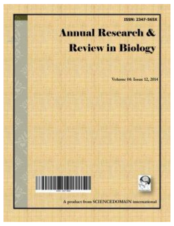

Annual Research & Review in Biology

21(6): 1-7, 2017; Article no.ARRB.38772
ISSN: 2347-565X, NLM ID: 101632869

Effects of Boron Applications on the Physiology and Yield of Lettuce

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SS, FG and NG designed the study, performed the experiments, statistical analysis and wrote the draft of the manuscript. Authors DK and SS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/38772
Editor(s):
(1) Bechan Sharma, Department of Biochemistry, University of Allahabad, Allahabad, India.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Ningappa M. Rolli, BLDEA's Degree College, India.
(2) Anibal Condor Golec, Canada.
(3) Raúl Leonel Grijalva Contreras, National Institute of Agricultural, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/22579

Original Research Article

Received 12th December 2017
Accepted 30th December 2017
Published 30th December 2017

ABSTRACT

Aim: The present study was conducted to determine the effects of boron applications on vegetative development, antioxidant activity and mineral composition of lettuce.
Study Design: In the greenhouse, treatments arranged according to a completely randomized block design, with four replications.
Methodology: Funnly F1 lettuce cultivar was used as the plant material and Etidot-67 (21% B) fertilizer was use as the boron source. Five different boron doses (0, 50, 100, 200 and 400 gr da-1) were applied and changes in lettuce plants were observed. Effects of different boron doses on root weights, head weights, water soluble dry matter (WSDM) contents, titratable acidity (TA), pH, vitamin C, leaf N, P, K and B concentrations were investigated. Besides, leaf ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POD) contents were also investigated.
Results: Root and marketable plant weights decreased with increasing boron doses. Boron

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treatments did not show significant effects on plant chlorophyll contents and biochemical characteristics. Leaf B concentrations increased with increasing B doses. Compared to control treatments, significant increases were observed in enzyme activities with boron treatments. APX, CAT, POD activities at the highest boron dose were respectively measured as 6.53, 527 and 0.387. Current results revealed that a boron dose of over 200 gr da-1 created stress conditions in lettuce plants.

Conclusion: Fertilizers should be selected and applied by taking boron sensitivity or resistance levels of plants into consideration.

Keywords: Antioxidant activity; ascorbate peroxidase; catalase; peroxidase; lettuce; boron applications.

1. INTRODUCTION

Boron is an essential element for the growth and development of plants and adequate B nutrition is crucial for crop production. Boron toxicity is a micro nutrient problem locally observed over the soils of arid and semi-arid regions [1]. Both toxic and deficient boron levels result in significant decreases in plant development and yields. Thus, boron should be applied to soil just at sufficient levels.

Beside plant growth, boron also plays a significant role in many physiological and biochemical processes of the plants. [2] listed the roles of boron as; 1- sugar transmission, 2- cell-wall synthesis, 3- lignification, 4- cell wall structure, 5- carbohydrate metabolism, 6- RNA metabolism, 7- respiration, 8- indole acetic acid (IAA) metabolism, 9- phenol metabolism and 10- membranes [3]. Boron is efficient upon pollen tube growth, reproduction and pollen germination in plant [4-5].

Boron toxicity of soils mainly comes from high boron concentrations of parent material, high boron concentrations of groundwater and excessive boron fertilizations. B toxicity of plant exerts different effects on very diverse processes in vascular plants, such as altered metabolism, reduced root cell division, lower leaf chlorophyll contents and photosynthetic rates and decreased lignin and suberin levels [6-7].

Boron toxicity also results in various alterations in antioxidant activity levels of the plants. Plants develop a series of defense mechanisms to overcome the oxidative stress. Antioxidant defense system of plant cells is composed of APX, CAT, POD and SOD-like enzymatic or ascorbate, α–tocopherol-like non-enzymatic components. Antioxidant system plays a defensive role in elimination of toxic radicals produced through oxidative stress [8] Increase or decrease in antioxidant activities is a direct adaptive respond of plants against metal toxicity. ROS eliminating enzyme, SOD, is a metalloprotein and converts O_{2^-} into H_2O_2 and oxygen. H_2O_2 is then metabolized with POD and CAT [9].

The objective of study was to investigate the effects of different boron doses on lettuce plant development, tissue boron concentrations of plant, resultant oxidative stress and antioxidant enzyme activities.

