Journal of Experimental Botany, Vol. 64, No. 4, pp. 1017–1024, 2013
doi:10.1093/jxb/ers376 Advance Access publication 10 January, 2013
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Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

CONSTANS-LIKE 7 regulates branching and shade avoidance response in *Arabidopsis*

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Received 8 November 2012; Revised 10 December 2012; Accepted 12 December 2012

Abstract

Branching is an important trait of plant development regulated by environmental signals. Phytochromes in *Arabidopsis* mediate branching in response to the changes in the red light:far-red light ratio (R:FR), the mechanisms of which are still elusive. Here it is shown that overexpression of *CONSTANS-LIKE 7* (*COL7*) results in an abundant branching phenotype which could be efficiently suppressed by shade or a simulated shade environment (low R:FR). Moreover, *col7* mutants develop shorter hypocotyls and *COL7* overexpression lines develop longer hypocotyls in comparison with the wild type in low R:FR, indicating that COL7 acts as an enhancer of the shade avoidance response. In shade or transient low R:FR, transcriptional and post-transcriptional expression levels of *COL7* are up-regulated and positively associated with rapid mRNA accumulation of *PHYTOCHROME INTERACTING FACTOR 3-LIKE 1* (*PIL1*), a marker gene of shade avoidance syndrome (SAS). Taken together, the results suggest a dual role for COL7 which promotes branching in high R:FR conditions but enhances SAS in low R:FR conditions.

Key words: Branching, COL7, light signal transduction, phytochrome B, PIL1, shade avoidance response.

Introduction

Branching is a significant developmental trait in agricultural and horticultural crops that determines the above-ground architecture of plants (Evers *et al*., 2011). As a lateral organ, a branch is developed from an axillary meristem. Several genes involved in axillary meristem initiation in *Arabidopsis* have been identified, such as *LATERAL SUPPRESSOR*, *REVOLUTA*, and *BLIND* (Schumacher *et al*., 1999; Otsuga *et al*., 2001; Schmitz *et al*., 2002). While their loss of functions dramatically impairs the formation of axillary meristems, little evidence exists on how environmental signals regulate branching via the initiation of axillary buds (Finlayson *et al*., 2010). Axillary branching is mainly regulated by breaking of bud dormancy and subsequent branch stem elongation, the processes of which are regulated by internal factors in responding to environmental cues such as light quality and intensity, nutrition, pruning, etc. (Leyser, 2009; Domagalska and Leyser, 2011).

As intrinsic factors, phytohormones play important roles in systemic control of branching. Auxin produced at the shoot apex is transported basipetally to inhibit shoot branching and establish apical dominance (Leyser, 2005). Strigolactone synthesized in the roots is transported acropetally, and also suppresses bud activity (Domagalska and Leyser, 2011). Cytokinins are mostly synthesized in the roots, and act within the bud to promote branch outgrowth (Chen *et al*., 1985; Nordstrom *et al*., 2004; Tanaka *et al*., 2006). Dozens of

genes, including *MORE AXILLARY GROWTH1* (*MAX1*), *MAX2*, *MAX3*, *MAX4*, *AUXIN RESISTANT 1* (*AXR1*), *BRANCHED1* (*BRC1*), and *BRC2*, are involved in the biogenesis, transport, or signal transduction of these phytohormones, and mutations of those genes lead to various abnormal branching phenotypes (Stirnberg *et al*., 2002; Sorefan *et al*., 2003; Booker *et al*., 2004, 2005; Aguilar-Martinez *et al*., 2007; Brewer *et al*., 2009; Domagalska and Leyser, 2011).

The shade avoidance syndrome (SAS) is characterized by adjustments in plant development in response to a low red light:far-red light ratio (R:FR) perceived by the plant. SAS in *Arabidospsis* triggers elongation of hypocotyls, stems, and petioles, elevation of leaf angles, suppression of branching, and promotion of flowering (Devlin *et al*., 2003; Casal, 2012). In dense growing conditions, the R:FR decreases as red light is absorbed by photoactive pigments of neighbouring plants, and far-red light is mainly reflected by and transmitted through the neighbouring plants (Ballare, 1999; Franklin, 2008; Hornitschek *et al*., 2009). In *Arabidopsis*, red light and far-red light signals are detected by phytochromes (phyA–phyE), which act as major sensors of light quality (Franklin and Quail, 2010).

