Red and blue light differentially impact retrograde signaling and photoprotection in rice

Liu Duan[1], M. Águila Ruiz-Sola[1], Ana Couso[1], Nil Veciana[1], Elena Monte[1,2,3]

[1]Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, Spain.

[2] Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain.

[3]Author for correspondence. Email: elena.monte@cragenomica.es

Author information:

Liu Duan

E- mail: liu.duan@cragenomica.es

ORCID: 0000-0003-4034-1152

M. Águila Ruiz-Sola

E-mail: aguila.ruiz@cragenomica.es

ORCID: 0000-0002-2281-6700

Ana Couso

E-mail: ana.couso@cragenomica.es

Nil Veciana

E-mail: nil.veciana@cragenomica.es

Corresponding author:

Elena Monte

E-mail: elena.monte@cragenomica.es

ORCID: 0000-0002-7340-9355

Abstract

Chloroplast-to-nucleus retrograde signaling (RS) is known to impact plant growth and development. In Arabidopsis, we and others have shown that RS affects seedling establishment by inhibiting deetiolation. In the presence of lincomycin, a chloroplast protein synthesis inhibitor that triggers RS, Arabidopsis light-grown seedlings display partial skotomorphogenesis with undeveloped plastids and closed cotyledons. In contrast, RS in monocotyledonous has been much less studied. Here, we show that emerging rice seedlings exposed to lincomycin do not accumulate chlorophyll but otherwise remain remarkably unaffected. However, by using high red (R) and blue (B) monochromatic lights in combination with lincomycin, we have uncovered a RS inhibition of length and a reduction in the B light-induced declination of the second leaf. Furthermore, we present data showing that seedlings grown in high B and R light display different non-photochemical quenching (NPQ) capacity. Our findings support the view that excess B and R light impact seedling photomorphogenesis differently to photoprotect and optimize the response to high light stress.

Keywords

Retrograde signaling, rice, photomorphogenesis, blue and red light, photoprotection, non-photochemical quenching (NPQ)

Introduction

Light is fundamental for plants as a source of energy and as an essential environmental cue. The quality, intensity, direction and duration of ambient light inform the plant about its immediate surrounding, time of day, and season. To capture this information, plants possess multiple photoreceptors sensitive to different wavelengths, including the red (R) and far red (FR) sensing phytochromes and the blue (B) /UV-A sensing cryptochromes. Rice, a model monocotyledonous plant, contains three phytochromes (phy) (phyA, phyB, and phyC) [1], and three cryptochromes (CRY) (OsCRY1a, OsCRY1b, and OsCRY2) [2, 3]. The contribution of each photoreceptor to light-regulated development has been elucidated through the study of deficient and overexpression mutants, which have shown similar but also distinct functions compared to their Arabidopisis counterparts [3-7].

Light or its absence is especially critical during germination and seedling establishment [8]. Early seedling growth and development is first fueled by the seed reserves, and this heterotrophic lifestyle can proceed in the dark for a few days. Seedlings in darkness exhibit skotomorphogenic development, characterized in rice by long coleoptiles, long first leaves, and elongation of the second internode. Upon light illumination, elongation of coleoptiles, first leaves, and internodes is inhibited, and seedlings switch to a photomorphogenesis pattern of growth with the development of fully functional chloroplasts and transition to autotrophy [5]. During this process, R, FR and B light contribute to the inhibition of elongation through the action of both phytochromes and cryptochromes. In contrast to Arabidopsis, rice phyA and phyC are both responsible for responses to FRc [4, 5], whereas phyB and phyC are involved in the responses to Rc, and CRY1a and CRY1b are the main CRYs involved in the deetiolation response to B [3]. Interestingly, B light promotes the declination of second leaf blades via CRY function, and phys (most prominently phyB) behave antagonistically [5]. Furthermore, supplemental B light has been shown to be essential for proper growth by enhancing photosynthesis and increasing the total nitrogen content of rice leaves [9].

In addition to providing environmental information, light can also be a source of stress for the plant. Whenever it is absorbed beyond photosynthetic capacity, excess energy becomes harmful causing oxidation of the photosynthetic apparatus and eventually cell death. In response, plants induce a photoprotective mode known as non-photochemical quenching (NPQ), which dissipates the excess energy as heat [10]. NPQ has been shown to be particularly important for plant fitness and productivity in field conditions [11, 12]. Mechanistically, NPQ is comprised of different components defined by their time-scales of induction and relaxation. The fastest component is qE, energy quenching in the antenna of photosystem II (PSII). qE relies on specific carotenoids from the xanthophyll group and on the qE protein effector PSBS [10]. PSBS is able to sense thylakoidal pH changes and activate the energy-dissipation mode by a mechanism that is not yet resolved [13, 14]. The slowest NPQ component is qI, photoinhibitory quenching that comprises all processes directly related to photoinhibition of PSII [15].

Assembly of fully functional chloroplasts during deetiolation requires exquisite coordination between the nucleus and the chloroplast. Most of the chloroplast components are encoded in the nuclear genome [16], and need to be imported into the chloroplast following their synthesis in the cytosol, a process referred to as anterograde regulation [17]. Chloroplast can in turn communicate with the nucleus in a process called retrograde signaling (RS), that adjusts nuclear gene expression to chloroplast status [18, 19]. This RS taking place during chloroplast biogenesis has been called “biogenic control”, and involves tight regulation of the expression of nuclear-encoded photosynthetic genes (*PhANGs*) such as those from the *LHCb* gene family [20].

Activation of RS takes place when chloroplast are damaged under stress conditions like high light, or with the use of chemicals like lincomycin that specifically inhibit plastid translation [21, 22]. Activation of RS causes repression of *PhANG* expression through a process that is mediated by the plastid-localized protein Genomes Uncoupled 1 (GUN1) and involves repression of *GOLDEN2-LIKE* (*GLK*) gene expression [20, 23-27]. In Arabidopsis, RS has been shown to optimize not only pigment accumulation and photosynthetic capacity but also morphogenic development to acclimate to high

light stress [21, 27, 28]. Accordingly, the *gun1* mutant is more sensitive to high light [21].

Our previous studies together with others [21, 27], have established in Arabidopsis that GUN1-mediated RS blocks photomorphogenic development during seedling deetiolation, particularly the inhibition of cotyledon separation. Indeed, light grown seedlings grown in the presence of lincomycin show a phenotype resembling dark-grown seedlings, with longer hypocotyls and appressed cotyledons with undeveloped plastids that do not green [27]. This response involves repression of *GLK1* expression and has been proposed to minimize the area exposed to potentially damaging light [27]. In contrast, RS in monocots has been much less studied [29-31], and whether it impacts photomorphogenesis has not been addressed. Here, we aim to start to characterize the RS response in rice to better understand how RS has evolved as a photoprotective mechanism for plants. We show that emerging rice seedlings activate RS in response to chloroplast stress, but their early development is remarkably unaffected by lincomycin and they do not exhibit characteristics of dark-grown seedlings, in clear contrast to Arabidopsis. Interestingly, seedlings grown under R or B monochromatic lights respond differently to lincomycin, and we uncover an inhibition of the length and the declination angle of second leaf blades in B light. Furthermore, we present data that indicate that seedlings grown in B and R light have distinct capacity to induce NPQ and display different photoprotection mechanisms to optimize growth in high light environments.

Methods

(a) Plant material and growth conditions

Rice (*O.sativa* cv. Nipponbare) was used in all experiments. After harvesting, rice seeds were incubated at 37ºC for two weeks to break dormancy. Before sowing, seeds were dehusked and sterilized for 1 min in 75% ethanol with shaking, followed by a 20-min treatment in 2.5% bleach and 5 washes with sterile water. Eight seeds were plated on 0.5 MS medium without sucrose in 14-cm high glass jars covered with 1 layer of transparent plastic film, except for the red light treatments where 7-cm high glass jars were used.

For constant light experiment, plants were grown at 25-28ºC for 5 days in darkness (D), or in continuous blue (B) (450 nm) (provided by Philips GreenPower LED research module blue), red (R) (660 nm) (provided by Philips GreenPower LED research deep red), or white light (W) at the specified light intensities. Light spectra were measured with a Flame-S spectrometer (Ocean Optics), and fluence rates were measured with a LI-190R quantum sensor (LI-COR).

In the deetiolation experiments, plants were first grown in continuous dark at 25-28ºC for 3 days, and then transferred to light for another 4 days. Lincomycin treatments were done by supplementing the media with 2 mM lincomycin (Sigma-Aldrich L2774). All the experiments were performed with at least two biological replicates as specified in each figure legend.

For seedling morphology measurements (See figure S1), seedlings were photographed using a digital camera Nikon D8000 and measurements were done using the NIH Image software (Image J, National Institutes of Health) [32].

(b) RNA extraction and quantitative RT-PCR

Shoot tissue from 5 day-old rice seedlings was harvested and immediately macerated in liquid N_2 in the dark prior to storage at -80 ℃. Frozen samples were mechanically ground with TissueLyser II (QIAGEN) into frozen powder, and extracted using Maxwell® RSC Plant RNA kit (Promega, AS1500) according to manufacturer instructions. cDNA synthesis was done using NZY First-Strand cDNA Synthesis Kit (NZYtech, MB12502), and cDNA samples were used for real-time PCR (Light Cycler 480; Roche) using SYBR Green I master (Roche Life Science) following previously described procedures [27]. For gene expression analyses, three independent biological replicates were assayed, each with two technical repeats. Primers are described in Table S1.

(c) Pigment extraction and quantification

For fresh weight chlorophyll (Chl) extraction, rice shoot samples were weighed, frozen, and ground in liquid nitrogen. Samples were extracted in Extraction buffer (45% ethanol, 45% acetone and 10% water) and then shaken for 3 h in the dark at 4 ºC.

Samples were centrifuged at 12,000g for 5 min, and the supernatant absorbance was measured at 645 nm and 663 nm in a Spectramax M3 plate reader (Molecular Devices). Chlorophyll content was calculated according to Arnon [33], following the equations Chl A = 12.72 *A663 - 2.59 * A645; Chl B = 22.88 * A645 - 4.67 * A663.

For dry weight Chl and carotenoid extraction, samples were ground and freeze-dried overnight in the dark using a Freeze-dryer ALPHA 2-4 LD machine (at -47 ℃ and 0.055 mbar). Each sample was weighed and extracted in pre-cooled 80% acetone for 1 h at 4 ℃ in the dark. Absorbance was measured at 663, 647 and 470 nm using a UV-Vis spectrophotometer (UV-2600, SHIMADZU). Chl and carotenoid content were calculated according to Lichtenthaler [34]: Chl A = 12.25 * A663-2.79 * A647; Chl B = 21.50 * A647-5.10 * A663; Total Chl = 7.15 * A663 + 18.71 * A647 or Chl A + Chl B; Carotenoids = (1000 * A470 - 1.82 * Chla - 85.02 * Chlb) / 198.

