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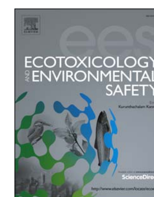

Alleviating effects of calcium on cobalt toxicity in two barley genotypes differing in cobalt tolerance

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ABSTRACT

Cobalt (Co) contamination in soils is becoming a severe issue in environment safety and crop production. Calcium (Ca)' as a macro-nutrient element, shows the antagonism with many divalent heavy metals and the capacity of alleviating oxidative stress in plants. In this study, the protective role of Ca in alleviating Co stress was hydroponically investigated using two barley genotypes differing in Co toxicity tolerance. Barley seedlings exposed to 100 μM Co showed the significant reduction in growth and photosynthetic rate, and the dramatic increase in the contents of reactive oxygen species (ROS), malondialdehyde (MDA), reduced glutathione (GSH) and oxidized glutathione (GSSG), and the activities of anti-oxidative enzymes, with Ea52 (Co-sensitive) being much more affected than Yan66 (Co-tolerant). Addition of Ca in growth medium alleviated Co toxicity by reducing Co uptake and enhancing the antioxidant capacity. The effect of Ca in alleviating Co toxicity was much greater in Yan66 than in Ea52. The results indicate that the alleviation of Co toxicity in barley plants by Ca is attributed to the reduced Co uptake and enhanced antioxidant capacity.

1. Introduction

Cobalt (Co) is biologically a metal of great importance as it is a component of biological enzymes in the most living organisms including higher plants (Chatterjee and Chatterjee, 2003). Once in larger amount however, Co causes irreversible damages to plant cells, resulting in the reduction of plant growth and decline in crop productivity and quality (Karuppanapandian and Kim, 2013). Co naturally occurs in most soils at the concentration of 8–25 mg/kg soil across the world, with 20 mg/kg soil Co being regarded as the permissible maximum in agricultural soils. In higher plants, Co concentration causing toxicity differs widely among species or genotypes within a species (Vanselow, 1965). Thus blackgum plants may normally grow in the soil containing over thousand mg/kg Co without any visual toxicity symptom, while citrus presents toxic symptoms in the soil only containing 11 mg/kg Co (Vanselow, 1965). The major sources of Co contamination in soil include ore smelting facilities, phosphate rocks, airport traffic and other industrial pollutions (Smith and Carson, 1981).

Commonly, reactive oxygen species (ROS) in plants could be excessively accumulated when exposed to abiotic or biotic stresses, causing damages to biomolecules as expressed by lipid peroxidation, membrane degradation, inactivation of several enzymes, decrease in photosynthesis, and DNA degradation (Mittler, 2002). Co as a transition metal may undergo Fenton reaction, resulting in excessive ROS generation (Tewari et al., 2002). Evidences on the participation of Co in ROS generation have been broadly reported in many plant species (Chatterjee and Chatterjee, 2000, 2003; Pandey and Sharma, 2002; Gopal et al., 2003; Li et al., 2009; Pandey et al., 2009). On the other hand, plants have developed sophisticated defense mechanisms to fight against oxidative stress, including development of non-enzymatic and enzymatic systems (Gopal et al., 2003; Karuppanapandian and Manoharan, 2008). The enzymatic ROS scavengers consisted of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR), and metabolites such as glutathione constitutes the non-enzymatic protectant. (Mansour, 2000; Mittler, 2002).

