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Effect of Boric Acid Application on Antioxidant Enzymes Activity and Gene Expression in Safflower (*Carthamus tinctorius* L.) Cultivars

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HIGHLIGHTS

- In this study, three safflower cultivar (Balcı, Dinçer, Remzibey) were used.
- Four different concentrations of boric acid (0, 5, 10, 15 mM) were applied to the plants.
- SOD, CAT, APX, GR activities of plant samples were investigated by spectrophotometric method and expression levels of these enzymes by RT-qPCR.
- The Remzibey variety was found to have the lowest antioxidant capacity to deal with oxidative stress compared to other varieties.

Abstract: Boron is one of the most important micronutrients for plants. Plants may suffer from deficiency or with boron toxicity. Boron plays a role in significant physiological and biochemical events in plants such as synthesis of the cell wall, membrane integrity, antioxidation, transport of photosynthesis products to other organs of the plant. The enzyme activities of ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) in three different safflower cultivars (Balcı, Dinçer and Remzibey) subjected to different boric acid concentrations (0, 5, 10, 15 mM) were measured spectrophotometrically, and the changes in the expression levels of the genes that encode these enzymes were obtained by quantitative RT-qPCR. When both the spectrophotometric measurements and the mRNA values were evaluated together, both the activity and mRNA values of APX and GR enzymes were found to be the highest in the Dinçer cultivar among the varieties treated with 15 mM boric acid, while the lowest values of these enzymes were determined in the Remzibey cultivar. According to the RT-qPCR results, the lowest SOD and CAT values were determined in Remzibey. The Dinçer cultivar was found to have the highest antioxidant capacity (APX, GR) to cope with oxidative stress caused by boric acid application at high concentrations. The sensitive Remzibey cultivar was found to have the lowest antioxidant capacity to cope with such oxidative stress. Balcı was found to be closer to Dinçer than to Remzibey in terms of boron tolerance. As a result, the boron-sensitive cultivar had low antioxidant activity.

Keywords: abiotic stress; superoxide dismutase; catalase; ascorbate peroxidase; glutathione reductase; RT-qPCR.

INTRODUCTION

Plants are under the influence of many stress factors (abiotic or biotic) during their life that hinders their growth, development and proliferation in the areas where they grow naturally [1]. One of the abiotic factors that cause these changes is sub-optimal levels of plant nutrients in the soil. The intake of these nutrients by plants depends on many factors, such as species, age, root growth, genotype, and soil nutrient availability. Boron, which is one of the most important micronutrients, is the nutrient element to which plants react the most [2]. Boron can be used by plants in the forms of H_3BO_3 (boric acid), $B(OH)_3$ or $B(OH)^{4-}$ (borate anion) [3].

There are many negative effects of boron deficiency, such as delayed or even arrested growth, short height, and decrease or complete cessation of fruit formation [4]. Though boron is an absolutely essential micronutrient for plants, the boron tolerance limits in plants cannot be determined precisely, and even a small amount of excess boron can cause severe damage to plants [5]. Plants exposed to excess boron show delayed growth, leaf scorch (chlorotic and necrotic burns on old leaves), leaf curl, decrease in the number, size and weight of fruits, decreased bud formation, irregular transpiration, and deformed leaves [6-8]. Boron toxicity leads to oxidative damage in addition to damage to the growth, development and permeability of membranes [9]. Boron toxicity, both naturally found in the soil and caused by human activities, is an important agricultural problem limiting plant yield in various regions of the world [10].

Boron plays an important role in many metabolic reactions, such as RNA metabolism, the regulation of water use and transport, oxine and phenol metabolism, the structural and functional properties of biomembranes, carbohydrate and protein metabolism, pollen germination and pollen tube growth, fruit ripening, root growth, nucleic acid, pectin and ATP synthesis, and the protection of conductive tissues [2, 11-14]. Although these roles are known, the role of boron in plant growth and development is still not fully understood.

It is known that cultivars of one species respond differently to the same stresses. Depending on the duration and amount of the stress, these differences may be at the ecological, physiological and molecular levels. These changes make the plant tolerant or sensitive to the environment in which it lives. Today, due to changing ecological conditions, it is important to produce stress-tolerant plants.

Safflower is a plant suitable for growth in arid regions [15, 16]. Safflower is mainly cultivated for the oil contained in its seeds, but the plant can also be used in many different fields, such as medicine and cosmetics, and for other industrial and ornamental purposes [17]. Safflower cultivation is important for the utilization of marginal soils due to the high growth capacity and low production cost of this culture. Determining varieties that are tolerant and sensitive to abiotic stresses is important as safflower cultivation will increase due to changing ecological conditions.

In our previous study, the effects of different boric acid concentrations on the ecological parameters of safflower plants were investigated [18]. According to the boric acid tolerance index, Remzibey (0.27) was the most sensitive cultivar to boron, and Balcı (0.63) was the most tolerant cultivar [18]. There are not enough data in the literature regarding the molecular reasons of the results obtained in our study above. In studies conducted so far, it has been determined that varieties with tolerance to high doses of micronutrient elements such as Al, Zn have higher antioxidant enzymes than sensitive varieties [19-21]. For this reason, there is a need to study whether boric acid tolerant varieties of safflower cultivars could show increased antioxidant enzyme activity in boron excess. Therefore, in this study, the changes in the activities of enzymes (APX, CAT, GR and SOD) and their gene expression levels were investigated in boron-tolerant and boron-sensitive safflower cultivars (Balcı, Dinçer and Remzibey) that were treated with boric acid applications at different concentrations (0, 5, 10, 15 mM). The aim of this study was to test the hypothesis that antioxidant enzymatic systems are up-regulated in boron toxicity and function to protect boron-tolerant safflower varieties in stress conditions.

MATERIAL AND METHODS

Three different safflower cultivars (Balcı-tolerant to Boron, Dinçer-tolerant to Boron, Remzibey-sensitive to Boron) were used [18]. Seeds were obtained from Eskisehir Passage Zone Agricultural Research Institute (ETAE) and were sterilized before planting. Randomly selected seeds were soaked in 10% NaOCl (Merck) for 10 minutes and then washed 3 times with distilled water [22]. Sterile seeds were subjected to imbibition

for 3 hours in boric acid (Merck) solutions (5, 10, 15 mM) before planting into the vials [23]. After sowing, the seeds were watered with these solutions every other day until the end of the experiment. The vials used in the experiment had 160 mL compartments, and 55 gr sterile peat soil was used for each compartment. Five seeds were placed in each vial compartment. After the sowing process was completed, the vials were kept at 25±1°C in a growth chamber for 10 weeks with a 16 hour day/8 hour dark photoperiod. Each vial compartment was watered with 20 mL of solution every other day. The leaves of the plant samples were collected after 10 weeks and stored at -80°C until they were used.

Extraction of leaf samples

For extraction, 0.5 grams of fresh leaf samples were ground to powder in liquid nitrogen and homogenised with 5 mL of extraction buffer (50 mM K_2HPO_4, 50 mM KH_2PO_4, 1% PVP, 1 mM EDTA; as well as 5 mM ascorbic acid for ascorbate peroxidase activity determination). The resulting homogenate was centrifuged at 20000 g for 20 minutes at +4°C (Thermal), and the supernatant was removed and held at -80°C (Thermo) for enzyme activity determination [24, 25].

Determination of lipid peroxidation

Lipid peroxidation in plants is expressed as malondialdehyde (MDA) content. Samples (0.5 g) were homogenised in an aqueous solution of trichloroacetic acid (10% w/v), and aliquots of the filtrates were heated in 0.5% thiobarbituric acid. The amount of MDA was determined from the absorbance at 532 and 600 nm.

Determination of protein amount

In this study, enzyme activities were calculated according to Bradford (1976), depending on their protein content [26]. For this reason, the protein content of all leaf extracts was determined first. Protein levels of leaf samples of Balcı, Dinçer and Remzibey cultivars were determined by using bovine serum albumin (BSA) (Sigma) standard in the control group and three different concentrations of boric acid (Merck) [26]. A graph was obtained with the absorbance values of the standards. The concentrations of leaf extracts were determined using the equation obtained from the graph ($y = 0.2308x - 0.2183$, $R2 = 0.9922$).

