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

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ABSTRACT

Background: In this study, boron which is an important element in many fields such as defense industry, energy, food and cleaning in the world, was studied. It is also an important plant nutritional element. However, like other important elements, it has some effects on plants and the environment. Türkiye has 76% of the world's boron reserves. In this study, proline applications were tried to determine the toxic effect of boron in soybeans, which is a strategic product and to reduce the damage.

Methods: This study was carried out to determine the effects of boron (0, 2.5, 5 and 10 ppm) and proline (0, 10 and 20 ppm) applications on plant growth parameters and biochemical properties in soybean (*Glycine max* L.). The study was carried out in the fully controlled climate room of our faculty's field crops. This trial was carried out according to the randomized plots experimental design in a factorial arrangement with 4 replications.

Result: In the research, after the applications, soybean plant height (22.7-33.3 cm), root length (26.2-38.7 cm), stem fresh weight (1.77-3.20 g), stem dry weight (0.59-0.71 g), root fresh weight (1.43-2.72 g), root dry weight (0.15-0.24 g), leaf area index (10.4-11.1 cm^2), ion leakage in leaf tissues (20.4- 24.9%), nitrogen balance index (47.3-92.2 dx), anthocyanin (0.04-0.08 dx), flavonoid (34.9-55.6 dx) and chlorophyll (22.5-42.4 mg cm^{-2}) were examined. In the results of working; Boron applications first increased and then decreased the values of plant height, root length, stem fresh and dry weight and root fresh and dry weight in soybean plants. There was a direct decrease in leaf area index, NBI and total chlorophyll values. Other parameters followed a fluctuating course. It has been determined that boron applications increase flavonoid and anthocyanin values. In this study, it was determined that proline applications had a positive and regulatory effect on root length, stem fresh weight, root fresh weight, root dry weight, nitrogen balance index and flavonoid values against boron toxicity.

Key words: Anthocyanıne, Boron, Proline, Soybean, Toxic.

INTRODUCTION

The genetic center of soybean (*Glycine max* L.), which has an important place in human and animal nutrition, is known as China and the Korean peninsula. The plant has been one of the main nutrients in the Asian continent from 5000 years ago to the present day (Liu, 2004). Although it came to Europe from the Asian continent in the early 17th century, it could not reach the desired yield potential due to some climatic reasons (Mert *et al.,* 2016). Soybean production in the world is about 356 million tons on an area of 131 million hectares.The country with the most cultivation area in terms of cultivation area is Brazil with 41.5 million hectares. (USDA, 2022). Soybeans have an important use in human and animal nutrition (Altuner, 2021). Soybean [*Glycine max* (L.) Merril] is an important oilseed and pulse crop rich in essential amino acids and is enriched with high-quality proteins. Besides improving soil health by nitrogen fixation from the atmosphere, this leguminous crop helps increasing productivity of succeeding crop (Sikka *et al.,* 2021). The seeds contain 36-40% protein, 18-24% fat (Omega-3 and similar fatty acids), vitamins B1, B2, K and E, Zn, Fe and Ca elements, 27% carbohydrates and 18% mineral substances. It allows its use in a wide range of applications. These products can be listed as children's foods, pastries, confectionery products, antiallergic milk and dairy products. Today, it is one of the important annual members of the legume family. The common feature of this family is that it can fix the free nitrogen of the air thanks to the rhizobium bacteria in the roots. Thanks to this feature, it can not only produce the nutrients the plant needs, but also create a suitable soil structure for the plants that will come after it. In soybean farming, the plant nutrients that the plants need must be met. In addition to the elements that are found in small or large amounts in all plants and that enable plants to survive, some other elements are also needed in minimal amounts. These are trace elements such as boron, iron, copper, manganese, zinc, molybdenum, cobalt, vanadium, Wolfram. These elements have very high coefficients and it is possible to provide optimum effect even in very small amounts (Güner, 2010). Among these elements, especially boron is known to be an important element that has been the subject of various

