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Transcriptomic analysis reveals light quality response and systemic nutrient allocation under monochromatic light environments in *Nicotiana tabacum*

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Abstract

Light quality plays a crucial role in plant growth and development. Nevertheless, mechanistic insights regarding the effects of monochromatic red light and monochromatic blue light on the development of tobacco seedlings remain limited. This study aims to elucidate how monochromatic red and blue light specifically affect the growth of tobacco seedlings, with a focus on systemic nutrient allocation from roots. By analyzing morphological, physiological and transcriptomic changes in both shoots and roots, we aim to fill the gaps in our understanding of the interaction between light quality and nutrient absorption mechanisms. Physiological results indicated that red light promoted seedling growth and enhanced nitrogen (N) and potassium (K) uptake, whereas blue light exerted the opposite effect by inhibiting growth and reducing nutrient uptake. Transcriptome analysis revealed that genes related to N uptake (e.g., *NRT1.1* was induced by red light and suppressed by blue light), N assimilation and K uptake were significantly regulated by monochromatic light in roots. Furthermore, various transcription factors (TFs) involved in phytohormone signal transduction were significantly regulated by blue and red light. These findings suggest that red and blue light may modulate phytohormone metabolism through TF-mediated pathways in tobacco roots. Overall, this study provides valuable insights into the effects of light quality on plant growth and underscores the importance of elucidating light-mediated mechanisms for improving crop yield and quality.

Keywords Tobacco, Light quality, Nitrogen uptake, Potassium uptake, Transcription factor

Introduction

Plant growth is shaped by light together with other environmental factors such as temperature and humidity. In addition to determining photosynthetic efficiency, specific wavebands within the spectrum strongly modulate photomorphogenesis, secondary metabolism, and phytohormone biosynthesis [1–3]. The solar spectrum spans red, green, blue, far-red, and ultraviolet regions. Red and blue light, in particular, have been interrogated extensively because they are efficiently absorbed by photosynthetic pigments. Distinct photoreceptors perceive these monochromatic signals: phytochromes sense red

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light, whereas cryptochromes and phototropins sense blue light [4]. Upon light perception, the receptors interact with the E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a central repressor of photomorphogenesis [5]. COP1 was first shown to directly interact with HY5 through its WD40 domain and mediate HY5 degradation in darkness via the ubiquitin-proteasome system [6]. Subsequently, a batch of light-signaling transcription factors were identified as COP1's targets such as BBX21, BBX24, and LAF1 [7–10]. Light also crosstalks with endogenous hormones, thereby coordinating plant growth. Several classical phytohormones—including ethylene (ETH), abscisic acid (ABA), gibberellins (GAs), cytokinins (CKs), jasmonates (JAs), and salicylic acid (SA)—respond to light quality [11, 12]. This reciprocal regulation weaves a complex light–hormone transcriptional network [13].

The absorption characteristics of primary photosynthetic pigments—chlorophylls and carotenoids—together with their associated photoreceptors determine the wavelength-dependent efficiency of both photosynthesis and photomorphogenesis [14]. In chicory, for example, the CO₂-assimilation rate is significantly lower under blue light than under red light [14]. Red light accelerates fruit ripening and carotenoid biosynthesis, whereas blue light inhibits hypocotyl elongation and promotes the accumulation of cytokinins and anthocyanins [15–17]. The *cryptochrome1/cryptochrome2* (*cry1 cry2*) mutant, defective in both cryptochrome 1 and cryptochrome 2, has been widely used to dissect blue- and red-light signalling pathways [17]. Nevertheless, most studies that address spectral quality have been performed with plants cultivated under monochromatic light. Closed-environment plant-production systems in modern agriculture now allow precise control of light, CO₂ concentration, nutrition, and water status.

Light-emitting diodes (LEDs) have emerged as the preferred illumination source because of their narrow emission spectra, long operational lifetime, low energy demand, and compact architecture. By selecting specific LED combinations, growers can tailor the spectral output to desired wave-bands, thereby increasing yield and improving quality traits in diverse crops [1]. Yorio et al. reported enhanced biomass in several vegetable species when monochromatic red light was supplemented with blue light [18]. Johkan et al. demonstrated that blue-LED irradiation of red-leaf lettuce seedlings improved seedling vigour and post-transplant performance [19]. Similarly, Samkumar et al. achieved a marked increase in bilberry anthocyanin content by providing supplemental red and blue light [20]. Collectively, these findings underscore the necessity of evaluating monochromatic-light effects on plant growth and development through integrated morphological, physiological, and biochemical analyses.

The effects of LED light quality have also been studied in tobacco. Tran et al. applied LEDs in their study and found that under green and blue light, the resistance of tobacco plants to *Pseudomonas syringae* pv. *Tomato* was significantly higher than that under white and red light [21]. Maity et al. showed that red and blue LEDs with a ratio of 1:1 remarkably enhance the in vitro flowering of tobacco [22]. However, how LED light application affects the physiology and transcription of tobacco seedlings is still unclear.

Previous work has shown that red and blue light significantly influence the abundance of photosynthetic pigments, leaf CO₂-assimilation rates, stomatal conductance, and the production of secondary metabolites such as phenylpropanoids, flavonoids, and anthocyanins [14]. At present, the vast majority of studies have focused on above-ground organs—leaves, stems, fruits, and sprouts [11, 12, 14, 20, 23]—whereas the influence of monochromatic light on root development, in particular on root architecture, biomass, and phytohormone content, remains largely unexplored [8, 24]. Roots are essential vegetative organs that acquire the mineral nutrients required for plant growth; among these, nitrogen (N) and potassium (K) are predominant. Nitrogen is absorbed as nitrate (NO₃[−]) or ammonium (NH₄⁺) and subsequently assimilated via the nitrogen-metabolism pathway [25], whereas potassium is taken up by K⁺-transport proteins [26]. However, little is known about how monochromatic light affects nutrient acquisition by roots.

To elucidate how monochromatic red and monochromatic blue light specifically affect early-stage growth of tobacco seedlings—with emphasis on systemic nutrient allocation from roots—we simultaneously profiled morphological, physiological, and transcriptomic responses in both shoots and roots. Our aim is to address the gaps in understanding the relationship between light quality and nutrient absorption mechanisms. Through integrating transcriptional data with physiological measurements, we have investigated the effects of monochromatic light on N metabolism and K absorption, while also analyzing the role of transcriptional regulation in light signaling. The results provide a theoretical foundation for the optimal utilization of monochromatic light to enhance crop yield and quality.

Materials and methods

Plant materials and light treatments

Common tobacco (*Nicotiana tabacum* L.) cultivar “Zhongchuan208 (ZC208)”, obtained from the Tobacco Genetics and Breeding Research (Northern) Center of China (Qingdao), was planted in trays filled with peat and vermiculite in a greenhouse under natural illumination (approx. 800–1200 μmol·m^{−2}·s^{−1} photosynthetic photon flux density, 14 h/10 h day/night cycle) with temperatures

maintained at 28 °C (day) and 22 °C (night). Twenty-five days after germination, uniformly sized tobacco seedlings were transplanted to a liquid medium (1/4 Hoagland's nutrient solution) and used for monochromatic light treatments. The pH of the solution was adjusted to 5.8 daily. The solution was fully replaced every three days, which adequately guaranteed oxygen availability for root ion absorption and eliminated the need for additional aeration. The seedlings were transferred to three chambers covered with photo-reflective sheets and equipped with top-mounted light-emitting diodes (LEDs), which generated white light, monochromatic blue light (peak wavelength: 448 nm), and monochromatic red light (peak wavelength: 660 nm), respectively. White light served as the control. All treatments were conducted under a 16 h light/8 h dark photoperiod, with daytime temperatures of 28 °C and nighttime temperatures of 22 °C. The dark period lasted from 10:00 PM to 6:00 AM the following day, during which the seedlings were grown in complete darkness. The light period ran from 6:00 AM to 10:00 PM, during which the seedlings were grown under red, blue, or white light. The above-canopy irradiance was maintained at 108 W/m². Five biological replicates were set up for each light treatment. Leaves and roots of the tobacco seedlings were separately harvested at 9:00 AM on the seventh day of light treatment. The samples were immediately frozen in liquid nitrogen and subsequently stored at -80 °C for subsequent analysis. The LEDs were purchased from Qingdao Cale Photoelectric Technology Co., Ltd., Qingdao, China.

Plant physiological analysis

Plant samples were dried at 70 °C for 72 h and weighed using a precision electronic balance (accuracy: 0.001 g) to determine dry weight (DW). To measure leaf area, the largest leaf from each tobacco seedling was selected, scanned at a consistent resolution, and its area was determined using Image J software. Root surface area was determined using the root analysis system WinRhizo (Regent Instruments, Montreal, QC, Canada), while the length of primary lateral roots was measured with a ruler and the numbers of primary and secondary lateral roots were counted; the average length of secondary lateral roots was calculated as (total root length - total length of primary lateral roots) / number of secondary lateral roots.

Determination of chlorophyll content

Clean and dry leaves of tobacco seedlings were used. The leaf veins were removed, and the leaves were cut into approximately 1 cm × 1 cm pieces and mixed thoroughly. Ten milliliters of 95% ethanol was added, followed by soaking in the dark for 24 h with 2–3 shakes during the period to ensure full infiltration of the sample. After the

sample tissue turned completely white, the supernatant was collected. Using 95% ethanol as the blank control, the absorbance was measured at 665 nm and 649 nm wavelengths (UV-1800, AOE Instruments Co., Ltd., Shanghai, China), respectively. Chlorophyll content was calculated using the Arnon formula: Chlorophyll a content (mg·g⁻¹) = (13.95 × A₆₆₅ - 6.88 × A₆₄₉) × V / (W × 1000); Chlorophyll b content (mg·g⁻¹) = (24.96 × A₆₄₉ - 7.32 × A₆₆₅) × V / (W × 1000). Wherein, "V" represents the volume of the extract (mL), and "W" represents the fresh weight of the sample (g). All analytical grade reagents were purchased from Shanghai Hushi Chemical Reagents Co., Ltd. (Shanghai, China).

