Boron Enhances Antioxidative Defense in the Leaves of Salt-affected *Pistacia vera* Seedlings

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Boron (B) toxicity and salt stress are widely observed in arid and semi-arid regions. Nonetheless, little is known about the interactions between B and salt stress with respect to plant defense systems. In this study, seedlings of *Pistacia vera* ‘Badami’—a valuable crop of arid lands in central Persia—were treated with different B concentrations in soil (0, 2.5, 5, 10, and 20 mg·kg⁻¹) to investigate oxidative injuries and antioxidative defense responses of the plants to salt stress (0, 800, 1600, and 2400 mg NaCl·kg⁻¹ of soil). Salt stress and application of 20 mg B·kg⁻¹ of soil intensified electrolyte leakage, lipid peroxidation, and lipoxygenase activity in pistachio leaves. Additional supplementation of B up to 5 mg·kg⁻¹ soil significantly decreased malondialdehyde (MDA) and H₂O₂ under salt stress. The alleviating effects of B on oxidative stress parameters were related to the improvement in antioxidant enzymes activity (ascorbate peroxidase and catalase), and the non-enzymatic antioxidant compounds (ascorbic acid and phenolic compounds), compared with those treated with either salt stress or a high concentration of B. However, application of 20 mg B·kg⁻¹ of soil exacerbated the oxidative damage induced by salt stress. On the contrary, applying mild salt stress mitigated the toxic effects of B on the plant, since oxidative stress due to B toxicity was significantly reduced by application of 800 mg NaCl·kg⁻¹ of soil. In conclusion, the optimization of B supply in the soil was suggested to alleviate the oxidative damage due to salt stress.

Key Words: antioxidative enzymes, ascorbic acid, hydrogen peroxide, oxidative damage, phenolic compounds.

**Introduction**

While the effects of salinity and boron (B) stresses on the growth and performance of plants are explained with good detail in the available literature (Karimi and Rahemi, 2012; Wimmer et al., 2005; Yermiyahu et al., 2008), concepts regarding plant antioxidative responses to B toxicity combined with salt stress are still unclear. This is despite the fact that these stresses often occur simultaneously in arid and semi-arid environments and have complicated interactions, as derived from the literature.

Boron is an essential element for stabilizing the cell wall pectin network which regulates cell wall pore size (Brown et al., 2002). In addition, B appears to play a major role in preserving the structural integrity of the plasma membranes through the association with membrane components. Accordingly, B may contribute to the protection of plasma membranes from peroxidative damage caused by reactive oxygen species (ROS). Therefore, it is not surprising that B-deficient plants are characterized by leaky plasma membranes (Cakmak and Romheld, 1997). However, toxic levels of B lead to ROS accumulation in tissues, thereby disrupting cell wall development, normal cell metabolism, cell division, and plant development (Reid et al., 2004). Salt stress, like imbalanced B nutrition, induces ROS accumulation in leaves and imposes oxidative stress on the plant (Cervilla et al., 2007; Mittler, 2002). Previous studies have suggested that tolerance to environmental stresses may be the result of enhanced resistance to oxidative stress (Hernandez et al., 2001; Mittler, 2002). A defense system including antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), or ascorbate peroxidase (APX), along with several non-enzymatic antioxidants develops in plant cells to detoxify ROS under environmental stress (Miller et al.,...
2008). SOD is among the first line in the detoxifying process and catalysis, contributing to the dismutation of $O_2^-$ to $H_2O_2$ and $O_3$ (Molassiotis et al., 2006). Peroxidases and catalase are responsible for the reduction of $H_2O_2$ to $H_2O$ (Mittler, 2002). Mitigation of oxidative damage and the enhanced tolerance to environmental stress often correlate with an efficient antioxidative system (Miller et al., 2008).

Most pistachio crops in the world come from marginal lands of central Persia, where soil salinity is observed in combination with B toxicity. There have been numerous studies on the evaluation of salt stress and B toxicity on plant antioxidative responses (Cervilla et al., 2007; Gunes et al., 2007). However, only a few studies have focused on evaluating B levels in the soil together with salt stress. Therefore, the current study was aimed at evaluating oxidative injuries and antioxidative responses of pistachio (Pistacia vera) seedlings to different concentrations of B under salt stress conditions in soil.