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studies on its presence and absence. Much work has been done on the function of boron in plants and it has important effects on growth and development. If we were to present a summary of all these studies, boron in plants; It has important and distinct functions in the transport of sugars, cell wall synthesis, lignification phenomenon, formation of cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, Indole acetic acid (IAA) metabolism, phenol metabolism in plants and the structural and functional properties of biological membranes (Lukaszewski and Blevins, 1996). Determined that nitrogen applied to soils with high boron content reduced boron uptake in citrus fruits and the toxic effect was eliminated. It is known that there is a remarkable balance between Potassium and Boron in plants. It is stated that light intensity has an effect on boron uptake and boron uptake in plants increases as a result of the prolongation of photosynthesis period and increase in transpiration rate depending on light intensity. Apart from these functions, boron is known to have a toxic effect on plants. However, there appears to be a wide variation among toxicity levels (Demirtaş, 2005). For this reason, it is one of the microelements with the most deficiency and toxic effects (Keren and Bingham, 1985). The limit between the amounts of boron required for plants and its toxic levels is quite narrow. Although this varies depending on plant species and varieties, even very low concentrations can have a toxic effect. In addition to this factor, arid and semi-arid climate types have been shown to increase the toxic effect. Toxicity increases are also observed in saline soils due to boron accumulation and excess. Very sensitive plants can tolerate toxic effects up to 0.7 ppm boron content. Toxic symptoms caused by boron in plants; It manifests itself as yellowing and subsequent drying, starting at the tips or other areas of the leaves and dead areas between the veins (Demirtaş, 2005). In nature, boron is found in low concentrations in all waters. Since they dissolve very quickly in water, they cause great harm to the environment. Especially with rainfall, it mixes with soil and water and turns into a compound structure with Pb, Cu, Co, Ni, Cd and other heavy metals, increasing its toxic effects (Boncukçuoğlu *et al*., 2003). In addition to its negative effects on the environment, the toxic effect causes yield and quality losses in plant production. There are toxic damages caused by Boron in the arid and semi-arid areas of the world, especially in Central and Eastern Anatolia in our country. Depending on the decrease in water balance in the soil, plants can take some measures to combat stress. Plants reduce CO_2 emissions by closing stomata and slowing down photosynthesis (Kutlu, 2010). It has also been reported that it increases the proline and SOD (superoxide dismutase) content, which reduces the effect of drought and differentiates plants as sensitive and resistant to stress (Sepanlo *et al.,* 2014). Similar studies have reported that the activity of proline applied to plants serves as a biochemical protection mechanism against the negative effects of drought (Ercan, 2008). Plants increase intracellular osmotic pressure positively by producing proline and some carbohydrate-based osmoregulators against physiological drought. It is reported in the literature that proline protects the plant against abiotic stress (drought) (Keren and Bringham, 1985).

The aim of this study was to investigate the effects of foliar applied boron and proline on the physiological and biochemical properties of soybean (*Glycine max* L.).

MATERIALS AND METHODS

This study was carried out in a fully controlled growing cabin belonging to the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University in (2024). Yeþilsoy variety obtained from Çukurova Agricultural Research Institute was used. The study was planted in 500cc pots in peat + perlite + soil mixture (1: 1: 2) and 65% humidity; 8/16 hour dark/light period; It was grown in a temperature environment of 25°C. The seeds were planted in the vials on 15.01.2024. After the real leaves emerged (3-4 leaf period), they were transferred to pots on 01.02.2024. Standard fertilization was applied to the plants in the pots on 02.02.2022. Proline application to plants started when plants had 8-10 leaves (12th day). Boron applications were started to plants in pots from foliar application on 16th day. Proline was stopped on 19th day. Boron applications continued until 23rd day. In this study, a total of 7 boron and proline applications were made every day. Proline was continued to be given together with 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).

