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LAZY1 controls rice shoot gravitropism through regulating polar auxin transport

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Tiller angle of rice (*Oryza sativa* L.) is an important agronomic trait that contributes to grain production, and has long attracted attentions of breeders for achieving ideal plant architecture to improve grain yield. Although enormous efforts have been made over the past decades to study mutants with extremely spreading or compact tillers, the molecular mechanism underlying the control of tiller angle of cereal crops remains unknown. Here we report the cloning of the *LAZY1* (*LA1*) gene that regulates shoot gravitropism by which the rice tiller angle is controlled. We show that *LA1*, a novel grass-specific gene, is temporally and spatially expressed, and plays a negative role in polar auxin transport (PAT). Loss-of-function of *LA1* enhances PAT greatly and thus alters the endogenous IAA distribution in shoots, leading to the reduced gravitropism, and therefore the tiller-spreading phenotype of rice plants.

***Keywords*:** *LAZY1*, gravitropism, auxin transport, tiller angle, plant architecture, *Oryza sativa* L.

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Introduction

Gravity is one of the fundamental factors that affect a number of biological processes. Plants, to compensate their sessile nature, adapt to gravity by directing shoots upwards and roots downwards termed negative and positive gravitropisms, respectively. Significant progresses on the molecular mechanisms of plant gravitropism have been achieved in the past years using *Arabidopsis* as a model [1]. Although genetic and phenotypic studies have indicated that the molecular mechanisms underlying the gravitropic response in roots, hypocotyles and inflorescence stems are not uniform [2], the gravitropic response is suggested to proceed through three major conserved steps: gravity perception, signal transduction and transmission, and growth response [3, 4].

In *Arabidopsis*, root cap and shoot endodermis are responsible for sensing gravity in plant roots and shoots, respectively [5, 6]. The displacement of amyloplasts is thought to be the main event that triggers further signal transduction. Phytohormones [7-10], cytosolic ions such as Ca^{2+} [11, 12], pH [13, 14], $InsP_3$ [15, 16], and several proteins such as DnaJ-like proteins [17, 18] were suggested to play important roles in the gravity signal transduction and transmission.

Since Cholodny and Went proposed that asymmetrical auxin distribution within plant organs might explain the mechanism of tropic growth [19, 20], auxin transport has been thought to be a central component of the gravitropic signaling. Auxin is transported along the shoot-root axis from cell to cell in a polar manner, which requires both influx and efflux carriers [21]. So far, auxin carriers have been identified and well characterized in *Arabidopsis*, especially for the efflux carriers. Eight members of PIN proteins have been demonstrated to facilitate auxin efflux [22]. Among them, PIN1 and PIN2/AGR1/EIR1/WAV6

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were first identified to participate in auxin transport because the phenotypes of *pin1* and *pin2* were similar to those of wild-type plants grown in the presence of auxin transport inhibitors [23, 24], and these two proteins are localized on plasma membranes in an asymmetric manner [25, 26]. PIN3, another regulator of auxin efflux, was subsequently reported to localize with the plasma membrane and vesicles that cycle in an actin-dependent manner and could relocalize laterally upon gravity stimulation [27]. Recent studies in *Arabidopsis* provided strong evidence to support the view that PIN polar localization within the auxin transport-competent cells determines the intercellular auxin flow [28-30]. In addition, AUX1 and AtPGP4 have been identified as auxin influx carriers in *Arabidopsis* [31-33].

More importantly, gravitropism is also a key factor to determine plant architecture, one of the most crucial agronomic traits that contributes to crop grain production and thus has attracted much attention of breeders for centuries. Several agravitropic mutants in crops have been reported such as *lazy* in maize [34], *lazy* (*la*) [35] and *spk*(*t*) (*spreading type of kasalath*) [36] in rice, and *serpentine* in barley [37]. Among them, the rice *la* mutant has been intensively studied for decades [35, 38-42]. Although enormous efforts have been made in the elucidation of the mechanisms underlying the *la* phenotype, the studies were mainly concentrated on its phenotypic description and preliminary physiological experiments, and the corresponding gene has not been identified, leading to the poor understanding on molecular mechanisms of shoot gravitropism in crops.

We report here the in-depth characterization of the rice mutant *la1-ZF802* (previously termed *la-2*) [43]. *LA1*, cloned through a map-based approach, is a novel grass-specific gene that functions as a negative regulator of PAT, the dysregulation of which likely leads to the reduced gravitropism and tiller-spreading phenotype of the mutant plants.

