C. R. Biologies xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

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Physiology/Physiologie

Manganese-induced cadmium stress tolerance in rice seedlings: Coordinated action of antioxidant defense, glyoxalase system and nutrient homeostasis

Anisur Rahman [a,b], Kamrun Nahar [a,c], Mirza Hasanuzzaman [b], Masayuki Fujita [a,*]

[a] *Laboratory of Plant Stress Responses, Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa 761-0795, Japan*
[b] *Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh*
[c] *Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh*

ARTICLE INFO

Article history:
Received 12 May 2016
Accepted after revision 22 August 2016
Available online xxx

Keywords:
Antioxidant defense glyoxalase system
Methylglyoxal
Metal accumulation
Oxidative damage
Reactive oxygen species
Trace elements

ABSTRACT

The accumulation of cadmium (Cd) alters different physiological and biochemical attributes that affect plant growth and yield. In our study, we investigated the regulatory role of supplemental manganese (Mn) on hydroponically grown rice (*Oryza sativa* L. cv. BRRI dhan29) seedlings under Cd-stress conditions. Exposure of 14-d-old seedlings to 0.3 mM $CdCl_2$ for three days caused growth inhibition, chlorosis, nutrient imbalance, and higher Cd accumulation. Higher Cd uptake caused oxidative stress through lipid peroxidation, loss of plasma membrane integrity, and overproduction of reactive oxygen species (ROS) and methylglyoxal (MG). The exogenous application of 0.3 mM $MnSO_4$ to Cd-treated seedlings partly recovered Cd-induced water loss, chlorosis, growth inhibition, and nutrient imbalance by reducing Cd uptake and its further translocation to the upper part of the plant. Supplemental Mn also reduced Cd-induced oxidative damage and lipid peroxidation by improved antioxidant defense and glyoxalase systems through enhancing ROS and MG detoxification, respectively.

1. Introduction

Plants grown under natural conditions are continuously subjected to various environmental stressors, which are the major constraints to crop production, because they are unpredictable, unavoidable, complex in nature, and change gradually [1–3]. Metal toxicity is one of the major threats to crop production because metals easily enter the food

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Abbreviations: AO, ascorbate oxidase; APX, ascorbate peroxidase; AsA, ascorbate; BSA, bovine serum albumin; Ca, calcium; Cd, cadmium; CAT, catalase; CDNB, 1-Chloro-2,4-dinitrobenzene; chl, chlorophyll; DAB, diaminobenzidine; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DTNB, 5,5′-Dithio-bis-(2-nitrobenzoic) acid; EDTA, ethylenediaminetetraacetic acid; Gly, glyoxalase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GPX, glutathione peroxidase; GST, glutathione *S*-transferase; K, potassium; LOX, lipoxygenase; MDA, malondialdehyde; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; MG, methylglyoxal; Mg, magnesium; Mn, manganese; NADPH, nicotinamide adenine dinucleotide phosphate; NBT, nitroblue tetrazolium chloride; Pro, proline; ROS, reactive oxygen species; SLG, *S*-D-lactoyl-glutathione; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

[*] Corresponding author.
E-mail address: fujita@ag.kagawa-u.ac.jp (M. Fujita).

http://dx.doi.org/10.1016/j.crvi.2016.08.002

Please cite this article in press as: A. Rahman, et al., Manganese-induced cadmium stress tolerance in rice seedlings: Coordinated action of antioxidant defense, glyoxalase system and nutrient homeostasis, C. R. Biologies (2016), http://dx.doi.org/10.1016/j.crvi.2016.08.002

chain, and because most are carcinogens [4]. Among toxic metals, cadmium (Cd) is considered to be of major environmental concern because it ranks seventh among the top-20 toxins and is easily absorbed by plants. Being a highly mobile element, Cd can be easily absorbed by plants, transferred to other parts of the plant, and accumulates in different parts of it [1,5]. Accumulation of Cd in different parts of the plant causes different physiological and biochemical changes and alterations of mineral nutrient uptake and of their metabolism [5,6]. The physiological and biochemical changes due to higher accumulation of Cd in plants are chlorosis, necrosis, leaf rolling, growth inhibition, decreased water potential, and cation efflux; the changes cause alterations in membrane functions, stomatal action, antioxidant metabolism, and activities of several key enzymes; induction of oxidative stress in plant tissues, and even death [1,7,8]. Although Cd is a redox non-active metal, it induces oxidative stress indirectly by increased production of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$), through activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9,10]. The Cd-induced higher production of ROS interacts with the antioxidant defense system [11,12] and disrupts the electron transport chain [13] as well as the metabolism and uptake of essential plant nutrients [6,14]. Moreover, Cd accelerates the generation of cytotoxic methylglyoxal (MG) through the glycolysis pathway and causes oxidative damage by impairing protein synthesis [12,15,16].

