Article:
Wang, F, Zhang, L, Chen, X et al. (8 more authors) (2019) SlHY5 Integrates Temperature, Light and Hormone Signaling to Balance Plant Growth and Cold Tolerance. Plant physiology, 179. pp. 749-760. ISSN 0032-0889

https://doi.org/10.1104/pp.18.01140

Running title: Tomato HY5 regulates ABA and GA biosynthesis

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Research area: Plant science and Ecology

Title: SlHY5 Integrates Temperature, Light and Hormone Signaling to Balance Plant Growth and Cold Tolerance

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One-sentence summary:

Tomato phytochrome-dependent SlHY5 signaling regulates ABA and GA biosynthesis by directly binding and activating the transcription of SlGA2ox4 and SlNCED6 to balance plant growth and cold tolerance.

Footnotes:

List of author contributions

Author contributions

Y.Z. conceived and designed the experiments. F.W., X.C., X.W. and Xu.X. performed the experiments. F.W., Y.Z., and J.Y. analyzed the data. L.Z. participated in preparing plant materials. J.Z., K.S. and X.J.X. provided technical and intellectual support. Y.Z., J.Y., and C.H.F wrote the article with contributions from F.W. The authors declare no competing interests.

Funding information:

This work was supported by the National Natural Science Foundation of China (grant nos. 31825023, 31672198, 31430076), the Key Research and Development Program of Zhejiang (2018C02010), and the Fundamental Research Funds for the Central Universities (2016XZZX001-07).

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ACKNOWLEDGMENTS

We are grateful to Prof. Jim Giovannoni of Cornell University and the Tomato Genetics Resource Center at the California University for tomato seeds. We also thank Prof. Michael Thomashow of Michigan State University for the valuable suggestion during the study and Dr. Zhenyu Qi of Zhejiang University for the help in cultivation of tomato plants.

ABSTRACT

During the transition from warm to cool seasons, plants experience decreasing temperatures, shortening days and decreasing red/far-red (R/FR) ratios of light. The mechanism via which plants integrate these environmental cues to maintain plant growth and adaptation remains unclear. Here, we report that low temperature induced the transcription of PHYTOCHROME A (SlPHYA) and accumulation of LONG HYPOCOTYL 5 (SlHY5, a bZIP transcription factor), especially under conditions of short days and low R/FR ratios of light, in tomato plants. Reverse genetic approaches and physiological analyses revealed that silencing of SlHY5 increased cold susceptibility in tomato plants, while overexpression of SlHY5 enhanced cold tolerance. By directly binding and activating the transcription of a gibberellin (GA)-inactivation enzyme gene, GIBBERELLIN 2-OXIDASE 4 (SlGA2ox4), and an abscisic acid (ABA) biosynthesis enzyme gene, 9-CIS-EPOXYCAROTENOID DIOXYGENASE 6 (SlNCED6), phyA-dependent SlHY5 accumulation resulted in an increased ABA/GA ratio, which was accompanied by growth cessation and induction of cold response. Furthermore, silencing of SlNCED6 compromises SD- and L-R/FR- induced tomato resistance to cold stress. These findings provide insight into the molecular genetic mechanism via which plants integrate environmental stimuli with plant hormones to coordinate plant growth with impending cold temperatures and reveal a molecular mechanism that plants have evolved for growth and survival in response to seasonal changes.

INTRODUCTION

Unlike animals, plants are sessile and must integrate environmental stimuli to optimize growth and development and survive under adverse environmental conditions. Plants experience reduced ambient temperatures, shorter days and decreased red to far-red ratios (R/FR) of light due to vegetative shading and longer twilight durations in cool seasons and vice versa in warm seasons (Franklin et al., 2007). Meanwhile, plants usually exhibit decreased growth and improved cold tolerance with gradual cooling after the start of the fall season. This acclimation process is associated with transcript reprogramming and altered homeostasis of plant hormones such as gibberellins (GAs) and abscisic acid (ABA), leading finally to growth cessation or dormancy with subsequent tolerance of plants to freezing (Wisniewski et al., 2011). However, the molecular mechanism responsible for this long-evolved phenomenon during seasonal changes is largely unknown.

Plant growth, development, and stress response are subject to regulation by light in a phytochrome-dependent manner (Kim et al., 2002). However, light-related effects, such as the effects of photoperiods, on plant growth, development and cold response are likely to be temperature and species dependent (Chen and Li, 1976; Cockram et al., 2007; Malyshev et al., 2014; Song et al., 2015). The effects of short days (SDs) on the induction of the transcription of C-repeat binding factors (CBFs) and on the subsequent tolerance to freezing are less notable in plants originating from low latitudes than in those from high latitudes (Li et al., 2003; Lee and Thomashow, 2012). Likewise, low R/FR ratios could induce the expression of the CBF regulon only at a temperature lower than the optimum growth temperature (Franklin and Whitelam, 2007; Wang et al., 2016). These results indicated that the induction or suppression of cold tolerance is associated with the interconversion between the R-light- absorbing form (Pr) and the FR-light- absorbing form (Pfr) of phytochrome A (phyA) and phyB in a temperature-dependent manner (Rockwell et al., 2006). Mutation of phyA has been shown to decrease the cold tolerance of Arabidopsis and tomato, while that of phyB1, phyB2 or phyD has increased the cold tolerance of these plants (Franklin and Whitelam, 2007; Wang et al., 2016). Recently, phytochrome B has been suggested to function as thermal sensor that integrate temperature information over the course of the night (Jung et al., 2016; Legris et al., 2016). However, the mechanism via which plants sense environmental cues and integrate these signals with plant physiological processes to balance growth and cold response during seasonal changes remains unclear.

LONG HYPOCOTYL 5 (HY5), a basic leucine zipper (bZIP) transcription factor, acts downstream of multiple photoreceptors and regulates a subset of physiological processes, such as photomorphogenesis, pigment biosynthesis, nutrient signaling and defense response (Oyama et al., 1997; Jiao et al., 2007; Gangappa and Botto, 2016). In addition to the regulation by photoreceptors, HY5 transcript and protein stability is also subject to regulation by low temperature in a CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1)-dependent manner (Catala et al., 2011), a RING-finger E3 ubiquitin ligase that targets HY5 for proteasome-mediated degradation (Osterlund et al., 2000). Interestingly, genome-wide ChIP-chip experiments demonstrated that HY5 regulates the expression of nearly one-third of genes in Arabidopsis (Lee et al., 2007). For example, HY5 can activate abscisic acid (ABA) signaling by directly binding to the promoter of ABA INSENSITIVE 5 (ABI5) during seed germination and cold stress in Arabidopsis and tomatoes (Chen et al., 2008; Xu et al., 2014; Wang et al., 2018). Moreover, LONG1, a divergent ortholog of the Arabidopsis HY5, has a central role in mediating the effects of light on the accumulation of gibberellin (GA) in pea (Weller et al., 2009). However, it remains unknown whether SlHY5 functions as a critical regulator of the trade-off between plant growth and cold response in response to light-quality, photoperiod and temperature signals during seasonal changes. Specifically, the molecular mechanism by which SlHY5 regulates ABA and GA biosynthesis to maintain plant growth and adaptation is unclear.

RESULTS

Roles of Phytochromes in Cold Acclimation, Short Days and Low R/FR-Induced Cold Tolerance

We previously found that phyA and phyB are positive and negative regulators, respectively, of cold tolerance in tomato (Wang et al., 2016). To reveal the mechanism of plant response to both light (light-quality and photoperiod) and temperature signaling, we tested the transcriptions of light signaling-, cold response- and plant growth- related genes, such as SlPHYA, SlPHYBs, SlCBF1 and SlDELLA genes. We found that the transcription of SlPHYA was induced while that of SlPHYB1 and SlPHYB2 was reduced in plants under SD (8 h) and low R/FR (L-R/FR, 0.5) conditions compared to those under long day (LD, 16 h) and high R/FR (H-R/FR, 2.5) conditions at 25 °C (Fig. 1, A and B; Supplemental Fig. S1A). Importantly, exposure to a suboptimal growth

temperature of 10 °C (cold acclimation, CA) further increased the transcript levels of SlPHYA but suppressed the transcription of SlPHYB1 and SlPHYB2, especially under SD and L-R/FR conditions. A combination of CA with SD and L-R/FR resulted in an 18-fold increase in the transcript levels of SlPHYA and in decreased transcription of SlPHYB1 and SlPHYB2 by 86% and 92%, respectively, compared to the values seen in plants grown at 25 °C under LD and H-R/FR light conditions. DELLA proteins, encoded by DELLA genes, play critical roles by inhibiting GA signaling in plant growth and cold response (Achard et al., 2008; Zhou et al., 2017). Gene silencing experiments demonstrated that a tomato SlDELLA gene called PROCERA (SlPRO) is the predominant gene among the tomato SlDELLA family genes (GA INSENSITIVE, SlGAIs) responsible for plant elongation (Supplemental Fig. S1, B and C; Jones, 1987). We found that the transcription of SlPRO was decreased in plants under SD with L-R/FR conditions compared to those under LD and H-R/FR conditions at 25 °C. Importantly, CA significantly induced the expression of SlPRO, especially in combination with SD and L-R/FR conditions (Fig. 1C). Meanwhile, transcription of GA-INSENSITIVE DWARF1 (SlGID1), the receptor of GA, was induced by either L-R/FR or SD at 25 °C but suppressed by low temperatures, especially under SD conditions (Supplemental Fig. S1D). While light quality and photoperiod had little effect on the transcription of SlCBF1 in plants grown at 25 °C, CA significantly induced the transcription of SlCBF1, especially under SD and L-R/FR conditions (Fig. 1D). These results indicated that light had greater effects on phytochromes, GA signaling and the CBF-pathway at low temperatures than at high temperatures. The low temperatures, short days and low R/FR ratios in cool seasons could efficiently induce SlPHYA and SlCBF1 expression but suppress SlPHYB expression and GA signaling.

