Physiological and Molecular Response of Cotton to Water Deficit

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ABSTRACT

The Differential Display technique (DD) was used to identify and isolate genes that may differ among four cotton genotypes (Gossypium hirsutum L.) with diverse response to water deprivation. The genotypes were first physiologically characterized in controlled environments where water and osmotic potential of roots and leaves, photosynthetic rate and relative water content (RWC) were some of the parameters analyzed during and after the water deficit applied. Cultivar Siokra L-23 and wild type T-1521, two drought tolerant genotypes, had significant ability to osmotically adjust to water deficit, maintaining photosynthetic rate and RWC near control values. However, cultivars CS 50 and Stoneville 506 were sensitive, confirming previous observations. Fifty-two cDNA fragments differentially expressed were isolated, cloned in pGEM-T vectors and sequences. Searches for homology in GenBank showed 17 clones with high homology to known genes and 20 clones with low homology while 15 clones had no homologous entries. Among the sequences that showed high homology was a heat shock protein that binds with calmodulin. This gene was expressed only in the water deficit tolerant genotypes during the stress. Trehalose-6-phosphate synthase, an enzyme that is part of the metabolic pathway for production of trehalose, a sugar known to osmoprotect cell membranes during desiccation, was identified by homology. The differentially expressed gene sequence could have use in screening germplasm banks for similar genotypes, in plant physiology studies and in molecular mapping of plant responses to water deficit.

Introduction

The objective of this work was to physiologically characterize four cotton genotypes with diverse responses to drought and to identify and isolate genes differentially expressed during the water deficit. Abiotic stresses such as drought can significantly reduce crop yields and restrict the latitudes and soils on which commercially important species can be cultivated. The implications of this are tremendous since not only are producers affected but the entire society feels the effects. According to USDA reports, the drought that affected United States in 1987/88 caused direct and indirect losses of more than US$ 39 billion. Unemployment, food price increases and financial market collapse were associated with the drought. The 1998 drought in Texas may have an even greater impact. Understanding how plants tolerate water deficit may significantly improve future drought events. Identifying and understanding mechanisms of water-stress tolerance is crucial to the development of new tolerant commercial cultivars.

The expression of novel genes in stress-tolerant genotypes could be used to study mechanisms of drought tolerance and to identify genotypes with similar characteristics. Resistance to drought in plants clearly is not a simple trait, but a complex of mechanisms working in combination to avoid or to tolerate water deficits. All physiological, morphological and developmental changes conferring drought tolerance in plants must have a molecular genetic basis. Thus, genotypes that differ in tolerance to water stress should have qualitative or/and quantitative differences in gene expression. Differential Display (DD) may be used to identify and isolate genes that differ among four cotton genotypes with diverse responses to water deprivation (Liang and Pardee, 1992, 1995). This technique uses sub-populations of the total mRNA pool as template to obtain representative cDNAs by reverse transcription. The cDNA sub-populations are then amplified by polymerase chain reaction (PCR), resulting in the generation of a PCR profile representative of the mRNAs within each population. PCR products are displayed side-by-side to identify treatment- or genotype-specific gene expression.

Materials and Methods

Two water-stress tolerant (Siokra L23 and T-1521) and two water-stress sensitive (Stoneville 506 and CS-50) cotton genotypes (*G. hirsutum*) were used. They were

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submitted to periods of water deficit induced in nutrient solution by polyethylene glycol (PEG 6000) and in pots with sand by withholding irrigation. They were characterized for osmotic adjustment, photosynthetic rate, relative water content, carbon discrimination and other physiological parameters. During and after the water stress, leaves of the four cotton genotypes were collected for RNA isolation. Total RNA was extracted after the last period of stress. A reverse transcription followed by PCR using anchor and 10-mer primers was done according to Liang and Pardee (1992, 1995). PCR products were separated by electrophoresis on a 6% denaturing polyacrylamide gel. Differentially displayed bands were analyzed and compared between stressed and non-stressed treatments in the four cotton genotypes. Bands that appeared differentially displayed were excised from the gel and reamplified. Reamplified, differentially displayed bands were cloned in a pGEM-T vector and sequenced using an ALF™ DNA sequencer (Pharmacia Biotech). Database searches were carried out using BLASTN2 and BLASTX2 provided by Bork Group's Advanced Search Services at the European Molecular Biology Laboratory (EMBL).