2. MATERIALS AND METHODS

2.1 Plant Growth and Boron Stress Treatments

The present study was conducted in an unheated greenhouse with fine gravel covered base located at Gaziosmanpaşa University Research and Experimental Farm, city of Tokat in Turkey, during the spring of the year 2014. Funnly F1 lettuce cultivar was used as the plant material of the study. Weekly fertilizers were applied through fertigation technique of a drip irrigation. Plants were supplied with 12 kg N da^{-1}, 8 kg P_2O_5 da^{-1} and 10 kg K_2O da^{-1} and Ca, Mg, Fe, Zn fertilizations were performed at optimal levels. Experiments were conducted in randomized plots design with 4 replications. Some physical and chemical characteristics of experimental soils are provided in Table 1. Five different boron (B) doses (0, 50, 100, 200 and 400 gr da^{-1}) were applied to root regions of the plants through Etidot-67 ($Na_2B_8O_{13}.4H_2O$-ETİDOT-67 (Disodyum Oktaborat Tetrahidrat) (21% B) fertilizer. Boron fertilizer was applied in two portions. (Etidot-67 fertilizer is produced by ETI mining operations). On-row plant spacing was 40 cm and there were 12 plants in each row and 48 plants in each plot. Seedlings were planted on 5th of March and plants were harvested on 30th of April.

Table 1. Some physical and chemical characteristics of experimental soils

Soil Characteristics		Soil Characteristics	
pH (1:2.5)	7.85	Total N (%)	0.05
Lime (%)	3.51	Available P_2O_5 (kg da^{-1})	3.75
Organic matter (%)	1.64	Available K_2O (kg da^{-1})	6.62
EC mmhos cm^{-1}	0.24	Iron (mg kg^{-1})	1.13
		Copper (mg kg^{-1})	0.34
Texture	Clay loam	Manganese (mg kg^{-1})	1.65
		Zinc (mg kg^{-1})	0.25
		Boron (mg kg^{-1})	0.12

2.2 Tissue Mineral Matter and Biochemical Analyses

Plant tissues were dried in an oven at 65˚C until a constant weight. Total nitrogen (N) was determined by using Kjeldahl method and available P and K levels were analyzed in an ICP device [10]. Boron levels of plant were determined according to the Azomethine-H method [11]. Leaf pH values and titratable acidity were measured from titrable acidity extracts [12]. Water soluble dry matter was measured in Hanna refractometer and spectrophotometric method was used to determine vitamin C contents [13].

2.2.1 Enzyme extraction

For enzyme activities, 0.3 g root and leaf samples were smashed in liquid nitrogen and homogenized in a mortar with 50 mM potassium phosphate buffer (pH=7) containing 1 mM EDTA and 1% (w/v) PVPP. Then the homogenate was centrifuged at + 4˚C and 15000xg for 20 minutes. Ultimately, catalase, ascorbate peroxidase and superoxide dismutase activities were determined spectrophotometrically from the resultant supernatant.

2.2.2 Catalase activity

Catalase activity was initiated by adding 50 µl crude extract into 50 mM phosphate buffer (pH=7) and 10 mM H_2O_2 mixture. Then the decomposition of H_2O_2 at 240 nm was monitored and changes in absorbances were read (E = 0,036 $mM^{-1}cm^{-1}$) [14] Peroxidase Activity.

For peroxidase activity, 30 µl enzyme extract was added to 50 mM phosphate buffer (pH=7), 30 mM guaiacol and 10 mM H_2O_2 reaction mixture. Then the activity was determined by measuring the increase in absorbance through oxidation of guaiacol at 470 nm (E = 26.6 mM^{-1} cm^{-1}) [15].

2.2.3 Ascorbate peroxidase

The reaction mixture contains 50 mM phosphate buffer (pH=7), 2.5 mM ascorbate, H_2O_2 and 30 µl enzyme extract. H_2O_2 was the last added compound and the activity was determined by measuring the oxidation of ascorbate at 290 nm (E = 2.8 mM^{-1} cm^{-1}) [16].

2.2.4 Statistical analysis

Statistical analyses and variance analysis (ANOVA) were performed through SPSS (Version 12.00; Chicago, IL, USA) software. Means were compared by Duncan's test.

3. RESULTS AND DISCUSSION

3.1 Plant Development, Biochemical Characteristics and Mineral Contents

Effects of boron treatments on plant root weights, head weights, head diameters and head lengths are provided in Table 2. Boron treatments resulted in significant differences in plant root and head weights ($P<0.01$). While the highest root and head weight were obtained from 100 gr da^{-1} B treatments respectively with 81 gr and 950 gr, the lowest values (68 and 824 gr) were obtained from 400 gr da^{-1} B treatments. Plant vegetative development exhibited a decreasing trend with 200 gr da^{-1} B treatment. Boron treatments did not yield significant differences in plant height, head diameter, WSDM, TA, vitamin C and leaf pH values.

