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Title page

Shade promotes phototropism through phytochrome B-controlled auxin production.

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28

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 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 optimize their growth strategy and hence photosynthetic light capture.

INTRODUCTION

Land plants respond to light cues with five photoreceptor families classified depending on 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 while 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, while 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 bHLH transcription factors as potential point of integration [7, 9-12]. PIFs regulate the expression of a broad range of genes in shade conditions including genes involved in auxin biosynthesis, transport and signaling [12-15].

During phototropism plants shift the growth axis of organs such as stems to reorient themselves towards the light source [16]. This response is controlled by phototropins, phot1 and phot2 in Arabidopsis. phot1 functions across a

broad range of blue-light fluence rates while phot2 is important in high light intensities [17, 18]. Members of NRL (NPH3/RPT2-Like) and PKS (PHYTOCHROME KINASE SUBSTRATE) protein families play a major role in early steps of phototropin signal transduction [19-25]. 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 [26, 27]. Auxin re-distribution 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 by modulating phototropin signaling at several steps [4, 28, 29]. In particular, phyA promotes the expression of positive regulators of this signaling cascade including *PKS1*, *RPT2,* and *ABCB19* [30-32]. Given that for the past 150 years etiolated seedlings have been the model of choice to study phototropism [16], 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 [33-35]. 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 [33, 34, 36, 37]. 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 red light [38]. 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 [39] 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 while FR light enhanced hypocotyl bending towards 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 towards 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 towards 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 towards 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 towards the blue light direction in both high and low R/FR, while *phot2* behaved like the wild type (Figure 1A). These results indicate that phot1 is the primary phototropin controlling hypocotyl growth re-orientation in green seedlings in our setup. The analysis of phytochrome mutants revealed that while *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 towards 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, while 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, PIN4 and PIN7 co-operate to enable hypocotyl phototropism in etiolated seedlings [40]. We observed that the *pin3pin4pin7* triple mutant was defective in phototropism in our 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 towards 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 of a low R/FR ratio *pif4pif5* showed a normal response, *pif7* showed reduced phototropism while 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

195 in high and low R/FR (Figure 2B). This epistatic relationship suggests that in
196 green seedlings these three PIFs act downstream of phyB to control
197 phototropism. A prediction of this model is that PIF over-expression would
198 result in a strong phototropic response irrespective of the R/FR ratio. Indeed,
199 the phototropic response of *PIF4* and *PIF5* overexpressing lines was higher
200 than that of the wild type and was no longer inhibited by a high R/FR ratio
201 (Figure 2C). Together these results indicate that PIF4/5/7 are essential for
202 phototropism in green but not etiolated seedlings (Figures 2, S2A, S2B).
203 Moreover, they suggest that phyB-mediated control of PIF4/5/7 underlies
204 shade modulation of phototropism (Figure 2).
205
206 **PIFs regulate phototropism by controlling the expression of *YUCCA***
207 **genes.**
208 PIFs mediate shade-regulated auxin production by controlling the expression
209 of *YUC2, YUC5, YUC8* and *YUC9* [12, 13] suggesting that in green seedlings
210 PIF-regulated auxin production may control phototropism. To test this
211 hypothesis, we first analyzed *YUC* expression in our experimental conditions.
212 Our data revealed that *YUC2, YUC5, YUC8* and *YUC9* expression was
213 induced by a low R/FR ratio in a PIF-dependent manner (Figure 3A).
214 Moreover, a *yuc2yuc5yuc8yuc9* (*yucQ*) mutant was strongly impaired in
215 shade-enhanced phototropism highlighting the importance of those four *YUC*
216 genes for this process in green seedlings (Figure 3B). Similarly, when
217 subjected to a blue light gradient in the absence of any additional R and/or FR
218 light the green *yucQ* seedlings showed a weak phototropic response (Figure
219 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 [41]. 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 while 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, 41], is a key step in the modulation of phototropism by shade. To test this hypothesis we 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 [42]. We found that while 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 while in de-etiolated seedlings “decapitation” leads to a stronger phototropic defect [43]. This difference might be explained by the requirement of auxin production for phototropism in green seedlings (Figure 3), while 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 [39] 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, 2). De-etiolated seedlings grown on vertical plates were placed outdoors under unilateral vegetative shade from tall grasses (Figures S4A, S4C, S4D). Wild-type and *phyB* seedlings re-orientated 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

