ACTA BIOLOGICA CRACOVIENSIA Series Botanica 56/2: 126–135, 2014
DOI: 10.2478/abcsb-2014-0027

INTERACTION BETWEEN PHOTORECEPTORS AND BR SIGNALING IN *ARABIDOPSIS*

ZHONG XIN ZHU[1#], XIAO FENG ZHU[2#], YU TING ZHU[3], DA NIAN YAO[1*] AND YUAN HU XUAN[3*]

[1]*Agricultural College, Anhui Agricultural University, Changjiangxi Road 130, Hefei, Anhui, China 230036*
[2]*College of Plant Protection, Shenyang Agricultural University, Dongling Road 120, Shenyang, Liaoning, China 110866*
[3]*College of Pharmaceutical Sciences, Wenzhou Medical University, Xueyuanxi Road 82, Wenzhou, Zhejiang, China 325035*

Received June 10, 2014; revision accepted October 3, 2014

Extensive studies have been performed to elucidate the role of brassinosteroids (BRs), an important class of phytohormone in plant growth, development, and photomorphogenesis. Different wavelengths of light recognized by photoreceptors play a crucial role in plant development. The role of different photoreceptors in BR signaling has not been analyzed. Here we used photoreceptor single mutants, double mutants and even a quadruple mutant to analyze BR-dependent hypocotyl growth and gene regulation. All the photoreceptor mutants differed from the controls in their response to BR, and hypocotyl elongation as well as BR marker gene regulation were inhibited by application of propiconazole (PCZ), a BR biosynthesis inhibitor. In addition, altered *Phytochrome* and *Cryptochrome* expression in *brassinosteroid insensitive 1* mutant *bri1-5* and *brassinazole-resistant 1* dominant mutant *bzr1-D* indicated that BR negatively regulates photoreceptors in transcriptional levels. This is the first study to investigate the connections between BR and photoreceptors in *Arabidopsis*.

Key words: *Arabidopsis*, brassinosteroids, morphogenesis, mutants, photpreceptors.

INTRODUCTION

A plant is a sessile organism that is flexible in adapting to diverse environments via morphological changes. Light is the most important environmental cue modulating plant growth and development, perceived by plant sensory photoreceptors such as phytochromes, cryptochromes and phototropins (Casal, 2013). Phytohormones are signal molecules that regulate most cellular processes in plants. Phytochrome A (PhyA) is solely responsible for sensing continuous far-red light, and PhyB is the major receptor for sensing red light (Quail et al., 1995). The *Arabidopsis* genome encodes two cryptochromes, cryptochrome 1 (CRY1) and cryptochrome 2 (CRY2), which mediate blue-light inhibition of hypocotyl elongation (Ahmad and Cashmore, 1993). The phototropins (phot1 and phot2) are the other blue-light receptors that function in mediating phototropism, chloroplast migration and stomatal opening in *Arabidopsis* (Briggs and Christie, 2002). Constitutive photomorphogenic 1 (COP1), a ring-finger-type ubiquitin E3 ligase, moves from the nucleus to the cytoplasm, resulting in disruption of proteasomal degradation and subsequent rapid accumulation of light-related transcription factors to stimulate photomorphogenesis (Ma et al., 2002). Long Hypocotyl 5 (HY5) is a transcription factor which is degraded in a COP1-dependent manner under dark conditions. HY5 is accumulated in the nucleus and required for hypocotyl growth inhibition in light (Oyama et al., 1997).

Phytohormones are plant hormones that regulate plant growth and development. Many reports have described the connections between light and the phytohormone signaling pathway. FAR-RED ELONGATED HYPOCOTYL3 (FHY3) and FAR-RED IMPAIRED RESPONSE1 (FAR1), two key transcription factors in the PhyA signaling pathway, directly bind to the ABA-insensitive (ABI5) promoter to modulate ABA signaling in *Arabidopsis* (Tang et al., 2013). PHYTOCHROME-INTERACTING FACTOR5

*e-mail: dnyao@163.com, yhxuan@wzmc.edu.cn # These authors contributed equally to this work.

PL ISSN 0001-5296

(PIF5) and COP1 have a role in ethylene biosynthesis and ethylene-regulated hypocotyl elongation (Khanna et al., 2007; Liang et al., 2012). Brassinosteroids (BRs) are one of the important groups of plant hormones that have been studied for their crucial role in regulating morphological processes, especially cell elongation (Wang et al., 2012). BRs are perceived by the receptor brassinosteroid insensitive 1 (BRI1), leading to dissociation of BRI1 kinase inhibitor 1 (BKI1) from the plasma membrane, and their co-receptor BRI1-Associated receptor Kinase 1 (BAK1), which interacts with BRI1 and mutually transphosphorylates to form an active BR receptor complex (Li and Chory, 1997; Li et al., 2002; Nam and Li, 2002; Yang et al., 2011). Brassinazole-resistant 1 (BZR1) and BRI1-EMS-Suppressor 1 (BES1) are two well-characterized transcription factors which regulate the expression of thousands of target genes (Sun et al., 2010). Protein stability was highly increased in *bes1-D* and *bzr1-D*, gain-of-function mutants of *BES1* and *BZR1*, in which a single proline is substituted by leucine within the PEST domain, and rescued *bri1*, a BR receptor mutant phenotype (Wang et al., 2002; Yin et al., 2002).

Studies have shown that BR biosynthesis or signaling-deficient mutants fail to maintain morphogenesis in the dark or respond to BR stimuli. Also, BR-deficient or signaling mutants exhibit short hypocotyls and dwarfism in the seedling and mature stages respectively (Clouse et al., 1996; Li et al., 1996; Clouse, 2002; Luccioni et al., 2002; Cluis et al., 2004). A number of studies have revealed that the hypersensitivity of *bzr1-D* to exogenously supplied BR under dark conditions causes direct activation of BZR1 to IAA19 and ARF7 (Zhou et al., 2013), and that a genetic lesion at the *Phytochrome B* gene could partially rescue a weak *bri1* mutant phenotype (Luccioni et al., 2002; Kim et al., 2014). Interactions between key light-signaling and BR-signaling proteins, such as BZR1 and PIFs or BZR1 and COP1, have been identified (Oh et al., 2012; Kim et al., 2014). However, the photoreceptor responses to BR stimuli have not been subjected to a comparative analysis.

In this study we used photoreceptor mutants, including *phytochrome* and *cryptochrome*, phototropin and light-signaling mutants, to examine the interaction between BR and light signaling. *Phytochrome* and *Cryptochrome* mutants exhibit longer hypocotyls under light conditions, similarly to BR-treated plants. In contrast, PCZ, a BR biosynthesis inhibitor (Hartwig et al., 2012), shows markedly inhibited hypocotyl elongation and BR-dependent gene expression. We examined BR and photoreceptor-dependent signaling interaction via analysis of hypocotyl elongation and transcript levels, using many combinations of photoreceptor mutants and the BR receptor mutant *bri1-5* or the *BZR1-d*ominant mutant *bzr1-D* which constitutively activates BR signaling. This is the first study of the interactive mechanism of BR and photoreceptors in *Arabidopsis*.

