SHORT HYPOCOTYL IN WHITE LIGHT1, a Serine-Arginine-Aspartate-Rich Protein in Arabidopsis, Acts as a Negative Regulator of Photomorphogenic Growth[1][W]

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Light is an important factor for plant growth and development. We have identified and functionally characterized a regulatory gene *SHORT HYPOCOTYL IN WHITE LIGHT1* (*SHW1*) involved in Arabidopsis (*Arabidopsis thaliana*) seedling development. *SHW1* encodes a unique serine-arginine-aspartate-rich protein, which is constitutively localized in the nucleus of hypocotyl cells. Transgenic analyses have revealed that the expression of *SHW1* is developmentally regulated and is closely associated with the photosynthetically active tissues. Genetic and molecular analyses suggest that SHW1 acts as a negative regulator of light-mediated inhibition of hypocotyl elongation, however, plays a positive regulatory role in light-regulated gene expression. The *shw1* mutants also display shorter hypocotyl in dark, and analyses of *shw1 cop1* double mutants reveal that SHW1 acts nonredundantly with COP1 to control hypocotyl elongation in the darkness. Taken together, this study provides evidences that SHW1 is a regulatory protein that is functionally interrelated to COP1 and plays dual but opposite regulatory roles in photomorphogenesis.

As photoautotrophs, plants are extremely sensitive to light environment (Huq and Quail, 2005; Jiao et al., 2007). Three distinct families of photoreceptors are known to perceive light: far-red light (FR) and red light (RL) by phytochromes (phyA to phyE), blue and UV-A light by cryptochromes (cry1 to cry3), and phototropins (phot1 and phot2). Recent studies have suggested that major activities of light signaling are concentrated in the nucleus (Chen et al., 2004; Huq and Quail, 2005). cry1 and cry2 are mostly in the nucleus, and recent studies suggest that cytoplasmic cry1 may have a distinct function other than the nuclear cry1 in photomorphogenic growth. Phytochromes are translocated into the nucleus upon light-mediated activation (Cashmore et al., 1999; Guo et al., 1999; Yamaguchi et al., 1999; Kircher et al., 2002; Lin, 2002; Nagy and Schafer, 2002; Schepens et al., 2004; Wu and Spalding, 2007).

Molecular characterization of genetically identified regulators has resulted in a dramatic progress in understanding the light signaling processes (Huq and Quail, 2005). Several regulatory proteins have been identified, which are involved in the transition of dark-grown skotomorphogenic growth to light-induced photomorphogenic growth (Chen et al., 2004; Jiao et al., 2007). The expression of about one-third of the total genes in Arabidopsis (*Arabidopsis thaliana*) genome is altered during the shift from skotomorphogenic to photomorphogenic growth (Ma et al., 2001; Tepperman et al., 2001). Photomorphogenesis is associated with several morphological and physiological alterations, which are otherwise suppressed during skotomorphogenic growth. These alterations include: inhibition of hypocotyl elongation, opening of apical hook, expansion of cotyledons, and accumulation of chlorophyll and anthocyanin (Whitelam et al., 1993; Neff et al., 2000; Wang and Deng, 2004).

COP1, a master repressor of photomorphogenesis, acts as an ubiquitin ligase and helps to degrade several photomorphogenesis promoting factors such as HY5, HYH, LAF1, and HFR1 in the dark (Ang et al., 1998; Wei and Deng, 1999; Osterlund et al., 2000; Holm et al., 2002; Seo et al., 2003; Yang et al., 2005). The *cop1* mutant seedlings show photomorphogenic growth in dark, hypersensitive responses to light and develop less lateral roots as compared to wild-type plants (McNellis et al., 1994; Wang and Deng, 2004). Recent studies have shown that COP1 interacts with SPA1, another repressor of photomorphogenesis, and SPA1 acts as an associated factor of COP1 in proteasome-mediated degradation of HY5, HFR1, and LAF1 (Hoecker et al., 1999; Saijo et al., 2003; Seo et al., 2003; Laubinger et al., 2004; Jang et al., 2005).

Although many genes showing light signaling functions have been reported in Arabidopsis, similar infor-

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mation is not available in crop plants. Recent studies have revealed that at least some orthologous genes in crop plants may have additional or altered functions that are not detectable in Arabidopsis and vice versa (Liu et al., 2004; Giliberto et al., 2005; Chatterjee et al., 2006). For example, *HP2* gene encodes the tomato (*Solanum lycopersicum*) homolog of *DET1* and dramatic variations between Arabidopsis *det1* and tomato *hp2* phenotypes have been observed. Most interestingly, *hp2* mutants do not display any strong visible phenotypes in darkness, however, *det1* mutants display strong deetiolated phenotype in the dark (Mustilli et al., 1999).

Guided by the above observations, we carried out subtractive hybridization and identified several regulatory genes from light-grown chickpea (*Cicer arietinum*) seedlings. In this study, we report the functional characterization of one such regulator, SHORT HYPOCOTYL IN WHITE LIGHT1 (SHW1), involved in Arabidopsis seedling development. *SHW1* encodes a Ser-Arg-Asp-rich protein that is localized in the nucleus both in dark- and light-grown Arabidopsis seedlings. Genetic and molecular analyses reveal that SHW1 acts as a negative regulator of light-mediated inhibition of hypocotyl elongation and is functionally interrelated to COP1, a master repressor of photomorphogenesis.

RESULTS

Identification and Molecular Cloning of *SHW1*

Recent studies have revealed that some of the well-characterized light signaling genes in Arabidopsis have additional or altered functions in crop plants (Mustilli et al., 1999; Chatterjee et al., 2006; Giovannoni, 2007). Intrigued by such observations, we reasoned that there might be some genes in crop plants that are otherwise difficult to identify in Arabidopsis through functional approaches. We therefore performed subtractive complementary DNA (cDNA) hybridizations to identify genes that are either up- or down-regulated in 5-d-old light-grown chickpea seedlings. We have identified several regulatory genes from chickpea, and some of these genes' functions are still unknown in Arabidopsis. Here, we report the identification and functional characterization of one such gene, *SHW1*, in Arabidopsis that encodes a Ser-Arg-Asp-rich protein with a consensus nuclear localization signal (Fig. 1A). *SHW1* (At1g69935) consists of three introns and four exons (Fig. 1C) and appears to be a unique gene in Arabidopsis genome. To determine the effect of light on the expression of *SHW1* during early seedling development in Arabidopsis, we used 5-d-old constant dark- or various light-grown seedlings for RNA gel-blot analysis. As shown in Figure 1B, although the expression of *SHW1* was barely detectable in dark and in various wavelengths of light such as RL, FR, and blue light (BL), it was highly expressed in white light (WL).

