Ecotoxicology and Environmental Safety 149 (2018) 101–107

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Signaling molecule methylglyoxal ameliorates cadmium injury in wheat (*Triticum aestivum* L) by a coordinated induction of glutathione pool and glyoxalase system

Zhong-Guang Li[a,b,c,*], Qian Nie[a,b,c], Cong-Li Yang[a,b,c], Yue Wang[a,b,c], Zhi-Hao Zhou[a,b,c]

[a] School of Life Sciences, Yunnan Normal University, Kunming 650092, PR China
[b] Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Kunming 650092, PR China
[c] Key Laboratory of Biomass Energy and Environmental Biotechnology, Yunnan Province, Yunnan Normal University, Kunming 650092, PR China

ARTICLE INFO

Keywords:
Cadmium toxicity
Glyoxalase system
Glutathione pool
Methylglyoxal
Wheat seedlings

ABSTRACT

Methylglyoxal (MG) now is found to be an emerging signaling molecule. It can relieve the toxicity of cadmium (Cd), however its alleviating mechanism still remains unknown. In this study, compared with the Cd-stressed seedlings without MG treatment, MG treatment could stimulate the activities of glutathione reductase (GR) and gamma-glutamylcysteine synthetase (γ-ECS) in Cd-stressed wheat seedlings, which in turn induced an increase of reduced glutathione (GSH). Adversely, the activated enzymes related to GSH biosynthesis and increased GSH were weakened by N-acetyl-L-cysteine (NAC, MG scavenger), 2,4-dihydroxy-benzylamine (DHBA) and 1,3-bis-chloroethyl-nitrosourea (BCNU, both are specific inhibitors of GR), buthionine sulfoximine (BSO, a specific inhibitors of GSH biosynthesis), and N-ethylmaleimide (NEM, GSH scavenger), respectively. In addition, MG increased the activities of glyoxalase I (Gly I) and glyoxalase II (Gly II) in Cd-treated seedlings, followed by declining an increase in endogenous MG as comparision to Cd-stressed seedlings alone. On the contrary, the increased glyoxalase activity and decreased endogenous MG level were reversed by NAC and specific inhibitors of Gly I (isoascorbate, IAS; squaric acid, SA). Furthermore, MG alleviated an increase in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in Cd-treated wheat seedlings. These results indicated that MG could alleviate Cd toxicity and improve the growth of Cd-stressed wheat seedlings by a coordinated induction of glutathione pool and glyoxalase system.

1. Introduction

Cadmium (Cd) is a highly toxic and nonessential heavy metal for plants. Even at low concentrations, plant injury by Cd can be observed at molecular, subcellular, cellular, organic, and even the whole plant level (Liu et al., 2016; Rizwan et al., 2016; He et al., 2017; Huang et al., 2017). Exposure of plants to Cd stress, sophisticated changes can be detected at biochemical, physiological, and molecular levels. Cd stress can disturb stomata movement, transpiration, water uptake, nutrition homeostasis, respiration, and photosynthesis, finally leads to growth retardation and the loss of crop productivity (Liu et al., 2016; Rizwan et al., 2016; He et al., 2017; Huang et al., 2017). In addition, Cd is a potential threat to human health when it enters into the food chain. Cd has adversely effects on many systems such as urinary, respiratory, reproductive, and skeletal (Rizwan et al., 2016; He et al., 2017). Due to its stability, non-degradation, and toxicity, Cd pollution has attracted much attention in the fields of plant stress biology and environmental science (Liu et al., 2016; Rizwan et al., 2016; He et al., 2017; Huang et al., 2017).

In general, Cd toxicity, similar to other abiotic stresses, usually leads to oxidative, methylglyoxal (MG) (one of the carbonyl stress), osmotic, ion, and nutrition stresses. Correspondingly, plants possess complexly and effectively adaptive mechanisms to Cd injury by stimulating the enhancement of reactive oxygen species (ROS) detoxification system (maintaining ROS homeostasis) and MG detoxification system (mainly glyoxalase system to remain MG at a low physiological level), osmoregulation (accumulating osmolytes such as proline, glycine betaine, and soluble sugar, to maintain turgor pressure), and ion and nutrition homeostasis (regulating uptake and transport) (Liu et al., 2016; Rizwan et al., 2016; He et al., 2017; Huang et al., 2017). During the process of

Abbreviations: BCNU, 1,3-bischloroethyl-nitrosourea; BSO, buthionine sulfoximine; Cd, cadmium; DHBA, 2,4-dihydroxybenzylamine; γ-ECS, γ-glutamylcysteine synthetase; Gly I, glyoxalase I; Gly II, glyoxalase II; Gly III, glyoxalase III; GPX, guaiacol peroxidase; GR, glutathione reductase; GSH, glutathione; IAS, isoascorbate; MDA, malondialdehyde; MG, methylglyoxal; NAC, N-acetyl-L-cysteine; NEM, N-ethylmaleimide; ROS, reactive oxygen species; SA, squaric acid

