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Far-red light enrichment affects gene expression and architecture as well as growth and photosynthesis in rice

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Abstract

Plants use light as a resource and signal. Photons within the 400–700 nm waveband are considered photosynthetically active. Far-red photons (FR, 700–800 nm) are used by plants to detect nearby vegetation and elicit the shade avoidance syndrome. In addition, FR photons have also been shown to contribute to photosynthesis, but knowledge about these dual effects remains scarce. Here, we study shoot-architectural and photosynthetic responses to supplemental FR light during the photoperiod in several rice varieties. We observed that FR enrichment only mildly affected the rice transcriptome and shoot architecture as compared to established model species, whereas leaf formation, tillering and biomass accumulation were clearly promoted. Consistent with this growth promotion, we found that CO_2-fixation in supplemental FR was strongly enhanced, especially in plants acclimated to FR-enriched conditions as compared to control conditions. This growth promotion dominates the effects of FR photons on shoot development and architecture. When substituting FR enrichment with an end-of-day FR pulse, this prevented photosynthesis-promoting effects and elicited shade avoidance responses. We conclude that FR photons can have a dual role, where effects depend on the environmental context: in addition to being an environmental signal, they are also a potent source of harvestable energy.

KEYWORDS
acclimation, light quality, light quantity, photobiology, shade avoidance, shoot architecture, transcriptome

Abbreviations: CPMs, counts per million; das, days after sowing; DEG, differentially expressed gene; difference in gene expression of treated to control group; FC, fold change; PFD, photon flux density; PFD is the number of photons that reach a given surface area each second in the range of 380–780 nm, which includes part of UV and FR, in addition to visible light, measured as µmol photons m^{-2} s^{-1}; PPFD, photosynthetic PFD = photosynthetic active radiation (PAR); PPFD is the number of photosynthetically active photons that reach a given surface area each second. It is defined as the photons in the range of 400–700 nm measured as µmol photons m^{-2} s^{-1}; R:FR, red to far-red light ratio; SAS, shade avoidance ayndrome; SLA, specific leaf area; is the ratio of leaf area per leaf mass, expressed as cm^2 g^{-1}; WL + EoD FR, end of day far-red light (EoD FR); FR light pulse after end of photoperiod (WL); WL, white light; WL + FR, white light with supplemental far-red light.
Parameters of gas-exchange measurements: g_s, stomatal conductance [mol H_2O m^{-2} s^{-1}]; P, photosynthesis [µmol CO_2 m^{-2} s^{-1}]; Pnet, net photosynthetic rate [µmol CO_2 m^{-2} s^{-1}] as the gross photosynthetic rate minus respiration.

1 | INTRODUCTION

Light is an essential resource for a plant to thrive, as it is the plant's sole source of energy. Therefore, it is not surprising that plants evolved responses to maximize exposure to light by avoiding shade from surrounding vegetation (Huber et al., 2020). In order for plants to detect surrounding vegetation, the plant-intrinsic alterations in reflected and transmitted light spectral composition are used. In the process of photosynthesis, especially red and blue light in the waveband of 400–700 nm (defined as photosynthetic photon flux density, PPFD) are absorbed, whereas light outside this wave band is mostly reflected or transmitted. Still, far-red light (FR) in the wavelength from 700 to 800 nm may contribute to photosynthesis under specific conditions (Emerson, 1958; Zhen et al., 2019) and is especially well known to carry important information about neighbour plant proximity (Casal, 2012; Roig-Villanova & Martínez-García, 2016; Ballaré & Pierik, 2017; Huber et al. 2020; Casal & Fankhauser, 2023). Even though FR thus plays a crucial role in plant physiology, currently little is known about the interplay between its well-established role as neighbour cue and its direct contribution to photosynthesis.

Neighbouring plants reflect FR light, but absorb red, thus decreasing the ratio of red to far-red (R:FR) with increasing neighbour proximity. The R:FR of sunlight is about 1.2 and can decrease to 0.3–0.4 in dense vegetation and drop to 0.1 under deep canopy shade (Roig-Villanova & Martínez-García, 2016). FR light enrichment is an early warning signal for approaching neighbouring plants, preceding true vegetational shade (Ballaré et al., 1987; Ballaré et al., 1990; Ballaré et al., 1991). When low R:FR is perceived by a shade-sensitive plant, it triggers a suite of responses referred to as the shade avoidance syndrome (SAS). SAS involves changes in shoot architecture that encompass upward leaf movement and rapid stem and internode elongation at the cost of leaf blade size and branching (Ballaré & Pierik, 2017; Casal, 2012; Fiorucci & Fankhauser, 2017; Franklin, 2008, Huber et al. 2020). These architectural changes take place before the plant is actually shaded and enable the plant to reach more light when vegetation grows dense and therefore give plants a fitness advantage.

The vast majority of our knowledge on mechanisms regulating shade avoidance comes from studies in the dicot model plant *Arabidopsis thaliana*. In short, FR detection in leaves inactivates phytochrome B photoreceptors, which then leads to the accumulation of active phytochrome interacting factor (PIF) transcription factors. PIFs in turn activate the auxin synthesis pathway, and auxin is subsequently transported to the elongating organs, promoting cell-expansion-driven elongation growth (Huber et al., 2020; Pierik and Ballaré, 2021). Rice (*Oryza sativa*), together with maize and wheat, is among the most important crops worldwide, yet, knowledge on SAS mechanisms in cereals, to date is limited. The sensitivity of rice to high density and low light is well documented, and effects include elongation of internodes, reduced tillering and reduced biomass, as well as reduced yield (Evers et al., 2006; Wu et al., 1998; Finlayson et al., 2007; Takano et al., 2001; Kikuchi et al., 2017; Warnasooriya & Brutnell, 2014). However, responses to low R:FR are poorly documented in rice, even though mutants for some phytochromes and phytochrome interacting factor-like (PIL) proteins have been described (Garg et al., 2006; Gu et al., 2011; Hirochika & Shinomura, 2005; Hu et al., 2020; Iwamoto et al., 2011; Izawa et al., 2000; Liu et al., 2016; Takano et al., 2001).

In addition to being information cues of neighbour proximity, FR photons can also affect photosynthesis through the so-called Emerson effect (Emerson, 1958), which describes the phenomenon of FR photons increasing the photochemical efficiency and photosynthetic rate of PSII, when present together with photons of shorter wavelength. This is due to FR photons preferentially exciting PSI, whereas photons of shorter wavelength (400–680 nm) can over-excite PSII (Evans, 1987; Laisk et al., 2014; Zhen et al., 2019). However, FR is not considered part of the photosynthetically active radiation, since applied on its own, plants cannot use FR for photosynthesis.

Various more recent studies (Li & Kubota, 2009; Stutte et al., 2009; Kalaitzoglou et al., 2019; Park & Runkle, 2017; Zhen & Bugbee, 2020a, 2020b; Zhen et al., 2022) have meanwhile revealed pronounced increases of biomass and rates of photosynthesis under supplemental FR treatment. Since enhanced biomass accumulation in supplemental FR could imply enhanced carbon fixation, we combined measurements of biomass and development with measurements of photosynthetic gas exchange under WL and supplemental FR light conditions in several rice varieties.

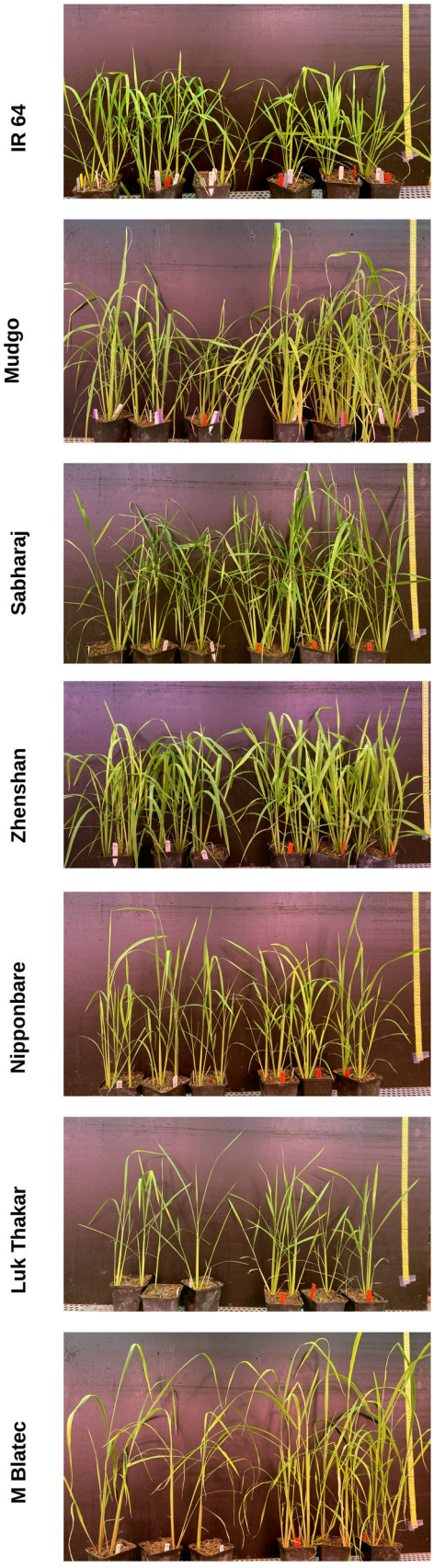
Here, we explore in detail how plant architecture, gene expression, and photosynthesis in multiple rice varieties respond to FR light enrichment. We show that rice has a surprisingly modest shade avoidance and transcriptome response to supplemental FR light, but at the same time, shows a particularly strong photosynthetic response to supplemental FR, resulting in substantial growth promotion.

