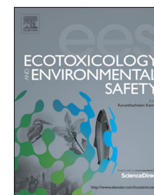
Ecotoxicology and Environmental Safety 105 (2014) 103–111

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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



Hydrogen-rich water confers plant tolerance to mercury toxicity in alfalfa seedlings

Weiti Cui [a], Peng Fang [a], Kaikai Zhu [a], Yu Mao [a], Cunyi Gao [a], Yanjie Xie [a], Jin Wang [b], Wenbiao Shen [a,*]

[a] College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China
[b] Laboratory Center of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form
6 April 2014
Accepted 9 April 2014
Available online 7 May 2014

Keywords:
Hydrogen-rich water
Medicago sativa
Mercury toxicity
Oxidative damage

ABSTRACT

In this report, the effect of hydrogen-rich water (HRW), which was used to investigate the physiological roles of hydrogen gas (H_2) in plants recently, on the regulation of plant adaptation to mercury (Hg) toxicity was studied. Firstly, we observed that the exposure of alfalfa seedlings to $HgCl_2$ triggered production of reactive oxygen species (ROS), growth stunt and increased lipid peroxidation. However, such effects could be obviously blocked by HRW. Meanwhile, significant decreases in the relative ion leakage and Hg accumulation were observed. Hg-induced increases in total and isozymatic activities of superoxide dismutase (SOD) were significantly reversed by HRW. Further results suggested that HRW-induced the activities of guaiacol peroxidase (POD) and ascorbate peroxidase (APX), two hydrogen peroxide-scavenging enzymes, was at transcriptional levels. Meanwhile, obvious increases of the ratios of reduced/oxidized glutathione (GSH), homoglutathione (hGSH), and ascorbic acid (AsA) and corresponding gene expression were consistent with the decreased oxidative damage in seedling roots. In summary, the results of this investigation indicated that HRW was able to alleviate Hg toxicity in alfalfa seedlings by (i) alleviating growth stunt and reducing Hg accumulation, and (ii) avoidance of oxidative stress and reestablishment of redox homeostasis.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Heavy metals (HMs) such as mercury (Hg) and cadmium (Cd) have become one of the major environmental contaminants that restrict plant productivity. The interrelationship between HMs toxicity and cellular redox imbalances leading to oxidative stress in plants has been intensely investigated (Sharma and Dietz, 2009). For example, besides Hg-induced growth stunt and the binding of Hg^{2+} to sulphydryl groups of proteins, another major toxicity of accumulated Hg in plants is that it induces the formation of reactive oxygen species (ROS) and triggers oxidative damage (Cho and Park, 2000; Gao et al., 2010; Wang et al., 2012). Therefore, the detection of Hg-triggered accumulation of ROS and thereafter oxidative stress can be used as biomarkers to indicate Hg toxicity in plants. Previous results also suggested that Hg-elicited oxidative stress modulates activities of antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1), guaiacol peroxidase (POD; EC 1.11.1.7), and ascorbate peroxidase (APX; EC 1.11.1.11), or corresponding gene expression (Gao et al., 2010; Meng et al., 2011).

Besides enzymatic antioxidant systems, plant cells usually contain nonenzymatic antioxidants, including ascorbic acid (AsA) and glutathione (GSH) (Foyer and Noctor, 2011). Subsequent work demonstrated that the balance between GSH and oxidized GSH (GSSG) and/or their homologs reduced/oxidized homoglutathione (hGSH/hGSSGh), which are more abundant than GSH/GSSG in alfalfa plants, as well as reduced AsA and its oxidized forms, such as dehydroascorbate (DHA), is important for the efficiency of plant antioxidant systems (Foyer and Noctor, 2011; Cui et al., 2012). The redox imbalance was due to oxidative burst triggered by Hg, which was paralleled by oxidation of GSH and/or hGSH, and an up-regulation of corresponding synthesis genes: *γ-glutamylcysteine synthetase* (*ECS*), *glutathione synthetase* (*GS*) and *homoglutathione*

Abbreviations: APX, Ascorbate peroxidase; AsA, Ascorbic acid; CAT, Catalase; DAB, 3,3′-Diaminobenzidine tetrahydrochloride; DHA, Dehydroascorbate; ECS, γ-Glutamylcysteine synthetase; GR, Glutathione reductase; GS, Glutathione synthetase; GSH, Glutathione; GSSG, Oxidized glutathione; H_2, Hydrogen gas; H_2O_2, Hydrogen peroxide; Hg, Mercury; hGSH, Homoglutathione; hGSSGh, Oxidized homoglutathione; HMs, Heavy metals; HRW, Hydrogen-rich water; ICP-OES, Inductively coupled plasma-optical emission spectrometer; MDHAR, Monodehydroascorbate reductase; POD, Guaiacol peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid reactive substances; UPLC, Ultra performance liquid chromatography

