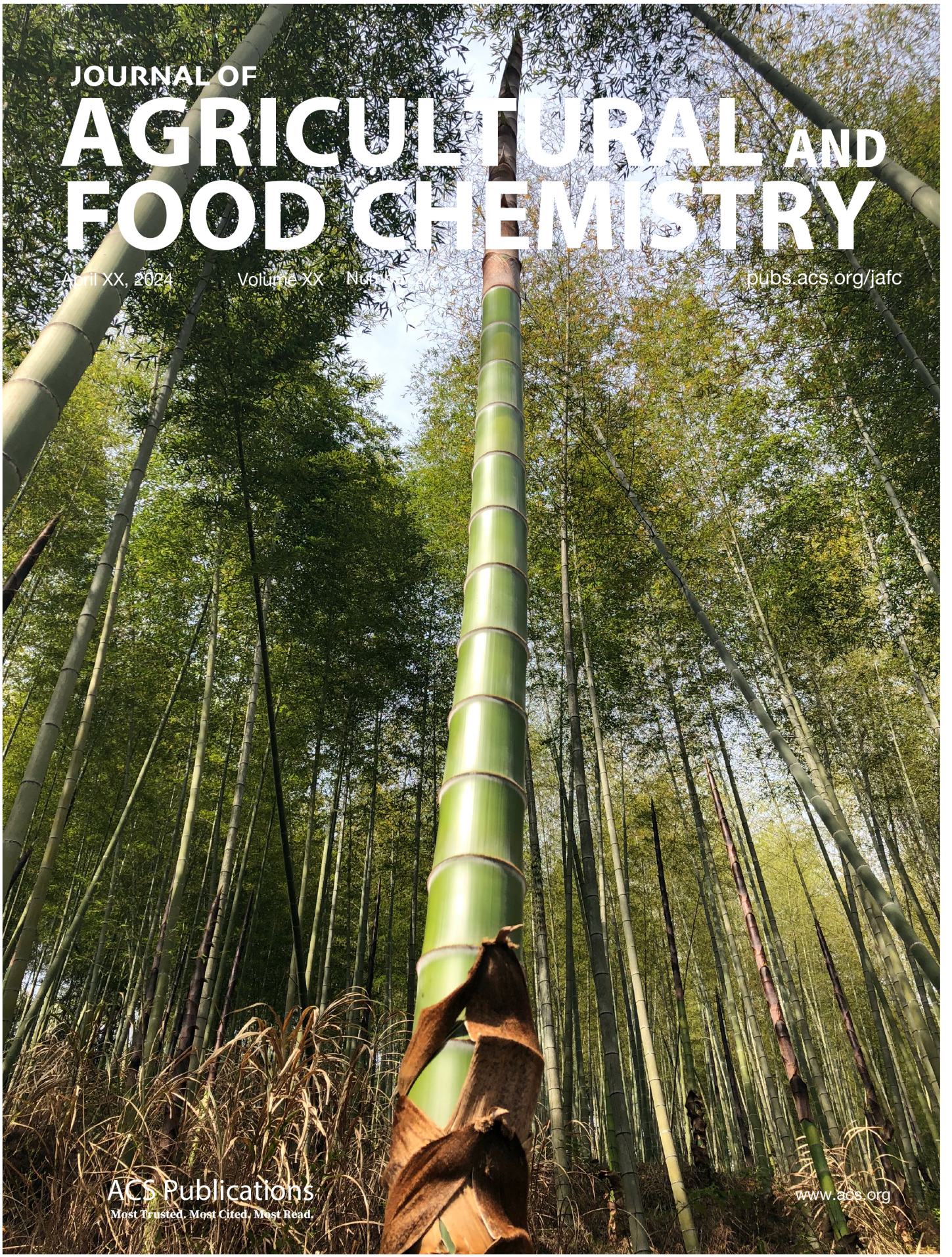
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Article

Integrating Physiological Features and Proteomic Analyses Provides New Insights in Blue/Red Light-Treated Moso Bamboo (*Phyllostachys edulis*)

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ABSTRACT: Bamboo is one of the most important nontimber forestry products in the world. Light is not only the most critical source of energy for plant photosynthesis but also involved in regulating the biological processes of plants. However, there are few reports on how blue/red light affects Moso bamboo. This study investigated the growth status and physiological responses of Moso bamboo (*Phyllostachys edulis*) to blue/red light treatments. The growth status of the bamboo plants was evaluated, revealing that both blue- and red-light treatments promoted plant height and overall growth. Gas exchange parameters, chlorophyll fluorescence, and enzyme activity were measured to assess the photosystem response of Moso bamboo to light treatments. Additionally, the blue light treatment led to a higher chlorophyll content and enzyme activities compared to the red light treatment. A tandem mass tag quantitative proteomics approach identified significant changes in protein abundance under different light conditions with specific response proteins associated with distinct pathways, such as photosynthesis and starch metabolism. Overall, this study provides valuable insights into the physiological and proteomic responses of Moso bamboo to blue/red light treatments, highlighting their potential impact on growth and development.

KEYWORDS: *Moso bamboo, blue light, red light, TMT quantitative proteomics*

1. INTRODUCTION

Phyllostachys edulis, commonly known as Moso bamboo, belongs to the monocot plant category within the Poaceae family and is one of the most widely planted and commonly seen bamboo species in China. Moso bamboo is known for its rapid growth, renewable characteristics, and exceptional physical and mechanical properties,[1] making it a versatile substitute for various materials. In addition, Moso bamboo possesses significant ecological value by purifying air, mitigating noise, preventing soil erosion, and maintaining soil fertility and also contributes to carbon cycle and ecosystem protection.[2−4] As a result, researchers have focused their attention on Moso bamboo, considering it an exceptionally important subject of study, and significant progress has been made in this regard.

Presently, research on Moso bamboo predominantly centers on both biotic and abiotic stresses that impact its yield, including factors such as disease infestation,[5] drought,[6] soil salinity,[7] temperature,[8] and high light.[9] Light, as a critical factor, serves not only as an energy source for photosynthesis in plants but also plays a crucial role in regulating the growth and development of plants.[10,11] Moso bamboo efficiently utilizes light energy to synthesize essential organic matter, contributing significantly to its rapid growth.[12] It has been reported that young bamboo shoots will perish within 10 days under complete shading treatment,[13] which highlights the importance of light for the growth of Moso bamboo. Currently, there have been notable advancements in the examination of light regulation in Moso bamboo. For instance, studies have delved into photosynthesis and related metabolism, elucidating the molecular mechanisms of photosynthesis in Moso bamboo under high light stress,[9] and the functional gene zeaxanthin epoxidase (ZEP) has been identified as playing a pivotal role in Moso bamboo's response to intense light,[14] and the violaxanthin de-epoxidase (VDE) acted as a positive regulator of response to high light intensity.[15] Furthermore, Zhao et al. generated gene expression profiles for Moso bamboo in response to high light,[9] and they identified genes associated with reactive oxygen species (ROS) scavenging, as well as transcription factors involved in the regulation of photosynthetic and related metabolic processes.

Specifically, red and blue light within the spectrum are pivotal in regulating various light responses in plants. It has been found that red light is involved in seed germination,[16] seedling morphogenesis,[17] inhibition of hypocotyl growth,[18] anthocyanin accumulation,[19] phototropic growth,[20] and induction of flowering,[21] while blue light plays a role in the regulation of the biological clock,[11] hypocotyl elongation,[22] stomatal opening and development,[23] flower formation,[24]

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stress response,[25] and the fruit developmental regulation,[26] among other functions. Therefore, investigating the impacts of blue and red light on Moso bamboo is imperative. Research has indicated that red and blue light impact photomorphogenesis in Moso bamboo seedlings postgermination, influencing parameters including root length, internode length, leaf width, and others.[27] At present, there is limited research on the effects of red and blue light on the growth and development of Moso bamboo seedlings, especially concerning the potential molecular mechanisms at the protein level. Proteins serve as the primary facilitators of life processes and essential cellular functions such as catalysis, synthesis, and functional regulation, which are unattainable without their involvement.[28] In recent years, quantitative proteomics based on mass spectrometry has emerged as a crucial tool employed in forestry trees, such as citrus,[29] mangrove,[30] and bamboo.[31] Tandem mass tag (TMT) technology combined with liquid chromatography tandem mass spectrometry (LC−MS/MS) offers greater ease and accuracy compared to other methods.[32,33]

In this study, we conducted physiological and TMT-based quantitative proteomics analyses on Moso bamboo seedlings exposed to red and blue light. The objective was to identify differentially abundant proteins involved in the photoregulation of Moso bamboo. This research will contribute to elucidating the mechanisms underlying the response of different light qualities and establishing a theoretical framework for the practical cultivation of Moso bamboo.

2. MATERIALS AND METHODS

2.1. Cultivation and Treatment of Plants. Moso bamboo seeds were gathered from GuiLin, GuangXi Province (coordinates: 28°28039.7400N, 112°11018.6200E), as previously documented.[34] The cultivation method of Moso bamboo was modified from the method previously described by refs 27 and 35. After they underwent dehulling, they were washed twice with double-distilled water and were placed inverted in a dark room and soaked for 48 h. Subsequently, the treated seeds were sown into nutrient soil covered with a transparent plastic lid. After 10 days, the germinated Moso bamboo were transferred to a greenhouse and kept at a temperature of 22 °C with a light/dark cycle (16 h of light and 8 h of darkness) for 22 days. Following this period, they were subjected to 3 days of treatment in darkness to eliminate the influence of normal light. Subsequently, they were exposed to either blue light or red light for 5 days with darkness serving as the control, and the intensity of red light and blue light set at 50 μmol m^{-2} s^{-1}. Basic growth indicators, including plant height, internode length, fresh weight, and dry weight, were measured. The plant height was measured as the distance from the ground to the ligules of the youngest fully developed leaf,[36] and the first internode length was defined as the distance from the second to the third node.[35] The area of the second leaf was determined using ImageJ.[37]

2.2. Determination of Net Photosynthesis. After light treatment, the key photosynthesis-related physiological index of Moso bamboo leaves, including the net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (μmol CO_2 mol^{-1}), and transpiration rate (mmol H_2O m^{-2} s^{-1}) were detected by using portable photosynthesis tester Li-6400XT (LI-COR Biosciences, Lincoln, USA) with the standard measurement method of the manufacturer.[38] In brief, the control of the fixed flow model was set to 500 μmol s^{-1}, and the desiccant was adjusted so that the reference relative humidity (RH_R) is maintained at about 65%, and finally the reference CO_2 concentration (400 μmol mol^{-1}) was set under ambient CO_2 using the CO_2 mixer. After waiting for the instrument to stabilize, the measurement was performed.

