Scientia Horticulturae 200 (2016) 178–185

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Bacillus pumilus alleviates boron toxicity in tomato (*Lycopersicum esculentum* L.) due to enhanced antioxidant enzymatic activity

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ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 8 January 2016
Accepted 14 January 2016
Available online 28 January 2016

Keywords:
Antioxidants
Boron
Chlorophyll
Shoot ions
PGPR

ABSTRACT

High soil boron (B) concentrations in arid and semi-arid regions of the world are responsible for decreased crop productivity. However, the use of plant growth promoting rhizobacteria (PGPR) can improve the plant growth under stress conditions like high B either due to limited uptake of B or enhanced antioxidants production, as PGPR have been shown to enhance the resistance in plants against various abiotic stresses. A pot experiment was conducted under green house conditions according to completely randomized design (CRD) and comprised of four levels of B (0.45, 10, 20, and 50 mg B kg^{-1} soil) either with or without PGPR. Plants were grown for 10 weeks after onset of experiment and analyzed for mineral composition including B and antioxidation activity. Shoot fresh weight, shoot dry weight and leaf chlorophyll contents were inhibited to increasing levels of B. Interestingly, shoot K^+ concentration tended to increase with increasing B levels, particularly at 50 mg B kg^{-1} soil; third level of B (B3). Similarly, highest shoot B concentrations were observed at high levels of B supply (B3) as compared to all other treatments. Likewise, with the increase in external B supply, antioxidant enzymes activities and proline contents in tomato shoot were enhanced. *Bacillus pumilus* inoculation significantly improved the shoot fresh weight and dry weight of unstressed (control) as well as B-stressed plants. In general, *B. pumilus* inoculation enhanced the plant antioxidation activity, particularly superoxide dismutase (SOD) and catalase (CAT) antioxidant enzymes. Contrary, the interaction between high B and *B. pumilus* remained insignificant for limiting the shoot B uptake except for second level of B (B2) which decreased the shoot B accumulation by 21% as compared to non-inoculated B2. Our results suggest that PGPR inoculation confer tolerance in B-stressed plants through the induced plant antioxidation activity. Additionally, PGPR inoculation reduces the shoot B accumulation, however, differential response of shoot B inhibition is expected to increasing B concentrations.

1. Introduction

Tomato (*Lycopersicum esculentum* L.) is one of the most widely grown vegetables worldwide, may face problems due to cultivation under elevated B concentrations (Cervilla et al., 2012). High B concentrations are common in arid and semi-arid regions of the world where natural soil B levels are high, however, other sources like irrigation water, B-fertilizers, mining, sewage sludge and fly ash may also contain additional B in soils (Nable et al., 1997). In addition, high B in agricultural systems can be resulted from municipal waste and industrial effluents (Tsadilas, 1997). Whilst low B concentrations in soils are essential for normal functioning of plants including tomato, higher B concentrations suppress plant growth (Alpaslan and Gunes, 2001). It is evident from the literature that soil B concentrations normally in the range of 1–4 mM affect the plant growth (Reid et al., 2004).

Boron toxicity symptoms in plants have been reported in the form of reduced vigor, stunted growth and development, lower leaf chlorophyll contents, leaf burn (chlorosis and necrosis beginning at the edges of mature leaves), and decreased number, size and weight of fruits (Nable et al., 1997). Further, B toxicity inhibits the roots and shoots yield (Nable et al., 1990). Toxic effects of high B

Abbreviations: B, boron; Ca, calcium; Mg, magnesium; K, potassium; Na, sodium; POD, peroxidase; SOD, superoxide dismutase; CAT, catalase; PGPR, plant growth promoting rhizobacteria.

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http://dx.doi.org/10.1016/j.scienta.2016.01.024
0304-4238/

are mainly attributed due to accumulation of B in plants, as yield of tomato was reduced when B concentrations were higher in plant matter (Francois, 1984). It was further revealed by Francois (1984) that 0.53 mM B in soil solution is threshold level of B and the addition of further each 0.1 mM B linearly decreases the relative yield of tomato by 3.7%. Despite the importance of this nutritional disorder, most of the reports on B tolerance of crops are inconclusive (Hayes and Reid, 2004; Miwa et al., 2007; Sutton et al., 2007), as based on incidence of B injury not on reduction in yields (Ben-Gal and Shani, 2003). Secondly, this antagonism might be resulted due to considerable genetic variations which exist among different plant species to tolerate high B levels (Cervilla et al., 2007). Therefore, it is important to determine certain biochemical indicators of tolerance for the selection of different varieties or developing transgenic plants (Cervilla et al., 2012). Among these, antioxidants and osmoprotectants are considered important biochemical indicators of plant tolerance to oxidative stress caused by various abiotic stresses (Juan et al., 2005; Sánchez-Rodríguez et al., 2010). During oxidative stress, production of reactive oxygen species (ROS) at high levels of B causes oxidative damage to plant membranes and eventually cell death (Cervilla et al., 2007). To avoid ROS damage, plants evolve antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and non-enzymatic antioxidants like ascorbate, glutathione, α-tocopherol (Mittler, 2002; Blokhina et al., 2003; Miller et al., 2008) and osmoprotective solutes like proline (Cervilla et al., 2012). In this regard, Gunes et al. (2006) suggested that antioxidant response mainly through antioxidant enzymes may alleviate B-toxicity in some plants. Similarly, Eraslan et al. (2007a) observed higher proline contents in leaves of pepper and tomato as an antioxidative adjustment under B toxicity. The enhanced antioxidants production scavenged the oxidative damage caused by various environmental stresses which in turn increased the resistance in plants (Cakmak et al., 1993).

