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Research article

Strigolactone promotes photomorphogenesis by B-box protein STH7 in an F-box protein MAX2-dependent manner

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Abstract

Photomorphogenesis is the process by which plants respond to light; it involves various signal molecules and phytohormones. This study explored the regulation of the signal transducer STH7, a member of the B-box protein, in photomorphogenesis. Hypocotyl shortening and cotyledon opening after GR24 (a strigolactone analog) and brassinazole (Brz, a brassinosteroid inhibitor) application were observed as photomorphogenesis responses of *Arabidopsis*. The expression level of the photosynthesis-related genes, *ELIP2*, *CHS*, *LHCB1* and *rbcS*, as well as the cell elongation-related genes, *SAUR-AC1*, *TCH4* and *PRE1*, were analyzed in wild-type *Arabidopsis* and *STH7* mutants. Overexpression of *STH7* (*STH7ox*) upregulated the expression of photosynthesis-related genes. However, the transcription levels of these genes were reduced in the strigolactone signaling mutant (*max2*) and *max2*×*STH7ox* mutants. Treatment with GR24 shortened the hypocotyl in *STH7ox*. This shortening was reduced in the *max2*×*STH7ox*. GR24-treated *max2*×*STH7ox* showed a decrease in *STH7*-downstream genes. The application of Brz reduced hypocotyl elongation and caused cotyledon opening in the *STH7ox* mutant. However, the functional suppression of *STH7* (*STH7-SRDX*), brassinosteroid gain-of-function (*bil1-1D*/*bzr1-1D*), *max2*, *max2*×*STH7ox* and *bil1-1D*×*max2* were weakly sensitive to Brz. Although *max2*×*STH7ox* mutants were treated with Brz, upregulations of cell elongation-related genes were observed. The results indicated that MAX2 regulates *STH7* which was upregulated by SL, promoting photomorphogenesis in *Arabidopsis*.

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Introduction

Light is an environmental stimulus that regulates the growth and development of plants, starting from seed germination through early seedling growth, regulates shade avoidance and stimulates circadian rhythm and flowering (Wu, 2014). During skotomorphogenesis in the soil, young seedlings may develop etiolation, exhibiting unopened hooks, closed cotyledons and elongated hypocotyls. When the seedlings protrude from the soil, they undergo photomorphogenesis or de-etiolation. Photomorphogenesis is the light-adapted development of plants that results in shortened hypocotyls, opened cotyledons, the development of true leaves and synthesis of photosynthetic pigments, such as chlorophyll and anthocyanin (Eckardt, 2001; Wu, 2014).

Several transcription factors have been identified as regulators in the photomorphogenesis process, with the basic transcriptional regulators including the basic leucine zipper transcription factor (bZIP), basic helix-loop-helix transcription factor (bHLH) and B-box zinc-finger transcription factor (BBX) families (Wu, 2014). In the bZIP family, HY5 and its homolog, HYH, are positive regulators of photomorphogenesis (Wei and Deng, 1999; Eckardt, 2001). HY5 functionally contributes to photomorphogenesis regulation by various light wavelengths, integrating light-signaling and plant hormone-signaling pathways (Lau and Deng, 2012). HY5 is repressed by COP1, which is a negative regulator of photomorphogenesis under darkness (Osterlund et al., 2000; Eckardt, 2001). In the bHLH transcription factor, phytochrome-interacting factors (PIFs) are the negative regulators of photomorphogenesis (Leivar et al., 2012). PIF1 and PIF3 directly activated HY5 and B-box genes and then repressed photomorphogenesis (Zhang et al., 2017). Strigolactone (SL), a lactone-type plant hormone (Xie et al., 2010; Al-Babili and Bouwmeester, 2015), promotes HY5 function (Tsuchiya et al., 2010). However, SL inhibited hypocotyl elongation in *Arabidopsis*, even in darkness, when HY5 was repressed by COP1 (Thussagunpanit et al., 2017). This indicates that SL not only regulates photomorphogenesis through crosstalk with HY5, but also regulates this process through other signal components.

The BBX protein family is another group of transcription factors that regulates photomorphogenesis (Gangappa and Botto, 2014). Among the BBX protein family, subfamily IV, consisting of BBX18–BBX25, plays an important regulatory role in photomorphogenesis (Sarmiento, 2013). BBX20/BZS1/STH7 (salt tolerance homolog 7), BBX21/STH2 and BBX22/LZF1 are positive regulators in photomorphogenesis. In contrast, BBX24/STO and BBX25/STH are negative regulators (Gangappa and Botto, 2014). Among BBX IV members, only *STH7* expression was upregulated by SL treatment (Wei et al., 2016).

