



Article

Far-Red Light Regulates the Circadian Rhythm Pathway to Accelerate Rice Flowering

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Abstract

Early flowering is a key element of the rice speed-breeding protocol that enables improved genetic gain and accelerates the cultivation of new varieties. Although far-red light (FR) is commonly used to modulate plant developmental processes, the mechanisms by which it influences flowering and growth in rice are poorly understood. In this study, the control treatment (CK) consisted of red-blue-green composite light at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, while two additional treatments were applied: one with the photon flux density (PFD) increased to $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ (HI—high intensity) under the same light spectrum as CK, and the other supplemented with $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of FR based on CK. The results demonstrated that both elevated PFD and supplemental FR significantly enhanced vegetative growth, as evidenced by increased plant height, tiller number, leaf area, and biomass accumulation, along with improved photosynthetic capacity and chlorophyll fluorescence. Under the FR treatment, flowering occurred 53 days after transplanting, which was 12 days and 9 days earlier than in the CK and HI treatments, respectively. Physiological profiling revealed that FR enrichment significantly increased leaf soluble sugar and starch levels, while simultaneously decreasing chlorophyll and carotenoid concentrations. FR also reshaped the endogenous hormonal profile, which was marked by elevated levels of gibberellin (GA3) and abscisic acid (ABA), and reduced auxin (IAA) content. Transcriptomic profiling revealed that FR enrichment activated the circadian rhythm pathway and upregulated genes associated with photoperiodic flowering and inflorescence development. In summary, FR promotes rice growth and early flowering through the integrated regulation of leaf area expansion, enhanced photosynthetic efficiency, hormonal rebalancing, and activation of flowering gene expression. This study provides a theoretical foundation and technical support for optimizing light environments and improving the economic viability of crop speed breeding systems in controlled environmental facilities.



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1. Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the global population; however, the current rate of genetic improvement falls behind the demand for future food security [1]. Conventional crossbreeding of crops relies on multiple generations of selfing and phenotypic selection, a process that significantly limits the rate of varietal

development and results in lengthy breeding cycles, often spanning 8–10 years [2]. Due to the global food challenges brought about by population growth and climate change, there is an urgent need to accelerate genetic gain and enhance breeding efficiency. To address this challenge, Ghosh et al. [3] proposed the speed-breeding protocol, which uses precisely controlled environmental conditions to accelerate plant development and rapid generation advancement in controlled facilities. Whereas field production yields only one or two generations per year, speed-breeding optimizes photoperiod and temperature to achieve four to six generations annually [4]. This approach significantly shortens the time required to obtain homozygous inbred lines and has shown considerable potential for improving breeding efficiency in short-day crops such as rice.

The core of the speed-breeding protocol is accelerating crop growth through the precise regulation of growth environmental factors, thereby shortening the growth period of crops. The total life cycle of rice is typically 100–150 days, with the period from sowing to flowering accounting for approximately 70% of this duration [5,6]. Therefore, controlling the timing of rice flowering is crucial for successful breeding. Flowering is the key developmental transition from vegetative to reproductive growth. It not only influences pollination success but also determines the environmental conditions during grain filling, ultimately affecting harvest time [7,8]. Light is a critical environmental factor for rice flowering that is regulated not only by the photoperiod but also by different light spectra [9]. Kabade et al. [10] developed a speed-flowering protocol that combined red-blue spectra with far-red light (FR) to shorten the flowering period to 52–60 days in both *japonica* and *indica* rice. However, this study only considered FR as a method for accelerating crop growth and development without systematically investigating the physiological response mechanisms of rice to FR signals.

Shade avoidance syndrome (SAS) induced by FR can significantly regulate morphogenesis as well as the growth and development processes of plants [11–13]. At low red-to-far-red (R/FR) ratios, plants initiate adaptive responses, including stem and leaf elongation, increased leaf angle, and regulation of downstream gene expression through phytochrome interaction factors (PIFs). In *Arabidopsis*, low R/FR stabilizes PIF4, PIF5, and PIF7 proteins, resulting in the upregulation of early flowering genes such as *FT* and *SPL*, thereby accelerating flowering [14]. Although the molecular pathway by which FR promotes flowering in *Arabidopsis* is relatively well characterized, how FR signaling regulates flowering in major cereal crops such as rice remains largely unknown.

Previously, we established a vertical speed-breeding system with hydroponics in a plant factory, achieving five generations of *indica* rice per year [15]. Here, we aimed to modulate rice flowering using FR to further improve speed-breeding efficiency. We also systematically investigated the effects of FR on flowering time, physiological metabolism, and the expression of key genes. Together, these results provide theoretical insights and methodological guidance for elucidating the physiological mechanisms underlying FR-induced flowering in rice and for optimizing light-environment-driven rapid crop-breeding technologies.

2. Results

2.1. Effects of FR on Rice Growth

Both supplementary FR and increased photon flux density (PFD) significantly promoted rice growth compared to the control treatment (CK). Rice plants grown under FR exhibited significantly greater plant height, tiller number, leaf area, and aboveground dry weight than those grown under CK (Figure 1), indicating that FR accelerated rice growth. However, this effect gradually diminished as rice development progressed. During the first 40 days after transplantation (DAT), all measured growth parameters were significantly

higher under FR conditions than under the high-intensity (HI) conditions. However, by day 70, plant height, tiller number, and leaf area in the HI treatment had surpassed those in the FR treatment, with no significant difference observed in the aboveground dry weight between the two treatments. These results suggest that FR primarily enhances early-stage growth with limited impact on final biomass accumulation. In addition, elevated PFD accelerated growth and tillering and significantly increased leaf area and aboveground biomass compared with CK (Figure 1).

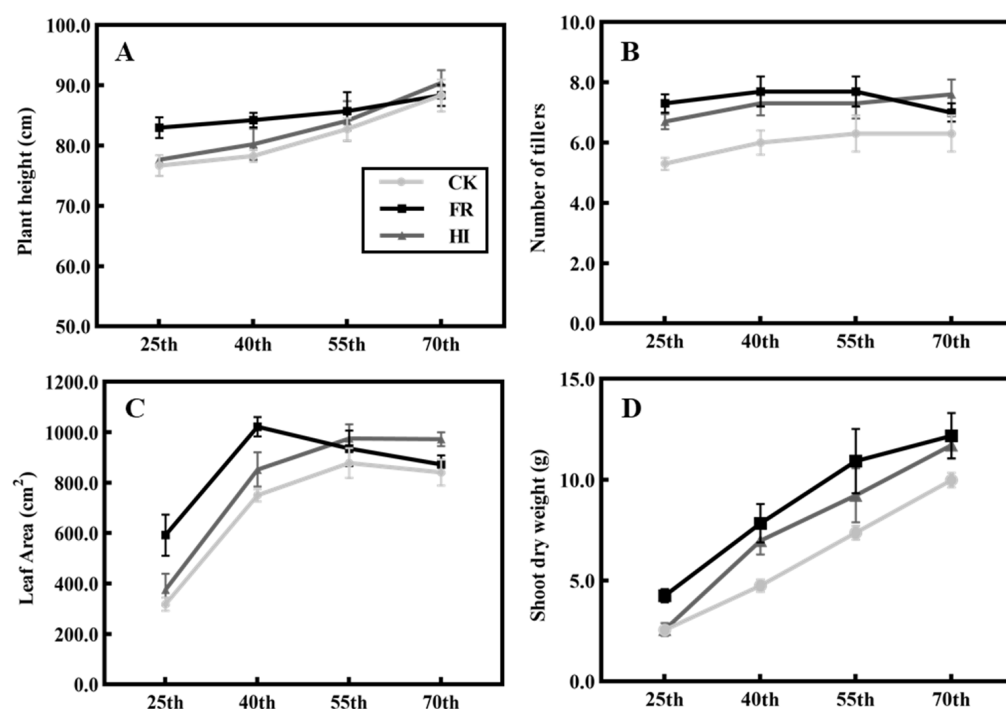


Figure 1. Rice plant growth performance in response to different light regimes. (A) Plant height. (B) Number of tillers. (C) Leaf area. (D) Shoot dry weight. All data are the means $\pm$ SD of three repeats. The x-axis represents days after transplantation (DAT). Abbreviations: CK, control treatment; FR, far-red light; HI, high intensity.

