PROTEOMIC ANALYSIS OF RESPONSES TO DROUGHT STRESS IN COTTON SEEDLINGS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS AND SPECTROMETRY

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Abstract

Xinjiang is the largest cotton-producing region in China and drought is a serious limiting factor in cotton production since it is located in the arid inland. It is imperative to study the genetic and molecular basis of drought resistance which will facilitate breeding for drought resistant cotton. The objective of this study was to identify drought responsive proteins in cotton seedlings when exposed to PEG treatment using two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) and mass spectrometry (MS). Using an improved protein extraction method, 113 differentially expressed (DE) proteins were identified from PAGE gels and 102 protein points were successfully analyzed using MALDI-TOF-TOF/MS. Further bioinformatic analysis showed that the DE proteins were mainly involved in carbohydrate metabolism, photosynthesis, cell defense, amino acids and protein synthesis, cells processing, molecular partner, and other processes.

Introduction

Xinjiang is the largest cotton-producing region in China and drought is a serious limiting factor in cotton production since it is located in the arid inland. It is imperative to study the genetic and molecular basis of drought resistance which will facilitate breeding for drought resistant cotton. The objective of this study was to identify drought responsive proteins in cotton seedlings when exposed to PEG treatment using two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) and mass spectrometry (MS).

Materials and Methods

Materials

The drought resistant cultivar “kk1543” (*Gossypium hirsutum*) was used in this study. Seed was surface sterilized and soaked in ddH2O for 24 h before transferred to filter paper for germination. Emerged uniform seedlings were transferred a hydroponic system containing ½ strength of Hoagland medium in a growth chamber. The seedlings were grown at temperature of a 28C- day/25C- night cycle under >10 h of light intensity of 12000 Lx per day for two weeks before the drought treatment.

PEG treatment

Seedlings of 2-week old from kk1543 were treated with 13% PEG 6000 for 0 h, 12 h, 24 h, 48 h, and 72 h.

Protein extraction

Proteins from the PEG treated seedlings and the controls from three biological replicates in each timepoint were extracted using an improved trichloroacetic acid (TCA)/acetone method.

Protein electrophoresis

After lysed and quantified, the extracted proteins were subjected to extracted proteins were resolved by two-dimensional (2-D) ployacrylamide gel electrophoresis (PAGE). The first dimension was based on their native isoelectric point using isoelectric focusing (IEF) and the second dimension was based on mass using ordinary SDS-PAGE. The gel was then stained using sliver staining and scanned.

MALDI-TOF–TOF analysis

Differentially expressed proteins were identified by comparing gels between PEG treated and control samples and then excised for MALDI-TOP-TOP analysis.

Data analysis

The MASCOT software (http://www.Matrixscience.com) was used to search for homologous proteins in the database.

Results and Analysis

Effects of PEG treatment on cotton seedlings

After 72 hours of PEG treatment, the cotton seedlings suffered serious wilting (Fig. 1).

Fig. 1. A comparison between seedlings from PEG treatment (right) and control conditions.

Repeatability of 2D electrophoresis

Differences were seen between 2D-PAGE maps from seedlings treated with PEG at different lengths (Fig. 2). Repeatability between biological samples from the same PEG treatment was high.