Materials and Methods

Plant materials

The original tiller-spreading mutant used in this work was acquired from Dr Khush in IRRI. We introduced its spreading character into an *indica* cultivar *ZF802* with 10 generations of backcrosses and named it as *la1-ZF802*. An allelic mutant *la1-Shiokari* was derived from *Shiokari* variety and used for gene transformations owing to its high transformation efficiency.

Analysis of plant gravity response

Rice gravity response under light and in the dark was measured using 5-day-old seedlings planted in plates containing 1/2 MS medium (pH 5.8). Rice seeds were dehusked and surface sterilized with 70% ethanol for 2 min and 30% bleach for 2 h, and then washed five times with autoclaved distilled water. The seedlings were then grown at 28 °C. Gravity response was determined by measuring the shoot curvature after seedlings were reoriented 90° at every 4 h interval with 20 seedlings for each time point.

DNA extraction and gel blotting

The rice genomic DNA and DNA gel blotting were performed as described [44]. In brief, the isolated genomic DNA was digested with proper restriction enzymes, separated in a 0.8% agrose gel, transferred onto a Hybond N^+ membrane (Amersham), and hybridized with ^{32}P-labeled probes.

RT- or RACE-PCR analysis

Total RNA was prepared using TRIzol® reagent according to the user manual (Cat. No. 15596-026, Invitrogen). One microgram of total RNA was treated with DNase I and used for complementary DNA synthesis with RT kit (Cat. No. A3500, Promega). 5′- or 3′-RACE of *LA1* was carried out by using a SMART™ RACE cDNA Amplification Kit according to the manufacturer's instruction (Cat. No. K1181-1, Clontech). The primer sequences used for the above studies were listed in Supplementary information, Table S1.

Subcellular localization of LA1

To determine the subcellular localization of LA1, CaMV35S::LA1-GFP, CaMV35S::LA1ΔN100-GFP, CaMV35S::LA1ΔNLS-GFP and CaMV35S::GFP were constructed. LA1ΔN100 is the truncated LA1 with a deletion of amino-acid residues1-100 that contain a predicted transmembrane domain. LA1ΔNLS refers to the LA1 truncated from amino-acid residues 286 to 312, a segment containing a putative nuclear localization signal (NLS) domain. The vectors were introduced into onion epidermal cells by using a bombardment-mediated gene transformation system (PDS-1000/He, BIORAD). After overnight incubation in the dark, GFP was examined under a confocal microscope at an excitation wavelength of 488 nm (FluoView 500, Olympus).

Histological analysis and mRNA in situ hybridization

The assay of β-glucuronidase (GUS) activity was performed as described [45]. For detecting the GUS-staining pattern in the rice vegetative shoot, the stained samples were trimmed, fixed, sectioned into 7 to 10 mm, observed under bright field through a microscope (Leica DMR), and photographed using a Micro Color charge-coupled device (CCD) camera (Apogee Instruments).

Shoot apexes of rice seedlings at the four-leaf stage were fixed with 4% (w/v) paraformaldehyde at 4 °C overnight, followed by a series of dehydration and infiltration, and embedded in paraffin (Paraplast Plus, Sigma). RNA *in situ* hybridization was performed as described previously [46]. The 996-1571 bp region of the *LA1* gene was subcloned into the T-easy vector and used as templates to generate sense and antisense RNA probes. Digoxigenin-labeled RNA probes were prepared using a DIG Northern Starter Kit (Cat. No. 2039672, Roche), according to the manufacturer's instruction. Slides were observed under bright field through a microscope (Leica DMR), and photographed with a Micro Color charge-coupled device (CCD) camera (Apogee Instruments).

Polar auxin transport assay

The polar auxin transport assays were performed according to the method described previously with some modifications [23]. Five groups of five 5-day-old dark-grown coleoptile segments (2 cm) were used for the assay. The segments were deprived of endogenous

