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Current Biology

Shade Promotes Phototropism through Phytochrome B-Controlled Auxin Production

Highlights

- Shade promotes phototropism in green seedlings
- Phytochrome B negatively regulates phototropism in green seedlings
- PIFs are essential for robust phototropism in green, but not etiolated, seedlings
- The developmental stage controls the crosstalk between phototropins and phytochromes

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In Brief

Goyal et al. show that foliar shade promotes growth toward the light (phototropism). This response involves the crosstalk between two classes of photoreceptors, the blue-light-sensing phototropin and red-/far-red-sensing phytochromes. The authors propose that this mechanism enhances access of plants to light and hence photosynthetic light capture.

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Shade Promotes Phototropism through Phytochrome B-Controlled Auxin Production

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SUMMARY

Phototropism is an asymmetric growth response enabling plants to optimally position their organs. In flowering plants, the phototropin (phot) blue light receptors are essential to detect light gradients. In etiolated seedlings, the phototropic response is enhanced by the red/far-red (R/FR)-sensing phytochromes (phy) with a predominant function of phyA. In this study, we analyzed the influence of the phytochromes on phototropism in green (de-etiolated) *Arabidopsis* seedlings. Our experiments in the laboratory and outdoors revealed that, in open environments (high R/FR ratio), phyB inhibits phototropism. In contrast, under foliar shade, where access to direct sunlight becomes important, the phototropic response was strong. phyB modulates phototropism, depending on the R/FR ratio, by controlling the activity of three basic-helix-loop-helix (bHLH) transcription factors of the PHYTOCHROME INTERACTING FACTORs (PIFs) family. Promotion of phototropism depends on PIF-mediated induction of several members of the *YUCCA* gene family, leading to auxin production in the cotyledons. Our study identifies PIFs and YUCCAs as novel molecular players promoting phototropism in photoautotrophic, but not etiolated, seedlings. Moreover, our findings reveal fundamental differences in the phytochrome-phototropism crosstalk in etiolated versus green seedlings. We propose that in natural conditions where the light environment is not homogeneous, the uncovered phytochrome-phototropin co-action is important for plants to adapt their growth strategy to optimize photosynthetic light capture.

INTRODUCTION

Land plants respond to light cues with five photoreceptor families classified according to their light absorption properties: UVR8 absorbing UV-B; phototropins, cryptochromes, and Zeitlupes absorbing blue/UV-A; and the phytochromes primarily absorbing red/far-red (R/FR) (reviewed in [1]). Some light responses are specifically mediated by a single photoreceptor, whereas others depend on photoreceptor coordination to integrate various light cues to optimize plant growth and development [2, 3]. For example, phytochromes and cryptochromes cooperatively promote de-etiolation, whereas phytochrome B (phyB) and cryptochrome 2 (cry2) antagonistically regulate the transition to flowering [2, 3]. Photoreceptor crosstalk also occurs during shade avoidance and phototropism, two growth responses enabling plants to maximize photosynthesis in low-light conditions [4, 5]. Vegetative shade is detected by phytochromes and cryptochromes because light under a canopy is characterized by a low R/FR ratio and low blue light [5, 6]. Shade responses are inhibited in the presence of UV-B by the UVR8 photoreceptor [7, 8]. Interestingly, these three photoreceptor families modulate the activity of PHYTOCHROME INTERACTING FACTORs (PIFs), identifying these basic-helix-loop-helix (bHLH) transcription factors as a potential point of integration ([7, 9–12]; see also the accompanying paper by de Wit et al. in this issue of *Current Biology* [13]). PIFs regulate the expression of a broad range of genes in shade conditions, including genes involved in auxin biosynthesis, transport, and signaling [12, 14–16].

During phototropism, plants shift the growth axis of organs, such as stems, to reorient themselves toward the light source [17]. This response is controlled by phototropins phot1 and phot2 in *Arabidopsis*. phot1 functions across a broad range of blue-light fluence rates, whereas phot2 is important in high-light intensities [18, 19]. Members of NRL (NPH3/RPT2-like) and PKS (PHYTOCHROME KINASE SUBSTRATE) protein families play a major role in early steps of phototropin signal transduction [20–26]. Subsequently, a lateral auxin gradient is formed across the hypocotyl by means of a complex process requiring auxin efflux carriers from the PINFORMED (PIN) family, the ABCB19 transporter, and regulation of the apoplastic pH [27, 28]. Auxin redistribution allows asymmetric growth in hypocotyls, leading to phototropic bending.