Root B and leaf B concentrations increased significantly with increasing B treatments ($P<0.01$). While B concentration in plant roots of control treatment was 9 ppm, the value increased to 32 ppm in 400 gr da^{-1} B treatment. Leaf boron concentrations were respectively observed as 15, 28, 45, 68 and 84 ppm (Table 3). Root N concentrations were not affected from boron treatments, but leaf N concentrations exhibited significant differences with boron treatments ($p < 0.05$). Root and leaf P and K concentrations did not exhibit significant differences with boron treatments (Table 3).

3.2 Antioxidant Enzyme Activity

Effects of boron treatments to plant growth medium on antioxidant defense system enzyme activities were determined in lettuce leaves. Compared to control treatment, significant increases were observed in CAT enzyme activity. Such an increase was almost 2.5 folds at high boron doses. POD enzyme activity also increased with boron treatments and realized as about 2.5 fold in 400 g da^{-1} treatment. APX enzyme activity increased significantly only at high boron dose and realized as 2 folds in high dose.

Significant differences were observed in leaf APX enzyme activities with different boron treatments ($P<0.01$) (Fig. 1). While APX activity of control treatment was 2.72, the value was observed as 6.53 at the highest boron dose. Significant increases were observed in leaf CAT and POD activity values with increasing boron doses ($P<0.01$) (Figs. 2 and 3). While leaf CAT activity of control treatment was 141, the value respectively increased to 162, 354, 507 and 527 with increasing boron doses. Leaf POD activity values varied between 0.162 - 0.387. Significant increases were observed in investigated antioxidant enzyme values with increasing boron doses ($P<0.01$).

Compared to control treatments, present results revealed significant increases in plant development parameters of lettuce with 50 and 100 gr da^{-1} boron treatments. Since the experimental soils had desired B levels, additional boron fertilizer increased the yields. However, the critical boron dose was determined to be 200 gr da^{-1} since yield decreases were observed beyond this dose. Optimal boron doses both increased the yields and improved quality parameters [17,18-19]. But the boron doses beyond the optimal levels are accumulated within the cells and create a toxic effect and ultimately recess plant physiological development. Root and head weights were sufficient until the toxic levels, but yield decreases were observed then because of boron toxicity and resultant damages in cell physiology and insufficient nutrient uptake.

Nutrient storage organs of foliated plants are the leaves. The nutrients are transported from the roots to leaves and accumulated there. In addition, plants take up and transport B through the transpiration stream [3].In this study, B concentrations of both the roots and the leaves increased with increasing boron doses. Such an increase was observed in 200 gr da $^{-1}$ treatments of the present study which was above the

Table 2. Effects of boron treatments on yield and quality parameters of lettuce plants

Doses	Head weight (gr)**	Root weight (gr)**	Height (cm)	Diameter (cm)	WSDM (%)	TA (%)	Vitamin C mg 100g^{-1}	pH
B0	823 c	73 c	18.1	32	2.21	2.38	14.12	3.23
B1	912 ab	78 b	17.9	34	2.32	2.45	13.23	3.45
B2	950 a	81 a	18.1	35	2.56	2.56	14.34	3.14
B3	867 b	72 c	18.1	32	2.34	2.41	13.13	3.09
B4	824 c	68 c	17.6	32	2.45	2.32	13.34	3.12
Means	886	74.4	17.8	33	2.37	2.42	13.63	3.20

WSDM: Water Soluble Dry Matter; TA: Titratable Acidity
***significant at p < 0.01*

Table 3. Root and leaf mineral concentrations of lettuce plants under boron treatments

Doses	Root B (mg kg $^{-1}$)**	Leaf B (mg kg $^{-1}$)***	Root N (%)	Leaf N (%)*	Root P (%)	Leaf P (%)	Root K (%)	Leaf K (%)
B0	9	15	1.25	2.41	0.26	0.38	1.25	2.56
B1	15	28	1.19	2.51	0.27	0.37	1.29	2.59
B2	21	45	1.21	2.54	0.26	0.35	1.31	2.61
B3	28	68	1.18	2.40	0.27	0.38	1.24	2.69
B4	32	84	1.22	2.38	0.24	0.33	1.27	2.65
Means	12	28.2	1.21	2.44	0.26	0.36	1.27	2.62

