

# Regulation of Strigolactone Biosynthesis by Gibberellin Signaling<sup>1</sup>[OPEN]

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Strigolactones (SLs) are a class of plant hormones that regulate diverse physiological processes, including shoot branching and root development. They also act as rhizosphere signaling molecules to stimulate the germination of root parasitic weeds and the branching of arbuscular mycorrhizal fungi. Although various types of cross talk between SLs and other hormones have been reported in physiological analyses, the cross talk between gibberellin (GA) and SLs is poorly understood. We screened for chemicals that regulate the level of SLs in rice (*Oryza sativa*) and identified GA as, to our knowledge, a novel SL-regulating molecule. The regulation of SL biosynthesis by GA is dependent on the GA receptor *GID1* and F-box protein *GID2*. GA treatment also reduced the infection of rice plants by the parasitic plant witchers weed (*Striga hermonthica*). These data not only demonstrate, to our knowledge, the novel plant hormone cross talk between SL and GA, but also suggest that GA can be used to control parasitic weed infections.

Strigolactones (SLs) are a group of terpenoid lactones that regulate shoot branching outgrowth and root development in various plant species (Gomez-Roldan et al., 2008; Umehara et al., 2008; Koltai, 2011a; Ruyter-Spira et al., 2011; Seto et al., 2012). SLs are also exuded

from roots into the rhizosphere as signaling molecules that stimulate the germination of root parasitic weeds and the branching of arbuscular mycorrhizal fungi (Cook et al., 1966; Akiyama et al., 2005). At present, two carotenoid cleavage dioxygenases (*D10* and *D17*), a carotenoid isomerase (*D27*), *Os01g0700900* (*Os0900*), and *Os01g0701400* (*Os1400*) are known to be involved in the biosynthesis of SLs in rice (*Oryza sativa*; Lin et al., 2009; Cardoso et al., 2014; Zhang et al., 2014; Seto et al., 2014; Abe et al., 2014). The activities of SLs depend on *D3* and *D14*: *D3* encodes the F-box protein and *D14* encodes SL receptor, which act in SL signaling. *D53*, which encodes a substrate of the SCF<sup>D3</sup> complex, was recently reported to be a repressor of SL signaling in rice, and the degradation of *D53* protein by SL, in cooperation with *D14* and *D3*, is considered as a key event in SL signaling (Jiang et al., 2013; Zhou et al., 2013; Yao et al., 2016; de Saint Germain et al., 2016).

Gibberellin (GA) is a plant hormone that regulates many aspects of plant growth and development during the life cycle of plants (Yamaguchi, 2008). Plants defective in GA biosynthesis or signaling show characteristic phenotypes, including dwarfism, small dark

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green leaves, prolonged germination dormancy, root growth retardation, suppression of flowering, reduced seed production, and male sterility (Yamaguchi, 2008). Several factors involved in GA perception have been identified. In rice, GA binds to the GA receptor *GID1*, and GA binding promotes the interaction between *GID1* and the transcriptional repressor DELLA protein, *SLR1*. GA-induced interaction of *GID1* and *SLR1* triggers the ubiquitination of *SLR1* by the *GID2* F-box protein, one of the components of an SCF-type E3 ubiquitin ligase that degrades *SLR1* through the 26S proteasomal pathway (Ueguchi-Tanaka et al., 2005; Sasaki et al., 2003). GA-dependent loss of the repressor activity of DELLA is considered a key step in GA signaling (Ueguchi-Tanaka et al., 2008).

Root parasitic weeds, such as broomrape (*Orobanche* spp.) and witchers weed (*Striga* spp.), are harmful plants in sub-Saharan Africa, the Middle East, and Asia that maintain seed dormancy in the absence of host plant (Spallek et al., 2013). The germination mechanisms of *Orobanche* and *Striga* spp. are very similar. Both species require germination stimulants released from the host plant. SLs are the major group of germination stimulants effective for almost all *Orobanche* and *Striga* spp. It has been reported that approximately 300 million people are affected economically by *Striga* spp. in Africa, with estimated losses of \$US 7 billion (Parker, 2009). Although many approaches to controlling these parasitic weeds have been explored, their success has been limited. One approach is the method called "suicidal germination", which induces the germination of the parasites' seeds in the absence of the host plants, because *Striga* and *Orobanche* spp. are obligate parasites. However, this approach is not cost effective for controlling these weeds expanded into a large area.

There is some cross talk between SLs and various hormones. For example, auxin, which regulates shoot branching and root morphology, acts both up- and downstream from SL signaling, in secondary growth and root hair elongation, respectively (Agusti et al., 2011; Koltai, 2011a). SL also inhibits auxin transport by reducing the accumulation of auxin efflux carrier component1 in xylem parenchyma cells (Crawford et al., 2010). Ethylene also affects root hair elongation by exerting epistatic effects on SLs (Kapulnik et al., 2011). Two reports have also directly demonstrated an interaction between SL and hormonal signaling. Wang et al. (2013) showed an interaction between the SL and brassinosteroid signaling pathways. *BES1*, an important transcriptional regulator in the brassinosteroid signaling pathway, interacts with *MAX2*, which is the ortholog of rice *D3*, and *BES1* degradation is accelerated by SLs in *Arabidopsis thaliana*. Nakamura et al. (2013) reported an SL-dependent interaction between *D14* and *SLR1* in rice. This data suggests the existence of cross talk between the GA and SL signaling pathways. However, contradicting results have been reported in which SLs act independently of GAs in stimulating internode elongation in the pea (*Pisum sativum*; de Saint Germain et al., 2013). Therefore, there

is as yet no physiological evidence of the existence of cross talk between the GA and SL signaling pathways, although Lo et al. (2008) reported that GA deficiency promotes tiller bud elongation in rice.