(d) Data analyses and statistics

R, Rstudio program, and GraphPad Prism version 7 for Mac (GraphPad Software, La Jolla California USA) were used to perform the data analyses and visualization. Seedlings whose total length was less than 0.4 times the mean length of their biological replicate were eliminated as poorly germinating seeds. Morphological data were analyzed by one-way ANOVA analysis, and the mean difference of each treatment in the multiple comparations was calculated using Tukey test. For two-group comparisons, such as low and high light treatment, or with and without lincomycin treatment, student's *t*-test was used. For gene expression and chlorophyll content comparations, data are the means ± s.e.m of biological triplicates (n = 3). Statistically significant differences were defined and labeled with *, **, and ***, corresponding to P values < 0.05, < 0.01, and < 0.001, respectively.

(e) Fluorescence measurements

In vivo chlorophyll fluorescence was measured at room temperature using a pulse modulated amplitude fluorimeter (MAXI-IMAGING-PAM, Heinz Waltz GmbH, Germany). Photosynthetic parameters including Fv/Fm, and non-photochemical quenching (NPQ) were assessed as described elsewhere [35]. Briefly, seedlings were

first dark adapted for 30 min. Next, a blue measuring light (450 nm, 0.5 µmol photons m^{-2} s^{-1}) was turned on and a very short saturating pulse (SP) (800 ms, 2700 µmol photons m^{-2} s^{-1}, 450 nm) was applied. Fluorescence signal before (Fo) and after (Fm or maximum F) the SP was recorded to estimate the maximum quantum yield of photosystem II (PSII), (Fv/Fm) defined as $Fv/Fm = (Fm - Fo)_{dark}/Fm_{dark}$ [36]. NPQ of chlorophyll fluorescence was then measured by exposing dark-adapted seedlings to 800 µmol photons m^{-2} s^{-1} during 10 min, followed by a 70 min dark recovery period. NPQ was calculated as NPQ = (Fm − F′m) / F′m [37], where Fm corresponds to maximum fluorescence from dark-adapted plants during a SP, and F'm to maximum fluorescence after SP applied every minute in the light as well as in the dark recovery period.

Results

(a) Lincomycin-induced retrograde signaling blocks pigment accumulation in rice and prevents growth and full separation of the second leaf

Rice seedlings were grown under continuous darkness (D), low (40 µmol m^{-2} s^{-1}) red light (R), low (10 µmol/m^2/s) blue light (B), and low (40 µmol m^{-2} s^{-1}) white light (W) for 5 days (figure 1a, b). Light intensities were selected based on similar coleoptile lengths. Different photomorphogenic features (coleoptile length, coleoptile angle, first and second leaf length, and leaf declination) were quantified as described in figure S1. Dark-grown seedlings exhibited vertical (angle ~6^o) elongated coleoptiles (2.1 cm) around the first and second leaves, which were 3.4 and 4.3 cm respectively (figure 1a, b). Light dramatically inhibited coleoptile elongation (to ~0.6 cm) and the growth of the first leaf (to 1.6 cm) to a similar extent in all three different light conditions (figure 1a, b). In contrast, elongation of the second leaf was inhibited in R and W compared to D (to ~2.7 cm), but not in B. Light also promoted opening of the coleoptile in all light conditions tested, and B and W were more efficient than R (to 72^o , 69^o and 55^o respectively) (figure 1a, b). Additionally, we observed that light-induced chlorophyll accumulation was ~2.6-fold higher in B compared to R (figure 1c). These data are in accordance to previous reports [3, 4, 38] and illustrate how light affects early rice seedling photomorphogenesis.

To investigate how retrograde signaling might affect this process, we first defined the optimal lincomycin concentration to use. Rice seedlings grown in W were treated from germination onwards with 0.25 mM, 0.5 mM, 1 mM and 2mM lincomycin in 0.5 MS media without sucrose. Seedlings showed a progressive decrease in pigmentation and were completely white in 2 mM lincomycin, which was chosen for further experiments (figure S2). At this concentration, we confirmed the block of chlorophyll accumulation in B and R (figure 1c) which correlated with gene expression repression of *OsLHCB* and *OsGLK1* (figure 1d), hallmarks of retrograde signaling (RS) [27], confirming that lincomycin induces RS in rice in both R and B light conditions.

We next compared the morphology of lincomycin treated with non-treated control seedlings grown under continuous D, R, B, and W light conditions (figure 1a, b). Besides the abovementioned blockage of pigment accumulation, lincomycin increased the coleoptile angle in light-grown seedlings, which was significant in B and W with an increase of ~20º. The treatment also had an effect on the growth of the first and second leaves in R and B, but not in W. Interestingly, lincomycin increased leaf length in R and the effect was opposite in B. Finally, RS did not affect coleoptile length in the dark or in any of the light treatments (figure 1a, b), which might be expected as the coleoptile does not develop functional chloroplasts.

To complete this characterization, we also analyzed the effect of light and lincomycin in 3-day dark-grown seedlings transferred to R and B for 4 days (figure 2). Under these conditions, B-light inhibited the elongation of the first and second leaves more efficiently than R, and lincomycin further repressed this growth (as observed above in continuous B conditions), whereas it did not have any significant effect in D or R light seedlings. We also observed separation of the second leaf specifically in B light, a response that has been described to increase with higher B intensities [5]. Indeed, we observed an increase in the second leaf angle declination when seedlings were transferred to high B (150 µmol m^{-2} s^{-1}) compared to low B (40 µmol m^{-2} s^{-1}). Interestingly, this increase was significantly affected in the presence of lincomycin (figure 2c, S3).

Together, these results indicate that lincomycin-induced chloroplast damage impacts two main processes during rice seedling deetiolation: (1) it blocks pigment accumulation, and (2) although it has modest morphologic effect in the overall growth of the emerging seedling, it prevents growth and full separation of the second leaf. These results contrast with Arabidopsis, where lincomycin-treated seedlings resemble dark-grown seedlings [27]. Interestingly, however, the observed inhibition of the second leaf growth and declination in rice, albeit of less importance, is reminiscent of the inhibition of cotyledon separation, the main developmental effect of RS in Arabidopsis seedlings [27, 39].

(b) High light treatment uncovers distinct effects of high red and high blue light in the photomorphogenesis of rice seedlings

The above results investigate the effects of monochromatic light on retrograde signaling in lincomycin-treated seedlings. However, because we wished to understand how each light quality by itself impacts development through retrograde signaling, we decided to use high intensity B and R light in untreated seedlings. To this end, we started by examining the effects on photomorphogenesis of rice seedlings subjected to continuous low (40 µmol m^{-2} s^{-1}) and high (450 µmol m^{-2} s^{-1}) R light, and low (10 µmol m^{-2} s^{-1}) and high (150 µmol m^{-2} s^{-1}) B light. High R and B intensities resulted in similar coleoptile length (figure 3a, b). High R blocked chlorophyll accumulation as described before [40], whereas, remarkably, high B did not (figure 3a, c). Even seedlings grown in higher B intensities (300 and 450 µmol m^{-2} s^{-1}) were green (figure S4). This result suggested that high B and R might have different capacities to induce RS. Indeed, high R repressed the expression level of *LHCB* and *GLK1*, whereas high B did not significantly impact their expression compared to low B (figure 3d), indicating that high R light but not high B can induce chloroplast damage triggering RS under these conditions. Interestingly, high R did not produce any apparent morphologic change other than lack of pigmentation, whereas high B induced clear inhibition of growth (figure 3a, b), an effect similar (albeit of weaker magnitude) to that induced by lincomycin (figures 1 and 2). This inhibition of growth was stronger in higher B light intensities (figure S4). These

results suggest that high B might induce a RS that selectively affects growth but not pigmentation. Alternatively, the inhibition of growth under these conditions might be independent of RS (e.g. only photoreceptor-mediated).

Again, to complete this characterization, we examined the phenotype of dark-grown seedlings transferred to high light (figure 4). In seedlings grown in the dark for 3 days, transfer to high R for 4 days prevented chlorophyll accumulation, while transfer to high B did not (figure 4a, b), similar to what we observed in continuous high light (figure 3, S4). No other phenotype was observed for high R-grown seedlings (figure 4a). In high B, we again observed inhibition of growth resulting in shorter leaf lengths (figure 4b, c, S3). As discussed above (figure 2c), second leaf declination was promoted by high B (figure 4b, c, S3).

Together, these results suggest that during rice seedling deetiolation, high R induces chloroplast damage triggering RS that results in a blockage of pigment accumulation while has little effect on morphology, whereas high B does not induce repression in the expression of the RS markers *LHCB* and *GLK1* under these conditions, whereas it clearly inhibits elongation growth. To our knowledge, this direct comparison between the effects of high R and high B in the accumulation of pigments and morphology is novel in rice or any other model plant.

(c) Blue light triggers higher accumulation of chlorophylls and carotenoids in rice seedlings compared to red light

Our results above prompted us to investigate further the differences in pigment accumulation between seedlings grown in R and B. Interestingly, chlorophyll levels were higher in B compared to R, both in low and high fluences (figures 1c, 3c), with similar Chlorophyll A/B ratio (figure 5a). Carotenoid quantification (figure 5b) revealed that carotenoid content in low B was also ~1.7-fold higher than that of low R, and did not change in high B, whereas high R induced blockage of carotenoid accumulation similar to what we observed for chlorophylls (figure 3c). In accordance to high pigment levels in B-light grown seedlings compared to R, expression of genes encoding the enzymes phytoene synthase 1 (PSY1), that catalyzes the carotenoid

biosynthesis rate limiting step, and Mg^2-chelatase/Genomes uncoupled 5 (CHLH/GUN5) involved in chlorophyll synthesis [38, 41], showed higher expression levels in B compared to R (figure 5c). These results are in general agreement with described light-specific transcriptional signatures of rice metabolism [42]. It is interesting to note that Nipponbare, the rice cultivar used in this work, lacks OsC1 function, a R2R3-MYB gene necessary for *OsCHS1* expression and anthocyanin production [43]. Thus, our seedlings do not accumulate anthocyanin even in B light, conditions where flavonoid production in cotyledons and leaves is enhanced in Arabidopsis or commonly seen in wild rice varieties [44].

