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An emphasis of hydrogen sulfide-cysteine cycle on enhancing the tolerance to chromium stress in *Arabidopsis*☆

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ABSTRACT

Increasing attention has been focused on the health of vegetables and grains grown in the contaminated agricultural soil, it is thus meaningful to find ways to reduce the heavy metals (HMs) accumulation in plants. As sulfur is considered to be an essential macronutrient for plant stress defenses, the important role of sulfur assimilation in plants responding to HMs stress has been followed. However, the potential mechanism of the only sulfur-containing gasotransmitter hydrogen sulfide (H_2S) and its main endogenously generated substrate, cysteine (Cys), in plant defense is poorly understood. The physiological and biochemical methods together with qRT-PCR were used to explore the response pattern of H_2S-Cys cycle in plants resisting to chromium (Cr^{6+}) stress. Our results suggested that Cr^{6+} stress inhibited *Arabidopsis* root elongation, increased the H_2S and Cys contents time-dependently, and H_2S production was activated earlier than Cys. Furthermore, H_2S increased Cys accumulation more quickly than Cr^{6+} stress. The qRT-PCR results revealed that H_2S up-regulated the Cys generation-related genes *OASTLa*, *SAT1* and *SAT5* expression levels, and that *SAT1* and *SAT5* expression was elevated for a longer duration. Data suggested that H_2S might regulate Cys metabolism-related genes expression to participate in Cr^{6+}-mediated Cys accumulation. H_2S and Cys relieved the root elongation inhibition caused by Cr^{6+} in *Arabidopsis*. Both H_2S and Cys enhanced glutathione generation and activated phytochelatins (PCs) synthesis by up-regulating *PCS1* and *PCS2* expression levels to fight against Cr^{6+} stress. Besides regulating the expression of PCs synthase encoding genes, H_2S might promote metallothioneins accumulation by significantly increasing the *MT2A* gene expression. Overall, H_2S and H_2S-induced Cys accumulation (H_2S-Cys system) was critical in imparting Cr^{6+} tolerance in *Arabidopsis*. This paper is the first to indicate that gasotransmitter H_2S induced Cys accumulation in *Arabidopsis* Cr^{6+}-stress defense and provides evidence for more extensive studies of the H_2S signaling pathway.

1. Introduction

Increasing anthropogenic and industrial activities have caused excessive emissions of toxic metals into the environment, which undoubtedly lead to soil contamination (Nriagu and Pacyna, 1988). Chromium (Cr) is the second most abundant inorganic contaminant in agricultural soil, hexavalent chromium (Cr^{6+}) and trivalent chromium (Cr^{3+}) species are the most stable species of chromium (Cr) to occur in the environment (Zhao et al., 2016). Because of its mutagenic and carcinogenic properties, the Cr^{6+} is a serious threat to organisms grown in soil. The contamination of agricultural soil has attracted critical concerns due to the potential adverse ecological effects (Seth et al., 2012). It is thus important to explore the mechanisms contributing to plants stress defense and find ways to reduce the heavy metals (HMs) accumulation in grains.

Excessive HMs adversely affect the growth and development of plants (Jonak et al., 2004). Generally, the overproduction of reactive oxygen species (ROS) is the primary response of plants to HMs. Lipid peroxidation is the most deleterious influence caused by

Abbreviations: ABRC, Arabidopsis Biological Resource Center; AsA, Ascorbic acid; CASC, β-cyanoalanine synthase; CDes, Cys desulfhydrases; Cr, Chromium; Cys, Cysteine; DES, desulfhydrase; GSH, Glutathione; HMs, Heavy metals; H_2S, Hydrogen sulfide; LCD, L-Cys desulfhydrase; MDA, Malondialdehyde; MTs, Metallothioneins; ½ MS, ½ Murashige-Skoog (medium); OAS, O-acetylserine; OASTL, O-acetyl-L-serine (thiol) lyase; PCs, Phytochelatins; PCS, Phytochelatins synthase; qRT-PCR, quantitative real-time PCR; ROS, Reactive oxygen species; SAT, Serine acetyltransferase; SCS, S-sulfocysteine synthase; UBQ, Ubiquitin.

