



Full-length transcriptome and RNA-Seq analyses reveal the molecular mechanism of light protection in *Hosta ventricosa*

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Abstract

Hosta ventricosa is a popular shade-tolerant ornamental perennial vulnerable to high light, whose molecular mechanisms of high-light stress response remain unclear. To clarify this, we measured leaf morphology, photosynthetic physiology, and performed integrated analyses using *PacBio* full-length and *Illumina* transcriptome sequencing on plants under full sunlight (FS), moderate shade (MS), and deep shade (DS) treatments. The results indicated that MS showed optimal growth, whereas FS and DS inhibited growth, each associated with distinct gene expression profiles. High light induced VDE by 5.68-fold to activate the xanthophyll cycle and repress light-harvesting complex II and ribulose-1,5-bisphosphate carboxylase/oxygenase genes, whereas deep shade inhibited the photosynthetic apparatus and triggered stress genes. In contrast, deep shade conditions suppressed the photosynthetic machinery and induced the expression of stress-related genes. This study elucidates the molecular mechanisms underlying light stress responses in *H. ventricosa*, providing valuable insights for future molecular breeding efforts.

Keywords: high-light stress response; *Hosta ventricosa*; hybrid transcriptome; photoinhibition; photoprotection.

Introduction

Light intensity is a primary environmental cue that governs plant growth, development, and survival. Plants have evolved a suite of adaptive responses to cope with fluctuating light conditions, ranging from morphological

plasticity (e.g., altered leaf area and plant height) to physiological and biochemical adjustments (e.g., changes in photosynthetic pigment composition and antioxidant capacity) (Zhang *et al.* 2022, Yan *et al.* 2025). However, when light exceeds the capacity of photosynthetic electron transport, excess excitation energy produces reactive

Highlights

- A full-length transcriptome of *Hosta ventricosa* was assembled to obtain 37,207 isoforms
- Transcript profiles were used to screen candidate genes related to light intensity
- Identified genes provide support for *H. ventricosa* breeding

Received 24 April 2026

Accepted 20 August 2026

Published online 17 September 2026

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Abbreviations: AQY – apparent quantum yield; BR – brassinosteroid; Chl *a(b)* – chlorophyll *a(b)*; DEGs – differentially expressed genes; FDR – false discovery rate; FLNC – full-length non-chimeric reads; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; FPKM – fragments per kilobase of transcript per million mapped reads; F_v – variable fluorescence; F_v/F_0 – variable-to-initial fluorescence ratio; F_v/F_m – maximal quantum yield of PSII photochemistry; *KEGG* – *Kyoto Encyclopedia of Genes and Genomes*; *KOG* – *Eukaryotic Orthologous Groups*; LCP – light-compensation point; LHCs – light-harvesting complexes; NPQ – nonphotochemical quenching; *NR* – *nonredundant protein*; P_N – net photosynthetic rate; P_{Nmax} – maximum net photosynthetic rate; q_p – photochemical quenching coefficient; qRT-PCR – quantitative real-time polymerase chain reaction; RIN – RNA integrity number; ROI – reads of insert; ROS – reactive oxygen species; SD – standard deviation; SMRT – single-molecule real-time; SSRs – simple sequence repeats; VDE – violaxanthin de-epoxidase; VIGS – virus-induced gene silencing; Φ_{PSII} – effective quantum yield of PSII photochemistry.

Acknowledgements: This research was funded by Wuzhou University research project (2023A1004) and the Achievement Transformation Project of the Ministry of Science and Technology (No. 2014GB2B100007).

Conflict of interest: The authors declare that they have no conflict of interest.

oxygen species (ROS) and damages PSII, a process known as photoinhibition (Shi *et al.* 2022). Photoinhibition reduces the maximum quantum efficiency of PSII (F_v/F_m) and net photosynthetic rate (P_N), ultimately limiting biomass accumulation and plant fitness (Lempiäinen *et al.* 2022, Takeuchi *et al.* 2025). Conversely, under prolonged low light, plants suffer from insufficient energy supply, impaired carbon fixation, and reduced growth (Iguchi *et al.* 2023).

To cope with high light stress, plants have evolved multiple photoprotective mechanisms, including thermal dissipation of excess light energy via the xanthophyll cycle (measured as nonphotochemical quenching, NPQ), reorganization of light-harvesting complexes (LHCs), and accumulation of protective secondary metabolites such as flavonoids and terpenoids (Yang *et al.* 2018, Liu *et al.* 2019). The violaxanthin de-epoxidase (VDE) enzyme, which converts violaxanthin to zeaxanthin, plays a central role in thermal dissipation (Biswal *et al.* 2023).

The genus *Hosta* (Asparagaceae) comprises approximately 40 shade-tolerant perennial herbs native to East Asia, widely cultivated as ornamental groundcovers (Yang and Kim 2025). Among them, *H. ventricosa* is valued for its attractive foliage but suffers from leaf chlorosis and scorching under full sunlight (Wang *et al.* 2021a). While morphological and photosynthetic responses to shading have been characterized, the molecular regulators, particularly genes involved in photoprotection and light-harvesting adjustment, remain elusive due to the lack of genomic resources for this non-model genus.

Hybrid transcriptome sequencing (*PacBio* SMRT long reads combined with *Illumina* short reads) offers a powerful solution for non-model species, enabling both full-length transcript reconstruction and precise expression quantification without a reference genome (Gao *et al.* 2019, Thirugnanasambandam *et al.* 2023). This approach has proven effective for uncovering photoprotective genes in shade-tolerant species such as *Paris polyphylla* (Hua *et al.* 2022) and *Panax notoginseng* (Zhang *et al.* 2020a). It has been successfully applied to ornamental species including *Rhododendron* (Jia *et al.* 2020) and *Camellia sinensis* (Wang *et al.* 2021b).

In this study, we integrated physiological measurements with hybrid transcriptome sequencing to investigate the response of *H. ventricosa* to three light intensities: full sunlight (FS, 100% transmittance, high-light stress), moderate shade (MS, 55% transmittance, optimal condition), and deep shade (DS, 16% transmittance, low-light stress). Our objectives were to: (1) characterize physiological, photosynthetic, and chlorophyll *a* fluorescence responses across the light intensities; (2) generate the first full-length reference transcriptome for the genus *Hosta*; and (3) identify differentially expressed genes and enriched pathways associated with photoprotection. These findings will provide candidate targets for breeding high-light-tolerant varieties and offer mechanistic insights into how shade-tolerant plants balance photoprotection and growth under contrasting light environments.

Materials and methods

Light treatment and sampling: *H. ventricosa* was planted in the Nursery for the Conservation and Utilization of Changbai Mountain Wild Resources at Jilin Agricultural University. Rhizomes exhibiting newly emerged buds were potted and subjected to a 30-d acclimatization period under tree shade. Subsequently, different shading gradients were established with black nylon shade nets. The steady-state photosynthetic photon flux density of the three light intensity treatments was quantified using a ZDS-10 illuminometer, with the measured values shown below: FS (full sunlight, 100% light transmittance): $1,850 \pm 19.93 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$, MS (moderate shade, 55.4% transmittance): $1,025 \pm 5.36 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$, DS (deep shade, 16.4% transmittance): $304 \pm 2.98 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$. Each treatment group comprised 30 pots, totaling 240 pots for the entire experiment. The pots measured 20×30 cm, and the potting medium consisted of a mixture of garden soil, peat, coal cinder, and deer manure in a volumetric ratio of 2:1:1:1. The physicochemical properties of the potting substrate were characterized by a pH of approximately 6.5 and nutrient contents of nitrogen (N), phosphorus (P), and potassium (K) at $122.42 \text{ mg kg}^{-1}$, 38.31 mg kg^{-1} , and $243.55 \text{ mg kg}^{-1}$, respectively.

After 60 d of shading treatment, intact healthy functional leaves were collected for subsequent analyses from three treatment groups (FS, MS, and DS). At least three biological replicates were sampled for each treatment. Sampling was conducted around 10:00 h, with senescent leaves and newly emerged young leaves excluded during collection. All leaf samples were immediately flash-frozen in liquid nitrogen and stored at -80°C for subsequent RNA sequencing analysis.

