

Transcriptome reveals effects of low red/far-red ratio and shade on soybean seedlings

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ABSTRACT

Light intensity and spectral composition regulate plant photomorphogenesis, with far-red light (FR) playing a vital role in shade avoidance. Soybean (*Glycine max* (L.) Merr.), a globally significant crop, is vulnerable to canopy shade, which decreases the red/far-red (R/FR) ratio and causes adaptive responses. We explored how FR supplementation modifies soybean seedling responses under shade stress using morphological, physiological, and transcriptome analysis. Four light treatments were applied: Normal light (NL, R = 125.62 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, FR = 91.68 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), normal light + FR (NF, R = 124.57 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, FR = 161.77 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), shade (SL, R = 4.62 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, FR = 4.58 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and shade + FR (SF, R = 4.58 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, FR = 16.35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Key characteristics assessed included plant height, stem thickness, chlorophyll concentration (SPAD), soluble sugars, antioxidant enzymes (superoxide dismutase SOD, peroxidase POD, catalase CAT), and transcriptome profiles at 7 and 15 d post-treatment. Results demonstrated that SF caused the most significant shade avoidance phenotype, with the highest plant height (22.7 cm), hypocotyl elongation (7.75 cm), and stem thinning (1.46 mm), associated with reduced chlorophyll concentration (SPAD: 25.3). The NF enhanced soluble sugar accumulation (2.66 g) and antioxidant enzyme activity (SOD: +38%, CAT: +29%), whereas SL/SF decreased these features compared to NL treatment group. Transcriptomic study showed 15 762 differentially expressed genes (DEGs) under SF, largely enriched in photosynthesis-antenna proteins, porphyrin-chlorophyll metabolism, and phytohormone signaling (abscisic acid, ethylene). These data indicate that FR possibly amplifies shade avoidance by coordinating photoreceptor signaling, hormonal dynamics, and photosynthetic repression. Shade avoidance is often characterized by stem and hypocotyl elongation, leaf expansion, and reduced chlorophyll content. These molecular insights are crucial for developing shade-resilient soybean cultivars.

Key words: Far-red light, *Glycine max*, photosynthesis, shade avoidance, soybean, transcriptome sequencing.

INTRODUCTION

Light is fundamental for plant growth, influencing not only the efficiency of photosynthesis but also plant development, stress resilience, and physiological responses (Ballaré and Pierik, 2017). Plants detect light at various wavelengths using photoreceptor systems, including photopigments, phytochromes, and cryptochromes. These systems govern plant physiological activities and developmental pathways (Legris et al.,

2016). Far-red light (FR, 700-800 nm) plays a distinct role in photomorphogenesis and plant responses to stress. It governs growth attributes like leaf shape, stem elongation, and root development by activating photosensitive pigments such as phytochrome A and phytochrome B. It may also influence plant stress tolerance and metabolic pathways.

Soybean (*Glycine max* (L.) Merr.), a significant global food and commercial crop, exhibits heightened sensitivity to variations in light conditions. Under shaded and spectrally variable conditions, the physiological activities and molecular responses of soybeans exhibit intricate regulation mechanisms. Research indicates that shade impedes photosynthesis in soybeans, adversely impacting their development and output (Hussain et al., 2019). Soybean, as a typical short-day plant, frequently encounters restricted photosynthesis and adverse growth and development in shaded environments. There is growing interest in the function of FR in shaded situations, since it not only influences plant photomorphogenesis but may also alleviate the negative impacts of shading by modulating the expression of photosynthesis-related genes (Tan et al., 2021). This is particularly significant for soybean research, as FR may facilitate plant adaptation to low-light environments by activating a network of endogenous genes that enhance photosynthetic efficiency and increase stress tolerance (Wang et al., 2023).

Light conditions for plants in natural habitats are typically intricate and fluctuating, with shade representing a common light environment. Shade impacts the quality and quantity of light accessible to plants, thereby affecting their growth and physiological responses (Pierik and de Wit, 2014). In agricultural production, shade is frequently generated by light-obstructing structures such as shade nets and greenhouse facilities. In natural habitats, plants often encounter shading caused by high vegetation density. Recent advancements in modern agricultural production methods have emphasized the manipulation of light conditions, particularly with the adaptive growth of plants in shaded environments. Shading often reduces light intensity and alters spectral composition, particularly by decreasing the red/far-red (R/FR) ratio, thereby affecting plant physiology and morphology. In soybeans, low R/FR ratios induce shade avoidance syndrome (SAS), marked by stem elongation, petiole lengthening, and disproportionate biomass allocation, ultimately leading to lodging and reduced yield (Lyu et al., 2020). In agroecosystems, darkness impedes plant growth and productivity by reducing photosynthetic efficiency through alterations in the production of photosynthetic pigments and the modulation of photosynthetic responses. A recent study has highlighted the significant potential of supplemental FR for commercial and agricultural applications. In soybean, a short-day crop, photoperiod modification using LEDs with spectral combinations abundant in blue and FR enhances breeding cycles, achieving maturity in 77 d and allowing for up to five generations per year (Jähne et al., 2020). In cucumber (*Cucumis sativus* L.) seedlings, the addition of FR (R/FR = 1.2) markedly enhanced fresh weight by 60.04% and plant height by 200.63%, while optimizing stomatal development and activating the Rubisco enzyme, highlighting its significance for commercial nursery production (Li et al., 2024a). Despite these advancements, the regulatory role of FR, particularly its adaptive mechanisms in shaded conditions, remains insufficiently understood, especially regarding the growth regulation of economically important crops like soybean in complex light environments (Wang et al., 2024).

The rapid progress of transcriptomics technology has provided us with powerful tools to analyze the molecular response of plants under different light conditions. The high-throughput RNA sequencing technique has facilitated the precise analysis of gene expression in plants. This has demonstrated the modification of plant transcriptomes under varying light conditions (Huang et al., 2019). Researchers have discovered that plants exposed to inadequate light modify the expression of genes associated with photosynthesis, pigment production for photosynthesis, antioxidant enzyme systems, plant growth and development, and the interactions between FR and shade (Meng et al., 2024). For example, the regulation of the phytochrome B gene allows FR to affect stomatal aperture, chlorophyll production, and overall plant development. Increased FR may assist plants in adapting to shade by modulating the expression of genes associated with photosynthesis and photoreceptive pigments (Liu et al., 2021).

Transcriptome analysis has comprehensively examined the metabolic responses of soybeans under many light circumstances (Yang et al., 2018a). Ye et al. (2023) assert that the transcriptomic response of soybean to diverse light and shadow conditions illustrates the complexity of their light-adaptive mechanisms. Far-red light therapy enhances soybeans' resilience to abiotic conditions such as drought and elevated temperatures by activating genes linked to photosynthesis and antioxidant pathways (Cao et al., 2018; Zhou et al., 2023).

Furthermore, FR has been demonstrated to enhance soybean development in low-light environments by augmenting the activities of antioxidant enzymes and reducing the photosynthetic pigments in shaded foliage (Wang et al., 2020).

While morphological shade responses in soybean have been shown, the transcriptome mechanisms governing the synergistic impacts of diminished irradiance and lowered R/FR ratio are inadequately understood. Prior research has predominantly concentrated on individual factors—such as low light or FR supplementation—rather than their interactions, and has mainly highlighted morphological characteristics over transcriptome regulation. Several transcriptome investigations indicated more extensive responses, such as contextualized shadow responses in soybean intercropping systems and light-quality-mediated hormonal modulation via GmCRY1s (Lyu et al., 2020). A comprehensive framework that integrates morphological, physiological, and transcriptome characteristics across varying light quality and quantity is yet to be established.

We propose that diminished R/FR signaling, under both normal light supplemented with FR (NF) and shade supplemented with FR (SF) conditions, alters soybean transcriptomes by influencing phytochrome-mediated signaling and hormonal pathways, resulting in shade-avoidance characteristics and simultaneous modifications in chlorophyll biosynthesis and antioxidant metabolism. This study aimed to examine how soybean seedlings respond to controlled combinations of irradiance level and FR supplementation. Specifically, four laboratory light regimes were established: Normal light (NL), NL supplemented with FR (NF), reduced irradiance (SL), and SL supplemented with FR (SF). These treatments were designed to compare the independent and combined effects of light quantity and FR enrichment under controlled conditions, rather than to fully reproduce the full spectral complexity of natural canopy light environments.

This study aimed to reveal the effects of FR and shade interaction on soybean seedlings' growth and transcriptome response. We are going to look into how FR affects soybean seedlings that are in the shade using high-throughput RNA sequencing technology along with physiological analyses. In particular, we will look at how FR and shade affect physiological indicators in soybeans, such as photosynthesis, chlorophyll concentration, and antioxidant enzyme activities. We will subsequently integrate these with transcriptome data to ascertain how alterations in various environmental circumstances influence soybean gene expression. We propose that the diminished R/FR ratio influences the morphology, metabolic characteristics, and gene expression of soybean seedlings experiencing shade stress by modulating their photosynthetic processes and antioxidant systems. This work will elucidate critical molecular mechanisms underlying light adaptation in soybeans within complicated light settings and give a theoretical foundation for light regulation technologies in agricultural production.

