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Evaluation of Enzymatic and Non-enzymatic Antioxidant Defense Responses of Durum Wheat (*Triticum durum* Desf.) in Coping with Boron Stress

Rıdvan Temizgül[1,a,*]

[1]*Department of Biology, Faculty of Science, Erciyes University, Kayseri, Türkiye*
[*]*Corresponding author*

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ABSTRACT

Wheat, one of the world's most important agricultural products, plays a vital role in meeting the nutritional needs of our growing global population. However, arid and semi-arid regions face a potential threat from boron (B) toxicity. While boron is an essential nutrient for plant growth and development, its excessive presence can be toxic. It disrupts physiological processes, causing chlorosis and necrosis, ultimately leading to yield loss or plant death. Although B deficiency is a problem in the soils of many countries, Türkiye is one of the few experiencing B toxicity problems in its agricultural areas. This study investigated the physiological and biochemical responses of durum wheat to various B concentrations (0-20 mg L^{-1}) under controlled air-conditioned cabin conditions. Durum wheat exhibited a decrease in chlorophyll content, phenolic content, and antiradical capacity at B doses exceeding 10 mg L^{-1}. However, carotene content increased steadily with increasing B concentrations. The activities of antioxidant enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione S-transferase (GST), increased up to a B dose of 15 mg L^{-1}. Catalase (CAT) and glutathione reductase (GR) activities increased up to 10 mg L^{-1} B dose but decreased at higher B levels. Proline content increased tenfold up to a B dose of 10 mg L^{-1}, indicating an attempt to mitigate stress. Conversely, malondialdehyde (MDA) accumulation increased continuously (approximately 150%) with increasing B doses, suggesting membrane damage. Despite being considered B-sensitive, this study demonstrated that durum wheat can effectively cope with B stress up to a B dose of 10 mg L^{-1} under controlled conditions. Beyond this threshold, physiological and biochemical changes indicate a decline in stress tolerance. Many osmoregulators, carotenes, alkaloids, flavonoids, tocopherols, phenolic compounds, non-protein amino acids, and several unidentified metabolites are activated to support antioxidant defense. The SOS pathway and the released ROS force gene regulatory systems come into play. Following these, the ROS released in the organism are neutralized, and ionic homeostasis and cellular stress resistance are achieved.

[a] *rtemizgul@erciyes.edu.tr* *https://orcid.org/0000-0002-1033-7067*

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Introduction

Wheat, as one of the most important agricultural products in the world, plays a crucial role in meeting the food needs of the growing world population. In Türkiye, wheat is also the most widely grown cereal crop (Taşçı et al., 2023). Given the increasing world population, it is reported that the cultivated area should be at least doubled by 2050 (Yilmaz & Tomar, 2022). According to the report of the International Grains Council (IGC), wheat production in the 2023/2024 marketing year decreased by 19 million tons compared to the previous production season, while consumption increased by 8 million tons in contrast (Anonymous, 2023a). According to the Turkish Grain Board (TMO) report, wheat production in Türkiye, which was 19.75 million tons in 2022, increased by 3.8% to 20.5 million tons in 2023 (Anonymous, 2023b). Boron mineral plays a crucial role in enhancing plant cell wall strength, maintaining membrane integrity, regulating phenol metabolism, facilitating carbohydrate transport, promoting the development of generative organs, and influencing pollen germination and pollen tube growth (Turan et al., 2018; Yalın et al., 2019). These effects highlight the vital importance of B in enabling various essential plant functions (Gunes et al., 2000; Avci & Akar, 2005).

While ocean evaporation serves as the primary source of B release in the biosphere (65-85%), rock weathering also contributes to the increase of B compounds in ecosystems (Princi et al., 2016). Soil B levels are further elevated through anthropogenic activities such as mining, fertilization, irrigation, and increased industrial use (Parks & Edwards, 2005; Rab & Haq, 2012). With its growing

importance as a strategic element in developing technologies, Turkey holds over 70% of the world's boron reserves (Ozturk et al., 2010). Effluent from mining operations generally flows into collection ponds, containing 14-18% boron oxide (B_2O_3). While an estimated 60,000 tons of waste are produced annually from boron mining sites, B concentrations in mine site collection ponds far exceed the limits set by the World Health Organization (WHO) (Torun et al., 2006; Ozturk et al., 2010).

Arid and semi-arid regions are inherently at risk of B toxicity due to the capillary action and evaporation of B-rich groundwater. Under these conditions, B concentrations can reach toxic levels for plants, contaminating agricultural areas and reducing crop yields (Tanaka & Fujiwara, 2008). Soils containing more than 5 mg L^{-1} of water-soluble B are considered B-toxic (Gupta, 2007). Borax [$Na_2B_4O_7.10H_2O$] and boric acid [$B(OH)_3$] are known as the most common water-soluble fertilizers containing B (Mattos Jr et al., 2017). B in the soil solution exists as boric acid and is easily leached from the soil due to its high solubility (Camacho-Cristóbal et al., 2018). It is adsorbed to the surface of soil particles according to the degree of adsorption depending on the soil type, pH, salinity, organic matter content, aluminum oxide content, iron and aluminum hydroxides content, and clay content. B is an essential micronutrient for plant growth and development. However, excessive boron can cause toxic effects, disrupting physiological processes (CO_2 assimilation, photosynthesis, root development, shoot development, water circulation and supply, carbohydrate metabolism, and antioxidant system) leading to chlorosis, necrosis, and ultimately yield loss or plant death (Miwa et al., 2007; Reid, 2013). While boron is crucial for plants, the required amount is very small (0.5-2.0 mgL^{-1}). Soils with boron levels exceeding 2.0 mg L^{-1} are considered boron-contaminated, leading to potential reductions in crop yield and product quality issues. The detrimental effects of B on agriculture and ecosystems have been extensively reported (Yau & Ryan, 2008; Stiles et al., 2011). While some countries, including Türkiye, South Australia, Egypt, Iraq, California, and Chile, face boron toxicity issues in agricultural areas, many others, such as Japan, China, the USA, and Brazil, have insufficient soil B concentrations for agricultural production and rely on B supplementation as fertilizer (Gupta et al., 1985; Yau et al., 1995).

Typical B toxicity symptoms manifest in the marginal areas of mature leaves, causing them to become chlorotic or necrotic (Soylu et al., 2005). Chlorosis (yellowing) develops at the leaf tips and progresses along the leaf margins and inwards. Leaf tissue necrosis results in a loss of photosynthetic capacity, ultimately reducing plant productivity (Lovatt & Dugger, 1984). Boron deficiency often limits the production of forage legumes and certain vegetable crops. Tolerant species include alfalfa, beets, cotton, various grains, sorghum, oats, sugar beets, and tomatoes. Conversely, beans, grapes, grapefruit, lemons, oranges, and wheat are known to be B-sensitive species (Landi et al., 2019).

Numerous studies have demonstrated that boron (B) toxicity triggers the excessive production of reactive oxygen species (ROS) in plants (Cervilla et al., 2007; Ardic et al., 2009). This B-induced oxidative stress leads to the generation of ROS, which can cause DNA damage and chromosomal abnormalities (Risom et al., 2005). To evaluate the extent of stress and damage caused by boron toxicity in plants, researchers employ a range of techniques: (1) measuring the levels of enzymatic and non-enzymatic antioxidants provides insights into the plant's antioxidant defense mechanisms against oxidative stress; (2) analyzing changes in photosynthetic components, including chlorophyll a, b, and total chlorophyll, reveals the impact of B toxicity on photosynthesis; (3) assessing the accumulation of proline-like compatible osmolytes, carotenoids, and phenolic compounds provides information about the plant's stress response mechanisms; (4) evaluating the antiradical capacity of plant extracts indicates their ability to scavenge free radicals and protect against oxidative damage, and (5) determining the extent of membrane damage caused by B-induced oxidative stress is crucial for understanding the overall cellular damage (Beddowes et al., 2003; Temizgul, 2024).

Durum wheat (*Triticum durum* Desf.), belonging to the cereal group, is a tetraploid (2n = 4x = 28, AABB) wheat species. Characteristically, durum wheat possesses a hard endosperm structure and a high vitreous kernel index (Temizgul et al., 2024). These qualities make durum wheat well-suited for pasta production. Additionally, durum wheat is distinguished by its high content of yellow-colored pigments and low lipoxygenase enzyme activity. Furthermore, durum wheat boasts a high protein content (Yüksel et al., 2011).

This study aimed to investigate the physiological and biochemical responses of durum wheat (*T. durum* Desf. cv. *karakilcik*) to varying B concentrations. Six B treatments were applied: 0, 1, 5, 10, 15, and 20 mg L^{-1} B, equivalent to boric acid [$B(OH)_3$] concentrations of 6.18, 30.92, 61.83, 92.75, and 123.66 mg L^{-1}, respectively. We tested the physiological [Chlorophyll a (chl_a), chlorophyll b (chl_b), chl a/b, total chlorophyll (total chl) and carotene] and biochemical responses [enzymatic and non-enzymatic; superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST), ascorbate peroxidase (APX), total protein content (TPC), total phenolic content (TP), antiradical capacity (AC), proline (PRO) and lipid peroxidation (LPO-MDA)] of durum wheat to different B concentrations under climatic chamber conditions.

Materials and Methods

Cultivation and Stress Treatments of Plants

Durum wheat (*T. durum* Desf. cv *karakilcik*) used in the study was obtained from the Aegean Agricultural Research Institute. Durum wheat seedlings were grown in a hydroponic system under controlled laboratory conditions in an acclimation chamber according to the method of Temizgul (2024). After the wheat was grown without any stress for 10 days, B stress was applied for 15 days. Control plants were grown with only hoagland solution (Hoagland & Arnon, 1950) for 25 days (hoagland solution contains 2.86 mg L^{-1} B) and the plant was prevented from becoming dehydrated by frequently adding hoagland. The solutions in the culture dishes were renewed daily. Six different B treatments were applied: 0, 1, 5, 10, 15, and 20 mg L^{-1} B, equivalent to 6.18, 30.92, 61.83, 92.75, and 123.66 mg L^{-1}

of boric acid [$B(OH)_3$], respectively. Each trial (6 sets) was set up with 3 repetitions (6×3=18 practices). Following 15 days of stress application, the roots and leaves of the plants were quickly harvested on ice molds and stored at -80°C until use.