MATERIALS AND METHODS

PLANT MATERIALS AND GROWTH CONDITIONS

Surface-sterilized *Arabidopsis* seeds were sown on Murashige and Skoog (MS) medium containing 1.2% agar (Sigma, Saint Louis, MO, USA). After two days of stratification at 4°C, seedlings were incubated at 22°C under a 16 h photoperiod. Ten-day-old seedlings grown on 0.5× MS medium were then transferred to soil and grown at 22°C under a 16 h photoperiod.

For 2,4-epibrassinolide (2,4-epiBL) or PCZ-treatment experiments, 30 seedlings were grown on 0.5× MS medium for 7 days with different concentrations of 2,4-epiBL (Sigma, 10, 50, 100, 200 nM) or PCZ (Sigma, 10, 100, 1000, 2000 nM) under continuous light or 24 h dark. Hypocotyl lengths were measured using ImageJ software.

Wassilewskija (WS2) was used as the control line for *bri1-5*, and Columbia (Col-0) was the control for *bzr1-D*, all photoreceptor and light-signaling mutants. Information on the mutants tested in this study is given in Table S1.

RNA EXTRACTION AND QUANTITATIVE RT-PCR ANALYSIS

Total cellular RNA was isolated with RNeasy Plant Mini Kits (Qiagen) or TRIzol (Takara, Dalian, Liaoning, China) and subsequently treated with RQ-RNase-free DNase (Promega, Madison, WI, USA) to eliminate genomic DNA contamination. The GoScript Reverse Transcription Kit (Reverse Transcription System, Promega) was used to reverse-transcribe 2 μg total RNA, following the manufacturer's protocol. Real-time PCR was performed, and gene expression was quantified. qRT-PCR products were quantified using Illumina Eco 3.0 software (Illumina, San Diego, California, USA), and the values were normalized against *Actin* levels from the same samples. The primers used for qRT-PCR are shown in Table S2 and the tested gene information is given in Table S1.

STATISTICAL ANALYSIS

Statistical calculations were performed with Prism 5 (GraphPad, San Diego, CA). All data are expressed as means ± SE. Comparisons employed the t test (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

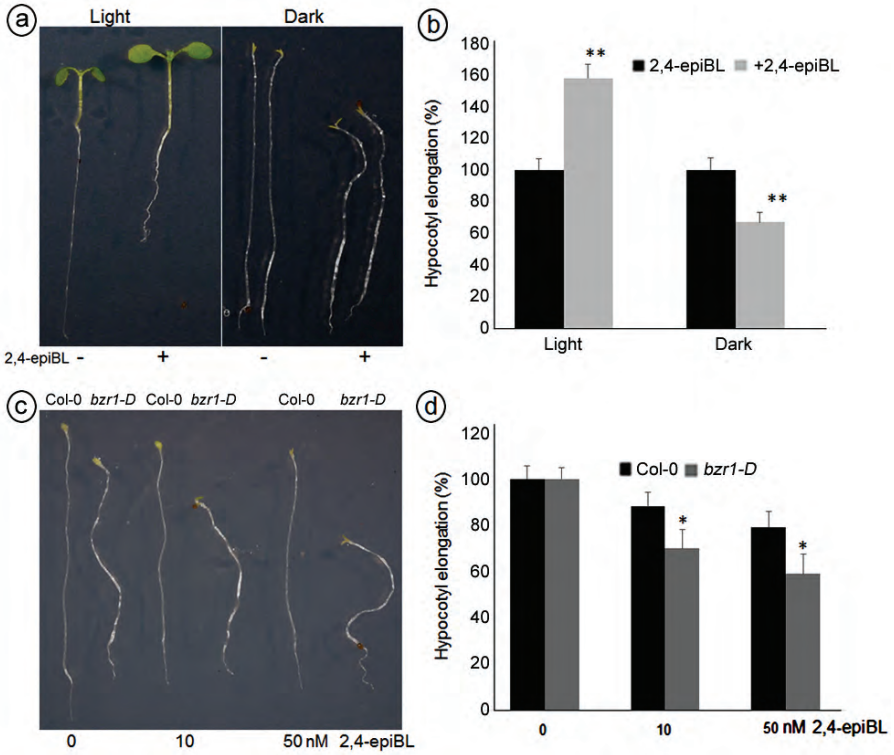

Fig. 1. Light-dependent hypocotyl elongation under BR treatment. (**a**) Col-0 plants grown with (+) or without (-) 100 nM 2,4-epiBL supply under light and in darkness, (**b**) Hypocotyl growth of plants shown in (a), (**c**) *bzr1-D* and Col-0 control plants grown under treatment with indicated concentrations of 2,4-epiBL, (**d**) Hypocotyl growth patterns of plants shown in (c). Error bars are SE of means of three independent experiments.

RESULTS

DIFFERENTIAL HYPOCOTYL GROWTH OF *cop1-4* AND *HY5* ox RESPONDS TO BR

Exogenously supplied BR led to significantly elongated hypocotyls (~54%) in light, but excess BR had the opposite effect (~36% shorter than control) under dark conditions (Fig. 1). *bzr1-D*, a dominant mutant of *BZR1*, an important BR signaling transcription factor, produced short, curved hypocotyls upon treatment with BR under dark conditions (Fig. 1) (Zhou et al., 2013). These data suggest that BR and light signaling are tightly connected. To test the effects of light on BR-mediated hypocotyl elongation, the Constitutive Photomorphogenic 1 (COP1) mutant *cop1-4*, Long Hypocotyl 5 (*HY5*) mutant *hy5*, Col-0, and *HY5* overexpression (*HY5 ox*) plants were analyzed for their BR-dependent hypocotyl growth in light or dark. *cop1-4* showed a normal BR response in hypocotyl elongation regardless of light conditions, while the *hy5* mutant was insensitive to BR in light conditions (Fig. 2) (Shi et al., 2011). *HY5 ox*, however, showed a BR response in the dark similar to that of the wild type and the *hy5* mutant (Fig. 2), which differed from that of the *cop1-4* mutant.

DIFFERENTIAL HYPOCOTYL GROWTH OF THE PHOTORECEPTOR MUTANTS RESPONDS TO BR AND PCZ

To further analyze the light-BR interaction, photoreceptor mutants were tested with different concentrations of 2,4-epiBL supplementation. Wild-type plants showed hypocotyl elongation correlated with BR concentration. Hypocotyl growth of *phyA211*, *phyA211/phyB9* and *phot1-2/cry1-2* (quadruple mutant in which *phot1*, *phot2*, *cry1* and *cry2* genes are mutated) was independent of exogenously supplied BR (Fig. 3). *phyB9* (~18% weaker than WT) and *cyr1-2* (*cry1* and *cry2* double mutant, ~25% weaker than WT) were slightly insensitive to BR, while *phot1-2* (*phot1* and *phot2* double mutant) showed a response to BR similar to that of wild-type plants (Fig. 3).