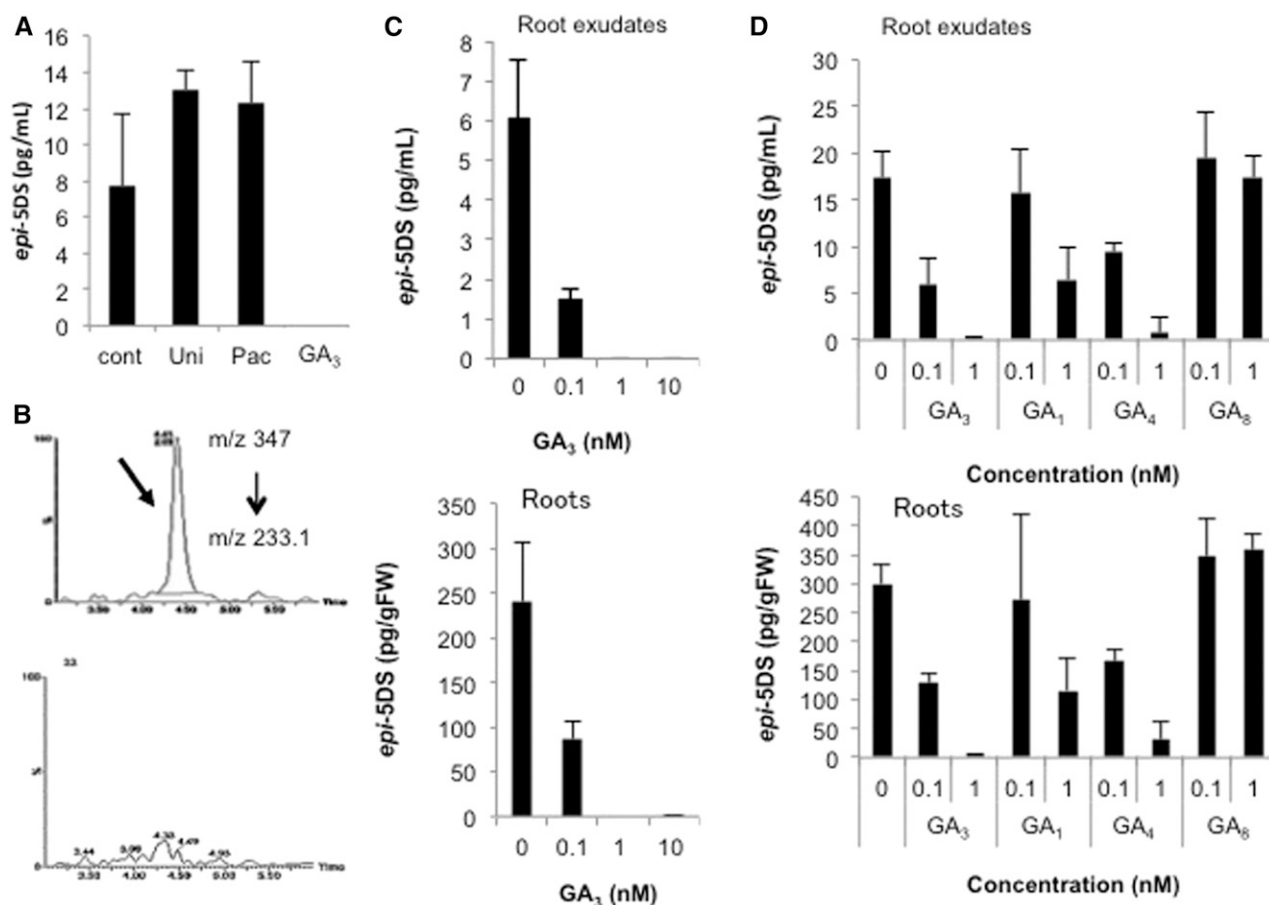
To identify, to our knowledge, a novel regulator of SL biosynthesis that controls the germination of root parasitic weeds in this study, we screened for chemicals that regulate SL biosynthesis. We found that GA is a regulator of SL biosynthesis, and that GA signaling controls the biosynthesis of SL by regulating the expression of SL biosynthesis genes. Moreover, GA-treated rice showed reduced *Striga* infection. Therefore, we demonstrate the cross talk between the GA and SL signaling pathways for the first time to our knowledge, and also present, to our knowledge, new insight into the management of root parasitic weeds.

## RESULTS

### GAs Regulate SL Biosynthesis

Because root parasitic weeds such as *Striga* and *Orobanche* germinate in response to SL in a concentration-dependent manner, low-SL-producing plants can be resistant to infection by these weeds (Jamil et al., 2012). In an attempt to find, to our knowledge, a novel SL biosynthesis regulator, we screened several chemicals, including plant hormones, plant growth regulators, and triazole derivatives previously constructed in our laboratory (Min et al., 1999; Sekimata et al., 2001, 2002). To evaluate their ability to regulate SL biosynthesis, we analyzed the levels of 2'-*epi*-5-deoxystriol (*epi*-5DS), an endogenous SL in rice, in root exudates, using liquid chromatography-tandem mass spectroscopy (LC-MS/MS). Because the SL levels in the root exudates of rice seedlings are elevated when inorganic P is depleted in the medium (Umehara et al., 2008), we examined the effects of these chemical treatments on SL levels under P-deficient conditions. With screening, we identified a plant hormone,  $GA_3$ , that strongly reduced the levels of *epi*-5DS in the root exudates (Fig. 1A). Another SL (orobanchol) in the root exudates was nearly undetectable in the  $GA_3$ -treated rice (Fig. 1B). The application of a GA biosynthesis inhibitor (1  $\mu$ M uniconazole-P or 1  $\mu$ M paclobutrazol) also slightly increased the levels of *epi*-5DS (Fig. 1A). To exclude the possibility that  $GA_3$  inhibits SL export and reduces the levels of *epi*-5DS in root exudates, we analyzed the endogenous *epi*-5DS levels in rice roots. Our data clearly indicate that  $GA_3$  strongly and dose-dependently reduces the levels of *epi*-5DS in both roots and root exudates in a concentration range of 0.1 to 10 nM (Fig. 1C). Moreover, the reduced levels of *epi*-5DS in a  $GA_3$ -treated *Lotus japonicus* root cell culture suggested that  $GA_3$  also reduces the levels of SL in in vitro culture systems and other plant species (Supplemental Fig. S1).