We then examined whether the light conditions required for growth are associated with cold sensitivity. By using relative electrolyte leakage (REL) as an indicator of cold tolerance, we found that the growth photoperiod and R/FR ratio before cold treatment did not alter the cold tolerance, since pretreatment with photoperiod and R/FR ratio before cold treatment did not alter the changes in REL (Supplemental Fig. S2A). However, the light conditions during chilling had significant effects on cold tolerance; plants subjected to SD, L-R/FR or both exhibited greater tolerance to chilling than those subjected to either LD or H-R/FR (Supplemental Fig. S2B). These results suggested that the integration of light signaling and cold stimuli is essential for the induction of cold tolerance. To determine whether the different responses, in terms of accumulation of the

phytochrome transcript, to variations in temperature, photoperiod and light quality are associated with cold tolerance, we exposed the WT and a set of phytochromes mutants (phyA, phyB1B2 and phyAB1B2) of tomato plants to LD or SD with L- or H-R/FR conditions at 25 °C or 10 °C for 7 d (CA), which was followed by chilling at 4 °C with identical light conditions for 7 d (Fig. 1E). The results indicated that phyA mutant plants were shorter while the phyB1B2 mutant plants were taller than WT plants at 25 °C (Supplemental Fig. S3). After chilling stress, phyA mutant plants always exhibited decreased chilling tolerance, while phyB1B2 plants always exhibited increased chilling tolerance relative to the WT plants, as indicated by the increased and decreased REL relative to the REL in WT plants (Fig. 1E). WT and phyB1B2 plants showed greater tolerance under SD and L-R/FR conditions relative to those under LD and H-R/FR conditions, respectively, regardless of CA. In contrast, CA and SD induced the tolerance of all plants to chilling stress; L-R/FR increased the tolerance of only WT and phyB1B2 plants but not of plants mutated in phyA (phyA and phyAB1B2). Based on these results, we conclude that the tomato phyA and phyB function antagonistically to regulate the adaptation of plants to the changes in temperature, photoperiod and light quality.

SlHY5 Inhibits Plant Growth and Induces Cold Tolerance by Integrating Both Light and Temperature Signaling

Multiple photoreceptors promote the accumulation of LONG HYPOCOTYL 5 (HY5) under specific light conditions, possibly by reducing the nuclear abundance of CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), an E3 ubiquitin ligase targeting HY5 for proteasome-mediated degradation in the dark (Osterlund et al., 2000; Yi and Deng, 2005). Here, we found that the effects of photoperiod and light quality on the SlHY5 and SlCOP1 transcript levels are largely dependent on growth temperature. Transcription of either SlHY5 or SlCOP1 was slightly altered by the photoperiod and by the R/FR ratio in plants at 25 °C (Supplemental Fig. S4). Interestingly, CA significantly induced the transcription of SlHY5 in WT and phyB1B2 plants, with the effect being more significant in phyB1B2 plants, especially under SD and L-R/FR light conditions (Fig. 2A). However, transcription of SlHY5 showed few changes in response to CA, photoperiod and R/FR in phyA and phyAB1B2 plants. In contrast, the CA-induced transcription of SlCOP1 was suppressed by either SD or L-R/FR in WT and phyB1B2 plants, especially in phyB1B2; and the transcription of COP1 was suppressed by SD but

not by L-R/FR in phyA and phyAB1B2 plants (Supplemental Fig. S5A). Finally, phyB1B2 plants had decreased transcript levels of SlCOP1 relative to those of WT plants throughout the treatment. Additional experiments with monochromic R and FR lights revealed that R light induced the transcription of SlCOP1 but suppressed the transcription of SlHY5, while FR induced the transcription of SlHY5 but suppressed the transcription of SlCOP1 at low temperatures; all of these effects were dependent on phyB or phyA (Supplemental Fig. S5, B and C). Therefore, efficient induction of the SlHY5 transcript is dependent on phyA in tomato plants in response to changes in growth temperature, photoperiod and light quality. By using an SlHY5-overexpressing line (SlHY5-OE) carrying a 3HA tag, we found that low temperatures increased the accumulation of the SlHY5 protein, which was increased under SD and L-R/FR conditions (Fig. 2B). These results suggested that SlHY5 levels are tightly controlled by temperature and light transcriptionally, via a phytochrome-dependent pathway, and posttranslationally, via protein stabilization.

To determine whether SlHY5 is involved in the integration of light and temperature stimuli to regulate plant growth and cold tolerance, we compared plant elongation and cold tolerance in tomato plants of the WT, SlHY5-RNAi and SlHY5-overexpressing (SlHY5-OE) lines in response to changes in growth temperature, photoperiod and R/FR ratio. We found that the SlHY5-RNAi plants were taller while the SlHY5-OE plants were shorter than WT plants at 25 °C or after CA (Fig. 2C). Meanwhile, SlHY5-RNAi plants exhibited increased while SlHY5-OE plants exhibited decreased sensitivity to chilling stress, as indicated by the changes in REL and maximum photochemical efficiency of PSII (Fv/Fm) regardless of CA (Fig. 2D; Supplemental Fig. S6A). While CA decreased REL and increased the Fv/Fm ratio, especially under conditions of SD, L-R/FR or both in the WT and SlHY5-OE plants, this positive effect on chilling tolerance was almost abolished in the SlHY5-RNAi plants. Meanwhile, CA induced transcript accumulation of SlCBF1 and associated genes (SlCOR47-like, SlCOR413-like), and in WT plants, the effects were highly significant under L-R/FR and SD conditions (Supplemental Fig. S6, B-D). Importantly, this induction was highly significant in SlHY5-OE plants and was mostly abolished in SlHY5-RNAi plants. Therefore, SlHY5 plays a positive regulatory role in the cold tolerance of tomato plants by integrating temperature, photoperiod and light quality signals.

SlHY5 Directly Activates SlGA2ox4 Expression and Suppresses the Accumulation of GAs

GAs play a critical role in plant growth and are also negative regulators of cold tolerance and growth cessation (Achard et al., 2008; Sun, 2011; Zhou et al., 2017). To determine whether SlHY5 participates in the regulation of GA homeostasis and subsequent plant growth, we analyzed the changes in GA levels in plants. The levels of active GAs (GA_1 and GA_4), their precursors (GA_{20} and GA_9) and their metabolites (GA_8 and GA_{34}) were higher in SlHY5-RNAi plants, and lower in SlHY5-OE plants, than in WT plants under H-R/FR and LD conditions at 25 °C (Fig. 3). Meanwhile, accumulation of these GAs decreased after CA under L-R/FR and SD conditions; in particular, the levels of GA_9 were too low to be detected. To determine whether SlHY5 participates in the regulation of GA homeostasis by deactivating GAs, we analyzed the expression of the major GA deactivation genes GA2-oxidases (SlGA2oxs) (Schomburg et al., 2003; Yamaguchi, 2008). Among these SlGA2ox genes, transcription of SlGA2ox4 was induced by low temperatures under SD and L-R/FR conditions, with SlHY5-RNAi plants exhibiting lower, but SlHY5-OE plants exhibiting higher, transcript levels of SlGA2ox4 than WT plants (Fig. 4A). However, such an SlHY5-dependent change in the transcript levels was not observed for other SlGA2ox genes (Supplemental Fig. S7A). Promoter analysis revealed that there are three ACGT-containing elements (ACE-boxes; nucleotides −115 to −112, nucleotides −338 to −335 and nucleotides −2347 to −2344), which are HY5-binding cis-elements (Lee et al., 2007), in the 2500-bp region of the SlGA2ox4 promoter (Supplemental Fig. S7B). Electrophoretic mobility shift assay (EMSA) showed that HY5 was able to bind to the biotin-labeled probes containing an ACE-box (nucleotides −124 to −104), leading to a mobility shift, but the binding ability to the SlGA2ox4 promoter was reduced, and even lost, when the promoter was mutated in the ACE elements (ACE-mut; Fig 4B; Supplemental Fig. S7C). ChIP-qPCR analyses showed that the GA2ox4 promoter sequence was significantly enriched in the 35S: SlHY5-HA (SlHY5-OE) samples pulled down by the anti-HA antibody compared to the WT control samples. No enrichment of the IgG control was observed (Fig. 4C). Therefore, HY5 directly associates with the promoter sequence of GA2ox4 and activates the expression of SlGA2ox4. These results suggested that SlHY5 is a hub for temperature, photoperiod and light quality stimuli, regulating plant growth via GA inactivation.

SlHY5 Binds to SlNCED6 Promoter, Activates Its Transcription and Promotes ABA Accumulation during Cold Stress

ABA plays a critical role in the response to cold stress and frequently functions as a regulator of bud formation in cool seasons (Knight et al., 2004; Ruttink et al., 2007; Lee and Luan, 2012; Tylewicz et al., 2018). We found little difference in ABA accumulation among WT, SlHY5-RNAi and SlHY5-OE plants at 25 °C (Fig. 5A). However, a decrease in growth temperature from 25 °C to 10 °C significantly induced the ABA accumulation and transcription of ABA pathway genes (SlAREB, SlABF4), especially under L-R/FR and SD conditions in WT plants (Fig 5A; Supplemental Fig. S8). However, such induction was greater in SlHY5-OE plants but attenuated in SlHY5-RNAi plants regardless of the photoperiod and light quality conditions applied. We then examined whether SlHY5 could bind to the promoters of ABA biosynthetic genes by analyzing the 2.5-kb promoter regions of a set of ABA biosynthetic genes. The G-box (CACGTG) was found in the upstream regions of four ABA biosynthesis genes, i.e., SlNCED1, SlNCED2/5, SlNCED6 and SlSit (Sitiens, an ABA aldehyde oxidase gene; Supplemental Fig. S9A). EMSA showed that SlHY5 was able to bind to two biotin-labeled probes of the SlNCED6 promoter (nucleotides −1780 to −1761 and nucleotides −168 to −149) and caused mobility shift but failed to bind to the probes of the SlNCED1, SlNCED2/5 and SlSit promoters (Fig 5B; Supplemental Figs. S9B and S10). When the core sequence of the G-box motif in the SlNCED6 probes was mutated in a single base (SlNCED6-G1/G2-mut2) or in multiple bases (SlNCED6-G1/G2-mut1), the binding ability of SlHY5 to the probes was reduced, and even lost (Fig 5B; Supplemental Fig. S10). Following ChIP-qPCR analysis with an anti-HA antibody, the SlNCED6 promoter was significantly enriched in 35S: SlHY5-HA samples compared to the WT control, whereas the IgG control was not enriched (Fig. 5C). Consistent with this result, SlNCED6 transcription was induced to a greater extent in SlHY5-OE plants than in WT plants by CA, especially under SD and L-R/FR conditions, but poorly induced in SlHY5-RNAi plants (Fig. 5D). These results indicated that SlHY5 positively regulated ABA biosynthesis by directly binding to the promoter of SlNCED6 and activating its transcription in response to cold stress.

