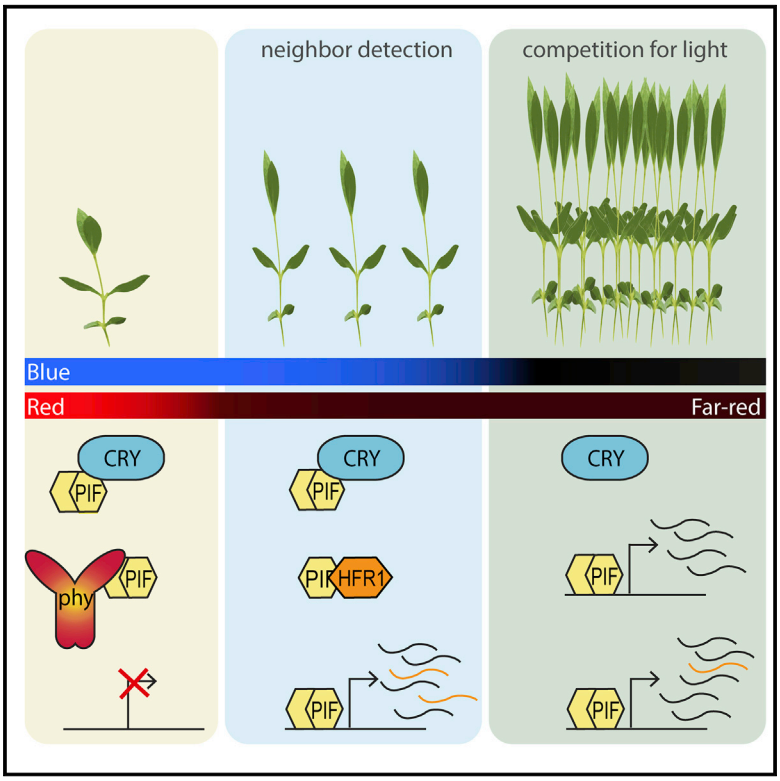
Report

Current Biology

Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light

Graphical Abstract



Authors

Mieke de Wit, Diederik H. Keuskamp, Franca J. Bongers, ..., Carmen Martínez-Cerón, Christian Fankhauser, Ronald Pierik

Correspondence

r.pierik@uu.nl

In Brief

de Wit et al. show that low blue light perception by cryptochromes enhances the shade avoidance response to low red:far-red light perceived by phytochromes in true competition. Increased elongation is mediated by higher abundance of the transcription factor PIF5 and increased gene expression, combined with reduced expression of negative regulators.

Highlights

- Blue light depletion combined with low R:FR mimics vegetation shade
- Low blue light perception enhances the low R:FR response through PIFs and COP1
- Low blue light perception counteracts a low R:FR-induced negative feedback loop

Accession Numbers

GSE87770

de Wit et al., 2016, Current Biology *26*, 3320–3326
December 19, 2016
http://dx.doi.org/10.1016/j.cub.2016.10.031

CellPress

Current Biology
Report

Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light

Mieke de Wit,[1,2] Diederik H. Keuskamp,[1] Franca J. Bongers,[1] Patricia Hornitschek,[2] Charlotte M.M. Gommers,[1] Emilie Reinen,[1] Carmen Martínez-Cerón,[1] Christian Fankhauser,[2] and Ronald Pierik[1,3,*]
[1]Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands
[2]Centre for Integrative Genomics, Lausanne University, Génopode Building, 1015 Lausanne, Switzerland
[3]Lead Contact
*Correspondence: r.pierik@uu.nl
http://dx.doi.org/10.1016/j.cub.2016.10.031

SUMMARY

Plants in dense vegetation perceive their neighbors primarily through changes in light quality. Initially, the ratio between red (R) and far-red (FR) light decreases due to reflection of FR by plant tissue well before shading occurs. Perception of low R:FR by the phytochrome photoreceptors induces the shade avoidance response [1], of which accelerated elongation growth of leaf-bearing organs is an important feature. Low R:FR-induced phytochrome inactivation leads to the accumulation and activation of the transcription factors PHYTOCHROME-INTERACTING FACTORs (PIFs) 4, 5, and 7 and subsequent expression of their growth-mediating targets [2, 3]. When true shading occurs, transmitted light is especially depleted in red and blue (B) wavelengths, due to absorption by chlorophyll [4]. Although the reduction of blue wavelengths alone does not occur in nature, long-term exposure to low B light induces a shade avoidance-like response that is dependent on the cryptochrome photoreceptors and the transcription factors PIF4 and PIF5 [5–7]. We show in *Arabidopsis thaliana* that low B in combination with low R:FR enhances petiole elongation similar to vegetation shade, providing functional context for a low B response in plant competition. Low B potentiates the low R:FR response through PIF4, PIF5, and PIF7, and it involves increased PIF5 abundance and transcriptional changes. Low B attenuates a low R:FR-induced negative feedback loop through reduced gene expression of negative regulators and reduced HFR1 levels. The enhanced response to combined phytochrome and cryptochrome inactivation shows how multiple light cues can be integrated to fine-tune the plant's response to a changing environment.

