GLUTATHIONE S-TRANSFERASE ACTIVITY IN COTTON PLANTS AND CALLUS SUBJECTED TO SALT STRESS M. C. Lucas, T. Fowler, and D. R. Gossett Louisiana State University-Shreveport Shreveport, LA

<u>Abstract</u>

Callus tissue of Coker 312 adapted to grow at 150 mM and 250 mM NaCl showed significant increases of glutathione S-transferase activity (GST) compared to callus tissue grown at 0 mM NaCl. Greenhouse-grown salt-tolerant Acala 1517-88 plants treated with either O or 150 mM NaCl were also analyzed for differences in GST activity. The Acala 1517-88 exhibited increased levels of GST when stressed. The NaCl-induced increase in the activity of GST suggests that this enzyme may increase the cell's ability to respond to lipid peroxidation induced by salt stress.

Introduction

Glutathione S-transferase (GST) catalyzes the conjugation of glutathione with a variety of xenobiotics, aiding their detoxification and metabolism. GST substrates include herbicides, insecticides, and organic peroxides (Guddewar and Dauterman, 1979; Mozer et al. 1983; Clark et al. 1986.; Ketterer and Meyer, 1989). GST activity has been demonstrated to be induced by herbicide antidotes (safeners) in corn (Mozer et al. 1983; Fuerst et al. 1993) and sorghum (Dean et al. 1990). Auxin induced GST's have also been found in tobacco (Droog et al. 1995). GST is also known to possess a residual glutathione peroxidase activity (GSTpx) capable of reducing organic hydroperoxides (Reddy et al. 1981).

Salt stress has been shown to elevate levels of certain antioxidants (i.e. glutathione reductase, peroxidase, and alpha-tocopherol) in cotton plants and callus (Gossett et al. 1994a; Gossett et al. 1994b). This study was designed to determine if salt stress has an effect on GST levels in callus and the whole plant.

Methods and Materials

Control and salt-adapted cotton callus tissue, derived from Coker 312, were grown and maintained according to the protocol of Gossett et al. 1994b. Cotton plants (Acala 1517-88) were grown and salt stressed according to the procedures of Gossett et al. 1994a. Glutathione Stransferase (GST) activity from leaf or callus tissue was assayed according to the method of Habig et al. (1974). One unit of enzyme activity was defined as the amount of enzyme required to couple one nmole of glutathione to 1chloro-2,4-dinitrobenzene (CDNB) per minute at 25° C. Data points are based on a mean of a minimum of four replicates.

Results and Discussion

Table 1 shows that there was a substantial increase in GST activity in callus adapted to grow at 150 mM or 250 mM NaCl compared to the 0 mM control. A similar increase was previously seen in antioxidant enzyme activities including superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, and glutathione reductase (Gossett et al. 1994b).

When plants of the salt-tolerant cultivar (Acala 1517-88) were subjected to salt stress (Table 2), the GST activity paralleled the results from previous antioxidant studies (Gossett et al. 1994a). The Acala 1517-88 appears to be able to induce GST activity in response to the stress.

Gossett et al. (1994a) demonstrated that increased lipid peroxidation occurred during salt stress of plants. Given the fact that GST is able to couple glutathione with lipid peroxides and/or reduce lipid peroxides with its residual glutathione peroxidase (GSTpx) activity, it is easy to understand the value of this enzyme to callus or whole plants undergoing salt stress.

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References

1. Clark, A. G., N. A. Shamaan, M. D. Sinclair, and W. C. Dauterman. 1986. Insecticide metabolism by multiple glutathione S-transferases in two strains of the housefly, *Musca domestica* L. Pestic. Biochem. Physiol. 25:169-175.

2. Dean, J. V., J. W. Gronwald, and C. V. Eberlein. 1990. Induction of glutathione S-transferase isozymes in sorghum by herbicide antidotes. Plant Physiol. 92:467-473.

3. Droog, F. N. J., P. J. J. Hooykaas, and B. J. van der Zall. 1995. 2,4-Dichlorophenoxyacetic acid and related chlorinated compounds inhibit two auxin-regulated type-III tobacco glutathione S-transferases. Plant Physiol. 107:1139-1146.

4. Fuerst, E. P., G. P. Irzyk, and K. D. Miller. 1993. Partial characterization of glutathione S-transferase isozymes induced by the herbicide safener benoxacor in maize. Plant Physiol. 102: 795-802.

5. Gossett, D. R., E. P. Millhollon, and M. C. Lucas. 1994a. Antioxidant response to NaCl stress in salt-tolerant

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and salt-sensitive cultivars of cotton. Crop Sci. 34: 706-714.

6. Gossett, D. R., E. P. Millhollon, M. C. Lucas, S. W. Banks, and M. M. Marney. 1994b. The effects of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.) Plant Cell Reports. 13:498-503.

7. Guddewar, M. B. and W. C. Dauterman. 1979. Purification and properties of a glutathione S-transferase from corn which conjugates S-triazine herbicides. Phytochemistry. 18:735-740.

8. Habig, W. H., M. J. Pabst, and W. B. Jakoby. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139.

9. Ketterer, B. and D. J. Meyer. 1989. Glutathione transferases: a possible role in the detoxication and repair of DNA and lipid hydroperoxides. Mutat. Res. 21: 33-40.

10. Mozer, T. J., D. C. Tiemeier, and E. G. Jaworski. 1983. Purification and characterization of corn glutathione Stransferase. Biochem. 22:1068-1072.

11. Reddy, C. C., C. D. Tu, J. R. Burgess, C. Ho, R. W. Scholz, and E. J. Massaro. 1981. Evidence for the occurrence of selenium-independent glutathione peroxidase activity in rat liver microsomes. Biochem. Biophys. Res. Comm. 101:970-978.

Table 1. Glutathione S-transferase (GST) activity (units/g fresh weight) in	
Coker 312 callus adapted to 0 mM, 150 mM, and 250 mM NaCl.	

mM NaCl	nmoles/min/g	
0	34 ± 5	
150	94 ± 19	
250	224 + 34	

leaves from salt stre	ssed Acala 1517-88 plants.	
mM NaCl	nmoles/min/g	
0	< 1	
150	8 ± 2	