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Polyamine and nitric oxide crosstalk: Antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems

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ABSTRACT

Cadmium (Cd) contamination is a serious agricultural and environmental hazard. The study investigates cross-protection roles of putrescine (Put, 0.2 mM) and nitric oxide (sodium nitroprusside; SNP, 1 mM) in conferring Cd ($CdCl_2$, 1.5 mM) tolerance in mung bean (*Vigna radiata* L. cv. BARI Mung-2) seedlings. Cadmium stress increased root and shoot Cd content, reduced growth, destroyed chlorophyll (chl), modulated proline (Pro) and reduced leaf relative water content (RWC), increased oxidative damage [lipid peroxidation, H_2O_2 content, $O_2^{\bullet-}$ generation rate, lipoxygenase (LOX) activity], methylglyoxal (MG) toxicity. Put and/or SNP reduced Cd uptake, increasd phytochelatin (PC) content, reduced oxidative damage enhancing non-enzymatic antioxidants (AsA and GSH) and activities of enzymes [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), and glutathione peroxidase (GPX)]. Exogenous Put and/or SNP modulated endogenous polyamines, PAs (putrescine, Put; spermidine, Spd; spermine, Spm), and NO; improved glyoxalase system in detoxifying MG and improved physiology and growth where combined application showed better effects which designates possible crosstalk between NO and PAs to confer Cd-toxicity tolerance.

1. Introduction

Rapid industrialization increases environmental pollution through overproducing considerable amounts of toxic metals, and cadmium (Cd) pollution is the most common. Due to high mobility and hydrophilic nature Cd is readily taken up and accumulated in a plant which is primary reason for Cd entering the trophic chain and a serious threat to living organism (Hasanuzzaman and Fujita, 2012). Some of the most common symptoms of Cd-induced toxicity in plants are stunted growth, chlorosis and leaf epinasty, repression of pollen germination and tube growth in phenotypic level. At physiological level altered chloroplast ultrastructure, photosynthesis inhibition, inactivation of enzymes in CO_2 fixation, induced lipid peroxidation, disturbance of the nitrogen (N) and sulfur (S) metabolism are common consequences of Cd toxicity (Benavides et al., 2005; Gill and Tuteja, 2011). Cd results in phytotoxicity by inducing complex changes at genetic, biochemical,

Abbreviations: AO, ascorbate oxidase; APX, ascorbate peroxidase; AsA, ascorbic acid (ascorbate); BSA, bovine serum albumin; CAT, catalase; CDNB, 1- chloro-2, 4-dinitrobenzene; chl, chlorophyll; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DTNB, 5,5′-dithio-bis (2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; Gly I, glyoxalase I; Gly II, glyoxalase II; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; LOX, Lipoxygenase; MDA, malondialdehyde; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; MG, methylglyoxal; NADPH, nicotinamide adenine dinucleotide phosphate; NTB, 2-nitro-5-thiobenzoic acid; PEG, polyethylene glycol; Pro, Proline; ROS, reactive oxygen species; RWC, relative water content; SLG, S-D-lactoylglutathione; TBA, thiobarbituric acid; TCA, trichloroacetic acid

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and physiological levels; it has affinity to phosphates, cysteinyl and histidyl side chains of proteins, purines, pteridines, and porphyrins that damage enzymes and nucleic acids, and disrupt oxidative phosphorylation. Cadmium is associated with oxidative stress generating reactive oxygen species (ROS: singlet oxygen, $^1O_2$; superoxide, $O_2^{\bullet-}$; hydrogen peroxide, H_2O_2; hydroxyl radical, OH•) which can damage biomolecules including proteins, lipids, and DNA (Hasanuzzaman and Fujita, 2012). Methylglyoxal (MG) is cytotoxic compound overproduced 2- to 6-fold (compared to non-stress condition) under abiotic stresses, including metal toxicity (Yadav et al., 2005a, 2008a; Suhartono et al., 2014). MG is a reactive oxidative compound which damages cellular ultrastructural components, and can cause DNA damage and mutation. Plants have an efficient glyoxalase system that detoxifies MG by the activity of two vital enzymes, glyoxalase I (Gly I) and glyoxalase II (Gly II) where GSH acts as co-factor (Yadav et al., 2008; Nahar et al., 2015).

Plants evolved protective mechanisms against Cd toxicity; metal chelation, binding, exclusion, active excretion, compartmentalization are common among those (Gratão et al., 2005). Antioxidant system cope with Cd-induced oxidative stress that include non-enzymatic antioxidants (ascorbic acid, AsA; glutathione, GSH; phenolic compounds, alkaloids, non-protein amino acids, α-tocopherol) and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; and glutathione *S*-transferase, GST) (Gill and Tuteja, 2010). Glyoxalase system consisting of glyoxalase I (Gly I) and glyoxalase II (Gly II) detoxifies MG with the help of GSH (Yadav et al., 2008).