Morphological and photosynthetic characteristics:

Following a 60-d shading treatment, key morphological parameters, including plant height, leaf area, and leaf count per individual, were directly measured. Concurrently, the total chlorophyll content, as well as chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) concentrations in leaves, were determined (Bauer *et al.* 1972).

Diurnal photosynthetic variations were assessed on clear, cloudless, and windless days utilizing the CIRAS-3 portable photosynthesis system. Measurements commenced at 6:00 h and were conducted at two-hour intervals, totaling seven observations and concluding at 18:00 h. For each treatment group, five plants were randomly selected, and one fully functional leaf with uniform light exposure was chosen per plant; each leaf was measured in triplicate. In data analysis, the MS group was set as the control, while the photosynthetic data of FS and DS were comparatively analyzed.

To evaluate the light-response curve, five uniformly developed plants per treatment were selected. The net photosynthetic rate of leaves was recorded across a gradient of light intensities ranging from 0 to $1,400 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$, with ten specified levels at 0, 50, 100, 150, 200,

400, 600, 800; 1,000; and 1,400 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$. Each measurement was conducted over approximately four minutes, with each leaf assessed three times. The resulting data were fitted using the leaf photosynthesis model (He *et al.* 2024) to generate light-response curves.

Chl fluorescence parameters were measured after a 30-min dark adaptation period for *Hosta* samples subjected to different treatments. Initial fluorescence (F_0) and maximum fluorescence (F_m) were recorded, followed by the calculation of Chl-fluorescence-related parameters, such as the maximum quantum efficiency of PSII (F_v/F_m), variable to initial fluorescence ratio (F_v/F_0), photochemical quenching coefficient (q_p), and nonphotochemical quenching (NPQ). NPQ was calculated by the formula: $\text{NPQ} = (F_m - F_m')/F_m'$. The actual photochemical efficiency of PSII (Φ_{PSII}) was determined under a steady actinic light of 200 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$. All physiological and photosynthetic data were subjected to one-way analysis of variance (ANOVA), and Duncan's multiple range test was used to compare significant differences between treatments at $P < 0.05$, and different lowercase letters in the table and figures represent statistically significant differences.

RNA extraction, library construction, and sequencing: Total RNA was extracted from leaf samples of the three treatment groups (FS, MS, DS) using the TRIzol reagent (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was evaluated with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and 1% agarose gel electrophoresis. Only samples with RNA integrity number (RIN) ≥ 7 and $\text{OD}_{260/280}$ between 1.8 and 2.0 were used for downstream applications.

For PacBio SMRT sequencing, equal amounts of total RNA from all three treatment groups were pooled. mRNA was enriched using Oligo(dT) magnetic beads and reverse-transcribed into full-length cDNA using the SMARTer™ PCR cDNA Synthesis Kit (Takara Bio, Kusatsu, Japan). Full-length cDNA was amplified by PCR, size-selected with the BluePippin system (Sage Science, Beverly, MA, USA), and subjected to additional PCR amplification. After end repair, SMRTbell adapters were ligated, and optional secondary size selections (3–6 kb and 5–10 kb fragments) were performed. The final libraries were sequenced on a PacBio RS II platform (Pacific Biosciences, Menlo Park, CA, USA). For Illumina RNA-seq, libraries were constructed separately for each treatment group (FS, MS, DS) using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's instructions. Sequencing was performed on an Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA) in paired-end mode (2×150 bp). All library construction and sequencing procedures for both PacBio SMRT and Illumina RNA-seq were entrusted to Berry Genomics Corporation (Berry Genomics Co., Ltd., Beijing, China).

Transcriptome assembly, clustering, and annotation: Raw PacBio reads (polymerase reads) were processed

using the SMRT Link ver. 5.0.1 software. Reads of insert (ROI) were extracted by removing short reads (< 50 bp) and low-quality reads (accuracy < 0.75). ROI were classified into full-length and non-full-length reads based on the presence of 5' and 3' adapters and a poly-A tail. Full-length nonchimeric reads (FLNC) were clustered using the Iso-Seq iterative clustering algorithm (ICE) to generate consensus isoforms, followed by polishing with non-full-length reads to obtain high-quality isoforms (accuracy $> 99\%$). Redundant isoforms were removed using CD-HIT (ver. 4.6.8) with a sequence identity threshold of 95%.

High-quality isoforms were aligned to the NCBI non-redundant protein (NR), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Eukaryotic Orthologous Groups (KOG) databases using BLASTX (E-value $\leq 1e^{-5}$). Gene Ontology (GO) annotation was performed using Blast2GO (ver. 5.2). Simple sequence repeats (SSRs) were identified with MISA (ver. 1.0).

Differential expression analysis: For each treatment group, clean Illumina reads were aligned to the PacBio-derived reference transcriptome using Bowtie2 (ver. 2.1.0). Transcript abundance was quantified as fragments per kilobase of transcript per million mapped reads (FPKM) using RSEM (ver. 1.2.15). Differential expression analysis was performed using edgeR (ver. 3.24.3). Transcripts with $|\log_2(\text{fold change})| > 1$ and a false discovery rate (FDR) < 0.05 were considered significantly differentially expressed.

Quantitative real-time PCR (qRT-PCR) validation: Sixteen candidate genes were randomly selected to validate the RNA-seq results. Total RNA (1 μg) from each sample was reverse-transcribed into cDNA using PrimeScript™ II Reverse Transcriptase (Takara Bio). qRT-PCR was performed using TB Green™ Premix Ex Taq™ II (Takara Bio) on a LightCycler® 480 System (Roche, Basel, Switzerland) under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The *Hosta ACTIN* gene (*Hosta_34120*) was used as the internal control. Relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. All reactions were performed in triplicate. Primer sequences are listed in Table 1S (Supplement).

Statistical analysis: All physiological measurements were performed with at least three biological replicates. Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were conducted using SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare means among treatments. Differences were considered significant at $P < 0.05$.

Results

Effects of light intensity on growth and photosynthesis: To evaluate the physiological responses of *H. ventricosa* to varying light intensities, we measured morphological

traits, chlorophyll content, photosynthetic parameters, and chlorophyll fluorescence under three light conditions: full sunlight (FS, 100% transmittance), moderate shade (MS, 55.4% transmittance), and deep shade (DS, 16.4% transmittance).

Morphological traits differed between light treatments. Plant height, leaf length, leaf width, and leaf number were highest under MS, reaching 37.45 cm, 15.98 cm, 9.94 cm, and 5.88 leaves per plant, respectively (Fig. 1A). Plants in the FS group showed the poorest growth, with reduced height (14.24 cm) and shorter leaves (12.18 cm length, 4.85 cm width). The FS leaves also turned yellow-green with scorched margins, indicating photodamage (Fig. 2S, Supplement). Deep shade plants (DS) showed intermediate morphological values but developed pale green, loosely arranged leaves. These results indicate that moderate shade promotes vegetative growth, whereas full sunlight suppresses growth and causes visible leaf injury.

In addition, shading altered leaf pigment composition. Both shade treatments increased Chl *a*, Chl *b*, and total Chl contents compared to full sunlight (Fig. 1B). Total chlorophyll concentration was the highest in MS (3.19 mg g⁻¹), followed by DS (2.55 mg g⁻¹), and FS (1.82 mg g⁻¹). The Chl *a/b* ratio was the lowest in MS (2.16) and higher in FS (2.57) and DS (2.50). This lower ratio under moderate shade suggests increased investment in Chl *b*-binding light-harvesting proteins, which may be an adaptive strategy to maximize light capture under limiting conditions.

Photosynthetic performance varied among treatments and across the diurnal cycle. Net photosynthetic rate (P_N) showed a bimodal pattern with peaks at 10:00 and 16:00 h and a midday depression, suggesting photoinhibition (Fig. 1S, Supplement). MS consistently produced the highest P_N values, peaking at 9.5 $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ at 10:00 h and 9.0 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ in the afternoon. FS showed lower peaks (7.5 and 6.8 $\mu\text{mol m}^{-2} \text{ s}^{-1}$) and a deeper midday depression, whereas DS maintained P_N below 2 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ throughout the day, indicating limited carbon assimilation.