We tested the hypocotyl growth of photoreceptor mutants treated with BR biosynthesis inhibitor PCZ because the photoreceptor mutants produced longer hypocotyls under normal conditions. *phot1-2/cry1-2* (~40% less sensitive to PCZ than WT) and *cry1-2* (~30% less sensitive to PCZ than WT) were clearly insensitive to PCZ, while *phyB* and *phyA211/phyB9* (~10% less sensitive to PCZ than WT) were slightly

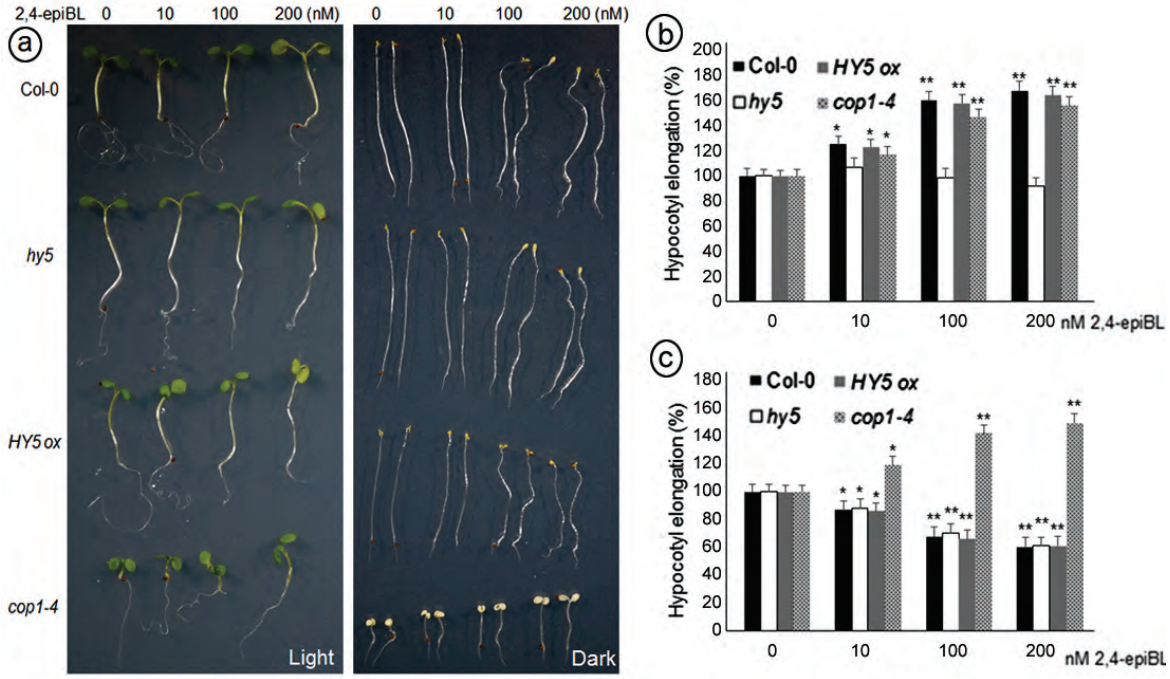

Fig. 2. 2,4-epiBL-mediated hypocotyl elongation in *hy5*, *HY5 ox* and *cop1-4* mutants and Col-0 control plants under light and in darkness. *hy5*, *HY5 ox* and *cop1-4* plants grown under treatment with indicated concentrations of 2,4-epiBL under light and in darkness (**a**), Hypocotyl elongation patterns under treatment with indicated concentrations of 2,4-epiBL treatment under light (**b**) and in darkness (**c**). Error bars are SE of means of three independent experiments.

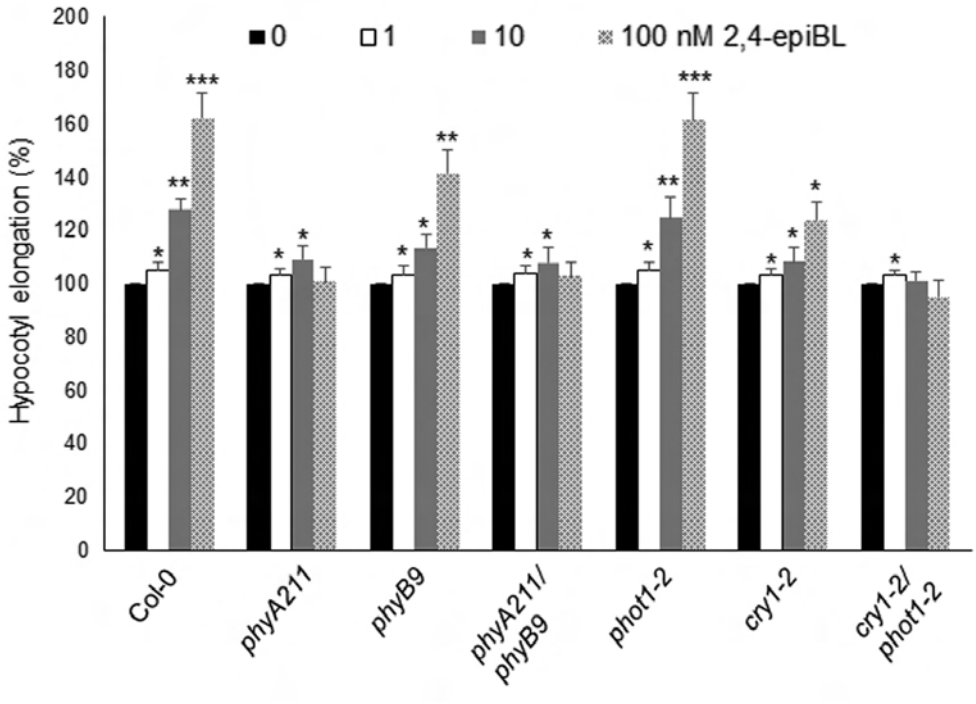

Fig. 3. 2,4-epiBL-mediated hypocotyl elongation in 7-day-old seedlings of photoreceptor mutants and Col-0 control plants. Hypocotyl elongation patterns under treatment with indicated concentrations of 2,4-epiBL under light. Error bars are SE of means of three replicates.