Determination of superoxide dismutase (SOD) activity

SOD activity was determined spectrophotometrically with a previously described method based on the reduction of nitroblue-tetrazolium (NBT) [27, 28]. The total SOD activity was calculated as U mg^{-1} protein. The activity of an enzyme unit is defined as the amount of SOD required to inhibit NBT reduction by 50%.

Determination of catalase (CAT) activity

The method used for the determination of CAT activity was based on the spectrophotometric monitoring of the absorbance at 240 nm as a result of the decomposition of hydrogen peroxide by water and oxygen due to CAT activity [29].

Determination of ascorbate peroxidase (APX) activity

For the determination of APX activity, 20 µL of enzyme extract was added to 1 mL of APX reaction buffer and mixed by vortexing. The kinetic measurement of this mixture was taken with the spectrophotometer (AgileSpec) at a wavelength of 290 nm for 3 times at 15 second intervals, and the reductions in absorbance were recorded [30].

Determination of glutathione reductase (GR) activity

To determine the GR activity, 20 µL enzyme extract was added to 1 mL GR reaction buffer and mixed by vortexing (Wisemix). The kinetic measurement of this mixture was taken with the spectrophotometer (AgileSpec) at 340 nm for 3 times at 15 second intervals, and the reductions in absorbance were recorded [31].

Total RNA isolation and cDNA synthesis

Total RNA was isolated from the fresh leaves of the plant samples. A total of 100 mg of fresh leaf sample was triturated with liquid nitrogen and taken into a sterile Eppendorf tube. Total RNA isolation was done using the trizol method. The quality of the RNA samples was determined with the nanodrop spectrophotometer (Shimadzu BioSpec-nano) and RNA samples of sufficient quality and quantity were converted to cDNA. Before converting RNA samples to cDNA, samples were treated with DNAase (ThermoFisher EN0521) to avoid DNA contamination. Isolated RNA samples were transformed into cDNA using the First Strand cDNA synthesis kit (BioLabs, Catalogue No: E6300S). 5µL of RNA was used for each cDNA sample.

Gene expression analysis

cDNA samples obtained by reverse transcription PCR were used as templates for RT-qPCR. Real-time quantitative PCR (RT-qPCR) was performed using the GoTaq 1-Step RT-qPCR System (Catalogue No: A6020) kit from Promega (Madison, USA). The materials required for RT-qPCR were prepared as described in the kit manual. RT-qPCR was performed for each cDNA sample with both specific primers belonging to the normalized GAPDH gene and SOD, CAT, APX, and GR genes. Δ-Ct values were calculated with regard to GAPDH (Figure 1). This experiment was carried out in three replicates.

Primary design for RT-qPCR

The primers specific to the genes to be used in PCR experiments were designed with the Primer3 program (Table 1) [32].

Statistical analysis and evaluation of results

All experiments were performed as three independent replicates. Experimental results with control groups and safflower cultivars exposed to three different concentrations of boric acid were statistically evaluated by using Tukey tests and one-way ANOVA (SPSS 21.0).

RESULTS

MDA content in safflower leaves

The most basic indicator of oxidative stress is lipid peroxidation. In the study, the lowest MDA level was determined in 5 mM and the highest MDA level in 15 mM boric acid application. An increase in MDA level was found in parallel with the increase in boric acid. The highest MDA level in 15 mM boric acid was determined in Balcı cultivar (3.93 nmol g^{-1} FW) and the lowest MDA level in Dinçer cultivar (3.68 nmol g^{-1} FW) (Figure 2).

Amount of total protein in safflower leaves

The protein content of safflower cultivars was determined following application of different concentrations of boric acid. It was determined that there were differences in the amount of protein when the cultivars and the concentrations were compared. Balcı showed 6.4 µg mL^{-1} protein in the 10 mM boric acid treatment, which was significantly higher (28%) than the control levels (5 µg mL^{-1} protein). However, compared with the control, Remzibey showed significantly lower protein contents (26.3%). The protein content of Remzibey was 2.8 µg mL^{-1} at the 10 mM boric acid concentration (Figure 3). When the boron concentration was increased to 15 mM, compared with that in the control, the protein content in Balcı and Dinçer decreased by 20% and 5%, respectively, and that of Remzibey increased by 10.5%. Balcı had a higher protein content than the other cultivars under all stress conditions.

Total SOD, CAT, APX and GR activities of leaf tissues

When SOD activities of the cultivars were compared with those of the control group, SOD activity decreased in the Balcı cultivar and increased in the Remzibey cultivar as the concentration of boric acid increased. The SOD activity of the Dinçer cultivar showed an increase with 5 and 10 mM boric acid application (145 and 169 U mg^{-1} protein, respectively) but showed a significant decrease with 15 mM boric acid application (22 U mg^{-1} protein) (Figure 4a). As a result, when 15 mM boric acid was applied, the SOD activity of Balcı decreased 65%, but the SOD activity of Remzibey increased 137.5% compared to the control levels.

While the SOD activity of Dinçer increased 62.5% under the 10 mM boric acid concentration, it decreased 78.8% under the 15 mM boric acid concentration.

CAT activities decreased in a concentration-dependent manner in all three cultivars at the 5 and 10 mM boric acid concentrations compared to the control levels. However, while the CAT activity of the Dinçer cultivar decreased by approximately 50% at the 15 mM boric acid concentration compared with the control and 10 mM concentration, Balcı and Remzibey showed an increase in CAT activity of approximately 2- and 4-fold, respectively, at 15 mM boric acid compared with levels at 10 mM boric acid (Figure 4b).

The APX activity of the Dinçer and Remzibey cultivars was found to be increased at all boric acid concentrations compared to the control levels. In the Balcı cultivar, when 5 and 10 mM boric acid was applied, APX enzyme activity increased compared to the control levels but decreased when 15 mM boric acid was applied (Figure 4c). APX activity increased with the application of 15 mM boric acid by 62.5-fold in Dinçer and 17-fold in Remzibey compared to the control levels. However, APX activity decreased 15.6% in Balcı with 15 mM boric acid application.

When the cultivars were compared, it was determined that there was no change in the GR activity of Balcı. In the Dinçer cultivar, decreases in GR activity at 5 and 10 mM (respectively 55.5%, 44.4%) and increases in GR activity at 15 mM (50%) were observed compared to those of the control. In Remzibey, GR activity increased 63%, 36%, 45.5% at the 5, 10 and 15 mM boric acid concentrations, respectively, compared to the control levels (Figure 4d). When assessed in general, it can be stated that SOD, CAT, APX and GR enzyme activities are higher in the Balcı cultivar than in the other two cultivars both when subjected to stress with boron and at control levels.

Change in SOD, CAT, APX and GR mRNA level in leaves of safflower cultivars

The levels of SOD mRNA in the Balcı cultivar decreased with the application of 5 mM and 10 mM boric acid (respectively, 0.03 and 0.02) and increased 8.5-fold with the application of 15 mM boric acid compared to the control levels (Figure5). With the application of 5 mM and 10 mM boric acid to the Dinçer cultivar, the level of SOD mRNA was increased 7- and 12.8-fold compared to the control levels, respectively, whereas SOD mRNA levels decreased 0.4-fold with the application of 15 mM boric acid compared to control (Figure 5). In the Remzibey cultivar, SOD mRNA levels increased 23-, 49- and 5-fold, respectively, at all boric acid concentrations compared to the control levels, whereas a 9-fold decrease was detected at 15 mM boric acid compared to that at 10 mM boric acid (Figure 5).

CAT mRNA levels decreased at all boric acid concentrations in all cultivars, except with the application of 5 mM boric acid to the Dinçer cultivar and 10 mM boric acid to the Remzibey cultivar. In the samples of Remzibey treated with 10 mM boric acid, there was an approximately 8-fold increase in CAT mRNA levels compared to those of the control; however, with the application of 5 and 15 mM boric acid, there was a decrease in CAT mRNA levels (1.8- and 15.5-fold, respectively) (Figure 6). The application of all boron concentrations to the Balcı cultivars led to a complete reduction in the CAT mRNA levels compared to those of the control. The levels decreased 147.5, 84.3 and 17.3-fold with application of 5, 10 and 15 mM boric acid, respectively, to the Balcı cultivar.

