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Green light augments far-red-light-induced shade response

Yihai Wang · Tingting Zhang · Kevin M. Folta

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Abstract Plants grown in shade exhibit changes in architecture and gene expression to best accommodate growth in photosynthetically challenging conditions. Adaptive changes in morphology include stem and petiole elongation and leaf hyponasty. These changes can be induced by low red to far-red ratio (R/Fr ratio) or by enrichment of green light relative to red and blue. In this report we demonstrate the relationship between far-red and green light in combination. Wild-type *Arabidopsis thaliana* plants were treated with a high and low R/Fr ratio background with or without supplemental green light. The addition of green light augmented the far-red response. Genetic analysis showed that the green effect operates independently of cry1, cry2, phot1, and phot2 receptors. Additive effects are not observed in *phyA* and *phyB* double mutants, but are observed in the phy signal transduction mutants *pif4*, *pif5*, *pif7*. The transcript levels of shade-associated genes (*PIL1*, *ATHB2*, and *HFR1*), are induced by low R/Fr ratio conditions and are reduced in the presence of green light, but not in *phyAphyB* mutants. The reduction in shade-related gene expression caused by supplementation of green light is inconsistent with the elongated petiole phenotype observed. These results suggest that phyA or phyB is required for the green light shade response, but they are not the main receptors because green light would increase the R/Fr ratio, leading to a non-shade phenotype.

Keywords Shade avoidance · Green light · Phytochrome · Cryptochrome · Adaptation

Introduction

Plants compete for light in the shaded environment by adjusting morphology and physiology to optimize growth. These changes include increased stem elongation, leaf hyponasty, and reduced leaf expansion. Long-term effects include early flowering with reduced yields. Together these alterations are termed "Shade Avoidance Syndrome" (Vandenbussche et al. 2005; Franklin 2008; Ballaré 2009; Ruberti et al. 2012). The shade avoidance response is a typical adaptive strategy that develops when the red to far-red light ratio (R/Fr ratio) is low. The response is also induced when blue light is reduced or when green light is enriched (Pierik et al. 2004; Millenaar et al. 2009; Keller et al. 2011; Zhang et al. 2011). The response to shaded environments provides an opportunity to study the interactions of multiple light signaling pathways as they converge to coordinate gene expression and adjust plant form.

The light spectrum is selectively filtered as it passes through or reflects off of vegetation. Red and blue light are efficiently absorbed by photosynthetic pigments and depleted from transmitted light. Far-red passes through and is relatively abundant in the shade of leaves, resulting in a lower R/Fr ratio. In response to this far-red enriched environment plants adjust their architecture and gene expression profiles. A low R/Fr ratio (<1) induces upward leaf angles and petiole elongation growth, which is mostly mediated by the phytochrome B (phyB) receptor, with

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Y. Wang · T. Zhang · K. M. Folta (✉)
Horticultural Sciences Department, University of Florida, 1301 Fifield Hall, PO Box 110690, Gainesville, FL 32611, USA
e-mail: kfolta@ufl.edu

Y. Wang · T. Zhang · K. M. Folta
The Graduate Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL 32611, USA

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phyD and phyE playing a minor role. The phyA receptor antagonizes the function of phyB, phyD, and phyE, modulating their effects to maintain an optimum architecture for light capture and photosynthesis (Franklin and Quail 2010).

The genetic mechanisms underlying shade avoidance adaptation are well understood from studies in *Arabidopsis thaliana*. Two bHLH transcription factors, PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5 represent a connection point for multiple signal inputs to direct shade avoidance responses (Huq and Quail 2002; Lorrain et al. 2008; Koini et al. 2009; Keller et al. 2011). PIF4 and PIF5 physically interact with active form of phyB, and are degraded via 26S proteasome pathway in high R/Fr ratio (>1) conditions. The shift from high R/Fr to low R/Fr ratio stabilizes PIF4 and PIF5 proteins, leading to upward leaf inclination and elongated petioles (Lorrain et al. 2008). Similarly, PIF4 and PIF5 are also required for the full expression of low-blue-light-induced shade symptoms (Keller et al. 2011). LONG HYPOCOTYL IN FAR-RED LIGHT (HFR1) opposes PIF4 and PIF5, and limits their activity by forming non-DNA-binding heterodimers (Hornitschek et al. 2009). Reports have shown that HFR1 acts as a positive component downstream of the phyA signaling pathway, modulating far-red-light-induced stem and petiole elongation growth (Fairchild et al. 2000; Soh et al. 2000; Fankhauser and Chory 2000; Sessa et al. 2005).

