GLUTATHIONE S-TRANSFERASE ISOZYMES IN CONTROL AND SALT-ADAPTED COTTON CALLUS

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Abstract

Previous studies have revealed increased levels of antioxidants such as peroxidase, catalase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase in NaCl stressed cotton callus (*Gossypium hirsutum* L.) and plant. Another study has revealed an elevation of the antioxidant glutathione S-transferase (GST) during salt stress in cotton callus and plant. This study was designed to determine the isozyme pattern of GST in callus grown at 0 mM NaCl and 150 mM NaCl using 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid (ECA) as substrates. GST revealed four major and two minor isozymes in the salt stressed callus versus one isozyme in the control callus using CDNB as substrate. Six major isozymes were evident in both the control and salt stressed callus when ECA was provided as substrate. Substrate specificity and both isozyme enhancement and induction are evident when the cotton callus is subjected to NaCl stress. GST's induction when salt stress is applied may impart an increased ability for the plant cell to respond to NaCl associated lipid peroxidation.

Introduction

Environmental stress in plants often results in oxidative stress as well. (Asada, 1994; Krause, 1994). Plants protect themselves from cytotoxic reactive oxygen metabolites (ROM) through antioxidants, and many investigators have shown plants with increased levels of these antioxidants, either constitutive or induced, can result in resistance to damage by ROM's evoked during different environmental stress conditions (Dhindsa and Matowe, 1981; Harper and Harvey, 1978; Wise and Naylor, 1987; Monk and Davies, 1989; Spychalla and Desborough, 1990; Mandamanchi and Alscher, 1991; Poole and Rennenberg, 1994).

Previous studies in salt stressed cotton plant (*Gossypium hirsutum* L.) and callus (Gossett *et al*., 1994a, 1994b), have indicated increased levels of antioxidants such as catalase, ascorbate peroxidase, peroxidase, glutathione reductase, and superoxide dismutase. Previous research has also indicated an elevation of the antioxidant enzyme glutathione S-transferase (GST) during NaCl stress in cotton plant and callus (Lucas *et al*., 1996).

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GST catalyzes the conjugation of the tripeptide glutathione to a large array of xenobiotics in order to detoxify them. Substrates for GST include insecticides, herbicides, and organic peroxides (Guddewar and Dauterman, 1979; Mozer *et al*. 1983; Clark *et al*. 1986; Ketterer and Meyer, 1989). Induction of GST by herbicide safeners was demonstrated in sorghum (Dean *et al*. 1990) and corn (Mozer *et al*. 1983; Fuerst *et al*. 1993). In tobacco, auxin induced GST's have also been found (Droog *et al*. 1995). In several plants, GST was shown to possess glutathione peroxidase activity (GSTpx) for reducing organic hydroperoxides (Reddy *et al*. 1981). Salt stress has been shown to elevate lipid peroxidation in cotton plants (Gossett *et al*. 1994a).

In light of this evidence, a study was designed to visualize the isozyme pattern of GST in response to NaCl stress in cotton callus using 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid (ECA) as substrates.

Methods and Materials

Callus tissue for the cotton cultivar Coker 312 was generated according to the method of Trolinder and Goodin (1987). A salt-tolerant Coker 312 cell line was developed according to the method outlined by Gossett *et al*. (1994b). 30 g callus samples were extracted according to the method of Habig *et al*. (1974). Protein purification and isozyme elution was carried out according to the method described by Mozer *et al*. (1983). Each column eluted fraction was assayed according to the method outlined by Habig *et al*. (1974). One unit of enzyme activity was defined as the amount of enzyme required to couple one hmole of glutathione to CDNB or ECA per minute at 25° C. Data points are based on a mean of a minimum of four replicates.

Results and Discussion

In Figure 1, when CDNB is used as the xenobiotic substrate for GST, four major and two minor isozymes of GST are evident in the 150 mM NaCl adapted cotton callus, versus one GST isozyme in the non-adapted cotton callus. This one isozyme in the control callus eluted at the same point as peak C in figure 1 and is significantly enhanced in the salt-adapted callus. Due to only one isozyme evident in the control callus, four isozymes of GST appear to be induced in the salt stressed callus.

Figure 2 reveals six major isozymes of GST using ECA as substrate in both the control and salt stressed callus. Each isozyme present in the control callus is enhanced three to four fold in the salt adapted cotton callus, while no isozymes appear to be induced by salt stress when ECA is used as substrate.

In comparing the salt stress induced isozymes of GST with substrates ECA and CDNB, it appears isozymes B and C in figure 2 are present when ECA is used, but are not apparent when CDNB is used. On the other hand, the minor

isozymes B_1 and B_2 in figure 1 respond only to CDNB and not ECA. This evidence appears to reflect substrate specificity for several isozymes of GST.

Isozymes of GST have been shown to be induced in many species of plants when various stresses other than NaCl are applied (Marrs, 1996). It appears several isozymes of GST are either induced or significantly elevated when NaCl stress is applied in the cotton callus using CDNB and ECA as substrates and that substrate specificity is evident for several of the NaCl induced isozymes of GST.

The NaCl-induced increase in the activity of the isozymes of GST suggest this enzyme may help the plant cell to respond to lipid peroxidation associated with salt stress.

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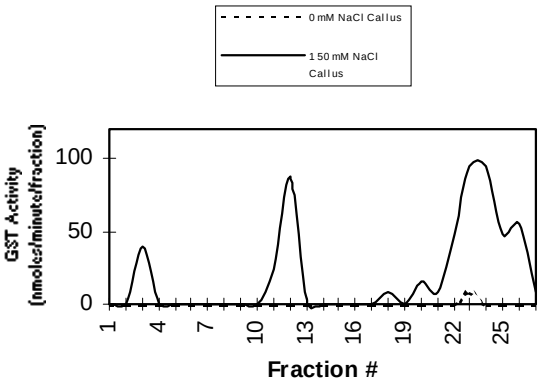

Figure 1 Isozyme profile of GST using CDNB as substrate

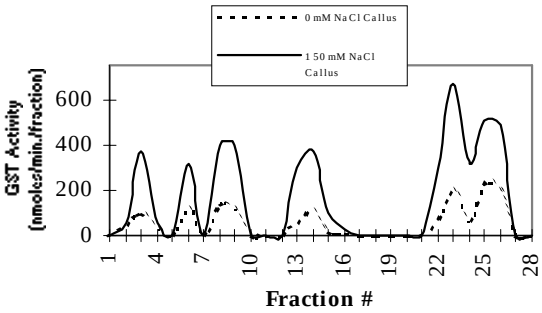

Figure 2 Isozyme profile of GST using ECA as substrate