In etiolated seedlings, phytochromes do not detect the light gradient per se; however, they manipulate the magnitude of the phototropic response [4]. Phytochromes, with a predominant function of phytochrome A (phyA), enhance phototropism

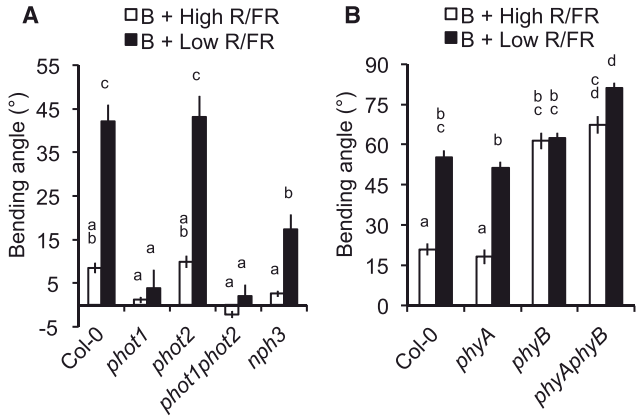

Figure 1. The Photoreceptors phot1 and phyB Regulate Phototropism in De-etiolated Seedlings

Three-day-old de-etiolated seedlings of WT (Col-0), phototropin, and *nph3* mutants (A) and phytochrome mutants (B) were subjected to blue light from the side, while red and far-red lights were provided from above to simulate control and shade conditions as described in the Experimental Procedures. Bending angles were measured after 24 hr of light treatments. Bars represent mean bending angle ± SE ($n \geq 20$). Small alphabetic letters above each bar indicate statistically significant groups at p value < 0.01 obtained by ANOVA followed by the post hoc Tukey's honestly significant difference (HSD). See also Figure S1.

by modulating phototropin signaling at several steps [4, 29, 30]. In particular, phyA promotes the expression of positive regulators of this signaling cascade, including *PKS1*, *RPT2*, and *ABCB19* [31–33]. Given that, for the past 150 years, etiolated seedlings have been the model of choice to study phototropism [17], we do not know whether phytochromes modulate phototropin-mediated responses in green seedlings. Photoautotrophic *Cucumis sativus* and *Boehmeria nipononivea* plantlets show a stronger reorientation of stem growth under canopy shade than in open places [34–36]. However, this could be the result either of stronger blue-light gradients in the presence of canopies or even of phytochrome perception of R/FR ratio gradients in de-etiolated tissues [34, 35, 37, 38]. Noteworthy, in sesame, blue-light-induced phototropism is promoted by red light given as a pretreatment to de-etiolate the seedling; however, red light given simultaneously with unilateral blue light inhibits bending compared to far-red light [39]. These results suggest that, in de-etiolated sesame seedlings, reduced phytochrome activity simultaneously with the exposure to a blue-light gradient enhances phototropism, which contrasts with what is typically observed in etiolated seedlings.

The aim of our study was to determine whether shade signals modulate phototropism in *Arabidopsis* and, if so, uncover the underlying molecular mechanisms. We found that the R/FR ratio has a strong impact on the phototropic potential of green *Arabidopsis* seedlings. phyB inhibits phototropism in open environments by limiting the activity of several members of the PIF family. In the shade, PIFs promote phototropism by enhancing auxin production. Our work uncovered new actors regulating phototropism, specifically in green seedlings, and novel mechanisms underlying photoreceptor crosstalk.

RESULTS

phyB Modulates Phototropism in Green Seedlings Depending on the Red/Far-Red Ratio in the Environment

Green (de-etiolated) *Arabidopsis* seedlings undergo phototropism [40], but whether phytochromes also regulate phototropin signaling at this developmental stage remains unknown. Unilateral blue (B) light, but not R or FR, provided a phototropic cue to both etiolated and de-etiolated seedlings (Figure S1A). When combined with B light, unidirectional R or FR did not modify phototropism of etiolated seedlings, however, modulated the phototropic response of green seedlings. In such photoautotrophic seedlings, phototropism was inhibited in the presence of R light whereas FR light enhanced hypocotyl bending toward the blue light source (Figure S1A). In natural environments, plants sense the R/FR ratio as a cue about the presence of competitors. Thus, we tested the impact of the R/FR ratio on the blue-light-induced phototropic response. The experiment was designed such that blue light was provided from the side and R/FR was provided from above in order to mimic growth toward a blue light maximum in open (e.g., sun in the morning) versus crowded habitats (e.g., canopy gap). We observed that de-etiolated seedlings were largely unresponsive toward the blue-light gradient in high R/FR (Figure 1), similar to the simultaneous unilateral irradiation with R and blue light (Figure S1A). On the contrary, hypocotyl bending toward blue light in low R/FR was strong (Figure 1) in accordance with unilateral application of blue and FR light (Figure S1A). We conclude that, in de-etiolated seedlings, phototropism toward blue light is modulated by the R/FR ratio.

To identify the primary photoreceptors regulating this response in our experimental conditions, we analyzed several phytochrome and phototropin mutants. The hypocotyls of *phot1* and *phot1phot2* seedlings failed to grow toward the blue light direction in both high and low R/FR, whereas *phot2* behaved like the wild-type (Figure 1A). These results indicate that phot1 is the primary phototropin controlling hypocotyl growth reorientation in green seedlings in our setup. The analysis of phytochrome mutants revealed that, whereas *phyA* seedlings displayed a largely wild-type response, *phyB* and *phyAphyB* seedlings did not show differential phototropic bending in response to different R/FR ratios (Figure 1B). Moreover, *phyB* mutants in the high R/FR ratio and wild-type in the low R/FR ratio showed a similarly strong reorientation toward blue light (Figure 1B). These findings suggest that phyB negatively regulates phototropism in the high R/FR ratio. We conclude that, in de-etiolated seedlings, phot1 is essential for phototropic bending, whereas phyB does not perceive the light gradient. However, phyB is key to modulate the phot1 response in different R/FR ratios.