(d) Photosynthesis efficiency and photoprotection in high red and high blue light

Given the differences in pigmentation between R- and B-light grown seedlings (figures 3-5), we hypothesized that each light quality might have a different impact on the photosynthetic efficiency and photoprotective capacities of the seedling. We first measured the maximum quantum yield of PSII (Fv/Fm), as an indication of PSII functionality. Fv/Fm levels of high B-light grown seedlings were unaffected compared to low B treatments (figure 6a), even in higher B light intensities (average Fv/Fm of 0.6-0.8) (figure S5). Interestingly, seedlings grown in low R displayed Fv/Fm levels similar to low and high B (figure 6a), even though the total chlorophyll content and carotenoid was significantly reduced in seedlings grown in low R compared to B (figures 1c, 3c, 5b). Finally, as expected due to the lack of pigments, seedlings grown in high R displayed greatly reduced Fv/Fm levels, of average 0.3, indicative of photoinhibition (figure 6a).

Because we detected a decrease of Fv/Fm specifically in high R but not in high B, suggestive of a differential capacity in activating photoprotective mechanisms, we next tested if light quality had an effect on the induction of non-photochemical quenching (NPQ), one of the main photoprotective strategies against high light (HL) in plants. Low B and R light-grown seedlings were dark adapted for 30 min and then exposed to 800 μmol m^{-2} s^{-1} of actinic light for 10 min to observe the induction and relaxation of NPQ. Strikingly, NPQ dynamics after exposure to HL were remarkably different in low

B light-grown seedlings compared to low R light (figure 6b, c). First, B light-grown seedlings showed significantly higher maximum NPQ levels in the light compared to R, and they were also faster in their response. Second, dark relaxation of fluorescence quenching was incomplete in R light-grown seedlings compared to B, indicating again photoinhibition. These NPQ values in B light-grown seedlings were not significantly changed in seedlings grown in higher B light intensities (figure S5). A more complete graph showing a longer dark relaxation period and levels of qE (energy quenching) and qI (photoinhibitory quenching) components is presented in figure S6. These NPQ differences correlated with the observed differences in carotenoid content (figure 5b). Moreover, transcript levels of the qE effector PSBS appeared also to be light regulated. In rice, PSBS protein is encoded by two genes, *PSBS1* and *PSBS2* [45]. In agreement with a role in NPQ [45-47], expression of both genes was elevated in B compared to R light grown seedlings (figure 6d).

Discussion

Our findings support a scenario whereby RS in rice plays distinct roles under red and blue light conditions to fine tune photomorphogenesis and photoprotection under potentially photodamaging conditions. Using monochromatic lights of different intensities and in combination with lincomycin treatment, we have uncovered a possible role for RS in rice to inhibit leaf growth and declination. Furthermore, our comparative data in R and B lights suggest that, whereas high R induces RS and can cause photobleaching, B light induces higher carotenoid and chlorophyll content, higher *PSBS* expression, and might equip plants with the capacity to withstand high light as exemplified by increased NPQ levels compared to R.

We show that, remarkably, disruption of chloroplast function in rice seedlings by treatment with lincomycin from germination onwards only affected the rice photomorphogenic development modestly (figures 1a, b, 2). This is in clear contrast to Arabidopsis, where lincomycin blocks deetiolation and seedlings resemble dark-grown seedlings with lack of pigmentation and appressed cotyledons [27]. However, our observation that in B-light grown seedlings lincomycin inhibited the length and the

small increase in declination angle of the second leaf once it unfolded (figure 2c, d) indicates that RS can reduce the area exposed to light, which might protect the seedling from damage in a high light environment. Together with previous results in Arabidopsis showing RS inhibition of cotyledon separation [27], we suggest that a common role of biogenic retrograde signals in dicots and monocots might be to minimize the area of the seedling exposed to potentially damaging light, by inhibiting cotyledon separation or seedling growth and second leaf length and declination.

Our data showing that high B and R lights have different effect on seedling pigmentation and development suggests that both lights induce distinct processes and might have different capacity to induce chloroplast damage in rice. This effect is probably at least partially masked when using monochromatic light in the presence of lincomycin, as evident in experiments under continuous B light with or without lincomycin: B light by itself, even of intensities high enough to inhibit growth almost completed, did not seem to affect greening, whereas addition of lincomycin in low B completely blocked seedling greening (figures 1a, 2a, c, 3a, c, 4b, S3). Therefore, the use of monochromatic high light has allowed us to dissect the ability of each wavelength to cause chloroplast damage and induce RS in rice. Interestingly, high R light inhibited pigment accumulation compared to low R, but seedlings otherwise looked unaffected (figures 3a, b, 4a). This suggests that RS induced by high R light specifically affects pigment accumulation and might not participate in tuning morphogenesis to the prevailing high light. In contrast, high B light-grown seedlings were able to accumulate pigments but were overall shorter (figures 3a, b, 4b, c). This elongation inhibition is similar to the effect that we saw with lincomycin (figures 1a, b, 2). However, because our results suggest that RS was probably not induced under this high B condition (figure 3d), and growth inhibition also took place in the presence of lincomycin (figures 1a, b, 2), our interpretation is that this effect might be photoreceptor-mediated and can take place whether RS is activated or not. Intriguingly, studies in Arabidopsis have suggested that plastid signals that affect photomorphogenesis are dependent on cryptochrome 1 [39].

Our data show a clear difference in the NPQ response when plants were grown in

continuous B or R light. B light-grown seedlings were able to induce higher NPQ levels, which correlated with higher transcript levels of the qE effector protein PSBS (figure 6b-d). Indeed, higher PSBS levels have been shown to increase qE capacity in many photosynthetic organisms, including rice [46]. At the same time, B light-grown seedlings also showed increased levels of total carotenoids compared to R-grown plants (figure 5b). Whether the increased NPQ in B light compared to R is due to enhanced PSBS expression and/or enhanced carotenoid content in B remains to be further studied. Interestingly, in the green microalgae *Chlamydomonas reinhardtii*, B light (and also UV-B independently) control NPQ induction through photoreceptor-mediated regulation of the expression of the main qE effectors [48, 49], which established for the first time a molecular link between photoreception and photoprotection. In this context, it is tempting to speculate that we have also uncovered a novel control of photoprotection by B light in higher plants that deserves further investigation. On the other hand, low R light-grown seedlings showed reduced NPQ levels, lower *PBSB* gene expression and lower carotenoid content compared to low B light-grown plants (figures 6b-d, 5b). When grown in high R light, they showed a complete albino phenotype (figure 3a), possibly as a consequence of decreased photoprotective capacities. Interestingly, low B light treatment allowed chlorophyll and carotenoid content restoration in high R-grown rice seedlings [40], suggesting a requirement for B light signaling in seedling photoprotection. Altogether, our results showing increased photoprotective capacities in B compared to R may explain why, under the conditions used, B light-grown rice seedlings may withstand HL without chloroplast damage and RS induction.

In Arabidopsis, we have previously shown that the RS regulating photomorphogenesis is mediated by Genomes Uncoupled 1 (GUN1), a plastid-localized member of the pentatricopeptide repeat family [25], and by Golden2-like 1 (GLK1), a transcription factor involved in the promotion of chloroplast development [27]. In rice, ectopic overexpression of *OsGLK1* was shown to induce chloroplast biogenesis in non-green cells, but the work was mostly done in calli and did not address possible morphogenic implications [50]. As for GUN1, no rice mutants are available to date. Future work will

address the molecular players of high light-induced RS in rice and how they mediate a different response to R and B light. Upcoming studies will also aim to decipher how B and R light-induced signals are mediated and interact to provide photoprotection, a relevant question in natural environments.

Authors' contributions

EM, LD and AR-S designed the work and analyzed data. LD, AR-S, AC and NV acquired the data. EM, LD and AR-S wrote the manuscript. All authors revised and approved the manuscript's content.

Competing interests

Authors declare no competing interests.

Acknowledgements

We thank Giovanni Finazzi (University of Grenoble Alps) for his comments on the manuscript. This work was supported by grants from FEDER / Ministerio de Ciencia, Innovación y Universidades – Agencia Estatal de Investigación (Project References BIO2015-68460-P and PGC2018-099987-B-I00) and from the CERCA Programme / Generalitat de Catalunya (Project Reference 2017SGR-718) to E.M. We acknowledge financial support from the Spanish Ministry of Economy and Competitiveness, through the "Severo Ochoa Programme for Centres of Excellence in R&D" 2016-2019 (SEV-2015- 0533)".

Figure Legends

Figure 1. Effect of lincomycin treatment on light-regulated development of rice seedlings under continuous light. A. Representative 5-day old rice seedlings grown under continuous dark (D), red light (R) (40 µmol m^{-2} s^{-1}), blue light (B) (10 µmol m^{-2} s^{-1}), and white light (WL) (40 µmol m^{-2} s^{-1}) at 25 ºC with or without lincomycin (linco, 2 mM) treatment. **B.** Quantification of coleoptile angle, and coleoptile, first and second leaf length in 5-day old rice seedlings (n= 16) under the conditions indicated in **A**. **C.** Chlorophyll content in R and B-light grown with or without lincomycin **D.** *GLK1* and *LHCB* and expression in seedlings grown as detailed in **A**. Gene expression and chlorophyll data correspond to the mean ± s.e.m. of independent biological triplicates.

Multiple groups in B were analyzed by one-way ANOVA analysis. Different letters denote statistically significant differences among means of each group by Tukey test. Student's t-test was used in **B**, **C**, and **D** for the analysis between lincomycin treated and non-treated groups in each light condition. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure 2. Effect of lincomycin treatment on light-regulated development of dark-grown rice seedlings transferred to light. A. Representative 7 day-old rice seedlings grown under continuous dark (D), or under D for 3 days and then transferred to red light (40 µmol m^{-2} s^{-1}) (DR) or blue light (10 µmol m^{-2} s^{-1}) (DB) for 4 days at 25 ºC with or without lincomycin (linco, 2 mM). **B.** Quantification of coleoptile angle, and coleoptile, first and second leaf length in rice seedlings (n= 16) grown under the conditions indicated in **A**. **C.** Representative seven day-old rice seedlings grown under dark for 3 days and then transferred to low blue light (10 µmol m^{-2} s^{-1}) (DB10) or high blue light (150 µmol m^{-2} s^{-1}) (DB150) for 4 days at 28 ºC with or without lincomycin. **D.** Quantification of second leaf angle and third leaf length in rice seedlings (n= 16) grown under the conditions indicated in **C**. Multiple groups in **B** were analyzed by one-way ANOVA analysis. Different letters denote statistically significant differences among means of each group by Tukey test. Student's t-test was used in **B** and **D** for mean comparison between two groups. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure 3. Effect of continuous high-intensity red and blue light on seedling development. A. Representative 5 day-old rice seedlings grown under low (40 µmol m^{-2} s^{-1}) (R40) or high (450 µmol m^{-2} s^{-1}) (R450) red (R) light, and low (10 µmol m^{-2} s^{-1}) (B10) or high (150 µmol m^{-2} s^{-1}) (B150) blue (B) light at 25 ºC. **B.** Quantification of coleoptile angle, and coleoptile, first and second leaf length in rice seedlings (n= 16) grown under the conditions indicated in **A**. **C.** Chlorophyll content in seedlings grown under the conditions indicated in **A**. **D.** *GLK1* and *LHCB* and expression in seedlings grown as detailed in **A**. Gene expression and chlorophyll data correspond to the mean ± s.e.m. of independent biological triplicates. Student's t-test was used in **B** and **D** for