Physiological Analysis

Chl_a, chl_b, chl a/b ratio, and total chl content were measured to assess photosynthetic efficiency. Carotenoid content was determined to evaluate the plant's antioxidant defense capacity. Chlorophyll and carotene contents of the leaves were determined by keeping 100 mg of fresh leaf samples in 10 ml dimethyl sulfoxide (DMSO) in a water bath at 65°C until the color disappeared, and then the optical densities of the samples were read against DMSO at 647, 663 and 470 nm (Yilmaz et al., 2020). The amount of chlorophyll is given as mg gr^{-1} fresh weight. The following formulas were used to calculate chlorophyll and carotene contents.

$$\text{Chl a}(\frac{\text{mg}}{\text{gr}}\text{fw}) = (12.25 \times \text{A663}) - (2.79 \times \text{A647})$$

$$\text{Chl b}(\frac{\text{mg}}{\text{gr}}\text{fw}) = (21.50 \times \text{A647}) - (5.1 \times \text{A663})$$

$$\text{Total Chl}\left(\frac{\text{mg}}{\text{gr}}\text{fw}\right) = (7.15 \times \text{A663}) + (18.71 \times \text{A647})$$

$$\text{Carotene}\left(\frac{\text{mg}}{\text{gr}}\text{fw}\right) = \frac{(1000 \times \text{A470}) - (1.82 - \text{Chl a}) - (85.02 \times \text{Chl b})}{198}$$

Biochemical Analyses and Enzyme Activities

The activities of SOD, CAT, GR, GST, and APX were measured to assess the plant's enzymatic antioxidant defense system. Total protein concentration (TPC), total phenolic content (TP), and antiradical capacity (AC) were measured to evaluate the plant's non-enzymatic antioxidant defense system. Proline (PRO) content was measured as an osmolyte, while lipid peroxidation (LPO-MDA) was assessed to evaluate membrane damage.

Determination of TPC and Preparation of Crude Enzyme Extracts

TPC were determined spectrophotometrically at 595 nm according to the Bradford (1976) method. Bovine serum albumin (BSA) fraction V was used as the standard. The results were recorded as µg ml^{-1} protein. Crude enzyme extracts were prepared according to the methods of Yilmaz et al. (2017). Crude enzyme extracts were stored at -20°C until use.

Catalase (CAT) (EC 1.11.1.6)

The catalase activity of the samples was determined using a modified version of the method described by Duman et al. (2011). The reaction was performed in 20 mM sodium hydrogen phosphate buffer ($NaHPO_4$, pH7.5) containing 50 µl crude enzyme extract. Shimadzu UV-1800 cooled spectrophotometer and 2 ml volume quartz cuvettes were used in the measurements. Buffer without enzyme extract was used as a blank. The reaction started with the addition of hydrogen peroxide (H_2O_2) to the cuvette and the decrease in absorbance was observed for 3 minutes at 25°C. For catalase, the molar absorption coefficient (Ɛ) of H_2O_2 at 240 nm wavelength is 40 $mM^{-1}cm^{-1}$. The specific activity (SA) of the samples was calculated using the following formula. The results were determined as unit mg^{-1} protein.

$$\text{SA}(\frac{\text{unit}}{\text{mg}}\text{protein}) = \frac{\Delta\text{Abs/min}}{40} \times \frac{\text{crude enzyme vol}}{\text{cuvette vol}} \times \frac{1}{\text{prot cons}} \times 1000$$

Superoxide Dismutase (SOD) (EC 1.15.1.1)

The SOD activity of the samples was determined using the method described by Temizgul (2024). The reaction mixture contained: 20 mM sodium phosphate buffer (pH 7.4), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM methionine, 0.1 mM nitro blue tetrazolium chloride (NBT), 0.005 mM riboflavin, and 50 µl crude enzyme extract. Samples and standards were placed 20 cm from a fluorescent lamp (400 lumens) and incubated for 15 minutes. Absorbance was measured at 560 nm using a spectrophotometer against a blank. The experiment was performed three times in duplicate.

Calculation of SOD Activity: Percent inhibition (Inh%) values were used to calculate SOD activity. One unit of SOD activity is defined as the amount of enzyme that inhibits the reaction by 50%. The percent inhibition was calculated using the following formula:

$$\text{Inh\%} = \frac{\text{Light Control Abs.} - \text{Sample Abs}}{\text{Light Control Abs.}} \times 100$$

A logarithmic graph was constructed using enzyme concentrations on the x-axis and % inhibition values on the y-axis. The logarithms of the standard SOD enzyme concentrations were used to create a new graph with the corresponding % inhibition values. The SOD activity was determined using the equation derived from this new graph. SOD activity was expressed in units per milligram of protein (Unit mg^{-1} protein).

Ascorbate Peroxidase (APX) (EC 1.11.1.11)

The APX activity of the samples was determined using a modified version of the method described by Duman et al. (2011). The reaction mixture contained: 0.2 mM ascorbate, 10 mM H_2O_2, 50 µl crude enzyme extract, and 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding H_2O_2 to the reaction mixture in a quartz cuvette at 25°C. The change in activity at 290 nm was monitored and recorded for 3 minutes. APX activity was expressed in units per milligram of protein (Unit mg^{-1} protein). Enzyme activity was calculated as the amount of H_2O_2 consumed using the following formula:

$$\text{SA}(\frac{\text{unit}}{\text{mg}}\text{protein}) = \frac{\Delta\text{Abs/min}}{2.8} \times \frac{\text{crude enzyme vol}}{\text{cuvette vol}} \times \frac{1}{\text{prot cons}} \times 1000$$

Glutathione Reductase (GR) (EC 1.6.4.2)

The GR activity was determined using the method described by Misra and Gupta (2006). The reaction mixture contained: 0.1 mM nicotinamide adenine dinucleotide phosphate (NADPH), 1 mM oxidized glutathione (GSSG), 0.1 mM EDTA disodium salt (Na_2EDTA), 50 µl crude enzyme extract, and 100 mM potassium phosphate buffer

(pH 7.5). The reaction was carried out in a quartz cuvette at 25°C for 5 minutes. The change in absorbance at 340 nm was monitored and recorded. The molar extinction coefficient (Ɛ) of nikotinamid adenin dinükleotit fosfat (NADPH) at 340 nm is 6.2 $mM^{-1}cm^{-1}$. GR activity was expressed in units per milligram of protein (Unit mg^{-1} protein). GR activity was calculated using the following formula:

$$\text{SA}\left(\frac{\text{unit}}{\text{mg}}\text{protein}\right) = \frac{\Delta\text{Abs/min}}{6.2} \times \frac{\text{crude enzyme vol}}{\text{cuvette vol}} \times \frac{1}{\text{prot cons}} \times 1000$$

Glutathione S-transferase (GST) (EC 2.5.1.18)

The GST activity was determined using the method described by Yilmaz et al. (2020). The reaction mixture contained: 0.1 mM EDTA, 0.1 mM NADPH, 1 mM glutathione (GSH), 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), and 100 mM potassium phosphate ($KHPO_4$, pH 7.5) buffer. The reaction was carried out in a quartz cuvette at 25°C and monitored at 340 nm. 50 µl of crude enzyme extract was added to the reaction mixture. The reaction was allowed to proceed for 5 minutes to eliminate non-specific activity. The change in absorbance at 340 nm was monitored and recorded for another 5 minutes. The molar extinction coefficient (Ɛ) of NADPH at 340 nm is 6.2 $mM^{-1}cm^{-1}$. GST activity was expressed in units per milligram of protein (Unit mg^{-1} protein). GST activity was calculated using the following formula:

$$\text{SA}\left(\frac{\text{unit}}{\text{mg}}\text{protein}\right) = \frac{\Delta\text{Abs/min}}{6.2} \times \frac{\text{crude enzyme vol}}{\text{cuvette vol}} \times \frac{1}{\text{prot cons}} \times 1000$$

Determination of Proline Content

The proline content of the samples was determined using the method described by Temizgul et al. (2016). The method involves extracting proline with sulphosalicylic acid, reacting it with ninhydrin to produce a colored product, and extracting the colored product with toluene. The absorbance of the toluene extract is measured at 520 nm using a spectrophotometer. A standard curve was prepared using 10 standards containing proline concentrations ranging from 0.01 µM to 1.5 mM. The same procedure was applied to the standards as to the samples, and the equation of the standard curve was used to calculate the proline content of the samples. The proline content of the samples was expressed in nmol g^{-1} fresh weight.

Determination of Lipid Peroxidation (LPO) (MDA)

The effect of B applications on lipid peroxidation was determined by evaluating the thiobarbituric acid reactive substances (TBARS) content in the tissues and using a 20% trichloroacetic acid (TCA) solution containing 0.5% 2-Thiobarbituric Acid (TBA) according to the method of Madhava and Sresty (2000). The results are given as TBARS ($nmolgr^{-1}$ fresh weight). TBARS analysis measures malondialdehyde (MDA) present in samples as well as malondialdehyde produced from lipid hydroperoxides by the hydrolytic conditions of the reaction (Janero, 1990).

Determination of Total Phenolics (TP)

Total phenolic compounds were obtained by dissolving 50 mg of dried sample in 80% methanol using the method of Sarafi et al. (2017). This solution was analyzed using the Folin-Ciocalteu reagent according to the method of Scalbert et al. (1989) and the results were expressed as mg gallic acid equivalents (GAE) g^{-1} FW.

Determination of Antiradical Capacity (ARC)

Antiradical capacities of the samples were determined according to the 2,2 diphenyl-1-picrylhydralysis hydrate (DPPH) method (Brand-Williams et al., 1995). 0.1 mL of each extracted sample was taken and analyzed in triplicate. Methanol was used as a control. 0.1 mM, 4.9 mL DPPH solution prepared with methanol was added to each tube at 20-second intervals, and after being kept at room temperature and in the dark for 20 minutes, measurements were made on a spectrophotometer at a wavelength of 517 nm. The following equation was used to calculate antiradical capacity (ARC %).

$$\text{ARC \%} = \frac{\text{Blank Abs.} - \text{Sample Abs.}}{\text{Blank Abs.}} \times 100$$

Statistical Analysis

In this study, one-way and two-way analysis of variance (ANOVA) was used to test the interactions between B doses and plant parts using the SPSS 28.01 (SPSS, Armonk, NY: IBM Corp) software package. Duncan's multiple range tests, least significant difference (LSD) test, and Tukey's test were used to test the significance between factors. All experiments were repeated three times with three replications. Values are expressed as mean ± SE. Different small letters (a, b, c, etc.) on tables and figures indicate statistical significance between means.