SlNCED6 is Essential for Cold Acclimation, Short Days and Low R/FR-Induced Cold Tolerance

Consistent with the regulation of SlHY5 by phytochromes, SD and L-R/FR, alone or in combination, significantly induced the transcription of SlNCED6 in WT and phyB1B2 plants, with the effect being greater in phyB1B2 plants under cold conditions (Supplemental Fig. S11A). However, the transcript levels of SlNCED6 showed little change in response to changes in photoperiod and R/FR ratio in phyA and phyAB1B2 plants. In addition, R light suppressed the transcription of SlNCED6 in WT and phyA plants but had little effect in phyB1B2 and phyAB1B2 plants (Supplemental Fig. S11B). In contrast, FR light induced the transcription of SlNCED6 in WT and phyB1B2 plants but had little effect in phyA and phyAB1B2 plants. Taken together, our results strongly suggest that phyA and phyB act antagonistically to regulate low temperature-, photoperiod- and light quality-dependent ABA biosynthesis in an SlHY5-dependent manner.

To assess the role of SlNCED6 in cold response, we generated SlNCED6-silenced (pTRV-SlNCED6) tomato plants (Supplemental Fig. S12A). pTRV-SlNCED6 plants exhibited a 75% reduction in the transcript levels and a 57% reduction in ABA accumulation relative to pTRV plants, but no differences in Fv/Fm and REL were observed between pTRV-SlNCED6 plants and pTRV plants grown under optimal growth conditions (Supplemental Fig. S12, B and C). However, nonacclimated pTRV-SlNCED6 plants showed increased sensitivity to chilling at 4 °C under LD and H-R/FR conditions compared with pTRV plants, as evidenced by the decreased Fv/Fm and increased REL (Fig 6, A and B; Supplemental Fig. S13A). When the same cold stress was imposed in cold-acclimated plants, expression of the key genes of the CBF pathway, such as SlCBF1, SlCOR47-like and SlCOR413-like, and ABA pathway genes (SlAREB and SlABF4) were highly attenuated in pTRV-SlNCED6 plants relative to pTRV plants (Fig. 6, C-F; Supplemental Fig. S13B). Therefore, SlNCED6 is essential for the induction of the SlCBF regulon and ABA signaling in response to changes in growth temperature and light conditions.

DISCUSSION

Plants must sense seasonal changes and respond it by integrating temperature, photoperiod and light-quality stimuli for growth and the correct induction of cold tolerance. Plants grow vigorously in spring and summer and exhibit decreased or even stop growth in fall and autumn with the changes in growth temperature, day length and R/FR ratio. For a long time, the role of phytochromes in the adaptation to the seasonal

changes has been ignored. Recently, phyB photoreceptor has been found to functions as a thermal sensor in the regulation of elongation growth in Arabidopsis at temperatures of 20~28°C (Jung et al., 2016; Legris et al., 2016). Warmer temperatures spontaneously accelerate the phyB switching it from an active Pfr state to an inactive Pr state, which promotes the activity of PIFs and its ability to activate gene expression to control plant expansion growth (Jung et al., 2016). Consistent with this, the phyB mutants were taller than WT at 25 °C (Supplemental Fig. S3). Notably, transcript of SlPHYA was significantly increased whilst that of SlPHYB1 and SlPHYB2 was significantly decreased in response to the decrease in growth temperatures, day length and the R/FR ratio (Fig. 1, A and B; Supplemental Fig. S1A), which was followed by increase in the transcript of CBFs and cold tolerance (Fig. 1, D and E). Recent studies have established the role of different phytochromes in cold response by regulating the expression of several COR genes through the CBF-pathway in different plant species (Williams et al., 1972; McKenzie et al., 1974; Franklin and Whitelam, 2007). In agreement with these studies, tomato phyA mutants had decreased chilling tolerance with decreased transcript of CBF1, while phyB1B2 mutants had increased chilling tolerance with increased transcript of CBF1 relative to the WT plants (Wang et al., 2016, Fig. 1E). Importantly, such a difference in cold tolerance or CBF1 transcript is day length- and R/FR ratio-dependent (Wang et al., 2016, Fig. 1E). These results suggested that plants have evolved phytochromes-dependent adaptation mechanism to cope with the changes in growth temperature, day length and R/FR ratio during the seasonal transmit. While phyB is important for plant elongation at modest growth temperatures, phyA is likely important for balancing plant growth and cold adaptation by integrating the seasonal cues like temperature, day length and R/FR ratio.

HY5 acts downstream of multiple photoreceptors and mediates light signaling in many physiological processes in plants (Gangappa and Botto, 2016). The finding that phyA and phyB have different roles in photoperiodic and light quality regulation of the SlHY5 transcript and thereby affect cold tolerance adds to the rapidly growing list of biological function for SlHY5 proteins in tomato plants (Figs. 1E and 2A). Previous studies indicated that low temperature could stabilize AtHY5 protein at posttranslational level through the nuclear exclusion of AtCOP1 (Catala et al., 2011), whilst AtHY5 induces its expression by directly binding to its own promoter (Abbas et al., 2014; Binkert et al., 2014). Moreover, once the AtHY5 protein levels have increased triggering the induction of anthocyanin biosynthesis genes, the transcription of

prefoldins (AtPFDs) genes would be activated (Perea-Resa et al., 2017). AtPFDs protein would accumulate in the nucleus via an AtDELLA-dependent mechanism, which then interacts with AtHY5 and promotes AtHY5 polyubiquitination and subsequent proteasome-mediated degradation via an AtCOP1-independent pathway (Perea-Resa et al., 2017). This regulation would ensure the appropriate levels of HY5 all along the cold acclimation response. In agreement with this finding, we found that gradual cooling accompanied by short days and decreased R/FR ratios initially induced a phyA-dependent SlHY5 accumulation (Fig. 2B). Meanwhile, changes to SD and L-R/FR ratio at low temperature induced a down-regulation of SlCOP1 (Supplemental Fig. S5A), allowing HY5 stabilization and the activation of light-responsive genes (Osterlund et al., 2000). To characterize the functions of SlHY5 in plant growth and cold response, SlHY5-suppressing tomato plants (SlHY5-RNAi) and SlHY5-overexpressing tomato plants (SlHY5-OE) were obtained (Liu et al., 2004; Wang et al., 2018). We found that silenced SlHY5 abolished CA, photoperiod and light quality signaling-induced cold tolerance, while overexpressing SlHY5 in tomato plants increased their cold tolerance (Fig. 2D; Supplemental Fig. S6). In addition, the SlHY5-RNAi plants were taller while the SlHY5-OE plants were shorter than WT plants at 25 °C or after CA (Fig. 2C). Based on the changes in SlHY5 levels with plant height and SlCBF1 transcript as well as plant growth and chilling tolerance in response to CA, photoperiod and R/FR ratio, we conclude that SlHY5 is involved in the integration of light and temperature stimuli to regulate plant growth and cold tolerance during the seasonal changes.

Plants usually grow fast in late spring and summer, slow in fall and stop growth in winter, when they require the greatest tolerance to cold stress. The development of tolerance or resistance is therefore at the expense of plant growth. ABA and GA are classic phytohormones, which antagonistically control diverse aspects of plant development and abiotic stress response (Razem et al., 2006; Shu et al., 2013, 2018a). It has been proposed that several key transcription factors, including AtABI4 and OsAP2-39, directly or indirectly control the transcription pattern of ABA and GA biosynthesis genes to regulate the balance between ABA and GA (Yaish et al., 2010; Shu et al., 2013, 2018b). GAs play a positive role in plant growth and a negative role in plant cold tolerance (Achard et al., 2008; Sun, 2011; Zhou et al., 2017). Interestingly, we found that SlHY5 could suppress the accumulation of GAs in tomato plants leading to plant growth cessation (Figs. 2C and 3). In agreement with a previous

study showing that pea mutants of long1 (a divergent ortholog of the Arabidopsis HY5) exhibited decreased GA accumulation (Weller et al., 2009), we found that SlHY5-OE had lower whilst SlHY5-RNAi plants had higher GA accumulation relative to WT plants (Fig. 3). EMSA and ChIP-qPCR assays both showed that SlHY5 directly binds to the conserved motif of SlGAox4, a major GA deactivation gene, activates its expression and negatively regulates bioactive GA accumulation (Fig. 4; Supplemental Fig. S7, B and C). Therefore, SlHY5 participates in the regulation of GA accumulation by GA deactivation in plants. Meanwhile, we found that SlHY5 levels and ABA accumulation were coincidently induced by SD and L-R/FR at low temperature (Figs. 2, A and B, and 5A). This increase is attributable to the SlHY5 directly binding to the promoter of NECD6, a key gene in ABA biosynthesis, and triggering its expression (Fig. 5, B-D; Supplemental Fig. S9 and S10). As in phyA plants, suppressed transcription of SlHY5 in SlHY5-RNAi plants abolished low temperature-induced, SD- and L-R/FR-promoted ABA accumulation, SlCBF1 transcription and cold tolerance (Fig. 6A; Supplemental Fig. S6, A and B). Our study also demonstrated the role of ABA biosynthesis in the development of cold tolerance as SlNECD6 is essential for low temperature-induced, SD- and L-R/FR-promoted ABA accumulation, SlCBF1 transcript and cold tolerance (Fig. 6; Supplemental Figs. S12 and S13). This finding is in agreement with earlier observation that ABA biosynthesis is important for the expression of COR genes in the cold response (Gilmour and Thomashow, 1991; Mantyla et al., 1995). All these results provided convincing evidence that SlHY5 is negative regulator of plant growth by activating the GA deactivation and a positive regulator of cold adaptation by activating ABA biosynthesis.

Our data suggest a new conceptual framework for understanding how plants integrate the seasonal stimuli with growth and environmental adaptation. Under optimal growth temperature, plants accumulate less SlHY5 with vigorous growth and high sensitivity to cold due to the high GA/ABA ratio (Fig. 7). Gradual cooling accompanied by short days and decreased R/FR ratios can induce phyA-dependent SlHY5 accumulation. Increased accumulation of SlHY5 resulted in a decrease in the GA/ABA ratio with growth cessation and an increase in cold tolerance. Phytochrome-dependent SlHY5 may function as a critical regulator of the trade-off between plant growth and stress response in plants. Our results not only explain the different growth potentials and cold sensitivities of plants growing in different seasons but also suggest that plants have evolved a phytochrome-dependent, SlHY5-mediated adaptation strategy by sensing and

integrating environmental cues with hormone signaling during seasonal changes. This mechanism is likely involved in the regulation of other physiological processes such as seed germination, diurnal growth rhythm and bud dormancy, which are controlled by temperature, light stimuli and hormones (Chen et al., 2008; Li et al., 2011; Tylewicz et al., 2018).

