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EFFECTS OF BORON ADDITION ON LETTUCE PLANTS GROWN IN THE SOIL AND HYDROPONICALLY

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BIOMASS
BORON
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SOIL

ABSTRACT. – Boron (B) is a crucial micronutrient needed for plant growth. This study investigated the effect of B addition (0.5 mg kg^{-1}-low, and 5 mg kg^{-1}-high) on lettuce (*Lactuca sativa* L.) plants grown in the soil and hydroponically. The results showed that B addition causes a differential increase in B concentration in lettuce leaves depending on the cultivation system. In soil-grown plants, a statistically significant increase in lettuce B concentration was shown with increasing B treatments compared to the control. Moreover, the B concentrations measured in lettuce were within the optimal level for healthy lettuce. While hydroponically-grown plants showed a statistically significant increase in lettuce B concentration at high B treatment compared to the control and low B treatment. However, the high B treatment in hydroponics caused a very high B concentration in lettuce (196.4 mg kg^{-1}) leading to toxicity expressed by the decrease in normalized difference vegetation index (NDVI) and chlorophyll content. Therefore, B concentration should be below 5 mg kg^{-1} for hydroponically-grown lettuce. Soil total and available B concentrations increased with B addition and considered a sufficient level. The other soil physicochemical parameters did not reveal a statistically significant difference with the B treatments, except a modest increase in pH, suggesting that the soil has a great homeostatic capacity. Additionally, the results showed that hydroponically-grown lettuce performs much better in terms of biomass production.

INTRODUCTION

Boron (B) is an essential micronutrient required for plant development (Shaaban *et al.* 2006, Ahmad *et al.* 2018). The role of B is as vital as that of macronutrients, such as nitrogen, phosphorus, and potassium (Aziz *et al.* 2019), since it is involved in many metabolic, physiological and biochemical, processes (Herrera-Rodríguez *et al.* 2010). Indeed, B plays a major role in sugar syntheses and transport, cell wall synthesis and structure, root elongation, RNA and carbohydrate metabolism, membrane transportation, and respiration (Saleem *et al.* 2011, Kabu & Akosman 2013, Aboyeji *et al.* 2019).

The soil total B content is in the wide range of 2-200 mg kg^{-1}, but less than 5 % of total soil B is hydrosoluble and available to plants (Niaz *et al.* 2013, Padbhushan *et al.* 2019, Javed *et al.* 2021). According to Brdar-Jokanović (2020), soil having less than 0.5 mg kg^{-1} of available B is considered as deficient. Factors affecting soil B availability are influenced by a wide array of parameters including drought and rainfall periods, soil properties, agronomic practices, and plant species (Al-Ameri *et al.* 2019, Javed *et al.* 2021). Among soil parameters affecting the concentration of available B, there are pH, texture, structure, content of calcium carbonate, moisture, organic matter, clay minerals, and sesquioxides (Matula 2009, Chowdhury *et al.* 2015, Tlili *et al.* 2019).

Compared with other nutrients, B has a narrow range between deficiency and toxicity (Matula 2009), and it is known that both low and excessive B concentrations limit crop growth and reduce the yield (Dridi *et al.* 2018). In fact, deficiency of B is the most widespread and common micronutrient deficiency problem in the world (Saleem *et al.* 2011, Ahmad *et al.* 2012; Niaz *et al.* 2013, Aydin *et al.* 2019). The symptoms of B deficiency can occur as denaturing, damage and cracking of cell wall structure, curd browning, hollow stem formation, and a reduction of the photosynthesis rates (Erdal *et al.* 2016, Brdar-Jokanović 2020). Besides changes in root morphology, limiting the absorption and distribution of nutrients in the plant (García-Sánchez *et al.* 2020). Toxic B concentrations lead to several physiological effects on plants, such as reduced root cell division, lower photosynthetic rates, and decreased leaf chlorophyll contents (Ouzounidou *et al.* 2013, Sahin *et al.* 2017, Nadeem *et al.* 2019). Soil B toxicity may be caused by irrigation with water that contains high concentrations of B, the excessive use of fertilization rich in B, or be of natural origin in the soil and/or the groundwater (Sahin *et al.* 2017).

