



Data in Brief

Transcriptome profiling of tobacco under water deficit conditions



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ABSTRACT

Drought is one of the limiting environmental factors that affect crop production. Understanding the molecular basis of how plants respond to this water deficit stress is key to developing drought tolerant crops. In this study we generated time course-based transcriptome profiles of tobacco plants under water deficit conditions using microarray technology. In this paper, we describe in detail the experimental procedures and analyses performed in our study. The data set we generated (available in the NCBI/GEO database under GSE67434) has been analysed to identify genes that are involved in the regulation of tobacco's responses to drought.

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Specifications

| | |
|---------------------------|---|
| Organism/cell line/tissue | Tobacco (<i>Nicotiana tabacum</i>) cv 'Burley 21', root and leaf |
| Sex | N/A |
| Sequencer or array type | NimbleGen custom oligo array (385 K) |
| Data format | Raw, pair and RMA |
| Experimental factors | Time of dehydration treatments |
| Experimental features | Time course profiling of gene expression in tobacco under dehydration |
| Consent | N/A |
| Sample source location | N/A |

1. Direct link to deposited data

Deposited data can be found here: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67434>.

2. Experimental design, materials and methods

2.1. Experimental scheme

Drought is a major environmental factor that limits crop production and affects 64% of the global land area [1]. In order to develop drought tolerant plants, we need to understand what mechanisms are involved when plants respond to drought. Transcriptome profiling provides

insights into important regulatory genes like transcription factors [2] that are involved in plant response to stress. In this study, transcriptome profiles revealed the dynamics of gene expression of various families of transcription factors as well as other important stress-induced genes [3]. Promoter analyses of these genes showed that they are also involved in other abiotic stresses [4].

2.2. Plant establishment and stress treatment

Tobacco (*Nicotiana tabacum*) cv. 'Burley 21' obtained from the University of Virginia (Charlottesville, VA, USA) was used in this study. Seeds were surface sterilized following a modified protocol of Li et al. [5] using a solution with 10% bleach and 0.1% Tween 80 (Thermo Fisher Scientific, Waltham, MA, USA) for 7 min. The seeds were then washed twice using sterilized water. The seeds were sown in sterile petri plates with modified Murashige and Skoog (MS) agar medium (Caisson Laboratories, North Logan, UT, USA) supplemented with 0.15 mM KH₂PO₄, and 3% (w/v) sucrose (Thermo Fisher Scientific, Waltham, MA, USA). The seedlings were then aseptically transferred into sterile MK–5 polycarbonate vessel (7.62 × 7.62 × 10.16 cm) (Caisson Laboratories, North Logan, UT, USA) with half-strength MS liquid medium (Fig. 1). Seedlings were grown for 2 weeks in a growth chamber set at 25 °C with constant light and later subjected to dehydration for 20, 40, 60, 120 and 240 min (Fig. 1). Sampling for each dehydration time point was done in three replicates with 20 plants per replicate to ensure enough tissues would be harvested. Dehydration was imposed by removing the plants from the liquid using the plastic holders (Fig. 1). In this way, the plants were never touched and

