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

Chao LIANG and Boon Leong LIM* vechao@hku.hk
School of Biological Sciences, the University of Hong Kong, Hong Kong, China

Abstract

Overexpression of *Arabidopsis thaliana* purple acid phosphatases *AtPAP2* in Arabidopsis can promote plant growth. The overexpression (OE) lines flower earlier, grow faster, and contain more ATP, sucrose and glucose. The seed yields and silique numbers of OE lines are also more than the control lines (Sun et al., 2012a). In this study, we compared the leaf transcriptomes of 20-d-old transgenic and wild-type Arabidopsis grown under long day (16h/8h) condition. 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 eight hours after light was turned on (t=8 hr). AtPAP2 is dually targeted to chloroplasts and mitochondria. To study the RNA encoded by chloroplast and mitochondrial genomes, ribosomal RNAs were removed before sequencing. Approximate 65 million clean reads (~6Gbp) were obtained by Illumina HiSeqTM 2000 sequencing from each library. In total, after assembly 29,435 transcripts are 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 8 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 transcriptome exerted by dark to light transition (external energy input) and high internal energy status.

Background

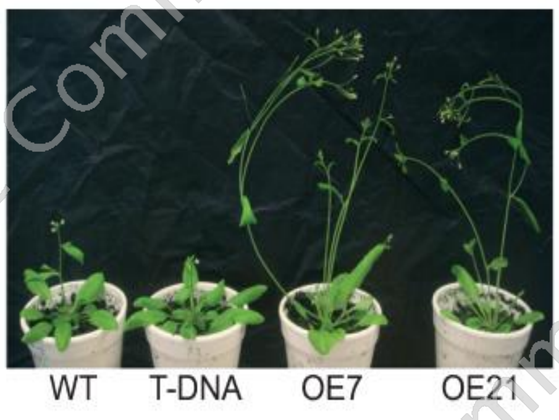


Fig. 1 Growth phenotypes of four-wk-old *Arabidopsis* under long day (16hr light/8hr dark)) conditions. WT, wild type; T-DNA, AtPAP2 mutant line; OE, AtPAP2 over-expression lines (Sun et al., 2012b).

OE lines of ATPAP2 contain higher levels of ATP in dark and in light conditions.

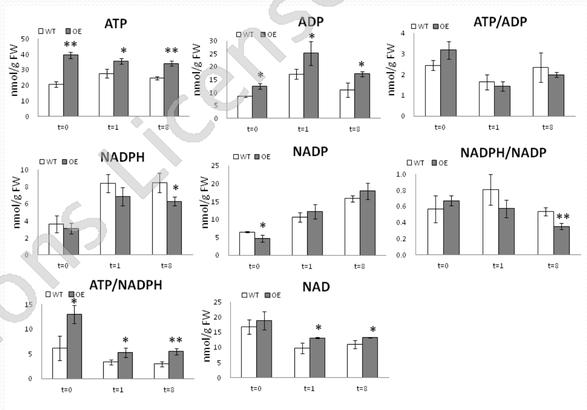
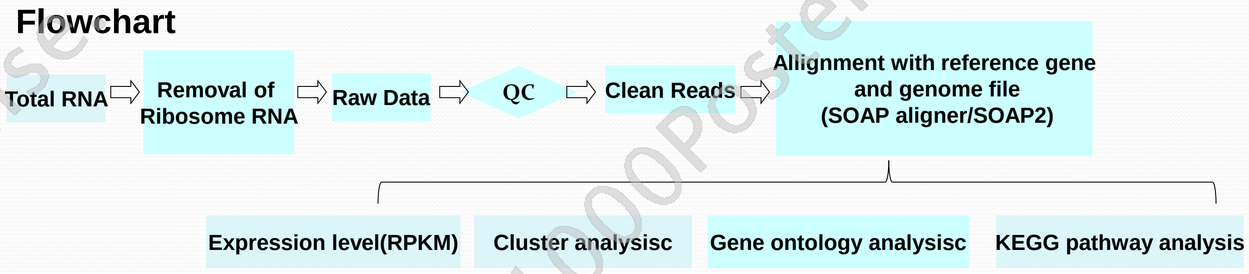