MATERIALS AND METHODS

Plant material and experimental design

The experiment employed the soybean (*Glycine max* (L.) Merr.) 'Heinong 48', the primary cultivar in Heilongjiang Province, distinguished by its significant resistance to lodging. This cultivar, noted for its disease resistance, high yield, and protein concentration, was provided by the Soybean Research Institute of the Heilongjiang Provincial Academy of Agricultural Sciences. The preliminary phase of the experiment was carried out in an artificial climate incubator (25 ± 2 °C, 60% RH). Soybean seedlings were grown in pots under controlled indoor settings. Round plastic pots measuring 14 cm × 13.5 cm × 9.5 cm were utilized, filled with a substrate composed of peat soil, perlite, and vermiculite mixed in a volume ratio of 7:2:1. Seeds of whole grain and consistent size were chosen for sowing. Following the emergence of soybean seedlings, four plants were retained and relocated to the artificial climate incubator for comprehensive light treatment, receiving continuous illumination for 16 h and darkness for 8 h daily. Light conditions were categorized into four treatments: Normal light (NL), NL + far-red light (NF), shade (SL), and SL + far-red light (SF), with three biological replicates for each treatment. In the present study, these four treatments were used as simplified and controlled laboratory light regimes to compare the effects of irradiance reduction and far-red (FR) enrichment on soybean seedlings. They were not intended to exactly reproduce all spectral characteristics of natural canopy light environments, which may additionally involve dynamic variation in blue light, green light, and other wavelength regions.

Table 1 delineates the light environment characteristics pertinent to soybean seedling culture. Light treatments commenced upon the complete expansion of the seedlings' opposite true leaves. At 7 and 15 d of light treatment, uniformly cultivated soybean seedlings were selected and partitioned into roots, stems, and leaves for the assessment of morphological indices. The leaves were preserved in a -80 °C ultra-low-temperature refrigerator for future biochemical index examination.

Table 1. Photosynthetically active radiation (PAR) and red/far-red (R/FR) ratios in different light environments. Different lowercase letters in the same column indicate significant differences at $P < 0.05$. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR. These treatments were established as controlled laboratory light regimes and should not be interpreted as exact equivalents of specific natural canopy light environments.

Treatment	PAR	R	FR	R/FR
	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$	$\mu\text{mol m}^{-2} \text{s}^{-1}$
NL	750.67 ± 2.08^a	125.62 ± 0.37^a	91.68 ± 0.21^b	1.37 ± 0.01^a
NF	751.33 ± 1.43^a	124.57 ± 0.11^a	161.77 ± 0.36^a	0.77 ± 0.01^b
SL	14.93 ± 0.35^b	4.62 ± 0.07^b	3.39 ± 0.05^d	1.36 ± 0.03^a
SF	14.68 ± 0.62^b	4.58 ± 0.13^b	16.35 ± 0.11^c	0.28 ± 0.01^c

Morphological and biochemical measurements

Plant height was assessed using a straightedge from the seedling cotyledon scar to the apical growth point. The length of the first internode of the soybean seedling stalk and the hypocotyl length were ascertained by measuring the distance from the seedling above ground to the cotyledon scar. Whole soybean seedlings, leaves, and stem thickness were measured using an electronic balance. The electronic balance was utilized to measure each component of soybean seedlings desiccated at 65 °C for 48 h till achieving a consistent mass. The diameter of the central portion of the first internode of the soybean seedlings was measured using a vernier caliper. The leaf area of the initial ternate compound leaf of the soybean seedlings was determined utilizing Digimizer scanning software (MedCalc Software Ltd, Ostend, Belgium). Chlorophyll SPAD values were measured with a handheld portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan) on the three youngest compound leaves of soybean seedlings in each treatment on the 7th and 15th days of light exposure. Six measurements were obtained for each leaf, and the mean was documented for six pots in each treatment.

The anthrone method was used to measure the soluble sugar content. Fresh soybean leaves (0.1 g) were homogenized in 10 mL distilled water and extracted in boiling water for 30 min. Upon cooling, the volume was calibrated to 25 mL. One milliliter of supernatant was combined with 0.5 mL anthrone-ethyl acetate reagent and 5 mL concentrated sulfuric acid, incubated in boiling water for 5 min, and subsequently measured at 630 nm. The soluble protein concentration was determined using the Coomassie brilliant blue G-250 method according to Bradford (1976). Leaf samples (0.3 g) were homogenized in 5 mL phosphate buffer (pH 7.8) and subsequently centrifuged. One milliliter of the supernatant was combined with Coomassie brilliant blue reagent, diluted to a total volume of 5 mL, and analyzed at 595 nm. The malondialdehyde concentration was quantified using the thiobarbituric acid (TBA) technique. Leaf tissue (0.3 g) was homogenized in 5 mL 5% trichloroacetic acid (TCA) and subsequently centrifuged. Two milliliters of supernatant were combined with 2 mL 0.67% TBA, heated for 30 min, and subsequently analyzed at 450, 532, and 600 nm.

Fresh plant samples (0.2 g) were weighed, 3 mL 50 mmol phosphate buffer added, and completely homogenized in an ice bath. Following centrifugation, 2 mL supernatant were obtained as the enzyme extract (Mousavi et al., 2022). The activity of superoxide dismutase (SOD) was assessed by the inhibitory effect of enzyme extracts on the photoreduction of nitroblue tetrazolium (NBT). The sample's absorbance was quantified at 560 nm with a spectrophotometer (T6, Beijing Purkinje General Instrument, Beijing, China). One unit of enzyme activity is defined as the quantity of enzyme that inhibits 50% of NBT photoreduction. The activity of peroxidase (POD) was determined by mixing the enzyme extract with 25 mM phosphate buffer, 1% guaiacol solution, and 20 mM H₂O₂, according to Maehly (1954). The absorbance of the samples was measured at 470 nm for 3 min. One unit of enzyme activity is defined as the quantity of enzyme that produces a 0.01 rise

in optical density at 470 nm per minute. The activity of catalase (CAT) was assessed by observing the reduction in absorbance of hydrogen peroxide at 240 nm, as described by Maehly. The enzyme extract was combined with 25 mM phosphate buffer and 100 mM H₂O₂ solution for a 3 min kinetic reaction. One unit of enzyme activity is defined as the quantity of enzyme that induces a 0.1 reduction in OD₂₄₀ per minute. Each treatment was replicated three times, and a blank control was necessary for assessing plant antioxidant enzyme-related indices and malondialdehyde levels.

Transcriptome sequencing and assembly

Transcriptome sequencing of soybean leaf samples subjected to a 15 d treatment was conducted by Shanghai Meiji Biomedical Technology Co. (Shanghai, China). The raw sequencing data were evaluated for quality and filtered using FastQC (Babraham Bioinformatics, Cambridge, UK) for quality control and Trimmomatic (Usadel Lab, Aachen, Germany) for adaptor removal and quality filtering, facilitating seamless future analyses. Following quality control, the integrity of the raw data was assessed and juxtaposed with the reference genome (*Glycine max* Wm82.a2.v1). DESeq2 (Bioconductor, Seattle, Washington, USA) was employed to detect differentially expressed genes in the samples, with the criterion for screening set as FPKM > 1, |log₂FoldChange| ≥ 1.5, and pad ≤ 0.05. Functional annotation was performed using BLAST (National Center for Biotechnology Information, Bethesda, Maryland, USA), Blast2GO (BioBam, Valencia, Spain), and InterProScan5 (EMBL-European Bioinformatics Institute, Hinxton, Cambridgeshire, UK), and annotated genes were further classified according to the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The TCseq R package (Wu and Gu, 2026) was employed to perform a K-means clustering analysis of the expression patterns of the differentially expressed genes.

Data analysis

Data collation was done using Microsoft Excel 2010, ANOVA by SPSS Statistics 25 (IBM, Armonk, New York, USA), and plotted with Origin 9.0 (OriginLab Corporation, Northampton, Massachusetts, USA) and Adobe Photoshop 2020 (Adobe Inc., San Jose, California, USA).

RESULTS

Effect of far-red light on phenotypic indicators of soybean seedlings

As demonstrated in Table 2, the different treatments showed a pattern of shade + FR (SF) > shade (SL) > normal light + FR (NF) > normal light (NL) impacts on the height of the plant, length of the first internode, and hypocotyl of soybean seedlings at different treatment times. Soybean seedlings treated with SL and SF, which had less light, grew substantially higher, with longer internodes and hypocotyls, compared to seedlings treated with NL. This effect became stronger as the number of treatment days increased. Decreased light intensity facilitated the elongation of soybean seedlings, resulting in increased plant height, internode length, and hypocotyl elongation. The incorporation of FR under both NL + FR (NF) and SL + FR (SF) conditions further augmented these characteristics. Notably, seedlings subjected to NF and SF treatments exhibited greater height and longer internodes compared to those under NL and SL treatments devoid of FR. The most significant alterations in seedling morphology were observed when both light intensity and the red/far-red (R/FR) ratio were diminished, with the SF treatment yielding the most substantial enhancements in plant height, internode length, and hypocotyl elongation (Figure 1).

The impact of various shade treatments on the stem thickness of soybean seedlings exhibited the hierarchy NL > NF > SL > SF, with all differences being significant. The stem thickness of soybean seedlings subjected to SL and SF treatments with diminished light intensity was markedly less than that observed under the NL treatment. This indicates that low-light intensity impedes the development of stem thickness in soybean seedlings. Augmenting FR in both standard and low light-intensity conditions led to significantly thinner stems in seedlings. This suggests that FR influences the thickness of plant stems, thereby inhibiting the growth of soybean seedling stems. Of the four treatments, the SF treatment resulted in the thinnest stem, which was markedly less than that of the NL treatment. This demonstrates that the SF treatment, characterized by low light intensity and R/FR ratio, significantly impeded stem biomass development in soybean seedlings.