mean comparison between two groups. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure 4. Effect of high-intensity red and blue light on the development of dark-grown rice seedlings transferred to high light. A. Representative 7 day-old rice seedlings grown under dark (D) for 3 days and then transferred to low (40 µmol m^{-2} s^{-1}) (DR40) or high (450 µmol m^{-2} s^{-1}) (DR450) red (R) light at 25 ºC. **B.** Representative 7 day-old rice seedlings grown under dark (D) for 3 days and then transferred to low (10 µmol m^{-2} s-1) (DB10) or high (150 µmol m^{-2} s^{-1}) (DB150) blue (B) light at 28 ºC. **C.** Quantification of coleoptile and second leaf angle, and coleoptile, first, second and third leaf length in rice seedlings (n= 16) grown under the conditions indicated in **B**. Student's t-test was used for mean comparison between two groups. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure 5. Red and blue light differently affect pigment accumulation. Rice seedlings were grown for 5 days under low (40 µmol m^{-2} s^{-1}) (R40) or high (450 µmol m^{-2} s^{-1}) (R450) red (R) light, and low (10 µmol m^{-2} s^{-1}) (B10) or high (150 µmol m^{-2} s^{-1}) (B150) blue (B) light at 25 ºC. **A.** Quantification of ChA/ChlB ratio (total content is shown in figure 3c) **B.** Quantification of carotenoid content. **C.** Relative gene expression of *PSY1* and *CHLH/GUN5*. Data are the means ± s.e.m of independent biological triplicates. Means were compared by student's t-test, and asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure 6. Photosynthesis efficiency and photoprotection capacities of rice seedlings grown red and blue light. Rice seedlings were grown for 6 days under low (40 µmol m^{-2} s^{-1}) (R40) or high (450 µmol m^{-2} s^{-1}) (R450) red (R) light, and low (10 µmol m^{-2} s^{-1}) (B10) or high (150 µmol m^{-2} s^{-1}) (B150) blue (B) light at 28 ºC. **A.** Maximum quantum yield of PSII (Fv/Fm). Values correspond to four independent seedlings. **B.** Representative colored images of NPQ in the second leaf data shown in **C**. The color scale is shown on the right. Left panel: NPQ induction after 5 min in high light. Right panel: NPQ relaxation after 20 min in dark. **C.** NPQ induction (light) and relaxation (dark). Values are the mean ± SD of six independent seedlings grown at the specified light conditions. **D.** Relative gene expression of *PSBS1* and *PSBS2*. Data are the means

± s.e.m of independent biological triplicates. Means were compared by student's t-test, and asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

References

[1] Mathews, S. & Sharrock, R. A. 1996 The phytochrome gene family in grasses (Poaceae): a phylogeny and evidence that grasses have a subset of the loci found in dicot angiosperms. *Molecular biology and evolution* **13**, 1141-1150. (DOI:10.1093/oxfordjournals.molbev.a025677).

[2] Matsumoto, N., Hirano, T., Iwasaki, T. & Yamamoto, N. 2003 Functional analysis and intracellular localization of rice cryptochromes. *Plant physiology* **133**, 1494-1503. (DOI:10.1104/pp.103.025759).

[3] Zhang, Y. C., Gong, S. F., Li, Q. H., Sang, Y. & Yang, H. Q. 2006 Functional and signaling mechanism analysis of rice CRYPTOCHROME 1. *The Plant journal : for cell and molecular biology* **46**, 971-983. (DOI:10.1111/j.1365-313X.2006.02753.x).

[4] Takano, M., Kanegae, H., Shinomura, T., Miyao, A., Hirochika, H. & Furuya, M. 2001 Isolation and characterization of rice phytochrome A mutants. *The Plant cell* **13**, 521-534. (DOI:10.1105/tpc.13.3.521).

[5] Takano, M., Inagaki, N., Xie, X., Yuzurihara, N., Hihara, F., Ishizuka, T., Yano, M., Nishimura, M., Miyao, A., Hirochika, H., et al. 2005 Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. *The Plant cell* **17**, 3311-3325. (DOI:10.1105/tpc.105.035899).

[6] Hirose, F., Shinomura, T., Tanabata, T., Shimada, H. & Takano, M. 2006 Involvement of rice cryptochromes in de-etiolation responses and flowering. *Plant & cell physiology* **47**, 915-925. (DOI:10.1093/pcp/pcj064).

[7] 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 of the United States of America* **106**, 14705-14710. (DOI:10.1073/pnas.0907378106).

[8] Gommers, C. M. M. & Monte, E. 2018 Seedling Establishment: A Dimmer Switch-Regulated Process between Dark and Light Signaling. *Plant physiology* **176**, 1061-1074. (DOI:10.1104/pp.17.01460).

[9] Matsuda, R., Ohashi-Kaneko, K., Fujiwara, K., Goto, E. & Kurata, K. 2004 Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. *Plant & cell physiology* **45**, 1870-1874. (DOI:10.1093/pcp/pch203).

[10] Muller, P., Li, X. P. & Niyogi, K. K. 2001 Non-photochemical quenching. A response to excess light energy. *Plant physiology* **125**, 1558-1566. (DOI:10.1104/pp.125.4.1558).

[11] Kulheim, C., Agren, J. & Jansson, S. 2002 Rapid regulation of light harvesting and plant fitness in the field. *Science (New York, N.Y.)* **297**, 91-93. (DOI:10.1126/science.1072359).

[12] Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S. T., Iwai, M., Niyogi, K. K. & Long, S. P. 2016 Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science (New York, N.Y.)* **354**, 857-861. (DOI:10.1126/science.aai8878).

[13] Li, X. P., Bjorkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S. & Niyogi, K. K. 2000 A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391-395. (DOI:10.1038/35000131).

[14] Ruban, A. V. 2016 Nonphotochemical Chlorophyll Fluorescence Quenching: Mechanism and Effectiveness in Protecting Plants from Photodamage. *Plant physiology* **170**, 1903-1916. (DOI:10.1104/pp.15.01935).

[15] Tikkanen, M., Mekala, N. R. & Aro, E. M. 2014 Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage. *Biochimica et biophysica acta* **1837**, 210-215. (DOI:10.1016/j.bbabio.2013.10.001).

[16] Li, H. M. & Chiu, C. C. 2010 Protein transport into chloroplasts. *Annual review of plant biology* **61**, 157-180. (DOI:10.1146/annurev-arplant-042809-112222).

[17] Jung, H. S. & Chory, J. 2010 Signaling between chloroplasts and the nucleus: can a systems biology approach bring clarity to a complex and highly regulated pathway? *Plant physiology* **152**, 453-459. (DOI:10.1104/pp.109.149070).

[18] Kleine, T., Voigt, C. & Leister, D. 2009 Plastid signalling to the nucleus: messengers still lost in the mists? *Trends in genetics : TIG* **25**, 185-192. (DOI:10.1016/j.tig.2009.02.004).

[19] Jarvis, P. & Lopez-Juez, E. 2013 Biogenesis and homeostasis of chloroplasts and other plastids. *Nature reviews. Molecular cell biology* **14**, 787-802. (DOI:10.1038/nrm3702).

[20] Waters, M. T., Wang, P., Korkaric, M., Capper, R. G., Saunders, N. J. & Langdale, J. A. 2009 GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. *The Plant cell* **21**, 1109-1128. (DOI:10.1105/tpc.108.065250).

[21] Ruckle, M. E., DeMarco, S. M. & Larkin, R. M. 2007 Plastid signals remodel light signaling networks and are essential for efficient chloroplast biogenesis in Arabidopsis. *The Plant cell* **19**, 3944-3960. (DOI:10.1105/tpc.107.054312).

[22] Kindgren, P., Kremnev, D., Blanco, N. E., de Dios Barajas Lopez, J., Fernandez, A. P., Tellgren-Roth, C., Kleine, T., Small, I. & Strand, A. 2012 The plastid redox insensitive 2 mutant of Arabidopsis is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. *The Plant journal : for cell and molecular biology* **70**, 279-291. (DOI:10.1111/j.1365-313X.2011.04865.x).

[23] Oelmuller, R., Levitan, I., Bergfeld, R., Rajasekhar, V. K. & Mohr, H. 1986 Expression of nuclear genes as affected by treatments acting on the plastids. *Planta* **168**, 482-492. (DOI:10.1007/bf00392267).

[24] Sullivan, J. A. & Gray, J. C. 1999 Plastid translation is required for the expression of nuclear photosynthesis genes in the dark and in roots of the pea lip1 mutant. *The Plant cell* **11**, 901-910. (DOI:10.1105/tpc.11.5.901).

[25] Koussevitzky, S., Nott, A., Mockler, T. C., Hong, F., Sachetto-Martins, G., Surpin, M., Lim, J., Mittler, R. & Chory, J. 2007 Signals from chloroplasts converge to regulate nuclear gene expression. *Science (New York, N.Y.)* **316**, 715-719. (DOI:10.1126/science. 1140516).

[26] Chan, K. X., Mabbitt, P. D., Phua, S. Y., Mueller, J. W., Nisar, N., Gigolashvili, T., Stroeher, E., Grassl, J., Arlt, W., Estavillo, G. M., et al. 2016 Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. *Proceedings of the National Academy of Sciences of the United States of America* **113**, E4567-4576. (DOI:10.1073/pnas.1604936113).

[27] Martin, G., Leivar, P., Ludevid, D., Tepperman, J. M., Quail, P. H. & Monte, E. 2016 Phytochrome and retrograde signalling pathways converge to antagonistically regulate a light-induced transcriptional network. *Nature communications* **7**, 11431. (DOI:10.1038/ncomms11431).

[28] Hills, A. C., Khan, S. & Lopez-Juez, E. 2015 Chloroplast Biogenesis-Associated Nuclear Genes: Control by Plastid Signals Evolved Prior to Their Regulation as Part of Photomorphogenesis. *Frontiers in plant science* **6**, 1078. (DOI:10.3389/fpls.2015.01078).

[29] Zhang, Y., Su, J., Duan, S., Ao, Y., Dai, J., Liu, J., Wang, P., Li, Y., Liu, B., Feng, D., et al. 2011 A highly efficient rice green tissue protoplast system for transient gene expression and studying light/chloroplast-related processes. *Plant methods* **7**, 30. (DOI:10.1186/1746-4811-7-30).