MAX2 (more axillary growth 2) or ORE9 (oresara 9), which encode an F-box protein with leucine-rich repeats (Woo et al., 2001), have also been reported to regulate photomorphogenesis (Shen et al., 2007). MAX2 is a downstream component of the SL signaling (Umehara et al., 2008) that mediates inflorescence branching and senescence pathways in *Arabidopsis* (Stirnberg et al., 2002). MAX2 is modulated by various plant hormones such as gibberellin and abscisic acid to regulate the photomorphogenesis process (Shen et al., 2012). Shen et al. (2007) reported that MAX2/ORE9 could regulate the de-etiolation of seedlings under red, far-red and blue light conditions. In addition, *max2* mutants downregulate the BBX protein subfamily IV, *STH7*/*BBX20* (Nelson et al., 2011). Both MAX2 and STH7 have been reported as positive factors in photomorphogenesis (Shen et al., 2012; Gangappa and Botto, 2014). In addition, the plant hormone brassinosteroid (BR) reportedly regulates MAX2. MAX2-mediated degradation of brassinazole-insensitive-long hypocotyl 1 (BIL1), brassinazole-resistant 1 (BZR1) and BRI1-EMS-suppressor 1 (BES1), which are transcription factors that are essential for BR signaling to control branching (Wang et al., 2013). BIL1/BZR1 is a BR-signaling molecule that acts as a positive regulator of BR signaling (He et al., 2005; Belkhadir and Jaillais, 2015) and *bil1-1D* causes constitutive expression of BIL1/BZR1 (Wang et al., 2002).

The mechanisms of the MAX2 regulation of plant hormones and other signal molecules to promote photomorphogenesis are still unclear. This study focused on BBX20/STH7 as the regulating factor of photomorphogenesis and the functions were examined of STH7 and MAX2 in photomorphogenesis by regulation of strigolactone and brassinosteroid.

Materials and Methods

Plant materials and growth conditions

The experiment used wild-type *Arabidopsis* ecotype Col-0, *STH7-overexpressing* (*STH7ox*), functional suppression of *STH7* (*STH7-SRDX*), SL signaling mutant *max2* and BR gain-of-function *bil1-1D*/*bzr1-1D* (hereafter *bil1-1D*) mutant. *Arabidopsis STH7ox* was generated by overexpression of

the B-box zinc finger protein *STH7*, driven by a CaMV35S promoter in the wild-type *Arabidopsis* ecotype Col-0 background (Thussagunpanit et al., 2017). The functional suppression of *STH7* was induced using chimeric repressor gene-silencing technology. The SRDX motif was fused to the C-terminal end of *STH7* and expressed under a CaMV35S promoter in wild-type *Arabidopsis* ecotype Col-0 (Hiratsu et al., 2003). The *max2* and *STH7ox* mutant (*max2*×*STH7ox*) was produced by *Agrobacterium* transformation of the *35S::STH7* vector onto the *max2 Arabidopsis* mutant using the floral dip technique, whereas the *bil1-1D* and *max2* mutant (*bil1-1D*×*max2*) was created through hybridization. Seeds were collected after *Agrobacterium* transformation or cross-pollination, respectively. The successful *max2*×*STH7ox* and *bil1-1D*×*max2* mutants were screened until homozygous lines were obtained. Then, they were used for the experiment.

Seeds were surface sterilized with ethanol and germinated on 1/2MS medium containing 3% sucrose and 0.8% Phyto agar (Duchefa; Haarlem, the Netherlands). *Arabidopsis* were stored at 4°C for 2 d, and then transferred to grow at 22°C under 1.75 µmol/m^2/s for weak light conditions or under dark conditions depending on the experiment.

Measurement of Arabidopsis hypocotyls

Arabidopsis was grown in 1/2MS medium supplemented with 10 µM GR24 (synthetic SL analog) or 0.1% (volume per volume, v/v) dimethyl sulfoxide (DMSO) for the control under weak light conditions at 1.75 µmol/m^2/s. After four days of growth, *Arabidopsis* hypocotyl elongation was measured using the ImageJ software (Schneider et al., 2012).

Quantitative real-time PCR

Arabidopsis plants were grown under weak light at 1.75 µmol/m^2/s in 1/2MS medium containing 10 µM GR24 (synthetic SL analog), 0.3 µM Brz (BR biosynthesis inhibitor) or 0.1% (v/v) DMSO (control) for 4 d. Plant samples were collected, frozen and homogenized in liquid nitrogen. Total RNA was extracted using a Total RNA Extraction Kit Mini for plants (RBC Bioscience; New Taipei City, Taiwan). The complementary DNA (cDNA) was synthesized using ReverTra Ace® qPCR RT Master Mix with gDNA remover (Toyobo; Osaka, Japan). Quantitative real-time PCR (qRT-PCR) was performed using KAPA SYBR® FAST One-Step qRT-PCR Master Mix (Kapa Biosystems; Cape Town, South Africa) with four replications per treatment. Data of relative gene expression were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The transcript level of each gene was normalized by that of *ACT7*, the constitutively expressed control gene. The *ACT7* amplification efficiency considered from various cDNA dilutions of wild-type *Arabidopsis* in mock to threshold cycles was 103.85%. The primers used for qRT-PCR are presented in Table S1.

Observation of cotyledon opening

Arabidopsis samples were grown in 1/2MS medium supplemented with 0.3 µM Brz or 0.1% (v/v) DMSO (control) under dark conditions. After 4 d growth, the *Arabidopsis* cotyledons were observed to determine if they were open or closed. Cotyledons that had opened more than 45° were considered as opened.

Statistically analysis

The data were analyzed using analysis of variance and mean differences among treatments were evaluated using Tukey's honestly significantly difference test. The tests were considered significant at $p < 0.05$.

