SPECIFIC ION EFFECTS ON THE INDUCTION OF ANTIOXIDANT ENZYMES IN COTTON CALLUS TISSUE

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Abstract

Callus tissue from a NaCl-tolerant cell line was transferred to aerated culture tubes containing 150 mM NaCl. Following a 2-hour preincubation, the tubes containing 150 mM NaCl were left untreated to serve as controls or amended with NaCl to a final concentration of 250 mM, 250 mM $NaNO_3$, 250 mM KCl, or 500 mM sucrose. The callus tissue was harvested at 30 min, 1 hr, 2 hr, 4 hr, and 8 hr intervals and analyzed for superoxide dismutase (SOD), peroxidase, catalase, ascorbate reductase (AP) and glutathione reductase (GR) activity. Treatment with NaCl resulted in significant increases in peroxidase, catalase, AP, and GR activities within 1 hour and SOD activity within 4 hours. $NaNO_3$ treatment failed to produce a significant peroxidase response, and KCl treatment failed to produce a significant GR response. The sucrose and KCl treatments resulted in significant increases in peroxidase activity; the sucrose and $NaNO_3$ treatments produced significant increases in GR activity; and the sucrose, $NaNO_3$, and KCl treatments caused significant increases in SOD activity. However, in each of these cases, the increases occurred significantly later in time than the increases observed with the NaCl treatment. The catalase response to the KCl treatment also occurred much later than the response to NaCl. These data suggests that the total antioxidant enzyme response observed during NaCl stress in cotton callus tissue is somewhat specific to the combined effects of the Na^+ and Cl^- ions.

Introduction

A number of antioxidants protect plant tissues against the cytotoxic species of activated oxygen, and plants with high levels of antioxidants, either constitutive or induced, are reported to be more resistant 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). NaCl stress invokes an antioxidant response in cotton plants and callus tissue (Gossett *et al.*, 1994 a, 1994b), and in previous studies involving a comparison of antioxidant activities in a control and a salt-tolerant cell line from the same cultivar (Coker 312), the activities of SOD, catalase, peroxidase, AP, and GR were significantly higher in the salt-tolerant cell line (Gossett *et al*., 1996).

The mechanism which imparts salt tolerance to non-halophytic plants has eluded definition. The plant response to salt stress most likely involves a cascade of events, and results from the aforementioned studies suggest that some of these events evoke the antioxidant defense system. Additional studies (Gossett *et al.*, 1998) have shown that treatment with NaCl, superoxide, and abscisic acid all result in a rapid increase in antioxidant enzyme activity and suggests that these molecules may be involved in the signal pathway associated with the upregulation of the of the antioxidant defense system. As for the involvement of NaCl in the signal transduction pathway, it is not known whether the upregulation of antioxidant enzyme activity was due a specific effect of the Na^+ ions, the Cl^- ions, a combination of both the Na^+ and Cl^- ions, or a change in the osmoticum. This experiment was designed to help provide additional information about the role of specific ion effects on the upregulation of the antioxidant system.

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*. (1996). At the beginning of each experiment, approximately 4 g of callus tissue from the NaCl-tolerant cell line was transferred to each of either a series of culture tubes containing media amended with 150 mM NaCl . Each culture tube was then connected to an aerator and allowed to preincubate for 2 hours. Following preincubation, the tubes containing 150 mM NaCl were left untreated to serve as controls or amended with NaCl to a final concentration of 250 mM, 250 mM $NaNO_3$ to serve as an alternate source of Na^+ ions, 250 mM KCl to serve as an alternate source of Cl^- ions, or 500 mM sucrose to serve as an osmotic stress. The callus tissue was harvested at 30 min., 1 hr, 2 hr, 4 hr, and 8 hr intervals and stored at -70^{o}C for subsequent antioxidant analyses.

Samples were prepared for enzyme analyses according to the method of Anderson *et al*. (1992) as modified by Gossett *et al*. (1994b). Total SOD was determined by the method described by Forman and Fridovich (1973). Peroxidase activity was assayed according to the method of Nickel and Cunningham (1969). Catalase activity was measured by the method of Beers and Sizer (1952). GR activity was determined by the method described by Schaedle and Bassham (1977). AP activity was assayed by the method described by Anderson *et al*. (1992). Data points are based on a mean of a minimum of three replicates. All data was subjected to one-way analysis of

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:540-542 (1999)
National Cotton Council, Memphis TN

variance, and significance was determined at the 95% confidence level.