Several recent studies have demonstrated that PIF family members (PIF4, PIF5, and PIF7) bind to the G-box-containing regions of the promoters of shade-associated genes, such as *PHYTOCHROME INTERACTING FACTOR 3-LIKE1* (*PIL1*), *HFR1*, *HOMEOBOX FROM ARABIDOPSIS THALIANA 4* (*HAT4*, also named *ATHB2*) (de Lucas et al. 2008; Hornitschek et al. 2009; Kunihiro et al. 2011; Leivar et al. 2012; Li et al. 2012; Hornitschek et al. 2012; Leivar and Monte 2014). Although green light can induce leaf hyponastic growth and petiole elongation, the transcriptional induction of *PIL1* and *ATHB2* by supplementation of green light to red and blue light is blocked by green-light-absorbing form of blue light receptor cryptochrome 1 (cry1) (Zhang et al. 2011). In addition, the green-induced shade avoidance is absent in *hat4* and *pil1* single mutant backgrounds. These observations suggest that multiple signaling pathways may independently contribute to hyponastic growth by adjusting a common set of regulators.

It was previously shown that green light can induce upward leaf inclination and petiole elongation when supplemented into the background of high red and blue light (Zhang et al. 2011). It is of interest to examine the interaction between green and far-red signals in the regulation of the same set of plant acclimation responses. Narrow-bandwidth LED light treatments can be used to at least partially isolate the effects of the individual sensory systems. The petiole elongation and shade-associated gene expression were examined to test the interaction between green and far-red signals in the regulation of shade response. Photoreceptor and several genetic shade avoidance regulator mutants were tested to separate the green effect from the known signaling pathways.

Materials and methods

Plant materials and growth conditions

The genotypes used were *A. thaliana* Col-0 ecotype, *cry1cry2* mutant (*cry1-304 cry2-1*; Mockler et al. 1999), *phot1phot2* (*phot1-5 phot2-1*; Sakai et al. 2001). The *phyAphyB* mutant line is the same *phyB-5* mutation (Ler) crossed into a *phyA* T-DNA insertion mutant (SALK_014575) in the Col-0 background, and then backcrossed to the *phyA* mutant twice and selected by red/far-red long-hypoctoyl phenotype. The *pif4* (SALK_140393), *pif5* (SALK_087012), *pif7* (*pif7-2*, CS875431, genotyped according to Leivar et al. 2008), *pif4pif5* (*pif4-2 pif5-3*, CS68096), and *hfr1* (SALK_037727) homozygous T-DNA mutants were obtained from the Arabidopsis Biological Resource Center. Experimental materials were grown in plastic trays with soilless media (ProMix BX, Premier Tech Horticulture, PA, USA). Seeds were distributed evenly to receive equal light distribution and stratified at 4 °C for 72 h. Seedlings were grown under white fluorescent light ($\sim$80 μmol m^{-2} s^{-1}, 16 h light/8 h dark) until they were at the appropriate developmental stage for experimental light treatment (typically 21–28 days, with four to five pairs of true leaves). Plants were then transferred to LED chambers containing the experimental light conditions for 3–5 days. The temperature in LED chambers was 22 ± 1.5 °C. Plants were watered approximately three times a week while under white fluorescent light and every other day under LED arrays with 0.1× Hoagland's solution. Plants were grown under constant illumination.

Light sources and treatments

Treatments were administered with LED light, with peak wavelengths of far-red, red, green and blue light at 730, 660, 525 and 470 nm, respectively (Table 1). Light fluence rates were measured using a photometer with a PAR sensor (LI-COR, Model LI-250, NE, USA). The far-red radiation was measured using a radiometer/photometer (International Light, Model IL1400A, MA, USA) and then converted to photon fluence rate. Four different light treatments were used. In all of the treatments, blue light fluence rates were kept constant at 25 μmol m^{-2} s^{-1}, and different R/Fr ratios

Table 1 Fluence rates (μmol m^{-2} s^{-1}) of light treatments used in this study

Light treatment	Light wavebands (nm)			
	Blue (470)	Green (525)	Red (660)	Far-red (730)
High R/Fr	25	0	15	5
High R/Fr + G	25	25	15	5
Low R/Fr	25	0	1.8	18
Low R/Fr + G	25	25	1.8	18

and green light combinations were added. The baseline treatment for comparison is 25 μmol m^{-2} s^{-1} blue, 15 μmol m^{-2} s^{-1} red, and 5 μmol m^{-2} s^{-1} far-red light (R/Fr ratio ≈ 3, the treatment is noted as "High R/Fr"). In the second treatment green light was added to the High R/Fr background at 25 μmol m^{-2} s^{-1} (noted as "High R/Fr + G") to test if green light affects shade symptoms in the presence of far-red light. In the third treatment, the R/Fr ratio was reduced to approximately 0.1 to induce shade symptoms (noted as "Low R/Fr") keeping blue light constant. The fourth treatment contained 25 μmol m^{-2} s^{-1} green light added to the same conditions as the third treatment (noted as "Low R/Fr + G").

