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RECIPROCAL REGULATION BY Z-BOX BINDING FACTORS GBF1 AND MYC2 CONTROLS STAGE-SPECIFIC DEVELOPMENT AND LIGHT-MEDIATED GENE EXPRESSION IN *ARABIDOPSIS*

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ABSTRACT

Arabidopsis MYC2 and GBF1 belong to a special class of transcription factors known as Z-box binding factors (ZBFs). MYC2 is a basic helix–loop–helix (bHLH) protein, whereas GBF1 belongs to the basic leucine zipper (bZIP) family of transcription factors. Both act as blue-light-specific negative regulators of photomorphogenic growth in *Arabidopsis* and mutually inhibit each other's activity during early seedling development. In contrast to MYC2, GBF1 exhibits a dual role and positively regulates cotyledon expansion. Although their antagonistic functions during seedling development are well characterized, their regulatory interplay at other developmental stages remains unclear. In this study, we demonstrate that an additional mutation of MYC2 in the *gbf1* background completely rescues its small-cotyledon phenotype. Likewise, mutation of GBF1 in the *myc2* background, which displays reduced adult plant stature, restores a wild-type-like adult phenotype in *myc2 gbf1* double mutants. Furthermore, loss of GBF1 suppresses the blue-light-mediated induction of light-responsive genes, *CAB1* and *RBCS1A*, in the *myc2* background. The expression of light-signaling pathway genes, including *SHW1* and *SPA1*, is also altered in the *myc2 gbf1* double mutants. Collectively, these results reveal that stage-specific reciprocal regulation between MYC2 and GBF1 fine-tunes *Arabidopsis* development.

Keywords : Z-box binding factor, photomorphogenesis, gene-expression, reciprocal regulation.

Introduction

Phytochromes (phyA to phyE) and cryptochromes (cry1, cry2 and cry3) are two most important classes of photoreceptors, which predominantly work in red-far/red and blue light conditions, respectively (Neff *et al.*, 2000; Briggs and Olney, 2001; Lin 2002; Huq and Quail, 2005; Jiao *et al.*, 2007). Many downstream components of phytochrome and cryptochrome signaling pathways have been identified and characterized (Jiao *et al.*, 2007; Liu *et al.*, 2011; Li *et al.*, 2011). These regulators of light signaling pathways affect the growth and development of plants in various ways. Among downstream signaling components, HY5, CAM7, HYH and HFR1 act as positive regulators whereas MYC2, GBF1, SHW1, and SPA1 act as negative regulators of light mediated inhibition of hypocotyl elongation (Oyama *et al.*, 1997; Ang *et al.*, 1998, Chattopadhyay *et al.*, 1998b; Hoecker *et al.*, 1998; Kushwaha *et al.*, 2008, Fairchild *et al.*, 2000; Holm *et al.*, 2002; Yadav *et al.*, 2005; Mallappa *et al.*, 2006; Bhatia *et al.*, 2008; Abbas *et al.*, 2014). HY5 (Elongated hypocotyl 5) is a bZIP protein, which acts as a positive regulator of photomorphogenic growth at various wavelengths of light (Oyama *et al.*, 1997). CAM7/ZBF3 (Calmodulin 7 or Z-box binding factor 3) also works as a positive regulator of photomorphogenic growth and light regulated gene expression (Kushwaha *et al.*, 2008). HYH (HY5 Homologue) is also a bZIP protein, which specifically works in blue light (Holm *et al.*, 2002, Singh *et al.*, 2012). HFR1 (Elongated Hypocotyl in Far-red 1) is a bHLH transcription factor that acts as positive regulator of photomorphogenic growth in far-red and blue light conditions (Fairchild *et al.*, 2000; Duek and Fankhauser, 2003).

et al., 1998, Chattopadhyay *et al.*, 1998b; Hoecker *et al.*, 1998; Kushwaha *et al.*, 2008, Fairchild *et al.*, 2000; Holm *et al.*, 2002; Yadav *et al.*, 2005; Mallappa *et al.*, 2006; Bhatia *et al.*, 2008; Abbas *et al.*, 2014). HY5 (Elongated hypocotyl 5) is a bZIP protein, which acts as a positive regulator of photomorphogenic growth at various wavelengths of light (Oyama *et al.*, 1997). CAM7/ZBF3 (Calmodulin 7 or Z-box binding factor 3) also works as a positive regulator of photomorphogenic growth and light regulated gene expression (Kushwaha *et al.*, 2008). HYH (HY5 Homologue) is also a bZIP protein, which specifically works in blue light (Holm *et al.*, 2002, Singh *et al.*, 2012). HFR1 (Elongated Hypocotyl in Far-red 1) is a bHLH transcription factor that acts as positive regulator of photomorphogenic growth in far-red and blue light conditions (Fairchild *et al.*, 2000; Duek and Fankhauser, 2003).

MYC2 is a bHLH protein, which acts as negative regulator of photomorphogenic growth in blue light. It acts as positive regulator of lateral root growth and flowering time (Yadav *et al.*, 2005; Gangappa and Chattopadhyay, 2010; Maurya *et al.*, 2015; Chakraborty *et al.*, 2019). GBF1 acts as a negative regulator of blue light-mediated inhibition of hypocotyl elongation, but positively regulates the cotyledon size and lateral root formation (Mallappa *et al.*, 2006). SHW1 (Short Hypocotyl in White light 1) is a unique serine-arginine-aspartate rich protein, which acts as a negative regulator of light mediated inhibition of hypocotyl elongation. SHW1 also acts as a negative regulator of photomorphogenic growth in the darkness. On the other hand, it acts as positive regulator of light mediated gene expression (Bhatia *et al.*, 2008). *SPA1* (Suppressor of phyA) belongs to *SPA* gene family. The other members of this family are *SPA2*, *SPA3* and *SPA4* (Hoecker *et al.*, 1998; Laubinger and Hoecker, 2003; Laubinger *et al.*, 2004). All these proteins act as negative regulators of photomorphogenic growth. *SPA1* has been shown to function in association with COP1, which is an E3-ubiquitin ligase and helps in the degradation of positive regulators of photomorphogenesis such as HY5, HYH, LAF1 and HFR1 in dark (Holm *et al.*, 2002; Saijo *et al.*, 2003; Seo *et al.*, 2003; Jang *et al.*, 2005; Yang *et al.*, 2005; Zhu *et al.*, 2008).