Table 2. Effects of different light environments on plant height, internode length, hypocotyl, and stem thickness of soybean seedlings. Values represent mean \pm standard error ($n = 3$). For vertical comparisons, different lowercase letters indicate significant differences between treatments ($P < 0.05$). NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Days of treatment	Treatment	Plant height cm	Interval length cm	Hypocotyl elongation cm	Stem thickness cm
7	NL	10.51 \pm 0.20 ^d	5.66 \pm 0.16 ^d	4.11 \pm 0.04 ^d	2.03 \pm 0.03 ^a
	NF	14.74 \pm 0.24 ^c	8.53 \pm 0.80 ^c	5.43 \pm 0.05 ^c	1.91 \pm 0.13 ^b
	SL	17.92 \pm 0.41 ^b	14.74 \pm 0.11 ^b	5.73 \pm 0.03 ^b	1.53 \pm 0.13 ^c
	SF	18.71 \pm 0.17 ^a	18.19 \pm 0.11 ^a	6.58 \pm 0.03 ^a	1.42 \pm 0.08 ^d
15	NL	12.98 \pm 0.25 ^d	12.43 \pm 0.25 ^d	6.06 \pm 0.03 ^d	2.40 \pm 0.01 ^a
	NF	16.44 \pm 0.44 ^c	17.01 \pm 0.30 ^c	7.11 \pm 0.06 ^c	2.12 \pm 0.05 ^b
	SL	18.48 \pm 0.64 ^b	17.98 \pm 0.32 ^b	7.49 \pm 0.03 ^b	1.77 \pm 0.04 ^c
	SF	22.70 \pm 0.24 ^a	22.56 \pm 0.28 ^a	7.75 \pm 0.10 ^a	1.46 \pm 0.01 ^d

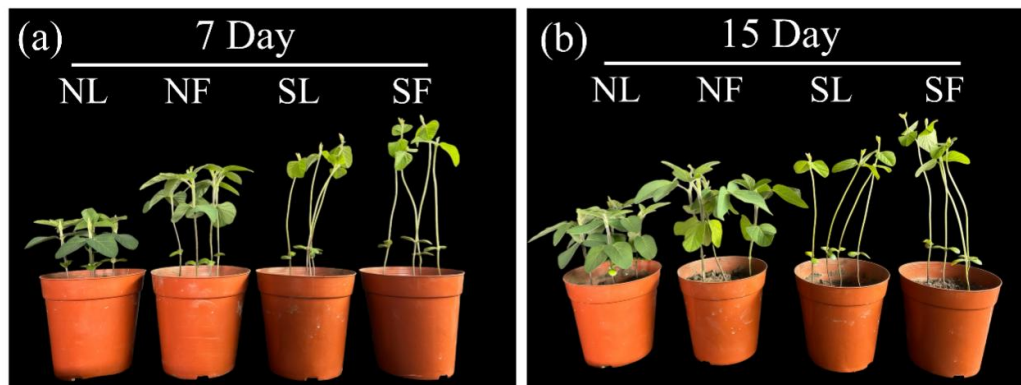


Figure 1. Growth of soybean seedlings at 7 d (a) and 15 d (b) under different light treatment conditions. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Reduced light intensity markedly diminished the leaf area and fresh dry mass of soybean seedlings. A diminished R/FR ratio adversely impacted the leaf area and fresh mass of soybean seedlings in both normal and low light conditions. Under low irradiance, SF plants exhibited a 14% augmentation in leaf area and a 33% enhancement in fresh mass relative to SL plants. Under standard irradiance, NF plants exhibited a 24% augmentation in leaf area and a 4% enhancement in fresh mass relative to NL plants. Following a 15 d treatment period, we discovered that the fresh mass of leaves and the stem thickness of soybean seedlings showed a similar trend, with the leaf area and fresh dry mass of soybean plants being highest under NF treatment and lowest under SL treatment (Table 3).

Table 3. Effect of different light environments on leaf area, fresh mass and dry mass of soybean seedlings. Values in the table represent mean \pm standard error ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$). NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Days of treatment	Treatment	Leaf area cm ²	Leaf fresh mass g	Leaf dry mass g	Stem fresh mass g	Stem dry mass g
7	NL	9.56 \pm 0.19 ^b	1.94 \pm 0.11 ^b	0.33 \pm 0.01 ^b	0.43 \pm 0.08 ^b	0.11 \pm 0.01 ^b
	NF	11.54 \pm 0.23 ^a	2.66 \pm 0.15 ^a	0.49 \pm 0.01 ^a	0.76 \pm 0.04 ^a	0.17 \pm 0.02 ^a
	SL	1.50 \pm 0.02 ^d	1.19 \pm 0.02 ^d	0.03 \pm 0.01 ^c	0.57 \pm 0.03 ^c	0.04 \pm 0.01 ^c
	SF	1.76 \pm 0.02 ^c	1.47 \pm 0.02 ^c	0.04 \pm 0.01 ^c	0.85 \pm 0.11 ^d	0.05 \pm 0.01 ^c
15	NL	11.22 \pm 0.21 ^b	2.98 \pm 0.02 ^b	0.40 \pm 0.05 ^b	0.89 \pm 0.06 ^b	0.35 \pm 0.11 ^b
	NF	13.93 \pm 0.18 ^a	3.11 \pm 0.04 ^a	0.63 \pm 0.01 ^a	0.27 \pm 0.17 ^a	0.42 \pm 0.21 ^b
	SL	1.60 \pm 0.02 ^d	1.22 \pm 0.03 ^d	0.10 \pm 0.08 ^c	0.10 \pm 0.02 ^c	0.05 \pm 0.01 ^c
	SF	1.82 \pm 0.02 ^c	1.62 \pm 0.02 ^c	0.07 \pm 0.01 ^c	0.17 \pm 0.03 ^d	0.06 \pm 0.01 ^c

Effects of FR on soybean seedlings and physiological indicators

On days 7 and 15 post-treatment (Figure 2a), the chlorophyll concentrations in soybean seedlings exhibited the hierarchy NL > NF > SL > SF. Between day 7 and day 15 post-treatment, the alteration in SPAD values was markedly more significant in the NL treatment group compared to the other three treatment groups. Under identical illumination circumstances, an increase in FR resulted in a significant reduction of SPAD values in soybean seedlings. This suggests that FR substantially influences soybean photosynthesis. The SPAD values of soybean seedling leaves were minimized under lower light intensity and R/FR ratio. The results indicate that both light intensity and FR affect the chlorophyll concentration in soybean seedlings.

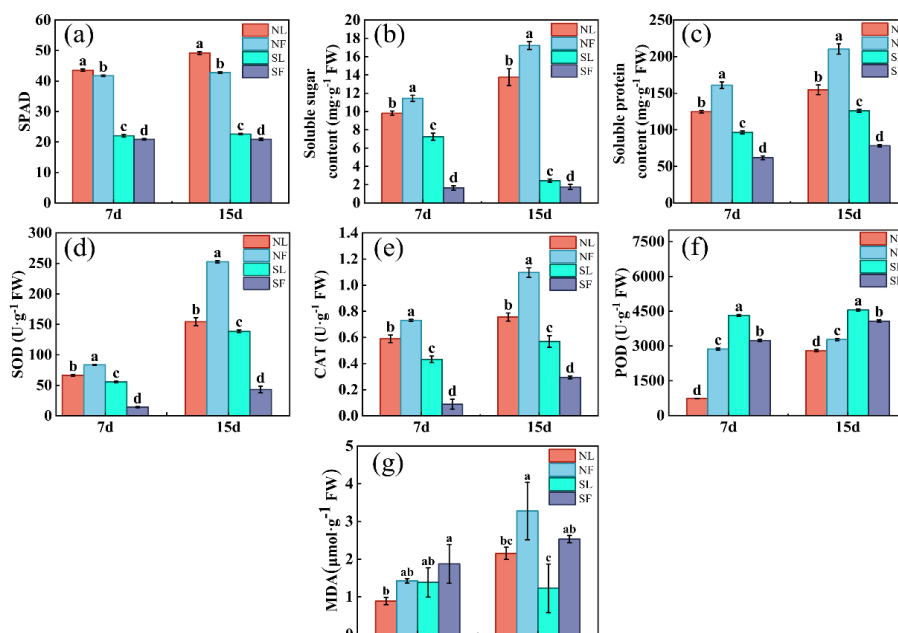


Figure 2. Effects of different light treatments on chlorophyll content, osmolytes, antioxidant enzyme activities, and malondialdehyde (MDA) content in soybean seedlings at 7 and 15 d. (a) SPAD value; (b) soluble sugar content; (c) soluble protein content; (d) superoxide dismutase (SOD) activity; (e) catalase (CAT) activity; (f) peroxidase (POD) activity; and (g) MDA content. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

With the extension of treatment duration, the concentrations of soluble sugar and soluble protein in soybean seedlings exhibited the sequence NF > NL > SL > SF, revealing significant disparities among the treatments. Seedlings subjected to NF (NL intensity with augmented FR) had the highest amounts of soluble sugars (Figure 2b) and soluble proteins (Figure 2c), demonstrating considerable disparities compared to the other treatments. This indicates that FR may promote the accumulation of soluble carbohydrates and proteins in soybean seedlings under standard light intensity. The concentrations of soluble sugar and protein in soybean seedlings were considerably diminished in the low light intensity SL and SF treatments compared to the control NL treatment, demonstrating that low light intensity greatly influenced the accumulation of these compounds. The quantities of soluble sugars and proteins in soybean seedlings subjected to SF treatment were markedly lower than those in other treatments.

