

# Strigolactones regulate rice tiller angle by attenuating shoot gravitropism through inhibiting auxin biosynthesis

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**Tiller angle, a key agronomic trait for achieving ideal plant architecture and increasing grain yield, is regulated mainly by shoot gravitropism. Strigolactones (SLs) are a group of newly identified plant hormones that are essential for shoot branching/ rice tillering and have further biological functions as yet undetermined. Through screening for suppressors of *lazy1* (*sols*), a classic rice mutant exhibiting large tiller angle and defective shoot gravitropism, we identified multiple *SOLS* that are involved in the SL biosynthetic or signaling pathway. We show that SL biosynthetic or signaling mutants can rescue the spreading phenotype of *lazy1* (*la1*) and that SLs can inhibit auxin biosynthesis and attenuate rice shoot gravitropism, mainly by decreasing the local indoleacetic acid content. Although both SLs and *LA1* are negative regulators of polar auxin transport, SLs do not alter the lateral auxin transport of shoot base, unlike *LA1*, which is a positive regulator of lateral auxin transport in rice. Genetic evidence demonstrates that SLs and *LA1* participate in regulating shoot gravitropism and tiller angle in distinct genetic pathways. In addition, the SL-mediated shoot gravitropism is conserved in *Arabidopsis*. Our results disclose a new role of SLs and shed light on a previously unidentified mechanism underlying shoot gravitropism. Our study indicates that SLs could be considered as an important tool to achieve ideal plant architecture in the future.**

**P**lant shoot gravitropism is a process in which plants perceive gravity stimuli and reorient the direction of growth during the plant growth and development. Gravitropism is a dynamic process, including the perception of gravity, transduction of the corresponding information into a biochemical signal, transmission of the biochemical signal to a response site, and organ curvature (1). Genetic studies have disclosed that shoot endodermal cells act as statocytes for shoot gravitropism (2). Amyloplast sedimentation transduces the gravitropic signal and leads to auxin redistribution, resulting in a higher auxin level on the lower side than on the upper side (1, 3). Although auxin redistribution upon gravistimulation plays an important role in shoot gravitropism, the mechanism by which auxin regulates shoot gravitropism is not yet understood.

In rice the tiller angle, which is defined as the angle between the tiller and main culm, plays a key role in determining rice plant architecture and thus grain yield (4). Several genes controlling the tiller angle have been identified previously in rice, including *LAZY1* (*LA1*), *TILLER ANGLE CONTROL1* (*TAC1*), *PROSTRATE GROWTH1* (*PROG1*), and *LOOSE PLANT ARCHITECTURE1* (*LPA1*) (5–9). Among these genes, *LA1* and *LPA1* are reported to regulate tiller angle through shoot gravitropism. In the *la1* mutant, the polar auxin transport (*PAT*) is enhanced, and the lateral auxin transport is decreased, resulting in a disturbance of auxin asymmetric distribution in the shoot base and therefore the tiller-spreading phenotype of rice plants (5).

Strigolactones (SLs), a group of terpenoid lactones, are newly discovered plant hormones that act as inhibitors of shoot branching in higher plants (10, 11). Previous studies have discovered a series of SL biosynthesis and signaling components, *MORE AXILLARY GROWTH* (*MAX*) in *Arabidopsis*, *DWARF* (*D*) in rice, *RAMOSUS* (*RMS*) in pea, and *DECREASED APICAL DOMINANCE* (*DAD*) in *petunia* (12). Among SL synthesis components, *D27* encodes a  $\beta$ -carotene isomerase that converts all-*trans*- $\beta$ -carotene into 9-*cis*- $\beta$ -carotene (13, 14). *MAX3/RMS5/D17/DAD3* and *MAX4/RMS1/D10/DAD1* encode the carotenoid cleavage dioxygenase 7 (*CCD7*) and *CCD8*, leading to the formation of carlactone, which is catalyzed further by *MAX1/OsMAX1* to yield SL compounds (12, 15, 16). For the SL signaling components, *MAX2/RMS4/D3* encodes an F-box protein participating in SL perception (17–19), *D14/DAD2* encodes a protein of the  $\alpha/\beta$ -fold hydrolase superfamily, a proposed SL receptor (20–25), and *D53* encodes a Clp protease family protein as a repressor (26, 27). Recently, SLs also have been found to be involved in regulating stem secondary growth, leaf senescence, seed germination, root development, and abiotic stress responses (28, 29). In this study, we found that mutants defective in the SL biosynthetic and signaling pathways can rescue the spreading phenotype of *la1* and further demonstrated

