Determinant effect of PAR on role of FR photons; FR light as a resource or signal for stress

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Research report
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Layman summary

Rice is one of the most important crops for agricultural purposes. The space required for farming (i.e., rice) will likely increase to feed a growing world population. Research on plant growth responses, may contribute to understanding factors limiting potential yield. Plants absorb mostly red and blue light for photosynthesis and reflect green and far-red light. Therefore, neighbouring plants, especially in high density growth conditions, lower ambient R:FR ratios. This triggers a range of responses referred to as the shade avoidance syndrome (SAS), in shade-avoiding plants. The SAS is important as it may negatively impact yield and limit the density of crops grown in agriculture. In this report, we discuss an observed response of rice to low R:FR light conditions that is atypical of what generally is observed in the SAS. The research question was: why is the observed response of rice under low R:FR light conditions so distinctly different from the typical SAS? We have seen increased or decreased shoot biomass and number of tillers (branches) and leaves in rice grown in FR light in autumn or winter, respectively. As an extension to existing literature, our results suggest that FR light and light that can be used for photosynthesis together, determine the growth response of rice under low R:FR light conditions. Additionally, we found that, even though it has not been described in other literature, rice also shows a (typical) SAS. We concluded this after a light treatment that removes the effect of FR light on photosynthesis. Then, we also investigated how different genes of interest were expressed in rice that was grown under low R:FR light conditions. We grew rice and exposed it to FR light 5 days after sowing, and harvested three tissues (meristem, culm and leaves) separately at three different timepoints (19, 24 and 29 hours after FR light exposure). We used these samples to determine different expression patterns in low R:FR grown rice compared to control grown rice in a quantitative real-time polymerase chain reaction (qPCR) assay. Our genes of interest likely play a role in plant hormone biosynthesis, cell division, the SAS pathway, photosynthesis, and flowering. We have found a potential role of plant hormones (auxin and ethylene) in mediating the response to low R:FR light conditions. Increased biomass or number of leaves and tillers may be due to increased cell division, based on *CYCB1;1* expression. Two genes encoding photosynthetic components (*PSBR* and *PSB1B2*) also showed a response in their expression, though the differential expression is not uniform. *FPFL3* is a gene that was most strongly differentially expressed, though its function is unknown. Lastly *PIL16*, an upstream component of the SAS pathway, was upregulated. This may indicate that even in rice that responds atypically to low R:FR light conditions, responses of the SAS are still present, and the pathway is still active, but it is probably attenuated or inhibited more downstream in the pathway.

Abstract

The shade avoidance syndrome (SAS) is a range of responses observed in plants when exposed to low red to far-red (R:FR) light conditions. The SAS is known to affect plant growth, development, and potential yield. The SAS in rice is very scarcely reported on, despite rice being one of the world's most important crops. Preliminary observations of rice growth under low R:FR light conditions contrast with commonly observed responses of plants under these conditions. In this study, we confirmed the preliminary observations and found that rice overall showed an increase in branching (leaves and tillers) and biomass and a general increase in culm height. We aimed to investigate the cause of this response to low R:FR light conditions and understand the underlying mechanism. Subsequent findings of rice under low R:FR light conditions in winter showed decreased branching traits and shoot biomass. The contrasting results were expected to be caused by far-red light (FR) driving photosynthesis, which is known as the Emerson effect. Based on these observations, in the next treatment end of day FR (+EoD FR) light was supplemented to remove the effect of FR light treatment on photosynthesis. +EoD FR treated rice plants also showed decreased branching traits and shoot biomass, and decreased culm height. Low R:FR treatment in winter and +EoD FR treatments show that rice can respond to FR light in a way typically described for the SAS. The response of rice to low R:FR light conditions in autumn suggest that FR light can also function as energy for photosynthesis if there is sufficient light in the defined range of photosynthetically active radiation (PAR). Morphological or physiological effects of FR light according to the SAS that have not been measured in this study may still occur in rice plants grown in low R:FR light and high PAR. A gene expression analysis, RT-qPCR, revealed a differential upregulation of *PIL16* in rice in low R:FR light in high PAR. This may indicate the role or influence of the SAS in rice in low R:FR light even in high PAR. Phytohormones auxin and ethylene are suggested to mediate the response of rice in low R:FR and high PAR. The gene *FPFL3*, related to a family of genes that play a role in flowering, and *PSBR* were strongly upregulated, indicating a potential role of the genes mediating the observed phenotypic response.

Introduction

Ever-increasing demand of agricultural produce puts a lot of pressure on an increase of production, driving deforestation. Agricultural land clearance is projected to cause habitat loss for over 87,7% of 19,859 species of terrestrial vertebrates according to (Williams et al., 2021). This is a call for increasing productivity of agricultural land to stop deforestation whilst still feeding the world population.

Rice is most frequently cited as the commercial and subsistence crop driving deforestation for 28 tropical conservation landscapes (Jayathilake et al., 2021). This is not surprising as rice is one of the most important crops for food production globally. It is grown on over 114 million farms, which is more than any other crop (Mohanty et al., 2013). Aims to increase rice yield may therefore have a very clear impact on expansion of agricultural land. Especially considering expected spatiotemporal changes in precipitation challenge suitability of rice agriculture (Özşahin & Ozdes, 2022), utmost effective use of land that is now and will remain suitable for rice cultivation is crucial.

Environmental factors which limit yield are extensively studied. Among these factors is competition for light among plants. A range of developmental (growth) responses of plants in response to neighbouring plants have been described and clustered under the name of the shade avoidance syndrome (SAS). The SAS includes a variety of changes to plant physiology and morphology including but not limited to: elongated hypocotyl, petioles and internodes, inhibited branching, hyponasty and accelerated flowering (Ballaré & Pierik, 2017). This reallocation of resources into plant organs that are not harvested leads to decreased yield (Boccalandro et al., 2003; Green-Tracewicz et al., 2011).

The signal for plants to initiate the changes typical of the SAS is a change of light quality, specifically a decreased ratio of red to far-red (R:FR) light perceived by plants through specialized proteins called phytochromes (Franklin & Whitelam, 2005). As common

notion states that plants utilize most of the visible light spectrum and especially red light, while not absorbing FR light, R:FR ratio decreases underneath canopies but also laterally due to neighbouring plants (Huber et al., 2021).

Lower R:FR light causes inactivation of phytochromes, which removes inhibition of growth promoting phytochrome interacting factors (PIFs), bringing about the SAS (Ballaré & Pierik, 2017). In Arabidopsis the phytochromes A to E have been identified, in rice phytochromes A, B and C have been described (Takano et al., 2009). Preliminary observations of rice growth under low R:FR light conditions (Huber, 2022) contrast with expected growth responses typical of the SAS. In this thesis, these results are to be confirmed and further investigated. It is also investigated why the observed response of rice under low R:FR light conditions is so distinctly different from the typical SAS. As experiments are performed in the greenhouse and therefore influenced by variable light quality and quantity, rice growth responses to FR light are studied regarding seasonality. Also, chlorophyll content is quantified to investigate possible effects of FR on photosynthetic processes. Additionally, (Huber, 2022) has analysed transcriptomic trends of rice grown in supplemental FR. Results of this RNA sequencing (RNAseq) assay show a relatively small transcriptomic response compared to the phenotypical response. By use of quantitative real-time polymerase chain reaction (qPCR), DEGs from RNA sequencing data of (Huber, 2022) are investigated as well as genes commonly associated with shade and cell division. We aim to understand the cause of the growth response observed in rice under low R:FR light conditions, and the mechanisms underlying this response.

Results

To confirm preliminary observations and elaborate on earlier findings (Huber, 2022), rice plant development and physiology are investigated. Specifically, morphological traits of low R:FR treated rice plants (culm height, apical dominance, biomass and number of leaves and tillers) were measured weekly for four weeks after sowing. The seven rice (*Oryza sativa*) varieties used for these experiments have also been used by (Huber, 2022). R:FR ratio in control conditions were 2.0 whereas R:FR ratios in FR conditions were 0.21. These experiments are conducted starting autumn 2021 until and including spring 2022 (detailed description can be found in materials and methods). Additionally, qPCR analyses for several genes of interest are conducted in the variety IR 64.

The phenotypical assessment of rice exposed to low R:FR

Low R:FR light treatment in autumn

Branching traits (number of leaves and tillers) were increased in low R:FR treated plants in spring. Number of leaves and tillers of four out of seven varieties were strongly increased at 21 days after sowing (das) (figure S1A and B). At 28 das all varieties, except for IR 64, showed significantly increased tillering, and increased number of leaves for four varieties (figure 1A and B). Culm height was strongly increased at 21 das in four varieties (figure S1C). At 28 das increased culm height was attenuated, and in case of two varieties culm height was decreased (figure 1C). Apical dominance, which is measured as culm height divided by number of tillers, was decreased under low R:FR light in four varieties 28 das (figure 1D and S3D).

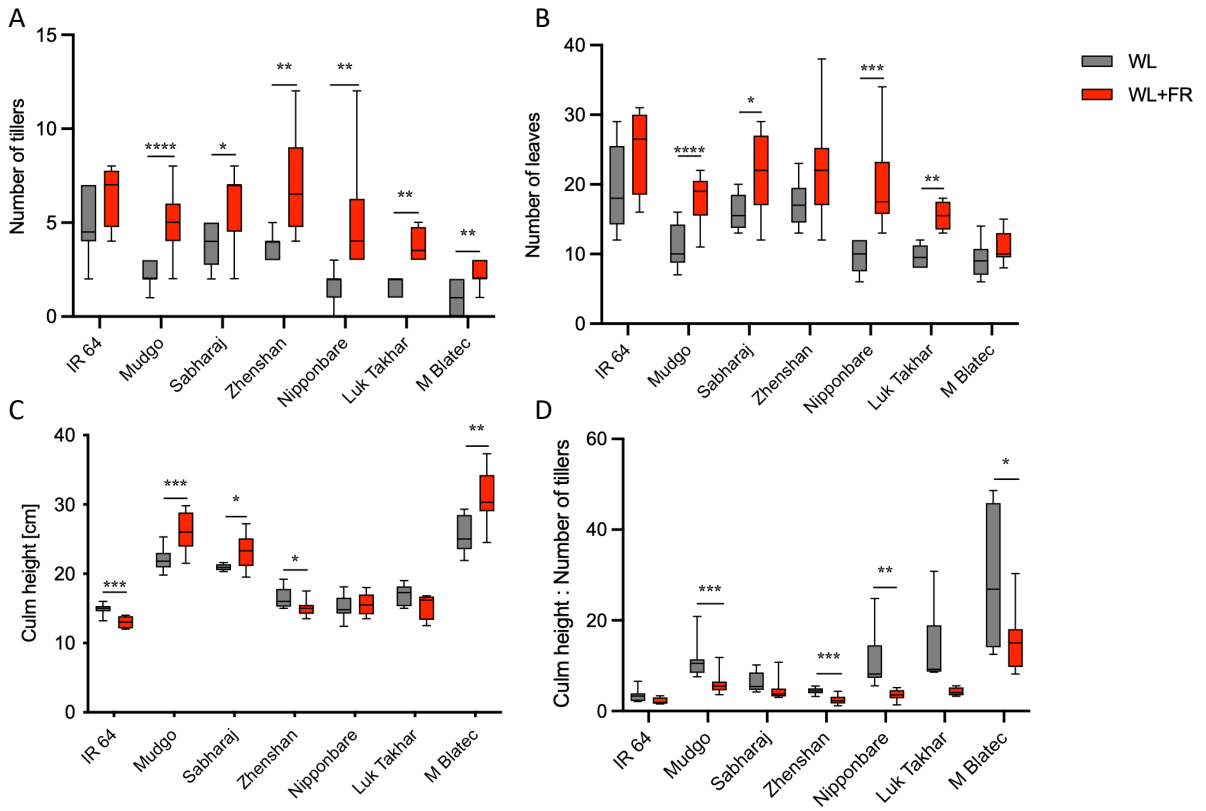


Figure 1. Growth response to low R:FR exposure in autumn of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 28 days after sowing (das), n=4-14. **Comparison of A. tillering, B. number of leaves, C. culm height and D. apical dominance.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown in control (WL) and supplemented with FR light during daylight (WL+FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

After phenotypical measurements described above (28 das), plants were harvested. In this experiment both root and shoot were harvested, to determine possible differential reallocation. Biomass measured as dry weight of low R:FR treated rice varieties was increased in four varieties in the root and in five varieties in the shoot (figure 2A and B). These significant increases were also represented in total biomass (figure 2C). Any reallocation of biomass due to treatment would become evident in a difference of shoot to root biomass (figure 2D). A general trend of decreased ratios of shoot/root biomass was observed, significant for four varieties.

