RESEARCH ARTICLE

TITLE: ATAF2 integrates Arabidopsis brassinosteroid inactivation and seedling photomorphogenesis

Hao Peng1, Jianfei Zhao1,2,3, and Michael M. Neff1,2,*

1Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, USA
2Molecular Plant Science Graduate Program, Washington State University, Pullman, WA 99164, USA
3Current Address: Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA

*Author for correspondence (mmneff@wsu.edu)
ABSTRACT

The *Arabidopsis thaliana* hypocotyl is a robust system for studying the interplay of light and plant hormones, such as brassinosteroids (BRs), in the regulation of plant growth and development. Since BRs cannot be transported between plant tissues, their cellular levels must be appropriate for given developmental fates. BR homeostasis is maintained in part by transcriptional feedback-regulation loops that control the expression of key metabolic enzymes, including the BR-inactivation enzymes CYP734A1/CYP72B1/BAS1 and CYP72C1/SOB7. In this research, the NAC transcription factor (TF), ATAF2, is found to bind the promoters of *BAS1* and *SOB7* to suppress their expression. ATAF2 restricts the tissue-specific expression of *BAS1* and *SOB7 in planta*. ATAF2 loss- and gain-of-function seedlings have opposite BR response phenotypes for hypocotyl elongation. ATAF2 modulates hypocotyl growth in a light-dependent manner, with the photoreceptor phytochrome A playing a major role. The photomorphogenic phenotypes of ATAF2 loss- and gain-of-function seedlings can be suppressed by treatment with the BR biosynthesis inhibitor brassinazole (BRZ). Moreover, the disruption of *BAS1* and *SOB7* abolishes the short-hypocotyl phenotype of ATAF2 loss-of-function seedlings in low-fluence-rate white light, which demonstrates an ATAF2-mediated connection between BR catabolism and photomorphogenesis. The expression of ATAF2 is suppressed by both BRs and light, which demonstrates the existence of an ATAF2-BAS1/SOB7-BR-ATAF2 feedback-regulation loop as well as a light-ATAF2-BAS1/SOB7-BR-photomorphogenesis pathway. ATAF2 also modulates root growth by regulating BR catabolism. Since ATAF2 was known to regulate plant defense and auxin biosynthesis, this TF acts as a central regulator of plant defense, hormone metabolism, and light-mediated seedling development.
KEY WORDS: Seedling development, Hypocotyl growth, Photomorphogenesis,
Brassinosteroids, Transcription factor, Cytochrome P450
Introduction

Multiple hormonal and light signals coordinately regulate plant morphogenesis (Neff et al., 2000; Gray, 2004). The *Arabidopsis thaliana* hypocotyl is a robust system for studying the interplay of light and plant hormones in the regulation of plant growth and development (Collett et al., 2000; Vandenbussche et al., 2005). Genetic screens for long-hypocotyl mutants in the light led to the isolation of genes involved in photomorphogenic signaling pathways including many of the photoreceptors (Neff and Chory, 1998). On the other hand, screens for short-hypocotyl mutants in the dark have also identified additional genes involved in photomorphogenesis-regulating pathways including those associated with hormone mediated development (Chory et al., 1989). For example, the dark-grown short-hypocotyl mutant *det2* uncovered the role of brassinosteroids (BRs) in seedling growth and development (Chory et al., 1991; Li et al., 1996).

Brassinolide (BL) is the endpoint of the BR biosynthetic pathway in many plant species and confers the strongest biological activity (Yokota, 1997). The BR signaling (Zhu et al., 2013) and metabolism pathways (Zhao and Li, 2012) have been well studied in Arabidopsis. Loss-of-function mutations in key BR biosynthesis genes leads to BR-deficient specific dwarfism of Arabidopsis plants, which can be rescued in a dose-dependent manner by exogenous BR (Zhao and Li, 2012). In contrast, a BR-deficient dwarf phenotype is conferred by overexpression (or gain-of-function) of BR catabolic enzymes, such as the cytochrome P450s CYP734A1/CYP72B1/BAS1 and CYP72C1/SOB7 (Neff et al., 1999; Turk et al., 2003; Turk et al., 2005; Thornton et al., 2010).

BRs cannot be transported between plant tissues (Savaldi-Goldstein et al., 2007; Symons et al., 2008). Since BRs are synthesized and perceived in the same tissues, homeostasis of this class of hormones is tightly regulated to ensure that their cellular levels are appropriate for a given
developmental fate. BR homeostasis is also important for the establishment of plant immunity (Belkhadir et al., 2012).

In Arabidopsis, BR homeostasis is maintained in part by transcriptional feedback-regulation loops that control the expression of key biosynthetic and catabolic enzymes (Tanaka et al., 2005). For example, TCP1, a putative bHLH transcription factor (TF), interacts with the promoter of the BR biosynthesis gene DWF4 (Choe et al, 1998) to modulate BR levels (Guo et al., 2010). In addition, the TF LOB (lateral organ boundaries) binds directly to the promoter of BAS1 as an activator, and consequently reduces BR accumulation to limit growth in organ boundaries (Bell et al., 2012).

In this research, we found that an Arabidopsis NAC (NAM, ATAF1,2, CUC2) family TF, ATAF2/ANAC081, binds the promoters of BAS1 and SOB7 to suppress their expression. ATAF2 regulates hypocotyl elongation and root growth by suppressing BR catabolism. The expression of ATAF2 is suppressed by both BRs and light. The photomorphogenic phenotypes of ATAF2 loss- and gain-of-function seedlings can be suppressed by treatment with the BR biosynthesis inhibitor brassinazole (BRZ) (Asami et al., 2000). Moreover, the disruption of BAS1 and SOB7 abolishes the short-hypocotyl phenotype of ATAF2 loss-of-function seedlings in low-fluence-rate white light, which demonstrates an ATAF2-mediated connection between BR catabolism and photomorphogenesis. We propose that ATAF2 plays a central role in integrating BR homeostasis and seedling development. Since ATAF2 has previously been identified as a regulator of plant defense (Delessert et al., 2005; Wang et al., 2009) and the auxin biosynthesis gene NIT2 (Huh et al., 2012), ATAF2 acts as a central regulator of plant defense, hormone metabolism, and light-mediated seedling development.
Results

ATAF2 binds to the promoters of *BAS1* and *SOB7*

In a yeast-one-hybrid (Y1H) screen for the potential protein interactors of the *BAS1* promoter, a truncated ATAF2 missing the first 65 amino acids (ATAF2t) was found to interact with a *BAS1* promoter fragment (-731 to -504) (supplementary material Table S1). This fragment, p*BAS1*-EE, contains only an Evening Element TF-binding site (EE; AAAATATCT or its reverse complement sequence) (Michael and McClung, 2002) when scanned with the Athena program (O'Connor et al., 2005). Thus the EE element was proposed to be the ATAF2 binding target in the *BAS1* promoter.