MATERIALS AND METHODS

Plant Materials and Constructs

Seeds of WT tomato (Solanum lycopersicum), 'cv. Ailsa Craig' and 'cv. Moneymaker', and the tomato phytochromes mutants, such as phyA, phyB1B2, and phyAB1B2 mutants in the cv. Moneymaker background were obtained from the Tomato Genetics Resource Center (http: //tgrc.ucdavis.edu). The HY5-RNAi lines in the cv. Ailsa Craig background were generously provided by Professor Jim Giovannoni (Cornell University, USA) (Liu et al., 2004). The SlHY5 overexpressing plants were generated as described previously (Wang et al., 2018). Tobacco rattle virus (TRV)-based vectors (pTRV1/2) were used for virus-induced gene silencing (VIGS) of the SlNCED6 gene and SlDELLA family genes (GA INSENSITIVE, SlGAIs) (Liu et al., 2002). The complementary DNA fragments of the SlNCED6 and tomato SlDELLA genes were amplified by PCR using the gene-specific primers listed in Supplemental Table S1. VIGS was performed as described previously (Wang et al., 2016). Tomato seedlings were grown in a growth room with 12 h photoperiod, temperature of 22 °C /20 °C (day/night), and photosynthetic photon flux density (PPFD) of 600 µmol m^{-2} s^{-1}.

Cold and Light Treatments

Plants at the 4-leaf stage were used for all experiments, which were carried out in controlled-environment growth chambers (Zhejiang QiuShi Artificial Environment Co., Ltd, China). To determine the effects of photoperiod and light quality on the subsequent cold tolerance, tomato plants were grown at 25 °C /22 °C under conditions of LD (16 h) or SD (8 h) with H-R/FR (2.5) light or L-R/FR (0.5) light for 7 d. After that all of them were transferred to a cold stress (4 °C) under white light (WL) with PPFD of 120 µmol m^{-2} s^{-1} for 7 d. For the light quality treatment, R light (λ_{max} = 660 nm, Philips, Netherlands) was maintained at a PPFD of 120 µmol m^{-2} s^{-1} and FR light (λ_{max} = 735 nm, Philips, Netherlands) was supplemented. The R/FR ratio was calculated as the quantum flux densities from 655 to 665 nm divided by the quantum flux density from 730 to 740 nm. To determine the effects of both photoperiod and light quality during cold stress, tomato plants were first grown at white light (WL) conditions under 25 ^{o}C for 7 d, then they were exposed to a low temperature of 4 °C under conditions of LD or SD with H-R/FR or L-R/FR light, respectively, for 7 d. To determine the combined effects of CA, photoperiod and light quality, plants were gown at 25 °C or 10 °C under

conditions of LD or SD with H-R/FR or L-R/FR light for 7 d before being subjected to a low temperature of 4 °C with the same light conditions as before.

Cold Tolerance Assays and Plant Height Measurement

Membrane permeability, in terms of relative electrolyte leakage (REL), was determined after plant exposure to cold stress for 7 d by a previously described method (Cao et al., 2007). The maximum quantum yield of PSII (Fv/Fm) was measured with the Imaging-PAMsetup (IMAG-MAXI; Heinz Walz, Germany) as previously described (Jin et al., 2014). The plant height was measured for least 10 tomato seedlings from each treatment.

Determination of ABA and GA Levels

Endogenous ABA was extracted and quantified from tomato leaves by LC/MS-MS on an Agilent 1290 Infinity HPLC system coupled to an Agilent 6460 Triple Quad LC-MS device (Agilent Technologies, USA), as described previously (Wang et al., 2016). GA levels were determined from 1-g samples of tomato leaves by a derivation approach coupled with nano-LC-ESI-Q-TOF-MS analysis as described previously (Chen et al., 2012; Li et al., 2016). For the determination of GA levels, the extraction solution was spiked with D_2-GA_1, D_2-GA_4, D_2-GA_8, D_2-GA_9, D_2-GA_{20} and D_2-GA_{34}.

Phylogenetic Analysis

Sequence alignment and phylogenetic tree construction were performed with the MEGA program (version 5.05). A consensus neighbor-joining tree was obtained from 1000 bootstrap replicates of aligned sequences. The percentage at branch points represents the posterior probabilities of amino acid sequences. Sequence alignments with different tomato (Solanum lycopersicum) reference sequences were from the Sol genomics network (available at: http://solgenomics.net/) or NCBI (available at: http://www.ncbi.nlm.nih.gov/).

RNA Extraction and qRT-PCR Analysis

Total RNA was extracted from tomato leaves using an RNAprep Pure Plant Kit (Tiangen Biotech Co., Ltd., Beijing, China) following the manufacturer's recommendations. The extracted RNA was reverse transcribed using a ReverTra Ace qPCR RT Kit with an enzyme for genomic DNA removal (Toyobo, Osaka, Japan). qRT-PCR experiments were performed on a LightCycler 480 II detection system

(Roche, Germany) with a SYBR Green PCR Master Mix Kit (TaKaRa, Japan). The PCR was performed with 3 min at 95 °C, which was followed by 40 cycles of 30 s at 95 °C, 30 s at 58 °C and 1 min at 72 °C. The tomato ACTIN2 gene was used as an internal control to calculate relative expression (Livak and Schmittgen, 2001). Gene-specific primer sequences can be found in Supplemental Table S2.

Immunoblotting Assays

35S:SlHY5-HA fusion proteins after CA or under normal conditions of LD or SD with H-R/FR or L-R/FR light for 5 d, were extracted from SlHY5-overexpressing tomato plants by homogenization in extraction buffer (50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 150 mM NaCl, 0.1% β-mercaptoethanol, 0.2% Triton X-100, 1 mM PMSF, and plant protease inhibitor cocktail). Protein concentrations were measured using Coomassie stain as described previously (Bradford, 1976). Equal amounts of total protein from each sample were subjected to SDS-PAGE (15% polyacrylamide) and electrotransferred to nitrocellulose membranes (BioRad, Hercules, CA, USA). The proteins were immunoblotted with anti-HA primary antibody (Cat. no. 26183; Pierce, USA) and subsequently with horseradish-peroxidase-conjugated secondary antibody (antigoat, Invitrogen, Sweden). The signals were detected with enhanced chemical luminescence (ECL).

Recombinant Proteins and Electrophoretic Mobility Shift Assay (EMSA)

The pET-32a-His-SlHY5 construct was generated using the full-length coding region of HY5 with the primers listed in Supplemental Table S1 and by restriction digestion using the BamHI and SacI sites of the pET-32a vector. The recombinant vector was transformed into Escherichia coli strain BL21 (DE3). The His-SlHY5 recombinant proteins were expressed and purified from E. coli following the manufacturer's instructions for the Novagen pET purification system. For the binding assay, probes were end-labeled with biotin following the manufacturer's instructions for the Biotin 3' End DNA Labeling Kit (Cat. no. 89818; Pierce, USA) and annealed to double-stranded probe DNA. EMSAs were performed using a LightShift Chemiluminescent EMSA Kit (Cat. no. 20148; Thermo Fisher Scientific, USA). The reaction mixture was loaded onto a 6% non-denaturing polyacrylamide gel in Tris-glycine buffer, electrophoresed at 100 V, transferred to a positive nylon membrane, and subjected to UV crosslinking. Finally, the protein-DNA signals were detected by chemiluminescence using the LightShift Chemiluminescent EMSA Kit (Cat. no. 20148; Thermo Fisher Scientific, USA) and

autoradiographed. The DNA probes used in the EMSA are shown in Supplemental Table S3.

Chromatin Immunoprecipitation (ChIP) Assay

ChIP assays were performed following the manufacturer's instructions for the EpiQuik^{TM} Plant ChIP Kit (Cat. no. P-2014; Epigentek, USA) as previously described (Li et al., 2011). Approximately 1 g of leaf tissue was harvested from SlHY5-OE#1 and WT plants, which were grown at 10 °C under conditions of SD with L-R/FR for 5 d and were treated with formaldehyde to crosslink the protein-DNA complexes. The chromatin samples were immunoprecipitated with an anti-HA antibody (Cat. no. 26183; Pierce, USA), and goat antimouse IgG (Cat. no. AP124P; Millipore, USA) was used as a negative control. Quantitative RT-PCR (RT-qPCR) was performed to identify enriched DNA fragments by comparing the immunoprecipitates with the inputs. Primers of the SlNCED6 and SlGA2ox4 promoters are listed in Supplemental Table S4.

Statistical Analysis

The experimental design was a completely randomized block design with three replicates. Each replicate contained ten plants. Analysis of variance (ANOVA) was used to test for significance. When interaction terms were significant ($P<0.05$), differences between means were analyzed using Tukey comparisons. Significant differences between treatment means are indicated by different letters.

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the accession numbers listed in Supplemental Tables S2, S3 and S4.

583 **Supplemental Data**

584 The following supplemental materials are available.

585 **Supplemental Figure S1.** Effect of temperature, photoperiod and light quality on genes
586 expression of SlPHYB2 and SlGID1, and plant height of tomato DELLA family genes
587 -silenced plants.

588 **Supplemental Figure S2.** Photoperiod and light quality regulation of cold tolerance
589 needs to be concurrent with low temperatures.

590 **Supplemental Figure S3.** The phytochromes mutants in tomato plants.

591 **Supplemental Figure S4.** Transcript levels of SlHY5 (A) and SlCOP1 (B) genes in
592 tomato plants grown at 25 °C for 5 d under long day (LD, 16 h) or short-day (SD, 8 h)
593 with high R/FR ratio (H-R/FR, 2.5) light or low R/FR ratio (L-R/FR, 0.5) light.

594 **Supplemental Figure S5.** Regulation of SlHY5 and SlCOP1 genes expression by cold
595 acclimation, photoperiod and light quality is phytochrome-dependent.

596 **Supplemental Figure S6.** The positive role of SlHY5 in tomato cold tolerance
597 regulated by temperature, photoperiod and light quality during the seasonal variation.

598 **Supplemental Figure S7.** Expression of SlGA2oxs family genes in WT, HY5-RNAi and
599 HY5-OE tomato plants and promoter analysis of tomato SlGA2ox4 gene.

600 **Supplemental Figure S8.** Regulation of SlAREB and SlABF4 genes expression by cold
601 acclimation, photoperiod and light quality in WT, HY5-RNAi and HY5-OE tomato
602 plants.

603 **Supplemental Figure S9.** The binding abilities of SlHY5 to the promoters of ABA
604 biosynthetic genes.