* Corresponding author at: School of Life Sciences, Yunnan Normal University, Kunming 650092, PR China.
E-mail address: zhongguang_li@163.com (Z.-G. Li).

https://doi.org/10.1016/j.ecoenv.2017.11.021
Received 7 September 2017; Received in revised form 1 November 2017; Accepted 9 November 2017

plants respond and adapt to Cd stress, glutathione (GSH, γ-glutamyl-cysteinyl-glycine) plays a key role by directly and indirectly scavenging excessive ROS, regulating redox homeostasis, binding Cd directly, and synthesizing phytochelatins and cysteine-rich proteins (Jozefczak et al., 2012; Hasanuzzaman et al., 2017a). In plants, GSH can be produced by de novo synthesis relying on both γ-glutamylcysteine synthetase (γ-ECS, a rate-limiting enzyme) and GSH synthetase, as well as the reduction of oxidized glutathione (GSSG) by glutathione reductase (GR) (Jozefczak et al., 2012; Hasanuzzaman et al., 2017a). In pharmacological experiments, therefore, buthionine sulfoximine (BSO) and N-ethylmaleimide (NEM) are used as specific inhibitor of GSH biosynthesis and its scavenger, respectively (Ma et al., 2007; Huang et al., 2008).

Methylglyoxal (MG) is a highly reactive ketoaldehyde with α, β-carbonyl. It is one of the reactive carbonyl species (RCS). RCS, similar to ROS, commonly include MG, malondialdehyde (MDA), glyoxal (GO), 4-hydroxy-2-nonenal (HNE), and 2E-hexenal (HE) (Hossain et al., 2016; Li, 2016; Sankaranarayanan et al., 2017). MG is an inevitable side-product of photosynthesis and respiration (mainly glycolysis) in plants (Li, 2016). For a long time, MG is considered to be a cytotoxin, which can rapidly react with biomacromolecules (protein, DNA, and RNA) and biomembrane, producing advanced glycation end products (AGE). AGE can further disturb cell metabolism, and even leads to cell death (Hossain et al., 2016; Li, 2016; Sankaranarayanan et al., 2017). Upon exposure to abiotic stress, plants can actively or passively accumulate MG, which shows an increase of two- ~ six-fold, approximately 140–420 μM in plant cells (about 30–70 μM under normal conditions). This increase maybe become a common stress marker or function as signaling molecule (Hossain et al., 2016; Li, 2016; Sankaranarayanan et al., 2017), which in turn triggers the tolerance of plants to abiotic stress. Under normal physiological conditions, the production and scavenging of MG in plant cells maintain homeostasis by the synergistic effect of GSH and glyoxalase system composed of glyoxalase I (Gly I), glyoxalase II (Gly II), and glyoxalase III (Gly III), and maintaining a low and nontoxic level. MG homeostasis or fluctuation within a certain range subtly regulates plant growth, development, reproduction, and the acquisition of stress tolerance (Hossain et al., 2016; Li, 2016; Hasanuzzaman et al., 2017b; Sankaranarayanan et al., 2017).

Recently, MG is found to be an emerging signaling molecule, which is involved in seed germination, pollen tube growth, reproduction, and stomata movement (Hossain et al., 2016; Li, 2016; Sankaranarayanan et al., 2017). Bless et al. (2017) reported that pretreatment with MG could promote seed germination and subsequent seedling growth of *Brassica rapa* L under zirconium stress. Our previous studies also showed that MG as signal molecule improved seed germination and subsequent seedling growth of wheat under NaCl stress. The acquisition of this salt tolerance was closely associated with MG and ROS detoxification systems, as well as osmolytes (Li et al., 2017a). In addition, MG could alleviate Cd toxicity in wheat seedlings (Li et al., 2017b), however the mechanism of which still remains unknown. Therefore, this study based on our previous research, using wheat seedlings as materials, further illustrated the mechanism of MG-induced Cd tolerance, and the new viewpoint of MG as signal molecule was highlighted.

