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Exogenous application of nitric oxide promotes growth and oxidative defense system in highly boron stressed tomato plants bearing fruit

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ABSTRACT

To assess the effectiveness of foliage spray of nitric oxide (NO) on some key physio-biochemical variables of tomato (*Lycopercison esculentum* cv. 'Target NF1') plants subjected to boron (B) toxicity, a glasshouse trial was established. A factorial experiment was conducted with three levels of B (0.5, 3.5 and 6.5 mg/L) and 0 or 0.1 mM NO as foliar spray. Boron toxicity caused marked decrease in dry matter and fruit yield in tomato plants as compared to non-stresses plants, but increased electrolyte leakage (EL), proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents coupled with superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC. 1.11.1.7), catalase (CAT; EC. 1.11.1.6) activities and total antioxidant activity (TAA). However, exogenous application of NO partly mitigated the damaging effects of B toxicity on key growth parameters which due to low membrane permeability, H_2O_2 and MDA contents, TAA and antioxidant enzyme activities. Leaf B was higher in tomato plants at B treatments than that in the control plants. High B reduced leaf Ca^{2+}, N and K^+ as compared to those in the control plants. Foliar application of NO lowered B concentration and increased Ca^{2+}, K^+ and N levels in the leaves. The study clearly reveals that exogenous NO can overcome the deleterious effects of B toxicity on tomato fruit yield and whole plant biomass by reducing the concentrations of B, MDA and H_2O_2as well as electrolyte leakage in the leaves.

1. Introduction

The source of high B in the soil is mainly from the fertilizers and mining (Nable et al., 1997). Boron toxicity is one of common problems for many crop plants growing on soil with high levels of boron and low rainfall (Reid, 2013). B toxicity has recently been contemplated as one of the major interesting research areas. Some of known effects induced by high B on plants are delayed development, leaf burn on old leaves, reduced vigor and deferral in fruit yield (Gunes et al., 2007).

Under B toxicity, higher activities of antioxidant enzymes are crucial for plants to improve tolerance to the stress (Gunes et al., 2006, 2007). The mitigation effects of antioxidants have been shown in some crops (Gunes et al., 2006). For overcoming the oxidative induced impairment, plant cells induce some key antioxidants (non-enzymatic/enzymatic) including SOD, catalase, and peroxidase (Ashraf, 2009). It is affirmed that change in levels/activities of these antioxidants could be used as an indicator of oxidative stress tolerance in crops (Mittler, 2002; Ashraf, 2009). These antioxidant responses enable plants to overcome oxidative disorders under environmental stresses (Ashraf, 2009; Akram and Ashraf, 2013; Shafiq et al., 2014). When plants are grown under oxidative stress conditions, they produce reactive oxygen species (ROS) which result in membrane damage ultimately leading to cell death. Analogous to the effects of most of ionic stresses on plants, B toxicity also results in the generation of ROS. For example, B stress causes oxidative stress and EL in some barley cultivars (Karabal et al., 2003). Likewise, in grapevine and apple it has been reported that excess B concentration caused oxidative stress by producing H_2O_2 and lipid peroxidation (Gunes et al., 2006; Molassiotis et al., 2006).

Nitric oxide (NO) is a universal signaling molecule in cells/tissues of plants which has a key role in plant resistance to a variety of stresses including heavy metal stresses, such as, Cd (Besson-Bard et al., 2009), Cu (Zhang et al., 2008), and Fe (Sun et al., 2007) deficiency/toxicity. Besides these three reports on different species and different elements, there is hardly any information available in the literature relating to the effect of exogenous NO on plants grown under high B regimes. Nitric oxide may exhibit a promising effect in this regard. So, the current

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research was carried-out to assess that up to what level boron toxicity had caused oxidative stress in tomato plants, as well as, whether foliage supply of NO could minimize the negative influences of B stress on antioxidant protective system, as well as, alter the distribution of B, N, Ca and K in different organs of tomato plants.