[30] Li, R. Q., Jiang, M., Liu, Y. H., Zheng, Y. C., Huang, J. Z., Wu, J. M. & Shu, Q. Y. 2017 The xantha Marker Trait Is Associated with Altered Tetrapyrrole Biosynthesis and Deregulated Transcription of PhANGs in Rice. *Frontiers in plant science* **8**, 901. (DOI:10.3389/fpls.2017.00901).

[31] Nishiyama, T., Sakayama, H., de Vries, J., Buschmann, H., Saint-Marcoux, D., Ullrich, K. K., Haas, F. B., Vanderstraeten, L., Becker, D., Lang, D., et al. 2018 The Chara Genome: Secondary Complexity and Implications for Plant Terrestrialization. *Cell* **174**, 448-464.e424. (DOI:10.1016/j.cell.2018.06.033).

[32] Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. 2012 NIH Image to ImageJ: 25 years of image analysis. *Nature methods* **9**, 671-675.

[33] Arnon, D. I. 1949 COPPER ENZYMES IN ISOLATED CHLOROPLASTS. POLYPHENOLOXIDASE IN BETA VULGARIS. *Plant physiology* **24**, 1-15. (DOI:10.1104/pp.24.1.1).

[34] Lichtenthaler, H. K. 1987 [34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in enzymology* (pp. 350-382, Elsevier.

[35] Maxwell, K. & Johnson, G. N. 2000 Chlorophyll fluorescence--a practical guide. *Journal of experimental botany* **51**, 659-668. (DOI:10.1093/jxb/51.345.659).

[36] Genty, B., Briantais, J. M. & Baker, N. R. 1989 The Relationship between the Quantum Yield of Photosynthetic Electron-Transport and Quenching of Chlorophyll Fluorescence. *Biochimica et biophysica acta* **990**, 87-92. (DOI:Doi 10.1016/S0304-4165(89)80016-9).

[37] Bilger, W. & Bjorkman, O. 1990 Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of Hedera canariensis. *Photosynthesis research* **25**, 173-185. (DOI:10.1007/bf00033159).

[38] Tran, L. H. & Jung, S. 2017 Effects of Light-Emitting Diode Irradiation on Growth Characteristics and Regulation of Porphyrin Biosynthesis in Rice Seedlings. *International journal of molecular sciences* **18**. (DOI:10.3390/ijms18030641).

[39] Ruckle, M. E. & Larkin, R. M. 2009 Plastid signals that affect photomorphogenesis in Arabidopsis thaliana are dependent on GENOMES UNCOUPLED 1 and cryptochrome 1. *The New phytologist* **182**, 367-379. (DOI:10.1111/j.1469-8137.2008.02729.x).

[40] Roy, A., Sahoo, D. & Tripathy, B. C. 2013 Involvement of phytochrome A in suppression of photomorphogenesis in rice seedling grown in red light. *Plant, cell & environment* **36**, 2120-2134. (DOI:10.1111/pce.12099).

[41] Jung, K. H., Lee, J., Dardick, C., Seo, Y. S., Cao, P., Canlas, P., Phetsom, J., Xu, X., Ouyang, S., An, K., et al. 2008 Identification and functional analysis of light-responsive unique genes and gene family members in rice. *PLoS genetics* **4**, e1000164. (DOI:10.1371/journal.pgen.1000164).

[42] Lakshmanan, M., Lim, S. H., Mohanty, B., Kim, J. K., Ha, S. H. & Lee, D. Y. 2015 Unraveling the Light-Specific Metabolic and Regulatory Signatures of Rice through Combined in Silico Modeling and Multiomics Analysis. *Plant physiology* **169**, 3002-3020. (DOI:10.1104/pp.15.01379).

[43] Chin, H. S., Wu, Y. P., Hour, A. L., Hong, C. Y. & Lin, Y. R. 2016 Genetic and Evolutionary Analysis of Purple Leaf Sheath in Rice. *Rice (New York, N.Y.)* **9**, 8. (DOI:10.1186/s12284-016-0080-y).

[44] Zheng, J., Wu, H., Zhu, H., Huang, C., Liu, C., Chang, Y., Kong, Z., Zhou, Z., Wang, G., Lin, Y., et al. 2019 Determining factors, regulation system, and domestication of anthocyanin biosynthesis in rice leaves. *The New phytologist* **223**, 705-721. (DOI:10.1111/nph.15807).

[45] Zulfugarov, I. S., Tovuu, A., Eu, Y. J., Dogsom, B., Poudyal, R. S., Nath, K., Hall, M., Banerjee, M., Yoon, U. C., Moon, Y. H., et al. 2014 Production of superoxide from

Photosystem II in a rice (Oryza sativa L.) mutant lacking PsbS. *BMC plant biology* **14**, 242. (DOI:10.1186/s12870-014-0242-2).

[46] Hubbart, S., Ajigboye, O. O., Horton, P. & Murchie, E. H. 2012 The photoprotective protein PsbS exerts control over CO(2) assimilation rate in fluctuating light in rice. *The Plant journal : for cell and molecular biology* **71**, 402-412. (DOI:10.1111/j.1365-313X.2012.04995.x).

[47] Hubbart, S., Smillie, I. R. A., Heatley, M., Swarup, R., Foo, C. C., Zhao, L. & Murchie, E. H. 2018 Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. *Communications biology* **1**, 22. (DOI:10.1038/s42003-018-0026-6).

[48] Allorent, G., Lefebvre-Legendre, L., Chappuis, R., Kuntz, M., Truong, T. B., Niyogi, K. K., Ulm, R. & Goldschmidt-Clermont, M. 2016 UV-B photoreceptor-mediated protection of the photosynthetic machinery in Chlamydomonas reinhardtii. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 14864-14869. (DOI:10.1073/pnas.1607695114).

[49] Petroutsos, D., Tokutsu, R., Maruyama, S., Flori, S., Greiner, A., Magneschi, L., Cusant, L., Kottke, T., Mittag, M., Hegemann, P., et al. 2016 A blue-light photoreceptor mediates the feedback regulation of photosynthesis. *Nature* **537**, 563-566. (DOI:10.1038/nature19358).

[50] Nakamura, H., Muramatsu, M., Hakata, M., Ueno, O., Nagamura, Y., Hirochika, H., Takano, M. & Ichikawa, H. 2009 Ectopic overexpression of the transcription factor OsGLK1 induces chloroplast development in non-green rice cells. *Plant & cell physiology* **50**, 1933-1949. (DOI:10.1093/pcp/pcp138).

[51] Ishikawa, R., Tamaki, S., Yokoi, S., Inagaki, N., Shinomura, T., Takano, M. & Shimamoto, K. 2005 Suppression of the floral activator Hd3a is the principal cause of the night break effect in rice. *The Plant cell* **17**, 3326-3336. (DOI:10.1105/tpc.105.037028).

[52] Welsch, R., Wust, F., Bar, C., Al-Babili, S. & Beyer, P. 2008 A third phytoene synthase is devoted to abiotic stress-induced abscisic acid formation in rice and defines functional diversification of phytoene synthase genes. *Plant physiology* **147**, 367-380. (DOI:10.1104/pp.108.117028).

[53] Yang, Y., Xu, J., Huang, L., Leng, Y., Dai, L., Rao, Y., Chen, L., Wang, Y., Tu, Z., Hu, J., et al. 2016 PGL, encoding chlorophyllide a oxygenase 1, impacts leaf senescence and indirectly affects grain yield and quality in rice. *Journal of experimental botany* **67**, 1297-1310. (DOI:10.1093/jxb/erv529).

Supporting Information

Figure S1. Morphological parameters measured in rice seedlings. Five (A) or seven (B) day-old grown Nipponbare seedlings were taken out of the jars, carefully positioned and pictured to measure coleoptile length and angle, the length of each leaf, and the second leaf declination as indicated using ImageJ software.

Figure S2. Eight day-old rice seedlings were grown in the dark (D) or low intensity light (white light, 1 µmol m^{-2} s^{-1}) (W1) at 21ºC in the presence of different lincomycin (L) concentrations as indicated. **A.** Representative rice seedlings grown under D, W1, and W1 with 0.25 mM (L0.25), 0.5 mM (L0.5), 1 mM (L1) lincomycin. **B.** Quantification of coleoptile angle, and coleoptile, first and second leaf length (n= 10) of seedlings grown as indicated in **A**. **C.** Representative rice seedlings grown under W1 with (L2) or without 2 mM lincomycin. **D.** Quantification of coleoptile angle, and coleoptile, first and second leaf length (n= 16) of seedlings grown as indicated in **C.** Student's t-test was used for the analysis between lincomycin treated and non-treated groups. Statistically significant differences were defined with P value < 0.05. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure S3. A. Representative 7 day-old rice seedlings grown at 28 ^{o}C under continuous dark (D) for 3 days and then transferred to blue light of different intensities: 10 (µmol m^{-2} s^{-1} (DB10), 150 µmol m^{-2} s^{-1} (DB150), 300 µmol m^{-2} s^{-1} (DB300), and 450 µmol m^{-2} s^{-1} (DB450). **B**. Quantification of coleoptile angle, and coleoptile, first, second and third leaf length, second leaf declination angle in seedlings (n = 24) grown as indicated in **A. C.** Chlorophyll A (CHLA), chlorophyll B (CHLB), total chlorophylls (CHLS) and carotenoid content in seedlings grown under the conditions indicated in **A**. Data correspond to the mean ± s.e.m. of independent biological triplicates. Multiple groups in **C** were analyzed by one-way ANOVA analysis. In **B**, different letters denote statistically significant differences among means of each lincomycin non-treated group by Tukey test, and student's t-test was used for the analysis between lincomycin treated

and non-treated groups. Asterisks denote statistically significant differences: P values < 0.05 (*), < 0.01 (**) and < 0.001 (***).

Figure S4. A. Representative 5 day-old rice seedling grown at 28 ºC under continuous high blue (B) light of different intensities: 10 (µmol m^{-2} s^{-1} (B10), 150 µmol m^{-2} s^{-1} (B150), 300 µmol m^{-2} s^{-1} (B300), and 450 µmol m^{-2} s^{-1} (B450). **B.** Quantification of coleoptile and second leaf angle, and coleoptile, first, second and third leaf length in seedlings (n = 8 for B10 and B150, n=30 for B300 and B450) grown as indicated in **A**. In **B**, different letters denote statistically significant differences among means of each group by Tukey test.