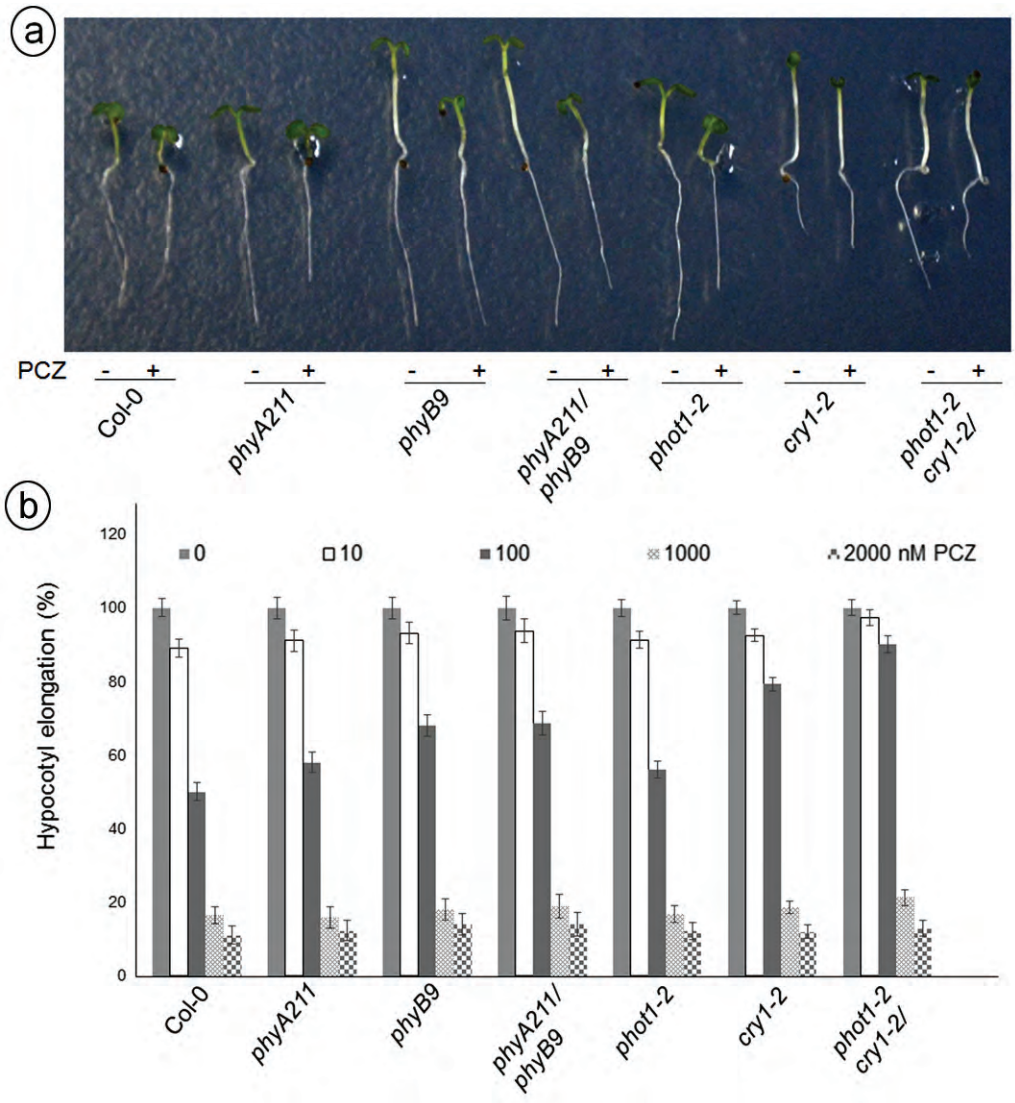

Fig. 4. Effects of the BR biosynthesis inhibitor PCZ on hypocotyl elongation in photoreceptor mutants. (**a**) Photoreceptor mutants (*phyA211*, *phyB9*, *phyA211*;*phyB9*, *phot1-2*, *cry1-2*, *phot1-2*;*cry1-2*) and Col-0 control plants grown with or without supply of 100 nM PCZ, (**b**) Photoreceptor mutants grown on media containing indicated concentrations of PCZ and hypocotyl growth. Error bars are SE of means of three independent experiments.

insensitive to PCZ (Fig. 4). The response to PCZ of *phyA211* and *phot1-2* mutants did not significantly differ from that of wild-type plants (Fig. 4).

We also examined the effect of BR on PCZ inhibition of hypocotyl elongation. Application of 1 μM PCZ drastically shortened hypocotyl length in both Col-0 and *phot1-2/cry1-2* plants (Fig 5). However, 2,4-epiBL treatment rescued hypocotyl growth inhibition by PCZ (Fig. 5).

We compared BR marker gene expression levels between wild-type, photoreceptor mutant and *bzr1-D* plants. Two BR biosynthetic genes (*BR6OX2*, *DWF4*) and signaling genes (*SAUR15*, *ACS5*) were analyzed for their expression levels in 7-day-old plants. The results showed that the *BR6OX2* level was lower in *phot1-2/cry1-2* (~77% lower than in WT) and *bzr1-D* (~68% lower than in WT), and higher in *phyA211* (~2.4-fold) and *phyA211/phyB9* (~1.5-fold) than in wild-type plants (Fig. 6a). The *DWF4* level was lower in *phyB9* (~60% lower than in WT), *phot1-2* (~60% lower than in WT), *cry1-2* (~57% lower than in WT), *phot1-2/cry1-2* (~82% lower than in WT) and *bzr1-D* (~74% lower than in WT) plants, and higher in *phyA211* (~1.5-fold) than in wild-type plants (Fig. 6b). The *SAUR15* level was lower in *cry1-2* (~56% lower than in WT) and higher in *phyA211* (~28% lower than in WT), (~28% lower than in WT), *phyB9* (~30% lower than in WT), *phyA211/phyB9* (~32%

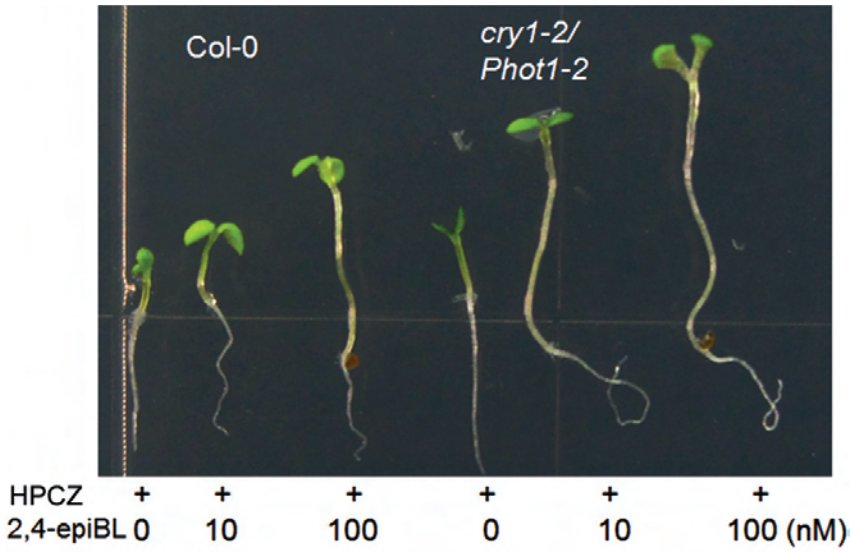

Fig. 5. PCZ and 2,4-epiBL effects on hypocotyl elongation in *cry1-2/phot1-2* and control Col-0 plants grown on media containing 1 μM PCZ together with indicated concentrations of 2,4-epiBL for 7 days.