Full-length ATAF2 interacted with p*BAS1*-EE in a targeted Y1H assay (Fig. 1A). The CCA1 binding site (CBS; AAAAATCT or its reverse complement sequence) is similar to EE in sequence. Both EE and CBS are the binding targets of the circadian clock protein CCA1 (Wang and Tobin, 1998; Michael and McClung, 2002; Harmer and Kay, 2005). Since CBSs are present twice in the *BAS1* promoter, and once in the *SOB7* promoter (Pan et al., 2009), three CBS-containing promoter fragments (supplementary material Table S1), p*BAS1*-CBS1 (-844 to -786), p*BAS1*-CBS2 (-1832 to -1768), and p*SOB7*-CBS (-1623 to -1524), were used as baits in targeted Y1H assays to test the hypothesis that ATAF2 binds the EE/CBS elements in the *BAS1* and *SOB7* promoters. CBS was the only predicted TF-binding site on all three fragments when scanned with the Athena program. ATAF2 interacted with both p*BAS1*-CBS1 (Fig. 1B) and p*SOB7*-CBS (Fig. 1C).

An electrophoresis mobility shift assay (EMSA) provided additional evidence that ATAF2 interacts with a CBS-containing DNA fragment (Fig. 1D). ATAF2 bound with biotin-labeled p*BAS1*-CBS1 suggesting the formation of an ATAF2-p*BAS1*-CBS1 complex. With increasing concentrations of the
unlabeled probes, the specific binding signal of the pBAS1-CBS1 by ATAF2 was gradually abolished
(Fig. 1D), which further suggests the physical interaction between ATAF2 and pBAS1-CBS1.

In order to further test whether the interaction between ATAF2 and BAS1/SOB7 promoters is
mediated by the EE/CBS elements, site-directed mutagenesis was performed for both pBAS1-EE (the
EE element mutated from AAAATATCT to AACATATCT, named as pBAS1-EEm) and pSOB7-CBS
(the CBS element mutated from AGATTTTT to AGATTCTT, named as pSOB7-CBSm). Neither
pBAS1-EEm (Fig. 1E) nor pSOB7-CBSm (Fig. 1F) was able to interact with ATAF2 in targeted Y1H
assays, which indicates that the EE/CBS elements are responsible for the binding of ATAF2.

Additionally, neither ATAF2 nor ATAF2t protein showed interaction with pBAS1-CBS2 in the
targeted Y1H assay (supplementary material Fig. S1), which indicates that the existence of EE/CBS
sequence alone is not sufficient to mediate interaction with ATAF2.

**ATAF2 suppresses the expression of BAS1 and SOB7**

ATAF2 can either activate (Wang et al., 2009; Huh et al., 2012) or suppress (Delessert et al., 2005) the
expression of downstream target genes. To test the effects of ATAF2 on BAS1 and SOB7 expression,
the transcript accumulation of BAS1 and SOB7 in ATAF2 gain- and loss-of-function seedlings was
examined by qRT-PCR. Unless otherwise stated, four-day-old seedlings grown in 25 °C were used for
all total RNA extraction and hypocotyl measurement experiments. BAS1 transcript accumulation was
significantly decreased in ATAF2 overexpression (ATAF2ox) lines and increased in ataf2 null-mutants
(ataf2-1, ataf2-2) in both darkness (Fig. 2A) and 80 μmol m⁻² s⁻¹ of continuous white light (Fig. 2B).
A similar relationship was observed in the case of SOB7 transcript accumulation except that SOB7
expression in ATAF2ox seedlings was not significantly reduced in white light (Fig. 2C,D).
The effects of ATAF2 on the expression of BR biosynthesis genes were also tested. Unlike the BR inactivation genes BAS1 and SOB7, two key BR biosynthesis genes, DWF4 (Choe et al., 1998) and CPD (Szekeres et al., 1996), did not show any dramatic changes of expression in the gain- or loss-of-function ATAF2 mutants (supplementary material Fig. S2A-D), which indicates that ATAF2 does not affect BR biosynthesis directly in the tested conditions. In darkness, the slight changes of DWF and CPD transcript accumulation in loss- and gain-of-function ATAF2 mutants (supplementary material Fig. S2A,C) may be caused by the transcriptional feedback regulation due to altered BR levels (Tanaka et al., 2005).

**ATAF2 restricts the tissue-specific expression of BAS1 and SOB7 in planta**

GUS analysis of ATAF2-GUS fusion transgenic Arabidopsis plants revealed that ATAF2 is expressed at varying levels in all plant tissues (Delessert et al., 2005). Similarly, GUS analysis showed that BAS1 and SOB7 have distinct as well as overlapping expression patterns, which are all limited to certain tissues, such as the shoot apex, root tip, or root elongation zone (Sandu et al., 2012). The inconsistency of expression patterns between ATAF2 and BAS1/SOB7 proteins indicates that ATAF2 may function as an *in vivo* repressor to restrict the expression of BAS1 and SOB7 in specific tissues. To test this hypothesis, pBAS1:BAS1-GUS and pSOB7:SOB7-GUS (genomic DNA translational fusions with 1.6 and 2.1 kb of their native promoters, respectively) transgenes were expressed in the *ataf2-2* background. The BAS1 promoter used includes the ATAF2-interaction-positive pBAS1-EE and pBAS1-CBS1 fragments, but not the ATAF2-interaction-negative pBAS1-CBS2 fragment. The SOB7 promoter used includes the ATAF2-interaction-positive pSOB7-CBS element. Approximately 10% of the T1 primary transformants showed BR-deficient dwarf phenotypes for both transgenes.
(supplementary material Fig. S3). Homozygous T₃ plants were identified from the BR-dwarf single-locus T-DNA insertion lines for each transgene.

