

Original Research

# Brassinosteroid Overaccumulation Differentially Modulates *Arabidopsis* Hypocotyl Growth in the Light and in the Dark

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## Abstract

**Background:** Brassinosteroids (BRs) are plant hormones that are critical for seedling growth. In *Arabidopsis thaliana* (L.) Heynh., BRs stimulate hypocotyl elongation in the light but inhibit it in darkness, whereas the BR biosynthesis inhibitor brassinazole (BRZ) suppresses hypocotyl growth under both conditions. Our earlier work using BR-deficient mutants suggested that endogenous BR levels are suboptimal for hypocotyl elongation in light-grown seedlings but nearly optimal in darkness. However, this model has not been validated using BR-overaccumulating lines. **Methods:** To increase endogenous BR levels, we used previously generated single, double, and triple knockout mutants of three well-characterized BR-inactivating genes (*BEN1*, *BAS1*, and *SOB7*). These mutants accumulate BRs in an additive manner. We examined their hypocotyl responses to exogenous brassinolide (BL) and BRZ under light and dark conditions, and analyzed the expression of selected BR- and light-responsive genes. **Results:** BR overaccumulation led to longer hypocotyls in the light but shorter hypocotyls in the dark. In the light, BL consistently promoted hypocotyl elongation, whereas, BRZ consistently inhibited it, in both wild-type and BR-overaccumulating seedlings. In darkness, both BL and BRZ suppressed hypocotyl growth across all genotypes at moderate and high concentrations. Notably, at high BRZ concentrations, BR-overaccumulating mutants developed longer hypocotyls than wild-type seedlings under the same dark conditions. Comparisons of relative hypocotyl length (% of untreated control) showed that BR overaccumulation increased hypocotyl responsiveness to high-dose BRZ in the light but conferred partial resistance to BRZ in the dark. **Conclusions:** These results support and refine our previous model of BR–light/dark interactions. BR overaccumulation enhances hypocotyl growth in the light, whereas excess BRs inhibit elongation in darkness. Gene expression analyses of wild-type and BR-overaccumulating seedlings identified GATA Transcription Factor 2 (GATA2) as a candidate integrator of BR and light signaling during hypocotyl development.

**Keywords:** *Arabidopsis*; brassinosteroids; etiolation; hypocotyl; light sensitivity

## 1. Introduction

Brassinosteroids (BRs) are a class of plant hormones structurally similar to animal steroids and well known for their indispensable roles in promoting cell expansion and growth [1]. Unlike many other plant hormones [2], BRs are relatively stable and do not undergo long-distance transport [3], making localized regulation of their synthesis and inactivation essential for proper developmental outcomes. Multiple feedback regulation loops, mediated through transcription factors [4,5], photoreceptors [6], and circadian oscillators [7], fine-tune BR metabolism to maintain appropriate hormone levels within specific tissues.

Among naturally occurring BRs, brassinolide (BL) exhibits the strongest biological activity [8,9]. Chemical inhibitors [10] such as brassinazole (BRZ) [11] and propiconazole (PCZ) [12] interfere with BR biosynthesis and are widely used to probe BR-dependent biological and physiological processes. Application of BL or BRZ has helped clarify BR functions in growth, development, and stress responses across different plant systems [13,14,15].

In *Arabidopsis thaliana* (L.) Heynh., BRs interact closely with light to regulate early seedling development [16]. Photomorphogenesis in the light [17] and skotomorphogenesis in darkness [18] produce distinct hypocotyl elongation patterns, and BR signaling contributes strongly to these differences [16]. Although BR-modulated root growth is largely independent of light [19], hypocotyl responses to exogenous BL vary substantially between light-grown [17] and etiolated [18] seedlings. In the light, BL enhances hypocotyl elongation in both wild-type plants and BR-deficient mutants [17]. In darkness, however, BL only promotes hypocotyl growth of BR-deficient mutants at low concentrations [18]. Higher concentrations of BL suppress elongation of dark-grown seedlings regardless of genotype [18].

Building on these observations, our previous work proposed a model in which endogenous BR levels are suboptimal for maximum hypocotyl growth in the light but are close to optimal in darkness [18]. In light-grown seedlings, exogenous BL boosts hypocotyl growth whereas BRZ reduces it [17]. In contrast, dark-grown wild-type seedlings exhibit reduced hypocotyl elongation when BR levels are



either increased or decreased, consistent with a narrow optimal BR range [18]. Dark-grown BR-deficient seedlings show a two-phase BL response. Application of exogenous BL first compensates for insufficient endogenous BR supply to promote elongation, but the effect eventually becomes inhibitory at higher doses [18]. BRZ further decreases their already insufficient BR levels and consistently suppresses growth [18].

One major limitation of this model has been the lack of evidence from BR overaccumulation lines. Because BR biosynthesis involves several rate-limiting steps [20], increasing endogenous BR content can be more effectively achieved by disrupting BR inactivating enzymes rather than by overexpressing individual biosynthetic genes. Three such BR inactivation enzymes, *phyB* Activation Tagged Suppressor 1 (BAS1/CYP734A1/CYP72B1) [4], Suppressor of *phyB-4* 7 (SOB7/CYP72C1) [4], and Brassinosteroid Insensitive 1 (BRI1)-5 Enhanced 1 (BEN1) [21], have been well characterized in *Arabidopsis*. Successive knockout of these genes elevates endogenous BR levels in an additive manner [22]. Using previously generated single (*ben1-2*), double (*bas1-2 sob7-1*), and triple (*bas1-2 sob7-1 ben1-3*) knockout mutants targeting *BEN1*, *BAS1*, and *SOB7* [22], we investigated how BR overaccumulation modulates hypocotyl responses to exogenous BL and BRZ under light and dark conditions. We also examined expression changes in selected BR- and light-responsive genes to identify regulatory factors linking BR homeostasis with light-mediated hypocotyl growth.

## 2. Materials and Methods

### 2.1 Plant Materials

All *Arabidopsis* lines used in this study were in the Columbia-0 (Col-0) background. All three BR overaccumulation mutants were previously described [22]. *ben1-2* was a CRISPR/Cas9-induced frameshift mutation in *BEN1* [22]. *bas1-2 sob7-1* was selected from an F2 population derived from crosses between *bas1-2* (SALK\_006781, [https://abrc.osu.edu/stocks/number/SALK\\_006781](https://abrc.osu.edu/stocks/number/SALK_006781)) and *sob7-1* (SALK\_120416, [https://abrc.osu.edu/stocks/number/SALK\\_120416](https://abrc.osu.edu/stocks/number/SALK_120416)) T-DNA insertion lines [22]. *bas1-2 sob7-1 ben1-3* was created by introducing a 38-nucleotide deletion in *BEN1* within the *bas1-2 sob7-1* background [22]. T-DNA insertion lines were ordered from the *Arabidopsis* Biological Resource Center. All mutant genotypes have been previously confirmed by gene sequencing [22]. These mutants successively eliminated BR-inactivation enzymes and therefore elevated endogenous BR levels in an additive manner [22]. Wild-type Col-0 served as the control for all experiments.

### 2.2 Seedling Growth and Hypocotyl Measurement

Seedlings were grown on solid half-strength Murashige and Skoog (1/2MS) medium (PhytoTech Labs M530, Lenexa, KS, USA) containing 15 g/L sucrose

(PhytoTech Labs S829) and 6 g/L Gellan gum (PhytoTech Labs G434). Media were supplemented with either BL (APExBIO A3265, Houston, TX, USA) or BRZ (TCI America B2829, Portland, OR, USA) at final concentrations of 0, 0.02, 0.1, 0.5, or 2.5  $\mu\text{M}$ . BL and BRZ were dissolved in N,N-dimethylformamide (DMF, Fisher Scientific D119-1, Pittsburgh, PA, USA), and equivalent DMF concentrations were added to all petri dishes.

Seeds were surface-sterilized, stratified at 4 °C in darkness for four days, and then exposed to white light (2 h, 25 °C) before being grown for four days under either continuous white light or complete darkness. Light intensity was set to 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for BL assays and 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for BRZ assays. All seedlings were maintained at 25 °C in controlled incubators (Percival Scientific I-30L, Perry, IA, USA).