Parameters examined

Physiological parameters

Root and stem length, which are morphological development parameters of the plant, were measured in cm with the help of digital calipers (06.03.2024). On the same day (06.03.2024), the fresh and dry weights of the root and stem of the plant were determined with the help of a precision scale (0.0001 g). The plants and roots, whose fresh weights were determined, were then dried at 40°C for 72 hours and their dry weights were determined (09.03.2024). Chlorophyll, Flavonoid, Anthocyanin content and nitrogen balance index (NBI) were determined according to Cerovic *et al*. (2015) measured using the Dualex scientific + (FORCE-A, France) device (06.03.2024). Ion leakage in leaf tissues Sairam, (1994) methods (06.03.2024).

Leaf area index

Leaf area index was measured using the Easy Leaf Area Program (06.03.2024).

Leaf tissues ion leakage

Arora *et al*. (2002) the amount of ion leakage in leaf tissues was determined.

Total flavonoid

Total flavonoid substance determination was performed by Quettier-Deleu *et al*. (2000). It was determined based on the method developed by. 2 ml of 2% AlCl 3 was added to 2 ml of extract and kept at room temperature and in the dark for 60 minutes. The prepared samples were measured with a spectrophotometer at a wavelength of 415 nm and calculated in mg QE/100 g using the calibration curve prepared using standard quercetin (QE).

Anthocyanin, NBI, chlorophyll

Anthocyanin, chlorophyll and nitrogen balance index (ABI) in leaves were measured non-destructively and in real time on the leaf with the Dualex scientific+ (FORCE-A, France) device (Şelem *et al*., 2022).

Data analysis

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 (Duzguneþ *et al*. 1987).

RESULTS AND DISCUSSION

Plant length

According to the data obtained at the end of the research, the effect of Boron and B×P applications on plant height in soybeans was found to be statistically significant, while proline was found to be insignificant ($P<0.05$, $P<0.01$). As a result of the boron doses applied in the study, the highest plant height was obtained from the B2 dose application with 32.0 cm, while the lowest plant height was measured with the B3 dose application with 25.0 cm (Table 1). In a study conducted on soybeans, 47.06% increase in plant height was detected in plants applied with 0.1 and 0.05 mM boron doses compared to the control group (Oluk and Latif, 2009). According to the results obtained in the experiment, boron started to decrease at a dose of 10 ppm. The limit between the amounts of boron required for plants and its toxic levels is in a very narrow range. Although this varies depending on plant species and varieties, even very low concentrations can cause toxic effects (Demirtaş, 2005). The highest plant height value obtained as a result of Boron×Proline applications was 34.7 cm in B2×P10

Table 1: Effects of boron and proline applications on some physiological characteristics in soybean.

Boron doses	Parameters				
	Proline doses	PL (cm)	RL (cm)	SFW (g)	SDW (g)
Control (B0)	P0 (Control)	30.0 ab	27.2	2.22 de	0.63 c
	P10	30.1 ab	33.4	2.64 cd	0.68 ab
	P20	25.9 b	34.7	3.12 ab	0.65 bc
B0 Mean		28.7 B	31.8 B	2.66 B	0.65 A
2.5 ppm (B1)	P0 (Control)	26.9 b	28.7	2.85 bc	0.64 c
	P10	29.7 b	34.6	2.78 c	0.67 b
	P20	33.3 a	38.7	3.20 a	0.69 ab
B1 Mean		29.9 B	34.1 A	2.94 A	0.67 A
5 ppm (B2)	P0 (Control)	32.3 ab	30.3	2.89 bc	0.68 ab
	P10	34.7 a	33.2	2.71 c	0.71 a
	P20	29.0 b	36.7	3.13 ab	0.67 b
B2 Mean		32.0 A	33.4 AB	2.91 A	0.69 A
10 ppm (B3)	P0 (Control)	26.3 b	26.2	2.12 e	0.62 cd
	P10	26.0 b	31.3	2.25 e	0.59 e
	P20	22.7 c	34.2	1.77 f	0.60 d
B3 Mean		25.0 C	30.6 B	2.04 C	0.60 B
P Doses Mean	P0 (Control)	28.9	28.1 C	2.54 C	0.64
	P10	30.1	33.1 B	2.62 B	0.66
	P20	27.7	35.7 A	2.83 A	0.65
CV (%)		9.95	6.55	6.47	4.72
Boron (B)		**	*	**	**
Proline (P)		NS	**	**	NS
B×P		*	NS	**	**