Both supplementary FR and increased PFD accelerated flowering compared with CK (Figure 2A). Under FR, flowering occurred 53 days after transplanting—12 days earlier than CK and 9 days earlier than HI (Figure 2B). HI also advanced flowering, occurring three days before CK.

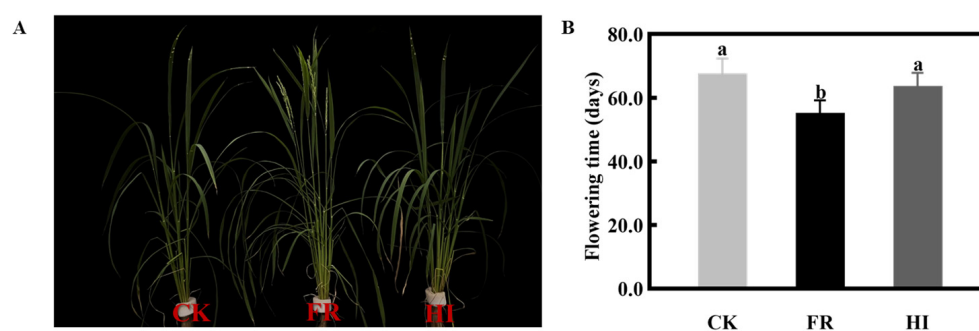


Figure 2. Rice flowering time in response to different light regimes. (A) Comparison of flowering in the first rice plant under different treatments. (B) First flowering time under different treatments. All data are the means $\pm$ SD of three repeats. Letters show statistically significant differences ($p < 0.05$).

2.2. Effects of FR on Rice Leaves Gas Exchange Parameters and Chlorophyll Fluorescence Characteristics

Different light treatments significantly affected photosynthetic and chlorophyll fluorescence parameters (Figure 3). Net photosynthetic rate (P_n) differed among treatments when PFD exceeded $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, with both FR and HI exhibiting significantly higher P_n than CK, indicating enhanced photosynthetic capacity. Across the $150\text{--}1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD range, FR consistently showed higher P_n than CK and HI (Figure 3A).

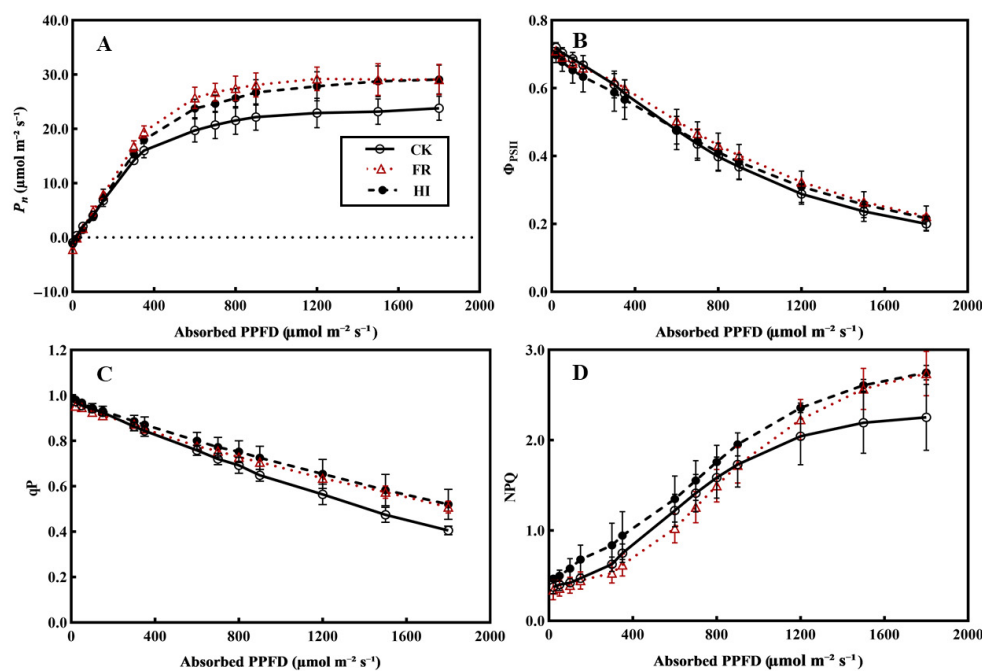


Figure 3. Effects of different light regimes on (A) the light response curve. (B) PSII operating efficiency. (C) Photochemical quenching. (D) Non-photochemical quenching of rice leaves. All data are the means $\pm$ SD of three repeats. PPFD represents the photosynthetic photon flux density of the photosynthetic instrument.

No significant differences were detected in the actual photochemical quantum yield (F_v/F_m) of PSII (Φ_{PSII}), although CK Φ_{PSII} values were slightly lower than those of FR and HI (Figure 3B). Photochemical quenching (qP) did not differ significantly between FR and HI (Figure 3C). Nevertheless, when PFD exceeded $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, qP in CK was significantly lower than in FR and HI as PFD increased, suggesting that supplementary FR or higher PFD helps maintain PSII reaction centers openness and supports electron transport and carbon assimilation.

Non-photochemical quenching (NPQ) was higher in FR and HI than in CK when PFD exceeded $900 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 3D), indicating enhanced thermal dissipation that likely helped mitigate photodamage. In contrast, within the $150\text{--}900 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD range, NPQ in FR was lower than in CK and HI, likely reflecting its higher photosynthetic efficiency and reduced need for strong energy-dependent photoprotection.

Supplementary FR applied to CK significantly enhanced the maximum photosynthetic rate, dark respiration rate, and maximum F_v/F_m of rice leaves (Table 1). In addition, a moderate increase in PFD significantly improved the leaves' maximum photosynthetic rate.

Table 1. Photosynthetic parameters of rice leaves in response to different light regimes. All data are the means $\pm$ SD of three repeats. Different lowercase letters within a row indicate significant differences (least significant difference [LSD], $p < 0.05$).

Photosynthetic Parameters	CK	FR	HI
Maximum photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	24.0 $\pm$ 2.3 b	29.87 $\pm$ 2.52 a	29.05 $\pm$ 5.59 a
Dark respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1.59 $\pm$ 0.44 b	2.61 $\pm$ 0.13 a	2.16 $\pm$ 0.10 b
Light saturation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1365.1 $\pm$ 65.5 a	1294.7 $\pm$ 70.5 a	1472.9 $\pm$ 73.3 a
Light compensation point ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	20.1 $\pm$ 2.3 a	27.9 $\pm$ 2.5 a	27.0 $\pm$ 3.9 a
F_v/F_m	0.827 $\pm$ 0.002 b	0.835 $\pm$ 0.005 a	0.828 $\pm$ 0.004 b

2.3. Effects of FR on Rice Leaves' Photosynthetic Pigments

The light treatments significantly influenced the rice leaves' photosynthetic pigment content (Figure 4). FR treatment significantly reduced chlorophyll and carotenoid contents in leaves but increased the carotenoid-to-chlorophyll ratio (Car/Chl) (Figure 4A–C). This ratio typically increased during leaf senescence, and under FR, the Car/Chl ratio was higher than under HI and CK (Figure 4D). However, no significant differences were observed in chlorophyll content and chlorophyll a/b ratios among HI and CK treatments (Figure 4A,D).