2.3. Analysis of Chlorophyll Content. The determination of chlorophyll content was conducted according to a modified method.[39] Fresh leaves were ground to a fine powder frozen in liquid N_2. Each sample was mixed with 10 mL of ice-cold 80% acetone and incubated overnight at 4 °C in the dark. After centrifuging, 1 mL of supernatant was measured for absorption at 646 and 663 nm against an 80% acetone blank by using SPAD502 Plus (Konica Minolta Investment Ltd., Tokyo, Japan).

2.4. Analysis of Chlorophyll Fluorescence Parameters. After treatment with blue or red light, the chlorophyll fluorescence parameters of Moso bamboo were analyzed with Imaging-PAM (Heinz Walz GmbH, Effeltrich, Germany) following the standard assay method. Briefly, healthy leaves with robust growth were selected under three conditions: control (dark), blue light, and red light, respectively. Three leaf pieces were randomly selected for each condition and immediately subjected to 30 min treatment in a darkroom. Initially, the instrument provided low light to the Moso bamboo leaves to determine the initial fluorescence amount (Fo). Subsequently, a light-saturated pulse intense flash within the instrument was utilized to measure the maximum fluorescence yield (Fm) as well as the maximum photochemical quantum. The maximum dark-adapted photochemical efficiency (Fv/Fm) of the Photosystem II (PSII) was measured using light-saturated pulse flashes in the instrument. Finally, the potential activity (Fv/Fo) and the non-photochemical quenching (NPQ) coefficient of PSII were determined.

2.5. Determination of Enzyme Activity. The activity of the NAD-malic enzyme (ME) and NADP-ME was determined using the methods described in Bohley et al.[40] 100 mg of tissue was weighed from the leaves of Moso bamboo and homogenized on ice. Subsequently, the samples were centrifuged at 8000g for about 10 min at 4 °C, and the initial absorbance $A1$ at 340 nm and the absorbance value $A2$ after 1 min of reaction were recorded immediately. The final calculations were performed according to the equations NAD-ME (nmol/min/g) = $(A2 - A1) \times 4823 \times W$ (fresh weight) and NADP-ME (nmol/min/g) = $(A2 - A1) \times 3215 \times W$ (fresh weight).

2.6. Protein Extraction, Digestion, TMT Labeling and Fractionation. Leaves were powdered in liquid nitrogen and then homogenized in extraction buffer [20 mM tris-HCl, pH 8.4,10 mM DTT, 8 M urea, 1% SDS, and 0.1% protease inhibitor (added freshly)] on ice for 30 min, and then samples were centrifuged to remove cellular debris. Subsequently, 6 times the volume of precooled 10% TCA-acetone was added to the supernatant and kept at −20 °C overnight to make a protein precipitate. The protein pellets were redissolved in lysis buffer (8 M urea, 100 mM tris-HCl, pH 8.0, 1 × protease inhibitor cocktail) and then measured by the BCA assay (ThermoFisher Scientific, MA, USA). Protein were digested with the filter-aided sample preparation (FASP) method, as previously described.[41] Briefly, lysates were loaded onto spin filter columns (Nanosep centrifugal devices with an Omega membrane, 30 kDa MWCO; Pall, NY, USA) and reduced by DTT, followed by alkylation with iodoacetamide (IAA). Afterward, lysis buffer was exchanged by washing the membrane 3 times with 50 mM tetraethylammonium bromide buffer. Proteins were digested overnight at 37 °C using trypsin (Promega, WI, USA) at an enzyme-to-protein ratio of 1:50 (w/w). The trypsin-digested proteins were labeled by the TMT-10Plex kit (Thermo Scientific, MA, USA) following the manufacture's instruction.[32] Dark-, blue light-, and red light-treated group samples (three replicates) were labeled with TMT-channels of 126−127C, 128N−129N, and 129C−130C, respectively. Subsequently, the pooled TMT sample mixes were isolated to 12 fractions using a high-pH reversed-phase HPLC system (U3000 UHPLC, ThermoFisher Scientific, MA, USA) equipped with a ThermoFisher Accucore C18 column (2.1 × 150 mm, 2.6 μm). The two-phase flow chromatographic condition was as follows: a linear gradient of 5% phase B to 45% phase B in 40 min (phase A, 20 mM ammonium formate in 80% H2O; phase B, 20 mM ammonium formate in 80% acetonitrile, pH 10.0, adjusted with ammonium hydroxide), flow rate of 300 μL/min, and column temperature of 30 °C.

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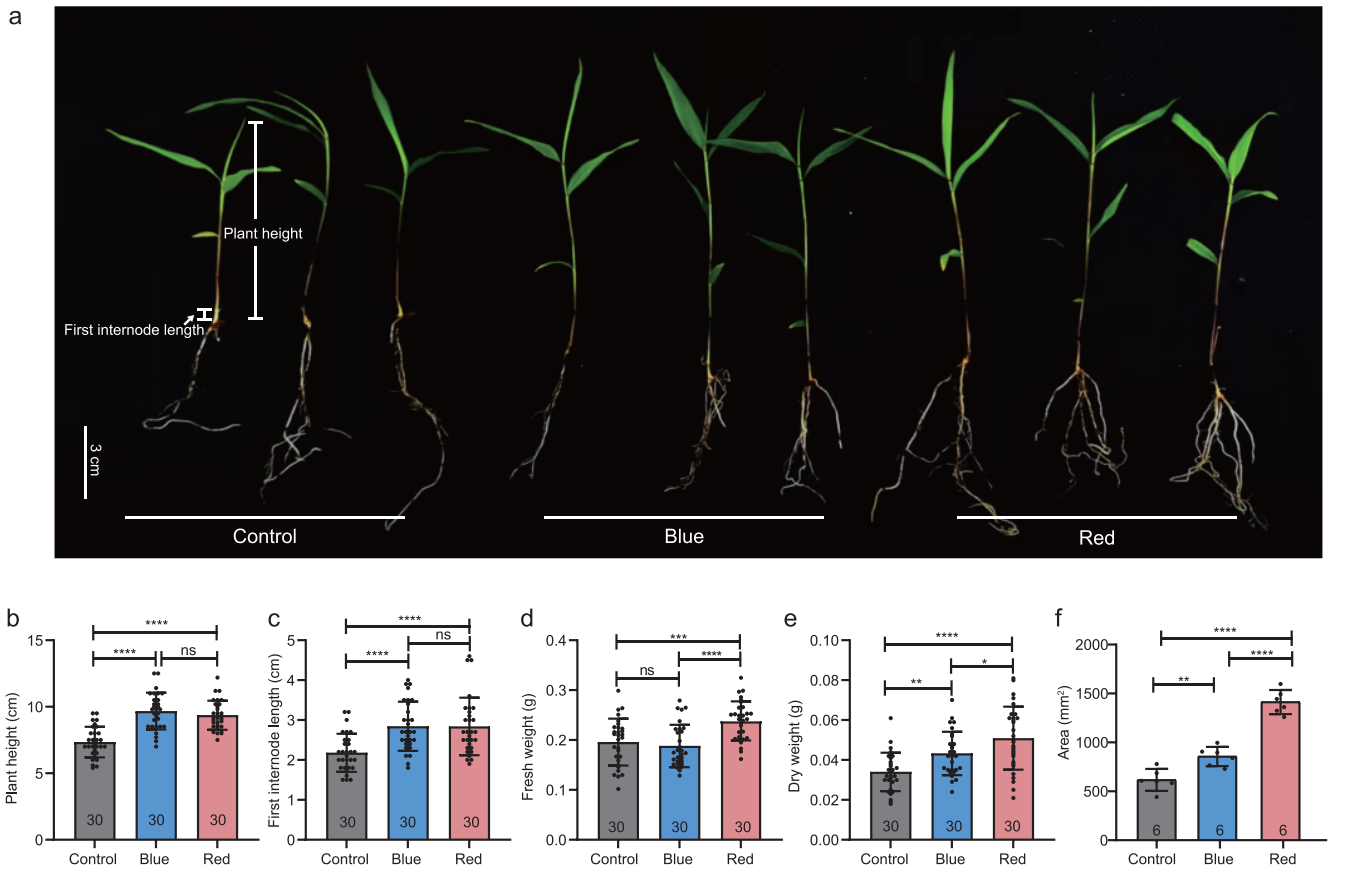

Figure 1. Basic growth indicators after blue/red light treatment. (a) Growth status of Moso bamboo under three light conditions. (b) Plant height of Moso bamboo seedlings. (c) First internode length of Moso bamboo seedlings. (d) Fresh weight of Moso bamboo seedlings. (e) Dry weight of Moso bamboo seedlings. (f) Area of the second leaf. Values are presented as means ± SEM from replicates, with bars representing SEM. The Student *t* test was employed to assess differences at the 0.05 probability level. An asterisk denotes a significant difference: "****": $P < 0.0001$; "***": $P < 0.001$; "**": $P < 0.01$; "ns": not significant with $P > 0.05$. The numbers within the bars represent the quantity of seedlings used for statistical analysis.