B-Boron; P- Proline; CV- Coefficient of variation; PL- Plant height; RL- Root length; SFW- Steam fresh weight; SDW- Steam dry weight; *Significant at $p<0.05$, **Significant at $p<0.01$, NS- Non-significant.

application and the lowest value was 22.7 cm in B3×P20 doses. Another study stated that harmful effects of boron may occur after a certain concentration in plants, water and soil (Yolcu *et al*., 2022). Considering the findings we obtained, a situation that supports this study has occurred.

Root lenght

Boron and Proline dose applications on root length were found to be significant and Boron × Proline was found to be statistically insignificant (P<0.05, P<0.01). The highest root length obtained as a result of boron doses was 34.1 cm after the 2.5 ppm dose and the lowest value was 30.6 cm after the control dose application. In a study similar to our findings, it was observed that the roots of the plant were 9% longer in boron deficiency than in the control and 4% shorter in its excess (Oluk *et al.,* 2004). The highest root length value obtained after proline application in soybeans was 35.7 cm after the 10 ppm dose and the lowest value was 28.1 cm after the control dose application (Table 1). It has been observed that proline applications increase root length values compared to the control dose. It has been reported that proline plays an important role in protein stabilization and activation of enzymes in plants. In a study conducted on maize, it was observed that high levels of proline accumulated in the roots under stress conditions and that it had positive effects on root elongation and weight (Mahboobi *et al.,* 2000).

Steam fresh weight

The effect of boron, proline and Boron × Proline applications on the fresh stem weight of the plant was found to be statistically significant (P<0.01). As a result of 4 different boron applications in the study, the highest stem fresh weight was measured in the B1 application with 2.94 g and the lowest value was measured in the B3 (2.04 g) dose. In this study, after an increase in trunk fresh weight at 2.5 and 5 ppm boron doses compared to the control, there was a decrease in the applied 10 ppm boron dose (Table 1). It has been understood that a dose of 10 ppm of boron has a toxic effect on root and stem length. In a similar study, it can be listed as a decrease in the stem and shoots of plants grown in soils with high boron content, a decrease in the conductivity of shoots and stomata and photosynthetic activity, a decrease in chlorophyll content, damage to the lipids and permeability of the cell membrane and a change in the plant defense system (Macho Rivero *et al.,* 2017). The highest stem fresh weight obtained as a result of proline applications in the plant was 2.83 g (P20) and the lowest value was 2.54 g (control) (Table 1). According to these results, it has been observed that proline application has a healing feature against the toxic effect of boron by reducing the dramatic losses in trunk fresh weight. Studies conducted on many plants such as rapeseed and wheat have shown that under conditions of increased toxic stress, the content of proline, which is synthesized from the plant and has a protective effect, increases (Knörzer *et al.,* 1999). As a result of B×P application in soybeans, the highest stem fresh weight was measured as 3.20 g (B1×P20) and the lowest value was 1.77 g (B3×P20). In a study similar to our findings, as a result of the application of four different boron doses (0.5, 7.5, 15, 22.5 mg B L^{-1}) in cotton varieties, a decrease of 29, 48 and 62%, respectively, occurred in the fresh stem weight compared to the control group (Harite, 2008).