After four days, seedlings were scanned at 1200 dots per inch (DPI) and hypocotyl length was measured using ImageJ v1.54g [23]. Relative hypocotyl length (% of untreated control) in response to BL/BRZ was calculated using the following formula: (individual hypocotyl length treated with 2.5  $\mu\text{M}$  of BL or BRZ)/(average hypocotyl length without BL or BRZ treatment)  $\times$  100%. Each data group represents measurements from 30 seedlings pooled from three biological replicates. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey honestly significant difference (HSD) test (<https://www.statskingdom.com/180Anova1way.html>). Groups labeled with different lowercase letters represent significantly different means ( $p < 0.05$ ).

### 2.3 Quantification of Relative Transcript Levels

To assess transcriptional responses, Col-0 and *bas1-2 sob7-1 ben1-3* seedlings were grown for four days in continuous white light (40  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) or complete darkness. Total RNA was extracted using the QIAGEN RNeasy Plant Mini Kit, followed by cDNA synthesis using the iScript Reverse Transcription Supermix from Bio-Rad Laboratories. Reverse transcription quantitative PCR (RT-qPCR) was performed with Bio-Rad SsoAdvanced Universal SYBR Green Supermix on a Bio-Rad C1000 Thermal Cycler and CFX96 Real-Time System.

Gene transcript levels were normalized to *Ubiquitin 10* (*UBQ10*) using the  $\Delta\Delta\text{Ct}$  calculation method. Each gene expression value reflects six measurements (three biological replicates  $\times$  two technical replicates). Statistical analysis was performed using one-way ANOVA and Tukey's HSD test. Groups labeled with different lowercase letters represent significantly different means ( $p < 0.05$ ). A group labeled with two or more letters means that it overlaps with distinct subsets. Its average value is statistically similar to multiple other groups that do not necessarily resemble each other. Primer sequences are provided in **Supplementary Table 1**.

### 3. Results

#### 3.1 Hypocotyl Responses of Wild Type and BR Overaccumulation Mutants to BL and BRZ Are Different Between Light and Dark Conditions

Col-0, *ben1-2*, *bas1-2 sob7-1*, and *bas1-2 sob7-1 ben1-3* seedlings were grown for four days on solid 1/2MS medium containing a range of BL or BRZ concentrations (0.02–2.5  $\mu\text{M}$ ) under continuous white light or complete darkness. All genotypes displayed clear and condition-dependent hypocotyl responses to BR and BRZ treatments (Fig. 1). In both light- and dark-grown seedlings, BL and BRZ altered hypocotyl length in dosage dependent patterns, and these trends were consistent across the single, double, and triple mutants. Representative seedling images illustrate these growth differences across treatments (Fig. 1A–D).

#### 3.2 In Light Conditions, BL Promotes and BRZ Inhibits Hypocotyl Growth in Both Wild Type and BR Overaccumulation Mutants

To enhance the visibility of treatment-dependent hypocotyl changes, BL and BRZ application experiments were conducted under two different light intensities of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for BL assays and 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for BRZ assays [17]. Four-day-old seedlings were chosen for measurement because this stage reliably shows distinct BR- and BRZ-responsive phenotypes [4,17,18].

Across all BL concentrations tested (0.02–2.5  $\mu\text{M}$ ), hypocotyls of Col-0 and all three BR overaccumulation mutants elongated significantly (Fig. 2A–D). These results indicate that BL supplied directly to the medium-hypocotyl interface is readily perceived by BR receptors and promotes cell expansion regardless of endogenous BR status [24].

Conversely, BRZ consistently reduced hypocotyl length across all genotypes and concentrations (Fig. 2E–H). Since BRZ inhibits BR biosynthesis and light already limits hypocotyl elongation, these reductions highlight that endogenous BR levels in light-grown seedlings remain below the threshold needed for maximal hypocotyl growth, even in the triple knockout mutant *bas1-2 sob7-1 ben1-3* (Fig. 2D,H).

Overall, hypocotyl length in the light correlated positively with available BR levels. Increasing exogenous BL enhanced growth, whereas BRZ-induced reductions in BR biosynthesis suppressed growth in both wild type and BR overaccumulation mutants.

#### 3.3 In Darkness, Both BL and BRZ Suppress Hypocotyl Growth in Wild Type and BR Overaccumulation Mutants

In dark-grown seedlings, BL consistently inhibited hypocotyl elongation in all genotypes across all tested concentrations (Fig. 3A–D). This inhibitory response aligns with the understanding that dark-grown wild-type seedlings already possess near-optimal BR levels for hypocotyl elongation [18]. Further increases in BRs shift seedlings beyond the optimal BR range and suppress growth [18].

BRZ treatments also suppressed hypocotyl growth in darkness at concentrations of 0.1  $\mu\text{M}$  and above (Fig. 3E–H). For BR-deficient seedlings, past work showed a biphasic BL response of initial promotion followed by inhibition as BL concentrations rise [18]. However, the BR overaccumulation mutants analyzed here already contain elevated endogenous BR levels, which push their BR supply beyond the optimal range even without exogenous BL supplementation. As a result, both BL and BRZ consistently suppressed their hypocotyl growth in the dark except for low concentration (0.02  $\mu\text{M}$ ) of BRZ (Fig. 3).

Notably, BRZ-treated BR-overaccumulation mutants grown in the dark did not exhibit the characteristic rise-and-fall hypocotyl pattern observed in dark-grown BR-deficient mutants treated with BL [18], indicating that BRZ cannot fully restore their hypocotyl elongation to wild-type levels (Fig. 3E–H).

#### 3.4 BR Overaccumulation Produces Opposite Hypocotyl Phenotypes in Light and Dark Environments

To compare genotypes directly under equivalent treatment conditions, hypocotyl measurements from Figs. 2,3 were reorganized as Fig. 4. In the light, hypocotyl length increased with endogenous BR levels regardless of BL or BRZ treatment concentrations (Fig. 4A,B). The triple mutant *bas1-2 sob7-1 ben1-3* was consistently taller than all other genotypes, followed by *bas1-2 sob7-1*, then *ben1-2*, and finally Col-0 (Fig. 4A,B).

The trend reversed in darkness (Fig. 4C,D). Seedlings with higher endogenous BR levels consistently exhibited shorter hypocotyls, except when subjected to high-dose BRZ treatments of 0.5 and 2.5  $\mu\text{M}$  (Fig. 4C,D). The triple mutant *bas1-2 sob7-1 ben1-3* was the shortest across all BL treatments, with *bas1-2 sob7-1* and *ben1-2* also shorter than Col-0 under every BL concentration tested (Fig. 4C). BRZ caused a more complex pattern (Fig. 4D) due to the combined effects of reduced BR biosynthesis and BRZ toxicity [25]. All three mutants remained shorter than the wild type at up to 0.5  $\mu\text{M}$  BRZ (Fig. 4D). 2.5  $\mu\text{M}$  of BRZ completely reversed hypocotyl length rankings (Fig. 4D), indicating that BR overaccumulation mutants retained more residual BR activity to support hypocotyl growth under severe biosynthesis inhibition.

Analyses of relative hypocotyl length (% of untreated control) treated with BL or BRZ clarified these patterns (Fig. 4E–H). Responses to high-dose (2.5  $\mu\text{M}$ ) BL were largely similar across genotypes in both light (Fig. 4E) and dark (Fig. 4G) environments. By contrast, BR overaccumulation increased sensitivity to high-dose BRZ in the light (Fig. 4F) but enhanced BRZ resistance in darkness (Fig. 4H).