**Materials and Methods**

**Plant material and growth conditions**

The experiments were conducted from autumn 2014 to spring 2016. The soil in these experiments was a fine loam, taken from 0 to 30 cm of natural-occurring soil in the field. Physicochemical properties of the soil are shown in Table 1. The soil was air-dried and smashed to pass through a 2-mm sieve. The soil was treated with nutritional elements. These were nitrogen and P in the form of NH$_4$NO$_3$, KH$_2$PO$_4$, CuSO$_4$·5H$_2$O, MnSO$_4$·H$_2$O, and ZnSO$_4$, respectively. Soil was placed in plastic pots (with volumes of 8-L each). Seeds of *P. vera* ‘Badami’ were sown in sand and kept at 30°C for one week. Four germinated seeds were planted in the pots and irrigated with deionized water twice weekly. After 20 days, the seedlings were reduced to two seedlings in every pot at the 4-leaf stage. A week later, B was applied to the pots by dissolving appropriate amounts of boric acid in irrigation water. The treatments were designed to obtain different concentrations of boron in the soil (0, 2.5, 5, 10, and 20 mg·kg$^{-1}$ soil). Then, in three subsequent irrigations (with a 4-day interval), NaCl was gradually increased in these pots by dissolving suitable amounts of NaCl in 0.3 L irrigation water to obtain 0, 800, 1600, and 2400 mg NaCl·kg$^{-1}$ of soil. The plants were grown under controlled environmental conditions. The environmental conditions were 25–30°C and relative humidity was 25–30% on a day-night basis. Photoperiod was maintained at 12 h. Light intensity at the leaf surface generally exceeded 1100 μE·m$^{-2}$·s$^{-1}$ at midday. The experiments were repeated twice and the treatments were arranged as a factorial experiment with 4 replications in a split plot design. After 120 days, the following measurements were made by sampling the 3rd leaf to the 8th leaf, which were actually fully developed young leaves, from the top of the plants.

**Table 1. Physical and chemical properties of the studied soil.**

| FC (%DM) | PWP (%DM) | pH | $EC_e$ (dS·m$^{-1}$) | CEC (Cmc·kg$^{-1}$) | N (%) | P (mg·kg$^{-1}$ soil) | K (mg·kg$^{-1}$ soil) |
|----------|-----------|----|---------------------|---------------------|-------|---------------------|---------------------|
| 22.0     | 9.0       | 7.9 | 1.3                 | 11.0                | 0.04  | 13.0                | 59.0                |

Field capacity (FC); permanent wilting point (PWP); electrical conductivity of saturated soil extract ($EC_e$); cation exchange capacity (CEC); nitrogen (N); phosphorus (P); potassium (K).
(pH 5.6) was added to the extract. The upsurge in absorbance was read at 234 nm. The extinction coefficient of (25 mM·cm⁻¹) was used in order to convert absorbance values to micromoles of conjugated diene. One unit of activity was taken as the quantity of enzyme that catalyzed the formation of 1 μmol of hydroperoxide (HPOD) per min.

Phenolic compounds in leaf tissue were extracted with 80% methanol. The extract reacted with Folin-Ciocalteau reagent and sodium carbonate solution. After being incubated in the dark, the absorbance was checked at 790 nm with the spectrophotometer (Shimadzu model 160A; Shimazu Co.) (AOAC, 1990). The quantity of phenolic compounds was stated as gallic acid equivalents (mg) using a linear equation according to a standard curve.

Concentration of ascorbic acid in leaf

Ascorbic acid (AA) was isolated with 6% TCA from leaf tissue. The extract was blended with dinitrophenylhydrazine, accompanied by the addition of one drop of 10% thiourea solution (in 70% ethanol). The mixture was heated to reach boiling temperature for 15 min in water, and was thereafter cooled down. The H₂SO₄ was added to the mixture at 0°C. The solution absorbance of the hydrazine complex was identified at 530 nm in a spectrophotometer (Shimadzu model 160 A; Shimazu Co.) (Mukherjee and Choudhuri, 1985).