In comparison to the standard light (NL) treatment, NF treatment (NL intensity with augmented FR) markedly enhanced the activities of malondialdehyde (MDA), superoxide dismutase (SOD) (Figure 2d), and catalase (CAT) (Figure 2e) in soybean seedling leaves, both after 7 and 15 d of treatment. Following 15 d of therapy, the activities of MDA, SOD, and CAT in the NF treatment group were significantly elevated compared to the NL treatment group, exhibiting notable differences between the two groups. Conversely, soybean seedlings subjected to SL and SF treatments, characterized by reduced light exposure, exhibited significantly lower MDA, SOD, and CAT activities compared to the NL treatment. The SF therapy yielded the lowest enzyme activity, demonstrating substantial changes compared to the NL treatment.

Furthermore, after 7 and 15 d of treatment, the peroxidase (POD) activity in soybean seedlings (Figure 2f) exhibited the following hierarchy: SL > SF > NF > NL. In comparison to the NL treatment, the POD activity of soybean seedlings under SL and SF treatments (characterized by diminished light intensity) exhibited a considerable increase. At 15 d, the POD activity of soybean seedlings under SL treatment peaked, significantly exceeding that of the NL treatment. Despite the enhancement in POD activity in soybean seedlings under the SF treatment relative to the NL treatment, the growth rate remained inferior to that of the SL treatment.

Transcriptome analysis of soybean seedlings in response to shade stress

The collected clean data were compared to the genome. Between 95.60% and 96.69% of the total readings were aligned with the reference gene ancestor. Of them, 2.27% to 3.22% corresponded to multiple locations on the reference genome. The mean ratio of uniquely localized sequences was 92.55%, 93.52%, 93.95%, and 94.36%, respectively. We conducted a comparative analysis of each sample of soybean seedling clean reads against the soybean reference genome to achieve genomic comparison. The comparative results in Table 4 indicated a comparison rate ranging from 80.58% to 93.29%, signifying the reliability of the transcriptome sequencing data. The cDNA library exhibited high quality, adequate for later bioinformatics analysis.

Table 4. Comparison of Reads to Reference Sequence. Values in the table represent mean ± standard error ($n = 3$). Different letters indicate significant differences between treatments ($P < 0.05$). NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Treatment	Total reads	Total mapped	Multiple mapped	Uniquely mapped
NL_1	44533158	42491758(95.42%)	1405467(3.16%)	41086291(92.26%)
NL_2	43568638	41644774(95.58%)	1264786(2.9%)	40379988(92.68%)
NL_3	52689468	50484331(95.81%)	1639364(3.11%)	48844967(92.70%)
NF_1	50502820	48874551(96.78%)	1566290(3.1%)	47308261(93.67%)
NF_2	47208392	45610744(96.62%)	1499436(3.18%)	44111308(93.44%)
NF_3	43497630	42049878(96.67%)	1400881(3.22%)	40648997(93.45%)
SL_1	41798658	40443927(96.76%)	1000312(2.39%)	39443615(94.37%)
SL_2	43542996	42189266(96.89%)	1066300(2.45%)	41122966(94.44%)
SL_3	43803758	42051348(96.0%)	1294379(2.95%)	40756969(93.04%)
SF_1	43657776	42214295(96.69%)	991297(2.27%)	41222998(94.42%)
SF_2	43930410	42508591(96.76%)	1022021(2.33%)	41486570(94.44%)
SF_3	43899204	42406775(96.6%)	1048161(2.39%)	41358614(94.21%)

Inter-sample correlation analysis

We computed Pearson's correlation coefficients for the 12 samples and produced a correlation heatmap (Figure 3a) to evaluate the consistency of results and variations among samples within each treatment group. The correlation coefficients varied from 0.08 to 1.0, with lighter colors representing low correlation and darker colors suggesting strong correlation. The results indicated that the correlation coefficients within the same treatment group were markedly greater than those between other groups. Samples from the NL and NF treatment groups demonstrated a more compact clustering and exhibited elevated correlations (both correlation coefficients exceeding 0.95). The correlations between the samples from the SL and SF treatment groups were comparatively low, with correlation coefficients between 0.7 and 0.9. The correlation coefficients between samples subjected to varied light treatments (SL vs. SF) were significantly lower than those under normal light treatments (NL vs. NF). This indicates that light intensity and FR substantially influence the transcriptomic profile of soybean seedlings.

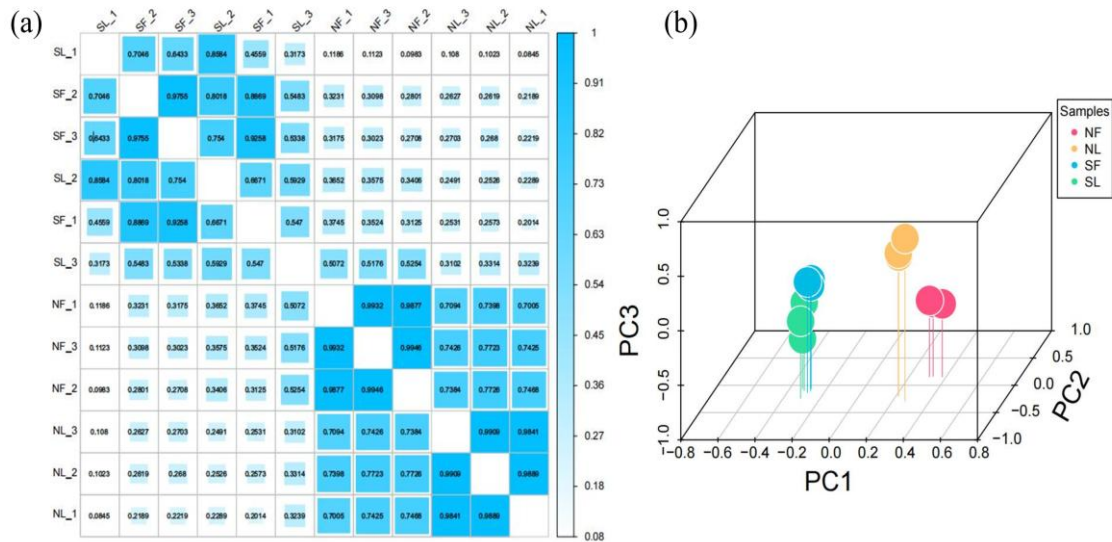


Figure 3. Heatmap of correlation of expression between different samples (a); Principal component analysis (PCA) correlation analysis (b). NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Principal component analysis

Principal component analysis (PCA) was employed to analyze the expression patterns of the 12 samples (Figure 3b), elucidating the effects of various light treatments on the transcriptome data of soybean seedlings. The results indicated that the samples are replicable independently. The PCA analysis (Figure 3b) indicated that the first principal component (PC1) accounted for 63.8% of the total variation, whereas the second principal component (PC2) accounted for 18.7%. PC1 predominantly represented variations in light intensity, whereas PC2 indicated the influence of FR on gene expression in soybean seedlings. The elevated reproducibility within the sample set validated the dependability of the experimental design and sequencing results.

Differential expressed gene analysis

Marked variations in gene expression were detected in soybean seedlings subjected to diverse shading conditions (Figure 4a). In contrast to the NL treatment, the NF treatment exhibited 8649 differentially expressed genes, comprising 3535 upregulated and 5114 downregulated genes. The SL therapy resulted in 13 206 differentially expressed genes, comprising 6898 upregulated and 6308 downregulated genes. The SF therapy exhibited the greatest quantity of differentially expressed genes, totaling 15 762, comprising 7010 upregulated and 8752 downregulated genes. We created a Venn diagram (Figures 4b and 4c) to compare the differentially expressed genes across the four treatment groups (NL, NF, SL, and SF) in order to examine the impact of various

light treatments on gene expression in soybean seedlings. The findings indicated that 1204 differential genes demonstrated significant alterations across all treatment groups. These genes reacted to both shading stress and FR treatments and were the principal genes assisting soybean seedlings in managing environmental stresses.

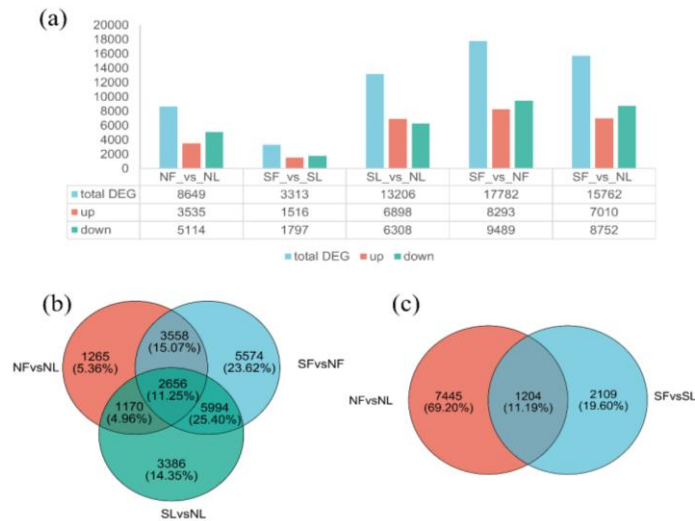


Figure 4. Number of differentially expressed genes (DEGs). NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Gene Ontology (GO) annotation analysis

To elucidate the impacts of FR, we conducted Gene Ontology (GO) functional annotation (Figure 5), which predominantly delineates the roles of associated genes across three dimensions: Biological process, cellular component, and molecular function. The twenty most enriched GO entries are presented. The findings indicate that low R/FR ratios and shading induce significant alterations in gene expression, especially for cell development, stress responses, and chlorophyll utilization. These molecular findings enhance comprehension of how soybean seedlings transcriptionally adjust to varying light exposures, with substantial implications for augmenting their resilience under fluctuating agricultural lighting settings.