Morphological measurements

To quantify the effects of green and far-red light on shade avoidance, the petiole length and leaf length were measured, and the petiole/leaf ratio was calculated. Whole plants were carefully removed from the growing medium, and then the third or fourth pair of true leaves were dissected and flattened on the adhesive side of a black electrical tape. Samples were imaged at 600 dpi resolution on a standard flatbed scanner and measured using ImageJ software (Version 1.44p, NIH, USA) with comparisons to an adjacent size standard. For experimental replication, at least three independent sets of 6–9 plants were measured for each light treatment.

RNA preparation and quantitative RT-PCR

Total RNA was isolated from the third or fourth pair of true leaves (for *phyAphyB* mutant plants, the second pair of true leaves was used, as the plants tended to flower from the absence of phyB soon after the second pair of leaves) from 3 to 4 individual plants using Qiagen RNeasy Mini kit (Qiagen, CA, USA) according to the manufacturer's protocol. 1 μg of RNA was reverse transcribed using Improm-II Reverse Transcriptase (Promega Inc., Madison, WI, USA). Quantitative RT-PCR was performed using the StepOne Plus system (Applied Biosystems, CA, USA) based on SYBR Green chemistry. All the primers were designed by the Primer Express 3.0 software (Applied Biosystems, CA, USA). *YLS8* (*AT5G08290*) was used as the reference gene (Czechowski et al. 2005). All primer sequences are listed in Table 2 (Supplemental Material). The relative mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). At least two independent experiments were performed and consistent gene expression patterns were observed.

Accession numbers

Sequence data from this report may be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *YLS8* (*AT5G08290*), *PIL1* (*AT2G46970*), *ATHB2* (*AT4G16780*), and *HFR1* (*AT1G02340*).

Results

Green light and far-red radiation additively regulate petiole elongation growth

Interactions between green and far-red light were assessed by measuring leaf characteristics in response to the individual wavelengths and wavelengths in combination. Representative leaf samples of wild-type Arabidopsis (Col-0) plants grown under the different light treatments (Table 1) are shown in Fig. 1. The petioles were increasingly longer as R/Fr ratio decreased, and green light increased the effect of the far-red response.

Leaf (petiole and leaf blade) and petiole length were measured in the fourth pair of true leaves. Six to nine plants were measured within each experiment. The same experiment was repeated at least three times with similar results. Petiole length as a function of total leaf length (petiole/leaf ratio) describes the condition observed, as the petiole elongates while the whole leaf length remains constant. Results in Fig. 1 show that under high R/Fr ratio conditions, wild type plants had an average petiole/leaf ratio of 0.376. Reduction of R/Fr ratio from 3 to 0.1 resulted in a significant increase in petiole/leaf ratio to 0.483. Supplementation of green light to "High R/Fr" and "Low R/Fr" conditions further increased this ratio to 0.433 and 0.534, respectively.