Activity of antioxidant enzymes

Plant material (leaf sample) was ground in sodium phosphate buffer at the pH of 7.8 for superoxide dismutase (SOD, E.C. 1.15.1.1), and pH 7.0 for catalase (CAT, E.C. 1.11.1.6) and ascorbate peroxidase (APX, E.C. 1.11.1.11). The homogenate was centrifuged at 4°C and the supernatant was used to gauge the activity of the enzymes. The protein content in the supernatant was identified according to the method explained by Giannopotitis and Ries (1977). One unit of enzyme activity was considered as the quantity of enzyme which causes 50% inhibition of the rate of decrease in nitro blue tetrazolium, assessed at 560 nm. CAT activity was identified based on the method used by Cakmak and Marschner (1992). The reaction mixture in a total volume of 2 mL was comprised of 25 mM sodium phosphate buffer (pH 7.0) and 10 mM H₂O₂. The reaction was initiated with the addition of 100 μL of the enzyme extract and the activity was assessed by identifying the initial rate of H₂O₂ degradation at 240 nm (E = 39.4 mM⁻¹·cm⁻¹) for 30 s. APX activity was determined according to Nakano and Asada (1981). The reaction mixture in a total volume of 2.5 mL contained 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H₂O₂, and 150 μL of the enzyme extract. The oxidation of ascorbate—which depended on H₂O₂—was accom-

panied by a reduction in absorbance at 290 nm (E = 2.8 mM⁻¹·cm⁻¹).

Statistical analyses

The experiment was performed twice, in 2014 and 2015, and was analyzed as a split plot experiment. Similar results were obtained in different years according to mixed ANOVA analyses, and therefore the data were pooled and exposed to a general linear model. ANOVA functioned as a factorial experiment based on completely randomized block design with 4 replications. In total, 80 pots with 2 seedlings in each were used. Mean values were separated by using Tukey’s test at the 5% probability level. The data presented here are the average of the two years of experiments. Data analyses were performed using SPSS v.19.0 software (SPSS Inc., USA).

Results

Oxidative damages

ELP was significantly affected by the interaction between salt stress and B concentration in the soil (P ≤ 0.05). Accordingly, application of 20 mg B·kg⁻¹ of soil significantly intensified ELP under non-saline control conditions; however, with application of 5 mg B·kg⁻¹ of soil, a significant reduction in ELP was observed under this condition (Fig. 1). Under 800–1600 mg NaCl·kg⁻¹ of soil, the ELP of plants treated with a high concentration of B was reduced significantly. A significant increase in ELP was observed by increasing the NaCl concentration by up to 2400 mg·kg⁻¹. Application of 20 mg B·kg⁻¹ of soil significantly enhanced ELP under this situation. In contrast, the lowest ELP was observed in the 5 mg B treatment under 1600 and 2400 mg NaCl stress. The leaf MDA content, which represents the degree of lipid peroxidation, was significantly affected by the interaction between salt stress and the soil’s B concentration (P ≤ 0.01). Application of 20 mg B·kg⁻¹ of soil significantly increased leaf MDA content under non-saline conditions (Fig. 1). By increasing the NaCl concentration in the soil, an increasing trend in leaf MDA content was observed. Under treatments of 800–2400 mg NaCl·kg⁻¹ of soil, the MDA content of the leaves managed to recover to the level of the respective control treatments. Generally, plants treated with 5 mg B·kg⁻¹ of soil had the lowest MDA concentration under different salt stress levels.

Leaf H₂O₂ content was affected by the interaction between salt stress and B (P ≤ 0.05). Under non-saline conditions, a significant increase in leaf H₂O₂ content was observed in plants treated with 10–20 mg B·kg⁻¹ of soil (Fig. 2). Under the treatment of 800–2400 mg NaCl·kg⁻¹ of soil, the H₂O₂ content of leaves in plants with excess amounts of B was restored to normal, reaching the H₂O₂ content of plants that were not treated with B. Application of 1600–2400 mg NaCl·kg⁻¹ of soil significantly increased H₂O₂ content in the leaves.
The lowest leaf $H_2O_2$ content was observed in the leaves of plants treated with 5 mg of B under different salt stress conditions.

The lipolytic enzyme activity was affected by the interaction between salt stress and B concentration in the soil ($P \leq 0.05$). Plants treated with 20 mg B·kg$^{-1}$ of soil displayed a drastic growth in LOX activity under non-saline salt stress treatment; however, under different NaCl treatments, LOX activity in the leaves of plants treated with 20 mg B was similar to plants with no B treatment. Moreover, a significant increase in LOX activity was observed in plants treated with 2400 mg NaCl·kg$^{-1}$ of soil. Nonetheless, the application of 2.5–5 mg B·kg$^{-1}$ of soil under severe salt stress restored the LOX activity to the degree observed in plants that were not treated with salinity (Fig. 2). Positive correlations between ELP and MDA ($R^2 = 0.72$), and ELP and $H_2O_2$ ($R^2 = 0.74$) were detected (Fig. 2).

