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.

Plant 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 gravitostimulation 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), PROSTATE 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 β-carotene isomerase that converts all-trans-β-carotene into 9-cis-β-carotene (13, 14). MAX3/RMS/D17/DAD3 and MAX4/RMS/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/RMS/D3 encodes an F-box protein participating in SL perception (17–19), D14/DAD2 encodes a protein of the α/β-fold hydrolase superfamily, a proposed SL receptor (20–25), and D53 encodes a Cip protease family protein as a repressor (26, 27). Recently, SLs also have been found to be involved in regulating stem secondary growth, leaf sentences, 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 Gravitropism/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. 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 F2 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 DARWF3 (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 D3RNAi plasmid was constructed and transformed into the la1 mutant mediated by Agrobacterium tumefaciens (Fig. S3A). The D3RNAi 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 D3RNAi 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 Gravitropic 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 those 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
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 gravistimulation (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 of the shoot base upon gravity stimulation after 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.

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 gravitropism 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...
SEM; n gravitropism independent of LA1. Therefore we performed an
Atla1 light-grown wild-type, represent the mean
a greater gravitropic response than the
shoot bent faster than the wild type. The
showed that
in
la1
without GR24 treatment upon gravistimulation. (Scale bar: 0.5 cm.) (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. 5 C 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

![Image](http://www.pnas.org/cgi/doi/10.1073/pnas.1411859111)

**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) Statistical 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 ± 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.

![Image](http://www.pnas.org/cgi/doi/10.1073/pnas.1411859111)

**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 wild-type, max2, max4, and Atla1 seedlings. 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 LA1 and LPA1, 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 sla1 mutants in rice.

Our results demonstrate that the SLs attenuate shoot gravitropism, which is in turn regulates tiller/branch angle. We show that SL biosynthetic and signaling mutants can rescue the sla1 phenotype in both rice (Figs. 1A and 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. 2F and 4A and B), but not in SL signaling mutants (Figs. 2D and E and 4A 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. 3D), 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 sla1 mutants. In the sla1 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 gravitropism, by which the shoot gravitropism is enhanced (Fig. 3 C and D). Shoot gravitropism is enhanced significantly in the dmax sla1 double mutant compared with the sla1 mutant but is attenuated compared with the dmax mutants (Fig. 4 C and D, Fig. 5A, and Fig. S5 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. 5F). 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 gravitostimulation. 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 gravitostimulation results in an enhancedgravitropic response and a compact plant. Although auxin biosynthesis is normal in the sla1 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 sla1 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 sla1 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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Supporting Information

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SI Materials and Methods

Plant Materials and Growth Conditions. Rice (Oryza sativa L.) plants were grown in paddy fields in Beijing in summer or in Hainan province in winter. The la1, sol1, and sos la1 mutants are in the ZH11 background, dl4 is in the Shikokari background, and d27 is in the Nipponbare background, all of which are japonica subspecies. Seedlings were grown on 0.4% agar plates at 28 °C under a 16-h light/8-h dark cycle.

Arabidopsis seedlings were grown in soil or on 1/2 Murashiga and Skoog (MS) medium plates containing 1% sucrose and 0.8% agar at 22 °C under a 16-h light/8-h dark cycle. All Arabidopsis mutants used in this study are in the Columbia (Col-0) background. The Atla1 (GABI 591A12) mutant was obtained from the Arabidopsis Biological Resource Center. The transfer DNA (T-DNA) insertion sites were verified by PCR and sequencing (Fig. S7), indicating that the genotype is identical to a previously reported allele (1). The max2 and max4 mutants were provided by Ottoline Leyser (University of Cambridge, Cambridge, UK) (2). Atla1 max2 and Atla1 max4 double mutants were generated from the genetic crosses of Atla1 × max2 and Atla1 × max4, and homozygous lines were confirmed by PCR genotyping and/or sequencing. Primers used for identifying T-DNA insertion sites and genotypes of double mutants are given in Table S1.