A key refinement to our previous hypocotyl growth model [18] emerges here. Although BRZ can partially counteract excessive BR accumulation, it cannot restore hypocotyl length in BR overaccumulation seedlings to the maximal lengths achievable by wild-type seedlings in dark-

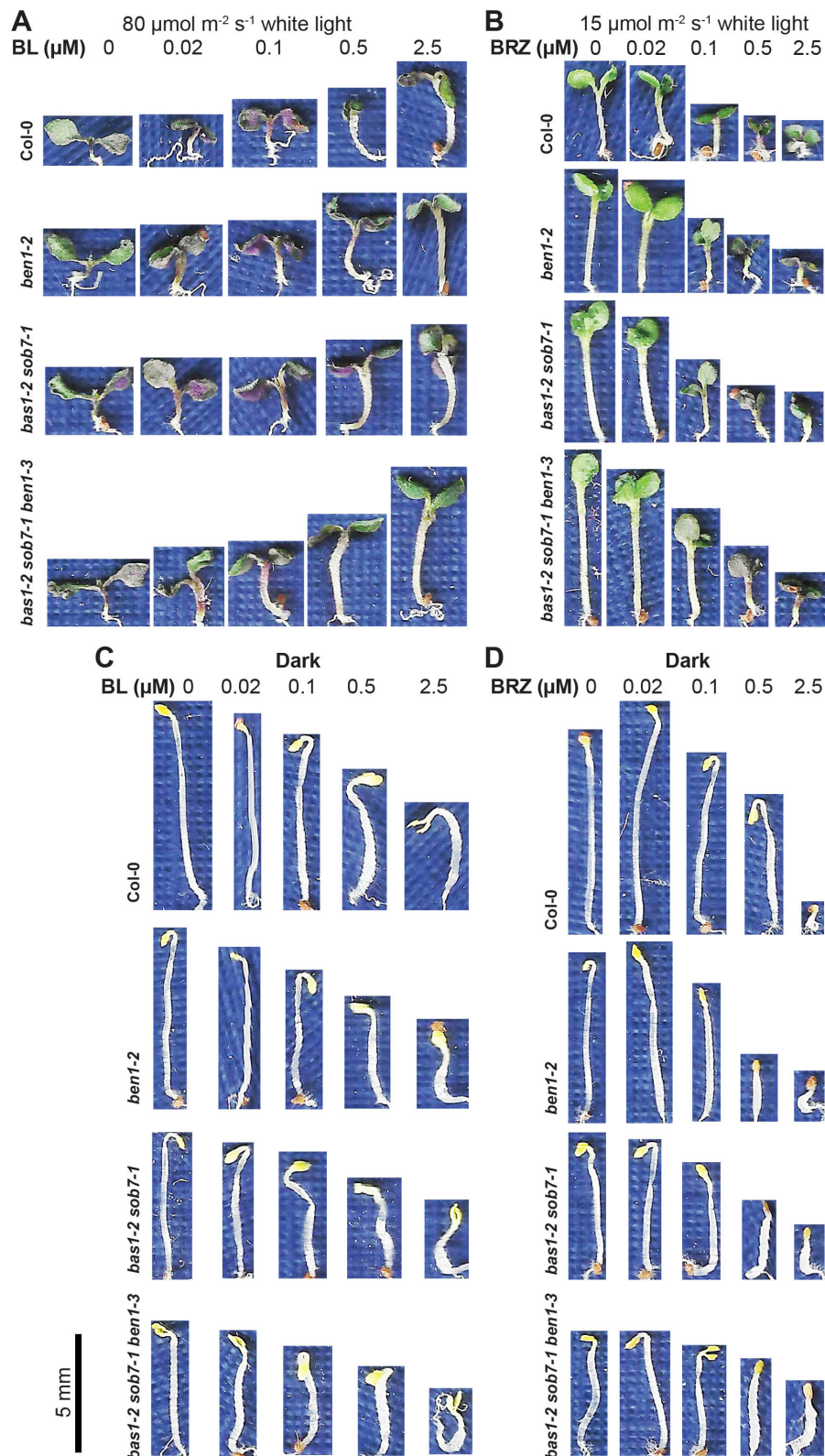


**Fig. 1.** Representative seedling images showing the hypocotyl phenotypes of wild-type *Arabidopsis* and BR overaccumulation mutants in response to exogenous BL or BRZ treatments under continuous light or complete darkness. Seedlings of Col-0, *ben1-2*, *bas1-2 sob7-1*, and *bas1-2 sob7-1 ben1-3* were cultivated on solid 1/2MS medium containing increasing concentrations of BL (A,C) or BRZ (B,D). Plants were photographed after four days of growth under either (A) 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light (BL treatments), (B) 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light (BRZ treatments), or (C,D) full darkness. Scale bar = 5 mm. BR, brassinosteroid; BL, brassinolide; BRZ, brassinazole; 1/2MS, half-strength Murashige and Skoog medium.

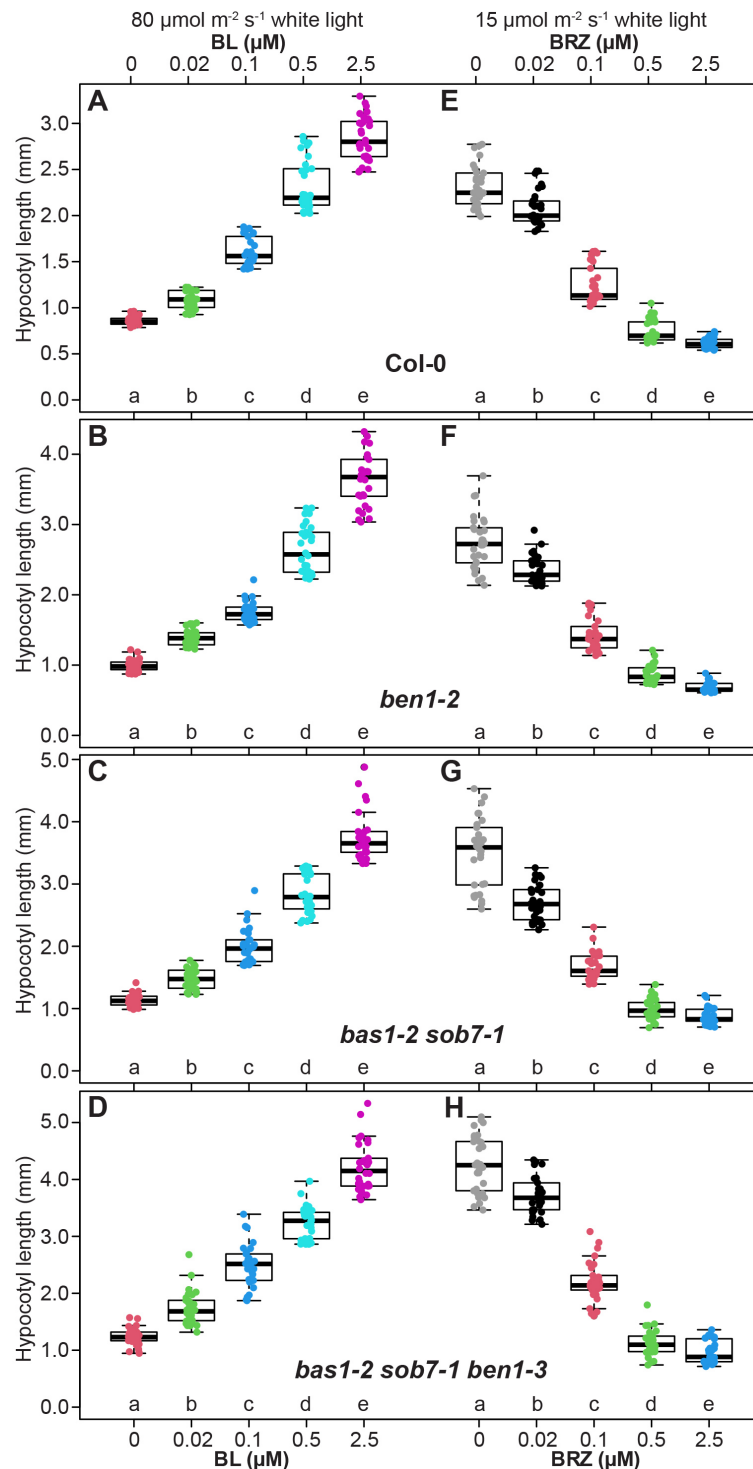


**Fig. 2. Hypocotyl length responses of wild-type plants and BR overaccumulation mutants grown in continuous light and treated with exogenous BL or BRZ.** Seedlings of Col-0 (A,E), *ben1-2* (B,F), *bas1-2 sob7-1* (C,G), and *bas1-2 sob7-1 ben1-3* (D,H) were grown for four days on solid 1/2MS medium supplemented with BL (A–D) or BRZ (E–H) at concentrations ranging from 0 to 2.5  $\mu\text{M}$ . Continuous white light of 80 and 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was used for BL and BRZ treatments, respectively. Hypocotyls were measured from 30 seedlings pooled from three biological replicates per genotype per treatment. Statistical significance within each genotype across treatment concentrations was assessed using one-way ANOVA followed by Tukey's HSD test. The horizontal line inside each box represents median (the middle value of the dataset). Each box represents the IQR. The whiskers represent limits based on  $1.5 \times \text{IQR}$ . Distinct lowercase letters above the horizontal axes denote statistically different groups ( $p < 0.05$ ). ANOVA, analysis of variance; HSD, honestly significant difference; IQR, interquartile range (the range between the 25th percentile and the 75th percentile).