Results

Physiological characterization of the four genotypes showed that Siokra L-23 and T-1521 maintained leaf photosynthetic rate under water-stress, whereas the rates significantly decreased in CS-50 and Stoneville 506 (Nepomuceno *et al.*, 1998). Also, the former two had a decrease in leaf osmotic potential at full turgor (osmotic adjustment) that did not appear in the latter two genotypes. The decrease in osmotic potential in the tolerant genotypes probably was responsible for the higher relative water content of their leaves which, consequently, maintained photosynthesis near normal. Genotypes Siokra L-23 and the wild type (T-1521) were more tolerant to water deficit compared with CS-50 and Stoneville 506.

A total of 109 cDNA fragments differentially displayed were identified. Each of these fragments represent genes that were expressed during water deficit. Sixty-five of these gene fragments were re-amplified, cloned in pGEM-T vectors and sequenced. Gene bank search showed 17 clones with high homology to known genes (partially presented in Table 1), and 20 clones with low homology, while 15 clones had no homologous entries. Among the sequences that showed high homology, a Heat Shock Protein (HSP) that binds to Calmodulin was found. This gene was expressed only in the water-deficit tolerant genotypes (Siokra L-23 and T-1521) during the stress. A sequence was found with high homology with trehalose-6-phosphate synthase, an enzyme in the metabolic pathway of trehalose. In this case the gene was expressed in all four genotypes but only in response to water stress. Trehalose is a sugar known to osmoprotect cell membranes during desiccation in yeast and bacteria. Several other differentially expressed gene sequences appeared to be involved in signal transduction.

Conclusions

The results obtained on the physiological and molecular characterization of water-deficit tolerant genotypes can have a tremendous impact in water-deficit tolerance selection in breeding programs. The differentially expressed gene sequences can be used as probes to screen germplasm banks to identify genotypes presenting similar water-deficit tolerance characteristics found in the genotypes used in this study. Physiological studies of plant responses to water deficit can be greatly improved when different treatments are compared in terms of gene expression. Also, the differentially expressed genes can help in molecular mapping of traits related to drought tolerance. Therefore, breeding selection for these traits can be accelerated and made more efficient. Furthermore, some of the identified genes show a high potential to be used in plant transformation studies. Transgenic plants could be created using genes that are differentially expressed during water deficit that are modified to enhance their activity.

Environmental forecasts signal increased global warming in the next few decades and drought is likely to accompany this event. The development of new plant genotypes more tolerant to longer periods of water deficit will be essential to feed and clothe an increasing world population. Plant physiology and molecular biology will play a key role in this process.

References

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Table 1. Clones of gene transcripts differentially expressed among cotton genotypes in response to water stress. Identification of the transcripts is based on sequence homology to genes identified in other organisms.

Clone #[1]	Homology[2]	Homology P(N)	Identity[3]
A12B15-5	NAD(P)H oxidase - *Oryza sativa*	1.7e-52	44(aa) 88%
A12B13-1	Trehalose-6-phosphate synthase	9.0e-37	89(aa) 87%
A12B15-6	heat shock protein, Calmodulin-binding	2.9e-32	57(aa) 69%
A5B1-13	GTP-binding protein, fusA-homolog (yihK)	1.2e-23	45(aa) 93%
A12B13-4	*Avena fatua* and *Petroselinum crispum* DNA-binding protein	1.3e-22	59(aa) 77%
A5B1-18	GTP-binding protein, fusA-homolog (yihK)	9.0e-22	58(aa) 83%
A12B11-2	Thioesterase homolog	1.9e-17	48(aa) 67%
A5B1-8	cf-9 protein precursor - tomato, gene for resistence to *Cladosporium fulvum*	5.4e-11	28(aa) 71%
A5B1-9	*A.thaliana* BAC IG005I10, weak similarity to *S.cerevisiae* B0B1 protein	3.4e-08	34(aa) 72%
A11B1-a	*Glycine max* TGACG-motif binding Protein (STGA1) Mrna	1.1e-05	34(aa) 70%
A12B15-8	Translation initiation factor EIF-4 gamma – human	1.4e-05	33(aa) 47%

[1] first letter+number represents anchor primer; second letter+number represents 10-mer; third number represents band position in the dried gel; greek letter represents band in non-denaturing gels.
[2] homology searches using the BLASTN2 and BLASTX2 programs provided by Bork Group's Advanced Search Services at EMBL
[3] aa - represents amino acid