Measurement of total N and K content

Nitrogen (N) and potassium (K) contents were determined according to the method described by [27]. In short, the desiccated plant samples were ground into powder; about 50 mg of the powder was digested by 5 ml of 98% H₂SO₄ and 1 ml of 30% H₂O₂ at 270 °C; after cooling, the digested sample was diluted to 100 mL with distilled water. All reagents were purchased from Shanghai Hushi Chemical Reagents Co., Ltd. (Shanghai, China). Potassium ion (K⁺) and total N concentrations were measured using an Optima 2100 DV inductively coupled plasma optical emission spectrometer (ICP-OES; PerkinElmer, USA) and an AutoAnalyser 3-AA3 continuous flow chemical analyzer (Bran + Luebbe, Norderstedt, Germany), respectively. For K⁺ detection with ICP - OES: RF power is 1300–1500 W, plasma gas (argon) flow 13–15 L·min⁻¹, auxiliary gas (argon) flow 0.2–1.0 L·min⁻¹, nebulizer gas flow 0.7–1.0 L·min⁻¹, uses axial viewing mode, analyzes K⁺ at 766.491 nm (primary) and 769.896 nm (secondary), sample uptake rate ~ 1.5 mL·min⁻¹, integration time 3–5 s, with µg·L⁻¹ level detection limit. For total N detection with AutoAnalyser 3 - AA3 continuous flow chemical analyzer: It oxidizes N to NO₃⁻ using alkaline potassium persulfate at ~ 120 °C for ~ 30 min, detects via colorimetry (540 nm, pink azo dye) or UV (220 nm, 275 nm correction), has reagent flow rate 1–3 mL/min with air - segmented flow, and ~ 0.1 mg·L⁻¹ detection limit.

Measurement of plasma membrane H⁺-ATPase activity

Fresh plant samples were crushed, and impurities were removed through filtration and centrifugation to obtain purified plasma membranes [28]. The activity of plasma membrane (PM) H⁺-ATPase was determined by quantifying the amount of inorganic phosphorus released during the reaction [29]. In short, 30 µL of purified plasma membranes suspension was incorporated into the 0.5 mL reaction system (30 mM BTP/MES, 5 mM MgSO₄, 50 mM KCl, 50 mM KNO₃, 1 mM Na₂MoO₄, 1 mM NaN₃, 0.02% (w/v) Brij 58, and 5 mM disodium-ATP

[substrate]), incubated at 30 °C for 30 min, then terminated with 1 mL of a mixture (2% [v/v] H₂SO₄, 5% [w/v] SDS, 0.7% [w/v] (NH₄)₂MoO₄) followed by 50 µL 10% (w/v) ascorbic acid. Ten minutes later, 1.45 mL of arsenite-citrate reagent (2% [w/v] sodium citrate, 2% [w/v] sodium arsenite, 2% [v/v] glacial acetic acid) was added, and after 30 min of color development, absorbance was measured at 720 nm. Activity—corrected against boiled-membrane controls—was expressed as phosphate liberated per g of membrane protein per h. All analytical grade reagents were purchased from Shanghai Hushi Chemical Reagents Co., Ltd. (Shanghai, China). All biological reagents used were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA), while H₂SO₄ and glacial acetic acid were obtained from Shanghai Hushi Chemical Reagents Co., Ltd. (Shanghai, China).

cDNA library preparation and transcriptome sequencing

Tobacco tissues from different light treatments were harvested for RNA sequencing (RNA-seq). Each sample had three biological replicates. RNA isolation and cDNA library construction were performed according to [30]. Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific, USA; Cat. No. 15596018). cDNA libraries were constructed using the TruSeq RNA Sample Preparation Kit (Illumina, USA; RS-930-2001). Transcriptome sequencing was carried out by Annoroad Gene Technology Co., Ltd. (Beijing, China) on an Illumina HiSeq 2500 platform with the 150 bp paired-end (PE) sequencing strategy.

RNA-seq data processing and transcriptome assembly

Low-quality bases, adapter sequences, and reads shorter than 40 bp were first removed with Trimmomatic v0.36 following the developer's guidelines [31]. STAR v2.7.8a software [32] was used to align the cleaned reads to the tobacco reference genome [33]. Transcript abundance was quantified and normalized with cuffquant and cuffnorm [34] and gene expression was reported as fragments per kilobase of transcript per million mapped fragments (FPKM). Genes with FPKM < 1 in all libraries were excluded from downstream statistical analyses.

RNA isolation and RT-qPCR assay

Roots RNAs were extracted following the treatment with monochromatic red and blue light, as detailed in Sect. 2.1. Approximately 100 mg of root tissue sample was grinded into powder and subjected to TRIzol reagent (Thermo Fisher Scientific, USA; Cat. No. 15596018). cDNAs were using generated via HiScript II Q RT Super-Mix kit with gDNA Wiper (Vazyme, Nanjing, China) following the manufacturer's instructions. RT-qPCR analysis were detected using ChamQ SYBR Color qPCR Master Mix (Vazyme) on a StepOne Plus Real-Time PCR

system (Applied Biosystems, Waltham, MA, USA). The tobacco ribosomal protein-coding gene *NtL25* (GenBank: L18908.1) [35] was used as a standard control. The relative transcript abundance was calculated using the 2^{-ΔΔCT} method. The data were presented as means ± se from 3 replicates, significance analysis was conducted using the Student's *t*-test. The primers used are listed in Table S1.

Statistical analysis

Differentially expressed genes (DEGs) were identified with DESeq2 using false-discovery rate (FDR) < 0.05 and |log₂(fold change)| > 1 as thresholds [36]. Gene Ontology (GO) enrichment was performed with the agriGO v2.0 toolkit [37] at FDR < 0.05, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was carried out with KOBAS 2.0 [38]. Principal-component analysis (PCA), volcano plots, heat maps, and hierarchical clustering dendrograms were generated with R software.

Results

Light quality regulates tobacco seedling growth and nutrient absorption

Light quality exerted a profound effect on the growth of tobacco seedlings (Fig. 1A).

Compared with the white light control, red light significantly increased root dry weight, whereas blue light reduced it (Fig. 1B). Above-ground biomass also responded spectrally: red light elevated shoot dry weight above control levels, but blue light elicited no significant change (Fig. 1C). Compared with the white-light control, red light significantly increased the leaf area of tobacco seedlings, while blue light exerted an inhibitory effect on leaf growth (Fig. 1D). In contrast, leaf number remained invariant across all treatments (Fig. S1A). Root nutritional status and plasma-membrane (PM) H⁺-ATPase activity were equally wavelength-dependent. As an important physiological indicator for assessing plant root absorption function, root surface area of tobacco seedlings was larger under red light and smaller under blue light (Fig. 1E). Compared with the white light control, red light increased the number of first and secondary lateral roots but reduced their length, whereas blue light exerted the opposite effects (Fig. 1F-I). Total nitrogen (N) and potassium (K) contents rose under red light and declined under blue light (Fig. 1J-K). Concordantly, PM H⁺-ATPase activity was upregulated by red light and downregulated by blue light (Fig. 1L). However, both red and white light suppressed chlorophyll accumulation in tobacco seedlings, with blue light exerting the strongest inhibitory effect (Fig. S1B, C).

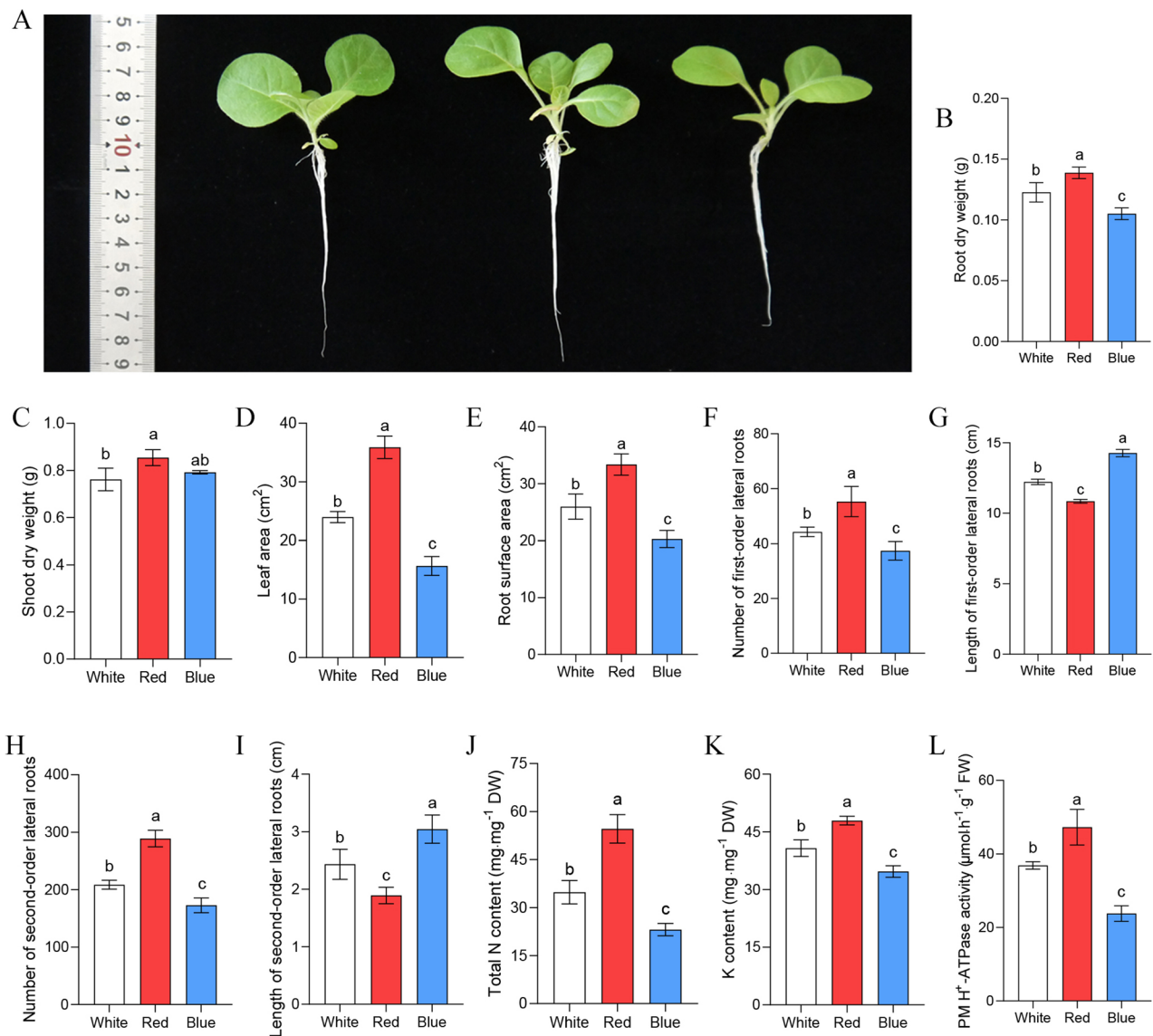


Fig. 1 Physiological responses of tobacco seedlings to monochromatic light treatments. **A** Morphology of tobacco seedlings grown under different light quality. **B, C** Biomass of root **B** and shoot **C** of tobacco seedlings treated with different light quality. **D, E** Leaf area **D** and root surface area **E** of tobacco seedlings treated with different light quality. **F-I** First-order and second-order lateral root number and length in response to different light qualities. **J-L** Total N content **J**, K content **K** and PM H⁺-ATPase **L** in activity of roots under different light conditions. $N \geq 3$, biological repetition. Different letters indicate significant differences between the data. Ordinary one-way ANOVA Tukey's multiple comparisons test, $p < 0.05$