Results

Expression of STH7 in wild-type and other mutants of Arabidopsis

The *STH7ox* mutant (overexpression of *STH7*) showed a significantly increased level of the *STH7* expression (Fig. 1A). In contrast, the *max2* mutant (SL signaling mutant) exhibited lower *STH7* expression compared to wild-type *Arabidopsis* (Fig. 1A). Notably, both lines of *max2*×*STH7ox* mutants exhibited lower *STH7* expression than *STH7ox* (Fig. 1A). The expression of key photosynthesis-related genes, including *ELIP2*, *CHS*, *LHCB1* and *rbcS*, were examined. Compared to wild-type *Arabidopsis*, these genes were upregulated in the *STH7ox* but *ELIP2* was significantly downregulated in the *STH7-SRDX* (transcriptional suppression of *STH7*) mutants, as shown in Fig. 1B, and expression of *CHS*, *LHCB1* and *rbcS* tended to reduce in the *STH7-SRDX* (Figs. 1C–E). The upregulation of photosynthesis-related genes was reduced in *max2* and *max2*×*STH7ox* mutants compared to *STH7ox* (Figs. 1B–E).

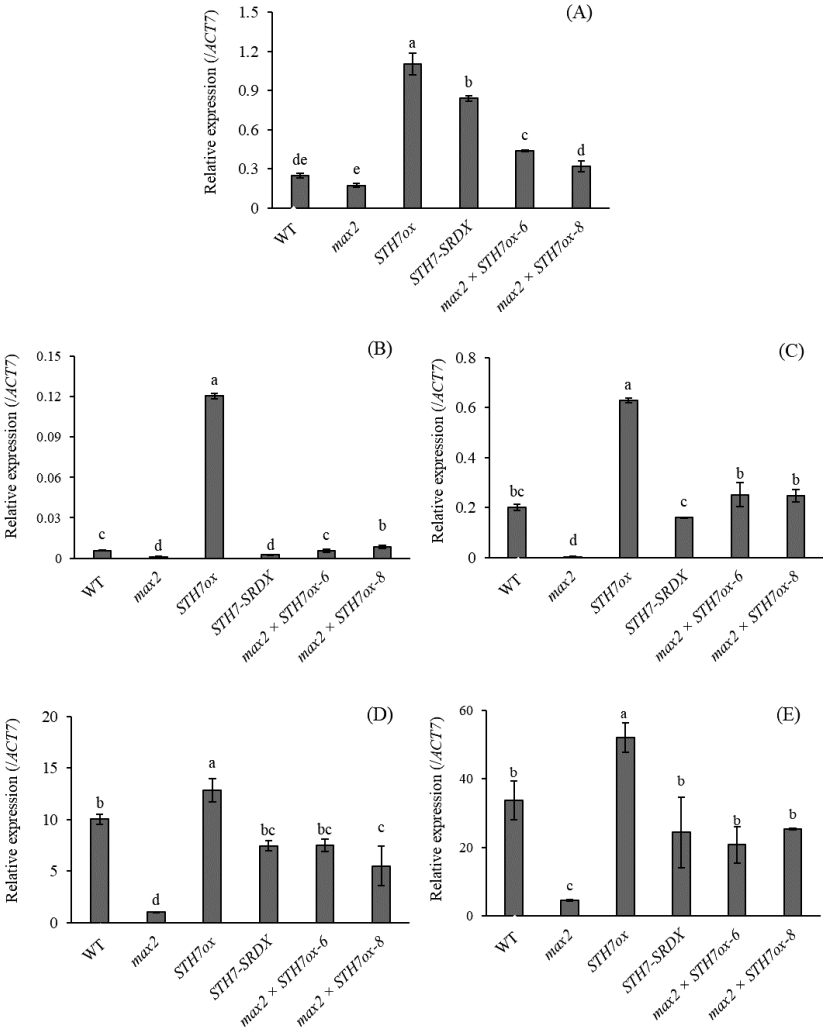

Fig. 1 Quantitative real-time polymerase chain reaction analysis of *STH7* and photosynthesis-related genes: relative transcript levels of *STH7* (A) and photosynthesis-related genes; *ELIP2* (B), *CHS* (C), *LHCB1* (D) and *rbcS* (E) in wild-type *Arabidopsis*, *max2*, *STH7ox, STH7-SRDX* and *max2*×*STH7ox* mutants were analyzed. Plants were grown in 1/2MS medium under weak light for 4 d. Transcript levels were normalized to those of *ACT7*. Data are presented as means ± SD ($n = 4$). Means superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Responses of wild-type and other mutants of Arabidopsis to strigolactone

GR24-treated wild-type *Arabidopsis* under the weak light condition showed 53% hypocotyl length of its control (Fig. 2). Without GR24, *STH7ox* exhibited significantly shortened hypocotyls compared to the wild-type, while treatment with GR24 decreased the hypocotyl length to 49% of its control. *STH7-SRDX* and *max2* mutants were insensitive to GR24 as the decrease in hypocotyl elongations were not significant in the GR24 treatments (Fig. 2). In addition, the shortened hypocotyls observed in *STH7ox* with GR24 were attenuated in the *max2*×*STH7ox* (Fig. 2).

To confirm that MAX2 was essential for STH7 expression in response to SL, qRT-PCR was used to analyze the expression of the *STH7* and *STH7*-downstream genes, *ELIP2* and *CHS*. Application of GR24 resulted in the upregulation of the *STH7* and *STH7*-downstream genes in the wild-type and the *STH7ox* mutant (Fig. 3). However, expressions of these genes, especially *ELIP2* (14.44 times) and *CHS* (4.07 times), were relatively lower in *max2*×*STH7ox*, than in the wild-type *Arabidopsis* (Figs. 3B and 3C).