Additionally, MS had the highest maximum net photosynthetic rate ($P_{N\text{max}}$) (8.37 $\mu\text{mol m}^{-2} \text{ s}^{-1}$), followed by FS (7.96 $\mu\text{mol m}^{-2} \text{ s}^{-1}$) and DS (7.89 $\mu\text{mol m}^{-2} \text{ s}^{-1}$), as shown in Table 1. The light-saturation point (LSP) was the highest under full sunlight [613.10 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$], indicating that FS plants could exploit higher irradiance but with reduced efficiency. In contrast, the light-compensation point (LCP) was the lowest in DS [16.11 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$], indicating greater capacity to utilize low light. Apparent quantum yield (AQY) was highest in MS (0.131), with FS (0.036) and DS (0.030) showing lower values.

Chlorophyll fluorescence measurements revealed differences in PSII function. In Table 1, the maximum quantum efficiency of PSII (F_v/F_m) remained high in MS (0.83) and DS (0.74) but declined in FS (0.56; $P < 0.05$), indicating photoinhibition under full sunlight. q_p reflects the proportion of open PSII reaction centers and followed

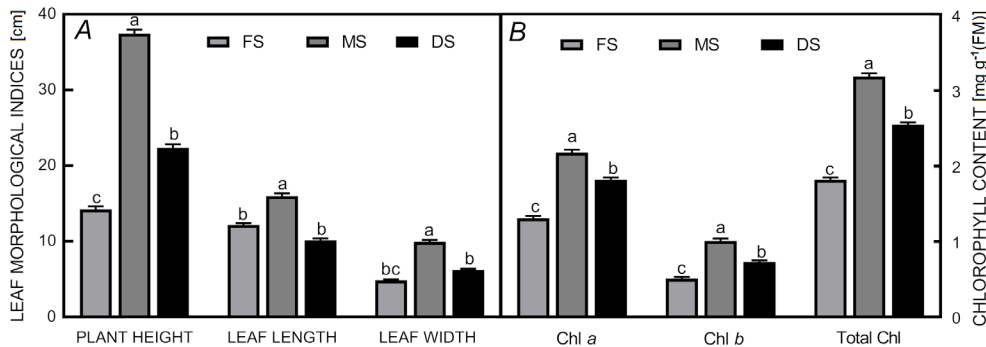


Fig. 1. Morphological and chlorophyll content of *Hosta ventricosa* under different light intensities, full sunlight (FS), moderate shade (MS), and deep shade (DS) conditions. (A) Quantitative comparison of plant height, leaf length, and leaf width [cm]; (B) concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total chlorophyll [Chl (*a*+*b*)] [mg g⁻¹(FM)]. Bars show mean values $\pm$ standard error (SE). Statistically significant differences between treatments were determined using one-way ANOVA and Duncan's multiple comparison test. Distinct lowercase letters on bars denote significant differences at the 0.05 probability level.

Table 1. Photosynthetic parameters and chlorophyll fluorescence characteristics of *Hosta ventricosa* under different light intensities. Different lowercase letters within the same column indicate significant differences among treatments at $P < 0.05$ based on Duncan's multiple range test, with moderate shading (MS) set as the reference control for pairwise comparisons. FS – full sunlight treatment; MS – moderate shade treatment; DS – deep shade treatment; $P_{N\text{max}}$ – maximum net photosynthetic rate; LSP – light-saturation point; LCP – light-compensation point; AQY – apparent quantum efficiency; Φ_{PSII} – actual photochemical efficiency of PSII; F_v/F_m – maximum photochemical efficiency of PSII; q_p – photochemical quenching coefficient; NPQ – nonphotochemical quenching coefficient.

Treatment groups	$P_{N\text{max}}$ [$\mu\text{mol m}^{-2} \text{ s}^{-1}$]	LSP [$\mu\text{mol m}^{-2} \text{ s}^{-1}$]	LCP [$\mu\text{mol m}^{-2} \text{ s}^{-1}$]	AQY	Φ_{PSII}	F_v/F_m	q_p	NPQ
FS	7.96 ^{bc}	613.10 ^a	21.72 ^a	0.036 ^a	0.12 ^c	0.56 ^c	0.14 ^c	0.29 ^b
MS	8.01 ^a	580.37 ^b	18.68 ^b	0.032 ^a	0.64 ^a	0.81 ^a	0.87 ^a	0.07 ^a
DS	7.89 ^b	569.66 ^c	16.11 ^c	0.030 ^a	0.31 ^b	0.74 ^b	0.73 ^b	0.08 ^b

a similar pattern, with values of 0.92 in MS and 0.14 in FS. NPQ peaked in FS (0.29) and reached a minimum in MS (0.08). These fluorescence parameters indicate that full sunlight caused PSII damage and increased thermal dissipation, whereas moderate shade maintained photosynthetic apparatus integrity and efficiency.

Full-length transcriptome of *H. ventricosa*: To obtain a comprehensive reference transcriptome for gene expression analysis under different light regimes, we performed *PacBio* single-molecule real-time (SMRT) sequencing on a pooled RNA sample from the three light treatments (FS, MS, DS). A total of 450,876 polymerase reads were generated, and after filtering, 299,037 high-quality reads remained (Table 2). The mean polymerase read length was 17,504 bp, with an N50 of 24,954 bp, demonstrating the long-read advantage of *PacBio* sequencing.

From the filtered reads, we extracted 376,542 reads of insert (ROI) with an average length of 1,819 bp and a mean read quality of 0.932. Classification of ROI yielded 158,225 FLNC reads, accounting for 42% of the total ROI. These FLNC reads had an average length of 1,525 bp. After iterative clustering and polishing, we obtained 37,207 high-quality consensus isoforms (accuracy >99%) with an average length of 1,617 bp (Table 2). Redundancy removal using CD-HIT reduced the number to 35,102 non-redundant full-length transcripts.

Functional annotation was performed by *BLASTX* searching against public databases. Among the 37,207 high-quality isoforms, 32,418 (87.1%) were successfully annotated in at least one database (Fig. 2A). Specifically, 31,245 (84.0%) transcripts had hits in the *NR* database, 28,761 (77.3%) in *Swiss-Prot*, 25,430 (68.4%) in *KOG*, and 22,156 (59.6%) in *KEGG*. *GO* annotation indicated that “cellular process” (40,504 transcripts) and “metabolic process” (38,368) dominated the biological

process category; “cell” (44,972) and “cell part” (44,621) dominated the cellular component category; and “binding” (31,580) and “catalytic activity” (28,749) dominated the molecular function category (Fig. 2B). *KOG* classification revealed that the largest categories were “Posttranslational modification, protein turnover, chaperones” (4,371 transcripts), “Signal transduction mechanisms” (3,090), and “Carbohydrate transport and metabolism” (2,619) (Fig. 2C). *KEGG* pathway analysis showed that the most enriched pathways were “Amino acid metabolism” (3,164 transcripts), “Folding, sorting and degradation” (2,433), and “Translation” (2,365) (Fig. 2D). This comprehensive transcriptome provides a high-quality reference for subsequent differential expression analysis.

Differentially expressed genes in *H. ventricosa*: To investigate the transcriptional basis of light stress in *H. ventricosa*, we performed *Illumina* RNA-seq on leaf samples from three light treatments, FS, MS, and DS. To address the high error rate inherent in *PacBio* single-molecule sequencing, *Illumina* short reads were used to polish the full-length transcripts. The corrected transcriptome was subsequently employed as the reference for *Bowtie2* (ver. 2.1.0) mapping. As shown in Table 2S (Supplement), overall mapping efficiencies ranged from 60.79% to 70.78% across samples, with 81.53–86.63% of mapped reads aligning to multiple locations – indicative of substantial redundancy in the transcriptome assembly. Furthermore, qRT-PCR was performed on nine randomly selected genes to validate the RNA-seq data. The expression profiles of the nine genes exhibited good consistency with the RNA-seq data in all three treatment groups (Fig. 3).

