Natural Variations in *SLG7* Regulate Grain Shape in Rice

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ABSTRACT Rice (*Oryza sativa*) grain shape, which is controlled by quantitative trait loci (QTL), has a strong effect on yield production and quality. However, the molecular basis for grain development remains largely unknown. In this study, we identified a novel QTL, *Slender grain on chromosome 7* (*SLG7*), that is responsible for grain shape, using backcross introgression lines derived from 9311 and Azucena. The *SLG7* allele from Azucena produces longer and thinner grains, although it has no influence on grain weight and yield production. *SLG7* encodes a protein homologous to LONGIFOLIA 1 and LONGIFOLIA 2, both of which increase organ length in *Arabidopsis*. *SLG7* is constitutively expressed in various tissues in rice, and the SLG7 protein is located in plasma membrane. Morphological and cellular analyses suggested that *SLG7* produces slender grains by longitudinally increasing cell length, while transversely decreasing cell width, which is independent from cell division. Our findings show that the functions of *SLG7* family members are conserved across monocots and dicots and that the *SLG7* allele could be applied in breeding to modify rice grain appearance.

KEYWORDS rice; quantitative trait loci; grain shape; cell elongation

RICE (*Oryza sativa* L.) is a staple food for half of the world's population (Khush 2001). Three major components, panicle number per plant, grain number per panicle, and grain weight, determine rice yield production. Grain weight is associated with grain size and shape, which are defined as grain length, grain width, and grain thickness (Duan *et al.* 2014). There is a striking diversity of grain size among the rice species worldwide. The grains of domesticated rice range from 3 to 11 mm in length and from 1.2 to 3.8 mm in width (Fitzgerald *et al.* 2009). Despite the influence of several environmental factors on plant growth and development, such as water supply and fertilizer level, the final grain size of rice is reasonably constant within a given species.

Rice grain traits are quantitatively inherited. In the past decade, several quantitative trait loci (QTL) controlling grain size and shape have been cloned. *GS3*, encoding a transmembrane protein containing four putative domains, was the first characterized QTL that regulates grain length (Fan *et al.* 2006). *qGL3* encodes a putative protein phosphatase with a Kelch-like repeat domain, and an aspartate-to-glutamate transition in the second Kelch domain leads to a long-grain phenotype (Zhang *et al.* 2012). *GW6* encodes a GNAT-like protein that harbors intrinsic histone acetyltransferase activity, and an elevated expression enhances grain length and weight by enlarging spikelet hulls and accelerating grain filling (Song *et al.* 2015). *GW2*, *GW5/qSW5*, *GS5*, and *GW8* were identified as regulators of rice grain width. *GW2* encodes a previously unknown RING-type protein with E3 ubiquitin ligase, which negatively regulates cell division by degrading its substrate(s) through the ubiquitin-proteasome pathway (Song *et al.* 2007). *GW5/qSW5* encodes a nuclear-located protein that physically interacts with polyubiquitin. Loss of function of *GW5/qSW5* leads to a significant increase in sink size, reflecting an increase in cell number in the outer glume of the rice flower (Shomura *et al.* 2008; Weng *et al.*

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2008). *GS5* encodes a putative serine carboxypeptidase (Li *et al.* 2011), and *GW8* encodes squamosa promoter-binding protein-like 16, which belongs to the SBP domain family of transcription factors (Wang *et al.* 2012). Both are positive regulators of grain width. Higher expression of these two genes contributed to larger grain size through their effects on the cell-cycle machinery. Additionally, the *GIF1* gene encodes a cell-wall invertase required for carbon partitioning during early grain filling (Wang *et al.* 2008), and *TGW6* encodes an indole-3-acetic acid-glucose hydrolase affecting the transition from the syncytial to the cellular phase of the endosperm (Ishimaru *et al.* 2013). *GIF1* and *TGW6* directly affect the endosperm size and consequently regulate grain size and weight. *GS3*, *qSW5/GW5*, *GS5*, *GW8*, *GIF1*, and *TGW6* have been artificially selected and widely used in rice-breeding programs. However, the advantageous alleles of *GW2*, *qGL3*, and *GW6* are present in rare varieties or subspecies and have not been actively exploited.