Concentrations of ascorbic acid (AA) and phenolics in leaves

Salt stress treatments did not affect the content of phenolics in pistachio leaves. However, the application of 5 mg B·kg$^{-1}$ of soil significantly reduced phenolics in the leaves ($P \leq 0.01$). The highest concentration of phenolics was observed in plants treated with 20 mg B·kg$^{-1}$ of soil (Table 2). Salt stress ($P \leq 0.01$) and the application of B in the soil significantly affected concentration of AA in pistachio leaves ($P \leq 0.05$). Total AA had a tendency to decrease, parallel to the increase in the soil’s NaCl concentration, since the lowest leaf AA content was found in plants treated with 2400 mg NaCl·kg$^{-1}$ of soil. However, the application of 2.5–10 mg B increased the AA concentration in the leaves. The highest AA concentration was observed in the leaves of plants treated with 5 mg B·kg$^{-1}$ of soil (Table 2).

Activity of antioxidative enzymes

Activity of SOD, CAT, and APX was significantly affected by salt stress ($P \leq 0.01$, $P \leq 0.05$, and $P \leq 0.01$, respectively) and B concentration in the soil ($P \leq 0.01$, $P \leq 0.01$, and $P \leq 0.05$, respectively). Activities of the antioxidant enzymes SOD, CAT, and APX significantly increased in the leaves of plants treated with 1600–2400 mg NaCl·kg$^{-1}$ (Table 3). The highest SOD activity was found in plants treated with 20 mg B, which is an excess amount of the element for the plant. Application of 5 mg B·kg$^{-1}$ of soil caused a noticeable decrease in SOD activity. The trends for CAT and APX activities in the presence and absence of B in salt stressed plants were contrary to those observed for SOD. The CAT and APX activities decreased significantly under high and low B concentrations in the soil. The application of 2.5 and 5 B increased the APX and CAT activities significantly, compared with B stress treatments.

Discussion

This study was performed to investigate the effects of the interaction between the soil’s B concentration and NaCl stress, and to understand the mechanism(s) by which B affects NaCl toxicity in P. vera. The interaction between a micronutrient and a stress factor—here salt—could be vital for understanding, analyzing, and improving plant defense strategies through various factors.
**Table 2.** The effects of boron concentrations and NaCl in soil on concentration of phenolics and ascorbic acid in the leaves of *Pistacia vera* ‘Badami’ seedlings.

| NaCl (mg·kg⁻¹ soil) | Phenolics (mg·g⁻¹) | Ascorbic acid (mg·g⁻¹) |
|---------------------|--------------------|------------------------|
|                     | Boron (mg·kg⁻¹ soil) | Mean       | Boron (mg·kg⁻¹ soil) | Mean       |
| 0                   | 0                  | 160.5      | 0.949              | 0.898     |
|                     | 2.5                | 141.0      | 1.143              | 0.942     |
|                     | 5                  | 133.2      | 1.048              | 0.952     |
|                     | 10                 | 171.1      | 0.923              | 0.903     |
|                     | 20                 | 181.9      | 0.884              | 0.921     |
| 800                 | 157.5              | 157.5      | 0.843              | 0.854     |
| 1600                | 146.7              | 151.0      | 0.638              | 0.861     |
| 2400                | 143.5              | 145.0      | 0.726              | 0.730     |

Differences among the treatments were analyzed by 4 salt stress × 5 boron levels ANOVA with 4 replications. Mean values followed by the same letters did not differ significantly in an intra-group comparison according to Tukey’s test at *P* < 0.05.

**Table 3.** The effects of boron concentrations and NaCl in soil on activity of antioxidative enzymes in leaves of *Pistacia vera* ‘Badami’ seedlings.

| NaCl (mg·kg⁻¹ soil) | Superoxide dismutase (unit·g⁻¹) | Catalase (µkat·mg⁻¹ prot·min⁻¹) | Ascorbate peroxidase (µkat·mg⁻¹ prot·min⁻¹) |
|---------------------|---------------------------------|---------------------------------|-----------------------------------------------|
|                     | Boron (mg·kg⁻¹ soil) | Mean                     | Boron (mg·kg⁻¹ soil) | Mean                     | Boron (mg·kg⁻¹ soil) | Mean                     |
| 0                   | 0                  | 206.6           | 219.1              | 0                 | 8.86           | 9.14              |
|                     | 2.5                | 205.7           | 248.9              | 800               | 10.0           | 10.37              |
|                     | 5                  | 207.6           | 274.4              | 1600              | 10.34          | 11.35              |
|                     | 10                 | 236.6           | 287.6              | 2400              | 232.2          | 12.75              |
|                     | 20                 | 239.0           |                    |                   | 260.8          |                   |
| 800                 | 0                  | 253.1           | 248.9              | 0                 | 9.06           | 9.14              |
| 1600                | 12.94             | 9.96            | 10.50              | 10.0              | 11.33          | 11.35              |
| 2400                | 11.33             | 11.10           | 13.41              | 12.73             | 13.84          | 12.75              |
| Mean                | 11.45             | 9.27            | 11.77              | 11.63             | 11.53          | 9.49               |
|                     |                    | 11.05           | 11.53              |                   | 11.15          |                   |
|                     |                    | 12.53           | 13.41              |                   | 11.16          |                   |
|                     |                    | 13.84           |                     |                   | 13.77          |                   |
|                     |                    | 14.11           |                     |                   | 11.77          |                   |
|                     |                    | 14.34           |                     |                   | 11.77          |                   |