## Significance

**Shoot gravitropism is a key determinant of tiller angle, one of the most important factors that affect ideal plant architecture and grain yield of cereal crops. Strigolactones (SLs) are newly identified plant hormones that play diverse roles in plant growth and development. In this study, we provide compelling evidences that SLs are involved in shoot gravitropism, showing that SLs attenuate shoot gravitropism by inhibiting auxin biosynthesis. Our study uncovers a new role of SLs and suggests a previously unidentified mechanism underlying shoot gravitropism and tiller angle. Based on our study, SLs could be considered an important tool for achieving ideal plant architecture in the future.**

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that SLs are involved in rice shoot gravitropism to affect rice tiller angle through an LA1-independent pathway.

## Results

**Isolation of *la1* Suppressors Reveals the Involvement of SLs in Shoot Gravotropism/Rice Tiller Angle.** To identify novel components in the LA1-dependent and -independent pathways that regulate shoot gravitropism, we screened for suppressors of *la1* (*sols*) from the ethyl methanesulfonate (EMS)-mutagenized *la1* progeny. One of the identified *sols*, named “*sol1*,” can mostly rescue the spreading phenotype of *la1* (Fig. 1A). The *la1 sol1* plant shows a phenotype of reduced plant height and increased tillers gradually standing up after seedling stages (Fig. S1A–C). From the mutagenized *la1* progeny, we found another *sol1* allelic mutant, which also is dwarf, high tillering, and erect (Fig. S1D–F). We therefore designated these mutants “*sol1-1*” and “*sol1-2*,” respectively.

We isolated the *SOL1* gene through a map-based cloning approach. In brief, by using 220 F<sub>2</sub> plants the *SOL1* locus was

placed within an 80-kb region between markers M8 and M10, and this 80-kb DNA fragment then was sequenced and compared in *la1* and *la1 sol1-1*. The result showed that one base was replaced at the first exon of LOC\_Os06g06050 in *la1 sol1-1*, resulting in an R702Q amino acid substitution (Fig. 1B), which is conserved across plant species (Fig. S2) and may be involved in substrate recognition (30). In addition, we found that a one-base deletion at the first exon of LOC\_Os06g06050 in *la1 sol1-2* resulted in a frame shift that leads to a premature translation termination in *la1 sol1-2* (Fig. 1B).

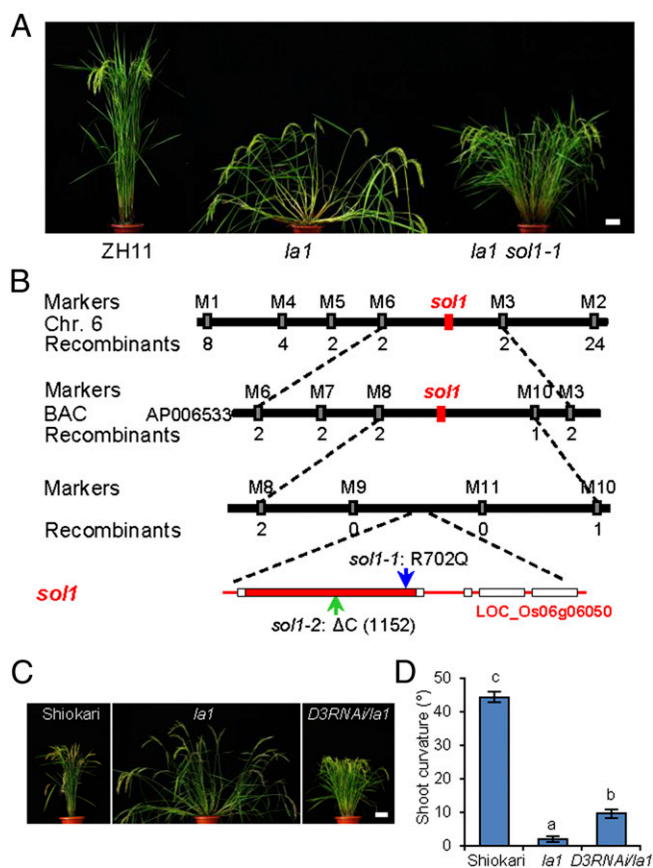
Sequence analysis revealed that LOC\_Os06g06050 is DWARF3 (D3), an F-box component of the SKP–Cullin–F box (SCF) E3 ubiquitin ligase complex that is essential for SL signal perception (17, 18). To verify the function of D3 in rescuing the *la1* phenotype, the *D3*RNAi plasmid was constructed and transformed into the *la1* mutant mediated by *Agrobacterium tumefaciens* (Fig. S3A). The *D3*RNAi transgenic plant was much more compact than the *la1* plant at the adult stage (Fig. 1C). Accordingly, quantitative RT-PCR (qRT-PCR) analysis showed that *D3* expression was reduced significantly in the transgenic plant (Fig. S3B). We also investigated the shoot gravitropic response of *D3*RNAi transgenic seedlings; their response to gravistimulation was enhanced significantly compared with that of the *la1* mutant (Fig. 1D and Fig. S3C), suggesting that the down-regulation of D3 can rescue the spreading phenotype of *la1*.