Following this, we quantified transcript abundance and identified differentially expressed genes (DEGs) for each pairwise comparison, applying thresholds of $|\log_2(\text{fold change})| > 1$ and $\text{FDR} < 0.05$. The comparison between FS and MS yielded 203 DEGs, of which 116 were upregulated

Table 2. *PacBio* SMRT sequencing statistics for *Hosta ventricosa*.

<i>Pacbio</i> sequencing statistics	Data
Polymerase read bases	5,726,792,618/5,234,504,396
Polymerase reads	450,876/299,037
Polymerase read N50	24,436/24,954
Polymerase read length	12,701/17,504
Polymerase read quality	0.612/0.822
Reads of insert (ROI)	376,542
Read bases of insert	685,228,702
Mean read length of insert	1,819
Mean read quality of insert	0.9322
Mean number of passes	8.0
Number of ROI	376,542
Number of non-full-length reads	168,526
Number of full-length reads	159,400
Number of full-length non-chimeric reads	158,225
Full-length percentage [%]	42
Average full-length non-chimeric read length	1,525

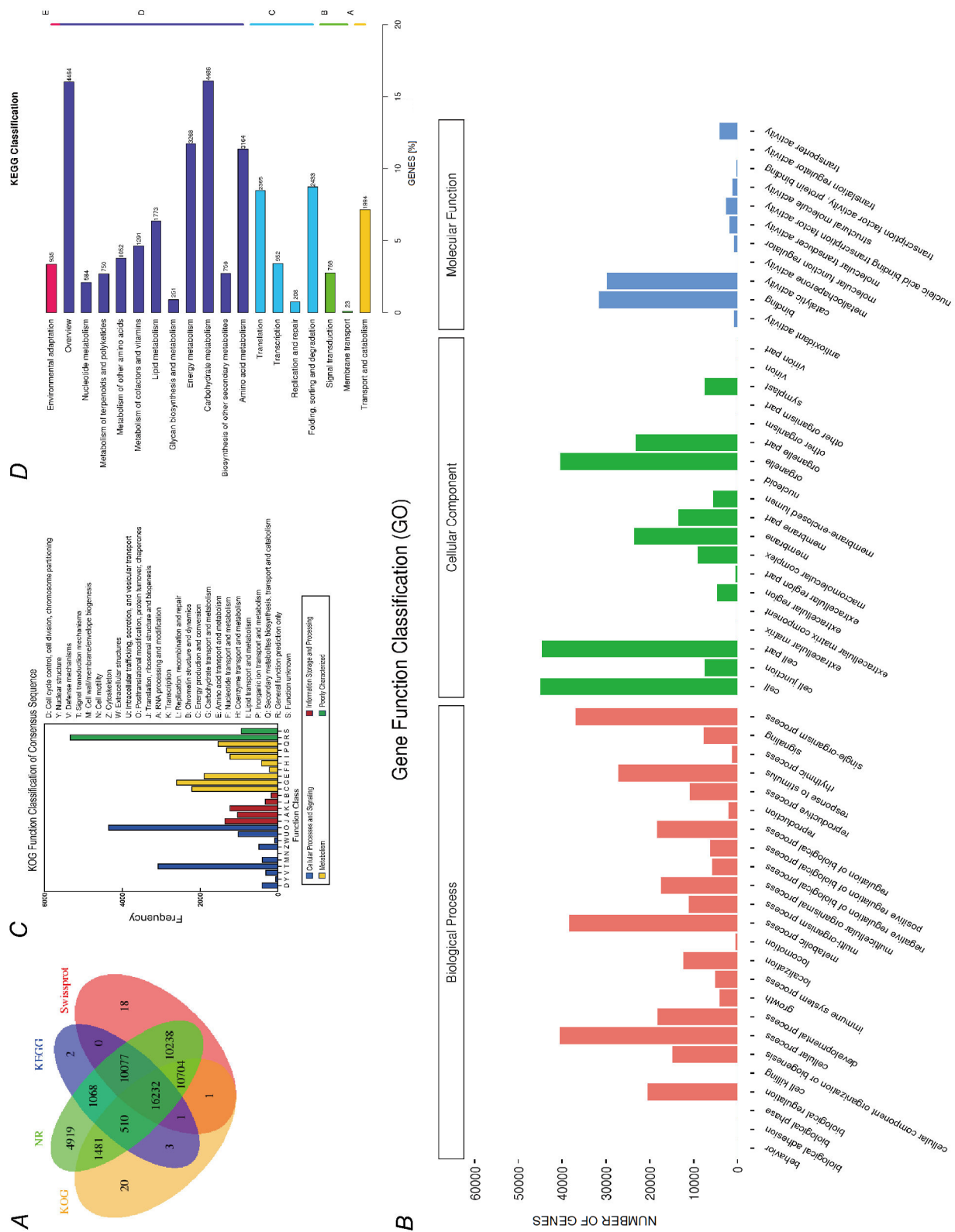


Fig. 2. Functional annotation of the *Hosta ventricosa* full length transcriptome. (A) Venn diagram showing the number of annotated transcripts in NR, Swiss-Prot, KEGG, and KOG databases. (B) GO annotation. (C) KOG classification of transcripts. (D) KEGG pathway classification.

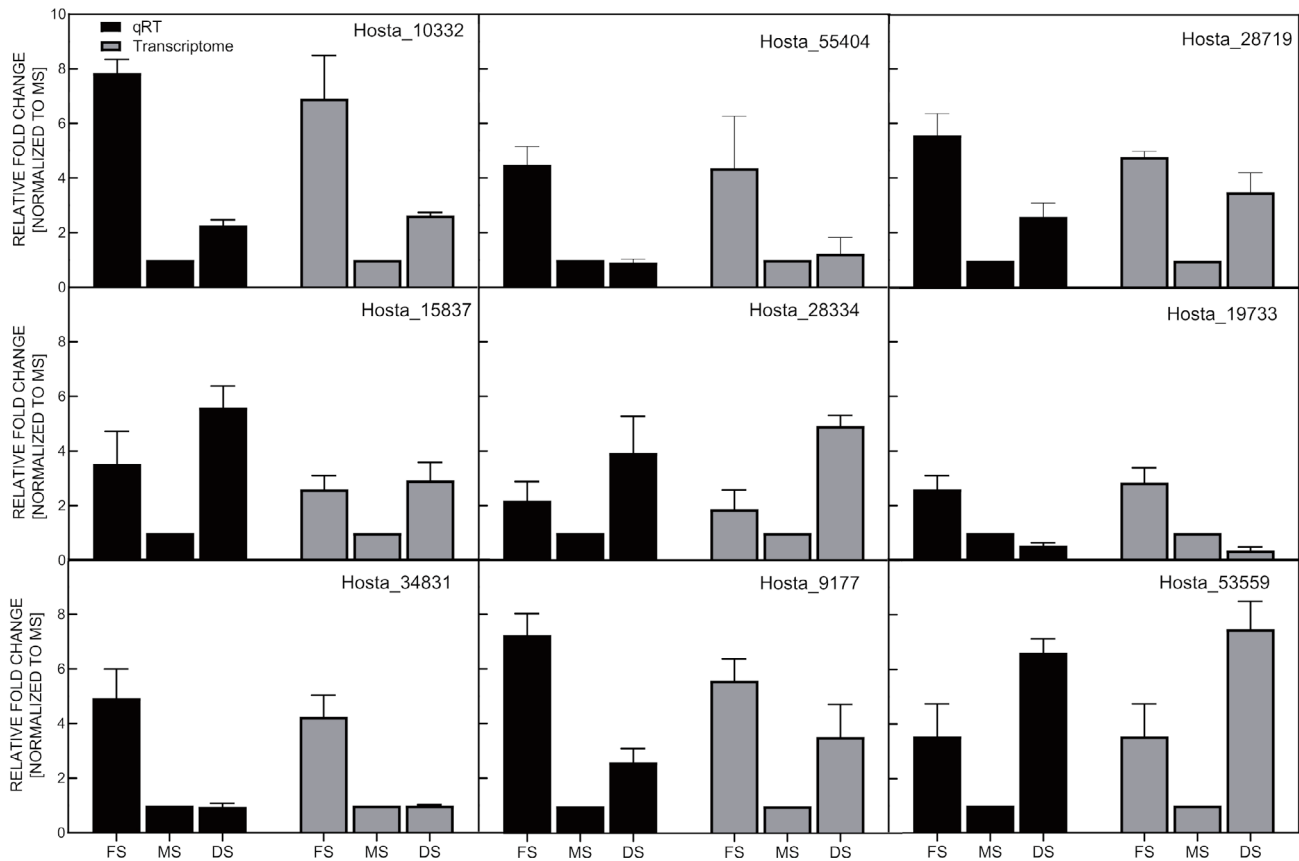


Fig. 3. Correlation of gene expression fold changes measured by qRT-PCR (black) and transcriptome (gray) for nine genes at FS, MS, and DS groups. Expression values were normalized to MS; error bars show SD. FS – full sunlight; MS – moderate shade; DS – deep shade.

and 87 downregulated in FS (Fig. 4A, top). Between DS and MS, we identified 449 DEGs, with 264 upregulated and 185 downregulated in DS (Fig. 4A, middle). The largest number of DEGs was observed between FS and DS, totaling 985 DEGs (567 upregulated in FS, 418 downregulated; Fig. 4A, bottom). These results indicate that distinct transcriptional reprogramming occurs under high-light and low-light conditions, with the most dramatic changes observed between the two extremes.

