Comparison of the Transcriptomic Responses of Two *Chrysanthemum Morifolium* Cultivars to Low Light

Shuang Han (✉ htshd_012@163.com)

Shangqiu Normal University

Qingchen Zhang

Shangqiu Normal University

Xiaoqin Zhu

Shangqiu Normal University

Dongli Pei

Shangqiu Normal University

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Abstract

Low light is a primary regulator of chrysanthemum growth. Herein, we conducted a transcriptomic analysis of leaf samples from the 'Nannonggongfen' and 'Nannongxuefeng' chrysanthemum cultivars following a 5-day exposure to optimal light (70%, control [CK]) or low-light (20%, LL) conditions. Gene Ontology (GO) classification of upregulated genes revealed these genes to be associated with 11 cellular components, 9 molecular functions, and 15 biological processes, with the majority being localized to the chloroplast, highlighting the role of chloroplast proteins as regulators of shading tolerance. Downregulated genes were associated with 11 cellular components, 8 molecular functions, and 16 biological processes. Heat map analyses suggested that basic helix–loop–helix domain genes and elongation factors were markedly downregulated in 'Nannongxuefeng' leaves, consistent with the maintenance of normal stem length, whereas no comparable changes were observed in 'Nannonggongfen' leaves. Subsequent qPCR analyses revealed that phytochrome-interacting factors and dormancy-associated genes were significantly upregulated under LL conditions relative to CK conditions, while succinate dehydrogenase 1, elongated hypocotyls 5, and auxin-responsive gene of were significantly downregulated under LL conditions. These findings suggest that LL plants were significantly lower than those of the CK plants. Low-light tolerant chrysanthemum cultivars may maintain reduced indole-3-acetic acid (IAA) and elongation factor expression as a means of preventing the onset of shade-avoidance symptoms.

Introduction

Chrysanthemum morifolium is among the most popular ornamental plants globally, and it is frequently grown in shaded greenhouses in China. The morphological characteristics of plants are profoundly impacted by light intensity such that lower levels of light exposure can affect key growth, anatomy[1–2], photosynthesis[3–5], antioxidant activity[6–7], and sucrose metabolism[8–9]. The molecular mechanisms governing the low-light (LL) responses of the 'NannongXuefeng' and 'Nannonggongfen' chrysanthemum cultivars are currently lacking. Transcriptomic analyses offer a powerful approach that can be used to explore gene expression in a wide range of plant species. As such, this study conducted such transcriptomic analyses in order to understand the mechanisms whereby chrysanthemum plants adapt to low light.

C. morifolium is an allohexaploid plant ($2n = 6 \times = 54$), and owing to such complexity there is no extant whole-genome sequence available for this plant[10]. RNA sequencing (RNA-seq), however, has been used to explore the biological properties of these plants, with the first transcriptomic study of *C. morifolium* responses to dehydration stress having been published in 2013[11]. Such analyses have also enabled researchers to identify differentially expressed genes (DEGs) in *C. morifolium* plants that are responsive to low-light conditions[12–14]. One deep transcriptomic analysis determined that phytochrome interacting factors (PIFs) likely serve as direct drivers of auxin biosynthesis, conjugation, transport, perception, and signaling-related genes[15]. Early termination of leaf proliferation can occur as a consequence of extended exposure to a low red to far-red light ratio (R/FR) through mechanisms

dependent upon the ATHB2 and ATHB4 HD-Zip II transcription factors[13]. Applied transcriptomic analysis methods have also been employed to explore *C. morifolium* responses upon exposure to UV-B light[16], the mechanisms regulating carotenoid accumulation[17], responses to dodder invasion[18], and other biologically relevant pathways. No studies, however, have explored *C. morifolium* transcriptomic responses to low-light exposure.

As the ability of these plants to grown under low-light conditions can impact the ornamental value of *C. morifolium*, there is clear commercial relevance to the elucidation of the molecular mechanisms governing such growth responses. Prior studies of *C. morifolium* have revealed shade-avoidance behaviors to emerge on day 7 of low-light treatment[19]. To better understand the transcriptomic changes associated with such behaviors, we herein used an RNA-seq approach to identify DEGs associated with exposure to supraoptimal (70%) and very low (20%) natural light conditions in the ‘NangnongXuefeng’ and ‘Nannonggongfen’ chrysanthemum cultivars. Leaves were collected from these plants at 11:00 AM on the fifth day of shade exposure, before the manifestation of overt shade-avoidance behaviors. In addition to identifying upregulated and downregulated DEGs in these plants, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses to understand the biological functions of these key low-light exposure-related genes. Together, these results may offer novel insights into the genetic determinants of low-light sensitivity in chrysanthemum cultivars, thereby guiding future research or selective breeding efforts.

