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THE EFFECT OF BORON ON THE MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF SUNFLOWER SEEDLINGS (*HELIANTHUS ANNUUS* L.)

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ABSTRACT

Boron, an essential element for many organisms, has a unique role in plant metabolism. Boron plays a vital role in cell propagation, plant growth, photosynthesis and various metabolic pathways. Although there have been different studies on the effects of boron on the sunflower, the number of studies of the effect of boron on its antioxidant defense mechanism is limited. This study aimed to determine the effect of boron on the morphological (leaf area) and physiological (chlorophyll contents and SOD, CAT, GR, and APX enzymes) characteristics of sunflower (*Helianthus annuus* L.) plants during the seedling growth period. The study used 250 g of turf contained in 15 cm × 13 cm pots. For each boron dose, six pots were used, and five seeds were planted in each pot, which were pressed down after covering with 75 g turf, and 100 ml of water was used for the first irrigation of each pot. Seedlings, which emerged in about a week, were irrigated every two days for two weeks with 100 ml of water containing boron ($Na_2O_5B_2O_3.10H_2O$) at different doses of 0 (control) and 0.5, 1.0, 1.5 and 2.0 mg l^{-1} per pot. The largest leaf areas (2nd and 3rd) were observed in the seedlings irrigated with water containing 1 mg l^{-1} of boron. Chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) decreased with increasing boron doses (10.39%, 31.78% and 31.27%, respectively), whereas GR and CAT activities increased (0.1% and 49.46%), and SOD and APX activities decreased (18.18% and 0.07%) with increasing doses of boron.

KEYWORDS:
Sunflower, boron (B), antioxidant enzymes, chlorophyll, leaf area

INTRODUCTION

Boron (B) is a micronutrient essential to the growth and development of plants and is absorbed by plants in the form of boric acid (H_3BO_3) from the soil [1]. The presence of boron in soil and irrigation water is an important determinant in agricultural production [2, 3]. All plants require boron in varying amounts for normal growth [4]. In dicotyledons, boron (B) deficiency inhibits root growth and causes degeneration in the meristematic regions [5]. Recent studies have shown that using boron-containing fertilizers for sunflowers, which are dicotyledonous plants, resulted in higher root and leaf development. Sunflower (*Helianthus annuus* L.) is the one of the main crops used for edible oil production in many countries of the world, including Turkey. Sunflower is an arable crop requiring high levels of boron (> 0.5 ppm), and thus, are highly susceptible to boron deficiency or boron excess [4, 6]. It was determined that fertilization with boron increased the yield of sunflower production by 10 to 20%. The leaf index reported that the critical boron level needed to reach the highest economic productivity was 32.4 mg kg^{-1} [7].

Boron (B) has a broad effect on the physiological processes in plants [8]. There is extensive evidence supporting its role in the cell wall, plasma membrane, and various metabolic pathways in cells [2]. There is no evidence proving its direct effect on photosynthesis [9, 10]. However, El-Shintinawy [11] reported that boron deficiency caused structural and functional disruption in the leaves of sunflowers (*Helianthus annuus* L.), and thus, directly or indirectly, affected photosynthesis. Other studies have also reported that the chlorophyll content of leaves in many plants showed a varying response to boron deficiency or excessive boron and may increase or decrease [10, 12].

The effect of boron on a plant varies based on the lack of or excessive boron. In plants, boron deficiency causes decreased product quality and product losses, while excessive boron causes toxicity [6]. Boron toxicity triggers the formation of reactive oxygen species (ROS), such as superoxide anions, hydroxyl radicals, hydrogen peroxide (H_2O_2) and singlet oxygen in plant tissues [13]. As in the case of other environmental stress factors (drought, salinity, cold, and heavy metals), a plant antioxidant defense

mechanisms interfere to eliminate the reactive oxygen species (ROS) formation due to boron toxicity. This system comprises enzymatic (CAT, SOD, APX, GR) and non-enzymatic (flavonoids, phenolic compounds, alkaloids, tocopherols and carotenoids) elements [14]. Furthermore, it has been reported that boron deficiency, an environmental stress factor, facilitated ROS formation, and thus, caused the structural and functional destruction of thylakoid membranes [11]. Many studies have investigated the morphological, physiological, biochemical and molecular responses of plants to boron (B) deficiency or excess [1, 5, 13, 15]. However, the studies on the effect of boron in the sunflower have been generally limited to the effect of boron applied to plant growth and development, the yield, oil content, boron requirement of the plant and fertilization of the plant with boron [7, 15]. Many physiological [6, 11] and molecular studies [16] were conducted pursuing different goals. However, there is a dearth of studies investigating the effect of boron application on the antioxidant system in sunflower plants. Keleş et al. [17] conducted a study on the effect of high boron concentration on the antioxidant system in sunflower. This study aimed to investigate the effect of boron (B) on the morphological and physiological characteristics of the sunflower (*Helianthus annuus* L.) plants during the seedling growth period.