Figure S5. Photosynthesis efficiency and photoprotection capacities of rice seedlings grown in blue light. Rice seedlings were grown for 5 days (**A** and **B**) or first grown in dark for 3 days then transfer to blue light for 4 days (**C** and **D**) under different blue light: 10 (µmol m^{-2} s^{-1} (B10), 150 µmol m^{-2} s^{-1} (B150), 300 µmol m^{-2} s^{-1} (B300), and 450 µmol m^{-2} s^{-1} (B450) at 28 ºC. **A.** Maximum quantum yield of PSII (Fv/Fm) of 5-day old seedlings. Chlorophyll fluorescence was measured after 30 min of dark adaptation using 800 µmol photons m^{-2} s^{-1} of actinic light. Values are the mean ± SD of at least eight independent seedlings grown at the specified light conditions. **B.** NPQ induction (light) and relaxation (dark) of 5-day old seedlings. **C.** Maximum quantum yield of PSII (Fv/Fm) of 7-day old seedlings. **D.** NPQ induction (light) and relaxation (dark) of 7-day old seedlings.

Figure S6. NPQ induction and relaxation kinetics of 6-day old rice seedlings grown in low red (40 µmol m^{-2} s^{-1}) (R40) and low blue (10 µmol m^{-2} s^{-1}) (B10) at 28 ºC. Chlorophyll fluorescence was measured after 30 min of dark adaptation using 800 µmol m^{-2} s^{-1} of actinic light. Values are the mean ± SD of six seedlings. The extent of the NPQ components qE (energy quenching) and qI (photoinhibitory quenching) is indicated.

Table S1. List of primers used for qRT-PCR.

Gene name	Gene number	Primer name	Sequence (5' -> 3')	Ref
Ubiquitin	LOC_Os03g13170	EMP1289	AACCAGCTGAGGCCCAAGA	[51]
		EMP1290	ACGATTGATTTAACCAGTCCATGA	[51]
LHCB	LOC_Os09g17740	EMP1167	GACCGCGTCCTCTACCTCG	[30]
		EMP1168	CCTTGAACCACACGGCCTC	[30]
GLK1	LOC_Os06g24070	EMP1169	TGCGAGATTTCCTGCTCCAC	
		EMP1170	CTCGCTTGATGGTTGAACCTG	
PSBS2	LOC_Os04g59440	EMP1301	ACGACAACGACGACCAATGA	
		EMP1302	GCTAGTCCACTTAACCGTCTCC	
PSBS1	LOC_Os01g64960	EMP1303	GATCGGTGAGGTAGCACGAG	
		EMP1304	CATTGGCTCCCGACACCAG	
PSY	LOC_Os06g51290	EMP1327	GGGAAGGCGAAGAAATTGCTA	[52]
		EMP1328	GTGAGTAGGGCATCAGCAATGA	[52]
CHLH	LOC_Os03g20700	EMP1336	AACTGGATGAGCCAGAAGAGA	[53]
		EMP1337	AAATGCAAAAGACTTGCGACT	[53]

Figure 1

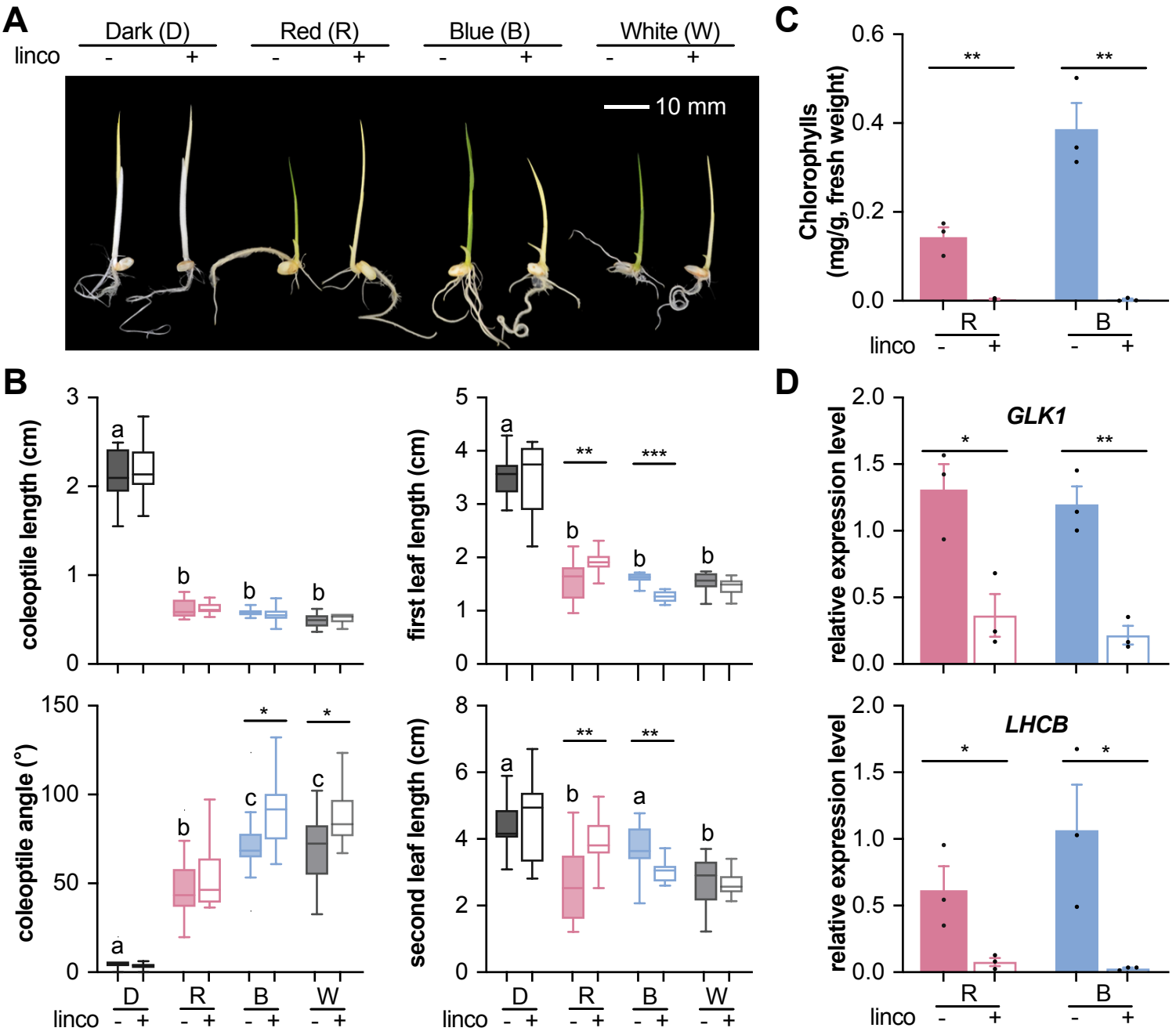

A
Dark (D)
Red (R)
Blue (B)
White (W)
linco
10 mm
B
coleoptile length (cm)
first leaf length (cm)
coleoptile angle (°)
second leaf length (cm)
D
R
B
W
C
Chlorophylls (mg/g, fresh weight)
D
GLK1
LHCB
relative expression level

Figure 2

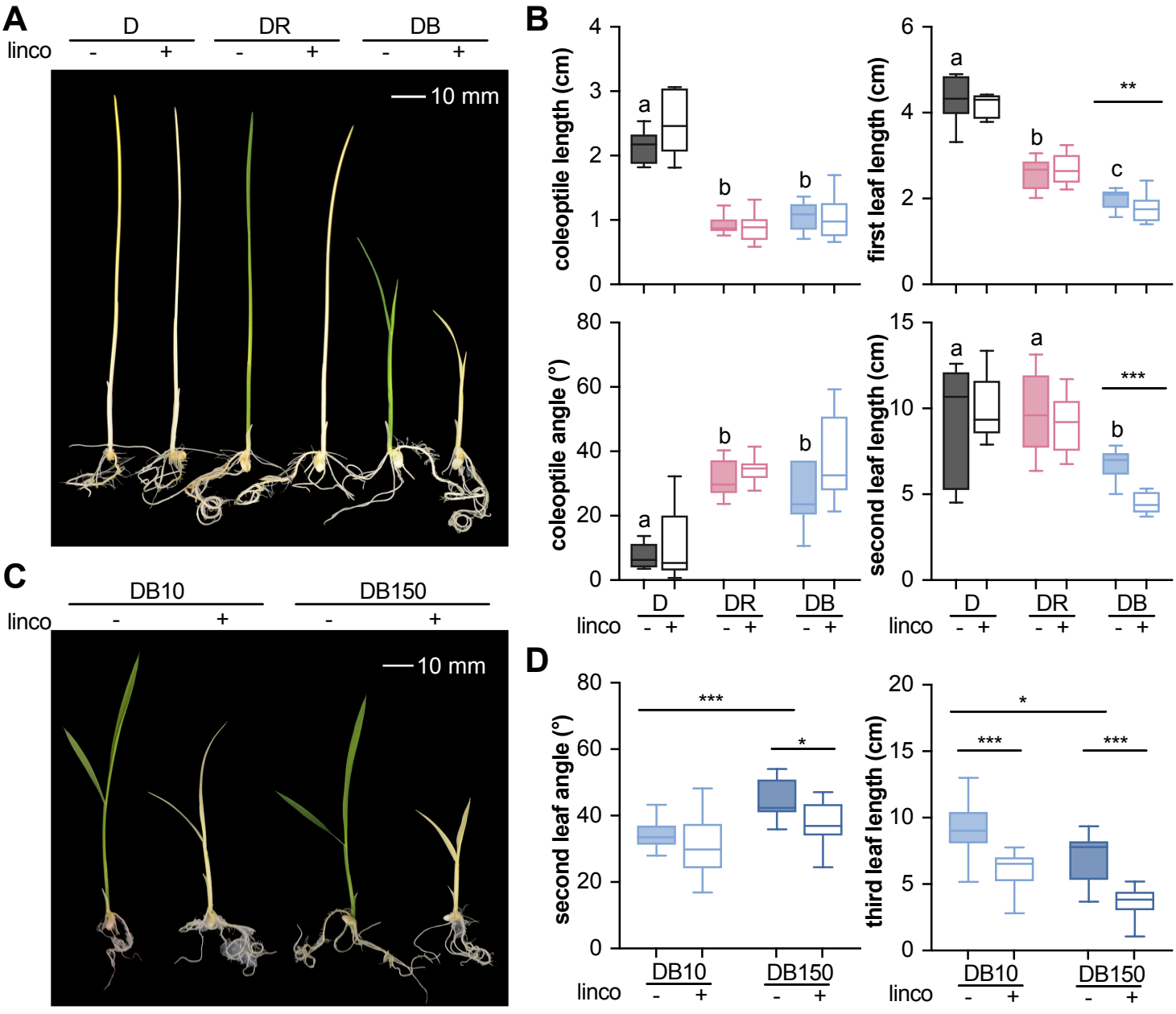

A
D
DR
DB
linco
- + - + - +
10 mm
B
coleoptile length (cm)
first leaf length (cm)
coleoptile angle (°)
second leaf length (cm)
a
b
b
a
b
c
**
a
b
b
a
a
b
***
D
DR
DB
linco
C
DB10
DB150
linco
- + - +
10 mm
D
second leaf angle (°)
third leaf length (cm)
***
*
***
*
***
DB10
DB150
linco