Many GAs are present in plants. In many cases, the intensity of GA responses is dependent on GA-*GID1* binding affinity (Ueguchi-Tanaka et al., 2005). To



**Figure 1.** Effects of GA on SL levels in 2-week-old rice. A, *Epi*-5DS levels in root exudate of rice treated with GA<sub>3</sub> and a GA biosynthesis inhibitor, detected with LC-MS/MS. B, Monitoring selected ions for orobanchol. Upper and lower panels show the chromatograms for the control and 10 nM GA<sub>3</sub>-treated root exudates, respectively. C, *Epi*-5DS levels in root exudates (upper) and roots (lower) of GA<sub>3</sub>-treated rice, determined with LC-MS/MS. D, *Epi*-5DS levels in root exudates (upper) and roots (lower) of rice treated with various GAs, determined with LC-MS/MS. Thirteen-day-old rice seedlings were treated for 1 d with chemicals. Data are means  $\pm$  SD ( $n = 3$ ). GA<sub>3</sub>, 10 nM GA<sub>3</sub>; Pac, 1  $\mu$ M paclobutrazol; Uni, 1  $\mu$ M uniconazole-P.

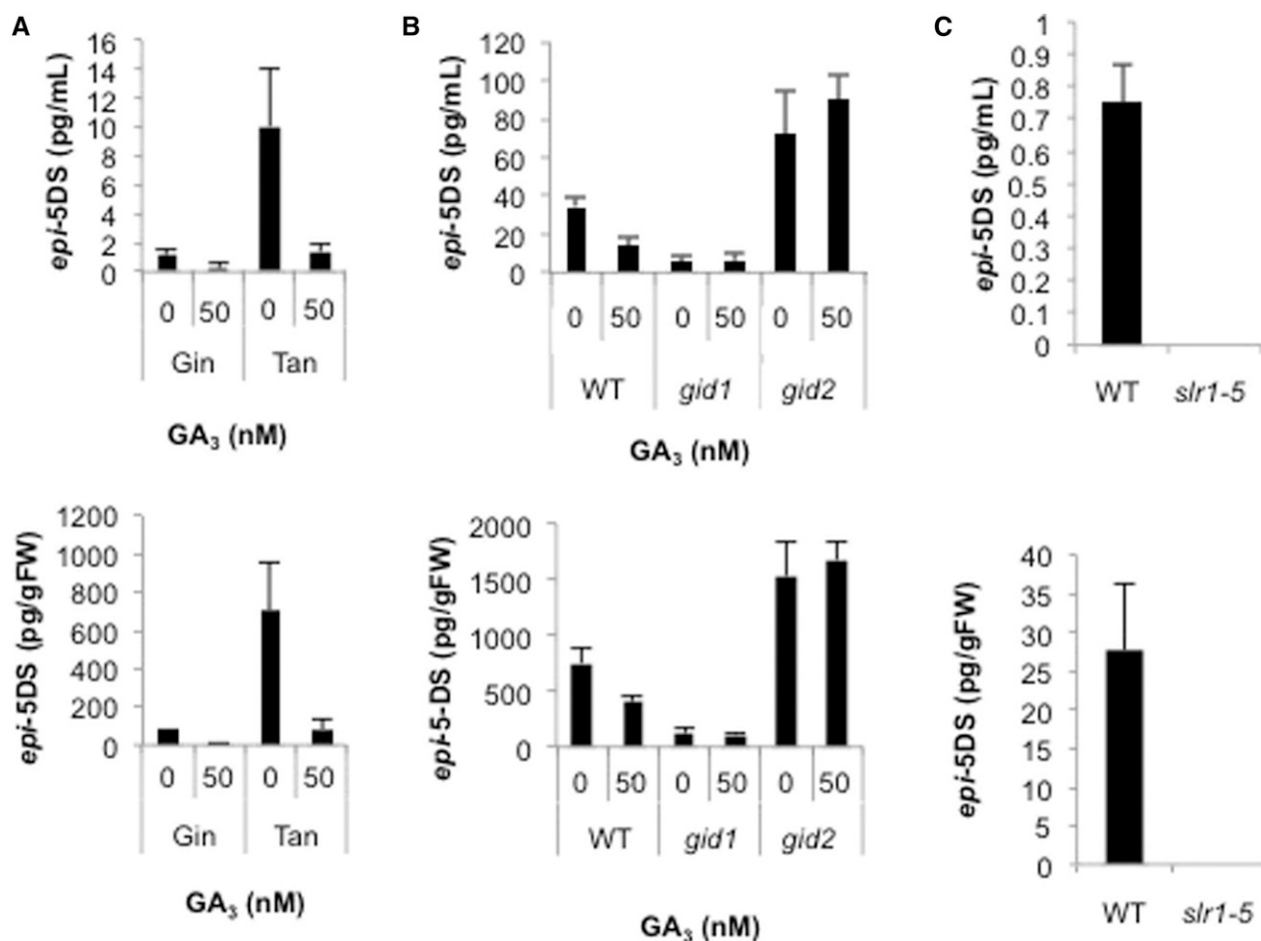
examine the effects of various GAs on SL production, we analyzed the levels of *epi*-5DS in rice treated with active (GA<sub>1</sub>, GA<sub>3</sub>, and GA<sub>4</sub>) or inactive (GA<sub>8</sub>) GAs. The effects of GAs on SL production were generally consistent with the physiological activities of each GA (Fig. 1D; Nishijima et al., 1994). These results indicate that bioactive GAs reduce SL production.

