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SALT STRESS AND GLUTATHIONE-DEPENDENT ENZYME ACTIVITIES IN BEAN (*PHASEOLUS VULGARIS* L.)

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Summary. The effects of two salts, NaCl and Na_2SO_4, applied at a concentration of 100 mM each in the root medium, on the antioxidant enzymes (GR, GPX and GST) and glutathione (GSH) homeostasis of hydroponically grown bean plants (*Phaseolus vulgaris* L.) were studied. An increased GPX activity as well as decreased GSH content in both root and leaf of salt-treated plants were well expressed. The other enzymes studied (GR and GST) responded organ- and salt-specifically. The results obtained showed that both salts provoked oxidative stress responses in the treated bean plants.

Key words: antioxidative enzymes; gutathione *S*-transferase; *Phaseolus vulgaris* L.; salt stress.

Abbreviations: ROS – reactive oxygen species, GSH – reduced glutathione, ASC – ascorbate, GR – glutathione reductase, GPX – glutathione peroxidase, GST – glutathione S-transferase, CDNB – 1-chloro-2,4-dinitrobenzene, NBC – 4-nitrobenzyl chloride.

INTRODUCTION

Salinization of agricultural areas due to intensive practices and irrigation is an important feature limiting crop yield and productivity. Inhibition of plant growth and even plant death by salinity is due to a reduction in water availability, sodium ion accumulation and mineral imbalances. In this context, ROS will be produced in both chloroplasts and mitochondria, which are highly reactive and often toxic to plant cells. Plant cells possess both enzymatic and non-enzymatic antioxidants to cope with oxidative damages (Jogeswar et al., 2006). The primary components of the cell antioxidative defence network are glutathione (GSH), ascorbate (ASC)

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and carotenoids as well as enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), peroxidases and the enzymes involved in the ASC-GSH cycle (Asada, 1992). The activities of the antioxidant enzymes are reported to increase under various environmental stresses, including salinity (Hernandez et al., 2000). Generally, salt-tolerant cultivars show higher activity of these antioxidant enzymes as compared to salt-sensitive ones (Sairam et al., 2002). Most salinity studies are focused on plant responses to NaCl stress, but also in many cases Na_2SO_4 salinity may be a major problem. The present paper aims to compare the effects of both salts on antioxidative defence network of young bean plants.

MATERIALS AND METHODS

The bean plants (cv. Lody) were grown in a greenhouse on perlite in pots and ½ Hoagland nutrient solution was added in the trays. Treatment with either 100 mM NaCl or 100 mM Na_2SO_4 was performed for 7 days starting at the appearance of the first trifoliate leaf unfolded. The extract preparation was performed according to published procedures (Götz and Schröder, 2005). Extracts were stored at -80°C until used**.** GR (EC 1.6.4.2) activity was quantified *in vitro* by the method of Schickler and Caspi (1999). GPX (EC 1.11.19) activity was estimated according to Dixon et al. (1998). For determination of GST (EC 2.5.1.18) activity three model substrates: 1-chloro-2,4-dinitrobenzene (CDNB), 4-nitrobenzyl chloride (NBC) and fluorodifen, were used (Habig et al., 1974; Scalla and Roulet, 2002). Protein was estimated according to Bradford (1976). The GSH content was measured by HPLC according to Siller-Cepada et al. (1991). Statistical analysis was performed using one-way ANOVA (for $P<0.05$). Based on ANOVA results a Tukey's test for main comparison at 95% confidential level was applied.

RESULTS AND DISCUSSION

In addition to its role in the transport and storage of sulfur in plants, the thiol tripeptide GSH participates in the removal of peroxides through the ASC-GSH cycle as well as in the control of cell redox status (Rennenberg, 1995). GSH is keeping ascorbate in its reduced form and the levels of GSH are critical in the protection of the chloroplast from oxidative damage (Hausladen et al., 1993). Glutathione is the substrate of GPX reactions and GST, which also serves in the removal of ROS and their reaction products (Tausz et al., 2004). GSTs are potent detoxification enzymes, catalyzing the nucleophilic attack of GSH at electrophilic pollutant molecules and products of oxidative stress (Schröder and Berkau, 1993) and the resulting complex is sequestered in the vacuole. Some of the GST isoforms are induced by changes in the thiol concentrations (Schröder and Pflugmacher, 1996). Results of several studies indicate that GST may be involved in plant tolerance to different abiotic and biotic stress factors. Induction of this enzyme has been reported in plant response to different xenobiotics, heavy metals (Lyubenova et al., 2007) as well as salt stress (Sudhakar et al., 2001).