MATERIALS AND METHODS

Plant Material. In the study, the edible "TARSAN-1018" sunflower variety (*Helianthus annuus* L.) was obtained from the "Trakya Agricultural Research Institute".

Culture Medium, Culture Conditions, and Boron Application. The study used 250 g of turf contained in 15 cm × 13 cm pots. For each boron dose, six pots were used and five seeds were planted in each pot, which was pressed down after covering with 75 g turf, and 100 ml of water was used for the first irrigation of each pot. All trials were carried out in climate-controlled growth cabinets under fluorescent light (27 µmol m^{-2} s^{-1}) and at 24 ± 1°C for a photoperiod of 16 hours of light and 8 hours of darkness.

Seedlings, which emerged in about a week, were irrigated every two days for two weeks with 100 ml of water containing boron ($Na_2O_5B_2O_3.10H_2O$) at different doses of 0 (control), 0.5, 1.0, 1.5 and 2.0 mg l^{-1} per pot.

Determination of Leaf Area. The leaf area in mm^2 was determined using a computer program and a desktop scanner.

Determination of Chlorophyll Content. The protocol proposed by Curtis and Shetty [18] was used to determine the chlorophyll a, chlorophyll b and total chlorophyll contents of the leaves of plants to which different doses of boron were applied. By following the protocol, 50 mg of green material was put into 3 ml of methanol and kept at 23°C in darkness for 2 hours to allow the chlorophyll in the green material to dissolve into methanol. Then, the optical density (OD) of 1.5 ml of the liquid part (the chlorophyll-containing methanol) was determined at 650 and 665 nm using spectrophotometry, and the chlorophyll a, chlorophyll b and total chlorophyll contents were expressed as "µg chlorophyll/g of fresh tissue."

Antioxidant Enzyme Analyses. To determine the enzyme changes in plants under drought and salt stress, approximately 1 g of fresh leaf samples in liquid nitrogen were ground in porcelain mortars and homogenized with 10 ml of a 50-mM phosphate buffer solution containing 0.1 mM of Na-EDTA (pH of 7.6). The homogenized samples were centrifuged at 15,000 g for 15 minutes, and the resultant precipitates were used in enzyme analyses. Samples were kept at +4°C until the enzyme analyses were performed. For the enzyme measurements, final volumes were obtained using the buffer solution.

Superoxide dismutase (SOD) activity was determined using the method proposed by Çakmak and Marschner [19], and Çakmak [20], based on the reduction of NBT (nitro blue tetrazolium chloride) by O_2- under light. All the solutions were added to the reaction medium in the following order: 0.1 mM of Na-EDTA containing 50 mM (pH: 7.6) phosphate (P) buffer, the enzyme extract (25 to 100 µl) followed by 0.5 ml of 50 mM Na_2CO_3 (pH of 10.2), 0.5 ml of 12 mM of L-methionine, 0.5 ml of 75 µm of p-nitro blue tetrazolium chloride (NBT) and 10 µm of riboflavin were each added to the medium to make up the final volume to 5 ml. The samples were kept under light for 15 minutes and measurements were taken at 560 nm.

Catalase activity (CAT) was measured based on the decomposition rate of H_2O_2 at 240 nm (E = 39.4 mM cm^{-1}) [19, 20]. In this enzyme analysis, the final volume of the reaction medium was adjusted to 1 ml by adding 0.1 mM of EDTA containing a 50-mM phosphate buffer (pH of 7.6), 0.1 ml of 100 mM of H_2O_2 and enzyme extract into the reaction medium.

Ascorbate peroxidase (APX) activity was measured using the method proposed by Çakmak and Marschner [19], and Çakmak [20], based on the oxidation of ascorbate at 290 nm (E = 2.8 mM cm^{-1}). By following the method, the final volume of the reaction medium was adjusted to 1 ml by adding 0.1 mM of EDTA containing a 50-mM phosphate buffer (pH of 7.6), 0.1 ml of 10 mM of EDTA containing 12 mM of H_2O_2, 0.1 ml of 0.25 mM of L-

ascorbic acid and enzyme extract into the medium, and then the ascorbate concentration was measured at 290 nm using spectrophotometry.