* Corresponding author. Fax: +86 25 84396542.
E-mail addresses: wbshenh@njau.edu.cn, 13851857655@139.com (W. Shen).

http://dx.doi.org/10.1016/j.ecoenv.2014.04.009
0147-6513/© 2014 Elsevier Inc. All rights reserved.

synthetase (*hGS*) (Sobrino-Plata et al., 2009), as well as the genes encoding enzymes involved in ascorbate–glutathione cycle, such as *monodehydroascorbate reductase* (*MDHAR*) and *glutathione reductase* (*GR*) (Sobrino-Plata et al., 2009; Foyer and Noctor, 2011). Further evidence demonstrated that abovementioned antioxidant systems play important roles in protecting plants against Hg-induced oxidative stress (Sobrino-Plata et al., 2009; Foyer and Noctor, 2011; Meng et al., 2011).

Hydrogen (H_2), a highly flammable gas, has been proved to be protective against injury to animal organs including the liver (Tsai et al., 2009), lung (Sun et al., 2011a, 2011b), brain (Chen et al., 2010), and eye (Oharazawa et al., 2010) through the inhibition of oxidative stress. To some extent, hydrogen is therefore similar to other gaseous signaling molecules, such as nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) (Li et al., 2012; Cui et al., 2012, 2013). However, hydrogen was not characterized as a toxic gas, and cannot be produced by mammalian cells (Zheng et al., 2011). Interestingly, the production of H_2 by bacteria, green algae and higher plants has been previously reported (Renwick et al., 1964; Tamagnini et al., 2002). Similar to the beneficial roles in animals, recent reports in plants illustrated that H_2 protects against stress conditions, such as salinity (Xie et al., 2012; Xu et al., 2013), drought (Jin et al., 2013; Zeng et al., 2013), paraquat exposure (Jin et al., 2013), and aluminum stress (Chen et al., 2014). It has been also demonstrated that H_2-enhanced plant tolerance to some abiotic stresses results from the changes in the expression of genes encoding antioxidant enzymes such as SOD, catalase (CAT; EC 1.11.1.6), and APX (Xie et al., 2012; Xu et al., 2013; Jin et al., 2013; Zeng et al., 2013). These H_2-modulated antioxidant enzymes may play key roles in above tolerance.

Alfalfa (*Medicago sativa* L.) is a valuable forage crop which is grown in areas of limited rainfall, high temperature and where the land is often HMs affected. Recently, we showed that H_2 could alleviate Cd toxicity in alfalfa seedlings, which is associated with the induction of antioxidant enzymes, elevated GSH/hGSH levels and alleviation of programmed cell death (PCD) (Cui et al., 2013). However, to the best of our knowledge, the impact of H_2 on Hg toxicity has never been addressed. Therefore, the aim of this study was to examine the role of HRW, which was previously used to characterize physiological roles of H_2 both in animals and recently in plants, in the regulation of Hg toxicity. Thus, this work will provide a basis for molecular breeding designed to improve plant tolerance against HMs, including the alleviation of seedling growth inhibition, Hg accumulation and oxidative stress caused by Hg exposure in alfalfa plants.

2. Materials and methods

2.1. Preparation of hydrogen-rich water (HRW)

Purified H_2 gas (99.99%, v/v) generated from a hydrogen gas generator (SHC-300, Saikesaisi Hydrogen Energy Co., Ltd., China) was bubbled into 1 L quarter-strength Hoagland's solution (pH 6.0) at a rate of 150 mL min^{-1} for 40 min. Afterwards, the corresponding hydrogen-rich water (HRW) was rapidly diluted to the required concentrations (1, 10, and 50%, [v/v]). In our experimental conditions, the H_2 concentration in freshly prepared HRW analyzed by gas chromatography (Bernardi et al., 2008) was 0.22 mM, and maintained at a relative constant level in 25 °C for at least 12 h.