#### Regulation of SL Biosynthesis via a GID-DELLA Signaling Pathway

To investigate the mechanism underlying the regulation of SL biosynthesis by GA, the levels of *epi*-5DS were measured in the roots and root exudates of mutants defective in GA biosynthesis or signaling, with LC-MS/MS (Fig. 2). In the GA biosynthesis mutant, Tanginbozu, which shows a semidwarf phenotype, elevated levels of *epi*-5DS were detected in the roots and root exudates, and were suppressed after GA treatment (Fig. 2A). In GA-insensitive mutants *gid1-3* and *gid2-2*,

the level of *epi*-5DS did not change after GA treatment (Ueguchi-Tanaka et al., 2005; Sasaki et al., 2003). The level of *epi*-5DS was also higher in the *gid2-2* mutant than in wild-type seedlings (Fig. 2B). SLR1 is a repressor of GA signaling and most GA-related responses are induced by the degradation of SLR1. One SLR1 mutant, *slr1-5*, is a constitutive GA response mutant. *epi*-5DS was undetectable in the roots and root exudates of the *slr1-5* mutant (Fig. 2C). These results suggest that the regulation of SL production by GAs is caused by a GID-DELLA signaling pathway.

SL biosynthesis is tightly controlled by endogenous SL levels through the negative feedback regulation of *D10* and *D17* expression (Umehara et al., 2008). To determine whether the regulation of SL production by GAs depends on SL signaling, we tested the effects of GA on SL levels in an SL signaling mutant. Although the *epi*-5DS levels were higher in the loss of function mutants in SL signaling (*d3-1* and *d14-1*) than in the wild type, presumably as a consequence of the feedback

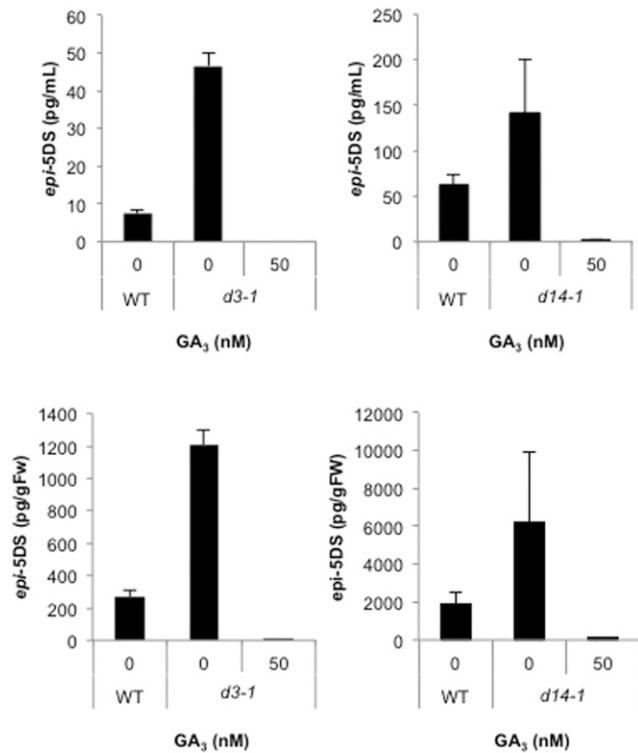


**Figure 2.** Effects of GA on SL levels in GA biosynthesis and signaling mutants. *Epi-5DS* levels in root exudates (upper) and roots (lower) of GA<sub>3</sub>-treated rice, determined with LC-MS/MS. A, GA biosynthesis mutant (Tanginbozu). B, GA signaling mutants (*gid1* and *gid2*). C, Constitutive GA signaling mutant (*slr1-5*). Thirteen-day-old rice seedlings were treated for 1 d with GA<sub>3</sub>. Data are means  $\pm$  SD ( $n = 3$ ). Gin, Ginbozu; Tan, Tanginbozu; WT, wild type.

regulation of the SL pathway, GA<sub>3</sub> reduced the levels of *epi-5DS* in both roots and root exudates (Fig. 3). These results indicate that the pathway through which GA<sub>3</sub> regulates SL biosynthesis is independent of *D3* and *D14*.

We used a quantitative reverse transcription PCR (qRT-PCR) analysis of the SL biosynthesis genes to clarify the mechanism underlying the regulation of SL biosynthesis by GA signaling (Fig. 4A). SLs are synthesized by OsD27, OsD10, OsD17, Os0900, and Os1400 in rice (Alder et al., 2012; Zhang et al., 2014; Supplemental Fig. S2). Our qRT-PCR analysis revealed that treatment with GA<sub>3</sub> for 24 h reduced these transcript levels in the wild-type roots (Fig. 4B). A time-course analysis of the SL biosynthesis genes was performed in roots exposed to 50 nM GA<sub>3</sub> for 0 to 24 h. All of the genes tested here showed similar expression patterns (Fig. 4C). In the control roots, the transcripts increased until 24 h, but remained low in the GA<sub>3</sub>-treated roots. This result suggests two possibilities: one is that GA

treatment reduces transcript levels of SL biosynthesis genes, and the other is GA suppresses the up-regulation of SL biosynthesis genes and maintains the transcription at basal level. To clarify these hypotheses, we performed the expression analysis of SL biosynthesis genes in the time course shown in Supplemental Figure S3A. The transcript levels of SL biosynthesis genes were reduced by GA treatment (Supplemental Fig. S3B). This result indicates GA down-regulates SL production through mediating the expression levels of SL biosynthesis genes. Next, we estimated the effects of a protein synthesis inhibitor, cycloheximide (CHX), on the regulation of SL biosynthesis by GA signaling. As mentioned above, treatment with 50 nM GA<sub>3</sub> reduced the transcript levels of the SL biosynthesis genes. On the other hand, CHX abolished the repression by GA on the transcript levels in comparison with the control (Fig. 4C). These results imply that the regulation of SL levels by GA signaling is attributable to the altered expression of the SL biosynthetic genes via de novo protein synthesis.