Materials And Methods

Plant materials, growth, and treatment

The ‘Nannongxuefeng’ (XF, low-light resistant) and ‘Nannonggongfen’ (GF, low-light sensitive) *C. morifolium* cultivars were obtained from an experimental field at Shangqiu Normal University. Uniform cuttings were collected for each cultivar and were grown in plugs containing a 1:1 peat and perlite mixture for 15 days, after which rooted seedlings were transferred to 4 L pots filled with a 2:1:1 combination of garden soil, perlite, and vermiculite that was treated with a nutrient solution. Seedlings were randomly selected to be subjected to either 70% or 20% natural light conditions (control and low-light groups; CK and LL, respectively). Commercial black shading nets were used to reduce light exposure in a net-house (2.5 m high × 4 m long × 4 m wide) for 21 days beginning on July 20, 2019, with 60 pots per treatment condition. No significant differences in temperature were observed between treatment conditions, with average canopy temperatures remaining within < 1.0°C of one another. other, and no consistent differences in temperature were detected between the treatments. The respective average maximum/minimum temperature, photoperiod (day/night), and humidity were 30°C/21°C, 14/10 h, and 70%. In addition to natural rainfall, supplemental water was applied to all plants as appropriate. On the fifth day, apex leaves from 10 plants per treatment condition were collected from 10:30 – 12:00 AM, snap-frozen, and shipped to the Huada Genetics Company for transcriptome sequencing.

Internode and petiole length measurements

Five plants per treatment condition from different pots were selected, and measurements of the internodes of the fourth and fifth fully expanded leaves and the petioles of the fourth fully expanded leaves were taken.

P_N–PAR response curve measurements

Leaf P_N –PAR response curves were assessed with a portable photosynthesis system (Li6400XT, Li-Cor, NE, USA) using a red-blue LED light source (6400-02B) on three sunny days at the same time the prior measurements had been made. Responses were evaluated at PAR values of 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, and 0 $\mu\text{mol (photon) m}^{-2} \text{s}^{-1}$ in a leaf chamber under identical conditions (ambient CO₂ concentration: 360–390 $\mu\text{mol [CO}_2\text{] mol}^{-1}$, leaf temperature: $30 \pm 3^\circ\text{C}$, relative humidity: 60–70%) from 09:00–11:30 AM.

RNA sequencing

The BGIS EQ-500 platform was used for sequencing, generating 122.59 Gb of raw data. Following assembly and the removal of redundant sequences, 104,769 unigenes were obtained, and 55,189 coding sequences (CDS) were detected using TransDecoder. Transcript expression was assessed based on the number of fragments per kilobase per million mapped reads (FPKM), with $\text{FPKM} \geq 1$ being indicative of reliability. Differences in expression levels between samples were assessed with DEseq2, with $P < 0.05$ serving as the threshold for the identification of differentially expressed genes (DEGs). DEGs were further classified into functional categories through GO and KEGG enrichment analyses conducted with the Phyper function in R. False discovery rate (FDR) approach was then used to correct P values, with $\text{FDR} \leq 0.01$ being indicative of significant enrichment. Samples were analyzed in triplicate.

qPCR

A total of 5 genes in three replicate plants were selected for qPCR-based validation of RNA-seq data using primers designed using the Primer 5.0 software (Applied Biosystems) and synthesized by Genewiz (Tianjin, China). A PrimeScript RT reagent kit (Takara, Dalian, China) was used to prepare cDNA from 1 μg of total RNA, and qPCR was subsequently conducted with a Thermo PR096 Real-Time PCR instrument (Applied Biosystems) and 2 $\times$ SYBR green PCR master mix (Applied Biosystems). Samples were analyzed in triplicate and results were reported as means $\pm$ SE ($n = 3$). Primers used in this study are shown in Additional File 1. The $2^{-\Delta\Delta\text{CT}}$ method was used to assess relative gene expression[20].