Light responsive elements (LREs) play important roles in the regulation of light-induced gene expression (Terzaghi and Cashmore, 1995; Arguello-Astorga and Herrera-Estrella 1998). Promoter sequence analysis of some light-inducible genes such as *CAB*, *RBCS*, and *CHS* have resulted in the identification of at least four commonly found LREs: G, GATA, GT1, and Z-box, which play important roles for light-mediated transcriptional activity (Ha and An, 1988; Donald and Cashmore, 1990; Terzaghi and Cashmore, 1995; Batschauer *et al.*, 1996; Puente *et al.*, 1996; Chattopadhyay *et al.*, 1998a; Yadav *et al.*, 2002; Gangappa *et al.*, 2013a). Transcription factors specifically interact with these LREs and regulate the expression of respective genes. Earlier studies have demonstrated that both MYC2 and GBF1 can bind to the Z- (ATACGTGT) or G-box (CACGTG) cis-elements present in the promoters of several genes (Schindler *et al.*, 1992; Boter *et al.*, 2004; Yadav *et al.*, 2005; Mallappa *et al.*, 2006, Dombrecht *et al.*, 2007; Gangappa *et al.*, 2010a, 2013b; Singh *et al.*, 2012; Hong *et al.*, 2012; Maurya *et al.*, 2015; Chakraborty *et al.*, 2019) and regulate their expression. Both Z- and G-box belong to E-box (CANNTG) family of LRE. Recently, by Chip-chip approach Ram *et al.*, 2013, has shown that GBF1 binds to the different types of cis-

acting elements present in the large number of plant promoters.

Here, we show that genetic interaction between *MYC2* and *GBF1* reciprocally regulates stage-specific development in *Arabidopsis*. *MYC2* mutation rescues the *gbf1* small-cotyledon phenotype, while *GBF1* loss restores adult stature in *myc2* mutants. *GBF1* also modulates blue-light-induced *CAB1* and *RBCS-1A* expression in *myc2* mutant background and also alters the expression of light signaling genes *SHW1* and *SPA1* levels in double mutants, revealing stage-specific cross-regulation fine-tuning plant development.

Materials and Methods

Arabidopsis seed plating, mutants and light conditions used

Seeds were surface sterilized with 1 ml of sterilization solution (2% sodium hypochlorite, 0.02% Triton X-100) by incubating for 10 minutes at room temperature with vortex two times, once in the beginning and once at the end. After 10 min, the sterilization solution was decanted in laminar air flow. The seeds were washed with sterile milli Q (MQ) water for five times and were finally suspended in a required volume of sterile water and plated onto the Murashige and Skoog (MS) plates using a pipette. The plates were kept at cold (4 °C) in dark for 3 to 4 days for stratification before transferring to the desired intensity and wavelength of light at 22 °C.

Wild-type, or mutant *Arabidopsis* plants were propagated in an identical manner. For the growth of plants, the white-light intensity used was 100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. The intensities of continuous light sources used for the growth of seedlings in this study are: white light (30 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) and blue light. Custom made LED chamber was used for the growth of seedlings under blue-light conditions. The wild-type *Arabidopsis thaliana* used in this study is Columbia (Col-0). The *atmyc2* and *gbf1* mutants are in Col-0 accession (Yadav *et al.*, 2005; Mallappa *et al.*, 2006 and Gangappa *et al.*, 2010a).

Nutrient media

The MS medium (Murashige and Skoog, 1962) was used for the growth of *Arabidopsis* seedlings. The MS salt mixture and the vitamin stock were from Sigma-Aldrich. The various components were mixed and then the pH was adjusted to 5.7 with 2M KOH, and after addition of agar (0.8-1%), the medium was autoclaved.

General sterilization procedures

Culture media, glassware's, chemical reagents and various tools were sterilized by autoclaving at 121 °C

at 15 PSI (pounds/inch²) pressure for 15 min. Antibiotics and other heat labile components used were filter sterilized using a syringe filtration unit (Tarsons, India) fitted with an autoclaved cellulose nitrate membrane filter of 0.45 µm pore size (Millipore, USA).

Plant growth procedure and conditions

The plastic pots were prepared by filling with soilrite or agropeat mixed with vermiculite till the top and watered enough so that all the soil in the pot will become wet after sometime by capillary uptake of water. In each pot, six day old seedlings either single or in multiple number were transferred and covered with plastic tray (trays were removed after ten days) and then kept in long-day (16hr light/8hr dark) conditions. The plant's growth was monitored and watered regularly.

Observation of epidermal cells of cotyledon

For the observation of size and shape of epidermal cells, imprints of cotyledons surfaces were prepared using clear nail polish. Images of cotyledons and imprints were taken using a Nikon P-III camera (Nikon, Japan) mounted on a Nikon, ALPHAPHOT-2 YS2 microscope (Nikon, Japan).

Measurements of epidermal cell length and expansion

Measurements of epidermal cell length and expansion of six-day-old seedlings were essentially carried out using stage and ocular micrometers by micrometry technique.

