Abstract: Boron (B) toxicity or deficiency are important abiotic stress factors that limit crop productivity mainly in arid and semi-arid regions of the world. High and deficient levels of B in the soil disrupt various physiological and biochemical processes in the plant. Nowadays, anti-stressors are being used to alleviate the effects of different abiotic stresses. The aim of this study was to examine the effects of externally applied selenium on various physiological and biochemical parameters of monocotyledonous and dicotyledonous plants grown in B-toxic and deficient conditions. As an outcome, the externally applied selenium reduced the stress-induced damage in the experimental monocot and dicot plants, and its protective roles have been determined.

Keywords: barley; boron deficiency; boron toxicity; selenium; soya

1. Introduction

Plants face adverse abiotic environmental conditions such as drought, heat and cold, nutrient deficiency and/or excess, excessive salt and toxic metal levels in the soil. Such stress factors limit the worldwide use of arable land and limit the productivity of sustainable farmland by affecting crop yields. However, the external application of some elements or compounds can increase the tolerance level of plants to such stresses via strengthening their protective mechanisms [1,2].

Externally applied elements are involved in the adaptive response of plants associated with the repair of stress-induced damage, rebalancing of cellular homeostasis, and increased growth [3,4].

Boron (B) is found in nature in the form of boric acid $\text{H}_3\text{BO}_3$, borate $[\text{B(OH)}_4]^{3-}$, or borosilicate mineral [5]. Several factors including texture, moisture, pH, temperature, and application methods affect the amount of B absorbed by the soil and its bioavailability in plants. There is a narrow range between B toxicity and deficiency in plants. High and low boron concentrations in plants affect many physiological and biochemical processes, including plant growth, lipid peroxidation, disturbed enzyme activities, and decreased photosynthetic efficiency [6].

Selenium (Se), a nonmetallic element, is very important for plants in many ways. Its most oxidized forms of selenium are water soluble and therefore both selenate ($\text{SeO}_4^{2-}$) and selenite ($\text{SeO}_3^{2-}$) have high bioavailability potential in nature [7]. Although it is an essential micronutrient, it has no direct requirements for plants. However, it has been
shown in many previous studies that additional application of a low concentration of Se has positive effects on plants grown in different abiotic stress conditions. However, there is no information in the literature about the effects of externally applied selenium on boron deficiency and toxicity in plants to date. In this direction, we tried to reveal the effects of selenium on the growth and antioxidant enzymes’ activities of monocot and dicot plants grown in B-deficient and toxic conditions.

2. Materials and Methods

In this study, monocot Barley genotypes (Tokak—tolerant to boron toxicity and sensitive to boron deficiency and Hamidiye—sensitive to boron toxicity and tolerant to boron deficiency) genotypes and dicot Soybean genotype (Sari Gelin) were used. The plant materials used in the research were obtained from the Department of Field Crops, Selcuk University.

2.1. Growth Medium, Application Doses, and Plant Sampling

The experimental seeds were sterilized and kept for germination. After germination, seedlings were grown under controlled conditions in a hydroponic system adjusted to 45–55% humidity, 16 h light and 8 h dark photoperiod, 22 ± 1 °C temperature, and 10,000 Lux/day light intensity during growth and development. In the study, toxic 1 mM and deficient 0 mM B doses were applied to the barley and soybean genotypes to compare with the Control (0.0033 mM B) group. Moreover, 0.01 and 0.05 mg kg”1 selenium applications were made individually and in combination with the boron applications. The study was set up with 4 replications in each group, and the nutrient solutions were renewed in every two days. Sampling was done after the plants started to show morphological changes in the boron-deficient and toxic conditions.

2.2. Growth Parameters

During the specified sampling periods, plants were harvested, and their roots and shoots were separated from each other to measure the shoot length. Moreover, samples were collected to estimate enzyme activity.

2.3. Superoxide Dismutase (SOD) Enzyme Activity

Superoxide dismutase (SOD) enzyme activity was measured according to the method described by Beauchamp and Fridovich [8]. SOD activity was determined as the amount of enzyme causing 50% inhibition of photochemical reduction of electron acceptor NBT measured in a spectrophotometer at 560 nm. The specific enzyme activity was determined as U/mg protein. One unit of SOD activity showed the amount of enzyme (SOD) that converted 1 µmol of substrate to product in 1 min at 25 °C.

2.4. Peroxidase (POX) Enzyme Activity

Peroxidase (POX) enzyme activity was determined according to the method described by Herzog and Fahimi [9]. Absorbance changes were read for 3 min depending on the amount of DAB (3’-3’-diaminobenzidine tetrahydrochloride) oxidized in the presence of H2O2 at 465 nm versus blank. Specific enzyme activity was expressed as µmol/mL H2O2 consumed per minute.