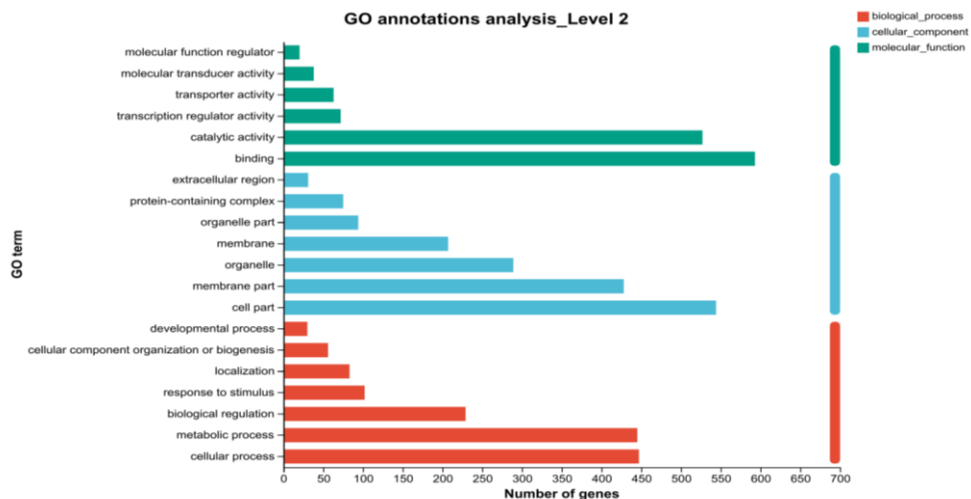


Figure 5. Gene ontology (GO) annotation analysis of differentially expressed genes co-expressed under shade and far-red light conditions.

GO Enrichment analysis

Differentially expressed genes influenced by various light treatments exhibited significant enrichment in GO categories pertaining to photosynthesis, hormone signaling, and antioxidants (Figure 6a). The NL and NF treatments notably enriched photosystem I (GO:0009522) and photosystem II (GO:0009523), whereas these were diminished in the SL and SF treatments. The SL and SF treatments demonstrated significant increases in the “abscisic acid-mediated signaling pathway” (GO:0009738) and the “ethylene response” (GO:0009723), indicating that low light and FR may augment plant stress responses and modify hormone signaling to assist plants in managing environmental stresses. Furthermore, the concentration of “chlorophyll binding” (GO:0016168) was significantly reduced, particularly in plants subjected to SF treatment, suggesting that shade and FR may impede photosynthesis by disrupting chlorophyll metabolism.

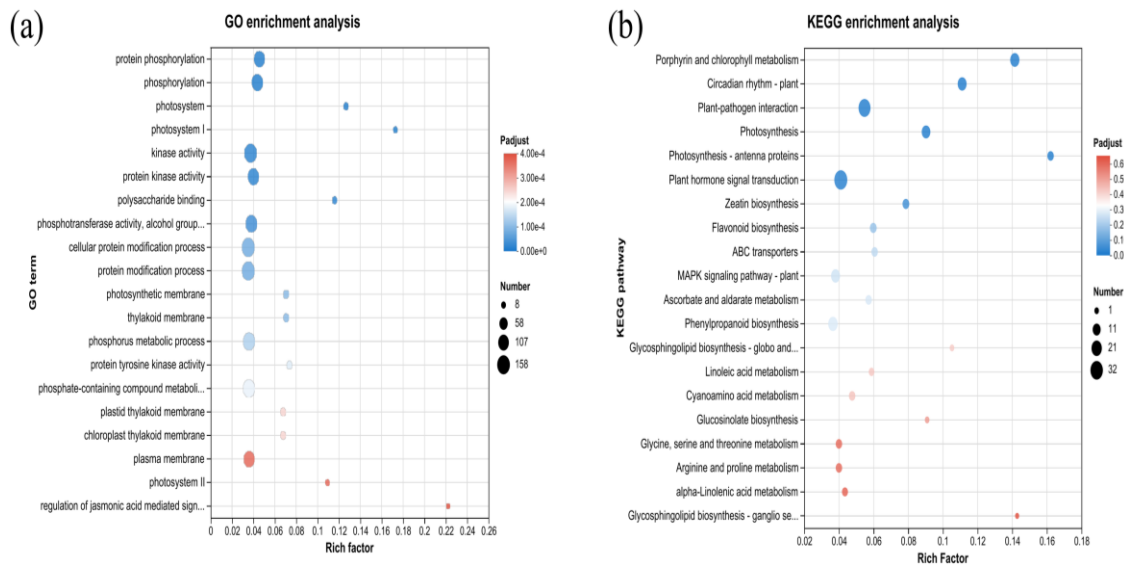


Figure 6. Gene ontology (GO) enrichment analysis (a) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (b) of differentially expressed genes co-expressed under shade and far-red light conditions.

KEGG Enrichment analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment studies (Figure 6b) identified numerous significantly enriched pathways predominantly associated with critical biological functions, including photosynthesis, biosynthetic metabolism, and plant signaling. The plant hormone signal transduction (PHT) enrichment analysis (Table 5) showed numerous genes associated with the signaling of growth hormones, including indole-3-acetic acid (IAA), gibberellin (GA), abscisic acid (ABA), cytokinin (CTK), and ethylene (ET). These genes may govern plant growth, development, stress response, and metabolic regulation. The chlorophyll biosynthesis and photosynthesis pathways are crucial for generating the energy and organic molecules necessary for plant development. Numerous essential genes (such as *psaA* and *psbA*) and light-harvesting complex genes (including the *Lhc* family) were identified in photosystem I and photosystem II of the photosynthesis pathway, suggesting that low light intensity and FR treatments markedly influenced the efficiency of the light reactions. The findings indicate that shade and FR may significantly influence soybean growth and development by modulating the three primary pathways of phytohormone signaling, chlorophyll metabolism, and photosynthesis. Consequently, we examined the aforementioned three pathways.

Table 5. Differentially expressed genes involved in the plant hormone signaling pathway. KO: Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology.

KO ID	Gene id	Description
K14484	Glyma.08G203100.Wm82.a4.v1	IAA, PTHR31734:SF5 - AUXIN-RESPONSIVE PROTEIN IAA27
	Glyma.20G210500.Wm82.a4.v1	IAA, PTHR31734//PTHR31734:SF11 - FAMILY NOT NAMED//AUXIN-RESPONSIVE PROTEIN IAA1-RELATED
	Glyma.13G354100.Wm82.a4.v1	IAA, PTHR31734//PTHR31734:SF11 - FAMILY NOT NAMED//AUXIN-RESPONSIVE PROTEIN IAA1-RELATED
	Glyma.04G066300.Wm82.a4.v1	IAA, K14484 - auxin-responsive protein IAA (IAA)
	Glyma.02G142600.Wm82.a4.v1	IAA, K14484 - auxin-responsive protein IAA (IAA)
	Glyma.13G127000.Wm82.a4.v1	IAA, PTHR31734//PTHR31734:SF6 - FAMILY NOT NAMED//AUXIN-RESPONSIVE PROTEIN IAA10-RELATED
	Glyma.19G161000.Wm82.a4.v1	IAA, K14484 - auxin-responsive protein IAA (IAA)
	Glyma.15G020300.Wm82.a4.v1	IAA, PTHR31734//PTHR31734:SF2 - FAMILY NOT NAMED//AUXIN-RESPONSIVE PROTEIN IAA18-RELATED
K14487	Glyma.10G019700.Wm82.a4.v1	GH3, PTHR31901//PTHR31901:SF18 - FAMILY NOT NAMED//INDOLE-3-ACETIC ACID-AMIDO SYNTHETASE GH3.9-RELATED
K14488	Glyma.04G006900.Wm82.a4.v1	SAUR, K14488 - SAUR family protein (SAUR)
	Glyma.03G029600.Wm82.a4.v1	SAUR, PTHR31374//PTHR31374:SF56 - FAMILY NOT NAMED//AUXIN-REGULATED PROTEIN-RELATED
K14490	Glyma.03G241700.Wm82.a4.v1	AHP, K14490 - histidine-containing phosphotransfer protein (AHP)
K14491	Glyma.17G076000.Wm82.a4.v1	ARR-B, PTHR26402//PTHR26402:SF423 - RESPONSE REGULATOR OF TWO-COMPONENT SYSTEM//TWO-COMPONENT RESPONSE REGULATOR ARR10-RELATED
	Glyma.17G030600.Wm82.a4.v1	ARR-B, PTHR26402//PTHR26402:SF535 - RESPONSE REGULATOR OF TWO-COMPONENT SYSTEM//SUBFAMILY NOT NAMED
K12126	Glyma.03G225000.Wm82.a4.v1	PIF3, K12126 - phytochrome-interacting factor 3 (PIF3)
K14497	Glyma.08G033800.Wm82.a4.v1	PP2C, PTHR13832//PTHR13832:SF357 - PROTEIN PHOSPHATASE 2C//SUBFAMILY NOT NAMED
	Glyma.14G162100.Wm82.a4.v1	PP2C, PTHR13832//PTHR13832:SF326 - PROTEIN PHOSPHATASE 2C//SUBFAMILY NOT NAMED
K14498	Glyma.14G176700.Wm82.a4.v1	SNRK2, PTHR24343//PTHR24343:SF207 - SERINE/THREONINE KINASE//SERINE/THREONINE-PROTEIN KINASE SRK2C
K14509	Glyma.10G008500.Wm82.a4.v1	ETR, ERS, PTHR24423//PTHR24423:SF530 - TWO-COMPONENT SENSOR HISTIDINE KINASE//ETHYLENE RESPONSE SENSOR 2-RELATED
K14516	Glyma.13G123100.Wm82.a4.v1	ERF1, K14516 - ethylene-responsive transcription factor 1 (ERF1)
	Glyma.19G248900.Wm82.a4.v1	ERF1, K14516 - ethylene-responsive transcription factor 1 (ERF1)
K13416	Glyma.05G119500.Wm82.a4.v1	BAK1, K13416 - brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1)
K14499	Glyma.04G038100.Wm82.a4.v1	BKI1, K14499 - BRI1 kinase inhibitor 1 (BKI1)
K14502	Glyma.02G069400.Wm82.a4.v1	BIN2, PF07714//PF11820 - Protein tyrosine kinase (Pkinase_Tyr)//Protein of unknown function (DUF3339) (DUF3339)
K14504	Glyma.17G065200.Wm82.a4.v1	TCH4, PTHR31062//PTHR31062:SF32 - FAMILY NOT NAMED//XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE PROTEIN 25-RELATED
K13464	Glyma.15G179600.Wm82.a4.v1	JAZ, PTHR33077:SF13 - PROTEIN TIFY 10A-RELATED
	Glyma.01G204400.Wm82.a4.v1	JAZ, PTHR33077:SF13 - PROTEIN TIFY 10A-RELATED
	Glyma.17G047700.Wm82.a4.v1	JAZ, PTHR33077:SF13 - PROTEIN TIFY 10A-RELATED
	Glyma.11G038600.Wm82.a4.v1	JAZ, K13464 - jasmonate ZIM domain-containing protein (JAZ)
	Glyma.09G071600.Wm82.a4.v1	JAZ, PTHR33077:SF13 - PROTEIN TIFY 10A-RELATED
	Glyma.13G112000.Wm82.a4.v1	JAZ, PTHR33077:SF13 - PROTEIN TIFY 10A-RELATED
K13422	Glyma.14G117300.Wm82.a4.v1	MYC2