Most genes involved in rice panicle development, such as *SP1*, *qPE9-1*, *EP2*, *DEP2*, and *DEP3*, influence grain size or shape (Li *et al.* 2009, 2010; Zhou *et al.* 2009; Zhu *et al.* 2010; Qiao *et al.* 2011). Moreover, based on the phenotypic analyses of null mutants, the genes involved in gibberellin and brassinosteroid biosynthesis or signaling also modulate rice grain size (Ashikari *et al.* 1999; Yamamuro *et al.* 2000; Hong *et al.* 2003; Tanabe *et al.* 2005; Nakagawa *et al.* 2012). These studies suggested that the control of grain size in rice is complicated. To further reveal the molecular mechanism that determines grain size, genes that affect grain development were identified using mutants and backcross populations as genetic materials. In this study, we isolated and characterized a QTL, *Slender grain on chromosome 7* (*SLG7*), which may produce longer and thinner grains and, if applied to breeding, will significantly improve rice grain yield and appearance quality.

Materials and Methods

Plant materials and growth conditions

A set of backcrossed introgression lines (ILs) derived from a cross between Azucena and 9311 was developed to clone QTL for grain size or shape. Their F_1 plants were backcrossed with 9311 for four times, and the BC_4F_1 seeds were selfed to produce BC_4F_4 lines (Supporting Information, Figure S1). One of the lines, IL5084, carrying an introgressed segment of chromosome 7 from Azucena, showed extremely slender grains. Rough mapping and fine mapping of *SLG7* was carried out using the segregating populations derived from IL5084 and 9311. From the BC_5F_3 generation, a pair of near-isogenic lines (NILs), NIL-*SLG7* and NIL-*slg7*, was developed to evaluate the genetic effect. NIL-*SLG7* carried a small segment including the Azucena *SLG7* allele in the 9311 background, whereas its matching line, NIL-*slg7*, had the homologous segment from 9311. All the plants were grown in the experimental field of Yangzhou University (E 119°25′/N 32°23′). Field management and control of diseases and pests followed the standard procedures to prevent yield loss during the growth period.

Mapping of SLG7

In each generation, short-grain plants (grain length $<$ 10.5 mm) in the derived segregating populations were used to screen recombinants. Fine mapping was performed using a total of 2500 short-grain plants in the BC_5F_3 and BC_5F_4 populations. *SLG7* was finally narrowed into a 60-kb interval between markers S7-272 and S7-258, and cosegregated with S7-257. The molecular markers used for fine mapping *SLG7* are shown in Table S3. The *SLG7* candidate genes from Azucena and 9311 genomic DNA were amplified and sequenced. The primers are listed in Table S4.

RNA extraction, complementary DNA synthesis, and qRT-PCR

Total RNA from various organs was isolated using an RNA extraction kit following the manufacturer's instruction (Beijing Tiangen Biotechnology, http://www.tiangen.com/). First-strand complementary DNA (cDNA) was reverse transcribed from ~1 μg gDNase-treated RNA using a FastQuant RT Kit following the manufacturer's instruction (Beijing Tiangen Biotechnology). Gene expression analyses were measured using quantitative real-time reverse transcription-PCR (qRT-PCR) with the rice *Actin* gene as control. The qRT-PCR was performed in a total volume of 25 μl, containing 2 μl of the cDNA, 0.2 mM of each primer, and 12.5 μl of 2× SYBR green PCR master mix (Takara, http://www.takara.com.cn). The qRT-PCR was carried out using an ABI ViiA7 real-time PCR system using the following program: 95° for 3 min and then 40 cycles of 94° for 30 sec, 55° for 30 sec, and 72° for 40 sec. Comparison of the expression level was done with the $2^{-\Delta\Delta}C_T$ method.

