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Supplementary phosphorus can alleviate boron toxicity in tomato

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ABSTRACT

The effect of supplementary phosphorus on growth and yield of tomato (*Lycopersicon esculentum* cv. Target F1) plants grown at high boron was investigated. The results showed that high B reduced dry matter, fruit yield and chlorophyll content. High B plus 0.5 or 1 mM P increased plant dry matter, fruit yield and chlorophyll concentrations as compared to high B treatments only. Membrane permeability was not increased significantly due to high B application. In the leaves of plants grown at high boron treatments, superoxide dismutase (SOD), peroxidase (POD) and polyphenol oxidase (PPO) levels were increased. However, supplementary P to nutrient solution containing high B reduced the activities of the earlier mentioned enzymes in leaves but their levels were still higher than those at the control treatments. The study revealed that B status affects the activities of some antioxidant enzymes examined. Boron (B) concentrations increased in leaves and roots in the highest external B treatment as compared to the control treatment. Concentrations of Ca, P and K were significantly lower in the leaves of plants grown at high B than those in the control plants. Supplemented nutrient solution containing high B with 0.5 or 1 mM P increased the tissue concentrations of nutrients. These results indicate that supplementary P can mitigate the adverse effects of high B on fruit yield and growth in tomato plants.

1. Introduction

Boron is often found in high concentrations in association with agriculture in arid regions where salt affected soils and saline irrigation water are prevalent. Municipal and other wastewater effluents used for irrigation are also rich sources of excess boron in agricultural systems (Tsadilas, 1997). High concentration of B may also be added to the soils from fertilizers and mining (Nable et al., 1997). However, high levels of boron in the soil or irrigation water suppress plant growth on soils of arid and semiarid regions in the world (Alpaslan and Gunes, 2001). In the recent years, B toxicity has gained an increasing interest because of the greater demand for desalinated water, in which B concentration may be very high for healthy irrigation (Parks and Edwards, 2005).

Yields are reduced in tomatoes as well as in other plant species when concentrations of boron in plant matter are high (Francois, 1984). In Francois' (1984) study it was found a linear decrease with a threshold level of 0.53 mM B in soil solution and a relative yield reduction of 3.7% with each additional increase of 0.1 mM B in the soil solution. However, most of the studies on B tolerance of crops are based on incidence of B injury and not on reduction in yields (Ben-Gali and Shani, 2003).

Considerable genetic variation in the plants in response to high B concentrations has prompted investigation into the mechanism operating in plants against B excess (Cervilla et al., 2007). These mechanisms are based on studies demonstrating an ability of plants to accumulate less B in shoots as has been earlier found in wheat and barley (Paull et al., 1992; Hayes and Reid, 2004). It has also been suggested that an antioxidant response mainly through the antioxidant enzymes system may reduce B-toxicity damage in some plants (Gunes et al., 2006). For protection against oxidative stress, plant cells contain antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase (POD; EC 1.11.1.7). Superoxide dismutase, the first enzyme in the detoxifying process, catalyzes the dismutation of $O_2^{\cdot -}$ to H_2O_2 and O_2 (Molassiotis et al., 2006). POD reduces H_2O_2 to H_2O using several reductants available in the cells (Mittler, 2002; Del Rio et al., 2003). Altered activities of these antioxidant enzymes and antioxidants commonly have been reported, and are used as indicators of oxidative stress in crops (Mittler, 2002).

Despite the considerable agronomic importance, our knowledge and understanding of B toxicity is rather limited (Mahboobi

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et al., 2001). However, some of the nutrient elements are additionally applied to the growth medium of crops to mitigate the adverse effects of boron toxicity. For example, addition of Ca in the irrigation water may reduce B accumulation in plants (Nable et al., 1997; Sotiropoulos et al., 1999).

For higher plants both P and B are essential nutrients and several reports suggest that interaction between these two nutrients is highly significant for many crop plants (Yamanouchi, 1980; Gunes and Alpaslan, 2000). High B is known to reduce P content in spinach and peanut leaves (Blamey and Chapman, 1979). In tomato and other crops, B concentration in leaves was reported to decrease with an increase in P supply (Yamanouchi, 1980). Some synergistic effects of P and B were observed on various metabolic phenomena in maize (Gunes and Alpaslan, 2000; Chatterjee et al., 1990). Besides this, there is hardly any information on this aspect. So, this led us to hypothesize that additional supply of P could mitigate B toxicity in tomato plants by regulating the uptake of essential nutrients and activities of some vital antioxidant enzymes.

