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Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate–glutathione cycle: Possible involvement of nitric oxide

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ABSTRACT

In plants, hydrogen sulfide (H_2S) is an emerging novel signaling molecule that is involved in growth regulation and abiotic stress responses. However, little is known about its role in the regulation of arsenate (As^V) toxicity. Therefore, hydroponic experiments were conducted to investigate whether sodium hydrosulfide (NaHS; a source of H_2S) is involved in the regulation of As^V toxicity in pea seedlings. Results showed that As^V caused decreases in growth, photosynthesis (measured as chlorophyll fluorescence) and nitrogen content, which was accompanied by the accumulation of As. As^V treatment also reduced the activities of cysteine desulfhydrase and nitrate reductase, and contents of H_2S and nitric oxide (NO). However, addition of NaHS ameliorated As^V toxicity in pea seedlings, which coincided with the increased contents of H_2S and NO. The cysteine level was higher under As^V treatment in comparison to all other treatments (As-free; NaHS; As^V + NaHS). The content of reactive oxygen species (ROS) and damage to lipids, proteins and membranes increased by As^V while NaHS alleviated these effects. Enzymes of the ascorbate–glutathione cycle (AsA–GSH cycle) showed inhibition of their activities following As^V treatment while their activities were increased by application of NaHS. The redox status of ascorbate and glutathione was disturbed by As^V as indicated by a steep decline in their reduced/oxidized ratios. However, simultaneous NaHS application restored the redox status of the ascorbate and glutathione pools. The results of this study demonstrated that H_2S and NO might both be involved in reducing the accumulation of As and triggering up-regulation of the AsA–GSH cycle to counterbalance ROS-mediated damage to macromolecules. Furthermore, the results suggest a crucial role of H_2S in plant priming, and in particular for pea seedlings in mitigating As^V stress.

Introduction

Arsenate (As^V), being a dominant As species in aerobic soils and due to its chemical similarity with inorganic phosphate, enters the plant cell through the phosphate transport systems (Zhao et al., 2009; LeBlanc et al., 2013; Singh et al., 2015). The co-transport of phosphate or As^V and protons through $H_2PO_4^-/H^+$ symporters requires at least $2H^+$ for each $H_2PO_4^-$ or $H_2AsO_4^-$ transported (Ullrich-Eberius et al., 1989). Once inside the plant cell, As^V is reduced efficiently to arsenite (As^{III}), thus suggesting that most plants have a high capacity for the reduction of As^V (Zhao et al., 2009). Consequently, As^{III} may be rendered non-toxic by forming a complex with phytochelatins or may be transported to the vacuoles (Cobbett, 2000; Indriolo et al., 2010). However, when plants are not

Abbreviations: As^V, arsenate; AsA–GSHcycle, ascorbate–glutathione cycle; APX, ascorbate peroxidase; DES, L-cysteine desulfhydrase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; F_m/F_0, electron transport rate through PS II; F_v/F_m, maximum quantum efficiency of photosystem II (PSII); F_v/F_0, activity of PS II; GR, glutathione reductase; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; •OH, hydroxyl radical; MDA, malondialdehyde; MSI, membrane stability index; MDHAR, monodehydroascorbate reducatase; NR, nitrate reductase; NO, nitric oxide; NPQ, non-photochemical quenching; GSSG, oxidized glutathione; qP, photochemical quenching; RCG, reactive carbonyl groups; ROS, reactive oxygen species; AsA, reduced ascorbate; GSH, reduced glutathione; NaHS, sodium hydrosulfide; SOR, superoxide radical.

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able to adjust As levels inside the cell, As toxicity occurs. In plants, adverse effects of As exposure are duration, dosage and species-dependent. Adverse impacts of As^V may occur at morphological, physiological, biochemical, proteomic, transcriptomic and genomic levels (Wojas et al., 2010; Requejo and Tena, 2012). Inhibition of seed germination and a decline in growth are common responses of As^V toxicity (Requejo and Tena, 2012). Reduction in photosynthetic pigments and, consequently, photosynthesis has also been reported in various photosynthetic organisms under As^V stress (Wang et al., 2012; Singh et al., 2013; Srivastava et al., 2013). Studies demonstrate that As^V exposure of plants alters the metabolisms of carbohydrate and protein, and nodule formation (Mishra and Dubey, 2006; Lafuente et al., 2010). Long-term exposure of humans to As results in skin disorders and cancer (Duker et al., 2005). Furthermore, there is much agricultural land that is contaminated with As. For India, As concentrations from 3.34 to 105 mg kg^{-1} soil have been reported (Patel et al., 2005). Considering the damage to plants and human health, there is an urgent need for reliable and cost-effective methods that can reduce As toxicity to plants and also curtail As levels in food products.

Although As^V is a metalloid without redox activity it may induce the generation of reactive oxygen species (ROS) (Singh et al., 2013; Srivastava et al., 2013) through its inter-conversion from one ionic form to another (Mylona et al., 1998). In the absence of protective mechanisms, ROS can damage the cell's structure and function by oxidizing lipids, proteins and nucleic acids. Thus, the over production of cellular ROS generally causes loss of functions of macromolecules (Singh et al., 2013). However, ROS-mediated oxidative damage to the cell can be minimized by the various pathways that operate to keep the cellular concentration of free metalloid to a minimum (primary detoxification, e.g., thiol-mediated metalloid complexation; Requejo and Tena, 2012; Leão et al., 2014) and also prevent ROS-mediated damage to macromolecules (secondary detoxification, e.g. quenching of ROS by antioxidants; Namdjoyan and Kermanian, 2013; Singh et al., 2013; Gomes et al., 2014). Among various pathways of ROS detoxification, the ascorbate–glutathione cycle (AsA–GSH) is of prime importance. The AsA–GSH cycle consists of various enzymes such as ascorbate peroxidase (APX), monodehydroascorbate reducatase (MDHAR), dehydrateascorbate reductase (DHAR) and glutathione reductase (GR) as well as non-enzymatic antioxidants such as glutathione and ascorbate (Foyer and Noctor, 2011). Various components of the AsA–GSH cycle act in a coordinated manner and protect the cell from oxidative damage.