In order to compare the expression strength of the exactly same pBAS1:BAS1-GUS or pSOB7:SOB7-GUS genome insertion sites in wild-type (Col-0) and ataf2-2 backgrounds, these selected homozygous single-locus T-DNA insertion BR-dwarf lines were crossed with Col-0 and homozygous pBAS1:BAS1-GUS/Col-0, pBAS1:BAS1-GUS/ataf2-2, pSOB7:SOB7-GUS/Col-0 and pSOB7:SOB7-GUS/ataf2-2 lines were identified from the segregating F₂ populations. While many pBAS1:BAS1-GUS/ataf2-2 and pSOB7:SOB7-GUS/ataf2-2 plants retained the BR-dwarf phenotype, no BR-dwarfs were identified in their pBAS1:BAS1-GUS/Col-0 and pSOB7:SOB7-GUS/Col-0 siblings (Fig. 3A,B). In addition, GUS analysis demonstrated that the disruption of ATAF2 allowed the expansion of BAS1 and SOB7 expression to almost all plant tissues (Fig. 3C,D). In conclusion, the disruption of ATAF2 dramatically increased the expression of BAS1 and SOB7 proteins demonstrating its role as a negative regulator of these BR-inactivating enzymes.

**ATAF2 loss- and gain-of-function seedlings have opposite BR response phenotypes**

The genetic state of BAS1 and SOB7 influences seedling growth in response to exogenous BRs (Neff et al., 1999; Turk et al., 2003; Turk et al., 2005; Thornton et al., 2010). As a transcriptional repressor of BAS1 and SOB7, the genetic state of ATAF2 is also likely to influence seedling growth in response to exogenous BRs. To test this hypothesis, Col-0, the ataf2 null- and the ATAF2ox mutants were grown on media with gradient concentrations of BL (0, 10, 100 and 1000 nM) in darkness or 80 μmol m⁻² s⁻¹ of white light. Under all three BL treatment concentrations, Col-0 hypocotyl growth was inhibited in darkness and enhanced in white light, which is consistent with Turk et al., (2003). When
compared with *Col-0*, the *ataf2* null-mutants were less responsive to all exogenous BL treatments with longer hypocotyls in the dark and shorter hypocotyls in the light. In contrast, the *ATAF2ox* mutants generally conferred the opposite response of shorter hypocotyls in the dark and longer hypocotyls in the light (Fig. 4A-D). The only exception being that in white light *ATAF2ox* mutants only showed significant hypocotyl-growth phenotype with 100-nM BL treatment. These results indicate that a 100-nM-BL treatment may be an ideal dose for the identification of hypocotyl elongation phenotypes for both dark and white light conditions.

Alteration of endogenous BR levels may also cause hypocotyl growth phenotypes in *ATAF2* mutants. When grown in the dark, *ataf2-1* and *ATAF2ox* seedlings showed opposite hypocotyl-growth phenotypes in response to treatments of the BR biosynthesis inhibitor BRZ (Fig. 4E,F). In all three BRZ treatment conditions (20, 100 and 500 nM), BRZ slightly promoted hypocotyl growth of *Col-0* and the *ATAF2ox* mutants, but inhibited that of *ataf2* null-mutants. Upon BRZ treatment, *ataf2* null-mutants always had a shorter hypocotyl than that of *Col-0* while *ATAF2ox* mutants were always taller than *Col-0*. BRZ treatment assays further demonstrated that the hypocotyl growth phenotypes of *ATAF2* mutants are due to the changes of BR levels.

BRZ has been reported to inhibit the hypocotyl growth of dark-grown seedlings in lower temperatures (Asami et al., 2000; Nagata et al., 2000). Thus the BRZ-treatment assays were also conducted on dark-grown seedlings in 20 °C (Fig. 4G). In this temperature, BRZ inhibited the hypocotyl growth of all three genotypes. *ATAF2ox* mutants were always less responsive to BRZ treatments than *Col-0* whereas *ataf2* null-mutants conferred the opposite phenotype. When grown at 20 °C *ATAF2ox* mutants were shorter than both *Col-0* and *ataf2* null-mutants in the absence of BRZ.

Since *ATAF2* has been reported to regulate the expression of the auxin biosynthesis gene *NIT2*
(Huh et al., 2012), the involvement of auxin in ATAF2-mediated seedling phenotypes were tested along with that of BRs. In contrast to the distinct hypocotyl growth phenotypes in ATAF2 mutants caused by BL and BRZ, treatments using either the auxin indole-3-acetic acid (IAA) or the auxin transport inhibitor naphthylphthalamic acid (NPA) (Ruegger et al., 1997) did not significantly affect the hypocotyl growth phenotypes of ATAF2 loss- and gain-of-function seedlings in darkness (supplementary material Fig. S4A,B). These results suggest that BRs are more critical than auxins in ATAF2-mediated hypocotyl growth.

**ATAF2 modulates root growth by regulating BR catabolism**

In addition to hypocotyl elongation, root growth is another important parameter for measuring seedling development. Thus the effects of ATAF2 on seedling primary and lateral root growth were also examined by BRZ and NPA treatments. With no BRZ applied, Col-0, ataf2 null- and ATAF2ox mutants showed similar primary root growth (Fig. 5A). Compared to the wild type and ATAF2ox mutant, ataf2-1 was more sensitive to exogenous BRZ treatments with respect to the elongation of the primary root (Fig. 5A). In contrast, ataf2-1, Col-0 and ATAF2ox seedlings showed similar primary root growth in response to exogenous NPA treatments (supplementary material Fig. S5A), indicating that BRs are more important than auxins in ATAF2-mediated primary root growth regulation.

Even in the absence of BRZ, ATAF2 loss- and gain-of-function seedlings showed opposite lateral root growth phenotypes (Fig. 5B). ataf2-1 had more lateral root growth than that of Col-0, whereas overexpression of ATAF2 inhibited lateral root growth (Fig. 5B). Exogenous BRZ treatments significantly attenuated the lateral root growth phenotype of ataf2-1, but not that of ATAF2ox (Fig. 5B). With BRZ concentration greater than 100 nM, the lateral root growth phenotype of ataf2-1 was
similar to that of the wild-type. An NPA treatment assay was carried out in the presence of 100 nM BRZ to investigate whether disruption of auxin transport would cause lateral root growth difference between Col-0 and ATAF2 mutants. The results showed that the lateral root growth phenotypes of ataf2-1 and ATAF2ox under 100 nM BRZ were not significantly affected by additional NPA treatments (supplementary material Fig. S5B).

**ATAF2 modulates hypocotyl growth in a fluence-rate dependent manner**

In a fluence-rate-response assay (Fig. 6A,B), the hypocotyl growth of ATAF2 loss- and gain-of-function and Col-0 seedlings had no significant difference in darkness or under high fluence rates of white light (80 and 100 μmol m⁻² s⁻¹), which was consistent with previous observations. However, ATAF2 gain- and loss-of-function mutants conferred opposite hypocotyl-length phenotypes in sub-saturating fluence rates of white light (10, 20, and 40 μmol m⁻² s⁻¹). Fluence-rate-response analysis demonstrated that ATAF2 stimulates hypocotyl growth in a light-intensity-dependent manner (Fig. 6A,B). ATAF2 acts as a suppressor of seedling photomorphogenesis under sub-saturating white light conditions.