Results

Effect of B Application on Chlorophyll and Carotenoid Content

The chl_a content of *T. durum* wheat increased up to the 15 mg L^{-1} dose (from 7.71 to 9.52 mg gr^{-1} fw) and decreased at the 20 mgL^{-1} dose (6.18 mg gr^{-1} fw) depending on B application. Chl_b content increased up to 10 mg L^{-1} B application and decreased regularly at 15 and 20 mg L^{-1} doses. The chlorophyll a/b ratio decreased compared to the control at 5 mg L^{-1} B application but increased at high B doses. Carotenoid content continuously increased in parallel with increasing B doses, ranging from 1.7 to 3.95 mg gr^{-1} fw depending on B doses (Table 1).

Effect of Boron Application on Total Protein Content

The effect of B application on the total protein content of durum wheat roots and stems was investigated. Significant differences were observed between application doses in both roots and stems ($p \leq 0.05$). The total protein content ranged from 424.8 to 489.1 mg gr^{-1} fw in roots and from 420.8 to 485.4 mg gr^{-1} fw in stems (Tables 2 and 3) depending on B application. The highest protein content was obtained with 15 mg L^{-1} B application (489.1 mg gr^{-1} fw in roots and 485.4 mg gr^{-1} fw in stems) (Figure 1f). The increase in protein content in roots and stems was approximately 15% with 15 mg gr^{-1} B application (Figure 3f).

Table 1. Effect of B application on chlorophyll and carotenoid content of T. durum wheat

B Doses (mgL^{-1})	Chl_a* mg gr^{-1} fw	Chl_b* mg gr^{-1} fw	Chl a/b	Total Chl* mg gr^{-1} fw	Carotene* mg gr^{-1} fw
Control	$7.71^{a.b}$	$3.33^{a.b}$	2.32^{a}	$11.04^{a.b}$	1.70^{c}
5	8.88^{a}	$3.93^{a.b}$	2.26^{a}	$12.82^{a.b}$	2.06^{bc}
10	9.81^{a}	5.99^{a}	1.63^{c}	15.80^{a}	2.69^{b}
15	9.52^{a}	$4.93^{a.b}$	1.94^{b}	14.45^{a}	3.33^{ab}
20	6.18^{b}	2.62^{b}	2.36^{a}	8.80^{b}	3.95^{a}
LSD	3.96	3.55	1.86	702.17	44.757

* Differences between the same groups are shown with the letters ^{abcde} and their significance level is $p < 0.05$; * Values were calculated by taking the average of three replicates.

Table 2. Effect of B applications on biochemical parameters in T. durum wheat stems

B Doses (mgL^{-1})	SOD* U mg-1	CAT* U mg-1	APX* U mg-1	GR* U mg-1	GST* U mg-1	MDA* nmol gr^{-1}	Proline* nmol gr^{-1}	Protein* mg gr^{-1}	TP* mg GAE gr^{-1} fw	ARC* Inh%
Control	0.919^{c}	0.032^{c}	1.948^{c}	0.491^{c}	3.272^{b}	13.833^{d}	243.8^{c}	420.8^{c}	12.400^{b}	62.998^{d}
5	5.204^{a}	0.045^{b}	2.186^{c}	1.175^{a}	4.866^{a}	17.032^{cd}	269.0^{c}	441.7^{b}	14.609^{b}	77.954^{c}
10	5.756^{a}	0.104^{a}	3.695^{b}	1.208^{a}	5.510^{a}	19.716^{bc}	2635.2^{a}	467.3^{ab}	26.497^{a}	80.982^{a}
15	6.077^{a}	0.047^{b}	4.439^{a}	0.900^{b}	5.537^{a}	21.505^{b}	2517.2^{a}	485.4^{a}	16.318^{c}	70.130^{b}
20	2.856^{b}	0.036^{bc}	2.042^{c}	0.835^{b}	5.323^{a}	33.239^{a}	2095.4^{b}	444.8^{b}	9.430^{d}	52.828^{e}
LSD	0.9091	0.013	0.4675	0.1412	0.7822	4.0149	223.37	456.2	0.5927	1.1737

* Differences between the same groups are shown with the letters ^{abcde} and their significance level is $p < 0.05$, * Values were calculated by taking the average of three replicates.

Table 3. Effect of B applications on biochemical parameters in T. durum wheat roots

B Doses (mgL^{-1})	SOD* U mg-1	CAT* U mg-1	APX* Umg-1	GR* U mg-1	GST* Umg-1	MDA* Nmol gr^{-1}	Proline* Nmol gr^{-1}	TP* Mg GAE gr^{-1}	ARC* Inh%	Protein* U mg-1
Control	1.009^{c}	0.071^{c}	2.801^{cd}	0.304^{b}	3.667^{c}	8.318^{c}	119.693^{d}	16.951^{c}	37.107^{c}	424.8^{c}
5	2.559^{b}	0.086^{b}	3.147^{c}	0.411^{a}	4.814^{b}	13.179^{b}	165.543^{c}	30.629^{a}	75.336^{a}	448.7^{b}
10	6.277^{a}	0.113^{a}	3.848^{b}	0.427^{a}	5.554^{a}	14.108^{b}	238.715^{a}	21.050^{b}	71.100^{b}	472.8^{ab}
15	6.606^{a}	0.059^{d}	4.402^{a}	0.225^{c}	5.646^{a}	14.417^{b}	192.267^{b}	10.557^{d}	32.818^{d}	489.1^{a}
20	3.336^{b}	0.047^{e}	2.501^{d}	0.151^{d}	5.346^{ab}	16.447^{a}	98.613^{e}	9.318^{e}	30.679^{e}	449.8^{b}
LSD	1.0689	0.0088	0.5038	0.0245	0.5708	1.3783	13.192	0.431	1.1394	463.7

* Differences between the same groups are shown with the letters ^{abcde} and their significance level is $p < 0.05$; * Values were calculated by taking the average of three replicates.

Effect of Boron Application on Antioxidant Enzyme Activities

The effect of B application on the antioxidant enzyme activities (CAT, GR, SOD, APX, and GST) in durum wheat roots and stems was investigated. Significant increases in CAT and GR enzyme activities were observed in both roots and stems up to 10 mg L^{-1} B dose (0.032-0.104 U mg^{-1} protein, respectively). SOD, APX, and GST enzyme activities increased in both roots and stems up to 15 mg L^{-1} B applications (0.919-6.077; 1.948-4.439 and 3.272-5.537 U mg^{-1} protein, respectively) (Tables 2 and 3; Figure 2). Compared to the control, the increase in enzyme activities due to B applications was 150-600% for SOD, -25-255% for CAT, -10-120% for APX, -50-150% for GR, and 30-75% for GST (Figure 3). While CAT, APX, and GR enzyme activities decreased by approximately 10-50% in roots with 15 and 20 mg L^{-1} B applications, no decrease in activity was observed in the stems of the plant.

Effect of Boron Application on Proline Accumulation

The effect of B application on proline accumulation in durum wheat roots and stems was investigated. The highest proline accumulation was obtained at 10 mg L^{-1} B application in durum wheat (238.72 nmol gr^{-1} fw in roots and 2635.2 nmol gr^{-1} fw in stems) (Tables 2 and 3; Figure 2a). Proline accumulation in roots increased by approximately 2-fold depending on the dose increase, while the increase in stems was more than 10-fold (Figure 4a).

Effects of Boron Application on Lipid Peroxidation (LPO) (MDA)

The effect of B application on lipid peroxidation (LPO) in durum wheat roots and stems was investigated by measuring malondialdehyde (MDA) content. MDA accumulation increased in both roots and stems of durum wheat in response to increasing B doses (8.318-16.447 nmol gr^{-1} fw in roots; 13.833-23.239 nmol gr^{-1} fw in stems) (Tables 2, 3; Figure 2b). Compared to the control, MDA accumulation increased by 50-100% in roots and 25-150% in stems with increasing B doses (Figure 4b).

Effect of Boron Application on Total Phenolic (TP) Content

The effect of B application on total phenolic content in durum wheat roots and stems was investigated. The highest total phenolic content was obtained at 5 mg L^{-1} B application in roots (30.629 mg GAE gr^{-1}) and at 10 mg L^{-1} B application in stems (26.497 mg GAE gr^{-1}) (Table 1, 3; Figure 2c). Total phenolic content in roots and stems of durum wheat increased by 20-113% compared to the control with 5 and 10 mg L^{-1} B applications, while it decreased by 20-45% with 15 and 20 mg L^{-1} B applications (Figure 4c).