2.7. Nano-HPLC-MS/MS Analysis. Fractioned peptides were analyzed with an Orbitrap Fusion mass spectrometer (Thermo Scientific, MA, USA) equipped with an Easy nLC 1000 liquid chromatography system (Thermo Scientific, MA, USA). Briefly, 1 μg of peptides per fraction were loaded to the precolumn at the flow rate of 5 μL/min and then isolated by the analytical column (Nano-EaseTM M/Z HSS C18, 75 μm × 25 cm, Waters) with a 120 min gradient at a flow rate of 300 nL/min and column temperature of 50 °C.

Peptides were ionized at a spray voltage of 2.2 kV, then analyzed by the mass spectrometer system under data acquisition in data dependent acquisition (DDA) mode with the following parameters, MS scan: a mass resolution of 60,000, scan range of m/z 350−1,550, maximum injection time of 50 ms, AGC target value of 4×10^5; MS/MS scan: a resolution of 60,000, AGC target of 8×10^4, maximum injection time of 120 ms, and HCD energy of 38%. The dynamic exclusion was set as $n = 1$, and the dynamic exclusion time was 45 s.

2.8. Data Processing and Bioinformatics Analysis. Raw data were analyzed using Proteome Discoverer 2.1 (Thermo Scientific, MA, USA) and Scaffold Q+ (version Scaffold_4.7.1) with the protein sequence database.[42] The false discovery rate of proteins and peptides was set at 0.01, and the minimum and maximum peptide length were 6 and 144, respectively. A decoy database search was employed to generate high ($P < 0.01$) and medium ($P < 0.05$) confidence peptide lists. We performed differential analysis of protein expression using a pairwise ratio-based calculation and Student's *t* test. Proteins with | Log2 fold change| > 0.585 and $P < 0.01$ were classified as differentially expressed proteins (DEPs). Gene ontology (GO) enrichment analyses were performed using OmicsBox software (https://www.biobam.com/omicsbox).[43] The DEPs specific to light were analyzed for protein−protein interactions (PPIs) using STRING version 12.0 (https://cn.string-db.org/) with reference to the protein library of *Arabidopsis thaliana*.[44] Weighted gene coexpression network analysis (WGCNA) was conducted using the R package.[45] Subcellular localization predictions were analyzed using an online Web site (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/).[46]

All statistical data were analyzed using GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). We performed ANOVA with two-tailed Student's *t* test.[47] The notation used for reporting significance is as follows: "ns": $P > 0.05$, "*": $P < 0.05$, "**": $P < 0.01$, "***": $P < 0.001$, and "****": $P < 0.0001$. All the data were reported as means ± SEM.

3. RESULTS

3.1. Growth Status after Blue/Red Light Treatment. The growth status of the plant is indicative, to a certain extent, of the level of photosynthetic accumulation, overall growth health, and the plant's responsiveness to environmental stress. The leaves of the Moso bamboo displayed increased openness after red light treatment (Figure 1a). Additionally, the plants exhibited significantly increased height under blue/red light treatments (Figure 1a,b), with no significant difference between the red and blue light treatments. The length of the first internode was measured, showing similarity initially but significantly increased after blue/red light treatment (Figure 1c). Fresh weight represents the total mass of the plant cells in

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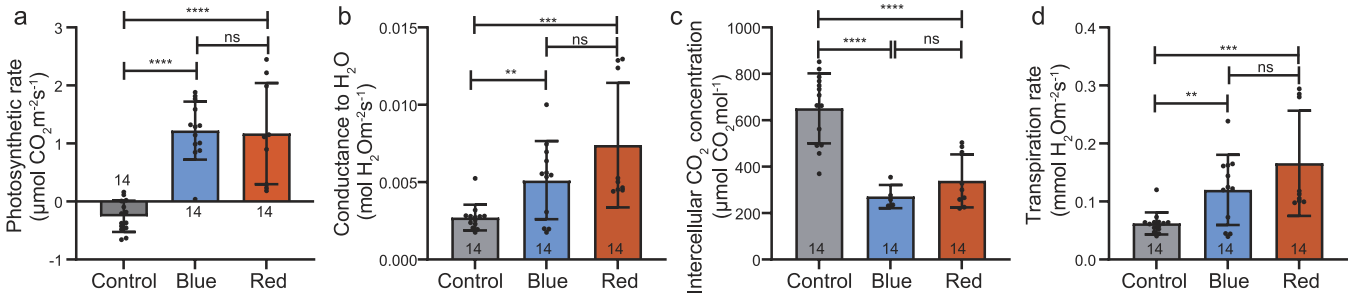

Figure 2. Photosystem indicators after blue/red light treatment. (a) Net photosynthetic rate of Moso bamboo under blue/red light treatment. (b) Stomatal conductance of Moso bamboo under blue/red light treatment. (c) Intercellular CO_2 concentration of Moso bamboo under blue/red light treatment. (d) Transpiration rate of Moso bamboo under blue/red light treatment. Values are presented as means ± SEM from replicates, with bars representing SEM. The Student *t* test was employed to assess differences at the 0.05 probability level. An asterisk denotes a significant difference: "****": $P < 0.0001$; "***": $P < 0.001$; "**": $P < 0.01$. Each group utilized 14 seedlings, as indicated on the bars.

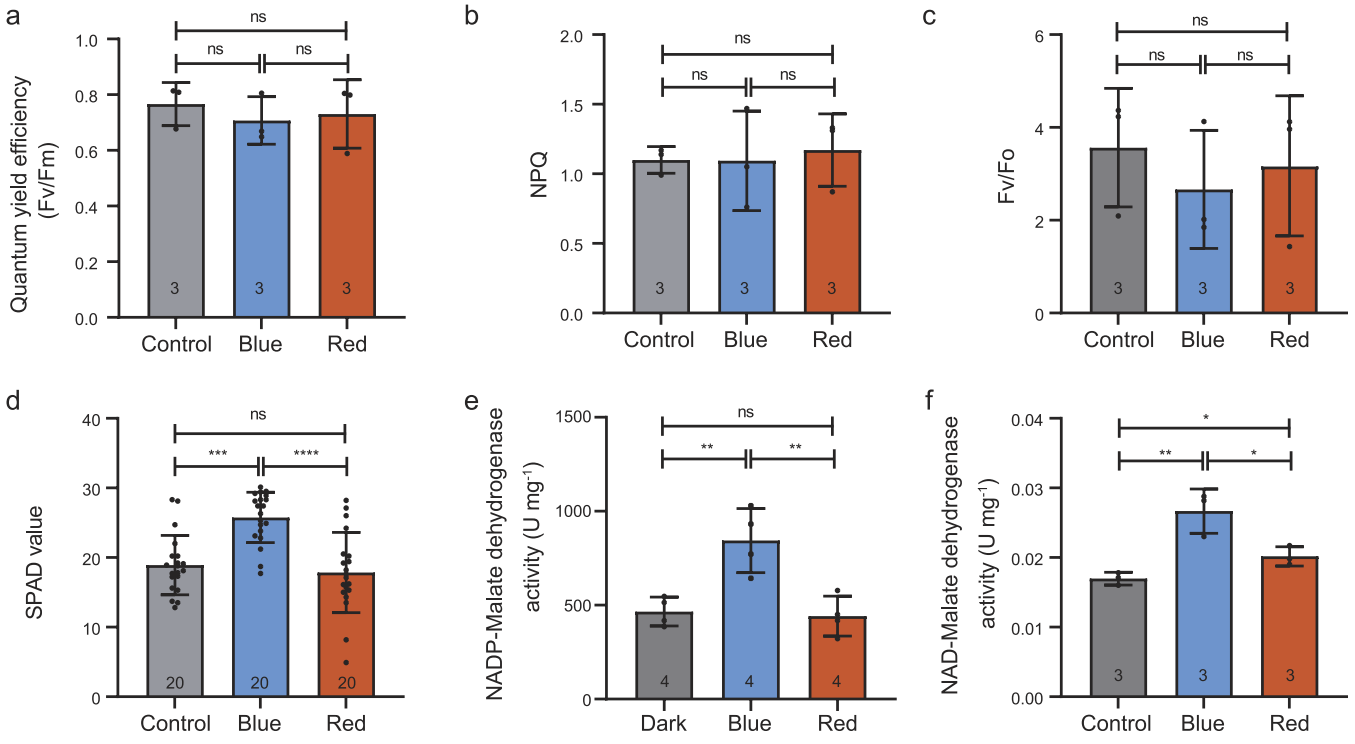

Figure 3. Effects of blue/red light treatments on Moso bamboo physiological parameters. (a) Maximum photochemical efficiency (Fv/Fm) of Moso bamboo leaves under different light conditions. (b) NPQ factor of Moso bamboo leaves after different light treatments. (c) Chlorophyll fluorescence parameter Fo of Moso bamboo leaves after different light treatments. (d) Relative chlorophyll content of Moso bamboo leaves represented by soil-plant analysis development (SPAD) values. (e) NADP-malate dehydrogenase activity in Moso bamboo leaves after different light treatments. (f) NAD-malate dehydrogenase activity in Moso bamboo leaves after different light treatments. Values are presented as means ± SEM from replicates, with bars representing SEM. The Student *t* test was employed to assess differences at the 0.05 probability level. An asterisk denotes a significant difference: "***": $P < 0.001$; "**": $P < 0.01$; "*": $P < 0.05$; "ns": not significant. The numbers within the bars indicate the number of seedlings utilized for statistical analysis.

their natural state, while the dry weight of the plant is related to the accumulation of its organic matter. Figure 1d illustrates a significant increase in the fresh weight of Moso bamboo after the red light treatment ($P < 0.0001$), while there was no significant increase in the fresh weight of the blue light-treated plants ($P > 0.05$). The dry weight, as shown in Figure 1e, indicated that the accumulation of organic matter in Moso bamboo after the red light treatment was higher than that of Moso bamboo after the blue light treatment. The leaf area significantly increased after treatment with different light qualities, particularly under red light ($P < 0.0001$), which is consistent with the observation from Figure 1a. These changes were found to significantly impact the Moso bamboo during red and blue light treatment, with the most noticeable effects being on the plant's height and dry weight.

