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Data in Brief

# Time-series analysis of the transcriptome of the re-establishment of desiccation tolerance by ABA in germinated *Arabidopsis thaliana* seeds

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ABSTRACT

Expression analyses of time series have become a very popular method for studying the dynamics of a wide range of biological processes. Here, we present expression analysis of a time series with the help of microarrays used to study the re-establishment of desiccation tolerance (DT) in germinated *Arabidopsis thaliana* seeds. Mature seeds of *A. thaliana* are desiccation tolerant (survive the loss of most of their water content), but they become desiccation sensitive while progressing to germination. Yet, there is a small developmental window during which DT can be re-established by treatment with the plant hormone abscisic acid (ABA). We studied germinated *A. thaliana* seeds at the stage of radicle protrusion during ABA incubation for 0 h, 2 h, 12 h, 24 h and 72 h. We describe in detail the methodology applied for generating and analyzing this expression data of time series. The microarray raw data (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62876) may be valuable for further studies on this experimental system, such as the construction of a gene co-expression network [1].

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| Specifications | |
|---|---|
| Organism/cell line/tissue | *Arabidopsis thaliana* (Columbia-0) seeds |
| Sex | NA |
| Sequencer or array type | Affymetrix ARAGene 1.1ST Array |
| Data format | Raw |
| Experimental factors | Time series during ABA incubation for 0 h, 2 h, 12 h, 24 h and 72 h |
| Experimental features | Germinated *A. thaliana* seeds at the stage of radicle protrusion were incubated in 6-cm Petri dishes containing 1.3 ml of an ABA solution (10 μM) on two sheets of white filter paper in the dark at 20 °C. Three replicates of approximately 1000 germinated seeds were used for each time point |
| Consent | NA |
| Sample source location | Wageningen, The Netherlands |

## 1. Direct link to deposited files

http://datalink.elsevier.com/midas/datalink/api/downloadfiles?items=15710.

## 2. Direct link to deposited genomic data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62876.

## 3. Experimental design, materials and methods

### 3.1. Experimental design

Germinated *Arabidopsis thaliana* seeds at the stage of radicle protrusion and these seeds incubated in 10 μM ABA for 2 h, 12 h, 24 h, and 72 h were used for RNA extraction (Fig. 1). Three replicates of approximately 1000 germinated seeds were used for RNA extraction for each timepoint. To assess the efficiency of the ABA treatment in re-establishing DT in these seeds, ABA treated and untreated germinated seeds were desiccated for 3 days and subsequently pre-humidified and rehydrated. The parameters evaluated were survival of their primary root, cotyledon survival and seedling survival.

## 4. Materials and methods

### 4.1. Plant growth conditions and germination assays

*A. thaliana* plants, accession Columbia (Col-0, N60000), were grown on Rockwool plugs (MM40/40; Grodan B.V., http://www.grodan.com), in a climate cell (20 °C day, 18 °C night, relative humidity (RH) of

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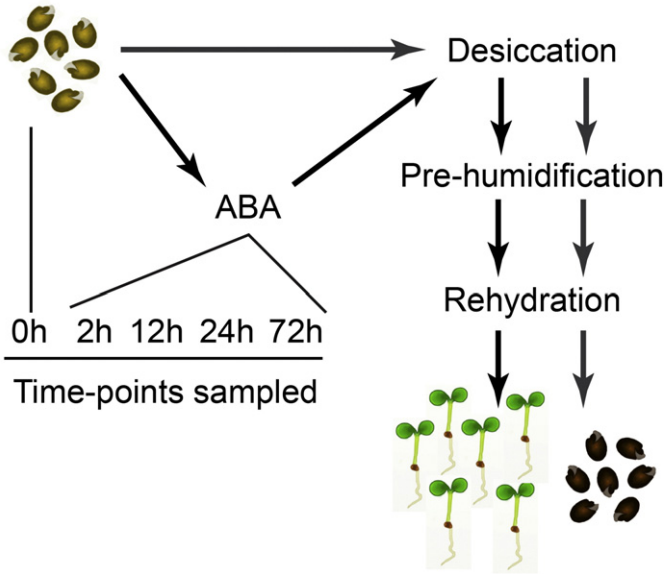


**Fig. 1.** General experimental flow. Germinated seeds at the stage of radicle protrusion and these seeds incubated in 10 μM ABA for up to 72 h were sampled for RNA extraction. To confirm the efficiency of the ABA treatment to re-establish DT in these seedlings, they were desiccated, pre-humidified, rehydrated and subsequently tested for survival.