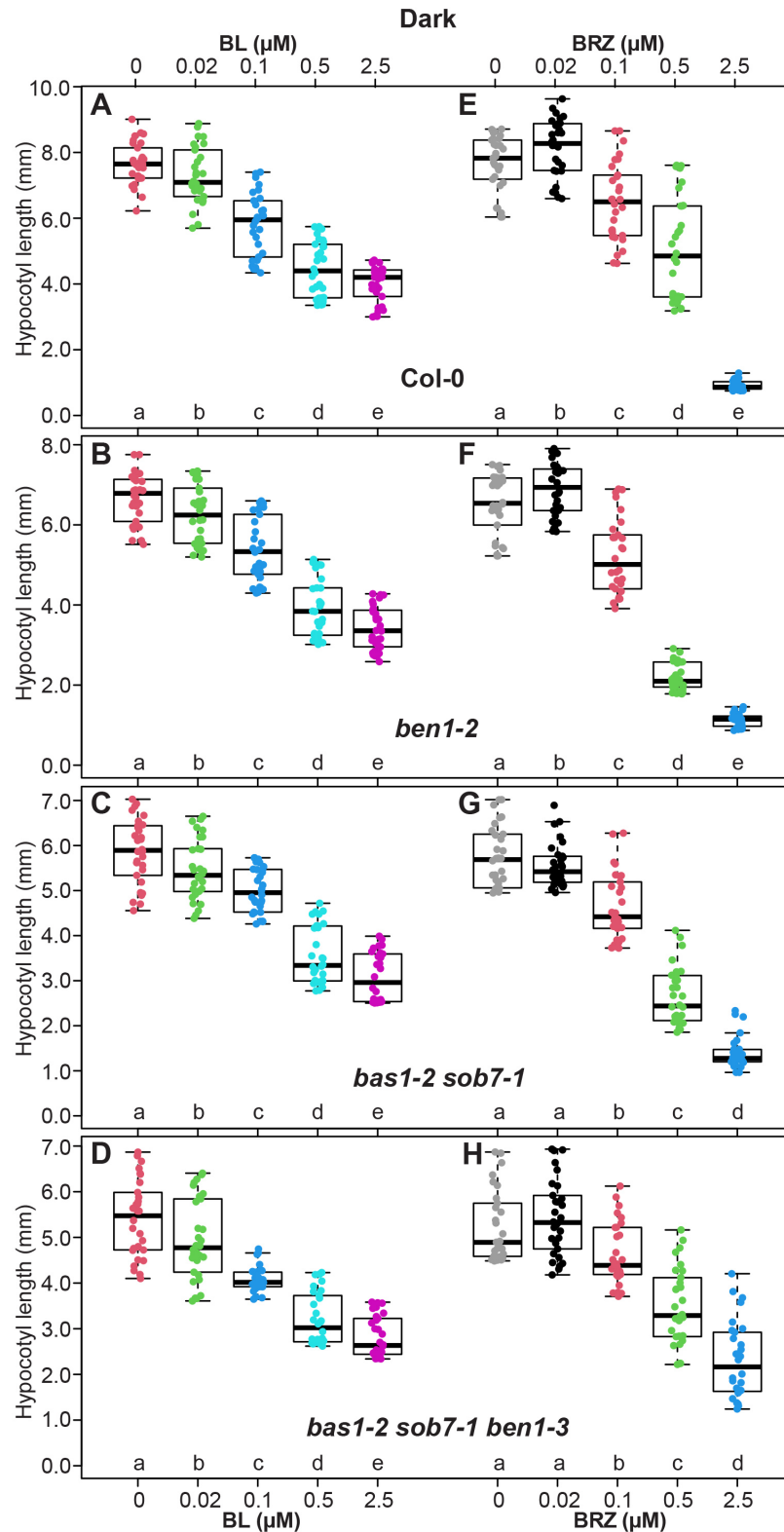


**Fig. 3. Hypocotyl growth responses of wild-type and BR overaccumulation mutants grown in darkness and treated with BL or BRZ.** Col-0 (A,E), *ben1-2* (B,F), *bas1-2 sob7-1* (C,G), and *bas1-2 sob7-1 ben1-3* (D,H) seedlings were grown for four days in the dark on solid 1/2MS medium containing BL (A–D) or BRZ (E–H) at concentrations ranging from 0 to 2.5  $\mu\text{M}$ . Each data group represents 30 measurements pooled from three biological replicates. One-way ANOVA with Tukey's HSD test was used to determine statistically significant differences among treatments within each genotype, indicated by different lowercase letters above the horizontal axes ( $p < 0.05$ ). The horizontal line inside each box represents median. Each box represents the IQR. The whiskers represent limits based on  $1.5 \times \text{IQR}$ .

