Transcriptional-profile responses to drought in the roots of contrasting genotypes of soybean

Electronic Supplementary – MIAME (Minimum Information about Microarray Experiment)

I Array design description

1) Array related information

- Array design name: The fluorescent signals were obtained using a GMS-417 Arrayer equipment (Affymetrix, Santa Clara, CA, USA).
- Platform type: spotted in CMT-GAPSII coated slides (Corning, New York, USA).
- Surface and coating specification: Amino-Silane coated slides that permitted covalent linkage of the NH^{4+} extremity with the PO^{4-} extremity of the DNA.
- Physical dimensions of array support (e.g. of slide): 25 X 75 mm, without barcodes.
- Number of features on the array: 2 genotypes – MG/BR46 (Conquista) and BR16 and 2 treatments – water stress and non water stress.
- production protocol for custom made arrays: yes

2a) For each reporter type

- the type of the reporter: PCR products
- single or double stranded: double strand

2b) For each reporter

- Sequence or PCR primer information:
- sequence or a reference sequence: Foward: 5'-CCGAGATCTGGACGAGCTT-3' and Reverse: 5'-GCTTAACCGGTTCACTCG-3´
- Approximate lengths if exact sequence not known: approximately 700bp in 753 clones double spotted in glass slides.
- Clone information, if relevant (clone ID, clone provider, date, availability): NCBI, ID in manuscript table 3 to 6.
- element generation protocol that includes sufficient information to reproduce the element for custom-made arrays that are not generally available:

  cDNA microarray: array construction, hybridization and analysis

  Three micrograms of each amplicon were placed in a 96-well polystyrene plate and mixed with DMSO at a proportion of 1:1 (v:v). For each period of time and genotype, total RNA was extracted from soybean plants using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For labeling, 20 µg total RNA from each sample was mixed with 40U RNAsin (Promega, Madison, WI, USA), 15 µg pd(N)6 random primer (Amershan Bioscience, Sunnyvale, CA, USA). The mixture was incubated at 70°C for 10 minutes and quickly chilled at 4°C. The RNA sample was mixed with reaction buffer (1x) (Invitrogen, Carlsbad, CA, USA), DTT 10 mM, dNTP mix (0.25 mM dATP, dGTP, dCTP; 0.1 mM dTTP), Cy3-dUTP or Cy5-dUTP 1.25 mM (Amershan Bioscience, Sunnyvale, CA, USA), and 20U of the RT-Superscript II reverse

transcriptase (Invitrogen, Carlsbad, CA, USA). The sample containing labeled cDNA was then purified in a Microcon YM-100 column (Millipore, USA) (Souza et al. 2006).

The pairs of cDNA targets were mixed in hybridization solution containing the RPN3601 liquid blocker (Amershan Bioscience, Sunnyvale, CA, USA), SSC (20X) and SDS 2%, and pre-denatured at 95°C for 2 minutes. Hybridization occurred in the GeneTac Hybridization station (Genetic Microsystems, Woburn, MA, USA), to which the glass slides of the microarray were attached. Each cDNA mixture was distributed on the slide and hybridized at 42°C for 12h. After hybridization, the slide was automatically and sequentially washed in 0.5% SSC (2X)/SDS, SSC (0.5X) and SSC (0.05X), at 25°C. Each washing lasted for a period of 15 minutes, with 10 seconds flow and 20 seconds incubation for 10 cycles. The slide was dried for 15 minutes.

After normalization, microarray data were processed by the SAM statistical tool (Significance Analysis of Microarray, Chu et al. 2001). This analysis was based on a series of specific t-tests for each gene, adapted for large scale detection of differentially expressed genes (Tusher et al. 2001).

3a) For each feature type

- Attachment: covalent

3b) For each feature

- Which reporter and the location on the array: The location and identification of each gene in the array were defined in a text file created with the help of the CloneTracker 2 program (Biodiscovery, El Segundo, CA, USA).

4) The reference sequence:

- Gene name and links to appropriate databases (e.g., SWISS-PROT, or organism specific databases), if known and relevant: NCBI (National Center for Biotechnology Information).