In recent years, the use of plant growth promoting rhizobateria (PGPR) have been emerged as an effective strategy for enhancing the abiotic stress tolerance in plants through the induced antioxidants synthesis (Shao et al., 2009; Islam et al., 2014). Numerous researchers have reported that several bacterial genera like *Pseudomonas*, *Bacillus*, *Pantoea*, *Burkholderia* and *Rhizobium* were beneficial in terms of providing resistance in pea, maize, wheat, grapevine and common bean against various abiotic stresses such as drought, temperature stress and salinity (Arshad et al., 2008; Marulanda et al., 2010; Egamberdiyeva and Hoflich, 2003; Barka et al., 2006; Figueiredo et al., 2010). Similarly, *Bacillus pumilus* has been shown to enhance the salinity tolerance in rice (Jha and Subramanian, 2013). This is possible that *B. pumilus* might use similar approach to cope high B stress, as operating mechanism is same in plants against abiotic stresses. To the best of our knowledge, this is first report to determine whether *B. pumilus* inoculation is responsible either for limited uptake of B or to enhance the tomato growth through the induced antioxidative defense system to B excess.

2. Materials and methods

2.1. Plant cultivation and growth conditions

A pot experiment was conducted in greenhouse, research facility of Quaid-i-Azam University, Islamabad, Pakistan located between 33.14° N latitudes and 73.13° E longitudes. The soil (top soil: 0–15 cm depth) used in our experiment was collected from the surrounding field and autoclaved at 121 °C for 20 min. Soil was analysed for EC 3.29 dS m^{-1}, pH 7.7, soil organic matter (SOM) 0.83%, total N = 730 μg g^{-1}, available P = 3.61 μg g^{-1}, extractable K = 197 μg g^{-1} and available B = 0.45 μg g^{-1} before filling the pots. The texture of the soil was clayey which was determined by USDA textural triangle method (Moodie et al., 1959). Each pot (14 × 18 cm) was filled with 1 kg of sterilized soil and mixed well with NPK fertilizers at the rate of 95 mg N kg^{-1} soil, 30 mg P_2O_5 kg^{-1} soil and 42.5 mg K_2O kg^{-1} soil viz urea (0.18 g pot^{-1}), di-ammonium phosphate (0.065 g pot^{-1}), and sulphate of potash (0.085 g pot^{-1}), respectively. Likewise, B was mixed in soil as H_3BO_3 with following four treatments; control (only native soil B; 0.45 mg/kg^{-1}), 10 mg/kg^{-1} dry soil (B1), 20 mg/kg^{-1} dry soil (B2) and 50 mg/kg^{-1} dry soil (B3). Tomato (*L. esculentum* L. cv. Rio-Grande) seeds were obtained from NARC, Islamabad, Pakistan and sown in plastic trays after treating them with 1 mM $CaSO_4{\cdot}2H_2O$ solution. After two weeks of seed emergence, three uniform seedlings were transplanted in each pot. Inoculum, *B. pumilus* with accession number KF875447 (Mufti et al., 2015) was prepared in 100 ml LB media and incubated overnight in shaker-incubator (ECELLA E23, California, USA), followed by centrifugation at 3000 rpm for 20 min. Afterwards, supernatant was discarded and bacterial pellet was diluted with sterile water. For inoculation, 1 ml of *B. pumilus* solution was injected in the rhizospheric soil of each tomato seedling using small syringe (1 m/cc 100 units). Half of the pots were inoculated with *B. pumilus*, whereas the other half not. The experiment was repeated twice which was consisted of 4 treatments with three replications of each either with or without PGPR inoculation.

2.2. Estimation of leaf chlorophyll contents and harvesting

Prior to harvesting, leaf chlorophyll contents were measured using chlorophyll meter (SPAD-502 Plus Konika Minolta Sensing, Inc., Japan). Plants were harvested after 10 weeks exposure to high B and plant samples (shoot) were either oven-dried at 65 °C or stored at −80 °C for subsequent analysis.

2.3. Shoot ion analysis

For the determination of ions from tomato shoot material, acid digestion was carried out using nitric acid and perchloric acid solution in the ratio of 3:1. Shoot digests were obtained by treating 1 g dry ground shoot material with 10 ml of digestion mixture. Afterwards, shoot digests were filtered through Whatman No. 42 filter paper and collected filtrates were used for the determination of minerals (K^+, Ca^{2+}, and Mg^{2+}) using atomic absorption spectrophotometer (AAS) (AA240FS, Varian, Australia).

2.4. Shoot B determination

For tomato shoot B analysis, 1 g oven-dried shoot material was processed for dry ashing at 550 °C. Afterwards, ash was dissolved in 10 ml of 0.36 N H_2SO_4 and filtered through Whatman No. 42 filter paper. The collected filtrates were used for B analysis using spectrophotometer (Gaines and Mitchell, 1979).

2.5. Determination of antioxidant enzymes activities and proline contents in tomato shoot

Antioxidant enzymes activities of SOD, POD and CAT in fresh shoot material of tomato on exposure to varying levels of B either alone or in combination with *B. pumilus* were determined using spectrophotometer procedures of Beauchamp and Fridovich (1971), Reddy et al. (1995) and Luck (1974), respectively. Similarly, proline contents in tomato shoot material were determined spectrophotometrically (Bates et al., 1973).