Furthermore, we found that other *sols* display phenotypes similar to *sol1* in the EMS-mutagenized *la1* progeny. Genetic-linkage analysis and sequencing showed that these *sols* result from mutations in the SL biosynthetic genes, including *D10*, *D17*, and *D27* (Fig. S4), suggesting that SLs may play an important role in regulating plant shoot gravitropism.

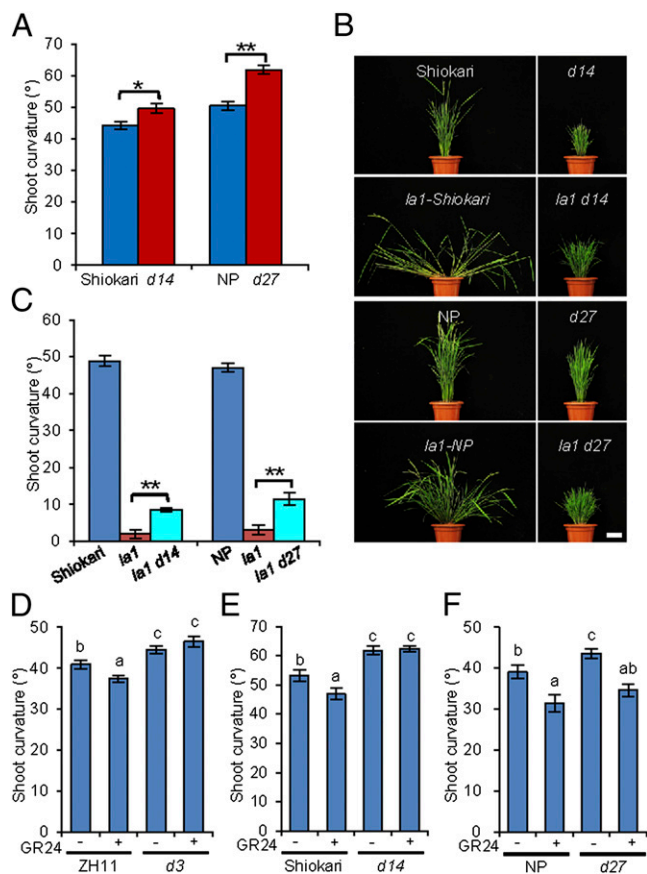
**SLs Attenuate Shoot Gravotropic Response in Rice.** To verify the involvement of SLs in regulating shoot gravitropism, we first examined the shoot gravitropic responses of seedlings of *d14* and *d27*, which were used to represent SL signaling and biosynthetic mutants, respectively. Our results showed that both *d14* and *d27* seedlings displayed enhanced gravitropic responses compared with the wild-type seedlings (Fig. 2A and Fig. S5A–D). Next, we constructed double mutants by crossing *la1* with *d14* and *d27*, respectively. The tiller angles of the double mutants *la1 d14* and *la1 d27* were significantly smaller than that of *la1* (Fig. 2B). Consistently, the gravitropic responses of *la1 d14* and *la1 d27* seedlings were enhanced significantly compared with *la1* (Fig. 2C and Fig. S5E–H), indicating that the deficiency in both SL biosynthesis and signaling could rescue shoot gravitropism and the spreading phenotype of the *la1* plant.