294 used a black filter placed on the north side (southern hemisphere) of the
295 seedlings used for control condition (high R/FR) and a combination of tall
296 grasses and an orange filter (cutting blue light) for low R/FR conditions
297 (Figures S4B, S4E). This way the seedlings were subjected to a similar lateral
298 blue light gradient but either in high R/FR (black filter) or low R/FR (vegetation
299 + orange filter) conditions (Figure S4C). As observed in laboratory conditions
300 wild-type seedlings showed enhanced phototropism in low R/FR (Figure 4B).
301 Also consistent with observations made in the laboratory the *phyB* mutant was
302 more phototropic than the wild type in high R/FR conditions. This trend was
303 also observed in low R/FR, a difference that we did not observe in the
304 laboratory (Figure 4B). However, as observed in the laboratory the response
305 of the wild type in low R/FR was similar to the response of *phyB* in a high
306 R/FR ratio. Finally, the *pif4pif5pif7* mutant had a reduced phototropic
307 response when the R/FR ratio was low (Figure 4B). Globally these
308 experiments confirmed the importance of the PIFs and phyB in the control of
309 phototropism in realistic environmental conditions.
310
311 The *phyB* mutant showed a residual enhancement of phototropism by true
312 canopy shade (Figure 4B), but not by low R/FR in the laboratory (Figure 2B).
313 phyB primarily controls shade responses elicited by a reduction of the R/FR
314 ratio that already occurs in the absence of direct shading (neighbor proximity)
315 [44]. Foliar shade leads to lower blue light levels and a further reduction of the
316 R/FR ratio, conditions that are sensed by phyB and the cryptochromes [5, 44].
317 The difference between laboratory and outdoors experiments therefore
318 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 while *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, since 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 [33-35]. However, it was not possible to discriminate whether this pattern resulted from the lower R/FR ratios, differences in the B light gradient, reduced photosynthetic light found in the shade or even phytochrome perception of R/FR gradients within the canopy [33, 34, 36, 37]. 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, S1A, 5 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) [33-36]. In cucumber phyB controls this response in part by mediating phototropism away from FR-rich light [33, 34]. Our work in Arabidopsis indicates that phyB regulation of phototropism towards 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 [44]. 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 2-4). 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, 13, 45]. 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, 46]. 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) [45]. 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, S3A) [41]. This reveals 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, 28-32]. In contrast, in photoautotrophic seedlings we observed no obvious role for phyA but phyB is a strong inhibitor of phototropism particularly in a high R/FR ratios (Figures 1, 4 and S5C). Such an antagonistic interaction between phyB and

389 the phototropins has also been observed in the control of leaf flattening [47].
390 In this situation the phyB response partially depends on PIF4 and PIF5, but
391 how those PIFs modulate leaf-flattening remains unknown [48]. PIF4 and
392 PIF5 were also proposed to inhibit phototropism in etiolated seedlings [49].
393 However, we did not observe a significant phototropic defect in etiolated
394 *pif4pif5pif7* triple mutant while previously it was reported that *pif4pif5* has a
395 very modest phenotype (Figure S2B) [49]. We conclude that in etiolated
396 seedlings PIF4, PIF5 and PIF7 play a minor role. In contrast, in green
397 seedlings these three PIFs are of great importance to enable phototropic
398 reorientation in all tested conditions (Figures 2-4, S2). Based on the
399 phenotypes of loss- and gain-of-function mutants we conclude that their role is
400 rate limiting in this process (Figure 2). Our study shows that PIFs promote
401 phototropism by YUC-mediated auxin production (Figure 3). Although it is
402 likely that PIFs also regulate this process by controlling the expression of
403 additional genes [13, 14, 49], our finding that *YUC3* induction in cotyledons
404 can complement the phototropic defect of *pif4pif5pif7* shows that auxin
405 production is an important aspect of PIF-mediated phototropic enhancement
406 (Figure 3). Interestingly, phytochromes control the expression of regulators of
407 the phototropic response in both etiolated and in green seedlings, but the
408 nature of these signaling elements is developmental stage-dependent
409 (Figures 3, 6) [30-32].
410

Experimental procedures

Plant material and growth conditions

Detailed descriptions of the 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 three-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 hours. 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 three-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 LEDs was provided from above to obtain the required R/FR ratios. Phototropic bending angles and growth rates determined by a customized MATLAB script developed in Fankhauser lab (see supplemental experimental procedures for details).

Outdoor phototropism analysis

For outdoor phototropism experiments three-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).

436 An orange acetate filter was used to allow the projection of shade on low
437 R/FR ratio (0.4)-treated seedlings.
438
439 **Supplemental Information**
440 Supplemental Information includes Figures S1-S5, figure legends and
441 supplemental experimental procedures.
442
443 **Author Contributions**
444 Conceptualization, A.G., J.C. and C.F.; Investigation, A.G., and E.K.;
445 Resources, V. C. G. and H. R.; Funding Acquisition, C.F.; Writing, A. G. and
446 C. F.; Supervision, J.C. and C.F.
447
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Figure legends

Figure 1

The photoreceptors phot1 and phyB regulate phototropism in de-etiolated seedlings.

A and B) 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 hours of light treatments. Bars represent mean bending angle ± S.E. (n≥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 S1

Figure 2

PIF transcription factors act downstream of phyB to regulate phototropism.

A-C) Three-day-old de-etiolated seedlings of the indicated genotypes were subjected to light conditions as described in figure 1. Bars represent mean bending angle ± S.E. (n≥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.

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 one hour. RNA was extracted from the untreated (W) and the light-treated seedlings and quantitative PCR was performed. Data are mean expression ± S.E. 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 of the indicated genotypes were treated with light conditions as described in figure 1. Bars represent mean bending angle ± S.E. (n≥20). In part C, the seedlings were induced with 10µM estradiol 16 hours prior to light treatments. 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.

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 hours before measurement of the phototropic bending angle.