2. Materials and methods

2.1. Plant culture and treatments

An experiment was conducted under glasshouse conditions in Mugla-Ortaca (Turkey) from February to April 2004 with tomato (*Lycopersicon esculentum* Mill.) cv. Target F1. Environmental conditions were typical of those for a small-scale tomato crop grown under glasshouse conditions. Temperature was controlled using a heater during the growing season for keeping daytime temperature in the 20–25 °C ranges and nighttime temperature above 10 °C. Three seeds of tomato were sown directly in plastic pots containing 8 kg of peat, perlite and sand mixture in equal ratios, and after germination they were thinned to one plant per pot. The pots were covered with black plastic to reduce evaporation. The basic nutrient solution used in this experiment was a modified Hoagland and Arnon (1940) formulation. The composition of the nutrient solution was (mg L^{-1}): 270 N, 31 P, 234 K, 200 Ca, 64 S, 48 Mg, 2.8 Fe, 0.5 Mn, 0.5 B, 0.02 Cu, 0.05 Zn and 0.01 Mo.

Twenty days after germination the different treatments were initiated. Treatments were: (1) control (C), normal nutrient solution including 0.5 mg L^{-1} B (boron), (2) B_1 treatment: 2 mg L^{-1} 1 boron, (3) B1 + P1: 2 mg L^{-1} B plus 0.5 mM P, (4) B1 + P2: 2 mg L^{-1} B plus 1 mM P, (5) B2 treatment: 4 mg L^{-1} B, (6) B2 + P1: 4 mg L^{-1} B plus 0.5 mM P and (7) B2 + P2: 4 mg L^{-1} B plus 1 mM P. Phosphorus was supplied as H_3PO_4. Each treatment was replicated three times and each replicate included five plants (i.e. 15 plants per treatment). The pH of the nutrient solution was adjusted each time to 5.5 with a minimum amount of 0.1 mM KOH. The volume of the nutrient solution applied to the root zone of plants ranged from 200 to 750 ml from February to April depending on plant age.

Plants were harvested first after fruit set to assess biomass and then after fruit ripening (4 weeks after fruit set) to determine some other parameters. At the fruit-set stage, two plants from each replicate were harvested and divided into shoots and roots for dry weight determination after drying at 70 °C for 48 h. At the fruit-harvest stage, fruits of the remaining three plants from each replicate were harvested and data for both individual and total fruit weight per plant were recorded.

2.2. Relative water content (RWC) and electrolyte leakage

Leaf relative water content was estimated based on the methods of Yamasaki and Dillenburg (1999). The electrolyte leakage (EL) was expressed following Dionisio-Sese and Tobita (1998).

2.3. Protein content

Protein content in the enzyme extracts was determined according to Bradford (1976) using Bovine Serum Albumin V as a standard.

2.4. Enzyme determination

Leaves (0.5 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrolidone (PVP). The homogenate was centrifuged at 20,000 × *g* for 15 min at 4 °C and the supernatant used for determining the activities of POD and SOD.

The activity of SOD was assayed by monitoring its ability to suppress the photochemical reduction of NBT (Beauchamp and Fridovich, 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome c by 50%.

The activity of POD was assayed by adding an aliquot of the tissue extract (100 μL) to 3 ml of assay solution, consisting of 3 ml of reaction mixture containing 13 mM guaiacol, 5 mM H_2O_2 and 50 mM Na-phosphate (pH 6.5) (Chance and Maehly, 1955). An increase in the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer. The POD activity was expressed as the change in absorbance min^{-1} mg^{-1} protein. The increase in A_{470} was measured for 3 min and the activity expressed as ΔA_{470}/mg protein/min.