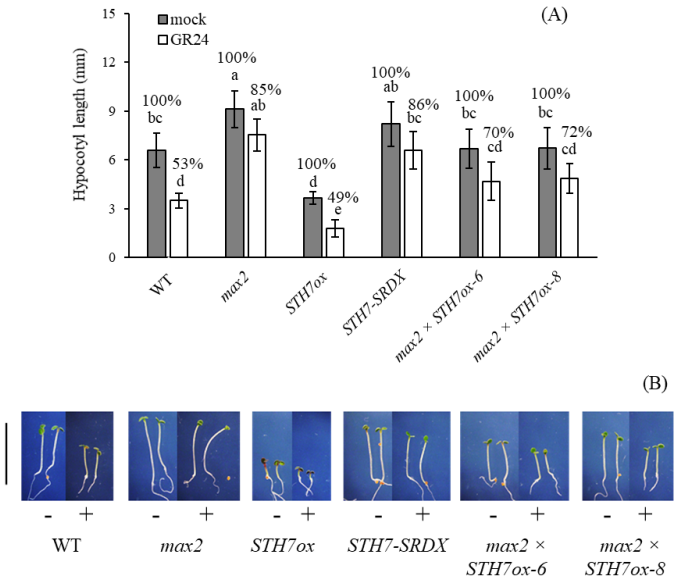

Fig. 2 Hypocotyl elongation response of wild-type *Arabidopsis* and *Arabidopsis* mutants (*max2*, *STH7ox*, *STH7-SRDX* and *max2*×*STH7ox*) on strigolactone treatment. Comparison of hypocotyl elongation in wild-type and mutant *Arabidopsis* (A). Four-day-old seedlings (B). Scale bar represents 10 mm. Plants were treated with 0.1% DMSO as the control (-) or 10 µM GR24 (+) under weak light conditions for 4 d. Data are presented as means ± SD ($n \geq 16$). Means superscripted with different lowercase letters are significantly ($p < 0.05$) different.

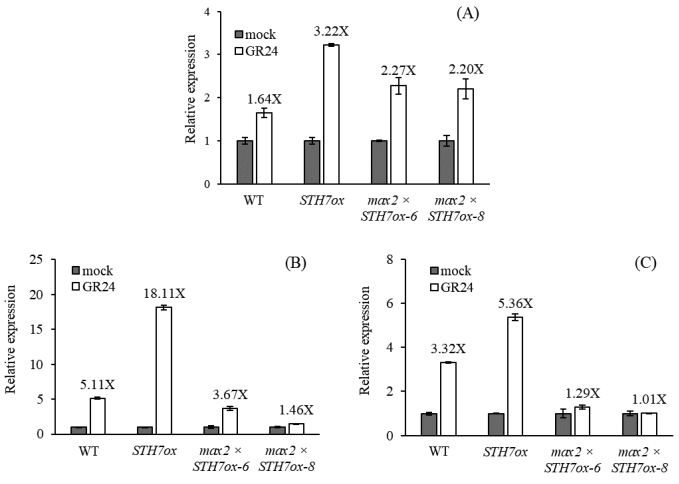

Fig. 3 Expression levels of *STH7* and *STH7* downstream genes under strigolactone treatment. Relative transcript levels of *STH7* (A) and *STH7* downstream genes; *ELIP2* (B) and *CHS* (C) in wild-type *Arabidopsis*, *max2*, *STH7ox* and *max2*×*STH7ox* mutants treated with 0.1% (v/v) DMSO as a control and 10 µM GR24 were analyzed. Plants were grown in 1/2MS medium under weak light for 4 d. The transcript levels were normalized to those of *ACT7*. Data are presented as means ± SD ($n = 4$).

Responses of wild-type and other mutants of Arabidopsis to brassinosteroid inhibitor

Brz reduced hypocotyl elongation in wild-type *Arabidopsis*, *STH7ox* and *STH7-SRDX* to 42%, 35% and 43%, respectively, of their controls. However, *STH7-SRDX* was less sensitive to Brz regarding hypocotyl shortening than *STH7ox* (Fig. 4). The BR gain-of-function *bil1-1D* showed a weak response to the Brz application. Brz-treated *bil1-1D* hypocotyls were decreased to 61% of their control (Fig. 4). Furthermore, *max2* and *bil1-1D*×*max2* had similar hypocotyl lengths to *bil1-1D*, indicating a weak response to the BR inhibitor (Fig. 4). The lack of MAX2 function reduced the effect of Brz, preventing hypocotyl elongation in *STH7ox*. The two lines of *max2*×*STH7ox* were weakly sensitive to Brz. After Brz application, they reduced hypocotyls by 39% and 46%, whereas hypocotyls of Brz-treated *STH7ox* decreased up to 65% (Fig. 4). Quantitative analysis of the expression levels of cell elongation-related genes was performed. It had been reported that Brz causes downregulation of *SAUR-AC1* and *TCH4* in wild-type *Arabidopsis*, however, this effect was not observed in *bil1-1D* (Figs. 5A and 5B). In the present study, the qRT-PCR results showed that Brz downregulated *SAUR-AC1*, *TCH4* and *PRE1* in *STH7ox* and *STH7-SRDX* (Fig. 5). However, Brz did not downregulate *SAUR-AC1* and *TCH4* in *bil1-1D*×*max2* and *max2*×*STH7ox* (Figs. 5A and 5B). In addition, Brz-treated *max2*×*STH7ox* exhibited high *SAUR-AC1*, *TCH4* and *PRE1* expression compared to the Brz-treated wild-type (Fig. 5).