2 | RESULTS

2.1 | Supplemental FR effects on rice shoot architecture

To investigate the shade avoidance responses in rice, *Oryza sativa*, we grew rice seedlings from seven different varieties in the greenhouse (Figure 1). The greenhouse light environment was supplemented with artificial white light (WL) to ensure a minimal light intensity of approximately 400 µmol PPFD photons m^{-2} s^{-1}, optimal for rice growth, with a red to far-red light ratio (R:FR) of approximately 2.0. The treatment group was exposed to supplemental FR light added to the WL background (WL + FR) lowering the R:FR to 0.2 (Supporting Information S4: Figure 1).

To analyze the effect of supplemental FR light on different traits related to shoot architecture and growth in addition to genetic background, we performed two-way ANOVA tests. Seedlings of 28 days produced more leaves and more tillers, when exposed to



supplemental FR as compared to WL (Figure 2a,b). The results of the two-way ANOVA show that FR light as well as varietal background both significantly affected leaf and tiller formation, and there was no significant interaction between these two main effects. For completeness, we performed a Tukey post hoc test, even though there was no significant treatment * variety interaction, and the FR main effect can be interpreted as is. The post hoc analysis revealed significant FR effects for plants of the variety Mudgo for leaf number, and in Mudgo and Sabharaj for tillering. Measurements of leaf formation and tillering at earlier timepoints showed that supplemental FR increases these traits already at 21 days after sowing (das) in the majority of varieties (Supporting Information S4: Figure 2). We also recorded the height and length of different plant organs (Figure 2c, Supporting Information S4: Figure 3), which are typical SAS traits, but we could not observe a clear elongation pattern. Response of culm height to supplemental FR varied strongly between varieties, as confirmed by the significant light treatment * variety interaction term, where some showed significantly shorter (IR 64 and Zhenshan) and others longer culms (Mudgo, M Blatec and Sabharaj) at 28 das. Interestingly, culm height at 21 das was larger in three varieties in FR-enriched light compared to control, and in two of these (Mudgo and Nipponbare), this coincided with an increased number of internodes (Supporting Information S4: Figure 3A). Responses of the internodes differed between varieties and between the developmental age of internodes (second and third youngest) that were measured (Supporting Information S4: Figure 3A). Similar patterns, where the effect on younger tissue is more pronounced, were observed for leaf length, where the difference between control and treated group is stronger in the younger leaves, but almost disappears in developmentally older leaves (Supporting Information S4: Figure 3B). An additional SAS trait is apical dominance, expressed as the ratio of culm height to the number of tillers. We observed a trend for the ratio to decrease under supplemental FR as compared to WL, but post-hoc comparisons upon finding a significant treatment * variety interaction, identified a significant difference only in the M Blatec variety, indicating more tillering, rather than apical dominance in FR-enriched conditions (Figure 2d).

Finally, although changes in leaf inclination angle are a well-established light response, we observed no clear supplemental FR effects on leaf inclination angle in most of the 21-day-old seedlings (Supporting Information S4: Figure 4A). Similarly, leaf erectness was marginally affected (Supporting Information S4: Figure 4B).

FIGURE 1 Phenotype of rice seedlings exposed to supplemental far-red of seven varieties, visualizing their differences in phenotypes under white light (white labels, left) and under supplemental far-red treatment during photoperiod (red labels, right), starting from the day of sowing until 28 days after sowing. [Color figure can be viewed at wileyonlinelibrary.com]

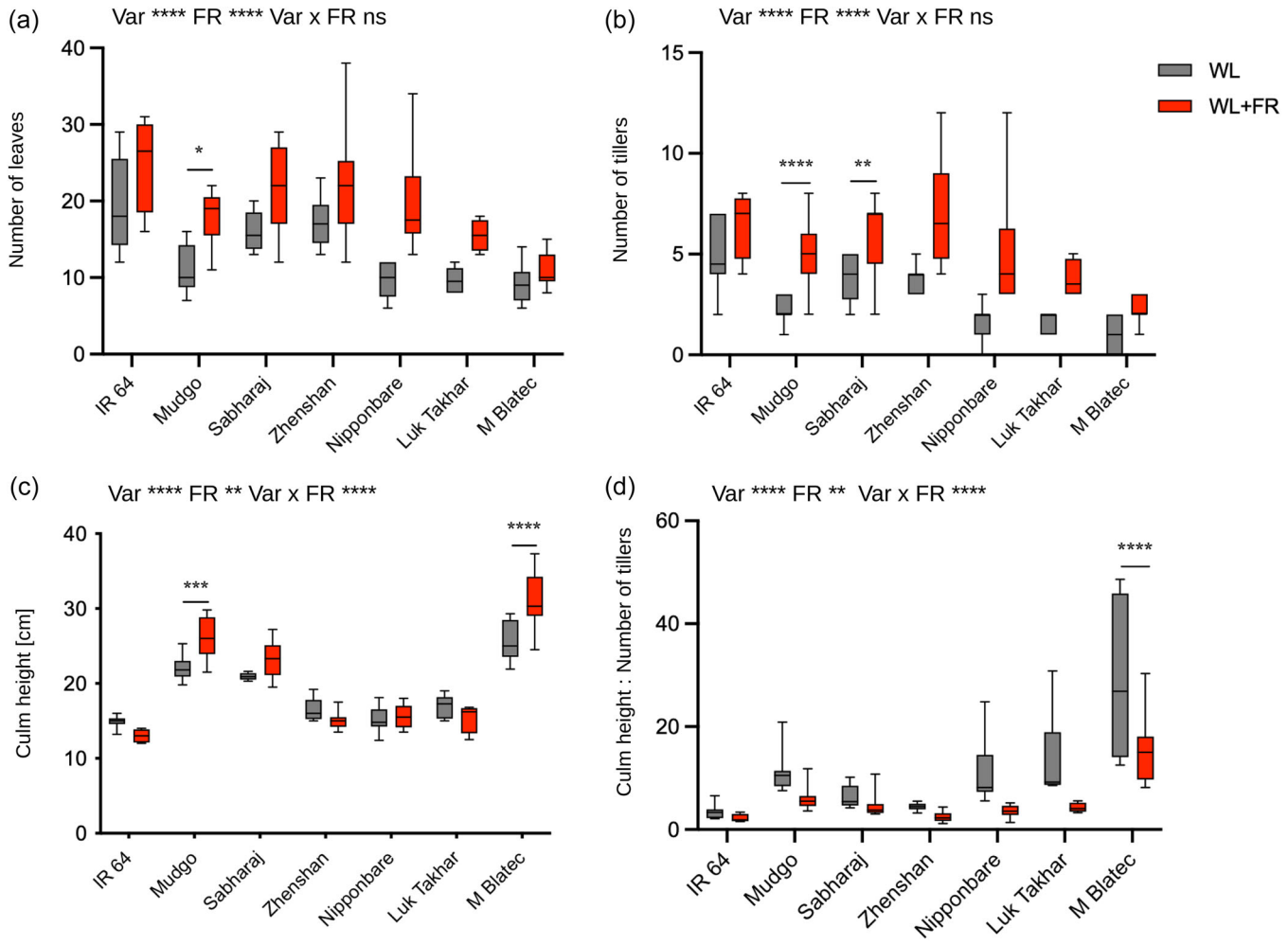


FIGURE 2 Comparison of different shoot traits in response to supplemental FR treatment in different rice varieties at 28 days after sowing. (a) Number of leaves, (b) tillering, (c) culm height, and (d) apical dominance, as the ratio of culm height to number of tillers, of plants grown in control (WL) and supplemental FR (WL + FR) light. Values are per plant; boxes indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for main effects of variety (Var) and FR light treatment (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of Tukey post hoc test on FR treatment effect. Detailed information of replicates can be found in Table S1. [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | Genome-wide transcriptome responses to supplemental FR

To get insight into transcript regulation under low R:FR, we carried out an RNAseq analysis on 5-day-old rice seedlings of six different varieties exposed for 24 h to supplemental FR or kept under control light conditions. A principal component analysis based on all expressed genes (counts per million, CPMs) per sample showed a strong separation of samples, primarily on the first axis (PC1), clearly distinguishing the different varieties (Supporting Information S4: Figure 5A). Hierarchical clustering of all expressed genes in each variety under WL or WL + FR, confirmed a primary clustering by variety and secondarily by treatment (Supporting Information S4: Figure 5B). We then calculated all differentially expressed genes (DEGs) from all varieties under supplemental FR as compared to WL. We assigned these 379 DEGs found as general FR-responsive genes, and PCA analysis of this subset (Figure 3a) shows that the variation of samples for this gene set was still mostly explained by variety (PC1) rather than light treatment (PC2), with 33.1% and 24.4%, respectively, and similar separation was found in a hierarchical clustering (Figure 3b). When we determined FR response DEGs per individual variety rather than over all varieties together, it became obvious that only a very minor subset of the transcriptome of each rice variety exhibited a response to supplemental FR treatment (Figure 3c,d), with some varieties, such as Luk Takhar, showing only four DEGs (Figure 3c,d, Table S2). Strikingly this shows that for the small number of genes that are responsive to treatment, they are largely unique to the variety, and only one single gene is shared for all the tested varieties (Figure 3c, Table S2). It is worth noting, that the response to supplemental FR is quite dependent on the variety itself. Some varieties, such as Luk Thakar or M Blatec, showed almost no response to supplemental FR, only two and nine misregulated genes,