Xanthophyll cycle, flavonoid/terpenoid biosynthesis, and brassinosteroid signaling: To investigate the molecular mechanisms underlying high light response in *H. ventricosa*, we performed a comparative transcriptome analysis between FS and MS treatments. Transcriptomic data revealed substantial differences in gene expression profiles between the FS and MS groups (Fig. 4A,B). A total of 203 DEGs were identified between the two conditions, with 116 genes upregulated and 87 genes downregulated in FS relative to MS (Fig. 4A). KEGG pathway enrichment analysis of the upregulated DEGs revealed significant enrichment in several metabolic pathways, including linoleic acid metabolism (ko00591, $P = 3.93 \times 10^{-10}$), α -linolenic acid metabolism (ko00592, $P = 7.16 \times 10^{-10}$), brassinosteroid biosynthesis (ko00905, $P = 1.00 \times 10^{-9}$), flavonoid biosynthesis (ko00941, $P = 1.55 \times 10^{-7}$), and steroid biosynthesis (ko00100,

$P = 6.71 \times 10^{-5}$) (Table 3). These pathways are known to be involved in photoprotection, stress signaling, and secondary metabolite production in plants exposed to high light stress.

The xanthophyll cycle, which plays a central role in nonphotochemical quenching and thermal dissipation of excess excitation energy, showed strong transcriptional activation under full sunlight. The gene encoding violaxanthin de-epoxidase (VDE, *Hosta_31445*), the key enzyme that catalyzes the conversion of violaxanthin to zeaxanthin, was markedly upregulated in FS with a log₂ fold change of 5.68 (FDR = 7.12×10^{-18}) (Table 4). This result suggests that *H. ventricosa* enhances its capacity for thermal energy dissipation as a primary defense mechanism against high light-induced photodamage.

Multiple genes involved in flavonoid biosynthesis were coordinately upregulated under full sunlight conditions, such as chalcone synthase-like (*Hosta_12414*), flavonol synthase (*Hosta_1905*), flavonoid 3'-hydroxylase (*Hosta_36175*), 4-coumarate-CoA ligase (*Hosta_18074*), and dihydroflavonol reductase (*Hosta_28662*). Flavonoids function as effective antioxidants and UV protectants, and their increased biosynthesis likely contributes to the scavenging of reactive oxygen species generated under high-light stress. In addition, multiple genes related to brassinosteroid biosynthesis and signaling, such as *Hosta_19733*, *Hosta_28566*, and *Hosta_24134*, were

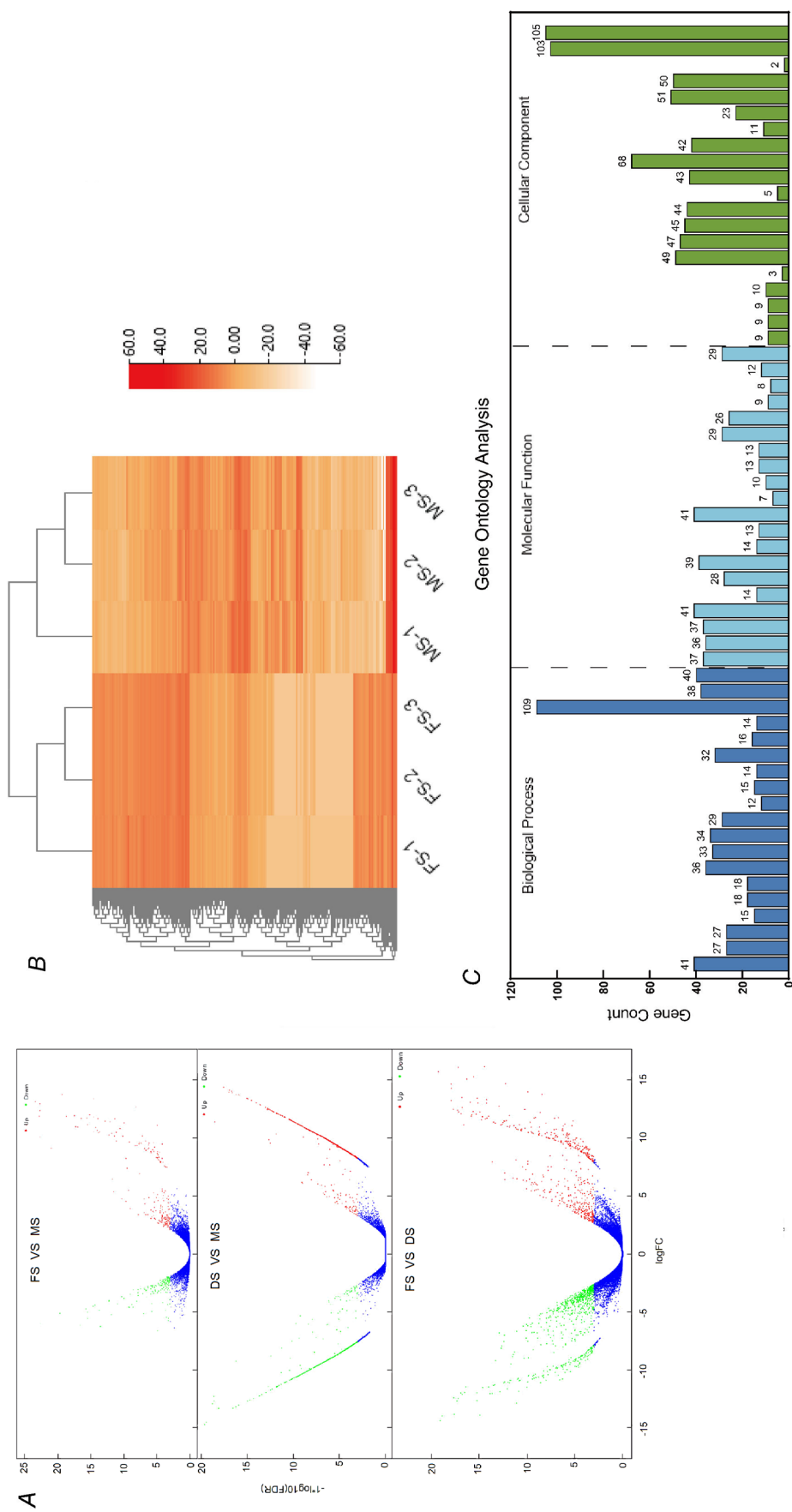


Fig. 4. Overview of differentially expressed genes (DEGs) among light treatments. (A) Volcano plots for the three pairwise comparisons: FS vs. MS (top), DS vs. MS (middle), and FS vs. DS (bottom). (B) Heatmap of differentially expressed genes between FS and MS treatments. (C) GO analysis of differentially expressed genes between FS and MS treatments. FS – full sunlight; MS – moderate shade; DS – deep shade.

Table 3. *KEGG* pathway enrichment analysis of differentially expressed genes between FS and MS treatments. FS – full sunlight treatment; MS – moderate shade treatment.

