DIFFERENTIAL DISPLAY OF GENE EXPRESSION DURING WATER DEFICIT STRESS

A. L. Nepomuceno, J. M. Stewart, D.M.Oosterhuis and W.A.Cress
University of Arkansas, Agronomy Department
Fayetteville, AR

Abstract

Genotypes which differ in tolerance to water stress should have qualitative or quantitative differences in gene expression. To identify and isolate genes which may differ among four cotton genotypes with diverse responses to water depravation, we used Differential Display (DD). DD is based on a reverse transcription of mRNA followed by a Polymerase Chain Reaction (PCR).

Introduction

Water deficits in plants elicit a complex of responses beginning with stress perception, which initiates a signal transduction pathway manifested in changes at many physiological/metabolic levels. The complex physiological and biochemical changes that occur during a period of water stress results, at least in part, from changes in the amounts or activities of various enzymes and other proteins present in the plant. Many of these protein differences reflect changes in gene expression and mRNA abundance. Therefore, a partial understanding of these developmental events may be obtained by analyzing and comparing mRNAs isolated from well-watered and water-stressed plants. Differential display is a relatively new technique used to compare gene expression (mRNAs) between cells under different conditions or tissues (Liang and Pardee, 1995).

Material and Methods

Two water-deficit-stress tolerant (Siokra L23 australian cultivar, and T-1521 wild type) and two water-deficit-stress sensitive (Stoneville 506 american cultivar, and CS-50 australian cultivar) genotypes were used. These genotypes were submitted to four periods (for four hours each, during dark time) of water deficit (at -0.3MPa) induced in nutrient solution by polyethylene glycol (PEG 6000), and in pots with sand by withholding irrigation. The reverse transcription was conducted according to Koonce and Haigler (personal communication) and Song et al. (1995). Five µL of the reverse transcription reaction cDNA was mixed in a 200µL tube with 5µL dNTP (100µM), 2.5µL 10x Taq buffer, 2.5µL $MgCl_2$ (25mM), 0.5µL 10-mer primer (50µM), 1µL anchor primer (50µM), 0.5µL ^{35}S-dATP (1250 Ci/mmol), 0.5µL Taq polymerase (Promega, Madison, WI, 5 U/µL) and DEPC-treated water to make a final volume of 25µL. PCR cycling conditions were: 94^0C for 30 sec, 40^0C for 1 min, 72^0C for 30 sec, 40 cycles, followed by 5 min final extension at 72^0C.

PCR products were separated by electrophoresis on a 6% non-denaturing polyacrylamide gel. Bands were analyzed and compared between stressed and non-stressed cultivars in the four cotton cultivars. Bands that appeared differentially displayed were excised from the gel and reamplified.

Reamplified differentially displayed bands were cloned in a pGEM-T vector (Promega, Madison, WI) for subsequent sequencing.

Results and Discussion

In the original protocol for differential display (Liang and Pardee, 1992) the annealing temperature was 42^0C, however we observed that the efficiency of the PCR reaction could be improved if we optimized the annealing temperature for each anchor primer used. We selected 40^0C as the best annealing temperature for the primers we use. Another modification from the original protocol was the use of a non-denaturing polyacrylamide gel to separate the PCR products. The use of a non-denaturing gel simplifies the process of extracting bands from the gel for reamplification. We selected until now 17 bands differentially displayd. They have been cloned in a pGEM-T vector for sequencing.

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