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Data in Brief

Time course transcriptional profiling of senescing barley leaves

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ABSTRACT

Cell senescence occurs as a part of developmental or stress-induced process. It is tightly regulated and involves a sequence of metabolic and structural alterations, eventually leading to cell death. Dark-induced leaf senescence is a useful model for studying senescence-related events. To facilitate the integration of physiological and molecular studies utilizing this model, we generated the microarray data set providing time course gene expression profiles in senescing barley leaves. Here, we describe the detailed procedures and data analysis scheme of our experiment. The entire data set (available at NCBI/GEO database under GSE62539) has been successively explored to find the genes differentially expressed during the senescence process as well as to identify genes with the invariant expression as reliable references for qPCR or ddPCR experiments.

Specifications	
Organism/cell line/tissue	*Hordeum vulgare* L. 'Nagrad'/primary leaf
Sex	N/A
Sequencer or array type	Barley gene expression microarrays, 4x44K, Agilent
Data format	Raw: gpr files; analyzed: txt files of log2 expression ratios
Experimental factors	Time of treatment (incubation in darkness)
Experimental features	Time course profiling of gene expression in barley leaves incubated in darkness, focused on identification of genes involved in the senescence process
Consent	N/A
Sample source location	N/A

Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62539

Experimental design, materials and methods

Experimental scheme

Senescence and cell death are genetically programmed processes turned on in animals and plants as a part of their normal development or in response to environmental challenge. A simple and well-controlled model for studying physiological and molecular aspects of leaf senescence has been developed [1]. In this model, the process is induced by continued incubation of the seedlings in the darkness. In barley, within 10–12 days of the senescence progress the leaves turn yellow and gradual degradation of cell components, mainly chloroplasts and nuclei can be observed [2]. To facilitate the integration of physiological and molecular studies utilizing the model of dark-induced senescence, we generated the microarray data set providing time course gene expression profiles in senescing barley leaves.

Plant materials, growth conditions and sampling

Barley (*Hordeum vulgare* L. 'Nagrad') seeds were surface-sterilized with ethanol (70%) and standard bleach (20%) for 10 min, followed with extensive washing. Seedlings were grown in soil under controlled conditions (day/night 16/8 h, 23 °C, light intensity 150 μmol m^{-2} s^{-1}, 60% humidity). The material for the Day 0 sample was then collected. Light limitation initiated the onset of senescence and allowed the leaves to senesce in the darkness for 3, 7 or 10 days. Plant material was collected at Day 3, Day 7 and Day 10.

The scheme of senescence induction and sample collection experiment is presented in Fig. 1. At a given time point, the Fv/Fm ratio (the maximum quantum yield of PSII in the dark adapted state, indicative of the photosystem's II physiological state) was measured for each plant with FluorPen FP100 (Photon System Instruments). Leaves of 15 plants per time point were then collected, which presented "typical" Fv/Fm values (~0.843 for Day 0, ~0.762 for Day 3, ~0.624 for Day 7 and ~0.116

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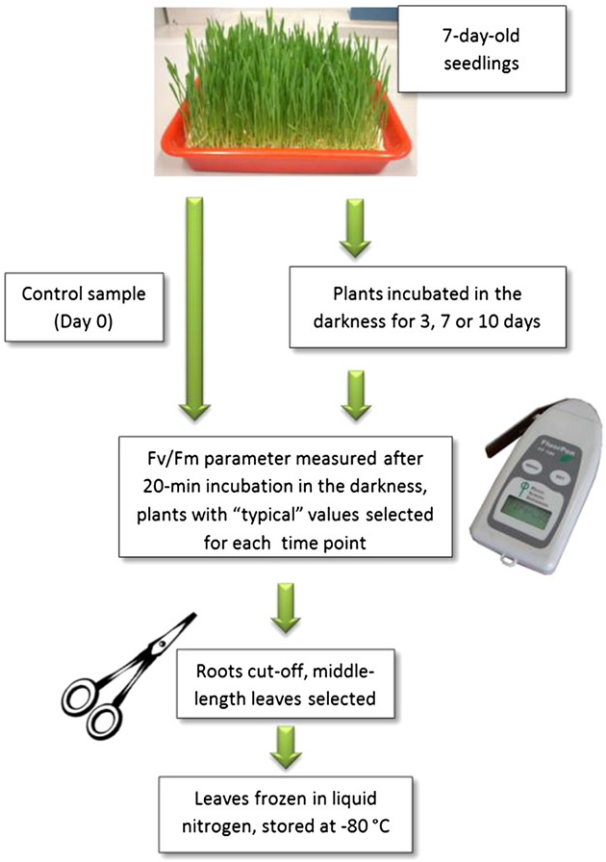

Fig. 1. Scheme of plant growth and experimental factor treatment.

for Day 10, as determined in a preliminary study). Leaves were pooled, frozen in liquid nitrogen and stored at −80 °C until use. The entire plant growth and sample collection procedure was repeated 3 times to obtain independent biological replicates of the senescing plants.

