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Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines

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ABSTRACT

Cadmium (Cd) stress causes several negative physiological, biochemical and structural changes due to the oxidative stress caused through the generation of ROS, leading to a reduction in plant growth. To look for an effective method to increase Cd tolerance of wheat seedlings, the effect of presoaking *Triticum aestivum* L. seeds in spermidine (Spd; 2 mM) or spermine (Spm; 2 mM) on seedling growth, physiological attributes and antioxidant defence system under 1 mM Cd stress were investigated. Spm or Spd alleviated the adverse effects of Cd stress to convergent degrees. Presoaking wheat seeds in either polyamine increased the seedling growth and the activities of antioxidant enzymes compared to the control, but other attributes were slightly affected. Under Cd stress, presoaking seeds in either polyamine significantly increased seedling growth, membrane stability index, relative water content, concentrations of protein, starch, ascorbic acid, total glutathione, Spm and Spd, and the activities of superoxide dismutase and catalase. In contrast, electrolyte leakage, concentrations of proline, total soluble sugars, malondialdehyde, hydrogen peroxide and Cd^{2+}, and the activities of peroxidase and ascorbate peroxidase were reduced compared to the control. These results are important as the potential of Spd or Spm to alleviate the harmful effects of Cd stress offer an opportunity to increase the resistance of wheat seedlings to growth under Cd stress conditions.

1. Introduction

Cereals are one of the most important crops and play a special role in human nutrition. Among cereal crops, wheat is most widely grown crop for human feed. Abiotic stresses, including metal toxicity decrease wheat growth and productivity (Shaikh et al., 2013).

Nowadays, environmental pollution by heavy metals has increased because of manifold industrial activities, solid waste management and agricultural improvements. The increased dependence of agriculture on chemical fertilizers, sewage wastewater irrigation and rapid industrialization has added toxic metals to agricultural soils, causing harmful effects on soil–plant environment system. Cadmium (Cd) considers a major environmental concern to the agricultural system as its residence time in soil is over thousands years (Kumar, 2013). Cd has been placed at seventh rank among the top toxins. Cd concentrations of uncontaminated soils are usually below 0.5 mg kg^{-1}, but can reach up to 3.0 mg kg^{-1} depending on the soil parent materials (Nazar et al., 2012). Some phosphatic fertilizers and phosphorites contain high concentrations of Cd (4.77 mg kg^{-1}) and are considered as the potential cause of increasing Cd contamination in rice (Khurana and Jhanji, 2014). The accumulation of Cd in plants may cause several physiological, biochemical and structural changes (Feng et al., 2010; Howladar, 2014; Yan et al., 2015). The toxicity of Cd to plant cells is related to the oxidative stress caused through the generation of reactive oxygen species (Yadav, 2010), the stimulation or inhibition of the antioxidant enzyme activities (Zheng et al., 2010), the production of oxidative damage, and the induction of lipid peroxidation (Howladar, 2014) and protein oxidation (Pena et al., 2006). On the other hand, carbohydrate metabolism (Gill and Tuteja, 2010), amino acid and proline contents (Sharma and Dietz, 2006), and polyamine levels (Groppa and Benavides, 2008) are altered.

Polyamines (PAs), widely present in living organisms, are now regarded as a new class of growth substances, including spermidine (Spd, a triamine) and spermine (Spm, a tetramine), which play a pivotal role in the regulation of plant developmental and physiological processes (Kusano et al., 2007). Exogenous application of PAs is effective approach for enhancing stress tolerance of crops for enhanced crop productivity. Exogenous application of

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PAs has been successfully used to enhance abiotic stress tolerance in plants (Gill and Tuteja, 2010; Aldesuquy et al., 2014).

In view of this, it was hypothesized that exogenous application of PAs (e.g., Spm and Spd) enhances plant growth and productivity when grown under Cd stress. Thus, the main objective of the present study was to assess up to what extent seed presoaking in Spm and Spd could improve growth, physiological attributes, and enzymatic and non-enzymatic antioxidant activities in wheat seedlings grown under Cd stress.