Metabolic process terms	Ko ID	<i>P</i> value	Genes
Linoleic acid metabolism	ko00591	3.93×10^{-10}	<i>Hosta_37046; Hosta_30826; Hosta_28958; Hosta_30990; Hosta_14913; Hosta_43452; Hosta_3887; Hosta_29623; Hosta_29668; Hosta_19449; Hosta_35570; Hosta_42113; Hosta_37563; Hosta_22544; Hosta_36042; Hosta_25933; Hosta_14323</i>
α -Linolenic acid metabolism	ko00592	7.16×10^{-10}	<i>Hosta_3887; Hosta_54616; Hosta_28667; Hosta_39283; Hosta_3076; Hosta_15356; Hosta_37563; Hosta_17082; Hosta_13896; Hosta_44650; Hosta_8265; Hosta_34859; Hosta_22544; Hosta_2002; Hosta_25851; Hosta_15529; Hosta_14913; Hosta_16171; Hosta_13636; Hosta_19449; Hosta_5547; Hosta_1441; Hosta_18227; Hosta_44117</i>
Brassinosteroid biosynthesis	ko00905	1.00×10^{-9}	<i>Hosta_48645; Hosta_18291; Hosta_10535; Hosta_15724; Hosta_58733; Hosta_19733; Hosta_44082; Hosta_36453; Hosta_35281; Hosta_34892; Hosta_33460; Hosta_28566; Hosta_27185; Hosta_24134; Hosta_17985</i>
MAPK signaling pathway	ko04010	1.04×10^{-7}	<i>Hosta_24911; Hosta_25204; Hosta_36263; Hosta_11704; Hosta_34008; Hosta_4052; Hosta_14754; Hosta_35676; Hosta_35578; Hosta_31490; Hosta_27412; Hosta_1245; Hosta_1059; Hosta_30688; Hosta_19688; Hosta_4639; Hosta_56557; Hosta_9021; Hosta_54002; Hosta_10622</i>
Flavonoid biosynthesis	ko00941	1.55×10^{-7}	<i>Hosta_10332; Hosta_15837; Hosta_36204; Hosta_3200; Hosta_21615; Hosta_12414; Hosta_28334; Hosta_45507; Hosta_34831; Hosta_1905; Hosta_23732; Hosta_35287; Hosta_57165; Hosta_36175; Hosta_18074</i>
Steroid biosynthesis	ko00100	6.71×10^{-5}	<i>Hosta_1017; Hosta_54806; Hosta_1034; Hosta_21228; Hosta_1016; Hosta_24509; Hosta_22834; Hosta_21830; Hosta_529; Hosta_58113; Hosta_34170; Hosta_5439; Hosta_12687; Hosta_46892; Hosta_35880; Hosta_532; Hosta_13954; Hosta_35624</i>
Stilbenoid, diarylheptanoid, and gingerol biosynthesis	ko00945	7.88×10^{-5}	<i>Hosta_24362; Hosta_15837; Hosta_32383; Hosta_28334; Hosta_13494; Hosta_16759; Hosta_3711; Hosta_36771</i>
Starch and sucrose metabolism	ko00500	8.65×10^{-5}	<i>Hosta_55404; Hosta_39431; Hosta_58074; Hosta_31530; Hosta_28719; Hosta_4477; Hosta_9177; Hosta_34195; Hosta_30808; Hosta_13907; Hosta_20025; Hosta_25364; Hosta_6561; Hosta_16731; Hosta_31735; Hosta_36976; Hosta_46021; Hosta_13689; Hosta_12984; Hosta_5703; Hosta_22533; Hosta_53559; Hosta_15843; Hosta_979; Hosta_51704; Hosta_39192; Hosta_7803; Hosta_15026</i>
Phenylpropanoid biosynthesis	ko00940	8.88×10^{-5}	<i>Hosta_55404; Hosta_58074; Hosta_28719; Hosta_4477; Hosta_9177; Hosta_34195; Hosta_15837; Hosta_30808; Hosta_18652; Hosta_25364; Hosta_36976; Hosta_35052; Hosta_28693; Hosta_13689; Hosta_12984; Hosta_15026; Hosta_53559; Hosta_20010; Hosta_10513; Hosta_12925; Hosta_979; Hosta_51704; Hosta_39192; Hosta_28334</i>
Polycyclic aromatic hydrocarbon degradation	ko00624	8.93×10^{-5}	<i>Hosta_24362; Hosta_32383; Hosta_13494; Hosta_16759; Hosta_3711; Hosta_36771</i>

Table 4. Differentially expressed genes related to photosynthesis screened by comparison between FS and MS treatments. $\log_2FC = \log_2(\text{expression in FS}/\text{expression in MS})$. FS – full sunlight treatment; MS – moderate shade treatment.

Gene ID	Annotation	$\log_2FC$	<i>P</i> value
<i>Hosta_12163</i>	Chlorophyll <i>a-b</i> binding protein of LHCII type 1	-12.65	3.41×10^{-12}
<i>Hosta_32230</i>	Chloroplast chlorophyll <i>a/b</i> -binding protein	-9.35	2.33×10^{-22}
<i>Hosta_33255</i>	Chlorophyll <i>a-b</i> binding protein 4	-7.73	3.23×10^{-21}
<i>Hosta_33517</i>	Type III chlorophyll <i>a/b</i> -binding protein	-10.81	3.94×10^{-21}
<i>Hosta_33602</i>	Chlorophyll <i>a-b</i> binding protein 13	-8.25	4.78×10^{-21}
<i>Hosta_33860</i>	Chlorophyll <i>a-b</i> binding protein 7	-10.78	7.77×10^{-21}
<i>Hosta_12121</i>	Photosystem II CP43 chlorophyll apoprotein (psbC)	6.94	7.93×10^{-21}
<i>Hosta_33429</i>	RuBisCO large subunit-binding protein subunit α	10.70	2.33×10^{-20}
<i>Hosta_280</i>	RuBisCO large subunit-binding protein subunit β	8.43	5.60×10^{-20}
<i>Hosta_34088</i>	Chloroplast RuBisCO activase	6.40	8.78×10^{-19}
<i>Hosta_31445</i>	Violaxanthin de-epoxidase (VDE)	5.68	7.12×10^{-18}

significantly upregulated in FS (Table 3). Brassinosteroids are plant steroid hormones implicated in various stress responses, and their enhanced expression suggests activation of hormone-mediated stress signaling pathways under high-light conditions.

Gene Ontology enrichment analysis provided additional functional classification of the DEGs (Fig. 4C). In the biological process category, upregulated genes were significantly enriched in terms related to response to light stimulus (*GO:0009416*), oxidative stress response (*GO:0006979*), flavonoid metabolic process (*GO:0009812*), and carotenoid biosynthetic process (*GO:0016117*). The molecular function category revealed enrichment in oxidoreductase activity (*GO:0016491*), chlorophyll binding (*GO:0016168*), and antioxidant activity (*GO:0016209*), consistent with the activation of protective mechanisms under high light. In the cellular component category, plastid-related terms including chloroplast (*GO:0009507*), thylakoid (*GO:0009579*), and photosystem II (*GO:0009523*) were prominent, reflecting the subcellular localization of photosynthetic and photoprotective processes. Together, the *GO* and *KEGG* enrichment results indicate a widespread transcriptional reprogramming underlying the high-light stress response in *H. ventricosa* under full sunlight conditions.

Low-light stress downregulates photosynthesis and carbon fixation: To characterize the molecular response to low-light conditions, we analyzed the 449 DEGs identified between the DS and the MS. Among these, 203 genes were upregulated, and 246 genes were downregulated in DS relative to MS (Fig. 4A, middle). *KEGG* pathway enrichment analysis of the upregulated DEGs in DS showed significant enrichment in carbon fixation in photosynthetic organisms (*ko00710*, $P = 2.37 \times 10^{-14}$), glyoxylate and dicarboxylate metabolism (*ko00630*, $P = 5.12 \times 10^{-12}$), photosynthesis (*ko00195*, $P = 8.48 \times 10^{-12}$), starch and sucrose metabolism (*ko00500*, $P = 8.73 \times 10^{-12}$), and anthocyanin biosynthesis (*ko00942*, $P = 1.12 \times 10^{-10}$) (Table 3S, Supplement).