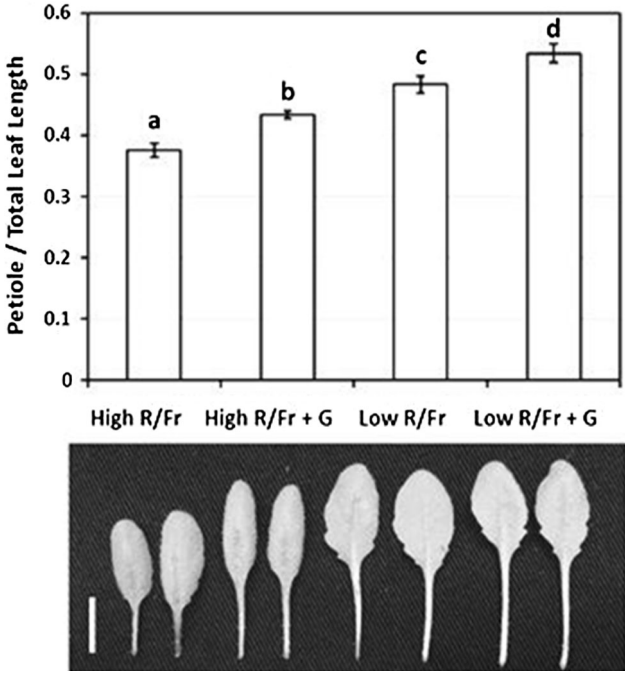

Fig. 1 Green and far-red light additively induce petiole elongation growth in wild-type Arabidopsis Col-0. Wild-type plants were grown under white light for approximately 3 weeks and then transferred to one of four light treatments: 25 μmol m^{-2} s^{-1} blue, 15 μmol m^{-2} s^{-1} red, and 5 μmol m^{-2} s^{-1} far-red (red/far-red ≈ 3, High R/Fr); 25 μmol m^{-2} s^{-1} blue, red/far-red ≈ 3, 25 μmol m^{-2} s^{-1} green light (High R/Fr + G); 25 μmol m^{-2} s^{-1} blue, 1.8 μmol m^{-2} s^{-1} red, 18 μmol m^{-2} s^{-1} far-red light (red/far-red ≈ 0.1, Low R/Fr); 25 μmol m^{-2} s^{-1} blue, red/far-red ≈ 0.1, 25 μmol m^{-2} s^{-1} green light (Low R/Fr + G) for 3–5 days. Individual plant rosettes were dissected, and conspicuous leaf attributes were quantified. Mean petiole length as a percentage of total leaf length of different light-treated plants was reported. The measurements were derived from the fourth pair true leaves from 6 to 9 individual plants. Same experiment was repeated at least three times with similar results. *Error bars* represent SE. Tukey's HSD test ($\alpha = 0.05$) was used to assess statistical significance. *Different letters* represent statistically different means. *Scale bar* 1 cm

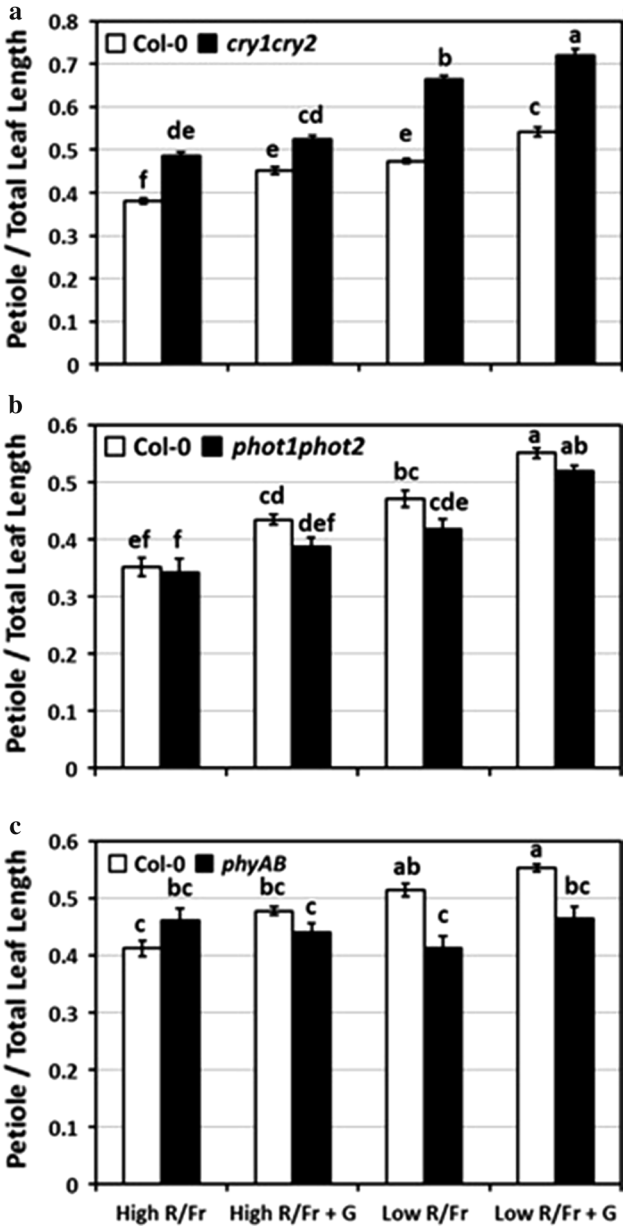

Fig. 2 Tests of green and far-red additive effects in *cry1cry2*, *phot1phot2*, and *phyAphyB* mutants. Mutant plants were grown and treated in the same conditions used in Fig. 1. **a** Mean petiole/leaf ratio of *cry1cry2* plants grown in the four light conditions. **b** Mean petiole/leaf ratio of *phot1phot2* plants grown in the four light conditions. **c** Mean petiole/leaf ratio of *phyAphyB* plants grown in the four light conditions. The measurements in **a** and **b** were derived from the third true leaves from 6 to 9 individual plants. The measurements in **c** were derived from the second true leaves from 6 to 9 individual plants. *Error bars* represent SE. Tukey's HSD test ($\alpha = 0.05$) was used to assess statistical significance. *Different letters* represent statistically different means