Steam dry weight

The effect of Boron and Boron×Proline applications on the trunk dry weight was found to be significant, while Proline was found to be statistically insignificant. As a result of boron dose applications, the highest stem dry weight value was measured in B2 application with 0.69 g. This value was in the same group with the results obtained as a result of control and B1 dose applications with 0.65 and 0.67 g. The lowest value was obtained from B3 dose application with 0.60 g (Table 1). As a result of B3 dose application, it was observed that the toxic effect reduced the stem dry weight values in the plant. In a study conducted on wheat, an average of 26, 51 and 67% decrease in dry stem weights of varieties was observed with B1, B2 and B3 applications, respectively, compared to the control (Taban and Erdal, 2000). The highest stem dry weight value obtained as a result of Boron×Proline applications in plants was 0.71 g (B2×P10) and the lowest value was 0.59 g (B3×P10). In another study conducted with corn varieties under greenhouse conditions regarding their sensitivity to boron toxicity; As a result of the application of boron boric acid (H_3BO_3) to the soil at levels of 0, 10 and 30 mg kg^{-1}, a decrease in stem and root weights occurred (Güneş *et al.,* 2000). In another similar study conducted on barley, it was determined that excessive boron doses caused a decrease in dry weight (Ayvaz, 2012).

Root fresh weight

The effect of B, P and B×P applications on the fresh root weight of the plant was found to be statistically significant (P<0.01). The highest root fresh weight obtained as a result of boron dose applications was 2.47 g at 5 ppm dose and the lowest value was 1.58 g at 10 ppm dose (Table 2). As with other physiological parameters, root fresh weight was negatively affected by the toxic effect of B3 dose. In a study conducted on sunflower, when boron deficiency was corrected, root fresh weight first increased and then decreased at later doses (Oluk and Latif, 2009). The highest root fresh weight obtained as a result of proline doses was 2.11 g (20 ppm) and the lowest was 1.78 g (control). It was observed that the effect of proline applications on root fresh weight in plants under boron stress was positive. In addition to being an essential component of protein biosynthesis in any growing tissue, proline also appears to play a role as a regulator of cell division, particularly in the root elongation zone (Wang *et al*., 2014). As a result of B×P dose applications, the highest root fresh weight was measured as 2.72 g at 5 ppm×20 ppm dose and the lowest value was measured as 1.22 g at 0 ppm×10 ppm

dose application. In a study conducted on sugar beet, by applying 0.3 kg of boron per decare in different soil types in soil+leaf, soil and leaf forms, root yield increased by 12.5%, 12.1% and 11.1% respectively and sugar yield increased by 8% and 8% 7, 18.3% and 3.5%, but they stated that the application of 0.6 kg da^{-1} boron resulted in lower sugar and root yield (Gökmen and Sezgin, 2010).

Root dry weight

As seen in Table 2, the effect of B and P doses on root dry weight was found to be significant and the effect of B×P applications was found to be statistically insignificant. According to boron doses, the highest value was measured in the B2 dose with 0.25 g and the lowest value was measured in the B3 and control groups in the same group, with 0.16 g and 0.17 g. As with the fresh weight of the root, the dry weight values also started to decrease at the B3 dose where the toxic effect was observed. In a study similar to our findings, boric acid (H_3BO_3) was applied to the soil of corn plants at levels of 0, 10 and 30 mg kg^{-1}. At the end of the experiment, a decrease was detected in the fresh and dry weights of the plants compared to the control group (Güneş *et al.,* 2000). According to proline dose applications, the lowest value was determined as 0.16 g in the control group and the highest value was 0.21 g in the P20 dose. Many studies similar to our findings have reported that proline has a protective biochemical effect against stress and toxic poisons (Eris, 1990; Kutlu, 2010).

Leaf area index

Boron and Boron×area index, while Proline applications were found to be insignificant. The highest leaf area index obtained as a result of boron dose applications was 11.5 cm^2 in the B2 dose and the lowest was 9.9 cm^2 in the B3 dose (Table 2). According to this result, boron doses caused the leaf area index to first increase and then decrease due to toxic effects. In a similar study, while boron doses applied to the soil had a toxic effect on rapeseed, lupine and willow trees at lower doses, the field index decreased by causing a 10% effect on the leaves at a dose of 900 B mg cm^{-2} in poplar (Rees *et al.,* 2011). The highest leaf area index obtained as a result of Boron×Proline application was determined as 11.7 cm^2 in the 5 ppm×20 ppm dose application and the lowest was 8.4 cm^2 in the 10 ppm×20 ppm application. In another study conducted under greenhouse conditions, boron (0, 0.5, 1.0 and 1.5 kg B ha^{-1}) and zinc (0 and 5 kg Zn ha^{-1}) first had a positive effect on root, stem development and leaf area index in corn plants and then on the leaf area index. It has been observed to have a toxic effect (Panhwar *et al.,* 2011).

