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Transcriptomic analysis of *Hevea brasiliensis* seedlings under supplemental LED night lighting

Xingcheng Yao, Hanqi Tu, Xinlong Wang, Jun Wang

Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan, 571101, China
Haikou Key Laboratory of Innovation of Seedlings of Tropical Plants
Hainan Provincial Key Laboratory of Cultivation and Physiology for Tropical Crops

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Abstract— *Hevea brasiliensis is an important economic crop which produces natural rubber. Supplemental LED night lighting improves its growth, however the underlying molecular mechanism remains unknown. The study analyzed the transcriptome of H. brasiliensis plants under the treatment of LED night lighting. The light treatment resulted in 1047 and 411 differentially expression genes (DEGs) during the day and night time, respectively. Functional group analysis showed that DEGs in the day time enriched into 185 metabolic pathways and that DEGs in the night time enriched into 116 metabolic pathways. A total of 92 DEGs were identified between night lighting and control plants. These DEGs were involved in regulation of pigment metabolism, photosynthesis, circadian rhythm, and carbohydrate metabolism. The genes associated with circadian rhythm were altered during the day and night time. The gene involved in carbohydrate metabolic process was upregulated and the related KEGG pathways associated carbohydrate metabolism were upregulated. These results concluded that supplemental LED night lighting improve growth of hevea plants by upregulating genes associated with photosynthesis and carbohydrate metabolism, so as to synthesize more carbohydrates.*

Keywords— ***rubber tree, seedling culture, photoperiod, circadian rhythm, molecular mechanism***

I. INTRODUCTION

The Para rubber tree (*Hevea brasiliensis* Müll. Arg.) is the sole source of commercial natural rubber worldwide. Due to its economic and industrial importance, China plants more than 10 million hectares of rubber trees. However, as China belongs to non-traditional zone for rubber tree growing, the growth speed of rubber tree in China is much lower than that in traditional zones. This is because China encounters low temperature in winter, which constrains the growth of rubber trees. It generally takes more than 8 years for the trees to attain the girth required for tapping (50 cm) in China. This is much longer than the time required in traditional zones (approximately 5–6 years). The low growth speed in China thus increases the time required for propagating planting materials (grafted seedlings). Around 18-20 months are needed for producing planting materials by green budding. It is much longer than the time needed in Southeast Asian countries.

Light is one of the key environmental factors regulating plant growth and development. As light provides energy for photosynthesis, limited light conditions could be a constraining factor for plant growing. Supplemental lighting during the night extends the photoperiod, which enables increased photosynthesis and thus promotes plant growth. Light-emitting diode (LED) lamps are the preferred source for supplemental lighting due to their low operating temperature, durability and low cost (Singh et al., 2015). Supplemental night lighting has been widely used in the production of crops and other plants to promote growth and quality (Fukuda et al., 2000, 2004, Okushima et al., 2012, Zhou et al., 2031, Kweon et al., 2016, Tewolde et al., 2016). Our earlier studies have been demonstrated that supplemental LED night lighting can improve the growth of rubber trees by accelerating rhythmic growth of shoots (Yao

et al., 2021a, b). The rhythmic growth of rubber tree is endogenously controlled (Hallé et al., 1978), however the underlying molecular mechanism is not clear. In this study, the transcriptome profile of plants under LED night lighting was investigated to uncover the molecular mechanism relative to growth accelerating under extended photoperiods.

II. MATERIALS AND METHODS

Plant materials and experimental design

One year old grafted plants (clone CATAS 73397) were subjected to LED night lighting from 20th December, 2021. The supplemental lighting time during the night was from 18:00 PM to 06:00 AM. LEDs were hung 0.5 m above the plants. The lamp spectrum was red and blue combined with a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1} measured at 50 cm from the LED lamps. Plants without night lighting were used as control plants. Three repeats were designed for night lighting and control plants, respectively. Each repeat was composed of 15 plants. After one month, leaves from top flush were collected for transcriptome sequencing. Sample were collected at two time points, 10:00 and 22:00. Leaves were immediately frozen in liquid nitrogen and then stored at -80°C until for RNA extraction. Three samples were collected from corresponding repeats.

RNA preparation, transcriptome sequencing, and assembly

Total RNAs of leaf samples were extracted with TRIzol reagent (Invitrogen) according to the manufacturer's protocol and quantified using Qubit® RNA Assay Kit and the Qubit® 2.0 Flurometer (Life Technologies). RNA degradation and contamination were monitored on 1% agarose gels; the purity and integrity were assessed using the NanoPhotometer® spectrophotometer (IMPLEN) and the RNA Nano 6000 Assay Kit of the Agilent Bio analyzer 2100 system (Agilent Technologies), respectively.