Map-Based Cloning of Suppressors of LA1. To isolate suppressors of LA1 (SOLS), we carried out map-based cloning. The sol1 mutants were crossed to la1 in the background of ZF802, an indica variety. The Indel and cleaved amplified polymorphic sequences markers were generated based on nucleotide polymorphisms between the genome sequences of Nipponbare and 93-11, an indica variety. The SOL1 locus was placed within an 80-kb region between M8 and M10 on chromosome 6 by using 220 F2 plants showing a compact plant type. The molecular lesions of sol1-1 and sol1-2 were identified by PCR amplification of the LOC_Os06g06050 (D3) genomic region from la1 and sol1-1 and sol1-2 mutant plants and sequence comparison using DNASTAR. The primer sequences used for mapping are listed in Table S1.

Assay of Shoot Response to Gravity. The gravitropic assay was carried out as described previously (3). For rice shoot gravitropism, 3- or 4-d-old light-grown seedlings were grown on 0.4% agar containing different concentrations of GR24 as indicated and then were transferred to darkness and reoriented by 90° for a series of time periods at 28 °C. To examine gene expression upon gravity stimulation by qRT-PCR analyses, 7-d-old light-grown seedlings were reoriented by 90° for 6 h, and then 1.5 cm of the basal shoot was dissected into lower and upper sides. For Arabidopsis shoot gravitropism, 4-d-old etiolated seedlings were grown on 1/2 MS medium with or without 2.5 μM GR24 and then were reoriented by 90° for up to 24 h, or 1-mo-old inflorescence stems were transferred to darkness with 24 h gravistimulation.

Generating D3RNAi Transgenic Lines. To construct the D3RNAi plasmid, two 350-bp DNA fragments were amplified from the D3 cDNA using two pairs of primers, D3RNAi-F1 and D3RNAi-R1 and D3RNAi-F2 and D3RNAi-R2 (Table S1) and were cloned into the binary vector i460. This recombined plasmid then was introduced into Agrobacterium tumefaciens EHA105, and the la1 mutant was transformed as previously reported (4). The phenotypes were scored in the homozygous T3 progeny.

Quantitative RT-PCR Analysis. Total RNA was extracted using a TRIzol RNA extraction kit (Invitrogen). One microgram of total RNA was treated with DNase I and used to synthesize cDNA with an Avian Myeloblastosis Virus Reverse Transcriptase (Promega). Quantitative RT-PCR (qRT-PCR) experiments were performed using the SsoFast EvaGreen Supermix kit (Bio-Rad) on the CFX96 real-time system (Bio-Rad) following the manufacturer’s instructions. The expression levels were normalized to the expression of a rice ubiquitin gene. The gene-specific primers are listed in Table S1.

Measurement of Free Indoleacetic Acid Content. Indoleacetic acid (IAA) extraction and measurement were performed as previously described (5) with minor modifications. Briefly, 7-d-old seedlings were reoriented by 90° for 12 h. After gravistimulation, 150-mg shoot tissues from the lower and upper sides of the 1.5-cm shoot base were collected for analysis. After extraction and purification, the samples were subjected to LC/MS-MS analysis using a system consisting of an Acquity Ultra Performance Liquid Chromatograph (Acquity UPLC; Waters) and a triple quadrupole tandem mass spectrometer (Quattro Premier XE; Waters).

Lateral Auxin Transport Assay. Lateral auxin transport was assayed as previously described with minor modifications (3). Briefly, 5-d-old dark-grown coleoptiles (1 cm) were harvested and deprived of endogenous IAA. The apical ends of coleoptiles were inserted horizontally into agar blocks that contain 100 nM 3H-IAA. After transport in darkness at 28 °C for 2.5 h, sections of the 0.5-cm segments from the apex were split evenly into upper and lower halves. After incubation in 2 mL scintillation liquid overnight, the radioactivity of each half was counted by a liquid scintillation counter (1450 MicroBeta TriLux; Perkin-Elmer).

