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Exogenous proline and glycinebetaine increase NaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells

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Summary

Up-regulation of the antioxidant system provides protection against NaCl-induced oxidative damage in plants. Antioxidants and activity of enzymes involved in the ascorbate–glutathione (ASC–GSH) cycle in tobacco Bright Yellow-2 (BY-2) were investigated to assess the antioxidant protection offered by exogenous proline and glycinebetaine (betaine from now on) against salt stress using cells grown in suspension culture. Reduced ascorbate (ASC) was detected in BY-2 cells but dehydroascorbate (DHA) was not. Large quantities of a reduced form of glutathione (GSH) and smaller quantities of an oxidized form of glutathione (GSSG) were detected in BY-2 cells. Salt stress significantly reduced the contents of ASC and GSH as well as activities of ASC–GSH cycle enzymes such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Exogenous proline or betaine increased the activities of all enzymes except MDHAR involved in NaCl-induced ASC–GSH cycle. Levels of ASC and GSH in BY-2 cells under salt stress were lower in the presence of

Abbreviations: APX, ascorbate peroxidase; ASC, reduced ascorbate; ASC–GSH, ascorbate–glutathione; Betaine, glycinebetaine; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; POX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase

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proline or betaine than in the absence of proline or betaine whereas there was no difference in redox status. Proline proved more effective than betaine in maintaining the activity of enzymes involved in NaCl-induced ASC–GSH cycle. Neither proline nor betaine had any direct protective effect on NaCl-induced enzyme activity involved in the antioxidant system; however, both improved salt tolerance by increasing enzyme activity. The present study, together with our earlier findings [Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. J Plant Physiol 2006;164:553–61.], suggests that proline offered greater protection against salt stress than betaine did because proline was more effective in increasing the activity of enzymes involved in the antioxidant system.

Introduction

Osmotic stress caused by salinity is one of the major abiotic factors limiting crop productivity because it affects almost all plant functions (Bohnert and Jensen, 1996). To counteract osmotic stress, many plants accumulate several kinds of compatible solutes such as proline, glycinebetaine (now on betaine), sugars, and polyols. Proline and betaine are well-known compatible solutes that play a pivotal role in the process of osmotic adjustment in different organisms including higher plants (Flowers et al., 1977; Greenway and Munns, 1980; Rhodes and Hanson, 1993; Hasegawa et al., 2000). Most plant species can accumulate proline but some cannot accumulate betaine because they are deficient in the enzymes involved in betaine biosynthesis (Rathinasabapathi et al., 1993; Holmström et al., 2000). Salt stress up-regulates the enzymes involved in proline and betaine biosyntheses in several plant species (Russell et al., 1998; Hare et al., 1999), and elevated levels of proline and betaine accumulated in plant cells correlate with enhanced stress tolerance (Chen et al., 2000; Munns, 2005).

Proline and betaine are considered to be involved in scavenging free radicals and in protecting enzymes in addition to their well-established roles as osmolytes. For example, Hong et al. (2000) suggest that the role of proline as a free radical scavenger is more important in overcoming stress than its role as a simple osmolyte. It has been shown that proline acts as a free radical scavenger (Okuma et al., 2004; Chen and Dickman, 2005) to alleviate salt stress, while betaine acts only as a simple osmolyte (Okuma et al., 2004). It is reported that proline and betaine act as enzyme protectants against abiotic stresses (Okuma et al., 2000; Sharma and Dubey, 2005) and protect higher plants against salt/osmotic stresses by stabilizing many functional units such as complex II electron transport (Hamilton and Heckathorn, 2001), membranes and proteins (Paleg et al., 1984; Hare et al., 1998; Mansour, 1998; McNeil et al., 1999; Holmström et al., 2000), and enzymes such as RUBISCO (Mäkela et al., 2000).

There is considerable evidence that exogenous proline and betaine mitigate the detrimental effects of salinity (Harinasut et al., 1996; Okuma et al., 2000, 2004). In earlier studies, we also demonstrated that both exogenous proline and betaine mitigated the inhibition of NaCl-induced growth of tobacco BY-2 cells but the mitigating effect of proline was more pronounced than that of betaine (Hoque et al., 2006). Moreover, exogenous proline improves salt tolerance by up-regulating stress-protective proteins (Khedr et al., 2003) and reducing oxidation of lipid membranes (Okuma et al., 2004). It is also reported that exogenous betaine improves stress tolerance by preventing photoinhibition (Ma et al., 2006) and reducing oxidation of lipid membranes (Chen et al., 2000; Demiral and Türkan, 2004) in a wide variety of accumulator/non-accumulator plants.

Salt stress induces the production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These ROS are necessary for inter- and intracellular signaling (Foyer and Noctor, 1999) but at high concentrations they seriously disrupt normal metabolism of plants through oxidation of membrane lipids, proteins, and nucleic acids in the absence of any protective mechanism (Noctor and Foyer, 1998; Hernández et al., 2001). Plants possess both enzymatic and non-enzymatic antioxidant defense systems to protect their cells against ROS. The ascorbate–glutathione (ASC–GSH) cycle plays a key role in this defense system (Jiménez et al., 1997; Noctor and Foyer, 1998). In this cycle, reduced ascorbate (ASC)