Sequencing library construction and Illumina sequencing were performed by Novogene Technology Co., Ltd (Beijing, China). After filtering out the adaptor sequences and deleting low-quality and contaminated reads, we assembled leaf transcriptomes using Trinity with min_kmer_cov set to 2 by default and all other parameters set default.

Differential gene expression and pathway analysis

Differential expression analysis of LED treatment and the control was performed using the DESeq2 R package (1.20.0). The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value <=0.05 found by DESeq2 were assigned as differentially expressed. Analysis of and enrichment was tested by Fisher's exact test. Clusterprofiler software was used to perform Gene Ontology (GO) function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Based on *Hevea* genome database, gene function annotation was performed using cluster transcriptome sequences and public databases, and the differential genes were annotated into gene sets in GO or KEGG databases.

III. RESULTS AND DISCUSSION

Transcriptome sequencing and sequence assembly

To investigate the genes associated with LED night lighting response, a total of 12 leaf samples were collected from two treatments at two time points for transcriptome analysis. Summary of sequence assembly after illumine sequencing was shown in Table 1. Generally, around 42-49 million clean reads comprising 6.32-7.32 Gb were obtained from 12 samples. The GC content of sequences was about 43% and the error rate was about 3%. More than 92% of the bases had a Phred quality score greater than 30 (Q30). These indicated that the quality of the sequencing data was acceptable and that this sequencing data could be used for transcriptome analysis.

Differentially expressed genes during the day and night times

The DEGs between LED night lighting and control plants were identified by the DEGseq of the R package (Version 1.12.0) with qvalue<0.005 and |log2FoldChange|>1 as the thresholds. A total of 1047 DEGs were identified during the day time, with 584 genes upregulated and 463 genes downregulated in night lighting plants compared with control plants (Figure 1-A). While during the night time, a number of 411 DEGs were identified, with 208 genes upregulated and 203 gene downregulated (Figure 1-B). These DEGs indicated that LED night lighting affected gene expression patterns in leaves, with larger changes occurring at the day time.

Analysis of GO terms

The GO was used for gene annotation and analysis, which included molecular functions, cellular components and biological processes three ontologies. The DEGs of day time were classified into 477 GO terms, of which 15 GO terms were significantly different. The upregulated DEGs of day time were classified into 370 GO terms, among of which 197 were related to biological processes, 30 were related to cell components, and 143 were related to molecular functions. Among the 370 GO terms, eight terms were significantly upregulated (Figure 2), with three relating to cell components (extracellular region, extracellular matrix, and extracellular region part), five

relating to molecular functions (iron ion binding, oxidoreductase activity, heme binding, tetrapyrrole binding, and UDP-glycosyltransferase activity). The downregulated DEGs of day time were classified into 308 GO terms, among of which 156 terms were related to biological processes, 9 terms were related to cell components, and 143 terms were related to molecular functions. Nine of the 308 GO terms were significantly upregulated, all belonging to molecular functions (Figure 3).

The DEGs of night time were classified into 331 GO terms, of which 10 GO terms were significantly different. The upregulated DEGs of night time were classified into 285 GO terms, among of which 157 were related to biological processes, 14 were related to cell components, and 114 were related to molecular functions. The downregulated DEGs of night time were classified into 104 GO terms, among of which 56 were related to biological processes, 13 were related to cell components, and 35 were related to molecular functions. Among the 104 GO terms, 16 terms were significantly upregulated, four were related to biological process and 12 were related to molecular functions (Figure 4).

Analysis of KEGG pathways

The DEGs were analyzed for KEGG pathway enrichment. A number of 611 differently expressed genes in the day time were enriched into 185 pathways, of which 4 pathways were significantly upregulated. The upregulated genes (378 genes) of day time were enriched into 151 pathways, three pathways being significantly upregulated (Figure 5). The most two upregulated pathways were photosynthesis-antenna proteins and circadian rhythm, both of which were related to photoperiod. This indicated that night lighting obviously affected the expression of genes involving photoperiod. The upregulated pathway of photosynthesis-antenna proteins suggest that the photosynthetic efficiency may enhance and more carbohydrates may be synthesized. The downregulated genes (233 genes) of day time were classified into 108 pathways. A total of 185 DEGs in the night time were enriched into 116 pathways. The upregulated genes (99 genes) in the day time were enriched in 63 pathways, while the downregulated genes (86 genes) were enriched in 71 pathways.

