Transcriptome and physiology analysis reveal key players of the shade-tolerant species *Panax notoginseng* in photosynthetic performance under both high and low light regimes

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**ABSTRACT**

The present study aimed to understand the molecular mechanism underlying the light-induced depression of photosynthetic capacity in a typically shade-tolerant species *Panax notoginseng* grown under different light intensities. Photosynthetic performance was declined in the low-light- and high-light-grown plants. The physiological plasticity was low, while the plasticity of morpho-anatomy traits, biomass allocation and biochemical traits was great in response to the light environment. High light enhanced the expression of genes related to reactive oxygen scavenging, pentose phosphate pathway and glycolysis. The expression of genes involved in the light reaction was suppressed in low light, along with a reduction in Rubisco activity. The expression of genes encoding Rubisco was repressed in high light. Photosynthetic capacity in low light might be inhibited by the deceleration on light reaction and by the increase of inactivated Rubisco, high-light-induced decrease of photosynthetic performance might result from the deceleration on Calvin cycle.

**Abbreviations:** \(A_{\text{net}}\), net assimilation rate; LCP, light compensation point; PQ, plastoquinone pool; LSP, light saturation point; PPFD, photosynthetic photon flux density; \(C_i\), internal leaf CO2 concentration; \(A_{\text{max}}\), maximum net photosynthesis rate; \(C_{\text{E}}\), carboxylation efficiency; \(J_{\text{max}}\), maximum electron transfer rate; \(V_{\text{c,max}}\), maximum carboxylation efficiency; \(R_{d}\), dark respiration rate; \(\Phi_{\text{PSII,}\text{m}}\), PSII photochemical quantum yield; \(\Phi_P\), photochemical quenching; \(F_F^{\prime}/F_F\), photochemical efficiency of PSII in the light; NPQ, non-photochemical quenching; \(\Phi_{A,}\text{fluorescence quenching dissipation}\), \(\Phi_{\text{NPQ, non-photochemical quenching dissipation}}\); \((V + A + Z)\), the xanthophyll cycle pigment pool; \(L\), lutein; \(N\), neoxanthin; \(\beta\)-Car, \(\beta\)-Carotene; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase

1. Introduction

Light is a vital resource for plant growth and development via triggering the expression of genes involved in photosynthesis (Li et al. 1995). The excess light energy may cause photosystem I (PSI) and photosystem II (PSII) of the plant to be restrained, reduce carbon assimilation, and even cause plant death in severe cases (Elsheery et al. 2008a; Vialet-Chabrand et al. 2017). The shortage of light energy will cause plants to photosynthesize at a rate that don't offset the rate of respiration consumption (Matuszyńska et al. 2019). Therefore, mechanisms to acclimate to the light condition are operated in maximizing plant fitness. Based on the light requirement, plants can be categorized into light-demanding and shade-tolerant plants (He et al. 2019; Poorter et al. 2019), and the former grows under open sites and flourishes under high light, and the latter grows under closed sites and prefers to low light. It has been generally believed that the shade-tolerant species is sensitive to high light and could not grow well or survive under full light. The shade-tolerant species show such eco-photosynthetic characteristics, presumably because it is the result of long-term acclimation to low light conditions (Mishanin et al. 2016).

It has aroused lots of people's interest in eco-photosynthetic characteristics of the shade-tolerant species for a long time. Thus many efforts have been made to elucidate plant features associated with shade tolerance. In comparison with the light-demanding plants, the shade-tolerant plants are characterized by low net assimilation rate \(A_{\text{net}}\), low light compensation point (LCP), high content of photosynthetic pigments, and low values of leaf mass per area and lamina thickness (Valladares et al. 2000). The shade-tolerant plants generally possess low \(A_{\text{net}}\) and low relative growth rate
(RGR) under high light, correspondingly, the light-demanding species hold the opposite (Valladares and Niinemets, 2008; Chen et al. 2016). Additionally, shade-adapted plants have possessed larger antenna than light-demanding plants, and increased content of Chl b in LHCII has been observed in the shade-adapted plants (Bailey et al. 2004; Hogewoning et al. 2012; Wientjes et al. 2013; Ware et al. 2015). Extremely low light would inevitably lead to energy shortage and also repress photosynthetic capacity and RGR in the shade-tolerant species. Nevertheless, plants have evolved an array of light acclimation strategies that rely on the regulation of relative amounts of LHCII and photosystem I (PSI) and PSII (Elsheery et al. 2007; Ferroni et al. 2016).

Photosynthesis-related gene expression contributes to photosynthetic performance in plants. The expression of genes involved in photosynthesis at transcriptional levels may be regulated by light. The turnover rates of photosynthetic proteins are accelerated at high-light intensity (Aro et al. 1993). High light results in the depression of genes encoding LHC components and subunits of two photosystems (Atienza et al. 2004; Murchie et al. 2005; Fischer et al. 2006; Kimura et al. 2007). High-light-induced inhibition on LHC genes has been presented in *Hordeum vulgare* and *Dunalellula salina* (Masuda et al. 2003; Atienza et al. 2004).

In contrast, the expression of genes involved in photoprotective proteins is elevated under high light (Kimura et al. 2007). Activation of a membrane-bound protein kinase that plays a role in the phosphorylation of LCHII is shown under low light when the cytochrome b6/f complex is reduced through plastoquinone pool (PQ) (Elsherey et al. 2008b; Pesaresi et al. 2009). The down regulation of photosynthesis-related genes might be regulated by photosynthesis-delivered carbohydrates, redox state of plastids and reactive oxygen species (ROS; Foyer et al. 2012).