Statistical analysis

Samples were selected randomly for quantitative evaluation. Data were compared via one-way ANOVAs using SPSS v21.0 (SPSS, IL, USA), with means being compared by Duncan's test at a 5% significance level.

Results

Chrysanthemum seedling responses to low-light conditions

On day 21 of low-light exposure, chrysanthemum plants of the GF cultivar exhibited thinner leaves and weaker growth relative to corresponding control (CK) plants, whereas no such morphological changes were evident for plants of the XF cultivar (Fig. 1). The low-light-sensitive GF plants exhibited increased internode and petiole lengths, in contrast to low-light-resistant XF (Fig. 2).

The response curves for different chrysanthemum varieties grown under low light treatment conditions are shown in Fig. 3. When photosynthetically active radiation was low ($\text{PAR} \leq 200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the net photosynthetic rate of leaves exhibited an almost linear increase with changing light intensity, and the net photosynthetic rate of XF CK plants was significantly higher than that of plants under low light treatment ($p < 0.05$). Similarly, GF CK plants exhibited a net photosynthetic rate that was significantly higher than that of plants under low light conditions ($p < 0.01$). When the photosynthetically active radiation was $1200\text{--}1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the net photosynthetic rate of XF LL plants tended to plateau and to reach the light saturation point. This trend manifested earlier in GF LL plants, indicating that GF plants were more significantly affected by low light.

Chrysanthemum gene expression profiles in response to low light

Relative to the low-light-resistant XF cultivar, the low-light-sensitive GF cultivar exhibited substantially more DEGs (both upregulated and downregulated) upon exposure to LL conditions (Fig. 4). When comparing shared DEGs between XF and GF plants (LL vs. CK conditions), 343 and 182 commonly up- and downregulated genes were identified, respectively (Figs. 4b-c).

GO classification of common DEGs

We next evaluated the transcriptomic responses of XF and GF plants to better understand how they respond to LL conditions. Overall, similar LL-induced DEG profiles were observed in leaf samples from both cultivars, with a slightly higher fraction of genes involved in extracellular responses, transcription regulator activity, and signal transduction activity being observed in GF plants relative to XF plants. A number of resistance-associated genes were among DEGs identified in both XF and GF plants (Fig. 5). Resistance-related genes that were preferentially expressed in XF plants included those encoding light-harvesting proteins and protein phosphatases. Enrichment analyses revealed upregulated genes to be primarily associated with photosynthesis, including 9 proteins related to photosystem I (GO: 0009522), 7 with pectate lyase activity (GO: 0030570), 9 with photosystem II-related functions (GO: 0009523), 9 associated with chlorophyll binding (GO: 0016168), 8 photosynthesis-related proteins (GO: 0009765), 9 involved in chromophore linkage (GO: 0018298), 9 associated with the chloroplast thylakoid membrane (GO: 0009535), 7 involved in pectin catabolic processes (GO: 0045490), 2 with ferroxidase activity (GO: 0004322), 2 capable of ferric iron binding (GO: 0008199), 2 with sigma factor activity (GO: 0016987), 2 linked to iron-ion transport (GO: 0006826), 10 involved in metal-ion binding (GO: 0046872), 2 pigment

binding-related proteins (GO: 0031409), 2 cell-related proteins (GO: 0005623), 2 related to cellular iron-ion homeostasis (GO: 0006879), 2 plastoglobule-associated proteins (GO: 0010287), 2 with isocitratelase activity (GO: 0004451), 3 extracellular proteins (GO: 0005576), and 2 involved in light harvesting in photosystem I (GO: 0009768) (Table 1). Proteins encoded by the downregulated genes were primarily related to glycometabolism and amino acid metabolism, including 12 linked to chorismate biosynthetic processes (0009423) and aromatic amino acid family biosynthetic processes (GO:0009073), 6 with 3-deoxy-7-phosphoheptulonate synthase activity (GO: 0003849), 20 chloroplast-related proteins (GO: 0009507), 4 with chorismate synthase activity (GO:0004107), 7 involved in FMN binding (GO: 0010181), 4 proteins with glycolipid biosynthetic process (GO: 0009247), 4 with prephenate dehydratase activity (GO: 0004664), 4 associated with L-phenylalanine biosynthetic processes (GO:0009094), 5 with phosphatase activity (GO: 0016791), 3 exhibiting strictosidine synthase activity (GO: 0016844), 4 with arogenate dehydratase activity (GO: 0047769), 8 related to biosynthetic processes (GO: 0009058), 2 associated with UDP-glucose metabolic processes (GO: 0006011), 2 sulfolipid biosynthetic process-related proteins (GO: 0046506), 3 with glucose-1-phosphate uridylyltransferase activity (GO: 0003983), 5 with transferase activity (GO: 0016758), 2 with 3-dehydroquinate synthase activity (GO: 0003856), 2 with receptor activity (GO: 0004872), and 2 hormone-related proteins (GO: 0009725) (Table 2).

