Article

Comparative Transcriptome Analysis of Gene Expression Patterns in Tomato under Different Light Intensity Distributions

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Abstract: Plants grown under fluctuating light impact plant developments compared with those grown under non-fluctuating light conditions. However, our knowledge on the underlying regulatory mechanisms is still quite limited, particularly from the transcriptional perspective. In order to investigate the influence of different light intensity distributions on tomato plant development, we designed three fluctuating light intensity distributions with the non-fluctuating light intensity as control and compared the transcriptional differences after five weeks of treatment. We found plant height and aerial/root weight were significantly reduced under all fluctuating light treatments. Transcriptome analysis revealed that the number of up and down regulated genes had a distinct distribution pattern between different treatments and control. The largest difference between the numbers of down and up regulated genes was found between treatment 1 and 3, reaching to a total of 416 genes. The number and type of the top 20 enriched pathways differed between treatments and control. The largest number of genes enriched was involved in the biosynthesis of secondary metabolites. These results provide insights into the transcriptional regulations of tomato under different light intensity distributions.

Keywords: transcriptome; *Solanum lycopersicum*; RNA-seq; light intensity distributions; differentially expressed genes.

1. Introduction

Artificial light sources are widely used in crop cultivation systems [1], especially in controlled environmental conditions for precise growth control in closed plant factories [2]. However, artificial light source requires excessive energy consumption and accounts for up to 85% of total power

consumption in a closed plant factory [3]. Increasing light intensity is an effective method to promote plant growth and development, as well as potentially reduce the energy cost [1,3–8]. Biomass production and quality can be improved by adjusting light intensity, light quality, light period, and light source, demonstrating the great potentials of wild applications [6,9–11].

Durations of monochromatic light distribution has an impact on plant biomasses [12,13]. Light fluctuations also impact plant phenotypes and acclamatory responses [5]. Light intensities of uniform spectra have difference influences on the growth and leaf development of young tomato plants under red and blue light-emitting diodes (LED) light combinations [9]. Proper management of light use efficiency could improve photosynthesis, resulting a higher yield potentials [5,14,15]. However, our knowledge on the light energy distribution under uniform daily light integral (DLI) and light period is still limited, especially the underlying regulation mechanisms.

Tomato is the highest-value fruit and vegetable crop worldwide [16]. This fruit contains diverse nutritional and health-promoting metabolites, including sugars, lycopene, organic acids, amino acids and volatiles [17–23]. However, the underlying regulations of these complex traits are quite complex [18,23–28]. Light intensity distributions showed potentials to improve plant biomass. However, the underlying mechanisms are quite complex and our understandings are still remain quite limited. High-through output RNA sequencing (RNA-seq) is a powerful and cost-effective tool to investigate the gene transcript profiling in various species [29–36]. In this study, RNA-seq technology was used to investigate the transcriptome differences between different light intensity distributions, in order to deepen our knowledge in the underlying regulatory mechanisms and promote artificial light applications and efficiencies.

2. Results

2.1. Experimental Design and Phenotypic Characterization

In this study, we divided the total photosynthetic photo flux Intensity during the day into different distributions (Figure 1A). All three treatments had the same total photosynthetic photo flux Intensity compared with the control. After five weeks of treatment, plant heights of all three treatments were significantly reduced, compared with the control (Figure 1B,C). In addition, both the fresh and dry weight of aerial and root were also significantly reduced, compared with the control (Figure 1D). These results showed that light Intensity distributions during the time had a significant impact on the morphological development of tomato.