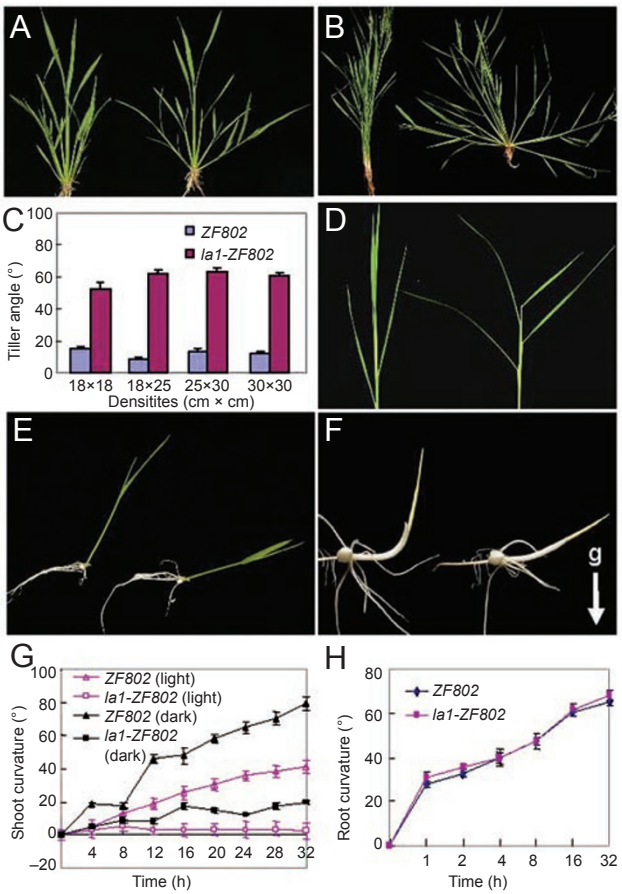

Figure 1 Phenotype of the *la1-ZF802* mutant. **(A, B)** Comparison of the tiller angle between *ZF802* (left) and *la1-ZF802* (right) at the **(A)** tillering stage and **(B)** ripening stage. **(C)** Tiller angles at different planting densities. **(D)** Leaf angles of *ZF802* (left) and *la1-ZF802* (right). **(E, F)** Five-day-old light-grown **(E)** and dark-grown **(F)** *ZF802* (left) and *la1-ZF802* (right) seedlings on stimulation of gravity for 24 h. The arrow marked with g indicates the direction of gravity. **(G, H)** Kinetic analyses of the gravitropic responses of shoots **(G)** and roots **(H)**. Error bars indicate ± SE (n = 18).

IAA by pre-incubation in 1/2 MS (pH 5.8) liquid medium for 2 h. *N*-1-naphthylphtalamic acid (NPA) was added to the medium as indicated. To avoid gravistimulation, the pre-incubation took place on a shaker. The apical or basal ends of the segments (for basipetal or acropetal transport assays, respectively) were then submerged in 10 μl of 1/2 MS liquid medium containing 0.35% phytogel, 500 nM ^{3}H-IAA and 500 nM free IAA in a 1.5-ml Eppendorf tube in the dark at room temperature, and ^{14}C-benzoic acid was applied as a control of IAA. The non-submerged ends were laid with a layer of lanolin. After 3 h, 5 mm sections from the non-submerged ends of segments were excised and washed two times with 1/2 MS liquid medium. After 18 h incubation in 2 ml scintillation liquid, the radioactivity of each section was counted by a liquid scintillation counter (1450 MicroBeta TriLux, Perkin-Elmer).

Lateral auxin transport assay

Five-day-old coleoptiles of seedlings grown in the dark were used for the assay of auxin lateral transport as described [47] with some modifications. Coleoptiles (1 cm) were harvested and deprived of endogenous IAA as mentioned above. The coleoptiles were laid horizontally on microscope slides with their apical ends contacting with 0.4 × 0.4 × 0.2 cm agar blocks that contain 500 nM ^{3}H-IAA and 500 nM IAA. After transport in the dark at room temperature for 2.5 h, sections of the 5-10 mm segments from the apex were evenly split into upper and lower halves. After 18 h incubation in 2 ml scintillation liquid, the radioactivity of each half (n = 15) was counted as described above.

Results

Characterization of rice spreading-grown mutant la1-ZF802

Although the phenotype of rice *la* mutant has been described in previous studies [35, 40, 48], the background of the materials used was not uniform and the analysis was not systematic and comprehensive. We therefore performed an in-depth analysis of the *la1-ZF802* mutant. Morphological analysis showed that the *la1-ZF802* mutant plant exhibited a tiller-spreading phenotype at different growth stages (Figure 1A and 1B) and the phenotype was not affected by planting densities (Figure 1C). Close examination revealed that the mutant phenotype was caused by the loss of ability to grow upright during the elongation of primary and tiller shoots, consistent with the previous report that the prostrate phenotype resulted from agravitropism of rice main shoots and tillers [41]. The gravitropic response was greatly reduced in both light- and dark-grown *la1-ZF802* mutant seedlings (Figure 1E and 1F), which was further confirmed by kinetic studies (Figure 1G). In addition, mutant plants also exhibited a larger leaf angle than those of wild type, especially at the reproductive stage (Figure 1D). However, mutant roots showed normal gravitropism (Figure 1H), indicating that *LA1* may be involved only in shoot and tiller gravitropism of rice plants.