RESULTS AND DISCUSSION

Low B Enhances the Low R:FR-Induced Petiole Response in a PIF-Dependent Manner

As reduction of specifically blue light (low B) does not naturally occur, we studied whether low B acts in concert with other shade signals. Adult plants exposed to 24 hr of low B displayed only a trend toward slight petiole elongation (Figure 1A). Interestingly, combination of low B with low red (R): far-red (FR) induced a stronger elongation response than low R:FR treatment alone, which was not further affected by reduced light intensity (green filter) (Figure 1A, light conditions in the Supplemental Experimental Procedures). This suggests that low B is perceived as a signal of increasing competition in the context of shade avoidance.

The phytochrome (phy) mutant *phyB* showed an exaggerated low B response, which was not enhanced in the combination with low R:FR (Figure 1B). Cryptochrome (cry) mutants similarly showed a compromised response to the combined light treatment (Figure 1B), but they retained a low R:FR response. These data indicate that the photoreceptors phyB and both cry1 and cry2 respectively mediate the R:FR and B signaling of the interaction.

PIF4, PIF5, and PIF7 are key regulators in low R:FR signaling [2, 3], and PIF4 and PIF5 play a role in low B responses [5, 7]. Petiole elongation in the different light treatments was abolished in the *pif4pif5pif7* mutant, but not in *pif4pif5* and *pif7* (Figures S1 and 1C), indicating that the enhanced response to low R:FR + low B depends on combined action of the PIF4, PIF5, and PIF7 transcription factors. Three direct PIF target genes showed an expression pattern consistent with enhanced elongation in low R:FR + low B. Genes encoding the positive regulators ATHB2 and IAA19 were more expressed in low R:FR + low B than in low R:FR alone, while gene expression of the negative regulator HFR1 was reduced in the combined light treatment (Figures 1D–1F). Together, these results show that simultaneous low B perception affects both low R:FR-induced gene expression and elongation.

Wild-type plants grown at high density (canopy) experience a reduction in R:FR, B, and light intensity over time, and they show a strong petiole elongation response ([8]; Figures 2A and 2B). In contrast, petiole elongation was largely reduced in *pif4pif5pif7*

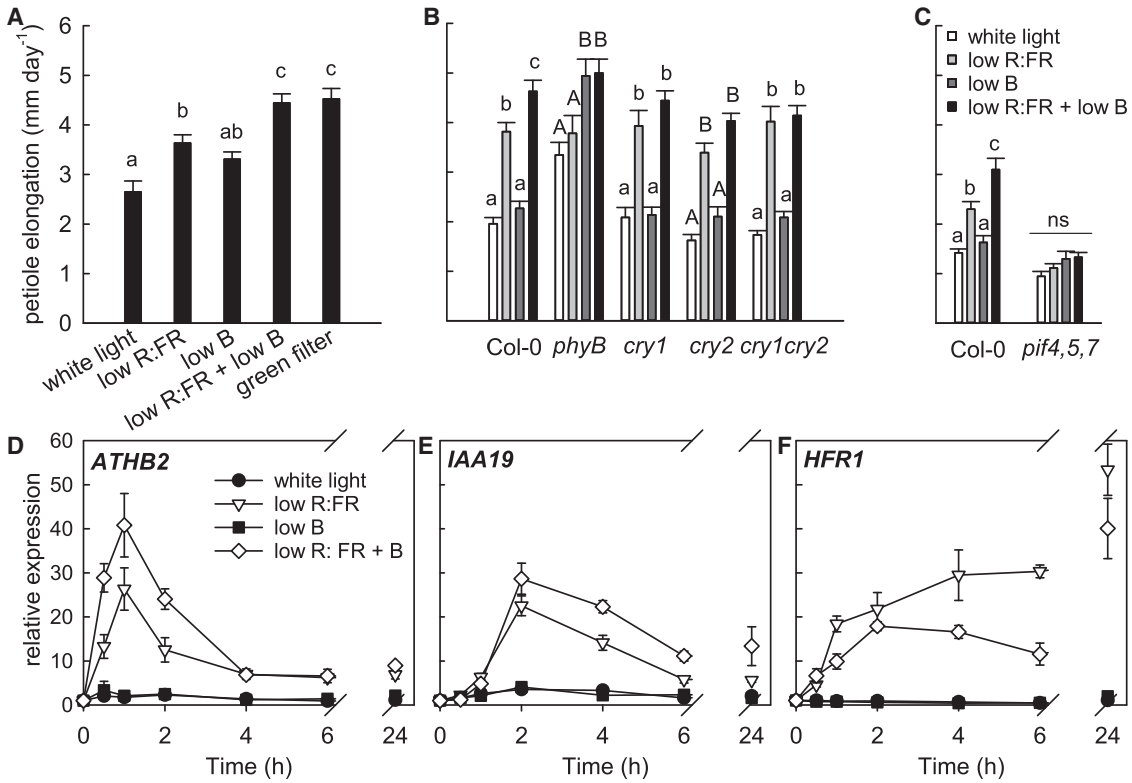


Figure 1. Low B Enhances the Low R:FR Response

(A–C) Elongation of ~5-mm-long petioles of 29-day-old Col-0 wild-type (A), photoreceptor mutants versus Col-0 (B), and *pif4pif5pif7* versus Col-0 (C) plants over 24 hr of light treatment (Supplemental Experimental Procedures) (n = 10). Green filter combines low R:FR, low B, and low light intensity.