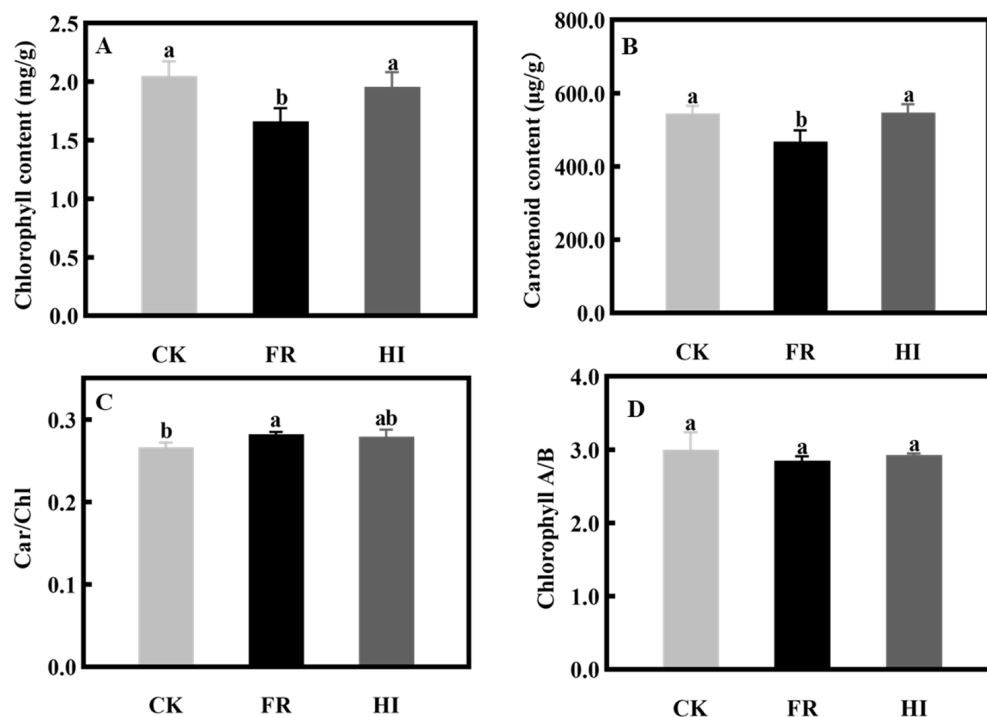


Figure 4. Rice leaves' photosynthetic pigment in response to different light regimes. (A) Chlorophyll content. (B) Carotenoid content. (C) Ratio of carotenoids/chlorophyll. (D) Ratio of chlorophyll A/B. All data are the means $\pm$ SD of three repeats. Letters show statistically significant differences ($p < 0.05$).

2.4. Effects of FR on Rice Leaves Biochemical Component Contents

FR significantly increased rice leaves' soluble sugar and starch contents (Table 2). The soluble sugar content under FR treatment was elevated by 67.2% and 48.4% compared to CK and HI, respectively, whereas the starch content increased by 19.0% and 8.0% relative to CK and HI, respectively. HI treatment also significantly increased the soluble sugar and starch contents—12.7% and 10.1%, respectively—compared with CK. However, total carbon and nitrogen contents did not differ significantly among the treatments.

Table 2. Leaf biochemical components in response to different light regimes. All data are the means $\pm$ SD of three repeats. Different lowercase letters within a row indicate significant differences (LSD, $p < 0.05$).

Biochemical Components	CK	FR	HI
Soluble sugar content (mg g ⁻¹)	24.06 $\pm$ 1.18 b	40.23 $\pm$ 2.25 a	27.11 $\pm$ 2.39 b
Starch content (mg g ⁻¹)	49.26 $\pm$ 2.84 c	58.82 $\pm$ 2.69 a	54.32 $\pm$ 0.71 b
Total nitrogen content (g kg ⁻¹)	42.42 $\pm$ 0.84 a	39.97 $\pm$ 0.90 a	42.81 $\pm$ 1.60 a
Total carbon content (g kg ⁻¹)	423.79 $\pm$ 6.31 a	423.53 $\pm$ 13.79 a	426.90 $\pm$ 13.22 a

2.5. Effects of FR on Rice Leaves Endogenous Hormone Contents

Plant hormones play crucial regulatory roles in the flowering process of crops, and different light treatments substantially affect the endogenous hormone levels in rice leaves. Compared with CK, FR greatly reduced auxin (IAA) content by 50.8 (Figure 5A) and increased gibberellin (GA3) and abscisic acid (ABA) contents by 175.0% and 59.5%, respectively (Figure 5B,C). Under HI, IAA and ABA decreased by 33.9% and 35.6%, respectively, compared with CK, while GA3 increased by 100.0%. Relative to HI, FR resulted in higher GA3 and ABA levels by 37.5% and 147.6%, respectively, and lower IAA by 25.5%.

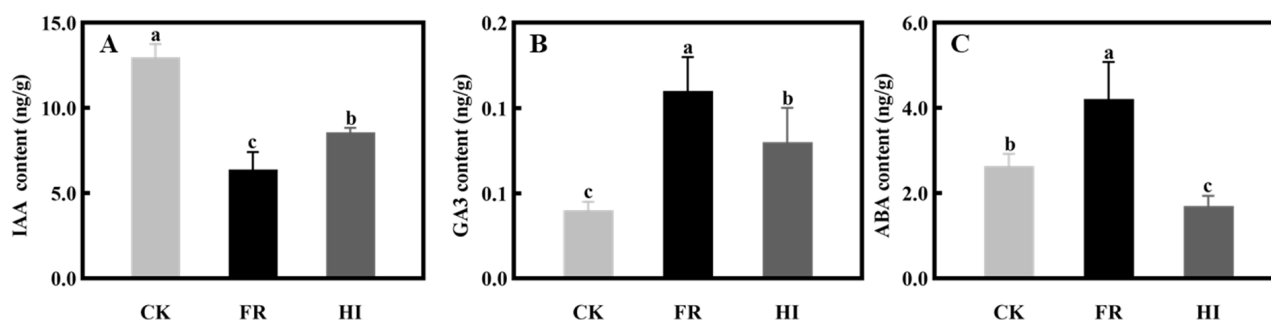


Figure 5. Leaf endogenous hormones in response to different light regimes. (A) IAA. (B) GA3. (C) ABA. All data are the means $\pm$ SD of three repeats. Letters show statistically significant differences ($p < 0.05$).

2.6. Rice Leaves Transcriptome Analysis

Transcriptome analysis was performed on the leaf samples from the three treatments, generating a total of 68.7 Gb of clean data across the nine samples. Each sample contained at least 6.3 Gb of clean data, with Q30 base percentages exceeding 87% and GC contents ranging from 57% to 59%. The overall sequencing error rate was 0.02% (Table S1). Principal component analysis (PCA) revealed a separation between different light treatments at the same growth stage (Figure 6A).

Differential expression analysis identified 803 differentially expressed genes (DEGs) in rice leaves under different light treatments at 30 DAT (Figure 6B). Substantial variations in DEG numbers were observed across treatment comparisons. The FR vs. CK comparison revealed the highest number of DEGs (400), comprising 137 upregulated and 263 downregulated genes (Figure 6C). In FR vs. HI, 256 DEGs were identified (114 upregulated and 142 downregulated). The HI vs. CK comparison yielded 147 DEGs, with 42 upregulated and 105 downregulated genes.

Gene Ontology (GO) enrichment analysis revealed that DEGs in the FR vs. CK comparison were significantly enriched in biological processes, molecular functions, and cellular components, with predominant enrichment in biological processes (Figure 6D). Within the biological process, terms directly associated with flowering included regulation

of the reproductive process, meristem transition from vegetative to reproductive phase, terpenoid biosynthetic process, photoperiodic flowering, and inflorescence development.