In the Balcı cultivar, with the application of 5 mM and 10 mM boric acid, the level of APX mRNA decreased by 3- and 2.5-fold, respectively, and it increased with 15 mM boric acid application (6.5-fold) compared to the control levels. In the Dinçer cultivar, the APX mRNA level increased in the 5 and 15 mM boric acid application groups compared to that of the control group (34.9 and 48.3, respectively), and it was the same in the 10 mM boric acid application group. It was determined that application of boric acid to the Remzibey cultivar at all concentrations increased the APX mRNA levels compared to those of the control group (Figure 7). In the presence of 15 mM boric acid, APX enzyme was synthesized at a high level of 6.4-fold that of the control in the Balcı cultivar and 3-fold that of the control in the Dinçer cultivar; there was a noteworthy increase of 544-fold in the Remzibey cultivar compared to the control levels (Figure 7). This is one of the most important results seen following the application of boric acid in all three safflower cultivars.

When the GR mRNA levels of the safflower cultivars that were treated with boric acid were compared, the level of GR mRNA in the Balcı cultivar decreased 15-fold, 9-fold and 23-fold with application of 5, 10 and 15 mM boric acid, respectively, compared to the control levels. GR mRNA levels increased with the application of 10 mM boric acid in the Dinçer cultivar compared to that of the control group (17-fold), but no significant change was observed at 5 and 15 mM boric acid. The GR mRNA level in Remzibey increased 13.7-fold compared to that of the control with 5 mM boric acid application, while it was observed to decrease 5-fold with 15 mM boric acid application (Figure 8).

Table 1. Primers used in DNA testing of safflower cultivars

Gene Symbol	Primer Sequences	Sequence ID	Efficiency (%)	Melting Temperature
GAPDH	F: ATGACTGCCACCCAAAAGAC R: TTCCGGTCAATTTCCCATTA	110940865	98.05	60.1 59.9
SOD	F: GGCAGTACCATCTTCGCCTA R: TTGTGGCCTTAAACCTGGAC	110872653	97.89	60.2 59.9
CAT	F: ATCGGAGGAACGAATCACAG R: GGGCTGCAAAGGTATGATGT	110934318	96.73	60.0 59.9
APX	F: GCATGATGCTGGAACATACG R: AAAGTCAACAGTAGGCCCAC	110918963	92.51	54.1 55.0
GR	F: GATGGGTTCCACTGTGAATC R: GGTTCGAGAATACTCGTCAAC	110884045	87.10	53.3 55.2

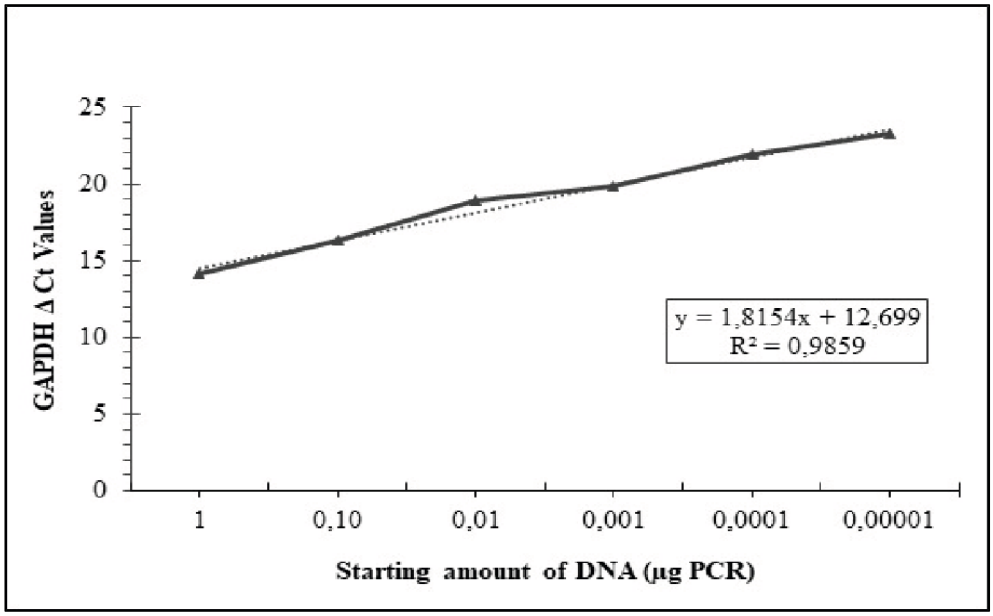

Figure 1. GAPDH Δ-Ct values.

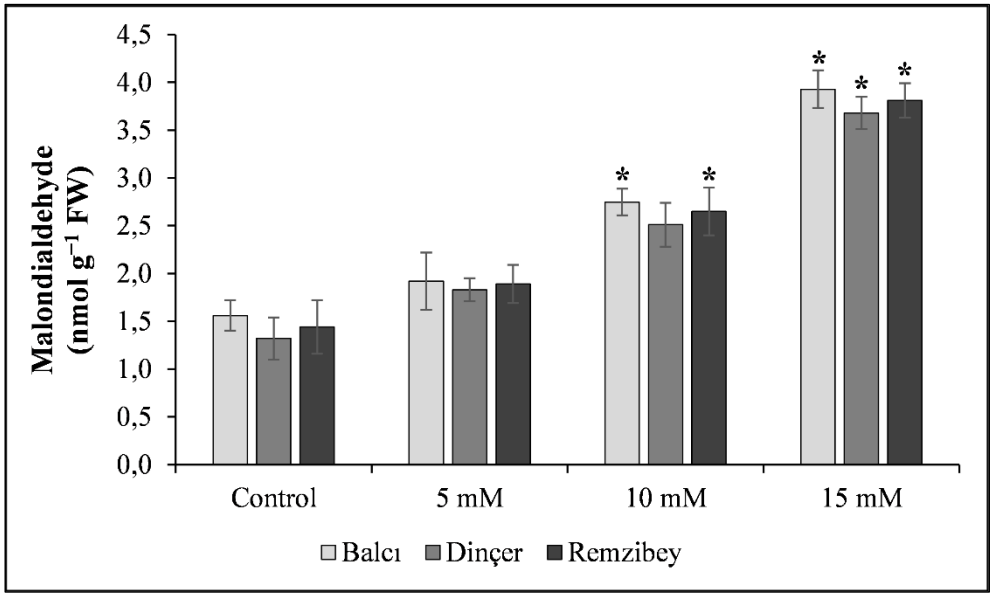

Figure 2. Malondialdehyde (MDA) concentration of safflower cultivars following boric acid application (*P<0.05).

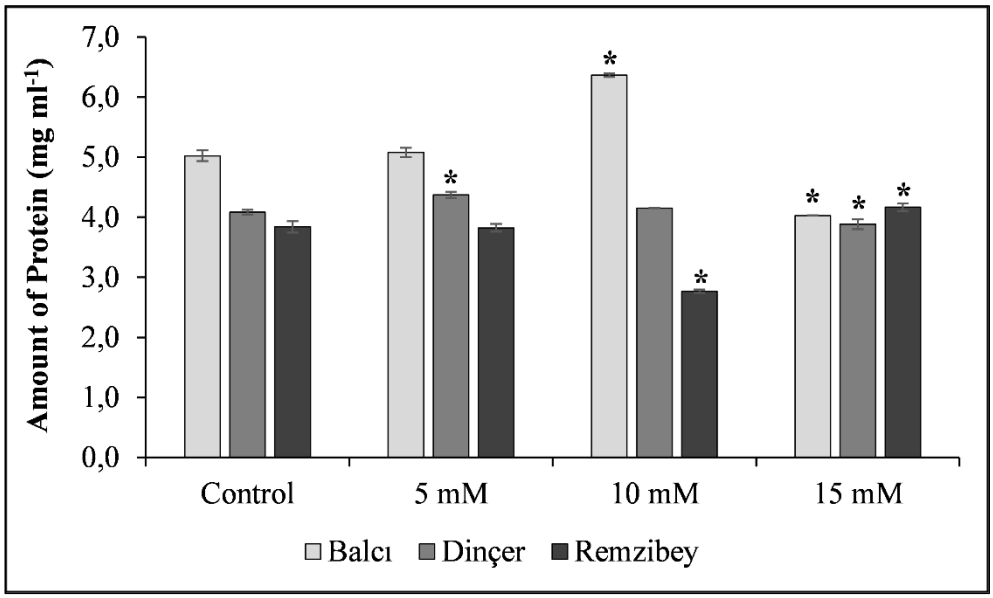

Figure 3. Protein content of safflower cultivars following boric acid application (*P<0.05).