SHW1 Is Predominantly Expressed in WL and Its Expression Is Developmentally Regulated

To examine the tissue-specific expression pattern of *SHW1*, Arabidopsis transgenic lines containing 1-kb upstream sequence of *SHW1* fused to the *GUS* reporter gene (*SHW1* promoter-*GUS*) were generated. The promoter reporter construct was introduced into ecotype Columbia of Arabidopsis (Col-0) plants by stable transformation and several homozygous transgenic lines were generated. A representative transgenic line (as revealed by the GUS stain) was selected for further studies.

To further substantiate the northern-blot results in Figure 1B, we monitored the light inducibility of the *SHW1* promoter in various light conditions. For this experiment, 4-d-old dark-grown seedlings were shifted

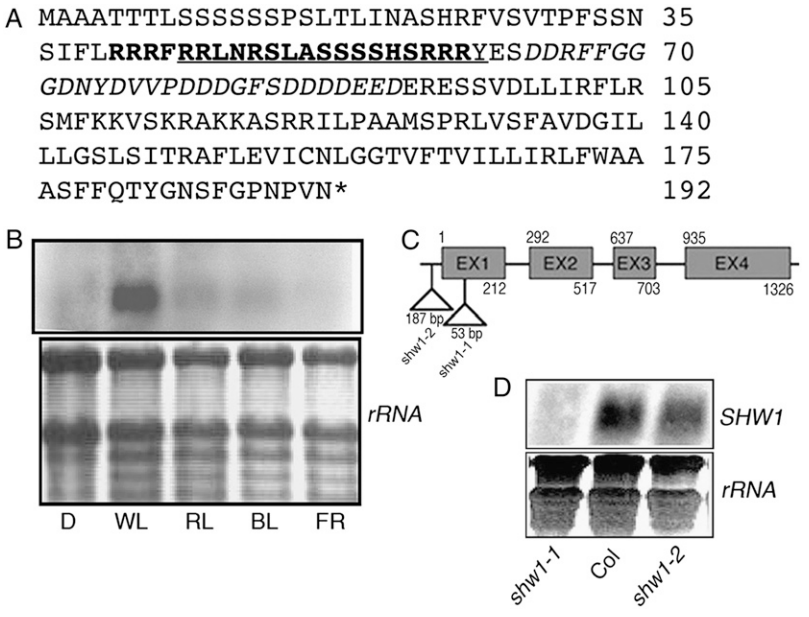

Figure 1. *SHW1* codes for a Ser-Arg-Asp-rich protein. A, The amino acid sequence of SHW1. The Ser-rich region includes 9 to 57 amino acids. Arg-rich region includes 40 to 60 amino acids (bold letters). The NLS region includes 44 to 61 amino acids (underlined letters). The Asp-rich region includes 64 to 91 amino acids (letters in italics). The star indicates the stop codon. B, The light-regulated expression of *SHW1*. Five-day-old seedlings were grown in constant dark (D) or in various light conditions (WL, RL, BL, and FR) for RNA gel-blot analyses. Total RNA (20 μg) was loaded in each lane; rRNA is shown as the loading control. C, The schematic diagram of the T-DNA insertion sites in *SHW1*. The inverted triangles show the T-DNA insertion sites. The exons (EX) are shown in gray boxes. The numbers indicate the starting and ending nucleotides of each exon. D, Northern blot of 20 μg of total RNA isolated from 5-d-old WL-grown wild type (Col), *shw1-1*, and *shw1-2* mutant seedlings; a 0.6-kb *SHW1* DNA fragment was used as the probe. rRNA is shown as the loading control.

to WL, specific wavelength or a combination of two wavelengths of light, and GUS activities were measured. The activity of the *SHW1* promoter was strongly induced in WL and found to be about 3-fold higher after exposure to 48 h (Supplemental Fig. S1A). The promoter was also slightly induced in FR (about 1.5-fold), however, no induction *SHW1* promoter was detected in RL or BL (Supplemental Fig. S1, B–D). On the other hand, the *SHW1* promoter was induced to about 1.5- to 2-fold when exposed to a combination of two wavelengths of light (Supplemental Fig. S1E). Taken together, these results suggest that the *SHW1* promoter is predominantly active in WL and in combination of at least two wavelengths of light.

To determine the tissue-specific expression, we stained the seedlings each day from the second day of germination in WL. As shown in Figure 2, A–D, the *SHW1* promoter-*GUS* transgene was expressed in hypocotyl and cotyledons up to the fifth day after germination. Afterward, the expression of the transgene in hypocotyl gradually decreased and became undetectable in 8-d-old seedlings (Fig. 2, E–G). Furthermore, expression of the transgene in the cotyledons started becoming patchy from 9-d-old seedlings (Fig. 2, H and I). The activity of the *SHW1* promoter was not detected in the roots at any stage of development (Fig. 2, A–I).

We then examined the expression of *SHW1* in various tissues of adult plants. The expression of *SHW1* promoter-*GUS* was detected in green tissues, including leaf, stem, sepal, and young siliques. Interestingly, as the siliques matured and accordingly the greenness of the organ reduced, the level of activity of the *SHW1* promoter also gradually decreased (Fig. 2, M–O). Most strikingly, expression of the *SHW1* promoter-*GUS* transgene was found to be patchy in older leaves and siliques with the expression confined to the green tissues (Fig. 2J, N, and O). Furthermore, the residual expression of the transgene was detected in a few seeds that were still partly green in an otherwise dried silique (Fig. 2O). Although the reason for the patchy expression pattern of the *SHW1* promoter-*GUS* transgene is presently unclear, collectively these data suggest that the expression of *SHW1* is developmentally regulated and more prominent in photosynthetically active tissues.