Identification of DEGs involved in LED night lighting

DEGs during the day and night time were further analyzed to identify genes involving LED night lighting. A total of 92 DEGs were found at both time points (Figure 6). Among these 92 DEGs, four genes were upregulated and 11 genes were downregulated (Figure 7). One downregulated gene (*scaffold0610_634905*) was annotated to be chlorophyllase, which involved in pigment metabolic process. Three downregulated genes (*scaffold1092_104788*, *scaffold0713_578123*, *scaffold0205_1475163*) were related to terpene synthase, which was associated with isoprene, a matter for rubber biosynthesis. Five downregulated genes were involved in eight GO terms, one belonging to biological process classification and 7 belonging to molecular function classification. Of the four upregulated genes, one (*scaffold0598_393375*) was a transcription factor (MYB 11), and one gene (*scaffold0528_875561*) was annotated to involve in carbohydrate metabolic process. The upregulated gene *scaffold0528_875561* was associated with 8 KEGG pathways, including carbon metabolism, carbon fixation in photosynthetic organisms, fructose and mannose metabolism, pentose phosphate pathway, and gluconeogenesis. Three downregulated genes were associated with three different KEGG pathways, one involving in chlorophyll metabolism, another involving in diterpenoid biosynthesis, the last involving in circadian rhythm.

Our earlier result showed that supplemental LED night could affect chlorophyll content in the leaves (Yao et al., 2021b). The transcription result in this study confirmed that the expression of genes associated with chlorophyllase and chloroplast were affected under LED night lighting. Therefore the change of chlorophyll content could be the result of gene expression. Photoperiod is associated with the genes of circadian rhythm. Supplemental LED night lighting lengthened the photoperiod and the expression of genes associated with circadian rhythm could be changed. The KEGG pathway of circadian rhythm-plant was different during the day and night time. Eleven genes involved in circadian rhythm-plant pathway were upregulated at the day time, but two genes were downregulated during the night time. Supplemental LED night lighting also may affected the process of rubber biosynthesis. Three downregulated genes at the day and night times were involved in terpene synthase, which associated with the GO term of terpene synthase activity. These genes also involved in diterpenoid biosynthesis pathway.

Our earlier studies demonstrated that the extended photoperiod by supplemental LED night lighting could promote *hevea* plants growth (Yao et al., 2021a, b). It could speculate that more carbohydrates could be synthesized because of supplemental LED night lighting. The transcript profile of this study showed that the gene involved in carbohydrate metabolic process was upregulated at the two time points. What is more, five KEGG pathways associated with carbohydrate metabolism were upregulated during the day and night time.

IV. CONCLUSIONS

Hevea brasiliensis grows slowly in the tropical regions of China because of relatively low temperature during winter. Supplemental LED night lighting could improves its growth. The transcription profile here identified the expression of genes associate with accelerated growth. The genes associated with pigment metabolism and carbohydrate metabolism were upregulated. The corresponding GO terms and KEGG pathways were thus upregulated. Therefore, more carbohydrates were synthesized and the growth were improved. The genes involved in circadian rhythm were also differently expressed at the day and night time. This study confirms the growth improvement of *hevea* young plants under supplemental LED night lighting from a molecular aspect. The study also suggests that it is recommended to apply supplemental LED night lighting to improve the growth of *hevea* seedlings during winter.

Table 1 Summary of sequencing data after filtering

sample	Raw reads	Clean reads	Raw bases	Clean bases	Total map %	Error rate	Q20 %	Q30 %	GC %
LD1	48,827,730	48,787,646	7.32G	7.32G	94.69	0.03	97.42	92.55	43.06
LD2	47,114,834	43,579,168	7.07G	6.54G	95.17	0.03	97.76	93.61	43.04
LD3	49,359,616	45,477,976	7.40G	6.82G	95.36	0.03	97.85	93.40	43.14
CD1	47,496,154	43,679,378	7.12G	6.55G	94.75	0.03	97.52	92.77	42.88
CD2	46,571,432	42,944,306	6.99G	6.44G	94.49	0.03	97.38	92.46	42.82
CD3	46,858,718	42,831,574	7.03G	6.42G	94.73	0.03	97.25	92.13	42.88
LN1	46,043,174	42,489,634	6.91G	6.37G	94.61	0.03	97.32	92.32	42.92
LN2	46,239,978	43,115,966	6.94G	6.47G	94.64	0.03	97.71	93.47	42.94
LN3	46,116,426	43,018,910	6.92G	6.45G	94.80	0.03	97.56	93.14	42.92
CN1	50,647,248	48,015,888	7.60G	7.20G	94.22	0.03	97.58	93.21	44.63
CN2	50,040,584	45,539,310	7.51G	6.83G	94.44	0.03	97.56	92.84	42.96
CN3	47,724,436	43,265,408	7.16G	6.49G	95.03	0.03	97.57	92.66	42.98