2) Control elements on the array

Position of the feature (the abstract coordinate on the array): map array file. The images obtained were overlaid and analyzed viewing the quantification of pixels intensity for each spot on the microarray, using the ImaGene software 5.5 version (Biodiscovery, El Segundo, CA, USA). A grid of independent cells, corresponding to each DNA spot on the arrays, was drawn on the image to designate each spot to be quantified. The quantification was calculated by the mean of the intensity of all the pixels referent to the signal of each spot. The pixels classified as background were automatically subtracted by the program.

- Control type (spiking, normalization, negative, positive): normalization by Gene Sight Software version 5.5 (Biodiscovery, El Segundo, CA, USA) applying lowess correction parameters (locally weighted linear regression/robust locally weighted regression/local polynomial regression) (Cleveland 1979), with a local normalization method (the algorithm was applied in physical subsets of the data).

II Experiment description

I) Experimental design

1a) Authors, laboratory, contact:

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2) ***Type of the experiment, for instance: treated vs. untreated comparison. The plants were subjected to*** drought for 0, 25, 50, 75, or 100 min; removing the roots of nutrient solution and keeping them in the dark, later roots were collected after each period of exposure for molecular analyses.

3) ***Experimental factors, i.e. parameters or conditions tested, for instance:*** Physiological and gene expression parameters in drought conditions

4) ***response to a treatment:*** drought response

5) ***How much hybridization in the experiment?*** Four hybridizations - T_0-Cy5 (control plants) plus T_1-Cy3 (bulked roots of plants submitted to 25 and 50 min of treatment), and 2) T_0-Cy5 (control plants) plus T_2 (bulked roots of plants submitted to 75 and 100 min of treatment) for each genotypes.

6) ***If a common reference is used for all the hybridizations?*** Yes, sample non water stress - T_0 (control plants)

7) ***Quality control steps taken:***

If any replicates done (yes/no), what type of replicates, description? Yes – three biological replicate for each sample, three slides were used for each experiment, meaning RNA extractions, cDNA synthesis and hybridizations were carried out in triplicate for each experiment performed.

- Whether dye swap is used (only for two channel platforms)? Yes, Cy3 and Cy5

II. Samples used, extract preparation and labeling

1) Bio-source properties

- organism (NCBI taxonomy): *Glycine max* (soybean)
- descriptors relevant to the particular sample, such as
- development stage: Vegetative (V_2)
- organism part (tissue): root
- animal/plant strain or line: plant genotypes – MG/BR46 (Conquista) and BR16, tolerant and sensitive to drought, respectively

- 2) Biomaterial manipulations: laboratory protocol, including relevant parameters, e.g.,
- growth conditions: hydroponic system
- in vivo treatments (organism or individual treatments): at greenhouse
- treatment type (e.g., small molecule, heat shock, cold shock, food deprivation): drought shock

8) 3) Hybridization extract preparation protocol for each extract prepared from the sample, including

- ***extraction method***: Total RNA was isolated from samples of roots

  01. Homogenize the tissue in liquid nitrogen, and add 1 mL of Trizol (room temperature);
  02. Centrifuge the material at 12,000 g for 10 min, 2 to 8 ° C
  03. Remove the supernatant and transfer to a new tube;
  04. Incubate for 5 min, 15 to 30 ° C;
  05. Add 200 mL of chloroform to each mL of Trizol used initially;
  06. Close the tubes and shake vigorously for 15 sec;
  07. Incubate for 2-3min, 15 to 30 ° C;
  08. Centrifuge samples at 12,000 g for 15 min, 2 to 8 ° C;
  09. Transfer the aqueous phase (approximately 60% of volume) to a new tube;
  10. Precipitate RNA by adding 0.5 mL of isopropanol alcohol for each mL of Trizol originally used;
  11. Invert the tubes up and down slowly, initiating the formation of the pellet of RNA precipitation;
  12. Incubate for 10 minutes, 15 to 30 ° C;
  13. Centrifuge samples at 12,000 g for 15 min, 2 to 8 ° C;
  14. Remove the supernatant and wash the RNA pellet once with 75% ethanol, adding at least 1 mL of ethanol 75% for each 1 mL of Trizol used initially;
  15. Centrifuge at 7500 g for 5 min at 2-8 ° C;
  16. Open the tubes and invert them on a absorbent paper on bench at room temperature for 5 minutes to dry the pellet (pellet careful not to slip through the wall of the tube);
  17. Add 50 mL of MilliQ water and let the pellets dissolve.
  18. After extraction, to quantify in Nanodrop or spectrum, running a gel to check integrity of the material and finally, store the RNA in a freezer at -80 ° C.