605 **Supplemental Figure S10.** SlHY5 directly binds to the G-boxes in the promoter of
606 SlNCED6.

607 **Supplemental Figure S11.** Regulation of SlNCED6 expression by cold acclimation,
608 photoperiod and light quality is phytochrome-dependent.

609 **Supplemental Figure S12.** The SlNCED6-silenced tomato plants.

610 **Supplemental Figure S13.** Tomato SlNCED6 positively regulates cold tolerance in
611 response to changes of temperature, photoperiod and light quality during seasonal
612 variation.

Supplemental Table S1. PCR primer sequences used for vector construction.

Supplemental Table S2. List of primer sequences used for qRT-PCR analysis.

Supplemental Table S3. Probes used in the electrophoretic mobility shift assays (EMSA).

Supplemental Table S4. Primers used for ChIP-qPCR assays.

FIGURE LEGENDS

Figure 1. Cold tolerance of tomato phytochrome mutants in response to the variation of temperature, photoperiod and light quality. A-D, Transcripts of phytochromes (SlPHYA, A; SlPHYB1, B), PROCERA (SlPRO, C) and SlCBF1 (D) genes as influenced by temperature, photoperiod and light quality in tomato plants. Plants grown at 25 °C or 10 °C under long-day (LD, 16 h) or short-day (SD, 8 h) conditions with high R/FR (H-R/FR, 2.5) light or low R/FR (L-R/FR, 0.5) light for 5 d. E, The relative electrolyte leakage was measured after wild-type (WT) and phytochrome mutants (phyA, phyB1B2 and phyAB1B2) in tomato plants were exposed to 25 °C or 10 °C under LD or SD with H-R/FR or L-R/FR light conditions for 7 d followed by cold temperature at 4 °C with identical light conditions for 7 d. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. Data are presented as the means of three biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 2. Temperature- and light signal- regulated SlHY5 is associated with plant growth and cold tolerance. A, Transcript of the SlHY5 gene after the tomato phytochrome mutants were exposed to a low temperature under long-day (LD, 16 h) or short-day (SD, 8 h) conditions with high R/FR (H-R/FR, 2.5) light or low R/FR (L-R/FR, 0.5) light for 5 d. B, Accumulation of SlHY5 protein in tomato HY5-overexpressing (HY5-OE) plants at 25 °C or 10 °C under LD or SD conditions with H-R/FR or L-R/FR light for 5 d. C, Plant height in WT, HY5-RNAi and HY5-OE after tomato plants were grown at two temperatures with different light conditions for 5 d (n=15). D, Fv/Fm of tomato wild-type (WT), HY5-RNAi and HY5-OE plants exposed to 25 °C or 10 °C under LD or SD conditions with H-R/FR or L-R/FR light for 7 d followed by cold treatment at 4 °C with identical light conditions for 7 d. The false-color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple), representing the level of damage in the leaves. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. Data are presented as the means of three biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 3. SlHY5 regulation of GA homeostasis in response to the variation of temperature, photoperiod and light quality. Levels of active GAs (GA_1 and GA_4), their precursors (GA_{20} and GA_9) and their metabolites (GA_8 and GA_{34}) in WT, HY5-RNAi

and HY5-OE tomato plants exposed to 10 °C under short-day (SD, 8 h) conditions with low R/FR (L-R/FR, 0.5) light or to 25 °C under long-day (LD, 16 h) conditions with high R/FR (H-R/FR, 2.5) light for 5 d. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. Data are presented as the means of three biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 4. SlHY5 directly binds to the SlGA2ox4 promoter and activates its transcription. A, Expression of SlGA2ox4 in WT, HY5-RNAi and HY5-OE tomato plants exposed to 10 °C under short-day (SD, 8 h) conditions with low R/FR (L-R/FR, 0.5) light or to 25 °C under long-day (LD, 16 h) conditions with high R/FR (H-R/FR, 2.5) light for 5 d. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. B, EMSA assay. The His-HY5 recombinant protein was incubated with biotin-labeled wild-type (GA2ox4-ACE-wt) or mutant (GA2ox4-ACE-mut) GA2ox4 oligos. The protein purified from the empty vector was used as a negative control. C, ChIP-qPCR assay. WT and 35S:HY5-HA tomato plants were grown at 10 °C under SD conditions with L-R/FR light for 5 d, and samples were precipitated with an anti-HA antibody. A control reaction was processed simultaneously using mouse IgG. The ChIP results are presented as percentages of the input DNA. Three independent experiments were performed with similar results. Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 5. SlHY5 induces ABA biosynthesis by directly binding to SlNCED6 promoter and activating its transcription under cold stress. A, ABA content in WT, HY5-RNAi and HY5-OE plants exposed to 25 °C or 10 °C under long-day (LD, 16 h) or short-day (SD, 8 h) conditions with high R/FR (H-R/FR, 2.5) light or low R/FR (L-R/FR, 0.5) light for 5 d. B, EMSA assay. The His-HY5 recombinant protein was incubated with biotin-labeled wild-type (NCED6-G1-wt) or mutant (NCED6-G1-mut1/2) NCED6 oligos. The protein purified from the empty vector was used as a negative control. C, ChIP-qPCR assay. WT and 35S:HY5-HA tomato plants were grown at 10 °C under SD conditions with L-R/FR light for 5 d, and samples were precipitated with an anti-HA antibody. A control reaction was processed simultaneously using mouse IgG. The ChIP results are presented as percentages of the input DNA. D, SlNCED6 gene expression in tomato plants exposed to 25 °C or 10 °C under LD or SD conditions with H-R/FR or L-R/FR light for 5 d. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. Data

are presented as the means of three biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 6. SlNCED6 is essential for cold acclimation, short days and low R/FR-induced cold tolerance of tomatoes. A and B, Fv/Fm (A) and relative electrolyte leakage (B) in tomato SlNCDE6-silenced plants after exposure to 25 °C or 10 °C under long-day (LD, 16 h) or short-day (SD, 8 h) conditions with high R/FR (H-R/FR, 2.5) light or low R/FR (L-R/FR, 0.5) light for 7 d followed by cold treatment at 4 °C with identical light conditions for 7 d. The false-color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple), representing the level of damage in leaves. C and D, SlCBF1 (C) and SlCOR413-like (D) gene expression in tomato SlNCDE6-silenced plants after exposure to 25 °C or 10 °C under LD or SD conditions with H-R/FR or L-R/FR light for 5 d. E and F, Transcripts of ABA-pathway genes (SlAREB, E; SlABF4, F) in tomato SlNCDE6-silenced plants after exposure to 25 °C or 10 °C under LD or SD conditions with H-R/FR or L-R/FR light for 5 d. For light-quality treatments, plants were maintained at R conditions (120 μmol m^{-2} s^{-1}) and supplemented with different intensities of FR. Data are presented as the means of three biological replicates (±SD). Different letters indicate significant differences ($P < 0.05$) according to Tukey's test.

Figure 7. A model for tomato phytochrome-dependent SlHY5 regulation of plant growth and cold tolerance in response to temperature and light during seasonal variations. During late spring and summer, environmental factors (such as warmth) do not favor the accumulation of SlHY5, leading to a high GA/ABA ratio and to the subsequent promotion of plant growth and decrease in cold tolerance. However, gradual cooling accompanied by the shortening of the days (short day, SD) and the decrease in the R/FR ratio (L-R/FR) in the fall induces phyA accumulation, leading to increased accumulation of SlHY5 protein. The transcription factor SlHY5 promotes abscisic acid (ABA) biosynthesis but suppress gibberellin (GA) accumulation by directly binding to the promoters of an ABA biosynthesis gene (SlNCED6) and a GA catabolic enzyme gene (SlGA2ox4) and activating the transcription of these genes. Consequently, the increased ABA/GA ratio resulted in growth cessation of tomato plants and induced cold response.

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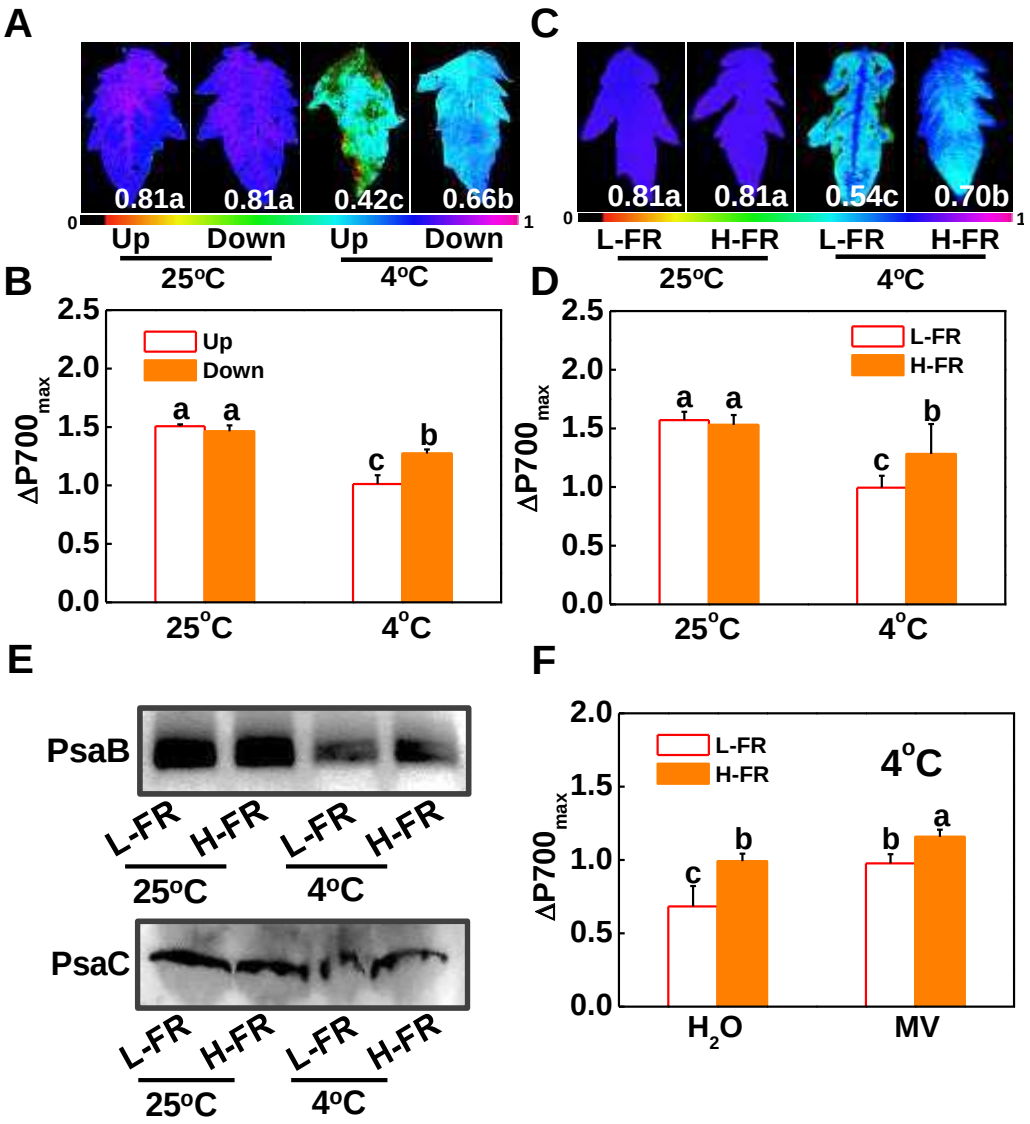