Among the five phytochromes, phyB plays a predominant role in SAS, and the *phyB* mutant displays constitutive SAS-like phenotypes including early flowering, hypocotyl elongation, and reduced branching (Reed *et al*., 1993). Furthermore, phyB exists in two photo-interconvertible forms: an inactive Pr form and an active Pfr form. Red light triggers conversion of phyB from the Pr to the Pfr form, while far-red light photoconverts phyB from Pfr to Pr. The Pfr form of phyB is able to interact physically with a subset of basic helix–loop–helix (bHLH) transcriptional factors, named PHYTOCHROME INTERACTING FACTORs (PIFs), which act as positive regulators to promote hypocotyl elongation (Hornitschek *et al*., 2009; Franklin and Quail, 2010; Li *et al*., 2012). In high R:FR, most phyB is in the Pfr form which interacts with PIFs and promotes their degradation through the 26S proteasome (Leivar and Quail, 2011). During low R:FR, phyB is converted into the Pr form and is disassociated from PIFs, leading to the accumulation of PIF proteins. Among the seven PIFs characterized in *Arabidopsis*, PIF4, PIF5, and PIF7 have been implicated in the regulation of SAS (Lorrain *et al*., 2008; Leivar and Quail, 2011; Li *et al*., 2012). In contrast to other unstable, light-sensitive PIF proteins, PIF7 shows no rapid light-induced degradation (Leivar *et al*., 2008; Li *et al*., 2012). Furthermore, PIF7 is a major positive regulator of SAS that undergoes dephosphorylation and directly binds to G-boxes of auxin biosynthesis genes to promote auxin biosynthesis and consequently enhances hypocotyl elongation in response to shade or a low R:FR (Li *et al*., 2012).

Additionally, phytochromes have been proposed to mediate branching by altering strigolactone signalling and polar auxin transport based on the observation that the inhibition of branching by phyB in response to low R:FR requires functional BRC1, BRC2, AXR1, MAX2, and MAX4 (Finlayson *et al*., 2010). These findings suggest a primary SAS signal transduction cascade from the perception of light quality to phytohormone biosynthesis, and finally to adjustments in growth and development. However, the network between those plant hormones and phytochrome-mediated control of branching remains elusive. In this study, it is shown that overexpression of *COL7* results in an abundant branching phenotype which can be efficiently suppressed by shade. It is demonstrated that mutation of *COL7* has suppressed, while overexpression of *COL7* has enhanced shade avoidance responses. This study suggests that *COL7* plays a positive role in branching and SAS signal transduction, and thus provides additional information about the SAS regulatory network.

Materials and methods

Plant material and growth conditions

The ecotype Col-4 of *Arabidopsis thaliana* was used as the wild type (WT) in this study. The *col7* (GABI-639C04) mutant was ordered from NASC. The *35S::COL7* and *35S::MYC-COL7* transgenic lines are in the Col-4 background. Quantification of branch phenotype analysis was performed using a modified method (Finlayson *et al*., 2010). Seeds were sown on soil, stratified at 4 °C in darkness for 3 d, and then transferred to long days (LDs) (16 h light/8 h dark, 100–150 μE m^{-2} s^{-1} of white light, R:FR ratio of 1.2) for 2 weeks. The plants were then left in white light or transferred to simulated shade (R:FR ratio of 0.1–0.3) provided by a combination of white light and far-red light (LED panel, 730 ± 30 nm). The number of primary rosette branches produced from the rosette buds was recorded when the first silique of *Arabidopsis* turned yellow. Hypocotyl lengths in continuous red light or far-red light were measured as described previously (Yu *et al*., 2007). Briefly, the seeds were surface sterilized with 10% bleach for 10 min, stratified at 4 °C in darkness for 3 d, treated with white light for 12 h, and grown in red light or far-red light (red light, 19 μE m^{-2} s^{-1}; far-red light 0.47 μE m^{-2} s^{-1}) for 7 d. The hypocotyl length of at least 20 seedlings was measured. Hypocotyl elongation assays in response to shade were performed as previously described (Li *et al*., 2012). The seeds were incubated in continuous white light (30 μE m^{-2} s^{-1}, R:FR ratio of 1.2) for 3 d, then either kept in white light or moved to simulated shade (LED: red light, 12 μE m^{-2} s^{-1}; blue light, 0.5 μE m^{-2} s^{-1}; and far-red light, 20 μE m^{-2} s^{-1}; R:FR ratio of 0.6) for 5 d. The hypocotyl length of ≥20 seedlings was measured.