(D–F) Expression relative to t = 0 of PIF-dependent genes *ATHB-2* (D), *IAA19* (E), and *HFR1* (F) over time in petioles of light-treated plants. Data represent means ± SE (n = 4). Different letters indicate significant difference ($p < 0.05$) within genotype. ns, not significant. See also Figure S1.

canopies (Figures 2A and 2B), confirming the importance of the PIF transcription factors in plant competition. To explore the transcriptional interaction between low R:FR and low B more broadly, we studied the genome-wide transcript profile of petioles from single-grown plants subjected to the different light treatments. We compared these with the transcript profile of canopy-grown plants of the same age. Of the light treatments, low R:FR single treatment overlapped best with low R:FR + low B treatment, both in number of differentially regulated genes (DEGs) and in direction of regulation (Figures 2C and 2D). In addition to light quality changes, canopy plants experienced a changed microenvironment, including reduced light intensity and mechanical stress [8]. These factors likely explain the larger number of DEGs in canopy-grown plants (Figure 2C). The combined low R:FR + low B treatment showed the best overlap in expression with the canopy profile, (Figures 2C, 2D, S2A, and S2B), suggesting that integration of low R:FR and low B signals indeed occurs in competition for light.

Approximately 60% of DEGs in each treatment was identified previously as PIF4/PIF5 targets in low R:FR-treated [9] or low B-treated [7] seedlings (Figure 2E). This indicates that there was a larger and partly unique set of PIF targets among the larger number of DEGs in the low R:FR + low B and canopy treatments (Figure 2E). R-activated phyB mediates PIF4 and PIF5 degradation and PIF7 inactivation [2, 3], and B-activated cry1 binds to PIF4 and PIF5 and inhibits PIF4 activity [7, 10]. Combined cry and phy inactivation may thus relieve inhibition of PIF abundance and activity, leading to increased regulation of PIF targets in shade.

Auxin and Brassinosteroid Positively Regulate Elongation in Low R:FR + Low B

Gene ontology (GO) terms for the plant hormones auxin and brassinosteroid (BR) were particularly enriched in the low R:FR + low B and canopy transcriptomes (Table S1). Auxin regulates both low R:FR and low B responses, and several auxin-related genes are direct PIF targets [3, 6, 9, 11]. BR, together with auxin, also is implicated in low R:FR and low B responses [5, 6, 12–14]. The transcription factors ARF6 (auxin related), BZR1 (BR related), and PIF4 directly interact and cooperatively induce genes involved in hypocotyl elongation [15], suggesting auxin and BR together can stimulate PIF-dependent growth.

To study whether auxin and BR mediate enhanced elongation in low R:FR + low B, we used a seedling hypocotyl assay to accelerate the experimental cycle and facilitate pharmacological manipulation. Although hypocotyls strongly responded to low B, they elongated more in combined R:FR and low B similar to petioles (Figure S3A). This response was dependent on PIF4, PIF5, and PIF7, with a more prominent role for PIF7 in low

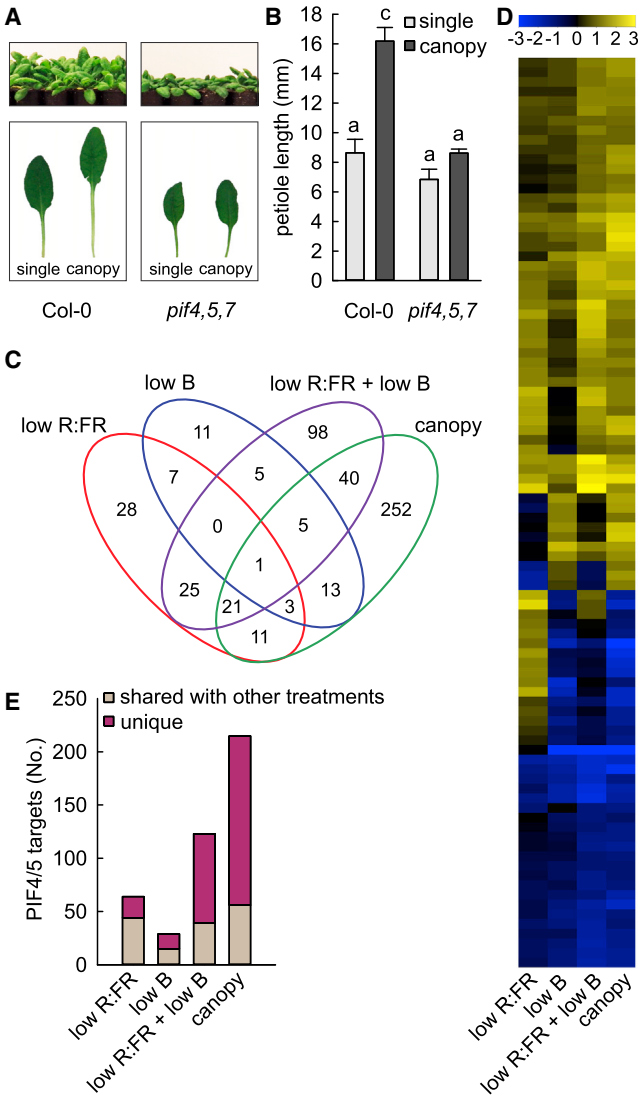


Figure 2. Genome-wide Transcript Analysis of Competition for Light

(A) Plants grown at low (single) or high (canopy) density are shown.