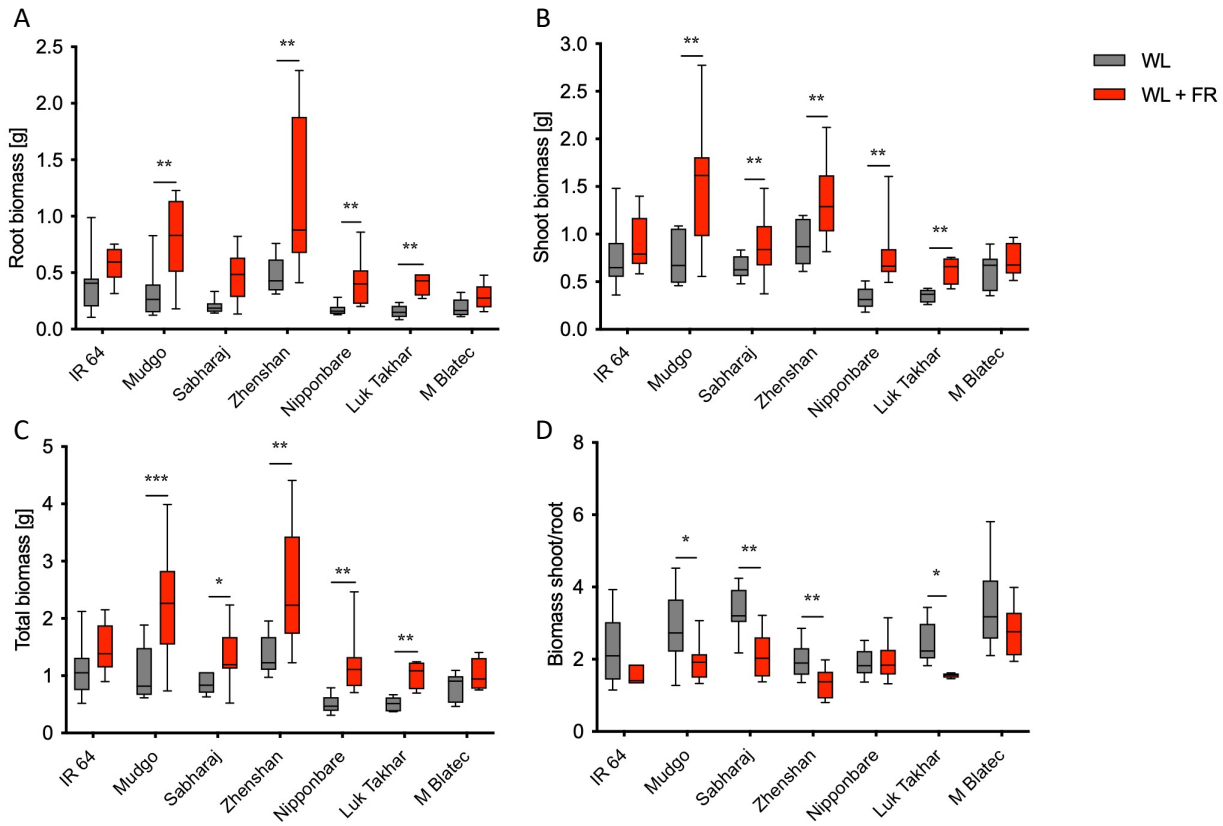


Figure 2. Growth response to low R:FR exposure in autumn of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 21 days after sowing (das), n=4-13. **Comparison of A. root biomass, B. shoot biomass, C. total biomass and D. shoot/root biomass ratio.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), supplemented with 12h of FR light during daylight (WL+FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

Low R:FR light treatment in winter

After the previously described experiment, rice plants (three cultivated *Oryza sativa* and two wild rice varieties *Oryza rufipogon* (NEP) and *Oryza nivara* (CAM)) were grown as either control, white light with supplemental 24h FR light (WL+FR_{24h}) or shifted from control to FR treatment upon onset of tillering. Additionally, FR filters were installed to prevent leakage of red light. Results of this experiment showed disappearance of most of the phenotypic response of the earlier experiment. No difference in culm height, shoot biomass or in number of leaves and tillers was observed for four out of five varieties (figure S2), except for culm height in Zhenshan. Only the wild rice variety CAM showed a very strong response to WL+FR_{24h} which was like observations in the first experiment, where CAM had a significantly increased number of tillers and leaves and strongly decreased culm height (figure S2).

Contrasting results to previous experiments, were expected to be caused due to changes of light treatment, and therefore an experiment with original light conditions was repeated for a subset of three varieties, IR 64, Mudgo and Nipponbare. Results of this experiment, which was conducted in winter are similar to the WL+FR_{24h} treated group. In contrast to the first experiment, differences in number of tillers and leaves were milder at 21 das, and significantly decreased in low R:FR treated plants 28 das in all varieties (figure 3A and B). Culm height response, however, was similar to observations of the first experiment, as IR 64 showed a decrease and Mudgo and Nipponbare an increase under low R:FR conditions (figure 3C). Increased apical dominance was found for Nipponbare, which contrasts with the earlier experiment (figure 3D). Shoot biomass was decreased under low R:FR treated conditions in this experiment (figure 3E).

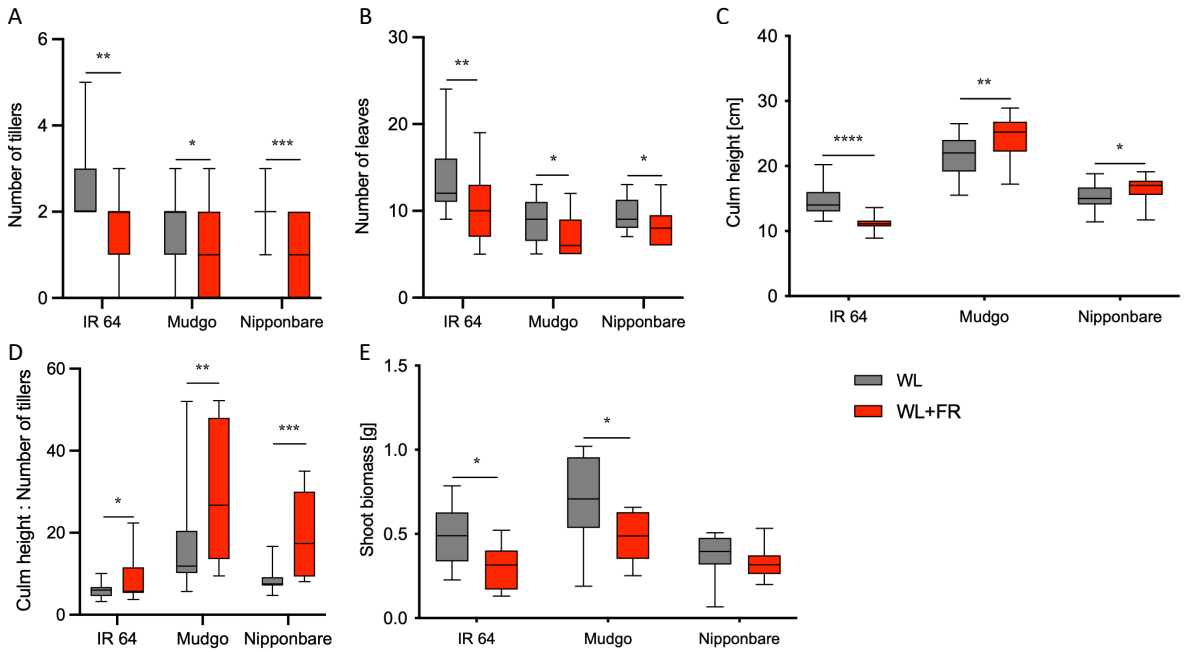


Figure 3. Growth response to low R:FR exposure in winter of cultivated rice varieties (IR 64, Mudgo and Nipponbare) at 28 days after sowing (das), n= 8-10 for shoot biomass, n=15-24 for other traits. **Comparison of A. tillering, B. number of leaves, C. culm height, D. apical dominance and E. shoot biomass.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), supplemented with 12h of FR light during daylight (WL+FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

End of day Far-red (+EoD FR) exposure

+EoD FR treatment in wintertime

Since far-red light could be expected to influence photosynthetic activity, end of day FR experiments were carried out. To detect any differences due to seasonality, the experiment was performed in both winter and spring, as plants are grown in a greenhouse and therefore influenced by external light. End of day FR treatment is performed with a short pulse of FR light just after the photoperiod. End of day FR treated (+EoD FR) rice plants are exposed to 15 minutes of FR light 10 minutes after the end of the photoperiod. In case of +EoD FR plants, tillering and number of leaves was decreased most strongly in IR 64, Mudgo and Luk Takhar 21 das (figure S5A and B). These branching traits are significantly decreased in case of all varieties, except for Zhenshan and Nipponbare at 28 das (figure 4A and B). Culm height was found to be decreased in three varieties at 21 das and in five at 28 das (figure S5C and 4C). Shoot biomass was found to be significantly decreased in four out of the seven varieties (figure 4D). Finally, apical dominance is increased in one and decreased in one other variety (figure S5D). A week later apical dominance is increased in the case of three varieties (figure 4E).

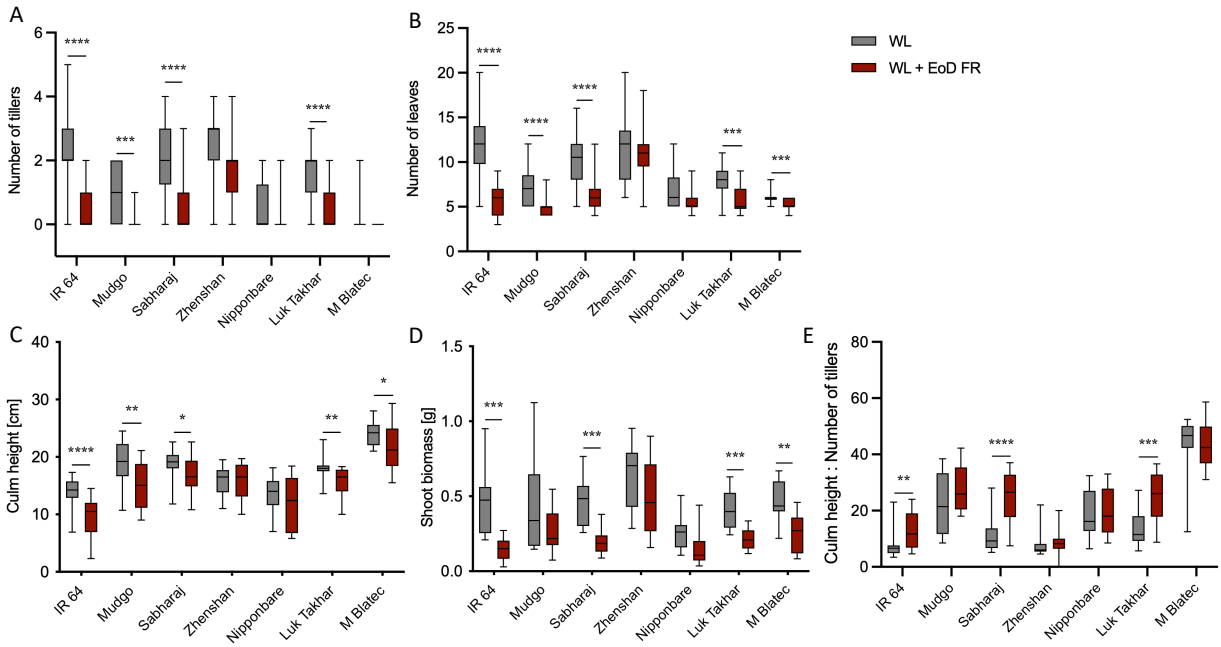


Figure 4. Growth response to +EoD FR exposure in winter of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 28 days after sowing (das), n=10 for shoot biomass, n=14-26 for other traits. **Comparison of A. tillering, B. number of leaves, C. culm height, D. shoot biomass and E. apical dominance**. The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL) grown as WL and exposed to 15 minutes of FR light 10 minutes after the end of the photoperiod (WL+EoD FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

+EoD FR treatment in spring

To exclude any effect of seasonality on +EoD FR treatment the experiment was repeated in spring, resulting in similar phenotypic responses. Number of leaves and tillers were decreased for all varieties except for IR64 at 21 das and a week later for all varieties except number of leaves in IR64 (figure S6A and B and 5A and B). Culm height is found to be significantly decreased only in two varieties 21 and 28 das (figure S6C and 5C). Shoot biomass is decreased in +EoD FR plants for four varieties (figure 5D). Finally, apical dominance was significantly increased in case of three varieties and decreased in one variety 21 das (figure S6D). A week later apical dominance is increased in four varieties (figure 5E).