The short-hypocotyl phenotype of ataf2-1 under low-fluence-rate (10 μmol m⁻² s⁻¹) white light was retained in monochromatic red (Fig. 6C), far-red (Fig. 6D) or blue (Fig. 6E) light. In order to investigate the contributions of photoreceptors to ATAF2-mediated photomorphogenesis, ataf2-1 was crossed with null-mutants of PHYB, PHYA and CRY1 to create corresponding double mutants. Under the same fluence-rate of monochromatic light (10 μmol m⁻² s⁻¹), the short-hypocotyl phenotype of ataf2-1 was retained but attenuated (supplementary material Table S2) when knocking out PHYB (Fig. 6F), PHYA (Fig. 6G), or CRY1 (Fig. 6H), respectively. These results indicate that ATAF2-mediated
photomorphogenesis is regulated by multiple photoreceptors. Among the three photoreceptors examined, PHYA played the most significant role in regulating ATAF2-mediated seedling photomorphogenesis (Fig. 6D,G; supplementary material Table S2).

In addition to far-red light, PHYA is known to respond to light in a wide range of wavelengths, including red (Tepperman et al., 2006; Franklin et al., 2007), blue and UV-A (Chun et al., 2001). Removal of PHYA was able to abolish the short-hypocotyl phenotype of ataf2-1 under 10 μmol m⁻² s⁻¹ red (Fig. 6I) or blue (Fig. 6J) light. These results indicate that the regulatory function of PHYA on ATAF2-mediated photomorphogenesis exists in light conditions of various wavelengths.

**ATAF2 is transcriptionally suppressed by both BRs and light**

Since ATAF2-regulated hypocotyl growth was shown to involve both BRs and light, the effects of these pathways on ATAF2 transcript accumulation were analyzed by qRT-PCR. The supplement of exogenous BL suppressed ATAF2 transcript accumulation in Col-0 seedlings grown in both darkness and continuous white light (Fig. 7A,B). In Col-0 seedlings, ATAF2 transcript accumulation was also suppressed by continuous white light in a fluence-rate dependent manner (Fig. 7C) with high fluence rates being more effective in suppressing ATAF2 transcript accumulation. Additionally, a one-hour white-light treatment for dark-grown seedlings was sufficient to significantly reduce ATAF2 transcript accumulation (Fig. 7D). The results above revealed that ATAF2 is transcriptionally regulated by both BR and light signals. The white-light-induced suppression of ATAF2 accumulation was retained in the same fluence-rate (10 μmol m⁻² s⁻¹) of monochromatic red, far-red or blue light (Fig. 7E), which is consistent with ATAF2-mediated photomorphogenesis being regulated by multiple photoreceptors and that the major regulator PHYA functions in a wide range of wavelengths.
The removal of PHYA did not affect ATAF2 transcript accumulation in dark-grown seedlings (Fig. 7F). The light-induced suppression of ATAF2 accumulation was attenuated by the removal of PHYA in 10 μmol m$^{-2}$ s$^{-1}$ of white, red, far-red or blue light (Fig. 7G). This result is also consistent with our observation that ATAF2-mediated photomorphogenesis is regulated by multiple photoreceptors with PHYA playing a major role in light conditions of various wavelengths (Fig. 6A-J).

**ATAF2 integrates BR catabolism and seedling photomorphogenesis**

To test whether the ATAF2-mediated photomorphogenic phenotype is caused by the changes of endogenous BR levels, Col-0 and ATAF2 loss- and gain-of-function seedlings grown under low-fluence-rate (10 μmol m$^{-2}$ s$^{-1}$) white light were treated with increasing concentrations (0, 20, 100 and 500 nM) of BRZ. Exogenous BRZ treatments attenuated the hypocotyl growth phenotypes of ATAF2 loss- and gain-of-function seedlings (Fig. 8A,B). The addition of 500-nM BRZ was sufficient to eliminate the hypocotyl-length differences of all three genotypes (Fig. 8A,B). In contrast, treatments using NPA did not affect the hypocotyl growth phenotypes of ATAF2 loss- and gain-of-function seedlings under the identical light condition (supplementary material Fig. S6). BRZ and NPA response assays demonstrated that BRs have a more important role than auxins in ATAF2-mediated seedling photomorphogenesis.

To test the function of BAS1 and SOB7 in ATAF2-mediated photomorphogenic phenotype, a bas1-2 sob7-1 ataf2-1 triple mutant was created and compared with the bas1-2 sob7-1 double mutant for hypocotyl growth in response to different fluence-rates (0, 10, and 80 μmol m$^{-2}$ s$^{-1}$, respectively) of white light (Fig. 8C). Removal of BAS1 and SOB7 completely abolished the short-hypocotyl phenotype caused by the disruption of ATAF2 (Fig. 8C). This result indicates that ATAF2 suppresses
seedling photomorphogenesis by suppressing the expression of the BR inactivation genes \textit{BAS1} and \textit{SOB7} (Fig. 8D).

**Discussion**

**ATAF2 binds A/T-rich \textit{cis}-acting elements to regulate the activities of downstream genes**

ATAF2 is a NAC family TF verified to be localized in the nucleus (Wang et al., 2009). NAC TFs share the features of a highly conserved N-terminal NAC domain and diverse C-terminal domains. The NAC domain can bind both DNA and protein. The C-terminal domains are responsible for the activation or suppression of downstream target genes (Ernst et al., 2004). ATAF2 has been reported to bind a 25-bp A/T-rich consensus sequence (Wang and Culver, 2012) and a 36-bp A/T-rich sequence (Huh et al., 2012). Here we have shown that ATAF2 also binds the A/T rich EE/CBS elements (Fig. 1A-F). Interestingly, ATAF2 interacts with two members of the AT-hook motif containing nuclear localized (AHL) protein family, which specifically bind A/T-rich DNA (Zhao et al., 2013). The shared binding-target similarity between ATAF2 and AHLs may facilitate their interactions \textit{in vivo}. Existing in all plant species, AHLs are involved in a wide range of biological processes, including regulating Arabidopsis hypocotyl growth (Zhao et al., 2013; Zhao et al., 2014). Together these observations suggest that ATAF2 and certain AHL proteins may work as functional partners in this fundamental plant development process.

