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Hydrogen sulfide – cysteine cycle plays a positive role in *Arabidopsis* responses to Copper Oxide nanoparticles stress

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ABSTRACT

Copper Oxide (II) nanoparticles (CuO NPs) are the most important nanomaterials, which also have a strong toxicity for plants. Hydrogen sulfide (H_2S) is a newly appreciated gasotransmitter participant in plant stress physiology. Here, we investigated the role of H_2S-cysteine (Cys) cycle in plant responses to CuO NPs stress. H_2S and Cys synthetic regulating related genes L-Cysteine desulfhydrase (*LCD*), L-Cysteine Desulfhydrase 1 (*DES1*) and O-acetylserine(thiol)lyase isoform A1 (*OASA1*) levels were successively induced by CuO NPs, and endogenous H_2S and Cys contents increased, suggesting that H_2S-Cys cycle responses to CuO NPs stress. The tolerance of CuO NPs weakened in *lcd*, *des1*, *lcddes1* or *oasa1* mutants. Subcellular distribution of Cu was disturbed, and organelle's Cu concentration increased in mutants. Chemical forms of Cu changed when H_2S-Cys cycle was blocked. Heavy metal chelator proteins were up-regulated under CuO NPs stress in Col-0, but the levels were weakened in mutants. H_2S-Cys cycle also regulated antioxidase activity and maintained ROS homeostasis in CuO NPs stress. The black particles appeared in root tip after CuO NPs treatment, which formed by CuO NPs. By contrast Col-0, the black particles significantly accumulated in mutants, suggesting that H_2S signal affects CuO NPs uptake and intracellular transport. Conclusion, H_2S-Cys cycle plays a positive role in plant responses to CuO NPs stress.

1. Introduction

Copper Oxide (II) nanoparticles (CuO NPs) are the most manufactured metal NPs, which have been widely used in electronics, chemical industry, machinery, and agriculture (Brumfiel, 2003; Service, 2003; Stampoulis et al., 2009). Along with the extensive use of CuO NPs in various products, the concentration of CuO NPs rises continuously in the soil, which significant consequences for plant growth (Nel et al., 2006; Bondarenko et al., 2013). Because of its physics characteristic, CuO NPs have stronger toxicity than the ordinary copper oxide, and which can penetrate plant cell wall and easier interact with the intracellular substance (Lacave et al., 2016). The mainly toxicity effects of CuO NPs for plants due to nanoparticle itself, dissolved Cu^{2+} ions contribute a minority of the toxicity of CuO NPs (Ke et al., 2017). It is reported that the elongation of root and shoot is inhibited by a low concentration of CuO NPs (Tang et al., 2016). The leaf of maize seedling was shown to minus green and inhibit growth in a high concentration of CuO NPs stress (Wang et al., 2012, 2016). However, to our knowledge, current data regarding the tolerance mechanism of CuO NPs is not available in plants.

Hydrogen sulfide (H_2S) plays the important physiological function in the regulation of nervous system and cardiovascular system, thus it is recognized as the third endogenous gasotransmitter, following the discovery of nitric oxide and carbon monoxide (Tan et al., 2010). In plants, cysteine (Cys) metabolism closely relate to H_2S generation and dispelling. Cys desulfhydrases (CDes), the key enzymes, contributes to H_2S generation (Papenbrock et al., 2007). L-Cys desulfhydrase (LCD) and D-Cys desulfhydrase (D-CDES) degrade Cys to H_2S, pyruvate, and ammonia (Kopriva, 2006). However, the activity of D-CDES is lower than LCD. O-acetylserine(thiol)lyase (OAS-TL) catalyzes Cys generation

Abbreviations: APX, aseorbateperoxidase; CAT, catalase; CuO NPs, Copper Oxide nanoparticles; Cys, cysteine; *DES1*, L-Cysteine desulfhydrase 1; GSH, glutathione; H_2S, hydrogen sulfide; *LCD*, L-Cysteine desulfhydrase; MTs, metallothioneins; *OASA1*, O-acetylserine(thiol)lyase isoform A1; PCs, phytochelatins; POD, peroxidase; SOD, superoxide dismutase

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by incorporating the sulfide into OAS (Sirko et al., 2004). Recently, a CS-LIKE (Cys synthase-like) enzyme was found through the sequence characteristics, which named DES1 (Alvarez et al., 2010). However, the function of DES1 is similar to LCD, which degrades Cys to generate H_2S. Based on these processes, H_2S-Cys cycle forms in plant cells.

Sulfur metabolism plays an important role in the stress resistance of plant. The products of sulfur metabolism, include H_2S, Cys, glutathione (GSH), phytochelatins (PCs) and metallothioneins (MTs) have biological functions in plant responses to heavy metal stress and oxidative stress (Takahashi et al., 2011; He et al., 2015; Luo et al., 2016). H_2S-Cys cycle is an integrant physiology process in sulfur metabolism. It is reported that H_2S-Cys cycle plays the positive role in the tolerance of chromium (VI) ion stress (Fang et al., 2016). Our previous work also validated that H_2S-Cys cycle enhances cadmium (II) ion tolerance in *Arabidopsis* (Jia et al., 2016). CuO NPs is a metal oxide NPs, which have much higher toxicity in comparison with other NPs such as TiO_2 and ZnO (Lacave et al., 2016). Previous researches revealed that many kinds of NPs can be assimilated through endocytosis in animal cell (Jani et al., 1990; Jung et al., 2000). Reasonable subcellular distribution of toxicant is a pivotal detoxification way for protecting organelle and reducing secondary damage (Zhang et al., 2014). CuO NPs toxicology research suggested that CuO NPs appear a selective subcellular distribution in helminth (*L. variegatus*) cells (Thit et al., 2016), implying that resistance system of CuO NPs stress maybe exist. However, the regulatory mechanism of the tolerance of CuO NPs is unknown in plant.