Polyphenol oxidase (PPO) activity was assayed according to the method of Zauberman et al. (1991) with 4-methylcatechol as a substrate. Half gram of fresh leaf was ground with 10 ml of 0.1 mol L^{-1} sodium phosphate buffer (pH 6.8) and 0.2 g of polyvinyl pyrolidone (PVP, insoluble). After centrifugation at 19,000 × *g* for 20 min, the supernatant was collected as the crude enzyme extract. The assay of the enzyme activity was performed using 1 ml of 0.1 mol L^{-1} sodium phosphate buffer (pH 6.8), 0.5 ml of 100 mmol L^{-1} 4-methylcatechol, and 0.5 ml enzyme solution. The increase in absorbance at 410 nm at 25 °C was recorded automatically for 5 min. One unit of enzyme activity was defined as an increase of 0.01 in absorbance per min per mg protein.

2.5. Dry weight determinations and chemical analysis

Three randomly selected plants per replicate were divided into leaves, stems, and roots, and dried in an oven at 70 °C for 2 days to determine dry weights and concentration of inorganic nutrients. Chemical analyses were carried out on dry weight basis. Ground samples were dry-ashed at 550 °C for 4 h, mixed with 2 M hot HCl, filtered, and then brought to a final volume of 50 mL with distilled water. P was determined in these sample solutions. P was analysed by a vanado-molybdate method using a UV/visible spectrophotometer (Chapman and Pratt, 1982). For B concentration measurements, the samples were dry ashed in a muffle furnace at 500 °C for 6 h. The carbon free residue was then dissolved in 0.1 M HCl and B was determined by the azomethine-H method (Wolf, 1971).

2.6. Statistical analysis

One way analysis of variance (ANOVA) was performed using SAS Institute program (SAS, 1996) and the data were declared significant if values were higher than *F* values at $P < 0.05$.

3. Results and discussion

3.1. Key growth parameters

Tomato exhibited visible symptoms of excess B more pronounced at adequate than high P when grown in excess B. The first

Table 1
Leaf relative water content (RWC), electrolyte leakage (EL), total, shoot, and root dry weights of tomato plants grown under high boron conditions in the presence of phosphorus (Different letter in each column represents significant difference at $P < 0.05$ level, based on *F*-test; ns – non-significant; $^{*}P \leq 0.05$).

Treatments	RWC (%)	EL (%)	Total DM (g/plant)	Shoot DM (g/plant)	Root DM (g/plant)
C	75.75 a	13.3	121.60 a	106.55 a	15.05 a
B_1	69.50 ab	14.5	95.66 c	85.46 c	10.02 bc
B_1+P_1	74.00 ab	14.3	110.35 b	98.60 b	11.75 bc
B_1+P_2	74.75 ab	13.2	106.14 b	92.82 b	13.32 b
B_2	70.25 ab	15.3	75.70 e	66.45 e	9.25 c
B_2+P_1	69.75 ab	15.3	85.89 d	74.30 d	11.59 bc
B_2+P_2	68.75 b	14.4	83.55 d	71.2 de	12.35 b
F-test	*	ns	*	*	*
Interaction BXP	ns	ns	*	*	*

Interaction: ns: not significant; $^{*}P \leq 0.05$. C: 0.5 mg L^{-1} boron; B_1 and B_2, 2 and 4 mg L^{-1} boron; P1 and P_2, 0.5 and 1 mM H_3PO_4 supplemented into the nutrient solution, respectively.

sign of boron toxicity was a yellow–green interveinal chlorosis, which developed first on the oldest leaves and progressed to the youngest. Later, small patches of necrotic tissue appeared between the minor veins and extended to the midribs resulting into a reduced photosynthetic leaf area. The chlorotic symptoms appeared 20 days after the initiation of the treatments, indicating that a significant B accumulation might have occurred before its adverse effects became apparent. It has also been reported that boron toxicity is crop specific, manifested by damage to tissues where it accumulates, and generally leads to chlorosis and necrosis beginning at edges of mature leaves (Brown and Shelp, 1997; Nable et al., 1997).

There were significant reductions in both shoot and root dry weights, and relative water contents in plants grown at both 2 and 4 mg L^{-1} B in nutrient solution compared with those in the control (Table 1). Supplementary P resulted in a slight increase in both dry weights and RWC. Both phosphorus treatments had statistically uniform effect on all growth parameters. Boron toxicity causes negative physiological effects including reduced cell division (Liu and Yang, 2000), decreased shoot and root growth (Nable et al., 1990; Reid et al., 2004). Interaction between B and P on RWC was not significant. There seems to be no report available on the effect of high B on RWC in the literature.