Anthocyanin estimation

Constant white light or blue light (30 µmol m⁻²s⁻¹) grown ~50 seedlings were collected in a microcentrifuge tube, weighed and 300 µl of extraction solution (1% HCL in methanol) was added and kept at cold-dark condition overnight. Next day, seedlings were crushed after addition of 200 µl of sterile water. Finally chlorophyll was removed by adding 500 µl of chloroform and debris was removed by centrifugation and supernatant was collected into a fresh microcentrifuge tube. Then, spectrophotometric estimation was carried out by taking readings at the wavelengths of 530 nm and 657 nm. The total Anthocyanin content was calculated with the help of the following formula: $(A_{530} - 0.33A_{657})/g$ of fresh weight.

Isolation of RNA using RNeasy plant mini kit

100 mg of tissue was ground to a fine powder in liquid nitrogen with the help of mortar and pestle. Then the powdered tissue was transferred to fresh

microcentrifuge tube and suspended in 450 µl of RLT buffer for the lysis. The lysate was vortexed vigorously and incubated at 56 °C for 2-3 min. Then, lysate was loaded onto the QIAshredder spin column (lilac) with a collection tube at the bottom and centrifuged at 13000 rpm for 2 min. The supernatant in collection tube was transferred to fresh microcentrifuge tube and 0.5 volume of 100% ethanol was added. This mixture was then loaded onto the RNeasy mini column (pink) placed in a collection tube and centrifuged at 13000 rpm for 15 sec. Then 700 µl of RW1 buffer was added to the column and centrifuged at 13000 rpm for 15 sec. The column was transferred to a fresh collection tube and then washed twice with 500 µl of RPE buffer. The final washing was for 2 min at 13000 rpm. The RNA was eluted by adding 40 µl of sterile RNase free water directly onto the membrane of the column and centrifuged at 13000 rpm for 1min.

Gene expression analysis by Real-Time PCR

Total RNA was isolated using RNeasy plant mini kit (Qiagen) extraction kit according to manufacturer's protocol. cDNA was synthesized from 1 µg of total RNA using RevertAid H Minus First Strand cDNA synthesis Kit (Thermo Scientific). Real-time PCR was performed using 7500 fast real-time system (ABI). Values were normalized with the amplification of the *actin* as a constitutively expressed internal control.

Results

Functional MYC2 is required for GBF1-mediated regulation of cotyledon expansion

GBF1 promotes cotyledon expansion at lower fluences of WL and BL (Mallappa *et al.*, 2006). However, *atmyc2* mutants do not show any altered cotyledon size (Yadav *et al.*, 2005). We ask whether MYC2 is able to modulate GBF1-mediated regulation of cotyledon expansion. Measurement of cotyledon area in wild-type, *atmyc2*, *gbf1* and *atmyc2 gbf1* backgrounds showed that cotyledon area of *atmyc2 gbf1* double mutants was similar to wild-type or *atmyc2* mutants (Figure 1A and B), suggesting that functional MYC2 is required for GBF1-mediated regulation of cotyledon expansion. We further examined the size of epidermal cells in wild type and various mutant backgrounds. Although *atmyc2* mutants did not show any altered cell size, *gbf1* mutants displayed less expanded epidermal cells (Figure 2A and B). As shown in Figure 2A and B, epidermal cells of *atmyc2 gbf1* double mutants were of similar size to wild type or *atmyc2* mutants. These results suggest that functional MYC2 is required for GBF1-mediated regulation of epidermal cell size.

MYC2 requires functional GBF1 to regulate adult phenotype

Earlier studies have shown that *atmyc2* adult plants display short stature at the time of flowering (Yadav *et al.*, 2005; Gangappa and Chattopadhyay, 2010). Therefore, we ask whether additional mutation in *GBF1* can modulate the altered adult phenotype of *atmyc2* mutants. As shown in Figure 3, visible adult phenotype of *atmyc2 gbf1* double mutant plants at the time of flowering under long day condition was similar to wild type, indicating that functional GBF1 is required for MYC2-mediated regulation of adult plant stature at the time of flowering.

MYC2 and GBF1 work in an antagonistic manner to regulate the expression of light inducible genes

To investigate the role of MYC2 and GBF1 in the regulation of light-induced gene expression, we determined the level of expression of light inducible genes such as *CAB1* and *RBCS-1A* (Huq and Quail, 2005). For this study, we used 6-day-old constant BL grown seedlings, and monitored the expression of *CAB1* and *RBCS-1A* by quantitative real-time PCR (qPCR). Earlier studies have shown that expression of *CAB1* is negatively regulated by MYC2, whereas GBF1 positively regulates *CAB1* expression in BL (Yadav *et al.*, 2005 and Mallappa *et al.*, 2006). As shown in Figure 4A, *CAB1* expression was found to be similar to wild type in *atmyc2 gbf1* double mutants. MYC2 and GBF1 negatively regulate *RBCS-1A* expression in BL (Yadav *et al.*, 2005 and Mallappa *et al.*, 2006). The expression of *RBCS-1A* in *atmyc2 gbf1* double mutant was also found to be similar to wild-type seedlings (Figure 4B). These results, taken together, suggest that MYC2 and GBF1 work in an antagonistic manner in the regulation of *CAB1* and *RBCS-1A* expression.