PIF4, PIF5, and PIF7, Acting Downstream of phyB, Are Necessary and Sufficient to Promote Phototropism

Next, we studied known signaling components acting downstream of phot1 and phyB to understand the mechanisms underlying this photoreceptor crosstalk. First, we tested the importance of key phototropism-signaling components in etiolated seedlings, such as NPH3 and the PINs. The *nph3* mutant showed a marked reduction in phototropic bending (Figure 1A), indicating that its activity is important to respond to a blue-light gradient in de-etiolated seedlings. It has been shown that PIN3,

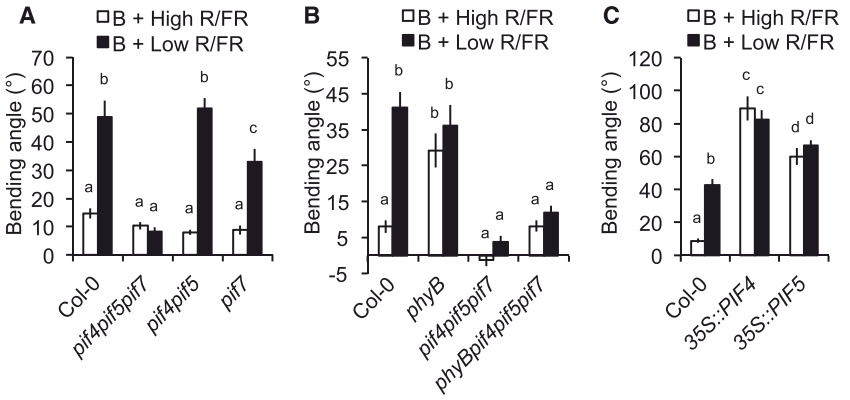

Figure 2. PIF Transcription Factors Act Downstream of phyB to Regulate Phototropism

Three-day-old de-etiolated seedlings were subjected to light conditions as described in Figure 1. Bars represent mean bending angle ± SE ($n \geq 20$). Small alphabetic letters above each bar indicate statistically significant groups at p value < 0.01 obtained by ANOVA followed by the post hoc Tukey's HSD. See also Figure S2.

(A) Col-0, *pif4pif5pif7*, *pif4pif5*, and *pif7*.

(B) Col-0, *phyB*, *pif4pif5pif7*, and *phyBpif4pif5pif7*.

(C) Col-0, 35S::PIF4, and 35S:PIF5.

PIN4, and PIN7 co-operate to enable hypocotyl phototropism in etiolated seedlings [41]. We observed that the *pin3pin4pin7* triple mutant was defective in phototropism in green seedlings (Figure S1B), suggesting that *PIN* activity is also important in de-etiolated seedlings, possibly to establish an auxin gradient. Thus, several phototropin-signaling elements that are essential in etiolated seedlings are also important for phototropism in green seedlings, irrespective of the R/FR condition.

PIF4, PIF5, and PIF7 play a major role downstream of phyB to promote shade-avoidance responses [5], prompting us to analyze their role during phototropism in green seedlings. Interestingly, in de-etiolated seedlings, the phototropic response toward B light was reduced in *pif7*, *pif4pif5*, and *pif4pif5pif7* mutants (Figure S2A). In contrast, the etiolated *pif4pif5pif7* triple mutant showed a phototropic response that was undistinguishable from wild-type seedlings (Figure S2B). Therefore, PIF4/5/7 promote phototropism in green, but not etiolated, seedlings. The phototropic response of all three de-etiolated *pif* mutants was similar to that of the wild-type under a high R/FR ratio (Figure 2A). However, under a low R/FR ratio, *pif4pif5* showed a normal response and *pif7* showed reduced phototropism, whereas the *pif4pif5pif7* triple mutant had strongly reduced phototropism that no longer responded to the R/FR ratio (Figure 2A). To determine whether these transcription factors act downstream of phyB in modulating phototropism, we generated a *phyBpif4pif5pif7* quadruple mutant. Similar to the *pif4pif5pif7* triple mutant, *phyBpif4pif5pif7* seedlings were largely insensitive to a blue-light gradient both in high and low R/FR (Figure 2B). This epistatic relationship suggests that, in green seedlings, these three PIFs act downstream of phyB to control phototropism. A prediction of this model is that PIF overexpression would result in a strong phototropic response irrespective of the R/FR ratio. Indeed, the phototropic response of *PIF4* and *PIF5* overexpressing lines was higher than that of the wild-type and was no longer inhibited by a high R/FR ratio (Figure 2C). Together, these results indicate that PIF4/5/7 are essential for phototropism in green, but not etiolated, seedlings (Figures 2, S2A, and S2B). Moreover, they suggest that phyB-mediated control of PIF4/5/7 underlies shade modulation of phototropism (Figure 2).