Table 1
GO enrichment analysis of common up-regulated gene in both XF and GF

Gene Ontology Term	Gene Num	$-\log_{10}(P\text{value})$
photosystem I (GO: 0009522)	9	12.5341
pectate lyase activity (GO: 0030570)	7	
photosystem II (GO: 0009523)	9	12.1079
chlorophyll binding (GO: 0016168)	9	11.7762
photosynthesis, light harvesting (GO: 0009765)	8	11.6658
protein-chromophore linkage (GO: 0018298)	9	9.7478
chloroplast thylakoid membrane (GO: 0009535)	9	9.6017
pectin catabolic process (GO: 0045490)	7	9.5221
ferroxidase activity (GO: 0004322)	2	8.1500
ferric iron binding (GO: 0008199)	2	4.1121
sigma factor activity (GO: 0016987)	2	4.1121
iron ion transport (GO: 0006826)	2	4.1121
metal ion binding (GO: 0046872)	10	3.8241
pigment binding (GO: 0031409)	2	3.8286
cell (GO: 0005623)	2	3.7338
cellular iron ion homeostasis (GO: 0006879)	2	3.5206
plastoglobule (GO: 0010287)	2	3.3802
isocitrate lyase activity (GO: 0004451)	2	3.2669
extracellular region (GO: 0005576)	3	2.6369
photosynthesis, light harvesting in photosystem I (GO: 0009768)	2	2.6269
*Pvalue of all GO terms are lower than 0.05. Conversely, $-\log_{10}(P\text{value})$ values of all GO terms are greater than 1.3010, that is, the greater $-\log_{10}(P\text{value})$ value, the better significance.		

Table 2
GO enrichment analysis of common down-regulated gene in both XF and GF

Gene Ontology term	Gene Num	$-\log_{10}(P\text{ value})$
chorismate biosynthetic process GO:0009423	12	20.33677
aromatic amino acid family biosynthetic process GO:0009073	12	19.80942
3-deoxy-7-phosphoheptulonate synthase activity GO:0003849	6	10.08535
Chloroplast GO:0009507	20	7.367669
chorismate synthase activity GO:0004107	4	6.505752
FMN binding GO:0010181	7	5.897949
glycolipid biosynthetic process GO:0009247	4	5.310687
prephenate dehydratase activity GO:0004664	4	4.714618
L-phenylalanine biosynthetic process GO:0009094	4	4.840234
phosphatase activity GO:0016791	5	4.703904
strictosidine synthase activity GO:0016844	3	4.759647
arogenate dehydratase activity GO:0047769	4	4.714618
biosynthetic process GO:0009058	8	4.356696
UDP-glucose metabolic process GO:0006011	2	3.894192

*Pvalue of all GO terms are lower than 0.05. Conversely, $-\log_{10}(P\text{ value})$ values of all GO terms are greater than 1.3010, that is, the greater $-\log_{10}(P\text{ value})$ value, the better significance.

Gene Ontology term	Gene Num	$-\log_{10}(P\text{ value})$
sulfolipid biosynthetic process GO:0046506	2	3.894192
UTP:glucose-1-phosphate uridylyltransferase activity GO:0003983	2	3.832329
transferase activity, transferring hexosyl groups GO:0016758	5	3.777114
3-dehydroquinate synthase activity GO:0003856	2	3.358706
receptor activity GO:0004872	2	3.358706
response to hormone GO:0009725	2	3.122524
*Pvalue of all GO terms are lower than 0.05. Conversely, $-\log_{10}(P\text{ value})$ values of all GO terms are greater than 1.3010, that is, the greater $-\log_{10}(P\text{ value})$ value, the better significance.		