Nitric oxide (NO) is highly diffusible gaseous free radical acting as cellular messenger (Neill et al., 2003). Di-amine putrescine (Put), tri-amine spermidine (Spd), tetra-amine spermine (Spm) are common polyamines (PAs) ubiquitously found in living organisms. PAs and NO have signaling function, they interact with hormones, perform biological functions including growth, development, and stress responses (Neill et al., 2003; Takahashi and Kakehi, 2010). PAs or NO induce metal stress tolerances acting as ROS scavengers, activating antioxidants, protecting biomembranes and biomolecules, inducing metal chelation (Hao and Zhang, 2010; Chen et al., 2013). Biosynthesis pathways of PAs and NO are over lapping; PAs induce NO production or directly converted to NO. There are few studies regarding interaction of PAs and NO. Many potential links between PAs and NO need to be verified (Yamasaki and Cohen, 2006). To the best of our knowledge, coordinated effect of Put and/or NO on antioxidant and glyoxalase systems in alleviating Cd toxicity on *Vigna radiata* L. has not been reported. We hypothesize that Put and/or NO could improve physiological processes to enhance Cd-toxicity tolerance.

2. Materials and methods

2.1. Plant materials and stress treatments

Six-day-old mung bean (*Vigna radiata* cv. BARI Mung-2) seedlings (grown in light, 350 µmol photon m^{-2} s^{-1}; temperature, 25 ± 2 °C; RH, 65–70%; hyponex was used as nutrient) were applied with different treatments for 48 h. One set was exposed to Cd ($CdCl_2$, 1.5 mM). Three sets of 5-day-old seedlings were grown with Put (0.2 mM), NO (applied as sodium nitroprusside, NO donor; SNP, 1 mM), and combination of Put with NO as pre-treatment (24 h). Pre-treated seedlings were exposed to Cd on the sixth day. Another three sets of seedlings were grown with Put and/or NO without stress. Control plants were grown with nutrient (hyponex) solution.

2.2. Determination of Cd content, biological concentration factor (BCF), translocation factor (TF), and biological accumulation coefficient (BAC)

Root and shoot Cd content were determined by acid digestion (HNO_3:$HClO_4$ at 5:1, v/v) of dried tissue using atomic absorption spectrophotometer (Z-5000; Hitachi, Japan).

BCF indicates Cd concentration ratio of roots to growing media (Malik et al., 2010).

$$BCF = \frac{[Metal]root}{[Metal]growing\ media}$$

TF indicates ratio of Cd in plant shoots to roots (Malik et al., 2010).

$$TF = \frac{[Metal]shoot}{[Metal]root}$$

BAC indicates ratio of Cd in shoots to that in growing media (Malik et al., 2010).

$$BAC = \frac{[Metal]shoot}{[Metal]growing\ media}$$

2.3. Histochemical detection of H_2O_2 and $O_2^{\bullet-}$

H_2O_2 (as brown spots) and $O_2^{\bullet-}$ (as dark blue spots) were localized histochemically (Chen et al., 2010) by staining leaves with 1% 3,3-diaminobenzidine (DAB) and 0.1% nitroblue tetrazolium (NBT) solution, respectively.

2.4. Lipid peroxidation

Lipid peroxidation was measured by estimating malondialdehyde (MDA, a product of lipid peroxidation) using thiobarbituric acid (TBA) (Heath and Packer, 1968).

2.5. Hydrogen peroxide content

Leaves were extracted in potassium–phosphate (K–P) buffer (pH 6.5) (centrifuging at 11,500*g*), then adding it to a mixture of $TiCl_4$ in 20% H_2SO_4 (v/v) and resulting solution was measured spectrophotometrically at 410 nm (Yu et al., 2003).

2.6. Measurement of $O_2^{\bullet-}$ generation rate

Leaves were homogenized in 65 mM K–P buffer (pH 7.8), centrifuged at 5000*g*. Supernatant was mixed with extraction buffer and 10 mM hydroxylamine hydrochloride. After 20 min, 17 mM sulfanilamide and 7 mM naphthylamine were added and measured at 530 nm (Yang et al., 2011).

2.7. Determination of growth parameters

Plant height, root length (cm), and leaf area (A, cm^2) were measured. Dry weight (DW, g) was taken drying seedlings (at 80 °C, 48 h).

2.8. Leaf relative water content (RWC) and succulence

Fresh weight (FW), turgid weight (TW), DW of leaves were measured, and RWC was calculated as: $RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$ (Barrs and Weatherley, 1962). Leaf succulence