Map-based cloning of LA1

Our previous studies placed the *LA1* gene in an interval of 0.4 cM between the AC35795 and AC6330 markers, near the centromere region of Chromosome 11 [43]. To fine-map the *LA1* locus, we generated three large F_2 mapping populations derived from crosses between *la1-ZF802* and three local cultivars in China. Of the F_2 plants, ~30 000 mutant individuals were used for fine-mapping. Screening with molecular markers (see Supplementary information, Table S2) placed *LA1* in a 68-kb region that was covered by two BAC clones (AC136378 and AC136787) between the pd56 and M265 markers, and no recombinants were found between the M13F marker and the *LA1* locus (Figure 2A). Within this region, there are 17 predicted genes/ORFs.

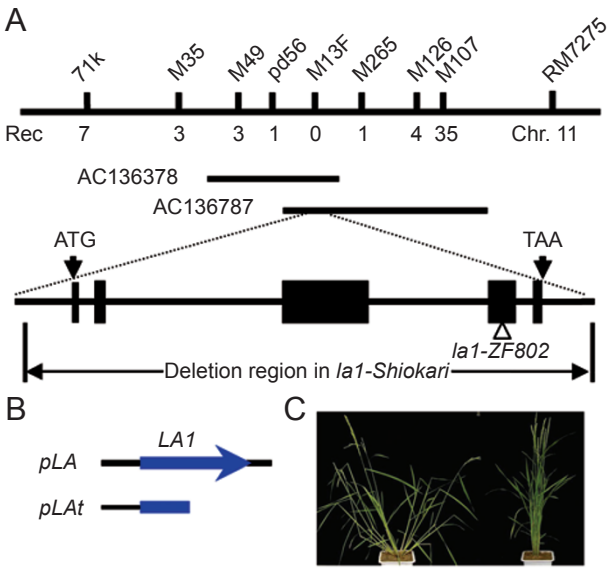

Figure 2 Cloning and functional confirmation of *LA1*. **(A)** Fine mapping and a schematic representation of *LA1*. Δ indicates the 8-bp deletion in *la1-ZF802* and the deletion of the entire *LA1* gene in *la1-Shiokari* is also shown. The start codon (ATG) and stop codon (TGA) are indicated. Boxes indicate the coding sequences and lines between boxes indicate introns. **(B)** Complementation plasmids containing the entire (*pLA*) or truncated (*pLAt*) *LA1*. **(C)** Phenotypes of transgenic lines of *pLAt* (left) and *pLA* (right).

Sequencing these genes/ORFs of *la1-ZF802* revealed an 8-bp deletion at the fourth exon of a putative gene/ORF, which leads to protein premature termination (Figure 2A and Supplementary information, Figure S1). No mutation could be found in the other predicted genes/ORFs (data not shown). Mutations were also identified in the *la1-Shiokari* allele with a prostrate phenotype similar to *la1-ZF802* (see Supplementary information, Figure S2). The predicted ORF was entirely lost in *la1-Shiokari* (Figure 2A), which was confirmed by DNA gel blot analysis (see Supplementary information, Figure S3). These results indicated that the predicted ORF was very probably the *LA1* gene.

The identity of *LA1* was further confirmed by genetic complementation. The plasmid *pLA*, containing the entire ORF, and *pLAt*, containing the partial coding region of the ORF (Figure 2B), were introduced into the *la1-Shiokari* mutant, and 8 and 11 independent lines were generated from these two constructs, respectively. All the eight lines containing the *LA1* transgene showed a complementation of the *la1-Shiokari* phenotype, whereas all the 11 lines of *pLAt* failed to rescue the *la1-Shiokari* mutant (Figure 2C). We therefore conclude that we have cloned the rice *LA1* gene, which is responsible for the tiller-spreading phenotype of mutant plants.

LA1 knockout/knockdown transgenic lines were generated by introducing the specific RNA interference (RNAi) construct into wild-type plants. The *LA1* RNAi transgenic plants displayed larger tiller angles compared to the wild plants (Figure 3A), and the phenotype was correlated with endogenous *LA1* expression levels (Figure 3B), suggesting that *LA1* is a potential useful target for rice tiller angle modification in molecular breeding.