KEGG pathway enrichment analysis of common DEGs

To explore the roles of DEGs in low-light resistance, we next conducted KEGG pathway enrichment analyses (Table 3). Upregulated genes were associated with 17 distinct metabolic pathways, including plant hormone signal transduction, MAPK signaling, purine metabolism, pyrimidine metabolism, phenylpropanoid biosynthesis, photosynthesis antenna proteins, galactose metabolism, RNA polymerase activity, circadian rhythms, porphyrin and chlorophyll metabolism, the pentose phosphate pathway, glutathione metabolism, nicotinate and nicotinamide metabolism, glycosylphosphatidylinositol-anchor biosynthesis, the synthesis and degradation of ketone bodies, and brassinosteroid biosynthesis. Processes associated with downregulated DEGs included phenylpropanoid biosynthesis, amino acid biosynthesis, flavonoid biosynthesis, starch and sucrose metabolism, phenylalanine/tyrosine/tryptophan biosynthesis, stilbenoid and phenylalanine metabolism, stilbenoid/diarylheptanoid/gingerol biosynthesis, phenylalanine metabolism, RNA degradation, isoflavonoid biosynthesis, circadian rhythms, monoterpenoid biosynthesis, ubiquinone and other terpenoid–quinone biosynthesis, the pentose phosphate pathway, anthocyanin biosynthesis, flavone and flavonol biosynthesis, diterpenoid biosynthesis, and glucosinolate biosynthesis (Table 4).

Table 3
KEGG pathway enrichment analysis of common up-regulated gene in both XF and GF

Pathway ID	Pathway Name	Gene Num	$-\log_{10}(P\text{value})$
ko00196	photosynthesis antenna proteins	16	10.3448
ko04075	plant hormone signal transduction	39	7.246061
ko00240	pyrimidine metabolism	21	2.952804
ko00402	benzoxazinoid biosynthesis	4	3.079756
ko00860	porphyrin and chlorophyll metabolism	9	3.007799
ko04712	circadian rhythm	13	2.935614
ko00052	galactose metabolism	15	2.746596
ko03020	RNA polymerase	14	2.565113
ko00030	pentose phosphate pathway	10	2.387964
ko00230	purine metabolism	22	2.336557
ko00760	nicotinate and nicotinamide metabolism	5	2.022957
ko00940	phenylpropanoid biosynthesis	17	1.880869
ko00563	glycosylphosphatidylinositol (GPI)-anchor biosynthesis	4	1.720203
ko04016	MAPK signaling pathway	23	1.5111
ko00480	glutathione metabolism	8	1.472649
ko00072	synthesis and degradation of ketone bodies	3	1.376332
ko00905	brassinosteroid biosynthesis	2	1.306046
* P value of all terms are lower than 0.05. Conversely, $-\log_{10}(P\text{value})$ values of all terms are greater than 1.3010, that is, the greater $-\log_{10}(P\text{value})$ value, the better significance.			

Table 4
KEGG pathway enrichment analysis of common down-regulated gene in both XF and GF

Pathway ID	Pathway Name	Gene Num	$-\log_{10}(P\text{value})$
ko00941	Flavonoid biosynthesis	43	28.56404
ko00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	27	19.0251
ko00940	Phenylpropanoid biosynthesis	56	17.62388
ko00400	Phenylalanine, tyrosine and tryptophan biosynthesis	29	12.48904
ko00943	Isoflavonoid biosynthesis	16	12.05271
ko00360	Phenylalanine metabolism	25	11.59716
ko00902	Monoterpenoid biosynthesis	15	9.850463
ko00942	Anthocyanin biosynthesis	12	9.275998
ko01230	Biosynthesis of amino acids	48	5.176359
ko00130	Ubiquinone and other terpenoid-quinone biosynthesis	14	5.040118
ko00944	Flavone and flavonol biosynthesis	6	4.746465
ko00500	Starch and sucrose metabolism	32	3.080086
ko00750	Vitamin B6 metabolism	6	3.003794
ko00030	Pentose phosphate pathway	13	2.338327
ko00906	Carotenoid biosynthesis	10	2.269926
ko04712	Circadian rhythm	15	2.132154
ko00904	Diterpenoid biosynthesis	6	1.883375
ko03018	RNA degradation	23	1.77389
ko00966	Glucosinolate biosynthesis	4	1.691318
ko00710	Carbon fixation in photosynthetic organisms	14	1.643104
ko00052	Galactose metabolism	16	1.473552
ko00740	Riboflavin metabolism	4	1.317761
* P value of all terms are lower than 0.05. Conversely, $-\log_{10}(P\text{value})$ values of all terms are greater than 1.3010, that is, the greater $-\log_{10}(P\text{value})$ value, the better significance.			