Photosynthesis-related gene expression has been conducted in *Arabidopsis thaliana* or in the crop plant, such as rice, barely, and maize (Hubbart et al. 2012; Wang et al. 2017). In the *A. thaliana*, mutants were commonly used to test photosynthesis-related gene function, and a wealth of global gene expression data is now available from *Arabidopsis* plants exposed to various light treatments (Feng et al. 2008). In the crop plants, the genome is available for rice, barely, and maize, thus these crop plants were also commonly used to investigate the photosynthesis-related gene function and expression (Hubbart et al. 2012; Wang et al. 2014). The model plant and crop plant all are light-demanding species or can tolerate high light. Surprisingly, the shade-tolerant species were rarely selected to elucidate the expression of genes involved in photosynthesis in response to varying light intensity. Shade-tolerant species is sensitive to high light and tolerant to low light. Moreover, the physiological phenotypic plasticity to a light gradient is low and the low-light- and high-light-grown plants show inferior photosynthetic performance than the optimal-light-grown ones (Chen et al. 2016). Unfortunately, the molecular mechanism for photosynthetic performance is not fully investigated, and, therefore, it is yet unclear.

*Panax notoginseng* is a Chinese medicinal herb, and this herb can tolerate low light and is less likely to survive in high light conditions (Huang et al. 2018). The light saturation point (LSP) for the shade-tolerant species *P. notoginseng* is from 100 to 150 μmol m⁻² s⁻¹, and this herb plant grows vigorously under about 10% of full light and could tolerate about 30% of full light to a maximum extent (Xu et al. 2018; Wu et al. 2021). However, nowadays little is known about light-induced photosynthesis-related gene expression, and about whether or not photosynthetic performance is in accord with the expression of genes involved in photosynthesis in the *P. notoginseng* grown under a light gradient. In the present study, *P. notoginseng* grows under high-light, moderate-light, and low-light conditions, photosynthetic capacity and transcriptome were simultaneously examined. This study aims to explore the coordination between photosynthetic performance and gene expression, and it is anticipated that the expression of genes might explain photosynthetic performance in the typically shade-tolerant species *P. notoginseng*. The molecular mechanisms of light adaptation in shade-tolerant plants will be further interpreted in this study.

2. Materials and methods

2.1. Plant materials and growth conditions

The experimental site was located at Yanshan county, Yunnan province, China (longitude 104°37′, latitude 23°56′). Yanshan county is characterized by the subtropical continental monsoon climate, and it is also one of the areas of origins for *P. notoginseng*. The shade-houses (4 m (length) × 4 m (width) × 2 m (height)) with different levels of light intensity were created by a permeable black plastic net. On sunny days, the Li-190R quantum sensor and Li-1500 quantum recorder (Li-Cor, USA) were used to collect the light intensity in each shade house every 10 s from 6:00 to 19:00. A typical diurnal curve of photosynthetic photon flux density (PPFD) for shade-houses and for the full sunlight was shown in Figure S1. Total photon exposure per day in screened growth house for three treatments was equivalent to 2.3%, 8.12%, and 34.3% of that in the full sunlight (FL), respectively. There are 8 shade-houses (plots) per treatment, 30 pots per plot, 4 plants per pot. Furthermore, the self-shading was precluded by a long distance among pots, and pots were rotated at a 15-day interval to correct for location effects. Healthy rhizomes of 1-year-old *P. notoginseng* were placed to a plastic pot that was placed in a controlled-light growth shade-houses. 30% FL is considered as the maximum transmittance that *P. notoginseng* can tolerate, 8% FL ~10% FL is considered as the moderate transmittance, and less than 3.0% FL would depress the growth of *P. notoginseng* (Xu et al. 2018; Wu et al. 2021).

*P. notoginseng* was used to determine leaf area and biomass, photosynthetic performance, photosynthesis-related pigments, antioxidant defense system analysis, and RNA-seq in August. The functional leaf (middle leaf of palmate compound leaves) normally has the greatest contribution to the whole plant, and therefore the fully expanded functional leaf was used for the determination of photosynthetic performance, photosynthesis-related pigments, antioxidant defense system analysis, and RNA-seq (Figure S2).

2.2. Determination of morpho-anatomical traits

For sampling, *P. notoginseng* were removed, cleaned soil and other debris, and 15 plants from each treatment were selected for measurement. Some morpho-anatomical traits (crown breadth, plant height, and steam diameter) were
2.4. Photosynthetic pigments

After photosynthetic parameters were measured, leaves were put into liquid nitrogen and stored at ~80°C. 0.5 g leaves were repeatedly extracted in the dark environment with 50 ml 80% cold acetone, and then the extract was filtered into the volumetric flask with filter paper. High-performance liquid chromatography (HPLC) according to Thayer and Björkman (1990) was used to determine the extracted pigments. Briefly, the extract was filtered by 0.45 μm filter membrane and then analyzed by HPLC (Agilent 1260, USA). The chromatographic column was Intersil ODS-3 (250 mm × 4.6 mm, 5 μm). The elution temperature was 25°C and the flow rate was 1 mL·min−1. The mobile phase A liquid was acetonitrile: Tris = 0.05 mol/L HCl buffer (70:3), and the mobile phase B liquid was methanol: n-hexane (5:1). Agilent 1260 single-wavelength detector was used for detection, and the detection wavelength was 445 nm. Standard products are purchased by Sigma Company.