3.2. Blue and Red Light Effects on the Photosystem in Moso Bamboo. The net photosynthetic rate represents the amount of CO_2 fixed per unit photosynthetic area and the release of oxygen or photosynthetic accumulation released per unit time. It serves as a crucial indicator of photosynthesis efficiency in plants and reflects the structural and functional integrity of plant leaves.[48] In this study, the net photosynthetic rate of Moso bamboo under blue/red light treatments remained positive and exhibited similar levels. In contrast, the net photosynthetic rate of the control was negative, reflecting the absence of photosynthesis (Figure 2a).

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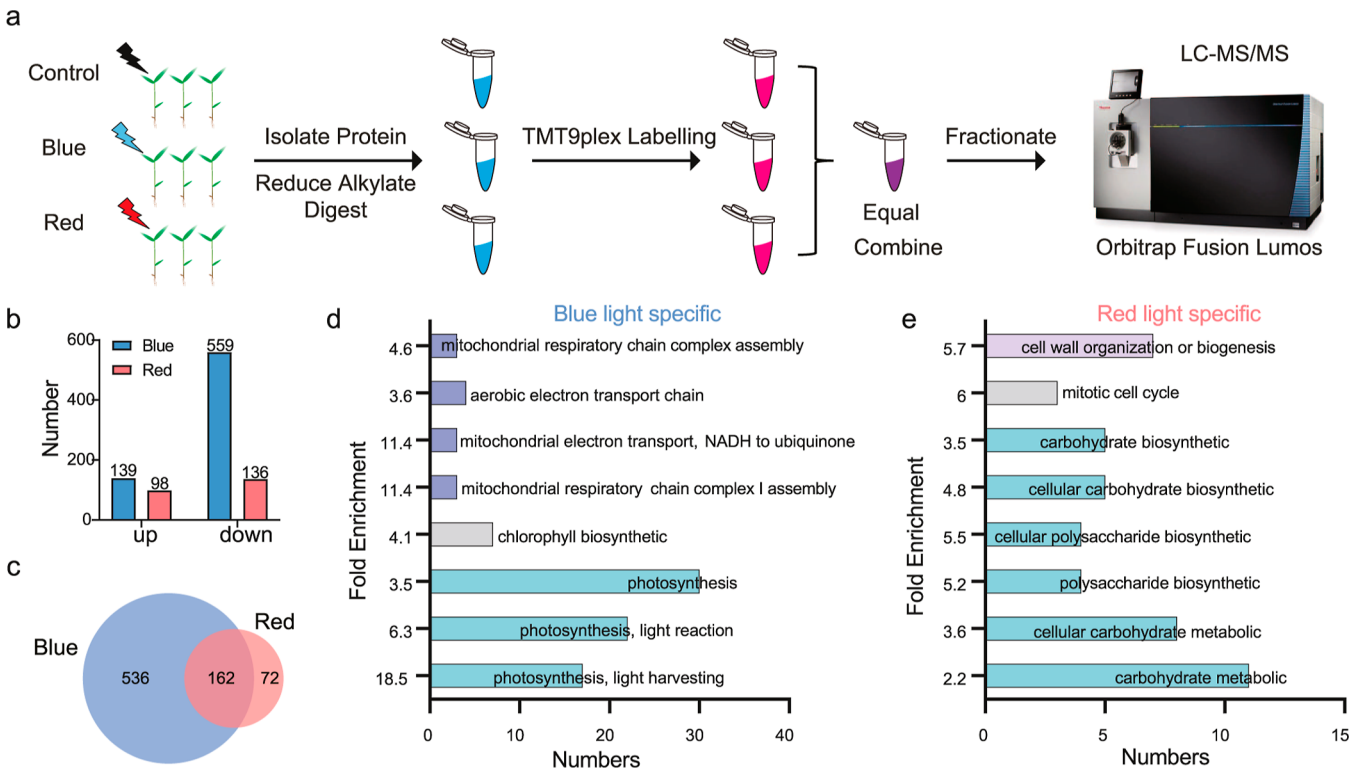

Figure 4. Quantitative proteomics analysis of Moso bamboo under different light treatments. (a) TMT-labeled quantitative proteomics approach employed in this study. (b) Differential protein abundance under blue and red light conditions. (c) Overlapping and specific DEPs. (d) Enrichment analysis for GO terms of proteins showing specific differential expression under blue light treatment. (e) Enrichment analysis for GO terms of proteins showing specific differential expression under red light treatment.

Stomata, which serve as common channels for the movement of CO_2 and water into and out of plant leaves, regulate the intensity of photosynthesis and transpiration. Stomatal conductance can be understood as the amount of water passing through a unit of leaf area per unit of time, per unit of water concentration gradient. It is influenced by environmental factors such as light, CO_2 concentration, air temperature, humidity, and soil moisture content as well as the genetic inheritance of the plant itself. The stomatal conductance of Moso bamboo plants changed significantly after different light treatments, with a more pronounced change observed in plants treated with red light compared to those treated with blue light (Figure 2b). Intercellular CO_2 concentration, which represents the ratio of the CO_2 assimilation rate to stomatal conductance, is generally positively related to net photosynthesis. A higher intercellular CO_2 concentration corresponds to a lower photosynthetic rate (Figure 2c). Following the blue/red light treatments, the intercellular CO_2 concentration of Moso bamboo plants was markedly lower compared with the control.

Transpiration is a crucial process of water exchange between the plant itself and the external natural environment and an integral part of the physiological and biochemical process of plant photosynthesis. It involves the plant vaporizing water through transpiration and diffusing the gases into the external environment. The intensity of transpiration in plants is primarily determined by genetic factors but also influenced, to some extent, by environmental factors. Thus, transpiration serves as a vital physiological indicator of a plant's water-regulating metabolism. In the present experiments, transpiration of Moso bamboo treated with red and blue light was found to increase significantly compared to control (Figure 2d).

3.3. Chlorophyll Fluorescence and Enzyme Activity Response of Moso Bamboo to Blue/Red Light Treatments. Moso bamboo leaves were quickly removed under different light treatment for 5 days, followed by 30 min of dark treatment. The maximum photochemical efficiency Fv/Fm, an important indicator of bamboo's response to environmental adversity and photoinhibition, was determined using Imaging-PAM. In this study, the values of Fv/Fm of bamboo leaves remained between 0.75 and 0.85 (Figure 3a). It was found that there was no significant difference in the Fv/Fm values of Moso bamboo after different light treatments (Figure 3a), indicating that Moso bamboo did not suffer from photoinhibition during the light treatment.

The chlorophyll fluorescence parameter Fo indicates the level of fluorescence exhibited when all of the primary electron acceptors of PSII are oxidized. The NPQ factor reflects the fraction of photon energy absorbed by PSII that is not available for photosynthetic electron transfer but can be dissipated in the form of heat, representing the amount of heat dissipation and a defense mechanism of Moso bamboo against the external environment. Fv/Fo is often used as a measure of the potential activity of PSII, and changes occurring due to environmental causes are more sensitive than Fv/Fm. The NPQ (Figure 3b) and Fv/Fo values (Figure 3c) of Moso bamboo changed slightly but not significantly after light treatment.

The relative chlorophyll content of Moso bamboo leaves, represented by the SPAD value, is an indicator of its photosynthesis strength. The SPAD values of plants of Moso bamboo after blue light treatment were significantly higher

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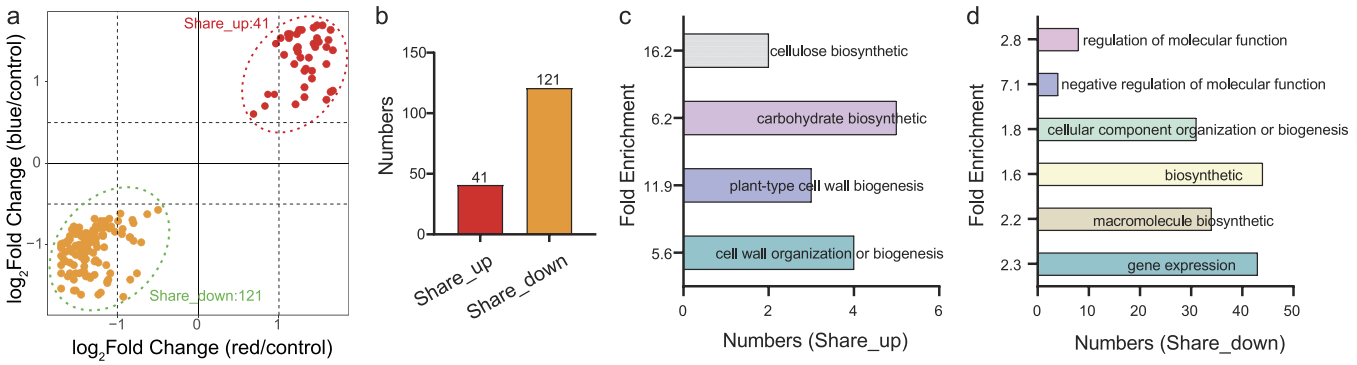

Figure 5. Analysis of proteins responding to both red and blue light in Moso bamboo. (a,b) Blue and red light jointly up-regulated and jointly down-regulated proteins. (c) GO functional analysis of jointly up-regulated proteins. (d) GO functional analysis of jointly down-regulated proteins.

than those after red light treatments (Figure 3d), indicating that the photosynthetic capacity of Moso bamboo under blue light treatment might be stronger than that under red light treatment.

NAD-malate dehydrogenase and NADP-malate dehydrogenase activities in Moso bamboo leaves were measured after different light treatments using microscopic methods. Malate dehydrogenase plays a pivotal role in facilitating the reversible biochemical conversion of malic acid to oxaloacetate in plants. Its participation in various physiological and biochemical processes, such as the TCA cycle, C4 cycle metabolism, photosynthesis, oxidative phosphorylation reactions, and other metabolic pathways, renders it of significant research interest. The NADP-malate dehydrogenase activity of the Moso bamboo plants remained at a high level after the blue light treatment, while it remained almost unchanged after the red light treatment, suggesting different metabolic responses in the two light treatments (Figure 3e). NAD-malate dehydrogenase showed higher enzyme activity in blue-treated plants than in red-treated plants, indicating a possible increase in oxidative phosphorylation and photosynthesis in the blue light-treated Moso bamboo plants (Figure 3f).