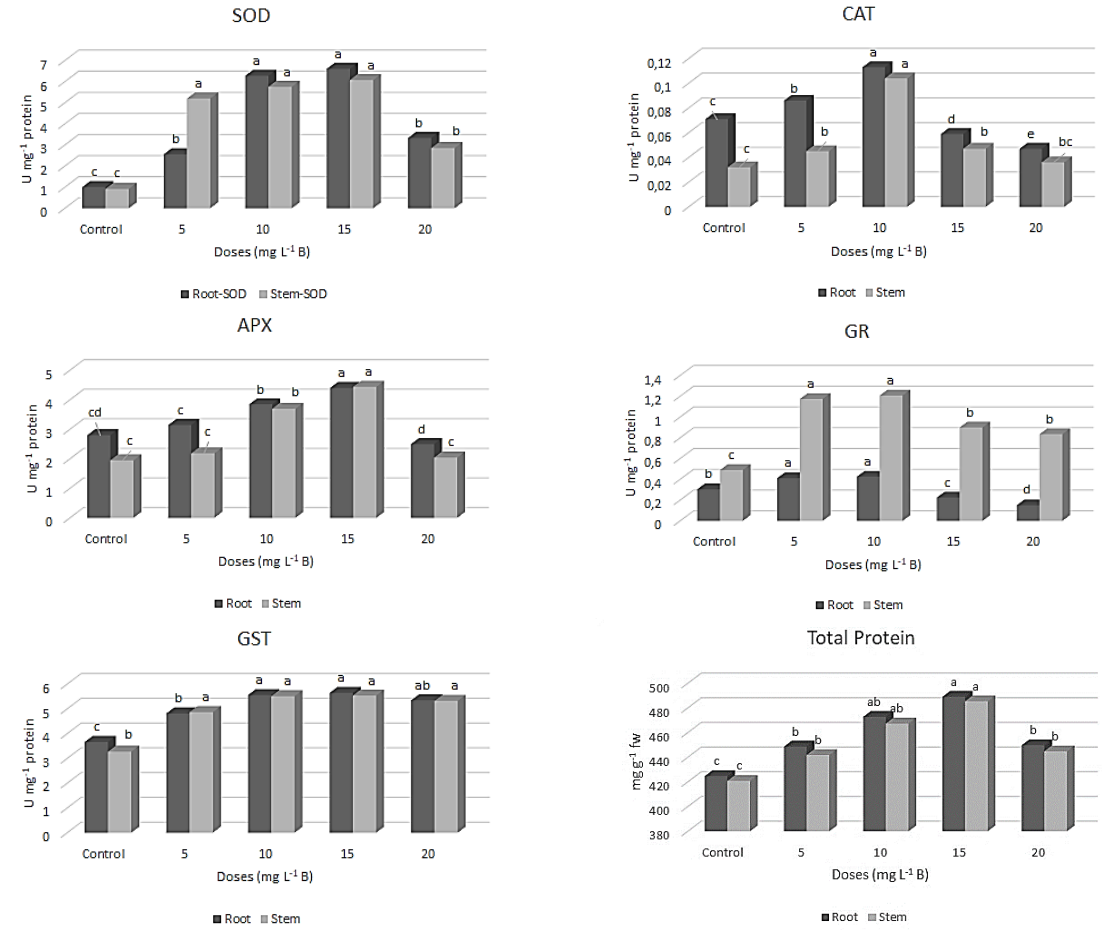


Figure 1. Effect of boron applications on antioxidant enzyme activities

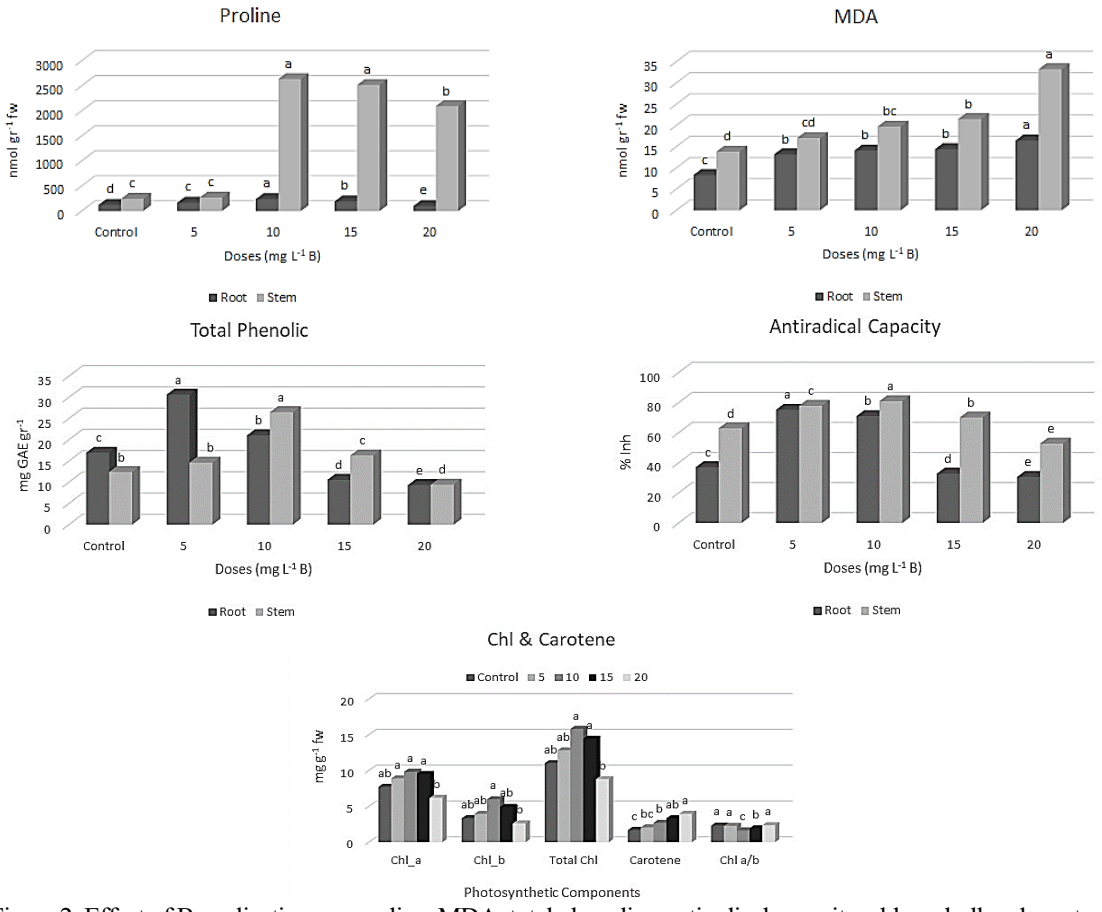


Figure 2. Effect of B applications on proline, MDA, total phenolics, antiradical capacity, chlorophyll and carotene

Effect of Boron Application on Antiradical Capacity (ARC)

The effect of B application on antiradical capacity (ARC) was investigated in durum wheat roots and stems. Similar to phenolic compounds, the highest antiradical capacity was obtained at 5 mg L^{-1} B application in roots (75.336% inh) and at 10 mg L^{-1} B application in stems (80.982% inh) (Table 1, 3; Figure 2d). Antiradical capacity in durum wheat roots ranged from 30.679 to 75.336% and in stems from 52.828 to 80.982% depending on increasing B doses. Antiradical capacity increased by approximately 100% in roots and 25% in stems compared to the control with 5 and 10 mg L^{-1} B doses, while it decreased by approximately 25% with 15 and 20 mg L^{-1} B doses (Figure 4d).

Discussion

There is considerable variation among plants in their tolerance to high boron levels. The visual B toxicity symptoms observed in plants are not directly related to the B concentration in their tissues (Mahalakshmi et al., 1995). B toxicity can alter various physiological processes, including delayed seedling emergence and leaf development, chlorosis and necrosis in leaves, membrane damage, structural disruptions in the cell wall, inhibition of cell division and elongation, and a range of metabolic disorders (Reid et al., 2004; Reid & Fitzpatrick, 2009; Savic et al., 2012; Açar et al., 2016). Interestingly, plants exposed to B toxicity also exhibit increases in antioxidant capacity and phenolic metabolism (Cervilla et al., 2007; Ardiç et al., 2009).

Effect of Boron Application on Chlorophyll Content

Photosynthesis is among the primary metabolic processes affected by B due to internal or external stomatal limitations (Shah et al., 2017; Papadakis et al., 2018). Studies have shown that B significantly influences transpiration rates, leading to a reduction in photosynthetic area, chlorophyll concentration, and consequently, photosynthetic rate as leaf margins die under B stress (Chen et al., 2014; Papadakis et al., 2015). B stress has also been reported to alter the content of photosynthetic pigments, including chlorophyll and carotenoids, and their ratios (Kayıhan et al., 2017; Sarafi et al., 2018). Excessive B can negatively impact plant characteristics related to photosynthesis, such as chlorophyll pigments and maximum quantum yield of chlorophyll fluorescence (Fv/Fm) (Kaya et al., 2020). This is associated with a decrease in the activity of certain CO_2 assimilation enzymes (ribulose-1,5-diphosphate carboxylase/ oxygenase and fructose-1,6-diphosphate phosphatase), disruption of ATP and NADPH coordination, and suppression of the electron transport system (Han et al., 2009). In our study, Chl_a and Chl_b levels increased with increasing B doses up to 10 mg L^{-1} B application (9.81 mg gr^{-1} fw and 5.99 mg gr^{-1} fw, respectively). However, significant decreases in both chlorophyll contents were observed with 15 and 20 mg L^{-1} B doses (approximately 25%) (Table 1, Figure 4e). The decline in chlorophyll content at high B doses is likely associated with leaf yellowing and necrosis caused by B accumulation in plant leaves.

Effect of Boron Application on Carotenoid Content

Taban and Erdal (2000) reported that durum wheat is more affected by boron (B) than bread wheat, and that while there is no significant yield loss in resistant varieties under high B exposure, sensitive varieties experience significant yield losses. Taban and Erdal (2000) also reported that low doses of B application increase carotenoid levels, but doses of 10 mg L^{-1} and above decrease them. Carotenoids exhibit antioxidant defenses by interacting with lipid peroxide products to terminate chain reactions, scavenging $^1O_2$, generating heat as a byproduct, reacting with excited chlorophyll to prevent $^1O_2$ formation, and dissipating excess excitation energy, thus protecting the photosynthetic system (Das & Roychoudhury, 2014). Indeed, many studies have shown that carotenoid synthesis is negatively affected by B stress due to excessive H_2O_2 production in plants under B stress (Choudhary et al., 2020). Contrary to the expected decrease in carotenoid content under B stress, our study did not observe any decrease in carotenoid content in wheat with increasing B doses. Instead, carotenoid content increased continuously with increasing B doses (150% increase compared to control at 20 mg L^{-1} B dose) (Figure 4e). The increase in carotenoid content of durum wheat against increasing stress with high B doses may be related to the plant's axillary antioxidant response capacity and the protective contribution of phenolic compounds it contains.