Figure 3

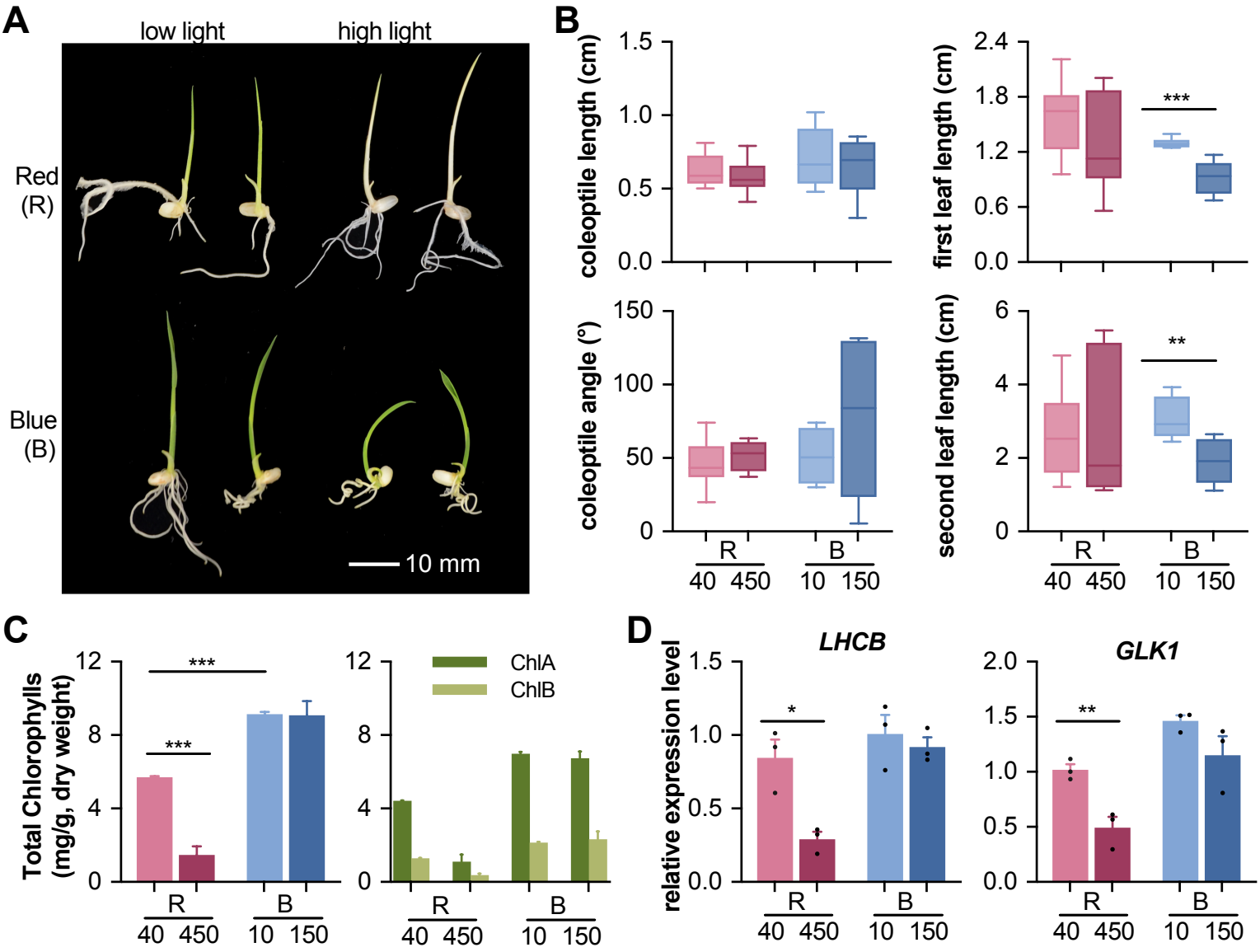
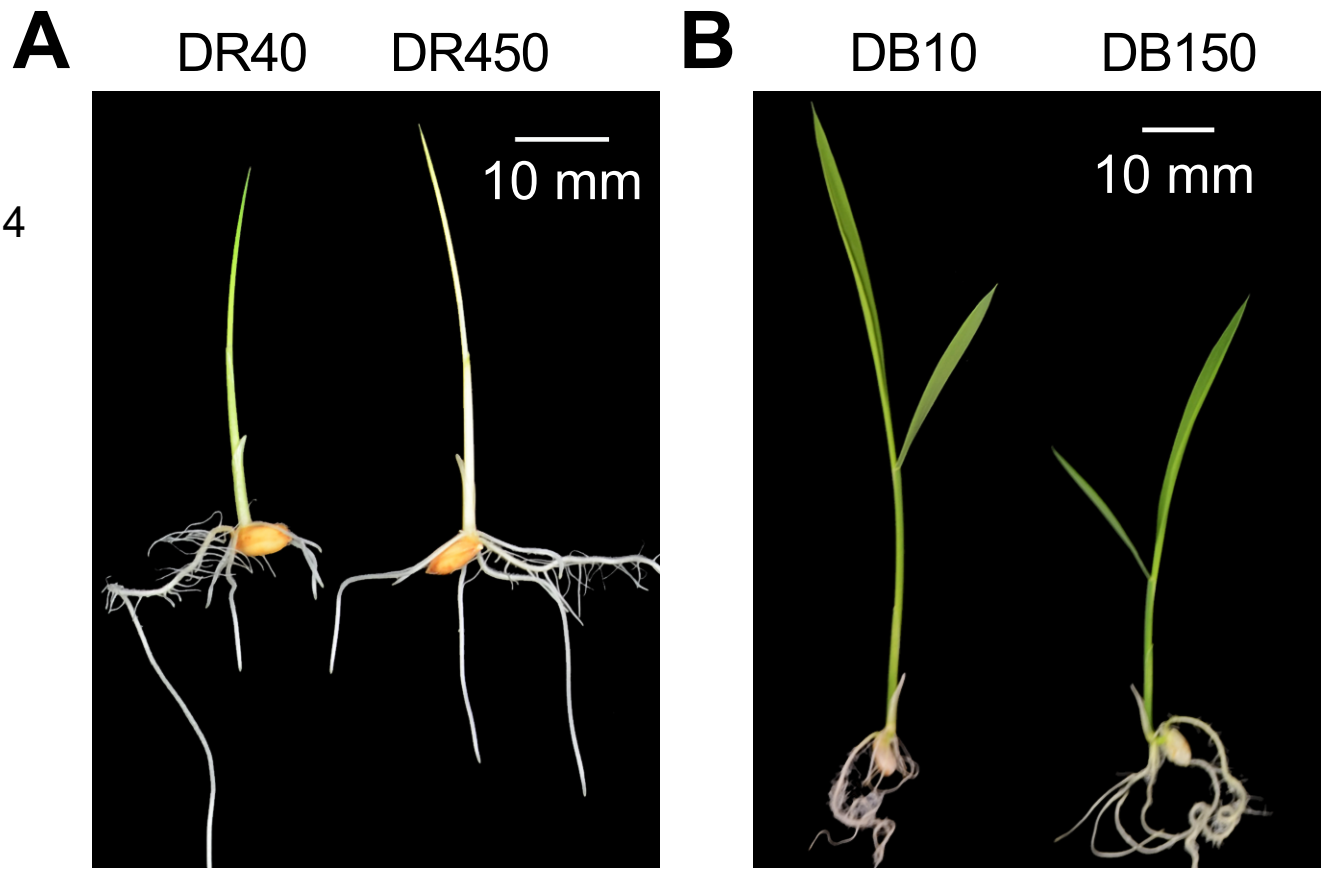

A
low light
high light
Red (R)
Blue (B)
10 mm
B
coleoptile length (cm)
first leaf length (cm)
coleoptile angle (°)
second leaf length (cm)
***
**
R
B
40 450
10 150
C
Total Chlorophylls (mg/g, dry weight)
ChlA
ChlB
D
LHCB
GLK1
relative expression level
*
**