Vector construction and plant transformation

To confirm the target gene of *SLG7*, the full coding regions of ORF1 and ORF2 were amplified from Azucena cDNA and were inserted into the p1300-Actin-3*flag vector to generate two overexpression constructs, p*Actin*::*ORF1*^{Azucena} and p*Actin*::*ORF2*^{Azucena}. For the complementary test, a DNA fragment ~2 kb upstream of the ORF1 translation start site was amplified from Azucena genomic DNA. Both segments were then cloned into the binary pCAMBIA1301 vector to generate a *pORF1*::*ORF1*^{Azucena} construct in which the Azucena ORF1 can be driven by its native promoter. All the constructs were transformed by *Agrobacterium tumefaciens*-mediated transformation (Hiei *et al.* 1994).

Subcellular localization

For subcellular localization analysis, the coding sequence of *SLG7* was fused in-frame with the green fluorescent protein (GFP) coding sequence in the *p163-GFP* vector to generate *CaMV35S*::*SLG7-GFP*. *CaMV35S*::*GFP* was used as a control. These two fusion constructs were transfected into rice Nipponbare protoplasts. The transformed protoplasts were examined using a confocal microscope (LSM 700, Leica).

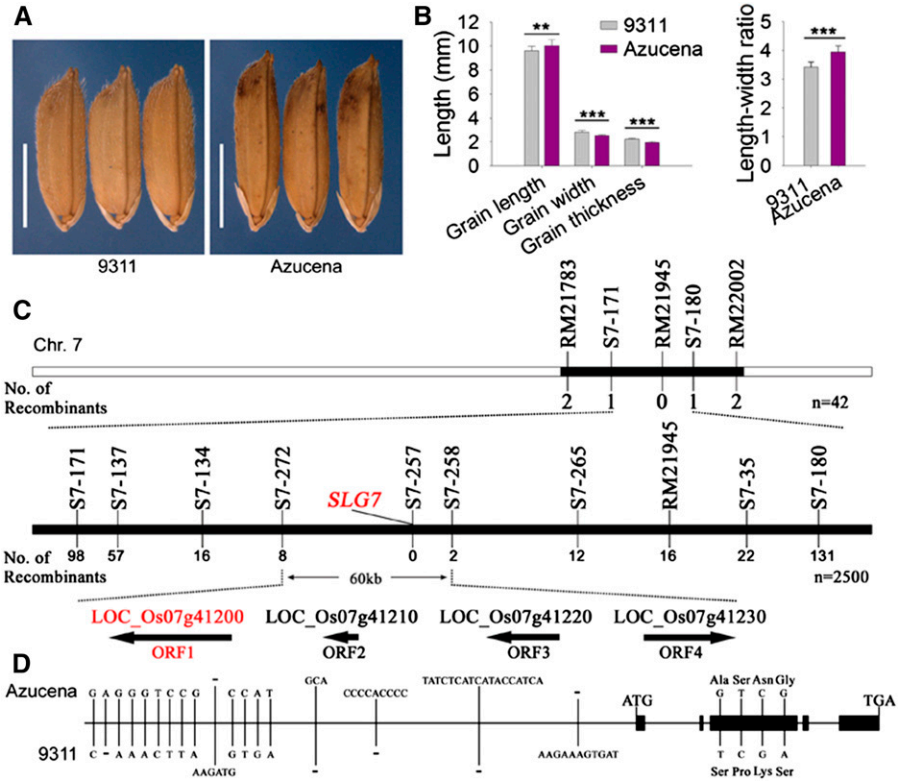

Figure 1 Mapping of *SLG7*. (A) Grain phenotypes of parents (9311 and Azucena). Bar, 5 mm. (B) Comparison of grain length, grain width, grain thickness, and length–width ratio between 9311 and Azucena. Data are given as mean ± SE ($n > 10$). Significant at ***0.1% and **1%. (C) Fine mapping of *SLG7*. *SLG7* was primarily mapped between markers S7-171 and S7-180 on chromosome 7 using 42 recessive plants from the BC_5F_2 segregating population. Then *SLG7* was fine-mapped into a 60-kb interval between markers S7-272 and S7-258 and cosegregated with S7-257, using 2500 recessive plants from the BC_5F_3 and BC_5F_4 populations. The numbers under each marker indicate the number of recombinants between *SLG7* and the molecular markers. Four candidate genes were located within this region, and the target gene is indicated in red. (D) Sequence polymorphisms of *SLG7* between 9311 and Azucena.