Figure 1. Spatial variation in photoinhibition is partially attributable to the changes in light quality conditions. A and B, Maximum photochemical efficiency of PSII (Fv/Fm, A), maximum P700 photooxidation level ($\Delta P700_{max}$, B) in leaves at the 9th (Up) and 5th (Down) ranks from the base in plants at 11-leaf stage under white light conditions after exposure to 4 °C for 7 d. C and D, Fv/Fm (C) and $\Delta P700_{max}$ (D) at 4th leaves of the tomato plants at 6-leaf stage grown in temperature-controlled chambers at 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple) represents the level of damage in leaves. E, Immunoblot detection of thylakoid proteins (PsaB and PsaC) separated by SDS-PAGE. Detached leaves were exposed to 25 °C or 4 °C for 3 d under L-FR or H-FR. F, Effect of methyl viologen (MV) on the $\Delta P700_{max}$ under cold stress in different light quality. After treated with 25 µM MV for 3 h in darkness at 25 °C, leaves were transferred to 4 °C for 6 h under different light quality conditions. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

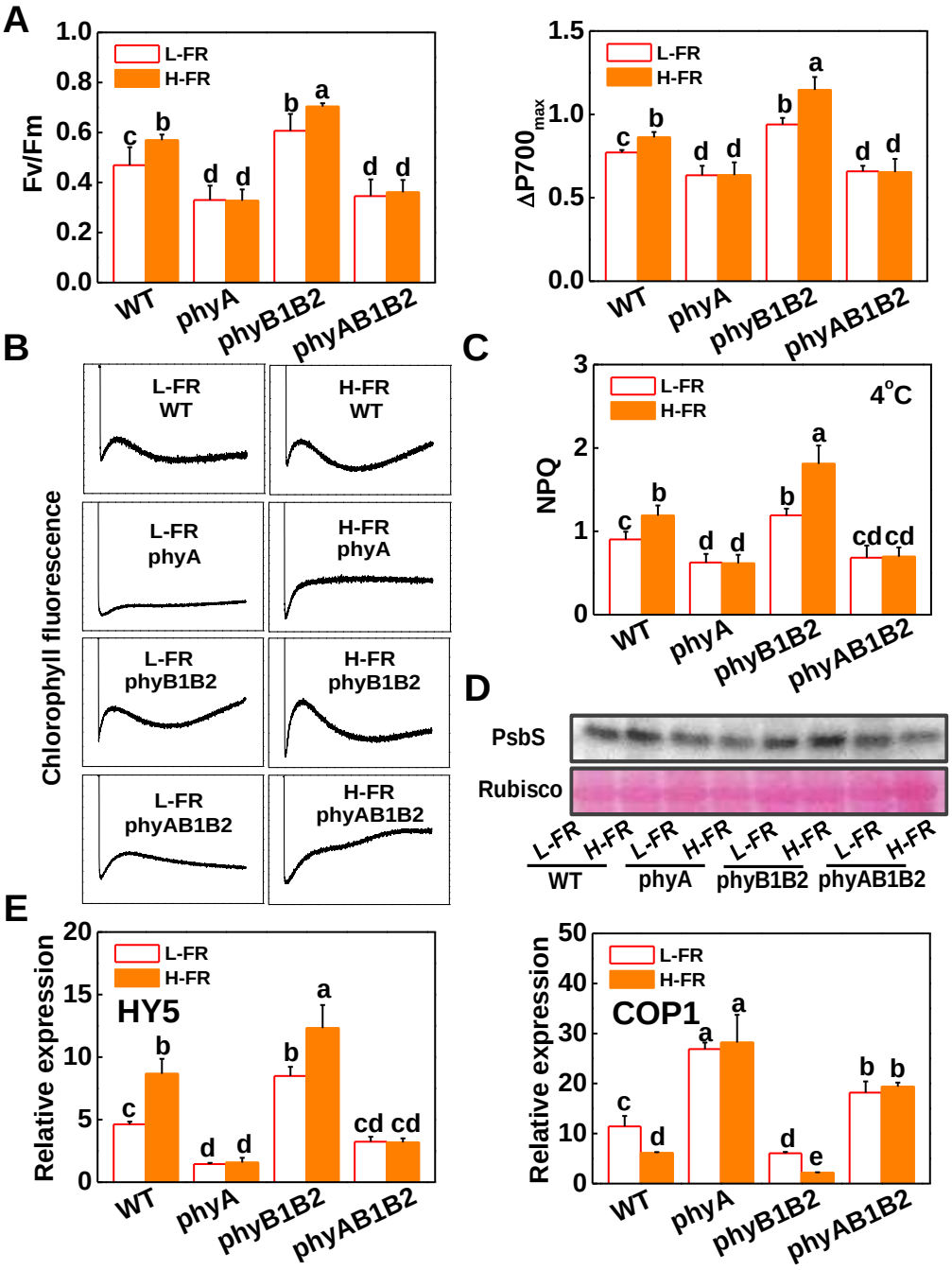

Figure 2. Role of tomato phytochromes in light quality regulation of photoinhibition and transcript levels of light signaling genes (HY5 and COP1). A, Fv/Fm and $\Delta P700_{max}$ of the tomato phytochrome mutant plants after exposure to a cold at 4 °C under L-FR or H-FR light conditions for 7 d. B, Post-illumination chlorophyll fluorescence (CEF around PS I) in tomato plants after exposure to a cold at 4 °C for 3 d under L-FR and H-FR conditions. C and D, Changes of NPQ (C) and PsbS protein (D) in wild type (WT) and phytochrome mutant plants under L-FR and H-FR light conditions at 4 °C for 3 d and 1 d, respectively. E, Transcript levels of HY5 and COP1 genes at 6 h after tomato phytochrome mutants were exposed to 4 °C under L-FR or H-FR light conditions. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

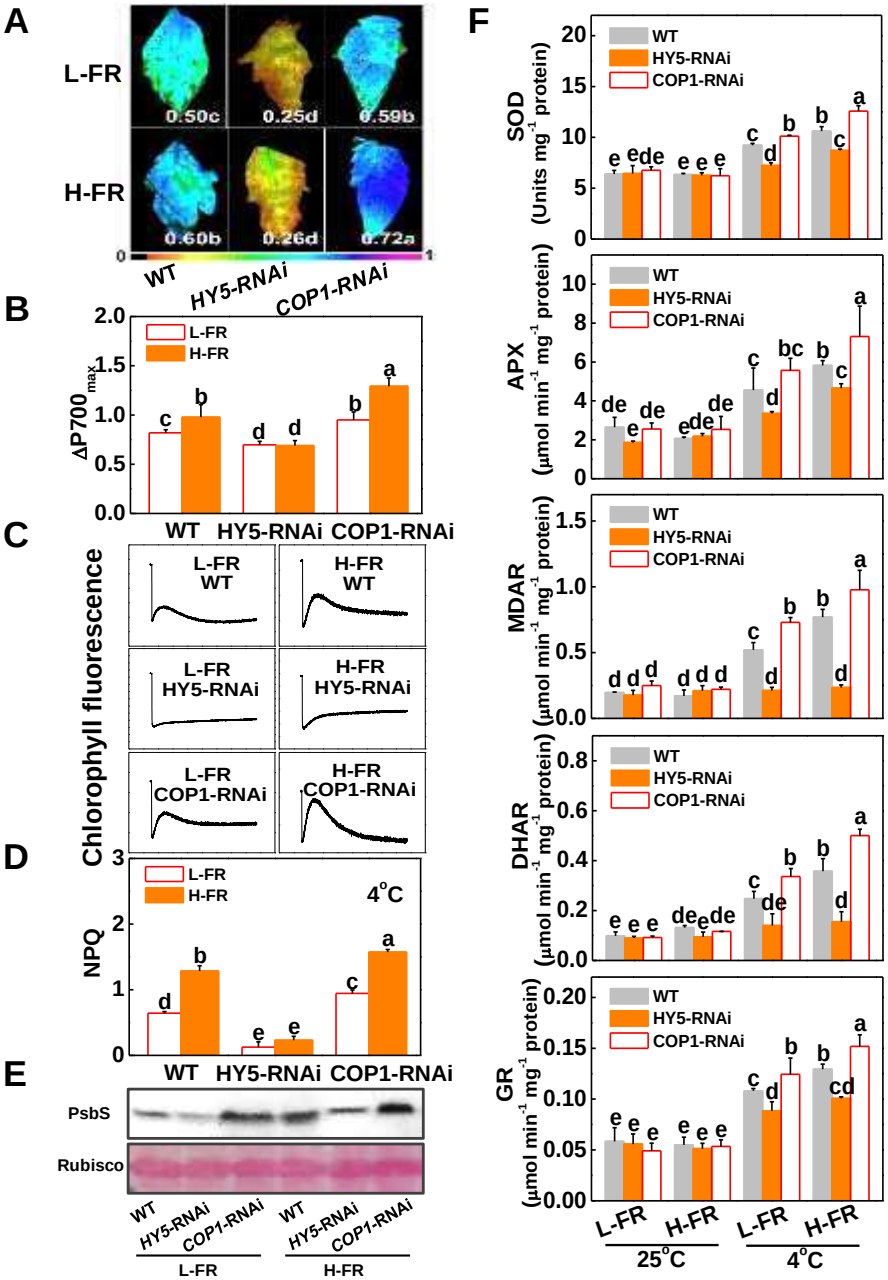

Figure 3. HY5 alleviated photoinhibition by induction of photoprotection. A and B, Fv/Fm (A) and $\Delta P700_{max}$ (B) of the wild type (WT), HY5-RNAi and COP1-RNAi tomato plants after exposure to a cold at 4 °C under L-FR or H-FR light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple) represents the level of damage in leaves. C and D, Post-illumination chlorophyll fluorescence (CEF around PSI, C) and NPQ (D) in WT, HY5-RNAi and COP1-RNAi tomato plants after exposure to 4 °C for 3 d under L-FR and H-FR conditions. E, Immunoblot analysis of PsbS in WT, HY5-RNAi and COP1-RNAi tomato plants after exposure to 4 °C for 1 d under L-FR and H-FR conditions. Samples were loaded at equal total proteins amounts based on Coomassie blue. F, Activity of antioxidant enzymes (SOD, APX, MDAR, DHAR and GR) involved in Foyer-Halliwell-Asada cycle after the WT, HY5-RNAi and COP1-RNAi tomato plants exposure to 25 °C or 4 °C under L-FR or H-FR light conditions for 3 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