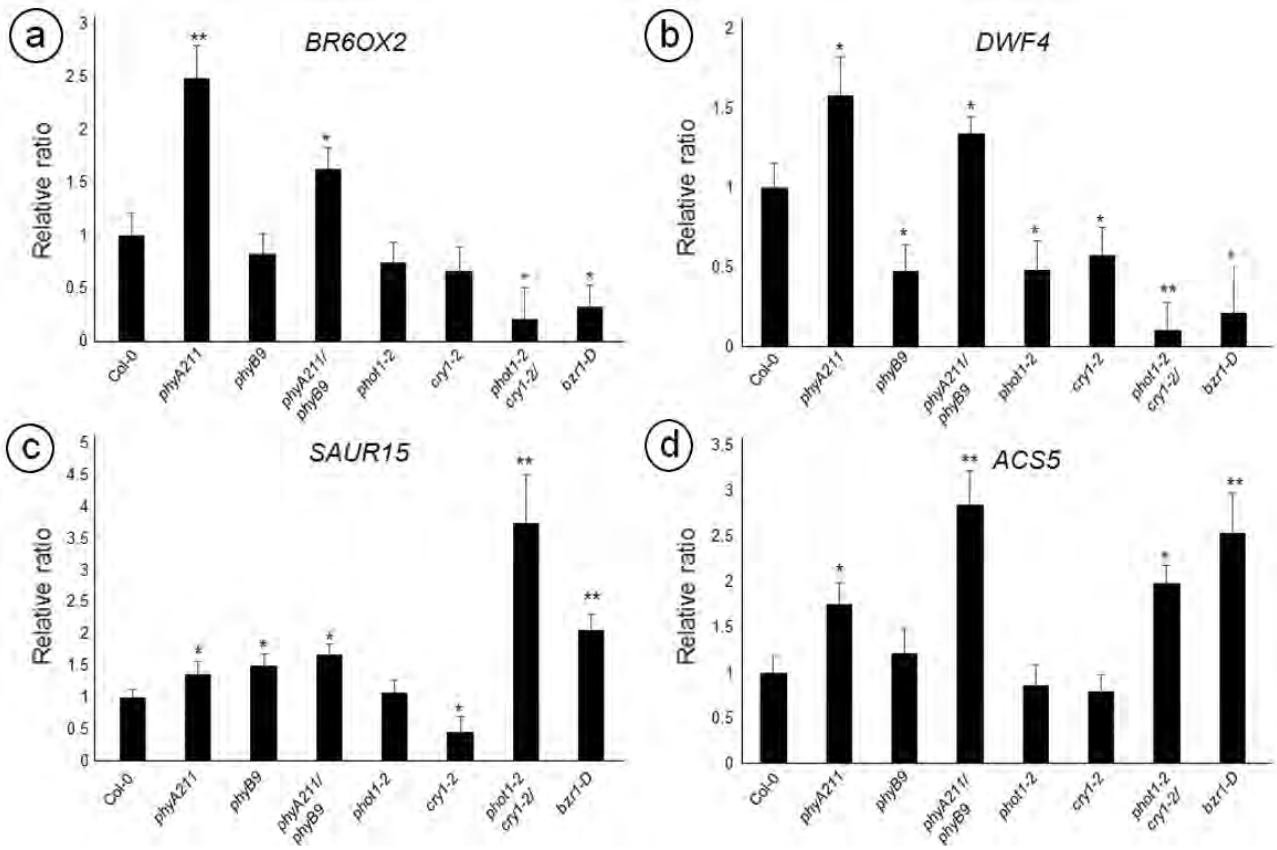

Fig. 6. Expression levels of BR biosynthetic and signaling genes in shoot tissues of photoreceptor mutants and *bzr1-D* mutants. (**a**, **b**) Expression levels of BR biosynthetic genes (*BR6OX2* and *DWF4*), (**c**, **d**) Signaling genes (*SAUR15* and *ACS5*) were monitored by qRT-PCR. Error bars are SE of means of three replicates.

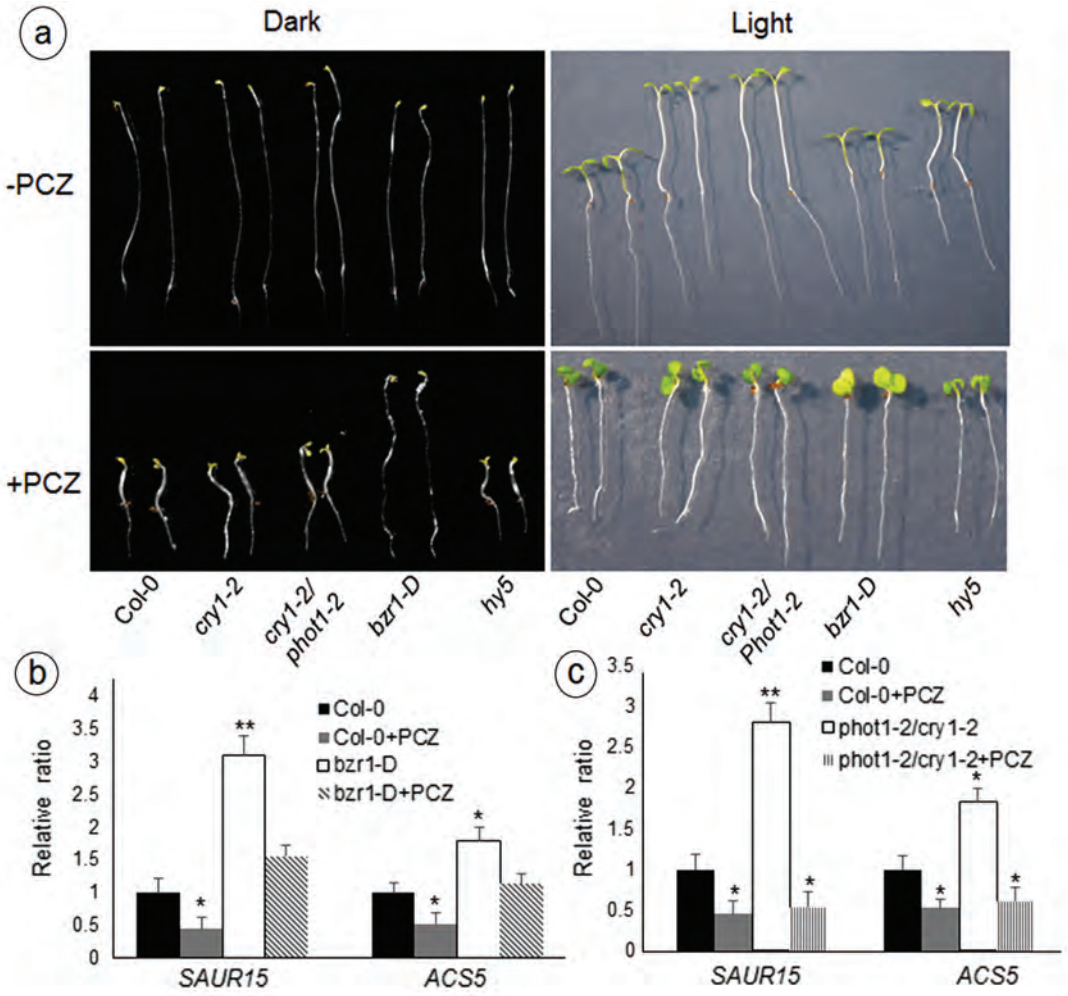

Fig. 7. PCZ-dependent hypocotyl elongation and marker gene expression in *phot1-2*;*cry1-2* mutant and Col-0 control plants. (**a**) cyr1-2, *cry1-2*;*phot1-2*, *bzr1-D* and *hy5* grown with or without 2 μM PCZ under light and in darkness, (**b**) *SAUR15* and *ACS5* expression levels in *bzr1-D* and Col-0 control plants grown for 5 days on 0.5× MS with or without 2 μM PCZ, (**c**) *SAUR15* and *ACS5* expression levels in *phot1-2*;*cry1-2* and Col-0 control plants grown with or without 2 μM PCZ. Error bars are SE of the means of three replicates.

lower than in WT), *phot1-2/cry1-2* (~3.5-fold) and *bzr1-D* (~1.9-fold) than in wild-type plants (Fig. 6c). The *ACS5* level was higher in *phyA211* (~1.7-fold), *phyA211/phyB9* (~2.7-fold), *phot1-2/cry1-2* (~1.8-fold) and *bzr1-D* (~2.3-fold) than in wild-type plants (Fig. 6d). These results demonstrate that the photoreceptors are involved in BR signaling.

