

Interaction of soil moisture and excess of boron and nitrogen on lettuce growth and quality

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Abstract

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A study of the effects of boron (10 mg/kg as H₃BO₃) and nitrogen (0-0.15-0.30-0.45-0.60 g/kg) fertilization in combination with soil water capacity (40% and 70%) on lettuce growth and nutritional value was performed. The effects of the simultaneous application of N and B depend on the soil moisture, since lower water in the soil reduced lettuce growth and quality. Linear correlation was found between N fertilization and lettuce yield and quality at the two water soil capacities. Only when N fertilization exceeded 0.45 g/kg the fresh and dry biomass, photosynthesis, sugars and ascorbic acid showed negative influence as compared to the control. The toxic effects of B in lettuce cultivation were alleviated by the presence of high N doses, since a competition between N and B ions was revealed. Overall, the management of irrigation and N fertilization is very important in soils with boron toxicity.

Keywords: ascorbic acid; boron toxicity; nitrogen fertilization; soil water capacity; sugars

Lettuce (*Lactuca sativa* L.) is considered one of the most economically important leafy vegetable crop in the world and is widely consumed in Western diets (CHIESA et al. 2009). Recently, the worldwide demand for romaine lettuce has been increased because of its crisp texture, pleasant aroma and flavour, fresh appearance as well as richness in phytochemicals, such as phenolic compounds (FU et al. 2012). It is also an important source of dietary antioxidants and fibres, sugars and minerals. Lettuce has relatively high water requirements, thus soil moisture and rainfall shortage would seriously

stunt growth and head quality. Irrigation greatly reduces risk of crop failure (STEFANELLI et al. 2011).

Nitrogen (N) is an indispensable element for the synthesis of proteins such as enzymes, which are responsible for the production of all the cellular components and secondary metabolites required for the development of the plant, and hence nitrate availability in the soil and plant tissue concentration can limit plant growth (LAWLOR 2002). Nitrate reduction catalysed by nitrate reductase (NR), is the primary step in the nitrate assimilation process and it is essential for the production of ammonium

to be incorporated into carbon skeletons for amino acid biosynthesis (MATAS et al. 2009). Very high nitrogen fertilization is often applied with the belief that it promotes vegetative development and yield. In the past years the use of N-P-K based fertilizers increased dramatically around the world, resulting in nitrates accumulation in leafy vegetables (DEMSAR et al. 2004). This can be deleterious to human health as nitrates can be converted to harmful nitrites after harvest (MAYNARD, BARKER 1979).

Boron (B) is an essential element for all vascular plants whose deficiency or toxicity causes impairments in several metabolic and physiological processes (HERRERA-RODRIGUEZ et al. 2010). The role of B in plant nutrition and physiology was investigated extensively and it was implicated in cell wall synthesis and structure. There is direct evidence for its involvement in cross-linking of cell wall rhamnogalacturonan II (RGII) and pectin assembly showing that boron is necessary for plant growth (O'NEILL et al. 2004). Another role attributed to B is its involvement in nitrogen metabolism (RUIZ et al. 1998), while foliar B improved seed set, yield and quality of alfalfa, sugar beet and sunflower (DORDAS 2006; DORDAS et al. 2007; BELLALOU et al. 2010). However, B toxicity can be observed in following cases: (a) in soils that are rich in B, (b) by the excessive use of the fertilizers that contain B and (c) the use of irrigated water with high B concentration (NABLE et al. 1997). In Greece toxicity problems were identified at some areas because of the high B concentration in irrigated water (OUZOUNIDOU et al. 2012). Toxic B concentration leads to different physiological effects during the life cycle of plants. Recent studies refer inhibition in the percentage germination of corn, carrots and tomatoes, while reduction of root cell division and decreased photosynthetic rates are also observed (HERRERA-RODRIGUEZ et al. 2010; BANON et al. 2012).

Minimizing N availability in lettuce, while maintaining yield and quality is a subject of recent studies (STEFANELLI et al. 2010, 2011). Fortification of leafy vegetables could be a successful strategy for improving human diets, resulting in an increase in the value and quality of the production. So far, little is known about the interaction of B and N supply and soil water capacity on lettuce development and quality. Therefore, the objective of this study was to examine the effects of a range of N concentration in lettuce yield and quality; while the impact of excess B application in N toxicity under two soil moisture levels was also assessed.