Isolation and Characterization of Mutations in *SHW1*

To determine the in vivo function of *SHW1*, we searched for mutants in T-DNA knockout collections (Alonso et al., 2003). Two independent mutant lines with T-DNA insertion at the first exon of *SHW1* or at the upstream promoter region of *SHW1* were identified and the corresponding alleles were designated as *shw1-1* and *shw1-2*, respectively (Fig. 1C). We carried out PCR genotyping analyses to determine plants that are homozygous or heterozygous for *shw1-1 or shw1-2* mutations. The segregation of self-fertilized plants

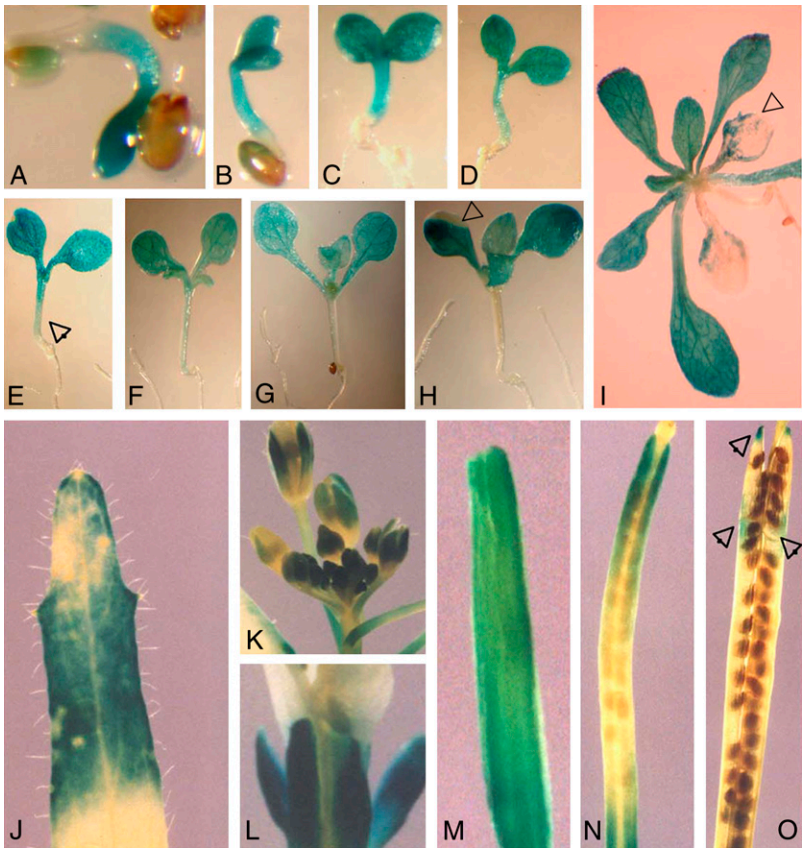

Figure 2. Tissue-specific expression of *SHW1*. All seedlings or adult plants carrying *SHW1* promoter-*GUS* transgene were grown in 14-h WL (30 μmol m^{-2} s^{-1}) and 10-h dark cycles and used for GUS staining using 20 to 30 seedlings in each sample (Yadav et al., 2002). A to H, Seedlings of 2- to 9-d-old, respectively. The arrowhead indicates partly stained hypocotyl (E) and cotyledon (H). I, Seedlings of 15-d-old. The arrowhead indicates partly stained cotyledons. J, Leaf of 30-d-old plant. K and L, Inflorescence and flowers buds of 30-d-old plant. M to O, Green, partly dried and mostly dried siliques, respectively. The arrowheads indicate the residual GUS stain.

heterozygous for *shw1-1* or *shw1-2* was monitored. The segregation ratios determined by the analyses of genotyping PCR in T2 progeny suggested that a single T-DNA locus was present in the *shw1-1* or *shw1-2* mutant line. To further test these results, we performed backcrosses of *shw1* mutant plants with the corresponding wild type (Col-0); and analyses of the segregation ratios in the F2 population confirmed a single genetic locus insertion of T-DNA in *shw1* mutant plants. The T-DNA was tightly linked to the mutation, and the drug resistance marker (kanamycin) of T-DNA segregated with the *shw1* mutation. The junctions of T-DNA and *SHW1* were amplified by PCR and the analysis of DNA sequences revealed that the T-DNA was inserted at nucleotide position 53 of exon1 in *shw1-1* or at nucleotide position 187 upstream to start codon in *shw1-2* (Fig. 1C). Whereas RNA gel-blot analyses could not detect any transcript encoded by *SHW1* in the *shw1-1* mutant, the level of transcript was significantly reduced in the *shw1-2* mutant background (Fig. 1D).

shw1 Mutants Exhibit Morphological Defects Both in Dark and WL

To determine whether *shw1* mutants have any morphological defect, we examined the growth of 6-d-old *shw1* and wild-type seedlings grown in constant dark or WL. The dark-grown *shw1* mutants displayed significantly ($P < 0.01$) shorter hypocotyl with drastic reduction in apical hook curvature compared to wild-type seedlings (Fig. 3A, K, and Q). In WL, the *shw1* mutants exhibited enhanced inhibition in hypocotyl elongation as compared to corresponding wild-type seedlings (Fig. 3, B–E, I, and J). Measurements of hypocotyl length revealed that 6-d-old WL grown *shw1* mutant seedlings had significantly shorter hypocotyl ($P < 0.01$) than wild-type seedlings (Fig. 3, M and L), and the enhanced inhibition of hypocotyl elongation in *shw1* mutants was more prominent at lower fluence rates of WL (Fig. 3, B–E, and M). To determine whether the *shw1* mutants have similar altered morphology in a particular wavelength of light, we examined the growth of 6-d-old seedlings under various wavelengths of light such as RL, FR, and BL (Fig. 3, F–H). However, no significant reduction in hypocotyl length was observed in any of these light conditions at various fluence rates tested (Fig. 3, N–P). Taken together, these results demonstrate that SHW1 acts as a negative regulator of photomorphogenic growth in dark and at lower fluence rates of WL irradiation. These results further demonstrate that the negative regulatory role of SHW1 is not specific to a particular wavelength of light.

We then asked whether the *shw1* mutants have any altered morphology in the later stage of development. Examination of growth of adult plants and the flowering time revealed that *shw1* mutation caused delayed flowering under long-day conditions (16-h light and 8-h darkness). Whereas long-day-grown wild-type plants started flowering after the formation of about eight rosette leaves, the *shw1* mutants flowered after 12 rosette leaves formed (Fig. 4, A and C). However, this effect was not observed in short-day-grown plants (8-h light and 16-h darkness). Examination of root growth of *shw1* mutant plants revealed that 16-d-old mutant plants developed significantly fewer lateral roots as compared to wild-type plants (Fig. 4, B and D). Taken together, these results suggest that although SHW1 acts as a negative regulator of photomorphogenic growth, it positively regulates flowering time and lateral root formation.