685 Data were pooled from 3 independent experiments. Bars represent mean
686 bending angle ± S.E. (n≥130).
687 B) Three-day-old de-etiolated seedlings of Col-0, *phyB* and *pif4pif5pif7* were
688 subjected to light gradients with the help of black (control) or orange filters +
689 vegetation (shade) placed on the north such that seedlings were exposed to
690 more light coming from south. Bending angle towards the south was
691 measured after 5 hours of the treatment. Data were pooled from six
692 independent experiments. Bars represent mean bending angle ± S.E.
693 (n≥110).
694 Small alphabetic letters above each bar indicate statistically significant groups
695 at p value < 0.05 obtained by ANOVA followed by the post-hoc Tukey's HSD.
696 See also Figures S4 and S5.
697
698 **Figure 5**
699 **Gradual enhancement of phototropism and *YUC* expression with a**
700 **declining R/FR ratio.**
701 A) Three-day-old de-etiolated Col-0 seedlings were subjected to similar white
702 light gradients in the presence of the indicated R/FR ratios. Our white light
703 source has a R/FR ratio of 1.4, which is close to the R/FR ratio of sunlight.
704 Bending angles were measured after 6 hours of light treatments. Bars
705 represent mean bending angle ± S.E. (n≥95). Small alphabetic letters above
706 each bar indicate statistically significant groups at p value < 0.01 obtained by
707 ANOVA followed by the post-hoc Tukey's HSD. B) Three-day-old de-etiolated
708 Col-0 seedlings were treated at ZT0 with the same light conditions as in the
709 panel A for one hour. RNA was extracted from the samples and quantitative

710 PCR was performed. Data are mean expression ± S.E. of *YUCCA* genes
711 normalized to two control genes (*UBC* and *YSL8*) and expressed relative to
712 R/FR ratio 1.4. Mean values are obtained from four biological replicates each
713 quantified with three technical replicates. Asterisks indicate the statistical
714 significance compared to R/FR ratio 1.4 (* p value < 0.05, ** p value < 0.01,
715 Student's t-test). See also Figure S5.
716
717 **Figure 6**
718 **Proposed model**
719 Schematic representation of a model of shade-regulated phototropism
720 suggested by our studies. On the left the seedling in an open (high R/FR)
721 environment where phyB is primarily in its active PfrB conformation. Active
722 phyB inhibits the PIFs thereby leading to reduced *YUC* gene expression
723 resulting in low auxin levels. On the right the seedling is in the shade (low
724 R/FR) where phyB is primarily in its inactive PrB conformation. PIFs are
725 released from the inhibitory activity of phyB leading to high expression of *YUC*
726 genes resulting in increased auxin levels which promotes phototropism in de-
727 etiolated seedlings.

Figure 1

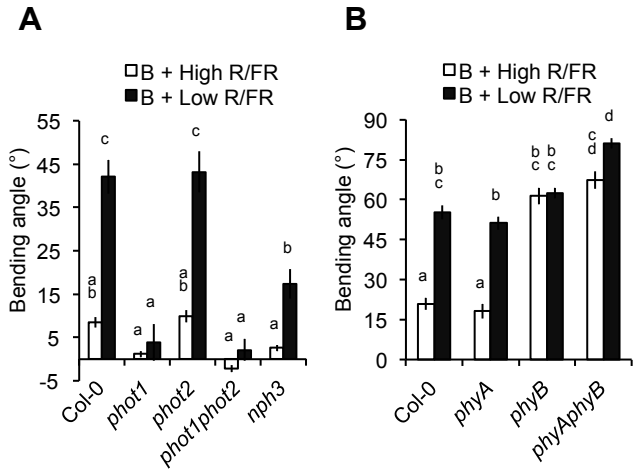

A
B
B + High R/FR
B + Low R/FR
Bending angle (°)
55
45
35
25
15
5
-5
90
75
60
45
30
15
0
Col-0
phot1
phot2
phot1phot2
nph3
phyA
phyB
phyAphyB

Figure 2

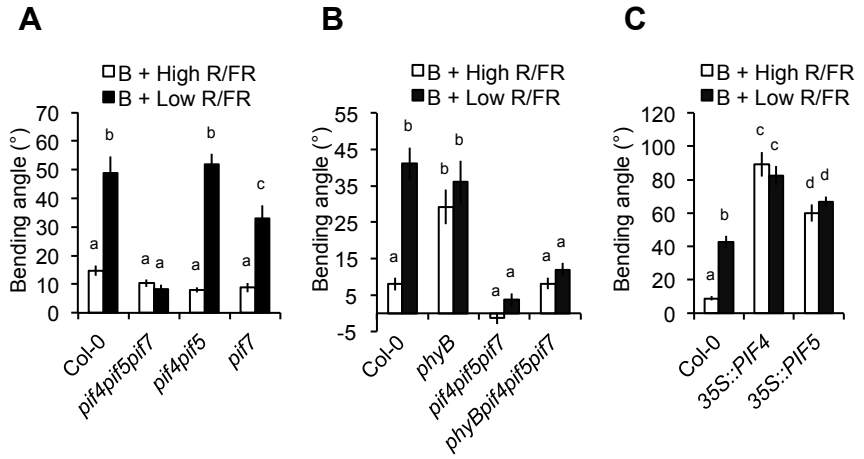

A
B
C
B + High R/FR
B + Low R/FR
Bending angle (°)
Col-0
pif4pif5pif7
pif4pif5
pif7
phyB
phyBpif4pif5pif7
35S::PIF4
35S::PIF5