Effect of Boron Application on Total Protein Content

Plants produce a variety of osmolytes, including proline, glycine betaine, and trehalose, as well as many other unidentified proteins, to cope with ionic, oxidative, and osmotic stress. Common osmotic response pathways for short- or long-term stress resistance trigger the synthesis of compounds known as compatible osmolytes, which can stabilize certain cellular structures and proteins to restore osmotic potential in cells (Sayed, 2011; Aamer et al., 2018; Yang & Guo, 2018; Dustgeer et al., 2021). Sayed (2011) previously reported that salt-tolerant bean varieties have lower protein content and higher proline and amino acids compared to salt-sensitive varieties. Öz et al., (2009) reported that 5 mM boric acid application caused a 2-fold increase in the expression level of 168 genes in wheat, while 10 mM boric acid application caused at least a 2-fold change in the expression of 312 genes. In this study, we observed an approximately 15% increase in protein content in roots and stems up to 15 mg L^{-1} B application, but a decrease in total protein content at 20 mg L^{-1} B application (Figure 3f). The results suggest that boron application can enhance the total protein content of durum wheat plants. This increase in total protein content with increasing B doses is likely due to the increase in compatible osmolytes and phenolic compounds with protective effects.

Effect of Boron Application on Antioxidant Enzyme Activities

Stress factors trigger the production of intracellular reactive oxygen species (ROS). If these reactive molecules are not controlled, they can damage the plant's morphological, physiological, and chemical processes (Maoka et al., 2001; Cerville et al., 2007; Zainab et al., 2021; Temizgul, 2024).

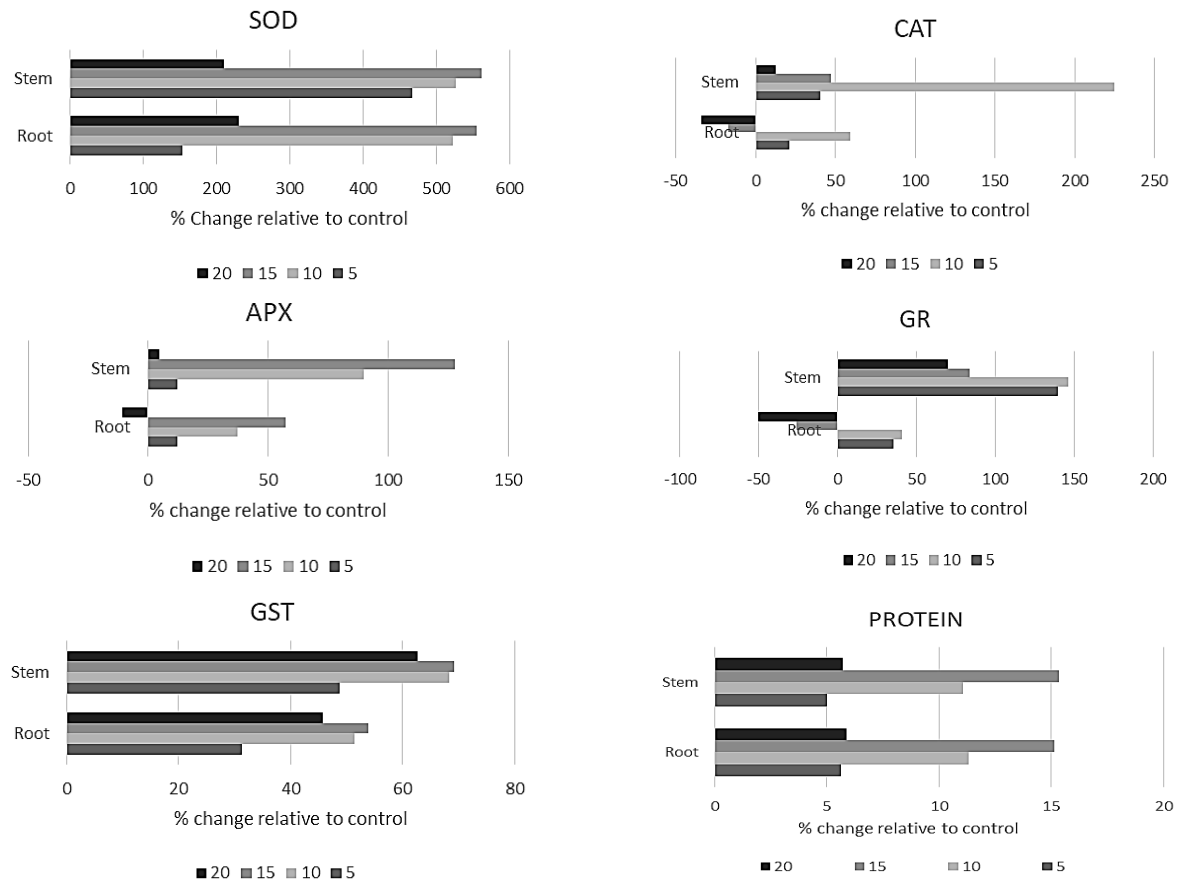


Figure 3. Change of antioxidant enzyme activities compared to control depending on boron applications

The main sites of production of these reactive molecules [hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), singlet oxygen ($^1O_2$), and superoxide anion (O_2^-)] are generally mitochondria, chloroplasts, and peroxisomes (Miller et al., 2010). Excess B also disrupts electron transport, leading to overproduction of ROS that causes membrane damage and ultimately cell death (Landi et al., 2019). The accumulation of H_2O_2 and increased lipid peroxidation in cells caused by B have been previously reported in many plants (Oboh et al., 2007; Farghaly et al., 2021). Non-enzymatic antioxidants (carotenoids, ascorbic acid, phenols, flavonoids, glutathione, and proline) that catalyze antioxidant reactions have also been reported (Das & Roychoudhury, 2014; Symes et al., 2018). Landi et al. (2019) reported that B tolerance has the potential to overstimulate the antioxidant system, which allows the plant to resist the oxidative stress caused by boron toxicity. Gill and Tuteja (2010) reported that this antioxidant system, which eliminates ROS, is activated by specific warning signals in stressed cells, while Das and Roychoudhury (2014) reported that these antioxidant enzymes are composed of various subcellular compartments.

Farooq et al. (2015) reported that when B and salt stress are applied together in rice, damage to biological membranes occurs, photosynthetic efficiency decreases, and plant biomass decreases. Sarafi et al. (2018) reported that in a study in which they applied 0.5-10 mg L^{-1} B to peppers, B toxicity decreased carbohydrate levels and increased carotenoids, flavonoids, phenolic compounds, and antioxidant enzyme capacity. Phenolic compounds, whose levels change significantly during fruit growth and ripening, not only enhance the taste and flavor of the fruit but also exhibit antioxidant properties (Serrano et al., 2010). Tepe and Aydemir (2011) reported that in a study in which they applied B to lentils and barley, SOD, GPX, and LOX activities in roots and shoots increased with B applications, while CAT and APX activities decreased. In our study, SOD, APX, and GST enzyme activities in both roots and stems of durum wheat increased up to 15 mg L^{-1} B application (SOD approximately 600%, APX 150%, and GST 90%). CAT and GR antioxidant enzymes increased up to 10 mg L^{-1} B dose (250% and 150%, respectively) and then decreased significantly (Table 2 and 3, Figure 3).

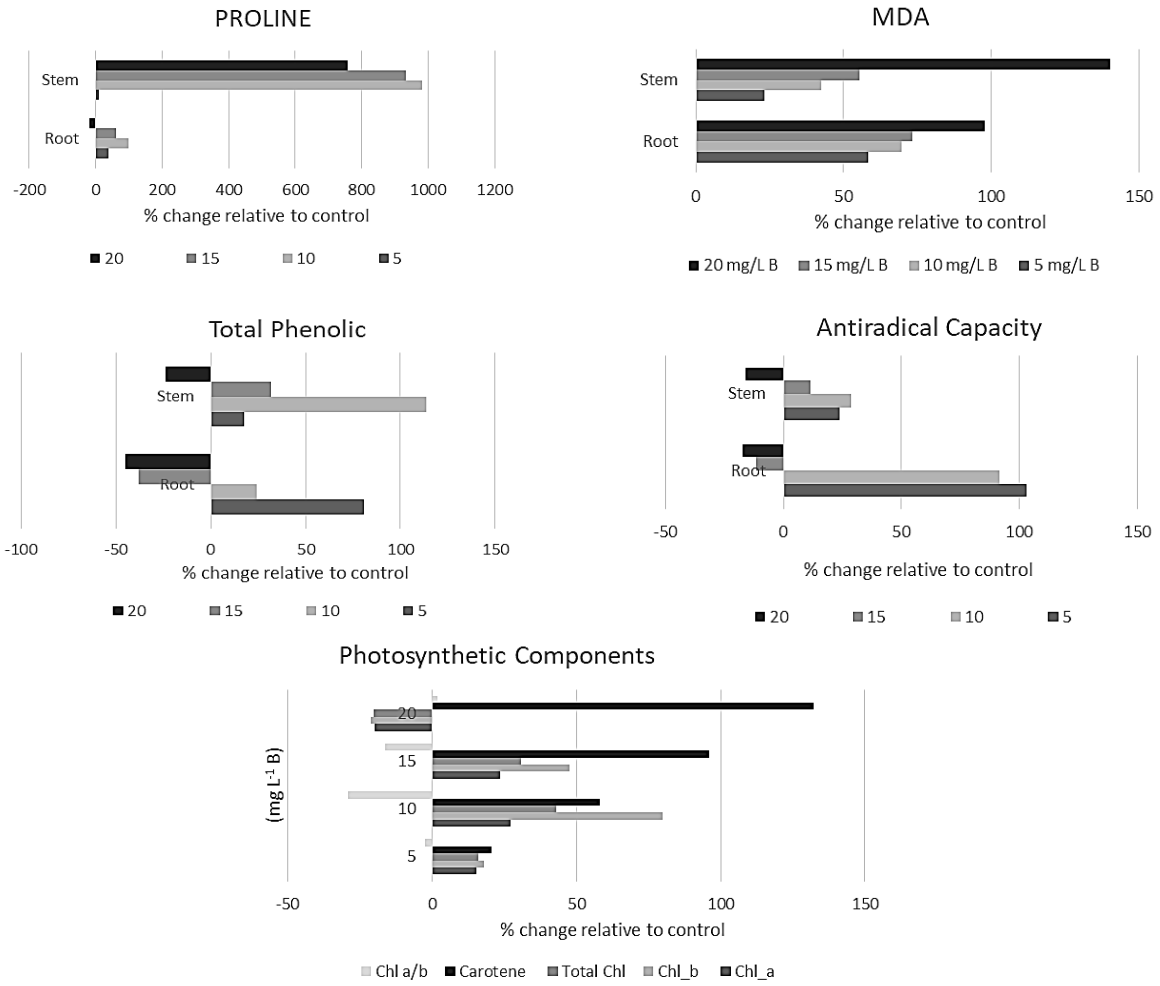
Cervilla et al. (2007) reported that ascorbic acid (AsA) plays a particularly important role in the antioxidant response to B stress. In their study analyzing the responses of tomato to B stress, they found that an increase in ascorbate concentration in tomatoes under B stress supported their hypothesis. These researchers also reported that the activities of antioxidant and ascorbate-metabolizing enzymes, particularly glutathione, were induced under B stress. Previous studies have also reported that AsA and GSH content increases in tomatoes (Farghaly et al., 2021) and canola (Metwally et al., 2018) under B stress. Eraslan et al. (2007) reported that nitrate reductase enzyme activity in particular increases under B stress. In our study, APX enzyme activity increased by approximately 60% in roots and 150% in stems in response to increasing B doses. However, GST enzyme activity increased by approximately 75% and GR activity increased by 150% compared to the control (Figure 3, Table 2 and 3).