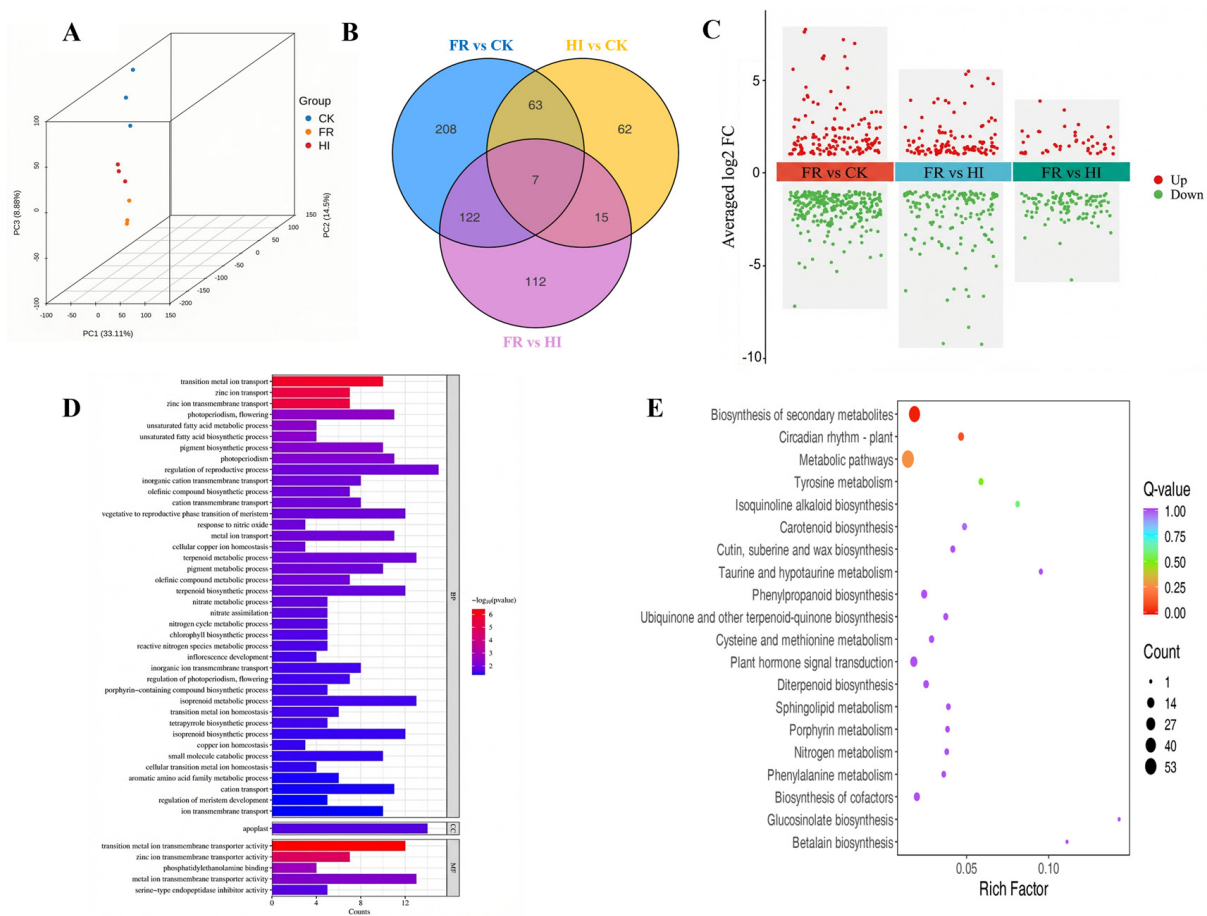


Figure 6. Rice leaf transcriptome analysis between FR and CK. **(A)** Transcriptome data principal component analysis. **(B)** Numbers of differentially expressed genes (DEGs) identified in each treatment. **(C)** DEGs volcano plot. **(D)** GO enrichment DEGs analysis bar chart for DEGs. **(E)** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis scatter plot for DEGs. All data were analyzed using independent sample *t*-tests, and statistical results were expressed as mean $\pm$ standard error.

For DEGs between the FR and HI, GO enrichment occurred mainly in biological processes and molecular functions (Figure S1A). Flowering-related biological processes included inflorescence development, regulation of meristem phase transition timing, regulation of the transition from vegetative to reproductive phase timing, regulation of meristem development, regulation of flower development, response to gibberellin, and terpenoid biosynthesis. Notably, three terms—inflorescence development, regulation of meristem development, and terpenoid biosynthesis—were enriched in both FR vs. CK and FR vs. HI. In contrast, DEGs between HI and CK were not significantly enriched in flowering-related GO terms (Figure S2A).

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (Q -value < 0.05) indicated that DEGs between FR and CK were enriched in the “biosynthesis of secondary metabolites” and “circadian rhythm-plant” pathways (Figure 6E). DEGs between FR and HI were enriched only in the “circadian rhythm-plant” pathway (Figure S1B), whereas those between HI and CK were enriched mainly in “nitrogen metabolism” (Figure S2B).

2.7. Expression Profiles of Flowering-Related Gene Response to Different Light Conditions

FR promoted rice flowering. To elucidate the underlying molecular mechanisms, flowering-related DEGs were identified through KEGG pathway analysis (Figure 7). These DEGs were primarily enriched in GA biosynthesis and signaling pathways and in the circadian rhythm pathway. In the GA biosynthesis pathway, FR upregulated *OsGA20ox2*, a key GA synthesis gene, relative to CK. In the GA signaling pathway, FR suppressed *GID1L2*, which encodes the GA receptor, and downregulated *DELLA* genes. Downstream of the GA pathway, the transcription factors *OsPIL15* and *OsPIL16* were markedly upregulated under FR. Because PIFs are pivotal regulators of photomorphogenesis and circadian integration, their increased expression suggests a functional role in mediating FR responses.

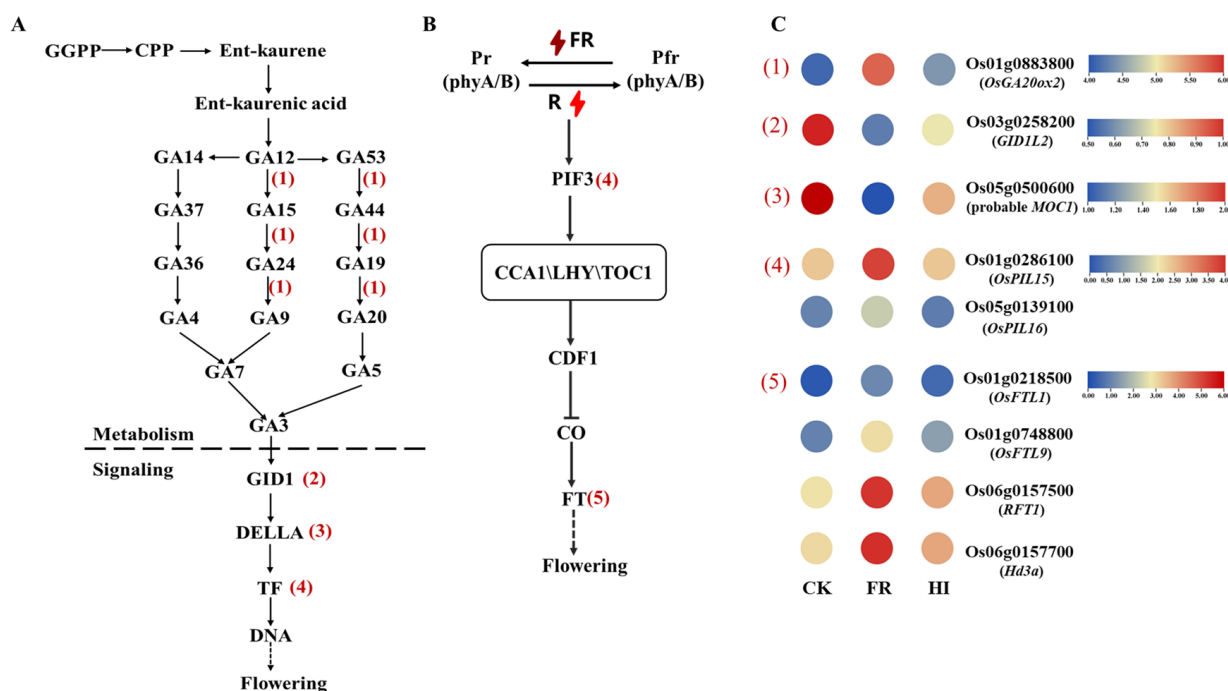


Figure 7. Heatmap of relative expression profiles of DEGs annotated in the GA and circadian rhythm pathways. (A) GA synthesis and signal transduction pathway. (B) Circadian rhythm pathway. (C) Heatmap of the relative expression of DEGs in the GA and circadian rhythm pathways. FR and R represent far-red light red light respectively.

