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Research article

Nitric oxide protects carbon assimilation process of watermelon from boron-induced oxidative injury

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ABSTRACT

Nitric oxide (NO) mediates plant response to a variety of abiotic stresses; however, limited information is available on its effect on boron (B)-stressed watermelon plants. The present study investigates the mechanism through which NO protects watermelon seedlings from B deficiency and toxicity stresses. Five days old watermelon seedlings were exposed to B (0, 0.5 and 10 mg L^{-1}) alone or with 75 μmole of NO donor sodium nitroprusside (SNP) for 30 days. Both low and high B concentrations in the media altered nutrient accumulation and impaired various physiological processes of watermelon seedlings, leading to a significant reduction in biomass production. The plants exposed to B deficient or toxic concentrations had 66 and 69% lower shoot dry weight, respectively compared with optimum B levels. B toxicity-induced growth inhibition of watermelon seedlings was associated with high B translocation to shoot tissues, which caused lipid membrane peroxidation (12% increase) and chlorophyll destruction (25% reduction). In contrast, B deficiency accelerated generation of reactive oxygen species (ROS), specifically OH^{-1} and induced cellular oxidative injury. Exogenously applied SNP promoted leaf chlorophyll, photosynthesis and consequently biomass production in B-stressed watermelon seedlings by reducing B accumulation, lipid membrane peroxidation and ROS generation. It also activated antioxidant enzymes such as SOD, POD and APX, and protected the seedlings from ROS-induced cellular burst.

1. Introduction

Boron (B) is an essentially required micronutrient for growth of most of the vascular plants. Many crop species are very sensitive to B supply, and experience serious growth and yield penalties under B concentration above or below certain limits. B deficiency is a widespread disorder in many high rainfall regions (Yan et al., 2006), acidic sandy (Shorrocks, 1997) and drought-stressed soils (Marschner, 1996). Similarly, use of B-contaminated irrigation water, poor drainage, and excessive B fertilizers application has resulted in buildup of high B concentrations [8–16 Kg of B ha^{-1} or 1 mM of $B(OH)_3$] in many agricultural soils (Roy and Basu, 2009). Poor growth, yield and grain quality has been observed in crops cultivated on B deficient or toxic soils, and the problem is more common in arid and semiarid regions, where is salts are accumulated in the soils. Excessive or deficient B concentration in soils may alter the bioavailbility of various nutrients in the soils, leading to growth inhibition (Tariq and Mott, 2007).

Due to its critical role in various cellular processes of plant such as cell division and elongation, protein synthesis and metabolism of nucleic acids and phenolics (Yan et al., 2006; Marschner, 1996), imbalanced B supply can lead to poor growth and overall crop productivity. Significant growth and yield losses have been reported in a wide range of plants species such as barley (Singh et al., 2010), wheat (Yau and Ryan, 2008), zucchini and cucumber (Pardossi et al., 2015) in response to B-induced stress. Inhibited or toxic levels of B in plant tissues can accelerate production of reactive oxygen species (ROS), impairing various physiological processes (Mishra et al., 2009; Landi et al., 2013). In response to excess ROS, plants activate an antioxidant defense system, comprising of enzymes e.g. superoxide dismutase (SOD), catalase (CAT),

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peroxidase (POD) and ascorbate peroxidase (APX), which can improve their survival under stressful environments (Mishra et al., 2009).

Watermelon is among the top 20 important cultivated and high value economic crops of the world. Similar to many other horticultural crops, watermelon yield and quality largely depend on proper nutrient management. Imbalanced B supply toxicity or deficiency in the soil or water media, is a critical issue in the major watermelon producing countries such as China (Liu et al., 1980) and Turkey (Munir Ozturk et al., 2010), which are ranked as the first and second top watermelon producers in the world (www.fao.org). However, limited information is available on the mechanisms of B toxicity- or deficiency-induced injury to watermelon (Hamurcu et al., 2015; Moustafa-Farag et al., 2016). Better understanding of the biochemical pathways through which imbalanced B supply inhibits watermelon growth may lead to development of management techniques for optimizing performance of watermelon crops under B stressed environments.

Nitric oxide (NO) is an important signaling molecule, which protects bioprocess in plants against harmful effects of abiotic stresses such as heat, cold (Uchida et al., 2002), salinity (Zhao et al., 2004), drought (Arasimowicz-Jelonek et al., 2009) and heavy metals (Mostofa et al., 2014). Inside plant tissues, NO interacts with ROS e.g. it changes superoxide anion to a less toxic product – peroxynitrite (ONOO) (Neill et al., 2003), and thus protects cells from oxidative injury (Shi et al., 2014). In addition, it can scavenge stress-induced ROS by activating antioxidant enzyme system (Aftab et al., 2012). Positive effects of NO application have already been reported on a range of plant species exposed to abiotic stresses but majority of the literature is focused on exploring the effect of NO on heavy metals or salinity stressed plants. However, limited information is available on the effect of NO on plants exposed to non-metals such as B toxicity or B deficiency.

We hypothesized that NO protects watermelon seedlings from B-toxicity/deficiency by activating anti-oxidative response mechanism. Therefore, the present study explores growth and physiochemical response of watermelon to B toxicity/deficiency and potential of NO to protect watermelon plants from B stresses.