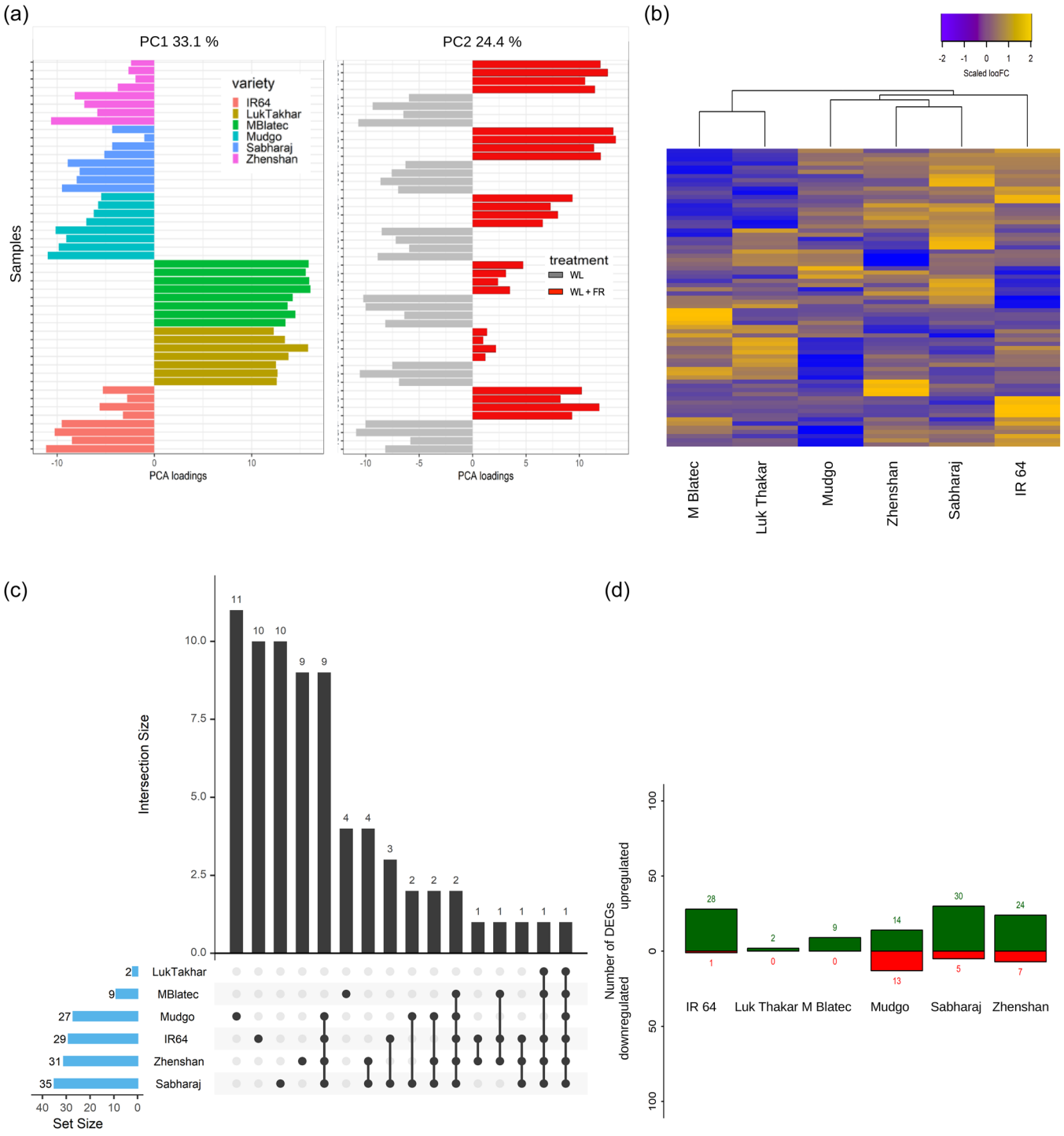


FIGURE 3 Differential gene expression upon supplemental FR exposure in rice seedlings. (a) Principal component (PC) Analysis of CPMs of 379 differentially expressed genes (DEGs) for FR response with FDR < 0.05 with colour code for variety in PC1 and colour code for treatment in PC2. Treatment groups with control plants grown in white light (WL) and supplemental FR light (WL + FR) grown plants. (b) Heatmap and clustering for log_2FC of differential gene expression upon supplemental FR treatment. Fold change of response genes calculated as the difference between white light-grown and supplemental FR light-grown plants (379 DEGs; with FDR < 0.05). Colour gradient for normalized and scaled log_2FCs from blue to yellow for strongest down to strongest upregulation. (c) DEGs separated per variety with an indication of genes that are shared in their response to supplemental FR, based on 379 DEGs for general FR response with FDR < 0.05. (d) Number of up and downregulated DEGs per variety. [Color figure can be viewed at wileyonlinelibrary.com]

respectively (Figure 3c,d, Table S2). Furthermore, only a few genes were found to be responsive in more than one variety: we found four DEGs only shared between M Blatec and IR 64, and three uniquely shared among Zhenshan and Sabharaj (Figure 3c, Table S2). Only one gene was commonly upregulated in all the varieties: LOC_Os09g27750, which encodes an ethylene-forming enzyme. We also found three other genes that were commonly misregulated in five out of the six tested varieties (LOC_Os04g58200, PROTOCHLOROPHYLLIDE OXIDOREDUCTASE A; LOC_Os04g41130, protein of phosphatidyl-ethanolamine-binding protein family; and LOC_Os03g37450, an unknown function gene) (Figure 3c, Table S2). The $\log_2$ fold changes of the DEGs expressed in a heatmap in Figure 3d give an overall impression of up versus downregulation of gene expression per variety. Hierarchical clustering analysis confirmed the strong influence of genetic background on gene regulation (Supporting Information S4: Figure 5), since all samples cluster for variety and only then by treatment. Biological process gene ontology (GO) enrichment analysis revealed that supplemental FR had a pronounced effect on genes involved in the regulation of photosynthesis and related processes, and to a lesser degree on genes involved in shade avoidance, including auxin signalling and response (Supporting Information S4: Figure 6A, Table S3). This correlated well with the cellular component GO. Additionally, cellular localization GO enrichment analysis indicated that most DEGs associated with a GO category are localized in the Chloroplast (Supporting Information S4: Figure 6B, Table S3). Taken together, these results showed that supplemental FR had an overall small effect on gene regulation and that it mostly affected genes involved in photosynthesis and those localized in the chloroplast.

2.3 | FR light accelerates growth and development

Despite the rather modest shade avoidance-like responses described above, we did observe accelerated leaf and tiller formation in supplemental FR-treated plants compared to their controls, consistently for all varieties (data presented for a subset of varieties in Supporting Information S4: Figure 2), leading to significantly more leaves and tillers at 28 das (Figure 2a,b). Similarly, the formation of internodes is accelerated in supplemental FR-treated plants (Supporting Information S4: Figure 3B). Consistent with these rates of organ development, we recorded higher biomass in supplemental FR, for shoots as well as roots (Figure 4a,b). Even though, this was only significant in one of the seven tested varieties, the increasing trend was consistent over all tested plants. In addition, we recorded the total plant leaf area (Figure 5a), which was increased in supplemental FR light-grown plants in all varieties. From the leaf area and leaf mass, we calculated specific leaf area (SLA); the ratio of leaf area per leaf mass. In four out of seven varieties, SLA was increased under supplemental FR treatment (Figure 5b), indicating that in some of the varieties, the leaves that are formed in supplemental FR have relatively less dry matter invested in their area.

To verify if supplemental FR light would potentially promote photosynthesis and contribute to increased biomass accumulation to an extent that it would mask any shade avoidance responses in rice, we performed an experiment with a 15 min end of day FR (WL + EoD FR) pulse just after the end of the photoperiod. This way, it is unlikely that FR would measurably promote photosynthesis and growth. WL + EoD FR-treated plants showed decreased tillering and number of leaves in all tested varieties (Figure 6a,b), opposite to what we

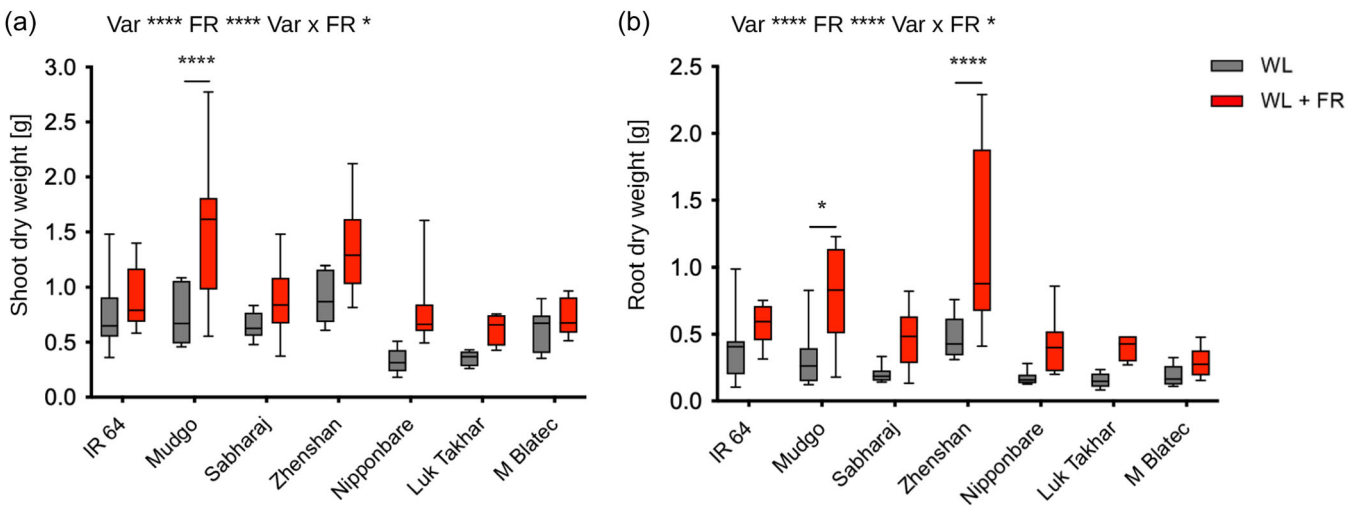


FIGURE 4 Increased biomass in rice seedlings exposed to supplemental FR. (a) Shoot and (b) root dry weight of different rice varieties grown in control (WL) and supplemental FR (WL + FR) light at 28 days after sowing. Values are per plant; boxes indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for main effects of variety (Var) and FR light treatment (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of Tukey post hoc test on FR treatment effect. Detailed information of replicates can be found in Table S1. [Color figure can be viewed at wileyonlinelibrary.com]

