Comparative transcriptome analysis of energy-rich Arabidopsis thaliana under dark and light conditions

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Abstract
Overexpression of Arabidopsis thaliana purple acid phosphatases AIPAP2 in Arabidopsis can promote plant growth. The overexpression (OE) lines flower earlier, grow faster, and contain more ATP, sucrose, and starch. The seed yields and siliqua numbers of OE lines are also higher than the control lines (Sun et al., 2012a). In this study, we compared the leaf transcriptomes of 20-day-old transgenic and wild-type Arabidopsis grown under long day (16:8 h) conditions. Total RNAs were collected at three time points: end of night (t=0 hr), one hour after light was turned on (t=1 hr), and after light was turned on (t=48 hr). AIPAP2 is mainly targeted to chloroplasts and mitochondria. To study the RNA encoded by chloroplast and mitochondrial genomes, ribosomal RNAs were removed before sequencing. Approximately 65 million clean reads (≈600bp) were obtained by illumina Hiseq TM 2000 sequencing from each library. In total, after assembly 29,485 transcripts were detected from the six libraries. More genes are suppressed in OE leaves (vs. WT) at all the three time points, 1,623 (down-regulated) versus 945 (up-regulated), 1,908 (down-regulated) versus 712 (up-regulated) and 1,642 (down-regulated) versus 824 (up-regulated) at t=0, 1 and 48 hours, respectively. Expression profiles of light-induced transcripts based on K-means clustering were analyzed. There are significant changes in the transcription levels of various components of photosystems, light-harvesting chlorophyll protein complexes (LHC), respiratory complexes, RNA polymerases and ribosomes. Our data provide systemic portraits of global changes in Arabidopsis transcriptomes caused by light to light transition (external energy input) and internal energy status.

Methodology
Arabidopsis thaliana ecotype Columbia (Col-0) (wild type: WT), AIPAP2 overexpressors (OE) in Col-0 background were used in this study (Sun et al., 2012b). Arabidopsis seeds of WT and OE7 lines were surface sterilized and plated on Murashige and Skogor medium supplemented with 2% (w/v) sucrose for 20 days before the seedling were transferred to soil under 16 hours light (22°C) / 8 hours dark (18°C) period in a growth chamber at a light intensity of 120-150 μmol m−2 s−1. Leaves of 20-day-old plants without bolting were immediately frozen in liquid nitrogen for RNA extraction. Leaves were harvested at three different time points: t=0 hr (end of night), t=1 hr (one hour after light turn on) and t=48 hr (eight hours after light turn on) respectively.

Flowchart
Total RNA
Removal of Ribosome RNA
Clean Reads
Expression level (RPMK)
Cluster analysis
Gene ontology analysis
KEGG pathway analysis

Results

Table 1: Numbers of unique number of sequencing reads mapped to Gene in TAR (103)

<table>
<thead>
<tr>
<th>Gene</th>
<th>TAR_TARGET</th>
<th>TAR_NONTARGET</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT 1</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 2</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 3</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 4</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 5</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 6</td>
<td>100.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>WT 7</td>
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<td>0.00%</td>
</tr>
<tr>
<td>WT 8</td>
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<tr>
<td>WT 9</td>
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<td>0.00%</td>
</tr>
<tr>
<td>WT 10</td>
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</tr>
</tbody>
</table>

Findings
- Our study presents the impacts of light (WTD. 1, 6) and energy (WT vs OE) on the leaf transcriptomes of Arabidopsis.
- Light induce the transcription of genes of PSI peripheral proteins, LHCCI, LHCCII and oxygen-evolving complexes. However, transcription of most genes of the NDH complexes and Lhca5 are downregulated in WT.
- The transcription of genes of Psll core (psaA/B/C), Plll core proteins and Cbyt bII complexes are not induced by light.
- Comparing to WT, the transcription of genes of certain Lhcbas, Lhca3, FAD2, Ndh0 are downregulated in the OE line under all 3 light conditions.
- The gene transcription of Cytochrome c, an electron transducer in lumen, is strongly induced in the OE lines.
- The transcription of genes of chloroplast genome is not significantly affected by light, but it is more obvious in some of the mitochondrial genomes.
- The impact of light to the transcriptional levels of 429 ribosomal protein genes were studied.
- The transcription of many ribosomal protein genes are downregulated in the OE lines at t=0, which is less obvious at t=1 and t=48 hr.

Fig. 1 Growth phenotypes of four-week-old Arabidopsis under long day (16:8 h) light condition. WT: wild type; T DNA: OE7 mutant line; OE: AIPAP2 over-expression lines (Sun et al., 2012b).

Fig. 2 Content of AIPAP2. NAD/MAP/PADPH measured at t=0, 1, and 6 hr. Data are expressed as means with ±SD of three biological replicates (unpublished data).

Fig. 3 Flowchart of this study.

Fig. 4 Differentially Expressed Genes (DEGs) were clustered in different group with PEAR (log fold change ≥10) and Fold Change of ≥2. The colors indicate the expression levels.

Fig. 6 Expression profiles are based on K-means clustering. Mean log2 expression ratios for genes that were significantly changed at least one time point were shown in WT (A) and OE (B) respectively. Each gene within a gene cluster is shown with a grey line and the mean expression profile for all genes is indicated with a single pink line. Number of genes in each cluster are shown above each panel.

Fig. 8 The expression levels of transcripts, indicated by KEGG thionine breakdown were up regulated (red frame) and down regulated (green frame) in OE vs WT. (A) DE_1 vs DE_2; (B) DE_3 vs DE_4; (C) DE_5 vs WT were compared.