Plant Physiology and Biochemistry 83 (2014) 180–184

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Short communication

Evidences for reduced metal-uptake and membrane injury upon application of nitric oxide donor in cadmium stressed rice seedlings

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ARTICLE INFO

Article history:
Received 13 May 2014
Accepted 21 July 2014
Available online 9 August 2014

Keywords:
Cadmium
Electrolyte leakage
Lipid peroxides
Rice
Sodium nitroprusside

ABSTRACT

Heavy metal Cadmium (Cd) contaminates the environment through various anthropogenic sources. Cadmium-induced productions of free radicals lead to oxidative stress and H_2O_2 formation in plants. Endogenous Nitric oxide (NO) acts as signal molecules in plant stress response and play a significant role in key regulatory pathways of plant development. This study investigates the effect of 50 μM exogenous sodium nitroprusside (SNP, NO donor), on roots and shoots of rice *cv.* HUR 3022 grown under 50 μM Cd-stress at 7 days of growth. Plants treated with Cd alone showed stunted growth, decreased length and weight, lower cell viability and less chlorophyll. An elevated lipid peroxidation complemented with more electrolyte leakage was noted. Levels of hydrogen peroxide and superoxide anion increased in Cd-exposed plants with corresponding increase in activity of antioxidant enzymes catalase and superoxide dismutase. Lower chlorophyll levels paralleled with more uptake of cadmium in Cd-treatments as compared to controls. Application of equimolar amount of SNP to cadmium-stressed rice in the growth medium inhibited Cd-uptake and reversed the Cd-induced toxic effects by restoring membrane integrity. The levels of H_2O_2 and $O_2^{\bullet-}$ were considerably recovered due to SNP treatment. The results indicate that exogenous NO diminishes the deleterious effects of Cd in rice plants.

1. Introduction

In recent times, nitric oxide (NO) has gained significance as a signaling molecule of archaic origin and ubiquitous importance (Durner and Klessig, 1999). NO mediated signaling has implications in many physiological responses such as pathogen response, programmed cell death, growth, germination, root organogenesis, phytoalexin production, internal iron availability and abscisic acid dependent stomatal closure (Lamattina et al., 2003; Groβ et al., 2013). One of the major challenges in management of crops in agriculture is the heavy metal contamination of soil (Saxena and Shekhawat, 2013). Many reports have suggested that application of exogenous NO as sodium nitroprusside (SNP), an NO donor, has enhanced tolerance of plants towards heavy metals (Chen et al., 2010; Xu et al., 2010; Panda et al., 2011; Wang et al., 2013; Xu et al., 2014). Uptake of heavy metal cadmium (Cd) by plants also inhibit the physiological processes including respiration, photosynthesis, cell elongation, plant–water relationship, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (Sanita' di Toppi and Gabbrielli, 1999). Cd toxicity in rice plants is reported to cause oxidative stress (Shah et al., 2001), inhibits seedling vigor, allows stunted growth (Shah and Dubey, 1995), decreases the activities of many key enzymes (Shah and Nahakpam, 2012) and induces the synthesis of certain novel proteins (Shah and Dubey, 1998) or leads to cell disruption and changes in membrane structure (Shah et al.,). Heavy metal induced toxicity is usually reported to be associated with metal uptake, its sequestration as well as accumulation inside the plants which causes imbalance in antioxidant levels and antioxidant enzyme activities in plants thereby essentially requiring their controlled management under metal stress (Hsu and Kao, 2004). NO acts either as a direct scavenger of ROS or by over expression of the antioxidant defense system. In the former case NO may take over functions of the antioxidant system and thereby prevent its activation whereas

Abbreviations: Cd, cadmium; ROS, reactive oxygen species; NO, nitric oxide; SNP, sodium nitroprusside; cPTIO, (2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide; SOD, superoxide dismutase; CAT, catalase.

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http://dx.doi.org/10.1016/j.plaphy.2014.07.018
0981-9428/

in the latter case NO might trigger antioxidant gene expression or activate antioxidant enzymes through post translational modifications (Groβ et al., 2013).

The present work aims to abridge our knowledge regarding effect of exogenously applied SNP (NO donor) on Cd-induced toxicity in rice seedlings with emphasis on uptake and membrane injury resulting from altered antioxidant defense system in rice seedlings grown with either Cd/SNP alone or in a combination.

2. Methods

2.1. Plant material and growth conditions

Rice seeds from *cv.* HUR 3022 were surface sterilized and imbibed in water for 24 h. Seeds were cultured in petriplates for 3 days to obtain seedling stage. Seedlings were then raised in sand culture for 7 days in plastic pots saturated with either Hoagland nutrient solution (Hoagland and Arnon, 1938) which served as controls or nutrient solution supplemented with either 50 μM $Cd(NO_3)_2$ or 50 μM SNP (NO donor) or in combination (50 μM $Cd(NO_3)_2$ + 50 μM SNP). Seedlings were maintained in the growth chamber at 28 ± 1 °C, 80% relative humidity and 12-h light/dark cycle (irradiance 40–50 μmol m^{-2} s^{-1}) as described earlier (Shah et al., 2001). Pots were maintained at field saturation capacity and irrigation was done when required. Seedlings were uprooted from 7 day plants, roots and shoots were separated and experiments were performed in triplicate.