2.5. Activities of antioxidant enzyme

0.5 g leaves were grounded with liquid nitrogen and crude enzyme extracts were prepared according to Chen et al (2011). The activity of catalase (CAT) was determined by referring to the method of Aebi (1984). The activity of peroxidase (POD): the assay solution was phosphate buffer (pH 7.0) at a concentration of 50, 10 mM 30% H2O2, 16 mM guaiacol and enzyme extract. The change in absorbance at 470 nm was measured at 25°C and the increase in absorbance was monitored for 5 min (Chen and Cao, 2008). The determination of superoxide dismutase (SOD) activity referred to the method of Nebot et al (1993). Since SOD is susceptible to acids, the extraction buffer pH was adjusted to 7.0. 3 ml assay solution consisted of 1.7 ml phosphate buffer, 0.3 ml methionine (MET), 0.3 ml nitroblue tetrazolium (NBT), 0.3 ml EDTA-Na2, 0.3 ml riboflavin and 0.1 ml enzyme solution. In addition, 200 μl enzymatic extract was used as the reference one. The absorbance of the reaction liquid was determined at 560 nm by an ultraviolet visible spectrophotometer.

Ascorbate peroxidase (APX) activity was measured according to the method of Mittler and Zilinskas (1991) with minor modification. For the determination of APX, the extract should contain 1 mM ascorbic acid, the assay solution should be phosphate buffer (pH 7.5) at a concentration of 50 mM. 3 ml reaction solution consisted of 50 μM enzyme solution and 2.95 ml assay solution. The change in absorbance at 290 nm was then measured at 25°C, and the decrease in absorbance was monitored for 5 min.

Glutathione reductase (GR) activity: the assay solution for the GR was phosphate buffer (pH 7.8) at a concentration of 50 and 2 mM EDTA-Na2, 0.15 mM NADPH-Na4, and 0.5 mM oxidized glutathione (GSSH). 3 ml reaction solution consisted of 50 μM enzyme solution and 2.95 ml assay solution. The change of absorbance at 340 nm was measured.
at 25°C, and the decrease in absorbance was monitored for 5 min (Chen et al. 2011).

Dehydroascorbate reductase (DHAR) activity: the increase in absorbance due to the generation of ascorbic acid at 265 nm was measured at 25°C. The reaction system contained 50 mM phosphate buffer (pH 7.0), 0.5 mM monodehydroascorbate, 5 mM reduced glutathione (GSH), and 10 μl enzyme extract. The change in absorbance at 265 nm was measured at 25°C for 3 min (Yang et al. 2007).

The activity of monodehydroascorbate reductase (MDHAR): the assay solution is 50 mM phosphate buffer (pH 7.8) containing 0.2 mM EDTA, 0.1 mM ascorbic acid, and 0.1 mM NADPH- Na4; 0.5 U of ascorbate oxidase should be prepared. 3 ml assay solution consisted of 2.75 ml assay solution, 50 μl enzyme solution, and 0.2 ml ascorbate oxidase. The absorbance at 340 nm decreased due to the oxidation of NADPH-Na4, and the decrease in absorbance was monitored for 5 min (Yang et al. 2007).

2.6. Determination of antioxidant metabolites and saponin content

Ascorbate (ASA) was determined as described by Hossain and Asada (1984). The determination of glutathione (GSH) was based on the method of Doulis et al (1997). Superoxide-free radical (O2) was determined according to the method of Misra (1974). The hydroxylamine oxidation can be used to determine O2 content. O2 reacts with hydroxylamine to produce NO2 and then NO2 reacts with p-aminobenzene sulfonic acid and α-naphthylamine to produce a pink azodye. The absorbance of the pink azodye was measured at a wavelength of 530 nm, and O2 content can be calculated from the absorbance achieved at a wavelength of 530 nm.

The saponin standards were dissolved in methanol and diluted to 10 ml. The solutions were mixed well to produce standard solutions at concentrations of 0.454, 0.355, 0.462, 0.466 and 0.449 mg/ml. Referring to the Chinese Pharmacopoeia for the determination of saponin content, 0.60 g dried P. notoginseng powder was weighed precisely and dissolved in 30 ml methanol for 30 min. The solution was extracted by ultrasonication for 1 h and diluted to 50 ml with methanol, accompanied by 30 min resting time. Finally, the solution was filtered. The saponin content of the samples was determined using an Agilent Zorbax SB-C18 (250 mm × 4.6, 5 mm) column on an Agilent HPLC (Agilent 1260, USA) with mobile phase (A) – water (B) and gradient elution. In addition, external standard method was used to determine the contents of notoginsenoside R1, ginsenoside Rd, Rb1, Rg1 and Re in the samples.

2.7. RNA extraction and library construction, sequencing

The extraction of RNA from leaves was conducted with RNA pre-pure Plant kit (BioTeke, Beijing, China). Prior to constrain cDNA library, the integrity and purity of RNA from each preparation were confirmed using agarose gel electrophoresis and a G:BOX F3 Gel Documentation System (Gene company, Hong Kong, China), the concentration of RNA was assessed in favour of Nano Drop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Inc.). The construction of the cDNA library was done by Gene Denovo Biotechnology Co., Guangzhou, China. dNTPs and DNA polymerase I and RNase H were used to synthesize double-stranded cDNAs, which were then purified with AMPure XP beads. The purified double-stranded cDNA was first repaired at the ends, then the A-tail was added and sequencing joints were ligated, and AMPure XP beads were used for fragment size selection, with only 150–200 bp fragments selected. Finally, PCR amplification was performed and PCR products were purified with AMPure XP beads to obtain the final library (Grabherr et al. 2011). After the libraries were constructed, the libraries were initially quantified using Qubit 2.0, and the libraries were diluted to 1.5 ng/μl.