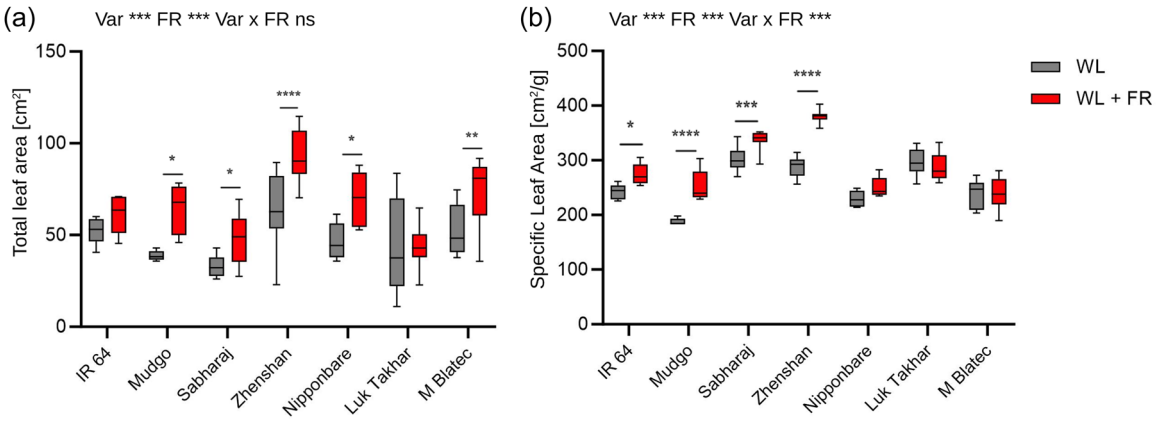


FIGURE 5 Supplemental FR treatment increased total leaf area (a) and specific leaf area (b) of rice seedlings grown in control (WL) compared to supplemental FR (WL + FR) at 28 days after sowing. Values are per plant; boxes indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for the main effects of variety (Var) and FR light treatment (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of Tukey post hoc test on FR treatment effect. Detailed information of replicates can be found in Table S1. [Color figure can be viewed at wileyonlinelibrary.com]

observed in WL + FR treatment during the photoperiod (Figure 2). Culm height is less affected, with shorter culms in plants of only two varieties (Figure 6c), and unlike in WL + FR during the photoperiod, apical dominance is increased in plants under WL + EoD FR (Figure 6e).

2.4 | FR enrichment evokes only marginal acclimation of photosynthetic traits

To understand whether plants grown in a FR-enriched environment would be able to accumulate more biomass and grow faster through enhanced photosynthesis, we first explored any effects on stomata and chlorophyll (morphology of stomata in Supporting Information S4: Figure 7). Stomatal density, defined as the number of stomata per section of vein, did not differ between treatment groups (Figure 7a), whereas stomatal length is higher for plants grown in WL than for plants in WL + FR (Figure 7b). Furthermore, we observed that the chlorophyll content of plants grown in WL + FR is lower than in WL, whereas the chlorophyll A/B ratio is not affected (Figure 7c,d). Thus, any observed differences in stomata and chlorophyll would predict lower, rather than higher photosynthesis in WL + FR as compared to WL.

We then set out to investigate if growth in supplemental FR light affects photosynthesis. We first measured light-response curves on plants that had been grown under WL + FR for 4 weeks, and compared them to control grown plants. These gas-exchange measurements were performed on plants of the variety Nipponbare (Figure 8), using a LI-COR 6400 with a closed leaf chamber with internal red and blue LEDs. Generally, our measured light response curves follow the typical trajectory of rice plants (Xiang-Sheng et al., 2006; Ye, 2007) and are similar for the two treatment groups (Figure 8). None of the quantitative parameters derived from the light curves showed a significant difference due to pretreatment of plants (Supporting Information S4: Figure 8). These data indicate that FR enrichment of the growth light environment does not drastically affect photosynthetic parameters and photosynthesis in WL.

2.5 | Supplemental FR light directly promotes CO_2 fixation

Since we still observed substantial growth promotion, we hypothesized that supplemental FR might directly contribute to photosynthesis. We, therefore, measured CO_2 fixation under our light treatment conditions.

Plants from both, the WL and WL + FR treatments, were placed under WL or WL with switchable supplemental FR light, similar to the growth conditions of the WL + FR treatment. CO_2 fixation was now measured with a LI6400XT portable photosynthesis system with a transparent top leaf chamber, allowing measurements at ambient light while including and excluding FR light (Supporting Information S4: Figure 9) on the same leaf mounted in the leaf chamber. Measurements were started with the light conditions each group of plants was pretreated with, that is, plants grown under WL were first measured with FR switched off and then on, whereas WL + FR treated plants were first measured with FR on and then off. The combined measurements were conducted in a time span of several minutes to minimize potential adjustment to non-native light conditions.

We observed a very strong instantaneous promotion of photosynthesis when FR lamps were switched on. In WL + FR, for some varieties, there was nearly a doubling of carbon fixation as compared to plants in WL. This stimulating effect of FR on CO_2 fixation was

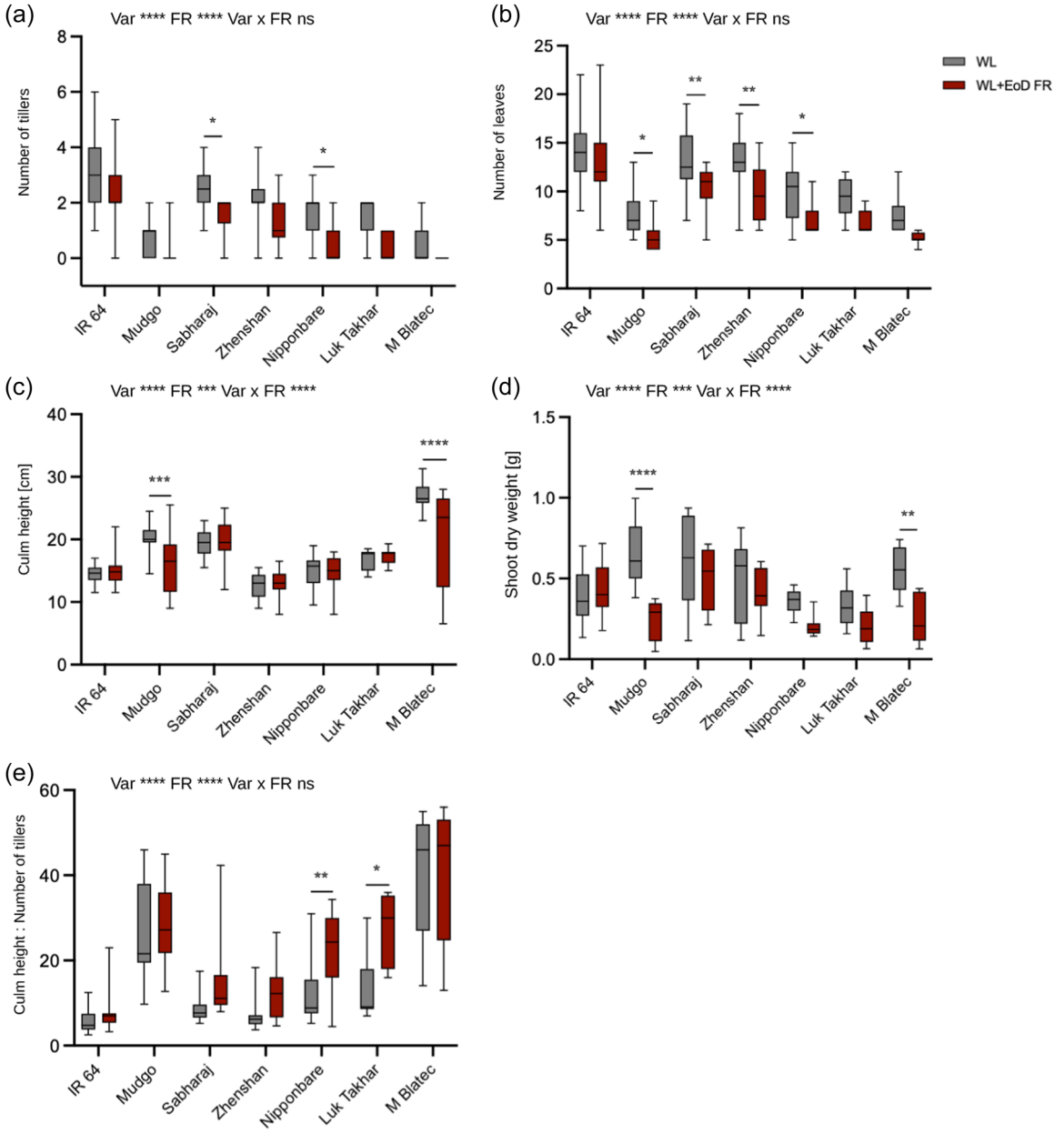


FIGURE 6 Growth response to FR light pulse after photoperiod in different rice varieties at 28 days after sowing. Comparison of (a) number of tillers, (b) number of leaves, (c) culm height, (d) shoot dry weight and (e) apical dominance of rice plants grown under white light (WL) and exposed to a 15-min pulse of far-red light (WL + EoD FR). Values are per plant with means of 10 plants for biomass and min 10 plants for other traits; boxes indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for main effects of variety (Var) and FR light treatment (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of Tukey post hoc test on FR treatment effect. [Color figure can be viewed at wileyonlinelibrary.com]

consistent between varieties and present both in plants that were pre-grown in WL and in plants grown in WL + FR (Figure 9a,b). When calculating the difference in CO_2 fixation rate between FR-on and FR-off during measurement, the response to switching on FR light is larger in the FR pretreated group (Figure 10) than in the group not pre-exposed to FR, irrespective of variety. Although the acclimation to FR light as a pretreatment has a significant effect on the responsiveness to instantaneous FR photons, this effect is small as compared to the overall large instantaneous effect of FR for driving photosynthesis.