2. Materials and methods

A research trial was carried out in a glasshouse using tomato (*Lycopersicon esculentum* Mill.) cv. 'Target NF1'. Before germination, seeds were sterilized using (1% v/v) sodium hypochlorite solution, followed by washing with dH_2O. Seeds (3) of tomato were sown into each pots filled with 8.5 kg of mixtures of equal quantity of perlite, sand and peat. After germination, one plant was maintained in each plastic pot. The plants were grown under typical atmospheric conditions suited for tomato crop on a small-scale. A heater used to control temperature with day temp. (20–30 °C) and night time temp. greater than 10 °C. To avoid evaporation, all pots were sheltered with a black plastic sheet. Nutrient solution was composed of N (270 mg/L) as KNO_3 and $Ca(NO_3)_2$ $4H_2O$, P (31 mg/L) as KH_2PO_4, K (234 mg/L) as KNO_3 and KH_2PO, Ca (200 mg/L) as $Ca(NO_3)_2$ $4H_2O$, S (64 mg/L) as $MgSO_4$ $7H_2O$), Mg (48 mg/L) as $MgSO_4$ $7H_2O$, Fe (2.8 mg/L) as NaFeEDTA $1.5H_2O$, Mn (0.5 mg/L) as $MnCl_2$ $4H_2O$, Cu (0.02 mg/L) as $CuSO_4$ $5H_2O$, Zn (0.05 mg/L) as $ZnSO_4$ $7H_2O$ and Mo (0.01 mg/L) as H_2MoO_4. All chemicals used were of "ANALAR" grade obtained from the British Drug House. Potassium hydroxide or sulfuric acid (0.01 M) was used to adjust the pH of the nutrient solution at 5.5 at each application. The experiment layout was a RCBD (Randomized Complete Block Design) with three replicates and every replicate consisted of five plants (fifteen plants/treatment). The amount of water applied to each pot ranged from 200 mL to 1000 mL based on plants' canopy during the experiment. A factorial experiment was conducted with three levels of boron (0.5, 3.5 and 6.5 mg/L) and 0 or 0.1 mM NO. Boron and nitric oxide treatments were started by adding boric acid (H_3BO_3) into nutrient solution and sodium nitroprusside (SNP) as donor of NO which was applied foliarly to 10-day-old seedlings. Plants were supplied with 0.1 mM NO as a foliar spray once a week (50 mL/pot) prepared in 0.01% T-20. Plants were harvested at the fruit set stage and recorded biomass and key growth parameters. Some other parameters were determined after fruit ripening (28 days after fruit set). At the fruit-set stage, two plants from each replicate were harvested. After recording fresh weights, shoots and roots were oven-dried at 75 °C for three days and dry weights recorded. At the fruit-harvest stage, fruits of the remaining plants were collected and total fruit weight/plant determined.

2.1. Relative water content (RWC)

Following the procedure proposed by Weatherley and Barrs (1962), RWC was determined.

2.2. Leaf free proline contents

Free proline in fresh leaves was determined by the method described by Bates et al. (1973). Fresh leaf material (500 mg) was triturated in 10 mL of 3% aqueous sulfosalicylic acid. An aliquot (2 mL) of the filtrate was reacted with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid (GAA). After heating the mixture for 1 h at 100 °C in a water bath, the mixture was extracted with 4 mL toluene. The chromophore containing toluene was aspirated, cooled down to room temperature, and the OD (optical density) recorded at 520 nm.

2.3. Electrolyte leakage (EL)

Fresh leaf (0.2 g) was cut into small pieces and placed in test tubes each containing 10 mL ddH_2O. After 2 h of incubation in the water bath, the initial electrical conductivity (EC1) was determined using an EC meter. Thereafter, the samples were autoclaved at 121 °C for 20 min to get released all electrolytes. The temperature of the samples was brought down to 25 °C and EC2 determined. The formula proposed by Dionisio-Sese and Tobita (1998) was used to determine EL.

2.4. Antioxidant enzymes

Fresh leaf (500 mg) was triturated in 50 mM Na–P buffer having 1% soluble polyvinyl pyrolidine. The mixture was centrifuged at 20,000 × *g* for 15 min at 4 °C and the supernatant was collected. CAT activity was estimated following Kraus and Fletcher (1994), whereas, that of SOD was determined following Beauchamp and Fridovich (1971). The POD activity was determined following the method described by Chance and Maehly (1955). The total soluble proteins were estimated following Bradford (1976).

2.5. Total antioxidant activity

The method described by Shimada et al. (1992) was used for determining free radical-scavenging activity of leaf extract in methanol. Each extract (0.2–10 mg/L) in methanol (2 mL) was mixed with 2 mL of freshly prepared methanolic solution containing 80 ppm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the equation below

$$\text{DPPH scavenging ability} = \left[1 - \frac{\left(A_i - A_j\right)}{A_c}\right] \times 100$$

where A_i is absorbance of extract + DPPH, A_j is absorbance of extract+ methanol, and A_c is absorbance of DPPH + methanol. A lower absorbance indicates a higher scavenging effect.

2.6. Leaf malondialdehyde (MDA)

With some modifications as suggested by Weisany et al. (2012), a product of lipid peroxidation, MDA was estimated adopting the procedure proposed by Cakmak and Horst (1991).

2.7. Hydrogen peroxide (H_2O_2)

The quantification of H_2O_2 in leaves was carried out following Loreto and Velikova (2001). Half gram of fresh leaves was triturated in 3 mL of 1% (w/v) TCA. The mixture was centrifuged and 0.75 mL of 10 mM K buffer and 1.5 mL of 1 M KI were added to an aliquot of 0.75 mL and then its absorbance recorded at 390 nm.

2.8. Chemical analyses

Total N was determined following the Kjeldahl method. For the analysis of other nutrients the dried and ground samples were ashed in a muffle furnace at 550 °C for 6 h. The white ash was dissolved in 5 mL of 2 M hot HCl, and maintained the final volume to 50 mL with dH_2O. Cation, such as, Ca and K were examined following Chapman and Pratt (1982). For estimating B contents, 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 determined by the azomethine-H method (Wolf, 1971).