The green and far-red light interactions persist in *cry1cry2* and *phot1phot2*, but not *phyAphyB* mutant plants

The blue light cryptochrome receptors can be inactivated by green light (Bouly et al. 2007; Banerjee et al. 2007). The green light induced further petiole elongation could be a result of green light negation of cryptochrome action. To test this possibility, the experiments in Fig. 1 were replicated in *cry1cry2* double mutants. The *cry1cry2* mutant plants showed higher petiole/leaf ratio compared with that of wild-type plants in all light conditions tested (Fig. 2a). The low R/Fr ratio led to a significant increase in petiole/leaf ratio in mutant plants as it did in wild-type plants. Adding green light to the background of "High R/Fr" or "Low R/Fr" conditions stimulated additional petiole elongation similar to what was observed in wild-type plants.

The same experiments were performed in *phot1phot2* and *phyAphyB* receptor mutants. Similarly, additional

green light applied to the *phot1phot2* double mutant plants induced further petiole elongation in a low or high far-red light background (Fig. 2b). However, no far-red or green effect was observed in *phyAphyB* mutant plants (Fig. 2c).

Roles of PIF4 and PIF5 in green and far-red light shade-inducing pathways

The green and far-red systems utilize separate light sensors to control a common response (Zhang et al. 2011). It is of interest to determine if the two are imparting their effect(s) through completely separate systems or if there is a point of convergence between the far-red and green shade-inducing pathways. Because *PIL1*, *ATHB2*, and *HFR1* are downstream targets of PIF4 and PIF5 in shade response (Lorrain et al. 2008; Sessa et al. 2005), the *pif4* and *pif5* mutants were tested in the same light conditions as described in Fig. 1. The *pif4* single mutant plants were phenotypically similar to wild-type plants in response to all conditions (Fig. 3a). The *pif5* mutants also showed similar responses to far-red and green (Fig. 3b). The *pif4pif5* double mutant plants were tested, and in all light conditions they exhibited lower petiole/leaf ratios compared to wild-type plants. The reduction in R/Fr ratio still induced significant petiole elongation growth in *pif4pif5*. This ratio increased when green light was added to the "Low R/Fr" condition (Fig. 3c). A role for PIF7 was assessed in green and far-red-induced shade responses. Results in Fig. 3d show attenuated petiole elongation growth of *pif7* mutant plants compared to wild-type plants in all light treatment conditions. However the *pif7* mutants retained the ability to respond to far-red and green shade-inducing signals.

Comparison of the transcript levels of shade-related genes

To further explore interaction networks between green and far-red shade responses, we examined the transcript levels of shade-associated genes after far-red and/or green light treatments. The expression of *PIL1*, *ATHB2*, and *HFR1* genes was compared in wild-type and mutant plants (*cry1cry2* or *phyAphyB*) in response to the far-red and green light treatments (Fig. 4). Transcripts were normalized to "High R/Fr"-treated wild-type plants. Green light did not induce any biologically significant changes in high R/Fr conditions in wild-type plants in all mutant backgrounds. However, in low R/Fr, the steady-state transcript levels of *PIL1*, *ATHB2*, and *HFR1* were significantly higher as expected. When green light was added to the same "Low R/Fr" condition, the steady-state transcript