PIFs Regulate Phototropism by Controlling the Expression of *YUCCA* Genes

PIFs mediate shade-regulated auxin production by controlling the expression of *YUC2*, *YUC5*, *YUC8*, and *YUC9* [12, 14], suggesting that, in green seedlings, PIF-regulated auxin production may control phototropism. To test this hypothesis, we first analyzed *YUC* expression in our experimental conditions. Our data revealed that *YUC2*, *YUC5*, *YUC8*, and *YUC9* expression was induced by a low R/FR ratio in a PIF-dependent manner (Figure 3A). Moreover, a *yuc2yuc5yuc8yuc9* (*yucQ*) mutant was strongly impaired in shade-enhanced phototropism, highlighting the importance of those four *YUC* genes for this process in green seedlings (Figure 3B). Similarly, when subjected to a blue-light gradient in the absence of any additional R and/or FR light, the green *yucQ* seedlings showed a weak phototropic response (Figure S2C).

We also tested the role of TRYPTOPHAN AMINOTRANSFERASE of *ARABIDOPSIS* 1 (TAA1), an enzyme acting upstream of YUCCA in the auxin biosynthetic pathway because of its importance for several shade-induced responses [42]. The *sav3/taa1* and *yucQ* mutant showed a similar shade-induced hypocotyl elongation defect (Figure S3A). However, we observed robust shade-regulated phototropism in *taa1*, but not in *yucQ* (Figure 3B). Moreover, the *taa1/sav3* mutant showed a marginal defect in responding to unidirectional blue light (Figure S2C). This indicates that modulation of phototropism under these experimental conditions did not depend on the activity of TAA1. Moreover, it suggests that shade modulation of the phototropic response is not simply a consequence of the growth potential of the seedlings in different conditions. To test this further, we examined phototropism in mutants defective in hypocotyl growth. The analysis of a *bri1* mutant revealed that, whereas it grew considerably slower than the wild-type, its phototropic response was similar to that of the wild-type in a low R/FR ratio (Figure S3B). On the contrary, the *hy5hfr1* mutant showed enhanced growth in a low R/FR ratio but had a reduced phototropic response (Figure S3B). Interestingly, the *hy5hfr1* mutant in a high R/FR ratio grew at a similar rate than wild-type in a low R/FR ratio, but we observed a large difference in hypocotyl bending (Figure S3B). These results indicate that the differences in phototropic bending triggered by the R/FR ratio cannot simply be explained by the growth potential in different conditions and/or genotypes.

Our *YUC* gene expression analysis and the phenotype of the *yucQ* mutant suggest that PIF-mediated *YUC* expression, which primarily occurs in cotyledons [12, 42], is a key step in the modulation of phototropism by shade. To test this hypothesis, we

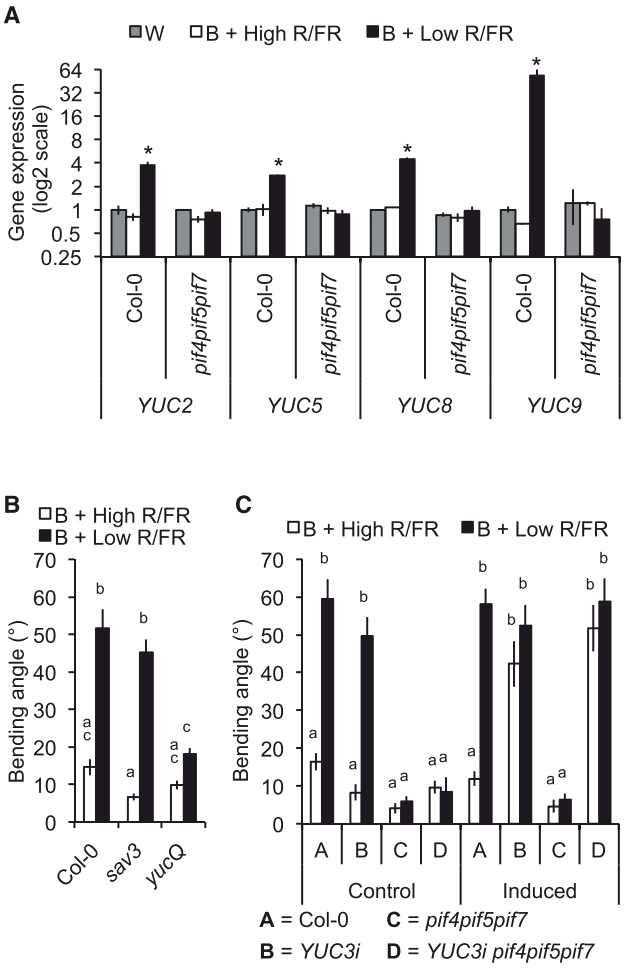

Figure 3. PIFs Modulate Phototropism by Transcriptional Regulation of *YUCCA* Genes

(A) Three-day-old de-etiolated seedlings of Col-0 and *pif4pif5pif7* were treated at ZT0 with light conditions as described in Figure 1 for 1 hr. RNA was extracted from the untreated (W) and the light-treated seedlings, and qPCR was performed. Data are mean expression ± SE of *YUCCA* genes normalized to two control genes (*UBC* and *YSL8*) and expressed relative to Col-0 in untreated condition. Mean values are obtained from three biological replicates, each quantified with three technical replicates. Asterisks indicate the statistical significance compared to Col-0 untreated (p value < 0.05; Student's t test).