LA1 encodes a novel grass-specific protein

Sequence analysis of 5′- and 3′- RACE cDNA products indicated that the *LA1* cDNA is 1 606 bp long, with an ORF of 1 251-bp, a 35-bp 5′-untranslated region (UTR), and a 320-bp 3′-UTR (see Supplementary information, Figure S1). Sequence comparison between genomic and complementary DNAs revealed that *LA1* is composed of five exons that encode a 416 amino-acid protein (Figures 2B and 4A). BlastP analysis revealed that LA1 is a novel protein, sharing no homology with any functionally known protein. However, homology analysis against TIGR plant transcript assemblies showed that LA1 shares high identities with deduced proteins only in cereal plants, including *Sorghum bicolor*, *Zea mays* and *Triticum aestivum* (Figure 4A), suggesting that LA1 might be a grass-specific protein.

Using the TMpred [49] and PredictNLS [50] programs, we found that the LA1 protein contains a transmembrane domain (amino-acid residues 62-83) and an NLS domain (amino-acid residues 286-312; Figure 4A), which were confirmed by transient expression analysis in onion epidermal cells (Figure 4B-F). In contrast to the GFP control that was

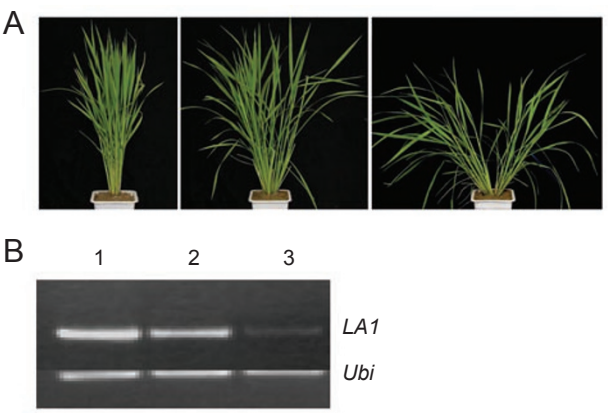

Figure 3 Analysis of *LA1* RNAi transgenic plants. **(A)** Comparison of tiller angles between the wild type (left) and *LA1* RNAi transgenic lines, *LAi-1* and *LAi-2* (middle and right). **(B)** Expression levels of endogenous *LA1* in the wild type (1), *LAi-1* (2) and *LAi-2* (3). *Ubi*, rice ubiquitin as a control.

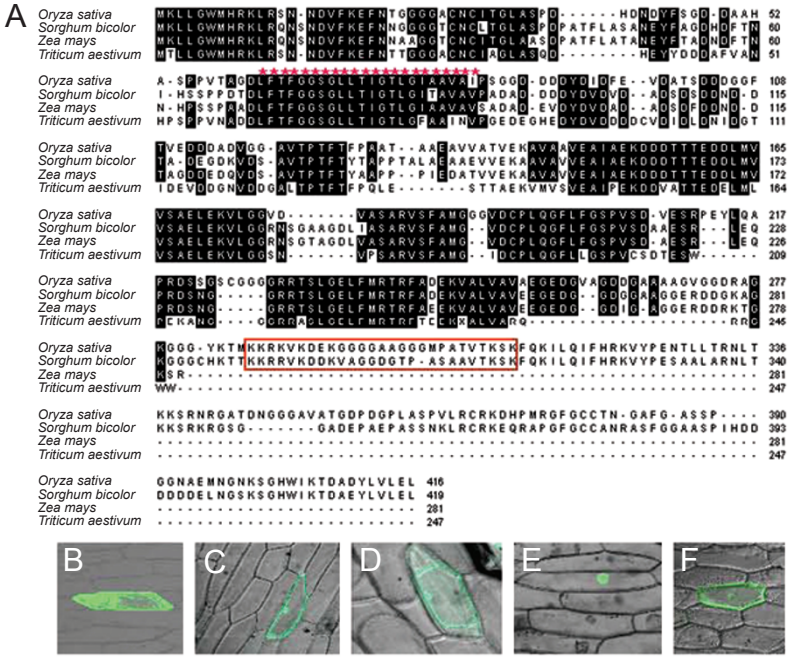

Figure 4 Sequence comparison and subcellular localization of LA1. **(A)** Alignment of deduced amino-acid sequence of LA1 with its homologs in *Sorghum bicolor*, *Zea mays* and *Triticum aestivum*. Shaded letters indicate the identical amino-acid residues. Asterisks indicate the predicted transmembrane domain and the squared letters indicate the putative NLS domain. **(B-F**) Subcellular localization of **(B)** CaMV35S:GFP, CaMV35S:LA1-GFP **(C)** before and **(D)** after plasmolysis, **(E)** CaMV35S:LA1ΔN100-GFP, and **(F)** CaMV35S:LA1ΔNLS-GFP in onion epidermic cells.

