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Investigations of glutathione S-transferase and peroxidase activities in auxin heterotrophic and autotrophic tobacco calli under salt stress conditions

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ABSTRACT Auxin autotrophic and heterotrophic lines of tobacco calli may differ not only in their indoleacetic acid (IAA) synthetizing abilities and sensitivities to exogenous auxins, but also in their stress tolerance. Auxin autotrophic callus tissues were generated and the growth of cultures was compared with that of the heterotrophic lines of the same tissues on MS medium containing different concentrations of NaCl. The growth remand usually higher in autotrophic calli than in heterotrophic calli with increasing NaCl concentrations. The glutathione S-transferase and peroxidase activities were higher in the autotrophic lines, but their level did not increase further with the salt treatment as much as in the heterotrophic tissues.
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KEY WORDS

salt stress
glutathione S-transferase
glutathione peroxidase
auxin autotrophic calli

Glutathione S-transferases (GSTs) are ubiquitous enzymes catalysing the addition of reduced glutathione (GSH) to electrophilic substrates, which tags them for vacuolar sequestration (Edwards et al. 2000). GSTs have direct cytoprotective activities and they might be essential for the preservation of plants during environmental stress and disease, as well as for the support of normal development (Marrs 1996). In addition to catalyzing GSH conjugation, GSTs also exhibit glutathione peroxidase (GSH-PX) activity, which suggests a role in protection against oxidative stress. Identification of GSH-PX activity in plants, isolation of plant genes with sequence homology to the animal analogs, isolation and characterization of the protein product proved that enzymes exist in plants belong to the phospholipid hydroperoxid glutathione peroxidase family. The function of phospholipid hydroperoxide glutathione peroxidases is reduction of alkyl hydroperoxides, such as fatty acid hydroperoxides (Eshdat et al. 1997). The investigations of these scavenging enzymes is important, because the level of highly cytotoxic alkyl hydroperoxides increase under different stress conditions.

Materials and Methods

The callus cultures were initiated from protoplasts of *Nicotiana tabacum* SR1 plants, as published earlier (Szabó et al. 1995). The cultures were kept in a growth chamber at 25 ºC and under 8.4 Wm^{-2} warm white fluorescent light (Tungsram F29 lamps, Hungary). GST activity was determined spectrophotometrically by using the artificial substrate CDNB according to Habig et al. (1974) with some modifications described earlier (Csiszár et al. 2001). Gluthatione peroxidase activity was measured according to Awasthi et al. (1975) with cumene hydroperoxide substrate. Guaiacol peroxidase activity was determined by the method of Upadhyaya et al. (1985).

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Results and Discussion

The growth rates of auxin-dependent (heterotrophic) and independent (autotrophic) calli as a function of the concentration of exogenous NaCl demonstrated that the auxin-synthetizing autotrophic calli were less sensitive to salt stress. In the autotrophic calli the NaCl-caused growth inhibition started later, and the growth level were higher than that in the heterotrophic lines. Comparison of the GST activities in the heterotrophic and autotrophic lines revealed higher GST activities in the autotrophic calli. The enzyme activities increased with the increasing NaCl concentrations in the heterotrophic calli, this was not found in the autotrophic tissues. The level of GSH-PX activity was also higher in the autotrophic calli. It is suggested, that the higher GST and GSH-PX activities of the autotrophic lines play a role in the elevated stress tolerance.

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