Analysis of low-light-related DEGs

To identify regulatory pathways related to low-light responses in these two chrysanthemum cultivars, heat map analyses were next conducted, revealing significant downregulation of the basic helix-loop-

helix domain genes (unigene 4998) and elongation factor (CL1856) in XF plants (Fig. 6), suggesting a role for these genes in the maintenance of normal stem length.

Candidate DEG validation. To validate our RNA-seq data, we next selectively assessed gene expression patterns for five DEGs via qPCR, including the plant hormone signal transduction-related genes XP_021983913.1 (phytochrome interacting factor 7, *PIF7*), XP_022023437.1 (elongated hypocotyls 5, *HY5*), and XP_022017432.1 (auxin-responsive protein IAA1-like, *IAA*) (KEGG Orthology), the mitochondrial electron transport-related gene XP_022017432.1 (succinate dehydrogenase assembly factor 1, *SDH1*) (Gene Orthology), and the dormancy-related gene *DOR* (Swissprot) (Fig. 6). We observed *PIF7* upregulation in response to LL conditions in both cultivars, although it was expressed at levels roughly four-fold higher in GF plants relative to XF plants (Fig. 7a). *HY5* was significantly downregulated in LL plant samples relative to CK plant samples (Fig. 7b), with low-light similarly significantly downregulating *IAA* (Fig. 7c) and *SDH1* (Fig. 7d) in both tested cultivars. *DOR*-associated gene expression was increased in response to LL conditions in both cultivars, with the increase in GF plants being roughly 32-fold higher than that observed in XF plants (Fig. 7e).

Discussion

Light exposure is the primary climatic determinant of plant growth [1, 19], with changes in irradiance levels having the potential to impact plant growth, morphology, and anatomy[21]. While many studies of how low-light exposure alters plant physiology have been published to date, the molecular basis for such physiological changes is not well understood. Herein, we found that proteins encoded by most upregulated LL exposure-related DEGs were associated with the chloroplast and involved in photosynthesis and oxidative stress-related pathways. In particular, these proteins are associated with photosystem I/II functionality and associated with the chloroplast thylakoid membrane, suggesting a direct role for these proteins as mediators of chrysanthemum low-light-related stress. In contrast, proteins downregulated under LL conditions were involved in processes associated with chorismate and aromatic amino acid biosynthesis and were also related to chloroplasts, indicating that these proteins may be involved in LL resistance. However, more research is necessary to understand the importance of the differential regulation of these genes in the context of chrysanthemum LL exposure and resistance.

We detected apparent relationships between chrysanthemum LL tolerance and altered plant hormone signal transduction and MAPK signaling pathway activities, in line with prior reports[22–23]. We also observed increases in succinate dehydrogenase, glycerol-3-phosphate transporter and chaperone protein biosynthesis, and E3 ubiquitin-protein ligase pathway activity-related proteins under LL conditions in the low-light tolerant XF cultivar, whereas the opposite was observed for the low-light sensitive GF cultivar. Light-activated photoreceptors are involved in downstream complex activation, repressing its E3 ubiquitin ligase activity and photoreceptor stabilization[24]. We observed several E3 ubiquitin ligase activity-related genes that were upregulated in XF plant leaves under LL conditions, whereas in GF plants these same genes were downregulated upon LL exposure. Increased E3 ubiquitin protein levels can promote photomorphogenesis under low light, benefiting short stem maintenance responses. In LL-sensitive

soybean cultivars, sucrose content and related synthase activity levels were markedly reduced in response to low-light stress[25], consistent with our data and suggesting that this may be a fundamental pathway involved in crop low-light tolerance.