Closer examination revealed that most genes directly involved in photosynthesis and carbon assimilation were downregulated in DS compared to MS, despite the appearance of these pathways in the enrichment analysis. This pattern can result from pathway enrichment algorithms detecting upregulation of a small subset of stress-responsive genes within otherwise downregulated pathways. Key photosynthetic genes showed substantially lower expression levels in the deep shade treatment, including light-harvesting chlorophyll *a/b* binding proteins (*Hosta_21452*, *Hosta_24170*, *Hosta_24421*, *Hosta_41286*), Rubisco large subunit binding protein subunits (*Hosta_33429*, *Hosta_280*), and Rubisco activase (*Hosta_34088*) (Fig. 2D). Expression levels of these core photosynthetic genes in DS were reduced to 30–60% of those in MS, indicating a general suppression of photosynthetic capacity under limiting light conditions.

A subset of stress-responsive genes showed moderate upregulation in DS, including heat shock proteins and peroxidases. This observation suggests that even under

deep shade, *H. ventricosa* experiences mild stress, possibly related to energy limitation or metabolic imbalance. Genes involved in plant hormone signal transduction displayed divergent expression patterns: *Hosta_37835* and *Hosta_41295* were upregulated in DS, whereas *Hosta_11888* and *Hosta_8799* were downregulated (Table 3S), indicating complex hormonal regulation during shade acclimation.

Overall, our transcriptomic data reveal distinct molecular strategies employed by *H. ventricosa* to acclimate to contrasting light environments. Under full sunlight, the plant activates multiple photoprotective mechanisms, including VDE-mediated thermal dissipation, enhanced biosynthesis of protective secondary metabolites (flavonoids and terpenoids), and brassinosteroid-mediated stress signaling, while concomitantly downregulating photosystem antenna proteins to reduce light capture and prevent photodamage. Conversely, under deep shade, the primary transcriptional response involves downregulation of photosynthetic machinery to match reduced light availability, accompanied by modest activation of stress-responsive genes and complex hormonal adjustments. These divergent molecular programs reflect the contrasting physiological challenges of excessive versus limiting light: the former requires dissipating excess energy and preventing oxidative damage, while the latter necessitates conserving resources and coping with energy deficit. Moderate shade appears to represent an optimal condition that minimizes the need for either photoprotection or resource conservation, which is consistent with the observed superior growth performance under this treatment.

Identification of light-responsive candidate genes in *H. ventricosa*: To further elucidate the molecular mechanisms underlying high-light stress responses in *H. ventricosa*, we screened candidate genes from the comparative transcriptome data between the FS and the MS treatments, focusing on genes implicated in photosynthesis and photoprotection (Table 4). The absolute $\log_2FC$ value indicates transcriptional variation between FS and MS. We identified 11 key DEGs, of which six were significantly upregulated in the FS group, and five were downregulated in the FS group. These DEGs include genes encoding light-harvesting complex proteins, photosystem core subunits, carbon fixation enzymes, and photoprotective proteins.

Among the light-harvesting complex II (LHCII)-associated genes, six chlorophyll *a/b*-binding protein genes showed significant differential expression. These included *Hosta_12163* (chlorophyll *a-b* binding protein of LHCII type I, $\log_2FC = 12.65$), *Hosta_32230* (chloroplast chlorophyll *a/b*-binding protein, $\log_2FC = 9.35$), *Hosta_33255* (chlorophyll *a-b* binding protein 4, $\log_2FC = 7.73$), *Hosta_33517* (type III chlorophyll *a/b*-binding protein, $\log_2FC = 10.81$), *Hosta_33602* (chlorophyll *a-b* binding protein 13, $\log_2FC = 8.25$), and *Hosta_33860* (chlorophyll *a-b* binding protein 7, $\log_2FC = 10.78$). The expression levels of these genes under full sunlight suggest active modulation of

the light-harvesting antenna system in response to high irradiance.

Genes encoding core components of PSII also exhibited significant differential expression. *Hosta_12121*, which encodes the PSII CP43 chlorophyll apoprotein, showed a $\log_2FC$ of 6.94, indicating substantial upregulation of PSII reaction center proteins in the full sunlight treatment. Regarding carbon assimilation, genes encoding Rubisco large subunit-binding protein subunits alpha (*Hosta_33429*, $\log_2FC = 10.70$) and beta (*Hosta_280*, $\log_2FC = 8.43$), as well as Rubisco activase (*Hosta_34088*, $\log_2FC = 6.40$), displayed pronounced differential expression patterns, suggesting altered regulation of carbon fixation machinery under high light conditions.

In the context of photoprotection, *Hosta_31445*, encoding VDE, was identified as a key candidate gene with a $\log_2FC$ of 5.68. This enzyme catalyzes the conversion of violaxanthin to zeaxanthin within the xanthophyll cycle, a critical mechanism for thermal dissipation of excess excitation energy and mitigation of photoinhibition.

The functional annotation of these candidate genes indicates that *H. ventricosa* employs an integrated molecular strategy to acclimate to high light stress, coordinating the regulation of light energy capture (LHCII proteins), photosynthetic electron transport (PSII core components), carbon assimilation (Rubisco-associated proteins), and photoprotective mechanisms (VDE). These differential expression patterns provide valuable insights into the molecular basis of high-light stress response in *H. ventricosa*.

Discussion

This study integrated physiological measurements with hybrid transcriptome sequencing to investigate strong light-response strategies in *H. ventricosa*, a shade-tolerant ornamental species. We demonstrated that moderate shade (55% transmittance) represents the optimal condition for growth and photosynthesis, whereas full sunlight induces severe photoinhibition and deep shade limits carbon assimilation. Transcriptomic analysis revealed distinct molecular programs under contrasting light regimes: high light activated photoprotective pathways including the xanthophyll cycle, flavonoid biosynthesis, and brassinosteroid signaling, while low light suppressed photosynthetic machinery and triggered stress responses. These findings establish a physiological and molecular framework for understanding shade plant adaptation and identify candidate genes for improving light tolerance in ornamental horticulture.

Optimal light intensity balances growth and photoprotection in *H. ventricosa*: Our results demonstrate that *H. ventricosa* achieves maximal growth and photosynthetic performance under moderate shade (55.38% transmittance), supported by improved morphology, higher chlorophyll accumulation, and optimal photochemical efficiency (Table 2; $F_v/F_m = 0.83$). This light intensity represents an ecological optimum that satisfies the plant's energy requirements while minimizing

photodamage. The reduced Chl *a/b* ratio (Fig. 1) under moderate shade reflects increased abundance of LHCII, a characteristic adaptation of shade-tolerant species that maximizes photon-capture efficiency under limiting irradiance (Mussnug *et al.* 2007). This adjustment allows *H. ventricosa* to maintain high apparent quantum yield and sustain elevated net photosynthetic rates throughout the diurnal cycle.

In contrast, exposure to full sunlight triggered severe photoinhibition, manifested as a drastic decline in F_v/F_m , elevated NPQ, and visible leaf scorching (Table 2). The 3.6-fold increase in NPQ indicates activation of thermal dissipation mechanisms; however, the sustained high values suggest that photoprotective capacity was exceeded, leading to chronic photodamage (Velitchkova *et al.* 2020, Endo *et al.* 2023). Deep shade imposed a different constraint: while plants avoided photoinhibition, carbon assimilation was severely limited, resulting in intermediate growth performance. These findings underscore the trade-off between light harvesting and photoprotection in shade-adapted species, where moderate shade minimizes the metabolic costs of stress responses while supporting robust vegetative growth (McKew *et al.* 2013, Jiang *et al.* 2026).