The observation that ATAF2 and CCA1 share common promoter-binding sites indicates the possibilities that they may interact \textit{in vivo} to regulate downstream gene expression coordinately (binding together to function) or antagonistically (competing for the same binding site or restricting each other from binding targets by direct protein-protein interaction). There is also a putative CBS site
(−577 to -570) on the ATAF2 promoter, which suggests that CCA1 may also regulate the expression of ATAF2 by directly binding its promoter.

Recently, more than 1000 CCA1-binding regions were identified via the ChIP-seq approach (Nagel et al., 2015) though BAS1, SOB7 and ATAF2 promoters were not identified as CCA1 targets. In Nagel et al. (2015) 10-day-old Arabidopsis plants were used whereas we used 3 to 4 day old seedlings. The age difference and/or growth conditions may explain the differences between the two studies. In addition, the ChIP-seq approach may not identify all CCA1-binding sites, especially ones where binding is transient or weak.

ATAF2 is indispensable for the spatial regulation of BR homeostasis.

ATAF2 acts as a repressor of BAS1 and SOB7 expression (Fig. 2A-D). ATAF2 expression is suppressed by BRs (Fig. 7A,B). Therefore, ATAF2-BAS1/SOB7-BR-ATAF2 forms a feedback-regulation loop. The BR biosynthesis genes, DWF4 and CPD are not involved in this ATAF2 regulatory loop in our growth conditions (supplementary material Fig. S2A-D). This result is consistent with the fact that no EE/CBS element is detected in the 2.5-kb region of DWF4 or CPD promoter when scanned with the Athena program. In addition to transcriptionally repressing BAS1 and SOB7, the emergence of BR-deficient dwarf plants in pBAS1:BAS1-GUS/ataf2-2 and pSOB7:SOB7-GUS/ataf2-2 transgenic populations (Fig. 3A,B; supplementary material Fig. S3) indicate that the disruption of ATAF2 dramatically increases the expression of BAS1 and SOB7 proteins. In addition, comparison of identical pBAS1:BAS1-GUS and pSOB7:SOB7-GUS transgene insertions in Col-0 and ataf2-2 backgrounds supports the hypothesis that ATAF2 modulates the expression patterns of BAS1 and SOB7, restricting their expressions to certain tissues, and controlling
their expressions within certain levels (Fig. 3C,D).

Although some pBAS1:BAS1-GUS/ataf2-2 and pSOB7:SOB7-GUS/ataf2-2 transgenic plants have BR-dwarf phenotypes, the original ataf2-2 mutant is not dwarf. These observations can be explained by at least four possible reasons. First, the transgenic plants harbor additional copies of BAS1 or SOB7. Second, the genome positions of the transgenes are different from those of the original BAS1 or SOB7. Third, the remote cis-regulatory elements may not be included in the promoter regions cloned in the constructs. Fourth, the fusion of ATAF2 to GUS protein may help to stabilize ATAF2. In Arabidopsis, there are examples that GUS and GFP tags increase the stability of several SMALL AUXIN UP RNA (SAUR) proteins (Chae et al., 2012; Spartz et al., 2012). In this case, BAS1-GUS or SOB7-GUS translational fusion proteins may have higher stability than that of the original BAS1 or SOB7, respectively. Increased accumulation of BAS1-GUS or SOB7-GUS may lead to the BR-deficient dwarf phenotypes in pBAS1:BAS1-GUS/ataf2-2 or pSOB7:SOB7-GUS/ataf2-2, respectively. In conclusion, all these possible reasons mentioned above may result in the expression of BAS1 or SOB7 in transgenic ataf2-2 strong enough to cause the dwarf phenotype due to increased BR inactivation.

The GUS expression patterns in pBAS1:BAS1-GUS/Col-0 and pSOB7:SOB7-GUS/Col-0 are similar but not exactly identical to what has been reported before (Sandu et al., 2012), which can also be caused by the T-DNA insertion position differences among ectopic expression lines. Additionally, the genetic background difference may affect the observed GUS expression patterns. Wild-type (Col-0) plants have been used to generate GUS expression lines in this work, whereas Sandhu et al. (2012) adopted a bas1-2 sob7-1 double mutant background for pBAS1:BAS1-GUS and pSOB7:SOB7-GUS expression.
The hypocotyl growth phenotypes of \textit{ATAF2} mutants can be triggered by either BR or light signals

The BL and BRZ response assay results (Fig. 4A-G) further support the hypothesis that ATAF2 acts as a repressor of BR catabolism. The reduced expression of \textit{ATAF2} in \textit{ataf2-null} mutants leads to higher transcript accumulation of \textit{BAS1} and \textit{SOB7}, which enhances the BR inactivation capacity in seedlings and confers more resistance to BL treatment. Similarly, higher expression of \textit{ATAF2} in \textit{ATAF2ox} mutants leads to lower levels of \textit{BAS1} and \textit{SOB7} transcript accumulation, which reduces the BR inactivation capacity in \textit{ATAF2ox} seedlings, conferring more sensitivity to BL treatment. In darkness, \textit{ATAF2ox} seedlings show a BL-oversensitive phenotype under all three BL-treatment conditions (Fig. 4A,B). In white light, high concentration of exogenous BL (1 μM) masks the BL-oversensitive phenotype of \textit{ATAF2ox} seedlings. A low concentration of exogenous BL (10 nM) is not sufficient to detect the phenotype (Fig. 4C,D). However, the hypocotyl phenotype was distinct under treatment of 100 nM BL (Fig. 4C,D). Both \textit{ataf2} null-mutants had BL-insensitive phenotypes under all six light- and dark-grown BL-treatment conditions (Fig. 4A-D). The relationship between BRs and hypocotyl growth phenotypes of \textit{ATAF2} mutants was further demonstrated by BRZ-response analysis (Fig. 4E-G).

In our assays, BRZ promotes the hypocotyl elongation of dark-grown seedlings (except \textit{ATAF2} loss-of-function mutants) in 25 °C (Fig. 4E,F) but inhibits the elongation in 20 °C (Fig. 4G). This observation suggests that the sensitivity of dark-grown hypocotyls to endogenous BR levels is temperature-dependent. Gray et al. (1998) reported that high temperature promotes hypocotyl elongation that is mainly mediated by auxin with a lesser involvement of BRs.
In all the BR, BRZ and NPA treatment assays conducted, only two distinct light conditions (darkness and 80 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) of white light) were used. These two conditions were selected because all hypocotyl lengths were similar for the non-treatment controls which facilitated the evaluation of BR-treatment effects. Since it is well known that seedling photomorphogenesis is modulated by the coordination of light and hormonal signals, the involvement of light intensity and quality in ATAF2-regulated seedling growth was investigated. The fluence-rate-response assay demonstrates that ATAF2 stimulates hypocotyl growth in a light-intensity-dependent manner (Fig. 6A,B). In darkness the hypocotyl-growth regulatory activity of ATAF2 was not significantly visible. Under high fluence rates of white light, the light intensities were strong enough to suppress the photomorphogenic activities of ATAF2.