70%) under 16 h of light (35 W $m^{-2}$) and watered with Hyponex nutrient solution (1 g $l^{-1}$, NPK = 7:6:19, http://www.hyponex.co.jp). Seeds were bulk harvested in three replicates of at least two plants. Seeds used in germination assays were cold stratified for 72 h at 4 °C in 9-cm Petri dishes on two layers of blue filter paper (Blue Blotter Paper, Anchor Paper Company, http://www.seedpaper.com) and 10 ml of distilled water. After stratification, seeds were transferred to germination cabinets with constant white light at 22 °C.

### 4.2. Re-establishment of DT using ABA

To assess the re-establishment of DT using ABA, germinated seeds at the stage of radicle protrusion (stage II, [2]) were selected using a stereomicroscope and incubated for a maximum of 3 days in 6-cm Petri dishes containing 1.3 ml 10 μM ABA on two sheets of white filter paper (grade 3hw, Biolab Products, Sartorius Stedim Biotec) in the dark at 20 °C. The incubation in ABA was done in the dark in order to reduce oxidative damage. After incubation, seeds were rinsed thoroughly in distilled water with the aid of a sieve, transferred to new Petri dishes with one dry sheet of white filter paper and dried for 3 days in a closed chamber at 40% RH (achieved by a saturated calcium chloride solution) at 20 °C, resulting in water content levels as low as 0.08 g H2O g-1 dry weight. Water contents were assessed gravimetrically for triplicate samples of 50 germinated seeds, by determination of the fresh weight and subsequent dry weight after 17 h at 105 °C. After drying seeds were pre-humidified in air of 100% RH for 24 h at 22 °C in the dark, in order to avoid imbibitional damage, and subsequently rehydrated for 10 days in water on a Copenhagen Table under a 12/12 h dark/light regime at 20 °C. Germinated seeds were evaluated according to the survival of their primary root, cotyledon survival (presence of green and fully expanded cotyledons) and seedling survival (growth resumption with both green and fully expanded cotyledons and development of a root system).

### 4.3. RNA extraction and microarray hybridization

Germinated seeds at the stage of radicle protrusion (control) and these seeds after four periods of incubation in ABA (2 h, 12 h, 24 h and 72 h) were used for RNA extraction. Total RNA was extracted from three replicates of approximately 1000 germinated seeds for each time point following a modified hot borate protocol [2,3]. The seeds were ground and mixed with 800 μl of extraction buffer (0.2 N Na borate decahydrate (borax), 30 mM EGTA, 1% SDS, 1% Na deoxycolate) containing 1.76 mg DTT and 52.8 mg PVP40, and heated to 80 °C. In the next step, 4 mg proteinase K was added to this solution before incubation for 15 min at 42 °C. After the addition of 64 μl of 2 M KCL, the samples were incubated on ice for 30 min and subsequently centrifuged for 20 min at 12,000 *g*. The supernatant was transferred to a new tube, 260 μl of ice-cold 8 M LiCl was added and the tubes were incubated overnight on ice. After centrifugation at 4 °C for 20 min at 12,000 *g*, the pellets were washed with 750 ml of ice-cold 2 M LiCl and resuspended in 100 μl milliQ water. The samples were DNAse treated (RQ1 DNase, Promega) as described by the manufacturer. Subsequently, 100 μl of phenol–chloroform (1:1) was added to the samples which were transferred to 2.0 mL tubes containing Phase Lock Gel (LPLG, Eppendorf Sci., Inc.), that had been spun for 30 s at maximum centrifuge speed immediately prior to use. The aqueous and organic phases were separated by centrifugation at 12,000 *g*, 4 °C for 5 min and the aqueous upper phase was further purified with RNeasy spin columns (Qiagen) according to the manufacturer's instructions. RNA quality and concentration were assessed by agarose gel electrophoresis and a NanoDrop ND-1000 spectophotometer (Nanodrop® Technologies, Wilmington, DE, USA). RNA was processed for the use on Affymetrix ARAGene 1.1ST arrays as described by the manufacturer. In brief, Biotin-labeled cDNA was prepared using the Affymetrix NuGEN PicoSL WTA v2 kit and Biotin Module from 50 ng total RNA. The ss-cDNA was fragmented, denaturized and used for hybridization on the Affymetrix ARAGene 1.1ST array plate. The Affymetrix HWS Kit for GeneTitan was used for hybridization, washing and staining of the array plates. The array plates were scanned using the Affymetrix Command Console v3.2 software.