Morphological and cellular analyses

Fresh spikelets from the panicles of NIL-*SLG7* and NIL-*slg7* at heading time were directly observed with a scanning electron microscope (SEM). For histology, spikelets were fixed in 2% glutaraldehyde, dehydrated in a graded ethanol series, and embedded in Spurr resin. The transverse sections of each spikelet were produced using an ultramicrotome (EM UC7, Leica), then stained with 0.5% toluidine blue, and viewed using a microscope (DM1000, Leica). The areas of the outer parenchyma cell layer of the spikelet palea and lemma were measured using Image J software.

Data availability

Materials and plasmids described here and in the Supporting Information are available upon request.

Results

Identification of a major QTL for slender grains on chromosome 7

To explore the genetic control of rice grain size and shape, we developed a set of backcrossed introgression lines derived from the cross between Azucena and 9311 (Figure S1). The average length and width of 9311 grains were 9.62 ± 0.36 mm and 2.82 ± 0.14 mm, respectively. By contrast, the average grain length and width of Azucena were 10.03 ± 0.50 mm and 2.54 ± 0.06 mm, respectively (Figure 1, A and B). Using this IL population, several QTL for grain weight and size were mapped (Y. Zhou and G. Liang, unpublished data). One of the BC_4F_4 lines, IL5084, produced longer but thinner grains than 9311. To reveal the genetic basis of this phenotypic variance, we made a cross between IL5084 and 9311 to generate the derived segregating populations. The distributions of grain traits in a random BC_5F_3 subpopulation of 400 individuals are illustrated in Figure S2. The ratio of the grain length and length–width ratio fitted well to the expected 3:1 ratio of single locus Mendelian segregation ($\chi^2_c = 1.92 < \chi^2_{0.05,1} = 3.84$; $\chi^2_c = 2.25 < \chi^2_{0.05,1} = 3.84$, respectively). However, no bimodal distribution was observed in grain width. We also found that grain length was negatively associated with grain width ($r = -0.325$). Linkage analysis suggested that a major gene, *SLG7*, located between molecular markers RM21783 and RM22002 on chromosome 7, was responsible for the grain-shape variation (Figure 1C).

Fine mapping and cloning of SLG7

As shown in Figure S2, the grain length showed a bimodal distribution with 10.75 mm as the boundary. Therefore, the plants with short grains (<10.5 mm in length) were considered as recessive individuals, which could be used to screen recombinants. *SLG7* was mapped between markers S7-171 and S7-180 using 42 recessive plants from the BC_5F_2 segregating population (Figure 1C). Fine mapping was further performed using 2500 recessive plants in the BC_5F_3 and BC_5F_4 populations. *SLG7* was finally narrowed down to a 60-kb interval between markers S7-272 and S7-258, with eight and two recombinants, respectively, and cosegregated with marker S7-257. Within this region, there are four predicted ORFs (Figure 1C). To define the candidate gene of *SLG7*, the genomic DNA sequences that corresponded to