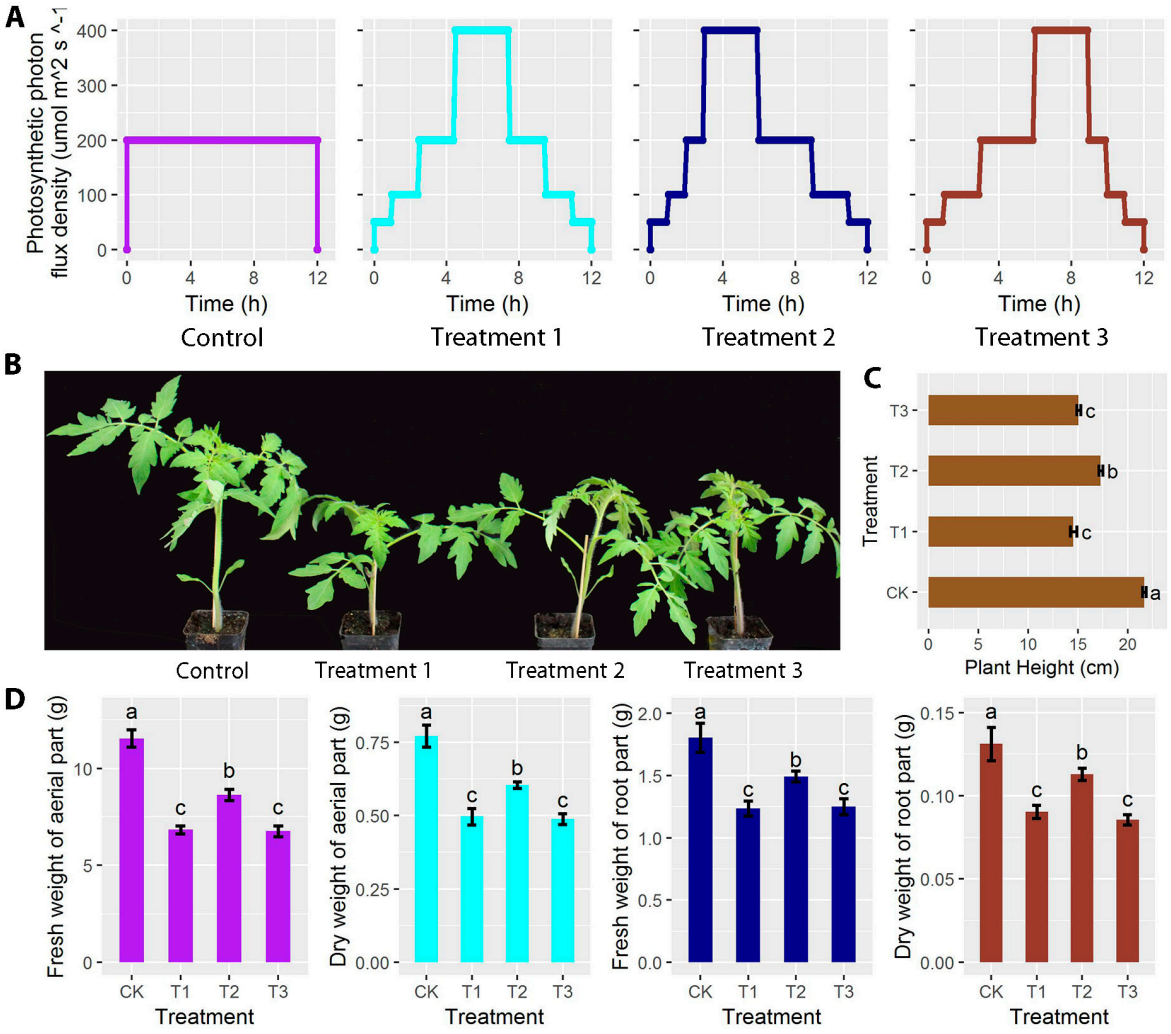

Figure 1. Experiment design and measurements of fresh and dry weight of aerial and root respectively. (**A**) Light distribution patterns of control (CK), treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3). (**B**) Morphological comparisons between different treatments after five weeks. (**C**) Comparison of plant height between different treatments after five weeks. (**D**) Comparisons of fresh weight of aerial part, dry weight of aerial part, fresh weight of root part and dry weight of root part between different treatments after five weeks.

2.2. Transcriptome Sequencing, Assembly and Annotation

In order to understand the mechanisms of the effects of light Intensity distributions on the development of tomato, we performed RNA-seq based deep transcriptome sequencing analysis, after five weeks of treatments. The sequencing quality for all the treatments and the control was quite high, after cleaning the raw sequencing data. The percentage of bases with Q20 (high sequencing quality) was close to 100 percent (Figure 2A). Gene coverage ranged from 80% to 100% accounted for approximately 80% of the total genes (Figure 2B). Within each control or treatment, the correlation coefficient between replicates was higher than 99.5%, indicating a high consistency between replicates (Figure 2C). These results showed that the transcriptome sequencing quality was sufficiently high for further analyses.