\textit{ATAF2} transcript accumulation is suppressed by light in a fluence-rate dependent manner (Fig. 7C,D), which at least partially explains why \textit{ATAF2} loss- and gain-of-function and \textit{Col-0} seedlings have no significant difference in hypocotyl length under high fluence rates of light. ATAF2 has been reported to activate the expression of the auxin biosynthesis gene \textit{NIT2} (Huh et al., 2012). Thus, both BRs and auxins could be involved in the photomorphogenic phenotypes of \textit{ATAF2} mutants. BRZ (Fig. 8A,B) and NPA (supplementary material Fig. S6) response assays demonstrate that BRs have a more important role in this response than auxins. The short-hypocotyl phenotype of \textit{ATAF2} loss-of-function mutants relies on the existence of \textit{BAS1} and \textit{SOB7} (Fig. 8C), which further demonstrates that \textit{ATAF2}-mediated photomorphogenic phenotypes depend on BR inactivation catalyzed by BAS1 and SOB7. Together, these results demonstrate the presence of a light-ATAF2-BAS1/SOB7-BR-photomorphogenesis signaling network. The BL-response phenotypes of \textit{ATAF2ox} seedlings were more dramatic in darkness than in white light (Fig. 4A-D). These results
are indirect evidence that light-mediated reduction of *ATAF2* expression may attenuate
ATAF2-mediated BR-response phenotypes.

**Multiple photoreceptors regulate ATAF2-mediated photomorphogenesis with PHYA playing a major role**
*ATAF2* loss- and gain-of-function seedlings retain their hypocotyl-length phenotypes in
monochromatic red, far-red and blue light (Fig. 6C-E). The phenotypes are attenuated (Fig. 6F-H;
supplementary material Table S2) by the removal of *PHYB*, *PHYA*, or *CRY1*, with the disruption of
*PHYA* being the most dramatic (Fig. 6D,G; supplementary material Table S2). These results suggest
that multiple photoreceptors are involved in ATAF2-mediated seedling photomorphogenesis, with
PHYA playing an important role. Turk et al. (2003) reported that the interaction between the light and
the BAS1-mediated BR-inactivation pathway is mainly dependent on far-red light. The regulation of
ATAF2-mediated photomorphogenesis is consistent with this previous observation and may constitute
part of the light-BR intersection. The observations that ATAF2-mediated photomorphogenic
phenotype under monochromatic red or blue light also requires PHYA (Fig. 6I,J) further demonstrate
the close relationship between PHYA and ATAF2 function.

**ATAF2 regulates root growth in part via BR catabolism**
BRZ treatments reduce both primary and lateral root growth (Fig. 5A,B), which is consistent with
previous observations that BRs can promote the growth of both tissues (Müssig et al., 2003; Bao et al.,
2004). There have been numerous reports revealing the complexity of BR activity in controlling root
growth and development (Hacham et al., 2011; Fridman et al., 2014; Vragović et al., 2015). BRZ (Fig.
5A,B) and NPA (supplementary material Fig. S5A,B) response assays demonstrate that BRs are responsible for the primary and lateral root growth phenotypes modulated by ATAF2. The root phenotypes of ATAF2 loss-of-function mutants indicate that ATAF2 acts as a repressor of lateral root growth while acting as an activator of primary root growth in the presence of BRZ. Thus, it is possible that ATAF2 has a role in determining resource allocation and differential growth between the primary and lateral roots.

**ATAF2 integrates Arabidopsis brassinosteroid inactivation and seedling photomorphogenesis**

Overall, we proposed that ATAF2 acts as an integrator of BR catabolism and seedling photomorphogenesis (Fig. 8D). ATAF2 suppresses the expression of the BR-inactivating genes BAS1 and SOB7. As a result, ATAF2 promotes the elevation of BR levels. In turn, BRs suppress ATAF2 expression to form an ATAF2-BAS1/SOB7-BR-ATAF2 feedback-regulation loop. On the other hand, ATAF2 promotes hypocotyl growth at least partially via the pathway of BAS1/SOB7-mediated BR degradation. Light suppresses hypocotyl growth through versatile pathways, one of which is by suppressing ATAF2 in a fluence-rate-dependent manner. PHYA plays a major role in ATAF2-mediated seedling photomorphogenic pathway. In this model, ATAF2 plays a key role in connecting seedling development with BR homeostasis and photomorphogenesis. Since ATAF2 was previously reported to regulate plant defense (Delessert et al., 2005; Wang et al., 2009) and auxin biosynthesis (Huh et al., 2012), ATAF2 acts as a central regulator of plant defense, hormone metabolism, and light-mediated seedling development.
Materials and Methods

Plant materials

Arabidopsis plants used in this study are in the Columbia (Col-0) background. T-DNA insertion mutants were obtained from the Arabidopsis Biological Resource Center (ABRC). For ataf2-1 (SALK_136355), the T-DNA insertion site is in the second exon. For ataf2-2 (SALK_015750), the T-DNA is located in the second intron. Quantitative RT-PCR (qRT-PCR) analysis, as described below, demonstrated that both ataf2-1 and ataf2-2 are null alleles. For the generation of ATAF2ox lines, the ATAF2 coding sequence (CDS) was overexpressed in Col-0 under the control of CAMV 35S promoter. The pBAS1:BAS1-GUS and pSOB7:SOB7-GUS constructs was described previously (Turk et al., 2003; Sandhu et al., 2012). Homozygous single-locus T-DNA insertion lines were selected for all three transgenic events. The histochemical GUS analysis was described previously (Sandu et al., 2012). Photoreceptor mutants (all in Col-0 background) phyB-9 (Reed et al., 1993), phyA-211 (Reed et al., 1994) and cry1-103 (Liscum and Hangarter, 1991) were crossed with ataf2-1 to isolate double mutants with ataf2-1 from F2 populations. The bas1-2 sob7-1 double mutant (Turk et al., 2005) was crossed with ataf2-1 to isolate the bas1-2 sob7-1 ataf2-1 triple mutant. The PCR identification of bas1-2, sob7-1, phyB-9, phyA-211 and cry1-103 mutations were described previously (Turk et al., 2005; Ward et al., 2005; Sandhu et al., 2012). Unless otherwise stated, four-day-old seedlings were used for total RNA extraction and hypocotyl measurements, and seven-day-old seedlings were used for root growth measurements. Unless otherwise stated, all the seedlings were grown in growth chambers at 25 °C for all physiological and molecular assays.
DNA-protein interaction assays