** *Significant at p < 0.01; * significant at p < 0.05*

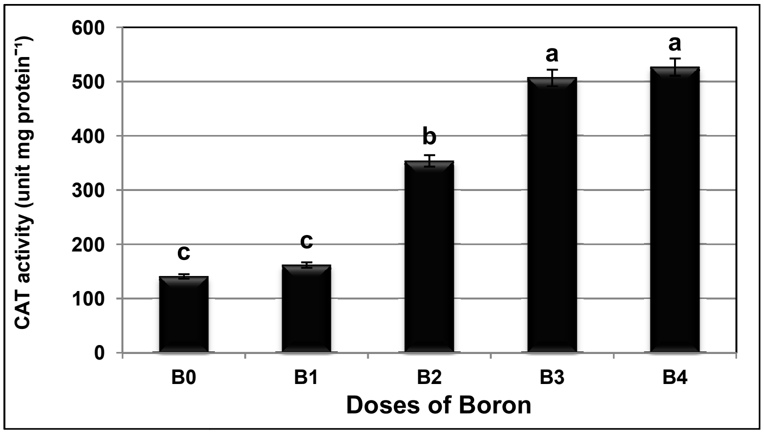

Fig. 1. Effects of boron treatments on CAT enzyme activity

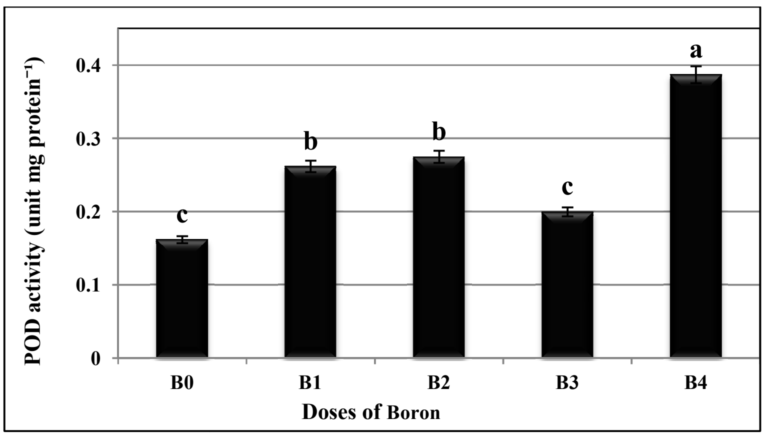

Fig. 2. Effects of boron treatments on POD enzyme activity

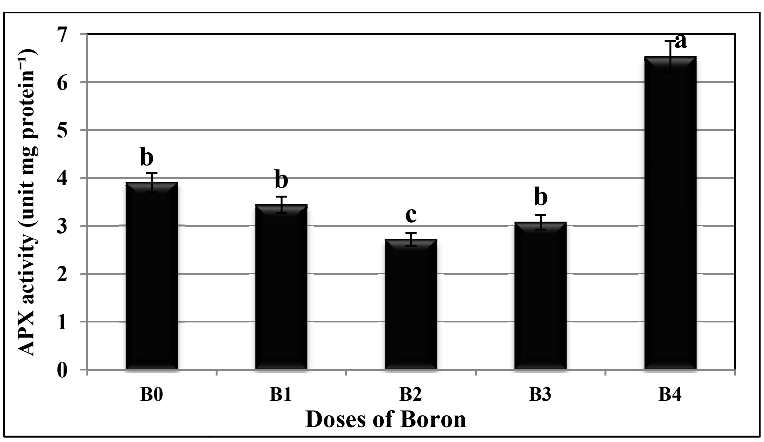

Fig. 3. Effects of boron treatments on APX enzyme activity

B sufficiency levels reported by [20] for lettuce. Boron plays a significant role in cell wall structures, membranes and membrane-related reactions. Various metabolic reactions increase the formation of reactive oxygen species (ROS) under stress conditions. Besides, plants have an efficient antioxidant system to eliminate ROS. Antioxidant enzymes play important roles as a part of this system [21]. Inner-cell H_2O_2 levels of plants are regulated by various enzymes and CAT and POD are the most significant ones of these enzymes. With the absence of antioxidant enzymes, higher H_2O_2 accumulations are observed. In this study, CAT activity significantly increased with boron treatments. POD activity also significantly increased in all boron treatments except for B3. APX activity on the other hand increased only at high doses.

The plants generally increase the enzymatic antioxidant activity in order to decrease the stress damage. All three enzymes played various roles in catalyzation of H_2O_2 into water and functioned to reduce the stress effects. Effects of boron toxicity on enzyme activity vary based on plant species. In a previous study carried out with lettuce, increased APX activity and constant CAT activity were reported [22], tomato. In tomatoes exposed to boron toxicity, increasing POD, CAT and APX activities were reported [23-24]. Similarly in barley and *Artemisia annua* L. plants, respectively increasing APX-CAT and POD-CAT activities were reported [21-25]. Considering these results, it is possible to say that the excessive B might cause oxidative stress in lettuce.

4. CONCLUSION

The present results revealed that boron levels over critical threshold values in soils or applied through boron fertilizers negatively affected plant development. Plant boron concentrations especially increased at toxic doses and resultant increase in boron concentrations of plant tissues increased antioxidant activities of the plants. Some plants are sensitive to boron and some others do not need much boron. In this study, development of medium-sensitive lettuce plants was negatively affected by toxic boron dose. Thus, soil boron levels and amount of boron fertilizers should be taken into consideration while boron fertilization of sensitive plants.

COMPETİNG İNTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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