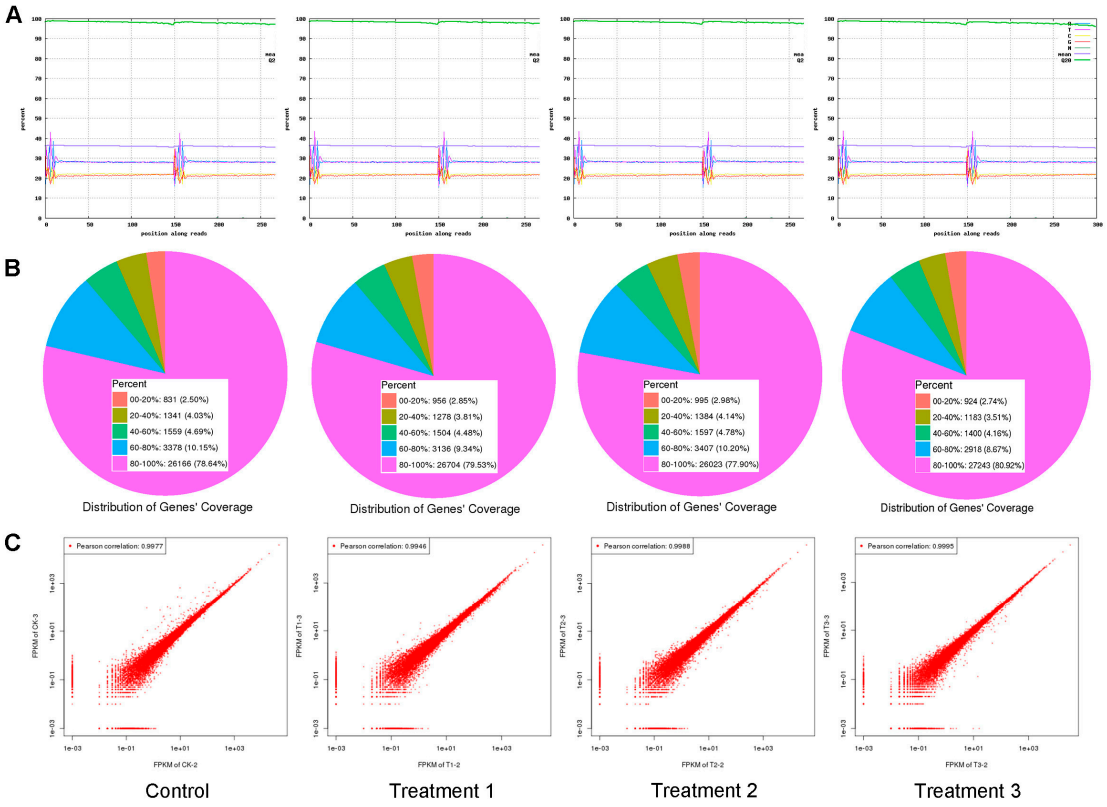

Figure 2. Transcriptome sequencing quality analysis between treatments and control. (**A**) Base composition and quality distributions. (**B**) Statistic maps of gene coverage. (**C**) Correlation coefficient maps between different sequencing repeat.

2.3. Gene Expression Difference Analysis

Difference analysis of gene expression between treatments was performed using edgeR software [37,38]. FDR and log2FC were used to screen differential genes. The screening conditions were FDR < 0.05 and |log2FC| > 1. Hundreds of genes were up or down regulated between control and treatments, as well as between different treatments (Figure 3A). In general, the number of up regulated genes was lower than that of down regulated genes, with the only exception between treatment 1 and control. In particular, the largest difference between the numbers of down regulated and up regulated genes was found between treatment 1 and 3, reaching to a total of 416 genes (Figure 3A). Volcano plots demonstrated that the number of up and down regulated genes had a distinct distribution pattern between different treatments and control (Figure 3B). For example, the distribution pattern of down regulated genes between treatment 1 were much higher than that of treatment 2 and 3, respectively. Though the number between down and up regulated genes between treatment 2 and 3 were smaller compared with other comparison, the distribution patterns were quite similar (Figure 3B). These results showed clear global gene expression patterns between different treatments and control.