Plant hormone signaling pathways

The heatmap results indicated that various light treatments markedly influenced the expression of genes associated with phytohormone signaling in soybean seedlings. In comparison to the NL treatment, the NF treatment markedly elevated the expression of genes associated with the gibberellin signaling system, such as *GID1* and *DELLA*, indicating that supplementary FR in conjunction with normal light may augment the GA signaling pathway. The expression of crucial genes associated with ABA, including *PYL* and *SnRK2*, was significantly elevated in plants subjected to SL and SF treatment. Likewise, the expression of genes associated with ethylene signaling, including *ETR* and *EIN3*, exhibited a notable rise. This suggests that exposing soybean seedlings to low light may improve their stress response by augmenting ABA and ET signaling. The genes associated with ABA and ET signaling were significantly increased under SF treatment, indicating that the synergistic impact of diminished light intensity and a low R/FR ratio intensified the modification of hormone signals. Significant alterations occurred in the expression of genes linked to several hormonal signals within the phytohormone signaling pathway (Figure 7). Under normal illumination conditions, *TIR1* and *ARF*, two genes essential for auxin signaling, were markedly increased in both NL and NF treatments. Conversely, their expression was markedly diminished in SL and SF treatments under low light conditions. The expression of GA signaling genes, including *GID1* and *DELLA*, was markedly elevated in NF treatments. In contrast, their expression was diminished in SL and SF treatments, attaining the lowest level in the SF therapy. Genes involved in ABA signaling, including *PYL*, *SnRK2*, and *ABF*, exhibited considerable upregulation in both SL and SF treatments, although their expression levels remained low in NL and NF treatments. Crucial genes in the ET signaling system, such as *ETR* and *EIN3*, exhibited significant upregulation in the SF treatment, suggesting that diminished R/FR ratios and low light intensity substantially influence ET signaling.

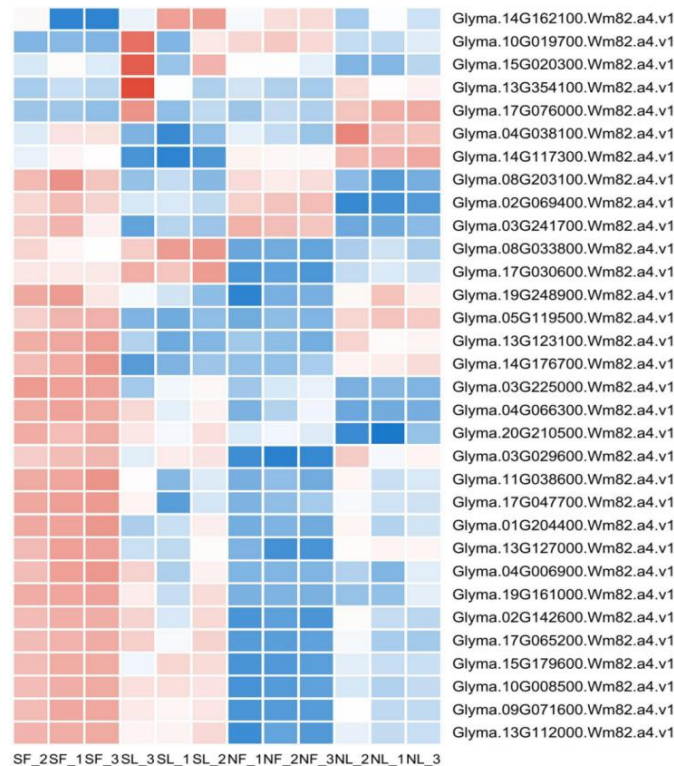


Figure 7. Heat map of differentially expressed genes co-expressed under shading and far-red light conditions in the phytohormone signaling pathway. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Porphyrin and chlorophyll pathways

The heatmap of the porphyrin and chlorophyll metabolic pathways (Figure 8) indicated that several treatments markedly influenced the expression of genes associated with chlorophyll biosynthesis. For instance, essential genes involved in chlorophyll synthesis, including *HEMA1* (Heme A Synthase 1), *CHLG* (Chlorophyllide a Reductase), and *POR* (Protochlorophyllide Oxidoreductase), exhibited considerable downregulation in the SF therapy. The expression of the *HEMA1* gene was significantly diminished in the SF treatment, suggesting that low light intensity and FR treatments markedly suppressed chlorophyll production. Conversely, genes associated with chlorophyll metabolism exhibited significantly more activity in the NL and NF treatments, indicating that normal irradiance is advantageous for sustaining chlorophyll metabolic equilibrium.

Utilizing physiological data (SPAD values) for chlorophyll concentration (Figure 2a), we observed that the SF group exhibited a significantly lower chlorophyll concentration compared to other groups on the 15th day post-treatment, thereby reinforcing the notion that diminished light intensity and a low R/FR ratio substantially influence chlorophyll synthesis.

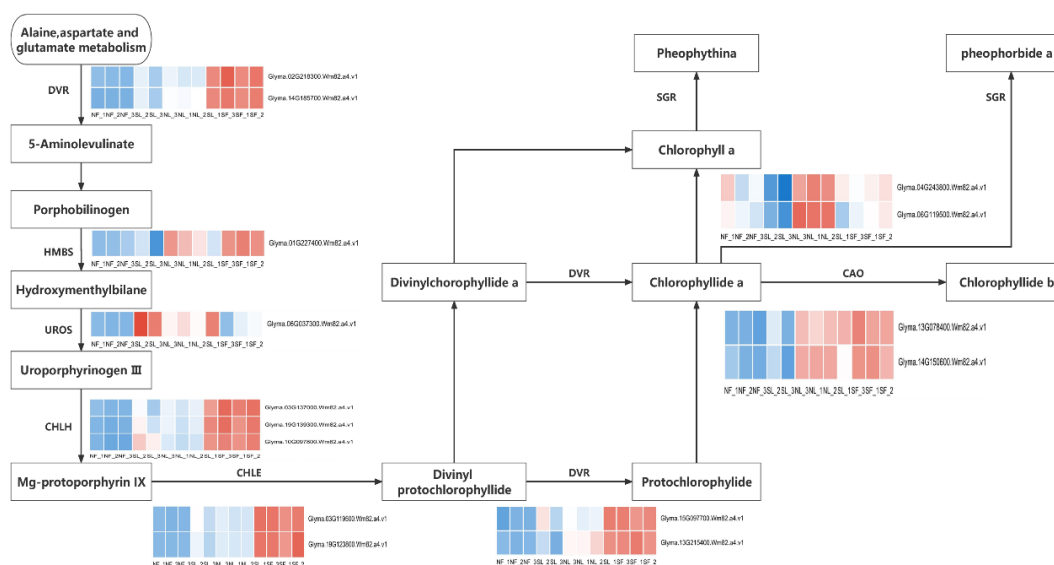


Figure 8. Heatmap of differentially expressed genes involved in the porphyrin and chlorophyll metabolism pathway under shading and far-red light treatments. The pathway framework was adapted from KEGG, and the heatmaps at each node were generated by the authors based on the data obtained in this study. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Photosynthesis pathway

Heatmaps of the photosynthetic pathway (Figure 9) indicated that essential genes associated with photosystem I and photosystem II (*psaA*, *psbA*, and *Lhca1*) were markedly downregulated in the SL and SF treatments, with gene expression attaining its nadir in the SF group. This indicates that low light intensities combined with FR significantly diminish the expression of light-responsive central genes. The expression of these genes was marginally elevated in the NF treatment relative to the NL treatment, suggesting that increasing FR at normal irradiance can enhance photosynthetic efficiency to a small extent. In conjunction with physiological data, we discovered that SL and SF treatments markedly influenced the leaf area and fresh mass of soybean seedlings, aligning with alterations in the expression of photosynthesis-related genes. This further illustrates that low R/FR certainly diminishes photosynthetic activity; however, the precise effect on overall plant development necessitates additional inquiry, including gas exchange analysis, to validate these findings.