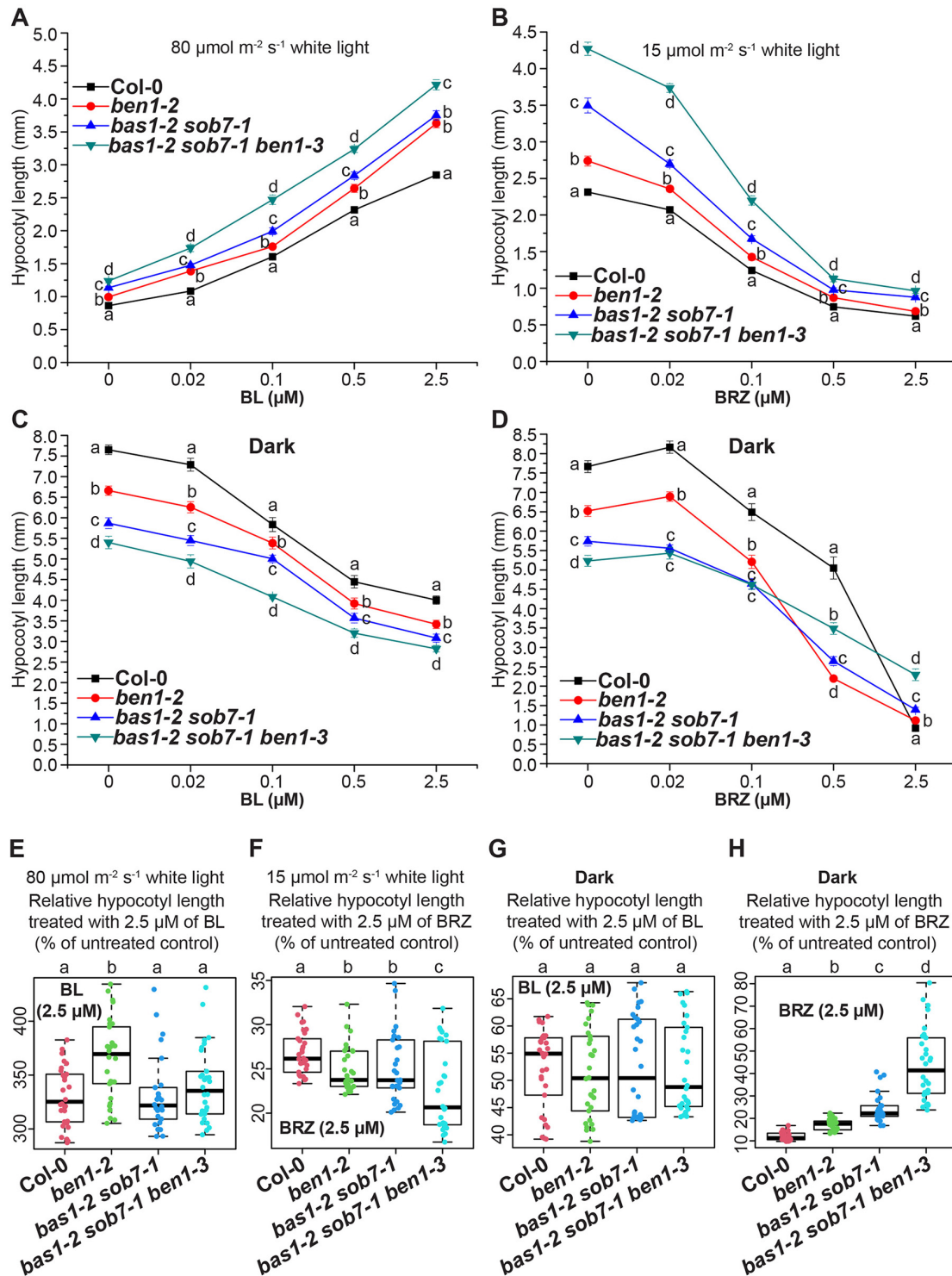


**Fig. 4. Comparative analysis of hypocotyl lengths in wild-type and BR overaccumulation mutants under light or dark conditions in response to BL or BRZ.** Panels show reorganized data from Figs. 2,3 to facilitate genotype comparisons under identical treatment conditions. (A) 0 to 2.5  $\mu\text{M}$  of BL treatments in strong light of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . (B) 0 to 2.5  $\mu\text{M}$  of BRZ treatments in dim light of 15  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . (C) 0 to 2.5  $\mu\text{M}$  of BL treatments in darkness. (D) 0 to 2.5  $\mu\text{M}$  of BRZ treatments in darkness. (E–H) Relative hypocotyl length (% of untreated control) with 2.5  $\mu\text{M}$  of BL or BRZ treatment. Data represent 30 seedlings per group. One-way ANOVA with Tukey’s HSD test was used to analyze genotype differences for each treatment condition. Error bars in panels (A–D) represent standard error of the mean (SEM). Distinct lowercase letters near the symbols or on top of the graphs denote significant differences ( $p < 0.05$ ).

ness (Fig. 4D). BRZ toxicity may prevent complete recovery of hypocotyl growth, even when endogenous BR levels in BR overaccumulation seedlings drop closer to the wild-type optimum upon BRZ treatments.

### 3.5 Light and BR Homeostasis Jointly Modulate the Expression of Hypocotyl-Growth Genes

To explore potential molecular mechanisms underlying the observed hypocotyl phenotypes, the expression of ten hypocotyl growth-related genes was examined in Col-0 and *bas1-2 sob7-1 ben1-3* seedlings grown in light or darkness (Fig. 5).

Five genes, including Phytochrome Interacting Factor 4 (*PIF4*), Increased Lamina Inclination 1 (IL1) Binding basic Helix-Loop-Helix (bHLH) 1 (*IBH1*), GATA Transcription Factor 2 (*GATA2*), Paclobutrazol Resistance 1 (*PRE1*), and Microtubule Destabilizing Protein 40 (*MDP40*), exhibited significant expression changes in the BR overaccumulation triple mutant, and these changes differed sharply between light and dark conditions (Fig. 5A). The remaining five genes, including two Expansin genes *EXPA1/8*, two Small Auxin-Up RNA genes *SAUR19/63*, and a DNA-binding bHLH protein-encoding gene *Homolog of BEE2 Interacting with IBH1* (*HBI1*), largely showed no strong association with altered BR homeostasis, except that statistically significant differences were observed in *EXPA1* and *SAUR63* under dark conditions (Fig. 5B).

Overall, these transcript patterns indicate that BR overaccumulation interacts with light signaling pathways to modulate the expression of key transcriptional regulators of hypocotyl elongation. Among these genes, *GATA2* displayed the strongest correspondence (Fig. 5A).

## 4. Discussion

The responses of BR overaccumulation mutants to BL and BRZ treatments clearly depend on hormone dosage, light intensity and availability (Figs. 1,2,3,4). These findings reinforce the concept that BR-regulated hypocotyl growth is tightly integrated with environmental light cues [26,27]. Previous studies have shown extensive crosstalk between BR signaling and pathways controlled by light and other hormones [28,29], and the present results provide additional support for that interconnected regulatory framework.

BRI1 Associated Receptor Kinase 1 (BAK1), initially characterized as a coreceptor of BRI1 in BR signaling, has emerged as a versatile regulatory hub that contributes to growth, defense, and environmental responses [30]. Its role in sensing light intensity and influencing growth independently of BR signaling highlights the multiple layers of interaction between BR and light responses in plants [31]. Physical interactions between BRI1 [32], BAK1, and Prohibitin 3 (PHB3) [33], observed in *Arabidopsis* and crop species such as tomato and potato, suggest that this signaling module is conserved across diverse plant lineages.

PIF4 is a central regulator of hypocotyl elongation, especially in darkness [34]. BRs stabilize PIF4 protein by inhibiting its phosphorylation [34]. Because *PIF4* gene expression is subject to threshold-dependent autorepression [35], elevated BR levels in the triple mutant *bas1-2 sob7-1 ben1-3* may enhance PIF4 protein accumulation and subsequently drive down *PIF4* transcript levels via this negative feedback regulation, which is consistent with our gene expression data (Fig. 5A). Several downstream genes, including *SAUR19/63* [36], *EXPA1/8* [37], *PRE1* [38], *GATA2* [39], and *IBH1* [40], are known components of the PIF4 regulatory network. However, not all of these genes appear to be responsive to BR overaccumulation in the hypocotyl context. For example, *SAUR19/63* and *EXPA1/8*, both associated with cell expansion and hormone responses [41], showed limited transcriptional changes in *bas1-2 sob7-1 ben1-3*, suggesting they do not play major roles in BR-modulated hypocotyl growth under the tested conditions (Fig. 5B).

PRE1 and IBH1 form an antagonistic pair of HLH/bHLH transcription factors that operate downstream of BR signaling component Brassinazole-Resistant 1 (BZR1) and have been implicated in BR-mediated cell elongation [42]. BZR1 directly targets *PRE1* promoter to maintain somatic cell identity [43]. PRE1 can also activate plant response to gibberellins (GAs), a main group of plant hormones associated with BRs in regulating a wide range of growth and developmental processes [44]. Nevertheless, the PRE1–IBH1–HBI1 module [45] generally promotes hypocotyl expansion in both light and dark [46], which is inconsistent with their gene expression levels (Fig. 5) and the growth suppression observed in BR overaccumulation *bas1-2 sob7-1 ben1-3* seedlings in darkness (Fig. 4C,D). Similarly, MDP40, known to promote BR-mediated microtubule reorganization and hypocotyl elongation [47], was upregulated in the triple mutant (Fig. 5A) despite its reduced hypocotyl length in the dark (Fig. 4C,D). These discrepancies illustrate the complexity of BR–light interactions and suggest that excessive BR levels may disrupt normal downstream regulatory balance.

Among all examined genes, *GATA2* stood out as a consistent indicator of hypocotyl growth outcomes across light and dark conditions. The zinc finger-containing GATA-family transcription factors [48] bind GATA motifs in promoters of light-responsive genes [49]. *GATA2* is known to integrate BR and light signaling pathways, inhibit hypocotyl elongation, and promote photomorphogenesis regardless of light availability [50]. In addition, *GATA2*–auxin interaction maintains the root transition domain to restrict cell division activity [51]. The triple mutant displayed reduced *GATA2* expression in the light, corresponding with longer hypocotyls, and increased expression in darkness, corresponding with shorter hypocotyls (Fig. 4A–D and Fig. 5A). This alignment between *GATA2* transcript levels and hypocotyl phenotypes across light/dark environments strongly suggests that *GATA2* acts as a key coordina-