- ***amplification (RNA polymerases, PCR):*** PCR reactions was composed individually by 25 ng of each DNA template, 0.1 mM dNTPs, 0.2 µM each primer (Foward: 5'-CCGAGATCTGGACGAGCTT-3' and Reverse: 5'-GCTTAACCGGTTCACTCG-3´), $MgCl_2$ 2.4 mM, DMSO (Dimethylsulfoxide) 0.25%, reaction buffer (1x) and 0.04U/µL Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). The amplification conditions were: 1) 10 minutes at 94°C; 2) 30 cycles at 94ºC for 30 seconds, 73°C for 30 seconds, and 68°C for 1 minute; and 3) 10 minutes at 72ºC. One aliquot of the PCR product was run in 1% agarose gel to verify the presence of a single band.
- ***Labeling protocol for each labeling prepared from the extract, including***
- amount of nucleic acids labeled: 20 µg total RNA from each sample
- label used (e.g., A-Cy3, G-Cy5, 33P, ….): Cy3 (green) and Cy5 (red)
- label incorporation method: direct cDNA method

III) Hybridization procedures and parameters

1) the hybridization protocol, normally including

- ***blocking agent:***

  The pairs of cDNA targets were mixed in hybridization solution containing the RPN3601 liquid blocker (Amershan Bioscience, Sunnyvale, CA, USA), SSC (20X) and SDS 2%, and pre-denatured at 95°C for 2 minutes.

- ***wash procedure:***

  Each cDNA mixture was distributed on the slide and hybridized at 42°C for 12h. After hybridization, the slide was automatically and sequentially washed in 0.5% SSC (2X)/SDS, SSC (0.5X) and SSC (0.05X), at 25°C. Each washing lasted for a period of 15 minutes, with 10 seconds flow and 20 seconds incubation for 10 cycles. The slide was dried for 15 minutes.

- ***quantity of labeled target used:***

  Three micrograms of each amplicon were placed in a 96-well polystyrene plate and mixed with DMSO at a proportion of 1:1 (v:v). For each period of time and genotype, total RNA was extracted from soybean plants using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For labeling, 20 µg total RNA from each sample was mixed with 40U RNAsin (Promega, Madison, WI, USA), 15 µg pd(N)6 random primer (Amershan Bioscience, Sunnyvale, CA, USA). The mixture was incubated at 70°C for 10 minutes and quickly chilled at 4°C. The RNA sample was mixed with reaction buffer (1x) (Invitrogen, Carlsbad, CA, USA), DTT 10 mM, dNTP mix (0.25 mM dATP, dGTP, dCTP; 0.1 mM dTTP), Cy3-dUTP or Cy5-dUTP 1.25 mM (Amershan Bioscience, Sunnyvale, CA, USA), and 20U of the RT-Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). The sample containing labeled cDNA was then purified in a Microcon YM-100 column (Millipore, USA) (Souza et al. 2006).

- ***description of the hybridization instruments:***

  Hybridization occurred in the GeneTac Hybridization station (Genetic Microsystems, Woburn, MA, USA), to which the glass slides of the microarray were attached.

IV) Measurement data and specifications of data processing

1) Image analysis and quantitation

Image analysis software specification and version, availability, and the description or identification of the algorithm and all the parameters used: The images obtained were overlaid and analyzed viewing the quantification of pixels intensisty for each spot on the microarray, using the ImaGene software 5.5 version (Biodiscovery, El Segundo, CA, USA). A grid of independent cells, corresponding to each DNA spot on the arrays, was drawn on the image to designate each spot to be quantified. The quantification was calculated by the mean of the intensity of all the pixels referent to the signal of each spot. The pixels classified as background were automatically subtracted by the program.