2.8. Biological information analysis

The raw data from high-throughput sequencing is filtered to obtain high-quality data. The valid data of all samples were combined and assembled, and Software Trinity was used to obtain unigene. The unigene is compared to the public database, and the BLAST (basic local alignment search tool) algorithm is used to functionally annotate the similarity of sequences. Similar sequences between two sequences are quickly found and regions are scored to determine the level of similarity. This study takes annotations with similarity ≥ 30% and e-value ≤ 1e-5, and merges all annotation details simultaneously. Gene expression differences between samples were calculated according to the FPKM method, and genes with a fold change ≥ 2 and a P-value ≤ 0.05 were defined as DEGs (differentially expressed genes). DEGs were then subjected to enrichment analysis of GO functions and KEGG pathways.

2.9. Real-time quantitative PCR (RT-qPCR) analysis

A set of DEGs should be randomly selected and the expression pattern of DEGs should be further confirmed by RT-qPCR. Primers for RT-qPCR were designed using Premier 3.0, and the primers are presented in Table S1. RT-qPCR was performed on a Quansstudio™5 Real-Time PCR instrument with three biological replicates. The RT-qPCR was conducted with three biological replicates using the Quansstudio™5 Real-Time PCR Instruments (Thermo Fisher Scientific). 20 μl Amplification reaction mixture contained 10 μl Eva Green 2X qPCR Master Mix, 1 μl cDNA template, 0.7 μl of each gene-specific primer and 7.6 μl ddH2O. PCR program was listed: 95°C for 4 min, 40 cycles of 30 s at 95°C, 50°C for 30 s and 72°C for 30 s. Actin was selected as an internal reference. Gene expression was determined by the cycle threshold (CT) obtained from the 2^-ΔΔCt calculation (Livak and Schmittgen, 2001).

2.10. Statistical analyses

Statistical analysis was conducted using the SPSS (Chicago, IL, USA) and Sigma Plot 14.0. Data are presented as the mean of 3–15 independent samples (bioinformatics analysis: 3 replicates; photosynthetic physiology: 7 replicates; biochemical analysis: 7 replicates; morpho-anatomical traits: 15 replicates). Significance between treatments was determined using One-way analysis. Pearson correlation was performed among morphological anatomy, photosynthetic physiology, biochemical indicators, and quality traits. In addition, a principal components analysis (PCA) of related traits was conducted (Valladare et al. 2000).
3. Results

3.1. Response of photosynthetic capacity to light levels

Leaf anatomical structure showed significant difference under different light intensities (Figure 1 and Table 1). The thickness of leaf anatomy reached the maximum under 34% FL condition. In addition, SLA decreased with increasing levels of growth irradiance (Table 1).

Responses of Anet to incident PPFD and internal leaf Ci were significantly different under different light regimes (Figure 2). Photosynthetic-related parameters calculated from corresponding curves differed significantly among three light levels (Table 2). The maximum values of $A_{\text{max}}$, $F_{\text{v}}$, and $V_{\text{cmax}}$ were recorded in plants grown under moderate light (8.12%FL), and $R_{\text{d}}$ in low-light-grown plants was highest (Table 2). $F_{\text{v}}/F_{\text{m}}$, $\Phi_{\text{PSII}}$, and qP decreased with the increase of PPFD (Figure 3a–c). The maximum values of $F_{\text{v}}'/F_{\text{m}}'$, $\Phi_{\text{PSII}}'$, and qP were always shown in moderate-light-grown plants, and the minimum $F_{\text{v}}/F_{\text{m}}$, $\Phi_{\text{PSII}}$, and qP were observed in low-light-cultivated plants (Figure 3a–c). NPQ increased with increasing PPFD (Figure 3d). High-light-grown plants showed the highest NPQ. $\Phi_{\text{NPQ}}$, elevated and $\Phi_{\text{PSII}}$, decreased with the elevation of PPFD (Figure 4). At each PPFD level, high-light-grown plants presented a higher value of $\Phi_{\text{NPQ}}$, and a lower value of $\Phi_{\text{PSII}}$. (Figure 4).

3.2. The xanthophyll cycle, antioxidant defense system, and the saponins content

The xanthophyll cycle pigment pool ($V + A + Z$) was larger in low-light-grown plants, whereas the ratio of ($A + Z$) to ($V + A + Z$) was higher in high-light-grown plants (Table 3). Chl content was reduced in high light conditions (Table 3). Lutein (L), neoxanthin (N), and $\beta$-Carotene ($\beta$-Car) contents were significantly higher in moderate-light-grown plants (Table 3). SOD, CAT, and POD activities were higher in high-light-cultivated plants (Table 4). Low light decreased the activity of APX and MDHAR. Plants grown in high light conditions possessed higher activity of DHAR and GR, accompanied with high concentrations of ascorbic acid and glutathione (Table 4). In addition, peak saponin content was achieved at 8.12% FL condition (Table 5).

3.3. Plasticity index

The plasticity index was used to respond to the responsive-ness of leaf traits to light. From the median of plasticity indices for morpho-anatomical traits, physiological traits, biochemical traits, biomass allocation, and qualitative traits, it is clear that the highest plasticity index was for qualitative traits and the lowest was for physiological traits. Among the morpho-anatomical traits, plant height, branching number, and spongy tissue had high plasticity indices; the plasticity indices of SOD, APX, CAT, and SOD/(APX + CAT) were elevated among the biochemical traits; the plasticity indices of root biomass showed the best; in the qualitative traits, the plasticity indices regarding saponin biosynthesis were all over 0.5 (Figure 5a).