(B and C) Three-day-old de-etiolated seedlings were treated with light conditions as described in Figure 1.

(B) Col-0, *sav3* and *yucQ*.

(C) Col-0, *YUC3i*, *pif4pif5pif7* and *YUC3i pif4pif5pif7*. For *YUC3* induction, the seedlings were induced with 10 μM estradiol 16 hours prior to light treatments. Bars represent mean bending angle ± SE ($n \geq 20$). Small alphabetic letters above each bar indicate statistically significant groups at p value < 0.01 obtained by ANOVA followed by the post hoc Tukey's HSD. See also Figures S2 and S3.

asked whether induction of *YUCCA* expression in green seedlings is sufficient to promote phototropism, as we observed in *PIF*-overexpressing lines. A cotyledon-specific estradiol-inducible *FRO6*::*XVE*::*YUC3* line (*YUC3i*) was analyzed to address this question [43]. We found that, whereas in control conditions, the *YUC3i* line behaved like the wild-type, upon induction of *YUC3*, we did not observe inhibition of phototropism by a high R/FR ratio, suggesting that *YUC3* expression in cotyledons was sufficient to promote phototropism (Figure 3C). In order to determine whether the phenotype of the *pif4pif5pif7* triple mutant can be complemented by PIF-independent *YUC* transcription, we crossed the *pif4pif5pif7* triple mutant with the *FRO6*::*XVE*::*YUC3* line and selected *YUC3i pif4pif5pif7* seedlings. We observed that induction of *YUC3* in the *pif4pif5pif7* triple mutant background rescued the inhibition of phototropism in both high and low R/FR (Figure 3C). This leads us to conclude that PIF-mediated *YUC* expression is a key step in PIF-mediated phototropism regulation.

We have previously shown that cotyledons, the major auxin production organs, are largely dispensable for phototropism in etiolated seedlings whereas, in de-etiolated seedlings, "decapitation" leads to a stronger phototropic defect [44]. This difference might be explained by the requirement of auxin production for phototropism in green seedlings (Figure 3), whereas in etiolated seedlings, redistribution of auxin present in the hypocotyls might be sufficient to promote phototropism. We therefore characterized etiolated *yucQ* seedlings and found that the *yucca* quadruple mutant displayed normal phototropism; if anything, the mutant reoriented growth more efficiently than the wild-type (Figure S2B). This suggests that auxin synthesis by YUC2, YUC5, YUC8, and YUC9 is important for phototropism specifically in photoautotrophic seedlings. Our characterization of the *pif4pif5pif7* and *yucQ* mutants and a previous analysis of phototropism in de-etiolated seedlings [40] reveal the existence of different signaling mechanisms controlling phototropism in etiolated versus green seedlings.

PIFs Are Important to Promote Phototropism in Natural Conditions

In order to verify the relevance of our observations obtained in laboratory conditions using monochromatic light sources, we decided to test the phototropic response of key genotypes in natural conditions where background light levels and temperature fluctuate. Because of their striking phenotype in the laboratory, we focused on the *phyB* and *pif4pif5pif7* mutants (Figures 1 and 2). De-etiolated seedlings grown on vertical plates were placed outdoors under unilateral vegetative shade from tall grasses (Figures S4A, S4C, and S4D). Wild-type and *phyB* seedlings reoriented hypocotyl growth away from the vegetative shade with a significantly stronger response in the *phyB* background (Figure 4A). In contrast, similar to our observation in the laboratory, the *pif4pif5pif7* triple mutant was severely defective in phototropic bending (Figure 4A). We further examined the impact of the R/FR ratio on phototropism by comparing phototropism in open field versus shade conditions. In order to create similar blue-light gradients in both conditions, we used a black filter placed on the north side (southern hemisphere) of the seedlings used for control condition (high R/FR) and a combination of tall grasses and an orange filter (cutting blue light) for low R/FR conditions (Figures S4B and S4E). This way, the seedlings were subjected to a similar lateral blue-light gradient but either in high R/FR (black filter) or low R/FR (vegetation + orange filter) conditions (Figure S4C). As observed in laboratory conditions, wild-type seedlings showed enhanced phototropism in low R/FR (Figure 4B). Also consistent with observations made in