After the salt treatment the GPX activity in both leaves and roots increased as compared with untreated control plants (Table 1). GST activity was measured using the three model substrates

Table 1. Effect of salinity on GST, GR and GPX activities in bean plants. Activity is expressed in [μkat/mg protein]. Each value represents the mean of six measurements ±SD.

Parameters	GST:CDNB	GST:NBC	GST: Fluorodifen	GR	GPX
			Leaves		
Control	1.99±0.19^{ab}	0.06±0.01^{a}	1.06±0.10^{a}	1.02±0.13^{b}	0.89±0.27^{a}
NaCl	1.95±0.15^{a}	0.11±0.02^{ab}	1.09±0.06^{a}	1.46±0.13^{c}	1.12±0.27^{a}
Na_2SO_4	2.24±0.22^{b}	0.19±0.11^{b}	2.26±0.12^{b}	0.53±0.29^{a}	1.00±0.07^{a}
			Roots		
Control	0.68±0.10^{a}	0.12±0.01	1.05±0.05^{c}	0.31±0.04^{a}	0.60±0.08^{a}
NaCl	0.65±0.16^{a}	No activity	0.26±0.05^{a}	0.35±0.07^{a}	0.89±0.12^{b}
Na_2SO_4	0.59±0.22^{a}	No activity	0.41±0.11^{b}	0.30±0.05^{a}	1.20±0.11^{c}

Within the same column values followed by the same letter (a, b or c) are not different for $P<0.05$.

mentioned above. A well-expressed response was detected only with the herbicide fluorodifen as a model substrate whereas the salinity stress did not induce significant differences in the enzyme activity with CDNB-GST and GST-NBC models. Whereas fluorodifen conjugation was stimulated in the leaves of Na_2SO_4-treated plants, the opposite tendency was observed in the roots of plants exposed to both salts. The detected GSH level in both leaves and roots of salt-treated bean plants was lower as compared to untreated ones, especially in the leaves (Fig. 1). To some extent this may be due to the lower leaf GR activity in Na_2SO_4-treated plants as it catalyzes the regeneration of GSH from its oxidized form (GSSG). The results obtained in this study correspond to the opinion of Roxas et al. (1997) that the expression of GST/GPX activity in plants represents cell metabolism acclimation to salt stress. In conclusion, the application of both salts (NaCl and Na_2SO_4) to the

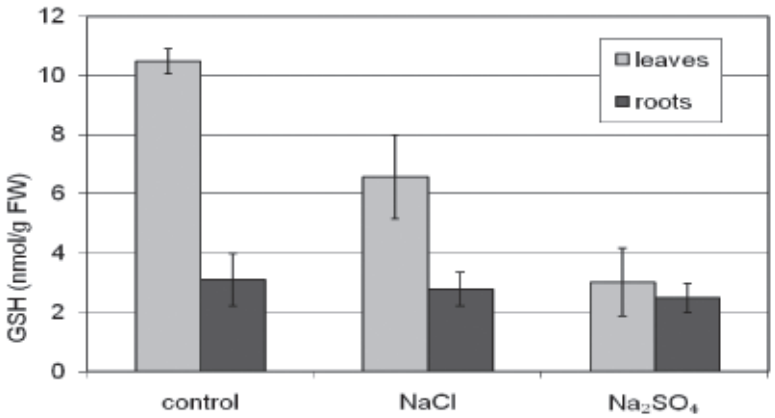

Fig. 1. Changes in GSH (nmol/g FW) content under salinity stress.

root medium generated oxidative stress responses in bean plants. The indicators of this physiological state were the lower GSH level and the enhanced activity of antioxidant enzymes like GPX. The responses of GST and GR were more specific and probably governed by other stress parameters.

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