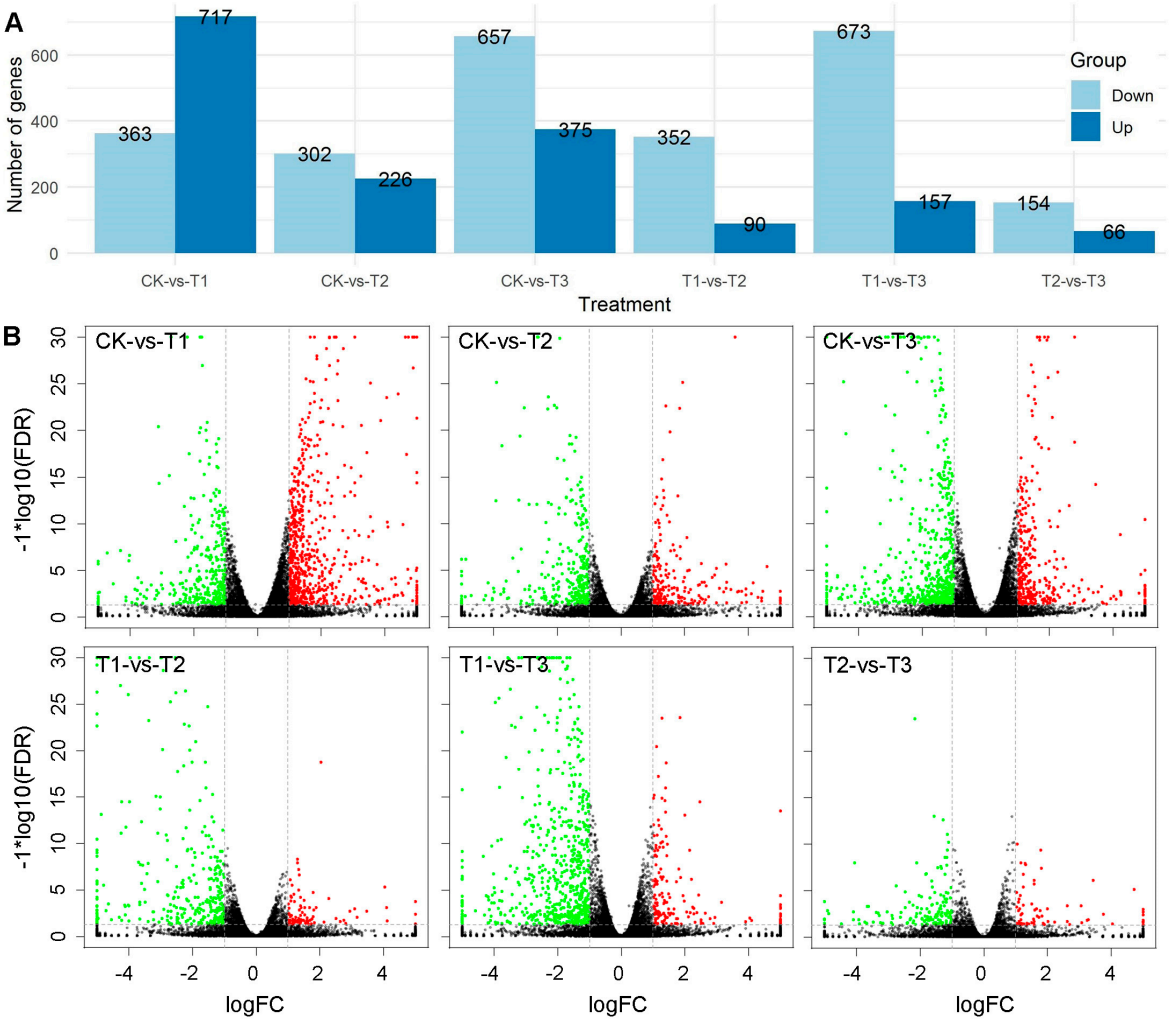

Figure 3. Difference analysis of gene expression between treatments. (**A**) Comparison of the number of up and down regulated genes. (**B**) Volcano plots between treatments and control. Red and green points represent up and down regulated genes, respectively.