All data are MIAME compliant as detailed on the MGED society website http://www.mged.org/Workgroups/MIAME/miame.html. The microarray data has been deposited on the NCBI's Gene Expression Omnibus [4] and is accessible through the GEO Series accession number GSE62876.

### 4.4. Quality control

The quality control was performed in the software Affymetrix Expression Console v.1.2.1. In order to minimize the effect of probe-specific affinity differences, the Robust Multichip Analysis (RMA-Sketch for gene level analysis) algorithm [5] was used for the summarization. Signal intensities were extracted and analyzed using the Bioconductor packages of R [6]. The data was normalized using the RMA algorithm with the TAIRG v17 cdf file (http://brainarray.mbni.med.umich.edu).

A box plot and a correlation heat map were used to detect possible outliers. One replicate of time point 2 h showed the lowest signal and both the box plot and the correlation plot indicated it as an outlier. Therefore, this replicate was removed for further analysis.

### 4.5. Microarray analysis

To equalize background noise, gene expression values less than four were replaced with four [7]. After this transformation, fold changes were calculated comparing each time-point with the control (non-treated germinated seeds at the stage of radicle protrusion). A gene was considered differentially expressed (the Differentially Expressed Genes: DEGs) if the difference between its mean expression in at least one time-point and the control was statistically significant at $P \leq 0.01$ after application of linear modeling with thresholds for absolute fold change of 2.0 (on a log2 scale).

### 4.6. Validation

To verify the accuracy of the microarray data, the expression of 20 genes with different expression patterns were analyzed by RT-qPCR.

For the RT-qPCR analyses, a new physiological experiment was done. Germinated seeds in the stage of radicle protrusion incubated in ABA for 0 h, 2 h and 24 h were sampled and RNA was isolated as describe before. Once the quality of the RNA and the absence of DNA contamination were assessed, cDNA was produced with the reverse transcriptase procedure (iScript cDNA Synthesis Kit; Bio-Rad). To do so, 600 ng of RNA was added to 4 μl iScript reaction mix, 1 μl reverse transcriptase and enough DEPC-treated water to reach 20 μl of solution. The cDNA synthesis protocol was as follows: 5 min at 25 °C, 30 min at 42 °C and 5 min at 85 °C. After the reaction, the samples were diluted with 180 μl DEPC-treated water.

The RT-qPCR was performed by mixing 5 μl cDNA, 10 μl iQ Sybr Green supermix (Bio-Rad), 0.5 μl primer mix (1:1 mix of 10 μM forward and reverse primer) and 4.5 μl DEPC-treated water. Samples were analyzed in duplicate and two genes were measured per plate to minimize technical variation between samples. The plates were placed in the MyiQ iCycler (Bio-Rad) and the reaction protocol was as follows: 3 min at 95 °C, followed by 50 cycles of 15 s at 95 °C and 1 min at 60 °C. Reaction products were confirmed by melting curve analysis.

## References

[1] M.C.D. Costa, K. Righetti, H. Nijveen, F. Yazdanpanah, W. Ligterink, J. Buitink, et al., A gene co-expression network predicts functional genes controlling the re-establishment of desiccation tolerance in germinated Arabidopsis thaliana seeds. Planta (2015) 1–15.

[2] J. Maia, B.J.W. Dekkers, N.J. Provart, W. Ligterink, H.W.M. Hilhorst, The re-establishment of desiccation tolerance in germinated *Arabidopsis thaliana* seeds and its associated transcriptome. PLoS ONE 6 (2011) e29123, http://dx.doi.org/10.1371/journal.pone.0029123.

[3] C.-Y. Wan, T.A. Wilkins, A modified hot borate method significantly enhances the yield of high quality RNA from cotton (*Gossypium hirsutum* L.). Anal. Biochem. 223 (1994) 7–12.

[4] R. Edgar, M. Domrachev, A.E. Lash, Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30 (2002) 207–210.

[5] R.A. Irizarry, B. Hobbs, F. Collin, Y.D. Beazer-Barclay, K.J. Antonellis, U.W.E. Scherf, et al., Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4 (2003) 249–264.

[6] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, et al., Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5 (2004) R80.

[7] B.J.W. Dekkers, S. Pearce, R.P. van Bolderen-Veldkamp, A. Marshall, P. Widera, J. Gilbert, et al., Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. Plant Physiol. 163 (2013) 205–215, http://dx.doi.org/10.1104/pp.113.223511.