MATERIAL AND METHODS

Plant material and growth conditions. This study was conducted in a non-heated greenhouse at Technological Educational Institute of Kalamata (Kalamata, Greece) in spring 2009. Greenhouse conditions were: temperature 12°C (min.), 32.5°C (max.) and 24.3°C (average); average relative humidity 65%; average photosynthetic photon flux density (PPFD) $890 \pm 80 \mu\text{mol}/\text{m}^2/\text{s}$. Ten-day old lettuce plants (*Lactuca sativa* var. Romana) were transferred in pots containing 4.5 l (four plants per pot) of soil having the following characteristics: mean texture (SL), pH 7.18, EC 757 $\mu\text{S}/\text{cm}$, CaCO_3 1.83%, organic matter 1.98%. The experiment was set up in a completely randomized block design with 20 different treatments and 4 replications. Eighty plants were grown in each replication and the harvest day was 58 days after transplantation. The experiment project includes five treatments with 70% soil moisture and other five with 40% soil moisture. Ten treatments involve non-additional boron fertilization; the B concentration in the pot soil was 0.5 mg/kg; and ten involve additional boron fertilization (10 mg/kg) supplied as boric acid (H_3BO_3). Five different N levels were used: 0, 0.15, 0.30, 0.45 and 0.60 g/kg, while at all treatments 0.15 g/kg P and K were added. The form of fertilizers to the basic fertilization was: superphosphate (0-20-0), potassium sulfate (0-0-50) and ammonium sulfate (21-0-0). The addition of fertilizer was as follows: the entire quantity of P, K and 70% of N were mixed with soil when filling the containers. The boron solution was incorporated in soil 7 days after the lettuce transplantation. The remaining amount of N (30%) was applied as ammonium nitrate (33.5-0-0).

Soil moisture was determined by the use of flood irrigation, on the basis of 100% water capacity of soil resulting from the weight difference between the ground saturated with water, drained and dry soil at 104°C. During the experiment the containers were weighed daily for calculation of soil moisture (DASSEL, NIELSEN 1986).

By the end of the cultivation period (almost three months), morphological (fresh and dry weight and height of the upper part of the plants, number of leaves per plant) and physiological (vitality index) measurements were recorded. The glucose, fructose, sucrose and ascorbic acid concentration in mature lettuce leaves were also measured.

In vivo chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured

Table 1. Effects of different nitrogen concentrations and soil moisture levels in growth and yield in lettuce plants grown under efficiency (–B) and toxicity (+B) of boron (10 mg/kg H₃BO₃)

Treatments	Dry weight (g/plant)		Leaves number/plant		Plant height (cm)	
	–B	+B	–B	+B	–B	+B
70% soil moisture						
N ₀ P ₁ K ₁	4.1 ^{a1}	3.5 ^{a*}	18 ^a	17 ^a	18.8 ^a	17.0 ^{a*}
N _{0.15} P ₁ K ₁	10.1 ^b	10.3 ^b	22 ^b	22 ^b	28.1 ^d	27.6 ^d
N _{0.30} P ₁ K ₁	13.1 ^c	14.4 ^{d*}	22 ^b	22 ^b	32.0 ^e	33.0 ^e
N _{0.45} P ₁ K ₁	17.2 ^d	17.3 ^f	25 ^c	27 ^{d*}	29.0 ^d	28.7 ^d
N _{0.60} P ₁ K ₁	13.0 ^c	18.9 ^{f*}	23 ^b	26 ^{d*}	28.6 ^d	32.5 ^{e*}
40% soil moisture						
N ₀ P ₁ K ₁	4.0 ^a	3.3 ^{a*}	17 ^a	17 ^a	17.5 ^a	17.0 ^a
N _{0.15} P ₁ K ₁	4.5 ^a	3.5 ^{a*}	17 ^a	18 ^a	17.0 ^a	16.5 ^a
N _{0.30} P ₁ K ₁	13.6 ^c	10.5 ^{bc*}	25 ^c	24 ^c	26.5 ^c	26.0 ^{cd}
N _{0.45} P ₁ K ₁	15.7 ^d	11.8 ^{c*}	26 ^c	23 ^{bc*}	23.0 ^b	25.3 ^{c*}
N _{0.60} P ₁ K ₁	9.8 ^b	9.5 ^b	22 ^b	22 ^b	22.0 ^b	22.0 ^b

¹each value is the mean of 15 different measurements; means in the same column followed by different letters are significantly different (Duncan's test $P < 0.05$); *significant differences ($P < 0.05$) between the efficiency and the excess of B for the same factor

using an open gas exchange portable system (LI-6400) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; both LI-COR, Inc., Lincoln, USA). *In vivo* chlorophyll fluorescence was measured on the upper surface of lettuce leaves at room temperature for all treatments. The ratio of the fluorescence decrease Fd, to the steady state fluorescence Fs (i.e. Rfd = Fd/Fs), known as vitality index, measuring the whole photosynthetic efficiency from the primary photochemical event to the activity of Calvin cycle enzymes (OUZOUNIDOU, ASFI 2012).