Vector construction and plant transformation

The open reading frame (ORF) of *COL7* (AT1G73870) was amplified by reverse transcription–PCR (RT–PCR) using primers *COL7*-CDS-F and *COL7*-CDS-R (primer sequences are provided in Supplementary Table S1 available at *JXB* online) and cloned into pDONR201 by BP reaction to generate pDONR201-*COL7* (Gateway, Invitrogen). Then the *COL7* coding sequence (CDS) was cloned into the binary vector pLeela (Liu *et al*., 2007) and *35S::MYC-GW* by LR reaction (Gateway, Invitrogen) to generate *35S::COL7* and *35S::MYC-COL7*, respectively. *Agrobacterium tumefaciens* strains GV3101(pMP90RK) and GV3101(pMP90) were used for *Arabidopsis* transformation with *35S::COL7* and *35S::MYC-COL7*, respectively, following the floral dip method (Clough and Bent, 1998).

PCR genotyping, RNA isolation, and mRNA expression analysis

Genomic DNA of the WT or *col7* mutant was used as template for PCR genotyping using prim-er P1, P2, and P3. Total RNA extraction and cDNA synthesis were performed as previously described (Yu *et al*., 2008). *COL7* mRNA abundance in the WT and *col7* mutant was evaluated by semi-quantitative RT–PCR using primer pairs P1 and P2 for *COL7* and P4 and P5 for *ACTIN2*. mRNA

levels of the indicated genes were measured by quantitative PCR (qPCR) using P6 and P7 for *COL7*, P8 and P9 for *PIL1*, and P10 and P11 for *ACTIN2*. Sequences of the above primers are provided in Supplementary Table S1 at *JXB* online.

Immunoblot

Seeds were sterilized, sown on Murashige and Skkog (MS) medium, grown, and moved into different treatment conditions. Samples were harvested, frozen in liquid nitrogen, and ground in 4× SDS protein extraction buffer for subsequent SDS–PAGE and immunoblot analysis probed with anti-MYC antibody (Millipore, Cat. #05-724). The same membrane was stripped and probed with anti-CRY1 antibody as internal control.

Results

Overexpression of COL7 in Col-4 results in an abundant branching phenotype

There are a total of 17 members of the *CO-LIKE* gene family which can be grouped into three phylogenetic clades (Khanna *et al*., 2009). The functions of some *CO-LIKE* genes belonging to clade I and II have been characterized, but the biological roles of the clade III *CO-LIKE* genes are still largely unknown (Hassidim *et al*., 2009). Here it was observed that overexpression of *COL7*, one of the clade III *CO-LIKE* genes, driven by the 35S promoter in WT Col-4 *Arabidopsis*, resulted in an obviously abnormal branching phenotype (Fig. 1). Multiple transgenic lines were obtained, and two representative lines, *35S::COL7* #10 and *35S::COL7* #11, in which the overexpression of *COL7* was verified by qPCR (Supplementary Fig. S1 at *JXB* online), were used for the subsequent studies. The phenotype of *35S::COL7* lines was compared with that of WT plants and the *col7* mutant in which the full-length mRNA expression of *COL7* was impaired by a T-DNA insertion at the second exon (Supplementary Fig. S1A–C). As shown in Fig. 1A, when the indicated plants were grown at a low density (one plant per pot) in LDs, *35S::COL7* lines generated more rosette branches than the WT and the *col7* mutant. To test if higher planting density can inhibit the abundant branching phenotype, the numbers of plants was increased and treatments of one, four, nine, or 20 seedling per pot in LDs were used for branch quantification. At low planting density of one plant per pot, the branch number of *35S::COL7* lines is ~5-fold greater than that of the WT and the *col7* mutant (Fig. 1B). However, the branch number of the overexpression lines declines dramatically with increased planting density and is almost as low as that of the WT and the *col7* mutant when the plants were grown at the highest density of 20 plants per pot. Since branching could be influenced by other factors such as nutrition, beside SAS, the branch numbers of each line grown at a low density of one plant per pot in low R:FR were compared. As show in Fig. 1C and D, sustained low R:FR efficiently suppressed the abundant branching phenotype of *35S::COL7* lines, indicating that the decline of R:FR at high planting density could result in the reduction of branch numbers of *35S::COL7* lines. In addition, when comparing the plants grown in high R:FR with those grown in low R:FR, the branch number of the *35S::COL7* lines decreases >4-fold, while that of the WT and *col7* decreased <2-fold, suggesting that *COL7* enhances the shade-induced suppression of branching.