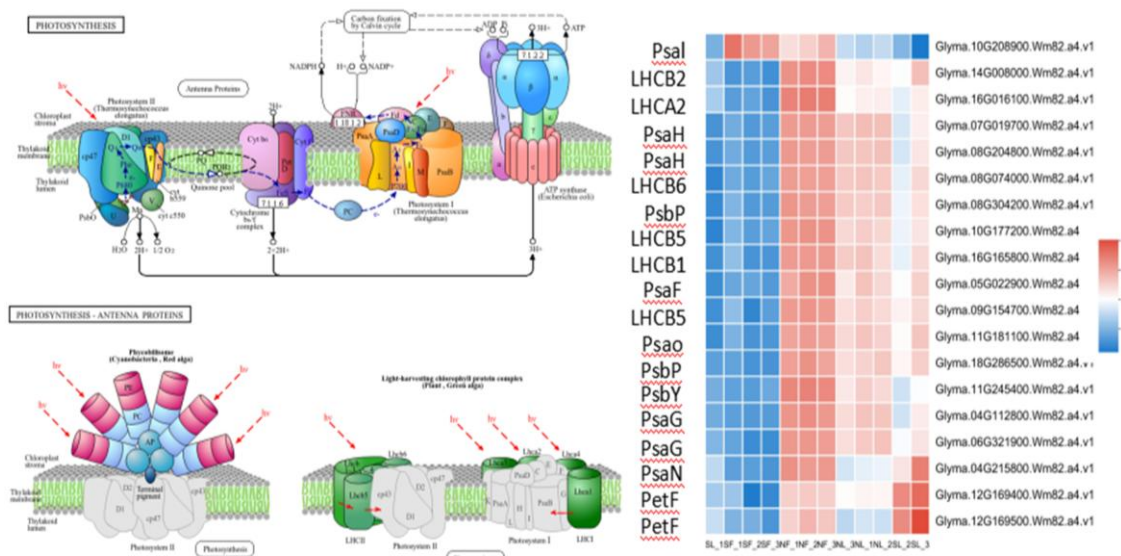


Figure 9. Heatmap of differentially expressed genes involved in the photosynthesis-antenna protein pathway under shading and far-red light treatments. The pathway diagrams on the left were adapted from KEGG, and the heatmap on the right was generated by the authors based on the data obtained in this study. NL: Normal light; NF: NL + far-red light (FR); SL: shade; SF: SL + FR.

Transcription factor family analysis

The statistical analysis of transcription factor families facilitated the construction of a network connecting these families to differentially expressed genes (Figure 10). Various transcription factors exhibited strong responses to FR therapy and its interaction with shade. The *WRKY* and *MYB*-related families exhibited the highest responsiveness, each governing several differentially expressed genes. These families are integral to plant light signal transduction and stress response, and may participate in FR-induced signaling mechanisms. The *NAC* (*NAM*, *ATAF*, and *CUC*), *bHLH* (basic Helix-Loop-Helix), and *ERF* (Ethylene Response Factor) are further notably enriched families. The gene regulatory networks of these families may collaborate to facilitate the adaptation of soybean seedlings to light and evade shade. The $-\log_{10}$ (p-value) significance test indicated that the majority of differentially expressed genes exhibited greater strength in FR-treated groups (NF, SF), highlighting their significance in the light signaling pathway induced by FR. The findings indicate that FR therapy triggers a cascade of gene expression alterations governed by particular families of transcription factors, influencing plant development and survival.

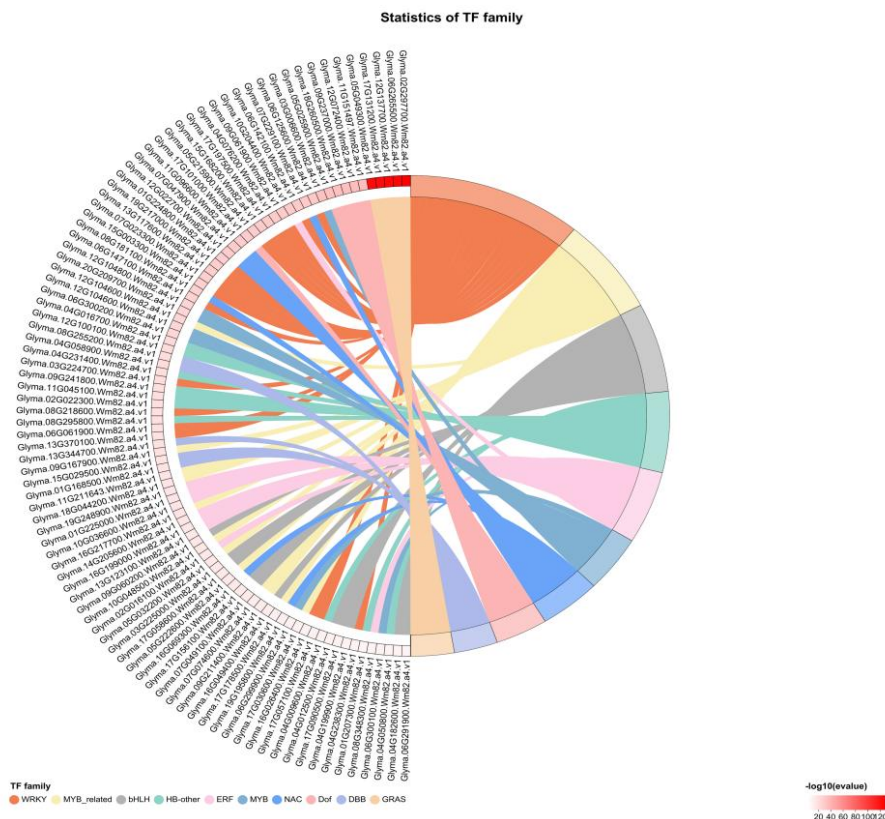


Figure 10. Statistical chord plots of predicted transcription factor families of differentially expressed genes co-expressed under shading and far-red light conditions.

DISCUSSION

Effect of shade stress and far-red light treatment on growth indexes of soybean seedlings

Light, as an abiotic element, is crucial for the growth and development of crops. While plants cannot select their environment, they can acclimatize to it through various adaptations. The photosynthetic patterns of plants may implicitly indicate their environmental adaptation, serving as a significant metric for evaluating plant growth, despite the absence of precise photosynthetic activity measures, such as gas exchange analyses, in this study (Lyu et al., 2020). The current experimental work revealed that far-red light (FR) treatment markedly enhanced the height of soybean seedlings and augmented plant fresh mass, aligning with the shade avoidance response mechanism posited by Park and Runkle (2017). The morphology of soybean plants in the FR treatment group suggested that the augmentation of aboveground biomass in soybean seedlings may be attributed to the shade avoidance response, leading to increased height, yet resulting in diminished stem thickness and a reduction in leaf count. This results in a decrease in the aboveground dry and fresh biomass of soybeans. In the present study, reduced irradiance and FR enrichment were manipulated independently and in combination under controlled laboratory conditions. Importantly, reduced irradiance in the shade (SL) treatment was not accompanied by a marked decrease in R/FR compared with normal light (NL), whereas NL + FR (NF) and SL + FR (SF) mainly represented conditions with additional FR and lower R/FR. Therefore, these treatments should be interpreted as simplified experimental light regimes for dissecting the effects of light quantity and FR supplementation, rather than as exact simulations of specific natural shade scenarios. Yang et al. (2017) employed analogous light circumstances to investigate the influence of light quality on plant growth, and their results corroborate our methodology in examining how light intensity and quality affect plant adaptation. The soybean seedlings exhibited reduced lateral stem growth and elongated stem nodes, resulting in the stem comprising a greater proportion of the plant's biomass. This transpired due to a reduction in stem

thickness concomitant with an increase in plant height. Simultaneously, to fulfil the demands for stem development, seedlings augment the ratio of nutrients derived from photosynthetic products to the stem, responding to the heightened FR, which can modulate the plant's photosynthetic pattern (Fan et al., 2019). This study investigated variations in plant height, internode length, stem thickness, and fresh mass of soybean seedlings between the NL and NF treatments under normal light, as well as SL and SF treatments under low light. The observed discrepancies are probably attributable to the diminished R/FR ratio. In typical lighting conditions, a low R/FR ratio facilitates leaf expansion, enhancing light absorption and photosynthetic products. Similar light-quality effects on commercially relevant traits have also been reported in purple cabbage microgreens, where the addition of red light to white light improved cotyledon development and yield (Cecilio Filho et al., 2026). In low light conditions, it enhances leaf growth while synchronizing stem and leaf development, hence facilitating improved seedling growth in shaded environments. Wos et al. (2022) utilized *Arabidopsis* leaves to investigate the effects of light quality on plant morphology. Their research showed that under low R/FR ratio conditions, although leaf size rose, biomass accumulation remained relatively unchanged. The variation in leaf area observed in shaded environments may be ascribed to species-specific reactions to FR. This study found that reduced light intensity led to an increased specific leaf area (SLA), a crucial metric for assessing plant development and C uptake. With increased shade, plants often augment their SLA to optimize light absorption and enhance photosynthetic efficiency in low light environments (Wen et al., 2021).