(B) Length of third youngest petiole of 37-day-old single and canopy-grown plants. Data represent means ± SE (n = 5). Different letters indicate significant difference ($p < 0.05$).

(C) Venn diagram of genes expressed differentially to control in petioles of 29-day-old light-treated (24 hr) single plants and canopy-grown plants. Microarray analysis used a cutoff of $p < 0.05$ and $|\log2FC| > 1$ (n = 3).

(D) Heatmaps of log2FC of significantly regulated genes in canopy and at least one of the light treatments.

(E) Number of differentially regulated genes in each of the treatments that are putative PIF4 and/or PIF5 targets. Different colors indicate targets shared with at least one other treatment and unique targets not expressed in the other treatments. See also Figure S2 and Table S1.

R:FR-induced elongation in hypocotyls than in petioles (Figures S1B and S3A). Simultaneous impairment of auxin and BR pathways was achieved by combining mutants with chemical inhibitors. Inhibition of both hormone pathways reduced the elongation response to low R:FR + low B more than inhibition of a single pathway, but it did not completely suppress it (Figure 3A). This suggests that, although auxin and BR indeed promote enhanced elongation in low R:FR + low B, further modes of regulation may exist, such as, for example, gibberellin [16].

Low B Enhances Low R:FR Response through a COP1-Dependent Mechanism

To study whether the increased number of PIF targets expressed in low R:FR + low B reflects increased PIF abundance, we studied PIF5 protein levels in *PIF5:PIF5-HA* seedlings. Indeed, PIF5 accumulated more in low R:FR + low B than in low R:FR after 1 hr, although PIF5 abundance did not significantly increase in low B alone (Figure 3B). PIF-dependent transcription could be enhanced further by counteracting low R:FR-induced negative feedback loops. Several negative regulators of the shade avoidance response are induced by low R:FR, such as *LONG HYPOCOTYL IN FAR-RED 1* (*HFR1*), *PHYTOCHROME RAPIDLY REGULATED 1* (*PAR1*) and *PAR2*, *LONG HYPOCOTYL 5* (*HY5*), and *HOMOLOG OF HY5* (*HYH*) [17–19]. These negative regulators may prevent exaggerated elongation in low R:FR, and they present a putative target for cross-talk. We therefore measured hypocotyl elongation in mutants of known negative regulators of shade avoidance.

Although *35S:PAR1-GFP* (*PAR1-G*) hypocotyls elongated less in all light treatments, both the *PAR1-RNAi* line (mildly reduced levels of *PAR1* and *PAR2* [18]) and the *par2-1* mutant maintained a wild-type-like low R:FR + low B response (Figures 3C and 3D), indicating that the *PAR*s do not play a major role. In contrast, the *hfr1* and *hy5hyh* mutants showed enhanced hypocotyl elongation. Whereas *hy5hyh* elongated more than wild-type in all light treatments, *hfr1* only did so in low R:FR and low R:FR + low B, suggesting a more specific interaction (Figure 3C). A line overexpressing a truncated stable version of HFR1 (G-BH-03 [20]) was impaired in all light treatments (Figure 3C), confirming that HFR1 can be a potent inhibitor of light quality-induced hypocotyl elongation [17, 21]. We therefore studied HFR1 protein levels in *HFR1:HFR1-HA* seedlings. After 1 hr, HFR1 abundance had increased in low R:FR, decreased in low B, and was similar to white light in low R:FR + low B (Figure 3E). This attenuated HFR1 accumulation in low R:FR + low B also was observed at later time points (Figure S3), and it is consistent with HFR1 protein destabilization during prolonged shade [22]. HFR1 forms non-DNA-binding heterodimers with PIF4 and PIF5, thereby inhibiting their transcriptional activity [21]. By reducing HFR1 abundance, low B signaling may thus increase availability of PIFs for transcription.

Regulation of protein abundance may occur at the transcript level, as was suggested by partially reduced *HFR1* expression in petioles in the combined light treatment (Figure 1F). Transcriptome analysis suggested that *HY5* expression was similarly reduced in low R:FR + low B, and qPCR analysis confirmed reduced expression of *HFR1*, *HY5*, and *HYH* in petioles of plants treated with low R:FR + low B compared to low R:FR (Figures 4A–4C). How transcript levels of these genes might selectively be reduced by the addition of a low B signal is currently not understood.