LD: samples collected from LED night lighting plants at day time; CD: samples collected from control plants at day time; LN: samples collected from LED night lighting plants at night time; CN: samples collected from control plants at night time.

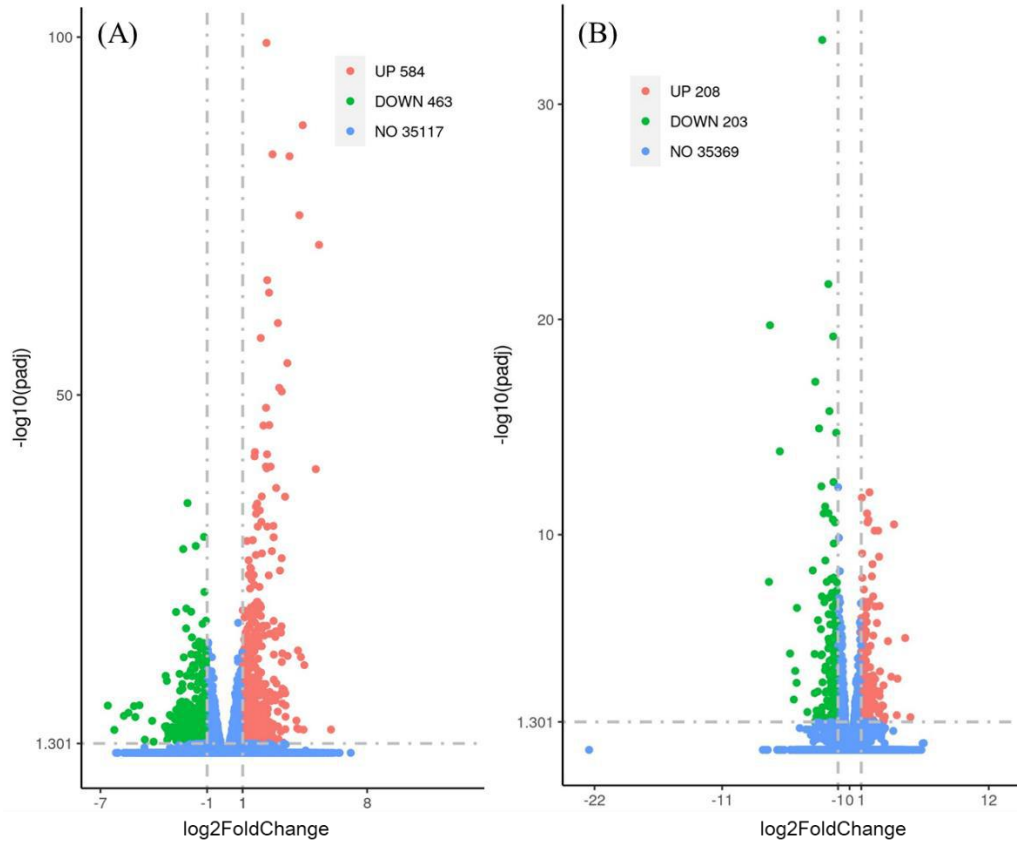

Fig.1. Volcano plots of DEGs at day time (A) and night time (B)