Pearson’s correlation among morpho-anatomical traits, physiological traits, biochemical traits, biomass allocation, and qualitative traits was presented. LMA was positively correlated with leaf anatomy, a significant positive association between SOD activity and the sum of APX and CAT was recorded in the present study. The content of saponin was positively correlated with the biomass allocation of roots, and they were correlated with the maximum photosynthetic rate under various light levels (Figure 5b).

Principal component analysis of 19 parameters of P. notoginseng under different light intensities showed that the cumulative contribution of PC1 and PC2 reached 70.61% and the eigenvalues were greater than 1. Therefore these two principal components could effectively explain the adaptation of P. notoginseng. Light regimes were separated along the first axis of the PCA (Figure 5c). In PC1, morpho-anatomical traits, biochemical traits, biomass allocation, and qualitative traits had larger weighting coefficients. The loading coefficients showed that plant height, leaf thickness, spongy tissue, APX, and total saponins had positive correlation with PC1 and contributed more to PC1.

3.4. Quality assessment of transcriptome sequencing

A total of 85.71 M reads with a total base number of 17.31 G were obtained through sequencing by transcriptome (Table 6).
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Compared to the 8.12% FL-grown plants, 2864 and 2385 genes were classed as DEGs in the 2.3%FL and 34.3% FL groups, respectively (Figure 6 and Table S6). Whereas, there were 1668 DEGs in both the 2.3%FL groups and 34.3%FL groups (Figure S3 and Table S5).

### 3.5. Differentially expressed genes (DEGs) identification

To gain insight into the genes related to photosynthesis and photoprotection, the expression profiles were analyzed in *Panax notoginseng* grown under different levels of light intensity. Compared to the 8.12% FL-grown plants, 2864 and 2385 genes were classified as DEGs in the 2.3%FL, 34.3%FL groups, respectively (Figure 6 and Table S6). Whereas, there were 1668 DEGs in both the 2.3%FL groups and 4.3%FL groups (Figure 6 and Table S6).

### 3.6. GO enrichment of DEGs

All DEGs were divided into eight categories, profile 5 and profile 6 were significant (Figure 7a). The two gene sets with significant differences were combined, and GO enrichment was performed (Figure 7c). Under the classification of cellular components, the ‘cell part’ and the ‘cell’ represented the dominant groups, accounting for 98.22% and 98.22%, respectively. In the molecular functions, ‘binding’ and ‘catalytic activity’ predominated, accounting for 78.18% and 68.48%, respectively. In the classification of biological process, ‘cellular process’, ‘metabolic process’, ‘single organism process’, ‘biological regulation’, and ‘response to stimulus’ were largely represented, accounting for 90.99%, 83.92%, 74.56%, and 68.89%, respectively (Figure 7c).

### 3.7. KEGG enrichment of DEGs

The top 13 KEGG pathways involved in photosynthesis were identified (Table 6). DEGs primarily related to starch and sucrose metabolism, carbon metabolism, and glycolysis/glucanoneogenesis accounted for 6.01%, 6.01%, and 5.46%, respectively; followed by photosynthesis (4.1%), ascorbate and aldolase metabolism (3.28%), nitrogen metabolism (3.01%), porphyrin and chlorophyll metabolism (2.73%) and glutathione metabolism (3.01%). The pathways with the least enrichment of DEGs were photosynthesis antenna proteins (2.19%), carotenoid biosynthesis (1.37%), pentose phosphate pathway (1.64%), carbon fixation in photosynthesis (1.09%), and oxidative phosphorylation (1.09%).

### 3.8. Genes expression involved in photosynthesis

To investigate how light levels affect photosynthetic capacity, we examined the effects of three light levels on the expression of genes participated in photosynthesis. The RT-qPCR displayed a similar expression trend with the bioinformatics analysis based on the transcriptome in *P. notoginseng* grown under different levels of light intensity (Figures 8–11). Genes (RPI2, RPI3, and RPI4) encoding ribose-5-phosphate isomerase were up-regulated at 2.3%FL condition, and they were downregulated at 34.3% FL condition (Figures 8 and 11). Furthermore, light levels significantly affected the expression of genes related to structural proteins of the photosystem (Figure 9). In high-light-grown groups, the...
expression of genes related to structural proteins of the photosystem parallel with the moderate-light-grown groups (less than twofold change; $P > 0.05$), except for $PsbS$. The unigene involved in $PsbS$ was downregulated in high-light-grown plants (Figure 9). In contrast, the expression of genes related to structural proteins of photosystems ($PsbP$, $PsbQ$, $PsbY$ and $PsaE$) was decreased in low-light-grown plants.

3.9. Light-induced genes related to photoprotection

$ZEP$ was upregulated by high light (Figure 11). Glutathione S-transferases (GSTs) exist in the cytoplasm in a soluble form and are involved in the antioxidant defense system (Edwards et al. 2000). High light positively enhanced the expression of GSTs in $P. notoginseng$ (Figure 10). Genes of Chl synthesis were downregulated in high-light-grown plants (Figure 10). Furthermore, the expression of genes ($GPX3$, $GST3$ and $GSTU10$) involved in reactive oxygen scavenging was up-regulated in high-light conditions (Figures 10 and 11). Genes involved in the glycolytic pathway were present in a higher expression in leaves of high-light-grown plants (Figure 10). Genes involved in pentose phosphate pathway PPP were upregulated in the high-light-grown plants (Figure 10).