As *HFR1* transcript levels are elevated in low R:FR + low B compared to white light while protein levels are similar, protein stability also may be regulated in the combined light treatment. HFR1, HY5, and HYH are targets of the COP1/suppressor of phytochrome (SPA) E3 ubiquitin ligase complex, which labels them for degradation [29]. COP1 is indeed involved in

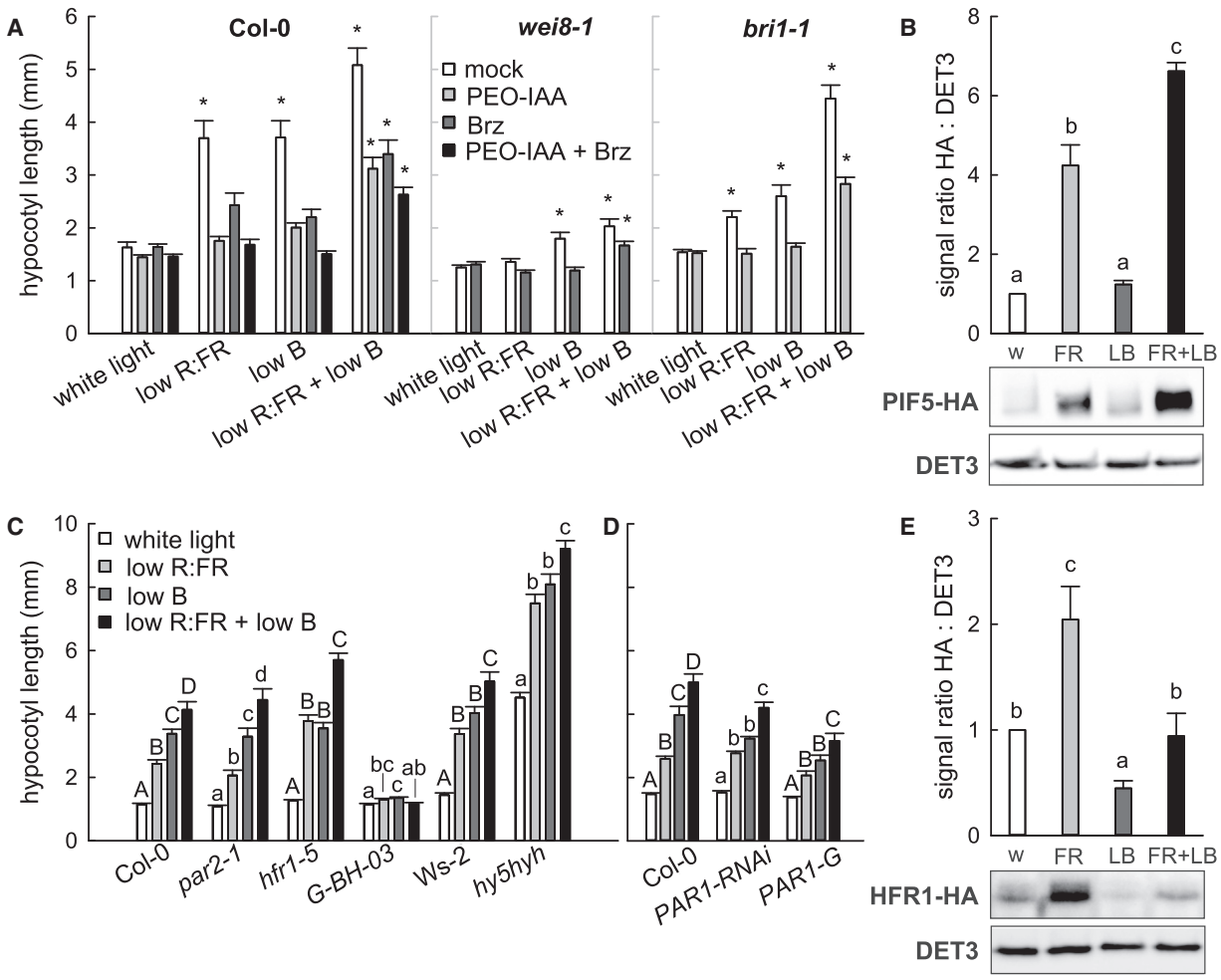


Figure 3. Effect of Auxin, BR, and Negative Regulators in Hypocotyl Elongation

(A, C, and D) Hypocotyl length of light-treated (A) auxin mutant *wei8-1* and BR mutant *bri1-1* and (C and D) negative regulator mutants ($n > 16$). Chemical inhibitors of auxin perception (50 μM α-(phenylethyl-2-one)-IAA [PEO-IAA]) and BR biosynthesis (0.5 μM Brassinazole [Brz]) were added to medium right before light treatments started.

(B and E) Protein accumulation in *PIF5*:*PIF5-HA* (B) and *HFR1*:*HFR1-HA* (E) seedlings detected with anti-HA antibody from total protein extract after 1 hr of light treatment, quantified, and normalized to DET3 signal ($n = 3$). Bands of representative blot correspond with bars in graph above. Data represent means ± SE. Asterisks indicate significant difference between light treatment and its respective chemical-treated control, and different letters indicate significant difference within genotype ($p < 0.05$). W, white light; FR, low R:FR; LB, low B; FR + LB, low R:FR + low B. See also Figure S3.

shade-induced elongation and accumulates in the nucleus both in low R:FR and low B [22, 24, 30]. Moreover, crys and phys are associated with the COP1/SPA complex, and their light activation inhibits COP1/SPA activity [25–28]. In low R:FR + low B, COP1 nuclear localization and relieved inhibition through deactivation of crys may thus provide more favorable conditions for degradation of COP1 targets than low R:FR alone. Accordingly, the *cop1-4* mutant did not show enhanced petiole elongation in combined low R:FR + low B (Figure 4D). This shows that low B stimulation of the low R:FR response is COP1 dependent, and it suggests that degradation of low R:FR-induced negative regulators is indeed required for enhanced elongation in low R:FR + low B.