Finally, we observed no clear acclimation effect for stomatal conductance towards WL + FR (Supporting Information S4: Figure 10). Plants grown in WL versus those grown in WL + FR had similar stomatal conductance in their respective native light environment, indicating that FR strongly promotes CO_2 fixation without affecting stomatal conductance.

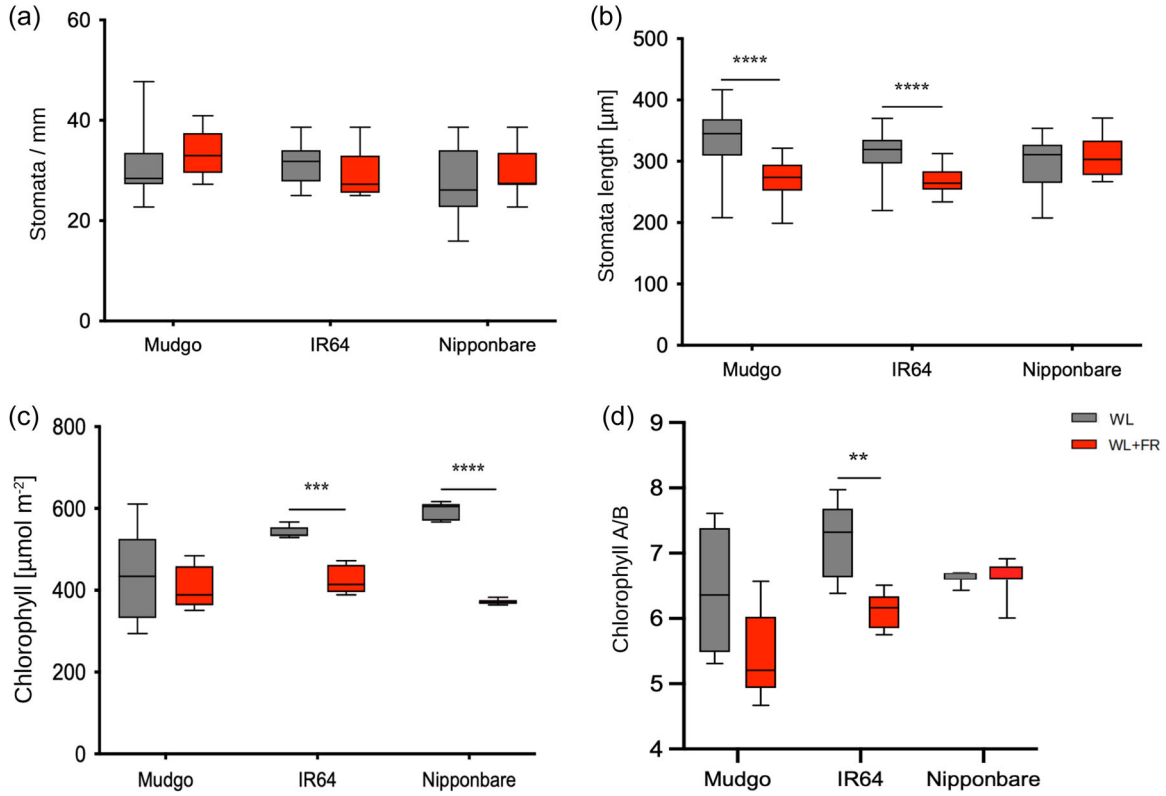


FIGURE 7 Acclimation of stomata and chlorophyll content of 28-day-old rice plants exposed to supplemented FR (WL + FR) and control group grown at WL. (a) Stomatal density is the number of stomata per mm of vein length and (b) stomatal length in µm. Measurements were taken on the third youngest leaf, for stomatal density n = min 12 counts in three biological replicates, for stomatal length n = min 16 counts in three biological replicates. (c) Total chlorophyll content is determined with a chlorophyll content metre as µmol m^{-2} and the (d) ratio of chlorophyll A/B determined with leaf disc extraction. Measurements were taken on the third youngest leaf, for both methods n = 5 biological replicates; boxes indicate IQ-range with error bars of 2.5–97.5 percentile. Significant differences following two-sample t-test are indicated with $p < 0.05$*, <0.01**, <0.001***, <0.0001****. [Color figure can be viewed at wileyonlinelibrary.com]

2.6 | Comparing the efficacy of PPFD and FR photons for photosynthesis

Importantly, we observed that at 400 µmol PPFD photons m^{-2} s^{-1}, the nett CO_2 fixation rates are higher than at the equivalent PFD, where part of PPFD is substituted with FR (170 PPFD + 230 FR µmol photons m^{-2} s^{-1}) (Figures 8 and 9).

Plants were grown at approximately 400 µmol PFD photons m^{-2} s^{-1} in WL and approximately 900 PFD µmol photons m^{-2} s^{-1} in WL + FR, with 500 µmol supplemented FR photons. When performing the gas exchange measurements, the actual light reaching leaves in the cuvette was only approximately 170 µmol PPFD photons m^{-2} s^{-1}. A similar 55% reduction of FR would translate into 400 PFD µmol photons m^{-2} s^{-1} at leaf level in the cuvette. At 170 µmol PPFD photons m^{-2} s^{-1} in the light-response curve, we see approximately 5 µmol CO_2 fixation m^{-2} s^{-1} (Figure 8), which is roughly the same as observed for Nipponbare control plants with FR lights off (Figure 9a). Similarly, when reading the CO_2 fixation at 400 µmol PPFD photons m^{-2} s^{-1} in the light response curve, this equals to 12.5–14 µmol CO_2 fixation m^{-2} s^{-1}. FR-acclimated plants measured with FR-light on, fixed 10.6 µmol CO_2 m^{-2} s^{-1} (Figure 9b). This comparison indicates that FR photons are not entirely as effective as PPFD photons in driving CO_2 fixation, but still substantially boost photosynthesis.

3 | DISCUSSION

In this study, we investigated how seven rice varieties respond to a FR-light-enriched environment at the level of plant architecture and growth. For this, we exposed rice seedlings to supplemental FR light and studied architecture, morphology, transcriptome and photosynthesis. We observed that although rice is not very FR-responsive in terms of architectural traits, it shows very pronounced photosynthetic responses and consistently, strong growth promotion. The impact of these findings is both scientifically relevant as well as of applied importance, since rice is the major staple crop for human consumption and is grown in high densities characterized by a FR-rich light climate.

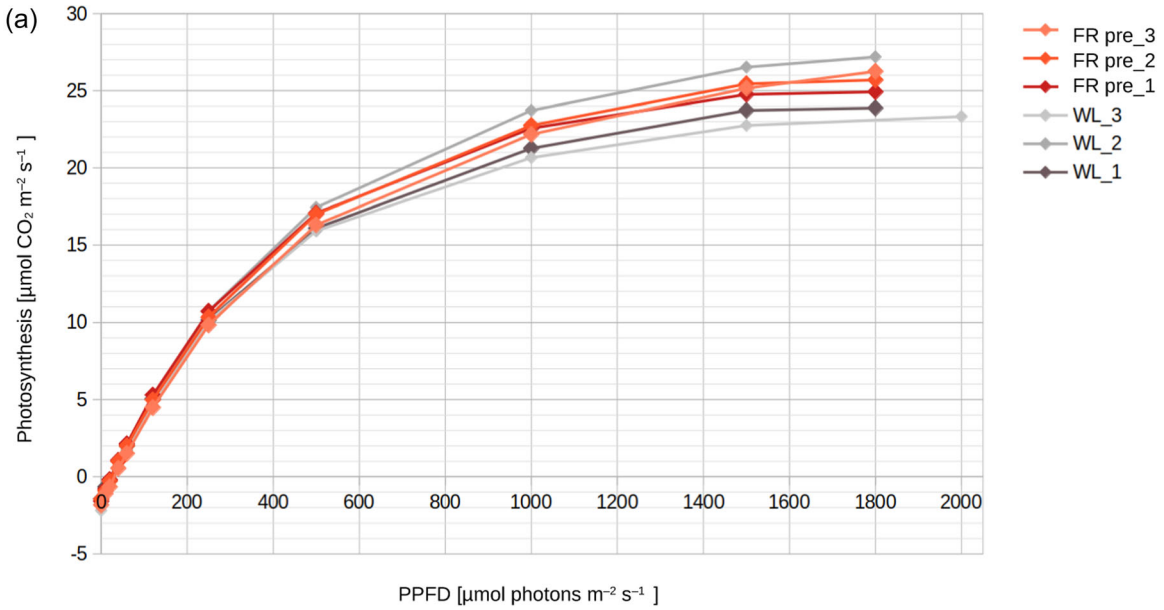


FIGURE 8 Photosynthetic rate in response to light intensity of control grown (WL) and FR pretreated (FR pre), 28-day-old rice plants of the variety Nipponbare. Light response curve showing the photosynthetic activity (µmol CO_2 m^{-2} s^{-1}) on the *y*-axis, at a given light intensity as µmol photons m^{-2} s^{-1} of 400–700 nm on the *x*-axis. *N* = 3 plants, with four technical replicates of measurements. [Color figure can be viewed at wileyonlinelibrary.com]

3.1 | Shade avoidance responses are not prominent in rice

At high planting densities, FR light is relatively more abundant as compared to the visible light, and this enrichment is monitored by plants as a cue for neighbour proximity. Under FR enrichment, plants typically display shade avoidance responses, which include enhanced elongation (height) growth at the cost of branching (Caton et al. 2003; Ballaré & Pierik, 2017; Casal, 2012; Franklin, 2008; Green-Tracewicz et al., 2011; Wang et al., 2013; Huber et al. 2020). What we observed in rice seedlings, however, was the opposite, with more leaves and tillers as an overarching general response under FR treatment, in contrast to mild elongation depending largely on the variety. Hence, a lack of shade avoidance responses over various varieties is likely partly associated with the simultaneous promotion of overall plant growth in these treatments. Also, the ratio of height to branching was lower in WL + FR treated plants compared to WL-grown plants, which is contrary to the known SAS manifestation. Measurements on different tissues of different age revealed that it was mostly the developmentally younger tissue which was responding stronger (Supporting Information S4: Figure 3). When we used a WL+EoD FR treatment instead, an established treatment to trigger SAS, we did observe a reduction of leaf formation and tillering. Although this suggests some degree of apical dominance, no observable height growth response occurred in any of the varieties tested. This is in line with the lack of an overall taller plant phenotype in rice *phyB* and *phyA phyB phyC* mutants as compared to their wild types under white light conditions (Takano et al., 2009).