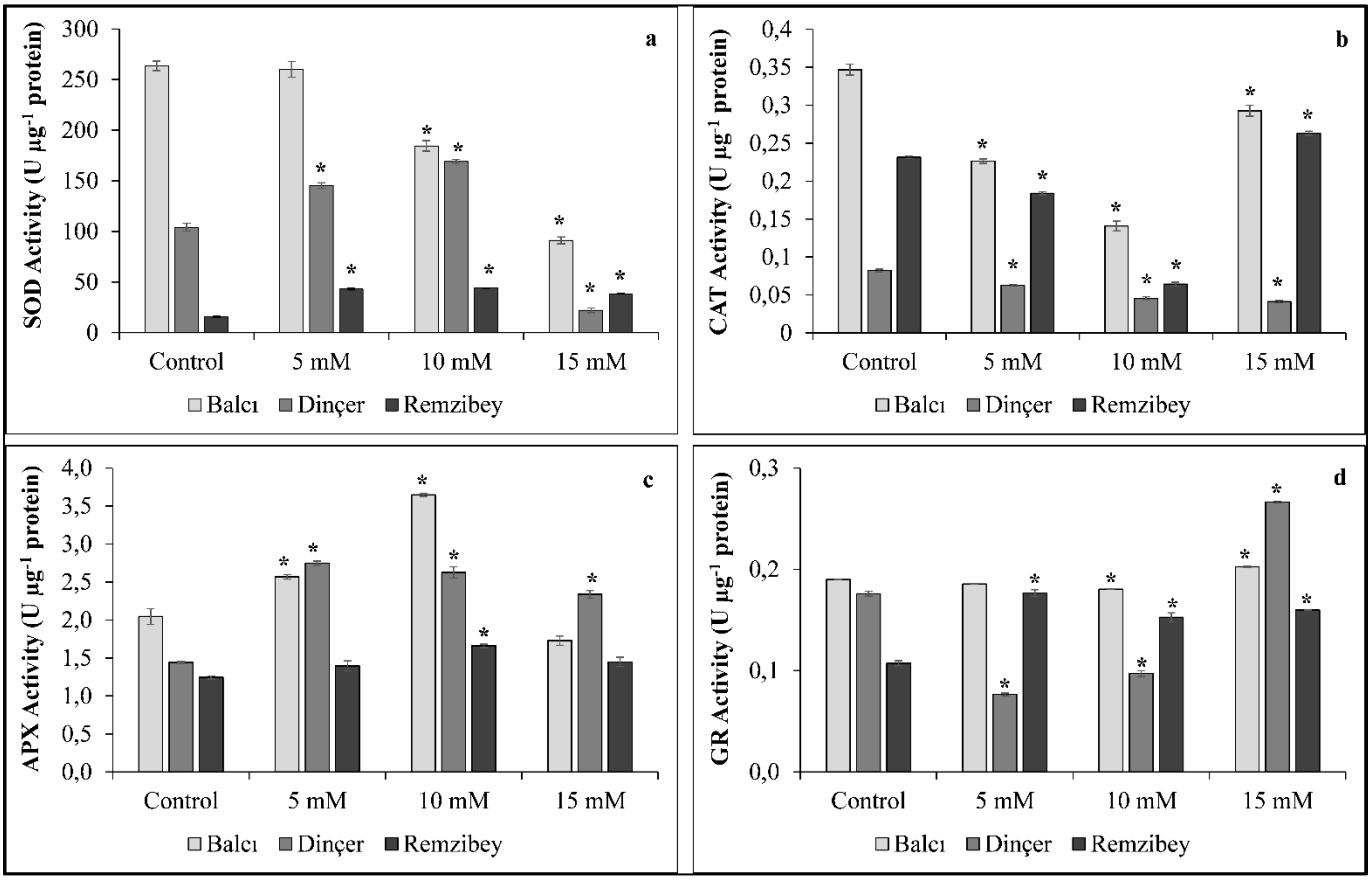

Figure 4. Total SOD (a), CAT (b), APX (c) and GR (d) activities in leaf tissue of safflower cultivars treated with boric acid (*P<0.05).

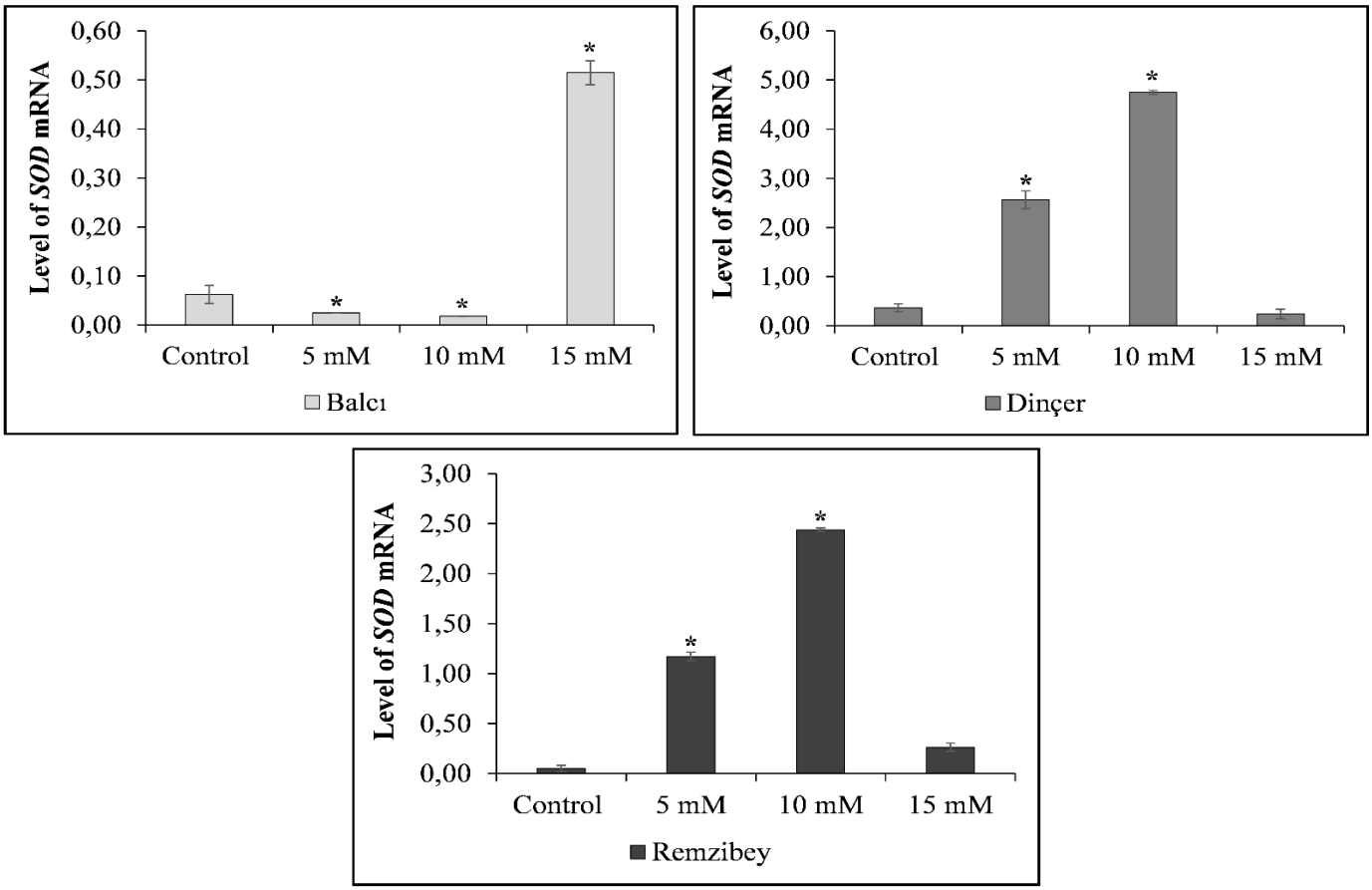

Figure 5. Total *SOD* mRNA levels in safflower cultivars treated with boric acid (*P<0.05).