Light quality reshapes the tobacco seedling transcriptome

Tobacco seedlings were planted in monochromatic red light, monochromatic blue light and white light environments, respectively. Shoots and roots were collected separately for RNA sequencing. The raw sequencing data of multiple samples shows high-quality metrics (Q20% $\geq 96.36\%$, Q30% $\geq 91.44\%$) with consistent read length and sufficient clean data, supporting subsequent bioinformatics analyses (Table S2). After filtering of genes with low expression levels (FPKM < 1 in all samples), a total of 29,094 genes were retained for analysis. Principal component analysis (PCA) analysis of all these genes revealed two distinct groups corresponding to shoot and

root samples, respectively (Fig. 2A). The first component (PC1; 62.57% variance explained) clearly separated the root from shoot samples, while the second component (PC2; 15.48% variance explained) discriminated among different light qualities. Pearson correlation matrix heatmap further confirmed distinct clustering patterns corresponding to different treatments (Fig. S2). Differentially expressed genes (DEGs) were identified between monochromatic lights and white light treatments in both shoots and roots (Table S3). Compared to the shoots under white light treatment (CKs), 1459 and 459 DEGs were identified in shoots under monochromatic red light

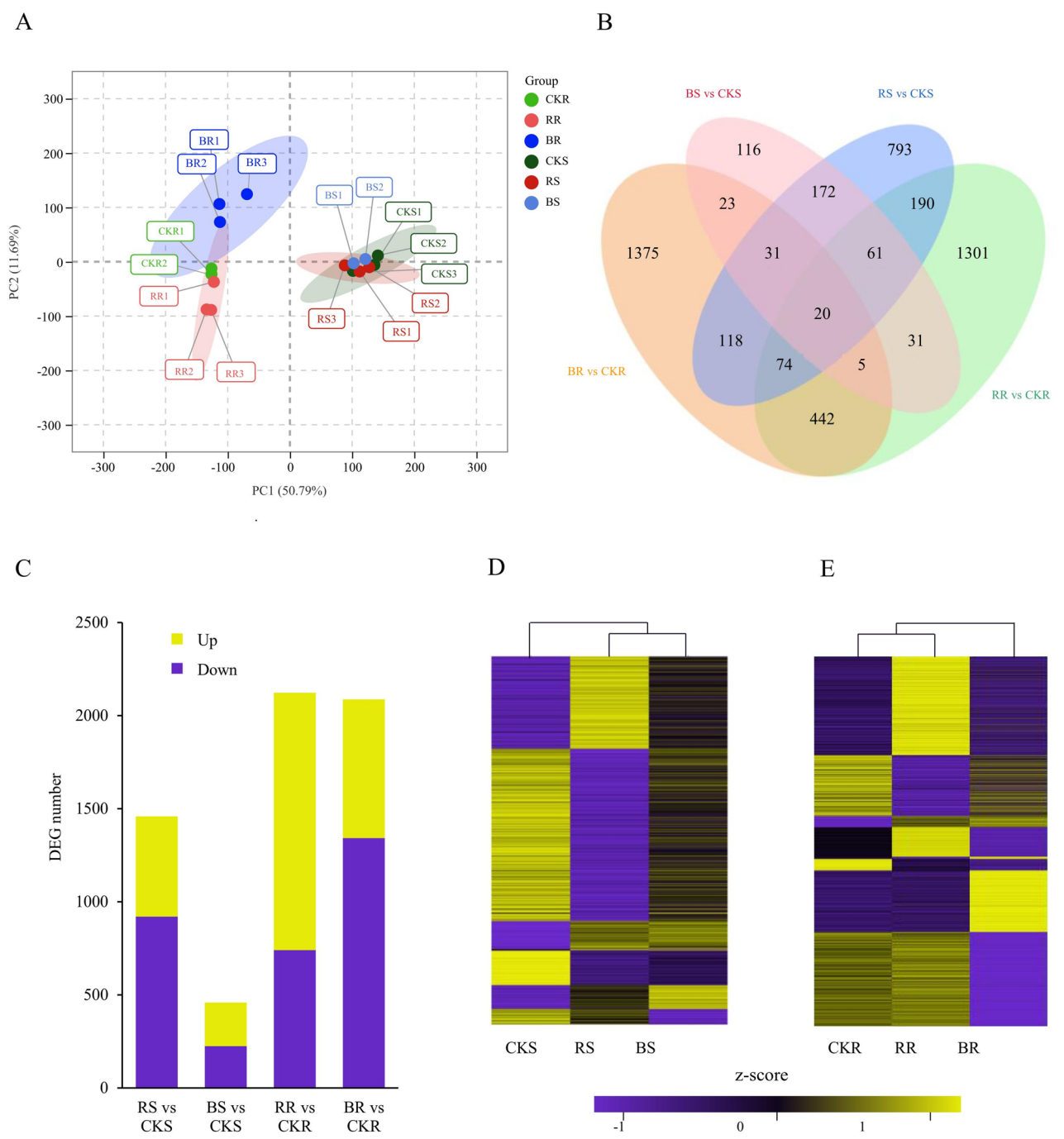


Fig. 2 Identification and analysis of DEGs under different light treatments. **A** PCA analysis of transcriptome data obtained from shoots and roots of tobacco seedlings under different light treatments. **B** Venn diagrams showing DEGs in different tissues under different light treatments. **C** Quantification of DEGs in different tissues under different light treatments. **D, E** Heatmaps depicting the expression levels of DEGs in shoots (**D**) and roots (**E**). The dendrogram is presented at the top. *Abbreviations:* CKS Shoots under white light treatment, RS Shoots under monochromatic red light treatment, BS Shoots under monochromatic blue light treatment, CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR roots under monochromatic blue light treatment

treatment (RS) and monochromatic blue (BS) light treatment, respectively (Fig. 2B). Compared to roots under white light treatment (CKR), 2124 and 2088 DEGs were identified in roots under monochromatic red light treatment (RR) and monochromatic blue (BR) light treatment, respectively (Fig. 2B). A total of 284 DEGs were common to RS and BS, while 541 DEGs were common to RR and BR (Fig. 2B). Compared with the CKS, 538 and 233 DEGs were upregulated in RS and BS respectively, whereas 921 and 226 genes were downregulated in RS and BS,

respectively (Fig. 2C). Compared with the CKR, 1382 and 745 genes were upregulated in RR and BR, respectively, with 742 and 1343 genes downregulated in RR and BR, respectively (Fig. 2C). The transcriptomic data were further subjected to hierarchical clustering analysis (Fig. 2D, E). Roots and shoots exhibited distinct dendrograms. For shoots, two distinct groups corresponded to the control and monochromatic light treatments, respectively (Fig. 2D). For roots, two distinct groups were identified: one corresponding to the red light and control treatments, and the other to the blue light treatment (Fig. 2E).

GO and KEGG analysis

GO analysis revealed that numerous GO terms were significantly enriched in the DEGs of RR/CKR, BR/CKR, RS/CKS, and BS/CKS (Table S4). Regarding biological processes, the GO term “transcription factor activity, sequence-specific DNA binding” was commonly enriched across all four tissue comparisons (Fig. 3A). Root-specific enriched terms (RR and BR) included “defense response”, “oligopeptide transport”, “oxidation-reduction process”, and “response to oxidative stress”, whereas “protein phosphorylation” was unique to red light treatments (RS and RR). The terms “protein ubiquitination” and “chitin catabolic process” were over-represented exclusively in RR, while “lignin catabolic process” and “nitrogen compound transport” were uniquely enriched in RS. Regarding molecular functions, “transcription factor activity, sequence-specific DNA binding” was the only GO term enriched in all four tissue comparisons (Fig. 3B). “Peroxidase activity”, “transporter activity”, and “xyloglucan: xyloglucosyl transferase activity” were enriched in BR, while “protein kinase activity”, “chitinase activity”, “DNA photolyase activity”, and “ubiquitin-protein transferase activity” were prevalent in RR. Furthermore, “protein dimerization activity” and “transferase activity” were enriched in BS and RS, respectively, whereas “oxidoreductase activity” was over-represented in both RR and BR. Regarding cellular components, DEGs in BR were significantly enriched in “extracellular region”, “cell wall”, and “membrane”, while DEGs in both RS and BS were mainly enriched in “extracellular region” (Fig. 3C).

GO analysis results indicated that transcriptional regulation played a central role in the response of both roots and shoots to monochromatic light. Monochromatic light treatment induced diverse physiological responses in roots, including oxidative stress response, defense response, altered transport activity, and oxidoreductase activity. Notably, numerous genes associated with “transport activity” were significantly regulated in BR, with the majority (81 out of 105) being downregulated (Table S4A, Fig. 3B).

Furthermore, KEGG pathway analysis revealed that the majority of enriched pathways were associated with metabolism, including “alpha-linolenic acid metabolism”, “phenylpropanoid biosynthesis”, “arginine and proline metabolism”, “starch and sucrose metabolism”, and “nucleotide metabolism” (Table S5, Fig. 4). Notably, several KEGG pathways in BR and RR were related to hormone signaling and biosynthesis (Table S5A, B, Fig. 4), such as “Plant hormone signal transduction”, “alpha-linolenic acid metabolism”, “zeatin biosynthesis”, and “carotenoid biosynthesis”, suggesting that the biosynthesis of endogenous hormones in tobacco roots is likely regulated by blue and red light. Additionally, “nitrogen metabolism” was enriched in both BR and RR (Table S5A, B, Fig. 4), indicating that nitrogen metabolism in roots may also be affected by monochromatic light.