Table 2: Effects of boron and proline applications on some physiological and biochemical characteristics in soybean.

Boron doses	Parameters				
	Proline doses	RFW (g)	RDW (g)	LAI (cm^2)	İLLT (%)
Control (B0)	P0 (Control)	1.74 f	0.19	10.4 f	21.3
	P10	1.66 g	0.16	10.9 de	20.7
	P20	1.43 hi	0.15	11.1 cde	20.4
B0 Mean		1.61 B	0.17 B	10.8 AB	20.8 C
2.5 ppm (B1)	P0 (Control)	1.95 e	0.20	10.7 e	21.2
	P10	2.14 d	0.22	11.1 de	21.4
	P20	2.35 bc	0.24	11.6 ab	20.9
B1 Mean		2.14 B	0.22 AB	11.1 A	21.2 B
5 ppm (B2)	P0 (Control)	2.21 cd	0.21	10.8 e	22.2
	P10	2.48 b	0.26	11.3 bcd	21.8
	P20	2.72 a	0.28	11.7 a	21.7
B2 Mean		2.47 A	0.25 A	11.3 A	21.9 B
10 ppm (B3)	P0 (Control)	1.22 I	0.14	11.5 abc	24.2
	P10	1.57 gh	0.15	9.9 f	23.9
	P20	1.94 e	0.19	8.4 g	24.9
B3 Mean		1.58 B	0.16 C	9.7 B	24.3 A
P Doses Mean	P0 (Control)	1.78 C	0.18 B	10.8	22.2
	P10	1.96 B	0.19 AB	10.7	21.9
	P20	2.11 A	0.21 A	10.7	22.0
CV (%)		5.38	9.63	2.95	2.78
Boron (B)		**	**	**	**
Proline (P)		**	*	NS	NS
B×P		**	NS	**	NS

B- Boron; P- Proline; CV- Coefficient of variation; RFW- Root fresh weight; RDW- Root dry weight; LAI- Leaf area; ILLT- Index: Ion leakage in leaf tissues; NBI-Nitrojen blance index, *Significant at p<0.05, **Significant at p<0.01, NS- Non-significant.

Ion leakage in leaf tissues

In the study, Proline and Proline×Boron applications were found to be statistically insignificant on ion leakage in leaf tissues, while Boron doses were found to be significant. In studies conducted on plant physiology, toxic elements cause deterioration in the integrity and permeability stability of the cell structure of plants under environmental factors such as drought, salt and heat stress (Blokhina *et al*., 2003). As a result of this deterioration, the amount of ion movement into and out of the cell is considered an important indicator in determining tissue damage. In this study, as a result of increasing Boron doses, the highest ion leakage in leaf tissues was determined as 24.3% in B3 dose and the lowest as 20.8% in control dose application (Table 2). It was observed that B3 dose caused toxic effects on plants. Studies conducted with elements having toxic effects have shown that various biochemical and physiological processes in cells and tissues are affected (Wang *et al.,* 2014). During these processes, ion leakage, malondialdehyde and reactive oxygen content, which are indicators of damage, also increase (Beligni and Lamattina, 2000).

