Journal of Plant Physiology **165** (2008) 813—824

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JOURNAL OF PLANT PHYSIOLOGY

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Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells

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Received 28 May 2007; received in revised form 23 July 2007; accepted 23 July 2007

KEYWORDS
Antioxidant defense;
Glycinebetaine;
Glyoxalase;
Proline;
Salt stress

Summary

Salt stress impairs reactive oxygen species (ROS) and methylglyoxal (MG) detoxification systems, and causes oxidative damage to plants. Up-regulation of the antioxidant and glyoxalase systems provides protection against NaCl-induced oxidative damage in plants. Thiol–disulfide contents, glutathione content and its associated enzyme activities involved in the antioxidant defense and glyoxalase systems, and protein carbonylation in tobacco Bright Yellow-2 cells grown in suspension culture were investigated to assess the protection offered by proline and glycinebetaine against salt stress. Salt stress increased protein carbonylation, contents of thiol, disulfide, reduced (GSH) and oxidized (GSSG) forms of glutathione, and the activity of glutathione-*S*-transferase and glyoxalase II enzymes, but decreased redox state of both thiol–disulfide and glutathione, and the activity of glutathione peroxidase and glyoxalase I enzymes involved in the ROS and MG detoxification systems. Exogenous application of proline or glycinebetaine resulted in a reduction of protein carbonylation, and in an increase in glutathione redox state and activity of glutathione peroxidase, glutathione-*S*-transferase and glyoxalase I under salt stress. Neither proline nor glycinebetaine, however, had any direct protective effect on NaCl-induced GSH-associated enzyme activities. The present

Abbreviations: betaine, glycinebetaine; CDNB, 1-chloro-2, 4-dinitrobenzene; DTNB, 5, 5′-dithiobis(2-nitrobenzoic acid); GPX, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-*S*-transferase; MG, methylglyoxal; ROS, reactive oxygen species; SDL, *S*-D-lactoylglutathione

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0176-1617/$ - see front matter
doi:10.1016/j.jplph.2007.07.013

study, therefore, suggests that both proline and glycinebetaine provide a protective action against NaCl-induced oxidative damage by reducing protein carbonylation, and enhancing antioxidant defense and MG detoxification systems.

Introduction

Osmotic stress caused by salinity is one of the major abiotic factors limiting crop productivity because it affects almost all plant functions. To counteract osmotic stress, many plants accumulate several kinds of compatible solutes such as proline, glycinebetaine (from now on betaine), sugars and polyols. Proline and betaine are the most common compatible solutes that contribute to osmotic adjustment (Greenway and Munns, 1980; Rhodes and Hanson, 1993; Hasegawa et al., 2000; Ashraf and Foolad, 2007), and stabilization and protection of membranes, proteins and enzymes (McNeil et al., 1999; Okuma et al., 2000, 2002; Ashraf and Foolad, 2007) from damaging effects of salt/osmotic stresses. In addition to their roles as osmoprotectants, proline and betaine might perform a protective function by scavenging reactive oxygen species (ROS). It has been reported that proline scavenges free radicals (Hasegawa et al., 2000; Hong et al., 2000; Okuma et al., 2000, 2004) and ROS (Chen and Dickman, 2005). Exogenous proline and betaine improve salt tolerance by up-regulating stress-protective proteins (Khedr et al., 2003) and reducing oxidation of lipid membranes (Demiral and Türkan, 2004; Okuma et al., 2004).

Environmental stresses including salinity induce the production of ROS (Hasegawa et al., 2000; Apel and Hirt, 2004) and methylglyoxal (MG) (Yadav et al., 2005a, b) in plant cells. ROS are highly reactive and toxic to plants and can lead to cell death by causing damage to proteins, lipids, DNA and carbohydrates (Noctor and Foyer, 1998; Apel and Hirt, 2004). MG can react with and modify other molecules including DNA and proteins (Yadav et al., 2005b). Proteins are one of the major targets of ROS, and oxidation of proteins can lead to the formation of protein carbonyl derivatives or peptide fragments. Plants possess both enzymatic and non-enzymatic antioxidant defense systems to protect their cells against ROS (Noctor and Foyer, 1998; Apel and Hirt, 2004). Thiol or thiol-containing compounds are chemically the most active groups found in cells and are known to act as antioxidants, and participate in the detoxification of xenobiotics. Reduced glutathione (GSH) is the most abundant low molecular weight thiol in plants and plays an important role in the detoxification of ROS (Noctor and Foyer, 1998; Noctor et al., 2002; Smirnoff, 2005). Glutathione peroxidase (GPX) is an antioxidant enzyme that catalyzes the reduction of H_2O_2, organic hydroperoxides and lipid peroxides using GSH. Recently, Miao et al. (2006) suggest that *Arabidopsis thaliana GPX3* might play a role in H_2O_2 homeostasis, acting as a general scavenger. The conjugation of GSH to a variety of hydrophobic, electrophilic and cytotoxic substrates is accomplished by a multifunctional enzyme glutathione-*S*-transferase (GST). GSH is also essential for MG metabolism in eukaryotes by the glyoxalase system, comprising two enzymes, glyoxalase I and glyoxalase II. Glyoxalase I catalyzes the formation of *S*-D-lactoylglutathione (SDL) from the hemithioacetal formed non-enzymatically from MG and GSH, while glyoxalase II catalyzes the hydrolysis of SDL to regenerate GSH and liberate D-lactate (Thornalley, 1990).

There are reports on the changes in content and activity of different components of the antioxidant defense and glyoxalase systems in plant responses to salt stress (Noctor and Foyer, 1998; Veena et al., 1999; Hasegawa et al., 2000; Shalata et al., 2001; Mittova et al., 2003a, b; Singla-Pareek et al., 2003; Saxena et al., 2005; Yadav et al., 2005a, b; Hoque et al., 2007a, b). It is expected that up-regulation of the components of the antioxidant and glyoxalase systems offered by proline and betaine protects plants against NaCl-induced oxidative damage. We have earlier shown that both proline and betaine improve salt tolerance in tobacco BY-2 cells by increasing the activity of enzymes involved in the antioxidant defense system (Hoque et al., 2007a, b). The role of proline and betaine in aspects of antioxidant defense has also been reported in plant cells and in fungal pathogenesis during various oxidative stresses (Khedr et al., 2003; Demiral and Türkan, 2004; Okuma et al., 2004; Chen and Dickman, 2005; Ma et al., 2006; Molinari et al., 2007). Thus, to gain better knowledge of the antioxidant defense mechanism as well as glyoxalase system provided by proline and betaine under salt stress, we investigated the effect of proline and betaine on the contents of thiol, disulfide and glutathione, and their redox states, activity of GSH-related enzymes involved in