Fig.4


A
DR40
DR450
10 mm
B
DB10
DB150
10 mm

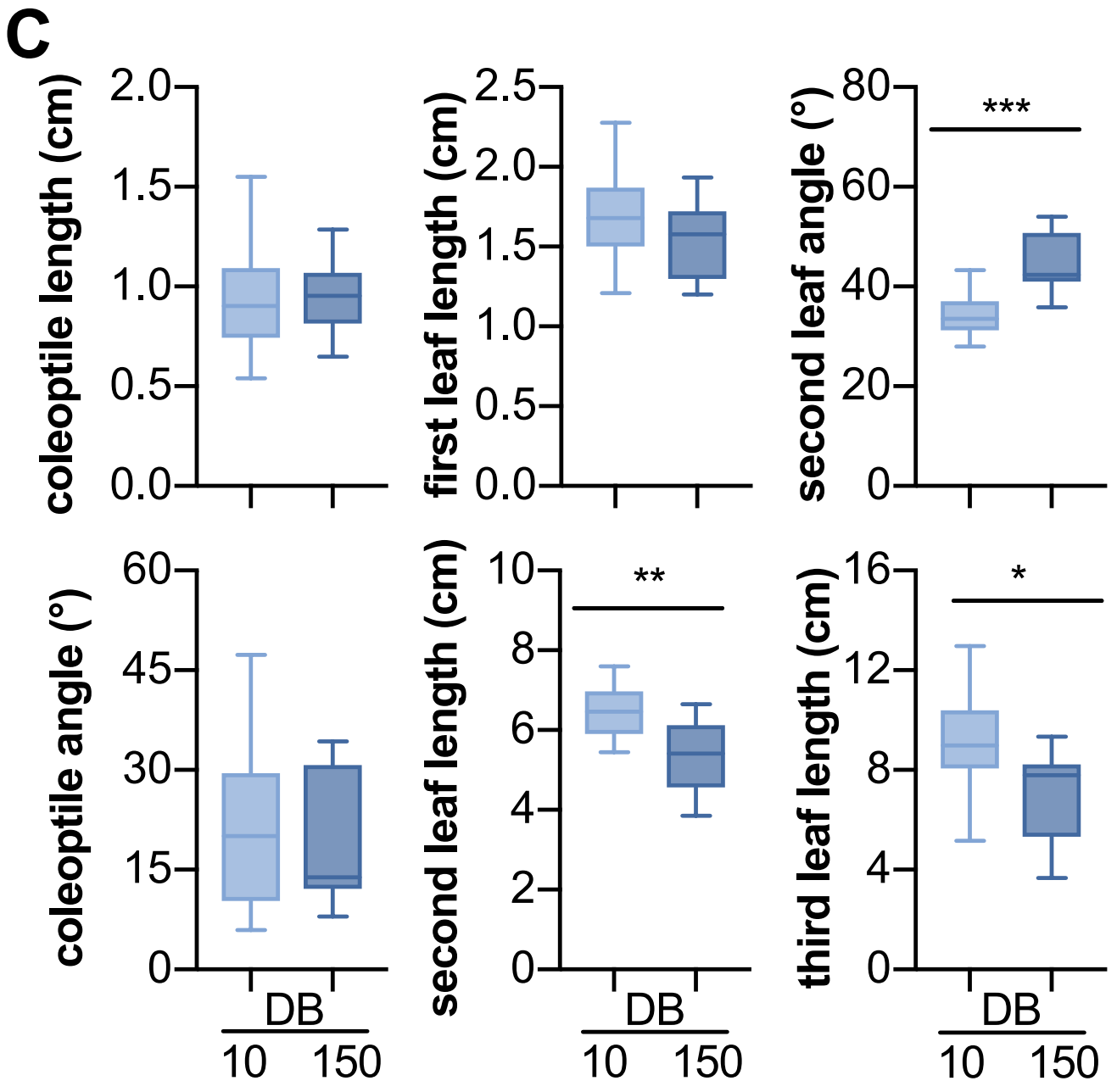

C
coleoptile length (cm)
2.0
1.5
1.0
0.5
0.0
first leaf length (cm)
2.5
2.0
1.5
1.0
0.5
0.0
second leaf angle (°)
80
60
40
20
0
***
coleoptile angle (°)
60
45
30
15
0
second leaf length (cm)
10
8
6
4
2
0
**
third leaf length (cm)
16
12
8
4
0
*
DB
10
150

Figure 5

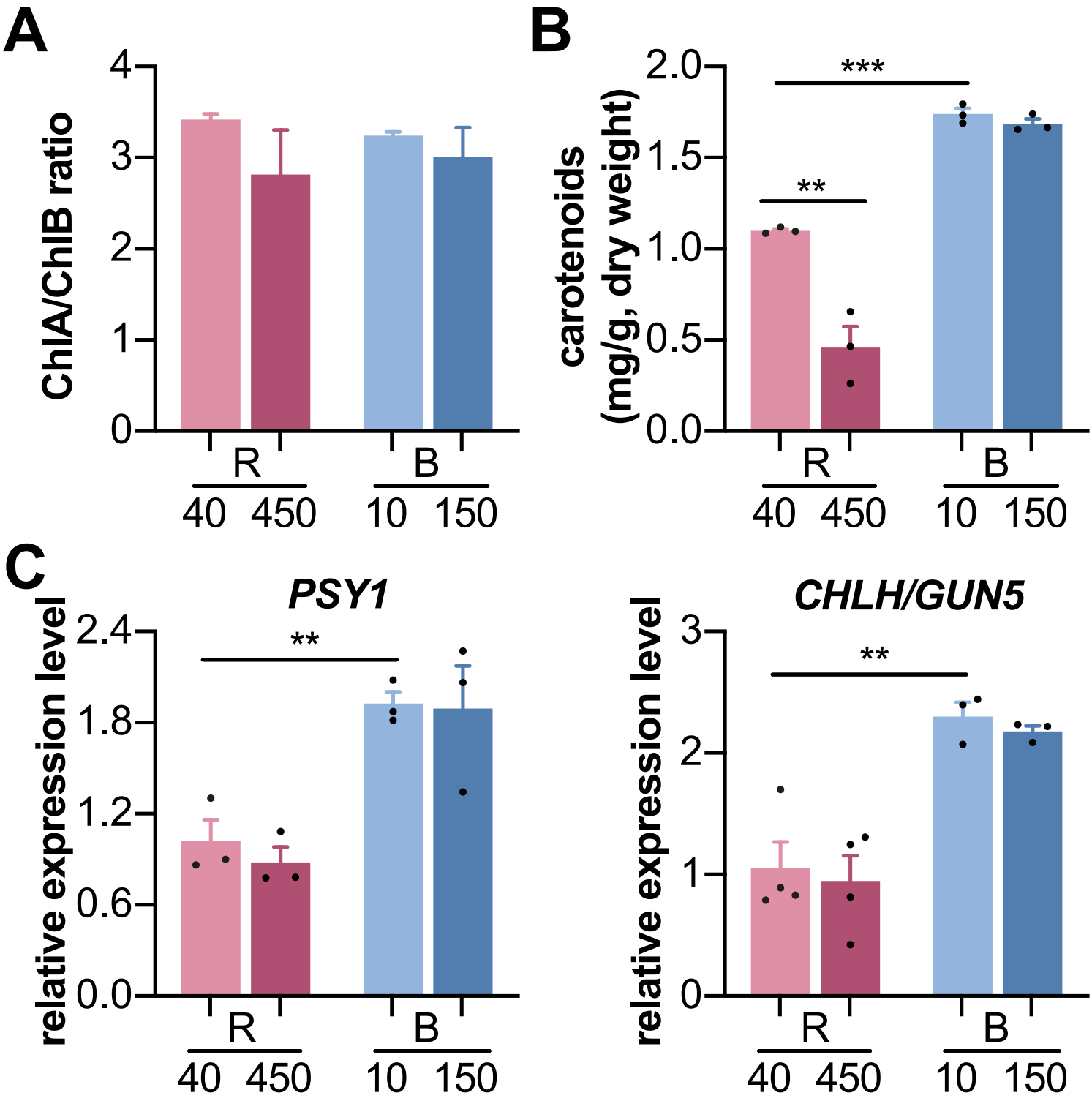
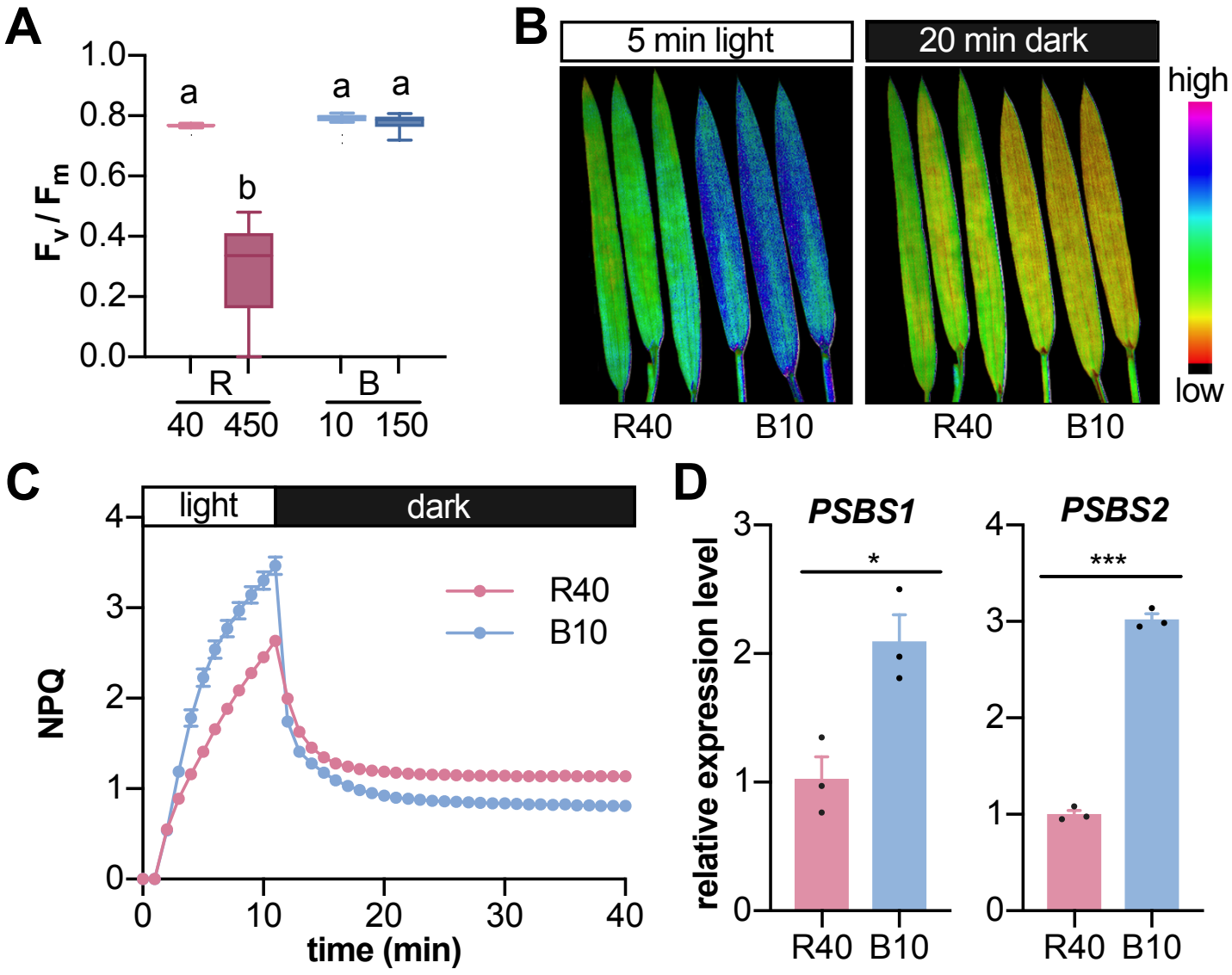

A
ChlA/ChlB ratio
4
3
2
1
0
R
B
40 450
10 150
B
carotenoids (mg/g, dry weight)
2.0
1.5
1.0
0.5
0.0
***
**
R
B
40 450
10 150
C
PSY1
relative expression level
2.4
1.8
1.2
0.6
0.0
**
R
B
40 450
10 150
CHLH/GUN5
relative expression level
3
2
1
0
**
R
B
40 450
10 150

Figure 6


A
1.0
0.8
0.6
0.4
0.2
0.0
F_v / F_m
a
b
a
a
R
B
40 450
10 150
B
5 min light
20 min dark
high
low
R40
B10
R40
B10
C
light
dark
NPQ
R40
B10
0
1
2
3
4
0
10
20
30
40
time (min)
D
PSBS1
PSBS2
relative expression level
*
***
0
1
2
3
4
R40
B10
R40
B10