Effect of Boron Application on Proline Accumulation

Proline is used as a non-enzymatic antioxidant in many plants to cope with the harmful effects of various ROS elements and increases significantly under stress (Verbruggen & Hermans, 2008; Das & Roychoudhury, 2014). Marco et al. (2015) also reported that proline accumulation in plants is induced either by the induction of the expression of proline synthesis genes or by the suppression of its degradation pathway genes. El-Shazoly et al. (2019) stated that the proline content of wheat seedlings increased by about 9 times under B stress. Eraslan et al. (2007) reported that increasing B levels (0.5-50 mg kg^{-1} B) in tomatoes and peppers significantly increased proline accumulation in plants. According to Tepe and Aydemir (2011), MDA, H_2O_2 and proline concentrations also increase under boron stress. In our study, proline accumulation increased significantly under B stress, especially at 10 mg L^{-1} B application, and decreased at higher B concentrations (Table 2 and 3). The increase in proline in roots was one fold compared to the control, while this increase was up to 10 fold in stems (Figure 4a). We evaluate that this high increase in proline in stems may be to prevent the loss of photosynthesis that may occur in leaves as a result of the transport of B to the leaf tips by xylem loading. In addition, the increased phenolic content with B stress may have triggered proline accumulation, especially in leaves.

Effect of Boron Application on Lipid Peroxidation (LPO) (MDA)

Abiotic stresses such as boron toxicity generally produce reactive oxygen species (ROS) in plant cells. In contrast, plants protect themselves against lipid peroxidation by activating their anti-radical properties (Maoka et al., 2001; Shetty, 2004; Oboh et al., 2007). Sarafi et al. (2018) reported that when pepper plants were subjected to B stress under greenhouse conditions, antioxidant activity and proline accumulation increased, but MDA accumulation was also high at high B doses. The authors also reported that both antioxidant capacity and MDA damage vary depending on the variety and B concentration. According to Balcı (2021), melatonin applications in strawberry seedlings grown in controlled and calcareous environments increase proline levels while decreasing lipid peroxidation (MDA). Tepe and Aydemir (2011) reported that MDA concentrations increase in plants under B stress. In the study by Eraslan et al. (2007), membrane permeability and MDA content increased significantly in tomatoes and peppers at 50 mg kg^{-1} B application. In our study, MDA accumulation increased continuously in roots and stems with increasing B doses (Table 2-3). MDA accumulation in stems increased by 150% compared to the control at 20 mg L^{-1} B application (Figure 4b).



Effect of Boron Application on Total Phenolic Content

Phenolics and flavonoids, which are secondary metabolites, are abundant in tissues and have antioxidant properties due to their ability to interact with free radicals as hydrogen donors (Sharma et al., 2019). It has been reported that boron increases phenolic accumulation while decreasing flavonoid content in tomato tissues (Cervilla et al., 2012; Farghaly et al., 2021). Sharma et al. (2019) and Sarabandi et al. (2019) have suggested that the phenylpropanoid synthesis pathway is catalyzed under abiotic/biotic stress, leading to the accumulation of phenolics with the potential to scavenge ROS. Additionally, Mishra and Sangwan (2019) reported that polyphenol oxidase helps to eliminate ROS and enhances plant tolerance to stress. Sarafi et al. (2017) reported that 5 mg L^{-1} B application increased the content of phenolic compounds in peppers in the first 7 days, but decreased after 28 days. This decrease was significantly different between sensitive and resistant varieties. Sarafi et al. (2018) reported that B application increased phenolic compounds in peppers, especially at the fruit ripening stage, and that these compounds contributed to the taste and flavor of the fruits as well as antioxidant defense. Similar results were reported by Asnin and Park (2013) in their studies on peppers treated with 0.5-10 mg L^{-1} B, where they reported that phenolic compounds increased by 3 times in mature pepper fruits under stress. In our study, the highest phenolic content was obtained in roots at 5 mg L^{-1} B application (30.629 mg GAE g^{-1}) and in stems at 10 mg L^{-1} B application (26.497 mg GAE g^{-1}) (Table 1 and 3). The increase in phenolic compounds under B stress was 80% in roots and 125% in stems compared to the control (Figure 4c).

Effect of Boron Application on Antiradical Capacity

In general, Sarafi et al. (2017) reported that antiradical capacity was observed to be low in short-term B applications, but increased in applications of 21 days or more (highest on day 28). Ardic et al. (2009) reported an increase in antioxidant capacity, phenolic metabolism, and antiradical capacity in plants under B toxicity. However, some researchers have reported no significant changes in phenol concentration and antiradical capacity in plants under high B (Keles et al., 2004). In our study, antiradical capacity was observed to be highest in roots at a B dose of 5 mg L^{-1}, as with phenolic compounds, and in stems at a B dose of 10 mg L^{-1} (75.336 and 80.982 %Inh, respectively). The increase in antioxidant capacity was 100% in roots and 25% in stems compared to the control, in contrast to phenolics (Figure 4d).

Conclusions

Although boron (B) is a very important microelement for plants, excess of it, as well as its deficiency, can cause significant metabolic disorders in plants, leading to yield and crop losses as well as plant deaths. However, the range between B deficiency and excess is very narrow and this range can vary greatly both within and between species. It is easier to correct B deficiency in agricultural lands than to eliminate B toxicity. To eliminate B toxicity in plants, B-tolerant genotypes are being screened, resistance genes are being transferred by genetic engineering, and different strategies are being tested to mitigate the effects of B stress. Due to the legal regulations and the long time required for results, the first two of these strategies are not as effective as identifying the effects of B stress in plants and developing strategies to mitigate these effects. However, testing the B stress coping potential of plants collected from genetic resources in the laboratory first and then creating greenhouse or field trials will allow for faster identification and introduction to agriculture of B-resistant species. Molecular studies that will reveal B resistance genes will also strengthen the hand of plant breeders in developing B-tolerant plants.

Declarations

Acknowledgment and/or disclaimers, if any

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data generated or analyzed during this study are included in this published article.

References

Aamer, M., Muhammad, U. H., Li, Z., Abid, A., Su, Q et al. (2018). Foliar application of glycinebetaine (GB) alleviates the cadmium (Cd) toxicity in spinach through reducing Cd uptake and improving the activity of antioxidant system. *Applied Ecology and Environmental Research* 16, 7575-7583. https://doi:10.15666/aeerr

Açar, I., Doran, I., Aslan, N. & Kalkancı, N. (2016). Boron affects the yield and quality of nonirrigated pistachio (*Pistacia vera* L.) trees. *Turkish Journal of Agriculture and Forestry* 40, 664-670. https://doi.org/10.3906/tar-1511-80

Anonimous (2023a). https://www.igc.int/en/ Access: 11 August 2023

Anonimous (2023b). https://www.tarimorman.gov.tr/sgb/Belgeler/SagMenuVeriler/TMO.pdf Access: 11 August 2023.

Ardic, M., Sekmen, A. H., Turkan, I., Tokur, S. & Ozdemir, F. (2009). The effects of boron toxicity on root antioxidant systems of two chickpea (*Cicer arietinum* L.) cultivars. *Plant and Soil* 314, 99-108. https://doi.org/10.1007/s11104-008-9709-y

Asnin, L. & Park, S. W. (2013). Isolation and analysis of bioactive compounds in Capsicum peppers. *Critical Reviews in Food Science and Nutrition* 55, 254-289. https://doi:10.1080/10408398.2011.652316

Avci, M, & Akar, T. (2005). Severity and spatial distribution of boron toxicity in barley cultivated areas of Central Anatolia and Transitional zones. *Turkish Journal of Agriculture and Forestry* 29, 377–382. https://journals.tubitak.gov.tr/agriculture/vol29/iss5/6

Balcı, G. (2021). Effects of melatonin applications on certain biochemical characteristics of strawberry seedlings in lime stress conditions. *Turkish Journal of Agriculture and Forestry* 45, 285-289. https://doi.org/10.3906/tar-2006-83

Beddowes, E. J., Faux, S. P. & Chipman, J. K. (2003). Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. *Toxicology* 187 (2–3), 101-115. https://doi.org/10.1016/S0300-483X(03)00058-1

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry* 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28 (1), 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Camacho-Cristóbal, J. J., Navarro-Gochicoa, M. T., Rexach, J., González-Fontes, A. & Herrera-Rodríguez, M. B. (2018). *Plant response to boron deficiency and boron use efficiency in crop plants. Plant micronutrient use efficiency.* Academic Press, Cambridge, pp 109-121. https://doi.org/10.1016/B978-0-12-812104-7.00007-1

Cervilla, L. M., Blasco, B., Rios, J. J., Romero, L. & Ruiz, J. M. (2007). Oxidative stress and antioxidants in tomato (*Solanum lycopersicum*) plants subjected to Boron toxicity. *Annals of Botany* 100, 747-756. https://doi:10.1093/aob/mcm156

Cervilla, L. M., Blasco, B., Rios, J. J., Rosales, M. A., S'anchez-Rodr'ıguez, E., Rubio-Wilhelmi, M. M., Romero, L. & Ruiz, J. M. (2012). Parameters symptomatic for boron toxicity in leaves of tomato plants. *Journal of Botany*, 1-17. https://doi:10.1155/2012/726206