Nitrogen balance index

Boron, Proline and Boron×Proline applications on the nitrogen balance index were found to be statistically significant. The highest NBI obtained as a result of boron dose applications was obtained from the control dose, with 84.0 dx and the lowest value was obtained from the B3 dose application, with 49.4 dx. Considering the findings, decreases in NBI values occurred as a result of the poisoning that occurred in parallel with increasing boron doses in soybeans (Table 3). The highest NBI value obtained from proline doses was 67.2 dx from the 20 ppm dose and the lowest value was determined from the control dose application with 60.2 dx. The increase in NBI values of proline applications compared to the control group is thought to be a reaction to poisoning. The highest NBI value obtained as a result of Boron×Proline application was 92.2 dx in the 0 ppm×20 ppm dose application and the lowest was 47.3 mg g^{-1} in the 10 ppm×0 ppm application. As the boron doses increased, the healing and regulatory effect of proline became insufficient, resulting in a decrease in NBI values. In a similar study, Yolci *et al.* (2022) reported in their study on fenugreek that the NBI value varied between 54.5-59.85 mg g^{-1} under drought stress and a decrease was observed with stress. In another study, they stated that the NBI value of soybean varies depending on stress conditions and this value increases between 70.64 and 82.90 mg g^{-1}. It is thought that the differences between our findings and the findings of other researchers are related to the severity, duration and quality of stress (Oral *et al.,* 2021).

Table 3: Effects of boron and proline applications on some biochemical characteristics in soybean.

Boron doses	Parameters				
	Proline doses	NBI (dx)	Flavonoid (dx)	Anthocyanin (dx)	Chlorophyll (mg cm^{-2})
Control (B0)	P0 (Control)	76.3 c	0.45	0.06	38.2 ab
	P10	83.6 b	0.44	0.08	42.4 a
	P20	92.2 a	0.39	0.07	37.2 bc
B0 Mean		84.0 A	0.43 C	0.07 B	39.3 A
2.5 ppm (B1)	P0 (Control)	65.8 de	0.46	0.07	37.9 b
	P10	64.3 e	0.42	0.06	36.3 c
	P20	67.6 d	0.38	0.08	37.5 b
B1 Mean		65.9 B	0.42 C	0.07 B	37.3 B
5 ppm (B2)	P0 (Control)	51.6 g	0.49	0.07	36.7 c
	P10	55.5 f	0.51	0.07	31.2 de
	P20	57.7 f	0.50	0.08	32.0 d
B2 Mean		54.9 C	0.50 B	0.07 B	33.2 C
10 ppm (B3)	P0 (Control)	47.3 h	0.63	0.12	28.3 f
	P10	49.4 g	0.70	0.10	22.5 g
	P20	51.5 g	0.69	0.09	29.4 e
B3 Mean		49.4 D	0.67 A	0.10 A	26.3 D
P Doses Mean	P0 (Control)	60.2 C	0.51	0.08	35.3 A
	P10	63.2 B	0.52	0.08	33.1 B
	P20	67.2 A	0.49	0.09	34.1 A
CV (%)		3.77	8.06	17.64	4.31
Boron (B)		**	**	**	**
Proline (P)		**	NS	NS	**
B×P		**	NS	NS	**

B- Boron; P- Proline; CV- Coefficient of variation; NBI- Nitrojen blance index, *Significant at p<0.05, **Significant at p<0.01, NS- Non significant.

Flavonoid

Boron doses were found to be important on the flavonoid content in the plant, but boron×proline and proline applications were found to be insignificant. The highest value was obtained from the B3 dose with 0.67 dx and the lowest was obtained from the B0 and B1 doses with 0.43, 0.42 dx. Phalavonoids are known as secondary metabolites in plants. It is known that they become active under biotic and abiotic stress conditions and protect plants (Shah and Smith, 2020). In another study supporting our findings, it was stated that flavonoid values increased in sage plant as a result of boron doses (0, 5, 10, 20 mM) compared to the control dose and changed to 7.93-10.29 mg QE 100 g^{-1} (Yolci *et al.,* 2022).