COL7 promotes hypocotyl elongation in response to shade

During SAS, the hypocotyls of *Arabidopsis* tend to elongate to reach a higher position in response to low R:FR (Casal, 2012). To test the role of *COL7* in the regulation of hypocotyl elongations, the hypocotyls of the *35S::COL7* line, the *col7* mutant, and the WT were analysed in red light, far-red light, high R:FR, and low R:FR, respectively. All the lines showed no obvious difference when grown in continuous red light for 3–6 d (Supplementary Fig. 2A, C at *JXB* online). When grown in continuous far-red light, *35S::COL7* lines developed longer hypocotyls and *col7* mutants developed shorter hypocotyls in comparison with the WT (Supplementary Fig. S2B, D), indicating that COL7 may suppress the far-red light-dependent inhibition of hypocotyl elongation. Furthermore, quantification of hypocotyl elongation in response to shade treatment shows that overexpression of *COL7* significantly promotes hypocotyl elongation, while loss of function of

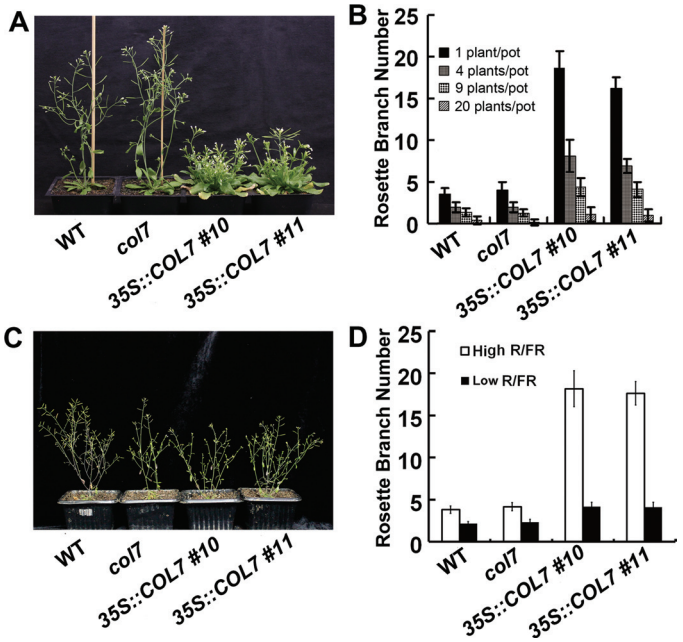

Fig. 1. Branching phenotypes of WT, *col7* mutant, and *35S::COL7* lines. (A) Branching phenotype of the WT, *col7* mutant, and *COL7* overexpression lines growing at the density of one plant per pot in LDs (16 h light/8 h dark). (B) Rosette branch number of each line as indicated grown at the density of one, four, nine, or 20 plants per pot, respectively. (C) Rosette branch phenotype of each line treated by low R:FR. Plants were grown in LDs for 3 weeks and then transferred to low R:FR. (D) Rosette branch number of each line grown in high R:FR or low R:FR. The number of the primary branches generated from the rosette axillary buds was measured. Means and standard deviations are representative of at least 20 plants. (This figure is available in colour at *JXB* online.)

COL7 in the *col7* mutant suppresses hypocotyl elongation when grown in low R:FR but not in high R:FR (Fig. 2A, B). These observations demonstrate that *COL7* enhances hypocotyl elongation in response to sustained shade, a typical phenotype of SAS.