2.5. Catalase (CAT) Enzyme Activity

Catalase (CAT) enzyme activity was performed according to the method described by Bergmeyer [10]. The analysis was performed by determining the reduction rate of H2O2 at 240 nm versus the blind in the UV light region. This enzyme activity was expressed as µmol H2O2 consumed per minute. The decrease in absorbance during the reaction was followed for 180 s. CAT activity was expressed as µmol H2O2 consumed per minute.
3. Results and Discussion

Boron, an important plant nutrient, has been reported in many previous studies to adversely affect plants in both deficient and toxic conditions. Excess and deficient B in leaves causes osmotic imbalance and increases the generation of reactive oxygen species (ROS), increasing electrolyte leakage and membrane damage [11]. However, the protective mechanism of antioxidant enzymes alleviate these symptoms and increase the tolerance level of plants. In this study, the shoot length of monocotyledonous and dicotyledonous plants was negatively affected in both boron deficiency and toxicity conditions, and decreased as compared to those of control conditions. However, on externally applying selenium doses, shoot length was improved as compared to those in the stressed conditions (Figure 1). The results obtained were in line with the several research studies where selenium was applied externally to plants exposed to various stresses [2,12]. SOD activity increased in all the treatments as compared to that of control. The selenium-treated groups showed increased SOD activity as compared to those of the non-selenium groups. Selenium applications also increased CAT, POX, and APX activities in plants grown under boron deficiency and toxicity conditions. These findings were in accordance with the previous studies reported by Habibi and Sarvary [13], Feng et al. [14], Shekari et al. [15], and Agbolade et al. [16]. The enhancement in the antioxidant enzymes’ activities of plants grown under B deficiency and toxicity conditions on selenium application determined the activation of a protective mechanism due to selenium.

![Graphs showing growth, SOD, and POX activities](image_url)

**Figure 1. Cont.**
References

1. Zhang, H.; Zhu, J.; Gong, Z.; Zhu, J.-K. Abiotic stress responses in plants. Nat. Rev. Genet. 2021, 23, 104–119. [CrossRef] [PubMed]
2. Sharifi, P.; Amirnia, R.; Torkian, M.; Bidabadi, S.S. Protective Role of Exogenous Selenium on Salinity-Stressed Stachys byzantine Plants. J. Soil Sci. Plant Nutr. 2021, 21, 2660–2672. [CrossRef]
3. Zhu, J.-K. Abiotic Stress Signaling and Responses in Plants. Cell 2016, 167, 313–324. [CrossRef] [PubMed]
4. Nabi, R.B.S.; Tayade, R.; Hussain, A.; Kulkarni, K.P.; Imran, Q.M.; Mun, B.-G.; Yun, B.-W. Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environ. Exp. Bot. 2019, 161, 120–133. [CrossRef]
5. Brdar-Jokanović, M. Boron Toxicity and Deficiency in Agricultural Plants. Int. J. Mol. Sci. 2020, 21, 1424. [CrossRef] [PubMed]
6. Princi, M.P.; Lupini, A.; Araniti, F.; Longo, C.; Mauceri, A.; Sunseri, F.; Abenavoli, M.R. Boron Toxicity and Tolerance in Plants: Recent advances and future perspectives. Plant Met. Interact. 2016, 115–147. [CrossRef]
7. Saha, U.; Fayiga, A.; Sonon, L. Selenium in the Soil-Plant Environment: A Review. Int. J. Appl. Agric. Sci. 2017, 3, 1–18. [CrossRef]
8. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 1971, 44, 276–287. [CrossRef]
9. Herzog, V.; Fahimi, H. A new sensitive colorimetric assay for peroxidase using 3,3′-diaminobenzidine as hydrogen donor. Anal. Biochem. 1973, 55, 554–562. [CrossRef]
10. Bergmeyer, N. Methoden der Enzymatischen Analyse; AkademieVerlag: Berlin, Germany, 1970; Volume 1, pp. 636–647.
11. Moussa, H.R.; Algamal, S.M.A. Does Exogenous Application of Melatonin Ameliorate Boron Toxicity in Spinach Plants? *Int. J. Veg. Sci.* **2016**, *23*, 233–245. [CrossRef]
12. Jiang, C.; Zu, C.; Lu, D.; Zheng, Q.; Shen, J.; Wang, H.; Li, D. Effect of exogenous selenium supply on photosynthesis, Na$^+$ accumulation and antioxidative capacity of maize (*Zea mays* L.) under salinity stress. *Sci. Rep.* **2017**, *7*, srep42039. [CrossRef] [PubMed]
13. Habibi, G.; Sarvary, S. The roles of selenium in protecting lemon balm against salt stress. *Iran. J. Plant Physiol.* **2015**, *5*, 1425–1433.
14. Feng, R.; Wei, C.; Tu, S. The roles of selenium in protecting plants against abiotic stresses. *Environ. Exp. Bot.* **2013**, *87*, 58–68. [CrossRef] [PubMed]
15. Shekari, F.; Abbasi, A.; Mustafavi, S.H. Effect of silicon and selenium on enzymatic changes and productivity of dill in saline condition. *J. Saudi Soc. Agric. Sci.* **2017**, *16*, 367–374. [CrossRef]
16. Agbolade, J.O.; David, O.; Ajiboye, A.; Kioko, J.; Jolayemi, O.; Olawumi, L.; Ojo, M.; Akomolafe, G.; Adekoya, M.; Komolafe, R. Morpho-physiological effect of selenium on salinity-stressed wheat (*Triticum aestivum* L.). *J. Biol. Res. Boll. Soc. Ital. Biol. Sper.* **2019**, *92*, 7650. [CrossRef]