2. Materials and methods

2.1. Plant materials, growth conditions, and treatments

Wheat seeds (*Triticum aestivum* L. cv. Yunmai 41) were sown on the cotton wool in culture vessels with the following solutions to imbibe and germinate after being sterilized according to previously described methods (Li et al., 2017b): (1) 0 (control), 150 μM $CdCl_2$ (abbreviated as Cd, the same as the following), 150 μM Cd + 700 μM MG (Cd + MG), 150 μM Cd + 700 μM N-acetyl-L-cysteine (NAC, a MG scavenger, Dhar et al., 2010) + 700 μM MG (Cd + NAC + MG), 150 μM Cd + 500 μM 2,4-dihydroxybenzylamine (DHBA, a specific inhibitor of GR, Fitzgerald et al., 1991) + 700 μM MG (Cd + DHBA + MG), 150 μM Cd + 500 μM 1,3-bischloroethyl-nitrosourea (BCNU, a specific inhibitor of GR, Fitzgerald et al., 1991) + 700 μM MG (Cd + BCNU + MG), 150 μM Cd + 700 μM NAC (Cd + NAC), 150 μM Cd + 500 μM DHBA, 150 μM Cd + 500 μM BCNU; (2) 150 μM Cd + 500 μM N-ethylmaleimide (NEM, a GSH scavenger, Huang et al., 2008) + 700 μM MG (Cd + NEM + MG), 150 μM Cd + 500 μM buthionine sulfoximine (BSO, a specific inhibitor of GSH biosynthesis, Ma et al., 2007) + 700 μM MG (Cd + BSO + MG), 150 μM Cd + 500 μM NEM (Cd + NEM), 150 μM Cd + 500 μM BSO (Cd + BSO); (3) 150 μM Cd + 500 μM isoascorbate (ISA, a specific inhibitor of Gly I, Ramaswamy et al., 1984) + 700 μM MG (Cd + ISA + MG), 150 μM Cd + 500 μM squaric acid (SA, a specific inhibitor of Gly I, Ramaswamy et al., 1984) + 700 μM MG (Cd + SA + MG), 150 μM Cd + 500 μM ISA (Cd + ISA), 150 μM + 500 μM SA (Cd + SA). In experiments, the optimal concentrations of chemicals were chosen in the light of our previous study (Li et al., 2017b) and preliminary experiments. And then, the imbibed seeds were sequentially germinated and the seedlings were grown on t in a plant growth chamber with the same solution above, 100 μmol m^{-2} min^{-1}, and 12 h photoperiod, as well as at 26 ℃ for 5 d. On the fifth day, the following parameters of seedlings were measured, respectively.

2.2. Growth parameters

Seedling height and root length of wheat seedlings treated with Cd alone or in combination with MG, NAC, DHBA, BCNU, NEM, BSO, IAS, and SA were assayed as per our previous methods (Li et al., 2017a, 2017b).

2.3. Extraction and assay of glutathione reductase

Glutathione reductase (GR, EC 1.6.4.2) in wheat seedlings treated with Cd alone or in combination with MG, NAC, DHBA, and BCNU was extracted. Its activity was measured according to our previous methods (Li et al., 2013b) and expressed as μmol g^{-1} fresh weight (FW) min^{-1}.

2.4. Gamma-glutamylcysteine synthetase activity assay

A Gamma-glutamylcysteine synthetase (γ-ECS, EC 6.3.2.2) in wheat seedlings treated with Cd alone or in combination with MG, NAC, DHBA, and BCNU was extracted and its activity was assayed as per the methods of Shan et al. (2017). Wheat seedlings were ground in liquid nitrogen, and then extracted with 0.1 M Tris-HCl (pH8.0). The extract was centrifuged at 20,000 × *g* for 15 min. The resulting supernatant was used for the assay of γ-ECS activity. The reaction mixture (1 mL) was composed of 0.1 M Tris-HCl (pH 8.0), 0.25 mM glutamate, 10 mM ATP, 1 mM dithioerythritol, 2 mM cysteine, and 50 μL of supernatant. Reaction was started after adding supernatant and maintained at 25 ℃ for 1 h. Then one milliliter of phosphorus agent (3 mM H_2SO_4: distilled water: 2.5% ammonium molybdate: 10% AsA = 1:2:1:1) was added and mixed. The mixture was incubated at 45 ℃ for 30 min. The absorbance at 660 nm was read. The activity of γ-ECS was calculated using molar coefficient of 5.6 × 10^3 M^{-1} cm^{-1} and expressed as μmol g^{-1} FW min^{-1}.

2.5. Extraction and estimation of reduced glutathione (GSH)

GSH in wheat seedlings treated with Cd alone or in combination with MG, NAC, NEM, and BSO was extracted. Its content was determined according to the methods of Griffiths (Griffiths, 1980) and expressed as μmol g^{-1} FW.

2.6. Glyoxalase assay

Gly I (EC 4.4.1.5) and Gly II (EC 3.1.2.6) in wheat seedlings treated with Cd alone or in combination with MG, NAC, IAS, and SA were extracted and their activities were determined as the procedure