Figure 3

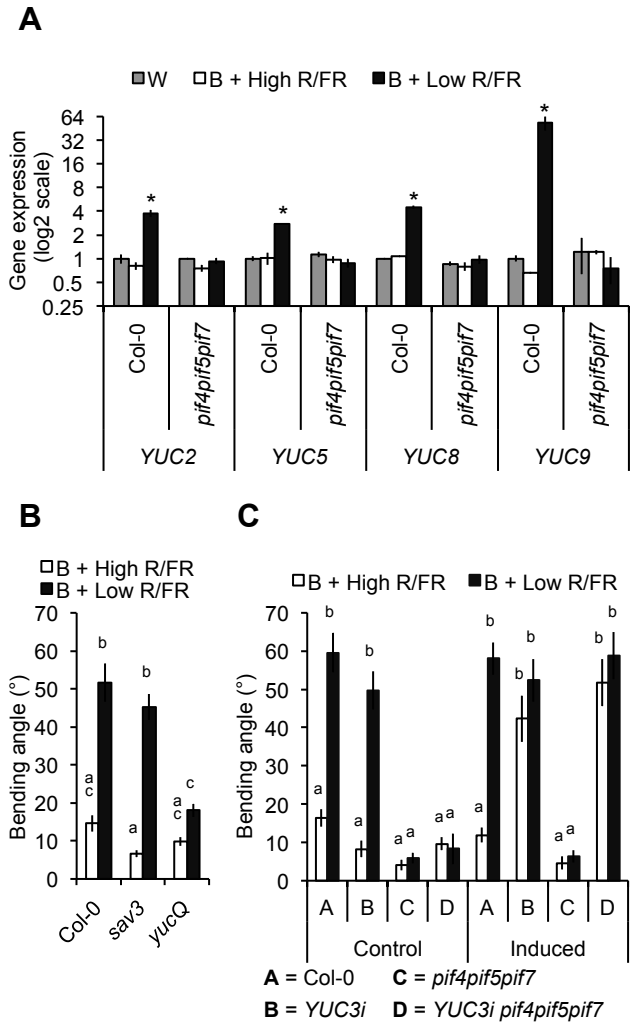

A
W
B + High R/FR
B + Low R/FR
Gene expression (log2 scale)
64
32
16
8
4
2
1
0.5
0.25
Col-0
pif4pif5pif7
YUC2
YUC5
YUC8
YUC9
B
B + High R/FR
B + Low R/FR
Bending angle (°)
Col-0
sav3
yucQ
C
B + High R/FR
B + Low R/FR
Bending angle (°)
A
B
C
D
Control
Induced
A = Col-0
B = YUC3i
C = pif4pif5pif7
D = YUC3i pif4pif5pif7

Figure 4

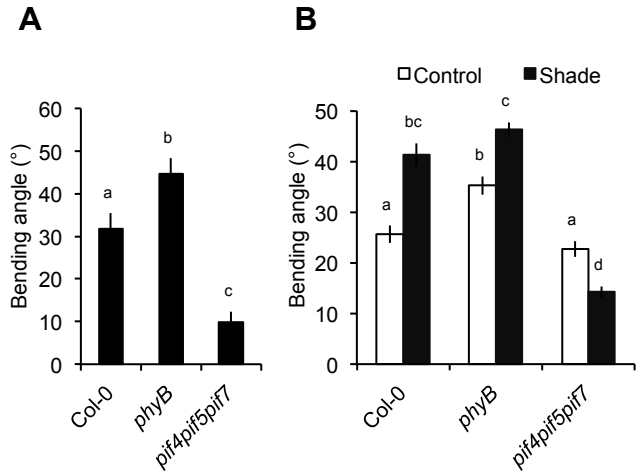

A
B
Control
Shade
Bending angle (°)
60
50
40
30
20
10
0
a
b
c
bc
d
Col-0
phyB
pif4pif5pif7

Figure 5

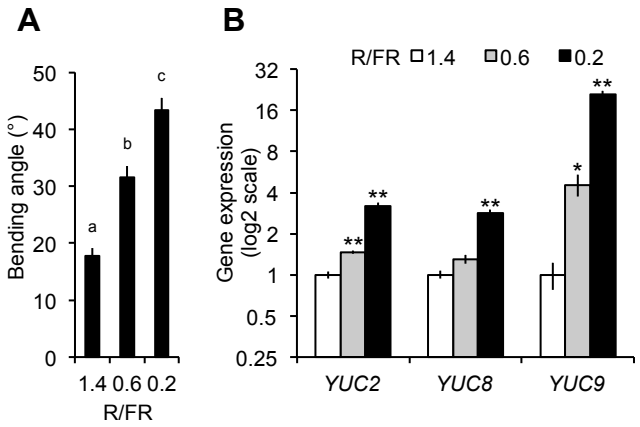
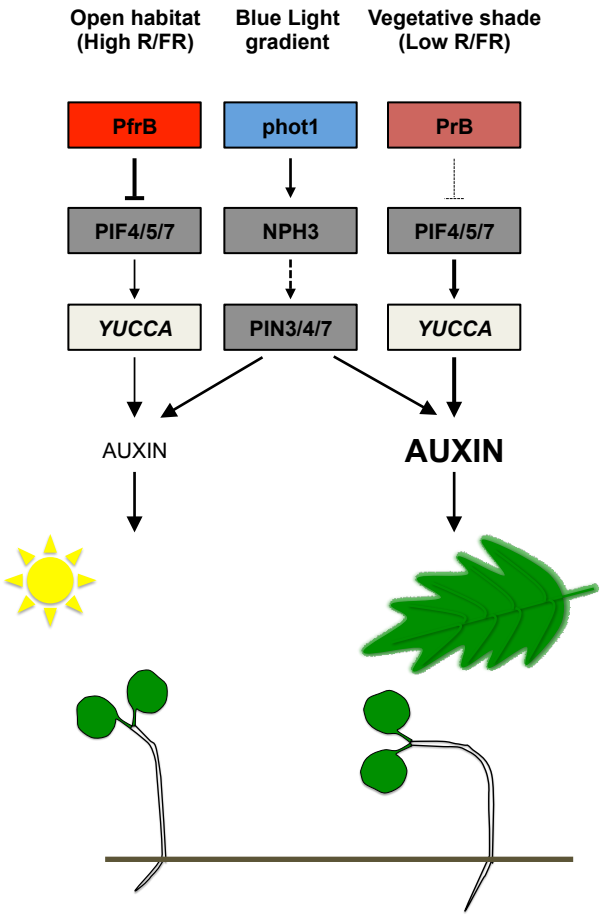