To ensure that the results obtained were due to exogenous SNP only all the experiments were performed with cPTIO, an NO scavenger. Experiments were setup in combination 50 μM Cd + 50 μM SNP + 50 μM cPTIO and results obtained were similar to those for 50 μM Cd treatments.

2.2. Extraction and quantification of cadmium and nitric oxide content

Fresh root and shoot samples were washed with 1 mM EDTA at 24 h interval to remove excess surface bound Cd and then oven dried at 70 °C for 5 days. Dried samples were weighed and acid digested. Cd was then estimated using Atomic Absorption Spectrophotometer and expressed in terms of mg g^{-1} dry wt. sample (Shah and Dubey, 1997). The amount of nitric oxide was measured as given by Zhou et al. (Zhou et al., 2005).

2.3. Determination of growth parameters, cell viability, electrolyte leakage and chlorophyll

The seedlings were treated for 7 days in Hoagland nutrient solution (control) supplemented with $Cd(NO_3)_2$ and SNP. After treatment, roots and shoots of 7 day old seedlings were separated and their length measured in centimeter. Fresh weight of three seedlings was measured in grams. The loss in % cell viability was estimated using the method of Evans blue (Baker and Mock, 1994). Electrolyte leakage was measured using a conductivity meter (CM-180, Elico, India) and % calculated (McNabb and Takahashi). Extraction, determination and calculation of chlorophyll was performed according to the method of Arnon (Arnon, 1949) and expressed as mg g^{-1} fresh weight (FW).

2.4. Assessment of oxidative damage: lipid peroxidation, hydrogen peroxide, superoxide anion and activities of enzymes catalase and superoxide dismutase

In 7 day old rice roots and shoots grown in control, Cd/SNP alone or in combination were used to measure oxidative damage and its restoration if any in presence of SNP. Lipid peroxides were determined as malondialdehyde (MDA) content, Davenport et al. (Davenport et al., 2003). H_2O_2 and superoxide anion ($O_2^{\cdot-}$) formation in root and shoot were measured as before as before (Rai et al., 2012). Antioxidant enzymes catalase (EC1.11.1.6) and superoxide dismutase (SOD, EC 1.15.1.1) were assayed as described earlier (Rai et al., 2012). In all the enzyme preparations protein was determined by the Lowry's method (Lowry et al., 1951) using bovine serum albumin (BSA, Himedia) as standard.

2.5. Statistical analysis

The data were analyzed by a simple one-way variance analysis (ANOVA) and significant differences were compared by *t*-test.

3. Results and discussion

3.1. Effect of SNP on uptake of Cd, NO and growth of Cd-stressed rice seedlings

Fig. 1 shows rice plants grown under 50 μM Cd alone/or in combination with 50 μM SNP. A reduced shoot length in Cd-treatments is evident. Application of SNP to Cd-stressed plants caused improvement in shoot lengths to an extent similar to that of controls. Table 1 summarizes the effect of 50 μM SNP on Cd-uptake and growth in rice seedlings grown under 50 μM Cd at 7 day of growth. Cd treatments alone on an average had only 2–4% reduction in seedling growth at day 7 as compared to all other treatments yet in Fig 1 this reduction in growth of rice seedlings in Cd-alone seem very significant perhaps because the picture is from one set of experiment and data in Table 1 is an average of three independent experiments. In support it is evident that the fresh weight of the rice seedlings decreased ~16% in rice roots and shoots in 50 μM Cd treatments alone. Supplementing the same plants with 50 μM SNP enhanced the root and shoot growth with corresponding increase in fresh weight of the seedlings.

Increased uptake of Cd in both roots and shoots as compared to control is noted in Cd-treated plants. Addition of 50 μM SNP in the growth medium lowered the uptake and accumulation of Cd by ~1.2–1.4 times in Cd + SNP treatment than that in plants grown under Cd alone (Table 1). Nitric oxide content as expected seems to be affected by SNP treatment. Addition of 50 μM SNP alone led to a 18% increase in NO levels in rice roots as compared to controls however no such increase could be observed in shoots of rice (Table 1). Rice plants grown in presence of Cd + SNP had 1.4 times increased levels of NO inside the rice roots as compared to the control plants raised in presence of SNP alone (Table 1) This could

Fig. 1. Effect of Sodium nitroprusside on cadmium toxicity in growth of rice plants.