4. Discussion
4.1. Phenotypic plasticity under different light regimes

Phenotypic plasticity has long been shown to be a major strategy for plant adaptation to rapid environmental variation (DeWitt and Scheiner, 2004; Arnold et al. 2019). Plants grown in high-light conditions produced significantly thicker leaves (increase by 23%) compared to those in moderate shade conditions, attributed to a 31.49% increase in palisade tissue and a 22.08% elevation in spongy tissue (Table 1). Leaf mass per unit leaf area ($LMA$) and leaf irradiance showed a positive correlation, plants grown in the shaded environment may enhance light capture by forming relatively small LMA (Markesteijn et al. 2007; Poorter, 2009). In the present study, LMA was positively correlated with leaf anatomy, which is similar to the results of Osnas et al (2013), indicating that LMA contributes to the variation of leaf thickness and leaf tissue density in response to light regimes. The difference in LMA may be due to the increased thickness of spongy tissue in plants grown under high-light environment (Kong et al. 2016; Baird et al. 2017; Coble and Cavalieri, 2017). Thus the thick sponge tissue in high light enhances the backscattering of diffuse light within the leaf, minimizing the damage to the leaf from high irradiance.
(Figure 1; Markesteijn et al. 2007), while previous and present studies have suggested that SLA functions in the adaptation to various light intensities, low-light-cultivated plants can increase light capture capacity by elevating SLA (Table 1; Lee et al. 2000; Hanba et al. 2002; Oguchi et al. 2005; Markesteijn et al. 2007). Moreover, leaf physiological traits showed low plasticity, including $A_{\text{max}}$ and PNUE (Figure 5a), and the low physiological plasticity is associated with the ability to utilize high light (Delagrange et al. 2004), indicating that *P. notoginseng* is sensitive to high light. In addition, *P. notoginseng* had the elevating plasticity of morpho-anatomical traits and the morphological plasticity to light functions in the resource acquisition (Figure 5a; Magyar et al. 2007).

Since light is highly heterogeneous as it fluctuates on short (seconds) and long (hours, days, seasons) time scales, and for plants, light is of pivotal importance for growth and development (Townsend et al. 2018), the plasticity of the antioxidant system is essential to balance intracellular redox homeostasis (Miller et al. 2008). It has been widely proved that SOD activity could elevate the tolerance of plants to stress (Zandalinas et al. 2017). The experimental results presented in transgenic *A. thaliana* confirmed the positive consequences of acceleration in SOD activity to oxidative stress tolerance.
have revealed that oxidative stress induced by changes in the light environment encompasses a trade-off between antioxidant enzymes. Moreover, it was found that the plasticity response of SOD, APX, and CAT changed with the increase of light intensity (Figure 5a). The plasticity of APX is stronger than that of CAT and SOD, suggesting that APX is more sensitive to the changes of the light environment. This result is consistent with the plasticity of the enzymatic antioxidants of Iris *pumila* grown under different light conditions studied by Vuleta et al. (2016). The balance between superoxide-free and antioxidant enzymes functions in attenuating the deleterious effects of oxidative stress (Omar et al. 2012; Naser et al. 2016; Kim et al. 2017). The present study estimated the plasticity of SOD to the sum of APX and CAT, the high plasticity of SOD (APX+ CAT) indicated that the equilibrium among SOD, APX, and CAT activities is a reliable indicator of the adaptation of shade-tolerant plant to changes in the light environment (Figure 5a; Lee et al. 2007).

Secondary metabolites are direct markers of biochemical activity and therefore more likely to reflect the plasticity of the plant to the dynamic environment (Rathi et al. 2019). The root of *P. notoginseng* has the effects of hemostasis, blood-breaking, dispelling blood stasis, anti-inflammatory, and pain-relieving. It is the main medicine for the treatment of fall and injury, all of which is attributed to the root saponins (Leung and Wong, 2010). Our results demonstrated that non-optimal light regimes had a debilitating effect on the concentration and yield of saponins (Table 5). Similarly, previous study showed that the peak of anthocyanin accumulation occurred at moderate light intensity (Narayan et al. 2005; Chan et al. 2010). Together, these data indicate that the accumulation of secondary metabolites can be positively improved by regulating the light intensity of the plant growth environment. It is worth noting that the content of saponin was positively correlated with the biomass allocation of roots, and they were correlated with the maximum photosynthetic rate under various light levels (Figure 5b), indicating that optimal shade treatment facilitated photosynthesis and increased biomass allocation of roots accompanied by elevated saponin concentration. This result is similar to the previous finding that proper shade accelerated carbon assimilation and secondary metabolite production in *Fritillaria cirrhosa* (Li et al. 2009). Thus beneficial light control could ameliorate the yields of saponins in *P. notoginseng*, and saponins possess strong plasticity to acclimate to rapid light variation (Figure 5a).

### 4.2. Photosynthetic pigments and antioxidants acting as photoprotective mechanisms under high light

The degradation of leaf Chl is a photoprotective mechanism in response to abiotic stress (Demmig-Adams, 1990; Hubbard et al. 2012). Chl was significantly decreased in high-light-grown plants (Table 3). Actually, excess Chl molecules would be detached from the photosynthetic reaction center during leaf development, and subsequently, Chl is degraded to substances that are not phototoxic, therefore, Chl...
Figure 5. Index of phenotypic plasticity for morphological, anatomical, biochemical, and physiological traits of *Panax notoginseng* (a). Pearson’s correlation among morpho-anatomical traits, physiological traits, biochemical traits, biomass allocation, and qualitative traits. *P* < 0.05 (b). Principal component analysis of 19 parameters of *P. notoginseng* under different light intensities. The colors of the points in the figure represent different light transmittances; the ellipse is the confidence interval of each parameter under different transmittance; the arrow represents the relationship between each index and the principal component (c).