Cotyledon opening is another plant response to photomorphogenesis. The *max2* mutant was insensitive to Brz, with most cotyledons remaining closed (Fig. 6). The Brz treatment stimulated 100% cotyledon opening in the dark of the *STH7ox* and wild-type *Arabidopsis*, whereas only 23% of the *STH7-SRDX* cotyledons opened (Fig. 6). In contrast with the wild-type, nearly all the *bil1-1D* and *max2* cotyledons remained closed in the Brz treatment (Fig. 6). However, Brz treatment opened only approximately 10% of the cotyledons in *bil1-1D*×*max2* and *max2*×*STH7ox* (Fig. 6). Similar to the results obtained under weak light conditions, Brz-treated *bil1-1D*×*max2* and *max2*×*STH7ox* in darkness exhibited longer hypocotyls than the Brz-treated wild-type (Fig. S1).

Working model for regulation of STH7 and MAX in photomorphogenesis

Application of strigolactone upregulated the *STH7* transcription. This study proposed that STH7 is related to the SL signaling (MAX2) and the positive regulator of brassinosteroids (BIL1/BZR1) to induce photomorphogenesis (Fig. 7A). With a lack of MAX2, such as *bil1-1D*×*max2* and *max2*×*STH7ox*, *STH7* could not promote photomorphogenesis (Fig. 7B).

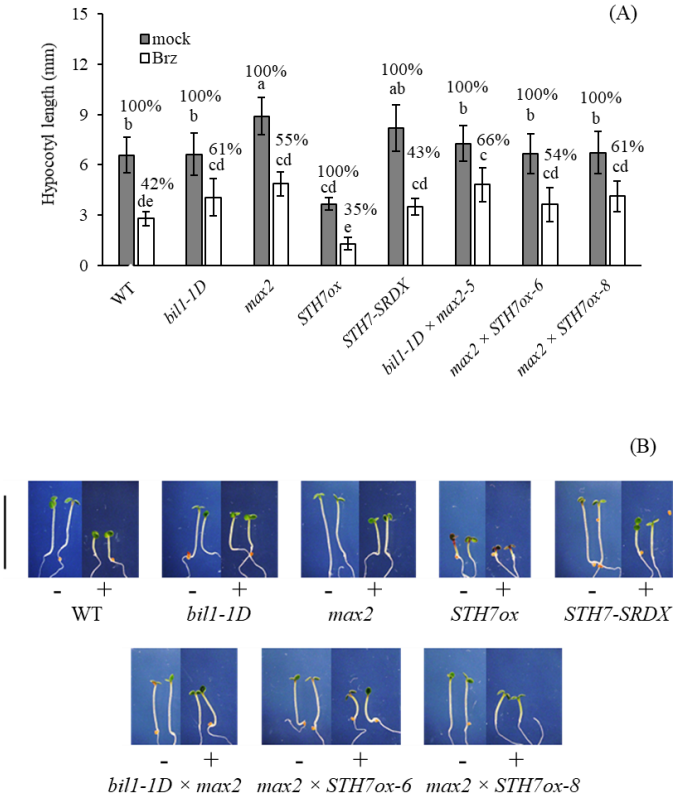

Fig. 4 Hypocotyl elongation response of wild-type and *Arabidopsis* mutants on brassinosteroid biosynthesis inhibitor treatment. Comparison of hypocotyl elongation in wild-type *Arabidopsis*, *bil1-1D/bzr1-1D*, *max2*, *STH7ox*, *STH7-SRDX*, *bil1-1D*×*max2* and *max2*×*STH7ox* mutants (A). Four-day-old seedlings, scale bar represents 10 mm (B). Plants were treated with 0.1% dimethyl sulfoxide as the control (-) and 0.3 µM Brz (+) under weak light conditions for 4 d. Data are presented as means ± SD ($n \geq 16$). Means superscripted with different lowercase letters are significantly ($p < 0.05$) different.