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HMs-induced ROS (Mithofer et al., 2004), and malondialdehyde (MDA), one of the decomposition products of lipid peroxidation, is considered to be an indicator of oxidative damage (Stohs and Bagchi, 1995). Unlike animals, higher plants are sessile and cannot escape from some stimuli, so they have developed strategies for stress avoidance (Xiong et al., 2002), such as activating the antioxidant glutathione (GSH) (Freeman et al., 2004, 2005; Semane et al., 2007) as well as the HMs chelators, phytochelatins (PCs) (Salt and Rauser, 1995; Vatamaniuk et al., 2004) and metallothioneins (MTs) (Hall, 2002; Cobbett and Goldsbrough, 2002).

Sulfur, an essential macronutrient in plants, acts as the functional component of various biochemical compounds, such as cysteine (Cys), GSH, PCs, MTs and hydrogen sulfide (H_2S), all of which play positive roles in plants HMs defense responses (Droux, 2004; Alvarez et al., 2010). H_2S, due to its unpleasant flavor, was previously widely regarded to be a toxic gas (see Lisjak et al., 2013; Jin and Pei, 2015). This changed when H_2S was reported to act as an endogenous neuromodulator in the brain (Abe and Kimura, 1996). Hereafter, H_2S was reported to be the only sulfur-containing gasotransmitter (Wang, 2002, 2012), and its central role in the physiological regulation and disease responses of mammals has been continuously implicated (Yang et al., 2008; Wang, 2012). Reports in plants indicate that the H_2S, with physiological concentration, is not only a crucial player in regulating plants growth and development (Zhang et al., 2008, 2009; Li et al., 2012a, 2012b), including root morphogenesis (Zhang et al., 2009) and flower senescence (Zhang et al., 2011), but is also a critical mediator in plant defense responses and tolerance acquisition (Zhang et al., 2010; Dawood et al., 2012; Li et al., 2012c, 2013; Shen et al., 2013; Shi et al., 2013, 2014; Fang et al., 2014; Cui et al., 2014).

In plants, H_2S can be generated endogenously through enzymatic pathways. The Cys desulfhydrases (CDes) occupy an irreplaceable position in H_2S generation. L-Cys desulfhydrase (LCD) is the most unambiguous CDes in *Arabidopsis*, which mediates L-Cys degradation into H_2S, ammonia and pyruvate (Romero et al., 2013; Jin and Pei, 2015). Interestingly, a novel enzyme was discovered and named DES1, which should be classified into O-acetyl-L-serine (thiol) lyase (OASTL) based on its sequence characteristics (Alvarez et al., 2010). However, the functional analysis revealed that DES1 had a higher affinity to L-Cys and degrades it to generate H_2S (Alvarez et al., 2010; Romero et al., 2013).

Besides being the main substrate for endogenous H_2S production, Cys is the first organosulfur compound of sulfur assimilation in plants and the major donor of reduced sulfur for organic sulfur compounds (Takahashi et al., 2011). Generally, the inorganic sulfate is taken up by plants, and then reduced and assimilated into Cys. Serine acetyltransferase (SAT) and OASTL are indispensable in this process (Harrington and Smith, 1980; Wirtz et al., 2004). SAT physically interacts with OASTL to form the Cys synthase complex, which controls the biosynthesis of Cys appropriately. Firstly, the SAT catalyzes the transfer of acetyl from acetyl-CoA to serine to form the intermediate O-acetylserine (OAS), and the OASTL then catalyzes Cys generation by incorporating the sulfide into OAS (Bonner et al., 2005; Heeg et al., 2008). There are two additional important enzymes in Cys metabolism, CASC1, a β-cyanoalanine synthase, which catalyzes the conversion of Cys and cyanide to H_2S and β-cyanoalanine, and SCS, a S-sulfocysteine synthase, which catalyzes the incorporation of thiosulfate to OAS to form S-sulfocysteine. All of these enzymes work together to maintain the Cys equilibrium (Gotor et al., 2014).