Effects of monochromatic light on root nitrogen metabolism genes

Key genes involved in N uptake and assimilation were analyzed (Fig. 5A). Three nitrate transporter (NTR) genes encoding *NRT1.1* were significantly regulated by monochromatic light, with red light inducing and blue light suppressing their expression, respectively (Fig. 5B). Ammonium transporter (AMT) genes, including *AMT1-1*, *AMT1-2*, and *AMT2*, were upregulated by red light but downregulated by blue light (Fig. 5B). Monochromatic light also modulated N assimilation, although red and blue light exerted distinct effects on genes encoding key enzymes. Red light positively regulated nitrate reductase (*NR*), glutamine synthetase (*GS*), glutamate dehydrogenase (*GDH*), and glutamate synthase (*GOGAT*) genes, though only the regulation of *NR* and *GDH* genes reached a significant level (Fig. 5B). In contrast, blue light negatively regulated *GS* and *GOGAT* but had no apparent effect on *NR* and *GDH* (Fig. 5B). Further RT-qPCR experiments were conducted for validation. The results indicated that *NRT1.1a*, *NRT1.1b*, *AMT1-1* and *AMT1-2* expression was upregulated by red light stimulation and downregulated by blue light inhibition (Fig. 5C). Collectively, genes associated with both N uptake and assimilation in tobacco roots were significantly affected by monochromatic light. To validate this, we performed a correlation analysis, which revealed a strong positive correlation between the expression levels of key genes (*NRT1.1*, *AMT*, *GS* and *GOGAT*) and the total nitrogen content in the roots (Pearson's $r > 0.85$, $p < 0.05$), directly linking the transcriptional changes to the observed physiological phenotype. These results indicate that red light likely promotes N metabolism by stimulating nitrogen uptake and assimilation, while blue light probably suppresses these processes.

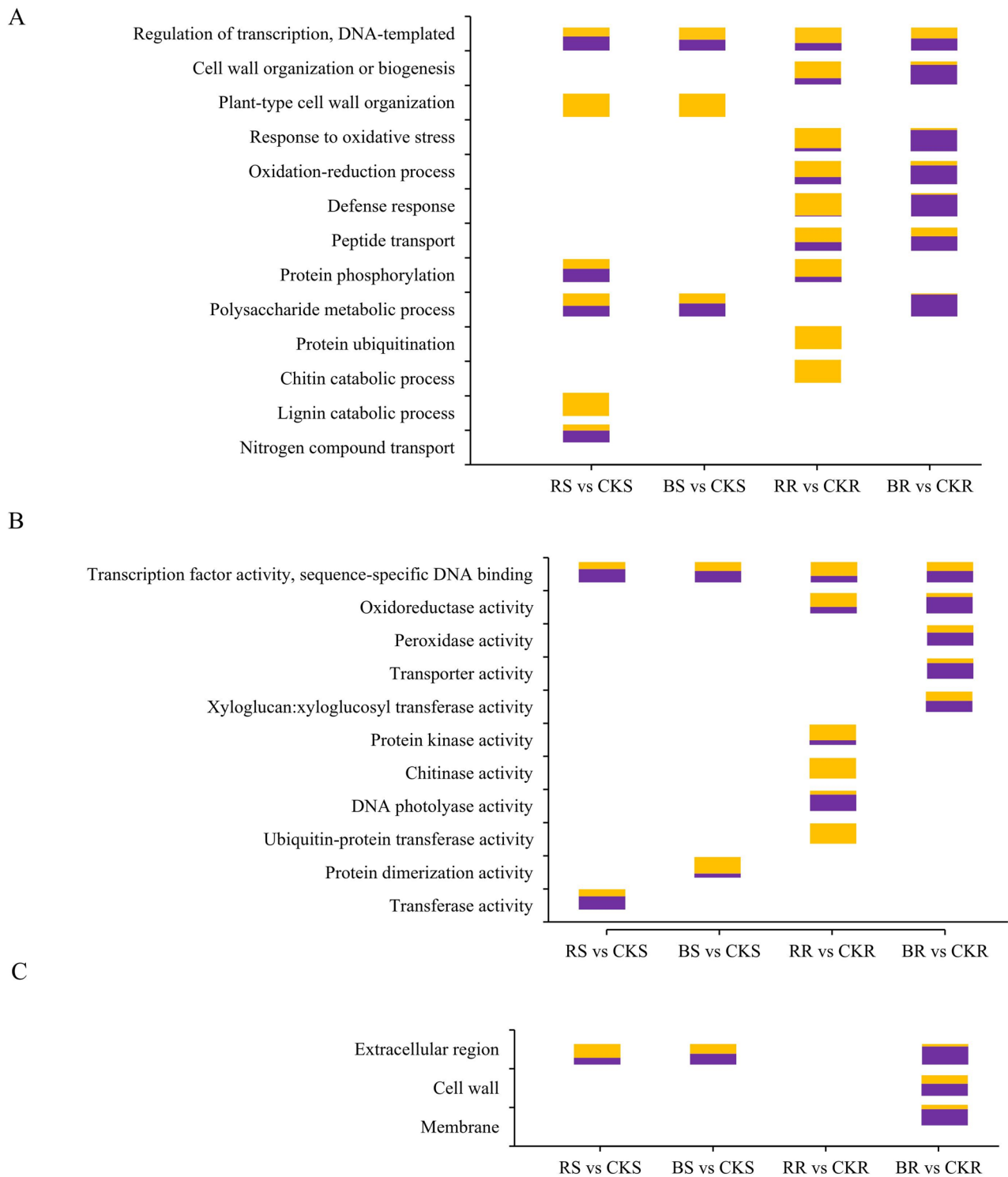


Fig. 3 Most representative GO terms enriched in DEGs. **A-C** Biological process (**A**), molecular function (**B**), cellular component (**C**). Orange represents the percentage of upregulated genes, while purple represents the percentage of downregulated genes. *Abbreviations:* CKS Shoots under white light treatment, RS Shoots under monochromatic red light treatment, BS Shoots under monochromatic blue light treatment, CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR Roots under monochromatic blue light treatment

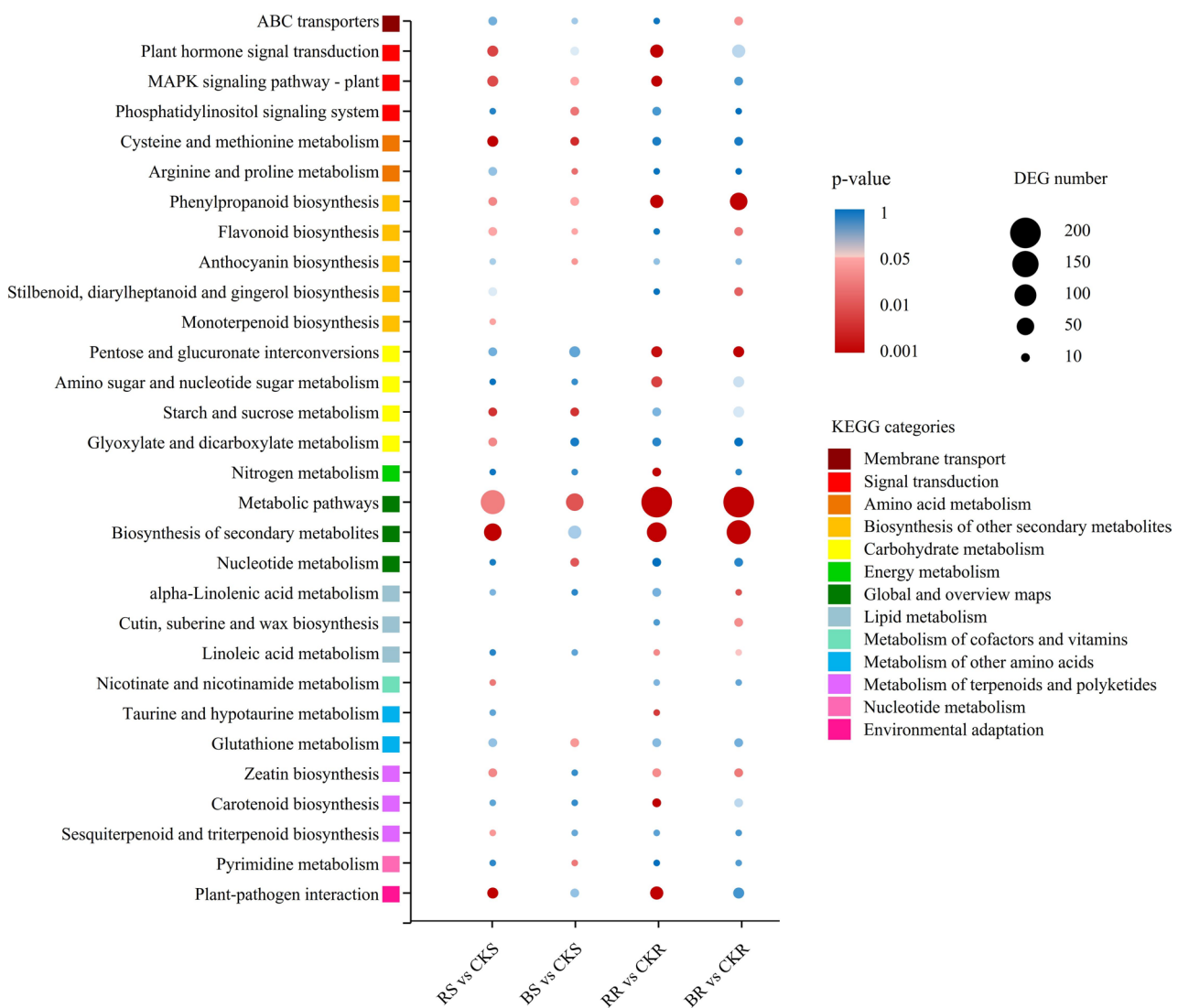


Fig. 4 KEGG pathway enrichment analysis of DEGs. Bubbles are colored by *p*-value as indicated in the color legend. Bubble size represents the number of DEGs involved in each KEGG pathway. The color of the square adjacent to each KEGG pathway denotes the corresponding KEGG category. Abbreviations: CKS Shoots under white light treatment, RS Shoots under monochromatic red light treatment, BS Shoots under monochromatic blue light treatment, CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR Roots under monochromatic blue light treatment

Effects of monochromatic light on root potassium metabolism genes

To investigate the effect of monochromatic light on K⁺ uptake, genes encoding K⁺ transport proteins were analyzed. The expression patterns of K⁺ channel genes (*AKT1*, *AKT2*, *KAT1*, *SORK* and *GORK*) and K⁺ transporter genes (*HAK5*, *KUP2*, *KUP3*, and *KUP7*) are presented in Fig. 6. The expression levels of *AKT1*, *AKT2* and *KAT1* in roots were extremely low (maximum FPKM < 5), precluding their use in subsequent analyses (Fig. 6A). Except for *HAK5* and *KUP3*, which were negatively regulated by blue light, other K⁺ transport genes were not affected by monochromatic light (Fig. 6A). Given that PM H⁺-ATPases also modulate K⁺ uptake [39], the transcription levels of PM H⁺-ATPase genes were determined.