In recent years, hydroponics has emerged worldwide as a soil-less alternative technology to traditional soil systems to grow plants, where soils are non-fertile or where space for agriculture is a constrain, like urban areas. Hydroponic systems allow growing plants without using soil as substrate, with nutrients being delivered directly to the root system through a nutrient solution: the minerals are thus readily available and quickly taken up by plants, resulting in faster plant growth.

The debate about whether hydroponically grown plants are to be preferred over soil-grown plants is still ongoing. Indeed, some studies (Sankhalkar *et al.* 2019, Majid *et al.* 2021, Dutta *et al.* 2023) have shown that hydroponic systems provide better nutrition than soil systems, while others (Gashgari *et al.* 2018) did not find any difference between these two systems or showed that plants grown in the soil have better nutritional parameters. Lettuce (*Lactuca sativa* L.) was selected for this research because lettuce is a fast-growing plant with a short life cycle (30-45 days), making it ideal for short-period experiments (Pink & Kerane 1993, Maucieri *et al.* 2019). Moreover, lettuce is a plant species that is sensitive to boron, it requires boron for optimal growth and development (Eraslan *et al.* 2007). In addition, lettuce can be easily grown in pots or other small containers, making it suitable for cultivation indoors as well as outdoors (Romero-Gámez *et al.* 2014).

Recent studies (Souza *et al.* 2019, Majid *et al.* 2021) evaluated the physiological differences between lettuce cultivated in the soil and hydroponically, and lettuce grown in hydroponic systems showed higher values for yield, chlorophyll, and dry matter and nutritionally superior quality products in less time (shorter growing cycles). Additionally, much lower water usage was reported.

Although several studies have shown that hydroponics has advantages over soil production, there are still some drawbacks to employing this new technology. The high costs of a hydroponic system allow little room for errors. Thus, in order to have good plant productivity from a hydroponic system, skill and appropriate knowledge of doses are mandatory to operate properly (Sublett *et al.* 2018). Research by Sublett *et al.* (2018) revealed that the yield and quality of lettuce grown in hydroponic systems are affected by season and plant variety, with the highest B concentration in lettuce leaves found in Spring for lettuce with green leaves and in the Fall for lettuce with red leaves.

The required concentration of B for optimal growth varies according to the plant species and environmental factors (Ghongtham *et al.* 2018), and as a matter of fact, it is crucial to know the range of adequate doses of B to be applied for enhancing both crop yield and quality under different conditions. Therefore, the objective of this study was to test the effects of B addition on lettuce (*Lactuca sativa* L.) grown in the soil and hydroponically, under controlled conditions.

MATERIALS AND METHODS

Soil-grown plants: For this experiment (soil-grown lettuce), the soil was collected from the field crop situated in the botanical garden at the University of Siena, Italy (43°18’47”N 11°19’49”E).

Soil samples were dried and sieved through a 2 mm diameter mesh. A pot experiment was carried out for 3 weeks in a climatic chamber at a constant temperature of 20° C and 12 h photoperiod at a PAR photon flux density of 350 µM m^{-2} s^{-1}. The pot experiment was laid out in a randomized block design (RBD) with three B treatments and five replicates for each treatment. The treatments comprised three levels of bioavailable B supplied as 100 ml of boric acid in a liquid form: 0 mg kg^{-1} (just water, control), 0.5 mg kg^{-1} (designated “low B”), and 5 mg kg^{-1} (designated “high B”). The soil was placed in 15 plastic pots (9.5 × 7 × 11 cm LxWxH) and one twenty-day-old lettuce plant (*Lactuca sativa* L.) bought from a local plant nursery was transplanted in each pot. B treatments were applied to soil-grown plants one *week* after planting lettuce at the scheduled time of irrigation. It was supplied as 100 ml of boric acid in a liquid form. Soil moisture was maintained at 80 % of field capacity by irrigating every two days with water. The soil was characterized before starting and at the end of the experiment.