Differences among the treatments were analyzed by 4 salt stress × 5 boron levels ANOVA with 4 replications. Mean values followed by the same letters did not differ significantly in an intra-group comparison according to Tukey’s test at *P* < 0.05.
It is commonly known that the lipid peroxidation of membranes—which is induced by ROS—reflects stress-induced damage at the cellular level (Jain et al., 2001). Preserving cell membrane integrity and function under environmental stresses are considered as tolerance mechanisms in plants. Many studies have shown that severe salt stress may affect cell membrane integrity in pistachio leaves, in agreement with our results (Karimi et al., 2009; Tavallali et al., 2009). Moreover, a similar trend in increasing ELP was observed by application of 10 and 20 mg B·kg⁻¹ of soil, which reflected cell membrane injury due to the high concentration of B in the soil. The positive correlations between ELP and MDA, and between ELP and H₂O₂ (Fig. 2), revealed that the loss of cell membrane integrity was mainly due to the accumulation of H₂O₂ in the leaves, which intensified lipid peroxidation. The level of MDA is often monitored as an indicator of oxidative destruction of plasma membranes (Karimi et al., 2017; Mittler, 2002). ELP and MDA were minimum in the moderate B level + salt stress condition (Table 2). B is known to play important roles in maintenance of membrane structure and membrane-associated reactions (Brown et al., 2002). In our study, application of 5 mg B·kg⁻¹ of soil in the presence of NaCl may have partially defended membranes against oxidative stress. This is because of the fact that B contributes to the preservation of the cell wall and the maintenance of plasma membrane integrity (Marschner, 1995). Similarly, sufficient amounts of B impeded the reduction in total lipid and phospholipid contents in the roots and leaves of Solanum lycopersicum (tomato) and Abelmoschus esculentus (okra) plants (Desiraju et al., 1993). When there is either a lack or excess of B supply in the soil, the lipid peroxidation becomes more evident under salt stress. These results are in accordance with previous studies on S. lycopersicum (Cervilla et al., 2007), Malus domestica rootstocks (Molassiotis et al., 2006), and Vitis vinifera (Gunes et al., 2006). Moreover, the increase in LOX activity due to salt stress and B toxicity confirms higher lipolytic activity of the membrane and oxidation of membrane-bound fatty acids, which intensify peroxidation of lipids (Lacan and Baccou, 1998).

Surprisingly, under 800–1600 mg salt stress, the oxidative stress of excess B application (20 mg B·kg⁻¹ of soil) was restored to the state of the control level. These observations suggest that low to moderate salt stress may reduce B toxicity pressure on the plant. Accordingly, Ferguson et al. (2002) mentioned that B toxicity in pistachio leaves under moderate salt stress was lower than B toxicity under non-saline conditions. Yermiyahu et al. (2008) stated that the reduced uptake of B in the presence of chloride may be associated with lower B toxicity under salt stress. In addition, as B absorption and translocation is a passive process in B-rich soils, a lower transpiration rate under salt stress may reduce B accumulation and its oxidative damage to the leaves (Edelstein et al., 2005).

We observed the lowest phenolics content in moderate B concentrations in the soil; however, opposite trends were observed in the absence, and high concentrations of B. Accordingly, Mondy and Munshi (1993) reported that moderate B fertilization significantly reduced the phenolics concentration of potato tubers. Increases in the concentration of phenolics in B-deficient and B-excess tissues are common issues (Marschner, 1995). Formation of cis-diols complexes between B and some sugars or phenolics can play a decisive role in the accumulation of phenolics in this situation. Consequently, under high and low B conditions, the substrate flux is shifted from glycolysis into the pentose phosphate pathway, resulting in an increased synthesis of phenol (Cakmak and Romheld, 1997).