Three PM H⁺-ATPase genes were expressed in roots. *PAM1* was not significantly regulated by either monochromatic red or blue light (Fig. 6A). One *PAM4* homolog was significantly induced by red light, while the other was significantly downregulated under blue light (Fig. 6A). Further RT-qPCR experiments were conducted for validation. The results indicated that *PAM4* expression was upregulated by red light stimulation and downregulated by blue light inhibition (Fig. 6B).

Key transcription factors involved in root responses to red and blue light

Compared with the white light control, 168 and 149 transcription factors (TFs) were differentially expressed in roots under monochromatic red light and

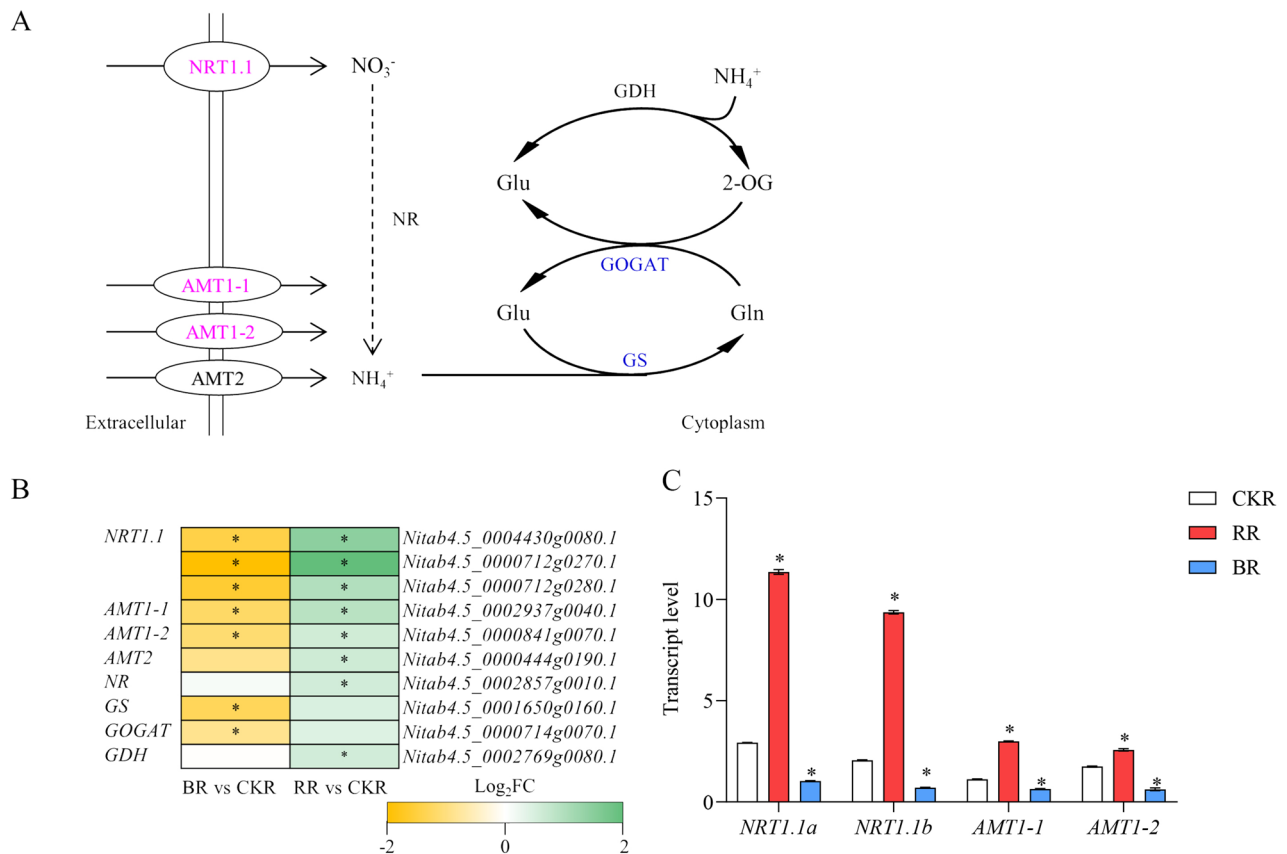


Fig. 5 Effects of monochromatic light treatments on N metabolism-related genes. **A** DEGs involved in the N metabolism pathway. Genes highlighted in blue were significantly regulated under blue light, while those highlighted in magenta were significantly regulated under both red and blue light. Solid arrows indicate specific steps or reaction processes in metabolic flux, and dotted arrows indicate multiple steps or reaction processes in metabolic flux. Abbreviations: AMT Ammonium transporter, GDH Glutamate dehydrogenase, GOGAT Glutamate synthase, GS Glutamine synthetase, NR Nitrate reductase, NRT Nitrate transporter. **B** Fold changes in the expression of N metabolism genes in response to monochromatic blue and red light treatments. * indicates significant differences (as specified in Materials and Methods). **C** Transcript levels of *NRT1.1a* (Nitab4.5_0004430g0080.1), *NRT1.1b* (Nitab4.5_0000712g0270.1), *AMT1-1* (Nitab4.5_0002937g0040.1) and *AMT1-2* (Nitab4.5_0000841g0070.1) were analyzed relative to that of *NtL25* (GenBank: L18908.1), a stable reference gene. * represents significant difference compared with white light treatment ($P < 0.01$, Student's *t*-test). Abbreviations: CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR Roots under monochromatic blue light treatment

monochromatic blue light treatments, respectively (Fig. 7A). Under red light treatment, the ERF family was the most abundant, followed by the WRKY, NAC, and MYB families. In contrast, the top three TF families under blue light treatment were the MYB, ERF, and bHLH families. A total of 45 TFs were co-regulated by both red and blue light treatments (Fig. 7B). The *BBX21* and *BBX24* genes were significantly regulated by blue light and red light, respectively. The *LAF1* gene (MYB family) was significantly upregulated in RR but downregulated in BR. Additionally, the *DOF15* gene (DOF family) and *SCL13* gene (GRAS family) were significantly induced by red light. All five genes are involved in light signal transduction. Furthermore, numerous phytohormone-responsive TFs were significantly regulated by monochromatic light (Fig. 7C). For instance, the *GAI* gene (GRAS family), *MYC1* gene (MYC family), and *TGA10* gene (TGA family) were induced by red

light, while the ABA-responsive element binding factor *ABF1* was induced by blue light. Notably, the *CRF2* gene (ERF family) was significantly suppressed in RR. Further RT-qPCR experiments were conducted for validation. The results indicated that *LAF* and *ERF2* expression was upregulated by red light stimulation and downregulated by blue light inhibition (Fig. 7D).

Discussion

Physiological analyses revealed that light quality significantly affects plant growth (Fig. 1). However, no notable difference in leaf number was observed among white, red, and blue light treatments (Fig. S1), indicating that seedlings under these light conditions were at the same developmental stage. Therefore, the influence of development-related genes [40] on transcriptomic analysis can be excluded.

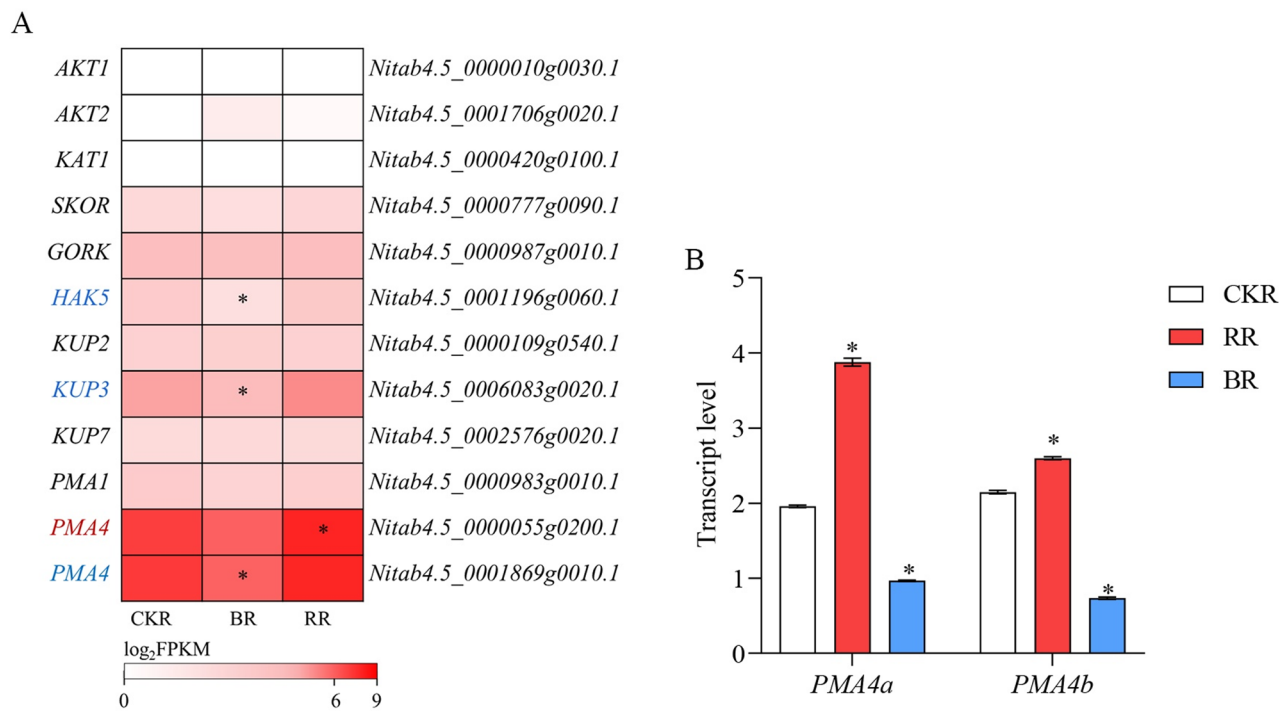


Fig. 6 Effects of monochromatic light treatments on K⁺ uptake-related genes. **A** Fold changes in the expression of K⁺ uptake-related genes in response to monochromatic blue and red light treatments. * indicates significant differences compared with the white light treatment (as specified in Materials and Methods). **B** Transcript levels of *PMA4a* (Nitab4.5_0000055g0200.1) and *PMA4b* (Nitab4.5_0001869g0010.1), were analyzed relative to that of *NtL25* (GenBank: L18908.1), a stable reference gene. * represents significant difference compared with white light treatment ($P < 0.01$, Student's *t*-test). Abbreviations: CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR Roots under monochromatic blue light treatment