Expression of COL7 is dynamically regulated at both the transcriptional and post-transcriptional level by shade

To test if the transcription of *COL7* is regulated by low F:FR, an mRNA expression analysis was performed via qPCR using the seedlings transferred from simulated shade to white light (Fig. 3A) or the seedlings transferred from white light to simulated shade (Fig. 3B). The results indicate that high R:FR results in a rapid decline of *COL7* mRNA within 15 min (Fig. 3A). In contrast, mRNA expression of *COL7* was rapidly up-regulated in 15 min and then fell back to its original level within 2 h after the seedlings were transferred from high R:FR to low R:FR (Fig. 3B). To test further the stability of COL7 proteins in different light conditions, the *35S::MYC-COL7* binary vector was constructed to transform *Arabidopsis*, and the *35S::MYC-COL7* transgenic line which showed a similar phenotype to *35S::COL7* transgenic lines was obtained (Supplementary Figs S1, S3 at *JXB* online). Seedlings of the *35S::MYC-COL7* line were grown on MS plates for 5 d in white light, transferred to dark for 3 d, and then treated by far-red light or red light. Immunoblots probed with anti-MYC antibody showed that COL7 protein accumulated to a high level within 1–2 h but then declined gradually when the seedlings were transferred from dark to red light or far-red light (Fig. 3C, D, G, H), indicating that both red light and far-red light could dynamically increase the stability of the COL7 protein. To explore the role of COL7 in SAS further, the fluctuation of COL7 protein in response to low R:FR was investigated. Seedlings grown in low R:FR were transferred into high R:FR (Fig. 3E, I) or vice versa (Fig. 3F, J). The results demonstrate that high R:FR destroys COL7 protein but low R:FR increases its stability. These data taken together reveal that the expression of *COL7* is dynamically up-regulated by shade at both the transcription and post-transcriptional level, suggesting that the regulation of *COL7* expression is a part of the SAS.

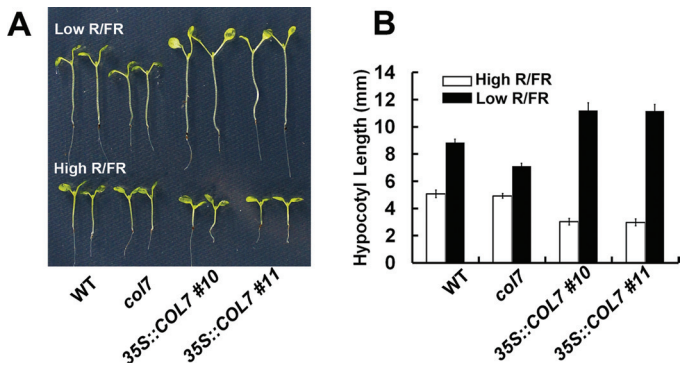

Fig. 2. *COL7* affects the hypocotyl elongation in low R:FR conditions. (A) The representative hypocotyl image of the WT, *col7* mutant, and *35S::COL7* lines grow in high R:FR (lower half of panel) or low R:FR (upper half of panel). The seedlings were incubated in white light for 3 d, then either kept in white light or transferred to simulated shade for 5 d. (B) Hypocotyl length of each indicated line as in (A). Similar results were obtained from three independent biological replicates and a representative one is shown. Means and standard deviations are representative of at least 20 seedlings. (This figure is available in colour at *JXB* online.)

COL7 promotes the expression of PIL1 mRNA in response to shade

PIL1 is a bHLH protein associated with the SAS (Salter *et al*., 2003). Because its mRNA level is rapidly up-regulated in response to low R:FR treatment, *PIL1* is widely used as a marker gene of SAS. To investigate if *COL7* is involved in the rapid regulation of *PIL1* expression by low R:FR, seeds of WT, *col7* mutant, and *35S::COL7* lines were sown on MS plates, grown in 24 h diurnal cycles (12 h light/12 h dark) for 5 d, and treated by low R:FR (Fig. 4A) from Zeitgeber time 1 (1 h after dawn) for the period indicated (0, 5, 10, 30, and 60 min). The results indicate that *PIL1* mRNA abundance increases ~40-fold in *35S::MYC-COL7* lines but increases only 25-fold in the WT and 17-fold in the *col7* mutant in response to low R:FR treatment for 1 h, indicating that the abundance of COL7 is positively correlated with the rapid increase in *PIL1* mRNA induced by low R:FR. To evaluate if COL7 sustainably affects the expression of *PIL1*, *PIL1* mRNA abundance was further analysed in the WT, *col7* mutant, and *35S::COL7* lines grown in sustained high R:FR, low R:FR, or continuous far-red light. *PIL1* mRNA showed a minor increase in the *col7* mutant and a decrease in the *35S::COL7* lines in comparison with the WT grown in high R:FR (Fig. 4B). In contrast, *PIL1* mRNA showed a slight decrease in the *col7* mutant and an increase in the *35S::COL7* lines in comparison with the WT in low R:FR and continuous far-red light (Fig. 4C, D). These results suggest that COL7 increases the *PIL1* mRNA abundance in continuous low R:FR or far-red light but decreases its abundance in continuous high R:FR.