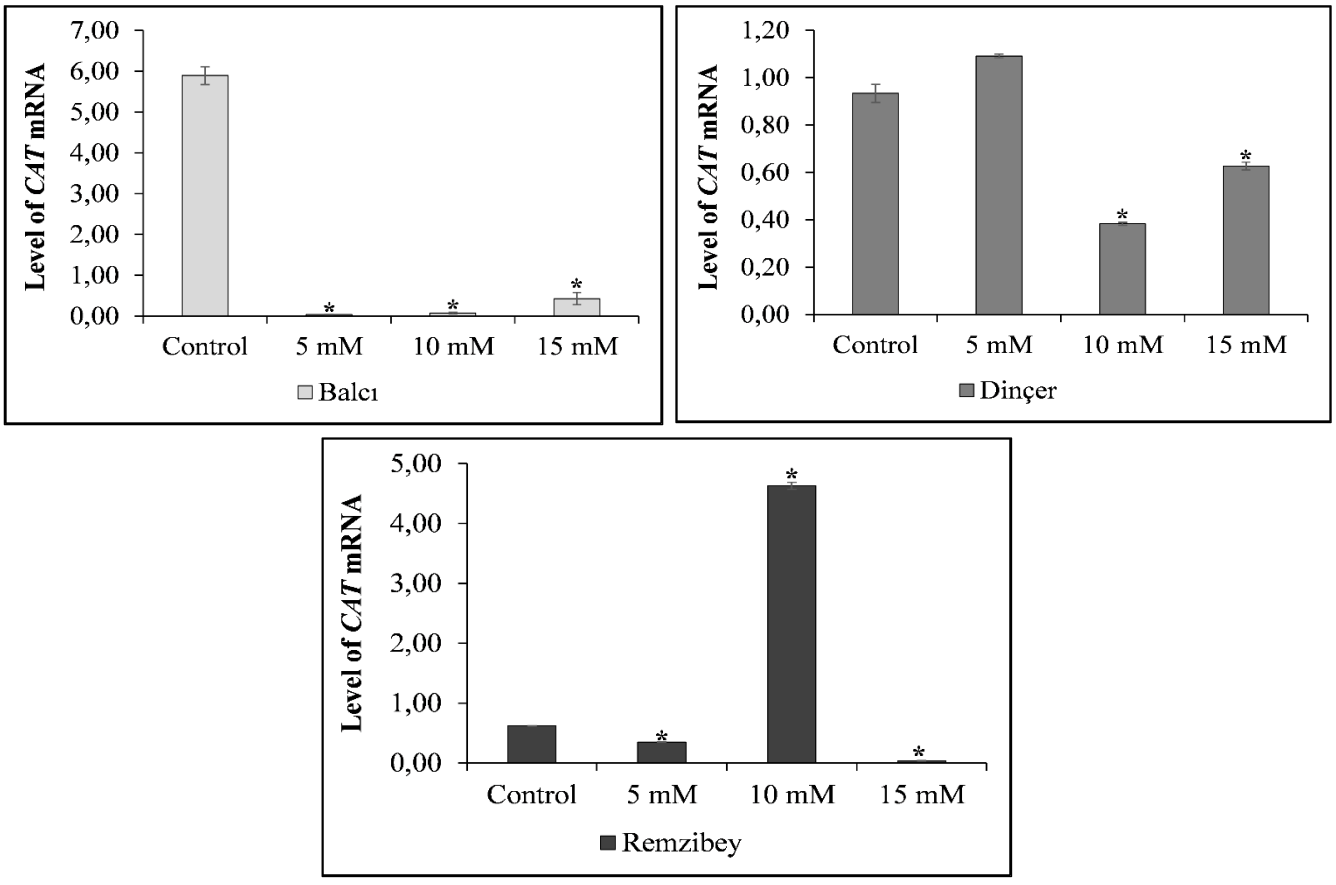

Figure 6. Total *CAT* mRNA levels in safflower cultivars treated with boric acid (*P<0.05).

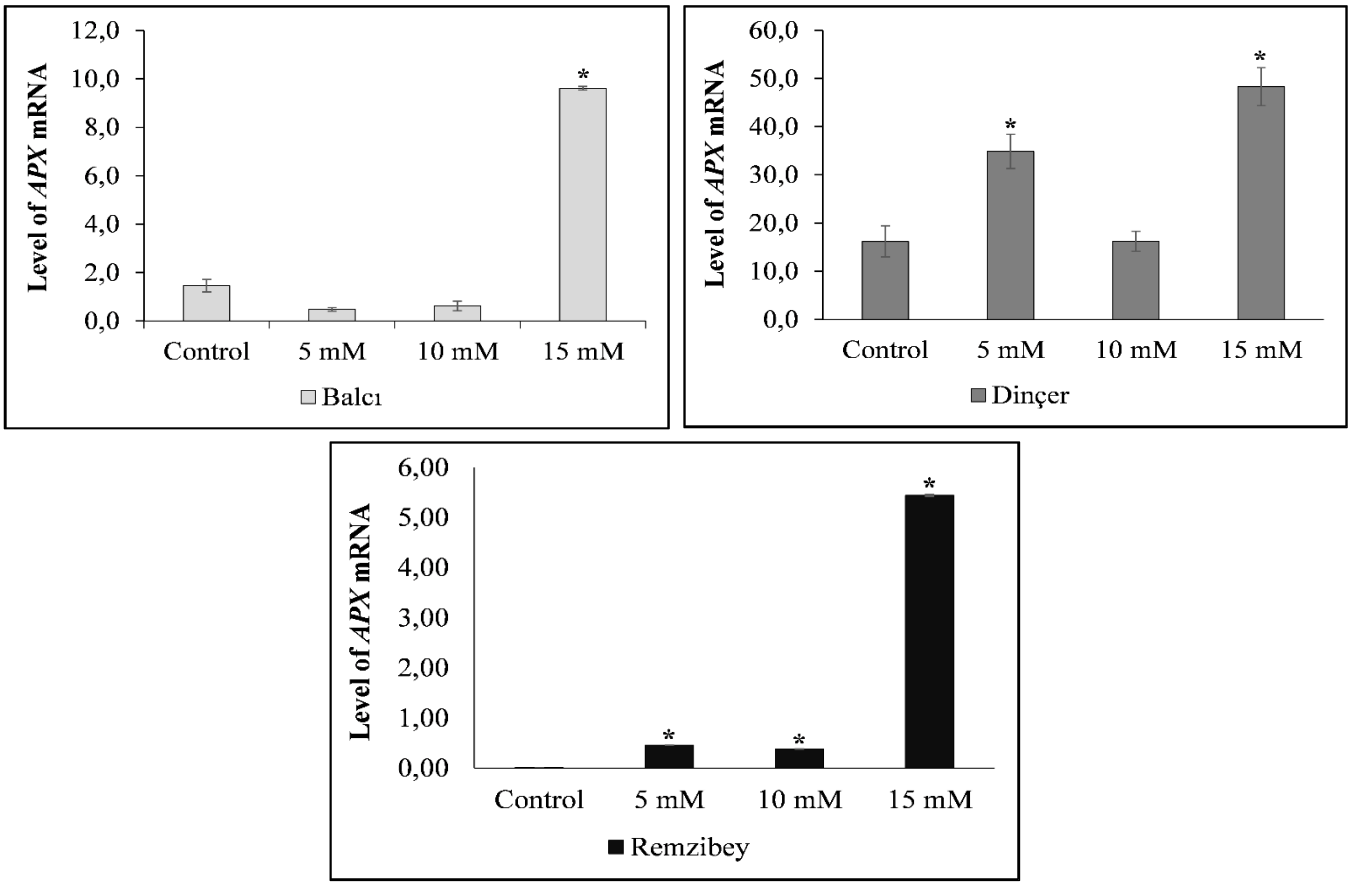

Figure 7. Total *APX* mRNA levels in safflower cultivars treated with boric acid (*P<0.05).