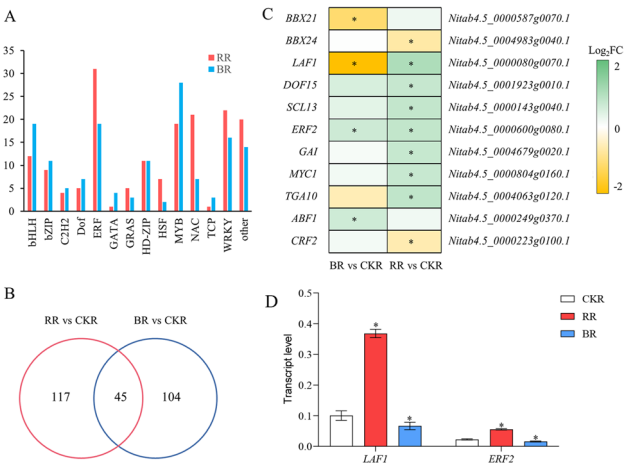


Fig. 7 Effects of monochromatic light treatments on the expression of TFs. **A** Categories of TFs regulated by monochromatic light. **B** Venn diagram of differentially expressed TFs. **C** Fold changes in the expression of differentially expressed TFs in response to monochromatic light treatments. * indicates significant differences compared with the white light treatment (as specified in Materials and Methods). **D** Transcript levels of *LAF1* (Nitab4.5_0000080g0070.1) and *ERF2* (Nitab4.5_0000600g0080.1) were analyzed relative to that of *NtL25* (GenBank: L18908.1), a stable reference gene. * represents significant difference compared with white light treatment ($P < 0.01$, Student's *t*-test). Abbreviations: CKR Roots under white light treatment, RR Roots under monochromatic red light treatment, BR Roots under monochromatic blue light treatment

Monochromatic light significantly influenced tobacco seedling growth

Roots exhibited greater sensitivity to monochromatic light than shoots, as evidenced by the higher number of DEGs in roots compared with shoots (Fig. 2B-C). In shoots, the number of DEGs under red light treatment was approximately three times that under blue light (1459 vs. 459), consistent with the observation that red light has a more pronounced impact on shoot biomass (Fig. 1C). In roots, the number of DEGs was comparable between red and blue light treatments (2124 vs. 2088), with only 541 DEGs shared by both light treatments. Monochromatic red and blue light exerted opposite effects on root growth (Fig. 1B). Interestingly, roots showed a greater transcriptomic response to light quality than shoots, with a higher number of DEGs. This heightened sensitivity in a non-photosynthetic organ could be attributed to several potential mechanisms. Shoots, as the primary light-perceiving organs, may transmit long-distance signals, such as auxin, cytokinin, or sucrose, to the roots, thereby modulating gene expression. It is also possible that roots possess their own photoreceptors that can perceive light transmitted through the soil or stem, although this signaling would be weaker than in shoots. The complex interplay of these systemic signals likely

leads to the extensive transcriptional reprogramming observed in the roots.

Red and blue light exert opposite effects on N metabolism in roots

Both transcriptomic and physiological data indicated that red light promoted N metabolism, whereas blue light inhibited it. KEGG pathway enrichment analysis revealed that both red and blue light treatments modulated N metabolism in tobacco roots (Fig. 4). This finding was further supported by significant alterations in total N content in the roots under different light treatments (Fig. 1J). N metabolism primarily involves N uptake and assimilation processes (Fig. 5A).

Plants acquire N from the soil primarily in the forms of nitrate (NO_3^-) and ammonium (NH_4^+), which are facilitated by NRTs and AMTs, respectively [41, 42]. Nitrogen assimilation initiates with the reduction of NO_3^- to NH_4^+ —a process sequentially catalyzed by NR and nitrite reductase (NiR) [43, 44]—and culminates in the incorporation of NH_4^+ into amino acids via the GS/GOGAT cycle [45]. NR, NiR, GS, and GOGAT are key enzymes involved in N assimilation. GDH, an enzyme catalyzing the reversible formation of glutamate from NH_4^+ and 2-oxoglutarate, also plays a prominent role in N assimilation [46].

Under blue light treatment, the expression of NO_3^- transporter genes (*NRT1.1*) and NH_4^+ transporter genes (*AMT1-1* and *AMT1-2*) was downregulated (Fig. 5B, C), indicating a reduction in N uptake [47, 48]. Conversely, red light treatment induced the expression of *NRT1.1*, *AMT1-1*, *AMT1-2*, and *AMT2*, suggesting that red light enhances N uptake [49–52]. The GS/GOGAT cycle is the primary pathway for N assimilation in crops [45, 53]; thus, the observed suppression of GS and GOGAT indicated that blue light negatively regulated N assimilation. NR, which catalyzes the initial step of nitrate assimilation and is upregulated by NO_3^- , functions as a rate-limiting enzyme in NO_3^- assimilation [43]. Red light positively regulated NR, suggesting that NO_3^- assimilation was enhanced by red light, rather than merely NO_3^- uptake. GDH expression was also induced by red light, likely facilitating the removal of excess NH_4^+ generated from increased NO_3^- reduction and NH_4^+ uptake, thereby preventing the accumulation of toxic NH_4^+ levels within cells [45, 54]. Collectively, these results indicate that red light likely promotes N metabolism by enhancing N uptake and assimilation, while blue light probably suppresses N uptake and assimilation, thereby inhibiting N metabolism.

Red and blue light exert opposite effects on K uptake in roots

Similar to N, K^+ is an indispensable nutrient for plant growth, and the uptake of N and K^+ in plants is subject to co-regulation [55, 56]. The activation of PM H^+ -ATPase under red light, which energizes the plasma membrane, provides the driving force for both K^+ channels and some N transporters, offering a mechanistic basis for their coordinated enhancement. K^+ transport proteins involved in transmembrane K^+ transport mainly belong to the Shaker K^+ channel family (AKT1, AKT2, KAT1, KAT2, SORK, and GORK) and the HAK/KUP/KT K^+ transporter family (HAK5, KUP2, KUP3, and KUP7) [26]. AKT1, SKOR, and AKT2 are responsible for K^+ uptake into root cells, loading into xylem sap, and transport through the phloem, respectively [57–60]. KAT1, KAT2, and GORK regulate stomatal movements by controlling K^+ fluxes in guard cells [61, 62]. Several KUP/HAK/KT transporters, including HAK5, KUP2, KUP3, and KUP7, were expressed in roots and contribute to K^+ uptake [26, 63]. PM H^+ -ATPases also modulate K^+ uptake [39].

Increased PM H^+ -ATPase activity leads to H^+ extrusion, causing extracellular acidification [64], which in turn activates K^+ channels and promotes K^+ transport and uptake [65–67]. PM H^+ -ATPase activity is positively associated with K^+ uptake efficiency [29, 67]. Compared with the control, red light significantly increased root K^+ content (Fig. 1K), yet none of the K^+ transport protein genes were significantly regulated (Fig. 6). Given that PM H^+ -ATPases can regulate K^+ uptake efficiency, the expression and activity of PM H^+ -ATPases were determined to clarify the mechanism underlying enhanced K^+ uptake. As expected, PM H^+ -ATPase activity was significantly increased (Fig. 1L), and the expression of PM H^+ -ATPase genes was significantly upregulated by red light (Fig. 6). In contrast to red light, blue light significantly downregulated *HAK5* and *KUP3* genes. Meanwhile, both the transcription and activity of PM H^+ -ATPases were suppressed by blue light (Figs. 1L and 6). Collectively, red light likely enhances K^+ uptake by activating PM H^+ -ATPases, while blue light probably reduces K^+ uptake by suppressing the transcription of K^+ transporters and the activity of PM H^+ -ATPases. This activation could be regulated at both transcriptional and post-translational levels. Our data show upregulation of a *PAM4* gene, but light-induced hormonal changes (e.g., auxin) are also known to trigger rapid, non-transcriptional activation of PM H^+ -ATPases through phosphorylation, providing another layer of control.

Monochromatic light regulate seedling growth via TF-mediated pathways

A total of 162 and 149 differentially expressed TFs were identified in RR and BR, respectively, with only 45 TFs

shared between the tissue comparisons (Fig. 7A–B). These results suggest that red and blue light likely regulate distinct biological processes. Among the identified differentially expressed TFs, BBX21, BBX24, LAF1, DOF15, and SCL13 are known to be involved in light signal transduction [7, 10, 68, 69]. BBX21, BBX24, and LAF1 function as regulators of seedling photomorphogenesis—particularly in root development—by interacting with COP1 and HY5, the major light signaling regulators in plants [68–71]. DOF15 and SCL13 participate in the transmission of phytochrome signals and regulate photomorphogenesis [72, 73]. These TFs were significantly regulated by red and blue light (Fig. 7C, D), underscoring the critical role of transcriptional regulation in light signal transduction in tobacco roots.

Numerous studies have reported that light quality modulates plant hormone levels [11, 12]. Herein, key TFs involved in plant hormone signal transduction were regulated by monochromatic light. For instance, ERF2, GAI, MYC1, TGA10, CRF2, and ABF1—critical regulators of ethylene (ETH), gibberellin (GA), jasmonic acid (JA), salicylic acid (SA), cytokinin (CT), and abscisic acid (ABA) signaling pathways, respectively [74–79]—exhibited differential regulation in response to red and blue light (Fig. 7C, D). KEGG pathway analysis revealed that pathways associated with hormone biosynthesis, such as alpha-linolenic acid metabolism (JA biosynthesis), zeatin biosynthesis (CT biosynthesis), and carotenoid biosynthesis (ABA biosynthesis), were enriched in DEGs from the RR/CKR and BR/CKR comparisons (Fig. 4, Table S5). Furthermore, the “plant hormone signal transduction” pathway was also enriched in both groups of DEGs. For instance, the induction of cytokinin response factor CRF2 under red light aligns with cytokinin’s known role in promoting cell division and biomass accumulation, consistent with the increased root and shoot dry weight observed. Conversely, the induction of ABA-responsive factor ABF1 by blue light could contribute to the observed growth inhibition, as ABA often acts as a growth antagonist. Collectively, these results indicate that monochromatic light likely regulates the biosynthesis and signaling of plant hormones through TF-mediated pathways.