Discussion

COL7 is a CO-LIKE protein, which belongs to a putative transcriptional factor family containing 17 gene members sharing two conserved domains, the B-box domain and CCT (CO, COL, and TOC1) domain. Among them, CO is the first B-box protein identified in *Arabidopsis* which plays a pivotal role in regulation of photoperiod flowering in *Arabidopsis*, and its expression is regulated by light at both the transcriptional and post-transcriptional level (Suarez-Lopez *et al*., 2001; Laubinger *et al*., 2006; Jang *et al*., 2008; Turck *et al*., 2008; Zuo *et al*., 2011). The presence of multiple *COL* genes in the genome of *Arabidopsis* suggests that they may share redundant functions in regulation of photoperiod flowering. Until now only COL5 was shown to induce flowering as CO does in SDs. Additionally, *col5* mutants were shown to flower

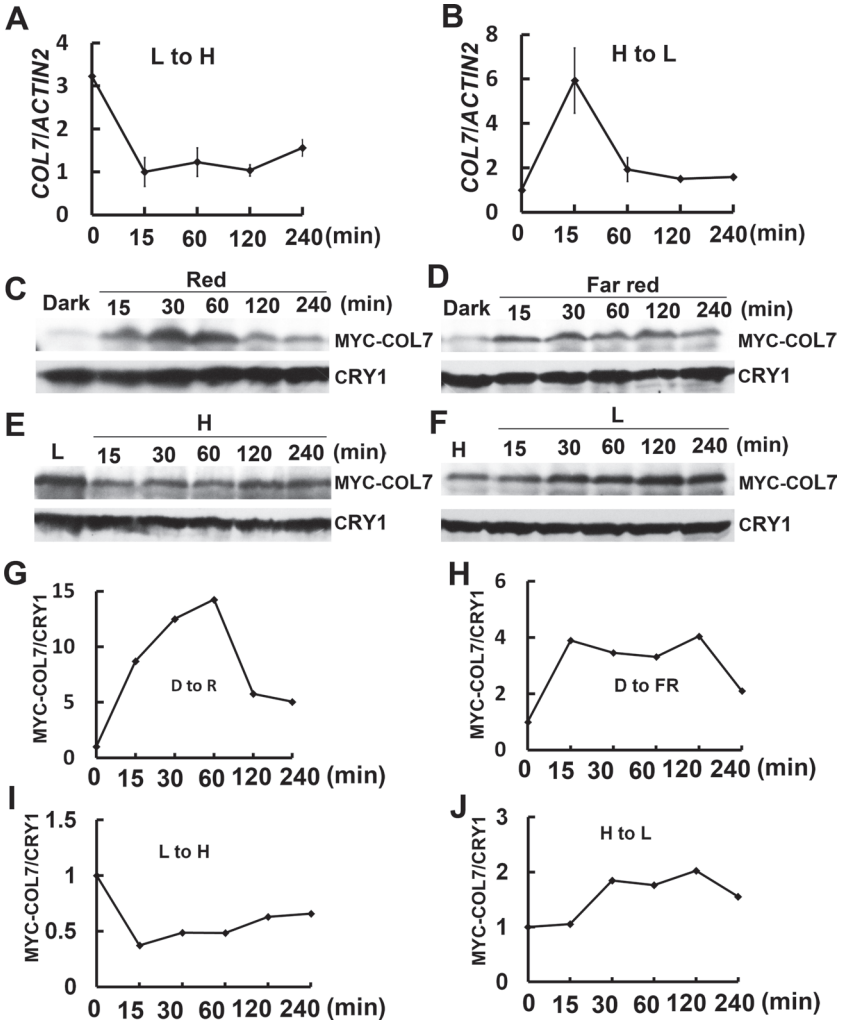

Fig. 3. Expression of *COL7* mRNA and protein. (A) *COL7* mRNA expression in response to high R:FR for the indicated period (15, 30, 60, 120, and 240 min). Seedlings were grown in white light for 3 d, moved to low F:FR for 4 d, and then transferred to high R:FR (white light, R:FR ratio of 1.2). (B) *COL7* mRNA expression in response to low R:FR. Seedlings were incubated in white light for 7 d and then transferred to low R:FR. Error bars represent the standard deviations of three independent replicates. (C, D) Representative immunoblot showing the level of COL7 protein in the *35S::MYC-COL7* line in response to red light (C) or far-red (D) light. Seedlings of the *35S::MYC-COL7* line were grown on MS plates for 5 d in white light followed by 3 d in the dark and then transferred to far-red light or red light. Immunoblots were probed with the anti-MYC antibody (MYC-COL7), stripped, and then probed with the anti-CRY1 antibody (CRY1). (G, H) The curves indicate the relative abundance of MYC-COL7 protein in samples