Quality characteristics. Sucrose, glucose and fructose were determined with an HP 1100 Series High Performance Liquid Chromatograph (Agilent Technologies, Waldbronn, Germany) (refractive index detector (RID)) using a reverse phase column 250 × 4 mm (Lichrosphere NH₂) bonded to micro-particulate silica of 5 μm diameter maintained at 37°C. Injection of 20 mm³ of sample solution into a mobile solvent of H₂O/AcCN (25:75; v/v) with a flow rate of 1.1cm³/min gave the optimum result (OUZOUNIDOU et al. 2011). The ascorbic acid content of lettuce leaves was estimated by macerating the plant sample mechanically with a stabilizing agent (5% metaphosphoric acid) and titrating the filtered extract with 2,6-dichlorophenolindophenol.

Statistical analyses. Data were subjected to the analysis of variance (ANOVA) using the SPSS 11.0.1 for Windows statistical package (SPSS, Chicago, USA). For comparison of the means, the Duncan's multiple range tests ($P \leq 0.05$) was employed.

RESULTS AND DISCUSSION

Growth and yield responses to soil moisture, boron and nitrogen concentrations

Lettuce plants cultivated under deficiency or very low dose of N (0 and 0.15 g/kg) were stunted, with chlorotic leaves and low yield, a fact more obvious in the lower soil water capacity (40%). In that treatment, the addition of intermediate N concentrations (0.30 and 0.45 g/kg), in conditions of normal B and excess of B, had a positive effect on plant growth and yield, whereas the highest N fertilization (0.60 g/kg) induced significant inhibition of lettuce development (Table 1, Fig. 1). In this lower water capacity, plants revealed slow growth, which means that fertilizers are not used if there is not adequate soil moisture (Table 1). Under 70% soil moisture and irrigation with N 0.15 g/kg, the dry weight of the upper part of the plant increased

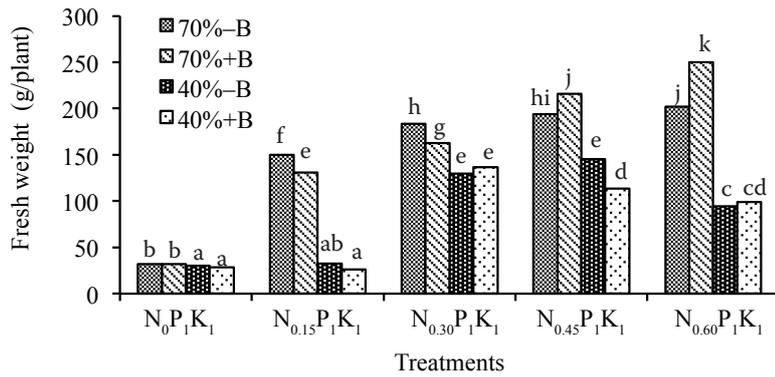


Fig. 1. Fresh biomass (g/plant) of lettuce plants grown under efficiency (0.5 mg/kg) and toxicity of B (10 mg/kg), two different soil water capacities (70 and 40%) and various N concentrations (0, 0.15, 0.30, 0.45, 0.60 g/kg) All data represent the means of 15 different measurements. Means that differ at $P < 0.05$ are shown by different letters

three times, the plant height was enhanced by about 57% and the fresh weight was about 4 times higher than the control depending on the presence or the absence of B. In the highest N dose, fresh weight continued to increase showing that N is a key element of growth. Our data are similar to NESTBY et al. (2005) working on strawberries. Lack or low N fertilization induces metabolic imbalance, where low N assimilation limits the synthesis of amino acids and proteins, thereby affecting photosynthesis, growth and chemical composition of the plant tissue. Nitrogen was correlated with positive effects on yield and fruit size depending on the time and rate of application, orchard density and possible alternate bearing of the crops (MARSCHNER 1995).