The florigen-encoding *FT* gene family also showed strong transcriptional activation: *OsFTL1*, *Hd3a*(*OsFTL2*), *RFT1*(*OsFTL3*), and *OsFTL9* were all significantly upregulated under FR. This coordinated induction of *FT*-like genes likely represents a key mechanism through which FR accelerates flowering in rice plants.

Twenty genes associated with “circadian rhythm,” “metabolic pathways,” and “diterpenoid biosynthesis” were selected for quantitative real-time PCR (qRT-PCR) validation. Their expression patterns were largely consistent with the RNA-Seq results (Figure S3).

3. Discussion

With the advancement of light-emitting diode (LED) lighting technology, FR has become increasingly used in controlled-environment agriculture, especially in plant factories, where it plays a key role in regulating plant growth and development. This study investigated the effects of FR on rice morphology, flowering time, photosynthesis, endogenous hormones, and transcriptome profiles under plant-factory conditions. Supplementary FR significantly accelerated flowering, enabling earlier harvests. These findings highlight the

significance of enhancing efficiency of crop speed breeding and economic sustainability of controlled facilities.

3.1. Supplemental FR Significantly Enhances Rice Growth and Accelerates Flowering

FR is a key signal inducing the shade-avoidance response. A reduced R/FR ratio typically promotes increased plant height, leaf expansion, and earlier flowering [16,17]. Here, supplementation with $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ of FR accelerated rice growth and development, increasing plant height, tiller number, leaf area, aboveground dry weight, and earlier flowering—consistent with a shade-avoidance response. Notably, even under constant total PFD, FR promoted growth and flowering by nine days compared with HI, indicating that its growth-promoting effect stems primarily from its photosensory function rather than additional light energy.

The growth-promoting effect of FR also showed clear temporal dependence (Figure 1). During early vegetative growth (before day 40), FR produced substantial increases in multiple growth parameters. However, this advantage gradually diminished as development progressed. By day 70, plant height and leaf area under FR were lower than under HI, and biomass no longer differed significantly. These results indicate that FR primarily accelerates developmental progression rather than sustaining biomass gains, consistent with Huber et al. [18].

3.2. Supplemental FR Enhances the Photosynthetic Capacity in Rice

Photosynthesis is influenced by both PFD and spectral composition. Here, rice leaves showed significantly enhanced photosynthetic capacity under either increased PFD or supplementary FR, with both treatments increasing the maximum photosynthetic rate. These improvements were consistent with higher qP. However, Φ_{PSII} was not markedly higher. The enhanced photosynthetic capacity under FR was likely linked to increased leaf area, which improved light interception, carbon assimilation, and biomass accumulation [19].

Chlorophyll and carotenoids (Chl/Car) are the main light-harvesting pigments. FR supplementation significantly reduced both pigment classes, and chlorophylls (a and b) also showed a decreasing trend, consistent with typical shade-avoidance responses [20]. The Chl/Car ratio was significantly higher under FR, a pattern often associated with leaf senescence. This likely resulted from accelerated growth and development under FR, producing physiologically older leaves at the same chronological stage and increasing the Chl/Car ratio. These physiological changes align with the earlier flowering observed under FR, collectively indicating accelerated developmental progression. Regarding the observation that photosynthetic capacity increases while photosynthetic pigment content decreases under FR, Huber et al. [18] believed that the augmentation of photosynthetic rate primarily derives from the Emerson enhancement effect and resultant optimization of photochemical efficiency, rather than from alterations in chlorophyll content.

Starches and soluble sugars are the primary photosynthetic products in higher plants. Here, both increased under FR, which is consistent with previous reports of carbohydrate accumulation in crops such as soybeans under low R/FR conditions [21,22]. Soluble sugars not only provide energy for growth and development but also act as signaling molecules involved in the regulation of various physiological processes. Sucrose functions as a long-distance signal in flowering induction and influences flowering time as an energy source. Elevated sucrose increases Trehalose-6-phosphate (Tre6P), which activates the key flowering gene *FT*, thereby promoting early flowering [23,24].

3.3. Supplemental FR Significantly Modulates Endogenous Hormone Levels in Rice Leaves

Endogenous phytohormones play important regulatory roles in plant shade avoidance responses through complex interaction networks [25–27]. In this study, compared with the

CK, FR treatment significantly increased the levels of GA₃ and ABA in rice plants, while the levels of IAA were significantly reduced. Gibberellin (GA) has been shown to play a critical regulatory role in the plant flowering process. Under low R/FR light conditions, the upregulation of the GA biosynthetic enzyme genes *GA20ox1* and *GA20ox2* enhances GA biosynthesis, thereby increasing GA content [28]. GA activates *SOC1*, which promotes *LFY* and upregulates the floral meristem identity gene *AP1*, accelerating floral transition [29,30]. Furthermore, the transcriptome analysis in this experiment indicated that FR affects flowering by regulating the GA biosynthesis and signaling (Figure 7A). Elevated levels of active GA enter the nucleus, bind to the *GID1* receptor, and form a GA–*GID1* complex. This complex further interacts with the key inhibitory factor *DELLA* in the signaling pathway, thereby triggering the ubiquitination and degradation of *DELLA*. The degradation of *DELLA* relieves their inhibition of transcription factors, such as PIFs, enabling activation of downstream genes that drive rice flowering, growth, and development. It is worth noting that while GAs are typically known to delay leaf senescence, their levels were elevated under FR treatment in our study. This can be attributed to the role of GA in mediating the shade avoidance syndrome, specifically in promoting internode elongation and accelerating the transition to flowering [31]. The FR-treated plants entered the reproductive stage earlier than the control; consequently, the observed decrease in chlorophyll and IAA, along with the increase in ABA, reflects an earlier onset of monocarpic senescence induced by this accelerated developmental progression, rather than a direct negative regulation failure of GA.

IAA is a key regulator of stem elongation in shade avoidance. A low R/FR ratio generally enhances IAA biosynthesis and signaling [32]. However, in this study, IAA in rice leaves decreased under FR. This discrepancy likely reflects IAA spatiotemporal distribution, as concentrations are higher in actively growing tissues (e.g., shoot apices) than in mature leaves [33].

ABA accumulation under low R/FR has been widely reported [34], which is consistent with our findings. However, ABA is often associated with delayed flowering [35]. In the present study, although FR increased ABA content in rice leaves, it simultaneously promoted early flowering. This can be explained by leaf senescence: ABA accumulation, reduced chlorophyll content, and an increased Car/Chl ratio are recognized as typical physiological indicators of leaf senescence [36]. FR treatment led to significantly higher ABA content, accompanied by decreased chlorophyll content and an elevated Car/Chl ratio, indicating advanced leaf physiological age. Therefore, higher ABA likely reflects leaf senescence driven by FR-accelerated development, with ABA passively accumulating in aging leaves rather than being directly regulated via the ABA signaling pathway in response to far-red light. This explains the coexistence of elevated ABA and early flowering. Besides, it is also possible that the elevation of ABA levels under FR is mediated by the stabilization of the transcription factor PIFs due to PhyB inactivation, which subsequently directly activates the expression of rate-limiting ABA biosynthetic genes, such as *NCED6* and *NCED9* [37,38], although this mechanism requires further investigation. Interestingly, although both FR and HI treatments accelerated flowering, they elicited opposite responses in leaf ABA content. This discrepancy can be attributed to the fundamental difference between light signaling and light energy effects. FR triggers SAS and induces a rapid developmental shift towards reproduction, where elevated ABA levels likely facilitate accelerated leaf senescence to support this rapid reproductive development [36]. Conversely, the HI treatment acts primarily by increasing photon availability for photosynthesis. Under high irradiance, plants tend to downregulate ABA or maintain low ABA levels to ensure maximum stomatal aperture for CO₂ fixation and biomass accumulation [39]. Thus,

the modest early flowering observed under HI is likely driven by enhanced assimilate accumulation rather than the stress-associated escape mechanism seen under FR.