To obtain deeper insight into the role of SLs in shoot gravitropism, we analyzed the shoot gravitropic response upon treatment with GR24, a synthetic analog of SLs (31). As shown in Fig. 2D–F, the gravitropic response of the wild-type seedlings was reduced significantly with GR24 treatment. In contrast, GR24 treatment could not affect the gravitropic response of the *d3* or *d14* mutant, suggesting that SLs decrease shoot gravitropism in a D3- and D14-dependent manner (Fig. 2D and E), as is consistent with the role of D3 and D14 in the perception of SLs. However, GR24 could reduce the gravitropic response of the *d27* seedlings (Fig. 2F). Taken together, these results suggest that SLs can attenuate shoot gravitropism and thus regulate the rice tiller angle.

**SL Regulation of Rice Shoot Gravitropism Is Dependent on Local Auxin Level.** It has been shown that the asymmetric distribution of auxin plays a key role in gravitropism (1–3). To characterize the involvement of auxin in SL-mediated gravitropism, we examined the expression levels of the auxin-responsive marker gene *OsIAA20* in the wild-type and *d3* seedlings upon GR24 treatment before and after gravistimulation. The results showed



**Fig. 1.** Cloning and characterization of *SOL1*. (A) Phenotypes of wild-type (ZH11), *la1*, and *la1 sol1-1* plants at the adult stage. (Scale bar: 10 cm.) (B) Map-based cloning of *SOL1*. *SOL1* was pinpointed in an 80-kb genomic region between molecular markers M8 and M10 and cosegregated with markers M9 and M11 on chromosome 6. Numbers under the markers indicate recombinants. The blue arrow indicates an R702Q amino acid substitution in *sol1-1*, and the green arrow indicates one base deletion at the first exon of LOC\_Os06g06050 that results in a premature translation in *sol1-2*. The closed box indicates an ORF, and open boxes indicate UTRs. (C) Comparison of tiller angle in the wild-type (Shiokari), *la1*, and *D3*RNAi transgenic plants. (Scale bar: 10 cm.) (D) The gravitropic response of *D3*RNAi/*la1* plants at the seedling stage after 24 h gravistimulation. Light-grown seedlings (3 d old) were transferred to darkness and reoriented by 90°. Error bars indicate SEM;  $n = 10$ . Means with different letters are significantly different ( $P < 0.01$ ; ANOVA).



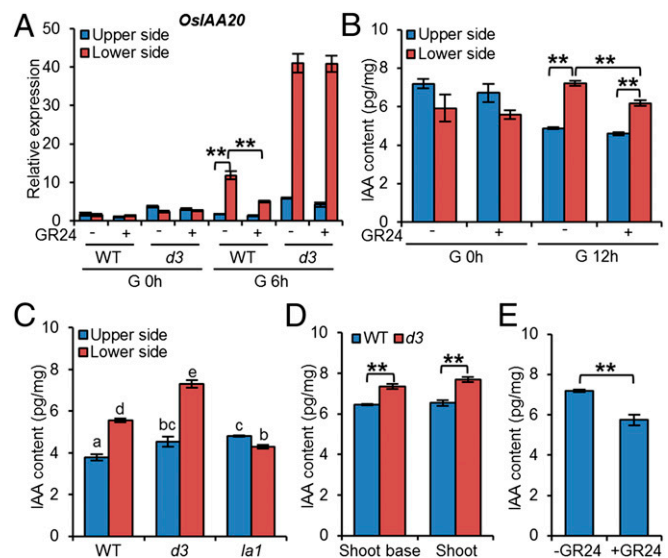
**Fig. 2.** SLs attenuate shoot gravitropic response in rice. (A) Shoot curvature of the wild-type plants [Shiokari and Nipponbare (NP)] and *d* mutants at the seedling stage after 48 h of gravistimulation. (B) The phenotypes of the *la1 d14* and *la1 d27* double mutants at the adult stage. (Scale bar: 10 cm.) (C) Shoot curvature of the *la1 d14* and *la1 d27* double mutants at the seedling stage after 24 h of gravistimulation. In A and C, \* $P < 0.05$ , \*\* $P < 0.01$ , Student *t* test. (D–F) The shoot gravitropic responses of *d3* (D), *d14* (E), and *d27* (F) seedlings upon GR24 treatment. The seedlings were grown under light with or without GR24 treatment for 72 h and then were transferred to darkness and reoriented by 90° upon gravistimulation. Means with different letters are significantly different ( $P < 0.05$ ; ANOVA). Error bars indicate SEM;  $n = 10$ .