A
B
Bending angle (°)
50
40
30
20
10
0
a
b
c
1.4 0.6 0.2
R/FR
R/FR 1.4 0.6 0.2
Gene expression (log2 scale)
32
16
8
4
2
1
0.5
0.25
**
**
**
*
**
YUC2
YUC8
YUC9

Figure 6


Open habitat (High R/FR)
Blue Light gradient
Vegetative shade (Low R/FR)
PfrB
phot1
PrB
PIF4/5/7
NPH3
PIF4/5/7
YUCCA
PIN3/4/7
YUCCA
AUXIN
AUXIN

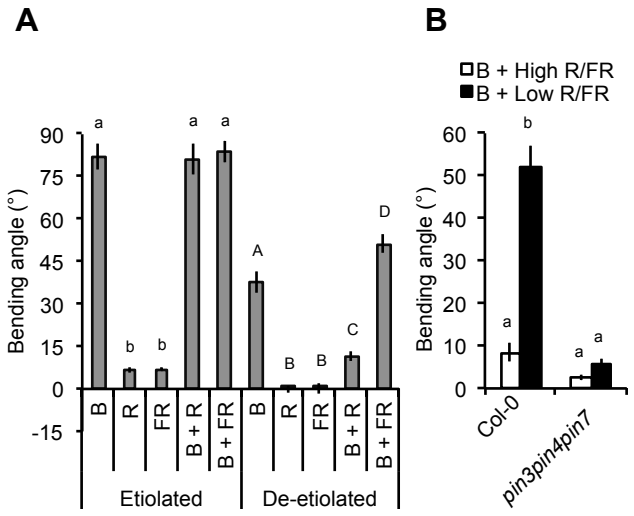


Figure S1. Modulation of phototropism by red and far-red light in Arabidopsis de-etiolated seedlings. Related to figure 1.

A) Three-day-old etiolated and de-etiolated Col-0 seedlings were subjected to the denoted combinations of unilateral lights (lateral illumination) for 24 hours. Bars represent mean bending angle ± S.E. (n>40). Key for graph legend: B = 10 µmol m^{-2} s^{-1} blue light; R = 10 µmol m^{-2} s^{-1} red light; FR = 10 µmol m^{-2} s^{-1} far-red light. Small and capital 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 for etiolated and de-etiolated seedlings, respectively.

B) Three-day-old de-etiolated seedlings of Col-0 and *pin3pin4pin7* were treated with unilateral blue light from the side, while red and far-red lights were provided from above to simulate control and shade conditions. Bending angles were measured after 24 hours of light treatments. Bars represent mean bending angle ± S.E. (n≥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 test.

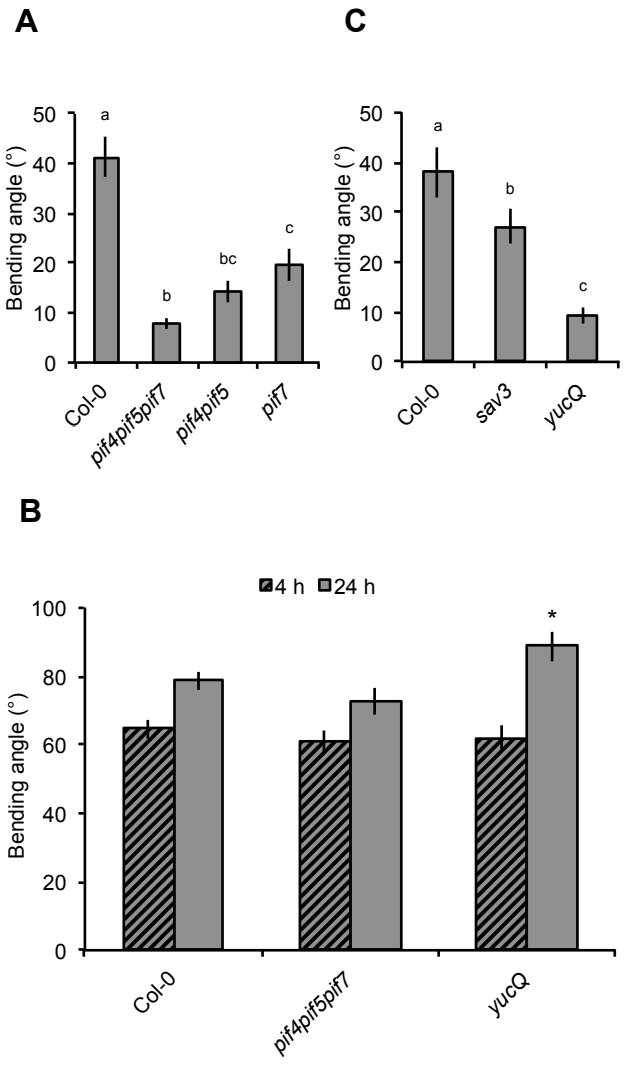


Figure S2. Regulation of phototropism by PIFs and YUCCAs is dependent on the developmental stage of Arabidopsis seedlings. Related to figures 2 and 3.