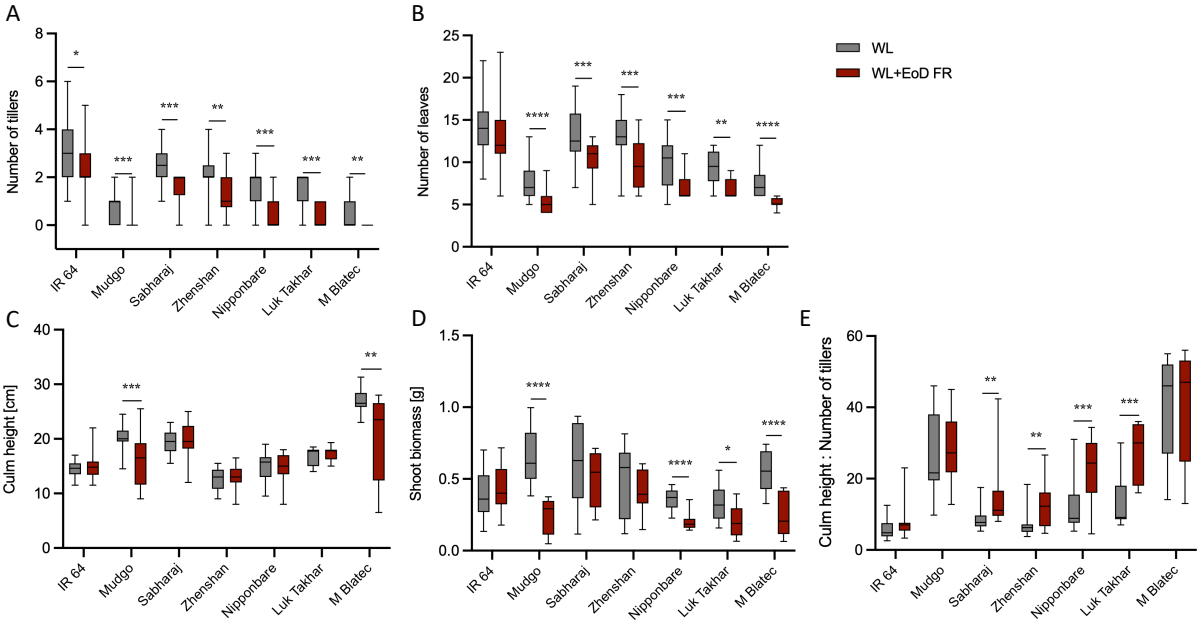


Figure 5. Growth response to +EoD FR exposure in spring of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 28 days after sowing (das), n=10 for shoot biomass, n=10-26 for other traits. **Comparison of A. tillering, B. number of leaves, C. culm height, D. Shoot biomass and E. apical dominance**. The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), or WL grown and exposed to a 15-minute pulse of FR light (WL+EoD FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

We speculated on a potential role of photosynthesis in the observed low R:FR phenotype. Therefore, chlorophyll content of rice plants was measured 21 and 28 das. A decreased chlorophyll content was found for Mudgo in the second and third youngest leaves 21das. For M Blatec also a decreased chlorophyll content was found in the third youngest leaves 21das (Figure 5A and B). No significant changes in chlorophyll content were found for second or third youngest leaves 28das (figure 5C and D).

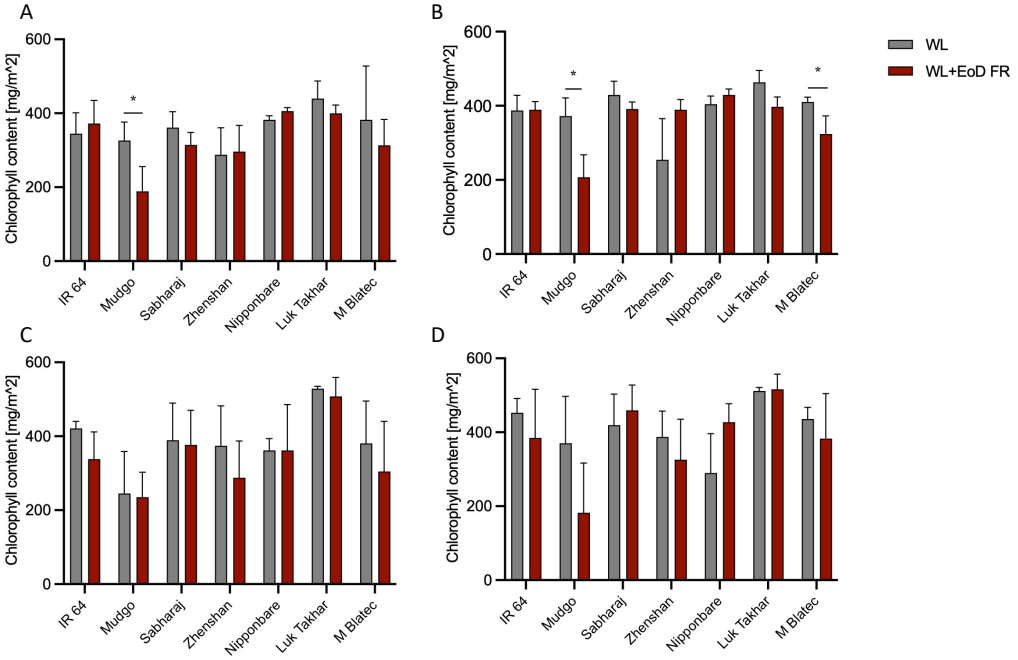


Figure 5. Chlorophyll content in +EoD FR exposure in spring of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 21 and 28 days after sowing (das), n=3. **Comparison of chlorophyll content in A. 2nd youngest leaf 21das, B. 3rd youngest leaf 21das, C. 2nd youngest leaf 28das and D. 3rd youngest leaf 28das**. The figure shows bars representing means with error bars representing SD. Rice plants were grown as control (WL), or WL grown and exposed to a 15-minute pulse of FR light (WL+EoD FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

Changes in gene regulation upon low R:FR treatment

An RNA sequencing assay of rice seedlings treated with low R:FR led to the detection of several differentially expressed genes (DEGs) upon treatment (Huber, 2022). Based on these results, certain genes were defined as genes of interest (GOIs), based on their magnitude of altered expression levels. GOIs from (Huber, 2022) include *IAA1*, *YUC6*, *ACO2*, *PSBR*, *PSB1B2*, *PIL16*, *FPFL3* as well as three genes that are commonly associated with the SAS or cell division regulation were investigated (*PHYB*, *CYCB1;1* and *OSH1*), a real-time quantitative polymerase chain reaction (qPCR) assay. Three separate tissues (meristem, culm, leaves) and three different timepoints (19 h, 24h, 29h after start of treatment) are represented in the qPCR assay. This was to determine whether DEGs were not found in the RNA sequencing assay due to dilution in the tissue or whether the timepoint of strongest differential expression was missed. qPCR data of genes

of interest (GOI) was normalized to three reference genes (*UBQ10*, *UEV1A* and *ACT1*) (see materials and methods). Subsequently, the absolute values were calculated with the PFAFFL method per treatment (Pfaffl, 2001). The $\log_2$ of these absolute values were then statistically compared per tissue per gene with one-way ANOVA and Tukey's post-hoc test to determine differences between control and low R:FR treated per timepoint. All qPCR data is visualized in figure 6 for each gene in the different tissues split per timepoint. The same data is also visualized with grouped timepoints, split per tissue in figure S7 to enable different comparisons.

IAA1 and *YUC6* are described to have a role in auxin hormone responses and biosynthesis (Thakur et al., 2001; Li et al., 2020; Yamamoto et al., 2007). *IAA1* was upregulated in meristems 29h after start of treatment. Expression of *YUC6* was also significantly upregulated in meristems 29h after start of treatment. Generally, *IAA1* and *YUC6* both showed different expression between the three timepoints for all tissues (two-way ANOVA, indicated above each graph for each gene). Additionally, YUC6 shows a significant interaction effect.

ACO2, responsible for ethylene synthesis (Iwai et al., 2006; Da Costa et al., 2021), was upregulated in culm and leaves 24h after exposure and even stronger upregulated in meristems and leaves 29h after treatment. For *ACO2* there is different expression between timepoints, and a strong interaction effect.

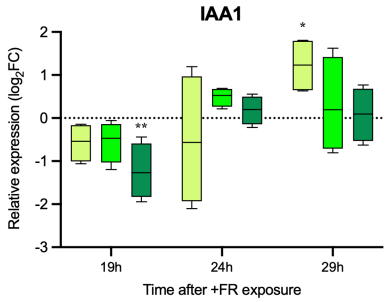
FPFL3, part of a family controlling flowering time in plants (Xu et al., 2005; Guo et al., 2020), was upregulated in leaves at all timepoints and in culm at 24h and 29h. Additionally, *FPFL3* was differentially expressed throughout the different timepoints and tissues.

PSBR and *PSB1B2* are both genes likely involved in photosystem II and thereby in photosynthesis (The UniProt Consortium, 2021; Sun et al., 2017). In all three tissues at 29h treatment, *PSBR* was upregulated. *PSBR* showed significant difference between timepoints and tissues. For *PSB1B2* at 29h treatment, upregulation was present in meristem whilst downregulation was found in leaves. There is no significant difference between tissues or timepoints, but there is a significant interaction effect.

PHYB and *PIL16* are likely key players in mediating the SAS (Takano et al., 2009; He et al., 2016). *PHYB* was not significantly differentially expressed. However, *PIL16* was upregulated, most strongly in leaves. *PIL16* showed significant difference in expression between timepoints and tissues, and a strong interaction effect.

Lastly, *CYCB1;1* is associated with cell division (Ma et al., 2009) and *OSH1* is associated with maintaining shoot apical meristem after germination (Tsuda et al., 2011). Expression of *CYCB1;1* was increased in meristems 29h in low R:FR treatment and was significantly differently expressed across timepoints. *OSH1* showed no differential expression throughout the treatment in any of the tissues.

Tissue: ns; timepoint: **; interaction: ns



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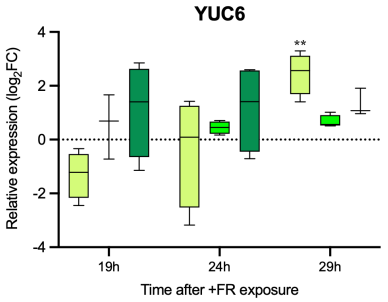

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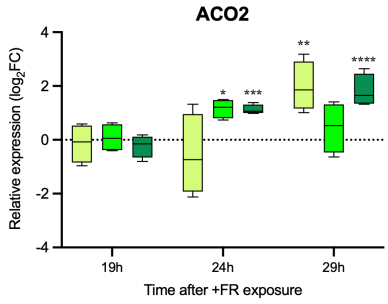

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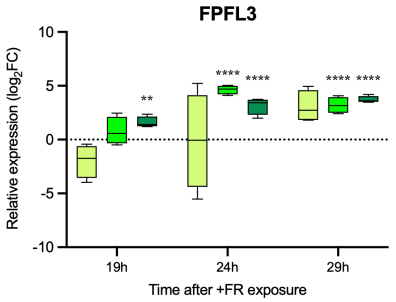

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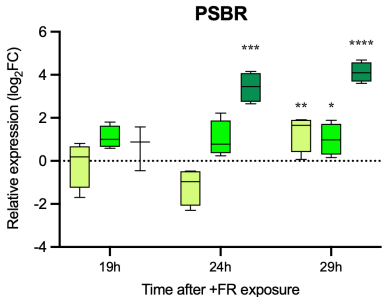

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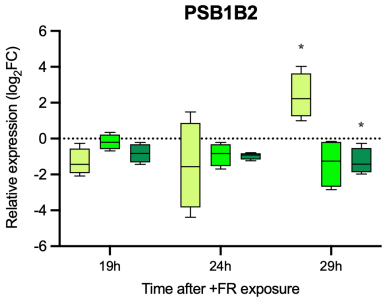

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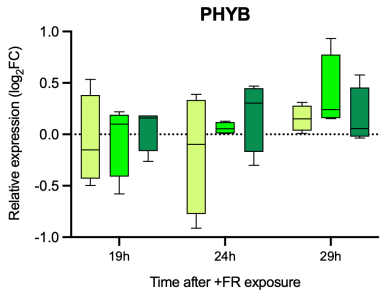

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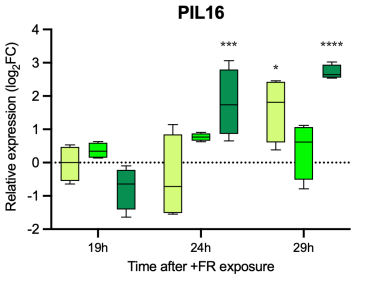

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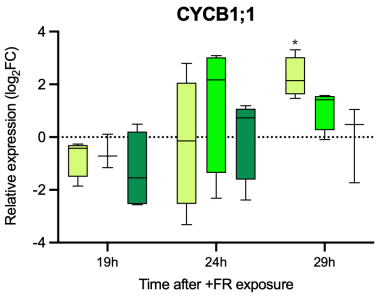

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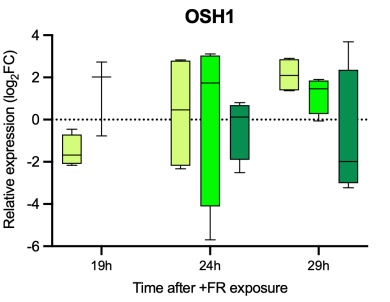


Figure 6. Gene expression upon low R:FR treatment in rice variety IR 64 compared to control grown plants. Gene expression was analysed for separated tissues (meristem, culm, leaves) at three timepoints (19 h, 24h, 29h after start of treatment) The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Boxes and whiskers represent log_2(FC), FC being calculated according to PFAFFL formula. Significant differences between treatment and control are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (one-way ANOVA + Tukey's post hoc test, on the log_2 of absolute values of control and treatment calculated with PFAFFL method). Significantly different expression between tissues and timepoints and their interaction effect is indicated above each graph (two-way ANOVA).

Discussion

The aim of this research was to confirm the observed growth response of rice under low R:FR light conditions and uncover underlying molecular mechanisms. For this purpose, additional phenotyping experiments with low R:FR light treatment and +EoD FR treatment have been conducted, throughout different seasons. Rice plants of several varieties have been phenotyped from one week after sowing up to four weeks after sowing. In addition to the phenotype, the aim was to get deeper insight into transcriptomic regulation, adding on previous analysis (Huber, 2022) of rice under low R:FR conditions. For transcriptomic analysis, a qPCR assay was conducted. For qPCR, five-day old seedlings were harvested at 19, 24 and 29 hours after start of treatment, separated into three tissues: meristem, culm, and leaves.