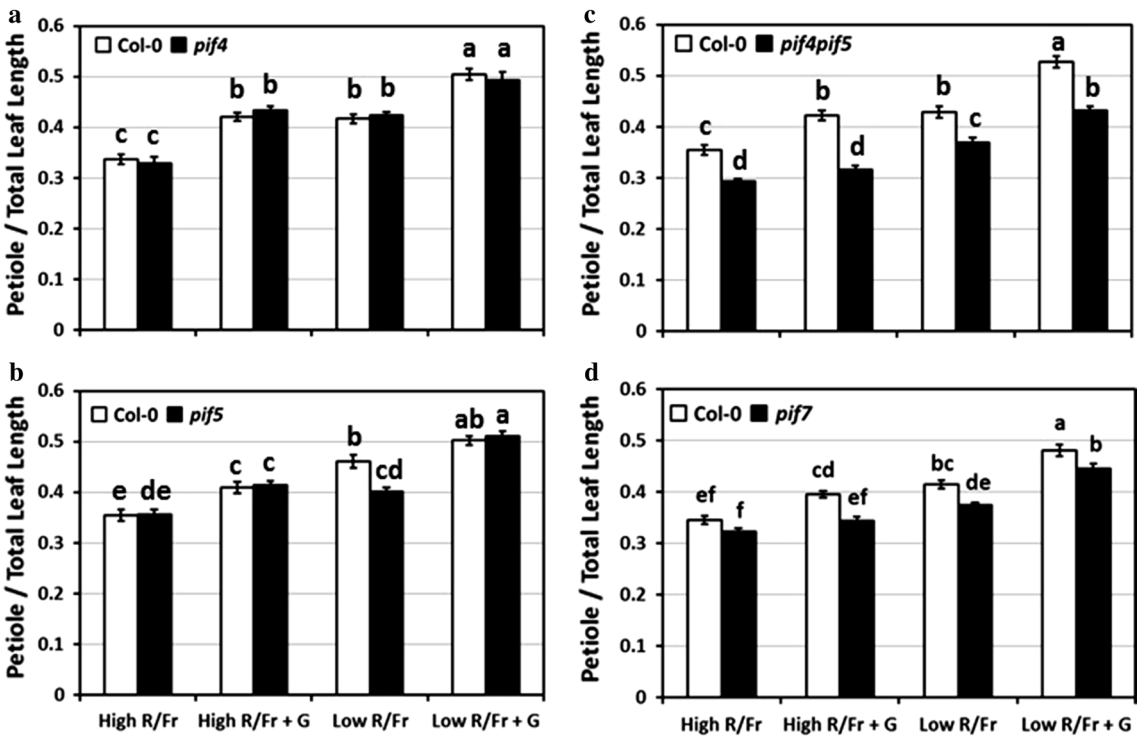

Fig. 3 PIF4, PIF5 and PIF7 are not solely required for the green and far-red additive shade response. Arabidopsis wild-type Col-0 plants and *pif* mutants were grown and treated in the same conditions used in Fig. 1. The mean petiole/leaf ratio of **a** *pif4* mutant plants, **b** *pif5* mutant plants, **c** *pif4pif5* double mutant plants, and **d** *pif7* mutants, under four different light conditions. Measurements were derived from the third true leaves from 6 to 9 individual plants. *Error bars* represent SE. Tukey's HSD test ($\alpha = 0.05$) was used to assess statistical significance. *Different letters* represent statistically discernable means