Hydroponically-grown plants: The hydroponically-grown plant experiment was carried at the University of Siena for 3 weeks in a climatic chamber at a constant temperature of 20° C and 12 h photoperiod at a PAR photon flux density of 350 µM m^{-2} s^{-1}. Twenty-day-old lettuce plants (*Lactuca sativa* L.) were washed to completely remove soil before placing being placed in 400 cc plastic cups containing 350 mL of Atami© B’Cuzz Hydro nutrient solution (pH 5.65), prepared by

Table I. – Chemical composition of the Atami© B’Cuzz Hydro nutrient solution.

Solution A	Amount
Nitrogen	4.85 %
Phosphorous	0.15 %
Potassium	4.73 %
Sodium	0.19 %
Calcium	3.79 %
Magnesium	1.32 %
Sulphur	0.11 %
Iron	0.04 %
Boron	0.001 %
Solution B	**Amount**
Phosphorus	4.1 %
Potassium	5.7 %
Boron	0.01 %
Manganese	0.03 %
Molybdenum	0.001 %
Zinc	0.039 %

diluting 2 mL L^{-1} of the nutrient solution A and B in deionized water (Table I) as better specified in (Vannini *et al*. 2021). The hydroponic experiment was laid out in a fully randomized block design (RBD) with three B treatments and 5 replicates for each treatment. Three B treatments were added as boric acid in a liquid form: 0 mg kg^{-1} (control), 0.5 mg kg^{-1} (designated "low B") and 5 mg kg^{-1} (designated "high B"). The solution was renewed weekly by draining out the old solution and replacing it with a fresh nutrient solution as described above. B treatments were applied to the hydroponically-grow plants in the second and third weeks when renewing the solution. For each treatment, the respective concentration of B was provided as boric acid in a liquid form mixed with the nutrient solution.

Plant Analysis: Photosynthetic parameters: The photosynthetic parameters were determined at the end of each experiment to investigate the effect of B on the vitality and health of the lettuce. Fifteen measurements of each photosynthetic parameter were taken at random for each plant. The normalized difference vegetation index (NDVI) was measured using the PlantPen NDVI (Photon Systems Instruments, Czech Republic). The total chlorophyll content was determined using a chlorophyll content meter (CCM-300, Opti-Science, Hudson, USA), and expressed as mg of chlorophyll content per m^2 of plant leaf (Gitelson *et al*. 1999). Photosynthetic efficiency was expressed in terms of maximum quantum yield of photosystem II photochemistry (Fv/Fm) and performance index (P_I) using chlorophyll fluorescence parameters: the leaves were dark-adapted for 15 min and subsequently flashed for 1 s with a saturation pulse (1800 µmol m^{-2} s^{-1}) of red light (650 nm) using a plant efficiency analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK).

Dry matter: The aboveground plant parts from each pot were cut and washed with deionized water and oven-dried at 70° C for 24 h. The dry matter was determined using the formula:

$$Dry\ matter\ (\%) = (Dry\ weight\ /\ Fresh\ weight) \times 100$$

Boron concentration: Lettuce leaves were ground using a stainless-steel mill and about 200 mg of powder were mineralized with a mixture of 3 mL of 70 % HNO_3 and 0.5 mL of 30 % H_2O_2 in a microwave digestion system (Milestone Ethos 900) at 280° C and 55 bars. Then, B was determined by inductively coupled plasma mass spectrometry (ICP-MS PerkinElmer-Sciex, Elan 6100). Analytical quality was checked with the certified reference material GBW07603.

Soil Analysis: The investigated soils were characterized for their main physicochemical properties. Soil pH was measured in 1:2.5 soil: water suspensions. Soil texture was determined using the Robinson pipette method (Robinson 1922). Total calcium carbonate ($TCaCO_3$) was measured using the volumetric method (Nelson 1982). Total organic carbon (TOC) was quantified by the modified Walkley-Black method (Nelson & Sommers 1982). The total nitrogen (TN) was analyzed by the modified Kjeldahl method (Bremner 1996). The exchangeable cations, namely potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^+) were extracted with ammonium acetate ($C_2H_7NO_2$) (Thomas 1982).