Anthohocyanin

In this study, the effect of Boron on anthocyanin content was found to be significant and the effect of Proline and Boron×Proline applications was found to be statistically insignificant ($P<0.01$). The highest anthocyanin content obtained as a result of boron applications was in the same group with the results obtained from the B3 dose with 0.10 dx and the lowest with the results obtained from the B0, B1 and B2 doses with 0.07 dx (Table 3). Considering the findings, increasing boron doses compared to the control caused a increase in the anthocyanin contents that protect the plant under stress conditions. It has been reported that chromium doses in beans increase anthocyanin contents compared to the control dose. (Mahdavian, 2021). It has been determined that arsenic doses reduce anthocyanin values in the hogweed plant (Gajic *et al.,* 2020). It has been observed that the environment, genetic structure, as well as the source, severity and duration of stress are important in the emergence of these effects of elements that are necessary for plants but have toxic effects in excessive doses.

Chlorophyll

In this study, Boron, Proline and Boron×Proline applications on total chlorophyll content were found to be statistically significant ($P<0.01$). The highest total chlorophyll value obtained as a result of boron dose applications was obtained from the control dose as 39.3 4 mg cm^{-2} and the lowest value was obtained from the B3 dose application as 26.34 mg cm^{-2} (Table 3). In parallel with the increasing doses, there was a decrease in the chlorophyll content, which has an important place in photosynthesis. In a similar study, the same results were obtained in the rice plant. The highest total chlorophyll value obtained from proline doses was 35.3 4 mg cm^{-2} and 34.14 mg cm^{-2} from the control and 20 ppm dose, respectively and the lowest value was 33.1 4 mg cm^{-2} from the 10 ppm dose application. It has been observed that increasing proline doses have a positive effect on the chlorophyll content, which is an indicator of the plant's protection mechanism against the negative effects of stress. Similar studies have reported that the activity of proline applied to plants serves as a biochemical protection mechanism against the negative effects of stress (Ercan, 2008). The highest total chlorophyll value obtained as a result of Boron×Proline application was seen in the B0×P10 application as 42.4 4 mg cm^{-2} and the lowest was seen in the B3×P10 dose application as 22.5 4 mg cm^{-2} (Table 3). In studies conducted on boron, it is known that it is an important element in increasing the chlorophyll content if it remains below the toxic level (Khan *et al.,* 2016). In the study conducted by Kayıhan *et al.* (2017) with rice, it was determined that the chlorophyll content increased in some varieties and decreased in others as a result of boron applications. When we compared our findings with the results obtained from these studies, it was understood that the nature of the environment, genotype and stress are important.

CONCLUSION

In this study, the effects of boron and proline applications on physiological and biochemical properties of soybean (*Glycine max* L.) were examined. A fluctuation was observed in the plant height, root length, stem fresh and dry weight, root fresh and dry weight values of 0, 2.5, 5, 10 ppm boron doses applied to soybeans, first increasing and then decreasing. Compared to the control group, at 10 ppm dose application, these parameters decreased by 12%, 3%, 23%, 7%, 1.8% and 5.8%, respectively. A direct decrease occurred in leaf area index, NBI and total in chlorophyll (decrease rates of 8%, 41% and 33%, respectively) in parallel with increasing doses. It was determined that boron applications increased the flavonoid and anthocyanin contents by 35% and 30%, respectively. In proline applications, root length, stem fresh weight, root fresh weight, root dry weight, nitrogen balance index and flavonoid values increased by 21%, 9%, 15%, 14%, 10% and 6%, respectively, at a dose of 20 ppm compared to the control doses has been detected. The effect of proline on other parameters was found to be statistically insignificant. According to these results, it was understood that although boron is an important element that plays a role in root and stem development in plants, it has a toxic effect in high doses. According to the results of the study, it is thought that proline has a positive effect on many parameters as well as a healing and regulating effect on the level of damage against poisoning. However, in order to reach more realistic results, this study needs to be tested under field conditions. For this purpose, it was concluded that conducting similar studies on elements with toxic effects would contribute to the literature and the solution of the problem.

Conflict of interest

I have no conflict of interest as I am the sole author. All analyses and work belong solely to me.

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