ANTIOXIDANT RESPONSE TO COLD STRESS DURING FIBER DEVELOPMENT

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Abstract

Ovules from flowers removed 2 d postanthesis (DPA) from field-grown Acala 1517-88 (AC-88) and Coker 312 (CO-312) were cultured either at a constant temperature of 30°C or at 18 DPA subjected to a temperature of 15°C for 15 h and then returned to 30°C. At 24 DPA the ovules were harvested and analyzed for antioxidant enzyme activity. Peroxidase and ascorbate peroxidase activities were similar in both cultivars, but cold stress appeared to cause a decrease in peroxidase activity in the ovules of both AC-88 and CO-312. Catalase, glutathione reductase, superoxide dismutase, and glutathione S-transferase activities ranged two- to three-fold higher in both the control and cold-stressed ovules of AC-88.

Introduction

Oxidative stress often results when plants are exposed to environmental stress (Asada, 1994; Krause, 1994). Plants possess a number of antioxidants for protection against the cytotoxic species of activated oxygen, and plant response to environmental stress most likely involves a cascade of events, some of which evoke the antioxidant defense system. A number of investigators have shown that high levels of antioxidants, either constitutive or induced, can result in resistance to damage by activated oxygen species generated 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).

Cotton fiber development is adversely affected by low temperatures (Gipson, 1985; Haigler *et al*., 1991). Decreasing night temperatures affect both fiber elongation and cell wall development, and night temperatures below 22°C increase the time required for fibers to reach potential maximum length (Gipson and Joham, 1969; Gipson, 1985). Chilling tolerance has often been associated with the complex biochemical and physiological changes associated with the acclimation response (Baker, 1994). However, chilling tolerance has also been correlated to increases in antioxidant activity (de Kok and Oosterhuis, 1983; Schoner and Krause, 1990; Spychalla and Desborough. 1990). Haigler *et al*. (1991) have shown that cultured ovules can serve as valid models for investigation of the regulation of the field cool temperature response. Thus, the objective of this project was to determine if cultured ovules subjected to cold stress during fiber development would produce an antioxidant response similar to that observed in vegetative tissue.

Methods and Materials

Flowers were removed 2 DPA from field-grown cotton plants, cv Acala 1517-88 (AC-88) and Coker 312 (CO-312). After the ovaries were surface sterilized, the ovules were dissected and cultured by floating on top of the medium according to the method of Beasley *et al*. (1974). Ovules were cultured in dark incubators set at a constant temperature of 30°C. At 18 DPA when the fibers were well into secondary wall formation, those replicates selected for subjection to cold stress were placed in a dark incubator set at 15°C for 15 h and then returned to the 30°C incubator. At 24 DPA the ovules were harvested and stored at -70°C for subsequent antioxidant enzyme analyses.

Samples were prepared for enzyme analyses according to the method of Anderson *et al*. (1992) as modified by Gossett *et al*. (1994). Catalase activity was determined by monitoring the disappearance of H_2O_2 according to the method of Beers and Sizer (1952). Total SOD activity was measured by determining the amount of enzyme required to produce 50% inhibition of the reduction of cytochrome C by superoxide generated by xanthine oxidase as outlined by Forman and Fridovich (1973). Glutathione reductase (GR) activity was determined by monitoring the glutathione-dependent oxidation of NADPH as described by Schaedle and Bassham (1977). Peroxidase activity was measured by monitoring the H_2O_2-dependent oxidation of reduced 2,3',6 trichloroindophenol according to the method of Nickel and Cunningham (1969). Ascorbate peroxidase (AP) activity was assayed by monitoring the ascorbic acid-dependent reduction of H_2O_2 as described by Anderson *et al*. (1992). Glutathione S-transferase (GST) activity was assayed according to the method of Habig, *et al*. (1974). For catalase, peroxidase, and AP, one unit of enzyme was defined as the amount necessary to decompose 1 µmole of substrate/min at 25°C. One unit of GR and GST was defined as the amount of enzyme required to reduce 1 nmole of substrate/min at 25°C. One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome C by 50%. Data points are based on a mean of a minimum of four replicates.

Results and Discussion

Table 1 shows that there was considerable difference in the antioxidant enzyme profiles of ovules from AC-88 and CO-312. SOD activity was from 192% to 252% higher in the cold-stressed and control ovules of AC-88 than in CO-312

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suggesting that AC-88 had a much higher capacity for dismutating superoxide. As for the enzymes which break down the H_2O_2 generated by SOD, catalase activity was from two to three times higher in the cold stressed and control ovules of AC-88 than in CO-312, but peroxidase activity was very similar in ovules from AC-88 and CO-312. Cold stress appeared to decrease peroxidase activity by 50% to 60% in both cultivars.

As for the enzymes associated with the ascorbate - glutathione cycle, AP activity was slightly higher in the control ovules from AC-88, but the activities were similar under both treatments in ovules from both cultivars. Although there was a slight decrease in the activity of this enzyme when AC-88 ovules were subjected to cold stress, the activity was as high as that recorded in the cold-stressed CO-312. GR activity was twice as high in the AC-88 control ovules as in the CO-312 control ovules. A slight cold-induced increase (26%) in GR activity in AC-88 resulted in the cold-stress AC-88 ovules having two and one-half times more GR activity than did the cold-stressed CO-312. GST, an enzyme with a primary role of detoxifying xenobiotics, has also been shown to exhibit antioxidant activity. GST activity was only slightly higher in the AC-88 control ovules than in the CO-312 controls, but cold stress appeared to reduce GST activity by 64% in CO-312 ovules and only 30% in AC-88 ovules. As a result of this difference in reduction, GST was 123% higher in the AC-88 cold-stressed ovules than in the CO-312 ovules subjected to cold stress.

Haigler *et al*. (1991) have shown that the adverse response of cotton fiber development to cool temperature is at least partly an ovule/fiber specific event. To date, the exact mechanism of this event is not understood. A range of possible mechanisms from changes in enzyme kinetics to feedback inhibition resulting from decreased substrate uptake have been postulated (Roberts *et al*., 1992). Changes in membrane fluidity has often been also been suggested as a general mechanism for chilling injury in sensitive plants (Hale and Orcutt, 1987). Changes in membrane form and function are often associated with the induction of oxidative stress. Hence, it may be that chilling injury results in a change in membrane fluidity which in turns results in the production of activated oxygen species which then somehow interferes with cellulose synthesis. If this be the case, increased antioxidant activity might result in a greater tolerance to chilling injury. Variations in tolerance to chilling among cultivars have been demonstrated (Gipson and Ray, 1969), and the results presented in this paper show that varietal differences in antioxidant enzyme expression also exist. Future research in our laboratory will focus on these latter issues.

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Table 1. Catalase (Cat), peroxidase (Per), ascorbate peroxidase (AP), Glutathione reductase (GR), superoxide dismutase (SOD), and glutathione S-transferase (GST) activities (units/g fresh weight) in 24 DPA Coker 312 and Acala 1517-88 control ovules and ovules subjected to 15°C for 15 hours at 18 DPA.

	Coker 312		Acala 1517-88	
Enzyme	**Control**	**Cold-Stressed**	**Control**	**Cold-Stressed**
Cat	17.7	14.7	36.3	41.0
Per	363.0	168.3	388.3	117.5
AP	511.7	530.3	691.7	482.0
GR	42.0	44.0	86.3	109.3
SOD	140.5	173.5	496.5	509.0
GST	295.2	105.7	335.3	235.4