Previous work has indicated important roles for hormones including ABA, auxin, cytokinins, ethylene, gibberellins, and brassinosteroid in shade-avoidance response (SAR) induction[26], with ethylene, gibberellic acid, auxin, and brassinosteroids interacting through complex mechanisms to control cellular elongation under varying light conditions [27]. Overexpression of brassinosteroid biosynthetic genes in tomatoes has been shown to result in brassinosteroid transcript upregulation, gibberellin and endogenous BRdownregulation, and enhanced germination, lateral root development, and CO₂ assimilation[28]. Herein, we identified certain auxin response-related genes that were associated with cell enlargement and tryptophan metabolism. We also observed lower levels of the key brassinosteroid signal transduction-related transcription factors EIN and BZR1 in the low-light tolerant XF cultivar relative to the low-light sensitive GF cultivar, suggesting that the reduced biosynthesis of these proteins may be linked to chrysanthemum low-light tolerance.

PIF transcription factors are required for the phytochrome-mediated SAR[29]. Elongation growth is inhibited by HY5, which competitively binds to PIF4 chromatin targets rather than regulating PIF4 transcription[30]. PIF7 promotes auxin biosynthesis and auxin redistribution[31], with auxins being essential mediators of the SAR such that auxin activation can modulate gene expression to promote shoot cell elongation[32–33]. AXR3/IAA17 mutant *Arabidopsis thaliana* plants exhibit reduced shade-induced hypocotyl growth[34]. Herein, we found LL treatment to enhance *PIF7* expression, with a roughly four-fold increase in the expression of this gene in GF plants relative to XF plants. GF plants also exhibited marked *HY5* downregulation, consistent with a clear impact of low-light conditions on GF plants. SDH functions at the interface between the oxidative phosphorylation and TCA cycle pathways[35], with lower *Arabidopsis* SDH levels serving to suppress primary root elongation with early lateral root emergence[36]. SAR behaviors may thus be suppressed in low-light tolerant chrysanthemum cultivars through the maintenance of low IAA and elongation factor expression levels (CL1856). In contrast, exposure of the low-light-sensitive plants to low-light conditions severely disrupted electron transfer and photosynthetic carbon assimilation, resulting in profound growth inhibition and other SAR symptoms.

Conclusions

In summary, the present transcriptomic analysis revealed 1620 and 1207 upregulated and downregulated genes, respectively, when comparing the GF LL and GF CK groups, while just 343 and 556 upregulated and downregulated genes were identified when comparing the XF LL and XF CK groups. GO and KEGG analyses of these DEGs revealed the majority of the upregulated genes to encode proteins localized to the chloroplast that were associated with a range of photosynthesis-related biological processes, suggesting that such proteins may be important regulators of chrysanthemum tolerance to low-light conditions. Proteins encoded by downregulated genes were associated with a range of biological functions including

hormone signal transduction, photosynthesis, and shading regulation pathways. Together these results provide novel insights into the transcriptional basis for low-light resistance in specific chrysanthemum cultivars, providing a foundation for future research and selective breeding efforts.

Declarations

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Availability of data and material: All data are fully available without restriction.

Authors' contributions: Performed the experiments: Shuang Han and Qingchen Zhang; analyzed the data: Xiaoqin Zhu; prepared and wrote the manuscript: Shuang Han; conceived and designed the experiment: Dongli Pei.

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Figures

Figure 1

Effects of different light treatments on chrysanthemum growth

Figure 2

Effects of different light treatments on internode and petiole lengths. (A) internode length (B) petiole length (Note: Data are given as mean±standard error [n=5]. Different letters above standard error bars indicate significant difference at $P<0.05$)

Figure 3

Effects of different light treatments on light response curve. (A 'Nannongxuefeng' B 'Nannonggongfen')

Figure 4

Gene expression profile of different chrysanthemum cultivars in response to low light. A Total number of upregulated and downregulated genes. B Venn diagram of upregulated genes. C Venn diagram of

downregulated genes. Three independent experimental replicates were analyzed for each sample, and data were indicated as means±SE (n=3).

Figure 5
Gene Ontology classification of common differential genes

Figure 6

Heat map of DEGs related to shade-avoidance syndrome ($RPKM \geq 1$, $\log_2[XuefengT/Xuefeng] \leq 0$ and $\log_2[GongfenT/Gongfen] \geq 0$; red and blue indicate high and low expression levels, respectively).

Figure 7

qRT-PCR analysis of gene expression compared with the RNA-seq data. (A) 'Nannongxuefeng' (B) 'Nannonggongfen'.