MYC2 and GBF1 coordinately regulate the expression of light signaling pathway genes

To investigate the role of MYC2 and GBF1 in the regulation of light signaling components involved in BL-mediated photomorphogenesis, we examined the expression of several light signaling pathway genes such as *SHW1* and *SPA1* in 6-day-old BL grown wild-type, *atmyc2*, *gbf1* and *atmyc2 gbf1* mutant seedlings by quantitative real-time PCR (Hoecker *et al.*, 1998; Bhatia *et al.*, 2008). Whereas *SHW1* expression was not altered in *gbf1* mutants as compared to wild type, the level of expression of *SHW1* was reduced in the *atmyc2* mutant background. The expression level of *SHW1* was similar to *atmyc2* mutant in *atmyc2 gbf1* double mutant (Figure 5A). These results indicate that whereas GBF1 does not have any role on the regulation

of *SHW1* expression, MYC2 positively regulates the expression of *SHW1*. While examined the transcript levels of *SPA1*, the accumulation of *SPA1* transcript was about 2.5-fold higher in *atmyc2* mutant, however its level was not altered in *gbf1* mutant as compared to wild type background. Further, the expression of *SPA1* in *atmyc2 gbf1* double mutant background was similar to wild type (Figure 5B). These results suggest that although GBF1 does not have any effect on the regulation of *SPA1* expression, MYC2-mediated negative regulation of *SPA1* requires functional GBF1. Taken together, these results demonstrate that MYC2 and GBF1 work in independent and interdependent manner to regulate the expression of key regulators of light signaling pathway genes such as *SHW1* and *SPA1*.

Genetic interactions of MYC2 and GBF1 modulate the anthocyanin accumulation during seedling development

To determine the effect of genetic interactions of *MYC2* with *GBF1* on anthocyanin accumulation, we examined the anthocyanin levels in wild-type, *atmyc2*, *gbf1* and *atmyc2 gbf1* backgrounds in WL and BL conditions. It has been shown that MYC2 negatively regulates the accumulation of anthocyanin, whereas GBF1 show no detectable effect on anthocyanin level in BL (Yadav *et al.*, 2005; Mallappa *et al.*, 2006, Mallappa *et al.*, 2008). No significant difference was observed in anthocyanin accumulation in *atmyc2 gbf1* double mutants as compared to single mutants in WL (Figure 6A). On the other hand, the level of anthocyanin accumulation was further increased in *atmyc2 gbf1* double mutants as compared to *atmyc2* single mutants in BL (Figure 6B). Thus, MYC2 and GBF1 appears to work synergistically to regulate anthocyanin accumulation in BL.

Discussion

The interplay of positive and negative regulators in light signaling pathways has important roles in controlling the photomorphogenic growth and light regulated gene expression. MYC2/ZBF1 (bHLH protein) and GBF1/ZBF2 (bZIP protein) belong to two different classes of transcription factors that work downstream to cry1 and cry2 photoreceptors and work as negative regulators of BL-mediated inhibition of hypocotyl elongation. However, GBF1/ZBF2 works as a positive regulator for the cotyledon expansion (Yadav *et al.*, 2005; Mallappa *et al.*, 2006). One interesting observation from this study is that, although GBF1 functions as a positive regulator of cotyledon expansion, an additional mutation in *MYC2* in the *gbf1* background suppresses the small-cotyledon phenotype of *gbf1* (Figures 1 and 2). Similar results were

observed for the adult phenotype of *myc2* mutants, where the additional mutation of *GBF1* completely rescued the small adult phenotype (Figure 3). Together, these results demonstrate that *GBF1* and *MYC2* are functionally interconnected and jointly regulate multiple developmental aspects of *Arabidopsis* growth.

MYC2 and *GBF1* are antagonistic to each other for the regulation of expression of light inducible genes such as *CAB1* and *RBCS-1A* (Figure 4A and B). Earlier studies demonstrated that both *MYC2* and *GBF1* bind to the promoters of *CAB1* and *RBCS-1A* and regulate the expression of these genes (Yadav *et al.*, 2005, Mallappa *et al.*, 2006 and Singh *et al.*, 2012). The expression level of these genes in *atmyc2 gbf1* double mutant is found to be similar to wild type. Thus one likely scenario at the molecular level might be that *MYC2* and *GBF1* inhibit each other functionally. One of the possibilities to perform such inhibitory function at the molecular level on each other might be through physical interaction and thereby not allowing each other to interact with the respective target promoters (Maurya *et al.*, 2015). Moreover, the heterodimerization of these two proteins increases the flexibility of regulation, either positive or negative manner, of gene expression. The regulation of anthocyanin accumulation by *MYC2* and *GBF1* is synergistic under blue-light conditions (Figure 6), suggesting a wavelength and context-specific developmental regulation mediated by these two proteins.

Conclusion

MYC2 and *GBF1* are two transcription factors that work downstream to *cry1* and *cry2* photoreceptors and work as negative regulators of BL-mediated inhibition of hypocotyl elongation. Although *MYC2* does not display any role in cotyledon expansion, *GBF1* plays a positive regulatory role in cotyledon expansion. This study demonstrates the functional interrelation of *MYC2* and *GBF1*, which belong to two different classes of transcription factors (bHLH and bZIP), in different developmental aspects at different stages and light-mediated gene expressions. The results obtained from this study show that the cotyledon area and epidermal cell-size of *atmyc2 gbf1* double mutants were similar to wild type or *atmyc2* mutants, suggesting that functional *MYC2* is required for *GBF1*-mediated regulation of cotyledon expansion and epidermal cell-size in BL. While determining the physiological responses of wild type and other mutants, we have found that *MYC2* and *GBF1* work synergistically during anthocyanin accumulation in BL. During the examination of the expression of light inducible genes in BL, we found the antagonistic role of *MYC2* and *GBF1* in the regulation of *CAB1* and *RBCS-1A* expression in BL. Gene expression analysis show that both *MYC2* and *GBF1* work in interdependent and independent manner to regulate the expression of light-signaling pathways genes *SPA1* and *SHW1*, respectively.