Atypical response of rice to low R:FR light

The aim of the first experiment was to confirm preliminary observations of rice growth under low R:FR light conditions. It was found that behaviour of branching traits (leaf and tiller formation) of rice growing under low R:FR conditions are in contrast with what is commonly described as the SAS. In this study we confirmed that branching was increased in most varieties instead of an expected decrease, according to a typical SAS phenotype (Carriedo et al., 2016). Culm height, in response to low R:FR, differs strongly depending on the variety, as it increases in some varieties but decreases significantly in others. As a consequence of the SAS it is described that culm height increases in low R:FR light (Morgan et al., 1980; Franklin, 2008). Apical dominance (i.e. increased height growth at the expense of branching) is typically described to be increased in the SAS (Smith & Whitelam, 1997; Whipple et al., 2011; Carriedo et al., 2016), however, here we found a decrease under low R:FR conditions in rice. Also, in contrast to the typical SAS, biomass in both root and shoot is increased in low R:FR light (figure 2). There may be reallocation of biomass in low R:FR from shoot to root as shoot/root biomass ratio significantly decreases under low R:FR conditions. However, whether this decreased ratio is directly due to low R:FR treatment or due to accelerated development in which tissue may be favoured over another, is not known. This experiment confirmed some of the earlier observations, which contrast with observations of typical plant growth in low R:FR ratios. The observed response could either be a very atypical form of the SAS, or the SAS is repressed or absent and a different mechanism is playing a role.

Research of (Takano et al., 2009) shows that there are three phytochromes (A, B and C) present in rice. These phytochromes are solely responsible for perception of red and far-red light, as triple mutants grown in red light or dark have very similar phenotype of the coleoptile. This indicates that rice in principle may under certain conditions show the SAS under low R:FR ratios. On the other hand, some studies also describe increased growth (increased tillering, number of leaves and biomass) in supplemental FR in other plant species such as pepper (Brown et al., 1995) or lettuce (Lee et al., 2015). Important to notice is, that in these studies, the treatment was not supplemented FR light reaching R:FR ratios lower than 1 and therefore not comparable to the treatment in the here presented study. However, more recent studies elaborately describe a synergistic effect of FR in combination with light of shorter wavelengths (in the range of photosynthetically active radiation) on photosynthesis which is also observed in R:FR ratios lower than 1 (Zhen & Bugbee, 2020a; Zhen & Bugbee, 2020b). In conclusion, rice showed a

phenotypic response under low R:FR light conditions in preliminary observations and in low R:FR light treatment conducted in autumn of this study. This growth response contrasts the expected SAS responses, as branching traits and biomass were increased. However, literature suggests a possible SAS response present in rice. Why this was not observed in autumn, may either be due to an atypical form of the SAS. Other mechanisms such as the synergy of FR light and PAR in driving photosynthesis may play an interacting role.

Role of FR light on photosynthesis and signal for the SAS

The mechanism behind the observed effect of FR on photosynthesis (and thereby on growth) can be explained by the Emerson effect, which has been described in 1958 by (Emerson, 1958). The Emerson effect explains increased photosynthesis due to addition of FR light to light with lower wavelengths.

The performed analysis indicates that enhanced formation of leaves and tillers and enhanced biomass is not solely due to low R:FR light conditions. Results of low R:FR experiments conducted during winter show attenuation or even a reversed response to observations in autumn, for certain traits. The R:FR ratio was maintained in winter, and yet phenotypical differences between rice growth under control or low R:FR conditions in winter contrast with rice growth in autumn. The differences in conditions between treatment in autumn and winter are maximum temperature and maximum PAR. Rice grown in lower temperatures and slightly higher PAR than in the experiment in winter, show a similar effect as observed in the low R:FR experiment in autumn (Huber, 2022). Therefore, these results suggest that the increased formation of tillers and leaves and accumulation of biomass in low R:FR light, might be dependent on the intensity of background PAR.

The range of background PAR, in which supplemental FR stimulates formation of tillers and leaves may be different between varieties. Under 24h FR treatment, which was also conducted in lower background PAR, the wild rice variety CAM showed a very strong response, in line with the trends seen for low R:FR treatment in autumn (Figure S1C). The response of rice in winter to supplemental FR light resembles the typical SAS phenotype. Whether this is what the SAS in rice looks like cannot be confirmed, as literature of rice growth in supplemental FR is scarce.

All in all, these results suggest the effect of low R:FR treatment strongly depends on other factors; rice may photosynthetically benefit from FR or perceive FR light as a signal for stress, depending on intensity of PAR, based on the observed behaviour of branching traits and shoot biomass.

Functional ambiguity of FR light

There was an obvious contrast in phenotypic response of rice in low R:FR light in autumn and winter. In winter, the effect of FR on photosynthesis in rice was investigated via gas exchange measurements by (Huber, 2022), showing that photosynthesis is increased in rice in low R:FR light. Following these findings, experiments were conducted with end of day FR treatment (+EoD FR). In +EoD FR treatment, plants are exposed to a short pulse of FR light after the photoperiod. The advantage of +EoD FR treatment is that it does not influence photosynthesis via the described Emerson effect as control light and FR light are temporally separate.

Results from +EoD FR treatment (in winter) have a similar trend to the results from low R:FR treatment in winter. If anything, the response is stronger in +EoD FR compared to low R:FR in winter. Culm height was decreased in most of the investigated varieties. Culm height was expected to increase, as stem elongation is one of the most prominent changes described in the SAS (Morgan et al., 1980; Franklin, 2008). However, this was not observed. Changes in culm height may therefore be more related to general growth or photosynthesis and not the SAS.

Repetition of the same +EoD FR treatment in spring lead to very similar results. Therefore, seasonality (or PAR) under these background light conditions may not have as much of a determinant effect on the growth response of rice. The growth response in +EoD FR treated in rice is stronger than that of low R:FR treated rice in winter. This may suggest an attenuating effect of FR light on the SAS through the Emerson effect.

Overall, +EoD FR treatment supports the hypothesis of involvement of the Emerson effect in the growth response of rice to low R:FR light treatment. The response of rice to +EoD FR treatment seems similar to the typical SAS. The phenotypical findings from this study are schematically visualized in figure 7. Low R:FR treated rice plants show increased tillering, number of leaves and biomass accumulation in higher background PAR, which will henceforth in this report be referred to as high PAR and FR (HPFR) phenotype.

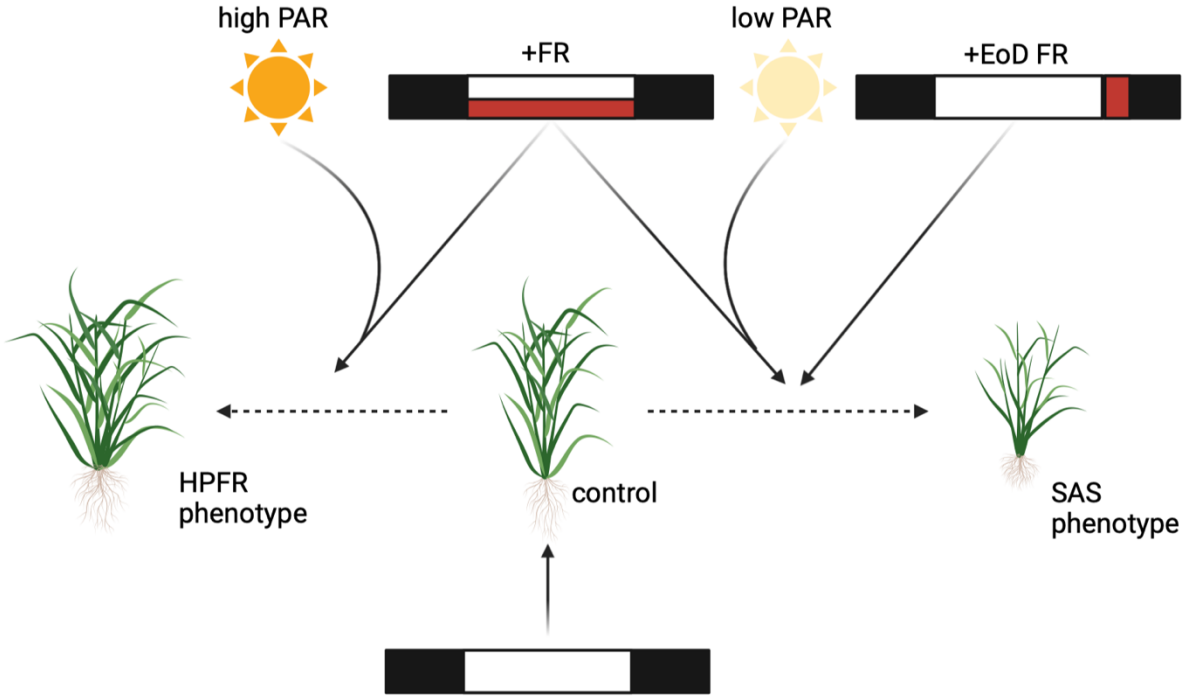


Figure 7. Overview of phenotypic responses of rice to supplemented FR (+FR) and end of day FR light (+EoD FR). in separate experiments plants have been grown under control, with low R:FR (+FR) or end of day FR treatment (+EoD FR). From results from treatment in autumn and winter it can be concluded that background PAR influences rice growth in low R:FR light. Low R:FR light treated rice in high PAR shows increased growth (increased branching, shoot biomass, and culm height), referred to as a high PAR and FR phenotype (HPFR). Plants under low R:FR treatment in low PAR shows overall decreased growth in line with the typical SAS. Phenotypic response of plants under +EoD FR treatment were independent of background PAR.

Insights into transcriptional trends of low R:FR treated rice

To provide an explanation for the low number of DEGs found in the RNA sequencing assay from (Huber, 2022), in-depth gene expression assessment using qPCR has been performed. Gene expression data from the genes in three different tissues at three different timepoints were analysed. Three genes showed a tissue-specific expression pattern (figure S7): *FPFL3*, *PSBR* and *PIL16*. Additionally, for most genes (seven out of ten) a significant difference between timepoints was found (figure 6). The expression at 29h after start of low R:FR treatment was strongest, across all tissues. This suggests that the timepoint for harvest was prior the maximum of gene expression induced in response to low R:FR treatment, therefore missing a part of DEGs playing a role in the response to low R:FR treatment. It is not clear yet whether the intensity and timepoint of the response of rice to FR treatment might depend on development, time after FR exposure, or circadian processes.

IAA1 is a gene which has an expression pattern influenced by presence of auxin hormone. Transcript levels were found to be increased after auxin supplementation, and decreased after auxin starvation (Thakur et al., 2001). *IAA1*-overexpressing transgenic plants have been found to have decreased culm height and less erect leaves (Song et al., 2009). Decreased culm height is not in line with

phenotypic data from this study (figure 1C), but less erect leaves have been described earlier by (Huber, 2022). In this experiment, *IAA1* expression was downregulated in leaves 19h after treatment and upregulated in meristems in 29h after treatment. Upregulation would be suspected as the SAS is auxin-mediated (de Wit et al., 2014).

YUC6 is an auxin biosynthesis gene, specifically functional in root and coleoptile tips, and belongs to a family of genes which function in auxin biosynthesis (Li et al., 2020; Yamamoto et al., 2007). Again, considering the role of auxin in the SAS, *YUC6* was expected to be upregulated. This can also be seen in results from this study as well as in meristems 29h after treatment.

ACO2 is a gene described to play a role in ethylene biosynthesis in rice (Iwai et al., 2006; Da Costa et al., 2021). As *ACO2* is upregulated in culm and leaves at 24 h, and meristems and leaves at 29 h after treatment, one may expect increased levels of ethylene in the rice plants. This would be in line to what has been described for the SAS responses to involve ethylene action, at least through gibberellic acid modulation (Pierik et al., 2004).

PSBR and PSB1B2 are components of photosystem II (The UniProt Consortium, 2021; Sun et al., 2017). If low R:FR light treatment in relatively higher background PAR is perceived as a high light treatment, (Huber et al., 2022) lower chlorophyll content could be expected. PSBR is upregulated in leaves at 24h, and in all tissues at 29h after low R:FR treatment. PSB1B2 is upregulated in meristems and downregulated in leaves 29h after low R:FR treatment. This suggests that a lower chlorophyll content does not necessarily have to correspond to a lower expression of photosystem component encoding genes. Additionally, the results show that different photosystem components are differently expressed, not only to a different extent but also in a different direction (up- or downregulated), suggesting a fine-tuned regulation system.

Generally, *PHYB* expression is expected to be linked to activity of the SAS pathway (Takano et al., 2009). Low R:FR ratio light conditions inactivate phytochrome B, however how this would impact its expression is unclear. qPCR data from this study shows no differential expression after FR treatment. Activity of PHYB can be linked to repression of *PIL16*, as *phyb* mutants have increased *PIL16* transcript levels (He et al., 2016). As PHYB photoreceptors would be expected to be inactive in low R:FR ratios, high *PIL16* expression would be expected. Upregulation of *PIL16* was indeed found, mostly in leaves. As *PIL16* probably acts relatively upstream in the SAS, attenuation of the SAS by the Emerson effect would likely be regulated more downstream in the SAS pathway.