Linear correlation was found between N fertilization and lettuce yield and quality (Tables 1, 2) at the two water soil capacities. Only when N fertilization exceeded 0.45 g/kg the morphological, physiological and quality parameters revealed a negative influence compared to the control (Tables 1, 2; Figs 1, 2). On exposure to excess of B, growth parameters of lettuce were more suppressed under low soil moisture than under adequate water capacity. This confirms that B recruitment is passive and it increases with plant transpiration, which is higher under drought conditions (BOWEN, NILSEN 1976). Generally, excess of B, results in increased membrane leakiness, peroxidation of lipids and proline accumulation, with a growth stunting as a consequence. It also

Table 2. Effects of different nitrogen concentrations and soil moisture levels in sugars (mg/100 g f.w.) and ascorbic acid content (mg/100 g f.w.) of mature lettuce leaves under efficiency (-B) and toxicity (+B) of boron (10 mg/kg H₃BO₃)

Treatments	Glucose		Fructose-		Sucrose		Ascorbic acid	
	-B	+B	-B	+B	-B	+B	-B	+B
70% soil moisture								
N ₀ P ₁ K ₁	120 ^{b1}	109 ^{b*}	39 ^b	47 ^{a*}	25 ^d	19 ^{b*}	9.35 ^b	9.33 ^b
N _{0.15} P ₁ K ₁	172 ^f	186 ^{d*}	62 ^f	72 ^{cd*}	15 ^a	13 ^a	10.95 ^c	10.15 ^{bc}
N _{0.30} P ₁ K ₁	177 ^{fg}	144 ^{c*}	78 ^g	90 ^{f*}	19 ^b	25 ^{c*}	12.98 ^e	12.17 ^c
N _{0.45} P ₁ K ₁	225 ^h	211 ^{e*}	70 ^h	75 ^{d*}	27 ^d	17 ^{b*}	14.98 ^f	13.50 ^{de}
N _{0.60} P ₁ K ₁	140 ^c	310 ^{g*}	63 ^f	75 ^{d*}	17 ^{ab}	27 ^{cd*}	9.15 ^b	14.40 ^{e*}
40% soil moisture								
N ₀ P ₁ K ₁	152 ^d	92 ^{a*}	57 ^d	49 ^{a*}	22 ^c	8 ^{a*}	7.35 ^a	7.13 ^a
N _{0.15} P ₁ K ₁	137 ^c	114 ^{b*}	35 ^a	68 ^{c*}	30 ^f	19 ^{b*}	11.50 ^d	9.21 ^{b*}
N _{0.30} P ₁ K ₁	185 ^g	227 ^{f*}	56 ^{cd}	73 ^{d*}	31 ^{cf}	29 ^d	13.24 ^e	11.17 ^{c*}
N _{0.45} P ₁ K ₁	164 ^e	236 ^{f*}	51 ^c	90 ^{f*}	25 ^d	47 ^{e*}	12.90 ^e	13.20 ^d
N _{0.60} P ₁ K ₁	97 ^a	153 ^{c*}	37 ^{ab}	63 ^{b*}	26 ^d	26 ^c	11.20 ^{cd}	12.86 ^{cd*}

¹each value is the mean of 3 different measurements; means in the same column followed by different letters are significantly different (Duncan's test $P < 0.05$); *significant differences ($P < 0.05$) between the efficiency and the excess of B for the same factor

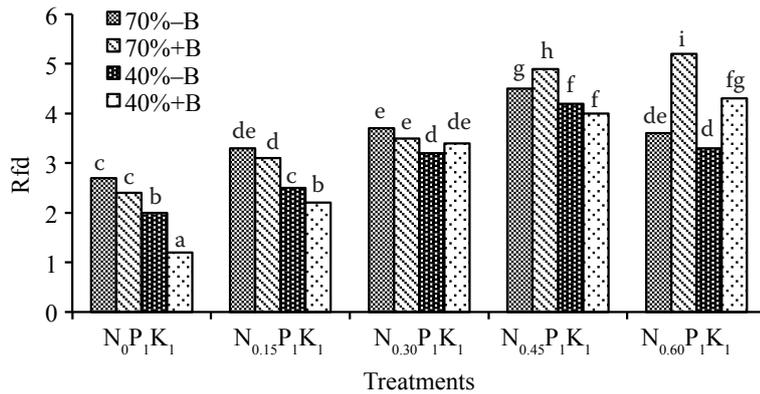


Fig. 2. Vitality index (Rfd) of mature leaves of lettuce plants grown under efficiency (0.5 mg/kg) and toxicity of B (10 mg/kg), two different soil water capacities (70 and 40%) and various N concentrations (0, 0.15, 0.30, 0.45, 0.60 g/kg)

All data represent the means of 5 different measurements. Means that differ at $P < 0.05$ are shown by different letters

negatively affects the enzymes related to the nitrogen assimilation, like nitrate reductase activity, as it was observed in tomato plants under boron toxicity (ERASLAN et al. 2007). The toxic effects of B in lettuce cultivation were alleviated by the presence of high N doses, since a competition between N and B ions was revealed. Nevertheless, the combined effect of N and B in plants appears to be very complex.