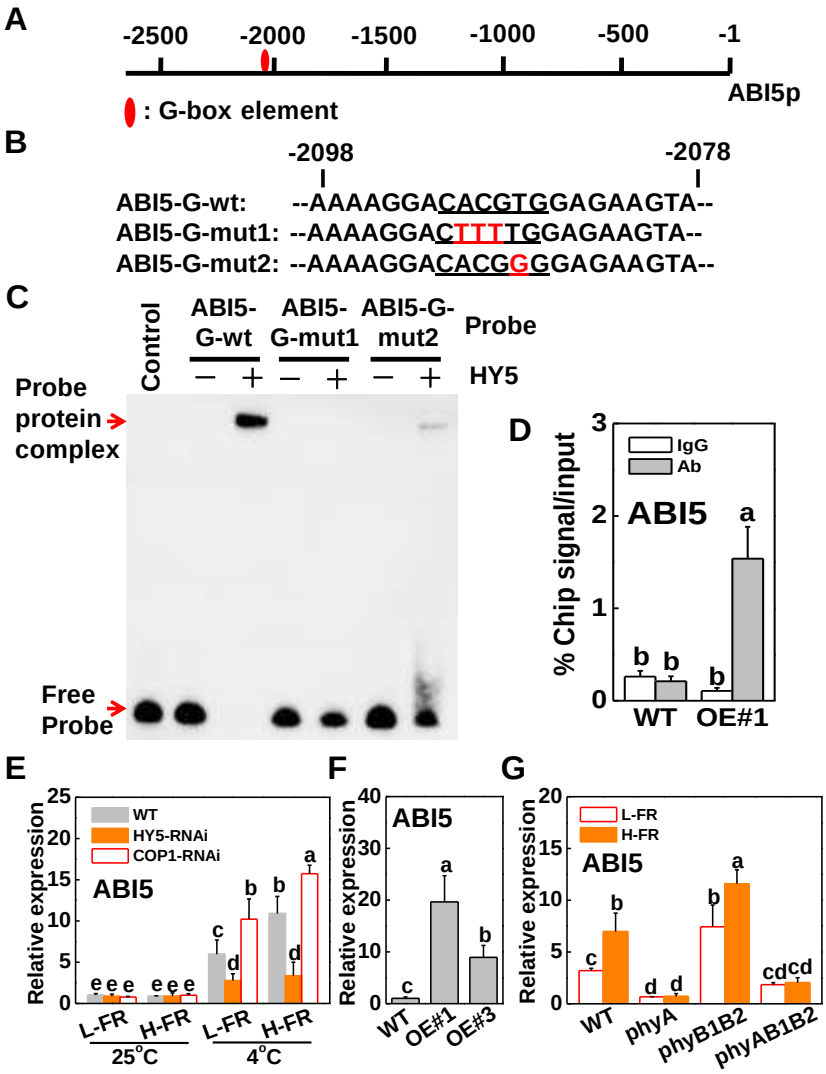

Figure 4. HY5 induced transcript level of ABI5 by binding to promoter of ABI5. A and B, G-box elements in the promoter of tomato ABI5 gene (A) and oligonucleotide used in the electrophoretic mobility shift assays (EMSA, B). Numbering is from predicted transcriptional start sites. The ABI5 probe contains one G-box (ABI5-G-wt), whereas in the ABI5-G-mut1 and ABI5-G-mut2 probes the G-box core sequence was mutated. The WT and mutated G-box sequences are underlined. The mutated bases were indicated in red. C, HY5 directly binds to the G-box of ABI5 promoter in vitro. Recombinant HY5 was purified from E. coli cells and used for DNA binding assays with probes of ABI5-G-wt, ABI5-G-mut1 and ABI5-G-mut2. The protein purified from empty vector was used as the negative control. D, Direct binding of HY5 to the ABI5 promoter was analyzed using ChIP-qPCR in 35S-HY5-3HA-overexpressing (HY5-OE#1) tomato plants. HY5-OE#1 plants at 6-leaf stage were exposed to 4 ^{o}C under H-FR condition and input chromatin was isolated from leaf samples at 6 h. The epitope-tagged HY5-chromatin complex was immunoprecipitated with an anti-HA antibody. A control reaction was processed side-by-side using mouse IgG. Input- and ChIP-DNA samples were quantified by qRT-PCR using primers specific for the promoter of the ABI5 gene. The ChIP results are presented as percentage of the input DNA. OE, overexpressing; #1, line of HY5-OE plants. E and F, Transcript level of ABI5 gene at 6 h after HY5-RNAi and COP1-RNAi tomato plants exposed to 25 °C or 4 °C under different R/FR light regimes (E), and two independent HY5 overexpressing transgenic tomato lines (HY5-OE#1, OE#3) exposed to 4 °C under H-FR conditions (F). G, Transcript level of ABI5 gene at 6 h after WT and phytochromes mutants of tomato exposed to 4 °C under different R/FR light regimes. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 µmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 µmol m^{-2} s^{-1}). Four independent experiments were performed with similar results. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

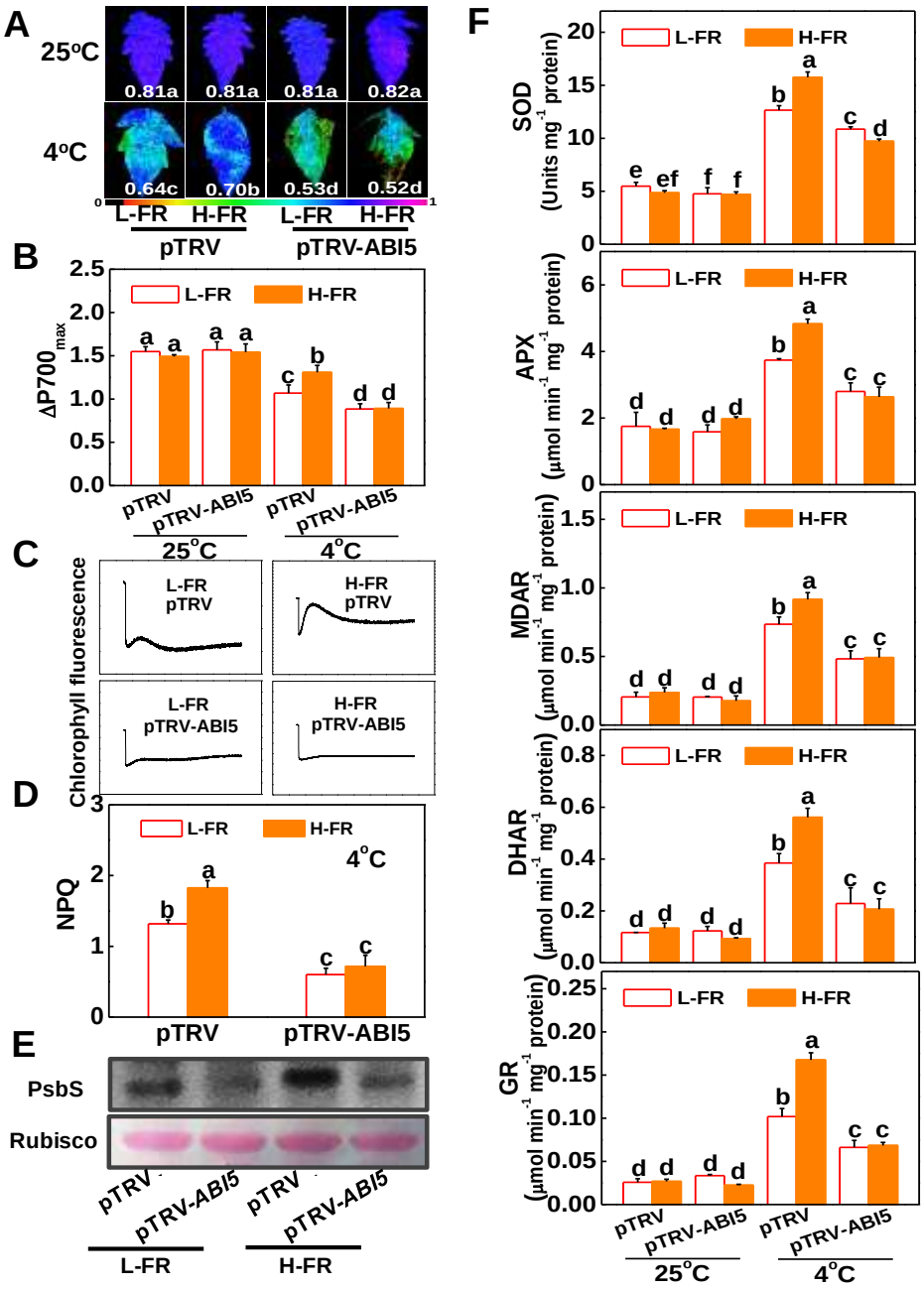

Figure 5. Role of ABI5 in light quality-regulated photoinhibition and photoprotection. A and B, Fv/Fm (A) and $\Delta P700_{max}$ (B) of the non-silenced (pTRV) and silenced (pTRV-ABI5) tomato plants grown in temperature-controlled chambers at 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple) represents the level of damage in leaves. C and D, Post-illumination chlorophyll fluorescence (CEF around PSI, C) and NPQ (D) in the pTRV and pTRV-ABI5 tomato plants after exposure to 4 °C for 3 d under L-FR and H-FR conditions. E, Immunoblot analysis of PsbS in pTRV and pTRV-ABI5 tomato plants after exposure to 4 °C for 1 d under L-FR and H-FR conditions. Samples were loaded at equal total proteins amounts based on Coomassie blue. F, Activity of antioxidant enzymes (SOD, APX, MDAR, DHAR and GR) involved in Foyer-Halliwell-Asada cycle after the pTRV and pTRV-ABI5 tomato plants exposure to 25 °C or 4 °C under L-FR or H-FR light conditions for 3 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