2.4. Global Gene Expression Patterns and Pathway Enrichment

Gene clustering heatmap revealed a distinct global gene expression pattern between control and treatments (Figure 4A). In particular, a large group of genes were identified with higher expressions, compared with the other treatments, as well as the control. For treatment 2 and 3, distinct genes with high expression levels were also identified (Figure 4A). We then performed GO expression enrichment analysis (Figure 4B). For each comparison, the top 20 enriched pathways differed in both the number and type of pathways and also the order of the most highly enriched pathways (Figure 4B). Similarly, the largest number of genes enriched was involved in the biosynthesis of secondary metabolites, though the degree of enrichment might not be the highest compared with the other top enriched pathways (Figure 4B).

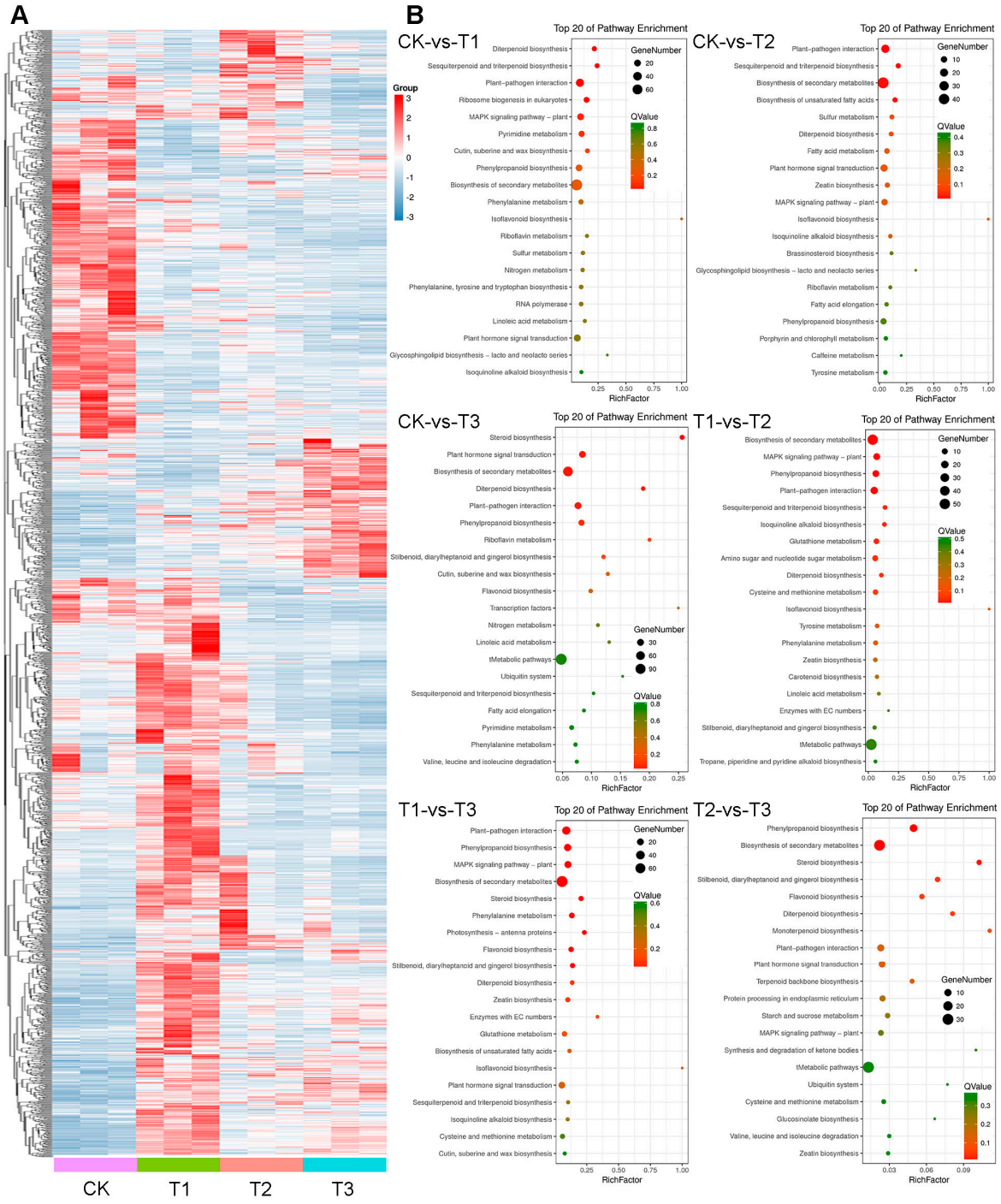

Figure 4. Gene expression clustering and GO pathway enrichment analysis. (**A**) Global gene expression patterns between control and treatments. (**B**) GO pathway enrichment analysis between control and treatments. RichFactor refers to the ratio of the number of transcripts in the pathway entry in the differentially expressed transcript to the total number of transcripts in the transcript that are located in the pathway entry. The larger the RichFactor, the higher the degree of enrichment. QValue is the PValue after multiple hypothesis test corrections, ranging from 0 to 1, the closer to zero, the more significant the enrichment. The figure is plotted using the Qvalue from small to large to sort the top 20 paths.

2.5. SNP/InDel Annotations

Transcriptome sequencing also identified various single nucleotide polymorphisms (SNPs) (Figure 5). Up to nine types of functional variations were identified for each control and

treatment, such as frameshift/nonframeshift deletion/insertion and nonsynonymous SNV (Figure 5A). Among these, nonsynonyous SNV and synonyous SNV represented the dominant functional variations, with an overall similar trend for all type of functional variations (Figure 5A). In addition, these SNPs were located in different locations, with the dominant locations in exonic and intronic locations, both of which were highest among all treatment and control (Figure 5B). All mutation types were identified in all each treatment, as well as the control, with two dominant types of transition and transversion (Figure 5C). These results demonstrated a comprehensive transcriptional regulation in tomato under different light Intensity distributions.