Mutation in *SHW1* Results in Reduced Chlorophyll Accumulation and Expression of Light-Regulated Genes

Accumulation of chlorophyll and anthocyanin are two physiological responses controlled by light signaling. To determine the role of SHW1 in chlorophyll and anthocyanin accumulation, we quantified chlorophyll and anthocyanin contents in *shw1* mutant seedlings and compared with the corresponding wild-type background. As shown in Figure 5, A and E, the accumulation of chlorophyll was significantly reduced in *shw1* mutants as compared to wild-type background, suggesting that SHW1 is required for the optimum level of chlorophyll accumulation. The level of anthocyanin remained similar to wild-type background in WL, however, there was a significant increase in the anthocyanin level in *shw1* mutants in dark (Figs. 5B and 7G).

We carried out RNA gel-blot analyses to determine the possible role of SHW1 in the regulation of light-inducible gene expression. For this experiment, 5-d-old constant dark-grown seedlings were transferred to WL for 2, 4, and 8 h and the transcript levels of *CAB* and *RBCS* were measured. As shown in Figure 5, C–D, the level of light-mediated induction of *CAB* and *RBCS* expression was significantly reduced in *shw1* mutants as compared to wild-type background. Taken together, these results indicate that SHW1 plays a positive regulatory role for WL-mediated induction of *CAB* and *RBCS* gene expression.

SHW1 Is Constitutively Localized in the Nucleus

The light-dependent shuttling of regulatory proteins between cytosol and nucleus has been shown to be crucial in controlling photomorphogenic growth in Arabidopsis (Jiao et al., 2007). SHW1 has a consensus nuclear localization signal (Fig. 1A). To determine the subcellular localization of SHW1, and also to investigate whether subcellular localization of SHW1 is light dependent, we generated Arabidopsis transgenic lines containing *SHW1-GUS* transgene expressed under a 1-kb upstream DNA fragment of *SHW1*. To test the functionality of SHW1-GUS fusion protein, we introduced *SHW1-GUS* transgene into the *shw1* mutant background by genetic crosses. The measurement of hypocotyl length revealed that the mutant transgenic