Soil available B was determined using hot-water extraction (Berger & Truog 1939). For the determination of the total B concentration, 200 mg of soil powder were mineralized with a mixture of 3 mL of 70 % HNO_3, 0.2 mL of 60 % HF and 0.5 mL of 30 % H_2O_2 in a microwave digestion system (Milestone Ethos 900) at 280° C and 55 bars. Total B was quantified by inductively coupled plasma mass spectrometry (ICP-MS PerkinElmer-Sciex, Elan 6100). The analytical quality was checked with the certified reference material GBW07411.

Statistical analysis: To disentangle the effect of B treatment on soil physicochemical properties, soil available and total B concentration, B concentration in lettuce leaves, photosynthetic variables, and dry matter, a linear mixed effects model (LMEM) was fitted for each parameter, with treatment as fixed effect and plant as a random factor. The significance of the models was checked with type III ANOVA (analysis of deviance) using the Wald chi-square test. A *posthoc* comparison ($p < 0.05$) among treatment means for each parameter was carried out using Duncan's test. All statistical analyses were run using SPSS v.25.0 (IBM, Chicago, IL, USA).

RESULTS

Soil-grown plant experiment

The main physicochemical properties of the experimental soils are shown in Table II. The soil texture was sandy clay loam. Control soil was moderately alkaline and intensely to moderately calcareous. The C/N ratio was indicative of mineralization, with enough nitrogen available for plant uptake. The exchangeable cations and

Table II. – Soil physicochemical properties (mean ± standard error). Different letters in each row indicate statistically significant ($p < 0.05$) differences between treatments.

	Control	Low B	High B
pH	8.07 ± 0.01 a	8.12 ± 0.01 b	8.14 ± 0.01 b
$CaCO_3$ (%)	20.3 ± 0.3 a	20.8 ± 0.7 a	19.8 ± 0.3 a
Carbon (%)	1.8 ± 0.1 a	1.8 ± 0.2 a	1.9 ± 0.1 a
Nitrogen (‰)	1.6 ± 0.1 a	1.3 ± 0.1 a	1.5 ± 0.1 a
C/N	11.8 ± 1.5 a	13.4 ± 0.8 a	12.7 ± 0.6 a
Ca^{2+} (mg kg^{-1})	4013 ± 19 a	3967 ± 28 a	4005 ± 9 a
Mg^{2+} (mg kg^{-1})	124 ± 1 a	120 ± 2 a	135 ± 12 a
Na^+ (mg kg^{-1})	324 ± 16 a	320 ± 27 a	288 ± 20 a
K^+ (mg kg^{-1})	168 ± 4 a	171 ± 1 a	173 ± 5 a
CEC (meq $100g^{-1}$)	23 ± 0.1 a	23 ± 0.2 a	23 ± 0.1 a

the cation exchange capacity (CEC) were at normal levels. These features did not show any statistically significant change with B addition, both at the low and high B treatment, with the only exception of a modest increase in pH.

Application of B increased the concentration of available B in the soil both for the low and high B treatment, but such a difference was statistically significant only for the high B treatment (Table III). Boron concentration in lettuce leaves of soil-grown plants increased with B treatment (Table IV), with differences being statistically significant in both cases. The values of NDVI, chlorophyll content, photosynthetic efficiency (Fv/Fm), and performance index (P_I) were very similar between control, low, and high B treatments (Table V), and statistically significant differences did not emerge.

Hydroponically-grown plant experiment

In hydroponically-grown lettuce plants, leaves B concentration increased with increasing B treatment (Table IV). Nevertheless, the difference between the control and the low B treatment was not statistically significant. The leaves B concentration in the high B treatment plants was ca. seven times higher than in control and ca. five times higher than in the low B treatment and both were statistically significant.