The Y1H assays were carried out using the Gateway-compatible system (Deplancke et al., 2006). Briefly, the promoter DNA fragments (baits) were amplified and fused to the reporter gene HIS3, and subsequently integrated into yeast genome. The yeast bait clones with the lowest self-activation levels were selected independently for each assay and used to either screen an Arabidopsis cDNA library for interacting protein preys or test interactions with the targeted prey. The activation of HIS3 was tested by yeast tolerance to 3-AT (3-aminotriazole, a competitive inhibitor of the His3p enzyme). Sequences of DNA fragments used for Y1H screen and targeted Y1H are listed in supplementary material Table S1. All constructs used in this research that were generated by PCR were sequence verified. Site-directed mutagenesis of baits was performed using the QuikChange Lightning kit (Agilent Technologies). His-tagged ATAF2 protein was purified for EMSA using procedures described previously (Zhao et al., 2013).

Transcript analysis

Total RNA was extracted using the RNeasy Plant Kit (QIAGEN). On-column DNase digestion was performed to eliminate genomic DNA contamination. Total cDNA was synthesized using the SuperScript III First-Strand Synthesis System (Invitrogen). qRT-PCR reactions were performed using the SsoFast EvaGreen Supermix with Low Rox Kit (Bio-Rad) and the Applied Biosystems 7500 Fast Real-Time PCR System. The relative expression levels were determined by normalizing to the transcript levels of UBQ10. For each qRT-PCR assay, four-day-old seedlings of the three biological
replicates were grown under the same treatment at the same time. All qRT-PCR primers are listed in supplementary material Table S3.

**Hypocotyl and root measurements**

Seed sterilization, media composition, plating, growth conditions, and hypocotyl measurement were previously described (Sandu et al., 2012; Zhao et al., 2013). For BL (Turk et al., 2003), BRZ (Asami et al., 2000), IAA (Collett et al., 2000) and NPA (Ruegger et al., 1997) treatment assays, seeds were put on the treatments from the beginning of the experiments. Thirty tallest seedlings from the group of three independent replicates were used for each sample in all hypocotyl measurements. For root growth measurement, seedlings were grown on vertical plates in white light. Ten seedlings with the longest primary or lateral root growth from the group of three independent replicates were used for each sample in all the root measurements. All seedlings were scanned/photographed and measured using NIH ImageJ (Schneider et al., 2012).
Acknowledgements

We thank Dr. Hanjo Hellmann (Washington State University) for providing the Arabidopsis cDNA prey library.

Competing interests

The authors declare no competing or financial interests.

Author contributions

H.P., J.Z., and M.M.N. designed the experiments, analyzed the data, and wrote the manuscript. J.Z. performed the electrophoresis mobility shift assay. H.P. performed all the other experiments.

Funding

This research was supported by the U.S. National Science Foundation project 1124749 (to M.M.N.). This research was also supported by the USDA National Institute of Food and Agriculture, HATCH project 1007178 (to M.M.N.).
References


**Wang, X., Goregaoker, S. P. and Culver, J. N.** (2009). Interaction of the Tobacco mosaic virus replicase protein with a NAC domain transcription factor is associated with the suppression of


