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Role of two-sided crosstalk between NO and H_2S on improvement of mineral homeostasis and antioxidative defense in *Sesamum indicum* under lead stress

Rayhaneh Amooaghaie[a,*], Faezeh zangene-madar[a], Shekoofeh Enteshari[b]

[a] Biology Department, Science Faculty, Shahrekord University, Shahrekord, Iran
[b] Biology Department, Science Faculty, Payame Noor University, Iran

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ABSTRACT

H_2S and NO are two important gasotransmitters that modulate stress responses in plants. There are the contradictory data on crosstalk between NO and H_2S in the studies. Hence, in the present study, the role of interplay between NO and H_2S was assessed on the Pb tolerance of *Sesamum indicum* using pharmacological and biochemical approaches. Results revealed that Pb stress reduced the plant growth and the content of photosynthetic pigments and Fv/Fm ratio, increased the lipid peroxidation and the H_2O_2 content, elevated the endogenous contents of nitric oxide (NO), H_2S and enhanced the activities of antioxidant enzymes (except APX). Additionally, concentrations of most mineral ions (K, P, Mg, Fe, Mn and Zn) in both shoots and roots decreased. Pb accumulation in roots was more than it in shoots. Both sodium hydrosulfide (NaHS as a donor of H_2S) and sodium nitroprusside (SNP as an NO donor) improved the plant growth, the chlorophyll and carotenoid contents and PSII efficiency, reduced oxidative damage, increased the activities of antioxidant enzymes and reduced the proline content in Pb-stressed plants. Furthermore, both NaHS and SNP significantly restricted the uptake and translocation of Pb, thereby minimizing antagonistic effects of Pb on essential mineral contents in sesame plants. NaHS increased the NO generation and many NaHS-induced responses were completely reversed by cPTIO, as the specific NO scavenger. Applying SNP also enhanced H_2S release levels in roots of Pb-stressed plants and only some NO-driven effects were partially weakened by hypotuarine (HT), as the scavenger of H_2S.These findings proposed for the first time that **two-sided interplay** between H_2S and NO might confer an increased tolerance to Pb stress via activating the antioxidant systems, reducing the uptake and translocation of Pb, and harmonizing the balance of mineral nutrient.

1. Introduction

According to the environmental protection agency (EPA), lead (Pb) is one of the most abundant heavy metals (Watanabe, 1997) contaminating soils, including agricultural soils due to its extensive use in fly ash and sludge, paints, explosives, petrol, industrial wastes, fertilizers and pesticides (Sharma and Dubey, 2005). It is known that lead (Pb) when enter to the environment, easily is adsorbed by plants and accumulated in their different parts and so readily is inserted in the food chain and can cause problems in terms of the health of human and animals (Sengar et al., 2008). Lead (Pb) can disturb water balance and hormonal status in plants (Malar et al., 2014; Pourrut al, 2011). A decreased uptake of micro and macronutrients has been known as a general consequence of Pb stress (Yilmaz et al., 2009; Lamhamdi et al., 2013). Lead also reduces photosynthesis by inhibiting synthesis of chlorophyll, plastoquinone, and carotenoids, disturbing electron transport, preventing the enzyme activities of the Calvin cycle; and causing CO_2 deficiency as a result of stomatal closure (Pourrut et al., 2011; Shu et al., 2012). The decreased content of photosynthetic pigments in Pb-treated plants may be the consequence of interaction of Pb to –SH group of enzymes involved in chlorophyll biosynthesis (Sengar et al., 2008), the increased activity of chlorophyllase activity (Drazkiewicz, 1994) or peroxidation of chloroplast membranes due to increased level of ROS generation in leaves.

Lead also induces oxidative stress via overproduction of reactive oxygen species (ROS) and disturbs the cellular redox and causes lipid peroxidation of membrane (Bharwana et al., 2014b; Malar et al., 2014). Pb stress can either suppress or increase the activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) depending on plant species (tolerant or susceptible) and metal concentration (Han et al., 2008; Sharma and Dubey, 2005; Sytar et al., 2013).

On the other hand, Liu et al. (2009) reported that Pb exposure altered the expression of > 1,310 genes in *Arabidopsis* seedlings that

* Corresponding author.
E-mail address: Rayhanehamooaghaie@yahoo.com (R. Amooaghaie).

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many of them were involved in the biosynthesis of signal molecules. Therefore, the endogenous signal molecules may be important in Pb sensing and can mediate the defensive responses of plants. In the last two decades, several lines of evidence have proven that three gasotransmitters of NO, CO and H_2S act as signal molecules which are modulating the plant physiological and developmental processes as well as modifying the plant defence responses against stresses (Amooaghaie and Nikzad, 2013; Amooaghaie et al., 2015; Amooaghaie and Rohollahi, 2016; Guo et al., 2016).

NO is a ubiquitous signal molecule in higher plants that is essential for the stress sensing. It is known that several hormones and the environmental cues elevate the internal NO generation in plants (Wendehenne and Hancock, 2011). Numerous studies have monitored role of NO in the plant physiological functions, including seed development, dormancy, germination, and plant stress responses (Baudouin and Hancock, 2014). Several experiments documented that the application of SNP, as a NO donor, can reduce the symptoms of many stresses including salinity (Ahmad et al., 2016), low temperature (Amooaghaie and Nikzad, 2013), nanoparticle toxicity (Amooaghaie et al., 2015), iron deficiency (Amooaghaie and Roohollahi, 2016) and toxicity of Cd (Kumari, 2010), Pb (Phang et al., 2011) and Cu (Dong et al., 2014).