Despite the COP1 dependency of the petiole response, the reduced gene expression of COP1 targets in petioles, and the obvious growth-inhibiting roles of HFR1 and HY5/HYH in hypocotyls, petiole elongation was not enhanced in *hfr1* adult plants subjected to light treatment or canopy growth (Figures 4E and 4F). Similarly, *hy5* responses to low R:FR and combined low R:FR + low B were not significantly different from wild-type ($p > 0.05$, Student's t test) (Figures 4E and 4F). This suggests that in adult plants other or a combination of COP1 targets inhibit elongation and that negative regulators of shade avoidance may depend on developmental stage.

A Model for Phy and Cry Signaling Integration in Plant Competition

The combination of low B with low R:FR is a specific signature of plant competition, posing a serious threat to light capture. Low B stimulation of the low R:FR response suggests that blue and red light signals are integrated to respond adequately to the transition from neighbor detection to real competition. This applies

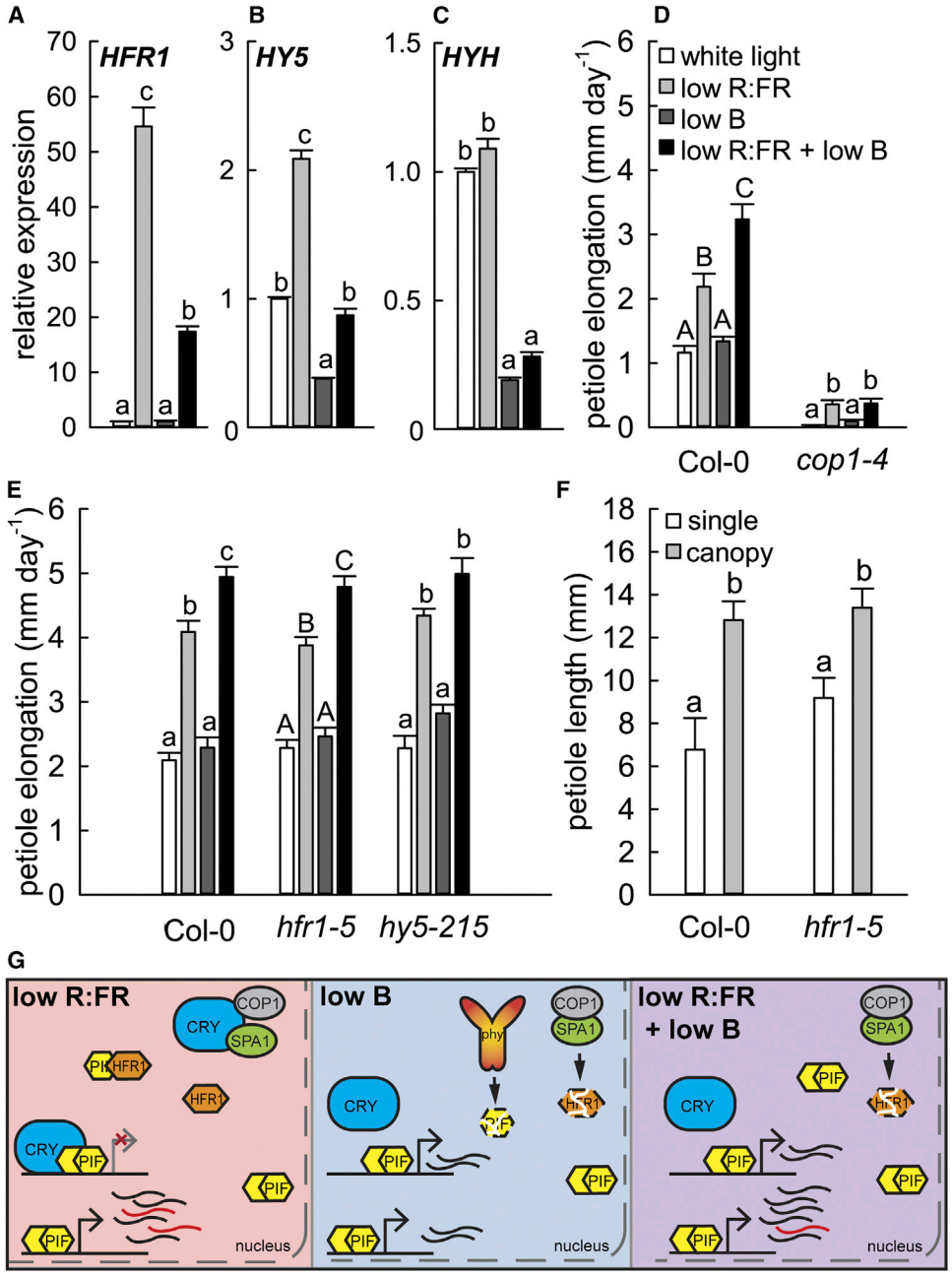


Figure 4. Interaction between Low R:FR and Low B Is COP1 Dependent

(A–C) Gene expression relative to white light in petioles of 4-week-old light-treated (4 hr) plants is shown for *HFR1* (A), *HY5* (B), and *HYH* (C) (n = 6).