Figure 6. Venn diagrams of differentially expressed genes (DEGs) in response to varied light levels. T1, T2, T3 are the irradiance of 34.3%, 8.12%, and 2.3% of full sunlight respectively. We identified genes with a fold change ≥2 and a false discovery rate (FDR) <0.05 in comparison as significant DEGs.
degradation becomes an important step to prevent photodamage (Hidema et al. 1992). It is noteworthy that the expression of Chl synthesis-related genes was downregulated in high-light-grown plants (Figure 10), and low Chl content is associated with the down regulation of genes related to Chl synthesis. Similarly, in a shade-tolerant plant *Norway spruce* grown under high light environment, the expression of genes related to Chl synthesis was also declined, accompanying with the obvious decline in Chl content (Ranade et al. 2019). *Cerasus pseudocerasus* was grown under high light conditions, the plants showed low content of Chl and correspondingly a reduction in expression of genes involved Chl biosynthesis as well as an enhanced activity of thermal energy dissipation (Tian et al. 2020). Hence, the down-regulated expression of Chl biosynthesis-related genes induces the decline in Chl content, showing the process of photoprotection in high-light-grown plants.

The dissipation of excess light as heat is related to the appearance of ΔpH across thylakoid lumen and to the activity of the xanthophyll cycle (Ma and Cheng, 2003). The PsbS protein and violaxanthin de-epoxidase play an important role in energy dissipation (Hubbart et al. 2012). PsbS protein has an important role in energy dissipation, owing to the low levels of quenching presenting in *A. thaliana* npq4 mutants lacking this protein (Li et al. 2002). In this study, the xanthophyll cycle pigment pool (V + A + Z) was larger in low-light-grown plants, whereas the ratio of (A + Z) to (V + A + Z) was higher in high-

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**Figure 7.** Dynamic transcriptome of *Panax notoginseng* under different light levels. T1, T2, T3 are the irradiance of 34.3%, 8.12%, and 2.3% of full sunlight respectively. (a) Cluster of gene expression patterns in response to different light levels. Short time-series expression miner (STEM) was used to analyze the gene expression pattern, and eight profiles exhibited significant clustering of gene expression patterns. The number of unigenes in each profile was labeled above the frame. The black line represents the general tendency in each profile. Expression patterns of 8 profiles at different light levels. (b) Enrichment of functional categories of each cluster with the significantly enriched KEGG pathways plotted for DEGs among the three levels of light. (c) GO functional annotation of DEGs under different light levels.

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**Table 6.** KEGG enrichment analysis of the first 13 pathways related to the mechanism about light protection and is widely annotated.

| Pathways                                      | DEGs genes with pathway annotation | Pathway ID |
|-----------------------------------------------|-----------------------------------|------------|
| Starch and sucrose metabolism                 | 22 (6.01%)                        | ko00460    |
| Carbon metabolism                             | 22 (6.01%)                        | ko01200    |
| Glycolysis/ Gluconeogenesis                    | 20 (5.46%)                        | ko00010    |
| Photosynthesis                                | 15 (4.1%)                         | ko00195    |
| Ascorbate and aldurate metabolism             | 12 (3.28%)                        | ko00053    |
| Nitrogen metabolism                           | 11 (3.01%)                        | ko00910    |
| Porphyrin and chlorophyll metabolism          | 10 (2.73%)                        | ko00860    |
| Glutathione metabolism                        | 11 (3.01%)                        | ko00480    |
| Photosynthesis- antenna proteins              | 8 (2.19%)                         | ko00196    |
| Carotenoid biosynthesis                       | 5 (1.37%)                         | ko00906    |
| Pentose phosphate pathway                     | 6 (1.64%)                         | ko00030    |
| Carbon fixation in photosynthetically         | 4 (1.09%)                         | ko00710    |
| Oxidative phosphorylation                     | 4 (1.09%)                         | ko00190    |
| Porphyrin and chlorophyll metabolism          | 10 (2.73%)                        | ko00860    |
light-grown plants (Table 3). Chl content was reduced in high-light conditions (Table 3). Sampling in our study was conducted at midday on a clear day, higher photoxicative stress occurs in high-light-grown plants, where the high de-epoxidation state of xanthophylls favors excessive dissipation of light energy. The expression of genes encoding ZEP was up-regulated in the high-light-grown plants, together with the up-regulation of PsbS genes (Figures 9 and 10). High activities of some antioxidants were shown in high-light-grown plants (Table 4), and

Figure 8. Calvin cycle pathways of *Panax notoginseng* and hierarchical cluster analysis of genes that were differentially expressed under different light levels. T1, T2, T3 are the irradiance of 34.3%, 8.12%, and 2.3% of full sunlight respectively. Firebrick indicates that the gene has a high expression in the light level; navy indicates that the gene has a lower expression in the light level.
Correspondingly, the expression of genes (GPX3, GST3, and GSTU10) involved in reactive oxygen scavenging was up-regulated in *P. notoginseng* grown under high light (Figure 10). Nevertheless, the ROS removal efficiency of plants grown under high light is low, mainly because of the high superoxide anion content (Table 4). Thus excess light...
Figure 10. Expression profiles of differentially expressed genes (DEGs) that regulate photosynthesis and photoprotection under different light levels. T1, T2, T3 are the irradiance of 34.3%, 8.12%, and 2.3% of full sunlight respectively.
Figure 11. Expression patterns and amplification plots of the genes of *Panax notoginseng* grown under light regimes. T1, T2, T3 are the irradiance of 34.3%, 8.12%, and 2.3% of full sunlight respectively. Actin was used as the reference one. **GST3**: Encoding glutathione transferase. **PsbP**: Encoding a 23 kD extrinsic protein that is part of photosystem II and participates in the regulation of oxygen evolution. **RPI4**: Ribose-5-phosphate isomerase 3. **PsaE**: Responsible for the final assembly of galactolipids in photosynthetic membranes. Provides stability to the PS I core complex. **RPI2**: Ribose-5-phosphate isomerase.
light energy or more electrons are dissipated or consumed by xanthophylls cycle, antioxidant pathway in the high-light-grown plants along with the low efficiency of PSII photochemistry.