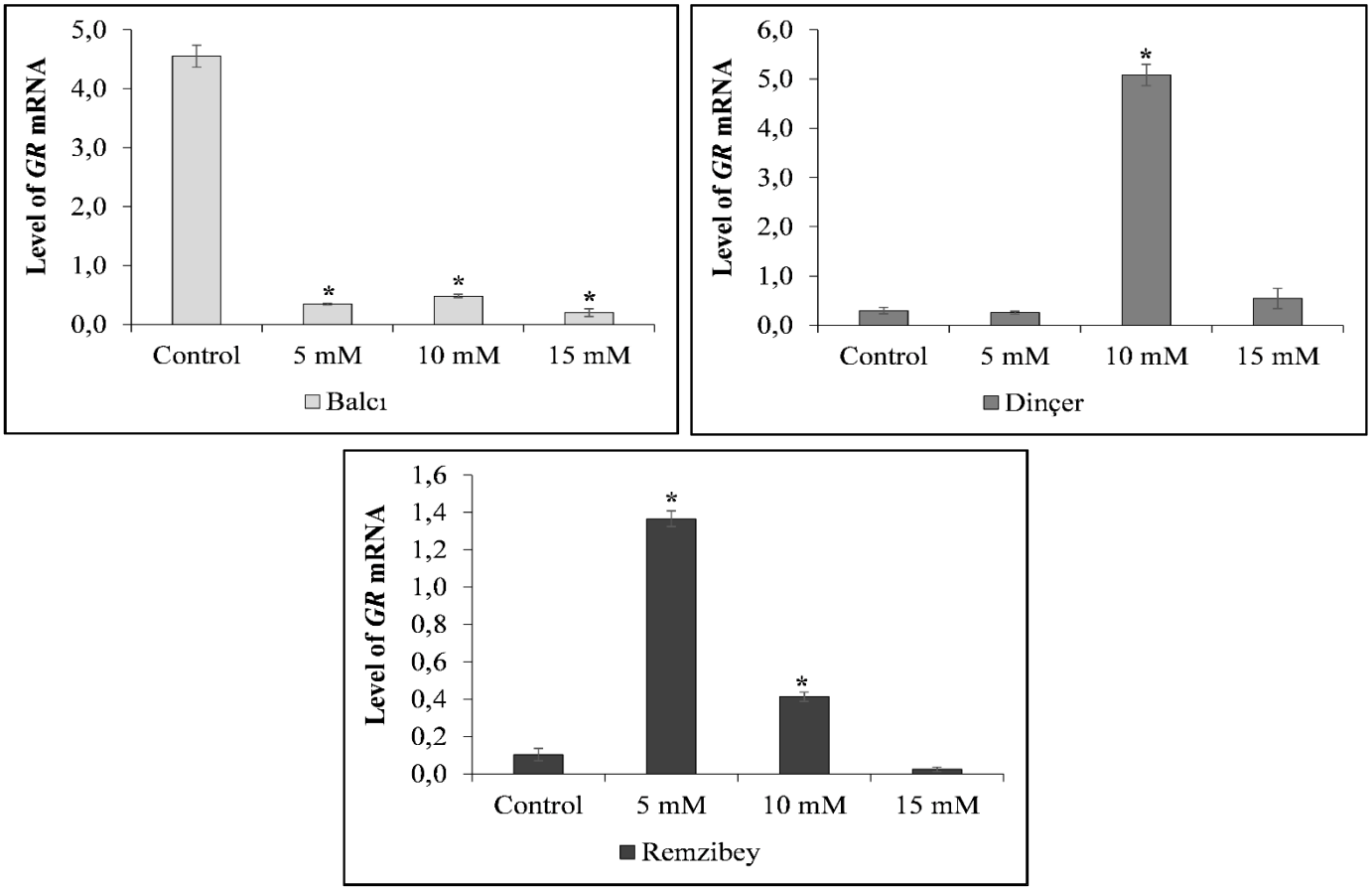

Figure 8. Total *GR* mRNA levels in safflower cultivars treated with boric acid (*P<0.05).

DISCUSSION

Many stress factors increase the activity of antioxidant enzymes and produce physiological changes in plants [1]. Boron is one of the micronutrient elements to which plants react the most. Boron is an essential nutrient element that is absolutely necessary for plants, and the levels representing boron deficiency and toxicity are quite close to each other [2]. Ecological and physiological changes in safflower plants under the influence of boric acid and physiological and molecular changes in antioxidant enzyme activities have not been previously reported in the literature. Therefore, ecological, physiological and molecular studies of the safflower plant response to boron provide important contributions to the literature and are worthwhile because of the economic value of safflower.

In the study, it was determined that the MDA level increased due to the increasing boric acid concentration in all three safflower varieties. This condition is an indicator of oxidative stress caused by boric acid [33-36]. Protein metabolism under stress conditions deteriorates at levels that prevent plant growth [37]. In this study, while the protein contents of Balcı and Dinçer cultivars increased at 5 and 10 mM boric acid concentrations, these contents decreased at 15 mM concentrations compared to the control levels. While the amount of protein at low boric acid concentrations was reduced in the Remzibey cultivar, it increased at high boric acid concentrations. Namjooyan and coauthors demonstrated a protein concentration 63% higher than the control levels at 100 µM Cd in *Carthamus tinctorius* callus cells [38]. Reid and coauthors reported that boron applications at low concentrations increased the protein content of wheat, similar to the findings of our study [39]. In the same study, they stated that high-concentration boron application (100 mM) slowed protein synthesis and cell activity and consequently decreased protein content in wheat cultivars. When the studies in the literature are reviewed, it is clear that boric acid has positive and negative effects on protein contents. It was reported that the protein content of tolerant barley cultivars increased at different boron concentrations and that the protein content of sensitive cultivars decreased [9, 40]. In our study, it was determined that while boron sensitive Remzibey cultivar has a low protein content, tolerant Balcı cultivar has high protein content. It is natural that a species grown in different environments can have different responses against the same stress.

Boron plays an important role in the change in oxidative stress enzymes [9]. SOD enzyme activity increases or decreases during oxidative stress in plants under different stress conditions [1]. There are no studies in the literature that show any association between boron administration and antioxidant enzymes in the *Carthamus* genus. In our study, there were differences in SOD enzyme activity among the safflower cultivars under different concentrations of boric acid. SOD enzyme activity in Balcı cultivar decreased as the concentration of boric acid increased. The Balcı cultivar had the highest activity of SOD compared with the other cultivars at 15 mM boric acid. The RT-qPCR results of the genes encoding SOD agreed with the spectrophotometric SOD activity results. When the amount of SOD transcribed and the amount of translated active enzyme was compared, it was determined that the Dinçer and Remzibey cultivars showed similar results at all concentrations. The transcriptional and translational production in the Balcı cultivar were in agreement, but the results were opposite those of both of the other cultivars. Both transcriptional and translational results showed that SOD plays an important role in eliminating the oxidative damage caused by boric acid in the safflower cultivars. Kaya and coauthors indicated that SOD enzyme activity increased significantly in tomato plants to which 2 and 4 mg L^{-1} boron were applied [41]. Cervilla and coauthors found that boron application at low concentrations increased SOD activity in two tomato cultivars in their study with sensitive and tolerant tomato cultivars [42]. They reported that while high-concentration boron application increased the SOD activity of tolerant cultivars, such an application decreased the SOD activity of sensitive cultivars. Karabal and coauthors obtained similar results in their study of boron sensitive and tolerant barley cultivars [9]. It was determined that there was a decrease in SOD activity in the sensitive barley cultivar, while there was no change in SOD activity in the tolerant barley cultivar after boron application. In this study, both mRNA production and enzymes activity of SOD was higher in boron tolerant Balcı cultivar according to sensitive Remzibey cultivar.

CAT is known to play an important role in the removal of H_2O_2 in plant tissues [43]. In this study, CAT activity decreased at 5 and 10 mM boric acid in three safflower cultivars, while CAT activity in Remzibey and Balcı was higher at 15 mM boric acid than the other concentrations. In addition, it was determined that the amount of CAT mRNA in the Balcı cultivar decreased with all three boric acid applications. In the Remzibey cultivar, interestingly, it was determined that there was an increase of approximately 100-fold at 10 mM compared to the control levels. The reason for the decrease in CAT activity in boric acid-applied samples is that oxidative stress causes H_2O_2 production at high boric acid concentrations. In this case, H_2O_2 is thought to inhibit the CAT enzyme [44]. Oluk and coauthors reported in their study with two tomato cultivars that boron

toxicity increased oxidative damage compared to that of the control group [45]. In addition, the fact that the CAT enzyme has an unstable structure and may be inhibited by high concentrations of H_2O_2 may also be a reason for the reduction in CAT mRNA levels with increasing boric acid concentrations in safflower.

