Plant Physiology and Biochemistry 108 (2016) 158–164

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

The effect of kinetin on wheat seedlings exposed to boron

Ahmet Eser, Tülin Aydemir*

Celal Bayar University, Faculty of Science and Letters, Department of Chemistry, 45140, Manisa Turkey

ARTICLE INFO

Article history:
Received 29 February 2016
Received in revised form
16 June 2016
Accepted 16 June 2016
Available online 18 June 2016

Keywords:
Wheat
Boron stress
Kinetin
Antioxidant enzymes

ABSTRACT

The objective of this study was to examine relationship between boron (B) induced oxidative stress and antioxidant system in boron sensitive and tolerant wheat cultivars Bezostaya and Kutluk, and also to investigate whether Kinetin (KN) enhances the level of antioxidant system, relative growth, concentration of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and proline and chlorophyll content in both cultivars exposed to B stress. B treatments diminished growth and chlorophyll content whereas, it enhanced accumulation of H_2O_2, MDA and proline, and various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and lipoxygenase (LOX) in the shoot and root of both cultivars. However, the follow-up application of KN to the B stressed plants improved growth and chlorophyll content and further enhanced the mentioned antioxidant enzymes and level of H_2O_2, MDA and proline. This study thus suggests that KN improves B tolerance of the studied cultivars grown under B toxicity.

1. Introduction

B toxicity is a common mineral nutritional problem in arid and semiarid regions, causing significant decreases in growth and yields as reported for many countries (Nable et al., 1997). Also in Turkey, B toxicity creates ecotoxicologicals problems because there are larger boron deposits (Gezgin et al., 2002). B is unusual among plant mineral nutrients in that it exists as an uncharged molecule, boric acid, at physiological pH. It is known that boric acid can easily pass directly across phospholipid bilayers (Dordas et al., 2000) and it has recently been shown that fluxes of boric acid can be further accelerated by movement through aquaglyceroporins, channellike proteins that permit the bi-directional movement of small neutral molecules such as arsenite, antimonite, glycerol and urea (Bienert et al., 2008). In general terms, boron is relatively unreactive but it can form strong complexes with a handful of metabolites that have multiple hydroxyl groups in the cis conformation (Reid, 2010). 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. Toxic effects of high B are mainly attributed due to accumulation of B in plants, as yield of tomato was reduced when B concentrations were higher in plant matter (Tombuloglu et al., 2012; Kaya and Ashraf, 2015) Furthermore, during oxidative stress, excess production of reactive oxygen species (ROS) such as superoxide radical (O_2^-) and hydroxyl radical ($OH^\cdot$) which are strong oxidizers of lipids, proteins and nucleic acids, leads to membrane damage that finally leads to cell death.Under B toxicity, ROS accumulation was reported in apple rootstock, mung bean and sweet basil plants (Molassiotis et al., 2006; Yusuf et al., 2011and Landi et al., 2013). Plants have multifunctional enzymatic and non-enzymatic antioxidant defense system to fight the oxidative effects of ROS. Among these, superoxide dismutase (SOD) dismutates O_2^- to O_2 and H_2O_2, which are further oxidized to molecular oxygen and H_2O by peroxidases (POX), catalase (CAT) and ascorbate–glutathione pathway enzymes like ascorbate peroxidase (APX) and glutathione reductase (GR) (Bonilla et al., 1990).

Plant hormones are chemical messengers produced in one cell or tissue and have been reported to act as active members of signal transduction cascade involved in plant stress responses (Mori and Schroeder, 2004). Cytokinins are a group of phyto-hormones that stimulate water uptake, increase cell division, promote organ development and lead to the regeneration and proliferation of shoots. The basis for application of cytokinins to field plants is inferred from the improved growth of plants in cytokinin-containing solutions. Cytokinins may not always be active unless other hormones are present. However, cytokinins alone can often

* Corresponding author. Celal Bayar Universty, Faculty of Science and Letters, Department of Chemistry 45140, Muradiye, Manisa, Turkey.
E-mail address: aydemirt@yahoo.com (T. Aydemir).

http://dx.doi.org/10.1016/j.plaphy.2016.06.024
0981-9428/

evoke a variety of physiological, metabolic, biochemical, and developmental processes when applied to plants (McCarty et al., 1987). Boron stress, in many plants, boron stress reduces cytokinin export from the root to shoot (Ali and Abbas, 2003). An adequate cytokinin supply is essential for normal plant development and stimulation of a great number of physiological processes which delay leaf senescence, and this can explain why exogenous applications of cytokinin can overcome the effect of salt stress on the growth of many plants. It has been shown that application of plant hormones including cytokinins in agricultural fields can improve crop yields (Al-Hakimi, 2007). Kinetin (6-furfurylaminopurin) is a synthetic cytokinin and there have been studies on the use of kinetin to mitigate the adverse effects of salt stress on plant growth (Gadallah, 1999).

B toxicity in plants occurs around the areas containing high B concentration in soil. This problem is further exacerbated due to excessive B-containing water for soil irrigation, which may have adverse effects on crop productivity. Kutluk which is a tolerant genotype of wheat against boron stress and Bezostaya which is a sensitive genotype of wheat against boron stress are two commercial wheat cultivars, but it chronically faces B toxicity during cultivation. The aim of the current work was therefore to investigate antioxidant enzyme and growth parameter response of the above cultivars to KN application under B toxicity.

