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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