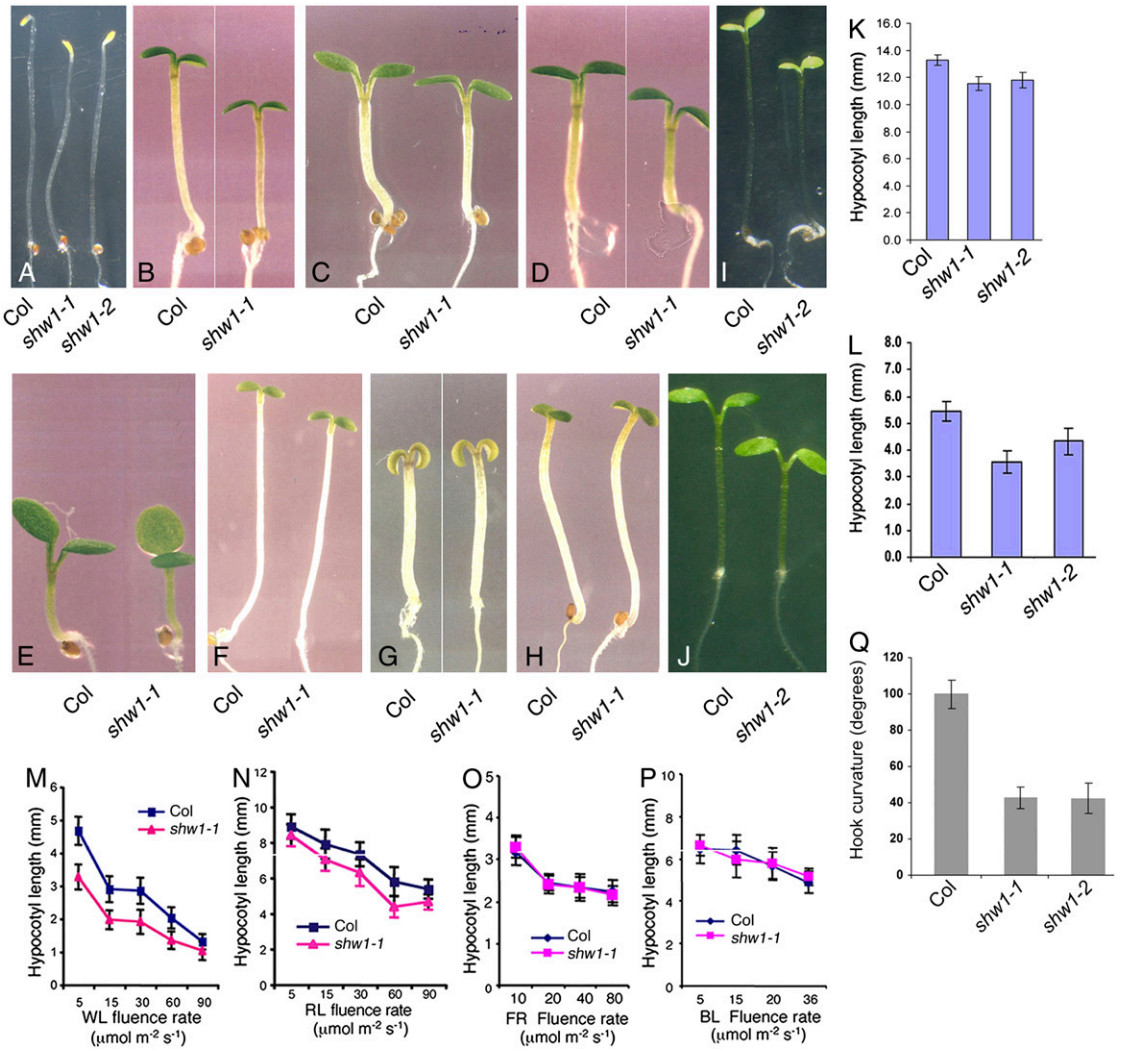

Figure 3. The *shw1* mutants show short hypocotyl phenotype. A, Six-day-old constant dark-grown seedlings. Wild-type (Col), *shw1-1*, and *shw1-2* seedlings are shown from left to right, respectively. B, Six-day-old constant WL (5 μmol m^{-2} s^{-1}) grown seedlings. C, Six-day-old constant WL (15 μmol m^{-2} s^{-1}) grown seedlings. D, Six-day-old constant WL (30 μmol m^{-2} s^{-1}) grown seedlings. E, Six-day-old constant WL (60 μmol m^{-2} s^{-1}) grown seedlings. F, Six-day-old constant RL (15 μmol m^{-2} s^{-1}) grown seedlings. G, Six-day-old constant FR (15 μmol m^{-2} s^{-1}) grown seedlings. H, Six-day-old constant BL (15 μmol m^{-2} s^{-1}) grown seedlings. I, Six-day-old constant WL (5 μmol m^{-2} s^{-1}) grown seedlings. J, Six-day-old constant WL (15 μmol m^{-2} s^{-1}) grown seedlings. K, Hypocotyl length of 6-d-old constant dark-grown seedlings. L, Hypocotyl length of 6-d-old constant WL (5 μmol m^{-2} s^{-1}) grown seedlings. M, Hypocotyl length of 6-d-old constant WL grown wild type (Col) and *shw1-1* mutant seedlings at various fluence rates. N, Hypocotyl length of 6-d-old constant RL grown wild type (Col) and *shw1-1* mutant seedlings at various fluence rates. O, Hypocotyl length of 6-d-old constant FR-grown wild type (Col) and *shw1-1* mutant seedlings at various fluence rates. P, Hypocotyl length of 6-d-old constant BL-grown wild type and *shw1-1* mutant seedlings at various fluence rates. Q, The quantification of curvature of apical hooks of 6-d-old wild type (Col), *shw1-1*, and *shw1-2* mutant seedlings grown in constant darkness.

lines displayed hypocotyl length similar to wild-type seedlings in WL (Supplemental Fig. S2). To determine the GUS staining pattern, we used hypocotyl cells of 3-d-old transgenic seedlings grown in constant dark or WL. In either dark- or light-grown seedlings, the GUS stain was exclusively visible in the nucleus (Fig. 6, A–D). These results demonstrate that SHW1 is constitutively localized in the nucleus either in dark- or light-grown Arabidopsis seedlings.

The Photomorphogenic Growth of *cop1* Mutants Is Further Enhanced by the Additional Loss of *SHW1* Function in Dark

A group of repressors, COP/DET/FUS, acts downstream to multiple photoreceptors to repress photomorphogenesis (Wei and Deng, 1999; Jiao et al. 2007). Among them, COP1 has been studied in detail and is considered as a key repressor of photomorphogenic growth. The *cop1* mutants display photomorphogenic

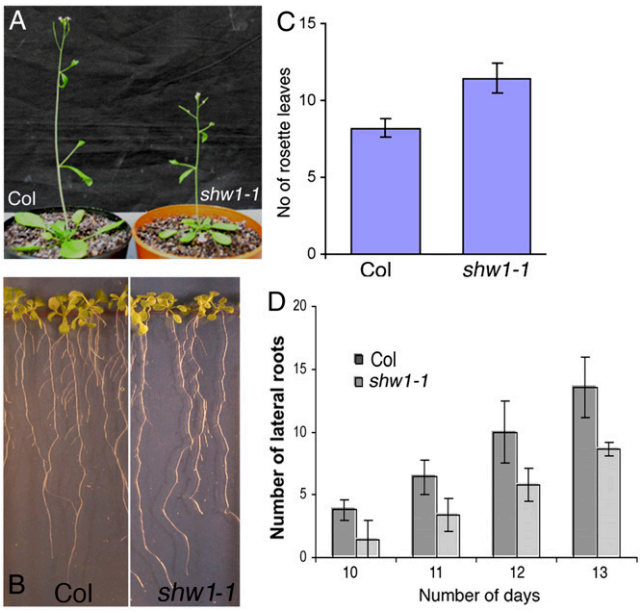

Figure 4. Phenotypes of *shw1* adult plants. A, Three-week-old wild type (Col) and *shw1-1* mutant plants grown under long-day cycles of 16-h WL (90 μmol m^{-2} s^{-1}) and 8-h dark. B, The root growth of 16-d-old wild type (Col) and *shw1-1* mutant plants grown in long-day cycles of 14-h WL (90 μmol m^{-2} s^{-1}) and 10-h darkness. C, Quantification of the number of rosette leaves formed at bolting. D, Quantification of lateral roots formed at various days.

growth in the dark and are hypersensitive to light. Since *shw1* mutants display shorter hypocotyl in the dark and enhanced inhibition in hypocotyl elongation in WL, we asked whether SHW1 was functionally related to COP1. To address this question, we constructed *shw1-1 cop1-6* double mutants. Since the null alleles of *cop1* are seedling lethal, we selected a relatively weak allele of *cop1* (*cop1-6*) for our studies (McNellis et al., 1994).

We examined the growth of *shw1 cop1* double mutants and the corresponding single mutants in dark and WL. The *shw1 cop1* double mutants exhibited shorter hypocotyl than *cop1* or *shw1* single mutants in constant darkness (Fig. 7, A and C). However, *shw1 cop1* double mutants exhibited similar hypocotyl length to *cop1-6* in WL (Fig. 7, B and D). These results indicate that *shw1* and *cop1* act in an additive manner in repressing photomorphogenic growth in dark. However, enhanced inhibition of hypocotyl elongation of *shw1* mutants in WL requires functional COP1 protein.

The *cop1* mutants exhibit dark-purple color fusca phenotype due to high-level accumulation of anthocyanin, however, such effects are not visible in *shw1* mutants. The percent of fusca phenotype, when examined, was dramatically increased in *shw1 cop1* double mutants as compared to *cop1* alone (Fig. 7, E and F). Consistent with this observation, the quantification of anthocyanin levels revealed a drastic increase in anthocyanin accumulation in *shw1 cop1* double mutants as compared to *cop1* single mutants either in dark or WL conditions (Fig. 7, G and H).

The *cop1* mutants are hypersensitive to light, and some of them are unable to turn green while dark-grown *cop1* seedlings are transferred to light. This physiological response of *cop1* mutants, known as COP1-mediated blocking of greening phenotype, becomes more severe with longer incubation in the darkness (Ang and Deng, 1994). To determine whether *SHW1* can modulate *cop1*-mediated blocking of greening phenotype, we examined the blocking of greening effects in *shw1 cop1* double mutants. A significantly higher percentage of *shw1 cop1* double mutants were able to turn green when 5-d-old dark-grown seedlings were transferred to light, suggesting that *shw1* can partly suppress the blocking of greening phenotype of *cop1* (Fig. 7I).