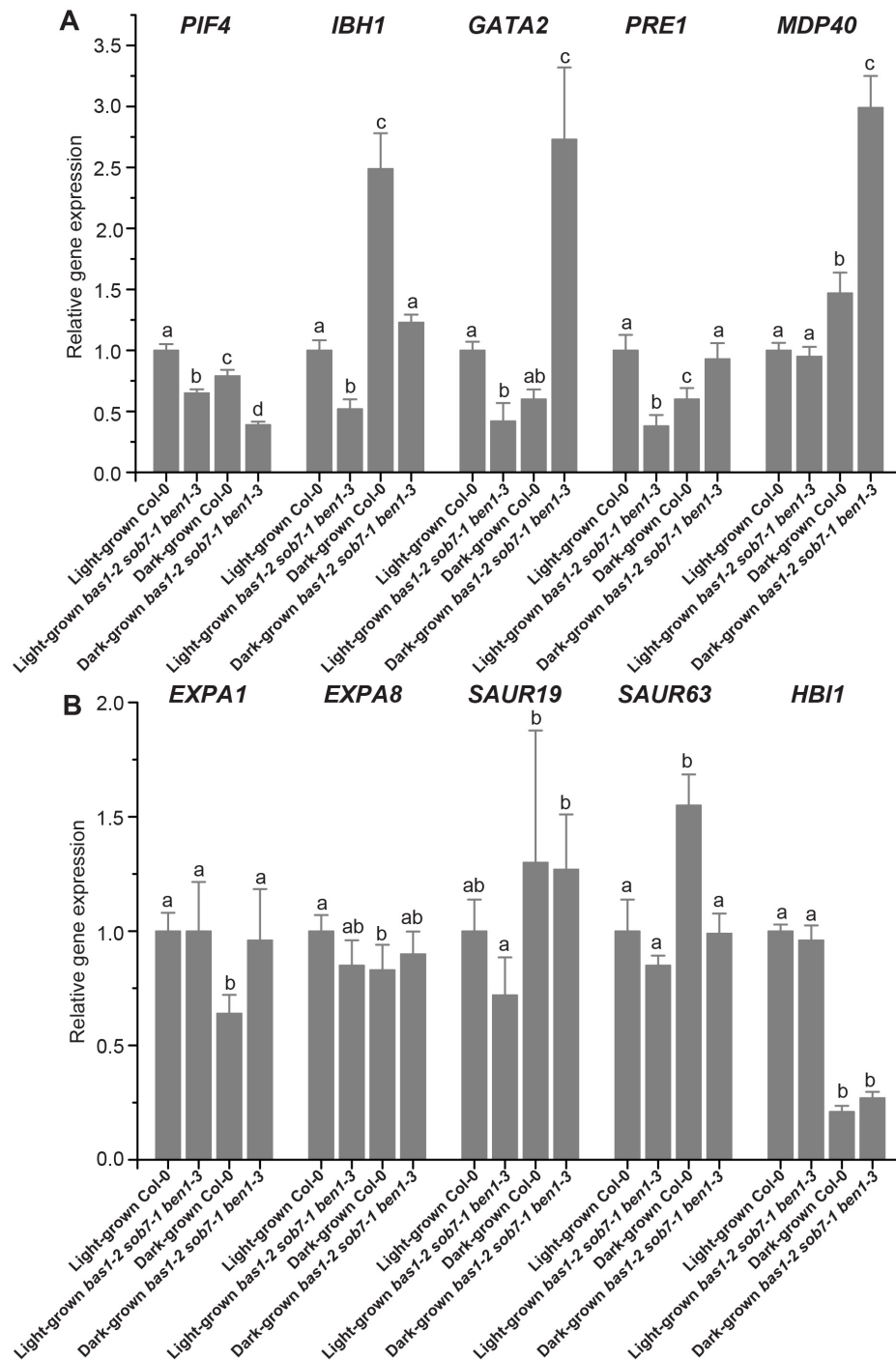


**Fig. 5.** Expression profiles of selected genes involved in hypocotyl growth regulation in light- and dark-grown seedlings of Col-0 and the BR overaccumulation triple mutant *bas1-2 sob7-1 ben1-3*. Panels show relative transcript levels of ten genes measured by RT-qPCR in four-day-old seedlings grown under continuous light ( $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or full darkness. (A) *PIF4*, *IBH1*, *GATA2*, *PRE1*, and *MDP40* exhibited significant expression changes in *bas1-2 sob7-1 ben1-3*, and these changes differed sharply between light and dark conditions. (B) Expression of *EXPA1/8*, *SAUR19/63*, and *HBI1* largely showed no strong association with altered BR homeostasis, except that statistically significant differences were observed in *EXPA1* and *SAUR63* under dark conditions. Data represent six measurements (three biological  $\times$  two technical replicates). Expression values were normalized to *UBQ10* using the  $\Delta\Delta\text{Ct}$  method. One-way ANOVA with Tukey's HSD test was used to assess differences among samples. Distinct lowercase letters on top of the error bars indicate statistically different groups ( $p < 0.05$ ). A group labeled with two or more letters means that it overlaps with distinct subsets. Its average value is statistically similar to multiple other groups that do not necessarily resemble each other. Error bars represent standard deviation (SD). RT-qPCR, reverse transcription quantitative PCR.

tor linking BR homeostasis with light-regulated hypocotyl growth.

Collectively, our findings highlight the central role of BR–light interactions in shaping hypocotyl development. BL/BRZ response assays on BR overaccumulation mutants reveal boundaries of BR-mediated growth control not visible in BR-deficient lines and underscore how excessive BR supply disrupts normal growth responses. The gene expression results further emphasize that BR signaling is part of a broader regulatory network in which transcription factors like GATA2 integrate hormonal and environmental signals to modulate hypocotyl elongation.

## 5. Limitations

This study focuses specifically on hypocotyl growth phenotypes in BR overaccumulation *Arabidopsis* seedlings and on expression changes in a selected group of growth-related genes. Although these data provide valuable insight into BR–light interactions during early seedling development, they represent only a subset of physiological and molecular processes influenced by BR overproduction. Extending these analyses to additional developmental stages would offer a more complete understanding of BR-regulated growth. However, such experiments are considerably more challenging. As plants mature, maintaining uniform growth conditions becomes more difficult, and phenotypic variability increases, making trait quantification less precise than in seedlings.

Our gene expression analysis, while informative, was limited to ten candidate genes. A broader transcriptomic approach would likely reveal additional components of the BR regulatory network and uncover interactions not captured in this targeted set. Future work employing whole transcriptome profiling could help establish a more comprehensive picture of BR-associated gene expression changes.

Finally, although BR overaccumulation mutants with combinations of BR-inactivating gene knockouts are available in *Arabidopsis*, similar mutants have not yet developed in other plant species. This restricts our ability to perform parallel BL/BRZ response assays in model crops such as rice and tomato, limiting broader comparative analyses across species.

## 6. Conclusions

This study demonstrates that BR overaccumulation produces distinct effects on *Arabidopsis* hypocotyl growth depending on light availability. In light-grown seedlings, both wild-type plants and BR overaccumulation mutants respond predictably to hormone treatments: BL enhances hypocotyl elongation, while BRZ suppresses it. In darkness, however, the relationship between BR levels and hypocotyl growth shifts. BL and BRZ both reduce hypocotyl length across all genotypes, indicating that dark-grown seedlings already possess nearly optimal endogenous BR levels for elongation, and deviations in either direction lead to inhibition of hypocotyl growth.

Analysis of BR overaccumulation mutants supports this model clearly. Although these mutants produce longer hypocotyls than wild-type plants under light, they exhibit shorter hypocotyls in darkness due to excessive BR supply. High concentrations of BRZ can partially counteract this, allowing dark-grown mutants to produce longer hypocotyls than wild-type seedlings under the same treatment. Likely due to BRZ-associated toxicity, BRZ cannot fully restore mutant hypocotyl length to the maximum values achievable in wild-type seedlings.

Gene expression analyses reveal that BR overaccumulation alters the transcription of multiple hypocotyl growth regulators and their responses depend strongly on light conditions. Among the genes examined, *GATA2* shows the clearest alignment with hypocotyl growth phenotypes and light/dark environments, suggesting its central role in integrating BR and light signals to modulate hypocotyl elongation.

Together, these results highlight how BR homeostasis and light signaling interact to shape early seedling development. They also illustrate the importance of considering hormone balance, not simply absolute hormone abundance, when interpreting BR-mediated growth responses. The findings contribute to a deeper understanding of BR–light crosstalk and underscore the value of utilizing BR overaccumulation mutants for revealing aspects of BR homeostasis that are not apparent in BR-deficient lines.

## Abbreviations

1/2MS, half-strength Murashige and Skoog medium; ANOVA, analysis of variance; BAK1, BRI1 Associated Receptor Kinase 1; BAS1, *phyB* Activation Tagged Suppressor 1; BEN1, BRI1-5 Enhanced 1; bHLH, basic Helix-Loop-Helix; BL, brassinolide; BR, brassinosteroid; BRI1, Brassinosteroid Insensitive 1; BRZ, brassinazole; Col-0, Columbia-0; BZR1, Brassinazole-Resistant 1; DMF, N,N-dimethylformamide; DPI, dots per inch; EXPA1, Expansin A1; EXPA8, Expansin A8; GATA2, GATA Transcription Factor 2; HBI1, Homolog of BEE2 Interacting with IBH1; HSD, honestly significant difference; IBH1, ILI1 Binding bHLH 1; ILI1, Increased Lamina Inclination 1; IQR, interquartile range; MDP40, Microtubule Destabilizing Protein 40; PHB3, Prohibitin 3; phyB, Phytochrome B; PIF4, Phytochrome Interacting Factor 4; PRE1, Paclobutrazol Resistance 1; RT-qPCR, reverse transcription quantitative PCR; PCZ, propiconazole; SAUR19, Small Auxin Up RNA 19; SAUR63, Small Auxin Up RNA 63; SD, standard deviation; SEM, standard error of the mean; SOB7, Suppressor of *phyB-4* 7; UBIQ10, Ubiquitin 10; USDA, United States Department of Agriculture.

## Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Author Contributions

Conceptualization: HP, YZ, MMN; Formal analysis: HP, YZ; Investigation: HP, YZ; Methodology: HP, YZ; Visualization: HP, YZ; Resources: HP, MMN; Writing — original: HP; Writing — review & editing: YZ, MMN; Supervision: HP, MMN; Project administration: HP. All authors have participated sufficiently in the work, agreed to be accountable for all aspects of the work, read and approved the final manuscript.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Declaration of AI and AI-Assisted Technologies in the Writing Process

AI-assisted technologies were used to improve English writing. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/FBL56314>.

## References

- [1] Nikolić B, Jovanović V, Knežević B, Nikolić Z, Babović-Dorđević M. Mode of Action of Brassinosteroids: Seed Germination and Seedling Growth and Development-One Hypothesis. *International Journal of Molecular Sciences*. 2025; 26: 2559. <https://doi.org/10.3390/ijms26062559>
- [2] Zhang Y, Berman A, Shani E. Plant hormone transport and localization: signaling molecules on the move. *Annual Review of Plant Biology*. 2023; 74: 453–479. <https://doi.org/10.1146/annurev-arplant-070722-015329>
- [3] An N, Zhang P, Zhang X. Mechanistic basis of plant hormone transport by ABC transporters: Insights from cryo-EM structures. *The Plant Journal : for Cell and Molecular Biology*. 2026; 125: e70782. <https://doi.org/10.1111/tpj.70782>
- [4] Peng H, Zhao J, Neff MM. ATAF2 integrates Arabidopsis brassinosteroid inactivation and seedling photomorphogenesis. *Development (Cambridge, England)*. 2015; 142: 4129–4138. <https://doi.org/10.1242/dev.124347>
- [5] Bell EM, Lin WC, Husbands AY, Yu L, Jaganatha V, Jablonska B, et al. Arabidopsis lateral organ boundaries negatively regulates brassinosteroid accumulation to limit growth in organ boundaries. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109: 21146–21151. <https://doi.org/10.1073/pnas.1210789109>
- [6] Sharma G, Bhatnagar A. A B-box protein BReaks brassinosteroid regulation of photomorphogenesis in the dark and light. *Plant Physiology*. 2026; 201: kiag593. <https://doi.org/10.1093/plphys/kiag593>
- [7] Zhang H, Aizezi Y, Bessho-Uehara K, Chaudhary A, Trinh CS, Xu SL, et al. Brassinosteroids promote sugar synthesis by inhibiting BIN2 phosphorylation of phosphoenolpyruvate carboxykinase. *Nature Plants*. 2026. <https://doi.org/10.1038/s41477-026-02379-5> (online ahead of print)
- [8] Nomura T, Kushihiro T, Yokota T, Kamiya Y, Bishop GJ, Yamaguchi S. The last reaction producing brassinolide is catalyzed by cytochrome P-450s, CYP85A3 in tomato and CYP85A2 in Arabidopsis. *The Journal of Biological Chemistry*. 2005; 280: 17873–17879. <https://doi.org/10.1074/jbc.M414592200>
- [9] Kim TW, Hwang JY, Kim YS, Joo SH, Chang SC, Lee JS, et al. Arabidopsis CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. *The Plant Cell*. 2005; 17: 2397–2412. <https://doi.org/10.1105/tpc.105.033738>
- [10] Bajguz A, Chmur M, Gruszka D. Comprehensive Overview of the Brassinosteroid Biosynthesis Pathways: Substrates, Products, Inhibitors, and Connections. *Frontiers in Plant Science*. 2020; 11: 1034. <https://doi.org/10.3389/fpls.2020.01034>
- [11] Asami T, Min YK, Nagata N, Yamagishi K, Takatsuto S, Fujioka S, et al. Characterization of brassinazole, a triazole-type brassinosteroid biosynthesis inhibitor. *Plant Physiology*. 2000; 123: 93–100. <https://doi.org/10.1104/pp.123.1.93>
- [12] Hartwig T, Corvalan C, Best NB, Budka JS, Zhu JY, Choe S, et al. Propiconazole is a specific and accessible brassinosteroid (BR) biosynthesis inhibitor for Arabidopsis and maize. *PloS One*. 2012; 7: e36625. <https://doi.org/10.1371/journal.pone.0036625>
- [13] Liu X, Yang H, Wang Y, Zhu Z, Zhang W, Li J. Comparative Transcriptomic Analysis to Identify Brassinosteroid Response Genes. *Plant Physiology*. 2020; 184: 1072–1082. <https://doi.org/10.1104/pp.20.00386>
- [14] Rusnak B, Oliver LS, Procopio K, Roeder AHK. Spatial and single-cell transcriptomics uncover brassinosteroid-mediated coordination of sepal elongation in Arabidopsis. *Genome Biology*. 2026; 27: 271. <https://doi.org/10.1186/s13059-026-04156-1>
- [15] Qin Z, Gao Y, Liu Y, Li S, Feng Q, Zhang Y, et al. IMB4 integrates into brassinosteroid signaling to regulate hypocotyl growth. *The Plant Journal : for Cell and Molecular Biology*. 2026; 125: e70749. <https://doi.org/10.1111/tpj.70749>
- [16] Rahul PV, Dwivedi S, Saha P, Datta S. A photomorphogenesis-promoting B-box protein, BReaks into the dark. *Plant Physiology*. 2026; 201: kiag370. <https://doi.org/10.1093/plphys/kiag370>
- [17] Peng H, Zhai Y. Grapevine CYP734A15 is a brassinosteroid-inactivating cytochrome P450 enzyme. *Biochemical and Biophysical Research Communications*. 2025; 775: 152128. <https://doi.org/10.1016/j.bbrc.2025.152128>
- [18] Peng H, Zhai Y. Optimal brassinosteroid homeostasis is required to maximize Arabidopsis hypocotyl growth in the dark.

