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Research article

Involvement of ABA- and H_2O_2-dependent cytosolic glucose-6-phosphate dehydrogenase in maintaining redox homeostasis in soybean roots under drought stress

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ABSTRACT

The roles of abscisic acid (ABA) and hydrogen peroxide (H_2O_2) in inducing glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity and the possible roles of G6PDH in regulating ascorbate-glutathione (AsA-GSH) cycle were investigated in soybean (*Glycine max* L.) roots under drought stress. Drought caused a marked increase of the total and cytosolic G6PDH activities and triggered a rapid ABA and H_2O_2 accumulation in soybean roots. Exogenous ABA or H_2O_2 treatment elevated the total and cytosolic G6PDH activities, whereas suppressing ABA or H_2O_2 production inhibited the drought-induced increase in total and cytosolic G6PDH activities, suggesting that ABA and H_2O_2 are required for drought-induced increase of total G6PDH activity, namely cytosolic G6PDH activity. Furthermore, ABA induced H_2O_2 production by stimulating NADPH oxidase activity under drought stress. Moreover, drought significantly increased the contents of AsA and GSH and the activities of key enzymes in AsA-GSH cycle, while application of G6PDH inhibitor to seedlings significantly reduced the above effect induced by drought. Taken together, these results indicate that H_2O_2 acting as a downstream signaling molecule of ABA mediates drought-induced increase in cytosolic G6PDH activity, and that enhanced cytosolic G6PDH activity maintains cellular redox homeostasis by regulating AsA-GSH cycle in soybean roots.

1. Introduction

Drought is a major limitation for plant growth and crop yield in many regions of the world (Shinozaki et al., 2003). It causes several interlinked physiological consequences that are deleterious to plant cells. A common effect of drought stress is the overproduction of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radical. The excess formation of ROS can enhance oxidative stress, leading to cellular damage and metabolic disorders (Mittler, 2002; Fan and Liu, 2012). To keep a balance between the generation and removal of ROS, plants develop anti-oxidative defence systems, including non-enzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), etc., and enzymatic scavengers such as superoxide dismutase, catalase, peroxidase, and the AsA-GSH cycle enzymes. The AsA-GSH cycle is an important and powerful pathway to remove hydrogen peroxide (H_2O_2) in plants through utilizing the AsA and GSH (Dipierro et al., 2005; Noctor and Foyer, 1998). Previous studies reported that transgenic plants overexpressing the genes encoding key enzymes in AsA-GSH cycle enhanced stress tolerance by increasing contents of AsA and GSH (Eltayeb et al., 2007; Foyer et al., 1995). However, the process of regulating AsA-GSH cycle under drought condition is still largely unknown in plants.

Glucose-6-phosphate dehydrogenase (G6PDH), widely present in plant tissues, catalyzes the first committed step of the oxidative pentose phosphate pathway that produces NADPH to meet cellular needs for reductive biosynthesis. Increasing evidence has demonstrated that G6PDH is involved in resistance to various stresses, such as salinity, heat, drought and pathogens (Corpas and Barroso, 2014; Dal Santo et al., 2012; Gong et al., 2013; Liu et al., 2007, 2013; Scharte et al., 2009). It has been reported that drought stress induced the activity and expression of G6PDH in soybean seedlings (Liu et al., 2013), and that overexpression of a G6PDH-encoding gene enhanced drought tolerance of transgenic tobacco plants (Scharte et al., 2009). However, it remains unclear how drought activates G6PDH activity and how G6PDH modulates drought tolerance in plants. Although NADPH is also needed in the AsA-GSH cycle for protection against oxidative damage (Noctor and Foyer,

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1998), there is no detailed study investigating the role of G6PDH on regulating the AsA-GSH cycle under drought stress.

G6PDH activity has been detected in both cytosol and plastids, and evidence has demonstrated that there are two cytosolic (Cyto) and four plastidic isoforms, belonging to plastidial classes P1 and P2 (Wakao and Benning, 2005; Wendt et al., 1999). In addition, the presence of G6PDH in plant peroxisomes has been also reported (Corpas et al., 1998; Mateos et al., 2003). In the last few years, the roles of G6PDH isoforms have also been investigated in plants. The plastidic isoforms have been reported to be involved in providing reducing power for nutrient assimilation, and P1-class G6PDH proteins are increased in leaves while P2-class G6PDH proteins are induced in roots in response to nitrogen supply (Bowsher et al., 2007; Esposito et al., 2001, 2005). Regarding cytosolic isoforms, the *Arabidopsis* cytosolic *G6PDH* mutants produce seeds with a higher oil content, suggesting that Cyto-G6PDH is essential for the metabolism of developing seeds (Wakao et al., 2008). In addition, Cyto-G6PDH isoforms are crucial in supply of NADPH which is required for plant defense responses to pathogen infection in tobacco (Scharte et al., 2009) and salt stress in *Arabidopsis* via phosphorylation (Dal Santo et al., 2012). Therefore, each G6PDH isoform seems to play a diverse role in stress tolerance and development in plants. However, the roles of G6PDH isoforms in plant resistance to stresses are only beginning to be understood, and the regulatory mechanism of each G6PDH type is still poorly characterized.