Consistent with the small phenotypic effects, supplemental FR also triggered a very mild transcriptome response as compared to studies in other species (Gommers et al., 2017; Liu et al., 2021; Pantazopoulou et al., 2017; Kohnen et al., 2016). Additionally, in our study, even that small number of responsive genes is not the same between the varieties. These observations thus indicate that rice, or at least the varieties studied here, is not very responsive to FR enrichment when it comes to shade avoidance and plant architecture. However, rice architecture is considered to be very responsive to planting density (Bahuguna et al., 2021; Heap, 2014; Zhao et al., 2007). It is possible that rice, unlike other species, is not (only) using FR light as a proxy for density. It would be possible that other light cues, or even chemical cues, are stronger cues for rice to detect neighbour proximity. Indeed, blue light has been associated with shade avoidance (Pierik & de Wit, 2014), and even volatile organic compounds hold potential for plant neighbour detection (Kegge et al., 2015; Pierik et al., 2003). Alternatively, it cannot be excluded that in the Green Revolution search for semi-dwarf varieties (Hedden, 2003; Kush & Khush, 2001; Wing et al., 2018), selection has also been against height growth in response to FR enrichment. This might be consistent with the observation that WL + EoD FR can still suppress tillering but not promote shoot elongation.

3.2 | Photosynthesis is enhanced in supplemental FR

Regardless of shade avoidance, we observed that rice can greatly benefit photosynthetically from FR light. The GO enrichment in our

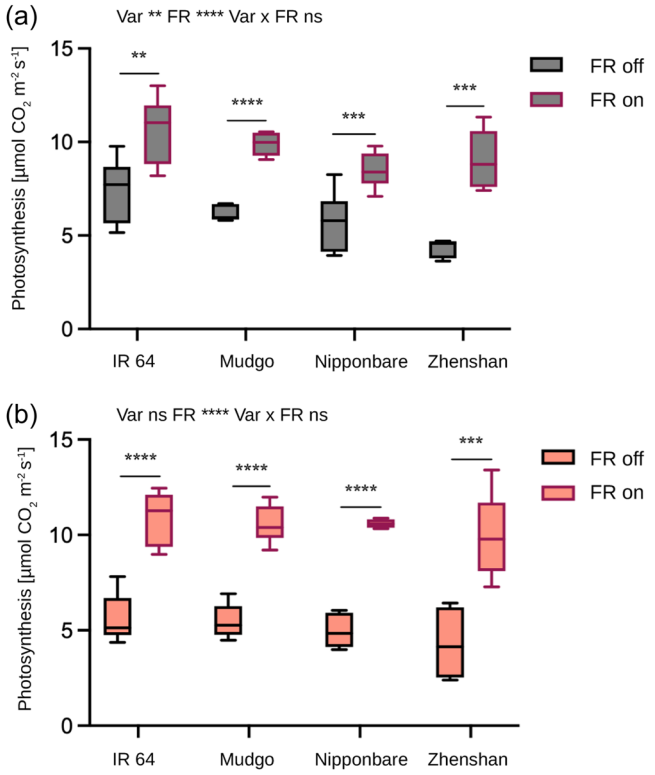


FIGURE 9 Instantaneous effect of supplemented FR on photosynthetic rate in 28-day-old rice plants of different varieties grown in WL and WL + FR given as µmol CO_2 m^{-2} s^{-1} in (a) control grown (WL) plants and in (b) FR pretreated plants. Measurement groups consist of two treatments: control grown in WL and treated plants grown with supplemental FR under two light settings: off and on. Values are means of six plants of each measurement group. Box-plots indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for main effects of variety (Var) and FR switched off/on (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of pairwise *t*-test on FR light effect. [Color figure can be viewed at wileyonlinelibrary.com]

transcriptome data of processes related to photosynthesis, and cellular localisation to the chloroplast hint at these processes being regulated by FR light in rice (Supporting Information S4: Figure 6). Our results show that rice plants grown in supplemental FR light conditions show an overall trend of accelerated rate of development as well as enhanced biomass accumulation. This has been observed as well in other studies, in different plant species, including vegetables and ornamentals, where the increase in dry weight under FR-enriched conditions was attributed to increased leaf area, providing higher light interception (Li & Kubota, 2009; Stutte et al., 2009; Kalaitzoglou et al., 2019; Park & Runkle, 2017; Zhen & Bugbee, 2020a, 2020b; Zhen et al., 2022). To investigate this in our study, we recorded leaf area and SLA, to get insight into what extent this contributes to the observed growth promotion. The data shows that total plant leaf area is increased under FR enrichment in all varieties, which will promote whole-plant carbon fixation, via increased light interception, and therefore likely indirectly also contribute to increased biomass. However, the pattern for SLA is less clear, it is found to be promoted in four out of seven varieties. Together this suggests that FR-supplemented plants typically have more and larger leaves, but with the exception of three varieties, these leaves require the same carbon investment per area as those formed in control WL conditions. Our photosynthesis measurements show that a major effect of supplemental FR light in rice is to promote CO_2 fixation per unit leaf area. Based on this, we propose that supplemental FR light can promote rice biomass accumulation through two mechanisms: (i) promotion of leaf area that can contribute to whole plant carbon fixation and (ii) direct promotion of CO_2 fixation per unit leaf area under FR-enriched light.

We observed two factors contributing to enhanced CO_2 fixation in WL + FR light conditions: strong instantaneous photosynthesis by FR photons and a modest long-term acclimation effect. The finding that WL + FR-grown plants showed a stronger CO_2 fixation response to WL + FR could not be explained by differences in stomatal density, and some varieties grown in WL + FR even had slightly smaller stomata. Furthermore, there were no significant differences in stomatal conductance between WL and WL + FR-grown plants. In addition, chlorophyll levels were even slightly reduced in WL + FR-grown plants as compared to control plants, as were the chlorophyll *a*/*b* ratio both indicative of a mild shade acclimation in these plants (Li & Kubota, 2009; Kalaitzoglou et al., 2019; Zhen & Bugbee, 2020b). The weak responses to supplemental FR pretreatment of stomatal conductance and chlorophyll under FR-enriched growth conditions would rather reduce than enhance photosynthesis. We, therefore, conclude that any acclimation responses in photosynthesis that we measured are not explained by chlorophyll and/or stomatal responses and may be associated with, for example, photosynthetic biochemistry or mesophyll conductance for CO_2.

When we determined CO_2 fixation rates in the presence or absence of supplemental FR photons, the vastly augmented rate of CO_2 fixation in WL + FR clearly indicated that FR photons can indeed be used to drive photosynthesis directly. This direct promotion of leaf-level photosynthesis by FR photons, combined with the increased investments into total leaf area under supplemental FR, likely explains the observed increase of biomass accumulation and accelerated rates of development in WL + FR. Although FR photons are typically not included within the definition of Photosynthetic Photon Flux Density, there are several other studies that show similarly strong impacts of FR supplementation on growth and photosynthesis. In a broad, multi-species study, Zhen and Bugbee (2020a) showed consistently for 14 species that enriching white light with supplemental FR photons boosted leaf-level carbon fixation quantitatively similar to enriching the light with equal fluence rates of shorter wavelength photons in the white light spectrum. Park & Runkle (2017) noticed that when they removed a portion of red light and substituted it with equal fluence rates of FR photons, their plants developed similar shoot dry weights. These studies are consistent with the observations presented here that FR photons can strongly contribute to photosynthesis.