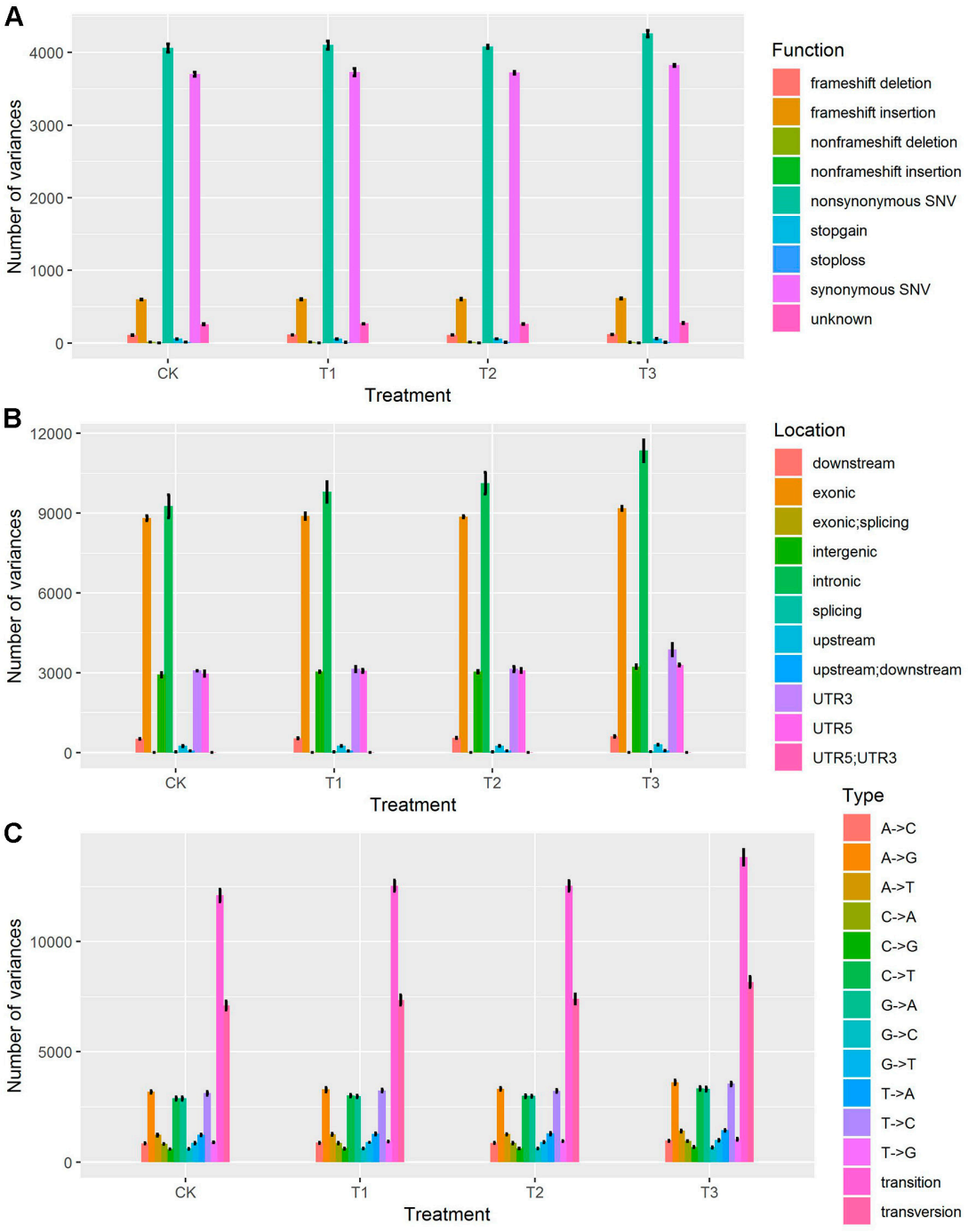

Figure 5. Gene expression clustering and GO pathway enrichment analysis. (A) Global gene expression patterns between control and treatments. (B) GO pathway enrichment analysis between control and treatments.

3. Discussion

The light environment acts as not only a photosynthetic driving force, but also a signal of plant morphological and physiological adaption under different environmental changes [39–44]. Plants grown under fluctuating light had significantly lower dry mass compared with those grown under non-fluctuating light conditions [5,12], which is consistent with the results obtained in this study. Results from this study also demonstrated the possibility to regulate plant heights by modifying the light intensity distributions [45,46].

The treatments in this study represent a miniature of natural daily light distributions, providing possible hints on the influence of circadian clock on plant development. In this study, many genes involved in hormone metabolism are circadian controlled, such as abscisic acid, auxin, and cytokines [47]. Transcriptome-based analysis in this study identified many differentially expressed genes belonged to diverse gene pathways. Notably, the largest number of genes enriched in different treatments and control was metabolite-related pathways, demonstrating the great influence of light intensity distributions on primary and secondary metabolites. As a central mediator in the coordination of metabolism, circadian clock in higher plants maintains