Fig. S1. Phenotypic comparison of wild-type (ZH11), la1, la1 sol1-1, and la1 sol1-2 plants. (A and B) Statistical analysis of plant height (A) and tiller number (B) of wild-type, la1, and la1 sol1-1 plants. (C) Phenotypic comparison of wild-type (ZH11), la1, and la1 sol1-1 plants after the seedling stage. DAG, days after germination. (D) Phenotypic comparison of wild-type (ZH11), la1, and la1 sol1-2 plants at the adult stage. (E and F) Plant height (E) and tiller number (F) of wild-type, la1, and la1 sol1-2 plants. Error bars indicate SEM; n = 10. **P < 0.01, Student t test.
Fig. S2. Sequence alignment of rice D3, Arabidopsis MAX2, pea RMS4, petunia PhMAX2A, and PhMAX2B. The conserved R702 is marked by the red box. The green box indicates the F-box, and the blue lines above the sequence indicate leucine-rich repeats.
Fig. S3. Confirmation of the involvement of D3 in shoot gravitropism. (A) Schematic representation of D3 and D3RNAi constructs. The closed box indicates an ORF, and open boxes indicate UTRs. The red arrows indicate the fragment selected from the corresponding sequence in D3. (B) qRT-PCR analysis of D3 expression levels in D3RNAi/la1 transgenic seedlings. Error bars indicate SEM; n = 4. **P < 0.01; Student t test. (C) Kinetic analysis of wild-type (Shiokari), la1, and D3RNAi/la1 shoot curvature upon gravistimulation. Three-day-old light-grown seedlings were transferred to darkness and reoriented by 90°. Error bars indicate SEM; n = 10.

Fig. S4. Identification of other la1 sols. The phenotypes of la1 sols and their mutation sites at the corresponding D loci. The black boxes represent exons. An 11-bp deletion occurred at the second exon in D10 in la1 sol14, resulting in premature translation. The 421st amino acid G of D17 is replaced by S in la1 sol5. The 369th base G, which occurs at the splicing site after the first exon, is replaced by A in D17 in la1 sol6. The 168th amino acid N of D17 is replaced by I in la1 sol7. A 60-bp deletion occurs in D27 in la1 sol17, resulting in a truncated protein.
Fig. S5. Analysis of gravitropic responses. (A and B) Photographs (A) and kinetic analysis (B) of shoot gravitropism of the wild type (Shiokari) and d14 mutants upon 48-h gravistimulation. (C and D) Photographs (C) and kinetic analysis (D) of shoot gravitropism of the wild type (NP) and d27 mutant upon 48-h gravistimulation. (E and F) Photographs (E) and kinetic analysis (F) of shoot gravitropism of the wild type (Shiokari), la1, and la1 d14 mutants upon 36-h gravistimulation. (G and H) Photographs (G) and kinetic analysis (H) of shoot gravitropism of the wild type (NP), la1, and la1 d27 mutants upon 36 h gravistimulation. The 3-d-old light-grown seedlings were transferred to darkness and reoriented by 90° for gravistimulation. Error bars indicate SEM. n = 10. (Scale bar: 2 cm.)
Fig. 56. Graphical representation of rice seedling upon gravistimulation. The blue and red boxes indicate the upper and lower sides of a shoot base, respectively, for gene-expression analysis and measurement of IAA content.

Fig. 57. Characterization of the Atla1 mutant. (A) Diagram of AtLA1 and the mutation site in the T-DNA insertion line. The sequence above the genomic structure shows the wild-type sequences flanking the T-DNA insertion site. Blue boxes indicate coding exons, the red box indicates the 5′ UTR, the black lines between the boxes indicate introns, and the green line indicates the promoter. The triangle represents T-DNA insertion, and the arrows indicate the positions of primers used for PCR genotyping. (B) T-DNA insertion was confirmed by the genomic PCR and sequencing. (C) The phenotype of the Atla1 mutant at the adult stage, showing the larger branch angle. (Scale bar: 2 cm.)
Fig. S8. Gene-expression analysis of LA1 and D3 by qRT-PCR and gravitropic responses of the wild type, d3, la1, and la1 d3. (A) Comparison of D3 levels expressed in the wild-type and la1 seedlings. (B) Comparison of LA1 levels expressed in wild-type and d3 seedlings. (C and D) Photographs (C) and kinetic analysis (D) of shoot gravitropism of wild-type, d3, la1, and la1 d3 seedlings upon 48-h gravistimulation. (Scale bar: 2 cm.) Error bars indicate SEM. n = 3 in A and B; n = 10 in D.
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