3.4. Supplemental FR Enhances the Circadian Pathway—Plant

FR plays a key role in regulating plant growth, and its positive effect on rice growth and development has been reported in numerous studies. However, the specific molecular mechanisms and key regulatory genes remain unclear. To investigate how FR induces early flowering in rice, we conducted transcriptome sequencing to analyze gene expression changes in rice leaves under FR treatment. Our findings showed that genes associated with flower development and related pathways were strongly upregulated by FR. KEGG enrichment analysis further revealed significant enrichment of DEGs in the plant circadian rhythm pathway, indicating that FR may regulate early flowering in rice by modulating this pathway.

The flowering process is precisely regulated by a complex network that integrates multiple endogenous signals, including hormones and developmental age, with environmental factors, such as photoperiod and temperature. The circadian rhythm system plays a central role in coordinating these signals and activating flowering genes [40]. It includes input pathways that detect external signals, core oscillators that generate circadian rhythms, and output pathways that regulate physiological processes [41,42]. Although endogenous, the circadian phase regulation setting and maintenance depend on environmental signals such as light [43].

Under FR, rice primarily perceives the signal through phytochrome A and transmits it to its core oscillator [44]. The negative feedback loop regulatory network formed by *CCA1*, *LHY*, and *TOC1* adjusts the circadian rhythm by regulating the expression of rhythmic genes, such as *OsGI*, thereby synchronizing the internal rhythm with the FR cycle [45,46]. Photoperiodic regulation of flowering is a key plant circadian output pathway [47,48]. Heading Date 1 (*Hd1*), a key transcription factor downstream of the rice circadian clock, is regulated by *OsGI*, other circadian clock genes, and light signals [49]. The *OsGI-Hd1-Hd3a* module is the primary pathway inducing rice flowering under short days and is highly conserved with the *GI-CO-FT* pathway in Arabidopsis [50]. Furthermore, *OsLHY* also binds CBS elements in the *OsGI* promoter, subsequently inhibiting its transcription and regulating flowering time, indicating that the *OsLHY-OsGI-Hd1* cascade constitutes a core circadian output pathway in rice [51].

In conclusion, FR transmits signals to the biological clock via photoreceptors and regulates downstream flowering genes via the photoperiodic pathway, thereby promoting early flowering in rice. However, key genes and their upstream and downstream regulatory components within this network still require further validation.

4. Materials and Methods

4.1. Cultivation Conditions

The experiment was conducted in a plant factory located in Chengdu (104°04' E, 30°40' N), Sichuan, China, using Nipponbare (*Oryza sativa* L. ssp. *japonica*) as the plant material. Rice seeds were surface-sterilized and soaked in distilled water at room temperature for 24 h. The seeds were then sown in seedling trays and placed in a dark environment until germination. After emergence, the seedlings were transferred to a growth chamber with red-blue LED and cultivated for 10 days until they reached the two-leaf-one-heart stage. The PFD at 50 cm below the LED panels was maintained at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a photoperiod of 11 h/13 h (light/dark) and a red:blue ratio of 1:1. The temperature during the seedling stage was set at 25 °C/21 °C (light/dark). The seedlings were transplanted into a double-layer hydroponic system fitted with LED lighting. Each cultivation trough measured

200 cm × 60 cm and was covered with four cultivation panels (50 cm × 60 cm). Each panel contained 15 planting holes, resulting in a total of 60 planting holes per trough, and a planting density of approximately 50 m⁻². The temperature after transplantation was set at 29 °C/20 °C (light/dark). The relative air humidity was maintained at (65% ± 5%) throughout the day, and the carbon dioxide concentration was maintained at (400 ± 10) µmol mol⁻¹ during the daytime. Plants were grown in a hydroponic nutrient solution based on Hoagland's formulation, prepared as described by Baiyin et al. [52]. The pH and electrical conductivity (EC) of the nutrient solution were maintained at approximately 5.8 ± 0.1 and 1.6 ± 0.1 dS m⁻¹, respectively. A 60 W water pump in each trough provided continuous 24 h circulation to ensure sufficient oxygen supply in the nutrient solution.

4.2. Experimental Light Regime Conditions

Three light treatments were applied after transplantation to investigate the effects of different light regimes on rice flowering and growth (Table S2). The CK consisted of a red–blue–green composite spectrum with a PFD of 300 µmol m⁻² s⁻¹ and a spectral ratio of red:blue:green = 1:1:1. The FR treatment was based on the CK, with an additional 50 µmol m⁻² s⁻¹ of FR radiation. In the HI treatment, total PFD was increased to 350 µmol m⁻² s⁻¹ with the same spectrum as the CK. The photoperiod of all three treatments was 11 h (8 am–7 pm). A multichannel LED panel (TYF Co., Ltd., Guangzhou, China) was used to deliver precise light spectra with peak emission wavelengths of 660 (red), 450 (blue), 550 (green), and 730 nm (far-red). The light environment was characterized using a spectrometer (AvaSpec-ULS2048x64-EVO, Avantes, Apeldoorn, The Netherlands) positioned 60 cm below the LED panels. The relative spectral distributions of the three treatments and the planting map are shown in Figure 8.

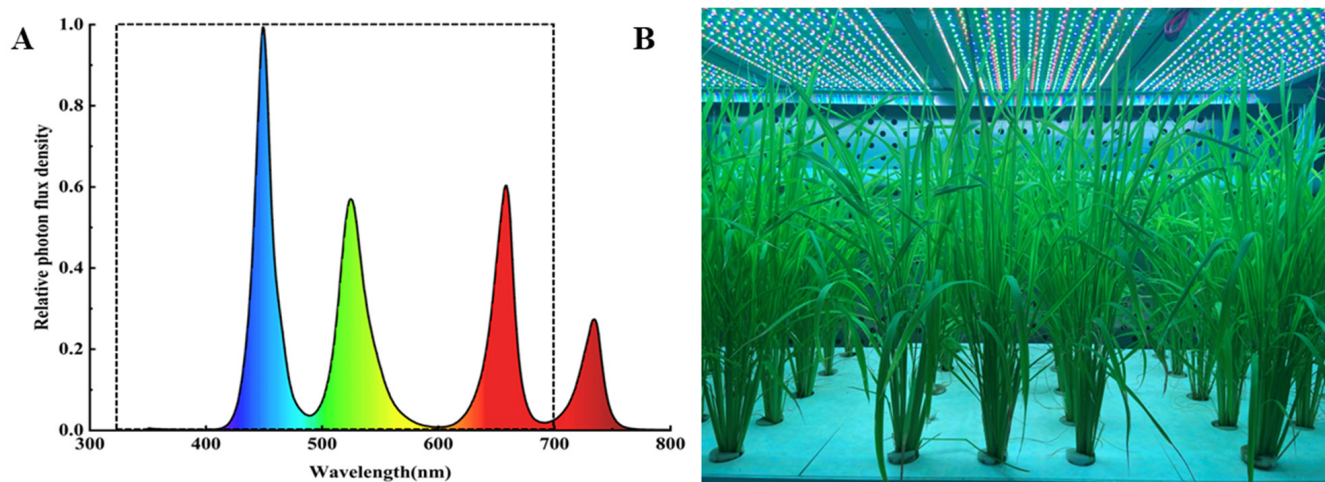


Figure 8. Experimental light regime settings. (A) The FR spectrum. (B) Rice growth under LED light. The box in (A) shows the spectra of the CK and HI. Different colors in A represent light spectra of light sources.

4.3. Measurement Methods

4.3.1. Sampling and Measurement of Morphological Indicators

Three rice plants were randomly selected from each treatment for growth parameter analysis at 25, 40, 55, and 70 DAT. The number of tillers and plant heights were recorded. Subsequently, the shoots were separated, and the leaf area was measured using a leaf area meter (LI-3100C, LI-COR, Inc., Lincoln, NE, USA). Finally, above-ground and below-ground biomass samples were labeled, placed in paper envelopes, and dried at 80 °C for 48 h until

constant weight was achieved. The dry weights were recorded. At 40 DAT, three additional plants per treatment were randomly sampled for subsequent biochemical analyses. Roots and petioles were removed, and fully expanded mature leaves were selected, flash-frozen in liquid nitrogen for 20 min, then stored at -80°C for the determination of biochemical components such as hormones, chlorophyll, and starch. The cultivation experiments were conducted independently in November 2023, April 2024, and September 2024, and data from one season were selected for analysis.