that the expression levels of *OsIAA20* in the lower side and upper sides were comparable in wild-type and *d3* seedlings with or without GR24 treatment before gravistimulation (Fig. 3A and Fig. S6). However, *OsIAA20* was expressed asymmetrically after gravity stimulus in the wild-type seedlings, and its expression was reduced significantly in the lower side of the shoot base upon GR24 treatment. *OsIAA20* expression was higher in the *d3* seedlings than in wild-type seedlings at both the upper and lower sides of the shoot base and did not change with GR24 treatment (Fig. 3A). These results indicate that the SLs may affect gravity-induced auxin redistribution. Furthermore, we measured the indoleacetic acid (IAA) content in the upper and lower sides of the shoot base upon gravity stimulation after GR24 treatment. The results demonstrated that GR24 decreased the IAA level in the lower side more significantly than in the upper side of the shoot base after 12 h of gravistimulation (Fig. 3B), indicating that SLs may regulate asymmetric auxin distribution after gravity stimulus in the shoot base.

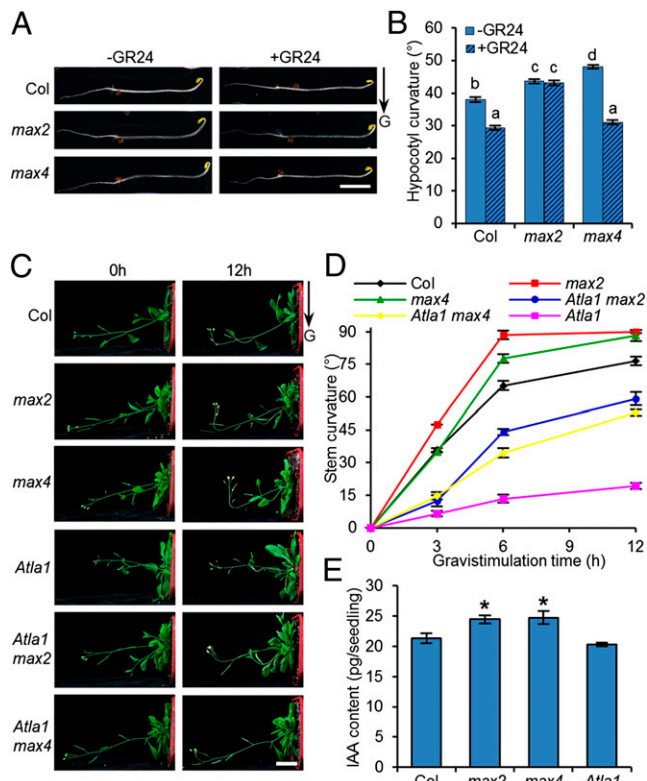
Furthermore, we measured the auxin contents of 7-d-old seedlings of the wild type, *la1*, and *d3* mutants after 12 h of gravistimulation. As shown in Fig. 3C, *d3* mutants accumulated more auxin in the lower side of the shoot base than did wild-type

seedlings. In contrast, in *la1* seedlings the auxin level was not elevated at the lower side of shoot base upon gravistimulation. Moreover, we found that in *d3* seedlings the IAA level was elevated not only in the shoot base but also in the shoot (Fig. 3D). In contrast, GR24 treatment resulted in decreased IAA levels in the shoots of rice seedlings (Fig. 3E). Collectively, these data demonstrate that SLs may attenuate shoot gravitropism by decreasing the local IAA content via regulating auxin biosynthesis.

**SL-Mediated Shoot Gravitropism Is Conserved in *Arabidopsis*.** Because the involvement of SLs in shoot branching is conserved in many plant species, we reasoned that the molecular mechanism of SL-mediated shoot gravitropism should be conserved in other species, such as the dicot *Arabidopsis*. To verify this notion, we examined the gravitropism of *Arabidopsis max2* and *max4* mutant seedlings. As shown in Fig. 4A and B, the gravitropic responses of etiolated hypocotyls in *max2* and *max4* mutants were enhanced significantly as compared with the wild type after 6 h of gravistimulation. Moreover, the hypocotyl gravitropism of the *max4* mutant was restored to the level in the wild type upon GR24 treatment, whereas the *max2* mutant exhibited consistent enhanced gravitropism upon GR24 treatment (Fig. 4A and B). Accordingly, the gravitropic responses of *max2* and *max4* inflorescence stems were enhanced significantly after gravistimulation (Fig. 4C and D). We then generated the double mutants of *Atla1 max2* and *Atla1 max4* and compared their gravitropic responses with the responses of *Atla1*, a gravitropism-defective mutant caused by a mutation in the *Arabidopsis LA1* gene (Fig. S7) (32). As shown in Fig. 4C and D, the gravitropic responses of the double mutants were enhanced compared with