Effects of shade stress and far-red light treatment on physiological indexes of soybean seedlings

Chlorophyll is a crucial pigment that enables plants to receive, transfer, and regulate light energy. The concentration of chlorophyll in leaves directly influences their capacity for photosynthesis. Researchers have discovered that diminished light or shade can impede leaf growth, resulting in thinner leaves, decreased leaf area, reduced fenestrated tissue thickness, and lower chlorophyll concentration, causing plants to absorb less light energy. In this investigation, the chlorophyll SPAD values of soybean seedling leaves in the SL and SF treatments (exposed to reduced light) were considerably lower than those in the NL treatments. This signifies that diminished light intensity impacts the photosynthesis of soybean seedlings. Under identical light circumstances, the SPAD values of soybean seedlings in the NF and SF treatments (with augmented FR) were inferior to those in the NL and SL treatments devoid of increased FR. This may be due to FR signals inducing oxidative stress, particularly the photo-oxidation of chlorophylls in soybean seedlings, leading to inadequate chlorophyll synthesis or chlorophyll degradation, a common indication of oxidative stress (Ma and Li, 2019).

Under standard light circumstances, the introduction of FR markedly enhanced the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as raised levels of malondialdehyde (MDA), soluble sugars, and soluble proteins in soybean seedlings. This phenomenon indicates that FR may augment the accumulation of photosynthetic products by enhancing the electron transfer efficiency of photosystem I (PSI), while concurrently resulting in excessive production of reactive oxygen species (ROS), thus triggering the negative feedback regulation of antioxidant enzymes (Rahman et al., 2025). A study on European spruce revealed that red light (660 nm) treatment markedly enhanced the net photosynthetic rate (P_N), while FR (730 nm) diminished the electron transfer efficiency of PSII but may indirectly affect ROS metabolism by maintaining ATP production via cyclic electron flow (CEF) (OuYang et al., 2021). In comparison to normal light, both low light (SL) and low light mixed with FR (SF) treatments led to reduced levels of SOD, CAT, soluble sugars, and soluble proteins, while POD activity showed an increasing tendency. This result can be ascribed to the reduced photosynthetic assimilation ability in low-light environments, with FR further inhibiting PSII photochemical efficiency and intensifying C assimilation deficits (Li et al., 2024b). *Fittonia albivenis* alleviates photoinhibition via PGR5/PGRL1-dependent CEF and non-photochemical quenching (NPQ) pathways under FR exposure; nevertheless, its shade-tolerance characteristics may not be applicable to light-demanding crops like soybean (Hao et al., 2025). Moreover, weak blue light collaborates with FR to hasten leaf senescence in soybean, with cryptochrome-mediated induction of GmWRKY100 expression being pivotal (Zhan et al., 2023). This suggests that FR may exacerbate plant stress resistance by interfering with blue light signaling pathways in shady environments. While SOD, CAT, and POD are integral components of the antioxidant enzyme system, the increase in POD activity in low light was juxtaposed with a decrease in the other enzymes. In a shade experiment on *Rhodiola rosea*, SOD activity dramatically increased under greater shading intensities, while MDA

concentration diminished, underscoring distinct adaptive strategies among plant species in reaction to light conditions (Yang et al., 2018b).

The surrounding light conditions significantly influence the impact of FR. In typical lighting conditions, FR facilitates the formation of ROS by augmenting photosynthetic electron transport, which in turn activates antioxidant defenses. Conversely, in shadowed environments, FR may exacerbate the imbalance in light energy distribution, hastening C assimilation deficits and senescence. Future research may incorporate transcriptomics and light-signaling mutants to further clarify the molecular mechanisms governing FR interactions with various light qualities.

Effect of shade stress and far-red light treatment on differential genes in soybean seedlings

This research utilized multidimensional analysis to illustrate the substantial effects of low light and FR on growth, photosynthesis, and transcriptome regulation in soybean seedlings. Principal component analysis (PCA) demonstrated that various light treatments significantly affected gene expression patterns, leading us to categorize data according to principal component dimensions. This suggests that the transcriptome of soybean seedlings saw significant alterations in response to different light conditions (Zhang et al., 2022), likely reflecting an adaptive regulation of plants to variations in light.

Gene ontology (GO) enrichment investigation identified light signaling, photosynthesis, and hormone regulation as significant processes influenced by light conditions. In accordance with Jesus et al. (2024), differentially expressed genes were primarily identified in the categories of “light response”, “chlorophyll-binding”, and “hormone signaling”. This indicates that low-light and FR treatments affect photoreceptor function, chlorophyll metabolism, and hormone signaling pathways, hence substantially modifying physiological and metabolic conditions. Significantly, low light conditions suppressed the expression of photosynthesis-related genes while enhancing the expression of redox-related genes, indicating a possible stress adaptation mechanism.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment study indicated that differentially expressed genes were predominantly enriched in essential metabolic pathways, such as photosynthesis, phytohormone signaling, and porphyrin/chlorophyll metabolism. Photosystem I/II functions were markedly modulated under varying light conditions, with *psbA* and *psaA* expression elevated under normal light (NL) and FR (NF) treatments, hence augmenting light energy absorption and biomass synthesis. In contrast, these genes were downregulated under low-light (SL and SF) conditions, diminishing the plants' capacity to absorb light and conduct photosynthesis, in accordance with physiological data indicating decreased P_N attributed to restricted Photosystem II activity and diminished electron transfer efficiency.

The substantial enhancement of phytohormone signaling pathways provides a novel understanding of the molecular mechanisms governing light-regulated plant growth. Genes associated with abscisic acid (ABA) signaling (PYL, SnRK2) exhibited upregulation, whereas genes related to auxin (AUX1, ARF) and gibberellin (GID1, DELLA) signaling demonstrated downregulation under low light conditions. This indicates a mechanism that slows growth through the inhibition of cell division and elongation, while simultaneously enhancing ABA-mediated stress tolerance (Pandey et al., 2019). This technique may be essential for plant adaptation to low-light environments, as enhanced ABA signaling has demonstrated improved survival under such stress (Chen et al., 2019).

Regulation of porphyrin and chlorophyll metabolic pathways demonstrated significant downregulation of chlorophyll biosynthesis genes (HEMA, CHLH, POR) under low-light and FR treatments, leading to reduced chlorophyll concentrations. This aligns with the measurement findings and indicates that alterations in the light environment probably influence both chlorophyll synthesis and photosynthetic efficacy. Certain studies ascribe this phenomenon to diminished R/FR ratios inhibiting the accumulation of chlorophyll precursors. The buildup of chlorophyll precursors is detrimental to the plant as these molecules disrupt the regular operation of the photosynthetic apparatus. Surplus chlorophyll precursors can interfere with light absorption and energy conversion, resulting in diminished photosynthetic efficiency. The accumulation of these precursors enhances the generation of ROS, which can harm cellular structures and adversely impact plant growth and development. Additionally, downregulation of photosynthetic pathway electron transport chain genes (PetE, PetF) shows reduced electron transport efficiency under insufficient light, a primary reason for lower photosynthetic rates.

Transcription factors, pivotal components in the regulation of gene expression, are important to plant responses to light signals and the modulation of metabolic pathways. This study's transcription factor prediction analysis discovered several differentially expressed families essential for regulating hormonal and light signaling

pathways. The bHLH, MYB, and WRKY families are principal regulators that respond to alterations in the light environment. The bHLH members were markedly elevated in shade and shade+FR treatments, likely facilitating plant adaptation to low-light environments through alterations in photopigment signaling pathways. The significant downregulation of MYB members indicates that alterations in the light environment may impede plant growth and food production via regulating chlorophyll biosynthesis and cell division genes.

The WRKY members were markedly elevated in shade treatments, presumably enhancing plant resilience to low-light stress by activating genes involved in stress responses and antioxidant defense systems (Ma and Hu, 2024).

This study demonstrates that varying light conditions greatly influence soybean seedling growth and metabolism by modifying essential processes such as light signal perception, photosynthesis, and hormone communication. Low light and FR acclimate to intricate light conditions by modulating photosynthesis-associated genes and chlorophyll metabolism/light-absorption pathways. Moreover, variable hormone signal expression also affects plant growth, development, and stress resilience. These findings provide a solid theoretical basis for comprehending plant molecular responses to alterations in light environments and indicate potential research avenues for enhancing crop performance under light stress. This study has some limitations. The light treatments were simplified laboratory regimes and did not fully reproduce the spectral complexity of natural canopy shade, particularly blue light variation. In addition, FR was applied continuously rather than as end-of-day FR (EOD-FR), which may better represent some vegetation proximity signals. Therefore, caution is needed when extrapolating these results to field shade conditions. Future studies should incorporate broader spectral designs and alternative far-red application modes.

CONCLUSIONS

This study carefully examined the impacts of light intensity and far-red light (FR) on the morphology, physiology, and molecular aspects of soybean seedlings. We elucidated the critical function of a low red/FR (R/FR) ratio in modulating the shade avoidance response of soybeans under shaded stress circumstances. Shade stress influenced plant morphology by enhancing height growth and inducing chlorophyll catabolism, as evidenced. The presence of FR, under both normal and low light circumstances, facilitated the recovery of soybeans from shade stress by affecting the pathways of photosensitive pigment signaling and hormone signaling. Nevertheless, the absence of control groups devoid of FR precludes us from asserting that these reactions were exclusively attributable to the presence of FR. This study offers novel insights into the mechanics of light signal control in crops and establishes a theoretical foundation for crop cultivation and management in shaded environments. Future research may investigate the precise processes of light-regulated essential genes and signaling pathways, especially the influence of the R/FR ratio in modulating these responses.

Author contribution

Conceptualization: X.Y-Z., Q.N-L. Writing-Original draft: X.Y-Z., Q.N-L. Formal analysis: Y.X. Resources: Y.X. Methodology: R.C., X.Y-S. Data curation: R.C., X.Y-S. Validation: G.G., L.H-Y. Investigation: G.G., L.H-Y. Project administration: Y.C-L., Y.G-W. Supervision: Y.C-L., Y.G-W.

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