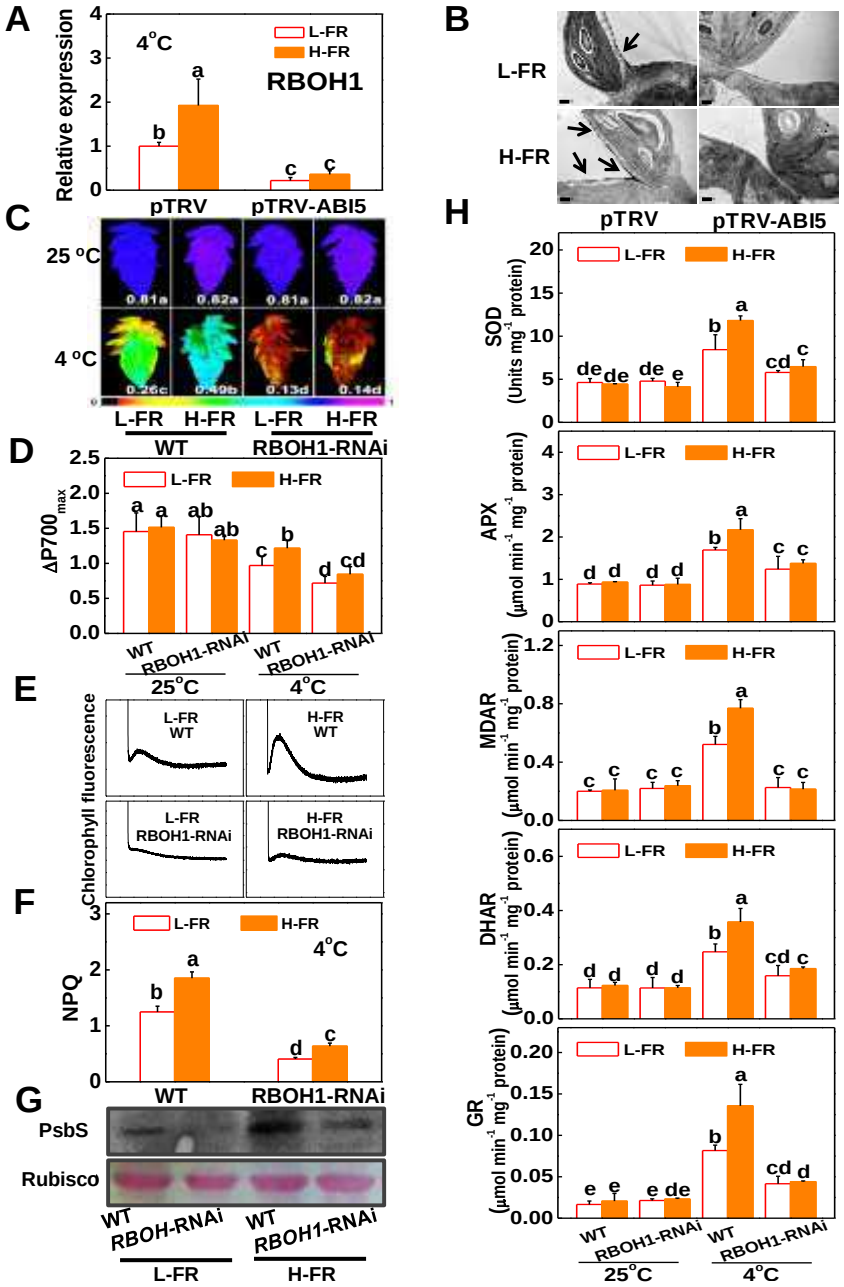

Figure 6. RBOH1-dependent ROS production prevents plants from photoinhibition by activating photoprotection. A and B, Transcript level of RBOH1 gene at 6 h (A) and cytochemical localization of H_2O_2 accumulation in leaf mesophyll cells at 1 d as visualized by $CeCl_3$ staining and TEM (B) after pTRV and pTRV-ABI5 tomato plants exposed to 4 °C under different R/FR light regimes. The arrows indicate $CeCl_3$ precipitates. Scale bars = 0.5 μm. C and D, Fv/Fm (C) and $\Delta P700_{max}$ (D) of the wild type (WT) and RBOH1-RNAi tomato plants were exposed to 25 °C or 4 °C under L-FR or H-FR light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple) represents the level of damage in leaves. E and F, Post-illumination chlorophyll fluorescence (CEF around PSI, E) and NPQ (F) in the WT and RBOH1-RNAi tomato plants after exposure to 4 °C for 3 d under L-FR and H-FR conditions. G, Immunoblot analysis of PsbS in WT and RBOH1-RNAi tomato plants after exposure to 4 °C for 1 d under L-FR and H-FR conditions. Samples were loaded at equal total proteins amounts based on Coomassie blue. H, Activity of antioxidant enzymes (SOD, APX, MDAR, DHAR and GR) involved in Foyer-Halliwell-Asada cycle after the WT and RBOH1-RNAi tomato plants exposure to 25 °C or 4 °C under L-FR or H-FR light conditions for 3 d. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR (133 and 400 μmol m^{-2} s^{-1}). Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

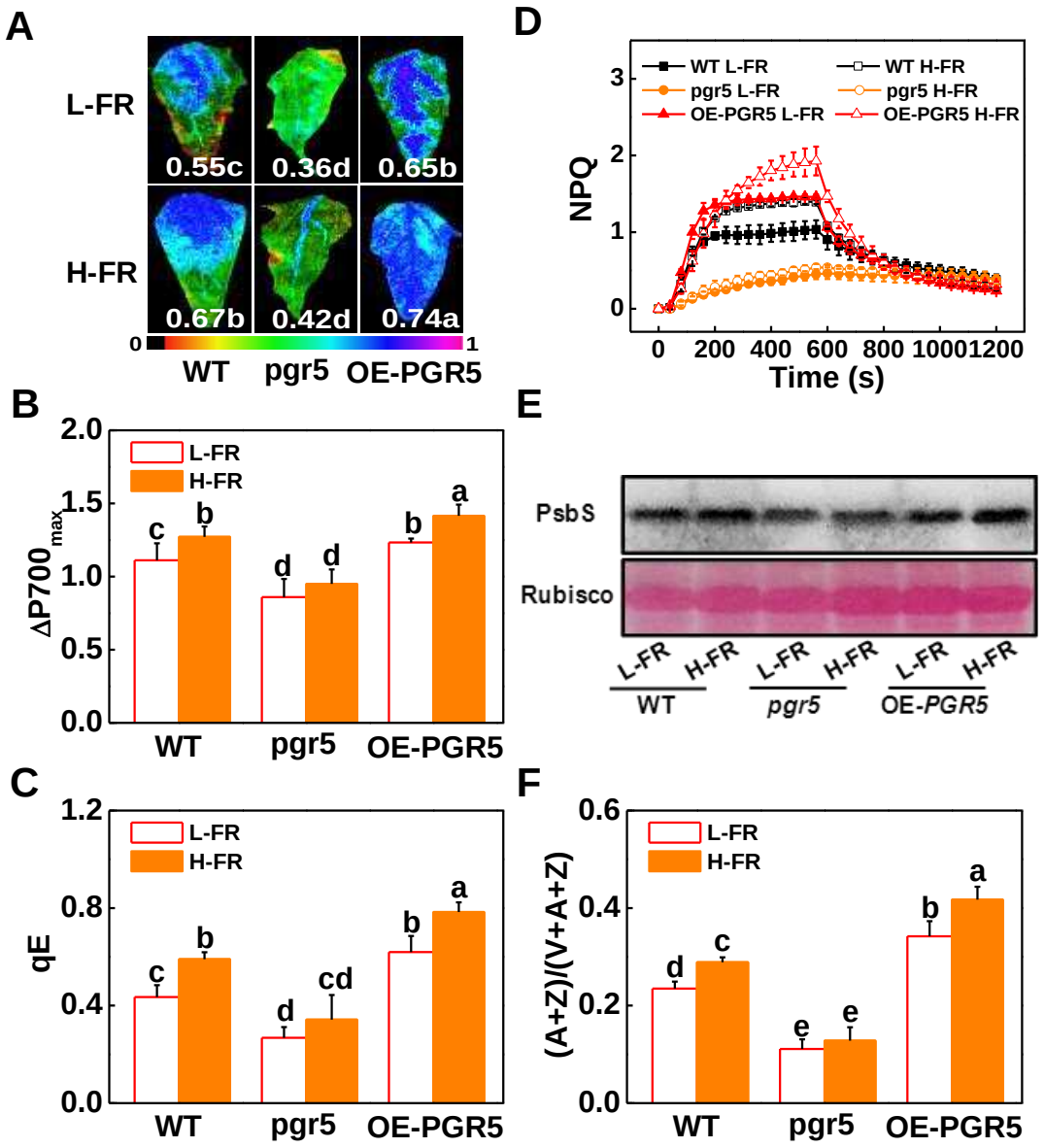

Figure 7. PROTON GRADIENT REGULATION5 (PGR5)-dependent CEF plays dual roles in preventing plants from photoinhibition. A and B, Fv/Fm (A) and $\Delta P700_{max}$ (B) of the wild type (WT), pgr5 mutant (pgr5#5) and PGR5-overexpressing (OE-PGR5#3) transgenic plants grown at 4 °C under L-FR or H-FR light conditions for 7 d. The false color code depicted at the bottom of the image ranges from 0 (black) to 1.0 (purple) represents the level of damage in leaves. C and D, qE (C) and NPQ (D) in the WT, pgr5#5 mutant and OE-PGR5#3 tomato plants after exposure to 4 °C for 3 d under L-FR and H-FR conditions. E and F, PsbS protein (E) and de-epoxidation state of the xanthophyll cycle (F) in the WT, pgr5#5 mutant and OE-PGR5#3 tomato plants after exposure to 4 °C for 1 d and 3 d, respectively, under L-FR and H-FR conditions. For the L-FR and H-FR, R/FR ratio at 1.5 and 0.5, respectively, plants were kept at R conditions (200 μmol m^{-2} s^{-1}) supplemented with different intensities of FR. Data are presented as the mean of 4 biological replicates (±SD) except for Fv/Fm which was the mean for 15 leaves from independent plants. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.