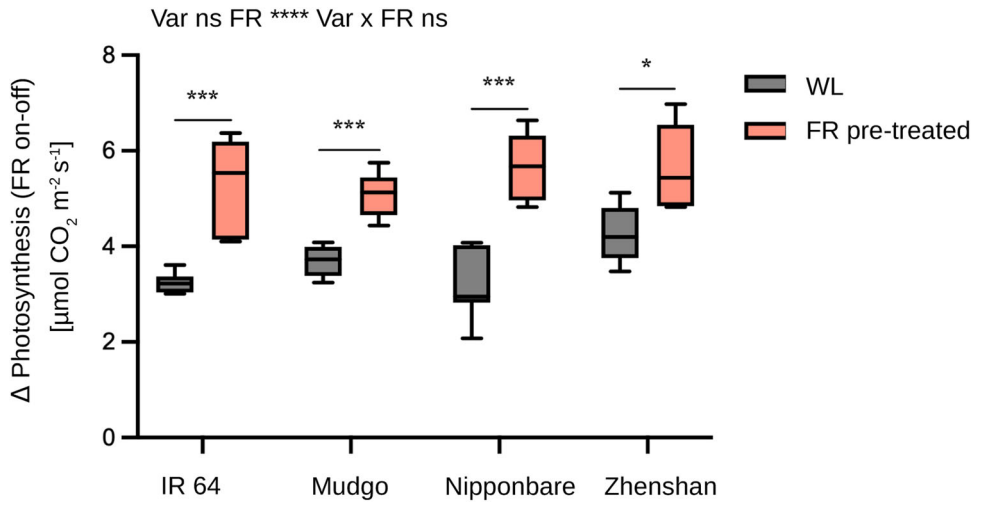


FIGURE 10 Acclimation effect on change in photosynthetic rate under supplemental FR as pairwise comparison of the difference in photosynthetic rate when FR lamps are switched on, for acclimated (FR pretreated) and control grown (WL) in 28-day-old rice plants of different varieties. Measurement groups consist of two treatments: control grown in WL and treated plants grown with supplemental FR (FR pretreated) under two light settings: off and on. Values are means of six plants of each measurement group. Box-plots indicate IQ-range with error bars of 2.5–97.5 percentile; significant differences following a two-way ANOVA for main effects of variety (Var) and FR pretreated (FR) and their interaction effect (Var × FR) are indicated with *p*-value > 0.05 ns, <0.05*, <0.01**, <0.001***; horizontal lines with asterisks indicate significant results of pairwise *t*-test on FR light effect. [Color figure can be viewed at wileyonlinelibrary.com]

These findings on FR-promoted photosynthesis are likely to be especially relevant for photosynthesis in leaves that are in lower zones of dense vegetation with substantial shading. Here, FR is proportionally much more abundant than photons of shorter wavelength and, therefore, proportionally adding a substantial part of energy for fuelling photosynthesis. At the same time, under high light conditions, FR photons can have a photo-protective effect by balancing out an overexcitement of Photosystem II. However, if FR photons are in strong excess of photons of shorter wavelength, their energy cannot be used

3.3 | Regulation of stomatal conductance under supplemental FR

The largely enhanced CO_2 fixation rates in light supplemented with FR were not accompanied by a change in stomatal conductance. This indicates that stomatal conductance is regulated independently of FR photons, even though these are powering CO_2 fixation. This is different from the common regulation of stomata, where with increasing PPFD levels and photosynthesis, stomata have increased opening position (Assmann & Jegla, 2016; Inoue & Kinoshita, 2017). The lack of enhanced stomatal opening in WL + FR indicates that apparently FR photons do not regulate stomatal opening, contrary to blue and red photons (Chen et al., 2012; Matthews et al., 2020). These findings imply that photosynthesis can be increased in supplemental FR without a further opening of stomata, which would allow plants to perform more CO_2 fixation without a penalty of losing water via transpiration.

3.4 | Two sides of the same coin—FR light as energy and signal

An important finding was that FR photons are not just a signal for proximity shade, but also an energy source for photosynthesis and biomass accumulation. Supplemental FR under our conditions appears to boost growth and photosynthesis without inducing shade avoidance-like responses. However, when removing the direct photosynthetic contribution of FR from the equation, using a FR pulse at the end of photoperiod, reduced tillering and reduced shoot biomass were observed. It is therefore possible, that rice does respond to low R:FR as a signal for neighbour proximity, but that under the strong FR enrichment conditions used here, this is masked by the strong promotive effect of FR on growth and development.

It remains to be studied how the contributions of FR and PPFD depend on the balance of each other. In a plant canopy, PPFD reduces from the top to the bottom of the canopy, but it is possible that up to a certain light level FR could compensate the effect of reduced PPFD on photosynthesis, since FR photons will be enriched where PPFD is depleted (Huber et al., 2020), still powering photosynthesis. FR can be absorbed only in Photosystem I (Zhen & van Iersel, 2017; Zhen et al., 2021), whereas PPFD can power both Photosystem I and II, with a bias to PSII. Thus, a strongly skewed balance between FR and PPFD fluence rates would have consequences for the balance between Photosystem I and II activation and, thus for coordinated electron transport.

It will be important to investigate the interdependencies of PPFD and FR fluence rates for photosynthesis in more detail to understand where in the canopies FR can and cannot compensate losses in PAR.

Such experiments could involve a matrix of FR * PPFD combination in individual plant photosynthesis and growth studies, that could then be paired to observations of PPFD and FR fluence rates throughout developing rice canopies at different planting densities. This is scientifically important but can also have important opportunities in cropping systems with full control over light quality and quantity, such as vertical farming solutions and greenhouses.

4 | MATERIALS AND METHODS

4.1 | Plant material and growth conditions

Seed material of different *Oryza sativa* varieties (IR 64, Nipponbare, Luk Takhar, M Blatec, Mudgo, Sabharaj and Zhenshan) was harvested from plants grown in the greenhouse at the International Rice Research Institute (IRRI), Los Baños, the Philippines, in wet season of 2018, stored at 6°C in the dark.

As a pre-germination treatment, seeds were kept at 37°C for 24 h, followed by 24 h at 21°C. For germination, seeds were put in Petri dishes on wet filter paper and incubated at 32°C for 24 h, which were planted with a tweezer 0.5 mm deep into the soil, with five seeds for each variety, per pot (10 × 10 × 11 cm) in a substrate mix of black soil, agra-vermiculite 0–1.5 mm and sand in a ratio of 5:3:2 together with Osmocote NPK-Mg 15-4-9 (+1) (2.4 g per L of soil) and 20% Yoshida nutrient solution (Yoshida, 1976) with a double iron dose (Sequestreen = Fe-EDTA) and pH 6.5 (1 L per kg substrate). Two weeks after seeding, maximally three plants per pot were retained.

4.2 | Light treatments

For experiments with different light treatments, plants were grown in the greenhouse facilities of the Botanical Gardens, Utrecht University, in The Netherlands, in the summer and autumn of 2021 (Supporting Information S4: Figure 1A). Experiments were conducted within one greenhouse compartment and on one growth table (2 m × 5 m). Temperatures were set to 30°C during the day and 25°C during the night and a 12 h photoperiod from 8 AM to 8 PM, with automatic watering twice-daily keeping the soil saturated. Pots were arranged at 10 cm distance. that kept the light intensity at min 400 µmol m^{-2} s^{-1}. An external light sensor above the growth table monitored sunlight and activated artificial light when light levels dropped below 400 µmol m^{-2} s^{-1}. The table was homogeneously lighted with uniform assimilation lighting (Valoya, Model Rx400 500 mA 5730, Spectrum AP673L). Half of the table was as further equipped with FR lamps (Valoya FR). We verified that FR light from one side did not reach the other side, and a 50 cm space between the two sides was kept open to achieve this. Light spectra of natural and artificial light were regularly recorded, at several locations at height of pot level, using a LiCor LI-180 spectrometer. Light intensity in PPFD range was the same between the control and treatment group. PFD was approx 400 µmol photons m^{-2} s^{-1} in control and 900 µmol photons m^{-2} s^{-1} where approximately 500 µmol m^{-2} s^{-1} FR light was added (Supporting Information S4: Figure 1B, C) for treatment. In this FR-enrichment treatment the R:FR ratio (determined as 650–670 nm waveband: 720–740 nm waveband) was approximately 0.2, which equals to approximately 0.4 Phytochrome Photostationary State (Sager et al., 1988). The PSS indicates the relative amount of active phytochrome in the far-red-absorbing Pfr form expressed as PSS = Σσr/(Σσr + Σσfr). Two recent papers have introduced two additional metrics to quantitatively express FR light in a WL background when using artificial light sources: FR fraction (FR/(WL + FR; Kusuma & Bugbee, 2021a) and percent FR (FR/ePPFD; Kusuma & Bugbee, 2021b). In the latter ePPFD is extended PPFD, defined as the photon flux within the 400–750 nm waveband. FR is defined here as 700–750 nm waveband. Expressing the light conditions using these metrics defines the WL control to have a FR fraction of 0.31 and a percent FR of 11%. The WL + FR treatment has a FR fraction of 0.85 and a percent FR of 53%.

For end-of-day FR (WL + EoD FR) treatment, control and treated group received the same light during photoperiod. WL + EoD FR group received a 15-min pulse of FR light (60–80 µmol m^{-2} s^{-1} FR light) 10 min after the end of photoperiod (set to 8 PM). Since, in the absence of background light, a minute amount of FR light can already affect the control plants, a vertical curtain was put up between the two treatment groups.

For leaf area measurements, plants were grown at the growth facilities in Radix Klima of Wageningen University Research, The Netherlands, in a growth chamber with the same settings as for previous experiments: 28°C during day and 25°C night temperature and a 12 h photoperiod from 8 AM to 8 PM and relative humidity were maintained at 75%. and same light settings with min 400 µmol m^{-2} s^{-1} PPFD and supplemented 500 µmol m^{-2} s^{-1} of FR, which were precisely recorded.

4.3 | Phenotypic measurements and analysis

The data for all macroscopic phenotypic traits presented, such as number of leaves, tillers, culm height, internode and leaf length, leaf inclination angle and shoot and root dry weight, are always per plant. Detailed information of replicates can be found in Table S1 and in respective figure captions. Leaves and internodes were recorded with numbering from the bottom up. Internode 1 thus is the oldest internode and the same numbering was used for leaves. Definitions of internodes and leaf number were followed as described by Izawa et al. (2000) and Liu et al. (2016). If a tiller was formed, then the leaf sheath of the first leaf on the tiller was recorded as the internode. The height of the highest node was noted as the culm height (Supporting Information S4: Figure 1D). Dry weight was recorded after plant material was dried in an oven at 80°C for min 3 days.

For statistical analysis, tissue of the same developmental stage was compared. If there was a tissue not (yet) formed under one of the treatment groups, the value 0 was assigned. Angles were determined in ImageJ using digital images taken from the side. For leaf erectness,

a smaller value refers to more droopy leaves, and 180° is a completely erect leaf. For leaf inclination, a smaller value shows a more vertical leaf, and with 90° the leaf bends off the culm horizontally. Statistical analysis was performed in R, and data visualization with GraphPad Prism.

