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Research article

Long- and short-term effects of boron excess to root form and function in two tomato genotypes

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ABSTRACT

Boron (B) is an essential plant nutrient, but when present in excess it is toxic. Morphological measurements were made to assess the impact of B toxicity on the growth of two different tomato hybrids, Losna and Ikram. Contrasting long and short-term B responses in these tomato hybrids, were observed. Losna showed less toxicity symptoms, maintaining higher growth and showing much less B content in both root and shoot tissues compared to Ikram. Root morphological differences did not explain the tolerance between the two hybrids. Under excess B supply, a significant inhibition on net nitrate uptake rate was observed in Ikram, but not in Losna. This effect may be explained by a decrease of nitrate transporter transcripts in Ikram, which was not measured in Losna. There was a different pattern of B transporter expression in two tomatoes and this can explain the contrasting tolerance observed. Indeed, Losna may be able to exclude or efflux B resulting in less accumulation in the shoot. Particularly, *SlBOR4* expression showed significant differences between the tomato hybrids, with higher expression in Losna explaining the improved B-tolerance.

1. Introduction

Boron (B) is an essential micronutrient in higher plants, although it is toxic in excess (Kot, 2008). The concentration range between B deficiency and toxicity is narrow and depends on plant species. However, both these extreme conditions severely reduce crop yield and quality worldwide (Takano et al., 2008). High B concentrations may occur naturally in the soils of arid and semi-arid areas arising from various anthropogenic sources such as mining, fertilizers, or irrigation water (Camacho-Cristóbal et al., 2008). Although B toxic symptoms are commonly expressed in above-ground organs causing delayed plant development and necrotic and/or chlorotic spots at the margin and tips of older leaves (Reid et al., 2004), root system appears to be also affected in many species, including tomato (Cervilla et al., 2009b), wheat (Kalayci et al., 1998), barley (Choi et al., 2007) and grapevine (Günes et al., 2006).

B excess caused root growth inhibition (Nable, 1988) and abnormal cell division in the root meristem (Liu et al., 2000). Thus, the genotypic variation in root elongation was used as indicator of B tolerance (Chantachume et al., 1995). The target site of B toxicity was identified in the root tips (Reid et al., 2004). Under B excess, the formation of hypodermis with increased suberin deposition in root cortical cell walls was also observed (Ghanati et al., 2002). Further, B excess caused root morphology modifications in different crops including high branching and fine root formation, changing in root distributions between top- and sub-soil (Choi et al., 2006) and less lateral root formation (Huang and Graham, 1990).

In contrast, there are limited information on how B excess changes root function, such as the uptake of nutrients, such as nitrate. There is a convincing evidence for a direct effect of B toxicity on nitrate assimilation. Boron excess inhibited NO_3^- reduction decreasing N organic concentration, but increasing NH_4^+ assimilation inducing GS/GOGAT and GDH activities in tomato (Cervilla et al., 2009a), sunflower (Kastori and Petrovic, 1989), barley and wheat (Mahboobi et al., 2002).

The ability to maintain much lower B levels in roots as well as in shoots by active B efflux was the tolerance mechanism in barley cultivar (Hayes and Reid, 2004). In *Arabidopsis*, B tolerance was associated with the *AtBOR4* overexpression, one of the six B transporters identified in the plant genome (Miwa et al., 2007).

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Indeed, BOR4 is considered the most important efflux transporter from the roots to the soil and, when B concentration becomes toxic, it is able to confer plant B-tolerance (Reid, 2014). Although, changes in *HvBOR2* and *TaBOR2* expressions were related to different level of B-excess tolerance in barley and wheat cultivars (Reid, 2007). In contrast, under high B supply, both the nodulin-like intrinsic protein (NIP5; 1) which facilitates B influx into root cells from the soil, and BOR1, an efflux B transporter for xylem loading, were degraded (Reid, 2014).

In this study, long- and short-term responses to B excess were evaluated in two tomatoes with contrasting sensitivity to boron. Root growth and morphology together with shoot B accumulation and chlorophyll content were examined after long-term B exposure. In the short-term experiment, the effect of B excess on net nitrate uptake and plasma membrane potential were analyzed. The expression patterns of *NRT2.1*, encoding for a putative high-affinity NO3- transporter, LHA1 and LHA8, encoding for PM H + -ATPase isoforms, were studied. Finally, the expression of B transporters, NIP5; 1, BOR1 and BOR4, involved in B uptake, xylem loading and B efflux, respectively, was analyzed. A possible mechanism of tolerance to B toxicity in tomato was suggested.