The contents of both B and P were calculated to show interaction between B and P on biomass. These results clearly show that there was an interaction between B and P on biomass. High Boron application increased B content and reduced P content per plant but application of supplement P reduced B content and increased P content (Table 4).

Electrolyte leakage was not significantly changed in the leaves of tomato plants grown under high boron concentrations compared to the control (unstressed) plants and other treatments (Table 1). Interaction between B and P on electrolyte leakage was not significant. There is an increasing evidence that B is required for membrane integrity and function (Dordas and Brown, 2005). B deficiency has been repeatedly associated with substantial and rapid alterations in ion fluxes (Cara et al., 2002). However, there are a few reports available in the literature on the effect of B toxicity on membrane permeability. For example, it has been reported that B toxicity increased membrane permeability in sorghum and maize plants grown under salinity (Ismail, 2003). Membrane permeability of the leaves did not differ significantly in presence of applied B under nonsaline conditions in tomato and cucumber (Alpaslan and Gunes, 2001; Eraslan et al., 2007), and onion (Inal and Tarakcioglu, 2001).

Fruit yield, number and average fruit weight decreased in the plants grown under high boron conditions. However, both the doses of supplementary P improved the yield parameters (Table 2). At the higher B (4 mg L^{-1}) treatment, supplementary 0.5 mM P was more effective in mitigating the effect of high B on fruit yield compared to 1 mM P (Table 3). Interactions between B and P were significant for fruit yield ($P \leq 0.05$), but not for number of fruit and average fruit weight. It has been reported that P is one of mineral elements for fruit yield in citrus (Lauer and Blevins, 1989; Adebooye and Oloyede, 2007).

3.2. Enzyme activities

In the present work, the activities of SOD, POD and PPO enzymes in the plants grown at high B suggest that oxidative stress may be an influential component of environmental stresses on tomato. Both high B concentrations caused significant increases in the activities of SOD, POD and PPO in tomato plants except the activity of SOD which was reduced at 4 mg L^{-1} B (Table 3). Interactions of B and P on SOD, POD and PPO enzymes were significant. It has been reported that high B concentration in the culture medium promotes oxidative damage in tomato leaves and induces a general increase in antioxidant enzyme activity (Cervilla et al., 2007). However, supplementary P applications reduced the activities of all antioxidant enzymes, but their levels were still higher than those in the non-stressed tomato plants. There are a number of reports in the literature which indicate the effect of B deficiency on the oxidative machinery (Cakmak et al., 1995; El-Shintinawy, 1999; Dordas and Brown, 2005), and also some other

Table 2
Fruit yield, number of fruits per plant, and average fruit weight of tomato grown under high boron conditions in the presence of phosphorus (Different letter in each column represents significant differences at $P < 0.05$ level, based on *F*-test; $^{*}P \leq 0.05$).

Treatments*	Fruit yield (g/plant)	Number of fruits/plant	Average fruit weight (g/fruit)
C	2881 a	42.25 c	68.19 a
B_1	2642 c	44.50 bc	59.37 bc
B_1+P_1	2860 ab	43.25 c	66.13 a
B_1+P_2	2770 b	48.25 a	57.41 c
B_2	2372 e	37.75 d	62.83 b
B_2+P_1	2729 b	39.75 d	68.65 a
B_2+P_2	2549 d	41.50 cd	61.42 b
F-test	*	*	**
Interaction BXP	*	ns	ns

Interaction: ns: not significant; $^{*}P \leq 0.05$. *C: 0.5 mg L^{-1} boron; B_1 and B_2, 2 and 4 mg L^{-1} boron; P1 and P_2, 0.5 and 1 mM H_3PO_4 supplemented into the nutrient solution, respectively.

Table 3
Superoxide dismutase (SOD: unit mg $protein^{-1}$), polyphenol oxidase (PPO: unit × 100/mg protein) and peroxidase (POD: ΔA_{470}/min/mg protein) levels in tomato grown under high boron conditions in the presence of phosphorus (Different letter in each column represents significant difference at $P < 0.05$ level, based on *F* test; $^{*}P \leq 0.05$).