APX mRNA levels were highest at the 15 mM boric acid concentration in all cultivars. Boron toxicity causes a metabolic reaction that activates and is directly related to APX. It has been reported that the activity of APX in boron susceptible barley plants increased at the 10 mM boric acid concentration, while APX activity decreased in the tolerant cultivar [9]. In some studies using different plants, ascorbate levels have been reported to decrease in cases of boron deficiency [46-48]. Accordingly, there is a relationship between APX and boron metabolism. It is also known that boron causes lipid peroxidation in plants [9]. Cervilla and coauthors reported that boron toxicity caused an increase in the concentration of H_2O_2 and ascorbate and APX activity in tomato cultivars [42]. Genes encoding APX are rearranged under various stress conditions, and gene expression changes [49-51]. Kayıhan and coauthors determined that APX activity and APX gene expression were similar in *Arabidopsis thaliana* following high-concentration boron application [52]. They indicated that APX activity and APX gene expression increased compared to that of the control group in following boron application. In different studies from the literature, it has been reported that APX mRNA levels and APX activities increased under stress conditions in many plants, such as mustard, wheat, lentil and bean [53-56]. The expression of genes encoding APX is known to be modulated differently by various abiotic stresses in different plant species [57]. The results of this study showed that boric acid application induces an increase in reactive oxygen species levels, which causes oxidative stress in safflower cultivars, and the increase in APX mRNA levels is a molecular response of safflower plants to eliminate these ROS [58-59]. It can also be suggested that APX enzymes, which are members of the class-I peroxidase family, may be potential biomarkers for B-contaminated environments [60-61].

In our study, GR mRNA levels increased significantly compared to those of the control group following the application of 10 mM boric acid in the Dinçer cultivar. In the Remzibey cultivar, GR mRNA levels were found to increase significantly at 5 mM and 10 mM boric acid concentrations compared to those of the control group. The increase in GR gene expression in Dinçer is thought to be a molecular response to eliminate the resulting H_2O_2. These results suggest that GR activity can be actively used as a defence mechanism against oxidative stress in the Remzibey cultivar. No study on the relation between boric acid and GR gene expression in safflower plants has been reported in the literature. It was reported that there was no significant difference in GR activity in leaf tissues of tolerant barley cultivars, but there was a significant increase in GR activity in leaf tissues of sensitive barley cultivars following boric acid application. The GR activity in these cultivars was not affected by 5 and 10 mM boric acid [9]. As a result, it is thought that GR is not as effective as other antioxidant defence mechanisms against boron stress in plants. As mentioned previously, in many studies, there were large differences between the genotypes of the same species in responses to boron toxicity [62-64]. It is thought that these differences may be due to many reasons, such as the genome structure and different tolerance limits among cultivars.

Day and coauthors carried out a study on the effects of boron toxicity with the Remzibey, Yenice, and Balcı cultivars [65]. They found that the Balcı cultivar has the potential to be used for the phytoremediation of B toxic soils (including alkaline soils and those with high available K levels). In our study, according to the ecological results, it was determined that the most tolerant cultivar to boric acid was Balcı [18].

According to the results of the study, it was determined that the transcriptionally produced mRNA was not transformed into the protein. In this case, the reading of mRNAs may have been blocked through posttranscriptional control mechanisms and the conversion to enzymes may have been prevented [39, 66, 67]. Thus, in physiological studies, quantitative analysis of transcriptional products along with enzyme activity will enable real assessment. There is not enough information in the literature about the boron toxicity and defense mechanisms, and this study aimed to contribute to the understanding of the molecular mechanism of the safflower-boron relationship.

CONCLUSION

According to the results obtained in our study, the cultivar Remzibey which is sensitive to boron toxicity had the lowest antioxidant capacity, while the tolerant Dinçer and Balcı varieties had higher antioxidant capacity. Thus, the ability to increase antioxidant system activity to limit cellular damages might be an important role in the B tolerance of safflower. It may be stated that Remzibey cultivar is sensitive the boron stress due to its low antioxidant enzyme activity. The tolerant cultivar was found to have a higher antioxidant capacity to cope with oxidative stress. However, antioxidant enzymes activity results alone may not be sufficient to explain tolerance or sensitivity. Therefore, while evaluating the results of ecological parameters,

besides antioxidant enzyme activity and gene expression, other biochemical events and metabolic reactions in which boron takes part should be investigated. Future studies shall investigate the role of factors such as the membrane permeability of stem cells, pectin and ATP synthetase activity, the role of the *BOR1* gene on boron tolerance of safflower varieties.

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REFERENCES

1. Hussain MI, Lyra DA, Farooq M, Nikoloudakis N, Khalid N. Salt and drought stresses in safflower: a review. Agron Sustain Dev. 2016;36:4.
2. Ahmad W, Zia MH, Malhi SS, Niaz A, Saifullah. Boron deficiency in soils and crops: A review. Crop Plant 2012;5:78-114.
3. Hu H, Brown PH. Absorption of boron by plants roots. Plant Soil 2005;193:49-58.
4. Altun F. Boron, National Boron Research Institute Publications, Ankara: Türkiye; 2005.
5. Taban S, Erdal İ. Effects of Boron on Growth of Various Wheat Varieties and Distribution of Boron in Aerial Part. Turk J Agric For. 2000;24: 255-62.
6. Nable RO, Banuelos GS, Paull JG. Boron toxicity. Plant Soil 1997;193:181–98.
7. Kaçar B, Katkat V. Plant nutrition, Nobel Publishing Ankara: Turkey; 2006.
8. Uluisik I, Karakaya HC, Koc A. The importance of boron in biological systems. J Trace Elem Med Biol. 2018;45:156-62.
9. Karabal E, Yücel M, Öktem AH. Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci. 2003;164:925-33.
10. Luis M, Cervilla JA, Begoña B, Rios JJ, Rosales MA, Sánchez-Rodríguez E, et al. Parameters Symptomatic for Boron Toxicity in Leaves of Tomato Plants. J Bot.2012;17:1-18.
11. Marschener H. Mineral nutrition of higher plants. 2nd Edt. Institute of Plant Nutrition University of Hohenheim: Germany; 1995.
12. Blevins DG, Lucaszewski KM. Boron in plant structure and function. Annu Rev Plant Biol. 1998;49:481-500.
13. Dannel F, Pfeffer H, Romheld V. Update on boron in higher plants-uptake, primary translocation and compartmentation. Physiol Plant. 2002;4:193-204.
14. Kaçar B, Katkat AV, Öztürk Ş. Plant Physiology, Nobel Publishing Ankara: Turkey; 2010.
15. Davis PH. Flora of Turkey and The East Aegaen Islands. Vol. 10. Edinburgh University Press Edinburgh: UK; 1988.
16. Weiss EA. Safflower in oilseed crops. Blackwell Sci Ltd Victoria: Australia; 2000.
17. Park M, Li Q, Shcheynikov N, Muallem S, Zeng W. Borate transport and cell growth and proliferation. Cell Cycle 2005;4:24-6.
18. Sülüş Ş, Leblebici S. The effect of boric acid application on ecophysiological characteristics of safflower varieties (*Carthamus tinctorius* L.), Fresenius Environ Bull. 2020;29 (09A):8177-85.
19. Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. J Plant Physiol. 2008;165:385–96.
20. Giannakoula A, Moustakas M, Syros T, Yupsanis T. Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line. Environ Exp Bot. 2010;67:487–94.
21. Gupta B, Pathak GC, Pandey N. Induction of oxidative stress and antioxidant responses in *Vigna mungo* by zinc stress. Russ J Plant Physiol. 2011;58:85-91.
22. Jabeen N, Ahmad R. Improvement in growth and leaf water relation parameters of Sunflower and Safflower plants with foliar application of nutrient solutions under salt stress. Pak J Bot. 2012a; 44(4):1341-5.
23. Çulha Ş. Investigation of the effect of salt stress on some physiological and biochemical parameters in safflower (Carthamus tinctorius L.) varieties. Master Thesis, Hacettepe University Graduate Scholl of Science and Engineering, Ankara: Turkey; 2011.
24. Sairam RK, Saxena DC. Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stres tolerance. J Agron Crop Sci. 2000;184:55-61.
25. Jebara S, Jebara M, Liman F, Aouani E. Changes in ascorbate peroxidase, catalase, guaicol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. J Plant Physiol 2005;162:929-36.