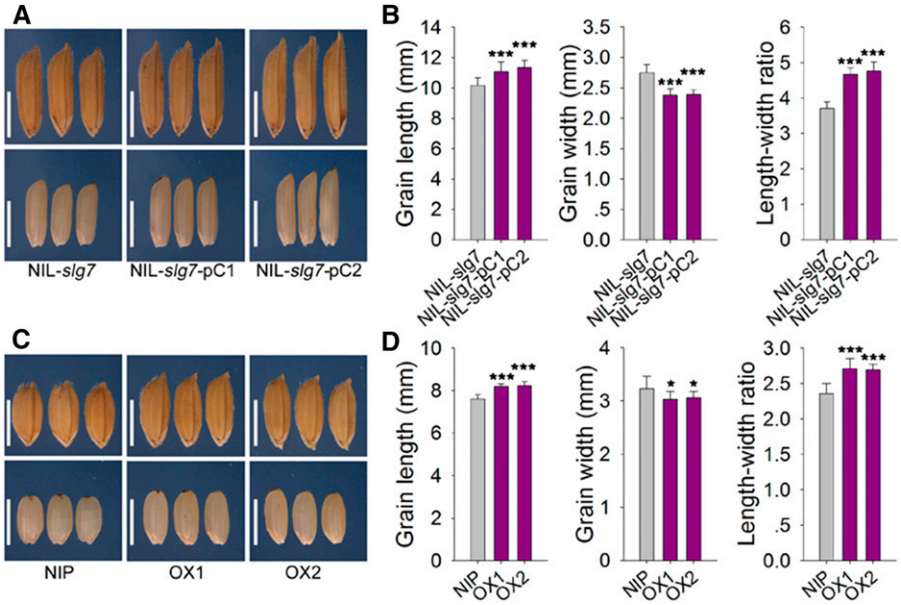

Figure 2 Complementary test and overexpression analysis. (A) Grains (top) and brown rice grains (bottom) of complementary transgenic plants. NIL-*slg7*-pC1 and NIL-*slg7*-pC2 are the transgenic lines with p*ORF1*::*ORF1*^{Azucena} construct under the NIL-*slg7* background. Scale bar, 5 mm. (B) Comparison of grain length, grain width, and length–width ratio between NIL-*slg7* and the transgenic lines. (C) Grains (top) and brown rice grains (bottom) of overexpression plants. OX1 and OX2 are the transgenic lines with p*Actin*::*ORF1*^{Azucena} construct under the Nipponbare background. Bar, 5 mm. (D) Comparison of grain length, grain width, and length–width ratio between Nipponbare and the overexpression lines of *ORF1*^{Azucena}. Data are given as mean ± SE ($n > 10$). Significant at ***0.1% and *5%.

these four ORFs from 9311 and Azucena were sequenced. There was no nucleotide difference between 9311 and Azucena in ORF3 and ORF4, respectively; however, multiple variances were found in ORF1 and ORF2 between the two parents. Thus, ORF1 and ORF2 were considered as good candidates for *SLG7*. A pair of NILs, NIL-*SLG7* and NIL-*slg7*, was also developed while fine mapping was carried out. NIL-*SLG7* carried a small segment including the Azucena *SLG7* allele in the 9311 background, whereas its matching line, NIL-*slg7*, had the homologous segment from 9311. Several constructs were generated and transformed to confirm the candidates. The transgenic NIL-*slg7* plants (NIL-*slg7*-pC1 and NIL-*slg7*-pC2) produced longer and thinner grains when expressing the Azucena ORF1-coding region under the control of its native promoter (Figure 2, A and B). We also overexpressed the Azucena ORF1-coding region using the *Actin* promoter in Nipponbare (OX1 and OX2) and found that the grains of the transgenic plants became slender (Figure 2, C and D; Figure S3). However, no visible change in grains was found in the transgenic Nipponbare plants expressing ORF2 using the constitutive *Actin* promoter (data not shown). Therefore, ORF1 must be the *SLG7* gene. Sequence analysis revealed that 12 SNPs and five indels in the promoter region and four SNPs in the coding region were found between 9311 and Azucena (Figure 1D and Table S1). All four SNPs are present in the third exon of *SLG7* and cause amino acid residue changes from alanine to serine (Ala462Ser), serine to proline (Ser518Pro), asparagine to lysine (Asn605Lys) and glycine to serine (Gly620Ser) (Figure 1D and Table S1).

SLG7 encodes a protein homologous to Arabidopsis LONGIFOLIA1 and LONGIFOLIA2

BLASTP searches revealed that the homologs of rice *SLG7* are present only in seed plants including gymnosperm and angiosperm. There are two homologs in rice genome, *SLG7-like1* (LOC_Os03g30530) and *SLG7-like2* (LOC_Os07g01860), having 52% and 30% identities with *SLG7*, respectively. Both monocot and dicot genomes contained multiple *SLG7-like* genes (Figure 3). *Arabidopsis* has four homologs of *SLG7*, but only two of them, *LONGIFOLIA1* (*LNG1*, At5g15580) and *LONGIFOLIA12* (*LNG2*, At3g02170), were functionally characterized. Both of them activate longitudinal organ expansion (Lee *et al.* 2006).