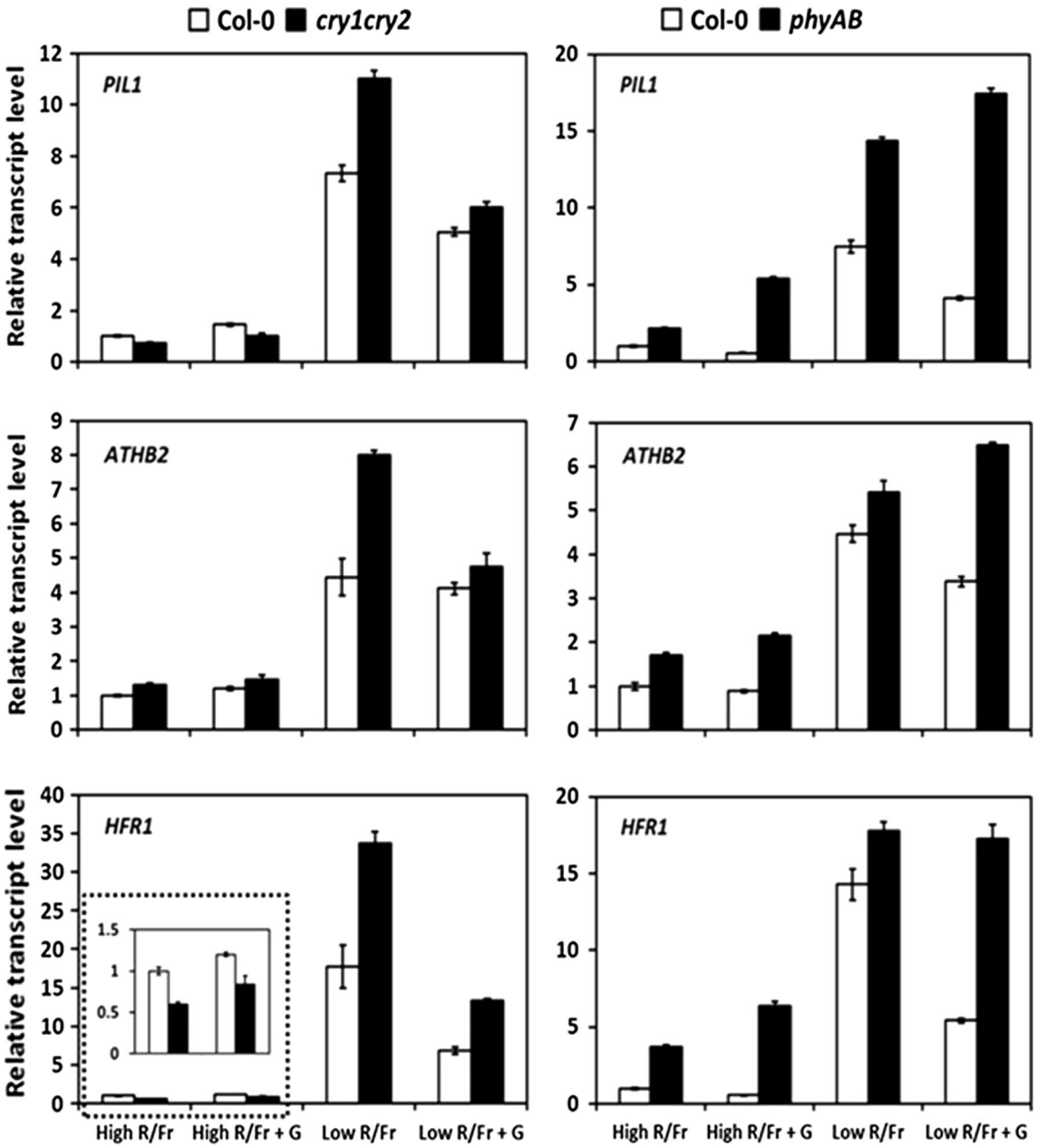

Fig. 4 Comparison of shade avoidance-related gene expression levels in wild-type, *cry1cry2*, and *phyAphyB* mutants. Plants were grown and treated in the same conditions used in Fig. 1. Gene expression levels were quantified using real-time qPCR. Transcript levels were normalized to the "High R/Fr" condition of wild-type Col-0 plants. *YLS8* (AT5G08290) was used as reference gene. *Error bars* represent SE from three technical replicates. At least two independent experiments were performed with similar expression patterns

levels of *PIL1* and *HFR1* were reduced to approximately half of the levels "Low R/Fr" condition with no added green. Only a modest decrease was observed in *ATHB2* transcripts. A similar trend was also observed in *cry1cry2* mutant plants. Here all three transcripts were induced by low R/Fr, and added green light resulted in lower accumulation. The *phyAphyB* mutants show the induction of the shade-related transcripts and the addition of green light does not reverse the trend.

Discussion

In the Arabidopsis rosette, a shift to low R/Fr ratio results in a conspicuous change in petiole elongation growth. Green light effects add to the far-red induced changes (Fig. 1). The same response is observed when green light is added to a background of red and blue (Zhang et al. 2011), so far-red light is not required to visualize the green light response. Figure 1 result shows that the green and far-red responses

can be induced together, and that the green response adds to the far-red response. It also indicates that the green light is not contributing meaningfully as R to the R/Fr ratio, as the morphological effect is more like that induced by Fr. This aspect becomes important when considering the results from gene expression experiments in Fig. 4.

Green light has been proposed to oppose blue responses, such as stem elongation and flowering, through the neutral semiquinone flavin of the receptor's chromophore (Banerjee et al. 2007; Bouly et al. 2007) or autophosphorylation of cryptochromes caused by a photolyase-like cyclic electron shuttle (Sancar 2003). The conditions used in Fig. 1 were used to test the response of *cry* mutants for far-red/green light interactions. The results in Fig. 2a showed that *cry* mutants have enhanced shade response compared with wild-type in all light conditions. The *cry* mutants responded normally to added green and/or far-red light with increased petiole elongation. The results shown indicated that the further elongation of petiole by added green to far-red is not attributable to inactivation of cryptochromes by green light, which is consistent with our previous report (Zhang et al. 2011). The *phot1phot2* mutant plants also have been shown to play a role in shade avoidance (Casal 2013), but here the results in Fig. 2b indicate they do not influence the effect of green light in low R/Fr conditions.

The results from phytochrome mutants were not easily interpreted. There are two forms of phytochromes, the biologically inactive Pr form and the biologically active Pfr form (Franklin and Quail 2010). The relative ratio of active (Pfr) versus total phytochrome (Ptot) is in a dynamic photoequilibrium determined by surrounding light environment (Casal 2013). It is possible that the added green light may affect the steady-state photoequilibrium of phytochromes in "High R/Fr" or "Low R/Fr" conditions. Indeed, green light can sufficiently stimulate phytochrome-mediated seed germination (Shinomura et al. 1996). Intuitively, the addition of visible light input would potentially drive the equilibrium toward a higher Pr/Pfr ratio, which would ultimately decrease shade responses. However, the opposite is observed.