FPFL3 is a gene which was found to be the most strongly upregulated gene in the RNA sequencing assay (Huber, 2022). Also, in qPCR data in this study, the gene is most strongly upregulated (in leaves at all three timepoints and in culms at 24 h and 29 h after low R:FR treatment). The function of the gene, in addition to being one of genes regulating root and flower development (Xu et al., 2005; Guo et al., 2020) is not clearly documented. It may therefore be interesting to study its role in future research.

CYCB1;1 encodes for a cyclin protein (Ma et al., 2009). It is therefore used in this qPCR assay as a marker gene for the cell division. As overall increased growth was found during phenotyping, upregulation in meristems was expected after low R:FR treatment. *OSH1* has been described as an autoregulating gene which is crucial for maintaining the indeterminate state of the shoot apical meristem (SAM) in rice (Tsuda et al., 2011). Differential regulation of this gene could have provided knowledge into meristem activity of rice after FR exposure, however its expression levels were not changed upon FR treatment

In conclusion, phytohormone mediated or biosynthesis genes (*IAA1*, *YUC6* and *ACO2*) of auxin and ethylene are overall upregulated, which suggests their involvement in mediating the response of rice to low R:FR light. *PSBR* is strongly upregulated, although *PSB1B2* has a more ambiguous response. Even though *PHYB* is not differentially regulated, *PIL16* which acts downstream to *PHYB* is upregulated. *FPFL3* is most strongly upregulated of all genes, though its function is not yet elucidated. Finally, *CYCB1;1* is upregulated in meristems, which would be in line to observed increased growth in low R:FR ratio in autumn. It

should be noted that there may also be differences on a post-translational level.

Future directions

The impact of low R:FR treatment on the development of rice growth in the first four weeks of growth has been studied in this report. In this study, the effect of FR on yield was not investigated. An indication is given by the HPFR phenotype, showing an increased number of tillers, which is commonly associated with a higher yield (Sakamoto & Matsuoka, 2008), which would be interesting to investigate further. Additionally, low R:FR treatments have now been performed in autumn and winter which unravelled different phenotypic responses. It would be valuable to determine the interplay at what intensity of background PAR, there is increased or a decreased growth (formation of leaves and tillers and accumulation of shoot biomass) due to low R:FR treatment. For such experiments, rice would have to be grown under controlled WL light conditions (such as a climate chamber) and not in a greenhouse. Apart from phenotypical traits studied in these experiments, there are other traits (physiological and morphological) that have been shown to change in response to low R:FR conditions. It would be important to determine whether the other changes typical of the SAS are also attenuated by supplemented FR treatment during daylight. An example of what could be studied is the trade-off between SAS and plant defence (Ballaré, 2014). This study found upregulation of phytochrome interacting factor like gene PIL16 despite seemingly repressed the SAS. This suggests that there might still be parts of the SAS pathway active in rice, but not strongly visible in the here reported traits. To provide more insight into the extent of the SAS attenuation, it would be interesting to bring more clarity, to the interplay between the SAS, photosynthesis, and its influence on plant development. One might expect a role of the sugar status of the plant, which has been described to modulate the SAS (Kozuka et al., 2005). Lastly, we have observed a potential natural variation in the response of rice to low R:FR treatment, since one investigated wild rice variety showed a HPFR phenotype despite lower background PAR.

Materials and methods

Plant material and growth conditions

The seeds of different varieties of *Oryza sativa* (IR64, Luk Takhar, M Blatec, Mudgo, Nipponbare, Sabharaj and Zhenshan) were acquired from the International Rice Research Institute (IRRI), Los Baños, the Philippines; plants were grown in wet season of 2018 and stored in the dark at 6°C. Seed material of the wild rice varieties *(Oryza nivara*, SRANGE from Cambodia (CAM); *Oryza rufipogon*, NABO from Nepal (NEP)) originate from the International Rice Genebank Center (IRGC) at IRRI.

In preparation for sowing, seeds were pre-germinated; seeds were kept at 37 °C for 24 h, then at 21 °C for 24 h and then transferred onto wet filter paper in petri-dishes, and incubated at 32 °C for 24 h. Afterwards seeds were directly sown into soil which was comprised of a mix of black soil, agra-vermiculite (0-1,5 mm) and sand in a ratio of 5:3:2, 6g Osmocote NPK-Mg 15-4-9 (+1) (2.4 g/L of soil) and 20% Yoshida nutrient solution (Yoshida et al. 1976) with a double iron dose (sequestreen = Fe-EDTA) and pH 6.5 (1l per kg substrate). The black soil and sand were provided by the Botanical Gardens, Utrecht University, in The Netherlands. Into each pot (10 x 10 x 11 cm) 5 seeds were sown.

Light treatment

For all experiments, the greenhouse facilities of the Botanical Gardens, Utrecht University, in The Netherlands, were utilized for growing rice plants in autumn 2021 throughout spring 2022 (table 1) (figure S8).

Table 1. Experiments, treatments, and dates.

Exp.	Treatment	Date
1	low R:FR light (12h)	September 16 until October 14, 2021
2	low R:FR light (24h)	October 22 until November 18, 2021
3	low R:FR light (12h)	(November 18 until December 16, 2021)
4	+EoD FR	January 1 until February 3, 2022
5	+EoD FR	April 1 until April 28, 2022

The minimum temperatures maintained in the greenhouse were 30 °C during the day and 25 °C in the night. Plants were grown in 12 h photoperiod, 8 am to 8 pm. The table on which the plants were grown, was flooded three times per day to keep the soil saturated by use of automatic watering. Pots were placed at an approximate distance of 10 cm from each other. Light intensity of sunlight for control groups was 400 µmol m^{-2} s^{-1} at minimum. Whenever the flux rate derived from sunlight dropped below 400 µmol m^{-2} s^{-1} during the day, artificial light (Valoya, Model Rx400 500mA 5730, Spectrum AP673L) switched on. FR treated groups were exposed to the same amount of light and in addition to this the group received FR light from LEDs (PAR range was the same, though 500 µmol m^{-2} s^{-1} FR light was added). R:FR ratio was 0.21. EoD FR treated groups received the same light as control but 10 minutes past 8pm the treated group was exposed to a 15-minute pulse of FR light (60-80 µmol m^{-2} s^{-1} FR light). To prevent transmission of FR during EoD experiments to the control group, a black plastic screen was put up in the middle of the table (figure S9).

On October 22nd five varieties (Mudgo, Zhenshan, Nipponbare, CAM and NEP) were sown and grown in a 12h photoperiod. The treated group was exposed to 24 hours of supplemented FR instead of 12 hours or EoD FR. An additional treated group (shift FR) was shifted to the FR side of the table upon formation of the first tiller in 50% of the group. The shift FR group was introduced to investigate whether initial tiller response to FR exposure was determined before or during initiation of the branching pathway. It should be noted that there was no screen between treated and control group so therefore the control group may have been exposed to reflected FR light during the night.

From October 22nd onwards, FR filters (Lee colour filter 120 Deep blue) were added to the FR lights, to cut very small amounts of red light that leaked from the FR lamps. R:FR ratio with filter was 0.18 (Smith, 1982). Additionally, semi-translucent curtains of the greenhouse were kept closed in winter to maintain the right temperatures, this led to a reduction of the external white light, and the WL lamps to be on continuously during the photoperiod.

Phenotypic measurements and analysis

In case of all experiments excluding the plants grown for the purpose of qPCR, branching

traits, culm height, apical dominance and shoot biomass were measured. Apical dominance was defined as culm height divided by number of tillers. Additionally, in the first FR exposure experiment leaf length and internode length were measured. For branching traits, number of leaves was counted from the bottom upwards, and number of tillers was counted. Upon formation of a tiller, the node is distanced from the culm and determination of internode is altered, thus the internode underneath or above the node were no longer measured thereafter. Culm height was defined as the height of the highest node. Youngest leaf or internode numbers were compared for statistical analyses in consideration of developmental stage of the plants. In case a tissue that was assessed in phenotyping had not yet been formed, it was valued as zero. Statistical analyses were performed in excel and Graphpad Prism. Data was visualized with GraphPad Prism (Version 9.3.1 (350), December 7, 2021). After the last phenotyping timepoint (28 das) plant shoots are harvested. Shoots are cut just above the root (visually defined by axillary root growth). In case of the first phenotypical experiment, root was also harvested after washing as much soil out as possible without damaging or losing parts of the root systems.

Chlorophyll content measurements

Chlorophyll content was measured on leaves 21 das and 28 das using CCM-300 Chlorophyll content meter (Opti-sciences). Three plants from different pots per variety per treatment were selected, based on how they visually represented the group. For each plant, the chlorophyll meter was placed onto the middle of the second and third youngest leaves.

Transcriptomic analysis

Plant material and experimental design

Plant material for the quantitative PCR analysis was grown following the same pre-germination and sowing procedure as described previously. Five days after sowing, the treated group was then at 13:30 exposed to FR for 24 h $\pm$ 5h. Plants were exposed five days after sowing (das) so enough tissue could be obtained while still capturing the early response to FR exposure. (Huber, 2022) found that four-week-old plants show no phenotypical difference between FR exposure starting at five das or FR exposure directly from sowing onwards. For preliminary qPCR, for each of the three timepoints (19, 24 and 29 h after exposure), three plants were harvested per variety. Out of each of the plants three tissues (meristem, culm, and leaves) are cut and the different tissues of the three plants are pooled, representing one single biological replicate. As soon as the tissues are harvested, they are flash frozen in liquid nitrogen and stored at -80 °C until the tissue was grinded. Grinding for preliminary qPCR is done by use of the Retsch grinder for leaves and culms. Grinding of meristems is done by use of the Tissuelyser II in Eppendorf tubes with metal beads. For the final qPCR analysis, IR64 is the only variety used. The plants are grown, treated, and harvested like mentioned before, obtaining four biological replicates are obtained with each biological replicate being comprised of tissue of four separate plants. All tissues are flash frozen in liquid nitrogen and stored at -80 °C before all tissues ground by use the Tissuelyser II in Eppendorf tubes with metal beads. From the ground tissue, RNA is extracted with the Sigma Aldrich Spectrum™ Plant Total RNA Kit with the Sigma Aldrich On-Column DNase I digestion set. RNA concentration and quality is checked with the N120 NanoPhotometer from Implen.

Primer design and efficiency testing

Primers were designed for genes selected from RNA sequencing data from (Huber, 2022), with genes of interest being defined as significantly up- or down-regulated or genes expected to play a role in either the SAS or cell division despite not arising from the RNA sequencing data. Primers were designed with the primer designing tool from NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers were provided by Integrated DNA technologies. Primers were tested for specificity by performing a qPCR for three concentrations of primers per gene per variety. Primer concentrations that only showed one clear peak in the melting curve plot, were selected to be used for the actual qPCR. The qPCR products derived from these same plates were collected and diluted 6,25*10^6 times with DEPC-treated water before making five serial dilutions (1/5) which will serve as points in the efficiency curve. Efficiencies ranging from 74%

until 207% are used for final calculations of expression data.

cDNA synthesis and qPCR

From the RNA isolated from the samples, cDNA is synthesized according to the protocol that can be found in the supplementary. Hereby, cDNA of 50ng/µL is obtained. 1µL (1ng/µL) cDNA is mixed with 0.25µL forward primer, 0.25µL reverse primer (concentrations in supplementary) and 2.5µL SYBR green mix (PCR Biosystems). For qPCR 5µL reaction solutions? in 384 well-plates are measured with the ViiA™ 7 Real-Time PCR System. qPCR data points without one clear peak in its melt curve plot are excluded. qPCR data is processed according to the PFAFFL formula and normalized with reference genes ACTIN 1, UEV1A, and RICE UBIQUITIN 2. Data is visualized in Prism Graphpad (Version 9.3.1 (350), December 7, 2021). Information about primer names, sequences, and concentrations used, can be found in the supplemental data (Table S1).