In this work, we found that H_2S-Cys cycle plays an important role in the tolerance of CuO NPs stress through genetics. When H_2S-Cys cycle is blocked up in *lcd*, *des1*, *lcddes1* or *oasa1* mutant, the resistance of plant on CuO NPs stress is weakened. Therefore, our aim is to know the interaction between H_2S-Cys cycle and CuO NPs stress and demonstrate the working mechanism of the H_2S-Cys cycle response to CuO NPs stress in *Arabidopsis*.

2. Materials and methods

2.1. Plant material and chemical treatments

This study was carried out on *Arabidopsis thaliana*, including Columbia (Col-0) and the *lcd* (SALK_082099), *des1-1* (SALK_103855), *lcddes1-1* (*lcd* and *des1* cross-fertilize) and *oasa1* (SALK_074242c) mutants (Jia et al., 2016). Details of Plant material and chemical treatments are provided in Supplementary information Materials and Methods.

2.2. RNA isolation and quantitative real time polymerase chain reaction (qRT-PCR)

The chemical treatments were the same as the measurements of root elongation assays. Roots of *Arabidopsis* seedling were harvested to extract total RNA for real-time PCR. All the primers used in this study were shown in Supplementary Table S1. Details are provided in Supplementary information Materials and Methods.

2.3. Root elongation assays and microscopic observation

4-days-old *Arabidopsis* seedlings grown on the vertical 1/2 MS agar plates were transferred to the 1/2 MS agar medium containing various chemicals for the different treatments. Root elongation and microscopic observation was measured after 3 days of various treatments. All experiments were repeated at least three times, and photographs taken from one representative experiment were shown. Then analyzed the root length with Image J. Microscopic observation of root cell used the laser confocal scanning microscope (Leica SM IRBE Multisyne FE 1250).

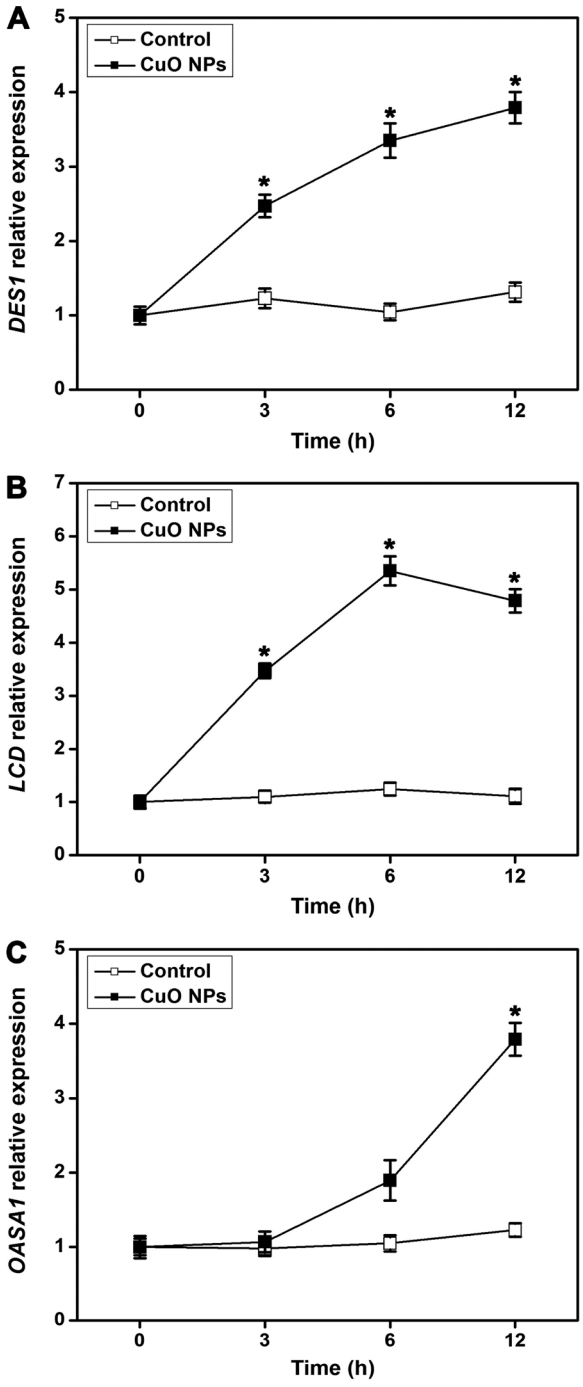

Fig. 1. qRT-PCR analysis the synthetic genes of H_2S and Cys in Col-0 roots. Relative expression levels were normalized with an internal standard *EF1a*. 4-d-old **(A)** Transcription level of *DES1* gene in 0–12 h. **(B)** Transcription level of *LCD* gene in 0–12 h. **(C)** Transcription level of *OASA1* gene in 0–12 h. *Arabidopsis* seedlings were grown on 1/2 MS agar plates supplied with 10 μg ml^{-1} CuO NPs or without CuO NPs for 0–12 h.

2.4. Electrolyte leakage and MDA assays

Lipid peroxidation of the roots was measured by estimating the MDA content according to the method of Heath and Packer (1965). Measurement of ion leakage was determined according to this method with some modifications (Sairam and Srivastava, 2002). Details are provided in Supplementary information Materials and Methods.