To prevent Cd-induced oxidative stress, plants have stress tolerance mechanisms such as detoxification of ROS and MG, maintenance of nutrient homeostasis, and reduction of Cd uptake [6,12,16,17]. In addition, plants produce proline (Pro) or other compatible solutes to maintain proper water balance and stabilize the protein complexes for ionic and osmotic homeostasis [18,19].

Plants have an antioxidant defense system comprising non-enzymatic antioxidants, such as ascorbate (AsA), glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids, and α-tocopherols, and enzymatic antioxidants, such as 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) to scavenge the overproduced ROS [2,20]. In addition, the glyoxalase enzymes [glyoxalase I (Gly I) and glyoxalase II (Gly II)] act coordinately with GSH to detoxify MG [15,21].

Regulating the antioxidant defense and glyoxalase systems, improving nutrient homeostasis, and reducing Cd uptake by applying an exogenous phytoprotectants might be an important strategy to reduce Cd-induced damage in rice seedlings. Manganese (Mn) is an essential trace element for plants and plays a role in several metabolic processes including photosynthesis, and respiration; synthesis of ATP, fatty acids, amino acids, lipids, proteins, and flavonoids; and activation of hormones [22,23]. Manganese also plays a vital role as cofactor in Mn-SOD and Mn-CAT, which participate in the plant's defense against oxidative stress [24]. Although the mechanism is not clear, it is also assumed that Mn act as a scavenger of $O_2^{\cdot-}$ and H_2O_2 [24]. Manganese is taken up by plants via an active transport mechanism as a divalent cation that can compete with divalent Cd, because they have some common routes of uptake and transportation [25,26]. This is the most important mechanism of Mn-induced reduction of Cd toxicity. Several studies revealed that supplemental Mn plays an important role in the adaptive responses of plant cells under environmental stresses [27–32]. Considering the strategies discussed, our present study was conducted to investigate the role of supplemental Mn in improving the antioxidant defense and glyoxalase systems, nutrient homeostasis, and Cd uptake reduction capacity in Cd-stressed rice seedlings.

2. Materials and methods

2.1. Plant materials and treatments

Rice (*Oryza sativa* L. cv. BRRI dhan29) seeds were surface-sterilized with 70% ethanol for 8–10 min, followed by washing several times with sterilized distilled water and soaking in distilled water in a dark place for 48 h. The imbibed seeds were then sown on plastic nets floating on distilled water in 250-mL plastic beakers kept in the dark at 28 ± 2 °C for 72 h. The uniformly germinated seeds were then transferred to a growth chamber (light, 350 μmol photon $m^{-2} \cdot s^{-1}$; temperature, 25 ± 2 °C; relative humidity, 65–70%) with the same pot providing a diluted (5000 times) commercial hydroponics nutrient solution (Hyponex, Japan). The nutrient solution contained 8% N, 6.43% P, 20.94% K, 11.8% Ca, 3.08% Mg, 0.07% B, 0.24% Fe, 0.03% Mn, 0.0014% Mo, 0.008% Zn, and 0.003% Cu. The nutrient solutions were renewed twice a week. Each pot contained approximately 60 seedlings. Fourteen-day-old seedlings were exposed to Cd-stress (0.3 mM $CdCl_2$) in the presence and absence of supplemental Mn (0.3 mM $MnSO_4$) with nutrient solution to verify the role of exogenous Mn under a Cd-stress condition. Control plants were grown in Hyponex solution only. Our experiment consisted of four treatments as follows: control, 0.3 mM $MnSO_4$ (Mn), 0.3 mM $CdCl_2$ (Cd), and 0.3 mM $CdCl_2$ + 0.3 mM $MnSO_4$ (Cd + Mn). The experiment was repeated three times under the same conditions. Data were taken after three days of treatment.

2.2. Observation of cadmium toxicity symptoms and seedlings growth

Seedling growth and Cd toxicity symptoms in rice seedlings were determined by careful observation and by measuring fresh weight (FW) and dry weight (DW). For DW, seedlings were oven-dried at 70 °C for 48 h. The plant height was measured from the base of the shoot to the tip of the longest leaf.

2.3. Determination of the relative water content in leaves

The relative water content (RWC) in the leaves was measured according to the method of Barrs and