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Exogenous glutathione alleviates salt-induced oxidative stress in tomato seedlings by regulating glutathione metabolism, redox status, and the antioxidant system

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ABSTRACT

The objective of this experiment was to investigate the effects of exogenous glutathione (GSH) on tomato (*Solanum lycopersicum* L. cv. Zhongshu No. 4) seedlings under NaCl stress (100 mM), particularly the effects on (1) redox status, (2) antioxidative metabolism, and (3) the gene expression and activity of five enzymes related to GSH synthesis and metabolism. Salt stress resulted in an oxidative burst in tomato seedling leaves, as evidenced by increases in hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and superoxide radical ($O_2^{\bullet -}$). Salt stress also reduced leaf GSH level and GSH redox homeostasis, whereas application of GSH reversed the negative effects of salt stress. Furthermore, application of GSH increased the transcript levels and activities of enzymes related to GSH synthesis and metabolism, including gamma-glutamylcysteine synthetase (γ-ECS), glutathione synthetase (GS), glutathione-S-transferase (GST), glutathione peroxidase (GPX), glutathione reductase (GR). Application of GSH also enhanced the activities of antioxidant enzymes such as superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT) and the enzymes involved in the ascorbate-glutathione cycle, and the contents of GSH and the ratios of reduced and oxidized glutathione (GSH/GSSG) in the salt-stressed plants or salt-stress plants treated with buthionine sulfoximine (BSO, inhibitor of GSH synthesis key enzyme gamma-glutamylcysteine synthetase). Taken together, our findings demonstrate that exogenous GSH application increases resistance to salt-induced oxidative stress by enhancing the antioxidant defense system, and regulating GSH synthesis and metabolism to maintain cellular redox homeostasis.

1. Introduction

Salt stress is one of the most harmful environmental factors limiting crop growth and yield. Salt stress causes water deficit and ion-specific toxicity, which in turn inhibits corp growth by disrupting plant physiological processes. Salinity can promote the accumulation of reactive oxygen species (ROS) in leaves when the antioxidant capacity to detoxify ROS is low (Khan and Panda, 2002; Apel and Hirt, 2004). The ROS can attack biomacromolecules, causing membrane lipid peroxidation, changes in antioxidative enzyme activity and antioxidant content, and finally cell death (Li et al., 2013). Plants have evolved detoxification strategies to cope with excessive ROS and maintain the ROS balance within cells (Mittler et al., 2004). Glutathione (GSH), its redox status (GSH/GSSG), and GSH-related enzymes, including glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase (GST), are found to play an indispensable role in detoxification of ROS (Anjum et al., 2012).

Reduced glutathione (g-Glu-Cys-Gly, GSH), a major low molecular-weight free thiol tripeptide, is ubiquitous in plant and

Abbreviations: GSH, reduced glutathione; GSSH, oxidized glutathione; BSO, L-buthionine-sulfoximine; AsA, reduced ascorbate; DHA, oxidized ascorbate; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); FW, fresh weight; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GPX, glutathione peroxidase; GST, glutathione-S-transferase; GS, glutathione synthetase; γ-ECS, gamma-glutamylcysteine synthetase; MDA, malondialdehyde; ROS, reactive oxygen species.

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animal cells and crucial to a variety of life processes, including the maintenance of protein thiol, thioldisulfide exchange, removal of hydroperoxides and free radicals, and amino acid transport across membranes (Mendoza-Cózatl et al., 2005; Nahar et al., 2015a). The GSH has unique redox and nucleophilic properties and is involved in the cellular defense against the toxic actions of xenobiotics, oxyradicals, salinity, acidity, and metal cations. The GSH can scavenge ROS either directly or indirectly via the ascorbate-glutathione cycle, thioredoxin (Trx) and glutaredoxin (Grx) systems, sulphur metabolism, regulation of cellular homeostasis, and signal transduction (through either glutathionylation of proteins or thiol bridge redox reactions) (Mittler et al., 2004; Riccillo et al., 2000; Romero-Puertas et al., 2004; Anjum et al., 2012). Thus, GSH is considered to be the major intracellular redox buffer (Foyer and Noctor, 2011). GSH can act as the first line of defense against metal toxicity by complexing metals before induced synthesis of phytochelatin (PC) reaches an effective level. GSH is also an immediate substrate for synthesis of PCs (Flores-Cáceres et al., 2015). Some reports suggest that intracellular GSH biosynthesis and accumulation can enhance the resistance and adaptation of plants to various environmental stresses (Foyer and Noctor, 2011; Rausch et al., 2007). Glutathione is synthesized from L-glutamate, L-cysteine and glycine in two ATP-dependent reactions catalyzed by gamma-glutamylcysteine synthetase (γ-ECS) and glutathionesynthetase (GS). Glutathione is oxidized to GSSG as part of its cellular antioxidant defense, and then, in turn, GSSG is reduced back to GSH by the action of GR using NADPH to maintain the highly reduced state of GSH/GSSG (Foyer and Noctor, 2011; Seth et al., 2012). Both GPX and GST use the GSH pool as a substrate to detoxify H_2O_2 and the xenobiotics by catalyzing their conjugation with GSH. Ramakrishna and Rao (2013) reported that exogenous HBR application regulated the activities of enzymes related to GSH synthesis and metabolism to keep H_2O_2 levels under control and maintained cellular redox homeostasis, allowing radish seedlings to cope better with Zn^{2+} stress.