Prospects for future research

The findings of this study provide valuable insights into the effects of monochromatic red and blue light on the growth and nutrient uptake of tobacco seedlings. However, it is important to acknowledge the study’s limitations and consider the implications for future research. This experiment was conducted under controlled environmental conditions, which may not fully simulate the complex scenarios encountered in natural or large-scale agricultural settings. Therefore, further studies are

warranted to evaluate the generalizability of our findings to other plant species and diverse environmental conditions. Additionally, integrating multi-omics data with physiological measurements will be crucial for developing a comprehensive understanding of light-mediated regulation of plant growth and nutrient dynamics. Functional validation approaches, such as mutant screening, gene knockout, and overexpression assays, are further essential to validate the proposed hypotheses. For long-distance mobile mRNAs transported from shoots to roots under red and blue light, grafting experiments may offer insights into the functions of specific genes in light responses and nutrient dynamics. Notably, the roles of phytochromes and cryptochromes in roots remain understudied, with most research focusing on shoot organs like leaves and stems. Future studies could explore their functions in root growth and environmental adaptation, offering new insights for optimizing cultivation via light regulation. Ultimately, these findings should inform the development of strategies to optimize light quality in agricultural practices, potentially leading to improved crop yield and quality.

From an agricultural perspective, these findings have practical implications. For example, in controlled environment agriculture like vertical farms, supplementing red light could be a strategy to enhance nutrient use efficiency, particularly for nitrogen and potassium, potentially reducing fertilizer costs and environmental runoff. Conversely, a higher proportion of blue light might be used to produce more compact seedlings for easier transplanting. Fine-tuning light spectrum could become a powerful tool to optimize crop growth, yield, and quality.

Conclusion

This study elucidates the distinct effects of monochromatic red and blue light on the growth and nutrient uptake of tobacco seedlings, demonstrating that red light promotes biomass accumulation and nutrient assimilation, whereas blue light exerts the opposite effect. Through transcriptomic analysis, we identified key genes and transcription factors regulated by light quality, suggesting the existence of a complex regulatory network modulating plant development. Despite being preliminary, our findings provide meaningful insights for the optimization of agricultural practices and further our knowledge of light-regulated plant growth and development.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-07974-w>.

Supplementary Material 1.

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Not applicable.

Authors' contributions

J.D.: conception, data curation, writing-original draft. Y.L.: data acquisition and analysis, writing-original draft. Z.X.: investigation, validation, methodology. X.H.: reviewed and edited the manuscript. H.S.: data acquisition and analysis. M.G.: investigation. Y.Z.: funding acquisition, reviewed and edited the manuscript. L.M.: design of the work, reviewed and edited the manuscript. All authors read and approved the final manuscript. J.D. and Y.L. contributed equally to this work. Correspondence to Y.Z. and L.M.

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Data availability

The raw sequence reads have been deposited in the NCBI sequence read archive (SRA) database under the accession number (Bioproject ID: PRJNA983570). All other data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

All experiments were conducted using *Nicotiana tabacum* L. cv. Zhongchuan208, a flue-cured tobacco variety obtained from the Tobacco Genetics and Breeding Research (Northern) Center of China. No wild plant material collection was performed, and thus no field permits, CITES certificates, or protected-species licenses were required. All experiments in this study complied with the General requirements for plant biosafety laboratory (GB/T 27428–2022).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Agarwal A, Gupta SD. Impact of light-emitting diodes (LEDs) and its potential on plant growth and development in controlled-environment plant production system. *Curr Biotechnol*. 2016;5(1):28–43.
- Murthy HN, Joseph KS, Paek KY, Park SY. Light as an elicitor for enhanced production of secondary metabolites in plant cell, tissue, and organ cultures. *Plant Growth Regul*. 2024;104(1):31–49.
- Zhang T, Zhang Y, Yang J, Wang X, Yang Q, Zhu X, et al. Transcriptome and targeted metabolome analysis revealed the effects of combined red and blue light on the growth and secondary metabolism of *Scutellaria baicalensis* Georgi. *Ind Crops Prod*. 2022;188:115598.
- Casal JJ. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochem Photobiol*. 2000;71(1):1–11.
- Osterlund MT, Wei N, Deng XW. The roles of photoreceptor systems and the COP1-targeted destabilization of HY5 in light control of Arabidopsis seedling development. *Plant Physiol*. 2000;124(4):1520–4.
- Han X, Huang X, Deng XW. The photomorphogenic central repressor COP1: conservation and functional diversification during evolution. *Plant Commun*. 2020;1(3):100044.
- Datta S, Johansson H, Hettiarachchi C, Irigoyen ML, Desai M, Rubio V, et al. LZFI/SALT TOLERANCE HOMOLOG3, an Arabidopsis B-box protein involved in light-dependent development and gene expression, undergoes COP1-mediated ubiquitination. *Plant Cell*. 2008;20(9):2324–38.
- Duan X, Xu S, Xie Y, Li L, Qi W, Parizot B, et al. Periodic root branching is influenced by light through an HY1-HY5-auxin pathway. *Curr Biol*. 2021;31(17):3834–e38473835.
- Jiang Y, Zeng B, Zhao H, Zhang M, Xie S, Lai J. Genome-wide transcription factor gene prediction and their expression tissue-specificities in maize. *J Integr Plant Biol*. 2012;54(9):616–30.
- Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, et al. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev*. 2003;17(21):2642–7.
- Li Y, Liu Z, Shi Q, Yang F, Wei M. Mixed red and blue light promotes tomato seedlings growth by influencing leaf anatomy, photosynthesis, CO₂ assimilation and endogenous hormones. *Sci Hort*. 2021;290:110500.
- Xiang N, Zhao Y, Wang S, Guo X. The modulation of light quality on carotenoids in maize (*Zea Mays* L.) sprouts. *Food Chem (Oxf)*. 2022;5:100128.
- Kilian J, Whitehead D, Horak J, Wanke D, Weini S, Batistic O, et al. The atgenexpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *Plant J*. 2007;50(2):347–63.
- Landi M, Zivcak M, Sytar O, Brestic M, Allakhverdiev SI. Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: A review. *Biochimica et biophysica acta (BBA) - Bioenergetics*. 2020; 1861(2):148131.
- Chen D, Li Z, Pan R, Wang X. Anthocyanin accumulation mediated by blue light and cytokinin in Arabidopsis seedlings. *J Integr Plant Biol*. 2006;48(4):420–5.
- Zhang J, Zhang Y, Song S, Su W, Hao Y, Liu H. Supplementary red light results in the earlier ripening of tomato fruit depending on ethylene production. *Environ Exp Bot*. 2020;175:104044.
- Zhao X, Yu X, Foo E, Symons GM, Lopez J, Bendehakalu KT, et al. A study of Gibberellin homeostasis and cryptochrome-mediated blue light inhibition of hypocotyl elongation. *Plant Physiol*. 2007;145(1):106–18.
- Yorio NC, Goins GD, Kagle HR, Wheeler RM, Sager JC. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience*. 2001;36(2):380–3.
- Johkan M, Shoji K, Goto F, Hashida SN, Yoshihara T. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience: Publication Am Soc Hortic Sci*. 2010;45(12):414–5.
- Samkumar A, Jones D, Karppinen K, Dare AP, Sipari N, Espley RV, et al. Red and blue light treatments of ripening Bilberry fruits reveal differences in signalling through abscisic acid-regulated anthocyanin biosynthesis. *Plant Cell Environ*. 2021;44(10):3227–45.
- Tran BQ, Nguyen AT, Jung S. Different light wavelengths differentially influence the progression of the hypersensitive response induced by pathogen infection in tobacco. *Antioxidants*. 2025;14(8):954.
- Maity TR, Samanta A, Jana D, Saha B, Datta S. *In vitro* flowering of tobacco induced by light emitting diode. *Indian J Biotechnol*. 2016;15(3):440–2.
- Kim EY, Park SA, Park BJ, Lee Y, Oh MM. Growth and antioxidant phenolic compounds in Cherry tomato seedlings grown under monochromatic light-emitting diodes. *Hortic Environ Biotechnol*. 2014;55(6):506–13.
- Meng L, Song W, Liu S, Dong J, Zhang Y, Wang C, et al. Light quality regulates lateral root development in tobacco seedlings by shifting auxin distributions. *J Plant Growth Regul*. 2015;34(3):574–83.
- Lea PJ, Mifflin BJ. Glutamate synthase and the synthesis of glutamate in plants. *Plant Physiol Biochem*. 2003;41(6):555–64.
- Wang Y, Wu WH. Potassium transport and signaling in higher plants. *Annu Rev Plant Biol*. 2013;64:451–76.
- Cai J, Chen L, Qu H, Lian J, Liu W, Hu Y, et al. Alteration of nutrient allocation and transporter genes expression in rice under N, P, K, and Mg deficiencies. *Acta Physiol Plant*. 2012;34(3):939–46.
- Yan F, Zhu Y, Müller C, Zörb C, Schubert S. Adaptation of H⁺-pumping and plasma membrane H⁺ ATPase activity in proteoid roots of white lupin under phosphate deficiency. *Plant Physiol*. 2002;129(1):50–63.
- Zhang M, Wang Y, Chen X, Xu F, Ding M, Ye W, et al. Plasma membrane H⁺-ATPase overexpression increases rice yield via simultaneous enhancement of nutrient uptake and photosynthesis. *Nat Commun*. 2021;12(1):735.
- He B, Meng L, Tang L, Qi W, Hu F, Lv Y, et al. The landscape of alternative splicing regulating potassium use efficiency in *Nicotiana tabacum*. *Front Plant Sci*. 2021;12:774829.
- Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics*. 2014;30(15):2114–20.
- Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15–21.
- Edwards KD, Fernandez Pozo N, Drake Stowe K, Humphry M, Evans AD, Bombarely A, et al. A reference genome for *Nicotiana tabacum* enables map-based cloning of homeologous loci implicated in nitrogen utilization efficiency. *BMC Genomics*. 2017;18(1):448.

34. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with tophat and cufflinks. *Nat Protoc*. 2012;7(3):562–78.
35. Schmidt GW, Delaney SK. Stable internal reference genes for normalization of real-time RT-PCR in tobacco (*Nicotiana tabacum*) during development and abiotic stress. *Mol Genet Genomics*. 2010;283(3):233–41.
36. Love MI, Huber W, Anders S. Moderated Estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
37. Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, et al. AgriGO v2.0: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res*. 2017;45(W1):W122–9.
38. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res*. 2011;39:W316–322. (Web Server issue).
39. Li J, Guo Y, Yang Y. The molecular mechanism of plasma membrane H⁺-ATPases in plant responses to abiotic stress. *J Genet Genomics*. 2022;49(8):715–25.
40. Tao S, Zhu Y, Pan Y, Zhang Z, Huang L. Enhancement of respiratory metabolism of the Pentose phosphate pathway (PPP) strengthens the chilling tolerance of postharvest Papaya fruit stored at 1 °C. *Postharvest Biol Technol*. 2022;191:111988.
41. Huang NC, Liu KH, Lo HJ, Tsay YF. Cloning and functional characterization of an Arabidopsis nitrate transporter gene that encodes a constitutive component of low-affinity uptake. *Plant Cell*. 1999;11(8):1381–92.
42. Loqué D, Von Wirén N. Regulatory levels for the transport of ammonium in plant roots. *J Exp Bot*. 2004;55(401):1293–305.
43. Campbell WH. Nitrate reductase structure, function and regulation: bridging the gap between biochemistry and physiology. *Annu Rev Plant Physiol Plant Mol Biol*. 1999;50:277–303.
44. Campbell WH, Kinghorn KR. Functional domains of assimilatory nitrate reductases and nitrite reductases. *Trends Biochem Sci*. 1990;15(8):315–9.
45. Lancien M, Gadal P, Hodges M. Enzyme redundancy and the importance of 2-oxoglutarate in higher plant ammonium assimilation. *Plant Physiol*. 2000;123(3):817–24.
46. Miyashita Y, Good AG. Glutamate deamination by glutamate dehydrogenase plays a central role in amino acid catabolism in plants. *Plant Signal Behav*. 2008;3(10):842–3.
47. Wang R, Xing X, Wang Y, Tran A, Crawford NM. A genetic screen for nitrate regulatory mutants captures the nitrate transporter gene *NRT1.1*. *Plant Physiol*. 2009;151(1):472–8.
48. Yuan L, Loqué D, Kojima S, Rauch S, Ishiyama K, Inoue E, et al. The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the Spatial arrangement and biochemical properties of AMT1-type transporters. *Plant Cell*. 2007;19(8):2636–52.
49. Hoque MS, Masle J, Udvardi MK, Ryan PR, Upadhyaya NM. Over-expression of the rice *OsAMT1-1* gene increases ammonium uptake and content, but impairs growth and development of plants under high ammonium nutrition. *Funct Plant Biol*. 2006;33(2):153–63.
50. Liu H, Zhang J, Hu H, Huang Y, Xu C, Hu Y, et al. Overexpression of *JrAMT2* in walnut (*Juglans regia* L.) rootstock enhances nitrogen level in grafted wild-type walnut scions. *Sci Hort*. 2021;280:109928.
51. Liu K, Huang C, Tsay Y. CHL1 is a dual-affinity nitrate transporter of *Arabidopsis* involved in multiple phases of nitrate uptake. *Plant Cell*. 1999;11(5):865–74.
52. Zhu Y, Huang X, Hao Y, Su W, Liu H, Sun G, et al. Ammonium transporter (BcAMT1.2) mediates the interaction of ammonium and nitrate in *brassica campestris*. *Front Plant Sci*. 2019;10:1776.
53. Mangelsen E, Wanke D, Kilian J, Sundberg E, Harter K, Jansson C. Significance of light, sugar, and amino acid supply for diurnal gene regulation in developing barley Caryopses. *Plant Physiol*. 2010;153(1):14–33.
54. Zhou Y, Bai L, Song C. Ammonium homeostasis and signaling in plant cells. *Sci Bull*. 2015;60(8):741–7.
55. Fang XZ, Liu XX, Zhu YX, Ye JY, Jin CW. The K⁺ and NO³⁻ interaction mediated by NITRATE TRANSPORTER1.1 ensures better plant growth under K⁺-limiting conditions. *Plant Physiol*. 2020;184(4):1900–16.
56. Ten Hoopen F, Cuin TA, Pedas P, Hegelund JN, Shabala S, Schjoerring JK, et al. Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: molecular mechanisms and physiological consequences. *J Exp Bot*. 2010;61(9):2303–15.
57. Gajdanowicz P, Michard E, Sandmann M, Rocha M, Corrêa LG, Ramírez-Aguilar SJ, et al. Potassium (K⁺) gradients serve as a mobile energy source in plant vascular tissues. *Proc Natl Acad Sci U S A*. 2011;108(2):864–9.
58. Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, et al. Identification and disruption of a plant shaker-like outward channel involved in K⁺ release into the xylem Sap. *Cell*. 1998;94(5):647–55.
59. Pilot G, Gaymard F, Mouline K, Chérel I, Sentenac H. Regulated expression of Arabidopsis shaker K⁺ channel genes involved in K⁺ uptake and distribution in the plant. *Plant Mol Biol*. 2003;51(5):773–87.
60. Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, et al. A protein kinase, interacting with two calcineurin B-like proteins, regulates K⁺ transporter AKT1 in *Arabidopsis*. *Cell*. 2006;125(7):1347–60.
61. Hiya HJ, Nakashima Y, Takeuchi A, Nakamura T, Nakamura Y, Murata Y, et al. Outward-rectifying potassium channels GORK and SKOR function in regulation of root growth under salt stress in *Arabidopsis Thaliana*. *J Plant Physiol*. 2024;302:154322.
62. Philippar K, Ivashikina N, Ache P, Christian M, Lüthen H, Palme K, et al. Auxin activates KAT1 and KAT2, two K⁺-channel genes expressed in seedlings of *Arabidopsis Thaliana*. *Plant J*. 2004;37(6):815–27.
63. Ankit A, Kamali S, Singh A. Genomic & structural diversity and functional role of potassium (K⁺) transport proteins in plants. *Int J Biol Macromol*. 2022;208:844–57.
64. Ren Z, Suolang B, Fujiwara T, Yang D, Saijo Y, Kinoshita T, et al. Promotion and upregulation of a plasma membrane proton-ATPase strategy: principles and applications. *Front Plant Sci*. 2021;12:749337.
65. Huang Y, Cao H, Yang L, Chen C, Shabala L, Xiong M, et al. Tissue-specific respiratory burst oxidase homolog-dependent H₂O₂ signaling to the plasma membrane H⁺-ATPase confers potassium uptake and salinity tolerance in *Cucurbitaceae*. *J Exp Bot*. 2019;70(20):5879–93.
66. Wang ZF, Xie ZM, Tan YL, Li JY, Wang FL, Pei D, et al. Receptor-like protein kinase BAK1 promotes K⁺ uptake by regulating H⁺-ATPase *AHA2* under low potassium stress. *Plant Physiol*. 2022;189(4):2227–43.
67. Zhang X, Wu H, Chen J, Chen L, Chang N, Ge G, et al. Higher ROS scavenging ability and plasma membrane H⁺-ATPase activity are associated with potassium retention in drought tolerant tea plants. *J Plant Nutr Soil Sci*. 2020;183(4):406–15.
68. Gangappa SN, Holm M, Botto JF. Molecular interactions of BBX24 and BBX25 with HYH, HY5 HOMOLOG, to modulate Arabidopsis seedling development. *Plant Signal Behav*. 2013;8(8):e25208.
69. Jiang L, Wang Y, Li QF, Björn LO, He JX, Li SS. Arabidopsis STO/BBX24 negatively regulates UV-B signaling by interacting with COP1 and repressing *HY5* transcriptional activity. *Cell Res*. 2012;22(6):1046–57.
70. Ballesteros ML, Bolle C, Lois LM, Moore JM, Vielle-Calzada JP, Grossniklaus U, et al. LAF1, a MYB transcription activator for phytochrome A signaling. *Genes Dev*. 2001;15(19):2613–25.
71. Datta S, Hettiarachchi C, Johansson H, Holm M. SALT TOLERANCE HOMO-LOG2, a B-box protein in *Arabidopsis* that activates transcription and positively regulates light-mediated development. *Plant Cell*. 2007;19(10):3242–55.
72. Fornara F, Panigrahi KC, Gissot L, Sauerbrunn N, Rühl M, Jarillo JA, et al. Arabidopsis DOF transcription factors act redundantly to reduce *CONSTANS* expression and are essential for a photoperiodic flowering response. *Dev Cell*. 2009;17(1):75–86.
73. Torres Galea P, Huang LF, Chua NH, Bolle C. The GRAS protein SCL13 is a positive regulator of phytochrome-dependent red light signaling, but can also modulate phytochrome A responses. *Mol Genet Genomics*. 2006;276(1):13–30.
74. Bassel GW, Zielinska E, Mullen RT, Bewley JD. Down-regulation of *DELLA* genes is not essential for germination of tomato, soybean, and Arabidopsis seeds. *Plant Physiol*. 2004;136(1):2782–9.
75. Boter M, Ruiz-Rivero O, Abdeen A, Prat S. Conserved MYC transcription factors play a key role in jasmonate signaling both in *tomato* and *Arabidopsis*. *Genes Dev*. 2004;18(13):1577–91.
76. Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M. Arabidopsis Ethylene-Responsive element binding factors act as transcriptional activators or repressors of GCC Box-mediated gene expression. *Plant Cell*. 2000;12(3):393–404.
77. Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ. A subset of Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. *Proc Natl Acad Sci U S A*. 2006;103(29):11081–5.
78. Schiermeyer A, Thurow C, Gatz C. Tobacco bZIP factor TGA10 is a novel member of the TGA family of transcription factors. *Plant Mol Biol*. 2003;51(6):817–29.
79. Amir Hossain M, Lee Y, Cho JI, Ahn CH, Lee SK, Jeon JS, et al. The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. *Plant Mol Biol*. 2010;72(4–5):557–66.

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