References

Ballaré, C. L. (2014). Light Regulation of Plant Defense. *Annual Review of Plant Biology*, *65*(1), 335–363. https://doi.org/10.1146/annurev-arplant-050213-040145

Ballaré, C. L., & Pierik, R. (2017). The shade-avoidance syndrome: Multiple signals and ecological consequences. *Plant, Cell & Environment*, *40*(11), 2530–2543. https://doi.org/10.1111/pce.12914

Boccalandro, H. E., Ploschuk, E. L., Yanovsky, M. J., Sánchez, R. A., Gatz, C., & Casal, J. J. (2003). Increased Phytochrome B Alleviates Density Effects on Tuber Yield of Field Potato Crops. *Plant Physiology*, *133*(4), 1539–1546. https://doi.org/10.1104/pp.103.029579

Brown, C. S., Schuerger, A. C., & Sager, J. C. (1995). Growth and Photomorphogenesis of Pepper Plants under Red Light-emitting Diodes with Supplemental Blue or Far-red Lighting. *Journal of the American Society for Horticultural Science*, *120*(5), 808–813. https://doi.org/10.21273/JASHS.120.5.808

Carriedo, L. G., Maloof, J. N., & Brady, S. M. (2016). Molecular control of crop shade avoidance. *Current Opinion in Plant Biology*, *30*, 151–158. https://doi.org/10.1016/j.pbi.2016.03.005

Da Costa, M. V. J., Ramegowda, V., Sreeman, S., & Nataraja, K. N. (2021). Targeted Phytohormone Profiling Identifies Potential Regulators of Spikelet Sterility in Rice under Combined Drought and Heat Stress. *International Journal of Molecular Sciences*, *22*(21), 11690. https://doi.org/10.3390/ijms222111690

de Wit, M., Lorrain, S., & Fankhauser, C. (2014). Auxin-mediated plant architectural changes in response to shade and high temperature. *Physiologia Plantarum*, *151*(1), 13–24. https://doi.org/10.1111/ppl.12099

Franklin, K. A. (2008). Shade avoidance. *New Phytologist*, *179*(4), 930–944. https://doi.org/10.1111/j.1469-8137.2008.02507.x

FRANKLIN, K. A., & WHITELAM, G. C. (2005). Phytochromes and Shade-avoidance Responses in Plants. *Annals of Botany*, *96*(2), 169–175. https://doi.org/10.1093/aob/mci165

Green-Tracewicz, E., Page, E. R., & Swanton, C. J. (2011). Shade Avoidance in Soybean Reduces Branching and Increases Plant-to-Plant Variability in Biomass and Yield Per Plant. *Weed Science*, *59*(1), 43–49. https://doi.org/10.1614/WS-D-10-00081.1

Guo, Y., Wu, Q., Xie, Z., Yu, B., Zeng, R., Min, Q., & Huang, J. (2020). OsFPFL4 is Involved in the Root and Flower Development by Affecting Auxin Levels and ROS Accumulation in Rice (Oryza sativa). *Rice*, *13*(1), 2. https://doi.org/10.1186/s12284-019-0364-0

He, Y., Li, Y., Cui, L., Xie, L., Zheng, C., Zhou, G., Zhou, J., & Xie, X. (2016). Phytochrome B Negatively Affects Cold Tolerance by Regulating OsDREB1 Gene Expression through Phytochrome Interacting Factor-Like Protein OsPIL16 in Rice. *Frontiers in Plant Science*, *7*, 1963. https://doi.org/10.3389/fpls.2016.01963

Huber, M., Nieuwendijk, N. M., Pantazopoulou, C. K., & Pierik, R. (2021). Light signalling shapes plant–plant interactions in dense canopies. *Plant, Cell & Environment*, *44*(4), 1014–1029. https://doi.org/10.1111/pce.13912

Iwai, T., Miyasaka, A., Seo, S., & Ohashi, Y. (2006). Contribution of Ethylene Biosynthesis for Resistance to Blast Fungus Infection in Young Rice Plants. *Plant Physiology*, *142*(3), 1202–1215. https://doi.org/10.1104/pp.106.085258

Izawa, T., Oikawa, T., Tokutomi, S., Okuno, K., & Shimamoto, K. (2000). Phytochromes confer the photoperiodic control of flowering in rice (a short-day plant). *The Plant Journal*, *22*(5), 391–399. https://doi.org/10.1046/j.1365-313X.2000.00753.x

Jayathilake, H. M., Prescott, G. W., Carrasco, L. R., Rao, M., & Symes, W. S. (2021). Drivers of deforestation and degradation for 28 tropical conservation landscapes. *Ambio*, *50*(1), 215–228. https://doi.org/10.1007/s13280-020-01325-9

Kozuka, T., Horiguchi, G., Kim, G.-T., Ohgishi, M., Sakai, T., & Tsukaya, H. (2005). The Different Growth Responses of the Arabidopsis thaliana Leaf Blade and the Petiole during Shade Avoidance are Regulated by Photoreceptors and Sugar. *Plant and Cell Physiology*, *46*(1), 213–223.

https://doi.org/10.1093/pcp/pci016
Lee, M., Park, S.-Y., & Oh, M.-M. (2015). Growth and cell division of lettuce plants under various ratios of red to far-red light-emitting diodes. *Horticulture, Environment, and Biotechnology*, *56*, 186–194. https://doi.org/10.1007/s13580-015-0130-1
Li, J., Yang, Y., Chai, M., Ren, M., Yuan, J., Yang, W., Dong, Y., Liu, B., Jian, Q., Wang, S., Peng, B., Yuan, H., & Fan, H. (2020). Gibberellins modulate local auxin biosynthesis and polar auxin transport by negatively affecting flavonoid biosynthesis in the root tips of rice. *Plant Science*, *298*, 110545. https://doi.org/10.1016/j.plantsci.2020.110545
Liu, H., Yang, C., & Li, L. (2016). Shade-induced stem elongation in rice seedlings: Implication of tissue-specific phytohormone regulation. *Journal of Integrative Plant Biology*, *58*(7), 614–617. https://doi.org/10.1111/jipb.12468
Ma, Q., Dai, X., Xu, Y., Guo, J., Liu, Y., Chen, N., Xiao, J., Zhang, D., Xu, Z., Zhang, X., & Chong, K. (2009). Enhanced Tolerance to Chilling Stress in OsMYB3R-2 Transgenic Rice Is Mediated by Alteration in Cell Cycle and Ectopic Expression of Stress Genes. *Plant Physiology*, *150*(1), 244–256. https://doi.org/10.1104/pp.108.133454
Mohanty, S., Wassmann, R., Nelson, A., Moya, P., & Jagadish, S. V. K. (n.d.). *Significance for food security and vulnerability*. 18.
Morgan, D. C., O'Brien, T., & Smith, H. (1980). Rapid photomodulation of stem extension in light-grownSinapis alba L.: Studies on kinetics, site of perception and photoreceptor. *Planta*, *150*(2), 95–101. https://doi.org/10.1007/BF00582351
Özşahin, E., & Ozdes, M. (2022). Determining the impact of climate change on land suitability for rice paddy cultivation using GIS and RS on FAO maximum limitation approach. *Theoretical and Applied Climatology*. https://doi.org/10.1007/s00704-022-04033-4
Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, *29*(9), e45. https://doi.org/10.1093/nar/29.9.e45
Pierik, R., Cuppens, M. L. C., Voesenek, L. A. C. J., & Visser, E. J. W. (2004). Interactions between Ethylene and Gibberellins in Phytochrome-Mediated Shade Avoidance Responses in Tobacco. *Plant Physiology*, *136*(2), 2928–2936. https://doi.org/10.1104/pp.104.045120
Sakamoto, T., & Matsuoka, M. (2008). Identifying and exploiting grain yield genes in rice. *Current Opinion in Plant Biology*, *11*(2), 209–214. https://doi.org/10.1016/j.pbi.2008.01.009
Smith, H., & Whitelam, G. C. (1997). The shade avoidance syndrome: Multiple responses mediated by multiple phytochromes. *Plant, Cell & Environment*, *20*(6), 840–844. https://doi.org/10.1046/j.1365-3040.1997.d01-104.x
Song, Y., You, J., & Xiong, L. (2009). Characterization of OsIAA1 gene, a member of rice Aux/IAA family involved in auxin and brassinosteroid hormone responses and plant morphogenesis. *Plant Molecular Biology*, *70*(3), 297–309. https://doi.org/10.1007/s11103-009-9474-1
Sun, C., Hu, Z., Zheng, T., Lu, K., Zhao, Y., Wang, W., Shi, J., Wang, C., Lu, J., Zhang, D., Li, Z., & Wei, C. (2017). RPAN: Rice pan-genome browser for ~3000 rice genomes. *Nucleic Acids Research*, *45*(2), 597–605. https://doi.org/10.1093/nar/gkw958
Takano, M., Inagaki, N., Xie, X., Kiyota, S., Baba-Kasai, A., Tanabata, T., & Shinomura, T. (2009). Phytochromes are the sole photoreceptors for perceiving red/far-red light in rice. *Proceedings of the National Academy of Sciences*, *106*(34), 14705–14710. https://doi.org/10.1073/pnas.0907378106
Thakur, J. K., Tyagi, A. K., & Khurana, J. P. (2001). OsIAA1 , an Aux / IAA cDNA from Rice, and Changes in Its Expression as Influenced by Auxin and Light. *DNA Research*, *8*(5), 193–203. https://doi.org/10.1093/dnares/8.5.193
The UniProt Consortium. (2021). UniProt: The universal protein knowledgebase in 2021. *Nucleic Acids Research*, *49*(D1), D480–D489. https://doi.org/10.1093/nar/gkaa1100
Tsuda, K., Ito, Y., Sato, Y., & Kurata, N. (2011). Positive autoregulation of a KNOX gene is essential for shoot apical meristem maintenance in rice. *The Plant Cell*, *23*(12), 4368–4381. https://doi.org/10.1105/tpc.111.090050
Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R., Schmidt, R., Doebley, J., Brutnell, T. P., & Jackson, D. P. (2011). Grassy tillers1 promotes apical dominance in maize and responds

to shade signals in the grasses. *Proceedings of the National Academy of Sciences*, *108*(33), E506–E512. https://doi.org/10.1073/pnas.1102819108
Williams, D. R., Clark, M., Buchanan, G. M., Ficetola, G. F., Rondinini, C., & Tilman, D. (2021). Proactive conservation to prevent habitat losses to agricultural expansion. *Nature Sustainability*, *4*(4), 314–322. https://doi.org/10.1038/s41893-020-00656-5
Xu, M.-L., Jiang, J.-F., Ge, L., Xu, Y.-Y., Chen, H., Zhao, Y., Bi, Y.-R., Wen, J.-Q., & Chong, K. (2005). FPF1 transgene leads to altered flowering time and root development in rice. *Plant Cell Reports*, *24*(2), 79–85. https://doi.org/10.1007/s00299-004-0906-8
Yamamoto, Y., Kamiya, N., Morinaka, Y., Matsuoka, M., & Sazuka, T. (2007). Auxin Biosynthesis by the YUCCA Genes in Rice. *Plant Physiology*, *143*(3), 1362–1371. https://doi.org/10.1104/pp.106.091561
Yoshida, S., Forno, D. A., Cock, J. H., & Gomez, K. A. (1976). *Laboratory manual for physiological studies of rice* (3rd ed.). The International Rice Research Institute
Zhen, S., & Bugbee, B. (2020a). Far-red photons have equivalent efficiency to traditional photosynthetic photons: Implications for redefining photosynthetically active radiation. *Plant, Cell & Environment*, *43*(5), 1259–1272. https://doi.org/10.1111/pce.13730
Zhen, S., & Bugbee, B. (2020b). Substituting Far-Red for Traditionally Defined Photosynthetic Photons Results in Equal Canopy Quantum Yield for CO2 Fixation and Increased Photon Capture During Long-Term Studies: Implications for Re-Defining PAR. *Frontiers in Plant Science*, *11*. https://www.frontiersin.org/article/10.3389/fpls.2020.581156

Supplementary

cDNA protocol

This protocol is used for cDNA synthesis from RNA by Reverse Transcriptase in a two-step reaction.
The reagents used for this protocol are:
- Random hexamer primers (48190011, Invitrogen)
- dNTP's (R0182, Fermentas)
- 5x first strand buffer (comes with reverse transcriptase)
- RevertAid H Minus RT - Reverse Transcriptase 200U/ul (EP0452 Thermo Scientfic)
- RiboLock RNase inhibitor 40U/ul (EO0382 Fermentas)

Before starting thaw reagents and RNA on ice
Enzymes should be kept @ -20°C at all times (do not keep them on ice).
PCR program: first step, initial denaturation, (RNA + Primers + dNTP's), 65°C – 5' Second step, annealing – cDNA synthesis – inactivation, 25°C – 10',
42°C – 60', 70°C – 10'

1. Add to a 0.2ml tube for each sample:

1ug RNA	X ul
RNase free H2O	Up to 11 ul
Random hexamer primers 50uM	2 ul
Total	13 ul

2. Spin down briefly
3. Place sample in the PCR machine, close lid and start the initial denaturation
4. Prepare a master mix (always make a bit extra mix)

reagent	1 x
5 x buffer	4 ul
dNTPs 10 mM	2 ul
RNase inhibitor (40 U/ul)	0.5 ul
RevertAid H Minus RT (200 U/ul)	0.5 ul
Total	7 ul

5. After the initial denaturation step in the PCR machine, place samples on ice 6. Spin down briefly
7. Add to each tube 7 ul master mix that you had just prepares at step 4
8. Mix carefully and spin down briefly, keep cold!
9. Place sample back in the PCR machine, close the lid and start the second step.
10. Samples can be stored at -20°C

Primer concentrations and nucleotide codes

Below (Table S1) you can find the nucleotide codes of the primers used and the concentrations in which they are used for qPCR for each of the genes used in (preliminary) qPCR including the MCU code.

Table S1. Genes with their RAP ID and primer nucleotide codes and concentrations.