in response to a period of red light (G) or far-red (H) light treatment, which was calculated by the formula (MYC-COL7/CRY1 [t_n])/(MYC-COL7/CRY1 [t_0]). The relative abundance of MYC-COL7 protein in the dark was set to 1. (E, F, I, J) COL7 protein level in the *35S::MYC-COL7* line in response to high R:FR (E, I) or low F:FR (F, J). The *35S::MYC-COL7* line was treated as mentioned in (A) and (B). 'H' represents high R:FR; 'L' represents low F:FR; 'R' represents red light; 'FR' represents far-red light; and 'D' represents dark. Similar results were obtained from three independent biological replicates.

normally, in contrast to *co* mutants that flower extremely late in LDs (Hassidim *et al*., 2009). *COL9* was shown to have the completely opposite effects to *CO*, where *COL9* overexpression lines were late flowering while co-suppression lines and *col9-t* mutant lines were early flowering in LDs (Cheng and Wang, 2005). Also *col3* mutants were also shown to flower early in both LDs and SDs (Datta *et al*., 2006). These unexpected observations imply that the *COL* genes have evolved wider roles in additiopn to regulation of flowering. It has been established that overexpression of *COL1* affects circadian rhythms (Ledger *et al*., 2001) and *COL3* regulates the formation of lateral roots, daylength, and light-dependent elongation and branching of shoots (Datta *et al*., 2006). However, the functions of other *COL* genes remain poorly understood.

In this study, the role of COL7 in branching, hypocotyl elongation, and marker gene expression in SAS was investigated. The results implicate a dual role for COL7 which acts oppositely in SAS signal transduction depending on the changes of R:FR. In high R:FR, COL7 promotes branching and suppresses hypocotyl elongation. In low R:FR, COL7 suppresses branching and enhances hypocotyl elongation and SAS marker gene expression. Unlike some of the other SAS