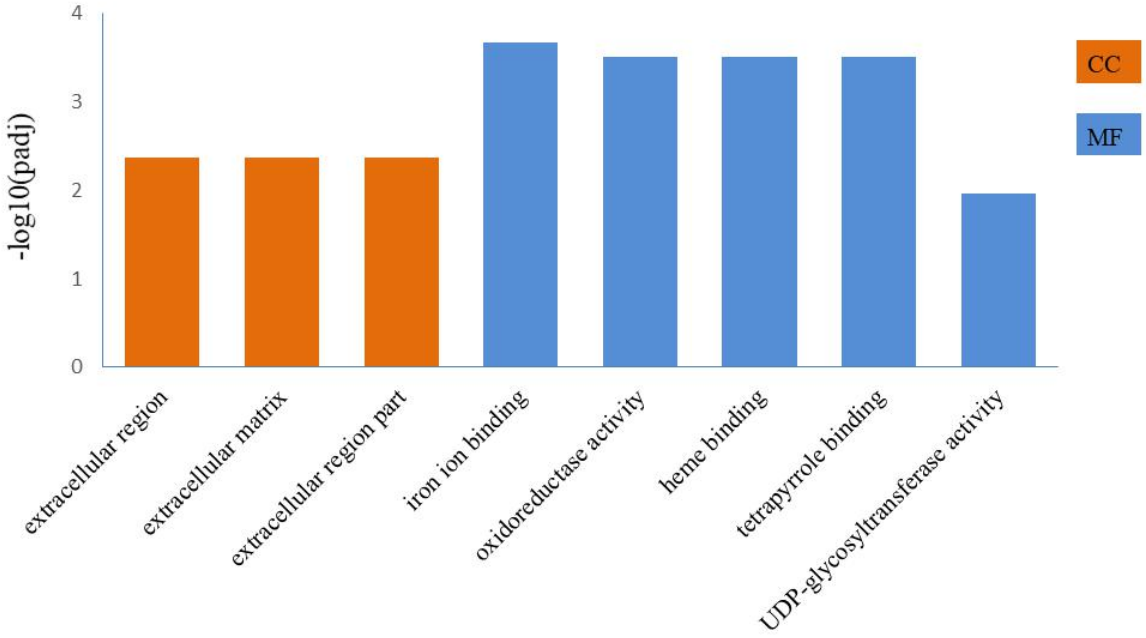

Fig.2. Upregulated GO terms at the day time

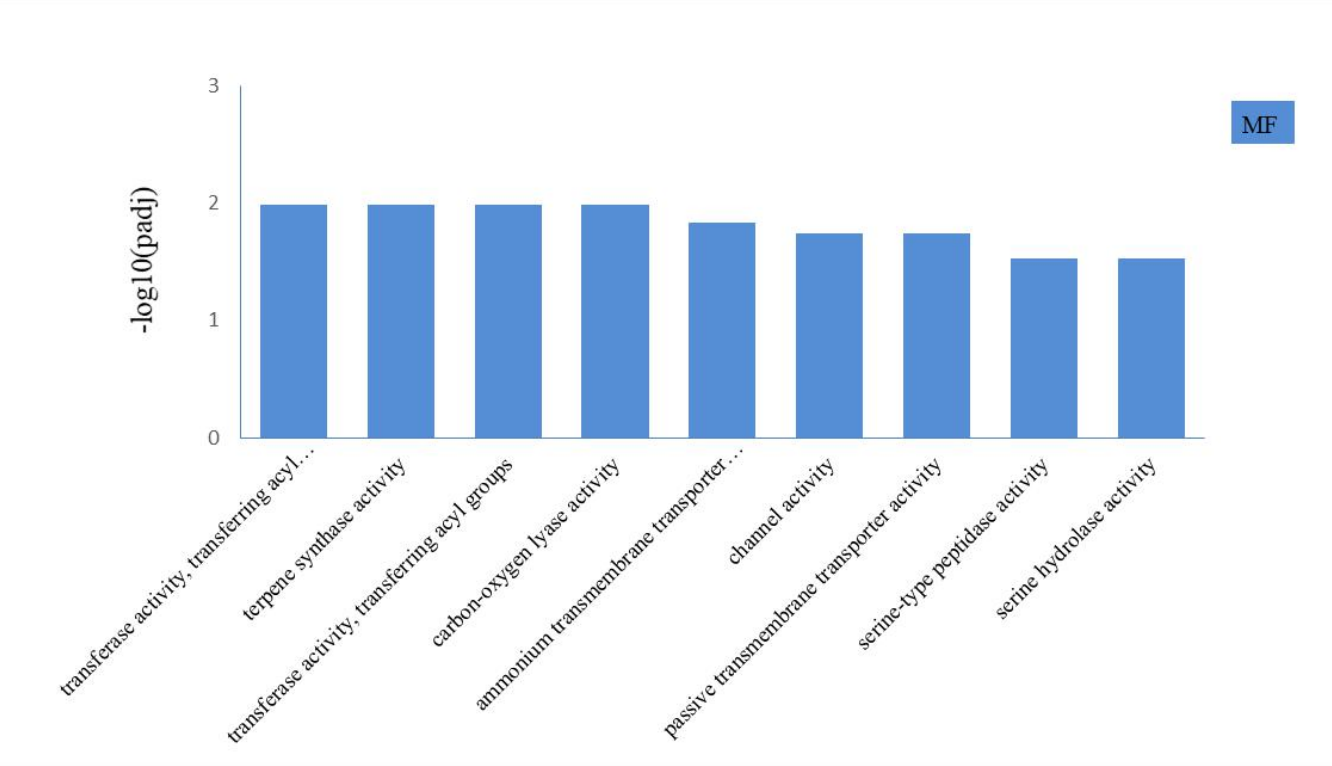

Fig.3. Downregulated GO terms at the day time

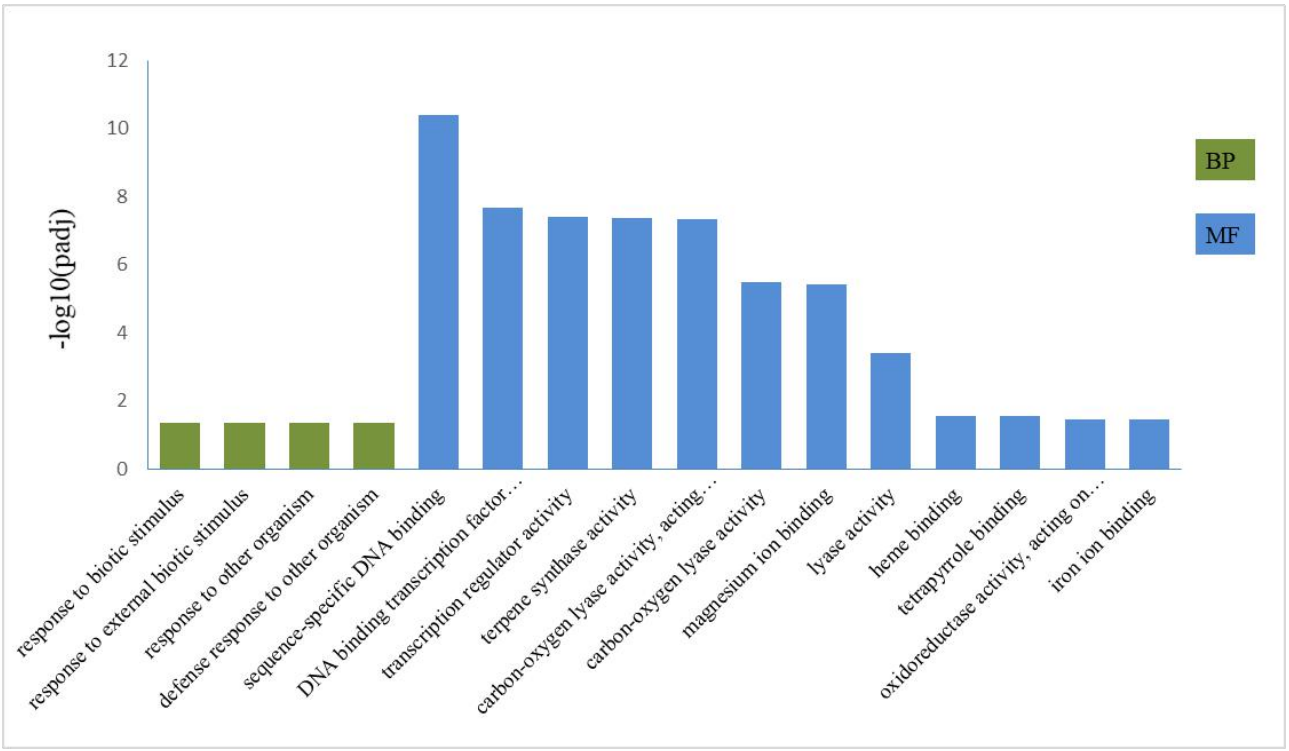