The experiments in the *phyAphyB* mutant plants were inconclusive. As shown in Fig. 2c, *phyAphyB* mutants did not respond to low R/Fr ratio, as anticipated. They also did not respond to supplementation with green light. One interpretation is that phyA or phyB mediate the green light response, but this has been tested before in the absence of far-red light (Zhang et al. 2011), and the green light response was observed. In the presence of far-red light, phyA and/or phyB are required to visualize the green light response, and this finding is difficult to reconcile. One hypothesis is that the other phytochromes (phyC–phyE) or downstream products exert far-red effects that block the green light elongation response, as roles in the low R/Fr environment for phyD and phyE are well described (Devlin et al. 1999; Franklin et al. 2003). Consistent with these data, the *hfr* mutants did not affect the normal green response (not shown). Future experiments will examine multiple phy mutants, yet preliminary experiments suggest these trials may not provide useful information. The plants show strong internode and petiole elongation responses and it is difficult to observe green-light-induced elongation when the basal mutant shows extensive elongation growth.

It was then of interest to see if core phy signaling molecules showed the green light effect. For instance the PIF4 and PIF5 transcription factors have been identified as central elements for phyB- and cry1-mediated shade avoidance responses (Lorrain et al. 2008; Keller et al. 2011). PIF7 also shows important function in shade response during early seedling development (Li et al. 2012). Results shown in Fig. 3 suggest that loss functions of *PIF4*, *PIF5*, or both did not affect the green or far-red effects on shade response. Similarly, *pif7* deficient mutant retains the ability to respond to green and far-red-induced petiole elongation growth (Fig. 3d). Although *pif4pif5* and *pif7* mutant plants showed reduced petiole elongation growth compared with wild-type plants in all light conditions, the far-red-induced petiole growth and the additional growth promoted by added green light were still statistically discernible. These results show that there is redundant effects of PIFs that did not block the response to far-red when mutagenized, and that the green effects were also visible in these mutants. This finding reinforces that PIFs can act redundantly, and do not genetically separate the far red and green responses.

To further explore other possible common signaling mechanisms we examined shade-related gene expression patterns of plants grown under the same green and far-red combined treatments (Fig. 4). In experiments with only red and blue light, added green-light did not induce shade transcripts, except in cryptochrome mutants, suggesting active repression of the pathway by green light (Zhang et al. 2011). The same test was performed in the presence of far-red light. In wild-type Arabidopsis plants the transcript levels of *PIL1*, *ATHB2/HAT4*, and *HFR1* were highly induced by decreasing R/Fr ratio, but were not induced by added green light (Fig. 4). Supplementation of green to "Low R/Fr" conditions actually reduced the expression levels of these three genes. Similar expression patterns were also observed in *cry1cry2* mutant plants, but not in *phyAphyB* mutants. There are several ways to interpret these findings. First it is possible that the reduction in the expression of shade-associated genes by adding green may be due to green light decreasing the Pr/Pfr ratio. However, this is unlikely, as there is no evidence of such a shift from the morphological leaf characteristics in Fig. 1.

Alternatively, this outcome suggests that the green wavelengths have a specific effect on shade transcripts, and

that it is not cryptochrome dependent (Fig. 4). The same response is absent in *phyAphyB* double mutants, where transcript levels still increase under low R/Fr conditions. Again, one interpretation (as in Fig. 2) is that the far-red response is being modulated by the remaining phytochromes, and that the green treatment is not able to suppress their action. This interpretation is more likely than phyA and/or phyB acting as the principle green light receptors, again because co-irradiation with far-red and green exaggerates the phy-mediated far-red response.

Canopy shade presents an environment where red and blue light are filtered, leaving an environment rich in far-red and some green light. Both green and far-red light have common effects on rosette morphology. The results of this study use LED illumination in the laboratory to reconstruct situations where green and far-red light may be enriched independently and together to test how these systems interact to guide shade responses. These results reinforce the hypothesis that green light induces shade avoidance responses through a pathway likely independent from the phy red/far-red sensing system, but requiring its function to exert its effect. This interpretation is based on observations that two independent signaling pathways may drive morphological changes that are greater in magnitude than either treatment alone, and one persists in the absence of the other. The findings support the interpretation that shade responses are an output distilled from information gathered throughout the spectrum, and that wavelengths thought to be benign may exert strong effects that augment the classical phytochrome response.

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