Until recently, hydrogen sulfide (H_2S) was known as a phytotoxic gas, but nowadays, it is considered as one of gaseous signal messengers that at lower concentrations modulates the developmental processes and increases the tolerance of plants to the environmental stresses (Guo et al., 2016). Hydrogen sulfide (H_2S) is generated in plants by the action of desulfhydrases localized in various cellular compartments or from cysteine via reversible O-acetylserine (thiol) lyase (OAS-TL) reaction (Papenbrock et al., 2007). Several studies revealed that H_2S is implicated in regulating a variety of plant physiological processes such as seed germination, root organogenesis, stomatal apertures and photosynthesis (Guo et al., 2016). It also was shown that NaHS, as a donor of H_2S, can alleviate the effects of different stresses such as: salt in strawberry (Christou et al., 2013), salt, osmotic and cold stresses in bermudagrass (Shi et al., 2014), lead (Pb) in canola (Ali et al., 2014), aluminum in barley (Dawood et al., 2012) and cadmium in rice (Mostofa et al., 2015).

Although the individual effects of NO and H_2S on the stress tolerance of plants have assessed in numerous researches, according to our literature review, there are little studies on cross-talk between H_2S and NO and the contradictory data were reported in them (Chen et al., 2015; Li et al., 2012, 2013; Shi et al., 2013). Therefore, in this study, we not only investigated effects of Pb stress on the changes of homeostasis of H_2S and NO in sesame but also using pharmacological and biochemical approaches, tried to elucidate the role of the link between NO and H_2S in the alleviation of Pb-induced oxidative damages and in the control of the uptake of Pb and mineral nutrients in sesame.

2. Materials and methods

2.1. Chemicals

Sesame seeds were supplied from the Medicinal Plants Research Center, Isfahan, Iran.

Sodium hydrosulfide (NaHS), as a donor of H_2S and Hypotaurine (HT), as a H_2S scavenger and Sodium nitroprusside (SNP), as an NO donor, 2-(4-carboxyphenyl) − 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (cPTIO), as a specific NO scavenger, were purchased from Sigma (St Louis, MO, USA).

2.2. Plants treatments

Sesame seeds were surface sterilized with 3% sodium hypochlorite for 5 min and washed with distilled water 3 times. Then seeds germinated for 2 day at 24 °C in the darkness. Uniform seedlings were grown in nutrient medium (quarter-strength Hoagland's solution) under a photoperiod of 12 h, photon flux density of 150 $\mu molm^{-2} s^{-1}$, and a temperature regime of 25/20 °C in day/night and 65% relative humidity. Treatments were started after 30 days pre-culture by addition of compounds to nutrient solution. Treatments were including of:

(1) Hoagland nutrient solution (CK, the control),
(2) 2 mM Pb^{+2}
(3) 2 mM Pb^{+2} + 200 μM NaHS
(4) 2 mM Pb^{+2} + 200 μM SNP
(5) 2 mM Pb^{+2} + 200 μM NaHS + 1 mM HT
(6) 2 mM Pb^{+2} + 200 μM SNP + + 1 mM HT
(7) 2 mM Pb^{+2} + 200 μM NaHS + 100 μMcPTIO
(8) 2 mM Pb^{+2} + 200 μM SNP + 100 μMcPTIO

It is worth noting that in a preliminary study sesame seeds exposed with various concentrations (0, .0.5, 1, 2 and 4 mM) Pb^{+2} (from $Pb(NO_3)_2$) and the results showed that 2 mM Pb^{+2} reduced the seed germination and the seedling growth approximately 50% when compared to control and 4 mM Pb^{+2} severely suppressed above traits (data not shown). Therefore, concentration of 2 mM Pb^{+2} was chosen as optimal stress conditions for this study. Also measurements with the concentration gradients of SNP and NaHS, revealed the concentrations of 200 μM SNP and NaHS were the best treatments for seedlings tolerance against 2 mM Pb^{+2} stress (data not shown). Thus these concentrations were applied in this experiment.

The root samples were harvested 48 h after above treatments for biochemical analyses and were snap-frozen in liquid nitrogen (−80 °C) until analysis.

2.3. The growth parameters and relative water content (RWC)

For determination of growth parameters, plants were harvested and separated into shoots and roots. The fresh weights (FW) were immediately measured. The samples were dried in a hot air oven for 48 h at 75 °C for determination of dry weights (DW). The relative water content (RWC) was also calculated using following formula:

$$RWC\% = [(FW–DW)/FW] \times 100$$

2.4. Chlorophyll content assays

The leaf samples (0.2 g of fresh weight) were homogenized with 5 ml acetone (80%, v/v) using a mortar and pestle and filtered through Whatman No. 2 filter paper. The absorbance was read using a UV–visible spectrophotometer (UV-2550, Shimadzu, Japan) at 663, 645 and 470 nm and the contents of chlorophyll a (Chl a), chlorophyll (Chl b) and carotenoids (Car) were calculated using following formula (Lichtenthaler, 1987).

$$Chl\ a = 12.21 \times A665–2.81 \times A649$$

$$Chl\ b = 20.13 \times A649–5.03 \times A665$$

$$Car. = (1000 \times A470–27 \times Chl\ a–104 \times Chlb)/229$$

2.5. Chlorophyll a fluorescence assay

In each treatment, at least 5 individual plants were evaluated for chlorophyll fluorescence. The chlorophyll a florescence was estimated in second leaf of sesame by portable Mini-PAM. All samples were kept in dark for 20 min prior to fluorescence assay for adaptation. The saturation pulse intensity was 6000 $\mu mol\ m^{-2} s^{-1}$ and its duration was 0.8 s. Light intensity during the assessment was 204 $\mu mol\ m^{-2} s^{-1}$ and natural light was applied as the actinic irradiance. After the first saturation pulse, the minimal fluorescence (F_o) and maximal fluorescence (F_m) were determined, then variable fluorescence (F_v) = F_m-F_o