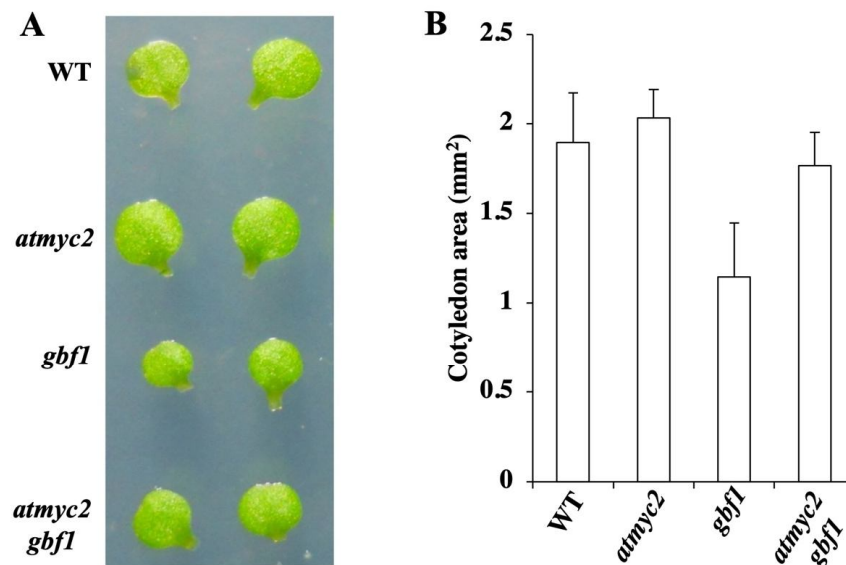


Figure 1. Role of MYC2 and GBF1 in the regulation of cotyledon size.

(A), Visible phenotype of cotyledons of 6-day old wild-type (Col), *atmyc2*, *gbf1* and *atmyc2 gbf1* mutant seedlings grown in constant BL (30 $\mu\text{mol m}^{-2}\text{s}^{-1}$). (B), Quantifications of cotyledon area. Error bars represent S.D. $n=3$ independent experiments with similar results.

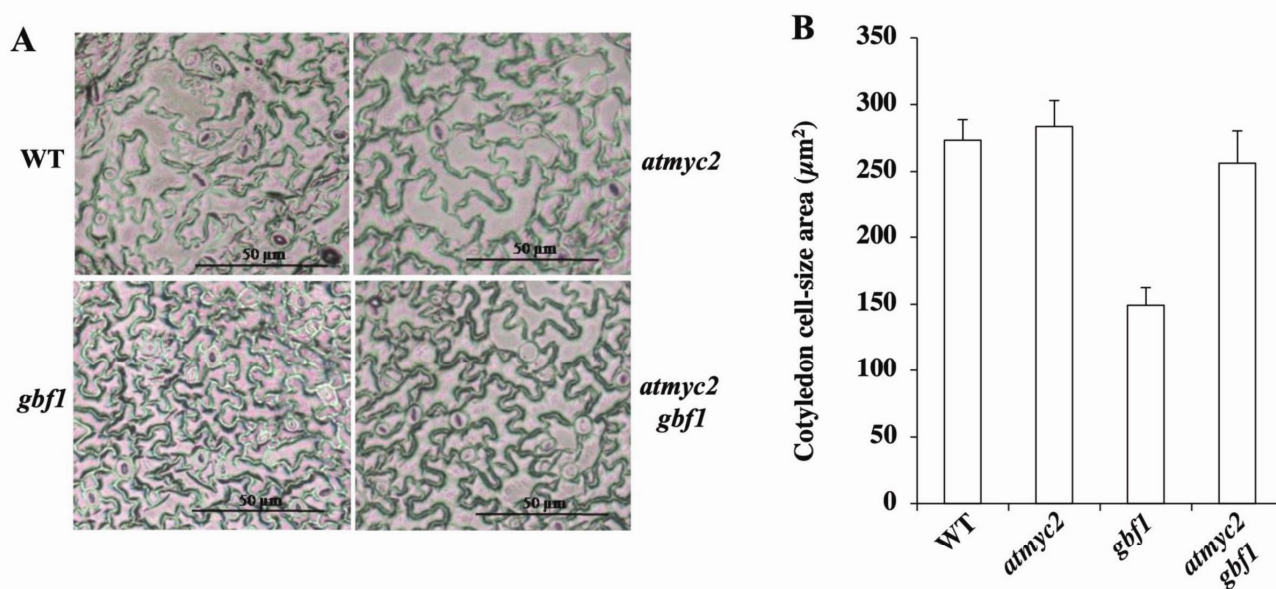


Figure 2. Role of MYC2 and GBF1 in the regulation of cotyledon cell size. (A), Epidermal imprints of cotyledons of 6-day old BL grown seedlings ($30 \mu\text{mol m}^{-2}\text{s}^{-1}$). (B), Quantification of epidermal cell size. Error bars represent S.D. $n=3$ independent experiments with similar results.



Figure 3. Adult phenotype of wild-type and different mutant lines.

(A-D), are visible adult phenotypes of wild-type (Col), *atmyc2*, *gbfl* and *atmyc2 gbfl* mutants plants, respectively, grown under long-day conditions (16 hr light/8 hr dark) in white light ($100 \mu\text{mol m}^{-2}\text{s}^{-1}$) and photographs were taken at the time of flowering.