BR IS REQUIRED FOR *phot1-2/cry1-2* PHENOTYPE AND GENE REGULATION

The *phot1-2/cry1-2* mutant was insensitive to PCZ, and BR marker gene expression in *phot1-2/cry1-2* was similar to that in *bzr1-D*. We further tested the effect of BR activity on *phot1-2/cry1-2*-mediated marker gene induction. Before testing gene expression, we monitored the effects of a high concentration of PCZ on hypocotyl elongation under dark and light conditions. In the dark, 2 μM PCZ treatment significantly inhibited hypocotyl elongation in de-etiolated wild-type, *cry1-2*, *phot1-2/cry1-2* and *hy5* seedlings but not in the *bzr1-D* mutant (Fig. 7a). Under light, hypocotyl elongation was completely inhibited by 2 μM PCZ application in all plants tested, but *bzr1-D* developed larger cotyledons than the other plants (Fig. 7a). Wild-type, *bzr1-D* and *phot1-2/cry1-2* plants were grown on 0.5×MS with or without 2 μM PCZ for 5 days and their *SAUR15* and *ACS5* levels monitored. *SAUR15* and *ACS5* expression was higher in *bzr1-D* (~3- and 1.6-fold respectively) and *phot1-2/cry1-2* (~2.7- and 1.5-fold respectively) than in the wild type (Fig. 7b, c). *SAUR15* and *ACS5* expression levels were higher in *bzr1-D* (~3- and 1.5-fold respectively) than in wild-type plants; their

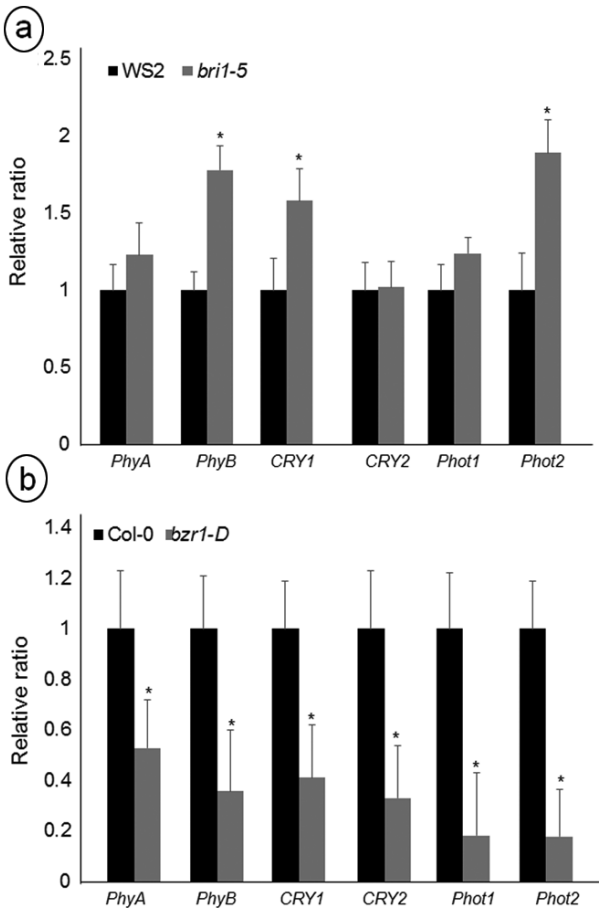

Fig. 8. Expression levels of photoreceptor genes in *bri1-5* and *bzr1-D*. RNA was extracted from 7-day-old plants and the expression levels of *PhyA*, *phyB*, *cry1*, *cry2*, *phot1* and *phot2* were monitored by qRT-PCR in *bri1-5* and WS2 control plants (**a**) or *bzr1-D* and Col-0 control plants (**b**). Error bars are SE of means of three replicates.

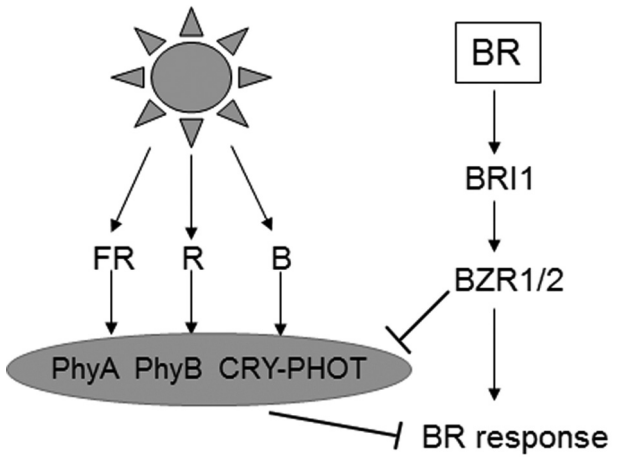

Fig. 9. Scheme of BR and light signaling interaction. Phytochrome A (PhyA), B (phyB), Cryptochrome (CRY) and Phototropins (PHOT) recognized far red (FR), red (R) and blue light (B) respectively. BRs are recognized by their receptor BRI1 to further activate downstream transcription factor BZR1/2. BZR1/2 negatively regulates photoreceptors, while photoreceptors negatively regulate BR-responsive genes.

levels in wild-type and *phot1-2/cry1-2* plants were similar after PCZ treatment (Fig. 7b, c). These data show that hypocotyl elongation under light in *phot1-2/cry1-2* requires BR activity.

BR SIGNALS NEGATIVELY REGULATE PHOTORECEPTORS

To further test the effects of BR signaling on photoreceptor gene expression, the weak BRI1 mutant *bri1-5* and the *bzr1-D* mutant were used to analyze the expression levels of *PhyA*, *PhyB*, *CRY1*, *CRY2*, *Phot1* and *Phot2*. In the *bri1-5* mutant we found higher levels of *PhyB CRY1* and *Phot2* (~1.7-, ~1.5- and ~1.8-fold higher respectively), while the levels of *PhyA*, *CRY2* and *Phot1* in *bri1-5* were similar to those in WS2 plants (Fig. 8a). In contrast, versus Col-0 we found lower levels of *PhyA* (~50% lower in *bzr1-D* than in Col-0), *PhyB* (~58% lower in *bzr1-D* than in Col-0), *CRY1* (~56% lower in *bzr1-D* than in Col-0), *CRY2* (~49% lower in *bzr1-D* than in Col-0), *Phot1* (~83% lower in *bzr1-D* than in Col-0) and *Phot2* (~84% lower in *bzr1-D* than in Col-0) in *bzr1-D* (Fig. 8b). These data indicate that BRI1 and BZR1 negatively regulate photoreceptor gene expression.