**Fig. 3.** SLs regulate rice shoot gravitropism by decreasing the local auxin level. (A) Expression levels of *OsIAA20* at the lower and upper sides of the shoot base in wild-type and *d3* seedlings with or without GR24 treatment upon gravistimulation for 0 and 6 h. Error bars indicate SEM;  $n = 3$ . (B) IAA contents at the lower and upper sides of the shoot base with or without GR24 treatment upon gravistimulation for 0 and 12 h. Light-grown 7-d-old seedlings were reoriented by 90° upon gravistimulation. Error bars indicate SEM;  $n = 4$ . (C) IAA contents showing an asymmetric auxin distribution between the upper and lower sides of shoot base in the wild-type and *d3* and *la1* mutant seedlings upon 12 h of gravistimulation. Error bars indicate SEM;  $n = 3$ . Means with different letters are significantly different ( $P < 0.05$ ; ANOVA). (D) The IAA contents in the shoot base and shoot of the wild-type and *d3* seedlings. Error bars indicate SEM;  $n = 4$ . (E) The IAA contents in the shoot of wild-type seedlings with or without GR24 treatment. Error bars indicate SEM;  $n = 4$ . \*\* $P < 0.01$ , Student *t* test.



**Fig. 4.** Involvement of SLs in shoot gravitropism is conserved in *Arabidopsis*. (A) Photographs of wild-type (Col-0), *max2*, and *max4* hypocotyls with or without GR24 treatment upon gravistimulation. (Scale bar: 0.5 cm.) (B) Statistic analysis of hypocotyl curvature in 4-d-old dark-grown wild-type, *max2*, and *max4* seedlings with or without GR24 treatment upon 6 h of gravistimulation. Error bars represent SEM;  $n = 50$ . Means with different letters indicate a significant difference ( $P < 0.01$ ; ANOVA). (C) Photographs of inflorescence stems of wild-type, *max2*, *max4*, *Atla1*, *Atla1 max2*, and *Atla1 max4* seedlings upon 12 h of gravistimulation. (Scale bar: 2 cm.) (D) Kinetic analysis of inflorescence stem curvature in wild-type, *max2*, *max4*, *Atla1*, *Atla1 max2*, and *Atla1 max4* seedlings upon gravistimulation. Values represent the mean  $\pm$  SEM;  $n = 10$ . (E) Quantification of IAA levels in 7-d-old light-grown wild-type, *max2*, *max4*, and *Atla1* seedlings. Error bars indicate SEM;  $n = 3$ . \* $P < 0.05$ ; Student *t* test.

those of *Atla1*. Furthermore, the IAA levels in *max2* and *max4* seedlings were higher than in the wild type, but the *Atla1* IAA level was comparable to that in the wild type (Fig. 4E). These results demonstrated that SLs can regulate shoot gravitropism by auxin biosynthesis in both dicot and monocot plants.