DISCUSSION

Analyses of *shw1* mutants have revealed that the light-mediated enhanced inhibition of hypocotyl elongation is restricted to WL with no visible effect in RL, FR, or BL. Whereas several photomorphogenic mutants show wavelength-specific phenotypes without displaying any morphological defect in WL, several other loss-of-function mutants exhibit phenotype both in WL and in specific wavelength of light. Additionally, mutants of several wavelength-specific negative regulators of seedling development including PIF3, SUB1, SPA1, MYC2, and GBF1 show hypersensitive responses in light, however, exhibit complete etiolation similar to wild-type seedlings in dark (Parks and Quail, 1993; Guo et al., 2001; Holm et al., 2002; Laubinger et al., 2004; Wang and Deng, 2004; Huq and Quail, 2005; Yadav et al., 2005; Mallappa et al., 2006). Therefore, this study reveals that SHW1 is a unique negative regulator of photomorphogenic growth.

The Ser- and Arg-rich domains are commonly found in the SR protein family, which plays important roles in constitutive and alternative splicing of pre-mRNA in eukaryotes (Black, 2003). Interestingly, whereas the Ser- and Arg-rich domains are well separated from each other in SR proteins, both these two domains are present at the N-terminal end and are overlapped in SHW1 protein. SR proteins are fairly conserved in higher plant and animal systems. However, SHW1 does not have any homolog in the animal system or in lower eukaryotes, suggesting it is likely to be evolved as a plant-specific protein. Recent studies have revealed that there are at least 19 and 20 SR proteins in Arabidopsis and rice (*Oryza sativa*), respectively, and SHW1 is not included in this family of proteins (Lorković and Barta, 2002; Reddy, 2004; Isshiki et al., 2006). *SHW1* appears to be a single-copy gene in the Arabidopsis genome, and the protein shows homology to unknown proteins from rice and grape (*Vitis vinifera*). The consensus sequences derived from these homologs, and the alignment of SHW1 with these proteins are shown in Supplemental Figure 3.

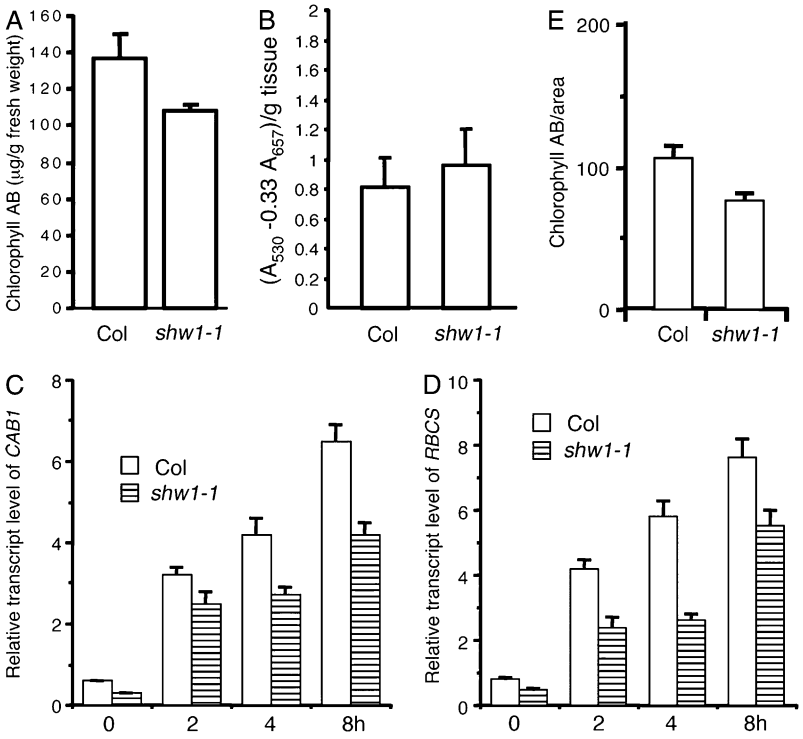

Figure 5. Chlorophyll and anthocyanin contents and the expression of light-inducible genes in *shw1* mutants. A, Accumulation of chlorophyll in 6-d-old constant WL (30 μmol m^{-2} s^{-1}) grown wild type (Col) and *shw1-1* mutant seedlings. B, Accumulation of anthocyanin in 6-d-old constant WL (30 μmol m^{-2} s^{-1}) grown wild type (Col) and *shw1-1* mutant seedlings. C, Quantification of RNA gel-blot analysis data of *CAB1* in wild type (Col) and *shw1-1* mutant seedlings. Five-day-old dark-grown seedlings were transferred to WL for 2, 4, and 8 h. Five-day-old dark-grown seedlings have been shown as 0 h. The experiment was repeated three times. The intensity of each band in autoradiograph was quantified by the Fluor-S-MultiImager (Bio-Rad) and ratios of the *CAB1* gene versus its corresponding *18S* rRNA band were determined and plotted (Fluor-S-MultiImager; Bio-Rad). D, Quantification of RNA gel-blot analysis data of *RBCS* in wild type (Col) and *shw1-1* mutant seedlings. For experimental detail see legend to Figure 5C. E, Accumulation of chlorophyll in the cotyledons after normalized by cotyledon size in 6-d-old constant WL (30 μmol m^{-2} s^{-1}) grown wild type (Col) and *shw1-1* mutant seedlings.

The morphological analyses of *shw1* mutants clearly demonstrate that the short hypocotyl phenotype of *shw1* seedlings is restricted to dark light and WL with no significant effect in RL, FR, or BL. It appears that *shw1* mutants may have a weak phenotype in RL, especially at 60 μmol m^{-2} s^{-1} fluences (Fig. 3N). However, measurement of hypocotyl length revealed that the difference in hypocotyl length between the wild type and *shw1* was not statistically significant ($n = 120$; $P \sim 0.08$). The negative regulatory role of SHW1 in light-mediated inhibition of hypocotyl elongation is thereby confined to WL, likely to be a cumulative effect mediated by multiple photoreceptors.

Examination of tissue-specific expression reveals that *SHW1* is expressed in all tissue types tested except in roots, and the gene is predominantly expressed in photosynthetically active tissues. Interestingly, although we did not detect any expression of *SHW1* in the root tissue, the microarray data available in the public domain apparently suggest that the gene is also expressed in roots (www.genevestigator.ethz.ch). It could be envisioned that the root-specific cis-acting elements involved in the expression of *SHW1* in roots are outside the length of the promoter used in this study. Alternatively, the expression level of *SHW1* is extremely low in roots and is beyond the sensitivity level of the reporter gene used in this study. Similar arguments could also be applicable for the weak activity of the *SHW1* promoter in the specific wavelength of light (Supplemental Fig. 1).