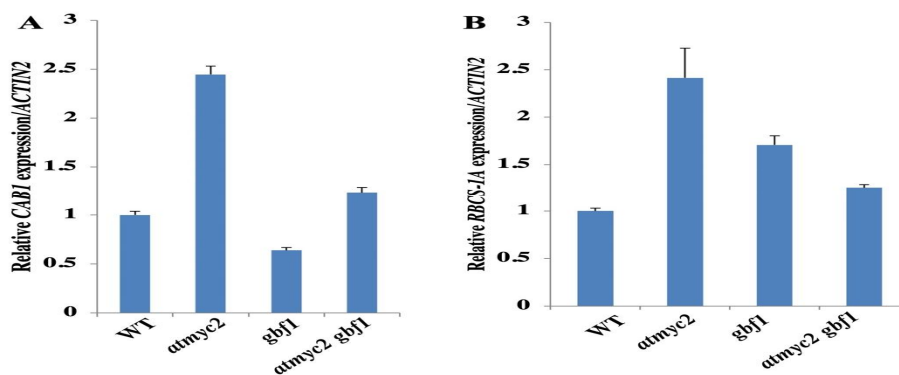


Figure 4. Expression of light inducible genes in wild-type and different mutant backgrounds under blue light.

(A and B), Real-time PCR analysis of *CAB1* and *RBCS-1A* transcript levels, respectively, from 6-day-old constant BL ($30 \mu\text{mol m}^{-2}\text{sec}^{-1}$) grown wild type (WT) and different mutant seedlings. *Actin 2* was used as control. Error bars represent S.D. $n=3$ independent experiments with similar results.

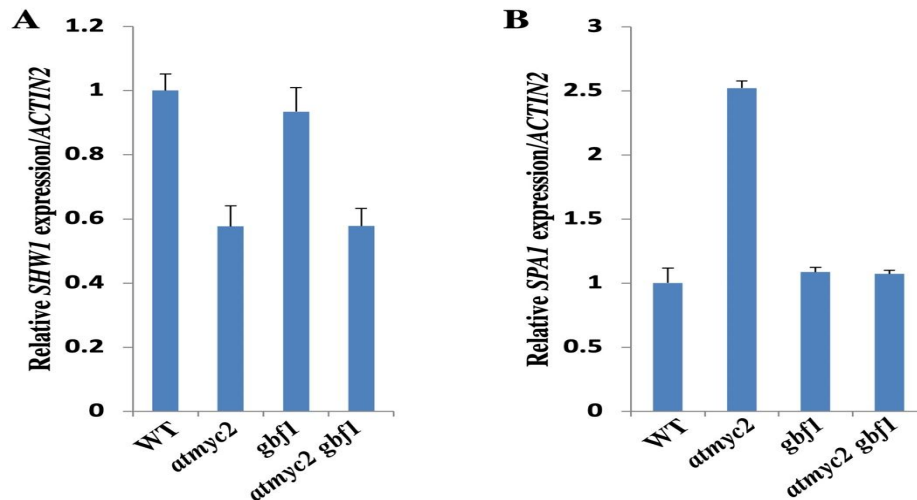


Figure 5. Expression of light signaling pathway genes in wild-type and different mutant backgrounds in blue light.

(A and B), Real-time PCR analysis of *SHWI* and *SPA1* transcript levels, respectively, from 6-day-old constant BL ($30 \mu\text{mol m}^{-2} \text{sec}^{-1}$) grown wild-type (WT) and different mutant seedlings. *Actin2* was used as control. S.D. $n=3$ independent experiments with similar results.

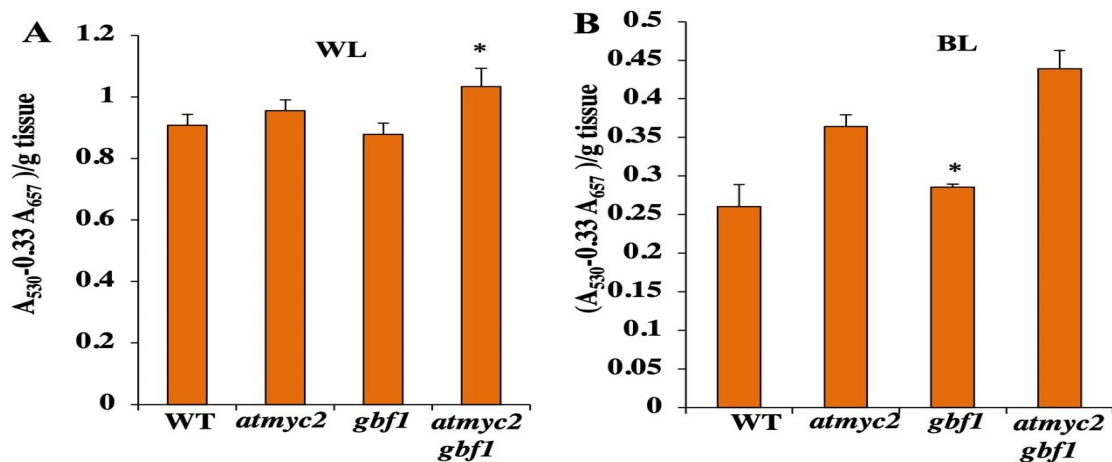


Figure 6. Anthocyanin accumulation in *atmyc2 gbf1* double mutants.

(A and B), Accumulation of anthocyanin in 6-day-old wild-type and various mutant seedlings grown in constant WL ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) and BL ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$). In figures A and C, asterisk (*) indicates no difference from wild-type and single mutants; in figure D, asterisk (*) indicates no difference from wild-type but significant difference from *atmyc2* and *atmyc2 gbf1* mutants ($P < 0.05$, Student's t-test). Error bars represent S.D. $n=3$ independent experiments with similar results.