Chen, M., Mishra, S., Heckathorn, S., Frantz, J. & Krause, C. (2014). Proteomic analysis of *Arabidopsis thaliana* leaves in response to acute boron deficiency and toxicity reveals effects on photosynthesis, carbohydrate metabolism, and protein synthesis. *Journal of Plant Physiology* 171(3-4), 235–42. https://doi:10.1016/j.jplph.2013.07.008

Choudhary, S., Zehra, A., Naeem, M., Khan, M. & Aftab, T. (2020). Effects of boron toxicity on growth, oxidative damage, antioxidant enzymes and essential oil fingerprinting in *Mentha arvensis* and *Cymbopogon flexuosus*. *Chemical and Biological Technologies in Agriculture* 7(1), 1–11. https://doi:10.1186/s40538-019-0175-y

Das, K. & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science* 2, 53. https://doi:10.3389/fenvs.2014.00053

Duman, F., Aksoy, A., Aydin, Z. & Temizgul, R. (2011). Effects of exogenous glycinebetaine and trehalose on cadmium accumulation and biological responses of an aquatic plant (*Lemna gibba* L.). *Water, Air and Soil Pollution* 217, 545-556. https://doi.org/10.1007/s11270-010-0608-5

Dustgeer, Z., Seleiman, M. F., Imran, K., Chattha, M. U., Alhammad, B. A. et al. (2021). Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 49(1), 12248. https://doi:10.15835/nbha49112248

El Sayed, H. E. A. (2011). Influence of NaCl and Na_2SO_4 treatments on growth development of broad bean (*Vicia faba* L.) plant. *Journal of Life Science*, 5, 513-523.

El-Shazoly, R. M., Metwally, A. A. & Hamada, A. M. (2019). Salicylic acid or thiamin increases tolerance to boron toxicity stress in wheat. *Journal of Plant Nutrition* 42(7), 702–22. https://doi:10.1080/01904167.2018.1549670

Eraslan, F., Inal, A., Gunes, A. & Alpaslan, M. (2007). Boron toxicity alters nitrate reductase activity, proline accumulation, membrane permeability, and mineral constituents of tomato and pepper plants. *Journal of Plant Nutrition*, 30(6), 981-994. https://doi.org/10.1080/15226510701373221

Farghaly, F. A., Salam, H., Hamada, A. M. & Radi, A. A. (2021). The role of benzoic acid, gallic acid and salicylic acid in protecting tomato callus cells from excessive boron stress. *Scientia Horticulturae* 278, 109867. https://doi:10.1016/j.scienta.2020.109867

Farooq, M. A., Saqib, Z. A. & Akhtar, J. (2015). Silicon mediated oxidative stress tolerance and genetic variability in rice (*Oryza sativa* L.) grown under combined stress of salinity and boron toxicity. *Turkish Journal of Agriculture and Forestry* 39, 718-729. https://doi.org/10.3906/tar-1410-26

Gill, S. S. & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry* 48(12), 909-30. https://doi:10.1016/j.plaphy.2010.08.016

Gunes, A., Alpaslan, M., Ozcan, H. & Cikili, Y. (2000). Tolerance to boron toxicity of maize (*Zea mays* L.) cultivars widely cultivated in Turkey. *Turkish Journal of Agriculture and Forestry* 24, 277-282. https://journals.tubitak.gov.tr/agriculture/vol24/iss2/17

Gupta, U. C., Jame, Y. W., Campbell, C. A., Leyshon, A. J. & Nicholaichuck, W. (1985). Boron toxicity and deficiency: a review. *Canadian Journal of Soil Science* 65, 381-409. https://doi.org/10.4141/cjss85-044

Gupta, U. C. (2007). *Boron. In Handbook of plant nutrition*, ed. A. V. Barker and D. J. Pilbeam, pp. 241-77. Boca Raton, Florida, USA: CRC press. https://doi.org/10.1201/9781420014877

Han, S., Tang, N., Jiang, H. X., Yang, L. T., Li, Y. & Chen, L. S. (2009). CO_2 assimilation, photosystem II photochemistry, carbohydrate metabolism and antioxidant system of citrus leaves in response to boron stress. *Plant Science* 176(1), 143–53. https://doi:10.1016/j.plantsci.2008.10.004

Hoagland, D. R. & Arnon, D. I. (1950). *The water-culture method for growing plants without soil.* University of California, Berkeley: College of Agriculture, 1938, 347. http://hdl.handle.net/2027/uc2.ark:/13960/t51g1sb8j

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine* 9(6), 515-540. https://doi:10.1016/0891-5849(90)90131-2

Kaya, C., Sarıoğlu, A., Ashraf, M., Alyemeni, M. & Ahmad, P. (2020). Gibberellic acid-induced generation of hydrogen sulfide alleviates boron toxicity in tomato (*Solanum lycopersicum* L.) plants. *Plant Physiology and Biochemistry* 153, 53-63. https://doi:10.1016/j.plaphy.2020.04.038

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. https://doi:10.1007/s11105-016-1008-9

Keles, Y., Öncel, I. & Yenice, N. (2004). Relationship between boron content and antioxidant compounds in Citrus leaves taken from fields with different water source. *Plant and Soil* 265, 345-353. https://doi.org/10.1007/s11104-005-0646-8

Landi, M., Margaritopoulou, T., Papadakis, I. E. & Araniti, F. (2019). Boron toxicity in higher plants: an update. *Planta* 250, 1011-1032. https://doi.org/10.1007/s00425-019-03220-4

Lovatt, C. J. & Dugger, W. M. (1984). Boron. In: Frieden, E. (eds) Biochemistry of the essential ultratrace elements. *Biochemistry of the Elements*, vol 3. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-4775-0_17

Madhava, R. K. & Sresty, T. V. (2000). Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science* 157, 113-128. https://doi.org/10.1016/S0168-9452(00)00273-9

Mahalakshmi, V., Yau, S. K., Ryan, J. & Peacock, J. M. (1995). Boron toxicity in barley (*Hordeum vulgare* L.) seedlings in relation to soil temperature. *Plant and Soil* 177, 151-156. https://doi.org/10.1007/BF00010121

Maoka, T., Mochida, K., Kozuka, M., Ito, Y., Fujiwara, Y., Hashimoto, K., Enjo, F., Ogata, M., Nobukuni, Y., Tokuda, H. et al. (2001). Cancer chemopreventive activity of carotenoids in the fruits of red paprika *Capsicum annuum* L. *Cancer Letters* 172, 103-109. https://doi:10.1016/s0304-3835(01)00635-8

Marco, F., Bitri'an, M., Carrasco, P., Rajam, M., Alc'azar, R. & Tiburcio, A. (2015). Genetic engineering strategies for abiotic stress tolerance in plants. In *Plant biology and biotechnology* pp. 579–609. New Delhi: Springer. https://doi:10.1007/978-81-322-2283-5_29

Mattos-Jr, D., Hippler, F. W., Boaretto, R. M., Stuchi, E. S. & Quaggio, J. A. (2017). Soil boron fertilization: The role of nutrient sources and rootstocks in citrus production. *Journal of Integrative Agriculture* 16(7), 1609-16. https://doi:10.1016/S2095-3119(16)61492-2

Metwally, A. M., Radi, A. A., El-Shazoly, R. M. & Hamada, A. M. (2018). The role of calcium, silicon and salicylic acid treatment in protection of canola plants against boron toxicity stress. *Journal of Plant Research* 131(6), 1015-28. https://doi:10.1007/s10265-018-1008-y

Miller, G., Suzuki, N., Ciftci-Yilmaz, S. & Mittler, R. O. (2010). Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, Cell and Environment* 33(4), 453-67. https://doi:10.1111/j.1365-3040.2009.02041.x

Mishra, B. & Sangwan, N. (2019). Amelioration of cadmium stress in Withania somnifera by ROS management: Active participation of primary and secondary metabolism. *Plant Growth Regulation* 87(3), 403-412. https://doi:10.1007/s10725-019-00480-8

Misra, N. & Gupta, A. (2006). Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkoloid content in *Catarantus roseus* seedlings. *Journal of Plant Physiology* 164, 11e18. https://doi.org/10.1016/j.jplph.2005.02.011

Miwa, K., Takano, J., Omori, H., Seki, M., Shinozaki, K. & Fujiwara, T. (2007). Plants tolerant of high boron levels. *Science* 318(5855), 1417. https://doi.org/10.1126/science.1146634

Oboh, G., Batista, J. & Bocha, T. (2007). Polyphenols in red pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *European Food Research and Technology* 225, 239-247. https://doi.org/10.1007/s00217-006-0410-1

Oz, M. F., Yilmaz, R., Eyidoğan, F., Graff, L. D., Yücel, M. & Oktem, H. A. (2009). Microarray analysis of late response to boron toxicity in Barley (*Hordeum vulgare* L.) Leaves. *Turkish Journal of Agriculture and Forestry* 33, 191-202. https://doi.org/10.3906/tar-0806-22

Ozturk, M., Sakcali, S., Gucel, S. & Tombuloglu, H. (2010). Boron and Plants. In: Ashraf, M., Ozturk, M., Ahmad, M. (eds) *Plant Adaptation and Phytoremediation*. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9370-7_13

Papadakis, I., Tsiantas, P., Tsaniklidis, G., Landi, M., Psychoyou, M. & Fasseas, C. (2018). Changes in sugar metabolism associated to stem bark thickening partially assist young tissues of *Eriobotrya japonica* seedlings under boron stress. *Journal of Plant Physiology* 231, 337-45. https://doi:10.1016/j.jplph.2018.10.012

Papadakis, I., Tsiantas, P., Gerogiannis, O., Vemmos, S. & Psychoyou, M. (2015). Photosynthetic activity and concentration of chlorophylls, carotenoids, hydrogen peroxide and malondialdehyde in loquat seedlings growing under excess boron conditions. *Acta Horticulturae* 1092, 221-226. https://doi:10.17660/ActaHortic.2015.1092.33

Parks, J. L. & Edwards, M. (2005). Boron in the environment. *Critical Reviews in Environmental Science and Technology* 35(2), 81-114. https://doi.org/10.1080/10643380590900200