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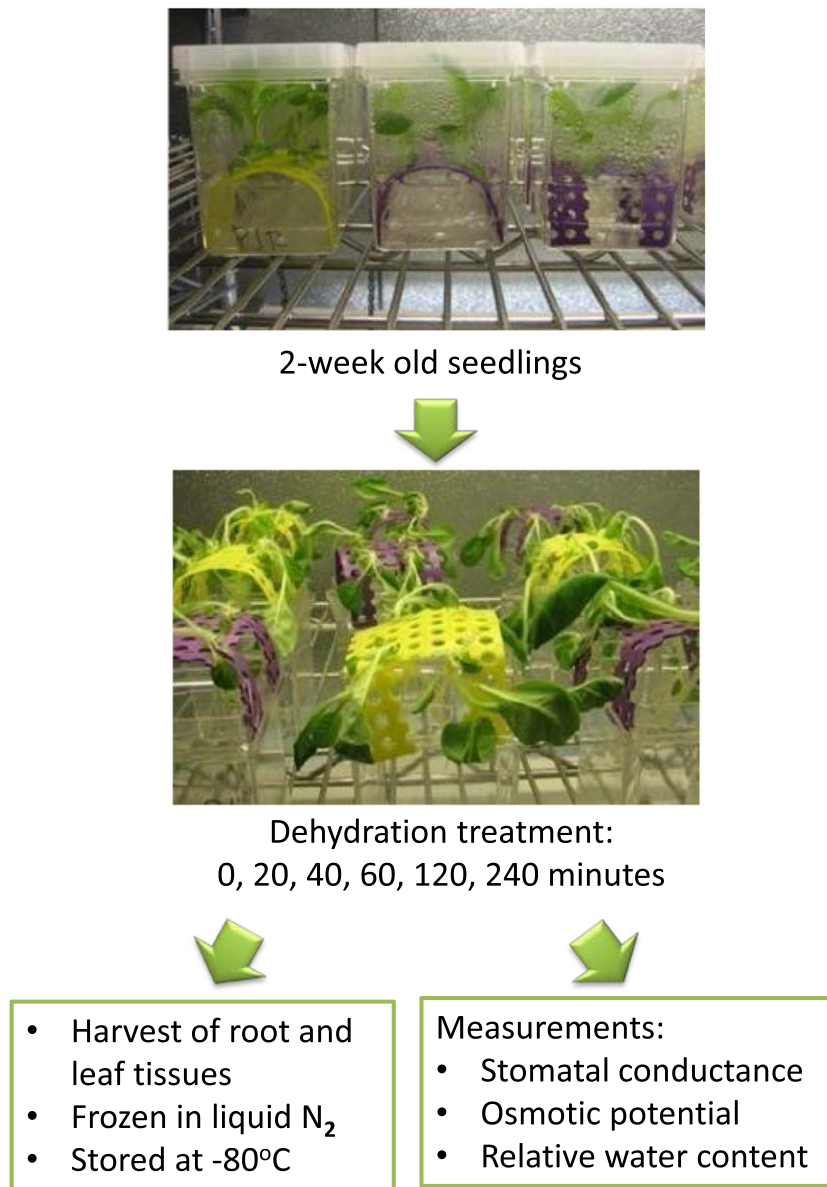


Fig. 1. Flow diagram of the experimental set up.

wound responses were therefore reduced. Roots and leaves were harvested rapidly after each treatment and placed into 50 mL centrifuge tubes (VWR International, Radnor, PA, USA) and immediately flash frozen in liquid nitrogen. Unstressed plants were also harvested to serve as controls. Samples were stored at -80°C until further use.

2.3. Total RNA extraction and quality control

RNA isolation was performed using an RNeasy kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Genomic DNA contamination was removed using an Ambion DNA-free and DNase removal kit (Life Technologies, Carlsbad, CA, USA). 10 μg total RNA from each sample was used for micro-array analysis. Quality of the extracted RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA) (Table 1).

2.4. Transcriptomics

A Nimblegen custom oligo array was used for transcriptome analyses in tobacco. The oligo array contained 385,000 probes with

Table 1

Concentration and RIN values of total RNA isolated from tobacco root samples.

| Sample | Concentration (g/ μL) | RIN |
|-----------|-----------------------------------|-----|
| 0 min–1 | 634.0 | 9.7 |
| 0 min–2 | 572.2 | 9.3 |
| 0 min–3 | 705.0 | 9.7 |
| 20 min–1 | 517.3 | 9.4 |
| 20 min–2 | 403.6 | 9.8 |
| 20 min–3 | 432.6 | 9.6 |
| 40 min–1 | 481.8 | 9.8 |
| 40 min–2 | 113.8 | 8.4 |
| 40 min–3 | 313.2 | 9.0 |
| 60 min–1 | 263.7 | 9.5 |
| 60 min–2 | 230.6 | 9.7 |
| 60 min–3 | 299.8 | 9.8 |
| 120 min–1 | 186.0 | 9.6 |
| 120 min–2 | 492.0 | 8.2 |
| 120 min–3 | 222.0 | 8.8 |
| 240 min–1 | 663.3 | 9.0 |
| 240 min–2 | 495.0 | 8.7 |
| 240 min–3 | 145.4 | 9.4 |