Attempts have been successfully made to alleviate the harmful effects of heavy metals including cadmium (Cd), nickel (Ni) and Lead (Pb) through the application of calcium (Farzadfar et al., 2013; Oukarroum et al., 2013; Ahmad et al., 2015; Aziz et al., 2015). Being an important macronutrient, Ca is known to play a vital role in signal transduction for regulating the plant cell metabolism (Hall, 2002; Ismai,

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2008). During oxidative stress, Ca is involved in the regulation of target proteins directly or indirectly via calcium-binding proteins, such as calmodulin (CaM), which, after binding to Ca, activates a number of protein kinases and other proteins in plant cells, including non-enzymatic and enzymatic antioxidants (Wang et al., 2007; Wang and Song, 2009). However, the mechanism behind the role of Ca in the regulation of antioxidants is yet to be elucidated. While some researchers support the contention that Ca up-regulates genes encoding antioxidant enzymes under oxidative stress (Gong et al., 1997; Jiang and Huang, 2001), others suggest that it may, in addition, participate in signal transduction (Bramm, 1992; Trofimova et al., 1999). Moreover, Ca not only controls the selective permeability and the movement of divalent cations across the cell membrane, but also competes with other transition metals for transporter sites and thus, hampers the metal accumulation in plant roots (Farzadfar et al., 2013). In addition, behaving as a messenger, Ca stabilizes cell membrane and prevents solute leakage by reducing lipid peroxidation of cell membranes and thereby, strengthens the plant tolerance (Antosiewicz and Hennig, 2004; Hirschi, 2004). It was reported that Cd accumulation in rice seedlings was reduced by the exogenous application of Ca in growth medium (Ahmad et al., 2015). Srivastava et al. (2015) found that the alleviation of growth inhibition by Ca application was paralleled with reduced Cd accumulation in rice seedlings. Although it was suggested a possible competition between Ca and Co for the transporter sites in yeast cells (Simonsen et al., 2012), the role of Ca in alleviating Co toxicity in higher plants is not yet investigated up to date.

Barley, ranking the fourth worldwide in terms of planting area and production, is one of the earliest cultivated crops. It is well known for its wider adaptation and higher tolerance to abiotic stress in comparison with other cereals (Harlan, 1976). However, barley is relatively sensitive to acidic soil (aluminum toxicity) and heavy metals such as Co. In our previous works, we examined the harmful effects of Co on growth and physiological characters (Lwalaba et al., 2017a) as well as the distribution of Co at the subcellular level (Lwalaba et al., 2017b) using three barley genotypes differing in Co tolerance. In the present study, we want to determine if the application of Ca in growth medium can alleviate Co-induced oxidative damages and possible mechanism of the alleviation.

2. Materials and methods

2.1. Plant material and hydroponic culture

Two barley genotypes differing in Co tolerance, namely Ya66 (tolerant) and Ea52 (sensitive) were used in this experiment, which was conducted in a greenhouse at Zijingang Campus, Zhejiang University, Hangzhou China. Seeds were surface sterilized with 1% H_2O_2 for 10 min, thoroughly rinsed with distilled water and germinated in petri dishes. The germination chamber was maintained at 22 °C/18 °C (light/dark temperatures, respectively), with a photoperiod of 16 h light/8 h dark, light intensity of 225 ± 25 µmol $m^{-2} s^{-1}$ and 85% of relative humidity. Seedlings were then transferred into 5-L plastic containers, which were filled with basal nutrient control (BNS) composed of (mg l^{-1}): $(NH_4)_2SO_4$ 48.2, $MgSO_4{\cdot}7H_2O$ 154.8, K_2SO_4 15.9, KNO_3 18.5, KH_2PO_4 24.8, $Ca(NO_3)_2{\cdot}4H_2O$ 86.17, $FeC_6H_6O_7$ 7, $MnCl_2{\cdot}4H_2O$ 0.9, $ZnSO_4{\cdot}7H_2O$ 0.11, $CuSO_4{\cdot}5H_2O$ 0.04, H_3BO_3 2.9 and H_2MoO_4 0.01. Each container was covered with a polystyrene plate with 6 evenly spaced holes (2 plants per hole) and placed in a greenhouse. The solution pH was daily adjusted to 5.8 ± 0.1 with HCl or NaOH, as required. The experiment was laid out as a completely randomized block with four replicates for each treatment.