Overall, these physiological indicators suggest that blue light treatment may lead to increased biochemical levels, including photosynthesis and oxidative phosphorylation, in Moso bamboo plants compared to red light treatment. However, red light treatment also showed some increase in enzyme activity, albeit not as pronounced as that with the blue light treatment.

3.4. Quantitative Proteomics Analysis. Many physiological indicators of Moso bamboo changed after light treatment, such as the plant height and gas exchange parameters. However, the impact of red and blue light on the protein levels of Moso bamboo remains unknown. Therefore, a TMT-labeled quantitative proteomics approach was employed to study the changes in protein levels of Moso bamboo after light treatment, with the aim of identifying key pathways and proteins affected by different light treatments (Figure 4a).

A total of 11,481 proteins were identified, and 8693 proteins were quantified (Supplementary Table 1). Their basic status of these proteins is shown in Figure S1. The coverage of the identified proteins (Figure S1a) shows that nearly 82% (10.95 + 15.92 + 22.89 + 31.84%) of the Moso bamboo proteins had a coverage below 40%, and over 50% (22.89 + 31.84%) of the proteins had a coverage below 20%. The number of unique peptides identified for the proteins is shown in Figure S1b, with 92% (17.62 + 74.4%) of the proteins having no more than 10 unique peptides. It was also observed that some proteins did not have unique peptides, which is one of the limitations of bottom-up proteomics.[49] The presence of some similar sequences in the proteins can lead to the existence of many identical peptides after cleavage of the proteins into peptides, making it challenging to determine the homologous protein. Proteome Discoverer 2.1 software was used to identify proteins by providing a library of Moso bamboo sequences, which were then theoretically cleaved to produce a peptide spectrum. This theoretical spectrum was matched with the actual spectrum to identify the protein, and some quality control calculations were performed, such as the spectrum quality tolerance and spectrum matching score. The quality tolerances of the spectra are shown in Figure S1c. Over 85% of the spectra were within 2 ppm of each other, indicating that the mass spectra were of good quality for proteomics data acquisition and that the collected data were accurate and did not affect the subsequent quantitative identification. The spectral match scores (Figure S1d) show that more than 40% (31.57 + 8.83 + 0.27%) of the spectra matched >70 points, and nearly 70% of the spectra matched >60 points, indicating that the quantitative MS/MS spectra were of relatively good quality.

A total of 698 proteins were significantly changed under blue light conditions, with 139 proteins being up-regulated and 559 proteins being down-regulated (Figure 4b, Supplementary Table 2). The down-regulated proteins accounted for more than 80% of the total, suggesting a possible stronger overall effect of blue light treatment on Moso bamboo. Under red light conditions, a total of 234 proteins, 98 up-regulated and 136 down-regulated, were differentially expressed (Figure 4b, Supplementary Table 2). There are 162 proteins that were significantly changed under blue/red light treatment, 536 proteins were specifically differentially changed under blue light treatment, while 72 proteins were specific under red light (Figure 4c, Supplementary Table 3), indicating that Moso bamboo have similar and different responses to red and blue light, with a more intense response to blue light.

To better understand the biological characteristics of the light response proteins, a GO enrichment analysis was conducted. Proteins related to photosynthesis, oxidative phosphorylation, light flutter, and repair of the photosystem were significantly enriched specifically under blue light treatment (Figure 4d). This indicates that blue light treatment mainly affects photosynthesis-related physiological functions in Moso bamboo. On the other hand, specific response proteins under red light treatment are mainly associated with biological

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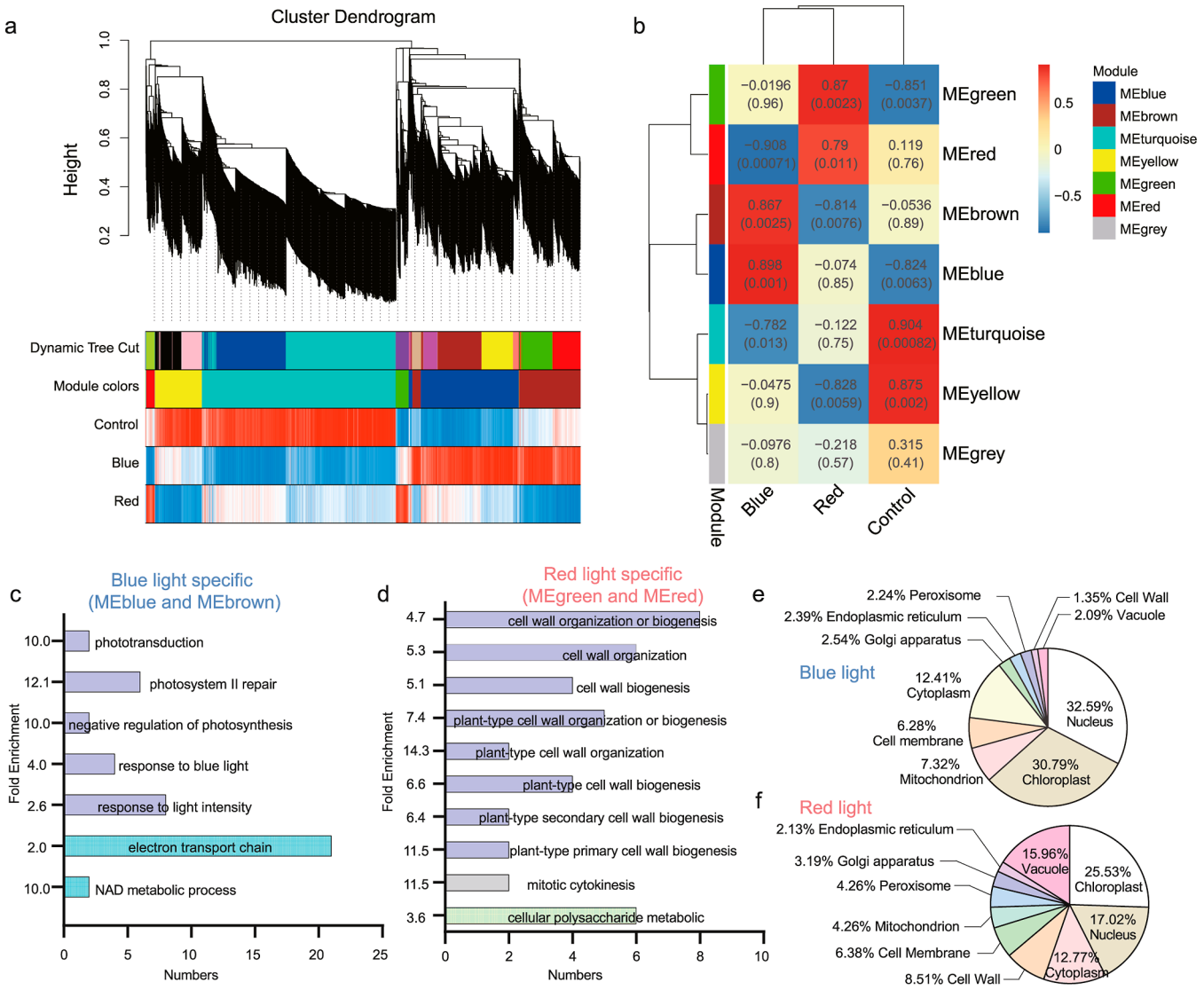

Figure 6. WGCNA exploring specific pathways and DEPs in Moso bamboo under red and blue light conditions. (a) WGCNA module gene−trait correlation graph. (b) Correlation coefficients between the specific modules and the samples. *P*-values are presented within parentheses and the color bar (−0.5 to 0.5) represents correlation coefficients. (c) GO enrichment analysis results for the blue light specific genes. (d) GO enrichment analysis results for the red light specific genes. (e) Subcellular location of DEPs (698 proteins) under blue light treatment. (f) Subcellular location of DEPs (234 proteins) under red light treatment.

processes such as carbohydrate synthesis, polysaccharide metabolism, cell wall genesis, mitosis, etc (Figure 4e). This finding suggests that red light treatment leads to changes in Moso bamboo that significantly differ from those occurring under blue light treatment.

To delve deeper into the alterations in Moso bamboo proteins following specific light treatment, a PPI network analysis was conducted (Supplementary Figure 2). Notably, interacting regions specific to blue light were prominently observed in processes related to photosynthesis, light reactions of photosynthesis, and chlorophyll biosynthesis. The light-harvesting complex (LHC) consisting of LHCB4.1 (PH02Gene07012), LHCB5 (PH02Gene42040), LHCB6 (PH02Gene18375), and LHCB2.1 (PH02Gene32299) acts as a light receptor, capturing and transferring excitation energy to closely associated photosystems. Subsequently, DEPs unique to red light treatment were analyzed, and PPIs including cellulose biosynthetic process and plant-type primary cell wall biogenesis were observed.

Subsequently, we analyzed the 162 proteins that responded to both red and blue light in Moso bamboo (Figure 4c). The findings revealed that blue and red light jointly up-regulated 41 proteins and jointly down-regulated 121 proteins (Figure 5a,b). Further, GO functional analysis was performed on these up- and down-regulated proteins. We found that the 41 up-regulated proteins were involved in processes such as cell wall synthesis (Figure 5c), while the 121 down-regulated proteins were associated with gene expression, biological functions, etc (Figure 5d). This result indicates that both types of light have consistent regulatory effects on certain functions of Moso bamboo.