distributed everywhere in onion epidermal cells (Figure 4B), the LA1-GFP fusion protein was mainly localized to the plasma membrane and nucleus (Figure 4C and 4D). However, the truncated LA1 without the predicted transmembrane domain (deletion of the amino-acid residues 1-100) was delocalized from the plasma membrane (Figure 4E), and that without the NLS-containing region was unable to direct GFP to the nucleus (Figure 4F). These results indicate that both predicted domains are functional.

Temporal and spatial expression of LA1

Expression analysis with RT-PCR showed that *LA1* transcripts were abundant in stems and etiolated coleoptiles, less in pulvini, and undetectable in panicles, mature leaves, sheaths and roots (Figure 5A). *LA1* mRNA *in situ* hybridization in longitudinal sections through the seedling apexes demonstrated that *LA1* was mainly expressed in vascular cells at the adaxial parts of the junctions of young rice leaves and stems (Figure 5B), consistent with the expectation for the LA1 action sites in regulating the angles of tillers. Cross-sections further showed that *LA1* was expressed specifically in the cells at the inner side of the vascular bundles of young leaf sheaths (Figure 5C and 5D) and peripheral cylinders of vascular bundles in the unelongated stems (Figure 5C and 5E). Taken together, we propose that *LA1* is a finely regulated temporally and spatially expressed gene, and that the region of its specific expression may play an important role in controlling the rice tiller angle.

Involvement of LA1 in auxin transport

Aberrations associated with gravitropism in *la1-ZF802* suggested that auxin homeostasis may be somehow perturbed in the mutant. Considering that the cereal coleoptile represents a model system for studying the regulation of cell growth and tropisms [51, 52], we measured the basipetal and acropetal transports of IAA in etiolated coleoptiles of wild type and mutant plants. We found that the basipetal PAT in *la1-ZF802* was highly elevated up to ~230% compared with that in the wild type, whereas the acropetal PAT of ^{3}H-IAA, basipetal transport of ^{3}H-IAA treated with a PAT inhibitor (30 μM NPA), or basipetal transport of a control compound ^{14}C-benzoic acid displayed no differences between wild type and mutant plants (Figure 6A). To find out whether the enhanced PAT caused by the deficiency in *LA1* affects the distribution of endogenous IAA, we visual-

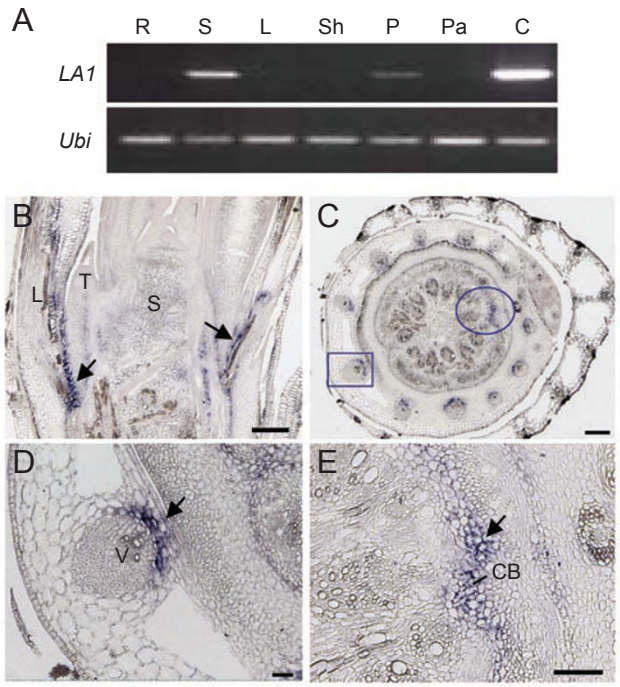

Figure 5 Expression pattern of LA1. **(A)** *LA1* expression pattern in various organs, including roots (R), stems (S), leaves (L), sheaths (Sh), pulvini (P), panicles (Pa), and etiolated coleoptiles (C). **(B-E)** *LA1* expression patterns revealed by mRNA *in situ* hybridization. **(D, E)** Magnification images of the squared and circled regions in **(C)**, respectively. L, leaf; T, tiller; S, stem; V, vascular bundle; CB, peripheral cylinder of vascular bundles. Arrows show the *LA1* expression sites. **(C)** Bars = 200 μm and **(B, D, E)** 100 μm.