Glutathione reductase (GR) activity was measured using the method proposed by Çakmak and Marschner [19] and Çakmak [20], based on the oxidation of NADPH at 340 nm ($E = 6.2$ mM cm^{-1}). By following the method, the final volume of the reaction medium was adjusted to 1 ml by adding 0.1 mM of EDTA containing a 50-mM phosphor buffer (pH of 7.6), 0.1 ml of 0.5 mM of oxidized glutathione (GSSG), 0.1 ml of 0.12 mM of NADPH and enzyme extract into the medium, and then the NADPH oxidation was measured at 340 nm.

Statistical Analysis. The trials were carried out using four repetitions in accordance with the "Completely Randomized Design". Variance analysis of the data was performed using "IBM SPSS Statistics 22" statistical software, and the averages of the treatments were compared to each other with Duncan's Test by using "MSTAT-C" software [21].

RESULTS

Table 1 shows that the application of 1 mg l^{-1} of boron increased the leaf area compared with the control group, as determined the morphological measurements. The highest results in the 2nd and the 3rd leaf areas (47.14, 15.96 mm^2) were obtained by applying 1 mg l^{-1} of boron to the seedlings. The highest boron concentration of 2 mg l^{-1} resulted in the 2nd leaf area being decreased by 49.03%, and the 3rd leaf area was increased by 113.36% (Table 1).

Table 2 shows the effect of boron doses on the chlorophyll content of the sunflower seedlings leaves.

TABLE 1
Effect of different boron doses on the leaf area of sunflower seedlings

Boron Dose (mg l^{-1})	2nd Leaf Area (mm^2)	Decrease Rate According to Control (%)	3rd Leaf Area (mm^2)	Increase Rate According to Control (%)
0.00 (Control)	31.63±1.17 b		7.48±0.63 c	
0.50	29.79±0.96 b		15.14±0.86 a	
1.00	47.14±1.13 a	49.03	15.96±0.99 a	113.36
1.50	32.83±0.73 b		9.71±0.73 c	
2.00	26.66±1.46 c		11.35±0.67 b	

Each data represents the average of four repetitions ± standard error.
The differences between the means represented with different letters in the same column are significant at $p \leq 0.05$.

TABLE 2
Effect of different boron doses on the chlorophyll content of the leaves of sunflower seedlings (µg chlorophyll/g fresh tissue)

Boron Dose (mg l^{-1})	Chlorophyll a Content	Decrease Rate According to Control (%)	Chlorophyll b Content	Decrease Rate According to Control (%)	Total Chlorophyll Content	Decrease Rate According to Control (%)
0.00 (Control)	843.81±16.34 a		309.94±12.44 a		459.51±13.76 a	
0.50	756.16±11.97 b		274.04±10.83 b		408.43±11.93 b	
1.00	788.80±12.12 ab	10.39	275.40±8.67 b	31.78	416.59±9.11 b	31.27
1.50	586.71±12.82 c		211.45±11.80 d		315.84±16.43 c	
2.00	597.34±14.77 c		241.10±8.73 c		320.50±8.02 c	

Each data represents the average of four repetitions ± standard error.
The differences between the means represented with different letters in the same column are significant at $p \leq 0.05$.

TABLE 3
Effect of different boron doses on antioxidant enzyme activity (GR, APX, CAT and SOD) in sunflowers (µmol min^{-1} mg^{-1} T.A.)

Boron Dose (mg l^{-1})	Glutathione Reductase (GR)	Increase Rate According to Control (%)	Ascorbate Peroxidase (APX)	Decrease Rate According to Control (%)	Catalase (CAT)	Increase Rate According to Control (%)	Superoxide Dismutase (SOD)	Decrease Rate According to Control (%)
0.00	80.44±1.45 a		5096.44±114.41 b		268.08±8.55		0.22±0.01	
0.50	78.78±1.95 b		5150.59±87.46 a		335.61±10.38		0.20±0.01	
1.00	76.89±2.38 b	0.1	5133.67±97.13 a	0.07	400.68±13.70	49.46	0.18±0.01	18.18
1.50	80.45±1.69 a		5093.06±75.46 b		147.55±8.88		0.18±0.02	
2.00	81.00±1.73 b		5079.53±79.02 b		302.62±14.33		0.16±0.01	

Each data represents the average of four repetitions ± standard error.
The differences between the means represented with different letters in the same column are significant at $p \leq 0.05$.