A) Three-day-old de-etiolated seedlings of the indicated genotypes were laterally illuminated with 10 µmol m^{-2} s^{-1} blue light for 24 hours. Bars represent mean bending angle ± S.E. (n≥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 test.

B) Three-day-old etiolated seedlings of the indicated genotypes were irradiated with 10 µmol m^{-2} s^{-1} unilateral blue light. Phototropic bending angle was measured at 4 hours and 24 hours. Data indicates mean ± S.E bending angle at each time point (n>50). Asterisk indicates statistical significance difference to Col-0 at the indicated time (* p value < 0.01, Student's t-test).

C) Same as part A.

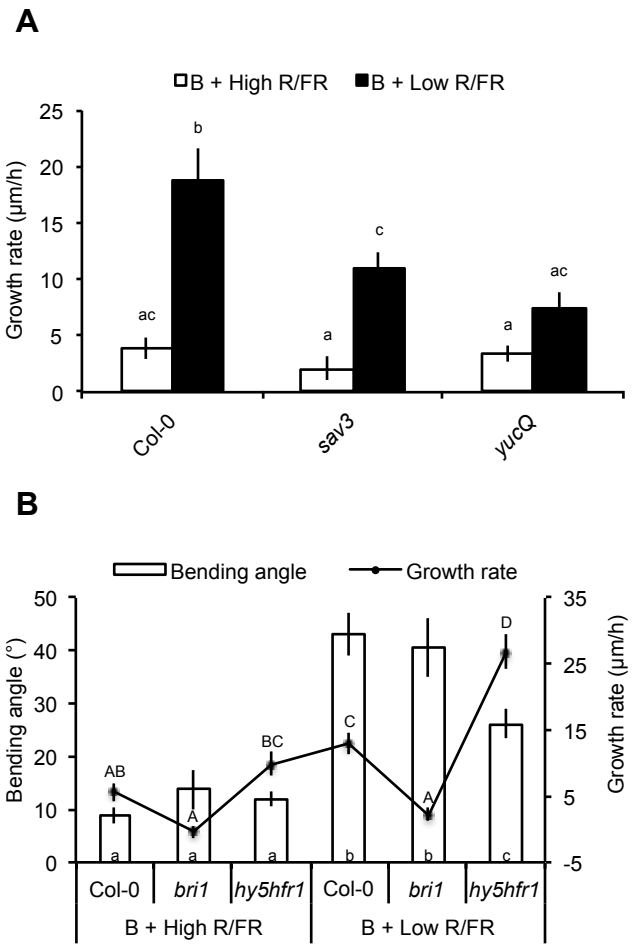


Figure S3. The growth potential of seedlings in high and low R/FR conditions does not strictly co-relate with the magnitude of phototropic bending. Related to figure 3.

A) The bar graph represents the hypocotyl growth rate ± S.E of the seedlings of the indicated genotypes used for measurement of phototropic bending angle in figure 3B. Small alphabetic letters indicate statistically significant groups according to growth rate at p value < 0.01 obtained by ANOVA followed by the post-hoc Tukey's HSD test.

B) Three-day-old de-etiolated seedlings of Col-0, *bri1*and *hy5hfr1* were subjected to light conditions as described in figure S1B. Bars represent mean bending angle ± S.E. (n>20). Line graph indicates mean hypocotyl growth rate ± S.E. during 24 hours of phototropism experiment for the same set of seedlings used to measure bending angles. Small and capital alphabetic letters indicate statistically significant groups according to bending angles and growth rate, respectively, at p value < 0.01 obtained by ANOVA followed by the post-hoc Tukey's HSD test.