26. Bradford MM. A rapid and sensitive method for the quantification of micro gram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
27. Beyer WF, Fridovich I. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem.1987;161(2):559-66.
28. Burke JJ, Oliver MJ. Differential temperature sensitivity of pea superoxide dismutases. J Plant Physiol 1992;100:1595–8.
29. Çakmak I, Horst JH. Effects of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). Physiol. Plant. 1991;83:463-8.
30. Lester NP, Shuter BJ, Abrams PA. Interpretingthe von Bertalanffy model of somatic growth in fishes: the cost of reproduction. Proceedings of the Royal Society of London 2004;271:1625–31.
31. Rao MV, Paliyath G, Ormrod DP. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. J Plant Physiol 1996;110:125–36.
32. Rozen S, Skaletsky H. Primer on the WWW for general users and for biologist programmers. Methods Mol Biol. 2000;132:365-86.
33. Jabeen N, Ahmad R. The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan. J Sci Food Agric. 2012b; 93(7):1699-705.
34. Golkar P, Taghizadeh M. In vitro evaluation of phenolic and osmolite compounds, ionic content, and antioxidant activity in safflower (*Carthamus tinctorius* L.) under salinity stress. Plant Cell, Tissue Organ Cult. 2018;134(3):357–68.
35. Chavoushi M, Kalantari KM, Arvin MJ. Effect of Salinity Stress and Exogenously Applied Methyl Jasmonate on Growth and Physiological Traits of Two *Carthamus tinctorius* Varieties, Int. J. Hortic. Sci. Technol. 2019;6(1):39-49.
36. Pourghasemian N, Landberg T, Ehsanzadeh P, Greger M. Different response to Cd stress in domesticated and wild safflower (*Carthamus* spp.). Ecotoxicol Environ Saf. 2019;171:321–8.
37. Köşkeroğlu S. Investigation of proline accumulation levels and stress parameters in maize plant (*Zea mays* L.) under salt and water stress. Master Thesis Muğla University Graduate School of Natural and Applied Sciences İzmir: Türkiye; 2006.
38. Namjooyan S, Khavari-Nejad R, Bernard F, Namdjoyan S, Piri H. The effect of cadmium on growth and antioxidant responses in the safflower (*Carthamus tinctorius* L.) callus. Turk J Agric For. 2012;36:145-52.
39. Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RDA. Critical analysis of causes of boron toxicity in plants. Plant Cell Environ. 2004;25:1405-14.
40. Barker AV, Pilbeam DJ. Handbook of Plant Nutrition, Taylor and Francis Group New York: USA; 2007.
41. Kaya C, Ashraf M, Sonmez O, Aydemir S, Tuna AL Cullu MA. The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity. Sci. Hortic. 2009;121:1–6.
42. Cervilla JA, Molina E, Rivera M, Torres-González F, Bellón JA, Moreno B, et al. The risk for depression conferred by stressful life events is modified by variation at the serotonin transporter 5HTTLPR genotype: evidence from the Spanish PREDICT-Gene cohort. Mol Psychiatry. 2007;12(8):748-55.
43. Kaushal J, Mehandia S, Singh G, Raina A, Arya SK. Catalase enzyme: Application in bioremediation and food industry. Biocatal Agric Biotechnol. 2018;16:192–9.
44. Foyer CH, Descourvières P, Kunert KJ. Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. Plant Cell Environ. 1994;17:507–23.
45. Oluk EA, Demiray H, Yardım D. In Vitro Effects of Excess Boron on Root Development and Anatomy of Sunflower (*Helianthus annuus* L.cv. Sambro No.5) Plant. Ege J Agricult Res. 2006;43:145-52.
46. Lukaszewski KM, Blevins DG. Root growth ınhibition in boron-deficient or aluminum-stressed squash may be a result of ımpaired ascorbate metabolism. J Plant Physiol. 1996;112(3):1135-40.
47. Çakmak I, Römheld V. Boron deficiency-induced impairments of cellular functions in plants. Plant Soil 1997;193:71–83.
48. Liu D, Jiang W, Zhang L, Li L. Effects of boron ions on root growth and cell division of broadbean (*Vicia faba* L.). Isr J Plant Sci. 2000;48:47–51.
49. Teixeira FK, Menezes-Benavente L, Galvão VC, Margis R, Margis-Pinheiro M. Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. Planta 2006;224:300–14.
50. Rosa SB, Caverzan A, Teixeira FK, Lazzarotto F, Silveira JA, Ferreira-Silva SL, et al. Cytosolic APx knockdown indicates an ambiguous redox responses in rice. Phytochemistry 2010;71:548–58.

51. Bonifacio A, Martins MO, Ribeiro CW, Fontenele AV, Carvalho FE, Margis-Pinheiro M, Silveira JA. Role of peroxidases in the compensation of cytosolic ascorbate peroxidase knockdown in rice plants under abiotic stress. Plant Cell Environ. 2011;34:1705–22.
52. Kayıhan DS, Kayıhan C, Çiftçi,YÖ. Excess boron responsive regulations of antioxidative mechanism at physio-biochemical and molecular levels in *Arabidopsis thaliana*. Plant Physiol Biochem. 2016;109:337-45.
53. Mobin M, Khan NA. Photosynthetic activity, pigment composition and anti-oxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J Plant Physiol. 2007;164:601-10.
54. Khan NA, Samiullah T, Singh S, Nazar R. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J Agron. Crop Sci. 2007;193:435–44.
55. Singh S, Khan NA, Nazar R, Anjum NA. Photosynthetic traits and activities of antioxidant enzymes in blackgram (*Vigna mungo* L. Hepper) under cadmium stress. Am J Plant Physiol. 2008;3:25-32.
56. Polidoros NA, Scandalios JG. Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize (*Zea mays* L.). J Plant Physiol. 1999;106:112-20.
57. Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. Genet Mol Biol. 2012;35(4):1011-19.
58. Landi M, Degl'Innocenti E, Pardossi A, Guidi L. Antioxidant and photosynthetic responses in plants under boron toxicity: A review. J Agric Biol Sci 2012;7(3):255-70.
59. Princi MP, Lupini A, Araniti F, Longo C, Mauceri A, Sunseri F, et al. Boron Toxicity and Tolerance in Plants. Plant Metal Interaction Reggio Calabria: Italy; 2016.
60. Cao Y, Han Y, Meng D, Li D, Jin Q, Lin Y, et al. Structural, Evolutionary, and Functional Analysis of the Class III Peroxidase Gene Family in Chinese Pear (*Pyrus bretschneideri*). Front Plant Sci. 2016;7:1874.
61. Gür N, Türker OC, Böcük H. Toxicity assessment of boron (B) by *Lemna minor* L. and *Lemna gibba* L. and their possible use as model plants for ecological risk assessment. Chemosphere 2016;157:1-9.
62. Paull JG, Cartwright B, Rathjen AJ. Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica 1998;39:137-44.
63. Huang C, Graham RD. Resistance of wheat genotypes to boron toxicity is expressed atthe cellular level. Plant Soil 1990;126:295–300.
64. Nable RO. Distribution of boron within barley genotypes with differing susceptibilities to boron toxicity. J Plant Nutr. 1991;14 (5):453-61.
65. Day S, Çıkılı Y, Aasim M. Screening of three safflower (*Carthamus tinctorius* L.) cultivars under boron stress. Acta Sci Pol-Hortoru. 2017;16(5):109-16.
66. Ara N, Nakkanong K, Lv W, Yang J, Hu Z, Zhang M. Antioxidant Enzymatic Activities and Gene Expression Associated with Heat Tolerance in the Stems and Roots of Two Cucurbit Species (*Cucurbita maxima* and *Cucurbita moschata*) and Their Interspecific Inbred Line Maxchata. Int J Mol Sci. 2013;14:24008-28.
67. Gonza´lez-Fontes A, Rexach J, Quiles-Pando C, Herrera-Rodrı´guez MB, Camacho-Cristo´bal JJ, Navarro-Gochicoa MT. Transcription factors as potential participants in the signal transduction pathway of boron deficiency. Plant Signal Behav. 2013;8(11):1–3.