The chlorophyll a, chlorophyll b and total chlorophyll contents of the control group were high in all cases being 843.81, 309.94 and 459.51 µg of chlorophyll/g of fresh tissue, respectively. Considering the different boron doses, the best result was obtained with the application of 1 mg l^{-1} of boron giving 788.80, 275.40 and 416.59 µg of chlorophyll/g of fresh tissue, respectively. The chlorophyll a, chlorophyll b and total chlorophyll contents of the group receiving the highest dose of boron decreased by 10.39%, 31.78%, and 31.27%, respectively compared with the control group (Table 2).

Table 3 shows the antioxidant enzyme (GR, APX, CAT, and SOD) activities in the control group and the groups receiving boron doses. The group treated with a 2 mg l^{-1} boron dose showed the greatest glutathione reductase (GR) activity of 81.00 µmol min^{-1} mg^{-1} (T.A.); the group treated with 1 mg l^{-1} boron dose showed the lowest glutathione reductase (GR) activity of 76.89 µmol min^{-} mg^{-1} (T.A.). GR activity increased with increasing boron (B) doses, and the increase in the group with the highest dose (2 mg l^{-1}) was 0.1% compared with the control group (Table 3).The group treated with a 0.5 mg l^{-1} boron showed the highest activity of ascorbate peroxidase of 5150.59 µmol min^{-1} mg^{-1} (T.A.), and the group treated with a 1.5 mg l^{-1} boron showed the lowest ascorbate peroxidase activity of 5093.06 µmol min^{-1} mg^{-1} (T.A.). Increasing doses of boron (B) resulted in decreasing ascorbate peroxidase (APX) activity. The group treated with the highest boron dose of 2 mg l^{-1}, caused a 0.07% decrease in the APX activity compared with the control group. The highest catalase (CAT) activity of 400.68 µmol min^{-1} mg^{-1} (T.A.) was observed with 1 mg l^{-1} boron, and the lowest activity of 268.08 µmol min^{-1} mg^{-1} (T.A.) was observed in the control group. The group receiving the highest boron dose (2 mg l^{-1}) in CAT activity showed a 49.46% increase compared with the control group. Superoxide dismutase (SOD) activity was the highest (0.22 µmol min^{-1} mg^{-1} (T.A.)) in the control group and the lowest (0.16 µmol min^{-1} mg^{-1} (T.A.)) in the group treated with 2.0 mg l^{-1} of boron. In the boron (B) dose applications, the highest value of SOD activity as 0.20 µmol min^{-1} mg^{-1} (T.A.) was obtained with 1 mg l^{-1} of boron application. The seedling group treated with a 2 mg l^{-1} boron showed an 18.18% decrease in the superoxide dismutase activity (SOD) compared with the control group (Table 3).

DISCUSSION

The 2nd and 3rd leaf areas of sunflower seedlings were affected by the different boron doses of 1 mg l^{-1}, 1.5 mg $l^{-1}$and 2 mg l^{-1} during the growth period (Table 1). The largest 2nd and 3rd leaf area results were observed in the seedlings irrigated with 1 mg l^{-1} of irrigation water containing boron (47.14 and 15.96 mm^2). Moreover, the 2nd and 3rd leaf area values decreased with increasing boron doses. Our results corroborate the study of Cervilla et al. [22], which reported a decrease in the foliar area of tomato plants with increasing boron doses. Moreover, boron deficiency resulted in a decrease in the leaf area of young sunflower plants as shown by Kastori et al. [12]. Ogunwole et al. [28] reported that in young corn seedlings, boron deficiency (3.30 ppm), and excessive boron (6.60 ppm) decreased the leaf area, and the study concluded that boron had a physiological role in leaf formation and/or growth. At higher boron doses, fresh and dry weights of sunflower seedlings were affected negatively [29].

Chlorophyll content is a major factor reflecting the photosynthetic ratios of cultured plants [28]. Previous studies have reported that boron may have a direct or indirect effect on chlorophyll content. In this study, chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) of young sunflower seedlings decreased with increasing boron doses (Table 2). Our results were confirmed by Karadavut et al. [30] who reported that increased boron gave rise to a significant decrease in the contents of chlorophyll a, chlorophyll b and total chlorophyll. Boron was reported to be a photosynthesis-limiting factor in sunflower plants as reported by Plesnicar et al. [23]. Seth and Aery [31] attributed the decrease in the chlorophyll content at high boron doses to the decrease in leaf area, as well as marginal necrosis and chlorophyll degradation. El-Shintinawy [11] reported that boron deficiency facilitated ROS formation, and may affect the chlorophyll synthesis system or the activity of chlorophyllase, and thus, directly or indirectly affect the initial or general processes of photosynthesis. Furthermore, they stated that boron deficiency significantly decreased the Hill reaction. Kastori et al. [12] reported that boron deficiency led to a decrease in the chlorophyll content of the leaves of young sunflower plants. Day [32] reported that at higher boron doses, net photosynthesis decreased in sunflower.