To further investigate the correlation between specific response pathways and different light treatments in Moso bamboo, we utilized WGCNA to explore the relationship between specific pathways and DEPs. All quantified proteins were clustered into modules based on dynamic tree cutting, which group proteins with similar alteration patterns together. Figure 6a shows the WGCNA module gene−trait correlation,

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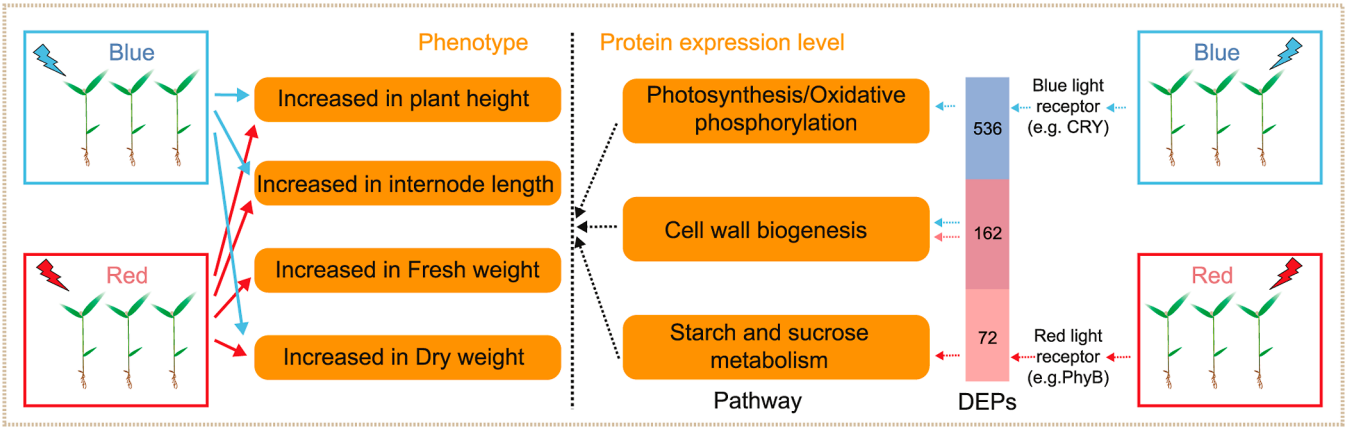

Figure 7. Schematic diagram illustrates how phenotype is related to protein expression in Moso bamboo under blue or red light treatment. Lines colored blue and red represent the effectors of blue and red light, respectively. CRY: Cryptochromes. PhyB: Phytochrome B. DEPs: Differentially expressed proteins. The numbers indicate the counts of DEPs.

where module colors corresponding to different light conditions are represented as yellow (MEyellow) and cyan (MEturquoise) for the control, blue (MEblue) and brown (MEbrown) for blue light conditions, and green (MEgreen) and red (MEred) for red light conditions (Supplementary Table 4). The correlation coefficients between the specific modules and the samples are highly correlated (Figure 6b). The results showed 787 genes (MEblue and MEbrown in Figure 6b, Supplementary Table 5) were present under blue light conditions and 100 genes were present under red light conditions (MEgreen and MEred in Figure 6b, Supplementary Table 6). Subsequently, the 787 genes and 100 genes were subjected to GO enrichment analysis separately. We found that the correlation coefficients of pathways such as photosynthesis, lipid metabolism, transport of substances across membranes, and NAD synthesis were higher under blue light conditions (Figure 6c). In contrast, there was a high correlation between pathways related to cell wall organization or biogenesis, cellular catabolism, and macromolecular catabolism under red light conditions (Figure 6d).

To gain a deeper understanding of the cellular compartments where the DEPs performed their functions (Supplementary Table 2), a subcellular location prediction analysis was conducted using Plant-mPLoc.[50] Results revealed that a majority of the specific DEPs under blue light treatment were predominantly located within the nucleus, chloroplasts, cytoplasm, and mitochondria, among other cellular compartments (Figure 6e). Similarly, it is evident that a significant portion of the distinct proteins in Moso bamboo, subjected to red light treatment, exhibited localization within chloroplasts, the nucleus, vesicles, and the cytoplasm (Figure 6f).

4. DISCUSSION

Previous studies of Moso bamboo showed that lignin content decreased sharply after UV irradiation,[51] and high light significantly changed the gene expression, such as genes of ROS in photosynthesis.[9] However, the protein expression profiles in response to red and blue light were elusive in bamboo. Previous study used a 14 day dark treatment of bamboo as a control and compared it with different light conditions.[27] Our germinated seeds were cultivated for 22 days under normal conditions. To avoid the severe stress that long-term dark treatment imposes on bamboo, we only compared bamboo subjected to a 3 day dark treatment. It is noteworthy that both fresh weight and dry weight showed slightly higher values under red light conditions than that under blue light (Figure 1), which was consistent with previous study.[27] In this study, we included additional physiological parameters for assessment, such as the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, maximum photochemical efficiency, NPQ, Fv/Fo, SPAD value, and NADP-malate dehydrogenase activity. Previous study also reported that blue light appears to enhance the expression of proteins involved in PSII, whereas red light is associated with the likely specific expression of cell wall synthetic proteins.[27] In this observation, we also noted an enrichment of proteins involved in photosynthesis specifically in the blue light treatment (Figure 6c), while proteins related to the cell wall were enriched in the red light treatment (Figure 6d). These findings suggest that blue light primarily affects the photosystem, while red light is more closely associated with cell wall synthesis in Moso bamboo. Additionally, we observed distinct PPI networks for blue- and red-light-specific processes, particularly evident in functions associated with photosynthesis and cell wall biogenesis, which represents a novel discovery not documented in prior studies.

The improvement in gas exchange parameters, such as the net photosynthetic rate and stomatal conductance, indicates an enhanced photosynthetic efficiency in the bamboo plants, contributing to their overall growth and response to environmental conditions, especially. Chlorophyll fluorescence parameters were assessed to understand the photosystem response of Moso bamboo to light treatments. The Fv/Fm values, which represent the maximum photochemical efficiency of PSII, remained consistent across all light conditions, indicating that Moso bamboo did not experience photoinhibition during the light treatments. Enzyme activity measurements revealed variations in malate dehydrogenase activities in Moso bamboo leaves after different light treatments. The higher NADP-malate dehydrogenase activity under blue light treatment suggests increased metabolic responses related to photosynthesis and energy production. On the other hand, the red light treatment showed some increase in enzyme activity, albeit not as pronounced as in the blue light treatment. These findings indicate distinct metabolic adjustments in response to different light conditions with blue light possibly promoting a more substantial biochemical response, particularly in photosynthesis and oxidative phosphorylation.

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The regulation of plant growth and development involves the utilization of red and blue light signals. Photoreceptors absorb light signals, transforming the physical light signal into a biochemical signal, which subsequently propagates downstream (Figure 7). The primary receptors for red light are phytochromes (PhyA to PhyE),[52] while for blue light, they are cryptochromes (CRY) and phototropins.[36,53] Our quantitative proteomics analysis identified specific response proteins associated with blue/red light treatments. The enrichment analysis revealed that blue light mainly influenced pathways related to photosynthesis and oxidative phosphorylation, while red light specifically induced proteins involved in starch and sucrose metabolism. The result implies distinct metabolic adjustments in the reaction to varying light wavelengths. Although studies concerning the synergism effects of red and blue light have been documented,[54] no such investigation has been previously reported in the context of bamboo. Notably, we observed a concurrent up-regulation of 41 proteins and a simultaneous down-regulation of 121 proteins when exposed to both blue and red light (Figures 4c, 5a,b, Supplementary Table 3). The 41 up-regulated genes that were shared exhibited a close association with cell wall biogenesis (Figure 5c), potentially leading to increased height growth following treatment with blue and red light (Figure 1a). These observations underscore the coherent regulatory impact of these two light types on specific functions of Moso bamboo. It is notable that after blue light treatment, the expression level of LONG HYPOCOTYL 5-like (HY5-like, PH02Gene25237) is significantly increased (approximately 1.9-fold, p-value = 0.00035) at the protein level, while it is also increased under red light treatment (about 1.13-fold, p-value = 0.047) (Supplementary Table 1). The abundance of HY5, a basic leucine zipper (bZIP) transcription factor, correlates with the degree of photomorphogenesis.[55] COP1, known as a repressor of photomorphogenesis, can directly engage with HY5 through their coiled-coil domains.[55] Evidence show that blue-light activation of CRY1 attenuated the association of COP1 with SPA1 in plants,[56] and the interaction between COP1 and SPA1 also plays a pivotal role in the regulation of HY5 activity mediated by PhyA.[57] HY5 serves as the central hub within the transcriptional network governing photomorphogenesis.[58] SPA1-like proteins SPA3 and SPA4 may also function to inhibit light responses in continuous far-red, red, and blue light by interacting with COP1.[59] It would be intriguing to explore whether HY5,[60] as well as SPA1 and SPA4, exhibits a response to blue light treatment in Moso bamboo. Of particular interest would be the investigation into whether SPA1, SPA3, and SPA4 also contribute to the enhancement of phyB signaling.

To explore the correlation between specific pathways and DEPs, WGCNA was employed. The gene−trait correlation showed highly correlated patterns for different light conditions, with specific modules corresponding to different light treatments. The correlation analysis between pathways related to photosynthesis and cell wall organization or biogenesis further supported the different effects of blue and red light on Moso bamboo. Blue light treatment exhibited significantly higher correlations with photosynthesis-related pathways (Figure 6c), while red light treatment showed stronger associations with pathways related to cell wall organization and catabolic processes (Figure 6d). However, we measured the proteomic data only at a specific time point. In the future, it would be beneficial to consider sampling at various time points and conducting a time-course WGCNA. This approach could yield a more precise identification of key light-responsive candidate genes in bamboo.