homeostasis under a predictable, changing environment [48], which involved in dynamic regulations of diverse physiological processes [40]. However, the highest light intensity of treatments still did not reach the tomato light saturation point, which is limited by the maximum number of lamps in the artificial green box. Further investigations are needed to identify the key regulated metabolites and the relationship between transcriptome and metabolome. It is also of great interests to investigate the influence of light intensity distributions during a complete tomato life circle, especially the key metabolic differences of tomato fruit quality at the red-ripe stage.

4. Materials and Methods

4.1 Materials and Plant Growth Condition

Tomato seeds (*Solanum lycopersicum*) (Jinpeng No.1) were used as the research material. Seeds were sown in a plastic seedling tray (53 cm × 27.5 cm × 4.5 cm) within the artificial climate chamber at south campus, Northwest A&F University, Yangling, China. The pH of nutrient solution was 5.5, with the concentration of N, P_2O_5 and K_2O at 28, 76 and 132 mg/L, respectively. The temperature and relative humidity during the time were 28°C and 65%, respectively, which decreased to 18°C and 55% at night. Three weeks after sowing, plants were transplanted into 7 cm × 7 cm × 8 cm black plastic pots. After three weeks' irrigation with a half dose Yamazaki nutrient solution (EC 1.0 ± 0.2 mS/cm), the dose of solution was doubled (pH 6.5 ± 0.5, EC 2.0 ± 0.5 mS/cm) till light Intensity treatment.

