Characterization of Cotton Gene Expression Related to Trehalose and Proline Metabolism in Response To Water-Deficit Stress

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RESEARCH PROBLEM

Stress resulting from cellular water-deficit is the most common challenge encountered by plants, and can result from a wide array of situations including drought, chilling and freezing, and saline soils. Through time, plants have evolved a complex mechanism of overlapping responses to water-deficit stress. The physiological changes that manifest as a result of water-deficit stress originate at the gene expression level. However, it is unclear if many of the morphological, physiological, and molecular responses induced by water-deficit stress actually enhance tolerance. One exception is osmotic adjustment, a process that is highly conserved in most organisms, and transgenic studies with model crops demonstrate that this phenomenon improves water-deficit stress tolerance.

BACKGROUND INFORMATION

Two solutes potentially contributing to osmotic adjustment that have recently received much attention in regards to water-deficit stress tolerance are the disaccharide, trehalose, and the amino acid, proline. The goal of these studies was to elucidate patterns of expression of genes directly involved in the metabolism of these osmotica. While proline is well-established as a compatible solute in higher plants including cotton, the occurrence of trehalose in most higher plants has only recently been documented. Expression of putative genes responsible for the synthesis of trehalose, trehalose-6-phosphate synthase (TPS) (Nepomuceno et al., 2002), and trehalose-6-phosphate phosphatase (TPP) are differentially expressed in response to water-deficit stress in cotton (Gossypium hirsutum L.). Neither the accumulation of trehalose nor the expression of trehalase, the enzyme that catabolizes trehalose, have previously been

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shown in cotton. Expression of genes related to proline metabolism is well-documented in model crops, but such studies are lacking in cotton. The present study sought to determine expression in cotton of the genes responsible for the metabolism of proline and trehalose.

RESEARCH DESCRIPTION

The Australian cultivar Siokra L-23, known for its high level of water-deficit stress tolerance (Nepomuceno et al., 1998), was grown in 2-L pots of Sunshine Mix under controlled conditions and subjected to slow stress induction (18 to 21 days) by withholding water to the point of moderate wilt. Plants were harvested at the point of maximum stress, when they were approximately six weeks old. A modified hot-borate method was used for the extraction of RNA, and northern analysis was used to determine expression of the various genes. Probes used in the northern analyses were cDNA clones of the respective genes from cotton or from other model plant species such as Arabidopsis or tomato.

RESULTS AND DISCUSSION

All three genes for trehalose metabolism (TPS, TPP, and trehalase) were present and regulated by water-deficit stress. One band (~1.5 kb) was obtained for TPS that was up-regulated under water-deficit stress conditions (Fig. 1). At least two genes for TPP (~2 and 3 kb) were present in cotton, the heavier of which was constitutively expressed while the lower molecular weight gene was induced by water-deficit stress. Three bands were present for trehalase (~1, 2, and 4 kb). The lowest molecular weight gene was noticeably up-regulated while the 2 kb band was slightly up-regulated by water-deficit stressed conditions. Expression of the largest gene apparently was not responsive to water deficit. Although two of the trehalase genes were up-regulated under water-deficit stressed conditions, they appeared to have a low level of constitutive expression in the well-watered plants. Since trehalose does not accumulate in cotton, the presence and role of these enzymes in response to water-deficit stress can only be conjectural at this point.

Among the genes responsible for proline metabolism, slight up-regulation was observed in some of the genes for \( \Delta 1\)-pyrroline-5-carboxylate reductase (P5CR) and \( \Delta 1\)-pyrroline-5-carboxylate synthetase (P5CS). These two enzymes are responsible for the synthesis of proline. On the other hand, the gene for proline dehydrogenase (PDH), the enzyme that degrades proline, was down-regulated. Only one gene product was detected for P5CR (~2.5 kb), with the stressed plants showing a very slight degree of up-regulation. Three gene products were obtained for P5CS (~1, 1.5, and 2.5 kb), which is responsible for the rate-limiting step in proline formation. While the differences between band intensity between water regimes were not dramatic, the two larger genes
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(~1.5 and 2.5 kb) appeared to be slightly down-regulated in the water-deficit stressed plants, while the smaller gene (~1 kb) was slightly up-regulated in this treatment. Water-deficit stress resulted in a notable down-regulation of PDH, with only one band being present for this gene (1.5 kb). These studies revealed that proline metabolism of cotton in response to water-deficit stress followed patterns similar to those exhibited in other plants. Namely, genes coding for enzymes involved in proline synthesis are slightly up-regulated while the gene coding for the proline-degrading enzyme is down-regulated in response to water-deficit stress. Logically this suggests that proline accumulates by increased synthesis, but especially by decreased degradation.

PRACTICAL APPLICATION

The responses to water-deficit stress are far-reaching and often overlapping. If plant production under adverse conditions is to be improved, underlying genetic mechanisms that improve resistance to stress must be understood so that these can be selected in breeding programs. Gene expression studies provide information on potentially useful systems of adaptation at the most basic molecular level. Results from the current study contribute to the field of knowledge of the effect of water-deficit stress on cotton gene expression related to compatible osmolyte synthesis, a component of osmotic adjustment. The observed expression of a putative trehalase gene as a complement to genes for enzymes involved in trehalose synthesis raises significant questions concerning the role of this disaccharide in resistance to water-deficit stress.

LITERATURE CITED

Fig. 1. Results of northern analysis in Siokra L-23 under well-watered (WW) and water-deficit stressed (WS) conditions for genes involved in trehalose metabolism. Left to Right: 1) EtBr stained membrane prior to hybridization; hybridization with cDNA probe corresponding to 2) TPS, 3) TPP, and 4) Trehalase.

Legend: WW = well-watered, WS = water-deficit stressed, TPS = trehalose-6-phosphate synthetase, TPP = trehalose-6-phosphate phosphatase, P5CR = 1-pyrroline-5-carboxylate reductase, P5CS = 1-pyrroline-5-carboxylate synthetase, PDH = proline dehydrogenase.