Leaf vitality index responses to soil moisture, boron and nitrogen concentrations

Investigation of Photosystem II electron transport revealed significant alterations in the photochemical reactions under low water soil capacity, lack of N and excess of N and B in lettuce leaves. This was firstly reflected by the decreased values in the vitality index (Rfd) of leaves in water deficiency and lack of N treatments (Fig. 2), pointing to disturbances in the photosynthetic electron transport or damage to the thylakoid structure in the donor side of Photosystem II and to an inhibition of enzymatic process in the Calvin cycle of the plants (ASFI et al. 2012; OUZOUNIDOU, ASFI 2012). Under normal soil moisture (70%), B normal conditions (-B) and intermediate N concentrations, a great assimilation of NO_3^- seemed to occur, expressed as higher leaf vitality index (Fig. 2). Highest N doses (0.60 g/kg) induced inhibition in Rfd ratio, showing a worse performance of lettuce physiology. The additional application of B alleviates the toxic effects of high N fertilization, which indicates that under the combination of the two stressors, the proportion of light absorbed by chlorophyll associated with PSII used in photochemistry was maintained.

Our data are in agreement with those of BONILLA et al. (1980) and MATAS et al. (2009) suggesting a specific action of B on N metabolic chain. Accord-

ing to HOWE (1998) and BANON et al. (2012) boron plays an important role in the photosynthesis and in the initial stages of metabolic path, sugar translocation and hormone action.

Quality characteristics responses to soil moisture, boron and nitrogen concentrations

A significant suppression of the total sugar content (glucose, fructose and sucrose) in lettuce plants cultivated at both water soil moistures, under excess of B and without N fertilization ($\text{N}_0\text{P}_1\text{K}_1$), was observed (Table 2). Nevertheless, fructose content was significantly higher under excess of B and lack of N fertilization at both soil moisture regimes. The lower total sugar content can be explained by the increased accumulation of B ions in plant tissues (data not shown) and their negative results in sugar formation. The application of increased level of N (0.30, 0.45 and 0.60 g/kg) had a positive impact on sugar concentration confirming the competition between N and B in plant uptake (MATAS et al. 2009). Under intermediate N fertilization at both soil moistures and without external B supply, sugars had the highest concentration suggesting that at 0.30 and 0.45 g/kg, there is an adequate balance between the assimilation of C and N, allowing leaves with higher accumulation of quality-related compounds (Table 2). The overall disturbances in many metabolic paths, induced by N toxicity (0.60 g/kg), are likely to influence directly and/or indirectly the sugars and ascorbic acid content. This response resulted in a loss not only in lettuce quality but in commercial value as well. On the other hand, the simultaneous application of excess B and excess N at 70% soil moisture significantly increased sugars and ascorbic acid showing an alleviation of the toxic effects induced by each of the elements applied separately.

Some quality indices of Crisp-head lettuce grown under high N (200 kg/ha), like dry matter, sugar and vitamin C contents, were declined (POULSEN et al 1995). Furthermore, Butter-head, Romaine and Oak leaf lettuce quality was maximized by as little as 80 kg/ha, significantly less than the normal recommended N application rate for field-grown lettuce (D'ANTUONO, NERI 2001; STEFANELLI et al. 2011).

CONCLUSION

In agricultural production, quality, yield and cost are the three most important criteria by which the optimization of environmental factors is conducted. Thus, these three factors should always be fully taken into consideration. In the present study, the following conclusions were obtained through the investigation of the effects of different soil moisture, B and N concentrations on quality and yield of lettuce.

The excessively low N concentration of 0 and 0.15 g/kg negatively affected the yield and quality of lettuce especially at low soil moisture (40%). Concentrations like 0.30 and 0.45 g/kg promoted the accumulation of sugars and ascorbic acid and enhanced plant development. Excessive use of N (0.60 g/kg) did not result in better lettuce quality. Thus, the fertilization of $N_{0.30}$ – $N_{0.45}$ and adequate (70%) soil moisture is recommendable for lettuce production. Also, an amelioration effect of high N concentration by the addition of B (10 mg/kg) was shown; this alleviation was more obvious at normal soil water capacity than at reduced water capacity.

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