Total RNA isolation and quality control

Total RNA extraction was performed using spin-columns (RNeasy Plant Mini Kit, QIAGEN), following the manufacturer's guidelines. For the common reference sample, the material from all time points and all replicates was pooled before RNA isolation. Up to 100 mg frozen material finely ground in liquid nitrogen with mortar and pestle was used per column. The RNA was eluted with 2 × 75 μl pure water, quality checked on Nanodrop 2000 and immediately subjected to DNase treatment using TURBO DNA-free kit (Ambion). Final RNA quality was again determined using Nanodrop 2000. All of the samples used for this study were of excellent purity (A260/A280 ≥ 1.9; A260/A230 ≥ 2) and showed no visible signs of degradation in 2100 Bioanalyzer RNA-Nano assay (Agilent). RNA samples were stored at −80 °C at a concentration of ~0.5 μg/μl.

Microarray experiments

Hybridization to microarrays was performed according to a common reference design: Cy5-labeled experimental samples representing barley leaf transcriptome in subsequent experimental time points (Day 0, Day 3, Day 7, Day 10) were hybridized each against Cy3-labeled common reference sample.

Amplified labeled cRNA samples were prepared from 200 ng total RNA each, using Quick Amp Labeling Kit (Agilent) and the manufacturer's guidelines. RNA Spike-In Kit (Agilent) was included in each total RNA sample before labeling, following the manufacturer's guidelines regarding the spike relative concentrations in Cy3 and Cy5 samples (Table 1). Labeled cRNA (825 ng) was used for the hybridization to Barley Gene Expression Microarrays, 4x44K (Agilent). A total number of 12 microarrays were used, as each condition was represented by 3 biological replicates. The hybridization, washing and drying steps were performed in A4x44k Quad Chambers in an HS 4800 Pro (Tecan) automatic hybridization station, according to the manufacturer's guidelines developed directly for that type of device [3]. Gene Expression Hybridization Kit (Agilent) and Gene Expression Wash Buffer Kit solutions (Agilent) were used for the hybridization and washing steps, respectively. The intensity data were collected with 4200AL GenePix scanner and GenePix Pro 6.1 software at 5 μm resolution, at green and red channel. Each microarray was scanned twice with auto PMT adjusted at low (0.01%) or high (1%) spot saturation levels.

Data processing

The scans were processed with GenePix Pro 6.1 software using morphological opening background method. Spots with >10% saturated pixels in both channels were flagged as "bad" and not considered during the differential analysis. All subsequent steps were conducted using R/Bioconductor limma package [4]. Both low- and high-saturation scanned data sets were background-corrected ("subtract," offset = 10), and the scans with low and high intensity were merged with mergeScansRG function. Data were normalized within arrays ("loess" method) and between arrays ("Aquantile" method). A series of quality plots were generated to evaluate the data normalization performance and assess the microarray data accuracy and dynamic range (Fig. 2). The analysis of Spike-in controls confirmed that the generated data set well reflected the theoretical Cy5/Cy3 RNA ratios across a broad range (up to 200-fold dilution, see Table 1) of template copy numbers (Fig. 3). It is therefore a reliable resource of information regarding transcripts of both high and low abundance in the experimental samples. Raw and normalized gene expression data were deposited in Gene Expression Omnibus repository [5] and are accessible through GEO Series accession number GSE62539.

Senescence-responsive genes

In order to identify the set of genes with differential expression during the dark-induced senescence, Baesian linear modeling was applied in a separate channel analysis mode. Time course profiles were generated by measuring gene expression at Day 3, Day 7 and Day 10, in comparison with Day 0. Additionally, early and late senescence stages were directly compared (Day 10 versus Day 3). Of 43,603 unique oligonucleotide probes present on the microarray, 3,014 exhibited differential gene expression, with moderated *F*-statistic p value < 0.0005 (after applying Benjamini and Hochberg's method to control the false discovery rate). For simplicity, we further assume that the number of differentially regulated genes equals the number of probes, although it cannot be

Table 1
Relative amounts of Spike-in controls added to total RNA before labeling.

Spike-in control	Relative copy number in Cy3 sample	Relative copy number in Cy5 sample	Expected Cy5/Cy3 ratio
E1A_r60_a22	10	100	0.1
E1A_r60_3	3	9	0.3
E1A_r60_a104	10	30	0.3
E1A_r60_a97	0.5	1.5	0.3
E1A_r60_1	10	10	1
E1A_r60_a20	100	100	1
E1A_r60_n11	1.5	0.5	3
E1A_r60_a107	30	10	3
E1A_r60_a135	9	3	3
E1A_r60_n9	100	10	10