Results and Discussion

SOD activity is shown is Table 1. The NaCl, $NaNO_3$, KCl, and sucrose treatments all resulted in significant increases in SOD activity above the control level; however, the increase in activity was observed within 4 hours after treatment with NaCl, while the increases with $NaNO_3$, KCl, and sucrose were not observed until 8 hours after treatment. The activity of the enzymes associated with the breakdown of the hydrogen peroxide generated by SOD activity are shown in Tables 2 and 3. Peroxidase activity (Table 2) increased significantly after 1 hour following treatment with NaCl. Sucrose and KCl treatments also resulted in significant increases in peroxidase activity, but these increases were not observed until 2 hours after treatment. Treatment with $NaNO_3$ failed to initiate a significant increase in peroxidase activity. NaCl, $NaNO_3$, and sucrose all resulted in significant increases in catalase (Table 3) activity within one hour after treatment, while the KCl treatment did not produce a significant increase in catalase activity until after 8 hours. Results for the antioxidant enzymes associated with the ascorbate-glutathione cycle are presented in Tables 4 and 5. All four treatments resulted in significant increases in AP activity within 1 hour (Table 4). NaCl induced a significant increase in GR (Table 5) activity within 1 hour after treatment, while the sucrose and $NaNO_3$ treatments resulted in increases in GR activity after 2 hours. No significant change in GR activity was observed with the KCl treatment.

The results from these experiments suggests that the total antioxidant enzyme response observed during NaCl stress in cotton callus tissue is somewhat specific to the combined effects of the Na^+ and Cl^- ions. If the increases in antioxidant activity observed during NaCl stress were due to the Na^+ ion, then the $NaNO_3$ treatment should have produced increases similar to those with the NaCl treatment. However, the $NaNO_3$ treatment did not induce an increase in peroxidase activity, and the increases in the SOD and GR activities observed with the $NaNO_3$ treatment occurred significantly later than the increases observed with the NaCl treatment. If the increases in antioxidant activity observed during NaCl stress were due to the Cl^- ion, then the KCl treatment should have produced increases similar to those with the NaCl treatment, but the KCl treatment failed to produce an increase in peroxidase activity. In addition, the increases in the SOD, peroxidase, and catalase activities observed with the KCl treatment were observed significantly later than the increases that occurred with the NaCl treatment. If the increases in antioxidant activity observed during NaCl stress were merely due to osmotic stress, then the sucrose treatment should have produced increases similar to those with the NaCl treatment. While the sucrose treatment did induce significant increases in all five of the antioxidant enzymes, the SOD, peroxidase, and GR responses occurred later than the increases observed with the NaCl treatment. It should be noted, however, that the sucrose treatment induced increases in the catalase and AP activities similar to those observed with the NaCl treatment. Hence, it may be catalase and AP activities are induced in response to changes in the osmotic concentration rather than the specific effects of the Na^+ and Cl^- ions.

Acknowledgments

We wish to thank Cotton Incorporated (91-723) and the The Louisiana Board of Regents Support Fund (BORSF) for support of this research.

References

Anderson J.V., B.I. Chevone, J.L. Hess. 1992. Seasonal variation in the antioxidant system of eastern white pine needles. Plant Physiol 98:501-508.

Beers, R.F. and I. Sizer. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195:133.

Dhindsa, R.S., and W. Matowe. 1981. Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. J Exp Bot 32:79-91.

Forman, F.J. and I. Fridovich. 1973. Superoxide dismutase: A comparison of rate constants. Arch. Biochem. Biophys. 158:396-400.

Gossett, D.R., E.P. Millhollon, and M.C. Lucas. 1994a. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. 34:706-714

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 cultivars of cotton. Plant Cell Rep. 13:498-503.

Gossett, D.R., S.W. Banks, E.P. Millhollon, M.C. Lucas. 1996. Antioxidant response to NaCl in a control and an NaCl-tolerant cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. Plant Physiol. 112:803-809.

Gossett, D.R., B. Bellaire, S.W. Banks, A. Manchandia, and M.C. Lucas. 1998. The influence of abscisic acid on the induction of antioxidant enzymes during salt stress. Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN. 1396-1399.

Harper, D.B. and B.M.R. Harvey. 1978. Mechanisms of paraquat tolerance in perennial ryegrass II. Role of superoxide dismutase, catalase, and peroxidase. Plant Cell Environ 1:211-215.

Mandamanchi, N.R. and R.G. Alscher. 1991. Metabolic bases for differences in sensitivity of two pea cultivars to sulfur dioxide. Plant Physiol 97:88-93.