Hydrogen sulfide (H_2S), a colorless, soluble and flammable gas, is known for its toxic effects to different types of organisms. Interestingly, H_2S has also been identified as an important metabolic regulator in plants within the last decade (Filippou et al., 2012; Christou et al., 2013; Li, 2013; Hancock and Whiteman, 2014). In mammals, it is considered third major endogenous gasotransmitter, besides nitric oxide (NO) and carbon monoxide (CO) (Wang, 2002; Olson, 2009; Li, 2013; Hancock and Whiteman, 2014) and also plays a central role as a stimulatory or inhibitory compound in gastrointestinal, inflammatory, cardiovascular, nervous, and endocrine systems by activating K^+-ATP channels and modulating endothelial Ca^{2+} concentration (Bauer et al., 2010). In plants, H_2S has also been reported to regulate growth and development (García-Mata and Lamattina, 2010; Hancock et al., 2011; Li, 2013; Li et al., 2013; Hancock and Whiteman, 2014). In plants, apart from sulfite reductase, there are at least four other enzymes capable of producing H_2S (Calderwood and Kopriva, 2014). Among these enzymes, cysteine desulfhydrase appears to play a central role in H_2S production and homeostasis (Riemenschneider et al., 2005). Besides developmental and regulatory roles, studies demonstrated that H_2S can alleviate toxicities of abiotic stresses such as heavy metal, salinity, osmotic stress, heat, hypoxia, drought, etc. by affecting levels of antioxidants (Jin et al., 2011; Dawood et al., 2012; Cheng et al., 2013; Li, 2013; Li et al., 2013; Duan et al., 2015; Hancock and Whiteman, 2014). Furthermore, Xie et al. (2014) demonstrated that H_2S can delay GA-mediated programmed cell death (PCD) in wheat aleurone layers by modulating glutathione homeostasis and expression of heme oxygenase-1. Although there are an increasing number of reports regarding the role of H_2S in alleviating abiotic stresses in plants, however, the exact mechanisms by which H_2S works remain unclear. Further, to our knowledge, there is no report on implications of H_2S in the management of As^V toxicity in plants.

In India, As contamination in soil and water is a relevant problem, which has been worsened recently due to use of As-containing pesticides and contaminated irrigation water. Pea, an important legume crop, is widely cultivated for its protein content. As-toxicity results in reduced yield and contaminated seeds (Päivöke, 2003). The present study investigates whether NaHS is involved in the regulation of As^V toxicity in pea seedlings. We have measured various physiological and biochemical parameters such as growth, chlorophyll fluorescence, activities of L-cysteine desulfhydrase and nitrate reductase, contents of H_2S, nitric oxide (NO) and cysteine, oxidative stress and damage, and components of the ascorbate–glutathione cycle (AsA–GSH cycle).

Material and methods

Plant material and growth conditions

Pea (*Pisum sativum* L. cv. Azad P-1) seeds were purchased from National Seed Corporation, New Delhi. Uniformly sized seeds were surface sterilized with 10% (v/v) sodium hypochlorite solution for 10 min, washed and soaked in distilled water for 4 h. After sterilization and soaking, healthy looking seeds were sown in plastic trays containing acid washed sterilized sand. Plastic trays were kept in the dark for seed germination at $26 \pm 1\,^{\circ}C$. After germination, seedlings were grown in a growth chamber (CDR model GRW-300 DGe, Athens) under photosynthetically active radiation (PAR) of 350 μmol photons m^{-2} s^{-1}, 16:8 h day–night regime and 66–70% relative humidity at $26 \pm 1\,^{\circ}C$ for 15 d.

NaHS and As^V treatments

The 15-d-old uniformly-sized seedlings were gently uprooted and acclimatized in half strength Hoagland's nutrient solution for 2 d. After this, arsenate (As^V, in the form of $Na_2HAsO_4 \times 7H_2O$) and sodium hydrosulfide (NaHS; a source of H_2S) treatments were given to the seedlings for next 15 d in plastic pots (20×30 cm^2; volume 150 mL) either alone or in combination. The As^V concentration (50 μM) that has been used in the present study is environmentally relevant, while the NaHS dose (100 μM) used is based on the dose-response curve (in terms of fresh weight). The treatments include: control (CK; no addition of As^V and NaHS), NaHS (100 μM), 50 μM As^V and 50 μM As^V + 100 μM NaHS. In case of As^V + NaHS treatments, seedlings were pretreated with NaHS for 48 h and then exposed to As^V (50 μM). Following As^V and NaHS treatments, seedlings were placed in a growth chamber under photosynthetically active radiation (PAR) of 350 μmol photons m^{-2} s^{-1}, 16:8 h day–night regime and 66–70% relative humidity at $26 \pm 1\,^{\circ}C$ for 15 d. During the 15-d growth of seedlings, solutions of selected treatments were changed five times at an interval of 3 d each i.e. first on the 3rd day, second on the 6th day, third on the 9th day, fourth on 12th day and fifth on 15th day in half strength Hoagland's solution. Thereafter, seedlings were harvested and secondary leaves were used for measurements. Nutrient solutions were aerated daily under aseptic conditions to avoid root anoxia.