4.4 | Transcriptome analysis

4.4.1 | Experimental design and plant material

Plants for transcriptome analysis were grown as described above. After 5 days in WL, when seedlings were big enough to provide enough tissue, the treatment group was exposed to supplemental FR light for 24 h. After this, the whole shoot of each of the 6 varieties (4 plants per variety, per treatment) was sampled for treatment and control groups. This was repeated on four independent occasions, resulting in 48 total samples (6 varieties × 2 treatments × 4 biological replicates). At harvesting, tissue was flash-frozen in liquid nitrogen and stored at −80°C. Harvested tissue was ground with a Retsch grinder. Total RNA was isolated from powdered tissue using the Qiagen RNeasy kit with on-column DNAse treatment, and quality was checked with the Bioanalyzer, before sequencing.

4.4.2 | High-throughput mRNA sequencing

RNA quality was checked with the Agilent Fragment Analyzer 5300 system using the RNA Kit (15nt) (Cat. DNF-471-1000). RNA quantity was measured with the Invitrogen™ Qubit ™ Fluorometer using the Qubit RNA HS Assay Kit (Cat. Q32855). 100 ng of total RNA was used to prepare TruSeq Stranded mRNA libraries (Cat. 20020594) following the manufacturer's protocol. With custom 384 xGen UDI-UMI adapters from IDT. After the library preparation, libraries were checked with the Fragment Analyzer system dsDNA 910 Reagent Kit (35–1500 bp) (Cat. DNF-910-K1000) and with Qubit dsDNA HS Assay Kit (Cat. Q32854). Sample libraries were pooled equimolar. Libraries were sequenced on a Nextseq. 2000 sequencer (Illumina) by using a P3 flowcell with 50 bp single-end reads, yielding around 20–30 million reads per sample. Sequencing was performed at USEQ, Utrecht, the Netherlands.

4.4.3 | Processing of RNA sequencing reads

Mapping was performed and optimized with Kallisto (Bray et al. 2016). Briefly, the reads were aligned to two available reference transcriptomes (*indica* and *japonica*; 'Osativa_323_v7.0. annotation_info. txt; based on MSUv7 [Feb. 7, 2012, retrieved from http://rice.plantbiology.msu.edu/]) using a range of Kmer lengths for the reference index. The mapping was equally high for all varieties and yielded the highest alignment rates with the Japonica transcriptome with a Kmer index of 17 (~90% mapped reads). Transcript abundances of the mapped reads were then quantified with Kallisto. Counts obtained from each sequencing lane were added together to obtain the total counts for each biological sample using custom R scripts.

Multidimensional scaling (MDS) analysis of the count data yielded very tight clusters by rice variant. However, some of the replicate 3 samples were localized in a cluster of a different variant. Additionally, LUK_C_R1 did not belong to any specific cluster. Given all misplaced samples were of rep 2 this suggested the samples were swapped. To test this, the genetic variants in these samples and those of rep 3 and LUK_C_R1 were identified. Briefly, fastq samples were aligned to the Japonica reference genome with Bowtie2 end-to-end alignment (Langmead & Salzberg, 2012), and subsequently, bcftools (Danecek et al., 2021) were used to call the genetic variants (bcftools call) from the resulting.bam files after making a pileup (bcftools mpileup). To remove noise, only variants supported by a read depth of at least 1000 and variant call in each sample, were considered. With the remaining 3662 variants, we determined the genetic relatedness between the samples by obtaining the proportion of variants that were shared. In the original sample assignment, this resulted in differing varieties being considered most similar. However, after making the corresponding swaps as predicted by the MDS plot, samples paired up corresponding to their relatedness. This also restored a clear separation between *Indica* and *Japonica* varieties. LUK_C_R1 did not genetically match any other sample. Delving deeper into the variant calling revealed that LUK_C_R1 was very high on heterozygous calls compared to the other variants, suggesting this might be the result of harvesting plants of differing varieties into one sample. Because of this, LUK_C_R1 was removed from further analysis.

4.4.4 | Differential gene expression analysis

CPMs were then obtained with the cpm() function of edgeR v3.38.4 (Robinson et al., 2010; Lun et al., 2016). Genes with more than 1 cpm in at least three samples were considered expressed and included in the analysis. This resulted in 25,925 out of 42,189 genes (61.45%) being considered for further downstream analysis. Before determining Fold Changes and significance, the counts were normalized (TMM, trimmed mean of *M*-values) by correcting for differences in library sizes and compositional biases. Fold Changes were subsequently determined with the Bioconductor R package edgeR v3.38.4. DEGs were estimated based on the response to treatment by each variety or regardless of variety ('general_shade'). The resulting *p-values* were adjusted for multiple comparisons with the Benjamini–Hochberg method yielding a false discovery rate (FDR) criterion. Genes with FDR values lower than 0.05 were considered differentially expressed. Detailed information about the statistics for each graph can be found in the respective figure legends.

4.4.5 | Functional enrichment analysis

Gene set enrichment analysis was performed using a combination of custom-written R scripts and the gprofiler2 package (Reimand et al., 2016). The 25,925 expressed genes were used as the universe gene set. Bubble plots were generated using R. GO enrichment data analysis by g:Profiler is from 19-05-2023.

4.4.6 | Accession numbers and data availability

Raw sequences (.fastq files) used in this paper have been deposited in the ArrayExpress (Kolesnikov et al., 2015) database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-13023. Pre-processed data is readily available for download in Zenodo https://doi.org/10.5281/zenodo.8117001. All custom R scripts are available at https://github.com/aromanowski/rice_shade. Alternatively, they are available upon request to the corresponding author.

4.5 | Measurements of stomatal morphology

For measurements on stomata, leaf samples of 28-day-old plants were fixed following the protocol of Sharma, 2017, with a 24 h incubation in 95% ethanol: acetic acid (7:1), followed by a 2× washing with 70% ethanol and incubation in 1 N potassium chloride. Samples of the third youngest leaf were observed at the abaxial side using a light microscope (Zeiss Fluorescence Stereo Macroscope) with 200-fold magnification for counting number of stomata and a 400-fold magnification for measuring stomatal length as described in Boer et al., 2016 (see Wu et al., 2020). Stomatal density was expressed as the number per vein length, that is, in one leaf segment, which is determined as 1 mm in length and extension in with between two veins; the stomata in one leaf segment do not differ. However, the size and number of segments were highly variable between varieties. The minimal stomatal density in 1 mm leaf section is the number of stomata in one segment multiplied by the total number of segments in the leaf (Supporting Information S4: Figure 7).

4.6 | Chlorophyll content

From the same experiment, samples of the third youngest leaf were analyzed for their chlorophyll content, with a minimum of five biological replicates, using two different methods. One is non-destructive, using a chlorophyll content metre (Gitelson et al., 1999; Buschmann, 2007). The second method is destructive, allowing to quantify not only total but also Chlorophyll a and b content, via extraction of leaf discs following the protocol described in Sharma (2017).

4.7 | Gas exchange measurements

Gas exchange was measured using a LI-COR 6400XT with a 2 × 3 cm measuring cuvette (LiCor Inc.) with a transparent top, allowing outside light to penetrate the leaves (Supporting Information S4: Figure 9A). The parameters of the infrared gas analyzer were set the same for both groups: a flow rate of 500 µmol s^{-1}, the CO_2 flow of the sample to 400 µmol m^{-2} s^{-1}, and the block temperature to 30°C. The relative humidity of the sample was approximately 70%. Measurement were recorded on the following groups:

1. Control plants grown in WL (WL) and treated plants grown with supplemental FR (FR pretreated).
2. Under two FR light settings: off and on.

The sequence of measurements was for each treatment group first under their 'native' light environment and then 'changed'. This means that for control grown plants first with FR lamps were off and then switched on, and for FR-acclimated plants vice versa, leading to four measurement groups: C-off, C-on, FR-on, and FR-off. Plants were grown and measured in the same setup as for phenotyping experiments; also light conditions were similar with approximately 400 µmol PPFD photons m^{-2} s^{-1} and 500 µmol FR photons m^{-2} s^{-1} (Supporting Information S4: Figure 1). The variables we investigated for statistical analysis were photosynthetic rate and stomatal conductance. We also compared leaf temperature, CO_2 of the sample, and internal PPFD inside the cuvette throughout the measurements (Supporting Information S4: Figure 9B–D), which give insight into potential confounding factors.

Light response curves were measured on plants of the variety Nipponbare of the two treatment groups grown under control and FR supplemental conditions using gas exchange using LI-COR 6400XT with a closed measuring cuvette equipped with LED light source (2 × 3 cm with red and blue LEDs). Following the protocol in Evans and Santiago (2014) with minor adjustments using a flow rate of 400 µmol s^{-1}. Measurements for light response curves were performed under ambient CO_2 concentration with set PPFD intensities starting at high and going to low light intensities: 1800 (first replicate was started at 2000), 1500, 1000, 500, 250, 120, 60, 40, 20, 10, 0 µmol photons m^{-2} s^{-1}. By following the analysis protocol described in de Lobo et al. (2013), we fitted equation 11 to determine the following parameters: light compensation point (Icomp), light saturation point (Isat), maximum gross photosynthetic rate (Pgmax), maximum net photosynthetic rate obtained at Imax (PN(Imax), dark respiration (RD) and quantum yield at the range between Icomp and I = 200 µmol photons m^{-2} s^{-1} (ϕ(Icomp – I200)).

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DATA AVAILABILITY STATEMENT

Pre-processed data is readily available for download in Zenodo https://doi.org/10.5281/zenodo.8117001. All custom R scripts are available at https://github.com/aromanowski/rice_shade. Alternatively, they are available upon request to the corresponding author. The RNAseq data that support the findings of this study are openly available in ArrayExpress at www.ebi.ac.uk/arrayexpress, reference number E-MTAB-13023.

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SUPPORTING INFORMATION

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