Table 1 : Primers used for RT-PCR and Real-Time PCR studies

SN	Gene	Primer Sequence (5'-3')
1	<i>Actin 2</i>	FP 5'-AAAGGCTTAAAAAGCTGGGG-3'
		RP 5'-GGGACTAAAACGCAAACGA-3'
2	<i>CABI</i>	FP 5'-GAGGAAGACTGTTGCCAAGC-3'
		RP 5'-CCCACCTGCTGTGGATAACTTC-3'
3	<i>RBCS-1A</i>	FP 5'-ACCTTATCCGCAACAAGTGG-3'
		RP 5'-TGGGGTACTCCTTCTTGAC-3'
4	<i>SPA1</i>	FP 5'-GCCATTGCGAGTCAGGCG-3'
		RP 5'-GCTGATGGTCGGGAGGAGGG-3'
5	<i>SHWI</i>	FP 5'-CGCGACGGATTTTGCCAGC-3'
		RP 5'-CGAAGCTGCCGCCAGAAC-3'

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Author contributions

VG and JPM carried out the experiments. VG, DK, and JPM contributed to data analysis and

manuscript preparation. JPM conceptualized the study and guided the overall research.

Declaration of interests

The authors declare no conflict of interest.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to make grammatical corrections only.

References

- Abbas, N., Maurya, J. P., Senapati, D., Gangappa, S. N. and Chattopadhyay, S. (2014). Arabidopsis CAM7 and HY5 physically interact and directly bind to the HY5 promoter to regulate its expression and thereby promote photomorphogenesis. *Plant Cell*, **26**, 1036–1052.
- Ang, L. H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A. and Deng, X. W. (1998). Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. *Molecular Cell*, **1**, 213–222.
- Arguello-Astorga, G. and Herrera-Estrella, L. (1998). Evolution of light regulated plant promoters. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 525–555.
- Batschauer, A., Rocholl, M., Kaiser, T., Nagatani, A., Furuya, M. and Schäfer, E. (1996). Blue and UV-A light-regulated CHS expression in Arabidopsis independent of phytochrome A and phytochrome B. *Plant Journal*, **9**, 63–69.
- Bhatia, S., Gangappa, S. N., Kushwaha, R., Kundu, S. and Chattopadhyay, S. (2008). SHORT HYPOCOTYL IN WHITE LIGHT1, a serine-arginine-aspartate-rich protein in Arabidopsis, acts as a negative regulator of photomorphogenic growth. *Plant Physiology*, **147**, 169–178.
- Boter, M., Rivero, O. R., Abdeen, A. and Prat, S. (2004). Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis. *Genes & Development*, **18**, 1577–1591.
- Briggs, W. R. and Olney, M. A. (2001). Photoreceptors in plant photomorphogenesis to date: Five phytochromes, two cryptochromes, one phototropin, and one superchrome. *Plant Physiology*, **125**, 85–88.
- Chakraborty, M., Gangappa, S. N., Maurya, J. P., et al. (2019). Functional interrelation of MYC2 and HY5 plays an important role in Arabidopsis seedling development. *Plant Journal*, **99**(6), 1080–1097.
- Chattopadhyay, S., Ang, L. H., Puente, P., Deng, X. W. and Wei, N. (1998b). Arabidopsis bZIP protein HY5 directly interacts with light responsive promoters in mediating light control of gene expression. *Plant Cell*, **10**, 673–683.
- Chattopadhyay, S., Puente, P., Deng, X. W. and Wei, N. (1998a). Combinatorial interaction of light-responsive elements plays a critical role in determining the response characteristics of light-regulated promoters in Arabidopsis. *Plant Journal*, **15**, 69–77.
- Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P. M., Manners, J. M. and Kazan, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. *Plant Cell*, **19**, 2225–2245.
- Donald, R. G. K. and Cashmore, A. R. (1990). Mutation of either G box or I box sequences profoundly affects expression from the Arabidopsis rbcS-1A promoter. *EMBO Journal*, **9**, 1717–1726.
- Duek, P. D. and Fankhauser, C. (2003). HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signaling. *Plant Journal*, **34**, 827–836.
- Fairchild, C. D., Schumaker, M. A. and Quail, P. H. (2000). HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes & Development*, **14**, 2377–2391.
- Gangappa, S. N. and Chattopadhyay, S. (2010). MYC2, a bHLH transcription factor, modulates the adult phenotype of SPA1. *Plant Signaling & Behavior*, **5**(12), 1650–1652.
- Gangappa, S. N., Maurya, J. P., Yadav, V. and Chattopadhyay, S. (2013a). The regulation of Z- and G-box containing promoters by light signaling components, SPA1 and MYC2, in Arabidopsis. *PLoS ONE*, **8**, e62194.
- Gangappa, S. N., Prasad, V. B. and Chattopadhyay, S. (2010a). Functional interconnection of MYC2 and SPA1 in the photomorphogenic seedling development of Arabidopsis. *Plant Physiology*, **154**, 1210–1219.
- Gangappa, S. N., Srivastava, A. K., Maurya, J. P., Ram, H. and Chattopadhyay, S. (2013b). Z-box binding transcription factors (ZBFs): A new class of transcription factors in Arabidopsis seedling development. *Molecular Plant*, **6**, 1758–1768.
- Ha, S. B. and An, G. (1988). Identification of upstream regulatory elements involved in the developmental expression of the Arabidopsis thaliana cab1 gene. *Proceedings of the National Academy of Sciences*, **85**, 8017–8021.
- Hoecker, U., Xu, Y. and Quail, P. H. (1998). SPA1: A new genetic locus involved in phytochrome A-specific signal transduction. *Plant Cell*, **10**, 19–33.
- Holm, M., Ma, L. G., Qu, L. J. and Deng, X. W. (2002). Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in Arabidopsis. *Genes & Development*, **16**, 1247–1259.
- Hong, G. J., Xue, X. Y., Mao, Y. B., Wang, L. J. and Chen, X. Y. (2012). Arabidopsis MYC2 interacts with DELLA proteins in regulating sesquiterpene synthase gene expression. *Plant Cell*, **24**, 2635–2648.
- Huq, E. and Quail, P. H. (2005). Phytochrome signaling. In W. R. Briggs & J. L. Spudis (Eds.), *Handbook of photosensory receptors* (pp. 151–170). Wiley-VCH.
- Jang, I. C., Yang, J. Y., Seo, H. S. and Chua, N. H. (2005). HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. *Genes & Development*, **19**, 593–602.
- Jiao, Y., Lau, O. S. and Deng, X. W. (2007). Light-regulated transcriptional networks in higher plants. *Nature Reviews Genetics*, **8**, 217–230.
- Kushwaha, R., Singh, A. and Chattopadhyay, S. (2008). Calmodulin7 plays an important role as transcriptional regulator in Arabidopsis seedling development. *Plant Cell*, **20**, 1747–1759.
- Laubinger, S., Fittinghoff, K. and Hoecker, U. (2004). The SPA quartet: A family of WD-repeat proteins with a central role in suppression of photomorphogenesis in Arabidopsis. *Plant Cell*, **16**(9), 2293–2306.