4.3.2. Gas Exchange and Measurement of Chlorophyll Fluorescence Parameters

The gas exchange and chlorophyll fluorescence parameters were measured using a portable photosynthesis system (LI-6800, LI-COR, Inc., Lincoln, NE, USA). Four plants were randomly selected from each treatment, and the uppermost fully expanded leaf of each plant was measured between 9 am and 6 pm.

The photosynthetic measurement system was configured with the following settings: an air flow rate of $500\ \mu\text{mol s}^{-1}$, a relative humidity of 60%, a CO_2 concentration of $400\ \mu\text{mol mol}^{-1}$, a leaf temperature of 29°C , and a leaf-to-air vapor pressure deficit ($\text{VPD}_{\text{leaf-air}}$) of 0.1 kPa.

A fluorescent leaf chamber was used to measure the light response curve of leaf photosynthesis. The actinic light source consisted of red (peak at 635 nm, 90%) and blue (peak at 465 nm, 10%) light. The light intensity sequence began at $1800\ \mu\text{mol m}^{-2}\text{s}^{-1}$ and was subsequently decreased in steps to 1500, 1200, 900, 600, 350, 300, 200, 150, 100, 70, 30, and $0\ \mu\text{mol m}^{-2}\text{s}^{-1}$. At each light intensity level, the steady-state photosynthetic rate was recorded after stabilization. The steady-state fluorescence yield (F_s), as well as the maximum (F'_m) and minimum (F'_0) fluorescence yields of light-adapted leaves, were simultaneously measured at each step.

To evaluate the effects of light treatment on gas exchange and chlorophyll fluorescence, plants were first dark-adapted for 2.5 h. Subsequently, the minimum (F_0) and maximum (F_m) fluorescence yields were measured using the LI-6800 fluorescence leaf chamber to determine the maximum quantum efficiency of PSII, calculated as $F_v/F_m = (F_m - F_0)/F_m$.

4.3.3. Measurement of Chlorophyll Content and Carotenoid Content

After grinding in liquid nitrogen, approximately 0.1 g of the powdered leaf sample was weighed and transferred to a tube containing 10 mL of the extraction solvent (anhydrous ethanol/acetone = 1/1). The mixture was vortexed thoroughly and then incubated in the dark for several hours to allow complete pigment extraction until the tissue residue at the bottom of the tube became completely white. A 200 μL supernatant aliquot was transferred to a 96-well microplate, with the extraction solvent used as the blank. The absorbance of the extract was measured at 663 nm and 645 nm (ABSPPlus, Molecular Devices, San Jose, CA, USA), recorded as A_{663} and A_{645} , respectively. The chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated using the following equations [53]:

$$\text{Chlorophyll a (mg/g)} = (12.72 \times A_{663} - 2.59 \times A_{645})/M \times V \times F$$

$$\text{Chlorophyll b (mg/g)} = (22.88 \times A_{645} - 4.67 \times A_{663})/M \times V \times F$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

where M is the fresh sample weight (g), V is the total volume of extraction solvent (0.01 L), and F is the dilution factor (if applicable).

The sample preparation and extraction procedures were consistent with those described for the chlorophyll analysis. Approximately 0.1 g of the powdered sample, ground in liquid nitrogen, was extracted with 10 mL of extraction solvent in the

dark for several hours until the plant residue became completely white. A 200 µL aliquot of the extract was then transferred to a 96-well microplate, with the pure solvent used as the blank. Absorbance was measured at 470 nm, 663 nm, and 645 nm (ABSPPlus, Molecular Devices, San Jose, CA, USA), and recorded as A_{470} , A_{663} , and A_{645} , respectively.

The concentrations of chlorophyll a (C_a) and chlorophyll b (C_b) were first calculated using the Arnon equations [54]:

$$C_a \text{ (mg/L)} = 12.72 \times A_{663} - 2.59 \times A_{645}$$

$$C_b \text{ (mg/L)} = 22.88 \times A_{645} - 4.67 \times A_{663}$$

The carotenoid concentration (C_c) was subsequently determined as follows:

$$C_c \text{ (mg/L)} = (1000 \times A_{470} - 3.27 \times C_a - 104 \times C_b) / 229$$

Finally, the carotenoid content in the tissue was calculated using the following formula:

$$\text{Carotenoid Content (mg/g FW)} = (C_c \times V \times F) / M$$

where M is the fresh sample weight (g), V is the volume of the extraction solvent (0.01 L), and F is the dilution factor.

4.3.4. Measurement of Total Starch Content

Approximately 0.1 g of powdered sample, ground in liquid nitrogen, was accurately weighed. The powder was treated with 1 mL of anhydrous diethyl ether, vortexed thoroughly, and centrifuged at $8000 \times g$ (H1750, Cence, Changsha, China) for 10 min at room temperature. The supernatants were discarded. Subsequently, 1 mL of 80% ethanol was added to the precipitate, and the mixture was vortexed to form a homogenate (if steel beads were used during grinding, they were removed at this stage). The mixture was incubated in a water bath at 80 °C for 30 min, followed by centrifugation at $8000 \times g$ (H1750, Cence, Changsha, China) for 10 min at room temperature, and the supernatant was discarded.

Next, 0.5 mL of distilled water was added to the precipitate, and the mixture was incubated in a boiling water bath for 15 min to ensure complete starch gelatinization. After cooling to room temperature, 6 mol/L HCl (0.5 mL) was added, and the solution was hydrolyzed in a 95 °C water bath for 30 min. The completeness of starch hydrolysis was confirmed by an iodine test, which showed no blue coloration. Subsequently, the reaction mixture was centrifuged at $8000 \times g$ (H1750, Cence, Changsha, China) for 10 min at room temperature, and the supernatant was carefully collected for further analysis.

The supernatant was diluted with distilled water. A 50 µL aliquot of the diluted supernatant was mixed with 50 µL of 6 mol/L NaOH to adjust the pH. Then, 50 µL of the resulting neutralized solution was transferred to a new tube, combined with 50 µL of DNS (3,5-dinitrosalicylic acid) reagent, and heated in a boiling water bath for 5 min. After cooling to room temperature, 200 µL of distilled water was added. Subsequently, 200 µL of the solution was transferred to a 96-well microplate for absorbance measurement at 540 nm.

A standard curve was generated using a 5 mg/mL glucose standard solution, which was serially diluted to yield concentrations of 1.0, 0.8, 0.7, 0.3, 0.2, and 0.1 mg/mL. The standard solutions were processed alongside the samples under identical conditions to ensure consistency and accuracy (ABS Plus; Molecular Devices, San Jose, CA, USA).

The total starch content was calculated using the following formula:

$$\text{Total Starch (mg/g)} = (C \times V \times F) / (M \times 0.9).$$

where C is the glucose concentration determined from a standard curve (mg/mL), V is the total volume of the extraction solution (1 mL), F is the dilution factor, M is the fresh weight of the sample (g), and 0.9 is the conversion factor of glucose to starch.

4.3.5. Measurement of Soluble Sugar Content

Approximately 0.1 g of powdered sample, ground in liquid nitrogen, was homogenized in 0.5 mL of distilled water. The homogenate was incubated in a 95 °C water bath for 10 min, cooled to room temperature, and centrifuged at $8000 \times g$ (H1750, Cence, Changsha, China) for 10 min. The resulting supernatants were collected for further analysis.

A 50 μ L supernatant aliquot was mixed with 10 μ L of 6 mol/L HCl and hydrolyzed in an 80 °C water bath for 30 min. Following hydrolysis, 10 μ L of 6 mol/L NaOH was added to neutralize the solution, followed by the addition of 30 μ L of distilled water. The mixture was thoroughly vortexed before measurement.