The physiological results of the hydroponically-grown plants (Table V) only showed statistically significant differences among treatments for NDVI and chlorophyll content. While the other physiological parameters (Fv/Fm, P_I, dry matter) remained quite stable and did not show any statistically significant change. Lettuce plants treated with low and high B concentrations showed a statistically lower NDVI and chlorophyll content compared with the control, with a clear decreasing trend with increasing B concentrations. However, while NDVI values were all statistically different from each other, the chlorophyll content was significantly decreased only under the high B treatment.

Table III. – Available and total boron concentration (mg kg^{-1}) in the soil (mean ± standard error). Different letters in each column indicate statistically significant ($p < 0.05$) differences between treatments.

	Available B	Total B
Control	0.55 ± 0.03 a	73.5 ± 4.62 a
Low B	0.72 ± 0.04 a	74.0 ± 4.52 a
High B	2.00 ± 0.17 b	85.8 ± 6.14 b

Table IV. – Boron concentration (mg kg^{-1} dw) in lettuce leaves (mean ± standard error). Lowercase and uppercase letters indicate statistically significant ($p < 0.05$) differences in soil and hydroponic systems, respectively.

	Soil-grown plants	Hydroponically grown plants
Control	25.2 ± 1.4 a	30.7 ± 0.9 A
Low B	42.0 ± 0.7 b	40.4 ± 3.0 A
High B	41.0 ± 3.0 b	196.4 ± 20.2 B

Table V. – Photosynthetic parameters and dry matter (mean ± standard error) of lettuce leaves. NDVI = normalized difference vegetation index; Fv/Fm = photosynthetic efficiency; PI = performance index. Lowercase and uppercase letters indicate statistically significant ($p < 0.05$) differences in soil and hydroponic systems, respectively.

	Soil-grown plants				
	NDVI	Chlorophyll (mg m^{-2})	Fv/Fm	PI	Dry Matter (%)
Control	0.40 ± 0.01 a	219 ± 11.48 a	0.80 ± 0.02 a	1.9 ± 0.27 a	4.5 ± 0.14 a
Low_B	0.38 ± 0.01 a	223 ± 1.73 a	0.81 ± 0.01 a	2.0 ± 0.28 a	4.8± 0.12 a
High_B	0.40 ± 0.01 a	220 ± 6.37 a	0.79 ± 0.02 a	1.9 ± 0.08 a	4.7 ± 0.12 a
	Hydroponically-grown plants				
	NDVI	Chlorophyll (mg m^{-2})	Fv/Fm	PI	Dry Matter (%)
Control	0.51 ± 0.01 C	220 ± 7.39 B	0.83 ± 0.00 A	2.6 ± 0.32 A	8.3 ± 0.55 A
Low_B	0.47 ± 0.01 B	213 ± 13.22 AB	0.84 ± 0.01 A	2.7 ± 0.17 A	8.8 ± 0.59 A
High_B	0.44 ± 0.01 A	199 ± 4.95 A	0.83 ± 0.00 A	2.6 ± 0.14 A	7.9 ± 0.64 A

DISCUSSION

Our results are in agreement with several studies which revealed that B concentration in leaves increased with increasing B supply (Petridis *et al*. 2013, Kapoor *et al*. 2016, Sahin *et al*. 2017). Such an increase was observed especially in the hydroponically-grown plants under the high B treatment, for which the recorded leaves B concentration (196.4 mg kg^{-1}) was one order of magnitude above the sufficient level of B for lettuce (20-23 mg kg^{-1}) and well in excess of the optimum level for healthy lettuce (25-50 mg kg^{-1}) (McHargue & Calfee 1933, MacKay *et al*. 1962). According to Mengel *et al*. (2001), B concentrations in lettuce leaves above 70-80 mg kg^{-1} (dry weight) are toxic. These data indicate that a B concentration of 5 mg kg^{-1} in the treatment solution (high B treatment) causes B uptake up to toxic levels in lettuce grown in hydroponic systems. Toxicity is confirmed by the negative impact found on plant physiology resulting in a decreased chlorophyll content and lower NDVI values. These results are consistent with the finding of many researchers (Herrera-Rodríguez *et al*. 2010, Banón *et al*. 2012, Gupta *et al*. 2014, Rehman *et al*. 2018, Nadeem *et al*. 2019, Choudhary *et al*. 2020) which they refer that B excess diminished photosynthesis parameter.