**Figure 3.** Effects of GA on SL levels in SL signaling mutants. *Epi-5DS* levels in root exudates (upper) and roots (lower) of the *d3-1* (left) and *d14-1* (right) mutants, determined with LC-MS/MS. Thirteen-day-old rice seedlings were treated for 1 d with GA<sub>3</sub>. Data are means ± SD ( $n = 3$ ). GA<sub>3</sub>, 10 nM GA<sub>3</sub>; WT, wild type.

#### Effects of SL Treatment on GA-Deficient Mutant

GA-deficient mutants show increased tiller bud outgrowth and dwarfism (Lo et al., 2008; Ito et al., 2010). To evaluate the effects of SL on GA-deficient rice, we used the GA-deficient mutant Tanginbozu. Tanginbozu displays dwarfism and greater tiller outgrowth in 5-week-old seedlings than the wild type (Ginbozu; Supplemental Fig. S4), although the length of the second tiller buds of Tanginbozu did not differ from that of Ginbozu in 2-week-old seedlings (Fig. 5). The length of the second tiller was suppressed in Ginbozu by the application of 2  $\mu$ M GR24, a synthetic SL analog, whereas no response was observed in the length of the second tiller bud of Tanginbozu. Plant height did not differ between the control and the GR24-treated plants of either Ginbozu or Tanginbozu. This result suggests that GA is required for the regulation of tiller bud outgrowth by SL.

#### GA-Treated Rice Is Infected by Fewer *Striga* Plants

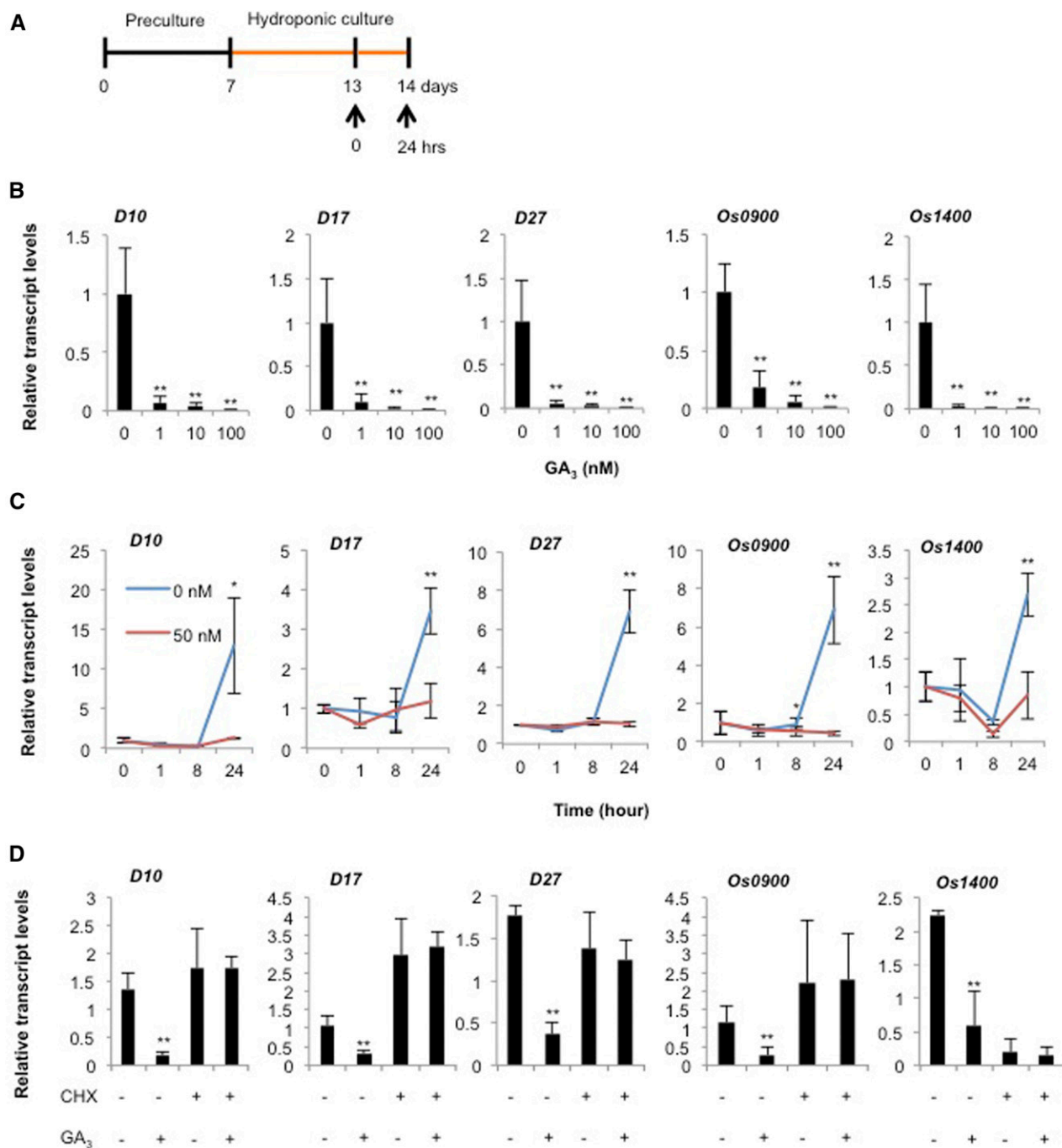
We have demonstrated that GA regulates SL biosynthesis. To explore the effects of GA treatment on the interaction of plants with root parasitic weeds, we performed *Striga* germination and infection assays. Consistent with the results of the *epi-5DS* level in GA-treated rice, the root

exudates of rice seedlings treated with 50 nM GA contained less germination-stimulating activity than those of the control plants (Fig. 6A). Moreover, fewer seeds germinated in the vicinity of the roots of 100 nM GA-treated rice than in the vicinity of the control roots (Fig. 6B). As a result of the reduced germination frequency, statistically fewer *Striga* established parasitism on the 100 nM GA-treated rice. When germinated *Striga* seeds that had been stimulated with strigol were incubated with 100 nM GA-treated rice, there was no significant difference in the frequency of successful parasitism between the control and 100 nM GA-treated rice plants (Fig. 6C). This result indicates that GA treatment does not affect the infectious processes except for germination process.