Fig.2 Content of ADP,ATP. NAD,NADP,NADPH measured at t=0, 1, and 8 hr. Data are expressed as means with ±SD of three biological replicates (unpublished data). Independent sample t-test using IBM SPSS Statistics 19 software. Asterisks indicate significant difference between WT and OE, *P<0.05, **P<0.01. FW, Fresh weight

Methodology

Arabidopsis thaliana ecotypes Columbia (Col-0) (wild type: WT), AtPAP2 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 Skoog medium supplemented with 2% (w/v) sucrose for 10 days before the seedling were transferred to soil under 16 hours light (22℃) / 8 hours dark (18℃) 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=8 hr (eight hours after light turn on) respectively.

Flowchart



Results

Table 1 Stastistics of total number of sequencing reads mapped to Gene in TAIR 10.0.

	OE_0		OE_1		OE_8		WT_0		WT_1		WT_8	
Map to Gene	Reads number	Percentage	Reads number	Percentage	Reads number	Percentage	Reads number	Percentage	Reads number	Percentage	Reads number	Percentage
Total Reads	65217952	100.00%	64885466	100.00%	67086798	100.00%	65856848	100.00%	68717410	100.00%	66618050	100.00%
Total BasePairs	5869615680	100.00%	5839691940	100.00%	6037811820	100.00%	5927116320	100.00%	6184566900	100.00%	5995624500	100.00%
Total Mapped Reads	49336837	75.65%	51529284	79.42%	51798246	77.21%	50220939	76.26%	54644882	79.52%	52800526	79.26%
perfect match	35412269	54.30%	37068080	57.13%	36637397	54.61%	39421448	59.86%	43404340	63.16%	42190196	63.33%
<=5bp mismatch	13924568	21.35%	14461204	22.29%	15160849	22.60%	10799491	16.40%	11240542	16.36%	10610330	15.93%
unique match	38628227	59.23%	40394837	62.26%	41849875	62.38%	40502486	61.50%	43070199	62.68%	42066874	63.15%
multi-position match	10708610	16.42%	11134447	17.16%	9948371	14.83%	9718453	14.76%	11574683	16.84%	10733652	16.11%
Total Unmapped Reads	15881115	24.35%	13356182	20.58%	15288552	22.79%	15635909	23.74%	14072528	20.48%	13817524	20.74%