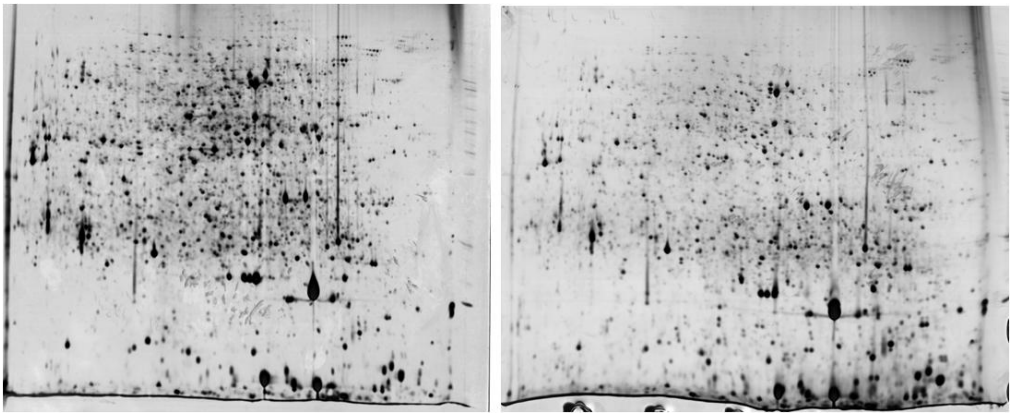

Fig. 2. The 2-DE maps of proteins from leaves of seedlings treated with PEG at different lengths.

Identification of differentially expressed proteins

113 significantly differential expressed (DE) protein points were excised (Fig. 3), resulting in identification of 103 proteins.

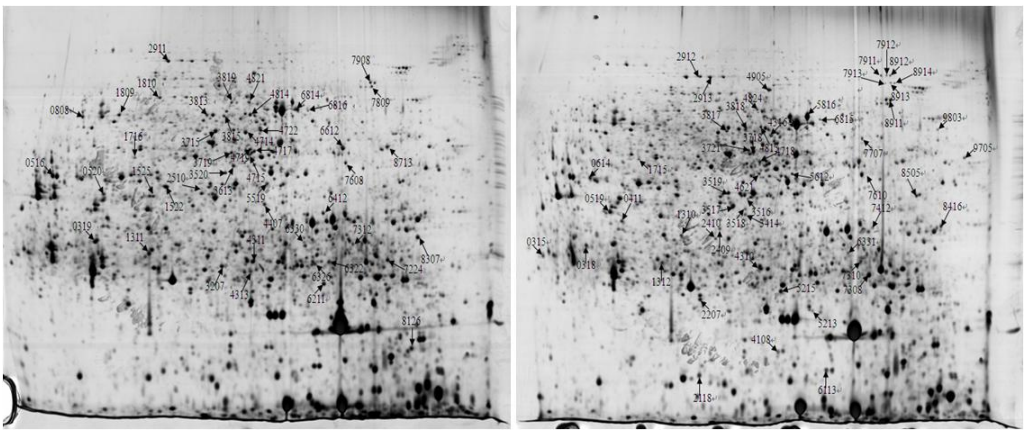

Fig. 3. Differentially expressed protein points were identified by comparing 2D-PAGE maps.

Bioinformatic analysis of DE proteins

The bioinformatic analysis showed that the DE proteins were mainly involved in carbohydrate metabolism, photosynthesis, cell defense, amino acids and protein synthesis, cells processing, molecular partner, and other processes (Fig. 4).

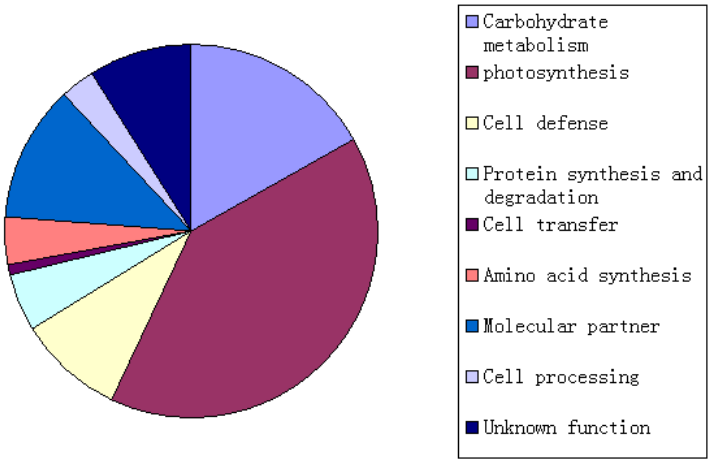

Fig. 4. Functional classification of differentially expressed proteins.