In soil-grown plants, leaves B concentration of treated lettuce (41-42 mg kg^{-1}) was within the optimal range according to several researchers (MacKay *et al.* 1962, Mills & Jones Jr 1996). Since values for control plants, although still at a sufficient level (McHargue & Calfee 1933, MacKay *et al.* 1962), were lower than those of treated plants, it is possible to suggest that an addition of 0.5 mg kg^{-1} of available B to the soil (or that a soil available concentration B of ca. 0.70 mg kg^{-1}) is optimal for lettuce growth. A boron addition of 0.5 mg kg^{-1} determined a 30 % increase in the concentration of soil B available for lettuce uptake. Also, Al-Ameri *et al.* (2019) found that B fertilization increased the concentration of available B in the soil and B uptake in straw and wheat.

Previous studies (Liu *et al.* 2005, Chowdhury *et al.* 2015, Aydin *et al.* 2019) showed that an adequate level of B boosts vegetation growth and improves the rate of photosynthesis and dry matter. This is at some variance with our results since we did not observe any change in photosynthetic parameters nor in dry biomass with B addition to the soil. Additionally, except for a modest increase in pH, also the physicochemical properties of the soil did not change with the addition of B. As a last consideration, consistently with the findings of Souza *et al.* (2019) and Dutta *et al.* (2023), the dry matter content of hydroponically-grown lettuce was almost twice that of soil-grown plants. This is because lettuce grown in hydroponics depends on the nutrient solution for moisture, which may affect plant growth and dry mass. In contrast, lettuce grown in soil have only access to the water stored in the soil.

The comparative study of lettuce plants grown in soil and hydroponic systems showed that boron addition (0.5 and 5 mg kg^{-1}) causes a differential increase in B concentration in lettuce leaves depending on the cultivation system. The hydroponically-grown plants showed a statistically significant increase in lettuce B concentration at high B treatment compared to the control and low B treatment. However, the addition of 5 mg kg^{-1} (high B treatment) in hydroponically-grown lettuce recorded an extremely high B concentration in the lettuce leaves (196.4 mg kg^{-1}) causing toxicity. It impairs plant physiological parameters resulting in a decreased chlorophyll content and lower NDVI values. Since B concentration in lettuce leaves was optimal with the low B addition (0.5 mg kg^{-1}), we conclude that the B concentration added to the nutrient solution of the hydroponically-grown lettuce should be below 5 mg kg^{-1} to avoid toxicity. Although lettuce B concentrations measured in soil-grown plants increased with the increase of B addition, it still belongs to the optimal level for healthy lettuce. Besides, no statistically significant difference in the physiological parameters was revealed with B addition in soil-grown plants. For the physicochemical soil properties, increasing the concentration of added boron increased soil pH value, the concentration of total and available B in the soil, this lead to an increase of B concentration in lettuce leaves. Thus, the soil has a great homeostatic capacity which prevents the toxicity of lettuce. It acts as a buffer against plant nutrient problems and disease. Additionally, the results also showed that lettuce grown hydroponically has a much higher performance in terms of biomass production.

To conclude, the B concentration added to the nutrient solution of the hydroponically-grown lettuce should be above 0.5 mg kg^{-1} and below 5 mg kg^{-1} to avoid toxicity that decreases photosynthetic parameters (especially NDVI and chlorophyll content). For the soil-grown lettuce, the B concentration added to the soil may be greater than 5 mg kg^{-1} because this concentration did not influence the lettuce physiological parameters and did not reveal toxicity. Further investigation should be established to determine the adequate range of B concentration that may be applied to the soil and hydroponically-grown lettuce that improve lettuce physiological parameters without causing toxicity.

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