Analyses of the light-regulated gene expression reveal that the rate of light-mediated induction of *CAB* and *RBCS* gene expression was significantly compromised in *shw1* mutants indicating that SHW1 is required for the optimal expression of light-inducible genes. Thus, SHW1 plays an opposite regulatory function in photomorphogenic growth and light-regulated gene expression. Several regulatory genes involved in seedling development have been described previously that function as positive as well as negative regulators of light responses (Ward et al., 2005; Kang and Ni, 2006; Jiao et al., 2007). For example, PIF3, a bHLH transcription factor, is a negative regulator of photomorphogenic growth, however, acts as a positive

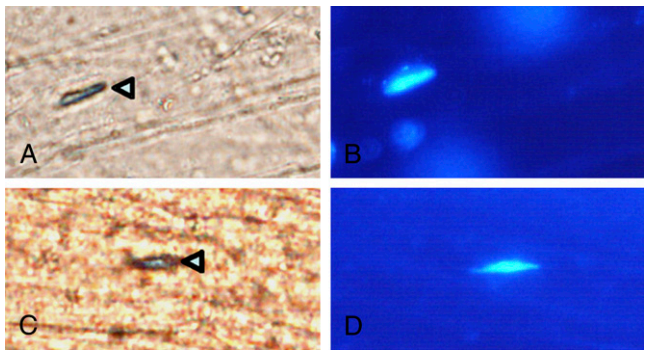

Figure 6. Subcellular localization of SHW1 in hypocotyl cells of transgenic seedlings. The hypocotyls of transgenic seedlings containing *SHW1-GUS* transgene expressed under *SHW1* promoter were stained for GUS (A and C), and for DNA using DAPI to identify nuclei (B and D). The arrowheads indicate nuclei. The hypocotyl cells of 3-d-old constant dark- or WL-grown seedlings were used for NLS studies (von Arnim and Deng, 1994). A and B, Hypocotyl cells of dark-grown seedlings. C and D, Hypocotyl cells of WL-grown seedlings.

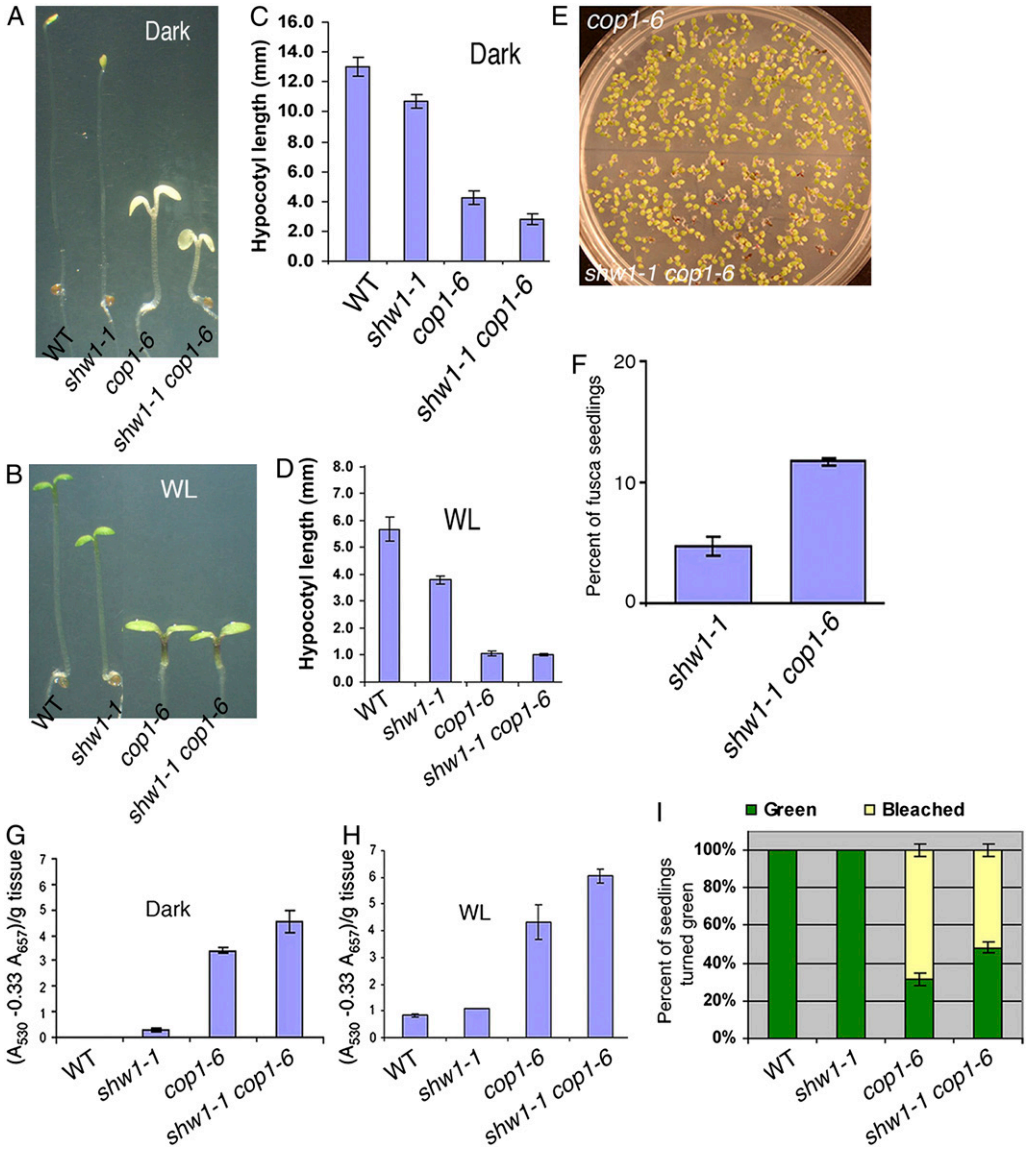

Figure 7. Characterization of *shw1 cop1* double mutants. A and B, Visible phenotype of 6-d-old wild type and various mutant seedlings (all in Col background) grown in constant dark and WL (15 μmol m^{-2} s^{-1}), respectively. C and D, Hypocotyl length of 6-d-old wild type and different mutant seedlings grown in constant dark and WL (5 μmol m^{-2} s^{-1}), respectively. E, Visible fusca phenotype in 6-d-old *cop1-6* and *shw1 cop1-6* mutant seedlings grown in constant WL (60 μmol m^{-2} s^{-1}). F, Percentage of fusca phenotype in WL (60 μmol m^{-2} s^{-1}) grown seedlings. G, Accumulation of anthocyanin in 6-d-old constant dark-grown wild type (WT) and various mutant seedlings. H, Accumulation of anthocyanin in 6-d-old constant WL (60 μmol m^{-2} s^{-1}) grown wild type (WT) and various mutant seedlings. I, Percentage of seedlings turned green whereas 5-d-old dark-grown seedlings were transferred to WL for 2 d.

regulator of light-regulated gene expression (Kim et al., 2003). GBF1/ZBF2, a bZIP transcription factor, acts as a negative regulator of inhibition of hypocotyl elongation, however, acts as a positive regulator of cotyledon expansion. Furthermore, GBF1/ZBF2 differentially regulates the expression of light-regulated genes (Mallappa et al., 2006).