#### DISCUSSION

SLs are important phytohormones required for plant growth and development. Because SLs are known to be involved in the regulation of diverse physiological phenomena, including shoot branching, root development, and leaf senescence, they are thought to be involved in physiological interactions with various hormones and environmental cues (Crawford et al., 2010; Dun et al., 2012; Kapulnik et al., 2011; Ha et al., 2014; de Jong et al., 2014; Mayzlish-Gati et al., 2012; Tsuchiya et al., 2010). However, the physiological cross talk between SL and GA has not been determined, despite their molecular interaction, in which the putative SL receptor, D14, interacts with the GA signaling repressor, SLR1 (Nakamura et al., 2013). Here, we provide evidence of the interaction between these two hormones, by showing that GA signaling regulates SL biosynthesis.

GA signaling negatively regulates the endogenous levels of SLs. Importantly, the application of an inactive GA metabolite (GA<sub>8</sub>) to wild-type plants or active GA to GA signaling mutants (*gid1-3* and *gid2-2*) did not induce this regulation, whereas the application of active GA to the wild type and a GA biosynthesis mutant (Tanginbozu) reduced their levels of SLs (Figs. 1 and 2). We also detected no SL in a constitutive GA response mutant (*slr1-5*). These results indicate that the regulation of endogenous SL level by GA signaling depends on the activity of the DELLA protein. The repressive activity of GA on SL biosynthesis did not correlate with the binding activity of GA to GID1 (Fig. 1D; Ueguchi-Tanaka et al., 2005). GA<sub>4</sub> shows the highest affinity for GID1. However, whereas GA<sub>1</sub> and GA<sub>4</sub> are inactivated by GA 2-oxidase in plants, GA<sub>3</sub> is not oxidized by GA 2-oxidase (Nakayama et al., 1990). These facts explain the inconsistency between the repressive activity on SL biosynthesis and the binding activity on GID1. The increased levels of SL in *gid2-2* are similar to those in Tanginbozu, suggesting that a reduction in GA signaling induces an increase in the level of SLs. Interestingly, however, the level of SL was lower in the *gid1-3* mutant than in the wild type (Fig. 2, A and B), whereas both the



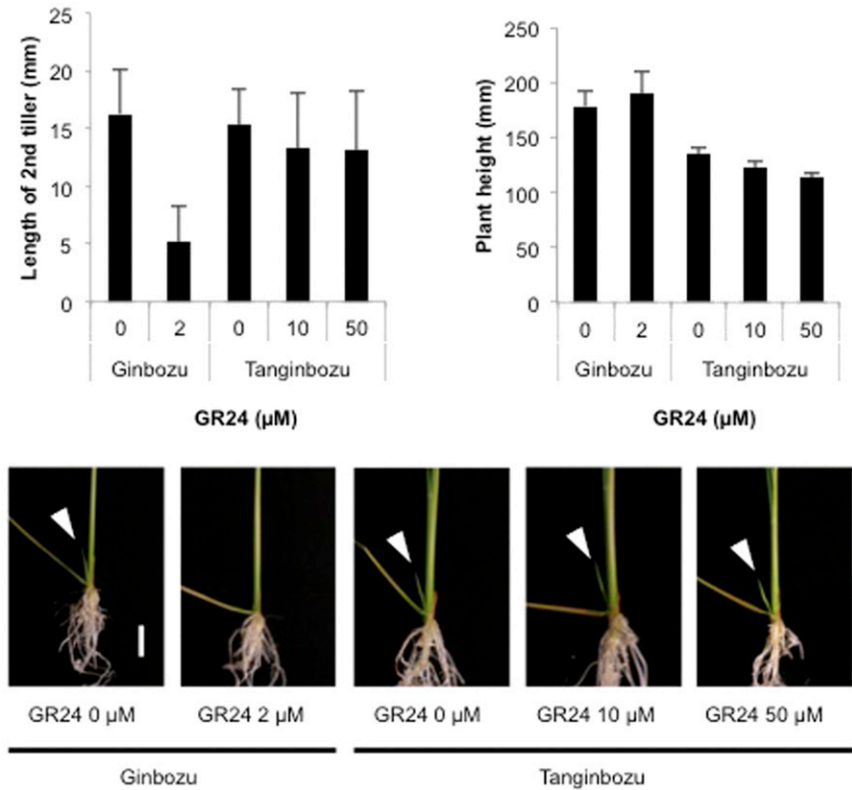
**Figure 4.** Effects of GA on transcript levels of SL biosynthesis genes in roots. A, Schematic diagram showing the experimental conditions. Orange bar indicates a hydroponic culture without P. B, Transcript levels of SL biosynthesis genes in GA-treated rice roots. C, Time-course analysis of the expression of SL biosynthesis genes after treatment with 50 nM GA<sub>3</sub>. D, Effect of protein synthesis inhibitor (CHX) on the expression levels of SL biosynthesis genes after treatment with 50 nM GA<sub>3</sub>. Thirteen-d-old rice seedlings were transferred to new vials containing fresh media with or without chemicals for 1 d (B and D) or the indicated times (C). Data are means  $\pm$  SD ( $n=3$ ). Asterisks (\*) and (\*\*) indicate signify differences from 0 nM GA<sub>3</sub>-treated plants ( $t$  test,  $P < 0.05$  and  $P < 0.01$ , respectively). GA<sub>3</sub>, 10 nM GA<sub>3</sub>.