Many studies have shown that exogenous GSH enhances the growth and the antioxidant response of plants subjected to abiotic stresses such as high temperature (Nahar et al., 2015a; Zhu et al., 2016), drought (Nahar et al., 2015b), isoproturon (Nemat Alla and Hassan, 2014) and heavy metal toxicity (Chen et al., 2010; Cai et al., 2011; Estrella-Gómez et al., 2012; Qiu et al., 2013). In a previous study, we observed that GSH application promoted the growth of salt-stressed tomato plants and enhanced salt-tolerance (Zhou et al., 2016), and also increased net photosynthetic rate by protecting PSII from damage caused by excess energy, thus increasing the photochemical efficiency of PSII and the activity of the photosynthetic dark reaction (Liu et al., 2014a,b).

However, there is no information about the effects of exogenous GSH on glutathione synthesis, glutathione metabolism, and redox homeostasis in plants under salt stress. Furthermore, little is known about the mechanism by which GSH acts. Thus, the objective of this study was to test the hypothesis that exogenous GSH application may regulate or provide an effective strategy for combating salt stress by stimulating the antioxidant defense system, GSH synthesis and metabolism, and by maintaining redox homeostasis.

2. Materials and methods

2.1. Plant materials and treatments

Hydroponic experiments were carried out in a solar greenhouse at the Shihezi University Agricultural Experiment Station, Shihezi University, China. Tomato seeds (*Solanum lycopersicum* L. cv. Zhongshu No. 4) were germinated on moist filter paper in the dark at 28 °C for 2 days. Then, the germinated seeds were sown in trays filled with a 2:1 peat:vermiculite (v/v). When the second leaves were fully expanded, the seedlings were transplanted into 12 L plastic containers (five seedlings per container) containing 10 L aerated full-strength nutrient solution.

The seedlings were pre-cultured for 7 days before treatments. The experimental plots included five treatments: (a) Control: no NaCl, no GSH (Roche, China), and no BSO (L-buthionine-sulfoximine, an inhibitor of GSH synthesis key enzyme γ-ECS) (Sigma, USA); (b) NaCl: 100 mM NaCl; (c) NG: 100 mM NaCl + 5 mM GSH; (d) NB: 100 mM NaCl + 1 mM BSO; and (e) NBG: 100 mM NaCl + 1 mM BSO + 5 mM GSH. The NaCl was added to the nutrient solution. For the NG and NB treatment, 150 mL GSH and 150 mL BSO were sprayed onto the seedling leaves at 10:00 a.m. every day for 15 days, respectively. For the NBG treatment, the entire foliar regions of salt-stressed plants were pretreated with 150 mL BSO for 1 h and subsequently treated with 150 mL GSH. The concentrations of GSH, and BSO were selected according to the results of preliminary experiments (Supplementary material). The containers were arranged in a randomized complete block with three replicates per treatment. There were three containers per treatment, giving a total of 15 containers. The light period in the greenhouses was about 14 h. The air temperatures were 24–30 °C during the day time and 17–20 °C at night. The nutrient solutions were replaced every 3 days. Leaf samples were analyzed at day 5, 10, and 15 after the treatments.

2.2. Measurement of leaf Na^+ and Cl^- content

For the determination of Na^+ and Cl^- contents, plant samples (0.2 g) were digested in Triacid mixture, a mixture of HNO_3 and H_2O_2 in the ratio of 2:1. The content of ions was extracted in distilled water by boiling for 30 min twice. The filtered extract was used to measure specific ions. The Na^+ content was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Scientific ICAP 6000 Series, Boston, USA), Cl^- content was determined by titration against 0.02 N silver nitrate solution using 5% K_2CrO_4 as an indicator as described by Nazar et al. (2011).

2.3. Lipid peroxidation

Lipid peroxidation was estimated by measuring leaf malondialdehyde (MDA) content. Leaf MDA was determined using the method of Health and Packer (1968) with thiobarbituric acid (TBA) as the reactive material. The MDA contents were calculated based on absorption at 532 and 600 nm, with an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.4. H_2O_2 content and $O_2^{\bullet -}$ formation rate

Leaf H_2O_2 was assayed according to the method described by Yu et al. (2003). H_2O_2 was extracted by homogenizing 0.5 g leaf tissue with 3 mL of phosphate buffer (50 m M, pH 6.5) at 4 °C. The homogenate was centrifuged at 6000 × *g* for 25 min. A supernatant of 3 mL was mixed with 1 mL of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 6000 × *g* for 15 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content (expressed as μmol g^{-1} fresh weight).

The O_2^- formation rate in the leaves was determined following Elstner and Heupel (1976) with some modifications. Fresh leaves (0.3 g) were homogenized in 3 cm^3 of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 5000 × *g* for 10 min. The supernatant was mixed with the extraction buffer and 10 mM hydroxylamine hydrochloride (at the ratio of 10.7:9.1:1) and incubated at 25 °C for 20 min. The solution was then mixed with 17 mM sulfanilamide and 7 mM naphthylamine (at the ratio of 1:1) and incubated again at 25 °C for 20 min. The absorbance was measured