Light-controlled shuttling of COP1 protein between the nucleus and cytoplasm is an important regulatory mechanism of light-mediated seedling development (von Arnim and Deng, 1994; Osterlund and Deng, 1998; Jiao et al., 2007). COP1 degrades several photomorphogenesis promoting factors in the dark to suppress photomorphogenic growth in the darkness (Jiao et al., 2007). It has been shown that mutations at *COP2*, *COP3*, and *COP4* result in cotyledon expansion in darkness (Hou et al., 1993). However, these loci are not involved in hypocotyl elongation and in light-regulated gene expression. It has been postulated from such observations that these less pleiotropic loci act as downstream-branched pathways of the highly pleiotropic photomorphogenic regulatory loci such as

COP1 and regulate subsets of seedling morphogenic responses to light. The analyses of *shw1 cop1* double mutants in this study suggest that SHW1 and COP1 function nonredundantly to control hypocotyl elongation in dark. On the other hand, in WL, enhanced inhibition in hypocotyl elongation in *shw1* mutants requires functional COP1 protein. The physiological responses of *cop1* mutants are also altered by additional loss of *SHW1* function. Thus, collectively, these data imply a role of SHW1 in downstream-branched pathways of COP1, similar to COP2, COP3, or COP4, in Arabidopsis seedling development.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) seeds were surface sterilized and sown on Murashige and Skoog plates, then kept at 4°C in darkness for 3 to 5 d, and transferred to specific light conditions at 22°C. The intensities of WL and various color lights (in the light-emitting diode chamber, Q-Beam 3200–A; Quantum Devices) used were described in Yadav et al. (2002).

To obtain the homozygous *shw1* mutant lines, plants heterozygous or homozygous for the *shw1-1* or *shw1-2* mutations were examined by PCR genotyping analyses. Individual plants were examined by PCR using the left border specific primer LBP (5′-GCGTGGACCGCTTGCTGCACCT-3′) and the *shw1-1* specific primers LP11 (5′-TGCAAAAGACACCTGCAAATCA-3′) and RP11 (5-′ATGCAA AGAACCGAGAGGTCG-3′); or *shw1-2* specific primers LP9 (TCACCACCGCCGAAGAATCTA) and RP9 (ACCCAATCGGTCCATGTCCTT).

For the generation of double mutants, such as *shw1-1 cop1-6*, homozygous *shw1-1* (Col-0) mutant plants were genetically crossed with *cop1-6* (Col-0) homozygous mutant lines. In the F2 generation, seedlings were grown in WL (90 μmol s^{-1} m^{-2}) for the identification of *cop1* homozygous lines, and short hypocotyl *cop1* mutants were selected and transferred to soil. To determine the genotype at *shw1* locus, about 40 seedlings from each line were tested by genomic PCR. F3 progenies that were homozygous for *shw1-1* mutant plants were further examined and considered as *shw1-1 cop1-6* double mutants.

GUS Assays and NLS Studies

For the generation of transgenic lines, a 1-kb upstream DNA sequence of *SHW1* gene was amplified by PCR using the primers (forward 5′-GGAATTCTTACGTTGAAGGAACATTC-3′ and reverse 5′-CATGCCATGGAATTAAAACGGACCTTTTTG-3′), and cloned into *Eco*RI + *Nco*I site of pCAMBIA recombinant vector containing *SHW1* in frame fused to *GUS* or *GUS* alone. *Agrobacterium* strain *GV3101* was transformed with the recombinant constructs, and wild-type ecotype Wassilewskija of Arabidopsis plants were transformed using *Agrobacterium* carrying recombinant pCAMBIA construct by the vacuum infiltration method. Transgenic plants were screened on 15 μg/mL hygromycin plates and several independent lines of homozygous transgenic plants containing the transgene were generated. Nuclear localization signal (NLS) studies were carried out as described in von Arnim and Deng (1994). GUS activity measurements were carried out essentially as described in Yadav et al. (2002).

Subtractive cDNA Library

Total RNA was isolated from 5-d-old constant dark- or light-grown chickpea (*Cicer arietinum*) seedlings using Tripure reagent (Tripure; Roche). The polyA-RNA was purified using the mRNA isolation kit (Roche) according to manufacturer protocol. The subtractive cDNA library was constructed from polyA-RNA using Clontech kit (Clontech) according to the manufacturer's procedure.

Northern-Blot Analyses

Total RNA was extracted using the RNeasy plant minikit (QIAGEN). Northern-blot analyses with 20 μg of total RNA for each sample was carried out essentially as described in Hettiarachchi et al. (2003). The 0.6-kb *SHW1* DNA fragment was used as probe after random prime labeling (Amersham). The DNA fragments of *CAB1* and *RBCS* genes were used for probes as described in Yadav et al. (2005). The *18S* ribosomal RNA was used as loading control. The intensity of each band in the autoradiograph was quantified by the Fluor-S-MultiImager (Bio-Rad) and ratios of the *CAB1* or *RBCS* gene versus its corresponding *18S* ribosomal RNA (rRNA) band were determined and plotted (Fluor-S-MultiImager; Bio-Rad).

Chlorophyll and Anthocyanin Measurements

Chlorophyll and anthocyanin levels were measured following protocols as described in Holm et al. (2002).

Sequence data from this article have been deposited with the EMBL/GenBank data libraries under accession number AM 419013.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Light-mediated induction of *SHW1* promoter.

Supplemental Figure S2. Functional analyses of SHW1-GUS fusion protein.

Supplemental Figure S3. Alignment of protein sequence of SHW1.

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