*gid1-3* and *gid2-2* mutants were insensitive to GA and had similar phenotypes. These data suggest that a GID1-independent GA signaling pathway could be present, similar to the GID2-independent GA signaling pathway

reported by Ueguchi-Tanaka et al. (2008), although this regulatory mechanism is still unclear. Alternatively, as GID1 and D14 competitively bind to SLR1, by the loss of function of GID1 binding to GA, D14 can dominantly



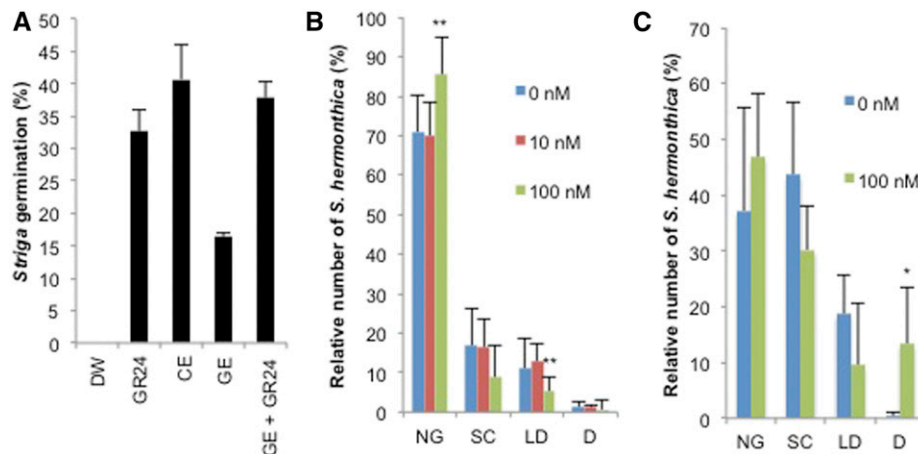
**Figure 5.** Effects of GR24 on tiller bud growth in GA biosynthesis mutant. Second tiller length and plant height in 2-week-old rice. Data are means  $\pm$  SD ( $n = 6$ ). White arrowheads indicate second tillers. Scale bar: 1 cm. Gin, Ginbozu; Tan, Tanginbozu.



bind to SLR1 and suppress SLR1 function. As a result, in the *gid1* mutant 5DS, the level decrease could be due to feedback regulation similar to in that in the *slr1* mutant.

SL biosynthesis is positively and negatively regulated by various hormones and environmental cues

(Yoneyama et al., 2007a, 2007b; Hayward et al., 2009; Koltai et al., 2011b; López-Ráez et al., 2010). Phosphate is a negative regulator of SL biosynthesis and its regulatory activity alters the expression of the SL biosynthetic genes (Umehara et al., 2010). A previous gene



**Figure 6.** Effects of GA on *Striga* germination and infection. A, *Striga* germination rate after treatment with culture medium. Data are means  $\pm$  SD ( $n = 3$ ). DW, distilled water; GR24, 1  $\mu$ M GR24; CE, culture medium for 0 nM GA<sub>3</sub>-treated rice; GE, culture medium for 100 nM GA<sub>3</sub>-treated rice. B and C, Ratio of *Striga* plants at each developmental stage 3 weeks after the inoculation of SL-treated (C) or untreated seeds (B). Concentrations in (B) and (C) indicate the GA concentrations of GA-treated rice. Data are means  $\pm$  SD; B,  $n = 8$  to 10; C,  $n = 2$  to 3. Asterisks (\* and \*\*) indicate significantly different from untreated rice ( $t$  test,  $P < 0.05$  and  $P < 0.01$ , respectively). NG, no germination; SC, penetration successful and seed coat remained attached; LD, leaf developed after the establishment of parasitism; D, died after penetration.

expression analysis indicated that P deficiency increased the transcription levels of *D10*, *D17*, *D27*, *Os0900*, *Os01g0701500*, and *Os02g0221900* (Umehara et al., 2010), whereas GA signaling regulated the expression of the *Os1400* gene, as well as *D10*, *D17*, *D27*, and *Os0900* (Fig. 2). These results suggest that GA signaling regulates the expression of the SL biosynthesis genes through a different pathway from P signaling. However, CHX treatment reduced the transcription level of *Os1400*, whereas the other SL biosynthesis genes (*D10*, *D17*, *D27*, and *Os0900*) were hardly affected by CHX treatment, suggesting that the regulatory mechanisms of *Os1400* expression by GA signaling were different from those of the other SL biosynthesis genes.

In the time course experiment, the expression levels of SL biosynthesis genes were increased at 24 h (Fig. 4C). We did not exchange hydroponic culture media from d 7 to d 13 and transferred rice seedlings into fresh media at d 13 (Fig. 4A). Removal of the accumulated SLs from media might lead the up-regulation of SL biosynthesis genes.

Tanginbozu showed the GR24-insensitive phenotype in tiller bud length (Fig. 5). In Tanginbozu, the endogenous level of *epi-5DS* was higher than that in Ginbozu (Fig. 2A). In addition, *d10-1* mutant is more sensitive to GR24 than the wild type (Umehara et al., 2008). Therefore, the result that Tanginbozu displayed a GR24-insensitive phenotype suggests the possibility that the SL signal is saturated with the accumulated SL in Tanginbozu. However, the overexpression of GA 2-oxidase also positively regulated the number of tillers, indicating the importance of GA signaling in shoot outgrowth in rice (Lo et al., 2008). Further physiological studies may contribute to the elucidation of the mechanism of shoot outgrowth.

In many parts of the world, *Striga* and *Orobanche* are serious agricultural pests. Reducing SL biosynthesis is one way to control these pests and low-SL-producing plants are actually resistant to root parasitic weed infections (Jamil et al., 2012; Umehara et al., 2008). In this study, we have shown that GA-treated rice was infected by fewer *Striga* plants than the control rice, at least under experimental conditions. Therefore, tissue-specific modification of GA signaling can be used to control root parasitic weed infection.