Gene name	RAP ID	Primer nucleotide code	Primer conc.
ACTIN 1 (ACT1)	Os03g0718100	FW: TCCTGACGGAGCGTGGTTAC REV: GAGGAGCTGGTCTTGGCAGT	10μM
UEV1A (UBC)	Os03g0712300	FW: TTGAGTAGCATCAGGCGCAA REV: TTGAGGCGACAGCACGGTTT	10μM
RICE UBIQUITIN 2 (UBQ10)	Os02g0161900	FW: TCCCGAGCCTCTGTTCGTCA REV: CTGCTGTCCCACAGGAAACTG	10μM
AUX/IAA PROTEIN 1 (IAA1)	Os01g0178500	FW: CCCATGACCGTGTGGTTGGA REV: CATATCACACGTGGGCGAACA	5μM
CYCLIN-B1-1 (CYCB1;1)	Os01g0805600	FW: GGCCAGGATTTGGCCTTTATGT REV: GGGATCGATTCAAGACACGAGA	2.5μM
AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OX-IDASE 2 (ACO2)	Os09g0451000	FW: GCGTGCATGGTCAGCTACTC REV: AGTACCACACGAGGACAGCC	5μM
FLOWERING PROMOT-ING FACTOR 1-LIKE 3 (FPFL3)	Os02g0460200	FW: CGTCAAGACCCGCAACGTCT REV: AATAGCAGGCAGGCAAGCTC	5μM
HOMEOBOX 1 (OSH1)	Os03g0727000	FW: AGCGGAGGAGAGACAGAGCTA REV: CCCACCAGTTGAGGAGCTGT	10μM
PHYTOCHROME B (PHYB)	Os03g0309200	FW: GAATCCTCTCAACGGTATCCGA REV: AAGCACCAAAGAGCCATCCTCA	5μM
PHYTOCHROME INTER-ACTING FACTOR_LIKE 16 (PIL16)	Os05g0139100	FW: TCAGAGCGGAGGAGAAGAGACC REV: TGGCTTCCTCCAGCATCGAC	5μM
PHOTOSYSTEM B1B2 LIGHT HARVESTING PROTEIN (PSB1B2)	Os01g0720500	FW: ACAACAACGCATGGGCCTAC REV: GAAAGCAATGGACCTCACCGC	10μM
PHOTOSYSTEM II SUBU-NIT R (PSBR)	Os07g0147500	FW: GGAAAGGGACAATGGCAAGGC REV: TGAACACCACTCCTCCAGCA	5μM
YUCCA-like gene 6 (YUC6)	Os07g0437000	FW: CATGGAACCACCAAACCGCC REV: GTTGAGTGCCATGTTGCGCC	5μM

24-hour exposure to FR

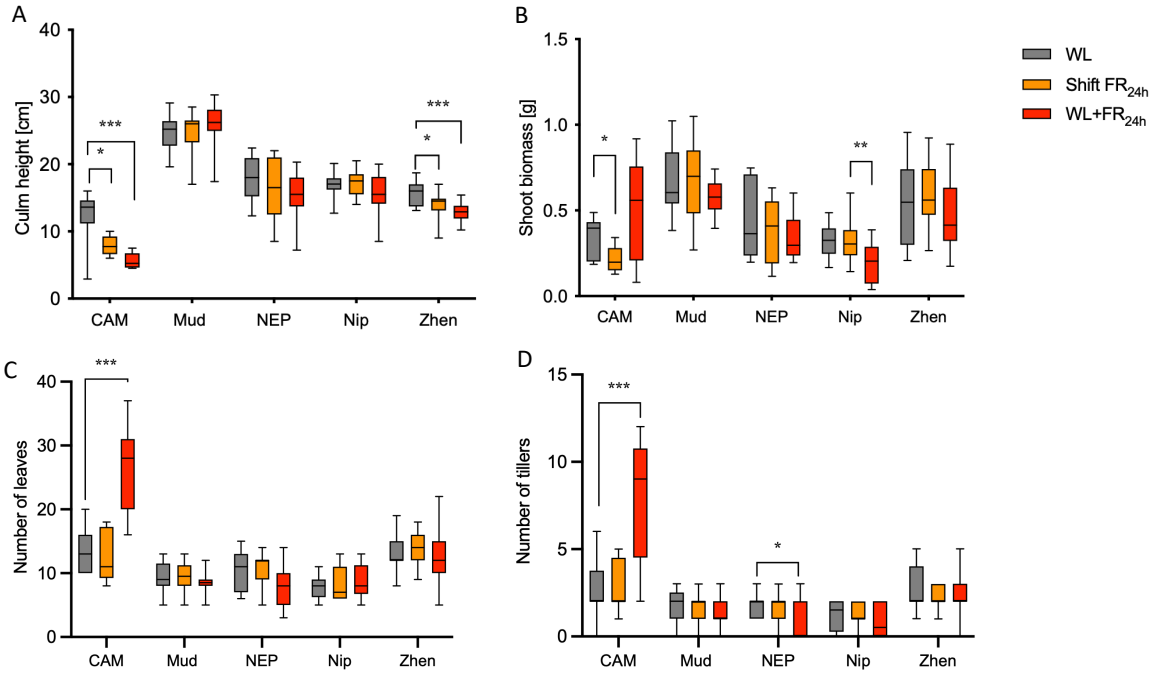


Figure S1. Growth response to 24h low R:FR light exposure of cultivated - Mudgo (Mud), Nipponbare (Nip), and Zhenshan (Zhen) - and wild rice varieties - SRANGE (CAM) and NABO (NEP) - at 28 days after sowing (das). **Comparison of A. culm height B. shoot biomass C. leaf number and D. tillering.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), supplemented with 24h of FR light ($WL+FR_{24h}$) or shifted upon formation of the first tiller in 50% of the group from control side to supplemented FR side (Shift FR_{24h}). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 *** (Student's t-test). If p≥0.05, findings are not considered significant.

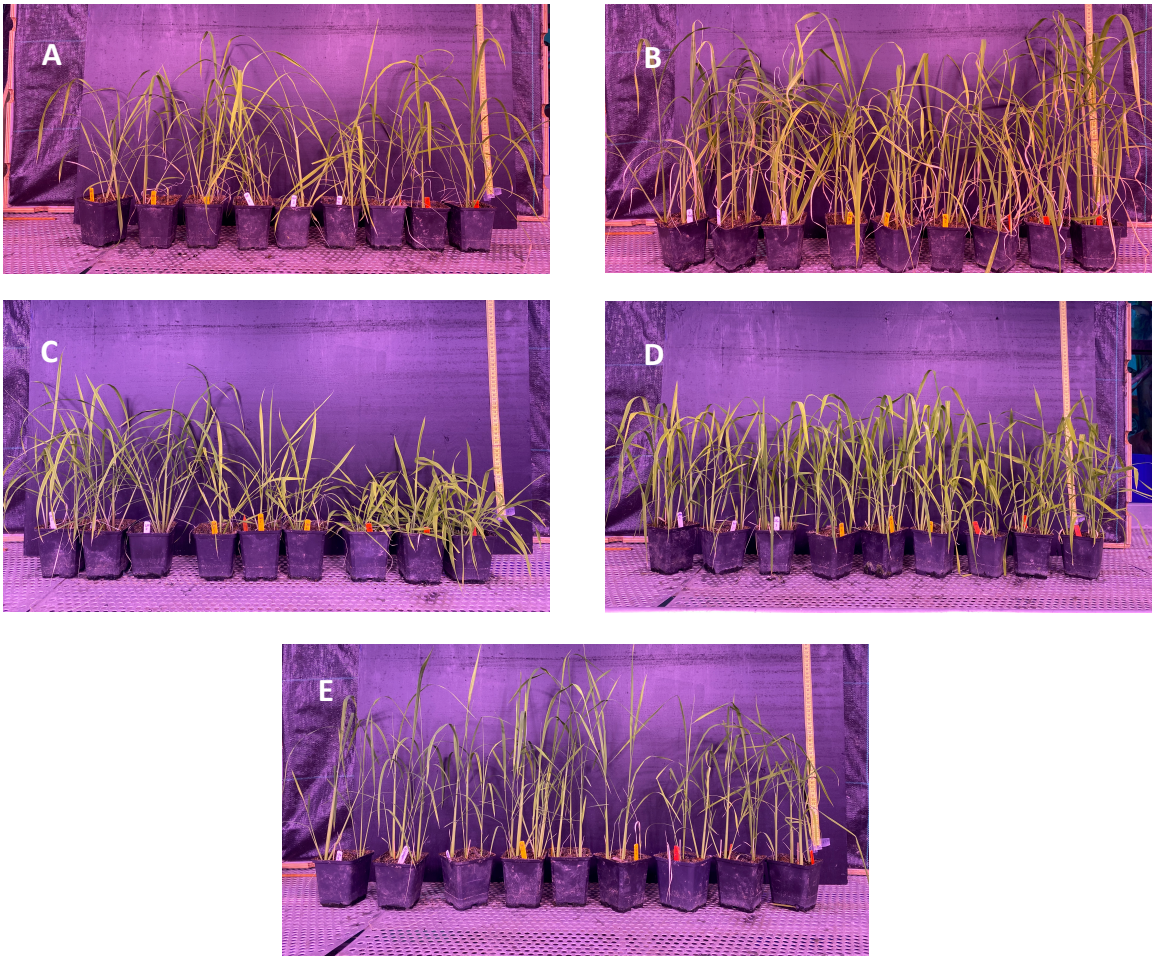


Figure S2. Growth response to 24h low R:FR exposure of (A) NEP, (B) Mudgo, (C) CAM, (D) Zhenshan and (E) Nipponbare at 28 days after sowing (das). Rice plants were grown as control (three left plants), supplemented with 24h of FR light (three right plants) or shifted upon formation of the first tiller in 50% of the group from control side to supplemented FR side (three middle plants).

Low R:FR 21das (Autumn)

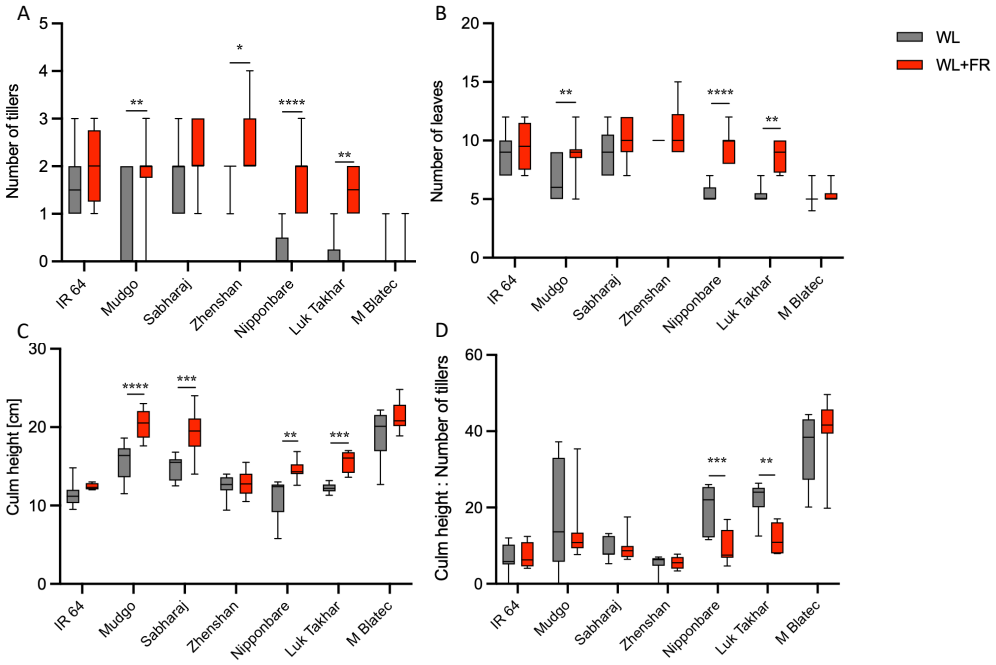


Figure S3. Growth response to low R:FR exposure in autumn of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 21 days after sowing (das). **Comparison of A. tillering, B. leaf number, C. culm height and D. apical dominance.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), supplemented with 12h of FR light (WL+FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

Low R:FR 21das (Winter)

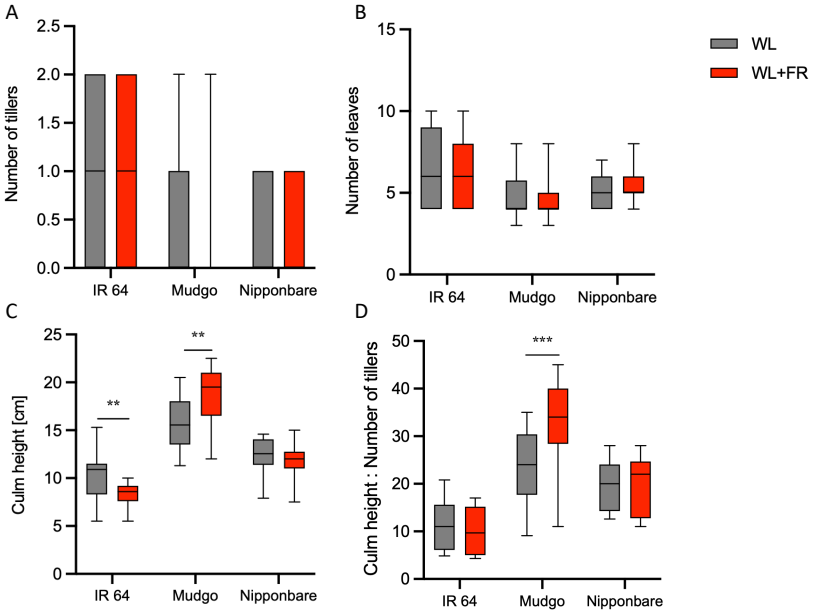


Figure S4. Growth response to low R:FR exposure in winter of cultivated rice varieties (IR 64, Mudgo and Nipponbare) at 21 days after sowing (das) (n=15-24). **Comparison of A. tillering, B. leaf number, C. culm height and D. apical dominance.** The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), supplemented with 12h of FR light (WL+FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