4.2 Light Intensity Distribution Design

Three different light Intensity distributions (treatment 1, 2 and 3, hereafter T1, T2 and T3) were designed in order to investigate their effects on tomato plant early stage development. The total daily light integral for each treatment and the control was the same during each day, with a total lighting period of 12 h. We first set a non-fluctuating light intensity at 200 μmol m^{-1} s^{-1} for 12 h as the control. We then modified the highest light intensity to 400 μmol m^{-1} s^{-1} for 2 h, after 4h30min (T1), 3h (T2) and 6h (T3) lighting (Figure 1A). Light intensity was measured using the PAR meter (Model MQ-100, Apogee Instruments Inc., Logan, Utah, USA). The nutrient solution and environmental conditions, except the light Intensity, was the same till the end of experiment.

4.3 Morphological Measurements

After five weeks of light treatments, fresh tomato plants were divided into two groups randomly. The fresh developed leaves from the first group with similar morphological shapes were immediately frozen with -80 °C liquid nitrogen for RNA sequencing. The remaining samples were used for measurements of some morphological traits, including plant height, fresh aerial and root weight, which were quickly dried at 105°C for 15 min and then kept at 60 to 80 °C for 48 h, till the samples were completely dried. The dried aerial and root weight were then measured to compare with the fresh weight.

4.4 RNA Extraction and Illumina Sequencing

Total RNAs were extracted from the frozen fresh tomato leaves using the EASYspin Plus Kit according to the manufacturer's instructions (Aidlab Biotechnologies Co. Ltd., Beijing, China). The quality and quantity of extracted RNAs was measured using agar gel electrophoresis and Nanodrop micro spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNAs from three biological repeats with the same concentration and volume were equally combined for RNA-seq. Library was constructed using the NEBNext Ultra RNA library prep kit (NEB#E7530, New England Biolabs, USA). The quality of the cDNA library was measured using DNA 1000 assay Kit (5067-1504, Agilent Technologies, Santa Clara, CA, USA) before sequencing on an Illumina HiSeq TM 2500 by Gene Denovo Biotechnology Co. (Guangzhou, China).

4.5 Sequence Quality Control and De Novo Assembly

Raw reads containing adapters, with more than 10% of unknown nucleotides and with more than 50% of low quality (Q-value ≤ 20) bases were filtered before mapping to ribosome RNA (rRNA) database in Bowtie2 [49]. Mapped rRNA reads were removed before mapping to reference genome by TopHat2 (version 2.0.3.12) [50]. The reconstruction of transcripts was carried out with software Cufflinks [51], together with TopHat2. Gene abundances were quantified by software RSEM [52]. The gene expression level was normalized by using FPKM (Fragments Per Kilobase of transcript per Million mapped reads) method. Single-nucleotide Polymorphism (SNP) were identified in GATK [53] and SNP/InDel annotation was done using ANNOVAR [54].

4.6 Differentially Expressed Genes (DEGs) Analysis

Differentially expressed genes across treatments and control were identified using the edgeR package (http://www.r-project.org/) in R. Genes with a fold change ≥2 and a false discovery rate (FDR) <0.05 were treated as significant DEGs. DEGs were then subjected to enrichment analysis of GO functions and KEGG pathways.

4.7 Gene and Pathway Enrichment Analysis

Gene Ontology (GO) enrichment analysis provides all GO terms that significantly enriched in DEGs comparing to the genome background. All DEGs were mapped to GO terms in the Gene Ontology database (http://www.geneontology.org/). Significantly enriched GO terms (FDR Correction p-value ≤ 0.05) were identified by hypergeometric test by comparing with the genome background. Pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [55]. Pathways with FDR corrected p-value ≤ 0.05 were defined as significantly enriched pathways in DEGs.

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