## MATERIALS AND METHODS

### Plant Material

The wild-type rice varieties (*Oryza sativa*) used in this study were Shiokari (*d3-1* and *d10-1*; Ishikawa et al., 2005), Nipponbare (*d10-2*; Arite et al., 2007), Taichung 65 (*slr1-5*, *gid1-3*, and *gid2-2*; Ueguchi-Tanaka et al., 2008), and Ginbozu (Tanginbozu; Itoh et al., 2004).

### Growth Conditions

Rice seedlings were grown hydroponically as described in Umehara et al. (2008). Surface-sterilized rice seeds were incubated in sterile water at 25°C for 2 d in the dark. For the analysis of SL, germinated seeds were transferred into

hydroponic culture medium (Kamachi et al., 1991) solidified with 0.7% agar and cultured at 25°C for 6 d under fluorescent white light with a photoperiod of 14 h light/10 h dark. Each seedling was transferred to a glass vial containing 12 mL of sterilized hydroponic culture solution and grown under the same conditions for 6 d. The seedlings were then transferred to a new glass vial containing the culture solution, with or without chemicals, for 1 d. For the tillering experiment, the germinated seeds were transferred into hydroponic culture medium with 0.7% agar, with or without chemicals, under the same conditions. Each seedling was transferred to a new identical vial with or without chemicals. The hydroponic solution was refreshed every 3 d.

### LC-MS/MS Analysis

The SL analysis was performed according to a previously described method (Umehara et al., 2008). Briefly, the hydroponic culture medium was extracted twice with ethyl acetate after the addition of *d<sub>6</sub>-epi-5DS* (200 pg) as the internal standard. The organic layer was dried and dissolved in 1 mL of ethyl acetate:*n*-hexane (15:85). The solutions were loaded onto a Sep-Pak silica 1 mL cartridge (Waters), washed twice with the same solution, eluted three times with ethyl acetate:*n*-hexane (35:65), and concentrated in vacuo. The roots were homogenized in acetone containing *d<sub>6</sub>-epi-5DS*. The filtrates were dried and dissolved in water. The solutions were extracted twice with ethyl acetate, dried, and dissolved in 10% acetone. The extracts were loaded onto Oasis HLB 3 mL cartridges (Waters), washed twice with water, eluted twice with acetone, and dried in vacuo. The concentrates were dissolved in 1 mL of ethyl acetate:*n*-hexane (15:85) and loaded onto Sep-Pak silica 1 mL cartridges, washed, eluted, and concentrated in the same way.

The SL-containing fractions were dissolved in 50% acetonitrile and subjected to an LC-MS/MS analysis in a system consisting of a quadrupole/time-of-flight tandem mass spectrometer (Triple TOF 5600 system; ABSciex) and an ultra-high-performance liquid chromatograph (Nexera; Shimadzu) equipped with a reversed-phase column (Acquity UPLC BEH-C18, 2.1 × 50 mm, 1.7 μm; Waters). The mobile phase was changed from 30% acetonitrile containing 0.05% acetic acid, to 40% acetonitrile at 5 min and to 70% acetonitrile at 10 min after injection, at a flow rate of 0.2 mL/min. The parent ions (*m/z*) were 331.2 for unlabeled *epi-5DS*, 337.2 for labeled *epi-5DS*, and 347.2 for orobanchol. The samples were quantified using fragment ions 234.13 for *epi-5DS* and 240.13 for *d<sub>6</sub>-epi-5DS*.

### Striga Germination and Infection Assays

The *Striga* germination and infection assays were performed as described by Sugimoto and Ueyama (2008) and Umehara et al. (2008), respectively. For the infection assay, 1-week-old rice seedlings were transferred to root-observing rhizotron chambers (140 mm × 100 mm square petri dishes filled with rockwool and nylon mesh) supplied with 50 mL of nutrient solution with low P (1 mM NH<sub>4</sub>NO<sub>3</sub>, 0.06 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.3 mM K<sub>2</sub>SO<sub>4</sub>, 0.3 mM CaCl<sub>2</sub>, 0.4 mM MgCl<sub>2</sub>, 45 μM Fe-EDTA with micronutrients; Makino et al., 1983) with or without GA<sub>3</sub>, and grown for 2 weeks in a greenhouse with a 12:12 h photoperiod (170 to 450 μmol/m<sup>2</sup>/s) with a day/night temperature cycle of 28°C/20°C. The *Striga* seeds were preconditioned on moist glass-fiber filter papers (GF/A; Whatman) at 26°C in the dark for 2 weeks, and treated with or without 10 nM strigol for 5 h in the dark. After they were rinsed with excess water, approximately 50 parasite seeds were carefully placed along the rice roots and the rhizotrons were incubated under the growth conditions described above. The germination, infection, and developmental status of the *Striga* plants were evaluated after cocultivation for 4 weeks (Yoshida and Shirasu, 2009).

### Gene Expression Analysis

Total RNA was extracted from the roots using Plant RNA Purification Reagents (Invitrogen). cDNA was synthesized with the PrimeScript RT Reagent Kit with gDNA eraser (Takara Bio). qRT-PCR was performed on a Thermal Cycler Dice Real Time System using SYBR Premix Ex Taq (Takara Bio). The specific primers used for qRT-PCR are listed in the Supplemental Table.

### Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers D10 (Os01g0746400), D17 (Os04g0550600), D27 (Os11g0587000), Os0900 (Os01g0700900) and Os1400 (Os01g0701400).



## Supplemental Data

The following supplemental materials are available.

**Supplemental Figure S1.** Effect of GA<sub>3</sub> on SL levels of *L. japonicus* root culture.

**Supplemental Figure S2.** SL biosynthesis pathway in rice.

**Supplemental Figure S3.** Effects of GA on transcript levels of SL biosynthesis genes in roots.

**Supplemental Figure S4.** Tiller number and plant height of GA biosynthesis mutant (Tanginbozu).

**Supplemental Table S1.** List of primers.

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