Princi, M. P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F. & Abenavoli, M. R. (2016). *Boron toxicity and tolerance in plants: recent advances and future perspectives. Plant metal interaction.* Elsevier, Amsterdam, pp 115-147. https://doi:10.1016/B978-0-12-803158-2.00005-9

Rab, A. & Haq, I. (2012). Foliar application of calcium chloride and borax influences plant growth, yield, and quality of tomato (*Lycopersicon esculentum* Mill.) fruit. *Turkish Journal of Agriculture and Forestry* 36, 695-701. https://doi.org/10.3906/tar-1112-7

Reid, R. J. & Fitzpatrick, K. L. (2009). Influence of leaf tolerance mechanisms and rain on boron toxicity in barley and wheat. *Plant Physiology* 151, 413-420. https://doi:10.1104/pp.109.141069

Reid, R. J. (2013). Boron toxicity and tolerance in crop plants. In N. Tuteja & S. S. Gill (Ed.), *Crop improvement under adverse conditions*. Springer. https://doi.org/10.1007/978-1-4614-4633-0_15

Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. C. R. & Graham, R. D. (2004). A critical analysis of the causes of boron toxicity in plants. *Plant, Cell and Environment* 25, 1405-1414. https://doi.org/10.1111/j.1365-3040.2004.01243.x

Risom, L., Møller, P. & Loft, S. (2005). Oxidative stress–induced DNA damage by particulate air pollution. *Mutation Research* 592, 119-137. https://doi:10.1016/j.mrfmmm.2005.06.012

Sarabandi, M., Farokhzad, A., Mandoulakani, B. & Ghasemzadeh, R. (2019). Biochemical and gene expression responses of two Iranian grape cultivars to foliar application of methyl jasmonate under boron toxicity conditions. *Scientia Horticulturae* 249, 355-63. https://doi:10.1016/j.scienta.2019.02.019

Sarafi, E., Siomos, A., Tsouvaltzis, P., Chatzissavvidis, C. & Therios, I. (2018). Boron and maturity effects on biochemical parameters and antioxidant activity of pepper (*Capsicum annuum* L.) cultivars. *Turkish Journal of Agriculture and Forestry* 42(4), 237-47. https://doi:10.3906/tar-1708-31

Sarafi, E., Siomos, A., Tsouvaltzis, P., Chatzissavvidis, C. & Therios, I. (2017). Boron toxicity effects on grafted and non-grafted pepper (*Capsicum annuum*) plants. *Journal of Soil Science and Plant Nutrition* 17(2), 441-460. https://doi:10.4067/S0718-95162017005000032

Savic, J., Römheld, V. & Nikolic, M. (2012). Oilseed rape (*Brassica napus* L.) genotypic variation in response to boron deficiency. *Turkish Journal of Agriculture and Forestry* 36, 408-414. https://doi.org/10.3906/tar-1109-43

Scalbert, A., Monties, B. & Janin, G. (1989). Tannins in wood: comparison of different estimation methods. *Journal of Agriculture and Food Chemistry* 37, 1324-1329. https://doi.org/10.1021/jf00089a026

Serrano, M., Zapata, P. J., Castillo, S., Guillen, F., Martinez-Romero, D. & Valero, D. (2010). Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. *Food Chemistry* 118, 497-503. https://doi.org/10.1016/j.foodchem.2009.05.006

Shah, A., Wu, X., Ullah, A., Fahad, S., Muhammad, R., Yan, L. & Jiang, C. (2017). Deficiency and toxicity of boron: Alterations in growth, oxidative damage and uptake by citrange orange plants. *Ecotoxicology and Environmental Safety* 145, 575-582. https://doi:10.1016/j.ecoenv.2017.08.003

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules* 24(13), 2452. https://doi:10.3390/molecules24132452

Shetty, K. (2004). Role of proline-linked pentose phosphate pathway in biosynthesis of plant phenolics for functional food and environmental applications: a review. *Process Biochemistry* 39, 789-804. https://doi.org/10.1016/S0032-9592(03)00088-8

Soylu, S., Sade, B., Topal, A., Akgün, N., Gezgin, S., Hakkı, E. E. & Babaoğlu, M. (2005). Responses of irrigated durum and bread wheat cultivars to boron application in a low boron calcareous soil. *Turkish Journal of Agriculture and Forestry* 29, 275-286. https://journals.tubitak.gov.tr/agriculture/vol29/iss4/7

Stiles, A. R., Liu, C., Kayama, Y., Wong, J., Doner, H., Funston, R. & Terry, N. (2011). Evaluation of the boron tolerant grass, *Puccinellia distans*, as an initial vegetative cover for the phytorestoration of a boron-contaminated mining site in southern California. *Environmental Science and Technology* 45(20), 8922-8927. https://doi.org/10.1021/es200879a

Symes, A., Shavandi, A., Zhang, H., Mohamed-Ahmed, I., Al-Juhaimi, F. & Bekhit, A. (2018). Antioxidant activities and caffeic acid content in New Zealand asparagus (*Asparagus officinalis*) roots extracts. *Antioxidants* 7(4), 52. https://doi:10.3390/antiox7040052

Taban, S. & Erdal, I. (2000). Effects of boron on growth of various wheat varieties and distribution of boron in aerial part. *Turkish Journal of Agriculture and Forestry* 24, 255-262. https://journals.tubitak.gov.tr/agriculture/vol24/iss2/15

Tanaka, M. & Fujiwara, T. (2008). Physiological roles and transport mechanisms of boron: Perspectives from plants. *Pflugers Archiv - European Journal of Physiology* 456(4), 671-677. https://doi.org/10.1007/s00424-007-0370-8

Taşcı, R., Özercan, B., Tarhan, S., Söylemez, E., Hamarat, T., Karabak, S. & Bolat, M. (2023).TAGEM tarafından geliştirilen buğday çeşitlerinin üreticiler açısından değerlendirilmesi; Kayseri ili örneği. *Ziraat Mühendisliği,* (377), 4-18. https://doi.org/10.33724/zm.1259739

Temizgul, R. (2024). Antioxidant defense responses of hulled wheat varieties to the addition of sodium and potassium salts and exogenous glycine-betaine, and evaluation of the usability of these hulled wheats in the remediation of saline soils. In Preprint, available at *Research Square* https://doi.org/10.21203/rs.3.rs-4368507/v1

Temizgul, R., Ciftci, B., Kardes, Y. M., Kara, R., Temizgul, S., Yilmaz, S. & Kaplan, M. (2024). Comparison of different hulled wheat genotypes in terms of yield, morphological, and nutritional properties. *Genetic Resources and Crop Evolution* https://doi.org/10.1007/s10722-024-01994-5

Temizgul, R., Kaplan, M., Kara, R. & Yilmaz, S. (2016). Effects of salt concentrations on antioxidant enzyme activity of grain sorghum. *Current Trends in Natural Sciences* 5, 171-178. https://api.semanticscholar.org/CorpusID:89938550

Tepe, M. & Aydemir, T. (2011). Antioxidant responses of lentil and barley plants to boron toxicity under different nitrogen sources. *African Journal of Biotechnology* 10(53), 10882-10891. https://doi:10.5897/AJB10.1076

Torun, A., Yazici, A., Erdem, H. & Çakmak, I. (2006). Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes. *Turkish Journal of Agriculture and Forestry* 30, 49-58. https://journals.tubitak.gov.tr/agriculture/vol30/iss1/6

Turan, M. A., Taban, S., Kayin, G. B. & Taban, N. (2018). Effect of boron application on calcium and boron concentrations in cell wall of durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat. *Journal of plant nutrition* 41(11), 1351-1357. https://doi.org/10.1080/01904167.2018.1450424

Verbruggen, N. & Hermans, C. (2008). Proline accumulation in plants: A review. *Amino Acids* 35(4), 753-759. https://doi:10.1007/s00726-008-0061-6

Yalın, S. B., Orman, Ş., Hüseyin, O. K. & Özgür, A. Z. (2019). Antalya ilinde yetiştirilen kışlık ekmeklik buğdayın bor beslenme durumunun belirlenmesi. *Mediterranean Agricultural Sciences* 32, 157-161. https://doi.org/10.29136/mediterranean.559042

Yang, Y. & Guo, Y. (2018). Elucidating the molecular mechanisms mediating plant salt-stress responses. *New Phytologist* 217, 523-539. https://doi.org/10.1111/nph.14920

Yau, S. K., Nachit, M. M., Hamblin, J. & Ryan, J. (1995). Phenotypic variation in boron–toxicity tolerance at seedling stage in durum wheat (*Triticum durum*). *Euphutica* 83, 185-191. https://doi.org/10.1007/BF01678128

Yau, S. K. & Ryan, J. (2008). Boron toxicity tolerance in crops: A viable alternative to soil amelioration. *Crop Science* 48(3), 854-865. https://doi.org/10.2135/cropsci2007.10.0539

Yılmaz, A. M. & Tomar, O. (2022). Türkiye'de buğdayın kendi kendine yeterlilik ve ithalata bağımlılık açısından değerlendirilmesi. *Avrupa Bilim ve Teknoloji Dergisi* (41), 449-456. https://doi:10.31590/ejosat.1192874

Yilmaz, S., Temizgül, R., Yürürdurmaz, C. & Kaplan, M. (2020). Oxidant and antioxidant enzyme response of redbine sweet sorghum under NaCl salinity stress. *Bioagro* 32(1), 31-38. https://revistas.uclave.org/index.php/bioagro/article/view/2684

Yilmaz, S. H., Kaplan, M., Temizgul, R. & Yilmaz, S. (2017). Antioxidant enzyme response of sorghum plant upon exposure to Aluminum, Chromium and Lead heavy metals. *Turkish Journal of Biochemistry* 42(4), 503-512. https://doi.org/10.1515/tjb-2016-0112

Yüksel, F., Koyuncu, M. & Sayaslan, A. (2011). Makarnalık buğday (*Triticum durum*) kalitesi. *Türk Bilimsel Derlemeler Dergisi*, (2), 5-31.

Zainab, Q., Tanees, C., Xiongming, D., Lori, H. & Tehseen, A. (2021). Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* L. in response to extreme abiotic conditions. *Journal of Cotton Research* 4(1), 1-9. https://doi:10.1186/s42397-021-00086-4