(D and E) Petiole elongation of 3-week-old (D: *cop1-4*, flowers early) or 4-week-old (E: *hfr1-5* and *hy5-215*) mutants involved in the COP1 signaling pathway over 24 hr of light treatment is shown (n = 10).

(F) Length of third youngest petiole in 35-day-old plants grown at low (single) or high (canopy) density (n = 5). Data represent means ± SE. Different letters indicate significant difference within genotype ($p < 0.05$).

(G) Model of phytochrome (phy) and cryptochrome (cry) signaling integration during competition for light. In low R:FR, phy is inactivated and resides in the cytosol [23]. This allows PIF accumulation in the nucleus and subsequent transcription of positive but also negative (red mRNA) regulators of shade avoidance [2, 17], such as HFR1 that forms non-DNA-binding heterodimers with PIFs [21]. In low B, PIFs may accumulate [7], and cry inactivation relieves its direct inhibition of PIF-mediated transcription [10]. In combined low R:FR and low B, PIF-mediated transcription is thus facilitated likely through both enhanced PIF abundance (this paper) and activity. Additionally, low R:FR + low B leads to reduced accumulation of negative regulators of shade avoidance, such as HFR1 (this paper), many of which are targets of the E3 ubiquitin ligase COP1. Low R:FR and low B both induce nuclear translocation of COP1 [24], while both cry and phy inactivation relieves their repression on the COP1/SPA complex [25–28]. This allows for enhanced degradation of COP1 targets. Furthermore, transcription of low R:FR-induced negative regulators is reduced in the combination with low B (this paper) through an unknown mechanism. See also Figure S3.

both to shade-induced unidirectional elongation (this paper) and phototropic movement (see the accompanying paper by Goyal et al. in this issue of *Current Biology* [31]). We propose that low B potentiates the low R:FR pathway through enhanced PIF action. In addition to increased abundance, PIF activity is likely enhanced directly through cry inactivation and indirectly through relieved inhibition of COP1, which increases degradation of negative regulators of PIF-mediated transcription, such as HFR1 (Figure 4G). As PIFs are thought to be signaling hubs [32], these may be common mechanisms through which plants adapt their growth to changing environmental conditions.

ACCESSION NUMBERS

The accession number for the microarray data reported in this paper is GEO: GSE87770.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.031.

AUTHOR CONTRIBUTIONS

Conceptualization, M.d.W. and R.P.; Investigation, M.d.W., D.H.K., F.J.B., C.M.M.G., E.R., and C.M.-C.; Resources, P.H. and C.F.; Funding Acquisition, M.d.W., R.P., and C.F.; Writing, M.d.W., R.P., and D.H.K.; Supervision, R.P.

ACKNOWLEDGMENTS

We thank Marleen Vergeer-van Eijk and Chrysa Pantazopoulou for sample collection, Debatosh Das for clustering analysis, and Anne-Sophie Fiorucci and Anupama Goyal for helpful comments. We are grateful to Séverine Lorrain for providing the *PIF5:PIF5-HA* line. Funding was provided by Netherlands Organisation for Scientific Research grants 86306001 and 86412003 to R.P., 021001030 to M.d.W., and 022001018 to C.M.M.G.; Swiss National Science Foundation grant FNS 31003A_160326 to C.F.; and the Universities of Lausanne and Utrecht.

Received: July 4, 2016
Revised: October 7, 2016
Accepted: October 19, 2016
Published: November 23, 2016