This study demonstrates that the DEPs triggered by blue light primarily participate in functions such as photosynthesis and oxidative phosphorylation. Photosynthesis predominantly operates within the chloroplast, while oxidative phosphorylation and other functions primarily take place in the cytoplasm and mitochondria (Figure 7). Concurrently, the study affirms that proteins in Moso bamboo influenced by blue light are predominantly localized within the chloroplasts and mitochondria (Figure 6e). Furthermore, the localization of these blue light-affected proteins within the chloroplasts and mitochondria reinforces the significance of these organelles in mediating the plant's response to blue light (Figure 6c). The enrichment of proteins in these organelles suggests their pivotal role in modulating photosynthesis and energy-related processes under blue light conditions. In contrast, the investigation into red light treatment showcases a distinct set of responses. The DEPs associated with Moso bamboo under red light treatment exhibit a pronounced engagement in pathways related to cell wall synthesis (Figures 4e and 6d). This observation suggests that red light triggers metabolic shifts that are primarily focused on carbohydrate utilization and cell structure reinforcement. In these processes, vesicles may play a noteworthy role. Of particular interest is the potential role of vesicles in these processes. The prominence of vesicles suggests their involvement in cellular transport mechanisms that facilitate the exchange of materials that are essential for sucrose and starch metabolism. These findings underscore the intricate interplay between different light wavelengths and the plant's intricate regulatory networks, ultimately shaping diverse metabolic and growth responses. However, in the future, it is advisable to conduct further subcellular localization analyses, such as GFP fusion and confocal microscopy, on key candidate proteins.

In conclusion, this study provides comprehensive insights into the growth, physiological responses, and proteomic changes of Moso bamboo under blue/red light treatments. The findings highlight the important role of light in influencing the growth and development of Moso bamboo and shed light on the potential applications of specific light treatments in forest species. Future studies may further explore the underlying molecular mechanisms and signaling pathways involved in the light-induced responses of Moso bamboo.

■ ASSOCIATED CONTENT

Data Availability Statement

Mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium via the iProX[61] partner repository with the data set identifier PXD045375.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c00724.

List of quantified proteins, list of proteins significantly changed under blue and red light, comparative analysis of DEPs, gene list according to WGCNA modules, gene list of specific modules under blue light, gene list of specific modules under red light, basic status of quantified proteins, and the PPI analysis (PDF)

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C.Z. and H.T. contributed equally to this work. H.Z., L.G., and Y.L. conceived and designed the research. C.Z. and H.T. performed experiments and proteomics. T.L., H.W., Y.G., J.Z., L.Z., and H.Z. performed bioinformatics. C.Z., H.T., L.G., Y.L., and H.Z. wrote the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Gupta, A.; Kumar, A. Potential of Bamboo in Sustainable Development. *Asia Pac. Bus. Rev.* **2008**, *4* (3), 100−107.

(2) Wang, B.; Wei, W.; Liu, C.; You, W.; Niu, X.; Man, R. Biomass and carbon stock in Moso bamboo forests in subtropical China: characteristics and implications. *J. Trop. For. Sci.* **2013**, *25*, 137−148.

(3) Liu, J.; Jiang, P.; Li, Y.; Zhou, G.; Wu, J.; Yang, F. Responses of N 2 O flux from forest soils to land use change in subtropical China. *Bot. Rev.* **2011**, *77*, 320−325.

(4) Song, X.; Zhou, G.; Jiang, H.; Yu, S.; Fu, J.; Li, W.; Wang, W.; Ma, Z.; Peng, C. Carbon sequestration by Chinese bamboo forests and their ecological benefits: assessment of potential, problems, and future challenges. *Environ. Rev.* **2011**, *19* (NA), 418−428.

(5) Liao, M.; Ren, X.; Gao, Q.; Liu, N.; Tang, F.; Wang, G.; Cao, H. Anti-fungal activity of moso bamboo (Phyllostachys pubescens) leaf extract and its development into a botanical fungicide to control pepper phytophthora blight. *Sci. Rep.* **2021**, *11* (1), 4146.

(6) Wu, M.; Liu, H.; Gao, Y.; Shi, Y.; Pan, F.; Xiang, Y. The moso bamboo drought-induced 19 protein PheDi19−8 functions oppositely to its interacting partner, PheCDPK22, to modulate drought stress tolerance. *Plant Sci.* **2020**, *299*, 110605.

(7) Liu, L.; Cao, X. L.; Bai, R.; Yao, N.; Li, L. B.; He, C. F. Isolation and Characterization of the Cold-Induced Phyllostachys edulis AP2/ERF Family Transcription Factor, peDREB1. *Plant Mol. Biol. Rep.* **2012**, *30* (3), 679−689.

(8) Huang, Z.; Zhong, X. J.; He, J.; Jin, S. H.; Guo, H. D.; Yu, X. F.; Zhou, Y. J.; Li, X.; Ma, M. D.; Chen, Q. B.; et al. Genome-Wide Identification, Characterization, and Stress-Responsive Expression Profiling of Genes Encoding LEA (Late Embryogenesis Abundant) Proteins in Moso Bamboo (Phyllostachys edulis). *PLoS One* **2016**, *11* (11), No. e0165953.

(9) Zhao, H.; Lou, Y.; Sun, H.; Li, L.; Wang, L.; Dong, L.; Gao, Z. Transcriptome and comparative gene expression analysis of Phyllostachys edulis in response to high light. *BMC Plant Biol.* **2016**, *16*, 34.

(10) Liu, H.; Yu, X.; Li, K.; Klejnot, J.; Yang, H.; Lisiero, D.; Lin, C. Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. *Science* **2008**, *322* (5907), 1535−1539.

(11) Wang, X.; Jiang, B.; Gu, L.; Chen, Y.; Mora, M.; Zhu, M.; Noory, E.; Wang, Q.; Lin, C. A photoregulatory mechanism of the circadian clock in Arabidopsis. *Nat. Plants* **2021**, *7* (10), 1397−1408.

(12) Peng, C.; Song, Y.; Li, C.; Mei, T.; Wu, Z.; Shi, Y.; Zhou, Y.; Zhou, G. Growing in mixed stands increased leaf photosynthesis and physiological stress resistance in Moso bamboo and mature Chinese Fir plantations. *Front. Plant Sci.* **2021**, *12*, 649204.

(13) Wang, Y.; Bai, S.; Binkley, D.; Zhou, G.; Fang, F. The independence of clonal shoot's growth from light availability supports moso bamboo invasion of closed-canopy forest. *For. Ecol. Manage.* **2016**, *368*, 105−110.

(14) Lou, Y.; Sun, H.; Li, L.; Zhao, H.; Gao, Z. Characterization and Primary Functional Analysis of a Bamboo ZEP Gene from Phyllostachys edulis. *DNA Cell Biol.* **2017**, *36* (9), 747−758.

(15) Lou, Y.; Sun, H.; Zhu, C.; Yang, K.; Li, X.; Gao, Z. PeVDE, a violaxanthin de-epoxidase gene from moso bamboo, confers photo-protection ability in transgenic Arabidopsis under high light. *Front. Plant Sci.* **2022**, *13*, 927949.

(16) Strasser, B.; Sánchez-Lamas, M.; Yanovsky, M. J.; Casal, J. J.; Cerdán, P. D. Arabidopsis thaliana life without phytochromes. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107* (10), 4776−4781.

(17) Arsovski, A. A.; Galstyan, A.; Guseman, J. M.; Nemhauser, J. L. Photomorphogenesis. *Arabidopsis Book* **2012**, *10*, No. e0147.

(18) Kirchenbauer, D.; Viczián, A.; Ádám, É.; Hegedűs, Z.; Klose, C.; Leppert, M.; Hiltbrunner, A.; Kircher, S.; Schäfer, E.; Nagy, F. Characterization of photomorphogenic responses and signaling cascades controlled by phytochrome-A expressed in different tissues. *New Phytol.* **2016**, *211* (2), 584−598.

(19) Shin, D. H.; Choi, M.; Kim, K.; Bang, G.; Cho, M.; Choi, S. B.; Choi, G.; Park, Y. I. HY5 regulates anthocyanin biosynthesis by inducing the transcriptional activation of the MYB75/PAP1 transcription factor in Arabidopsis. *FEBS Lett.* **2013**, *587* (10), 1543−1547.