ized the difference in the endogenous IAA distribution by comparing GUS expression levels in the transgenic plants that carry the auxin reporter *DR5::GUS* in the background of *la1-Shiokari* and wild-type *Shiokari*, respectively. In *Shiokari* coleoptiles, a higher GUS expression level was detected in the apical than that in the middle or basal part (Figure 6B), whereas in *la1-Shiokari* coleoptiles the GUS expression level in the apical part turned out to be lower than that in the middle or basal part (Figure 6C). Moreover, in longitudinal sections through apexes of *DR5::GUS* transgenic seedlings, the altered GUS expression profile could also be observed in *la1-Shiokari* (Figure 6D and 6E). In wild-type plants, GUS expression was mainly accumulated at the peripheral area of shoots (Figure 6D); in *la1-Shiokari*, however, the GUS expression was more intense and covered a broader area in the unelongated stem (Figure 6E), indicating that the enhanced basipetal PAT resulted in altered endogenous IAA distribution in the *la1-Shioka*ri mutant plant. Furthermore, we measured the lateral IAA transport in rice coleoptiles upon gravity stimulation. After 2.5-h gravistimulation, the radioactivity ratio between the lower and upper halves of the coleoptiles in *la1-ZF802* was significantly lower than that in *ZF802* (Figure 6F), suggesting that the asymmetric IAA distribu-

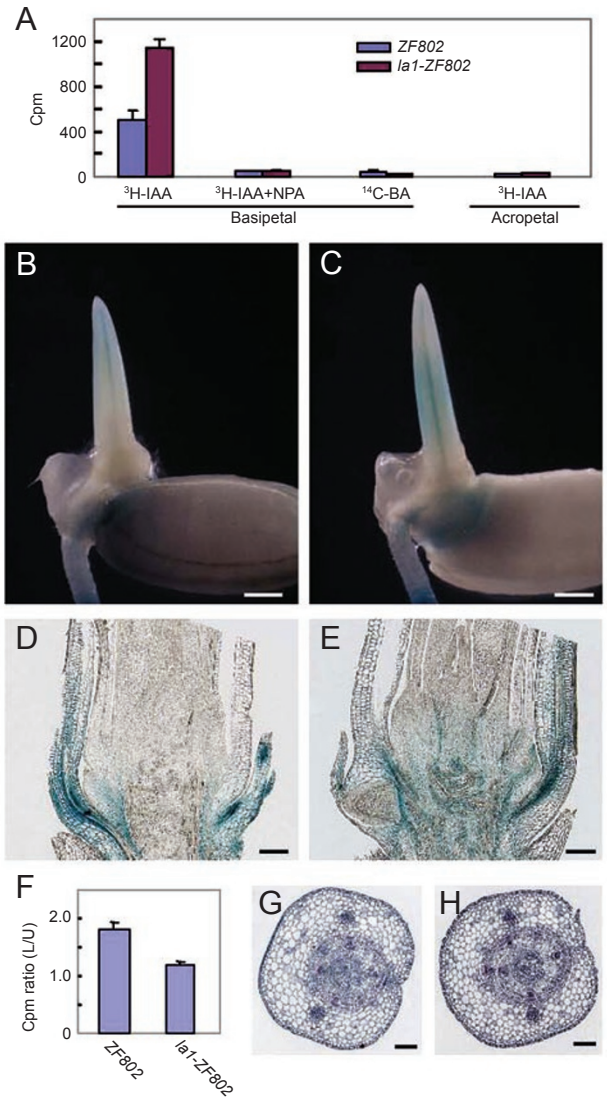

Figure 6 Comparison of auxin transport between wild-type and *la1-ZF802* plants. **(A)** Comparison of PAT between *ZF802* and *la1-ZF802* in dark-grown coleoptiles. Error bars indicate ± SE (n = 5). **(B, C)** *DR5::GUS* expression patterns in dark-grown coleoptiles of **(B)** wild type and **(C)** *la1-Shiokari*. **(D, E)** *DR5::GUS* expression patterns in wild type **(D)** and *la1-Shiokari* **(E)** unelongated stems. **(F)** Comparison of lateral IAA transport in coleoptiles between *ZF802* and *la1-ZF802*. Error bars indicate ± SE (n = 15). The cpm ratio represents the radioactivity ratio between the lower half and the upper half of coleoptiles upon gravity stimulation. **(G, H)** Cross-sections of **(G)** *ZF802* and **(H)** *la1-ZF802* coleoptiles. **(B, C)** Bars = 1 mm and **(D, E, G, H)** 200 μm.

tion is impaired by the *LA1* mutation. The structures of wild type and *la1-ZF802* coleoptiles are very similar (Figure 6G and 6H), which rules out the possibility that the difference in IAA transport arises from their structural difference.