REFERENCES

1. Ballaré, C.L., Scopel, A.L., and Sánchez, R.A. (1990). Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science *247*, 329–332.
2. Lorrain, S., Allen, T., Duek, P.D., Whitelam, G.C., and Fankhauser, C. (2008). Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J. *53*, 312–323.
3. Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.S., et al. (2012). Linking photoreceptor excitation to changes in plant architecture. Genes Dev. *26*, 785–790.
4. Casal, J.J. (2013). Photoreceptor signaling networks in plant responses to shade. Annu. Rev. Plant Biol. *64*, 403–427.
5. Keller, M.M., Jaillais, Y., Pedmale, U.V., Moreno, J.E., Chory, J., and Ballaré, C.L. (2011). Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant J. *67*, 195–207.
6. Keuskamp, D.H., Sasidharan, R., Vos, I., Peeters, A.J.M., Voesenek, L.A.C.J., and Pierik, R. (2011). Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in Arabidopsis seedlings. Plant J. *67*, 208–217.
7. Pedmale, U.V., Huang, S.S., Zander, M., Cole, B.J., Hetzel, J., Ljung, K., Reis, P.A.B., Sridevi, P., Nito, K., Nery, J.R., et al. (2016). Cryptochromes interact directly with PIFs to control plant growth in limiting blue light. Cell *164*, 233–245.
8. de Wit, M., Kegge, W., Evers, J.B., Vergeer-van Eijk, M.H., Gankema, P., Voesenek, L.A.C.J., and Pierik, R. (2012). Plant neighbor detection through touching leaf tips precedes phytochrome signals. Proc. Natl. Acad. Sci. USA *109*, 14705–14710.
9. Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., et al. (2012). Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant J. *71*, 699–711.
10. Ma, D., Li, X., Guo, Y., Chu, J., Fang, S., Yan, C., Noel, J.P., and Liu, H. (2016). Cryptochrome 1 interacts with PIF4 to regulate high temperature-mediated hypocotyl elongation in response to blue light. Proc. Natl. Acad. Sci. USA *113*, 224–229.
11. Tao, Y., Ferrer, J.-L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., et al. (2008). Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell *133*, 164–176.
12. Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H., and Nagatani, A. (2010). Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol. *153*, 1608–1618.
13. Bou-Torrent, J., Galstyan, A., Gallemí, M., Cifuentes-Esquivel, N., Molina-Contreras, M.J., Salla-Martret, M., Jikumaru, Y., Yamaguchi, S., Kamiya, Y., and Martínez-García, J.F. (2014). Plant proximity perception dynamically modulates hormone levels and sensitivity in Arabidopsis. J. Exp. Bot. *65*, 2937–2947.
14. Procko, C., Burko, Y., Jaillais, Y., Ljung, K., Long, J.A., and Chory, J. (2016). The epidermis coordinates auxin-induced stem growth in response to shade. Genes Dev. *30*, 1529–1541.
15. Oh, E., Zhu, J.-Y., Bai, M.-Y., Arenhart, R.A., Sun, Y., and Wang, Z.-Y. (2014). Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. eLife *3*, e03031.
16. Djakovic-Petrovic, T., de Wit, M., Voesenek, L.A.C.J., and Pierik, R. (2007). DELLA protein function in growth responses to canopy signals. Plant J. *51*, 117–126.
17. Sessa, G., Carabelli, M., Sassi, M., Ciolfi, A., Possenti, M., Mittempergher, F., Becker, J., Morelli, G., and Ruberti, I. (2005). A dynamic balance between gene activation and repression regulates the shade avoidance response in Arabidopsis. Genes Dev. *19*, 2811–2815.
18. Roig-Villanova, I., Bou-Torrent, J., Galstyan, A., Carretero-Paulet, L., Portolés, S., Rodríguez-Concepción, M., and Martínez-García, J.F. (2007). Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. EMBO J. *26*, 4756–4767.
19. Ciolfi, A., Sessa, G., Sassi, M., Possenti, M., Salvucci, S., Carabelli, M., Morelli, G., and Ruberti, I. (2013). Dynamics of the shade-avoidance response in Arabidopsis. Plant Physiol. *163*, 331–353.
20. Galstyan, A., Cifuentes-Esquivel, N., Bou-Torrent, J., and Martinez-Garcia, J.F. (2011). The shade avoidance syndrome in Arabidopsis: a fundamental role for atypical basic helix-loop-helix proteins as transcriptional cofactors. Plant J. *66*, 258–267.
21. Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O., and Fankhauser, C. (2009). Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. EMBO J. *28*, 3893–3902.
22. Pacín, M., Semmoloni, M., Legris, M., Finlayson, S.A., and Casal, J.J. (2016). Convergence of constitutive photomorphogenesis 1 and

phytochrome interacting factor signalling during shade avoidance. New Phytol. *211*, 967–979.

23. Sakamoto, K., and Nagatani, A. (1996). Nuclear localization activity of phytochrome B. Plant J. *10*, 859–868.
24. Pacín, M., Legris, M., and Casal, J.J. (2013). COP1 re-accumulates in the nucleus under shade. Plant J. *75*, 631–641.
25. Liu, B., Zuo, Z., Liu, H., Liu, X., and Lin, C. (2011). Arabidopsis cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes Dev. *25*, 1029–1034.
26. Lian, H.-L., He, S.-B., Zhang, Y.-C., Zhu, D.-M., Zhang, J.-Y., Jia, K.-P., Sun, S.-X., Li, L., and Yang, H.-Q. (2011). Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. Genes Dev. *25*, 1023–1028.
27. Zuo, Z., Liu, H., Liu, B., Liu, X., and Lin, C. (2011). Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. Curr. Biol. *21*, 841–847.
28. Sheerin, D.J., Menon, C., zur Oven-Krockhaus, S., Enderle, B., Zhu, L., Johnen, P., Schleifenbaum, F., Stierhof, Y.-D., Huq, E., and Hiltbrunner, A. (2015). Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. Plant Cell *27*, 189–201.
29. Huang, X., Ouyang, X., and Deng, X.W. (2014). Beyond repression of photomorphogenesis: role switching of COP/DET/FUS in light signaling. Curr. Opin. Plant Biol. *21*, 96–103.
30. Rolauffs, S., Fackendahl, P., Sahm, J., Fiene, G., and Hoecker, U. (2012). Arabidopsis COP1 and SPA genes are essential for plant elongation but not for acceleration of flowering time in response to a low red light to far-red light ratio. Plant Physiol. *160*, 2015–2027.
31. Goyal, A., Karayekov, E., Galvão, V.C., Ren, H., Casal, J.J., and Fankhauser, C. (2016). Shade promotes phototropism through phytochrome B-controlled auxin production. Curr. Biol. *26*. Published online November 23, 2016. http://dx.doi.org/10.1016/j.cub.2016.10.001.
32. Leivar, P., and Quail, P.H. (2011). PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. *16*, 19–28.