2.2. Plant materials, growth condition and treatments, and determination of seedling growth

Alfalfa (*M. sativa* L. cv. Biaogan) seeds were surface-sterilized with 5% NaClO for 10 min, rinsed comprehensively in distilled water and germinated for 1 d at 25 °C in the darkness. Uniform seedlings were then chosen and transferred to the plastic chambers and cultured in nutrient medium (quarter-strength Hoagland's solution, pH 6.0) for another 4 d in the illuminating incubator (MGC-300B, Shanghai Yiheng Technology Co., Ltd., Shanghai, China) at 25 ± 1 °C, with a light intensity of 200 μmol m^{-2} s^{-1} and 14 h photoperiod.

Afterwards, 5-day-old seedlings were incubated with quarter-strength Hoagland's solution with or without the indicated concentrations of HRW, or varying concentrations of $HgCl_2$ alone for the indicated time points, followed by another 24 h or the indicated time points of incubation in 10 μM $HgCl_2$. The sample without chemicals was the control (Con). After different treatments, the root tissues of 30 seedlings were harvested, and fresh weight (FW) and dry weight (DW) were determined. Meanwhile, alfalfa seedlings were photographed. The roots of seedlings were harvested for used immediately or flash-frozen in liquid nitrogen, and stored at −80 °C for further determination.

2.3. Determination of thiobarbituric acid reactive substances (TBARS) contents

Lipid peroxidation was estimated by measuring the amount of TBARS as previously described (Cui et al., 2013).

2.4. Determination of root ion leakage

Root ion leakage was determined by relative conductivity assay following the previous method (Xiong et al., 2012) with a slight modification. After various treatments, 0.3 g fresh root were washed twice with deionized water, then kept immersion in a test tube with 10 ml deionized water at 25 °C. After 5 min of incubation, the electrical conductivity (EC0) of solution was determined with a conductivity meter (DDS-12A; Shanghai Kangyi Instrument Co., Ltd., China). After another 12 h of incubation, the electrical conductivity (EC1) of solution was determined again. Samples were then boiled for 30 min and cooled to 25 °C, and the electrical conductivity (EC2) of the solution was measured once more. Relative conductivity = (EC1 − EC0)/EC2 × 100%.

2.5. Determination of Hg content

After various treatments, fresh seedlings were washed two times with EDTA-Na_2 solution and rinsed briefly in deionized water. Then, root tissues were oven-dried at 60 °C, followed by the digestion with HNO_3 using a Microwave Digestion System (Milestone Ethos T, Italy). The Hg contents were determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Perkin Elmer Optima 2100DV).

2.6. Histochemical analyses

Reactive oxygen species (ROS) production was detected by 3,3′-diaminobenzidine tetrahydrochloride (DAB) staining (Thordal-Christensen et al., 1997). Histochemical detection of lipid peroxidation and loss of plasma membrane integrity in root apexes was performed with Schiff's reagent and Evans blue respectively, as described previously (Yamamoto et al., 2001; Cui et al., 2013). All the roots stained with DAB, Schiff's reagent or Evans blue were washed thoroughly, then observed under a light microscope (model Stemi 2000-C; Carl Zeiss, Germany) and photographed on color film (Powershot A620, Canon Photo Film, Japan).

2.7. Assay of enzyme activity

Activities of superoxide dismutase (SOD) and guaiacol peroxidase (POD) were analyzed by the methods described previously (Xu et al., 2012). Ascorbate peroxidase (APX) activity was also measured (Nakano and Asada, 1981). Protein content was determined by the previous method (Bradford, 1976).

2.8. Gel electrophoresis

The isozymes of SOD, POD and APX were separated according to previous methods (Cui et al., 2013). For each lane, 30 μg of protein extract was applied. SOD, POD and APX isozymatic activities on the gel were visualized by activity staining according to the procedure described previously (Beauchamp and Fridovich, 1971; Jin et al., 2013). For the determination of the relative activity of different isozymes, gels were scanned and the intensity of bands was calculated by using the Quantity One v4.4.0 software (Bio-Rad, Hercules, CA, USA).

2.9. Glutathione and homoglutathione analysis by ultra performance liquid chromatography (UPLC)

Glutathione and homoglutathione and their corresponding disulfides contents were measured according to the methods previously reported (Queval and Noctor, 2007; Cui et al., 2012) with minor modification. The monobromobimane (mBBr) derivatives were filtered through a 0.22 μm filter, 4 μl of the mixture was subjected to UPLC analysis (Agilent Technologies, 1290 series). Thiol derivatives were separated on a ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8-Micron; Agilent) at a flow rate of 0.2 mL min^{-1}. The linear gradient was from 0% solution B to 10%

Download English Version:

https://daneshyari.com/en/article/4420068

Download Persian Version:

https://daneshyari.com/article/4420068

Daneshyari.com