(20) Sullivan, S.; Hart, J. E.; Rasch, P.; Walker, C. H.; Christie, J. M. Phytochrome A Mediates Blue-Light Enhancement of Second-Positive Phototropism in Arabidopsis. *Front. Plant Sci.* **2016**, *7*, 290.
(21) Trupkin, S. A.; Debrieux, D.; Hiltbrunner, A.; Fankhauser, C.; Casal, J. J. The serine-rich N-terminal region of Arabidopsis phytochrome A is required for protein stability. *Plant Mol. Biol.* **2007**, *63* (5), 669−678.
(22) Ahmad, M.; Cashmore, A. R. HY4 gene of A. thaliana encodes a protein with characteristics of a blue-light photoreceptor. *Nature* **1993**, *366* (6451), 162−166.
(23) Kang, C. Y.; Lian, H. L.; Wang, F. F.; Huang, J. R.; Yang, H. Q. Cryptochromes, phytochromes, and COP1 regulate light-controlled stomatal development in Arabidopsis. *Plant Cell* **2009**, *21* (9), 2624−2641.
(24) El-Din El-Assal, S.; Alonso-Blanco, C.; Peeters, A. J.; Raz, V.; Koornneef, M. A QTL for flowering time in Arabidopsis reveals a novel allele of CRY2. *Nat. Genet.* **2001**, *29* (4), 435−440.
(25) Mao, J.; Zhang, Y. C.; Sang, Y.; Li, Q. H.; Yang, H. Q. From The Cover: A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102* (34), 12270−12275.
(26) el-Assal, S. E.; Alonso-Blanco, C.; Hanhart, C. J.; Koornneef, M. Pleiotropic effects of the Arabidopsis cryptochrome 2 allelic variation underlie fruit trait-related QTL. *Plant Biol.* **2004**, *6* (4), 370−374.
(27) Li, K.; Ji, L.; Xing, Y.; Zuo, Z.; Zhang, L. Data-Independent Acquisition Proteomics Reveals the Effects of Red and Blue Light on the Growth and Development of Moso Bamboo (Phyllostachys edulis) Seedlings. *Int. J. Mol. Sci.* **2023**, *24* (6), 5103.
(28) Aebersold, R.; Mann, M. Mass-spectrometric exploration of proteome structure and function. *Nature* **2016**, *537* (7620), 347−355.
(29) Jiang, J.; Hou, R.; Yang, N.; Li, L.; Deng, J.; Qin, G.; Ding, D. Physiological and TMT-labeled proteomic analyses reveal important roles of sugar and secondary metabolism in Citrus junos under cold stress. *J. Proteomics* **2021**, *237*, 104145.
(30) Xing, J.; Pan, D.; Wang, L.; Tan, F.; Chen, W. Proteomic and physiological responses in mangrove Kandelia candel roots under short-term high-salinity stress. *Turk. J. Biol.* **2019**, *43* (5), 314−325.
(31) Yu, X.; Wang, Y.; Kohnen, M. V.; Piao, M.; Tu, M.; Gao, Y.; Lin, C.; Zuo, Z.; Gu, L. Large Scale Profiling of Protein Isoforms Using Label-Free Quantitative Proteomics Revealed the Regulation of Nonsense-Mediated Decay in Moso Bamboo (Phyllostachys edulis). *Cells* **2019**, *8* (7), 744.
(32) Thompson, A.; Schafer, J.; Kuhn, K.; Kienle, S.; Schwarz, J.; Schmidt, G.; Neumann, T.; Hamon, C.; Mohammed, A. K.; Hamon, C. Tandem mass tags: a novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. *Anal. Chem.* **2003**, *75* (8), 1895−1904.
(33) Muntel, J.; Kirkpatrick, J.; Bruderer, R.; Huang, T.; Vitek, O.; Ori, A.; Reiter, L. Comparison of Protein Quantification in a Complex Background by DIA and TMT Workflows with Fixed Instrument Time. *J. Proteome Res.* **2019**, *18* (3), 1340−1351.
(34) Wang, W.; Li, Y.; Cai, C.; Zhu, Q. Auxin response factors fine-tune lignin biosynthesis in response to mechanical bending in bamboo. *New Phytol.* **2024**, *241* (3), 1161−1176.
(35) Zhang, H.; Wang, H.; Zhu, Q.; Gao, Y.; Wang, H.; Zhao, L.; Wang, Y.; Xi, F.; Wang, W.; Yang, Y.; et al. Transcriptome characterization of moso bamboo (Phyllostachys edulis) seedlings in response to exogenous gibberellin applications. *BMC Plant Biol.* **2018**, *18* (1), 125.
(36) Singh, I.; Kumar, U.; Singh, S. K.; Gupta, C.; Singh, M.; Kushwaha, S. R. Physiological and biochemical effect of 24-epibrassinoslide on cold tolerance in maize seedlings. *Physiol. Mol. Biol. Plants* **2012**, *18* (3), 229−236.
(37) Collins, T. J. ImageJ for microscopy. *Biotechniques* **2007**, *43* (1S), S25−S30.
(38) Long, L.; Pedas, P. R.; Kristensen, R. K.; Schulze, W. X.; Husted, S.; Zhang, G.; Schjoerring, J. K.; Yuan, L. High light intensity aggravates latent manganese deficiency in maize. *J. Exp. Bot.* **2020**, *71* (19), 6116−6127.
(39) Wu, L.; Wu, M.; Liu, H.; Gao, Y.; Chen, F.; Xiang, Y. Identification and characterisation of monovalent cation/proton antiporters (CPAs) in Phyllostachys edulis and the functional analysis of PheNHX2 in Arabidopsis thaliana. *Plant Physiol. Biochem.* **2021**, *164*, 205−221.
(40) Bohley, K.; Schroder, T.; Kesselmeier, J.; Ludwig, M.; Kadereit, G. C4-like photosynthesis and the effects of leaf senescence on C4-like physiology in Sesuvium sesuvioides (Aizoaceae). *J. Exp. Bot.* **2019**, *70* (5), 1553−1565.
(41) Wisniewski, J. R.; Zougman, A.; Nagaraj, N.; Mann, M. Universal sample preparation method for proteome analysis. *Nat. Methods* **2009**, *6* (5), 359−362.
(42) Peng, Z.; Lu, Y.; Li, L.; Zhao, Q.; Feng, Q.; Gao, Z.; Lu, H.; Hu, T.; Yao, N.; Liu, K.; et al. The draft genome of the fast-growing non-timber forest species moso bamboo (Phyllostachys heterocycla). *Nat. Genet.* **2013**, *45* (4), 456−461.
(43) Conesa, A.; Gotz, S.; Garcia-Gomez, J. M.; Terol, J.; Talon, M.; Robles, M. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* **2005**, *21* (18), 3674−3676.
(44) Szklarczyk, D.; Kirsch, R.; Koutrouli, M.; Nastou, K.; Mehryary, F.; Hachilif, R.; Gable, A. L.; Fang, T.; Doncheva, N. T.; Pyysalo, S.; et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* **2023**, *51* (D1), D638−D646.
(45) Langfelder, P.; Horvath, S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf.* **2008**, *9*, 559.
(46) Chou, K. C.; Shen, H. B. Cell-PLoc: a package of Web servers for predicting subcellular localization of proteins in various organisms. *Nat. Protoc.* **2008**, *3* (2), 153−162.
(47) Schmittgen, T. D.; Livak, K. J. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* **2008**, *3* (6), 1101−1108.
(48) Kitaya, Y.; Tsuruyama, J.; Kawai, M.; Shibuya, T.; Kiyota, M. Effects of air current on transpiration and net photosynthetic rates of plants in a closed plant production system. In *Transplant Production in the 21st Century: Proceedings of the International Symposium on Transplant Production in Closed System for Solving the Global Issues on Environmental Conservation, Food, Resources and Energy*; Springer, 2000; pp 83−90.
(49) (a) Dupree, E. J.; Jayathirtha, M.; Yorkey, H.; Mihasan, M.; Petre, B. A.; Darie, C. C. A critical review of bottom-up proteomics: the good, the bad, and the future of this field. *Proteomes* **2020**, *8* (3), 14. (b) Cassidy, L.; Kaulich, P. T.; Maaß, S.; Bartel, J.; Becher, D.; Tholey, A. Bottom-up and top-down proteomic approaches for the identification, characterization, and quantification of the low molecular weight proteome with focus on short open reading frame-encoded peptides. *Proteomics* **2021**, *21* (23−24), No. e2100008.
(50) Wang, T.; Wang, H.; Cai, D.; Gao, Y.; Zhang, H.; Wang, Y.; Lin, C.; Ma, L.; Gu, L. Comprehensive profiling of rhizome-associated alternative splicing and alternative polyadenylation in moso bamboo (Phyllostachys edulis). *Plant J. Cell Mol. Biol.* **2017**, *91* (4), 684−699.
(51) Yu, H.-x.; Pan, X.; Xu, M.-p.; Yang, W.-m.; Wang, J.; Zhuang, X.-w. Surface chemical changes analysis of UV-light irradiated Moso bamboo (Phyllostachys pubescens Mazel). *R. Soc. Open Sci.* **2018**, *5* (6), 180110.
(52) Casal, J. J.; Mazzella, M. A. Conditional synergism between cryptochrome 1 and phytochrome B is shown by the analysis of phyA, phyB, and hy4 simple, double, and triple mutants in Arabidopsis. *Plant Physiol.* **1998**, *118* (1), 19.
(53) Liu, H.; Liu, B.; Zhao, C.; Pepper, M.; Lin, C. The action mechanisms of plant cryptochromes. *Trends Plant Sci.* **2011**, *16* (12), 684−691.
(54) Sellaro, R.; Hoecker, U.; Yanovsky, M.; Chory, J.; Casal, J. J. Synergism of red and blue light in the control of Arabidopsis gene expression and development. *Curr. Biol.* **2009**, *19* (14), 1216−1220.
(55) Osterlund, M. T.; Hardtke, C. S.; Wei, N.; Deng, X. W. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. *Nature* **2000**, *405* (6785), 462−466.

https://doi.org/10.1021/acs.jafc.4c00724
J. Agric. Food Chem. XXXX, XXX, XXX−XXX

(56) Lian, H. L.; He, S. B.; Zhang, Y. C.; Zhu, D. M.; Zhang, J. Y.; Jia, K. P.; Sun, S. X.; Li, L.; Yang, H. Q. Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. *Genes Dev.* **2011**, *25* (10), 1023−1028.
(57) Saijo, Y.; Sullivan, J. A.; Wang, H.; Yang, J.; Shen, Y.; Rubio, V.; Ma, L.; Hoecker, U.; Deng, X. W. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes Dev.* **2003**, *17* (21), 2642−2647.
(58) Gangappa, S. N.; Botto, J. F. The Multifaceted Roles of HY5 in Plant Growth and Development. *Mol. Plant* **2016**, *9* (10), 1353−1365.
(59) Laubinger, S.; Hoecker, U. The SPA1-like proteins SPA3 and SPA4 repress photomorphogenesis in the light. *Plant J.* **2003**, *35* (3), 373−385.
(60) Holm, M.; Ma, L.-G.; Qu, L.-J.; Deng, X.-W. Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Gene Dev.* **2002**, *16* (10), 1247−1259.
(61) (a) Chen, T.; Ma, J.; Liu, Y.; Chen, Z.; Xiao, N.; Lu, Y.; Fu, Y.; Yang, C.; Li, M.; Wu, S.; et al. iProX in 2021: connecting proteomics data sharing with big data. *Nucleic Acids Res.* **2022**, *50* (D1), D1522−D1527. (b) Ma, J.; Chen, T.; Wu, S.; Yang, C.; Bai, M.; Shu, K.; Li, K.; Zhang, G.; Jin, Z.; He, F.; et al. iProX: an integrated proteome resource. *Nucleic Acids Res.* **2019**, *47* (D1), D1211−D1217.

https://doi.org/10.1021/acs.jafc.4c00724
J. Agric. Food Chem. XXXX, XXX, XXX−XXX