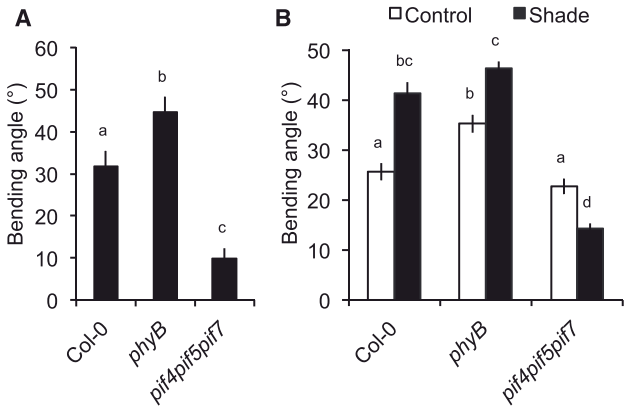

Figure 4. PIF4/5/7 Are Important for Robust Phototropism in Natural Environments

(A) Three-day-old de-etiolated seedlings of Col-0, *phyB*, and *pif4pif5pif7* grown on vertical plates were placed on the south side of vegetative shade from tall grasses for 5 hr before measurement of the phototropic bending angle. Data were pooled from three independent experiments. Bars represent mean bending angle ± SE ($n \geq 130$).

(B) Three-day-old de-etiolated seedlings of Col-0, *phyB*, and *pif4pif5pif7* were subjected to light gradients with the help of black (control) or orange filters + vegetation (shade) placed on the north such that seedlings were exposed to more light coming from south. Bending angle toward the south was measured after 5 hr of the treatment. Data were pooled from six independent experiments. Bars represent mean bending angle ± SE ($n \geq 110$).

Small alphabetic letters above each bar indicate statistically significant groups at p value < 0.05 obtained by ANOVA followed by the post hoc Tukey's HSD. See also Figures S4 and S5.

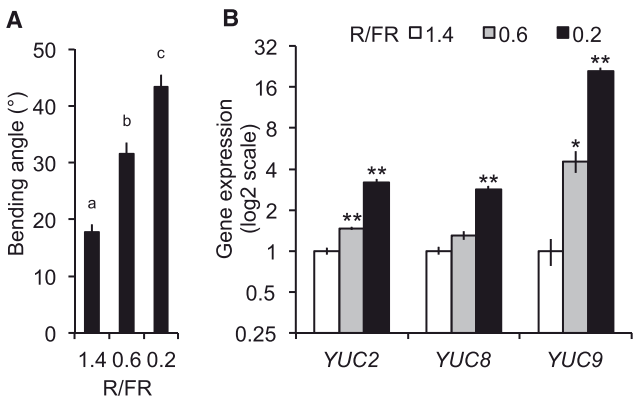

Figure 5. Gradual Enhancement of Phototropism and *YUC* Expression with a Declining R/FR Ratio

(A) Three-day-old de-etiolated Col-0 seedlings were subjected to similar white light gradients in the presence of the indicated R/FR ratios. Our white light source has a R/FR ratio of 1.4, which is close to the R/FR ratio of sunlight. Bending angles were measured after 6 hr of light treatments. Bars represent mean bending angle ± SE ($n \geq 95$). Small alphabetic letters above each bar indicate statistically significant groups at p value < 0.01 obtained by ANOVA followed by the post hoc Tukey's HSD.

(B) Three-day-old de-etiolated Col-0 seedlings were treated at ZT0 with the same light conditions as in (A) for 1 hr. RNA was extracted from the samples, and qPCR was performed. Data are mean expression ± SE of *YUCCA* genes normalized to two control genes (*UBC* and *YSL8*) and expressed relative to R/FR ratio 1.4. Mean values are obtained from four biological replicates, each quantified with three technical replicates. Asterisks indicate the statistical significance compared to R/FR ratio 1.4 (*p < 0.05; **p < 0.01; Student's t test). See also Figure S5.

the laboratory, the *phyB* mutant was more phototropic than the wild-type in high R/FR conditions. This trend was also observed in low R/FR, a difference that we did not observe in the laboratory (Figure 4B). However, as observed in the laboratory, the response of the wild-type in low R/FR was similar to the response of *phyB* in a high R/FR ratio. Finally, the *pif4pif5pif7* mutant had a reduced phototropic response when the R/FR ratio was low (Figure 4B). Globally, these experiments confirmed the importance of the PIFs and phyB in the control of phototropism in realistic environmental conditions.