- Biochemical and Biophysical Research Communications. 2026; 794: 153062. <https://doi.org/10.1016/j.bbrc.2025.153062>
- [19] Peng H, Zhai Y. Light and brassinosteroids differentially modulate *Arabidopsis* seedling root growth in a largely independent manner. *Biochemical and Biophysical Research Communications*. 2026; 810: 153538. <https://doi.org/10.1016/j.bbrc.2026.153538>
- [20] Zebosi B, Vollbrecht E, Best NB. Brassinosteroid biosynthesis and signaling: Conserved and diversified functions of core genes across multiple plant species. *Plant Communications*. 2024; 5: 100982. <https://doi.org/10.1016/j.xplc.2024.100982>
- [21] Yuan T, Fujioka S, Takatsuto S, Matsumoto S, Gou X, He K, et al. BEN1, a gene encoding a dihydroflavonol 4-reductase (DFR)-like protein, regulates the levels of brassinosteroids in *Arabidopsis thaliana*. *The Plant Journal : for Cell and Molecular Biology*. 2007; 51: 220–233. <https://doi.org/10.1111/j.1365-3113X.2007.03129.x>
- [22] Tayengwa R, Westenskow SR, Peng H, Hulbert AK, Neff MM. Genetic interactions between BEN1- and cytochrome P450-mediated brassinosteroid inactivation. *Physiologia Plantarum*. 2024; 176: e14141. <https://doi.org/10.1111/pp1.14141>
- [23] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 2012; 9: 671–675. <https://doi.org/10.1038/nmeth.2089>
- [24] Planas-Riverola A, Gupta A, Betegón-Putze I, Bosch N, Ibañes M, Caño-Delgado AI. Brassinosteroid signaling in plant development and adaptation to stress. *Development* (Cambridge, England). 2019; 146: dev151894. <https://doi.org/10.1242/dev.151894>
- [25] Nagata N, Asami T, Yoshida S. Brassinazole, an inhibitor of brassinosteroid biosynthesis, inhibits development of secondary xylem in cress plants (*Lepidium sativum*). *Plant & Cell Physiology*. 2001; 42: 1006–1011. <https://doi.org/10.1093/pcp/pce122>
- [26] Lin F, Cao J, Yuan J, Liang Y, Li J. Integration of Light and Brassinosteroid Signaling during Seedling Establishment. *International Journal of Molecular Sciences*. 2021; 22: 12971. <https://doi.org/10.3390/ijms222312971>
- [27] Gautrat P, Matton SEA, Oskam L, Shetty SS, van der Velde KJ, Pierik R. Lights, location, action: shade avoidance signalling over spatial scales. *Journal of Experimental Botany*. 2025; 76: 695–711. <https://doi.org/10.1093/jxb/erae217>
- [28] Cheng R, Xiao Q, Gong J, Sun R, Du Y, Zhao W, et al. Comprehensive Identification and Unveiling Key Nitrate-Transporting Proteins in Oat (*Avena sativa* L.). *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 39679. <https://doi.org/10.31083/FB.L39679>
- [29] Vilain L, Fauconnier ML, Genva M. Towards an integrated molecular understanding of plant hormones. *Journal of Integrative Plant Biology*. 2026; 68: 2787–2826. <https://doi.org/10.1111/jipb.70238>
- [30] Wu Y, Ma Y, Wang W, Zhang S, Wu W. Mastering the balance: BAK1's dual roles in steering plant growth and immunity. *Horticulture Research*. 2025; 12: uhaf206. <https://doi.org/10.1093/hr/uhaf206>
- [31] Zhang S, Li C, Ren H, Zhao T, Li Q, Wang S, et al. BAK1 Mediates Light Intensity to Phosphorylate and Activate Catalases to Regulate Plant Growth and Development. *International Journal of Molecular Sciences*. 2020; 21: 1437. <https://doi.org/10.3390/ijms21041437>
- [32] Deng R, Huang S, Du J, Luo D, Liu J, Zhao Y, et al. The brassinosteroid receptor StBRI1 promotes tuber development by enhancing plasma membrane H<sup>+</sup>-ATPase activity in potato. *The Plant Cell*. 2024; 36: 3498–3520. <https://doi.org/10.1093/plcell/koae163>
- [33] Li C, Zhang S, Li J, Huang S, Zhao T, Lv S, et al. PHB3 interacts with BRI1 and BAK1 to mediate brassinosteroid signal transduction in *Arabidopsis* and tomato. *The New Phytologist*. 2024; 241: 1510–1524. <https://doi.org/10.1111/nph.19469>
- [34] Bernardo-García S, de Lucas M, Martínez C, Espinosa-Ruiz A, Davière JM, Prat S. BR-dependent phosphorylation modulates PIF4 transcriptional activity and shapes diurnal hypocotyl growth. *Genes & Development*. 2014; 28: 1681–1694. <https://doi.org/10.1101/gad.243675.114>
- [35] Das S, Garhwal V, Mondal K, Das D, Gangappa SN. Threshold-dependent negative autoregulation of PIF4 gene expression optimizes growth and fitness in *Arabidopsis*. *PLoS Genetics*. 2025; 21: e1011758. <https://doi.org/10.1371/journal.pgen.1011758>
- [36] Pereyra ME, Costigliolo Rojas C, Jarrell AF, Hovland AS, Snipes SA, Nagpal P, et al. PIF4 enhances the expression of *SAUR* genes to promote growth in response to nitrate. *Proceedings of the National Academy of Sciences of the United States of America*. 2023; 120: e2304513120. <https://doi.org/10.1073/pnas.2304513120>
- [37] Lee HY, Back K. PIF4-dependent 2-hydroxymelatonin and 3-hydroxymelatonin biosynthesis is involved in skotomorphogenic seedling growth in *Arabidopsis thaliana*. *The Plant Journal : for Cell and Molecular Biology*. 2025; 123: e70394. <https://doi.org/10.1111/tjp.70394>
- [38] Hao Y, Oh E, Choi G, Liang Z, Wang ZY. Interactions between HLH and bHLH factors modulate light-regulated plant development. *Molecular Plant*. 2012; 5: 688–697. <https://doi.org/10.1093/mp/sss011>
- [39] Wang Q, Zhu Z, Ozkardesh K, Lin C. Phytochromes and phytohormones: the shrinking degree of separation. *Molecular Plant*. 2013; 6: 5–7. <https://doi.org/10.1093/mp/sss102>
- [40] Zhiponova MK, Morohashi K, Vanhoutte I, Machemer-Noonan K, Revalska M, Van Montagu M, et al. Helix-loop-helix/basic helix-loop-helix transcription factor network represses cell elongation in *Arabidopsis* through an apparent incoherent feed-forward loop. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111: 2824–2829. <https://doi.org/10.1073/pnas.1400203111>
- [41] Miao P, Li X, Wan Y, Zhang K, Yu H, Li Y, et al. Study of the Function and Expression of the *AhRUVBL2* Promoter in *Arabidopsis*. *Frontiers in Bioscience (Landmark Edition)*. 2025; 30: 38940. <https://doi.org/10.31083/FBL38940>
- [42] Zhang LY, Bai MY, Wu J, Zhu JY, Wang H, Zhang Z, et al. Antagonistic HLH/bHLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and *Arabidopsis*. *The Plant Cell*. 2009; 21: 3767–3780. <https://doi.org/10.1105/tpc.109.070441>
- [43] Cai H, Huang Y, Liu L, Su H, Xi X, Liu Y, et al. The KLU-PRE module provides positional cues that maintain somatic cell identity around the megasporocyte cell in *Arabidopsis*. *The Plant Cell*. 2026; 38: koag008. <https://doi.org/10.1093/plcell/koag008>
- [44] Ritonga FN, Zhou D, Zhang Y, Song R, Li C, Li J, et al. The Roles of Gibberellins in Regulating Leaf Development. *Plants (Basel, Switzerland)*. 2023; 12: 1243. <https://doi.org/10.3390/plants12061243>
- [45] Fan M, Bai MY, Kim JG, Wang T, Oh E, Chen L, et al. The bHLH transcription factor HBI1 mediates the trade-off between growth and pathogen-associated molecular pattern-triggered immunity in *Arabidopsis*. *The Plant Cell*. 2014; 26: 828–841. <https://doi.org/10.1105/tpc.113.121111>
- [46] Lee S, Lee S, Yang KY, Kim YM, Park SY, Kim SY, et al. Overexpression of PRE1 and its homologous genes activates Gibberellin-dependent responses in *Arabidopsis thaliana*. *Plant & Cell Physiology*. 2006; 47: 591–600. <https://doi.org/10.1093/pcp/pcj026>
- [47] Wang X, Zhang J, Yuan M, Ehrhardt DW, Wang Z, Mao T. *Arabidopsis* microtubule destabilizing protein40 is involved in brassinosteroid regulation of hypocotyl elongation. *The Plant Cell*. 2012; 24: 4012–4025. <https://doi.org/10.1105/tpc.112.103838>

- [48] Jeong MJ, Shih MC. Interaction of a GATA factor with cis-acting elements involved in light regulation of nuclear genes encoding chloroplast glyceraldehyde-3-phosphate dehydrogenase in *Arabidopsis*. *Biochemical and Biophysical Research Communications*. 2003; 300: 555–562. [https://doi.org/10.1016/s0006-291x\(02\)02892-9](https://doi.org/10.1016/s0006-291x(02)02892-9)
- [49] Teakle GR, Manfield IW, Graham JF, Gilmartin PM. *Arabidopsis thaliana* GATA factors: organisation, expression and DNA-binding characteristics. *Plant Molecular Biology*. 2002; 50: 43–57. <https://doi.org/10.1023/a:1016062325584>
- [50] Luo XM, Lin WH, Zhu S, Zhu JY, Sun Y, Fan XY, et al. Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in *Arabidopsis*. *Developmental Cell*. 2010; 19: 872–883. <https://doi.org/10.1016/j.devcel.2010.10.023>
- [51] Jiang K, Yung V, Chiba T, Feldman LJ. Longitudinal patterning in roots: a GATA2-auxin interaction underlies and maintains the root transition domain. *Planta*. 2018; 247: 831–843. <https://doi.org/10.1007/s00425-017-2831-4>