+EoD FR 21das (Winter)

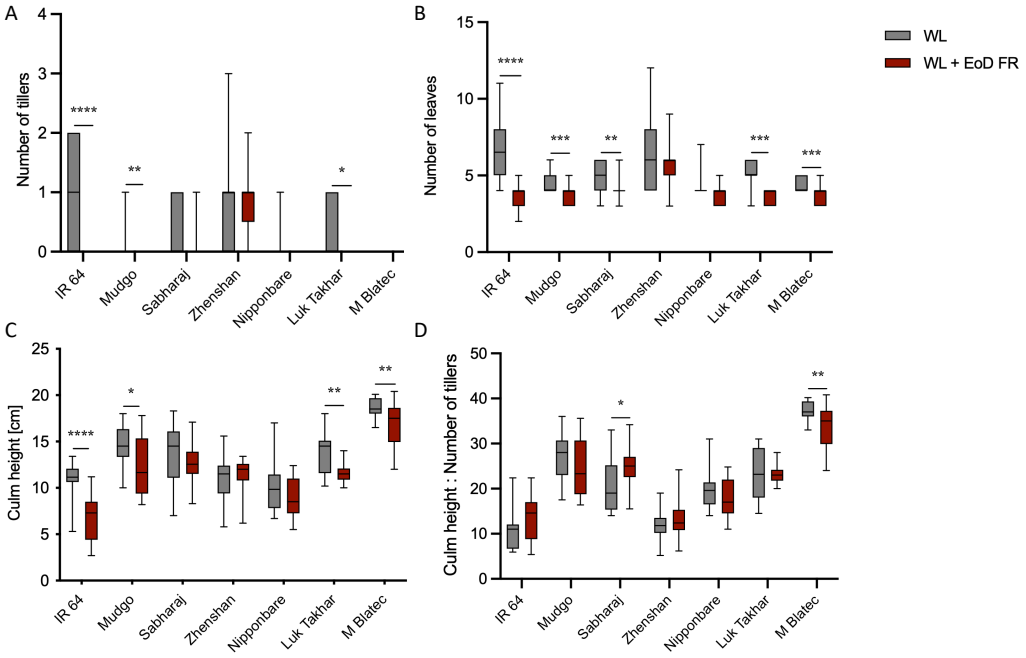


Figure S5. Growth response to +EoD FR exposure in winter of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 21 days after sowing (das) (n=14-26). **Comparison of A. tillering, B. leaf number, C. culm height and D. apical dominance**. The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), or WL grown and exposed to a 15-minute pulse of FR light (WL+EoD FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

+EoD FR 21das (Spring)

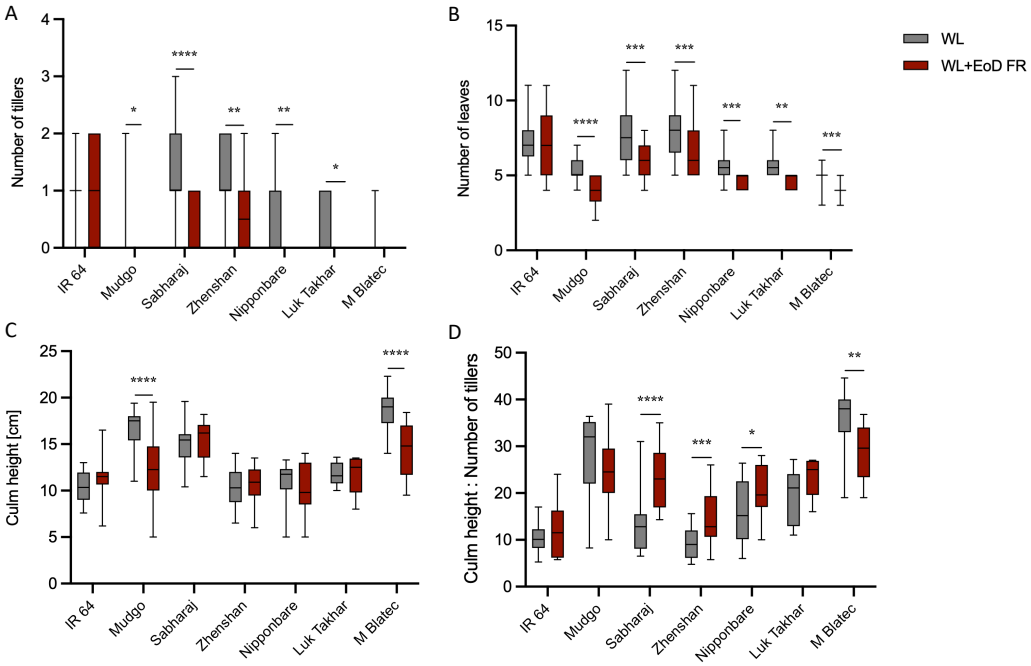
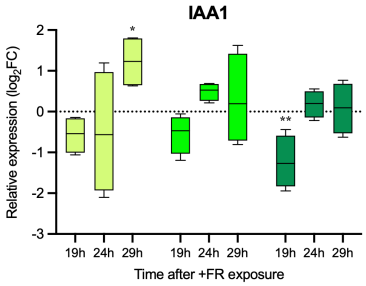


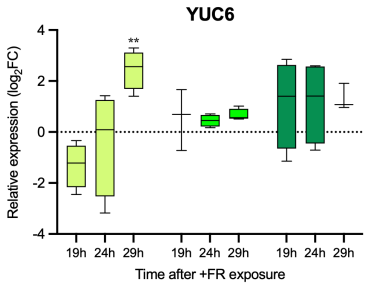
Figure S6. Growth response to +EoD FR exposure in spring of cultivated rice varieties (IR 64, Mudgo, Sabharaj, Zhenshan, Nipponbare, Luk Takhar, M Blatec) at 21 days after sowing (das). **Comparison of A. tillering, B. leaf number, C. culm height and D. apical dominance**. The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Rice plants were grown as control (WL), or WL grown and exposed to a 15-minute pulse of FR light (WL+EoD FR). Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (Student's t-test).

IR 64 qPCR split per tissue at different timepoints

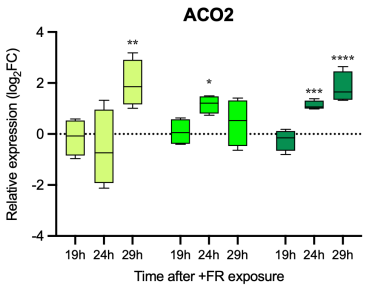
Tissue: ns; timepoint: **; interaction: ns



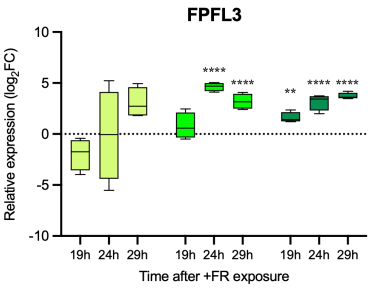
Tissue: ns; timepoint: *; interaction: *



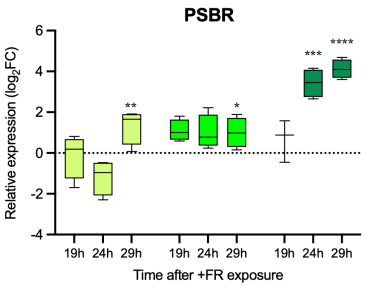
Tissue: ns; timepoint: ***; interaction: ***



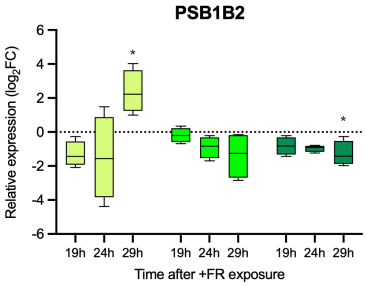
Tissue: **; timepoint: ***; interaction: ***



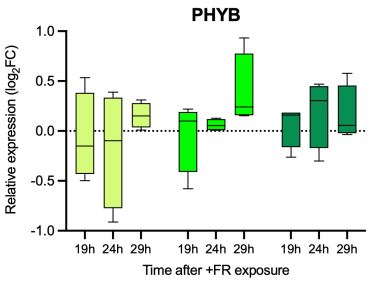
Tissue: ****; timepoint: ***; interaction: ***



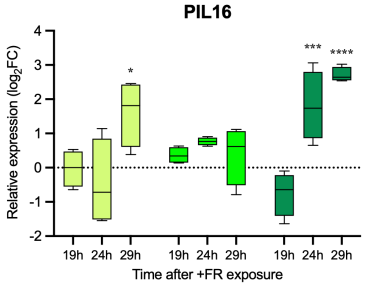
Tissue: ns; timepoint: ns; interaction: ***



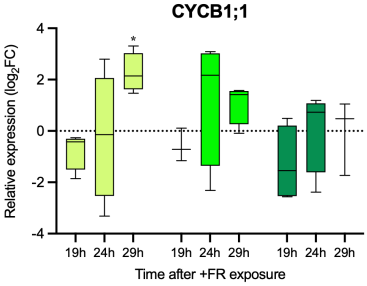
Tissue: ns; timepoint: ns; interaction: ns



Tissue: *; timepoint: ****; interaction: ***



Tissue; ns; timepoint: *; interaction: ns



Tissue: ns; timepoint: ns; interaction: ns

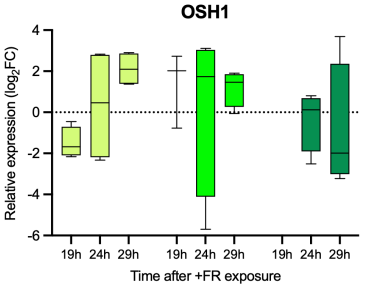


Figure S7. qPCR results visualized per tissue for rice variety IR 64 grown under low R:FR conditions compared to control. The figure shows boxplots with box (median and first and third quartile) with whiskers (2.5 and 97.5 percentiles). Data represents log_2(FC), FC being calculated according to PFAFFL formula. Significant differences between groups are indicated with p-value < 0.05 *, < 0.01 **, < 0.001 ***, < 0,0001 **** (one-way ANOVA + Tukey's post-hoc test). If p≥0.05, findings are not considered significant. Significantly different expression between tissues was found for PIL16 (*), PSBR (****), FPFL3 (**) (two-way ANOVA).

Light treatment conditions and experimental setup

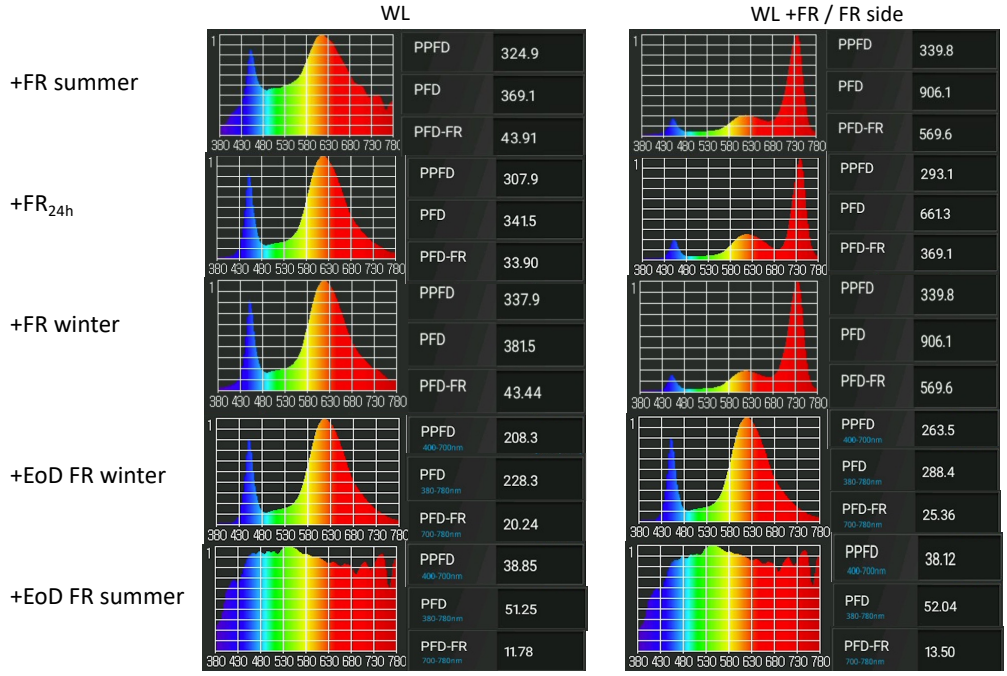


Figure S8. Light treatment conditions throughout experiments. Figure shows light treatment conditions during the photoperiod in WL and low R:FR or +EoD FR. Photosynthetic photon flux density (PPFD) (400-700 nm), photon flux density (PFD) (380-780 nm) and PFD-FR (700-780 nm) values for each treatment per experiment are shown.

Figure S9. Experimental setup for rice growth, without (above) and with screen between treatment groups (under). Table setup with screen as shown below is used for growth of +EoD FR treatment.