The *phyB* mutant showed a residual enhancement of phototropism by true canopy shade (Figure 4B), but not by low R/FR in the laboratory (Figure 2B). phyB primarily controls shade responses elicited by a reduction of the R/FR ratio that already occurs in the absence of direct shading (neighbor proximity) [45]. Foliar shade leads to lower blue light levels and a further reduction of the R/FR ratio, conditions that are sensed by phyB and the cryptochromes [5, 45]. The difference between laboratory and outdoors experiments therefore suggested that the cryptochromes may also inhibit phototropism. When analyzed in natural shade conditions, we found that *cry1* and *cry1cry2* double mutants also displayed an exaggerated phototropic response (Figure S5A). We further investigated the role of the cryptochromes in a controlled environment where seedlings were grown in the presence of white light supplemented or not with additional FR light to mimic shade signals (Figure S5B). Under these conditions, *cry1* displayed an enhanced phototropic response both in high and low R/FR whereas *phyB* displayed a constitutively strong bending response that was not enhanced by a reduction of the R/FR ratio (Figure S5C). Collectively, these results confirm a role for the cryptochromes in the modulation of phototropism by shade. Moreover, because true shade leads to a stronger decline of the R/FR ratio than the presence of non-shading neighbors, these results suggest that shade-induced phototropic enhancement may be a gradual response with a stronger impact as the R/FR ratio declines. We tested this hypothesis by analyzing phototropism in seedlings exposed to white light with different R/FR ratios. Indeed, the phototropic response was inversely proportional to the R/FR ratio (Figure 5A). Moreover, in agreement with the importance of shade-induced *YUC* expression promoting phototropism (Figure 3), we observed that particularly *YUC2* and *YUC9* expression gradually increased with a declining R/FR ratio (Figure 5B). We conclude that shade regulation of phototropism is a gradual response that is presumably tuned to the degree of shading.

DISCUSSION

Our results show that the R/FR ratio modulates phototropism under both controlled and field conditions (Figures 1, 4, and 5). Field observations and laboratory experiments have suggested that plants under vegetative shade show more pronounced phototropic responses compared to open field environments [34–36]. In cucumber, part of this response is due to growth away from reflected FR, mediated by phyB

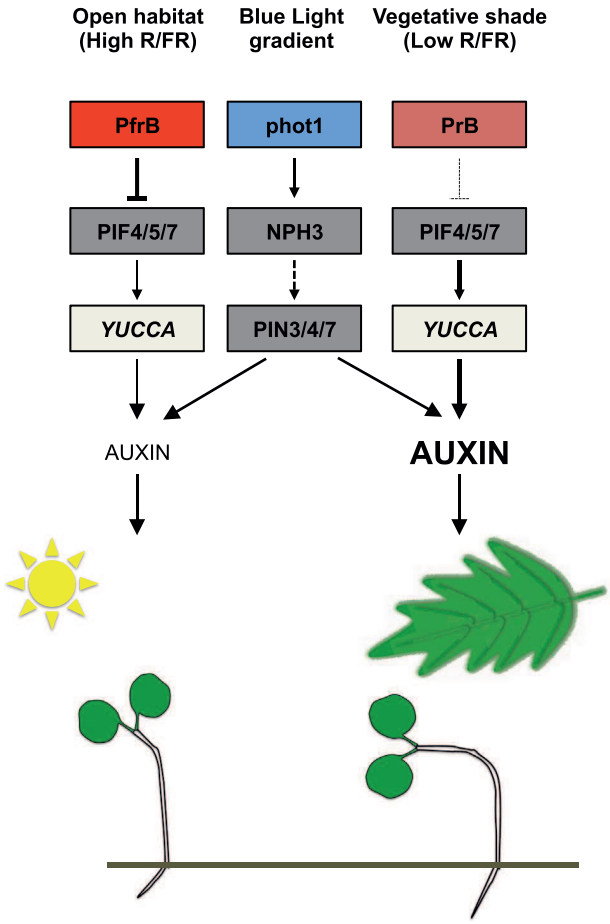

Figure 6. Proposed Model

Schematic representation of a model of shade-regulated phototropism suggested by our studies. On the left, the seedling in an open (high R/FR) environment where phyB is primarily in its active PfrB conformation is shown. Active phyB inhibits the PIFs, thereby leading to reduced *YUC* gene expression, resulting in low auxin levels. On the right, the seedling is in the shade (low R/FR), where phyB is primarily in its inactive PrB conformation. PIFs are released from the inhibitory activity of phyB, leading to high expression of *YUC* genes, resulting in increased auxin levels, which promotes phototropism in de-etiolated seedlings.

[34]. However, whether phytochrome perception of a low R/FR ratio promotes growth towards blue light was unknown [34, 35, 37, 38]. The experiments that we performed in the laboratory circumvent this problem and allowed us to test the effect of the R/FR ratio on phototropism by keeping the blue light stimulation and the amount of photosynthetic light equal (Figures 1, 5, S1A, and S5C). We propose that phyB-mediated regulation of growth orientation in photoautotrophic plantlets contributes to their ability to fill canopy gaps, an important physiological response in dense plant communities (Figures 1, 2, 4, and S5C) [34–37]. In cucumber, phyB controls this response in part by mediating phototropism away from FR-rich light [34, 35]. Our work in *Arabidopsis* indicates that phyB regulation of phototropism toward blue light promotes growth out of the shade (Figures 1, 2, 4, and S5C). This enhancement of the phototropic response gradually increases with a declining R/FR ratio (Figure 5). This suggests that this response is more pronounced in true shade compared to non-shading neighbors that moderately lower the R/FR ratio by reflecting FR light [45]. Moreover, the involvement of cryptochromes in the regulation of phototropism in light-grown seedlings also supports the view that phototropic enhancement is particularly strong in true shade, where blue light and the R/FR ratio are strongly reduced (Figure S5).