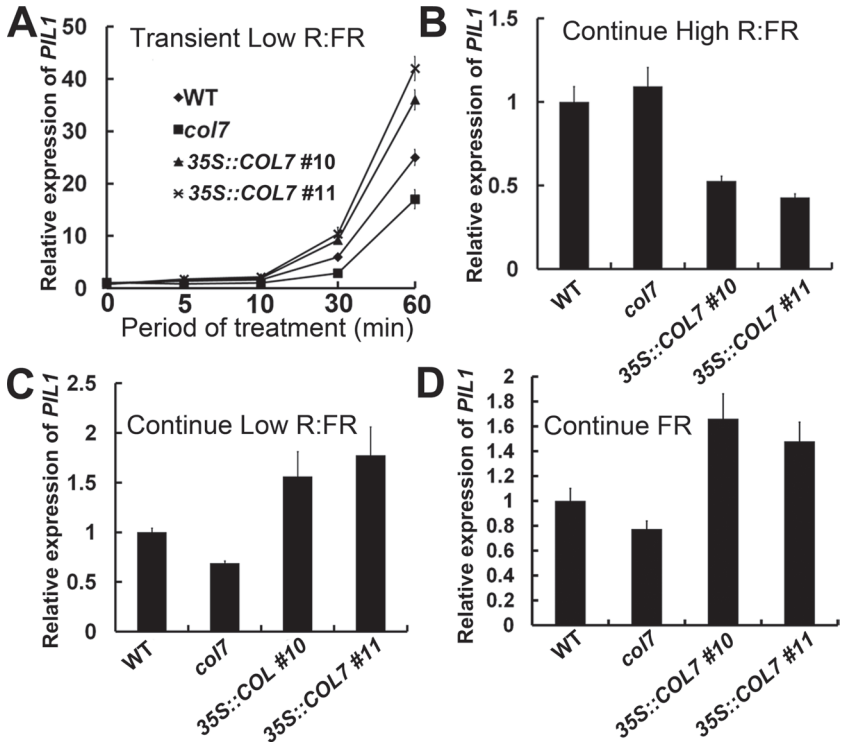

Fig. 4. *COL7* regulates *PIL1* mRNA abundance. (A) The qPCR results showed the relative expression of *PIL1* in the WT, *col7* mutant, and *35S:: COL7* lines. Seedlings were grown in diurnal periods (dark 12 h/light 12 h) for 5 d and transferred to simulated shade. Then the seedlings were sampled at 0, 5, 10, 30, and 60 min from ZT1 (1 h after the light exposure). The relative mRNA level of *COL7* at Zeitgeber time 1 (0 min, just before low R:FR treatment) was set to 1. (B–D) The relative mRNA level of *PIL1* in each line is indicated. The relative mRNA level of *COL7* in the WT was set to 1. Error bars represent the standard deviations derived for three biological replicates. (B) qPCR of *PIL1* in the seedlings grown in white light for 7 d. (C) qPCR of *PIL1* in seedlings grown in white light for 3 d and transferred to simulated shade for 4 d before sampling. (D) qPCR of *PIL1* in seedlings grown in far-red light for 7 d.

regulators, COL7 positively expands the capacity for shade avoidance. For instance, an elevated level of phyB or PIFs in engineered overexpression lines results in a shorter or longer hypocotyl phenotype, respectively, but both overexpressing phyB and PIFs attenuate the extent of shade-induced hypocotyl elongation (Roig-Villanova *et al*., 2006; Lorrain *et al*., 2008; Leivar and Quail, 2011). In contrast, hypocotyl growth of *35S::COL7* lines was suppressed by high R:FR but promoted by low R:FR (Fig. 2), demonstrating an increased capacity for shade-induced hypocotyl elongation. Overexpression of *COL7* also promotes branching in high R:FR and increases the sensitivity of SAS to suppress branching in low R:FR. Such an elastic branching trait of *COL7* overexpression lines in response to varying light qualities may be utilized to optimize the agricultural architecture and planting density of crops to improve production in the field.

How *COL7* dynamically regulates branching in response to competing neighbours and/or light quality is still not known. Plant branching is regulated by genes associated with the biogenesis, transport, and signal transduction of phytohormones such as auxin, strigolactone, and cytokinin. Phytohormone-associated mutants, such as *max1*, *max2*, *max3*, *max4*, *axr1*, *brc1*, and *brc2*, all showed an abundant branching phenotype similar to that displayed by *35S::COL7* lines (Stirnberg *et al*., 2002; Sorefan *et al*., 2003; Booker *et al*., 2004, 2005; Aguilar-Martinez *et al*., 2007; Brewer *et al*., 2009; Domagalska and Leyser, 2011). Low R:FR or disruption of *phyB* function can efficiently suppress the branch production in WT *Arabidopsis* plants but has little effect on the abundant branching phenotype of the phytohormone-associated mutants, indicating that SAS requires normal function of those phytohormone-associated genes to inhibit branching (Finlayson *et al*., 2010; Casal, 2012). Whether the abundant branching phenotype of *35S::COL7* lines could be suppressed by those phytohormone-associated genes in perception of low R:FR has yet to be investigated. It is summarized here that COL7 is involved in a complicated network of branching regulation and shade avoidance signal transduction, the mechanism of which need to be illustrated by further genetic and molecular investigations.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Abundance of *COL7* mRNA in the wild type, *col7* mutant, and overexpression lines.

Figure S2. *COL7* affects the hypocotyl elongation in far-red light.

Figure S3. Hypocotyl phenotype of the *35S::MYC-COL7* line.

Table S1. Sequences of primers used in this study.

Acknowledgements

This work is supported in part by the National Natural Science Foundation of China (grant no. 31171352 to BL and 30570162 to HL), the National Transgenic Crop Initiative (grant no. 2010ZX08010-002) and the China National Doctoral Fund Project (no. 755228001).

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