Treatments*	SOD	PPO	POD
C	50.5 c	4.24 d	3.24 d
B_1	53.19 b	6.26 b	6.40 c
B_1+P_1	22.72 g	3.26 e	12.24 b
B_1+P_2	37.16 e	4.20 d	14.87 b
B_2	31.40 f	6.60 a	25.35 a
B_2+P_1	43.86 d	5.38 c	13.66 b
B_2+P_2	63.00 a	6.52 a	13.12 b
F test	*	*	*
Interaction BXP	**	*	**

Interaction: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$. *C: 0.5 mg L^{-1} boron; B_1 and B_2, 2 and 4 mg L^{-1} boron; P1 and P_2, 0.5 and 1 mM H_3PO_4 supplemented into the nutrient solution, respectively.

Table 4
Content and concentrations of B and P in leaves and root of tomato grown under high boron conditions in the presence of phosphorus (Different letter in each column represents significant difference at $P < 0.05$ level, based on F-test; $^{**}P \leq 0.05$).

Leaf					Root	
Treatments*	B (mg/kg)	P (g/kg)	B content (mg/plant shoot)	P content (mg/plant shoot)	B (mg/kg)	P (g/kg)
C	44 e	4.5 b	4.49 f	479 a	55 de	8.5 b
B_1	87 bc	2.8 c	8.32 a	239 e	64 cd	6.5 d
B_1+P_1	81 cd	4.1 c	7.99 b	404 b	64 cd	8.5 b
B_1+P_2	74 d	4.3 b	6.86 d	399 b	49 e	9.7 a
B_2	109 a	2.5 c	8.25 a	166 f	78 ab	4.5 e
B_2+P_1	97 b	4.3 b	7.20 c	319 d	82 a	5.9 d
B_2+P_2	90 bc	5.2 a	6.41 e	370 c	71 bc	7.8 c
F test	**	**	**	**	**	**

*C: 0.5 mg L^{-1} boron; B_1 and B_2, 2 and 4 mg L^{-1} boron; P1 and P_2, 0.5 and 1 mM H_3PO_4 supplemented into the nutrient solution, respectively.

studies are available on the oxidative damage of plants under excess B. For example, it has been reported that high boron concentration in the growth medium stimulated SOD level in apple rootstock (Molassiotis et al., 2006). Furthermore, increased SOD in barley (Karabal et al., 2003) and in tobacco leaves (Garcia et al., 2001) under B-toxic conditions.

It has been reported that the POD activity was increased in the rootstock of apple grown at high B concentration (Molassiotis et al., 2006). On the other hand, there are no reports available on the relationship between excess B and PPO activity. The induction of PPO activity can lead to an increased concentration of quinones, produced by oxidation of phenolics (Lopez-Gomez et al., 2007).

3.3. Nutrient contents

Boron concentration increased in plant tissues with increasing B concentration in the nutrient solution, however, leaf P decreased in plants grown at high boron (Table 4). It was also reported that high B reduced P concentration in wheat (Singh et al., 1990), kiwifruit plants (Sotiropoulos et al., 1999), and maize genotypes (Gunes and Alpaslan, 2000), but Mouhtaridou et al. (2004) found that high B in tissue culture increased P content in apple rootstock. However, in the present study, supplementary P in the growth medium increased P concentration and decreased B concentration in the leaves of tomato plants (Table 4). It was reported that excess supply of P reduced B concentration (Sinha et al., 2003).

In conclusion, high B concentrations reduced dry weight, fruit yield and P concentration in tomato plants, but supplementary P application improved dry biomass, fruit yield and P concentration in tomato plants. High B also increased the activities of SOD, POD and PPO enzymes, but the effect of additional supply of P on these enzymes was not consistent with respect to the varying levels of B and P. Since the data presented here suggest the involvement of oxidative stress enzymes in excess B, thus these data will provide a basis for further studies to uncover how these key enzymes are up-regulated. Our data also depict a strong leaf P response to excess B and additional supply of P, indicating that this study might be a useful tool for elucidating the underlying physiological/biochemical phenomena in plants subjected to excess boron and phosphorus.

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