DISCUSSION

Light is a cue for plant growth and development, and is recognized by photoreceptors. BR hormones regulate numerous aspects of plant growth and development, including photomorphogenesis and the interaction between light and BR signaling (Wang et al., 2012). The *cop1-4* and *HY5 ox* mutants differed in their BR responses under dark conditions, suggesting that BR-promoted *cop1-4* hypocotyl elongation in the dark is not due to overexpression of *HY5*. It might be due to the interaction between COP1 and BZR1, recently identified (Kim et al., 2014). The responses of photoreceptor mutants to BR demonstrated that *phyA211*, *phyA211/phyB9* and *phot1-2/cry1-2* are significantly insensitive to exogenously supplied BR (Fig. 3). The response of photoreceptor mutants, especially *phyB9*, *phyA211/phyB9*, *cry1-2* and *phot1-2/cry1-2*, was similar to that of BR-treated plants growing under light. This suggests that BR treatment is not suitable for examining the relationship between photoreceptors and BR signaling.

To further analyze BR effects on photoreceptor mutants we applied the BR biosynthesis inhibitor PCZ. The *cry1-2* and *phot1-2/cry1-2* mutants were clearly insensitive to PCZ, while *phyB9* and *phyA211/phyB9* were slightly insensitive to 100 nM PCZ (Fig. 4). However, a high PCZ concentration (2 μM) completely inhibited *cry1-2* and *phot1-2/cry1-2* hypocotyl elongation growing under light (Fig. 7a), indicating that hypocotyl growth in *cry1-2* and *phot1-2/cry1-2* mutants requires BR activity. Analysis of BR marker gene expression in the photoreceptor mutants showed that BR biosynthetic and signaling genes are regulated differently in the photoreceptor mutants (Fig. 6), suggesting that photoreceptors somehow regulate the BR signaling pathway. Without PCZ treatment, BR marker gene expression in *phot1-2/cry1-2* was similar to that in the *bzr1-D* mutant, but with PCZ treatment the *SAUR15* and *ACS5* expression of *phot1-2/cry1-2* was completely suppressed; in *bzr1-D*, on the other hand, PCZ did not completely inhibit *SAUR15* and *ACS5* expression (Fig. 7b, c). These data are correlated with the results on PCZ-mediated hypocotyl elongation in *phot1-2/cry1-2* and *bzr1-D* (Fig. 7a). The results also suggest that blue-light receptors negatively regulate BR signaling. In contrast, photoreceptor expression in *bri1-5* (BR-insensitive) and *bzr1-D* (BR-sensitive) mutants indicated that BR signaling negatively regulates photoreceptors at transcription level (Fig. 8).

This is the first time the interaction between BR and light signaling has been tested using photoreceptors and BR mutants. Taken together, our results suggest that light signaling tightly regulates the BR response, which may occur partially via photoreceptors (Fig. 9). Further experiments should elucidate the exact role of each photoreceptor in BR signaling.

CONCLUSIONS

BRs play different roles in hypocotyl elongation under light (promotion) and darkness (inhibition). The key light-signaling gene mutant *cop1* exhibited light-independent BR-mediated hypocotyl elongation. The photoreceptor mutants *phyA211*, *phyA211/phyB9* and *cry1-2/phot1-2* exhibited insensitive responses to BR, while the blue-light receptor mutants *cry1-2* and *cry1-2/phot1-2* were more insensitive than other photoreceptor mutants to PCZ, a BR biosynthesis inhibitor. Expression of BR biosynthetic genes was higher in *cry1-2/phot1-2*. However, supply of PCZ reduced the higher expression of BR biosynthetic genes in *cry1-2/phot1-2*. BRI1 and BZR1 negatively regulated the transcription of photoreceptor genes.

AUTHORS' CONTRIBUTIONS

ZXZ, YHX and DNY designed research; ZXZ, XFZ, YTZ and YHX performed research; ZXZ, XFZ, YHX, and DNY analyzed data; ZXZ, YHX and DNY wrote the paper.

ACKNOWLEDGEMENTS

This work was supported by the National Nature Science Foundation of China (31371615 to DNY) and by Wenzhou Medical University startup funding to YHX. *bzr1-D* and *bri1-5* mutant seeds were obtained from the Wang Lab, and *phyA211*, *phyB9*, *phyA211*;*phyB9*, *cry1-2*, *phot1-2* and *cry1-2*;*phot1-2* seeds were obtained from the Briggs Lab (Carnegie Institution for Science) as a kind gift. *cop1-4*, *hy5* and *HY5 ox* seeds were obtained from the Hong Lab (Gyeongsang National University).

SUPPLEMENTARY MATERIAL

Supplementary material (Tables S1 and S2) for this article can be found in the online version at doi: 10.2478/abcsb-2014-0027

REFERENCES

AHMAD M, and CASHMORE AR. 1993. HY4 gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature* 366: 162–166.

AIHARA Y, TABATA R, SUZUKI T, SHIMAZAKI K, and NAGATANI A. 2008. Molecular basis of the functional specificities of phototropin 1 and 2. *Plant Journal* 56: 364–375.

BELKHADIR Y, DURBAK A, WIERZBA M, SCHMITZ RJ, AGUIRRE A, MICHEL R, ROWE S, FUJIOKA S, and TAX FE. 2010. Intragenic suppression of a trafficking-defective brassinosteroid receptor mutant in *Arabidopsis*. *Genetics* 185: 1283–1296.

BRIGGS WR, and CHRISTIE JM. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends in Plant Science* 7: 204–210.

CASAL JJ. 2013. Photoreceptor signaling networks in plant responses to shade. *Annual Review of Plant Biolology* 64: 403–427.

CANAMERO RC, BAKRIM N, and BOULY JP. 2006. Cryptochrome photoreceptors cry1 and cry2 antagonistically regulate primary root elongation in *Arabidopsis thaliana*. *Planta* 224: 995–1003.

CLOUSE SD. 2002. *Arabidopsis* mutants reveal multiple roles for sterols in plant development. *Plant Cell* 14: 1995–2000.

CLOUSE SD, LANGFORD M, and MCMORRIS TC. 1996. A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiology* 111: 671–678.

Cluis CP, Mouchel CF, and Hardtke CS. 2004. The *Arabidopsis* transcription factor HY5 integrates light and hormone signaling pathways. *Plant Journal* 38: 332–347.

Franklin KA, and Quail PH. 2010. *Phytochrome* functions in *Arabidopsis* development. *Journal of Experimental Botany* 61: 11–24.