Fig.4. Downregulated GO terms at the night time

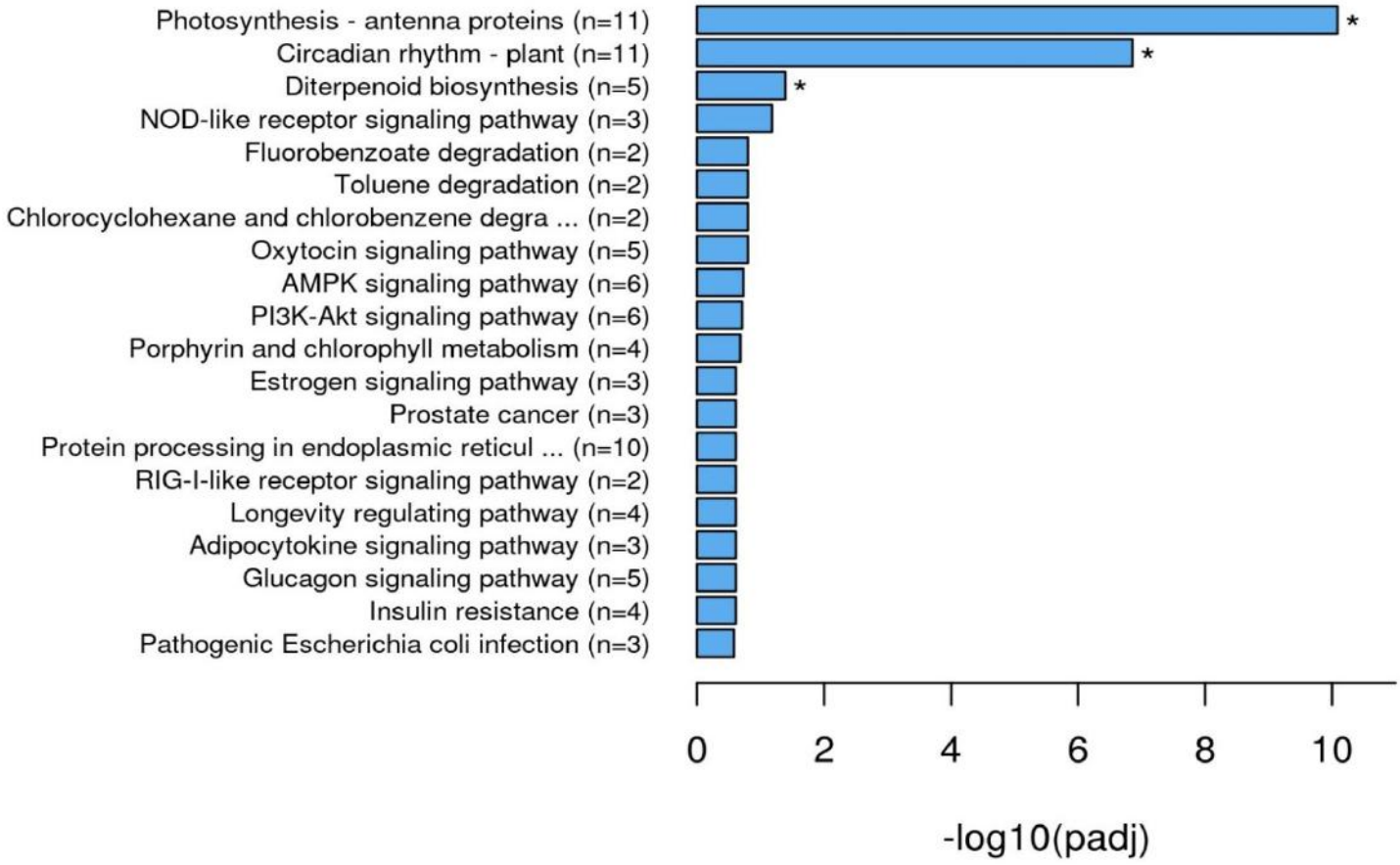

Fig.5. KEGG pathway enrichment map of DEGs at the day time

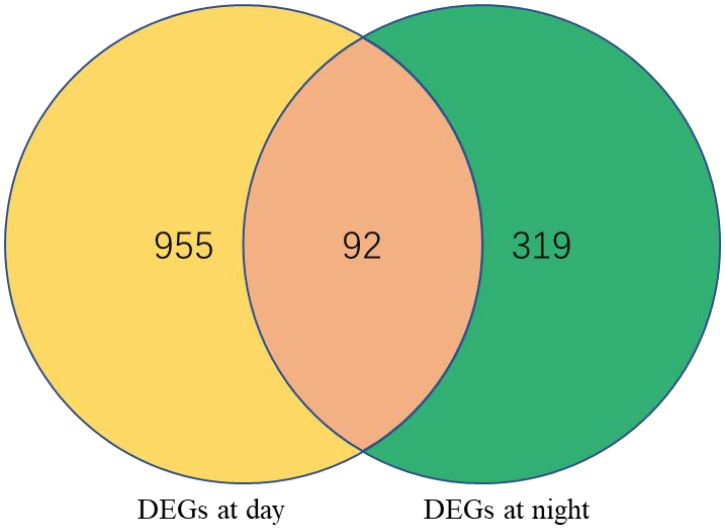

Fig.6. Venn diagram showing all DEGs at day time and night time

(each row corresponds to a gene, the color of every cell indicates the expression level based on z-score normalization.)