In plants, the activity of enzymatic antioxidants usually increases the plant's tolerance to the destructive effects of stress factors [33]. In this study, APX and SOD activities decreased (0.07% and18.18%, respectively) and GR and CAT activities increased (0.1% and 49.46%, respectively) with increasing boron doses (Table 3). CAT and GR play a primary role in H_2O_2 elimination in cytoplasm and chloroplasts [17]. At the highest boron concentration of 2.00 mg l^{-1}, the activity of these two enzymes became prominent in the young, growing sunflower seedlings. The increase in the activities of GR and CAT, which are among the most effective antioxidant enzymes, indicate increasing ROS activity in the leaves receiving a high boron concentration of 2.00 mg l^{-1}. Our results agree with those of Cervilla et al. [13] and

Oluk et al. [34] that determined that high boron doses (2.00 mM) increase the CAT and GR activities in tomato varieties. Conversely, it was reported that under conditions with soil boron doses above 40 μg g^{-1}, the activities of both enzymes were particularly repressed [17]. Tassi et al. [6] showed that GR activity was decreased in the leaves of sunflowers grown in soil containing high doses of boron. Moreover, Çakmak and Römheld [24] and Dube et al. [26], reported that boron deficiency in sunflower (*Helianthus annuus*) plants (< 0.33 mg l^{-1} of boron) decreased the activity of CAT. Superoxide dismutase (SOD) catalyzes the conversion of O_2 to H_2O_2, and ascorbate peroxidase (APX) catalyzes the conversion of H_2O_2 to H_2O [24]. Keleş et al. [17] reported that, in the leaves of young sunflower seedlings, SOD activity increased with increasing boron doses. Tassi et al. [5] showed that, depending on boron toxicity in the leaves of sunflower, SOD activity increased in addition to necrosis and chlorosis. Conversely, Çakmak and Römheld [24], and Dube et al. [26] reported that APX activity increased, although SOD activity was not affected. The effect of boron on the enzyme activity in leaves varies greatly depending on the boron concentration and the tolerance of plant varieties to boron [8, 13]. High boron doses significantly affected the antioxidant enzyme activity in potato [8], tomato [13], and chickpea [1], whereas low boron doses significantly affected the antioxidant enzyme activity in tea [27].

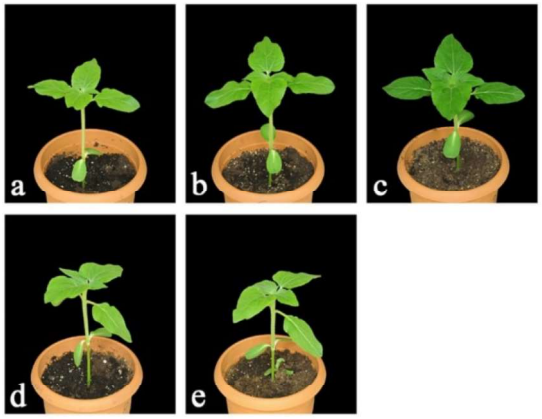

FIGURE 1
The effect of different boron doses (a.0.00 mg l^{-1}, b. 0.50 mg l^{-1}, c. 1.00 mg l^{-1}, d. 1.50 mg l^{-1} and e. 2.00 mg l^{-1}) on the growth of sunflower seedlings (*Helianthus annuus* L.)

Thus, our study showed that seedlings irrigated with water containing 1 mg l^{-1} of boron developed better and had the highest values regarding the morphological and physiological characteristics investigated. Increasing doses of boron had an adverse effect on plants (Figure 1). In summary, regarding the investigated parameters, the leaf area increased until it reached a particular boron concentration (1 mg l^{-1}) and then decreased with increasing concentration, while chlorophyll content decreased with increasing boron concentration. GR and CAT activities increased with increasing boron concentration, whereas APX and SOD activities decreased. Boron was also speculated to have a physiological effect on the development of the leaves of sunflower seedlings.

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