SLG7 inactivates grain and panicle elongation but does not affect yield production

NIL-*SLG7* and NIL-*slg7* lines were developed to investigate the effect of *SLG7* on rice agronomic traits. We confirmed the allele variances of six reported grain-size genes, *GS3*, *GIF1*, *GW2*, *GW8*, *GL3*, and *GW6*, in NIL-*SLG7* and NIL-*slg7* and found that this pair of NILs shared common alleles at these loci (Table S2). The grain and other agronomic traits of NIL-*SLG7* and NIL-*slg7* were evaluated under field conditions (Figure 4). The grains of NIL-*SLG7* were 19.0% longer and 14.3% narrower than those of NIL-*slg7* (Figure 4, C–G). No difference was detected in 1000-grain weight between NIL-*SLG7* and NIL-*slg7* (Figure 4H), suggesting that the two NILs did not differ from one another with respect to grain weight although their grain shapes were clearly distinct. NIL-*SLG7* also produced longer panicles (+14.2%) and flag leaves (+15.8%) compared with NIL-*slg7* (Figure 4, A, B, J, and K). No significant differences in plant height and two other rice yield components, grain number per panicle and panicle number per plant, were observed between NIL-*SLG7* and NIL-*slg7* (Figure 4, A, I, L, and M). As a result, there was no difference in grain yield per plant between the two NILs (Figure 4N). These data indicated that the *SLG7* allele from Azucena produces slender grains but has no effect on grain weight and yield production.

SLG7 promotes cell expansion but has no effect on cell division

To investigate whether cell number or cell size contributes to the development and patterning of grain shape, we compared