Khanna R, Shen Y, Marion CM, Tsuchisaka A, Theologis A, Schäfer E, and Quail PH. 2007. The basic helix-loop-helix transcription factor PIF5 acts on ethylene biosynthesis and phytochrome signaling by distinct mechanisms. *Plant Cell* 19: 3915–3929.

Kim B, Jeong YJ, Corvalán C, Fujioka S, Cho S, Park T, and Choe S. 2014. Darkness and *gulliver2/phyb* mutation decrease the abundance of phosphorylated BZR1 to activate brassinosteroid signaling in *Arabidopsis*. *Plant Journal* 77(5): 737–747.

Hartwig T, Corvalan C, Best NB, Budka Js, Zhu Jy, Choe S, and Schulz B. 2012. Propiconazole is a specific and accessible brassinosteroid (BR) biosynthesis inhibitor for *Arabidopsis* and maize. *PLos One* 7: e36625.

Li J, and Chory J. 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* 90: 929–938.

Li J, Nagpal P, Vitart V, McMorris TC, and Chory J. 1996. A role for brassinosteroids in light-dependent development of *Arabidopsis*. *Science* 272: 398–401.

Li J, Wen J, Lease KA, Doke JT, Tax FE, and Walker JC. 2002. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* 110: 213–222.

Liang X, Wang H, Mao L, Hu Y, Dong T, Zhang Y, Wang X, and Bi Y. 2012. Involvement of COP1 in ethylene- and light-regulated hypocotyl elongation. *Planta* 236: 1791–802.

Luccioni LG, Oliverio KA, Yanovsky MJ, Boccalandro HE, and Casal JJ. 2002. Brassinosteroid mutants uncover fine tuning of phytochrome signaling. *Plant Physiology* 128:173–181.

Ma L, Gao Y, Qu L, Chen Z, Li J, Zhao H, and Deng XW. 2002. Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in *arabidopsis*. *Plant Cell* 14: 2383–2398.

Nam KH, and Li J. 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* 110: 203–212.

Oh E, Zhu JY, and Wang ZY. 2012. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. *Nature Cell Biology* 14: 802–809.

Oyama T, Shimura Y, and Okada K. 1997. The *Arabidopsis HY5* gene encodes a BZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes and Development* 11: 2983–2995.

Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, and Wagner D. 1995. Phytochromes: photosensory perception and signal transduction. *Science* 268: 675–680.

Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, Ma L, Hoecker U, and Deng XW. 2003. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes and Development* 17: 2642–2647.

Shi Qm, Yang X, Song L, and Xue HW. 2011. *Arabidopsis* MSBP1 is activated by HY5 and HYH and is involved in photomorphogenesis and brassinosteroid sensitivity regulation. *Molecular Plant* 4: 1092–1104.

Sun Y, Fan Xy, Cao DM, Tang W, He K, Zhu JY, He JX, Bai MY, Zhu S, Oh E, Patil S, Kim TW, Ji H, Wong WH, Rhee SY, and Wang ZY. 2010. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in *arabidopsis*. *Developmental Cell* 19: 765–777.

Tang W, Ji Q, Huang Y, Jiang Z, Bao M, Wang H, and Lin R. 2013. FAR-RED ELONGATED HYPOCOTYL3 and FAR-RED IMPAIRED RESPONSE1 transcription factors integrate light and abscisic acid signaling in *Arabidopsis*. *Plant Physiology* 163: 857–866.

Wang ZY, Bai MY, Oh E and Zhu JY. 2012. Brassinosteroid signaling network and regulation of photomorphogenesis. *Annual Review of Genetics* 46: 701–724.

Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T, and Chory J. 2002. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. *Developmental Cell* 2: 505–513.

Yang CJ, Zhang C, Lu YN, Jin JQ, and Wang XL. 2011. The mechanisms of brassinosteroids' action: From signal transduction to plant development. *Molecular Plant* 4: 588–600.

Yin Y, Wang ZY, Mora-Garcia S, Li J, Yoshida S, Asami T, and Chory J. 2002. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. *Cell* 109: 181–191.

Zhou X, Song L, and Xue HW. 2013. Brassinosteroids regulate the differential growth of *Arabidopsis* hypocotyls through auxin signaling components IAA19 and ARF7. *Molecular Plant* 6: 887–904.

TABLE S1. Gene information

Gene name	Symbol	Mutant	Phenotype	Physiological effect	Literature
BRI1	BR receptor	*bri1-5*	Dwarf	Insensitive to BR	Belkhadir et al., 2010
BZR1	BR signaling transcription factor	*bzr1-D*	Broad leaves, thick stem	Insensitive to BR inhibitor PCZ	Wang et al., 2002
PhytochromeA	Far red light receptor	*phyA211*	No obvious phenotype	Fails to respond to far red light	Franklin et al., 2010
Phytochrome B	Red light receptor	*phyB9*	Long petiole	Fails to respond to red light	Franklin et al., 2010
Cryptochrome 1/2	Blue light receptor	*cry1/2*	Long petiole	Fails to respond to blue light	Canamero et al., 2006
Phototropin 1/2	Blue light receptor	*phot1/2*	Phototropism insensitive	Fails to respond to blue light	Aihara et al., 2008
BR6OX2	BR biosynthesis enzyme				Oh et al., 2012
DWF4	BR biosynthesis enzyme				Oh et al., 2012
SAUR15	BR inducible gene				Oh et al., 2012
ACS5	BR inducible gene				Oh et al., 2012
COP1	Light signaling E3 ligase	*cop1-4*	Dwarf	Light response in the dark	Ma et al., 2002
HY5	Light signaling transcription factor	*hy5*	Long hypocotyl	Constitutive light response	Saijo et al., 2003

TABLE S2. Primer sequences

Primer	Sequence
BR6OX2 F	ATGGCGGCGATGAAATACAAAGGA
BR6OX2 R	TGTTCCTCCATCAATCTTCTTCTC
DWF4 F	TGGCGGTGTACGGTTTAAGAT
DWF4 R	TGGCGGTGTACGGTTTAAGAT
SAUR15 F	AAGAGGATTCATGGCGGTCTATG
SAUR15 R	GTATTGTTAAGCCGCCCATTGG
Actin F	TCCAAGCTGTTCTCTCCTTG
Actin R	GAGGGCTGGAACAAGACTTC
ACS5 F	GCGATGCTTTCCTTTTGCCTACTC
ACS5 R	TTTCTGGGCTTGTTGGTAAGCTTGT
CRY1 F	GACCTGAAGAAGACGAAG
CRY1 R	ACTCGGGGACTATGCCTC
CRY2 F	GGCCTTAGGGGCTAATAC
CRY2 R	ATACCTTCCAGATTCTTC
PHOT1 F	AGTTTCCAGCTAGCATTC
PHOT1 R	TAGCTCAGGATCAACAAC
PHOT2 F	CAGTTGATCAACACGTTG
PHOT2 R	ATTCACAAGCACTCCATC
PhyA F	CTTGCTAATCTAGAGATC
PhyA R	GTTTGCTGCAGCGAGTTC
PhyB F	GCCCTGAAGGTTTAGGTC
PhyB R	CATCATCAGCATCATGTC