We identify PIF4, PIF5, and PIF7 as key factors promoting phototropism in low R/FR (Figures 2, 3, and 4). Moreover, we show that four *YUC* genes whose expression is rapidly induced in a PIF-dependent manner are important PIF target genes regulating this process (Figures 3, 5, and S2C) [12, 14, 46]. PIF4/PIF5 and PIF7 play a predominant role in shade-regulated hypocotyl elongation in response to low B and low R/FR, respectively [6, 9, 13, 47]. The low R/FR promotion of phototropism is also controlled by PIF7 with a clear contribution of PIF4 and PIF5 (Figure 2). Collectively, YUC2, YUC5, YUC8, and YUC9 are essential for low-R/FR-induced hypocotyl growth and phototropism (Figure 3) [46]. However, TAA1/SAV3, which is very important for low-R/FR-induced hypocotyl elongation, plays a minor role in the regulation of phototropism (Figures 3 and S3A) [42]. This reveals an interesting difference between both low-R/FR-induced responses and shows that promotion of phototropism in such conditions does not simply correlate with the growth potential (Figure S3).

Our study illustrates how development modifies the regulation of phototropism signaling and photoreceptor crosstalk. In etiolated seedlings, phytochromes promote phototropism, with phyA playing a predominant role [4, 29–33]. In contrast, in photoautotrophic seedlings, we observed no obvious role for phyA, but phyB is a strong inhibitor of phototropism, particularly in high R/FR ratios (Figures 1, 4, and S5C). Such an antagonistic interaction between phyB and the phototropins has also been observed in the control of leaf flattening [48]. In this situation, the phyB response partially depends on PIF4 and PIF5, but how those PIFs modulate leaf flattening remains unknown [49]. PIF4 and PIF5 were also proposed to inhibit phototropism in etiolated seedlings [50]. However, we did not observe a significant phototropic defect in etiolated *pif4pif5pif7* triple mutant, whereas previously it was reported that *pif4pif5* has a very modest phenotype (Figure S2B) [50]. We conclude that, in etiolated seedlings, PIF4, PIF5, and PIF7 play a minor role. In contrast, in green seedlings, these three PIFs are of great importance to enable phototropic reorientation in all tested conditions (Figures 2, 3, 4, and S2). Based on the phenotypes of loss- and gain-of-function mutants, we conclude that their role is rate limiting in this process (Figure 2). Our study shows that PIFs promote phototropism by YUC-mediated auxin production (Figure 3). Although it is likely that PIFs also regulate this process by controlling the expression of additional genes [14, 15, 50], our finding that *YUC3* induction in cotyledons can complement the phototropic defect of *pif4pif5pif7* shows that auxin production is an important aspect of PIF-mediated phototropic enhancement (Figure 3). Interestingly, phytochromes control the expression of regulators of the phototropic response in both etiolated and in green seedlings, but the nature of these signaling elements is developmental stage dependent (Figures 3 and 6) [31–33].

EXPERIMENTAL PROCEDURES

Plant Material and Growth Conditions

Detailed descriptions of plant material and growth conditions used in this study are provided in the Supplemental Experimental Procedures.

Physiological Analysis of Phototropism and Measurements

For phototropism experiments, 3-day-old de-etiolated seedlings grown in continuous white light were shifted at ZT0 to 10 μmol m^{-2} s^{-1} blue light from one side and 10 μmol m^{-2} s^{-1} red light from above at 22°C for 24 hr. Supplementary far-red light provided from above was adjusted such that R/FR ratio was 6.6 in high-R/FR and 0.18 in low-R/FR conditions. White light gradient experiments were performed by shifting 3-day-old de-etiolated seedlings in 180 μmol m^{-2} s^{-1} white light at 22°C in a black box opened from only one side (Figure S5B). Varying amount of supplemental FR from light-emitting diodeswas provided from above to obtain the required R/FR ratios. Phototropic bending angles and growth rates were determined by a customized MATLAB script developed in C.F. lab (see Supplemental Experimental Procedures for details).

Outdoor Phototropism Analysis

For outdoor phototropism experiments, 3-day-old de-etiolated seedlings were shifted at ZT4 to fields in Buenos Aires, Argentina. The seedlings were either placed to the south side of a grass canopy or a tilted screen was placed between the grasses and the seedlings (Figure S4). A black screen was used to prevent the projection of grass shade on control seedlings (R/FR ratio: 1.2). An orange acetate filter was used to allow the projection of shade on low R/FR ratio (0.4)-treated seedlings.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.001.

AUTHOR CONTRIBUTIONS

Conceptualization, A.G., J.J.C., and C.F.; Investigation, A.G. and E.K.; Resources, V.C.G. and H.R.; Funding Acquisition, C.F.; Writing, A.G. and C.F.; Supervision, J.J.C. and C.F.

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