Monk, L.S. and H.V. Davies. 1989. Antioxidant status of the potato tuber and Ca^{2+} deficiency as physiological stress. Physiol. Plant. 75:411-416.

Nickel, R.S. and B.A. Cunningham. 1969. Improved peroxidase assay method using leuco 2,3',6 trichloroindophenol and application to comparative measurements of peroxidase catalysis. Ann Biochem. 27:292-299.

Polle, A and H. Rennenberg. 1994. Photooxidative stress in trees. *In* CH Foyer, PM Mullineaux, eds, *Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants* CRC Press. Boca Raton, FL, pp 199-218.

Schaedle, M. and J.A. Bassham. 1977. Chloroplast glutathione reductase. Plant Physiol 59:1011-1012.

Spychalla, J.P. and S.L. Desborough. 1990. Superoxide dismutase, catalase, and alpha-tocopherol content of stored potato tubers. Plant Physiol 94:1214-1218.

Trolinder, N.L. and J.R. Goodin. 1987. Somatic embryogenesis and plant regeneration in *Gossypium hirsutum* L. Plant Cell Rep 6:231-234.

Wise, R.R. and A.W. Naylor. 1987. Chilling-enhanced photooxidation: Evidence for the role of singlet oxygen and endogenous antioxidants. Plant Physiol 83:278-282.

Table 1. Superoxide dismutase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 250 mM NaCl, 250 mM $NaNO_3$, 250 mM KCl, and 500 mM sucrose

	Time					
Treatment	0	0.5 h	1 hr	2 hr	4 hr	8 hr
Control	28±4	20±4	20±6	33±4	41±6	20±2
NaCl	28±4	22±2	21±5	42±7	103±6	57±5
$NaNO_3$	28±4	22±5	21±2	35±4	29 ±9	64±5
KCl	28±4	21±5	25±5	45±7	42±7	53±5
Sucrose	28±4	15±4	21±5	38±5	35±4	61±4

Table 2. Peroxidase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 250 mM NaCl, 250 mM $NaNO_3$, 250 mM KCl, and 500 mM sucrose

	Time					
Treatment	0	0.5 h	1 hr	2 hr	4 hr	8 hr
Control	119±42	164±45	129±25	131±45	179±39	144±10
NaCl	119±42	164±31	358±38	348±54	197±28	165±40
$NaNO_3$	119±42	288±83	197±46	207±51	281±67	132±40
KCl	119±42	30±20	273±73	346±39	325±30	303±41
Sucrose	119±42	171±35	173±13	443±53	191±40	268±64

Table 3. Catalase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 250 mM NaCl, 250 mM $NaNO_3$, 250 mM KCl, and 500 mM sucrose

	Time					
Treatment	0	0.5 h	1 hr	2 hr	4 hr	8 hr
Control	15±2	13±1	18±1	14±1	16±1	13±1
NaCl	15±2	14±1	38±3	16±1	15±2	18±1
$NaNO_3$	15±2	12±1	26±1	16±1	18±2	10±1
KCl	15±2	12±1	18±2	14±1	14±1	33±1
Sucrose	15±2	13±1	28±2	24±2	26±1	30±1

Table 4. Ascorbate Peroxidase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 250 mM NaCl, 250 mM $NaNO_3$, 250 mM KCl, and 500 mM sucrose

	Time					
Treatment	0	0.5 h	1 hr	2 hr	4 hr	8 hr
Control	217±8	223±31	187±29	272±41	200±33	139±20
NaCl	217±8	703±17	558±43	263±43	190±39	305±23
$NaNO_3$	217±8	968±85	824±30	754±22	1524±69	904±20
KCl	217±8	505±47	589±33	1640±5 0	803±43	966±61
Sucrose	217±8	487±65	607±49	716±52	869±66	993±54

Table 5. Glutathione reductase activity (units/g fresh weight ±SE) in salt-tolerant callus tissue treated for 0, 0.5, 1, 2, 4, and 8 hrs with 150 mM NaCl (controls), 250 mM NaCl, 250 mM $NaNO_3$, 250 mM KCl, and 500 mM sucrose

	Time					
Treatment	0	0.5 h	1 hr	2 hr	4 hr	8 hr
Control	31±2	41±4	57±5	66±5	59±6	69±4
NaCl	31±2	58±6	321±24	235±8	362±17	103±1
$NaNO_3$	31±2	41±1	45±3	184±13	47±4	63±5
KCl	31±2	31±9	18±3	37±6	60±2	85±8
Sucrose	31±2	33±3	52±3	151±4	42±4	87±2