2.2. Treatments and sampling

At the 10 d after seedlings were transferred into nutrient solution, three treatments were applied: (T0), BNS which was used as control; (T1), 100 µM $CoCl_2{\cdot}6H_2O$ alone and (T2), a combination 100 µM $CoCl_2{\cdot}6H_2O$/2 mM $CaCl_2$ in growth medium. The solution was continuously aerated with air pumps and renewed daily. After 15 days of the treatments, seedlings were harvested and separated into roots and shoots, and their growth parameters (shoot and root height) were immediately measured. The plant tissues were dried in an oven at 105 °C for 3 h, followed by 80 °C for 48 h, and weighed. The roots were soaked in 20 mM Na_2-ethylene diamine tetra-acetic acid (EDTA) for 3 h to remove any adhering cations, and then washed with distilled water, and blotted on filter papers. Fresh samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.3. Cobalt concentration and translocation factor

Approximately 100 mg of grounded tissue samples were used to determine Co concentration. The samples were ashed at 500 °C for 24 h, and digested in HNO_3-$HClO_4$ (3:1, v/v) for 48 h at room temperature. After digestion, the solution was diluted to 15 ml with distilled water. The Co concentration was determined using an inductively coupled plasma-optical spectrometer (ICP-OES; Optima 8000DV; PekinElmer). The translocation factor was determined according to Aziz et al. (2015) and the used formula is presented as follows:

Translocation factor = [(ShootCo/Root Co) *100]
Shoot Co: accumulation of Co in shoot
Root Co: accumulation of Co in root

2.4. Pigments and photosynthetic parameters

The concentration of leaf chlorophylls (a and b) and carotenoids was determined as described by Lichtenthaler (1987) with minor modification. Fresh leaf tissues were cut into small pieces (1 cm^2) and then placed into 80% acetone in the ratio of 1:15 w/v for 24 h until leaf tissues were completely discolored. The extract was centrifuged at 4000 × *g* for 15 min to remove all possible residues. The color intensity of clear supernatant was measured at 663.2, 646.8 and 470 nm for chlorophyll a, chlorophyll b and carotenoids, respectively, and 80% acetone was used as blank. Prior to plant harvesting, the photosynthetic parameters including net photosynthetic rate (Pn), and transpiration were measured on the second fully expanded leaves using a LiCor-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). The measurements were performed at a sunny day with air temperature of 25.8–28.8 °C, relative humidity of 50–70%, CO_2 concentration of 400 mmol mol^{-1} and photosynthetic photon flux density (PPFD) of 1000 mmol $m^{-2} s^{-1}$. The total five readings per treatment were taken from randomly selected plants.

2.5. Lipid peroxidation and reactive oxygen species

Lipid peroxidation (LP) was analyzed in terms of malondialdehyde (MDA) according to Zhou and Leul (1999). To determine hydrogen peroxide (H_2O_2) content, leaf and root samples (100 mg) were extracted with 1.5 ml of trichloroacetic acid (0.1%TCA) in a cool bath, and the homogenate was centrifuged at 12,000*g* for 15 min under 4 °C (Velikova et al., 2000). The 100 µl supernatant was mixed with 100 µl of 10 mM potassium phosphate buffer (pH 7.0) and 200 µl of 1 M KI. The absorbance was read at 390 nm and H_2O_2 content was calculated by using a standard curve. Superoxide radical (O^-_2) was determined according to Jiang and Zhang (2001) with minor modification. The leaf and root samples (100 mg) were homogenized in 1.5 ml of 65 mM potassium phosphate buffer (pH 7.8) and then homogenate was centrifuged at 5000*g* for 10 min at 4 °C. Then the supernatant (200 µl) was mixed with 180 µl of 65 mM potassium phosphate buffer (pH 7.8) and 200 ml of 10 mM hydroxylamine hydrochloride, and incubated at 25 °C for 20 min. After incubation 200 µl of 17 mM sulphanilamide and 200 µl of 7 mM a-naphthylamine were added into

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