2. Materials and methods

2.1. Plant material and growth conditions

In this study two Turkish cultivars of wheat, Bezostaya and Kutluk, were used. The seeds were obtained from Eskisehir Anatolia Agricultural Research Institute. Seeds were first surface-sterilized with 30% sodium hypochlorite solution for 10 min. Then, they were washed and imbibed in distilled water for 1 day. After this process, approximately 15–20 seeds were planted onto plastic trays covered with filter paper and cotton containing half-strength Hoagland's solution (Hoagland and Arnon, 1950). They were grown for 10 days in a growth chamber at 23 ± 2 °C with 16-h light: 8-h dark photo-cycle at a light intensity of 40 mmol m^{-2} s^{-1}.

On 10 days stage, the seedlings were supplemented with different concentrations (5 and 10 mM) of B and different concentrations of B plus KN (5 mM B + 1 μM kN and 10 mM B + 1 μM kN) along with nutrient solution for 7 days. The shoot and root tissues of control (no B treatment), B treated and B plus KN treated plants were then freshly used in the experiments.

2.2. Boron concentration of the plant tissues

Tissue samples of wheat seedlings were washed, dried and prepared for analysis. B concentration of the tissues was determined on nitric-perchloric acid digests of plant tissues by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3. Growth measurements

At the initiation of the experiment and 7 days, 1 g plants for each group were taken at random and divided into separate shoot and root fractions. The fresh weights of shoots and roots were weighed. After measuring the fresh weights of seedlings the same tissues were let to dry in an oven at 70 °C for 24 h, and then the dry weights of the samples were recorded. The relative growth rate was calculated as the ratio of fresh weight difference between fresh weight at the end of 7 days of incubation and fresh weight at the beginning of the experiment over fresh weight at the beginning of the experiment $\left[\frac{Wt_7 - Wt_0}{Wt_0} \times 100\right]$. The experiment was repeated three times.

2.4. Determination of proline and H_2O_2 content

The amount of proline was determined according to a modified method of Bates (1973). Approximately 0.5 g of shoot and root tissues were cut into small pieces and homogenized by the addition of 1 ml of 5-sulphosalycylic acid solution in ice bath. The homogenates were centrifuged at 13,000 g for 10 min at 4 °C.

For each sample, an eppendorf tube containing 0.2 ml acid ninhydrine (0.31 g ninhydrine, 7.5 ml acetic acid, and 5 ml 6 M phosphoric acid), 0.2 ml 96% acetic acid and 0.1 ml 3% 5-sulphosalycylic acid were prepared. Supernatant (0.1 ml) from each homogenate was added to the tubes. Tubes were incubated at 96 °C for 1 h in a hot block and after incubation 1 ml of toluene were added to each tube. Then, tubes were mixed and centrifuged at 13000*g* for 10 min at 4 °C. The toluene phase was carefully pipetted out into a glass test tube and the absorbance was measured at 520 nm in a spectrophotometer. The concentration of proline was calculated from a proline standard curve. The concentration of proline was expressed as μmol/g FW.

The hydrogen peroxide content was determined according to Jana and Choudhuri (1981). Aliquots of fresh shoots and roots were homogenized in 50 mM potassium phosphate, pH 6.5 and centrifuged at 10,000*g* for 25 min. The solution was mixed with 1% titanium chloride (in concentrated HCl) and then centrifuged at 10,000*g* for 15 min The absorbance of the supernatant was measured at 410 nm and the H_2O_2 content calculated using 0.28 μM^{-1} cm^{-1} as extinction coefficient.

2.5. Determination of MDA content

MDA content was determined spectrophotometrically as described by Heath and Packer (1968). Samples were homogenized in 1% trichloroacetic acid and then centrifuged at 10,000*g* for 15 min. Supernatant was heated with 0.05 thiobarbituric acid for 30 min at 95 °C. The heated supernatant was re-centrifuged at 5000*g* for 5 min and the absorbance was measured at 532 and 600 nm on UV–VIS Spectrometer (UV–Vis 530, Jasco, Japan). The non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance.

Table 1
Effect of 5 and 10 mM B on B level in shoot and root of wheat cvs. Kutluk and Bezostaya.

Kutluk		Bezostaya	
Shoot	B (mg/gDW)	Shoot	B (mg/gDW)
Control	0.10 ± 0.02^{a}	Control	0.12 ± 0.02^{a}
5B	0.27 ± 0.03^{b}	5B	0.38 ± 0.05^{b}
10B	0.56 ± 0.07^{c}	10B	0.69 ± 0.09^{c}
5B + K	0.20 ± 0.02^{ab}	5B + K	0.29 ± 0.04^{ab}
10B + K	0.44 ± 0.03^{bc}	10B + K	0.55 ± 0.06^{bc}
Root			
Control	0.05 ± 0.006^{a}	Control	0.06 ± 0.01^{a}
5B	0.09 ± 0.008^{b}	5B	0.19 ± 0.02^{b}
10B	0.18 ± 0.03^{c}	10B	0.32 ± 0.04^{c}
5B + K	0.07 ± 0.004^{b}	5B + K	0.12 ± 0.02^{ab}
10B + K	0.12 ± 0.02^{bc}	10B + K	0.29 ± 0.03^{c}

Values are means ± S.E. Values with different superscripts within same column show significant differences at $P < 0.05$ level between treatments.