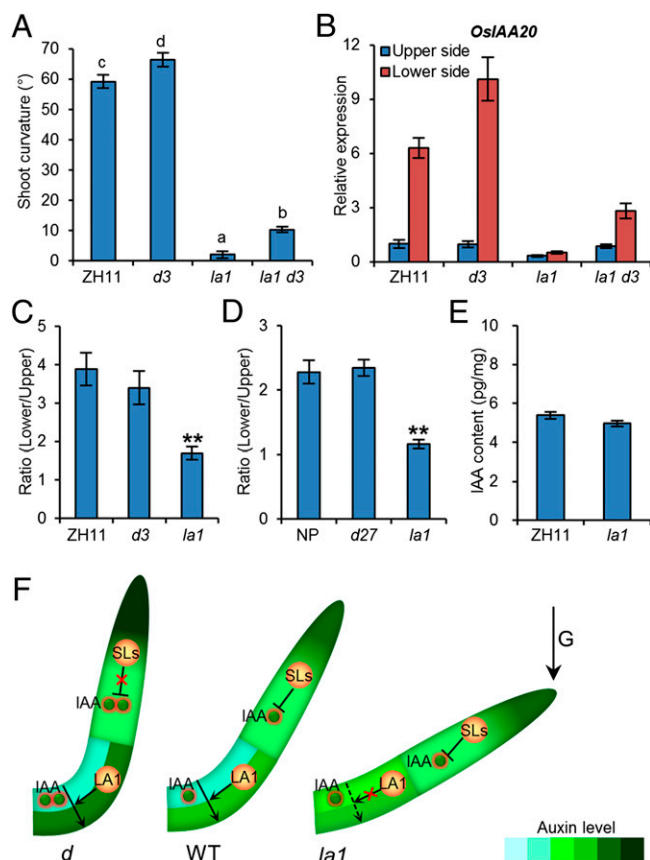
**SL Regulation of Rice Shoot Gravitropism Is Independent of LA1.** To reveal the relationship between the SLs and LA1 in the regulation of shoot gravitropism, we first examined the *D3* expression in *la1* and the *LA1* expression in *d3*. The qRT-PCR results showed that *D3* expression was comparable in wild-type and *la1* seedlings (Fig. S8A), and the expression of *LA1* was unchanged in *d3* seedlings (Fig. S8B), implying that *D3* may regulate shoot gravitropism independent of LA1. Therefore we performed an in-depth analysis of the shoot gravitropism in *d3*, *la1*, and *la1 d3* seedlings. Upon gravistimulation at the seedling stage, the *d3* shoot bent faster than the wild type. The *la1 d3* seedling showed a greater gravitropic response than the *la1* plant, but this response still was weaker than those of the wild-type and *d3* seedlings (Fig. 5A and Fig. S8C and D). Accordingly, the expression of *OsIAA20* at the lower side of the *la1 d3* shoot base after 6 h of gravistimulation was significantly higher than in the *la1* seedling but was lower than in the wild-type and *d3* seedlings

(Fig. 5B). These results indicate that *D3* and LA1 may have parallel but opposite functions in regulating shoot gravitropism through an asymmetric distribution of auxin.

As previously reported, LA1 could regulate the asymmetric auxin distribution through lateral auxin transport (5). In this study, we found that, unlike *la1* mutants, mutants defective in the SL biosynthetic and signaling pathways exhibited normal lateral auxin transport (Fig. 5C and D). We also found that the IAA level was not affected in *la1* seedlings (Fig. 5E) but was elevated in the SL-defective mutants (Fig. 3D). These data strongly suggested that SLs may regulate auxin asymmetric distribution and thus shoot gravitropism mainly through auxin biosynthesis, without an obvious effect on lateral auxin transport. Therefore, the SL biosynthetic and signaling pathways very likely are involved in a negative regulation of rice shoot gravitropism and tiller angle in a genetic pathway that is distinct from that of LA1.

## Discussion

Rice tillers are specialized branches, and the tiller angle is an important agronomic trait that contributes to plant architecture



**Fig. 5.** SLs regulate shoot gravitropism in an LA1-independent manner. (A) Comparison of shoot gravitropism in wild-type (ZH11), *d3*, *la1* and *la1 d3* seedlings. Light-grown 3-d-old seedlings were transferred to darkness and reoriented by 90° upon gravitropism for 2 d. Error bars indicate SEM;  $n = 10$ . Means with different letters are significantly different ( $P < 0.05$ ; ANOVA). (B) Expression levels of *OsIAA20* at the lower and upper sides of the shoot base after gravity stimulation for 6 h. Error bars indicate SEM;  $n = 3$ . (C and D) Comparison of lateral IAA transport in wild-type, *la1*, and *d3* seedlings (C) and *d27* mutants (D). The ratio indicates the radioactivity of the lower side to that of the upper side of coleoptiles upon gravity stimulation. Error bars indicate SEM;  $n = 10$ . \*\* $P < 0.01$ ; Student *t* test. (E) Comparison of IAA levels in wild-type and *la1* seedlings. Error bars indicate SEM.  $n = 3$ . (F) A proposed model of SL- and LA1-dependent shoot gravitropism.

and grain production (4). In the past years, several genes that determine rice tiller angle have been cloned and characterized (5–9). Some of them, for example *LAI* and *LPAI*, are involved in shoot gravitropism and thus regulate tiller/branch angle in different plant species (5, 9, 32, 33), indicating that shoot gravitropism is the key component dictating the proper positioning of shoot branches. Although the asymmetric distribution of auxin has long been regarded as the main factor that affects shoot gravitropism, the underlying molecular mechanism still remains to be elucidated.