Fig. 1. ATAF2 binds to the promoters of *BAS1* and *SOB7*. (A) ATAF2 interacted with p*BAS1*-EE in a targeted Y1H assay. (B) ATAF2 interacted with p*BAS1*-CBS1 in a targeted Y1H assay. (C) ATAF2 interacted with p*SOB7*-CBS in a targeted Y1H assay. (D) ATAF2 bound to biotin-labeled p*BAS1*-CBS1 in an EMSA. (E) ATAF2 did not interact with p*BAS1*-EEm in which the EE element has been mutated. (F) ATAF2 did not interact with p*SOB7*-CBS in which the CBS element has been mutated. For each tested Y1H interaction, yeast integrated with the indicated bait sequence in the genome and transformed with the indicated prey plasmids were plated on selection medium supplemented with 3-AT of indicated concentrations. Four independent clones were shown for each sample. For EMSA, 20 fmol of p*BAS1*-CBS1-Biotin probe was used in the reaction (lanes 1, 3, 4, 5...
and 6). With the increasing concentration of the cold unlabeled pBAS1-CBS1 probe (lane 4: 4 pmol; lane 5: 10 pmol; lane 6: 50 pmol), the binding capacity ATAF2 protein to biotin-labeled pBAS1-CBS1 was gradually abolished.
Fig. 2. ATAF2 suppresses the expression of BASI and SOB7. BASI accumulation was decreased in ATAF2ox seedlings and increased in ataf2-1 and ataf2-2 mutants under both dark (A) and 80 μmol m⁻² s⁻¹ white-light conditions (B), as was the case with SOB7 accumulation in dark (C). In white light, only the ataf2-1 and ataf2-2 mutants showed significantly increased SOB7 accumulation (D). Each qRT-PCR value is the mean of results from three biological replicates. Error bars denote the SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired one-tailed student’s t-test). n = 3 for each value. t-tests were performed between the indicated sample group and the control group of Col-0.
Fig. 3. ATAF2 restricts the tissue-specific expression of BAS1 and SOB7 in planta. Ectopic expression of pBAS1:BAS1-GUS and pSOB7:SOB7-GUS could cause BR-deficient dwarfism in ataf2-2. Single-locus T-DNA insertion pBAS1:BAS1-GUS/ataf2-2 and pSOB7:SOB7-GUS/ataf2-2 lines were crossed with Col-0. Homozygous lines were selected from the two F2 segregation populations for morphology comparison. (A) The pBAS1:BAS1-GUS/ataf2-2 line was dwarf, while its pBAS1:BAS1-GUS/Col-0 sibling was relatively normal. (B) The pSOB7:SOB7-GUS/ataf2-2 line was dwarf, while its pSOB7:SOB7-GUS/Col-0 sibling was relatively normal. GUS analysis demonstrates that ATAF2 restricts the tissue-specific expression of BAS1 and SOB7 in planta (C,D). Scale bars: 1 cm in (A,B); 1 mm in (C,D).
Fig. 4. ATAF2 loss- and gain-of-function seedlings have opposite BR-response phenotypes. (A,B)
In darkness, *ATAF2* loss- and gain-of-function seedlings conferred BL-insensitive and -oversensitive phenotypes, respectively, under all three BL-treatment conditions. (C,D) In white light, *ataf2-1* and *ataf2-2* seedlings conferred BL-insensitive phenotypes under all three BL-treatment conditions. *ATAF2ox* seedlings conferred significant BL-oversensitive phenotypes only under the treatment of 100 nM BL. (E,F,G) *ataf2-1* and *ATAF2ox* seedlings showed opposite hypocotyl-growth phenotypes in response to BRZ treatments. Four-day-old seedlings were used for all the assays. The seedlings were grown in either 25 °C (A-F) or 20 °C (G). Each sample value (A,C,E,G) represents the average of measurement results from thirty seedlings. Error bars denote the SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired two-tailed student’s *t*-test). *n* = 30 for each value. When a single significance-level label is used for several similar genotypes, it means that all these samples’ significance levels are identical. *t*-tests were performed between the indicated sample group and the control group of *Col-0*. Scale bars: 1 mm in (B,D,F).
Fig. 5. *ATAF2* modulates root growth via regulating BR catabolism. (A) *ataf2-1* showed reduced primary root growth in response to exogenous BRZ treatments. (B) *ATAF2* loss- and gain-of-function seedlings showed opposite lateral root growth phenotypes. Exogenous BRZ treatments attenuated the lateral root growth phenotype of *ataf2-1*. Each sample value represents the average of measurement results from ten seven-day-old seedlings. Error bars denote the SEM. *P* < 0.05, **P** < 0.01, ***P*** < 0.001 (unpaired two-tailed student’s *t*-test). *n* = 10 for each value. *t*-tests were performed between the indicated sample group and the control group of *Col-0*. 
Fig. 6. *ATAF2* modulates hypocotyl growth in a fluence-rate dependent manner. (A,B) *ATAF2* loss- and gain-of-function seedlings had reduced differences of hypocotyl responsiveness when increasing white-light fluence-rates. (C-H) *ATAF2*-mediated photomorphogenesis is regulated by multiple photoreceptors. The short-hypocotyl phenotype of *ataf2-1* under low-fluence-rate (10 μmol
m² s⁻¹) white light was retained in monochromatic red (C), far-red (D) or blue (E) light. Under the same fluence-rate of monochromatic light conditions, the short-hypocotyl phenotype of *ataf2-1* was retained but attenuated (supplementary material Table S2) by the removal of *PHYB* (F), *PHYA* (G), or *CRY1* (H). Removal of PHYA abolished the short-hypocotyl phenotype of *ataf2-1* under 10 μmol m⁻² s⁻¹ red (I) or blue (J) light. In (C-J), all measurements were from three-day-old seedlings grown in 25 °C. Each sample value (A,C-J) represents the average of measurement results from thirty seedlings. Error bars denote the SEM. *P < 0.05, **P < 0.01, ***P < 0.001* (unpaired two-tailed student’s *t*-test). *n* = 30 for each value. When a single significance-level label is used for several similar genotypes, it means that all these samples’ significance levels are identical. In (A, C-E), *t*-tests were performed between the indicated sample group and the control group of *Col-0*. In (F) and (H), *t*-tests were performed between the indicated sample group and the control group of *phyB-9* and *cry1-103*, respectively. In (G,I,J), *t*-tests were performed between the *phyA-211 ataf2-1* sample group and the control group of *phyA-211*. Scale bars: 1 mm in (B).
Fig. 7. *ATAF2* is transcriptionally suppressed by both BR and light signals. In *Col-0* seedlings, *ATAF2* accumulation was significantly suppressed by 100 nM BL treatment in both darkness (A) and white light (B). (C) *ATAF2* accumulation was suppressed by white light in a fluence-rate dependent manner. (D) *ATAF2* accumulation was significantly suppressed by one-hour treatment of white light. (E) The white-light-induced suppression of *ATAF2* accumulation was retained in monochromatic red, far-red or blue light. (F) The removal of *PHYA* did not affect *ATAF2* transcript accumulation in
dark-grown seedlings. (G) The light-induced suppression of ATAF2 accumulation was attenuated by
the removal of PHYA in 10 μmol m⁻² s⁻¹ of white, red, far-red or blue light. Error bars denote the SEM.

*P < 0.05, **P < 0.01, ***P < 0.001 (unpaired one-tailed student’s t-test). n = 3 for each value in
(A,B,F,G). n = 6 for each value in (C-E). In (A,B), each qRT-PCR value is the mean of results from
three biological replicates, and t-tests were performed between the BL-treated sample group and the
untreated control. In (C-E), each qRT-PCR value is the mean of results from six replicates (three
biological replicates with each having two technical replicates), and t-tests were performed between
the indicated sample group and the control group of Col-0 under dark. In (F), each qRT-PCR value is
the mean of results from three biological replicates, and t-test was performed between the phyA-211
group and the control group of Col-0 under dark. In (G), each qRT-PCR value is the mean of results
from three biological replicates, and t-tests were performed between the indicated sample group and
the control group of phyA-211 under dark.
ATAF2 promotes hypocotyl growth by elevating BR level in seedling, which is accomplished by suppressing the expression of BR inactivating genes BAS1 and SOB7. BRs suppress A TAF2 expression to form a feedback regulation loop. White light suppresses hypocotyl growth through BR catabolism and seedling photomorphogenesis. (A,B) Exogenous BRZ treatments attenuated the hypocotyl growth phenotypes of ATAF2 loss- and gain-of-function seedlings under low-fluence-rate (10 μmol m⁻² s⁻¹) white light. (C) There were no significant differences of hypocotyl lengths between bas1-2 sob7-1 double mutant and bas1-2 sob7-1 ataf2-1 triple mutant in response to different fluence-rates (0, 10, and 80 μmol m⁻² s⁻¹) of white light. (D) The model of ATAF2 function in connecting hypocotyl growth to BR catabolism and light fluence-rates.
versatile pathways. One of which is by suppressing ATAF2 to modulate BR catabolism. Four-day-old seedlings grown in 25 °C were used for all the assays. Each sample value (A,C) represents the average of measurement results from thirty seedlings. Error bars denote the SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (unpaired two-tailed student’s t-test). n = 30 for each value. In (A), t-tests were performed between the indicated sample group and the control group of Col-0. In (C), t-tests were performed between either the ataf2-1 group and the control group of Col-0, or the bas1-2 sob7-1 ataf2-1 group and the control group of bas1-2 sob7-1. Scale bars: 1 mm in (B).