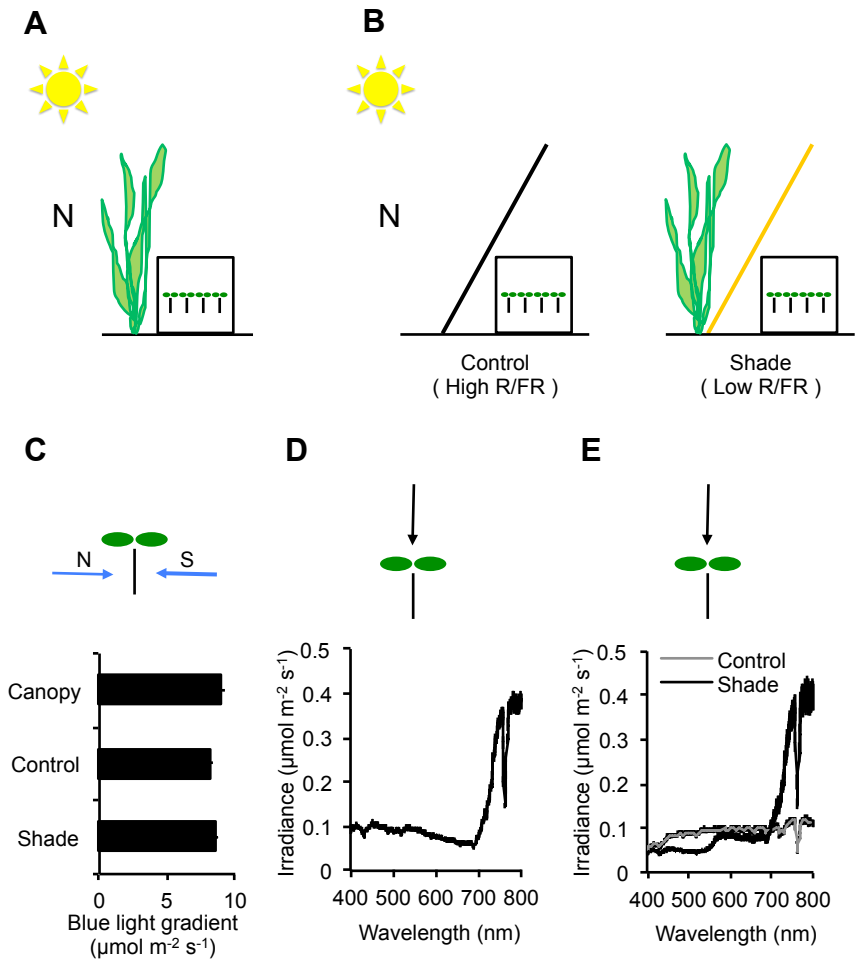


Figure S4. Schematic representation of outdoor experimental set up. Related to figure 4.
The figure schematically describes the experimental set up used for outdoor phototropism experiments. The experiments were performed in an open field in Buenos Aires, Argentina (southern hemisphere).
A) In this set up, three-day-old de-etiolated seedlings grown in lab conditions were shifted on the south side of a grass canopy (R/FR ratio 0.4) at ZT4.
B) In this set-up, de-etiolated seedlings were either subjected to control condition (High R/FR ratio = 1.2) by placing a black screen on the north side of seedlings or vegetative shade (Low R/FR ratio = 0.4) by placing an orange screen, at an angle of approximately 45°, between seedlings and tall grasses. While the black screen blocked the projection of grass shade on control seedlings, the orange screen enabled seedlings to be subjected to vegetative shade of a grass canopy. The blue light gradient was similar in both conditions (panel C), such that seedlings were subjected to more light from the south side.
C) Horizontal blue light gradient in the experimental setups. Canopy corresponds to the set-up shown in A. Control and shade correspond to the respective conditions presented in B. The horizontal blue light gradient was determined by subtracting the blue light irradiance coming from North from the irradiance coming from South. The light intensity was determined by placing the light sensor facing away from the filter (South) or towards the filter (North). Blue light irradiance is the integral of a Gauss curve with λmax at 450 nm and band width of ±15 nm at 0.5 the irradiance of λmax. Six measurements were made in each direction; data are the average +/- S.E.
D) Scan of the light reaching the seedling from above (sensor facing upwards) in the set-up shown in A. Data are average +/- S.E. of six measurements.
E) Scan of the light reaching the seedling from above (sensor facing upwards) in the control (black filter) and shade (orange filter) of the set-up shown in B. Data are average +/- S.E. of six measurements.

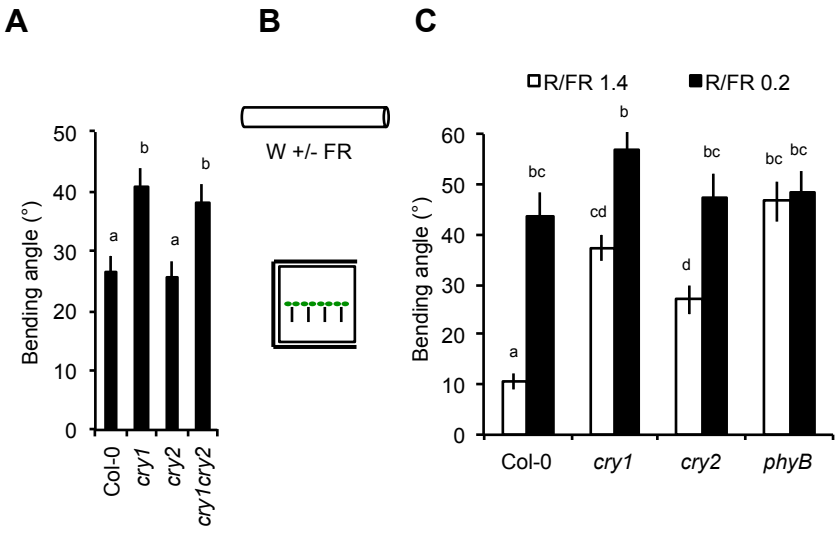


Figure S5. Cryptochromes negatively regulate phototropism in de-etiolated Arabidopsis seedlings. Related to figures 4 and 5.

A) Three-day-old de-etiolated seedlings of Col-0, *cry1*, *cry2* and *cry1cry2* grown on vertical plates were placed on the south side of vegetative shade from tall grasses for 5 hours as described in figure S4A. Phototropic bending angle was then measured. Data were pooled from 3 independent experiments. Bars represent mean bending angle ± S.E. (n≥230).