Transcriptomic reprogramming underlies divergent light response strategies: The integration of *PacBio* SMRT sequencing with *Illumina* RNA-seq revealed comprehensive transcriptional networks that mechanistically explain the physiological observations. Under full sunlight, the pronounced upregulation of VDE represents a critical adaptive response to enhance the xanthophyll cycle-dependent thermal dissipation capacity. VDE catalyzes the conversion of violaxanthin to antheraxanthin and zeaxanthin, facilitating the formation of dissipative energy traps within the PSII antenna that safely dissipate excess excitation energy as heat and high irradiance stress (Guan *et al.* 2015, Yang *et al.* 2015). The functional role of VDE in photoprotection has been supported by evidence that overexpression of *HvVDE* improved light protection in transgenic tobacco (Qu *et al.* 2024). Consistently, the magnitude of VDE induction observed here exceeds that reported in other shade-tolerant species such as *Panax notoginseng* and *Paris polyphylla* under comparable high light stress (Zhang *et al.* 2020b, 2025), suggesting that *H. ventricosa* possesses robust transcriptional plasticity to activate photoprotection, albeit insufficient to prevent chronic damage under continuous full sun exposure.

High light coordinately upregulates flavonoid biosynthetic genes, activating phenylpropanoid metabolism to eliminate photosystem-derived ROS. Vacuolar and chloroplastic flavonoids act as antioxidants and UV-B filters, shielding macromolecules from oxidative injury (Sako *et al.* 2021). Concurrent induction of brassinosteroid (BR) synthesis and signaling genes (*BR1* and *BZR1* homologs) indicates hormone-regulated stress acclimation. BRs boost stress resistance *via* antioxidant activation, photosystem stabilization, and ABA/ROS-dependent stomatal regulation (Zhang *et al.* 2011, Nascimento *et al.* 2015, Zhu *et al.* 2015). During high-

light stress responses in *H. ventricosa*, hormones including BR and ABA participate in stress adaptation, possibly by triggering ROS signaling and metabolite biosynthesis in response to light stress. This hypothesis requires further experimental verification.

Under deep shade, the widespread downregulation of photosynthetic genes reflects a fundamental shift from carbon acquisition to energy conservation. The suppression of LHCII isoforms and Rubisco-associated chaperones likely reduces the ATP and NADPH demands of protein turnover, allowing the plant to maintain positive carbon balance despite low assimilation rates (Pawlak *et al.* 2020, Sheng *et al.* 2021). This “thriftness” strategy, characterized by reduced investment in photosynthetic machinery, has been documented in shade-adapted understory species such as *Alocasia macrorrhiza* (Sims and Pearcy 1989, Sun *et al.* 2022). The modest upregulation of heat shock proteins and peroxidases under low light suggests that energy limitation itself constitutes a metabolic stress, potentially triggering unfolded protein responses or oxidative stress in mitochondria due to altered redox poise (Knack *et al.* 1992, Essemine *et al.* 2007, Sun *et al.* 2022). The divergent expression patterns of hormone signaling genes (e.g., upregulation of *Hosta_37835* and *Hosta_41295* vs. downregulation of *Hosta_11888* and *Hosta_8799*) imply complex hormonal crosstalk involving auxin, gibberellin, and ethylene pathways that fine-tune shade avoidance vs. tolerance responses (Chuang *et al.* 2015, Gangappa and Botto 2016, Yin *et al.* 2023). These transcriptional adjustments collectively enable *H. ventricosa* to survive in deeply shaded environments by minimizing metabolic costs while maintaining cellular homeostasis.

Candidate genes and molecular targets for horticultural improvement: Our comprehensive screening of light-responsive candidate genes has identified specific molecular targets with significant potential for marker-assisted breeding and genetic improvement of *Hosta* cultivars. The marked differential expression of multiple LHCII isoforms, particularly strong downregulation of *Hosta_12163*, *Hosta_33517*, and *Hosta_33860* under full sunlight, suggests that these genes encode antenna proteins that are actively degraded or transcriptionally suppressed to reduce excitation pressure on PSII reaction centers (Sheng *et al.* 2021). In *Arabidopsis thaliana*, the LHCB1, LHCB2, and LHCB3 subunits are known to undergo phosphorylation-dependent relocation from grana to stroma lamellae (state transitions) or proteasome-mediated degradation during prolonged high light exposure, thereby minimizing photodamage (Damkjær *et al.* 2009, Luciński and Jackowski 2013, Adamiec *et al.* 2015, Crepin and Caffarri 2015). The identification of *H. ventricosa* orthologs with extreme fold changes provides candidate markers for selecting genotypes with enhanced capacity for antenna remodeling, a trait that could extend the light tolerance threshold in landscape plantings.

The upregulation of PSII core protein psbC and Rubisco-associated chaperones under high light reveals

active attempts to repair and maintain photosynthetic function despite photoinhibitory conditions. The D1 protein (PsbA) of the PSII reaction center is particularly susceptible to photo-oxidative damage and requires rapid turnover facilitated by the FtsH and Deg proteases, coordinated with *de novo* synthesis of replacement proteins (Bhattacharjee *et al.* 2025). The co-expression of psbC with Rubisco activase suggests coordinated maintenance of both photochemical and carbon assimilation machineries under stress. These genes are promising candidates for overexpression. Transgenic tobacco overexpressing Rubisco activase exhibits enhanced photosynthesis under combined heat and high light (Song *et al.* 2022), implying that promoting photosynthetic enzyme assembly may improve *Hosta* high-light tolerance.

Most significantly, VDE emerges as a central hub connecting physiological NPQ capacity to its transcriptional basis. In shade-tolerant species such as *Alocasia odora* and *Tradescantia fluminensis*, VDE content and activity are typically lower than in sun-adapted species, correlating with their limited photoprotective capacity (Demmig-Adams *et al.* 2014, Kromdijk *et al.* 2016). The strong transcriptional induction of VDE in *H. ventricosa* under full sunlight suggests that enhancing VDE expression through genetic engineering or allele selection could significantly improve photo-tolerance without compromising shade performance.

Limitations and future perspectives: While this study establishes the first transcriptomic resource for *H. ventricosa*, several limitations merit consideration. Transcriptional profiling captures only gene expression changes, leaving post-transcriptional regulation and metabolic flux unresolved. Integrating proteomics and metabolomics would clarify how observed mRNA patterns translate into functional photoprotective capacity. The greenhouse experiments, conducted over a single growing period, may not fully capture seasonal acclimation dynamics or field performance under natural fluctuating conditions. Long-term studies across multiple growing seasons would provide more robust assessments of light adaptation dynamics. Additionally, functional approaches such as virus-induced gene silencing (VIGS) or stable transformation are needed to validate the proposed roles of candidate genes. Extending comparative transcriptomic analyses to other *Hosta* species with contrasting shade tolerances would further reveal conserved regulatory modules and accelerate marker-assisted breeding for improved light adaptation.

Conclusion: In this study, we integrated physiological measurements with PacBio full-length transcriptome and Illumina RNA-seq to investigate the response of *H. ventricosa* to different light intensities. Moderate shade (55.38% transmittance) promoted vegetative growth, increased chlorophyll content and photosynthetic efficiency, and maintained high PSII photochemical activity, whereas full sunlight caused photoinhibition and leaf scorching, and deep shade (16.41% transmittance) suppressed photosynthesis and carbon fixation. Using

PacBio SMRT sequencing, we generated the first full-length transcriptome for the genus *Hosta*, yielding 37,207 high-quality isoforms (average length 1,617 bp). Comparative transcriptomic analysis revealed that under high light, *H. ventricosa* activates a coordinated photoprotective program including strong upregulation of violaxanthin de-epoxidase to enhance thermal dissipation (NPQ), induction of flavonoid and terpenoid biosynthesis pathways for reactive oxygen species scavenging and UV screening, activation of brassinosteroid signaling, and downregulation of light-harvesting complex and PSII core proteins to reduce light absorption. In contrast, deep shade led to widespread downregulation of photosynthesis-associated genes (e.g., LHCII, Rubisco, Rubisco activase), reflecting a strategy to match carbon assimilation capacity with limited energy supply. Key genes such as VDE (*Hosta_31445*), flavonoid synthase (*Hosta_12414*), and terpenoid synthase (*Hosta_1017*) represent promising targets for molecular breeding of high-light-tolerant *Hosta* cultivars and for engineering photoprotection in related ornamental plants. Collectively, our findings provide a valuable transcriptomic resource and offer mechanistic insights into high-light stress response in a shade-tolerant plant, with practical implications for ornamental horticulture.

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