2. Materials and methods

2.1. Plant material and growth condition

Tomato (*Solanum lycopersicum* L.) seeds of Ikram and Losna hybrids (kindly provided by *Syngenta,* Italy) were surface sterilized for 10 min in 10% (v/v) sodium hypochlorite solution and then rinsed with deionized water. Seeds were germinated on filter paper moistened with 0.5 mM $CaSO_4$, at 24 °C in darkness, for 5 days. Individual seedlings, selected by uniform size, were transferred into plastic containers (diameter 7 cm, 110 ml volume), filled with silver sand and then placed in a growth chamber with a 16-h photoperiod, a photon flux density of 350 μmol m^{-2} s^{-1}, 25 °C and 70% RH. Seedlings (one per pot) were watered, twice a week, with a complete nutrient solution containing KNO_3 (6 mM), $NH_4H_2PO_4$ (1 mM), $MgSO_4$ (2 mM), $Ca(NO_3)_2$ (4 mM), KCl (50 μM), H_3BO_3 (25 μM), $MnSO_4$ (2 μM), $ZnSO_4$ (2 μM) $CuSO_4$ (0,5 μM), $(NH_4)_2MoO_4$ (0.5 μM) and Fe-EDTA (20 μM), pH 5.8, for 7 days. Later, four seedlings were transferred into a growing unit containing 4.3 L of aerated nutrient solution having the same above composition, for 7 days. The nutrient solution was replaced twice a week, and the pH adjusted to 5.8 with 1.0 M KOH.

2.2. Long- and short-term boron experiments

For long term experiments, tomato seedlings (19 d-old) were transferred to aerated nutrient solution containing the same composition as above adding by 25 (control), 320, 640 or 1280 μM H_3BO_3, for 7 days. The boric acid addition did not change the pH of the nutrient solution. Root morphological analysis, chlorophyll and boron contents were evaluated.

For short-term experiment, tomato seedlings (19 d-old) were transferred to aerated N-free nutrient solution (0.5 mM $CaSO_4$) for 24 h. Afterwards, N-starved seedlings were transferred to aerated nutrient solution and exposed to 200 μM NO_3^- and 25 (control), 320 or 640 μM H_3BO_3 for 0, 4, 8, 24, and 48 h. The highest B concentration (1280 μM) applied in the long-term experiment was not used here. Net NO_3^- uptake and plasma membrane H^+-ATPase assays, membrane potential measurements, gene expressions and root morphological analysis were carried out.

2.3. Morphological analysis

At the end of treatment, five seedlings of each genotype for each B treatment (long-term experiments) and for both each exposure period and B treatment (short-term experiments) were collected and separated into roots and shoots. Then, roots were stained using 0.1% (w/v) toluidine blue (Sigma-Aldrich, #89640) to improve the contrast during the scanner acquisition. Briefly, stained roots were positioned on the scanner, and an image was captured at 600 dots per inch (dpi) of resolution. The root length (cm), root volume (cm^3), root area (cm^2) were measured using WinRhizo Pro system v. 2002a software (Instruments Régent Inc., Quebec, Canada), and lateral roots number was counted manually from the image (Lupini et al., 2014, 2016). Shoots and roots were then placed in an oven at 70 °C until reaching the constant weight for the determination of the dry weights (SDW and RDW, g, respectively). Root length ratio (RLR, root length/whole plant dry weight, g cm^{-1}), root mass ratio (RMR, root dry weight/whole plant dry weight, g g^{-1}), root fineness (RF, root length/root volume, cm cm^{-3}) and root tissue density (RTD, root dry weight/root volume, g cm^{-3}) were measured and calculated as described by Abenavoli et al. (2016).

Shoot and root growth rates (SGR and RGR, g DW day^{-1}, respectively) were also analyzed by linear regression using the increase of the biomass over short-term experiments.

2.4. Chlorophyll content

After 7 days of B exposure (long-term experiment), relative absorbance measurements using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Japan) were taken (read, detected, recorded) in tomato plants (21 d-old) from the lower, middle and upper true leaves of the fully expanded frond. SPAD readings were then averaged for each part of leaves and each tomato plant.

2.5. Boron content

After 7 days of B exposure (long-term experiment), leaf and root samples were collected, rapidly washed with deionized water, and dried at 80 °C for 72 h. The total B concentration was measured after digestion of milled 0.15 g dry weight of leaf or root samples with a mixture of HNO_3 (98% v/v) and $HClO_4$ (30% v/v) at 230 °C for 1 h. Boron was determined by the Azomethine-H method (Wolf, 1974), the absorbance was read using a UV–vis spectrophotometer (Perkin Elmer Lambda 35, Walthman, MA, USA) at 420 nm, and B concentration was expressed as g kg DW^{-1}.

2.6. Net NO_3^- uptake assay

The net nitrate uptake rate (NNUR) was defined as net influx across the plasma membrane (Lupini et al., 2016). For each B treatment (200 μM NO_3^- *plus* 25, 320, 640 μM H_3BO_3) and exposure period (0, 4, 8, 24 and 48 h) of short-term experiment, three tomato N-starved seedlings (19-d-old) were collected. Their intact roots were rinsed with 0.5 mM $CaSO_4$ for 20 min. The seedlings were then immersed in 40 ml of continuously aerated nutrient uptake solution containing 200 μM KNO_3 and 0.5 mM $CaSO_4$ at pH 6.0. Samples were taken from the uptake solution at 5 min intervals over a 50 min period, and nitrate concentration was measured spectrophotometrically at 210 nm (Goldsmith et al., 1973) using a UV–Vis spectrophotometer (Perkin Elmer Lambda 35, Walthman, MA, USA). The NNUR was calculated from the linear phase of the nitrate depletion curve and expressed as μmol NO_3^- g^{-1} FW h^{-1}.