B) The figure schematically describes the experimental set up used for white light gradient experiment. White light and varying amount of supplemental FR was provided from above to obtain the required R/FR ratios. Square plates with de-etiolated seedlings were put into a black box opened only from one side and kept under the light, thereby, subjecting seedlings to a gradient of light.

C) Three-day-old de-etiolated seedlings of Col-0, *cry1*, *cry2* and *phyB* were subjected to light conditions as described in the panel B. Bending angles were measured after 6 hours of light treatments. Bars represent mean bending angle ± S.E. (n>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 test.

Supplemental Experimental Procedures

Plant material and growth conditions

The Columbia (Col-0) ecotype was used as the wild-type control. The mutants used in the study described previously are: *cry1-b104* (outdoor experiment) [S1], *cry1-301* (indoor experiment), *cry2-1* [S2], *FRO6::XVE::YUC3* [S3], *hy5-215hfr1* [S4], *nph3-6* [S5], *phot1-5*, *phot2-1, phot1-5phot2-1, phyA*-211, *phyB*-9, *phyA-211phyB-9* [S6], *pif4pif5pif7* [S7], *pin3-3pin4-101pin7-101* [S8], *sav3-2* [S9], *yuc2yuc5yuc8yuc9* [S10], *35S::PIF4* and *35S::PIF5* [S11]. The *bri1-235* mutant contains a point mutation at the nucleotide position 467 (C to T) in the *BRI1* protein coding sequence resulting in the substitution of S156F. The *phyBpif4pif5pif7* and *YUC3i pif4pif5pif7* mutants were obtained by crossing *pif4pif5pif7* with *phyB-9* and *FRO6::XVE::YUC3*, respectively.

Seeds were surface sterilized and plated on half-strength Murashige and Skoog medium with 0.8 % (w/v) agar and kept at 4 °C in the dark for 3 days. Square plates were then transferred to continuous white light (50 µmol m^{-2} s^{-1}) for 3 days at 22-22.5 °C in Percival AR-41L2 incubator to obtain de-etiolated seedlings. For outdoor experiments seedlings were grown in LD (16 h light / 8 h darkness) for 3 days in 50 µmol m^{-2} s^{-1} white light (fluorescent lamps) at 22 °C on vertical plates. Etiolated seedlings were obtained by inducing germination in white light (150 µmol m^{-2} s^{-1}) for 6 hours after 3 days of cold and dark treatment and subsequently shifting plates to dark for 64 hours at 22 °C. For experiments involving induction of *YUC3* expression, de-etiolated seedlings were grown on nylon mesh (160 mm, Micropore) placed on the surface of plates as described above. The nylon mesh was transferred to a new plate containing 0.1% ethanol (control) or 10 µM estradiol (Sigma) in 0.1% ethanol (induced) for performing the phototropism experiments.

Indoor unilateral light phototropism and white light gradient experiments were performed in Percival I-33NL and Percival SE-41L incubators, respectively. The LED light sources were from CLF Plant Climatics GmbH: blue (λmax, 462 nm), red (λmax, 664 nm) and far-red (λmax, 730 nm). Light intensities were determined with an International Light IL1400A photometer (Newburyport, MA) equipped with an SEL033 probe with appropriate light filters or with an Ocean Optics (Dunedin, FL, USA) USB2000+ spectrometer. In the field, the vertically and horizontally incident radiation (R/FR ratio, R, FR and blue light) were measured for each experiment with the SKR 1850 four-channel sensor probe of a Skye Instruments SKL 904/I SpectroSense2 meter, respectively facing upwards or towards north and south. For a more detailed characterization we produced scans with an Ocean Optics USB4000-UV-VIS spectrometer configured with a DET4-200-850 detector and QP600-2-SR optical fiber in one of the experiments (Figures S4D, S4E).

Phototropism experiment and measurements

De-etiolated seedlings for phototropism experiment were treated as described in the experimental procedures. Etiolated seedlings were subjected to unilateral light from the side. Plates were photographed at the indicated times in infra-red light.

The phototropic bending angles of de-etiolated seedlings were calculated by subtracting average angle of orientation of upper region (70% to 95% of total length) of each hypocotyl with respect to horizontal before and after blue light treatment determined by a customized MATLAB script developed in Fankhauser lab. Growth rates were determined by measuring hypocotyl length of the same seedlings used for phototropism measurements using the same MATLAB script.

Bending angles for etiolated seedlings were calculated by measuring hypocotyl angles relative to growth direction prior to light treatments by using National Institutes of Health ImageJ software version 1.45s.

RNA extraction and RT-qPCR

50 de-etiolated seedlings were harvested and frozen in liquid nitrogen for each light condition. Total RNA was extracted using TRIzol reagent (Life Technologies) following the manufacturer's instructions. Samples were further treated with DNase I (New England Biolabs) and cleaned up using RNeasy Mini Kit (Qiagen). cDNA was prepared from 250 ng RNA per sample using Superscript II Reverse Transcriptase (Invitrogen, Life Technologies, Carlsbad, CA, USA) and random primers. RT-qPCR was performed in three technical replicates for each sample (ABI prism 7900HT sequence detection system, Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using FastStart Universal SYBR green Master mix (Roche, Mannheim, Germany). Primer sequences used were described previously [S7]. The data was analyzed using the Biogazelle qbase software.

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