2. Materials and methods

2.1. Plant material and boron application

A moderately B stress tolerant watermelon cultivar Zhemi-5(*Citrullus lanatus Thumb*) selected from pre-experiments was used for this study. This cultivar is a widely cultivated in most part of China. Seeds were thoroughly washed using distilled water, sterilized with 20% (v/v) sodium hypochlorite for 10 min and soaked into sterile distilled water for 24 h at 25 °C. The seeds were germinated on filter papers in dark at 25 ± 1 °C temperature and 85% humidity for 5 days. The seedlings were transplanted into plastic boxes (10 plants per box), containing 5 L Hoagland solution. The chemical composition of growth media was (concentrations in mg L^{-1}) 80 NH_4NO_3, 506 KNO_3, 945 $Ca(NO_3)_2{\cdot}4H_2O$, 493 $MgSO_4{\cdot}7H_2O$, 2.13 $MnSO_4{\cdot}H_2O$, 0.22 $ZnSO_4{\cdot}7H_2O$, 0.08 $CuSO_4{\cdot}5H_2O$, 30 EDTA-Na_2Fe, 0.02 $(NH_4)Mo_7O_2{\cdot}4H_2O$, 136 KH_2PO_4. Boric acid (H_3BO_3) was added in the solution to achieve final B concentrations as 0, 0.5 and 10 mg L^{-1}. Zero or 75 μmol L^{-1} of NO donor, sodium nitroprusside (SNP), were added to the growth media immediately after transplanting the seedlings. Each treatment contained 20 plants. The plants were allowed to grow for 30 days, under controlled conditions as: 20–25/15–20 °C day/night temperature and 60% humidity. Different leaves from the third middle part of watermelon were used to determine the gas exchange, chlorophyll fluoresces and biochemical analyses.

2.2. Morphological measurements

After 30 days of growth in the treated media, 10 seedlings were randomly selected from each treatment, harvested and separated into shoots and roots. Seedlings were dried in a forced-draught oven at 80 °C for at least 72 h and weighed to record dry biomass. The dry leaf and root tissues were used for determining concentrations of various nutrients using inductively coupled plasma mass spectrometry (ICPMS).

2.3. Photosynthetic gas exchange parameters

Data on photosynthetic gas parameters such as photosynthetic rate (Pn) and stomatal conductance (Gs) were recorded using a portable photosynthesis system LI-COR 6400 (Lincoln, Nebraska, USA). Intrinsic water use efficiency (water use efficiency $WUE_{intrinsic}$) was calculated from the ratio of rate of photosynthesis and stomatal conductance (Medrano et al., 2015). Measurements were taken from five fully expanded leaves located in the 3rd middle part of the shoot 30 days after treatment. Before measurements, IRGA was calibrated, and zero was adjusted approximately every 45 min during measurement period. All the measurements were performed from 10.00 to 12.00 a. m. Variables inside the sensor head were adjusted as 20 °C temperature, 1000 μmol m^{-2} s^{-1} light intensity, and 60–80% relative humidity.

2.4. Chlorophyll and carotenoids determination

Fresh leaf-tissues (0.5 g) of the interveinal area were ground in liquid nitrogen using pestle–mortar. Methanol (95%) was added to the samples and the mixture was centrifuged for 5 min at 4500 rpm at 20 °C. The absorbance of the extracted solution was recorded at wavelengths of 470, 652, 665 and 750 nm to estimate chlorophyll *a* chlorophyll *b* and carotenoids, respectively, using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

2.5. Chlorophyll fluorescence

Chlorophyll fluorescence parameters e.g. maximal quantum yield of PSII (Fv/Fm), initial fluorescence (F0) and maximum fluorescence (Fm) were measured using an imaging pulse amplitude modulation (PAM) device (IMAG-MAXI; Heinz Walz, Effeltrich, Germany). The topmost fully expanded leaves were preconditioned in dark for 30 min and then used for chlorophyll fluorescence measurement. All the measurements were taken from four leaves of each treatment and were averaged.

2.6. Malondialdehyde (MDA) and reactive oxygen species

Oxidative damage to lipid membranes was determined in terms of malondialdehyde (MDA) production. Five samples from fresh watermelon leaves (0.5 g) were homogenized in 8 mL of 0.25% thiobarbituric acid (TBA) prepared with 10% trichloroacetic acid (TCA). The extract was heated at 95 °C for 30 min and subsequently cooled on ice. The samples were centrifuged at 5000×*g* for 10 min and absorbance of the mixture was taken at 532 nm. MDA level was expressed as mmol g^{-1} fresh weight, using an extinction coefficient of 155 mM cm^{-1}.

Hydrogen peroxide (H_2O_2) contents were determined using Velikova et al. (2000) method. In brief, fresh leaf samples (0.5 g) were extracted with 5.0 mL of TCA (0.1%, w/v), and the homogenate was centrifuged at 12000 *g* for 15 min. The supernatants were used for determining H_2O_2 content. Extra-cellular hydroxyl radicals (OH) from the leaf samples were estimated following the protocol of Halliwell et al. (1987).