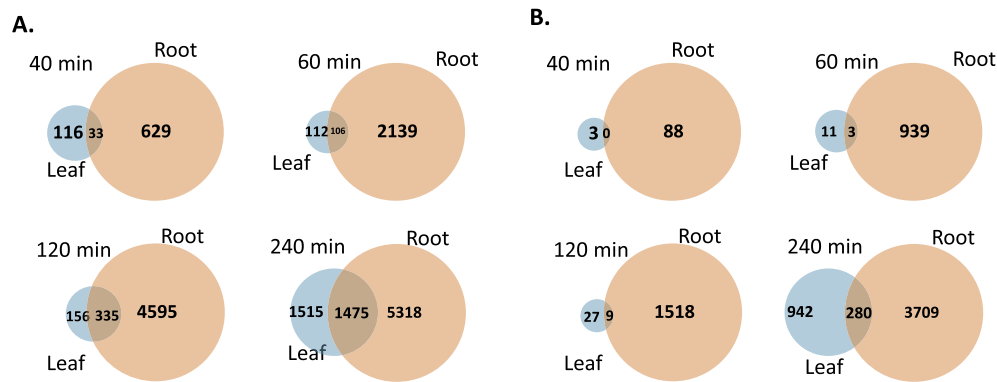


Fig. 2. Venn diagram of upregulated (A) and down-regulated (B) genes in tobacco roots and leaves at different dehydration time points.

approximately seven 60-mer probes for each gene sequence. The sequences came from three different sources. Firstly, 40,000 individual genomic survey sequence reads (GSSs) from the Tobacco Genome Initiative that had the highest E-value hits to proteins in the NCBI nr database (A data set of 1,159,022 genomic survey sequences was downloaded from the TGI <http://solgenomics.net/http://www.tobaccogenome.org/> in 2008), secondly all TOBFAC transcription factors (<http://compsysbio.achs.virginia.edu/tobfac/>), and thirdly the Version 4.0 DFCI Tobacco Gene Index EST sequences (NTGI4; ftp://occams.dfci.harvard.edu/pub/bio/tgi/data/Nicotiana_tabacum/; file name: Source files/NTGI.071508.fasta). The genome survey sequences have subsequently been deposited at The National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>; file name: Source files/TGSS_expressed.fasta) although these deposited sequences were the result of re-reading to extend the length of the sequences and have more errors. Oligoarray experiments were performed at MOgene LC (St. Louis, MO, USA) using their standard protocols. Data analysis was performed using ArrayStar v4 (DNASTAR Inc., Madison, WI, USA). Differential expression was calculated using 90% confidence (FDR Benjamini Hochberg) and 8-fold change as the cut off. We chose a high fold inducibility because under the experimental conditions very high inducibilities were obtained and a cut off value of 4-fold gave several thousand genes at the later time points. 8-fold was chosen to focus on only the most highly induced or repressed genes. 90% confidence was chosen because at early time points some genes failed the 95% confidence limit but as the time course progressed, these and other genes typically passed the 95% or even the 99% confidence limit. Pathway visualization was performed using our tobacco MapMan mapping [6]. Fig. 2 shows the overview profile of upregulated and down regulated genes in leaf and root tissues.

3. Discussion

This study describes a microarray data set that provides a time course of the relative levels of transcripts in tobacco leaves and roots during dehydration. This data set has revealed a large number of

differentially expressed transcripts and represents the discovery phase of our continuing research. The availability of this microarray data will facilitate meta-analyses and has already proved useful by providing direct comparisons with a similar data set that we have produced using soybean plants. Comparisons of the two data sets have revealed both differences and similarities in drought stress responses between these two families of plants (legumes and solanaceae) [3].

Conflict of interest

The authors have no conflicts of interest.

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