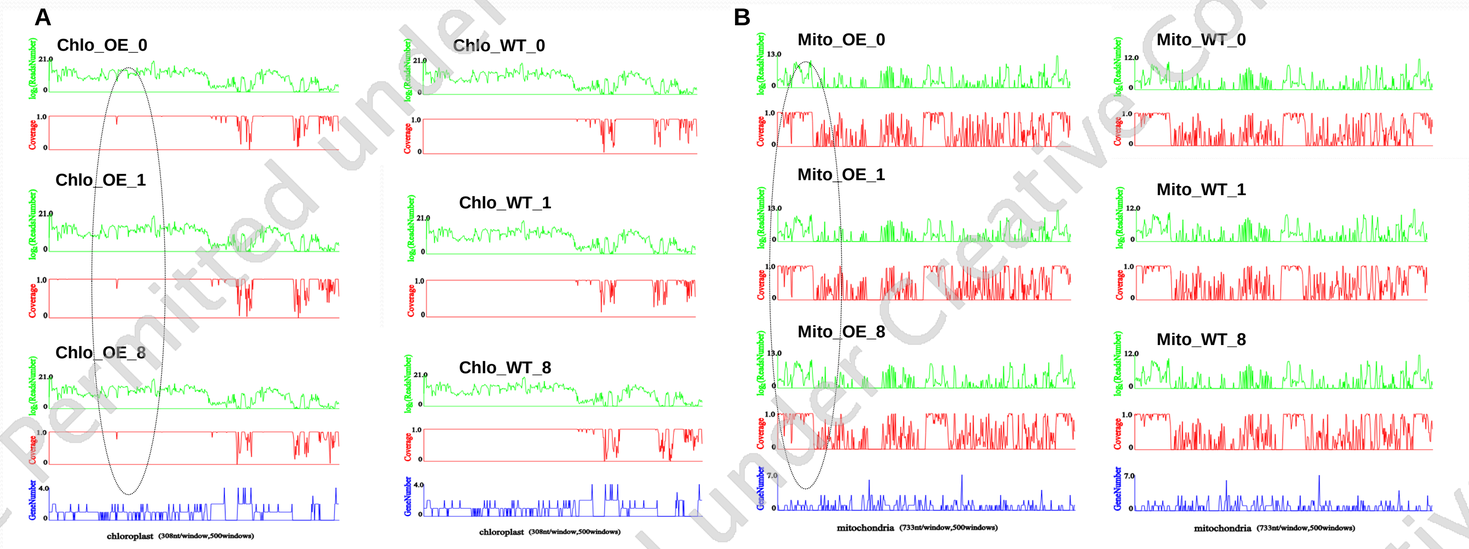


Fig.3 Differential gene expressions of chloroplast (A) and mitochondrial (B) genomes of WT and OE lines at t=0,1 and 8 hr.

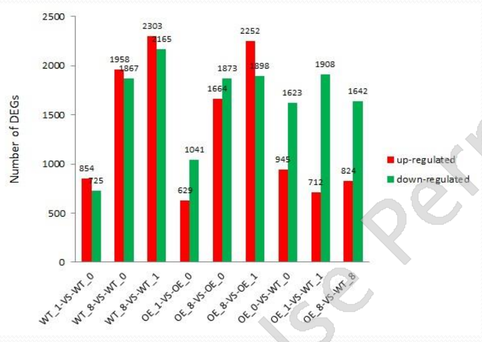


Fig.4 Differentially Expressed Genes (DEGs) were shown in different groups with False Discovery Rate (FDR) ≤0.001 and Fold Change of 2 folds. .