Abscisic acid (ABA) is considered as an important signal involved in the adaptive response to various stresses, especially in response to drought stress (Cutler et al., 2010; Wang et al., 2015). ABA levels increase under drought conditions and stimulate the expression of genes associated with drought resistance (Seki et al., 2007; Zhang et al., 2015). It was also reported that the activity and expression of G6PDH were both increased in ABA-treated barley plants (Cardi et al., 2011). However, whether ABA is involved in regulating G6PDH activity under drought condition needs clarification. H_2O_2 is another important molecule involved in regulating the response of plants to various stresses. In addition to functioning as an endogenous oxidant, H_2O_2 has been suggested as a key signal for inducing the expression of genes associated with plant defense responses (Chen et al., 1993; Wang et al., 2010). In several studies, H_2O_2 is reported to be able to induce the activity and expression of G6PDH in plants (Liu et al., 2013; Wang et al., 2008). Although ABA and H_2O_2 seem to be possibly involved in inducing G6PDH activity, the roles of ABA and H_2O_2 in the regulation of the G6PDH activity under environmental stresses remain unknown.

Based on the above observations, the aims of this work were to investigate the roles of ABA and H_2O_2 in regulating G6PDH isoforms in roots of soybean under drought stress. In addition, the possible role of G6PDH under drought stress in regulating the AsA-GSH cycle was also investigated. The data obtained demonstrate that ABA and H_2O_2 mediate drought-induced increase in cytosolic G6PDH activity, and H_2O_2 may act downstream of ABA in this process. Furthermore, cytosolic G6PDH enhanced drought tolerance through regulating the AsA-GSH cycle in soybean roots.

2. Materials and methods

2.1. Plant culture and treatments

Seeds of soybean (*Glycine max* L.) were placed on wet sponge for 2 days to germinate at 25 °C in the dark. After germination, the seeds of similar size were chosen to be cultured in a container filled with 400 ml of 1/4 Hoagland solution medium. Seedlings were grown in a controlled growth chamber (25 °C, photosynthetic flux of 300 μmol m^{-2} s^{-1}, and 16/8 h light/dark photoperiod). After 3-d of culture, the seedlings were transferred to 1/4 Hoagland medium containing various treatment reagents. Different concentrations of polyethylene glycol 6000 (PEG6000, drought stress), abscisic acid (ABA) and hydrogen peroxide (H_2O_2) were added to the medium for various treatment. Glucosamine 6-phosphate (GN6P) was used to inhibit G6PDH activity (Liu et al., 2013; Scharte et al., 2009). Diphenylene iodonium (DPI) is an effective inhibitor of plasma membrane NADPH oxidase (Zhang et al., 2009). Fluridone and sodium tungstate were used as inhibitors of ABA biosynthesis (Seiler et al., 2011). Catalase (CAT, from bovine liver) was used to a scavenger of H_2O_2 (Yang et al., 2007). These reagents were obtained from Sigma chemical (St Louis). To investigate the effects of various inhibitors or scavengers, the roots of seedlings were pretreated with 0.5 mM GN6P, 20 μM DPI, 50 μM fluridone, 200 μM tungstate and 50 U/ml CAT prior to drought treatment. After collection and washing, the roots were used for parameters assay.

2.2. Determination of G6PDH activity

G6PDH (EC 1.1.1.49) extraction and assay were performed as described by Hauschild and von Schaewen (2003). Briefly, soybean roots (0.2 g) were ground in liquid nitrogen and extracted in 2 ml extraction buffer consisting of Hepes-Tris (50 mM, pH 7.8), $MgCl_2$ (3 mM), EDTA (1 mM), and phenylmethylsulfonyl fluoride (1 mM). The homogenate was then centrifuged at 12,000 *g* for 20 min at 4 °C. The supernatant (crude extract) was used to determine enzyme activity. To determinate the total G6PDH activity, a 100 μl aliquot of the extract was added to the assay buffer containing Hepes-Tris (50 mM, pH7.8), $MgCl_2$ (3.3 mM), glucose-6-phosphate (0.5 mM), and $NADPNa_2$ (0.5 mM). G6PDH activity was assayed at 25 °C by monitoring the reduction of $NADP^+$ to NADPH at 340 nm. DTT has been reported to be able to inhibit plastidic G6PDH, and the inhibition can be reversed if DTT is removed (Johnson, 1972). For the assay of cytosolic G6PDH activity, the sample was incubated with DTT_{red} (final concentration 62.5 mM) for 5 min at room temperature prior to measuring enzyme activity (Wenderoth et al., 1997). Plastidic G6PDH activity was calculated as the difference between total and cytosolic G6PDH activities.

2.3. Measurement of H_2O_2 content

H_2O_2 assay was performed as described by the method of Patterson et al. (1984). Roots (0.2 g) were ground with liquid nitrogen and extracted with 2 ml of cooled acetone. After centrifugation at 5000 *g* for 10 min at 4 °C, 1 ml of the supernatant was mixed with 0.1 ml of concentrated ammonia and 0.1 ml of 5% $TiSO_4$. After centrifugation at 3000 *g* for 10 min, the titanium-peroxide complex was resuspended in 4 ml of 2 M H_2SO_4. H_2O_2 content was measured by reading the absorbance at 415 nm with a spectrophotometer.

2.4. Determination of plasma membrane (PM) NADPH oxidase activity

PM isolation was performed according to the method of Yang et al. (2003). Briefly, root sample (5 g) was homogenized in 15 ml isolation buffer containing 250 mM sucrose, 25 mM Hepes-Tris (pH 7.6), 1 mM DTT, 1 mM EDTA, 1.5% polyvinylpyrrolidone, 1 mM PMSF. The whole isolation procedures were performed at 4 °C. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 *g* for 20 min. The supernatant was centrifuged at 80,000 *g* for 30 min to obtain a microsomal pellet, which was resuspended in a buffer containing 250 mM sucrose, 1 mM EDTA, 1 mM DTT, and 2 mM Hepes-Tris (pH 7.2). The extract was used for PM H^+-ATPase activity determination.