/10.18016/ksutarimdoga.vi.1699900

Kutlu, İ. (2010). Drought Stress in Cereals. *Turkish Journal of Scientific Reviews, (1)*, 35-41.

Lichtenthaler, H.K., & Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions, 11*, 591– 592. https://doi.org/10.1042/ bst0110591

Lukaszewski K.M., & Blevins, D.G., (1996). Root Growth inhibition in boron defici en tor aluminium stressed scuash may be aresult of Impaire D'ascorbate metabolism. *Plant physiology*, *112*(3), 1135-1140. https://doi.org/10.1104/pp.112.3.1135

Mert, M., & İlker, E., (2016). Studies on Adaptation Abilities of Some Soybean (Glycine max (L.) Merill) Varieties and Lines Under Main Crop Conditions of Aksaray Region. *Biotech Studies, 25* (2), 176-181. https://doi.org/10.21566/tarbitderg.281322

Nable, R. O., Bañuelos, G. S., & Paull, J. G. (1997). Boron toxicity. *Plant and soil*, 193(1), 181-198.

Obanda M., & Owuor P.O. (1997). Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *Journal of the Science of Food and Agriculture*, *74*, 209-215. https://doi.org/10.1002/(SICI)1097-0010(199706)74:2<209::AID-JSFA789>3.0.CO;2-4

Phung, T. H., Jung, H. I., Park, J. H., Kim, J. G., Back, K., & Jung, S. (2011). Porphyrin biosynthesis control under water stress: sustained porphyrin status correlates with drought tolerance in transgenic rice. *Plant physiology*, *157*(4), 1746-1764.

Quettier-Deleu C., Gressier B., Vasseur J., Dine T., Brunet J., Luyck M., Cazin M., Cazin J.C., Bailleul F., & Trotin F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal Ethnopharmacology*, *72*, 35-40. https://doi.org/10.1016/S0378-8741(00)00196-3

Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. R., & Graham, R. D. (2004). A critical analysis of the causes of boron toxicity in plants. *Plant, Cell & Environment*, *27*(11), 1405-1414.

Riaz, M., Kamran, M., El-Esawi, M.A., Hussain, S., & Wang, X. (2021). Boron-toxicity induced changes in cell Wall components, boron forms, andantioxidant defense system in rice seedlings. *Ecotoxicology and Environmental Safety*, *216*, 112-192. https://doi.org/10.1016/j.ecoenv.2021.112192

Sakal, R., & Singb, A.P. (1995). *Boron re search and agricultural production. In micronutrient res. Agric. Prod.* (Ed. Tondon, Hıs) p: 1-31. Fert Dev. And Cons. argo New Delhi. India

Sarraf, M., Bansal, R., Shackira, A. M., Yadav, V., Zarbakhsh, S., Roychowdhury, R., ... & Hasanuzzaman, M. (2026). Magnesium-mediated stress adaptation in plants: from physio-biochemical insights to climate-resilient agriculture. *Frontiers in Plant Science*, *17*, 1715501.

Schoeter, H., Williams, R.J., Martin, R., Iversen, L., & Rice-Evans, C.A. (2000). Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. *Free Radical Biology and Medicine, 29*, 1222-1233. https://doi.org/10.1016/S0891-5849(00)00415-9

Sepanlo, N., Talebi, R., Rokhzadi, A., & Mohammadi, H. (2014). Morphological and physiological behavior in soybean (*Glycine max* L.) genotypes to drought stress implemented at pre- and post-anthesis stages. *Acta Biologica Szegediensis, 58*(2), 109-113.

Shah, A., & Smith, D.L. (2020). Flavonoids in agriculture: chemistry and roles in, biotic and abiotic stress responses and microbial associations. *Agronomy, 10*(8), 1-26. https://doi.org/10.3390/agronomy10081209

Stewart, W., N., & Rothwell, G. W. (1983). *Paleobotany and the evolution of plants.* Cambridge University Press. Vermicomp., part B: verms and Vermicomposting, pp1-7.

Szabados, L., & Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends in Plant Science*, *15*(2), 89-97.

Trebst A. (2003). Function of ß-carotene and tocopherol in photosystem II. *Zeitschrift für Naturforschung, 58*, 609-

620.

Wimmer, M. A., & Eichert, T. (2013). Mechanisms for boron deficiency-mediated changes in plant water relations. *Plant science*, *203*, 25-32.

Vurarak, Y. (2024). Determining the possible effects of different storage conditions on biological material after harvesting: soy example. *Journal of Tekirdag Agricultural Faculty*, *21*(1), 219-232.

Yolci, M. S., Tunçtürk, R., & Tunçtürk, M. (2022a). Tıbbi Adaçayı (*Salvia officinalis* L.)'ında Bor Toksisitesi ve Rizobakteri (PGPR) Uygulamalarının Fide Gelişimi ve Fizyolojik Özellikleri Üzerine Etkileri. *Journal of the Institute of Science and Technology*, *12*(2), 1102-1113. https://doi.org/10.21597/jist.1039238

Yolci, M.S. Tunçtürk, M., & Tunçtürk, R.(2022b). The Effects of Organic Fertilizer and Boron Applications on Seedling Growth and Biochemical Parameters of Chamomile (*Matricaria recutita L.*). *SPEC Journal of Agr. Sciences 6*(1), 20-31. https://doi.org/10.46291/ISPECJASvol6iss1pp20-31

Zhang, L., & Becker, D. F. (2015). Connecting proline metabolism and signaling pathways in plant senescence. *Frontiers in Plant Science*, *6*, 552.

Zhang, Q., Feng, Y. X., Tian, P., Lin, Y. J., & Yu, X. Z. (2022). Proline-mediated regulation on jasmonate signals repressed anthocyanin accumulation through the MYB-bHLH-WDR complex in rice under chromium exposure. *Frontiers in plant science*, *13*, 953398.

Zhang, Y., Liu, X., Wang, J., & Chen, L. (2023). Proline metabolism and its role in plant antioxidant defense under abiotic stress. *International Journal of Molecular Sciences*, *24*(4), 3562. https://doi.org/10.3390/ijms24043562

Zulfiqar, F., & Ashraf, M. (2023). Proline alleviates abiotic stress induced oxidative stress in plants. *Journal of Plant Growth Regulation*, *42*(8), 4629-4651.