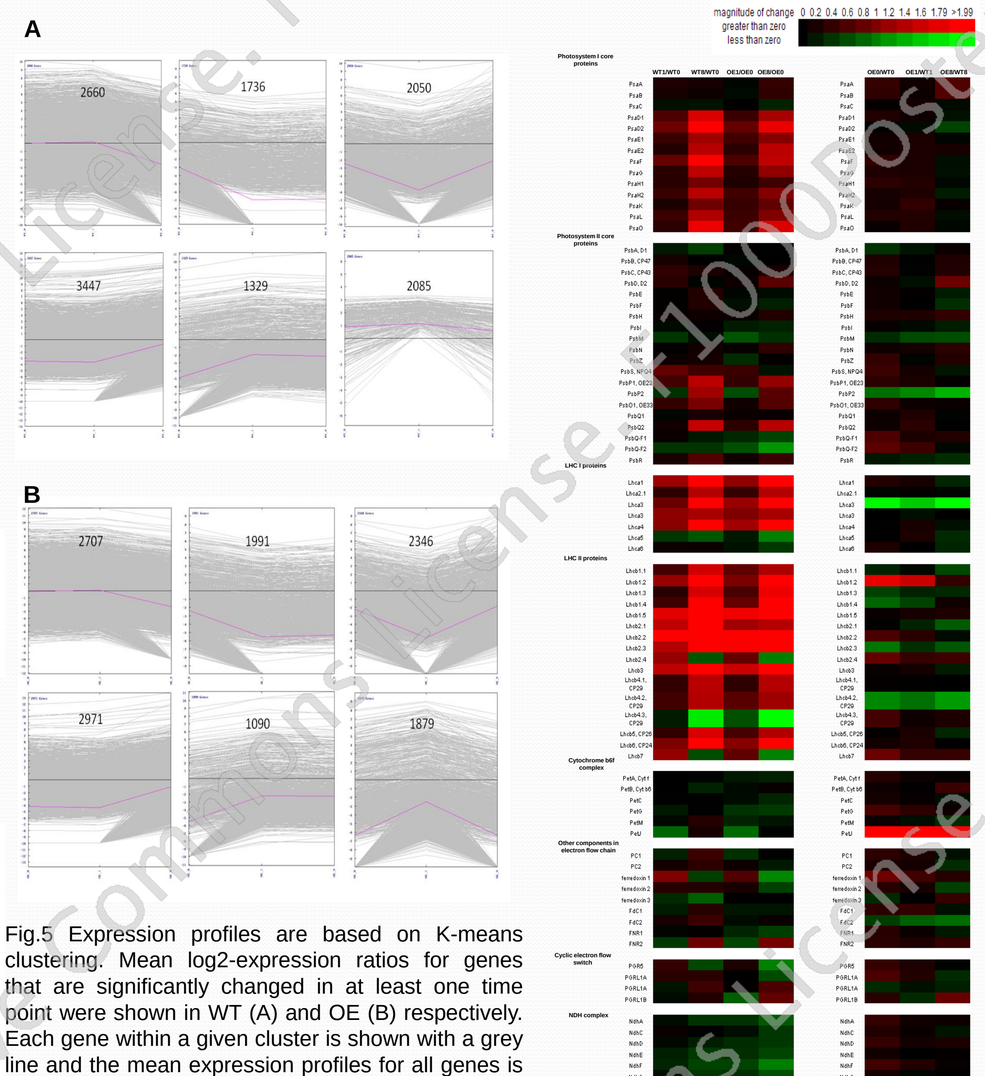


Fig.5 Expression profiles are based on K-means clustering. Mean log2-expression ratios for genes that are significantly changed in at least one time point were shown in WT (A) and OE (B) respectively. Each gene within a given cluster is shown with a grey line and the mean expression profiles for all genes is indicated with a single pink line. Number of genes in each cluster are shown above each panel.

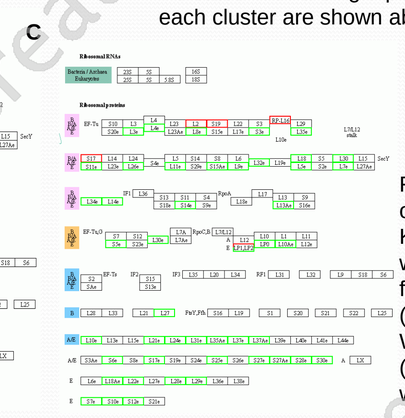


Fig.7 The expression levels of transcripts involved in KEGG ribosome biogenesis were up regulated (red frame) and down regulated (green frame). OE_0 vs WT_0 (A), OE_1 vs OE_1 (B) and OE_8 vs WT_8 (C) were compared respectively.

Fig. 6 Heatmaps of transcripts for the photosystem, values were calculated by log2-expression ratios.

Findings

- **Our study presents the impacts of light (WT0, 1, 8) and energy (WT vs OE) on the leaf transcriptomes of Arabidopsis.**
- **Light induce the transcription of genes of PSI perpheral proteins, LHCI, LHCII and oxygen-evolving complexes. However, transcription of most genes of the NDH complexes and Lhca5 are down-regulated by light.**
- **The transcription of genes of PSI core (psaA/B/C), PSII core proteins and Cyt b6f complexes are not induced by light.**
- **Comparing to WT, the transcription of genes of certain Lhcbs, Lhca3, FdC2, NdhO are downregulated in the OE line under all 3 light conditions.**
- **The gene transcription of Cytochrome c6, 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 for some genes in the mitochondrial genome.**
- **The impact of light to the transcription levels of 429 ribosomal protein genes were studied.**
- **The transcription of many ribosomal protein genes are downregulated in the OE lines at t=8, which is less obvious at t=0 and t=1.**

Sun F, Carrie C, Law S, Murcha MW, Zhang R, Law YS, Suen PK, Whelan J, Lim BL (2012a) AtPAP2 is a tail-anchored protein in the outer membrane of chloroplasts and mitochondria. Plant signaling & behavior 7
Sun F, Suen PK, Zhang Y, Liang C, Carrie C, Whelan J, Ward JL, Hawkins ND, Jiang L, Lim BL (2012b) A dual-targeted purple acid phosphatase in Arabidopsis thaliana moderates carbon metabolism and its overexpression leads to faster plant growth and higher seed yield. The New phytologist 194: 206-219