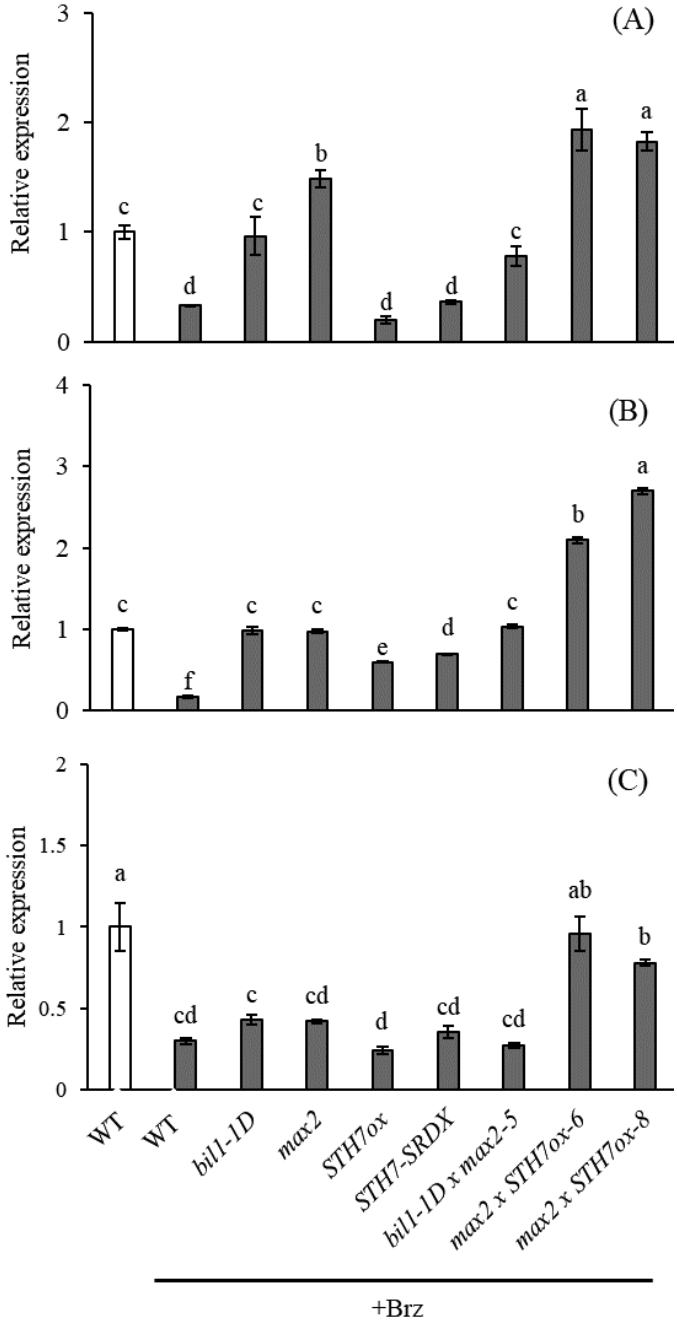

Fig. 5 Expression levels of cell elongation-related genes under treatment with brassinosteroid biosynthesis inhibitor. Relative transcript levels of *SAUR-AC1* (A), *TCH4* (B) and *PRE1* (C) in wild-type *Arabidopsis* treated with 0.1% (volume per volume) dimethyl sulfoxide as a control and wild-type *Arabidopsis*, *max2*, *STH7ox*, *STH7-SRDX*, *bil1-1D*×*max2* and *max2*×*STH7ox* mutants treated with 0.3 µM brassinazole (Brz) were analyzed. Plants were grown in 1/2MS medium under weak light for 4 d. The transcript levels were normalized to those of *ACT7*. Data are presented as means ± SD (n = 4). Means superscripted with different lowercase letters are significantly ($p < 0.05$) different.

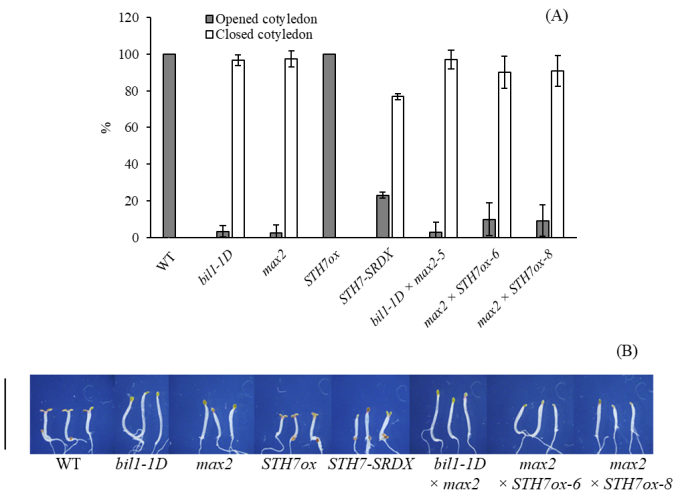

Fig. 6 Cotyledon opening response of wild-type *Arabidopsis* and *Arabidopsis* mutants on brassinosteroid biosynthesis inhibitor treatment. Comparison of cotyledon opening in wild-type *Arabidopsis*, *bil1-1D/bzr1-1D*, *max2*, *STH7ox*, *STH7-SRDX*, *bil1-1D*×*max2* and *max2*×*STH7ox* mutants (A). Plants were treated with 0.1% dimethyl sulfoxide as the control and 0.3 µM Brz under dark conditions for 4 d. Scale bar represents 10 mm are shown (B). Data are presented as means ± SD ($n \geq 16$).