Discussion

Gravitropism, a complicated multi-step process that directs plants to adapt to the fundamental environment factor gravity, has fascinated plant biologists for more than a century. Although significant progress in elucidating mechanisms involved in gravitropism has been made recently in *Arabidopsis*, the mechanisms in monocot remain largely unknown.

Plant shoots typically grow upwards and lateral organs are generated at a defined angle termed gravitropic set-point angle, which determines plant architecture at large extent and is mainly maintained by gravitropism [53]. Tiller angle, the angle between the main culm and its primary tillers, is an important agronomic trait that contributes to the rice plant architecture [54]. Neither the extremely spreading nor the compact plant type is beneficial to rice grain production [54, 55]. Two single recessive mutations, *lazy* (*la*) and *erecta* (*er*), were reported to confer spreading and compact phenotypes, respectively [35, 56]. However, no corresponding genes have been cloned and the molecular mechanism controlling rice tiller angle remains unclear. In this article, we described molecular genetic characterization of the tiller-spreading mutant *la1-ZF802* and the isolation and functional analyses of the *LA1* gene. By in-depth analysis of the rice classical mutant *la1* and map-based cloning of *LA1*, we have shown that *LA1* plays an essential role in regulating rice shoot gravitropism, which in turn controls the tiller angle, an important agronomic trait determining rice grain production.

The expression pattern of *LA1* is consistent with the morphological phenotype of *la1* mutant. *LA1* is specifically expressed at the adaxial parts of the junctions of young rice leaves and stems (Figure 5B), where the shoot bending happens, and in the cells at the inner side of the vascular bundles of young leaf sheaths (Figure 5C and 5D) and peripheral cylinders of vascular bundles in the unelongated stems (Figure 5C and 5E), where it correlates with PAT. Additionally, *LA1* expression strictly depends on the developmental process and its expression level is correlated with the degree of tiller angles (Figure 3).

Although LA1 is a novel protein that shows no homology to any functionally known proteins, proteins sharing high homology with LA1 are encoded by corresponding cDNAs in grass species (Figure 4A), implicating that the grass species may respond to gravity and transduce the signal through a distinct pathway from that in dicots. The transient expression in onion epidermal cells demonstrates that LA1 contains both the transmembrane and NLS domains (Figure 4A-F). The LA1-GFP fusion protein was mainly found in the plasma membrane and nucleus, whereas the truncated LA1 lacking the transmembrane domain or without the NLS-containing region was unable to direct the GFP signal to the membrane or nucleus. Therefore, LA1 may represent a new type of regulating proteins that shuttle between the plasma membrane and the nucleus. Further investigations of LA1 functions will allow for a better understanding of the mechanism underlying the monocotyledonous shoot gravitropism.

The Cholodny-Went hypothesis suggested that auxin might be the main phytohormone involved in gravitropism [19, 20]. So far, ample evidences that have been gathered with the asymmetrical distribution and polar or lateral transport of auxin support its major role in gravitropism, especially in *Arabidopsis* roots. Consistently, our results showed that the reduced gravitropism in *la1-ZF802* shoots results from the defective PAT. As shown in Figure 6A, the capacity of basipetal auxin transport in *la1-ZF802* is about two-fold higher than that in wild type, leading to an alteration in IAA distribution in the mutant as visualized by the DR5::GUS analysis (Figure 6B-E). Furthermore, IAA lateral transport upon gravity stimulation is impaired in *la1-ZF802* (Figure 6F). These results are consistent with the previous finding that the unequal distribution of auxin on either side of the organ is the premise of its bending upon gravistimulation [47, 52, 57]. Therefore, we conclude that the mutation in *LA1* results in a significant increase in PAT and thus impairs the IAA differential distribution in *la1-ZF802*, which ultimately leads to a reduced gravitropic response in the mutant shoots.

Our findings demonstrate that *LA1* is an essential regulator of tiller angle of rice, opening a promising way for breeders to develop elite rice cultivars and other cereal crops with optimal plant architecture.

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Accession number

LA1 sequence data from this article can be found in the GenBank data libraries under accession number DQ855268.

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