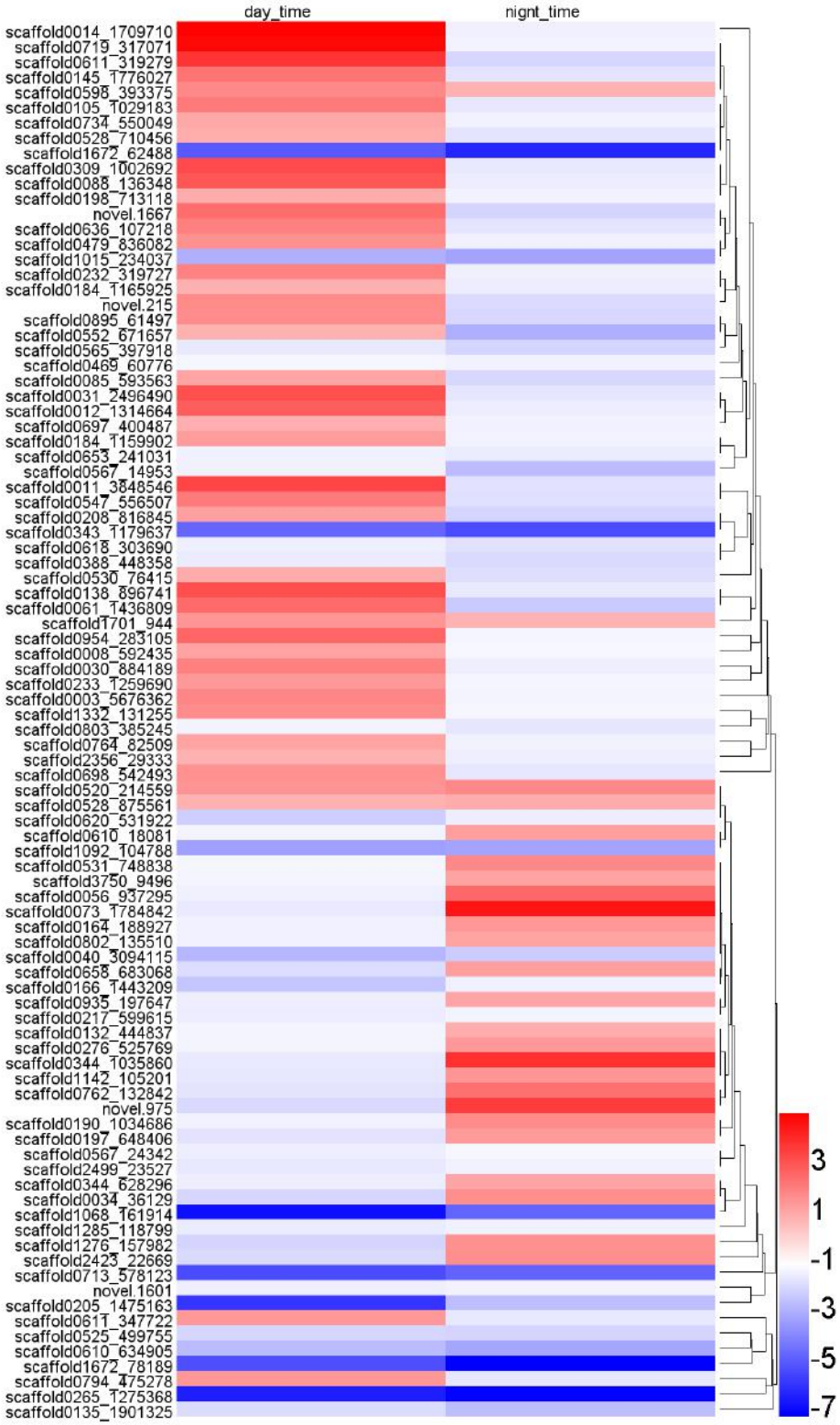

Fig.7. Heatmap of 92 DEGs at the day and night time.

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