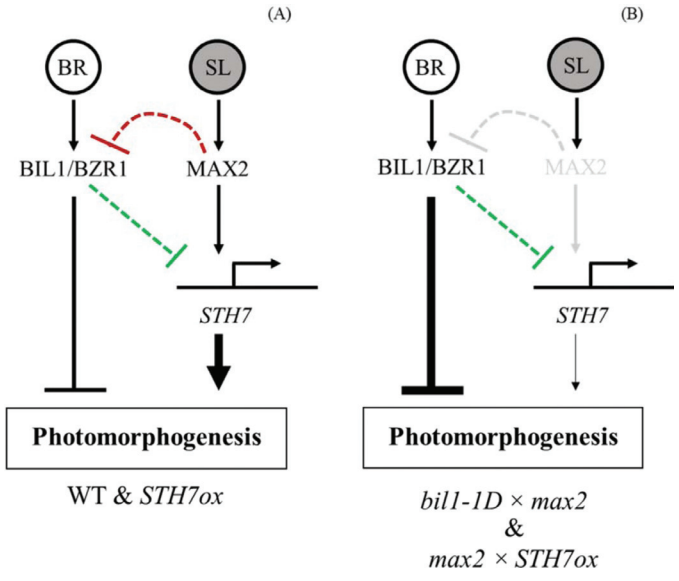

Fig. 7 Proposed working model for role of STH7 and MAX in photomorphogenesis. *STH7* is upregulated by strigolactone (SL). STH7 is related to MAX2, which is SL signaling, and BIL1/BZR1, which is the positive regulator of brassinosteroids (BR), to promote photomorphogenesis (A). However, STH7 could not induce photomorphogenesis without the contribution of MAX2 (B), where red dashed line indicates BIL1/BZR1 interaction with MAX2 and BIL1/BZR1 will be degraded (Wang et al., 2013) and green dashed line indicates BIL1/BZR1 repressed *STH7* transcription (Fan et al., 2012).

Discussion

Photomorphogenesis or light-adapted development is regulated by various signal molecules related to light signals and phytohormones (Wu, 2014). Among the phytohormones, SL plays a positive role in photomorphogenesis, as shown by the inhibition of hypocotyl elongation and the increases in the chlorophyll and anthocyanin contents (Tsuchiya et al., 2010; Thussagunpanit et al., 2017). Another study demonstrated that STH7, a transcription factor belonging to the double B-box zinc finger family, is a positive regulator of photomorphogenesis (Thussagunpanit et al., 2017). To investigate the role of MAX2 and STH7 in the regulation of photomorphogenesis, the expression of *STH7* and photosynthesis-related genes, including *ELIP2*, *CHS*, *LHCB1* and *rbcS*, were analyzed. *ELIP2* produces early light-induced proteins (Harari-Steinberg et al., 2001); *CHS* encodes chalcone synthase involved in anthocyanin biosynthesis (Albert et al., 2009); *LHCB1* encodes the light-harvesting chlorophyll a/b binding protein (Meehan et al., 1996); and *rbcS* encodes the *small subunit* of rubisCO (Dhingra et al., 2004). The present study produced an upregulation of the photosynthesis-related genes *ELIP2*, *CHS*, *LHCB1* and *rbcS*, in *STH7ox* but not in *STH7-SRDX* (Figs. 1B–E), indicating that SL induces photomorphogenesis in *Arabidopsis* in an *STH7*-dependent manner.

GR24, a synthetic SL analog, was applied to *Arabidopsis* and after 4 d growth, the hypocotyl length was measured. GR24 reduced hypocotyl elongation in the *STH7ox* and wild-type *Arabidopsis* (Fig. 2), indicating a photomorphogenesis response. However, STH7 functions were attenuated in the SL-signaling *max2*×*STH7ox*. The present study found that the expression level of *STH7* was reduced in *max2* and *max2*×*STH7ox* mutants compared to *STH7ox* itself (Fig. 1A) implying that *MAX2* works upstream of *STH7*. However, GR24 treatment could increase *STH7* transcriptions in *max2*×*STH7ox* but the expression levels were less than for *STH7ox* (Fig. 3A) indicating that *max2*×*STH7ox* still had functions of *STH7* overexpression. Photosynthesis-related gene expression was analyzed to confirm that STH7 was related to photomorphogenesis. While *STH7ox* upregulated the expression levels of *ELIP2*, *CHS*, *LHCB1* and *rbcS*, lowered transcription levels were observed in the *max2*×*STH7ox* mutants (Figs. 1B–E). These results indicated that MAX2 has a relationship with STH7 to promote photomorphogenesis.

Since MAX2 has been reported to promote photomorphogenesis in response to SL (Nelson et al., 2011), investigation was undertaken of hypocotyl elongation in *STH7ox* and *max2*×*STH7ox*. When treated with the SL analog GR24, *STH7ox* exhibited a shortened hypocotyl; however, the *max2*×*STH7ox* mutant hypocotyls were significantly longer than that of *STH7ox* (Fig. 2), implying that STH7 alone does not respond to SL to reduce hypocotyl elongation. In addition, when treated with GR24, *max2*×*STH7ox* exhibited lower transcription levels of *STH7* and downstream genes of *STH7*, *ELIP2* and *CHS,* than *STH7ox* (Fig. 3). Low expression of *STH7* reduced STH7 proteins, which may have led to decreased transcription of *STH7* downstream genes. STH7 could not regulate photosynthesis-related genes and could not respond to SL without the presence of MAX2. This evidence was consistent with another report that MAX2 acts as a positive regulator of photomorphogenesis to enhance seedling de-etiolation (Nelson et al., 2011). Shen et al. (2012) reported that MAX2 affected photomorphogenesis independent of SL. However, the present study proposed a model in which MAX2 was associated with STH7 to induce photomorphogenesis in an SL-dependent manner (Fig. 7).