Additionally, Cys acts as a functional precursor for numerous essential biomolecules (Noctor et al., 2012), such as GSH and PCs, both of which play important roles in the acquisition of HMs tolerance in plants. GSH, a sulfur and thiol containing tri-peptide, synthesized by γ-glutamylcysteine synthetase and glutathione synthetase (Wachter and Rausch, 2005; Seth et al., 2012), is an important defender in the organisms fighting against ROS, and it has been reported to eliminate ROS by its own oxidation to glutathione disulfide in a redox signaling pathway. Moreover, GSH regenerates the reduced ascorbic acid (AsA) through the GSH-AsA cycle signaling pathway, which maintains a higher reduced AsA state. Both reduced GSH and AsA act as key regulators of antioxidant defenses (Anjum et al., 2012; Fang et al., 2014). Moreover, GSH is a substrate for PCs synthesis, which is catalyzed by phytochelatins synthase (PCS). As a set of novel HMs-binding peptides, PCs carry toxic HMs to insensitive regions mediated by compartmentalization. Furthermore, the crucial role of PCs in HMs detoxification has been indicated in numerous studies (Cobbett and Goldsbrough, 2002; Seth et al., 2012; Fang et al., 2014).

In the present study, we used physiological and biochemical methods to explore the response mode of the H_2S-Cys system in *Arabidopsis* that responds to chromium (Cr^{6+}) stress. This study proposes a signaling pathway for the gasotransmitter H_2S protecting *Arabidopsis* against Cr^{6+} stress, and it provides some evidence for understanding the mechanism of plant-stress defenses.

2. Materials and methods

2.1. Plant materials and treatments

Arabidopsis thaliana ecotype Col-0 (wild-type, Wt), the *LCD* defective mutant *lcd* (SALK_082099) and the *DES1* defective mutant *des1* (SALK_205358C) were obtained from the Arabidopsis Biological Resource Center (ABRC). The *LCD* over-expression mutant OE-*LCD* (the transgenic line of 35S:*LCD*) and the *DES1* over-expression mutant OE-*DES1* (the transgenic line of 35S:*DES1*) were generated as described previously (Qiao et al., 2015). These seeds were sterilized with 75% ethanol for 50 s and 6% sodium hypochlorite for 8 min under sterile conditions. After rinsed with sterile water three times, the seeds were sown on ½ Murashige-Skoog (½ MS) medium, and then the Petri dishes were sealed with parafilm. These Petri dishes were cultivated under a 16 h/8 h (light/dark) photoperiod with a light illumination of 160 $Em^{-2}s^{-1}$ at 23 °C and 60% relative humidity.

For selecting the Cr^{6+} concentration of stress exposure, ten-day-old *Arabidopsis* seedlings were transferred aseptically to Cr^{6+} containing (0, 100, 200, 300, 400 and 500 μmol/L Cr^{6+}) ½ MS medium.

For the H_2S fumigation pretreatment, the one-week-old seedlings were successively fumigated with H_2S released by NaHS. The NaHS solution-containing tube was placed in the Petri dish mentioned above and the H_2S fumigation concentration was 50 μmol/L. For Cys pretreatment, the Cys was added directly to the ½ MS medium, and the concentration of Cys is 1 mmol/L. After pretreated for 3 d, these seedlings were transferred aseptically to the stress condition of the 300 μmol/L Cr^{6+}-containing (150 μmol/L $K_2Cr_2O_7$) ½MS medium.

2.2. MDA and GSH content assays

The MDA content was determined by the thiobarbituric acid reaction based on published methods (Qiao et al., 2015). The GSH content was measured based on a previously described method (Fang et al., 2014).

2.3. Measurement of the endogenous H_2S content

To determine the regulation of Cr^{6+} stress on endogenous H_2S generation, the content of endogenous H_2S was measured according to previously described methods (Qiao et al., 2015).