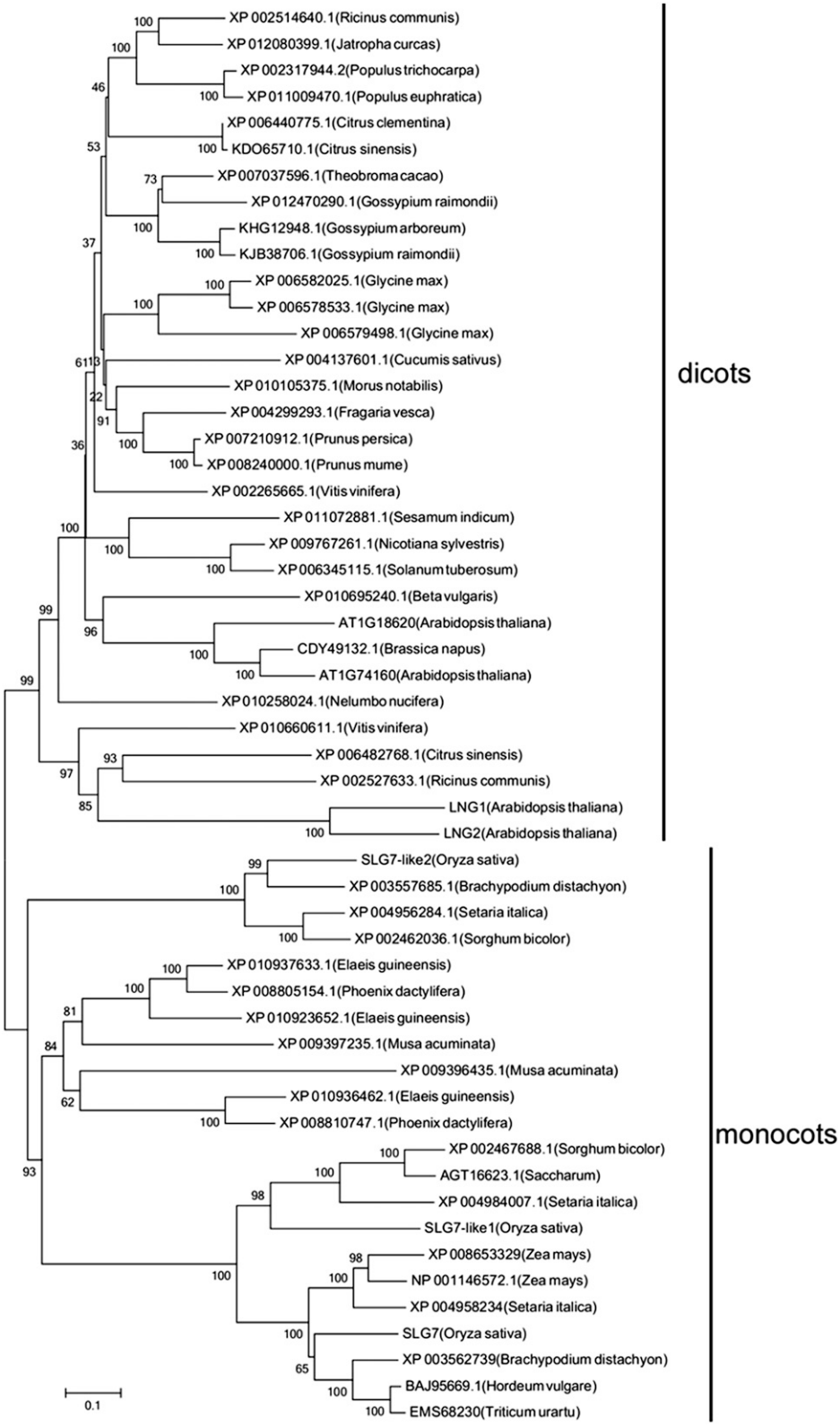

Figure 3 Phylogenetic tree of SLG7-like proteins of the subfamily defined by SLG7. The tree was constructed using MEGA 4.0 based on the neighbor-joining method.

the length and width of the outer glume epidermal cells using SEM. The outer epidermal cells of NIL-*SLG7* were longer but narrower than those of NIL-*slg7* (Figure 5A). We further examined the cross sections of spikelet hull central parts between NIL-*SLG7* and NIL-*slg7* (Figure 5, B and C). The number of outer parenchyma cell layers in NIL-*SLG7* did not differ from that in NIL-*SLG7* (Figure 5D). However, the cell sizes of outer parenchyma cell layers of the palea and lemma in NIL-*SLG7* were 10.8 and 20.8% smaller, respectively, than those in NIL-*slg7* (Figure 5E). These results suggested that