4.3. The photosynthesis under non-optimal light regimes

Leaf N is an essential component of Rubisco (Li et al. 2013), photosynthesis was inhibited in low-light-grown plants possibly as a result of low Rubisco catalytic capacity and low CE. In the present study, CE was decreased in low-light-grown plants, accompanying with up-regulated expression of genes (RPI2, RPI4, and RPI4) encoding Rubisco (Figures 8 and 11). V_{cmax} is positively correlated with the potential carboxylation capacity of Rubisco and the regeneration rate of ribulose-bisphosphate (RuBP) (Farquhar et al. 1980; Elsheery et al. 2020). In this study, V_{cmax} was lowest in the low-light-grown plants (Table 2), suggesting that Rubisco activity might be considerably depressed. It was in line with previous findings that low light led to the decrease of photosynthetic capacity and Rubisco catalytic capacity in Manihot esculenta (De Souza et al. 2019). Rubisco has only 25% potential catalytic capacity in C3 plants, and a large proportion of Rubisco serves as N storage and exists in an inactivated form (Cheng and Fuchigami, 2000; Warren et al. 2000). Under low light conditions, most Rubisco is shown as N storage proteins (Sage et al. 2002), and a high percentage of inactivated Rubisco is found especially in low light plants.

Photosynthetic assimilation decreased considerably in the low-light-grown and high-light-grown plants (Figures 2 and 3), and it is thought to arise from the interrupted synthesis of photosynthetic proteins. In our studies, light levels significantly affected the expression of genes encoding structural proteins of the photosystem (Figure 9). In the high-light-grown groups, the transcriptional level of structural proteins of the photosystem parallel with the moderate-light-grown groups, but photosynthetic capacity still declined in high-light-grown groups (Figure 2), potentially as a result of a decline in regimes of F-type ATPase subunits (Zhang et al. 2018). In contrast, the transcriptional level of PsbP, PsbQ, PsbY, and PsaE, was decreased in the low-light-grown plants. There was a positive relationship between the transcriptional level of structural proteins of photosystems and photosynthetic capacity (Bonardi et al. 2005; Song et al. 2017). Thus low light depresses the expression of genes involved in photosynthesis, consequently constraining photosynthetic performance.

4.4. The pentose phosphate pathway and glycolysis contribute to photoprotection

Any potential decreases in NADPH would have an impact on the energy and carbon balance (Flamholz et al. 2013). Interestingly, glycolytic pathway and pentose phosphate pathway (PPP) functions in providing energy for various metabolic activities of cells, and in solving the problem of energy and carbon imbalance imposed by abiotic stresses (Scheible et al. 2004; Christodoulou et al. 2018). The expression of different glycolytic transcripts was upregulated to maintain homeostasis in Bigeloviwella natans (Rangsrikitphotithi and Durnford, 2019) and in A. thaliana (Dyson et al. 2015) under high light conditions. The genes involved in the glycolytic pathway were present in a high expression when plants were grown under high light conditions (Figure 10). The results indicated that the active glycolytic pathway might compensate for NADPH to enhance ΔpH and then reinforce xanthophyll cycle. Besides glycolysis, PPP is also an effective pathway for glucose oxidation in plant cells (Yang et al. 2018), NADPH produced in this pathway is involved in numerous biosynthetic reactions. In the current study, high-light-grown plants induced the up-regulation of genes involved in PPP (Figure 10). Enzymes involved in PPP may provide vital metabolic flexibility to promote plant development and adaptation to environmental stresses (Gary, 2018). Thus the active glycolytic pathway and PPP could compensate for the shortage of ATP and NADPH to facilitate the formation of ΔpH across thylakoid lumen, and then drive photo-protection.

5. Conclusion

The non-optimal light regimes significantly depress photosynthetic performance in a typically shade-tolerant plant, as in P. notoginseng. Low light induces high expression of genes encoding Rubisco and low carboxylation activity of Rubisco, leading to a reduction in electron transport and carbon assimilation, consequently suppressing photosynthetic capacity. The expression of genes (PsbP, PsbQ, PsbY, and PsaE) involved in the light reaction is repressed in low-light-grown plants. On the other hand, excess light energy or more electrons are dissipated or consumed by xanthophylls cycle, antioxidant pathway, and chlorophyll degradation in the high-light-grown plants along with the low efficiency of PSI photochemistry. Meanwhile, high-light-grown plants protect photosynthetic apparatus against photodamage by thick leaf anatomic structure and active NPQ. Moreover, high light also inhibits the expression of genes (RPI2, RPI3, RPI4) involved in the Calvin cycle. The optimal light level represents an effective strategy for preserving the integrity and the activity of the photosynthetic apparatus. Anyway, photosynthetic capacity and photosynthesis-related genes expression are generally coordinated in a shade-tolerant species grown under different light intensity as suggested by our study.

Author contributions

The data of photosynthetic physiology were analyzed by Jin-Yan Zhang. The manuscript was written by Jin-Yan Zhang. Jun-Wen Chen conceived the experiment and article. Shuang-Bian Kuang and Zhu Cun performed the leaf anatomy and photosynthetic physiology. Sheng-Pu Shuang, Long-Gen Li, and Zhen-Gui Meng participated in the construction of plant growth shade-house and pot experiments.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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