- Laubinger, S. and Hoecker, U. (2003). The SPA1-like proteins SPA3 and SPA4 repress photomorphogenesis in the light. *Plant Journal*, **35**, 373–385.
- Li, J., Li, G., Wang, H. and Deng, X. W. (2011). Phytochrome signaling mechanisms. *The Arabidopsis Book*, **2011**, e0148.
- Lin, C. (2002). Blue light receptors and signal transduction. *Plant Cell*, **14**, S207–S225.
- Liu, H., Liu, B., Zhao, C., Pepper, M. and Lin, C. (2011). The action mechanisms of plant cryptochromes. *Trends in Plant Science*, **16**, 1360–1385.
- Mallappa, C., Yadav, V., Negi, P. and Chattopadhyay, S. (2006). A basic leucine zipper transcription factor, G-box-binding factor 1, regulates blue light-mediated photomorphogenic growth in *Arabidopsis*. *Journal of Biological Chemistry*, **281**, 22190–22199.
- Maurya, J. P., Sethi, V., Gangappa, S. N., Gupta, N. and Chattopadhyay, S. (2015). Interaction of MYC2 and GBF1 results in functional antagonism in blue light-mediated *Arabidopsis* seedling development. *Plant Journal*, **83**(3), 439–450.
- Neff, M. M., Fankhauser, C. and Chory, J. (2000). Light: An indicator of time and place. *Genes & Development*, **14**, 257–271.
- Oyama, T., Shimura, Y. and Okada, K. (1997). The *Arabidopsis* HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes & Development*, **11**, 2983–2995.
- Puente, P., Wei, N. and Deng, X. W. (1996). Combinational interplay of promoter elements constitutes the minimal determinants for light and developmental control of gene expression in *Arabidopsis*. *EMBO Journal*, **15**, 3732–3743.
- Ram, H., Priya, P., Jain, M. and Chattopadhyay, S. (2013). Genome-wide DNA binding of GBF1 is modulated by its heterodimerizing protein partners, HY5 and HYH. *Molecular Plant*, **6**, Article sst143. <https://doi.org/10.1093/mp/sst143>
- Saijo, Y., Sullivan, J. A., Wang, H., Yang, J., Shen, Y., Rubio, V., Ma, L., Hoecker, U. and Deng, X. W. (2003). The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. *Genes & Development*, **17**, 2642–2647.
- Schindler, U., Menkens, A. E., Beckmann, H., Ecker, J. R. and Cashmore, A. R. (1992). Heterodimerization between light-regulated and ubiquitously expressed *Arabidopsis* GBF bZIP proteins. *EMBO Journal*, **11**, 1261–1273.
- Seo, H. S., Yang, J. Y., Ishikawa, M., Bolle, C., Ballesteros, M. L. and Chua, N. H. (2003). LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature*, **423**, 995–999.
- Singh, A., Ram, H., Abbas, N. and Chattopadhyay, S. (2012). Molecular interactions of GBF1 with HY5 and HYH proteins during light-mediated seedling development in *Arabidopsis thaliana*. *Journal of Biological Chemistry*, **287**, 25995–26009.
- Terzaghi, W. B. and Cashmore, A. R. (1995). Light-regulated transcription. *Annual Review of Plant Physiology and Plant Molecular Biology*, **46**, 445–474.
- Yadav, V., Kundu, S., Chattopadhyay, D., Negi, P., Wei, N., Deng, X. W. and Chattopadhyay, S. (2002). Light-regulated modulation of Z-box containing promoters by photoreceptors and downstream regulatory components, COP1 and HY5, in *Arabidopsis*. *Plant Journal*, **31**, 741–753.
- Yadav, V., Mallappa, C., Gangappa, S. N., Bhatia, S. and Chattopadhyay, S. (2005). A basic helix-loop-helix transcription factor in *Arabidopsis*, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. *Plant Cell*, **17**, 1953–1966.
- Yang, J., Lin, R., Sullivan, J., Hoecker, U., Liu, B., Xu, L., Deng, X. W. and Wang, H. (2005). Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in *Arabidopsis*. *Plant Cell*, **17**, 804–821.
- Zhu, D., Maier, A., Lee, J. H., Laubinger, S., Saijo, Y., Wang, H., Qu, L. J., Hoecker, U. and Deng, X. W. (2008). Biochemical characterization of *Arabidopsis* complexes containing Constitutively Photomorphogenic1 and Suppressor of Phytochrome proteins in light control of plant development. *Plant Cell*, **20**, 2307–2323.