For colorimetric determination, 50 μ L of the processed sample was combined with 50 μ L of DNS reagent in a 1.5 mL microcentrifuge tube. The mixture was heated in a boiling water bath for 5 min, cooled to room temperature, and diluted with 200 μ L of distilled water. After mixing, 200 μ L of the solution was transferred to a microplate, and absorbance was measured at 540 nm.

A standard curve was prepared using a 5 mg/mL glucose solution serially diluted with distilled water to yield concentrations of 1.0, 0.8, 0.5, 0.4, 0.3, and 0.2 mg/mL. These standard solutions were processed alongside the samples under identical conditions to ensure analytical consistency (ABS Plus, Molecular Devices, San Jose, CA, USA).

The soluble sugar content was calculated as follows:

$$\text{Soluble Sugar (mg/g)} = (C \times V \times F) / M$$

where C is the sugar concentration determined from the standard curve (mg/mL), V is the volume of the extraction solution (0.5 mL), F is the dilution factor, and M is the fresh weight of the sample (g).

4.3.6. Measurement of Total Carbon and Nitrogen Content

Approximately 0.05 g of the sample was accurately weighed, wrapped in tin foil, and introduced into an elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). After a 10 min stabilization period, measurements were performed, and the data were recorded. Sulfanilamide was used as the standard reference material.

4.3.7. Measurement of Endogenous Plant Hormones

Standard Curve Preparation: Stock solutions of each hormone standard were prepared in methanol at a concentration of 500 μ g/mL. Appropriate aliquots of each stock solution were diluted with methanol to prepare a mixed working solution containing 5 μ g/mL of each target analyte and 0.1 μ g/mL of the corresponding internal standard. The mixed working solutions were serially diluted with methanol to generate calibration standards at concentrations of 0.1, 0.2, 0.5, 2, 5, 20, 50, and 200 ng/mL. An equal volume of the internal standard solution was added to each calibration level, maintaining a constant internal standard concentration of 20 ng/mL across all standards.

Sample Extraction and Purification: Rice leaf samples were ground into a fine powder in liquid nitrogen. Exactly 0.2 g of the homogenized powder was weighed and extracted with 2.0 mL of acetonitrile and 30 μ L of the internal standard working solution. The mixture was vortexed and centrifuged at $7000 \times g$ (H3-18KR, Ke Cheng, Changsha, China) for 5 min at 4 °C. The supernatant was collected, and the residue was re-extracted with an additional 2.0 mL of acetonitrile. The combined supernatants were mixed with 200 mg of C18 sorbent,

vigorously vortexed for 30 s, and centrifuged at $7000\times g$ (H3-18 KR, Ke Cheng, Changsha, China) for 5 min. The resulting supernatant was collected, evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 150 μ L of methanol. The final extract was filtered through a 0.22 μ m organic membrane filter into an autosampler vial prior to UPLC-MS/MS analysis (QTRAP 5500, Allen-Bradley, Marlborough, MA, USA).

Chromatographic Conditions: Separation was achieved on a Waters XSelect[®] HSS T3 column (2.1 mm $\times$ 150 mm, 2.5 μ m) maintained at 30 °C. The mobile phase consisted of (A) water containing 0.1% formic acid and (B) acetonitrile delivered at a flow rate of 0.35 mL/min. The injection volume was set to 5 μ L. The gradient program was as follows: 0–0.5 min, 10% B; 0.5–5.0 min, linear increase from 10% to 95% B; 5.0–5.1 min, decrease from 95% to 10% B; 5.1–8.0 min, 10% B for re-equilibration.

Mass spectrometry conditions analysis was performed using multiple reaction monitoring. The curtain gas pressure was set to 35 psi, the ion spray voltage to ± 4500 V (positive/negative ionization mode), the nebulizer gas pressure to 60 psi, the auxiliary gas pressure to 60 psi, and the source temperature to 600 °C.

4.4. Transcriptome Analysis

Fully expanded leaves were collected from the plants in the three treatment groups at 40 DAT for transcriptome analysis, with three biological replicates per group. Total RNA was extracted from the leaf tissues using the CTAB-PBIOZOL method [55]. Sequencing was performed using a NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Raw sequencing data were processed using FASTP to generate clean reads, which were subsequently aligned to the reference genome (IRGSP-1.0) using HISAT2. Gene expression levels were quantified as fragments per kilobase of transcript per million mapped reads using feature-Counts. Differential expression analysis between groups was performed using DESeq2, and *p*-values were adjusted using the Benjamini–Hochberg method to control the false discovery rate (FDR). DEGs were identified based on an FDR threshold of <0.05 and an absolute fold change of ≥ 2 . Functional enrichment analysis of DEGs was conducted using the GO and KEGG databases, with significantly enriched terms or pathways defined as those with a *p*-value ≤ 0.05 . Table S3, Table S4, and Table S5 present the DEGs of FR vs. CK, FR vs. HI, and HI vs. CK, respectively.

4.5. qRT-PCR Analysis

Twenty DEGs were randomly selected for validation by quantitative reverse transcription PCR (qRT-PCR). Total RNA was extracted using the MolPure[®] Plant RNA Kit, and cDNA was synthesized using the Evo M-MLV RT Mix Kit with gDNA Clean for qPCR Ver.2. *OsActin* (Os04g0177600) was used as the endogenous reference gene for normalization. The relative expression levels of the target genes were calculated using the $2^{-\Delta\Delta CT}$ method [56]. Table S6 presents all the primers used.

4.6. Statistical Analysis

Data were analyzed using SPSS (V25.0, IBM Corp., Armonk, NY, USA), and the effects of treatments on the measured plant variables were assessed using analysis of variance (ANOVA), followed by Fisher's protected least significant difference (LSD) test at a 95% confidence level. GraphPad Prism 9 (V9.0, GraphPad Software Inc., San Diego, CA, USA) was used for data visualization.

5. Conclusions

In this study, we systematically investigated the effects of FR on rice growth and development, photosynthetic characteristics, endogenous hormone levels, and transcriptomic profiles. Our findings demonstrated that supplementary FR significantly increased plant

height, tiller number, leaf area, and biomass accumulation during the vegetative growth stage, while enhancing photosynthetic capacity and chlorophyll fluorescence parameters. FR application accelerated growth and development and shortened flowering time.

These findings indicate that the growth-promoting effects of FR are primarily mediated through photoregulatory signaling, rather than through increased light energy input. Transcriptome analysis revealed that FR activates the circadian rhythm-plant pathway and upregulates the expression of genes associated with photoperiodic flowering and inflorescence development.

In summary, FR promotes rice growth and early flowering by expanding the leaf area, enhancing photosynthetic efficiency, and modulating hormonal homeostasis, and the circadian rhythm system likely plays a key role in this process. Supplemental FR induces early developmental progression in rice and shortens the harvest cycle, representing an effective strategy for reducing energy consumption in speed-breeding systems and improving economic viability.

This study provides a theoretical basis and technical support for light-regulation strategies to accelerate crop breeding, offering valuable insights for improving the economic viability and sustainability of controlled environmental speed-breeding protocols.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27041683/s1>.

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Abbreviations

The following abbreviations are used in this manuscript:

CK	Control treatment
FR	Far-red light treatment
HI	High light intensity treatment
PFD	Photon flux density
SAS	Shade avoidance syndrome
R/FR	Red light to far-red light ratios
PIF	Phytochrome interaction factor
DAT	Days after transplantation
Pn	Net photosynthetic rate
Φ_{PSII}	Photochemical quantum yield of PSII
qP	Photochemical quenching

NPQ	Non-photochemical quenching
F_v/F_m	Maximum photochemical quantum yield
Car/Chl	Carotenoid to chlorophyll ratio
IAA	Indole-3-acetic acid
GA3	Gibberellin 3
GA	Gibberellin
ABA	Absciscic acid
PCA	Principal component analysis
DEG	Differentially expressed gene
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LED	Light-emitting diode
Chla/b	Chlorophyll A to chlorophyll B
FDR	False discovery rate

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