2. Materials and methods

2.1. Plant material, growing conditions and treatments

Wheat (*Triticum aestivum* L.) seeds (cv., Giza-168) were obtained from the Field Crops Research Institute (FCRI), Agricultural Research Center, Giza, Egypt. Seeds were surface sterilized using 0.1% $HgCl_2$ for 1 min, and then thoroughly washed several times with sterile deionized water. Sterilized seeds were presoaked for 6 h at room temperature, either in distilled water (as a control), 2.0 mM spermine (Spm), or 2.0 mM spermidine (Spd). Each category of these three categories was then divided to two divisions of which one division was treated with Cd. These polyamine concentrations were selected based on the best response in our preliminary studies (data not shown). After air-drying overnight, 20 seeds were sown, on 10 November 2012 and 18 November 2013, in each plastic pot (40 cm in diameter, 50 cm depth), previously filled by sand (15 kg for each) washed with acid, and then with deionized water several times. Pots (n=120) were arranged for growing plants in an open greenhouse. The average day and night temperatures were 19 ± 3 °C and 10 ± 2 °C, respectively. The relative humidity ranged from 62.0% to 65.1%, and day-length ranged from 10 to 11 h. Half-strength Hoagland's No. 2 nutrient solution (containing macronutrients: KNO_3, Ca $(NO_3)_2 \times 4H_2O$, $MgSO_4 \times 7H_2O$, $NaH_2PO_4 \times 2H_2O$ and micronutrients: $FeCl_3$, H_3BO_3, $MnSO_4 \times 4H_2O$, $ZnSO_4 \times 7H_2O$, $CuSO_4 \times 5H_2O$, NaCl, $(NH_4)_6Mo_7O_{24} \times 2H_2O$; Hoagland and Arnon, 1950) free from $CdCl_2$ was supplied every 2 days to all pots up to complete emergence (15 days). Excess solution was drained through holes in the base of the pots. After seedlings were thinned to 10 in each pot (15 days), 1 mM Cd, using $CdCl_2$, was added to the half-strength Hoagland's nutrient solution. Each pot was supplemented every 3 days with 1 L of Cd^{2+}-containing Hoagland's nutrient solution. The dose of 1 mM Cd, which greatly affected wheat seedling growth, was also selected based on our preliminary studies (data not shown). The Cd concentration in the medium was maintained at 1 mM by using an inductively coupled plasma atomic emission spectrometry (ICP-AES, IRIS-Advan type, Thermo, USA). Initial soil pH was 5.5, but it was corrected to 6.8 by adding 3 g of $CaCO_3$ per pot. The pH of the nutrient solution was adjusted to 7, with dilute HCl or NaOH. The experimental layout was completely randomized design with 20 replicates/pots (each pot represents one replicate) for each of the six treatments (6 treatments × 20 replicates/pots × 10 seedlings in each pot). The experiment was terminated after 60 days from sowing after exposing the seedlings to Cd stress for 45 days. The 60-day-old seedlings from each treatment were collected for various measurements.

2.2. Plant growth analysis and cadmium (Cd^{2+}) determination

The sixty-day-old wheat seedlings were removed from the pots and moved smoothly to remove the adhering sand particles by dipped them in a bucket filled with water. The length of shoots was measured by using a meter scale. Leaves area was recorded by using a digital leaf meter (LI-3000 Portable Area meter Produced by LI-COR Lincoln, NE, USA). Seedlings were weighed to record their fresh weight, then placed in an oven at 70 °C to reach a constant dry weight (DW). The powdery dried shoots were used to determine the concentartion of Cd^{2+} by using a Perkin-Elmer, Model 3300, atomic absorption spectrophotometer (Chapman and Pratt, 1961).

2.3. Determination of membrane stability index, electrolyte leakage, and relative water content

Membrane stability index (MSI) was estimated as described by Premchandra et al. (1990) and modified by Rady (2011). Duplicate 0.2 g samples of leaf tissue were placed in test tubes containing 10 ml of double-distilled water. One sample was heated at 40 °C in a water bath for 30 min, and the electrical conductivity of the solution was recorded using a conductivity bridge (C_1). The second sample was boiled at 100 °C for 10 min, and the conductivity was measured (C_2). The MSI was calculated using the formula:

$$\text{MSI } (\%) = \left[1 - (C_1/C_2)\right] \times 100$$

The total leakage of inorganic ions from leaves was determined using the method of Sullivan and Ross (1979). Twenty leaf discs were placed in a boiling tube containing 10 ml deionized water and the electrical conductivity (EC_1) was recorded. The contents were then heated to 45 – 55 °C for 30 min each in a water bath and the electrical conductivity (EC_2) was recorded. The sample was boiled at 100 °C for 10 min and the electrical conductivity (EC_3) was recorded. Electrolyte leakage was calculated using the formula:

$$\text{Electrolyte leakage } (\%) = \left[(EC_2 - EC_1)/EC_3\right] \times 100$$

Excluding the midrib, fresh 2 cm-diameter fully-expanded leaf discs were used to determine the relative water content (RWC) as described by Weatherly (1950). The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70 °C for 48 h. The RWC was then calculated using the formula:

$$\text{RWC } (\%) = [(FM - DM)/(TM - DM)] \times 100$$

2.4. Determination of free proline, total soluble sugars and starch concentrations

Proline concentration in fresh leaves was measured by the rapid colorimetric method of Bates et al. (1973). Each sample of 0.2 g fresh leaf tissue was extracted by grinding in 10 ml of 3% (v/v) sulphosalicylic acid. The mixture was then centrifuged at 10,000 × g for 10 min. Two ml of the supernatant was added to a test-tube and 2 ml of freshly prepared acid–ninhydrin solution was then added. Each tube was incubated in a water bath at 90 °C for 30 min. The reaction was terminated in an ice-bath. Each reaction mixture was extracted with 5 ml of toluene and vortex mixed for 15 s. The tubes were allowed to stand for ≥20 min in dark at room temperature to allow separation of the toluene and aqueous phases. Each toluene phase was then collected carefully into a test tube and the absorbance of the toluene fraction was read at 520 nm. The proline concentration in each sample was determined using a standard curve of analytical-grade proline.

Total soluble sugars were extracted and determined according to Irigoyen et al. (1992). A 0.2 g sample of fresh leaves was homogenized in 10 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol. The extract was centrifuged at 3500*g* for 10 min