SLs are a group of terpenoid lactones that were known first for their functions in rhizosphere parasitic and symbiotic interactions (31, 34). Recently, SLs have been found to act as plant hormones playing diverse roles in plant growth and development (12). In this study, we revealed a novel role of SLs in regulating shoot gravitropism by screening for *sols* in rice.

Our results demonstrate that the SLs attenuate shoot gravitropism, which in turn regulates tiller/branch angle. We show that SL biosynthetic and signaling mutants can rescue the *la1* phenotype in both rice (Figs. 1*A* and 2*B* and Fig. S4) and *Arabidopsis* (Fig. 4). Consistently, SL-deficient mutants have enhanced shoot gravitropism in both rice seedlings (Fig. 2*A* and *D* and Fig. S5) and *Arabidopsis* hypocotyls and inflorescence stems (Fig. 4). GR24 treatment, on the other hand, reduces the shoot gravitropic response in the wild-type seedlings and SL biosynthetic mutants (Figs. 2*F* and 4*A* and *B*), but not in SL signaling mutants (Figs. 2*D* and *E* and 4*A* and *B*). Furthermore, SLs regulate the asymmetric distribution of auxin in the shoot base after gravity stimulus (Fig. 3*A–C*). Further evidence showed that SL-deficient mutants have increased IAA content in both the shoot base and whole seedlings (Fig. 3*D*), but lateral auxin transport is not altered (Fig. 5*C* and *D*). Therefore we concluded that SLs attenuate shoot gravitropism mainly by inhibiting auxin biosynthesis.

As previously reported, PAT is enhanced in both SL-defective and *la1* mutants. In the *la1* mutant, the enhanced PAT disrupts the lateral auxin transport, leading to an abnormal asymmetric distribution of auxin and shoot gravitropism (5) (Fig. 5*A–E*). However, in the SL-defective mutants, the lateral auxin transport is normal (Fig. 5*C* and *D*), although PAT is also enhanced (14, 35). The increased auxin biosynthesis in SL-defective mutants leads to an increased IAA level at the lower side of shoot base upon gravistimulation, by which the shoot gravitropism is enhanced (Fig. 3*C* and *D*). Shoot gravitropism is enhanced significantly in the *d/max la1* double mutant compared with the *la1*

mutant but is attenuated compared with the *d/max* mutants (Fig. 4*C* and *D*, Fig. 5*A*, and Fig. S8*C* and *D*), suggesting that the SL biosynthetic and signaling pathways may antagonize LA1 in regulating shoot gravitropism in an LA1-independent manner.

Based on our present results, we proposed a model of rice tiller angle control (Fig. 5*F*). In the wild-type rice plant, SLs inhibit auxin biosynthesis and maintain a moderate auxin level to generate an asymmetric auxin distribution at the shoot base upon gravistimulation. In SL-defective mutants, SLs seem unable to inhibit the auxin biosynthesis; the increased auxin level at the lower side of the shoot base upon gravistimulation results in an enhanced gravitropic response and a compact plant. Although auxin biosynthesis is normal in the *la1* mutant, the PAT is enhanced, and the lateral auxin transport is defective, so that the lower side of shoot base cannot accumulate auxin, eventually resulting in a spreading phenotype. In the *la1 d* double mutants, the increased auxin level at the lower side of shoot base of *d* mutants can compensate to some extent for the lack of auxin at the lower side of shoot base of *la1* mutants, leading to a compromised tiller angle. As a major hormone in regulating shoot branching/tillering, SLs recently have been shown to interact with auxin by triggering rapid deletion of the auxin efflux protein PIN1 (36). Notably, the tropic response of shoots in *pin1* is normal (37). Therefore we proposed that SLs are likely to regulate shoot branching and gravitropism in parallel by auxin transport and biosynthesis.

Taken together, our results uncover a previously unidentified mechanism underlying the involvement of SLs in the control of shoot gravitropism and thus the tiller/branch angle, illustrating a potentially widely conserved strategy by which plants optimize growth and development under diverse environmental conditions.

## Materials and Methods

Plant growth, map-based cloning, qRT-PCR, and shoot gravitropic assays were performed as described previously (5, 32), and rice was transformed as in a previous report (38). Free IAA content and lateral auxin transport were measured as previously described (5, 39), with minor modifications. Primers used in this study are listed in Table S1. Details are provided in *SI Materials and Methods*.

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