Ascorbic acid (AA) as a non-enzymatic antioxidant reacts with superoxide radicals (O₂⁻), hydroxyl radicals (OH⁻), and lipid hydroperoxidases (Reddy et al., 2004). It also is linked to H₂O₂ scavenging via APX (Sairam et al., 1998). Moreover, AA involves in the regeneration of another non-enzymatic antioxidant, α-tocopherol (Sairam et al., 2005). The AA–GSH cycle is considered the chief mechanism for ascorbate regeneration against ROS formation in plants (Mittler, 2002). A role for increased AA content in amelioration of oxidative stress has been reported by Sairam et al. (2005). In our study, AA content was increased following application of 5 mg B·kg⁻¹ of soil, while high B and NaCl stress reduced the AA content. In agreement with our results, several reports have indicated that an adequate B supply can increase the concentration of AA in other species (Cakmak and Romheld, 1997; Mondy and Munshi, 1993). Moreover, Han et al. (2009) and Ferro et al. (2009) reported that the AA content was reduced in plants with excess amounts of B. Furthermore, B induced an increase in ascorbate levels in sunflower and maize plants subjected to aluminum stress (Corrales et al., 2008), which supports the view that adequate B supply stimulates antioxidant responses in stressed plants.

As mentioned before, several antioxidant enzymes participate in the detoxification of ROS. The SOD activity was enhanced in response to NaCl that was combined with both the excessive level of B, and the lack of it, in the soil. However, a noteworthy decrease was observed for adequate B concentrations at all salt stress levels (Table 3). Similar trends were reported for M. domestica rootstocks (Molassiotis et al., 2006), Citrus grandis (Han et al., 2009), and in Helianthus annuus and S. lycopersicum (Keleş et al., 2011) under the excess presence of B. The increased SOD activity under stressful conditions may increase the ability of plants to scavenge O₂⁻, but the byproduct of this process is H₂O₂ which is a potent oxidizing agent. H₂O₂, like other ROS, can be expected to be responsible for lipid peroxidation, so plants need to detoxify it by CAT
and APX (Sairam et al., 2005). In the present study, the activity of CAT and APX was induced by salt stress up to 139.5% and 152.7%, respectively. Interestingly, under low salt stress, no significant increase in APX was detected, which suggests that the enhanced CAT activity is probably enough to detoxify low levels of H$_2$O$_2$. According to the H$_2$O$_2$ data, the enhanced CAT under 800 mg NaCl·kg$^{-1}$ of soil was effective to keep H$_2$O$_2$ levels as low as the control treatment. Moreover, application of B up to 10 mg·kg$^{-1}$ soil enhanced CAT and APX activity in the leaves. These observations suggest that the protective effects of B application against salt stress is in part due to the enhancement of antioxidative enzymes. Additionally, APX was found to be more important than CAT in the detoxification of H$_2$O$_2$ under the high salt stress treatment. The upsurge in APX, as induced by salt stress, has previously been described by Hernandez et al. (2000). Moreover, the defense capacity of cells against toxic reactive O$_2$ species was strengthened in tissues containing sufficient amounts of B, which is because of the augmented ascorbic acid levels and H$_2$O$_2$ scavenging enzymes (Cakmak and Romheld, 1997). In our study, the high B concentration in the soil (20 mg·kg$^{-1}$ soil) reduced CAT and APX activities, especially in plants under NaCl stress. Similar results have been reported in V. vinifera (Gunes et al., 2006) and in S. lycopersicum (Ferro et al., 2009) and also in relation to the catalase activity in C. sinensis leaves (Keleş et al., 2004) and in C. grandis (Han et al., 2009).

In conclusion, we found a modification of antioxidative activity. This can be explained by the effects of the interaction between salt stress and boron. The stress factor—NaCl—caused a greater oxidative stress compared to the other stress factors, i.e. the excessive presence or lack of B. Our major observation was that moderate B concentrations in the soil, by up to 5 mg·kg$^{-1}$ soil, can alleviate salt stress. Moreover, the results surprisingly revealed that the presence of a mild to moderate condition of salt stress (800–1600 mg NaCl·kg$^{-1}$ of soil) may reduce B toxicity pressure on pistachio plants. Therefore, a sufficient B supply is useful for increasing the ability of plant cells to tolerate oxidative damage and in maintaining the structural integrity of plasma membranes and the cell wall.

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