While SL has a positive effect on photomorphogenesis, BR suppresses this process through the activation of BIL1/BZR1, a master transcription factor of BR (Sasse, 2003; Clouse, 2011). The BR biosynthesis inhibitor, brassinazole (Brz), was applied to *Arabidopsis* to investigate the effect of BL on photomorphogenesis. Brz reduced hypocotyl elongation in wild-type *Arabidopsis* and *STH7ox* (Fig. 4). However, *STH7-SRDX* and the BR gain-of-function *bil1-1D* were weakly sensitive to Brz (Fig. 4); therefore, Brz had a positive effect on photomorphogenesis. Fan et al. (2012) reported that BZS1/STH7 mediated the crosstalk between the light signal and the BR pathway. This together with the present result confirmed that STH7 and BIL1/BZR1, which was a BR signal molecule, were involved in the photomorphogenesis response. BIL1/BZR1 is not only important for the BR response but also integrates with several other signal molecules of plant hormones (Sun et al., 2010). The present results showed that *max2* and *max2*-related mutants, including *bil1-1D*×*max2* and *max2*×*STH7ox*, also exhibited weak sensitivity to the decrease in hypocotyl elongation caused by Brz, similar to *bil1-1D* (Fig. 4). Generally, *bil1-1D* hypocotyls were not shortened by the low concentration of Brz but *bil1-1D* hypocotyls slightly reduced after application with Brz higher than 0.1 µM (Wang et al., 2002). The result in the *max2*-related mutants indicated that STH7 works with MAX2, which is SL signaling, and with BIL1/BZR1, which is the BR transcription factor, to control photomorphogenesis by decreasing hypocotyl elongation. The result was concordant with Wang et al. (2013). MAX2 could interact with BIL1/BZR1 when SL was added and their homolog BES1 and then BIL1/BZR1 was degraded, triggering responses to SL, such as a decrease in inflorescence branching (Wang et al., 2013).

Based on the present observation of the hypocotyl elongation response to Brz and Wang et al. (2013) reporting that MAX2 inhibits BIL1/BZR1 and then reduces hypocotyl elongation, it was hypothesized that MAX2 works in combination with STH7 (Fig. 7A). To confirm this hypothesis, the present study examined the transcription levels of cell elongation-related genes, consisting of an early auxin-inducible gene (*SAUR-AC1*), a gene encoding a xyloglucan-endotransglycosylase (*TCH4*) and *PRE1*, which has been identified as a positive regulator of cell elongation. *TCH4* and *SAUR-AC1*, which are BR-specific expression genes, are downregulated by the BR inhibitor (Iliev et al., 2002; Nakamura et al., 2003). Generally, Brz causes downregulation of *SAUR-AC1* and *TCH4* in wild-type *Arabidopsis*. In *max2* and *max2*-related mutants, consisting of *bil1-1D*×*max2* and *max2*×*STH7ox*, Brz could not downregulate *TCH4* and *SAUR-AC1*. Furthermore, Brz-treated *max2*×*STH7ox* showed higher expression levels of the cell elongation-related genes than the wild-type (Fig. 5). These results clearly indicated that MAX2 is essential for the expression of cell elongation-related genes. In the mutant plants that are deficient in MAX2 function, such as *bil1-1D*×*max2* and *max2*×*STH7ox*, STH7 could not promote hypocotyl elongation or inhibit BIL1/BZR1, leading to suppression of photomorphogenesis (Fig. 7B).

In this context, photomorphogenesis was investigated by observing cotyledon openings. Brz was added to the planting media to activate cotyledon opening in darkness. Generally, the BR inhibitor causes cotyledons to open in the dark, which can be prevented by BR treatment (Asami et al., 2000). The present results showed that *STH7-SRDX* had a weak response to Brz because it exhibited some closed cotyledon, similar to *bil1-1D* (Fig. 6). This result suggested that *STH7* affected cotyledon opening. However, STH7 function was attenuated in *max2*×*STH7ox*, as shown by the increase in closed cotyledons under the Brz treatment (Fig. 6). Furthermore, *max2* and *bil1-1D*×*max2* had more closed cotyledons than the wild-type after Brz application (Fig. 6), suggesting that MAX2 works with STH7 to control cotyledon opening. Together with the results in hypocotyl shortening, promotion of photomorphogenesis by STH7 is related to the MAX2 function.

Photomorphogenesis was enhanced by MAX2 and brassinosteroid signal transduction. The findings in the present study critically illustrated that this process is promoted by STH7 in a MAX2-dependent manner. Nevertheless, the molecular mechanisms are still unclear on how MAX2 inhibits BR-signaling, which suppresses photomorphogenesis. This question would be clarified by further investigation into whether direct interaction of MAX2 with BIL1/BZR1 is observed in the regulation of branching (Wang et al., 2013). In addition, how MAX2 cooperates with STH7 in photomorphogenesis remains unrevealed. Accordingly, further studies at the translational level (proteomic analysis and protein-protein interaction) should be carried out, as well as investigating the double mutant generated by hybridization crossing between *STH7ox* and *max2* mutant, to compare with *max2*×*STH7ox* used in the present study. Generally, as a subunit of E3 ubiquitin ligase, MAX2 negatively regulated downstream factors. Any MAX2-downregulated unknown factors that suppress the STH7 function should be identified and characterized.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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