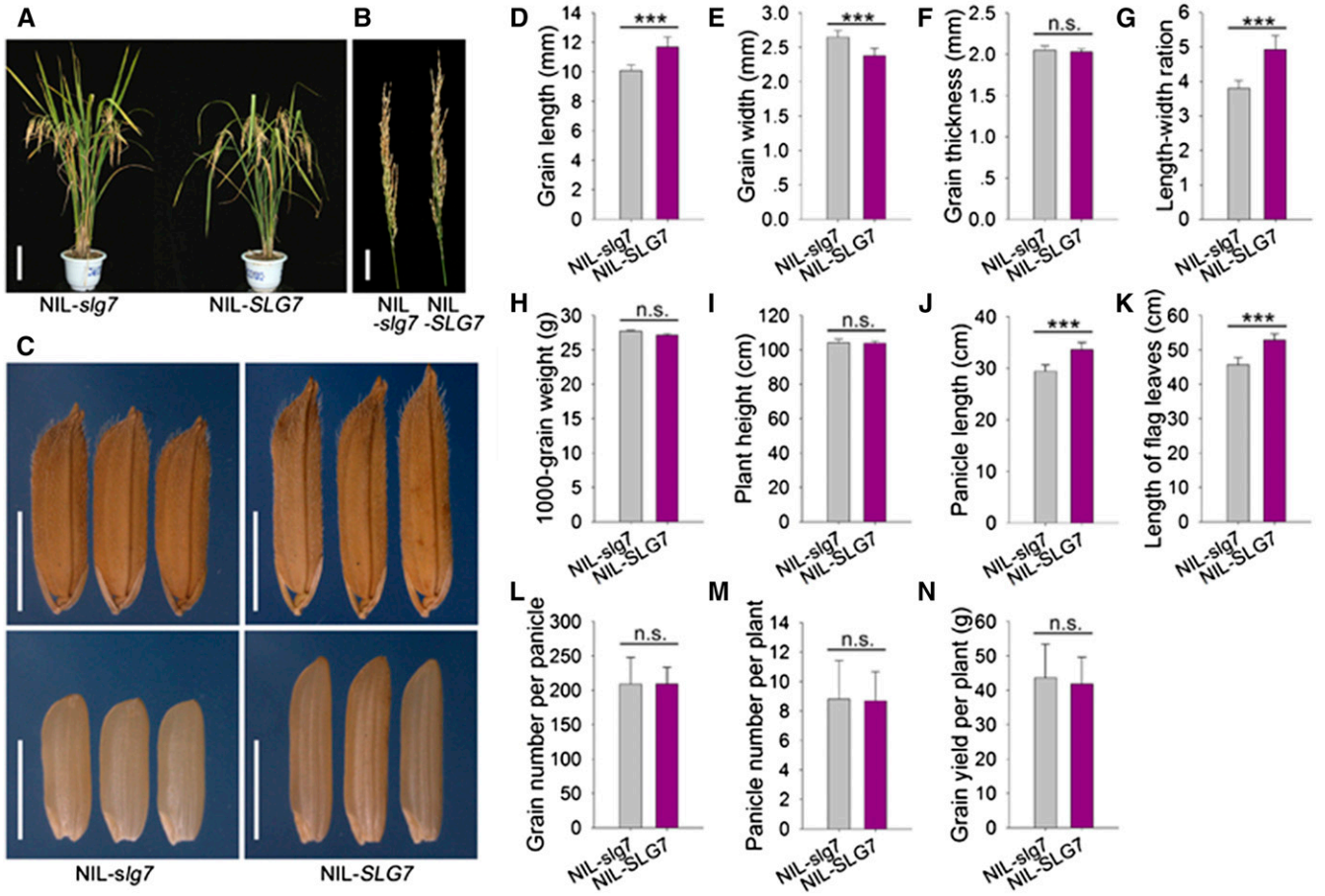

Figure 4 Comparison of the agronomic traits in NIL-*slg7* and NIL-*SLG7* plants. (A) Plant architecture of NIL-*slg7* and NIL-*SLG7* plants. Bar, 20 cm. (B) Panicle phenotype of NIL-*slg7* and NIL-*SLG7* plants. Bar, 5 cm. (C) Phenotypic characterization of grains (top) and brown rice grains (bottom) in NIL-*slg7* and NIL-*SLG7* plants. Bar, 5 mm. (D–N) Comparison of 11 traits in NIL-*slg7* and NIL-*SLG7* plants. (D) Grain length. (E) Grain width. (F) Grain thickness. (G) Length–width ratio. (H) 1000-grain weight. (I) Plant height. (J) Panicle length. (K) Length of flag leaves. (L) Grain number per panicle. (M) Panicle number per plant. (N) Grain yield per plant. All data are given as mean ± SE ($n > 10$). Significant at ***0.1%. n.s., not significant.

SLG7 produces slender grains by longitudinally increasing cell length while transversely decreasing cell width, which is independent from cell division.

Expression pattern of SLG7 and subcellular localization of the SLG7 protein

qRT-PCR was used to investigate the expression pattern of *SLG7*. As shown in Figure 6A, *SLG7* was constitutively expressed in leaf, panicle, stem, sheath, node, and root. The transcripts in panicle accumulated to a higher level than those in other tissues, which was consistent with the biological function of *SLG7* in the regulation of rice inflorescences and grain development. Additionally, the difference of expression level was observed throughout the whole panicle developmental process (Figure 6B). These results implied that the sequence variations in promoter may affect the expression level of *SLG7*. The expression pattern of *SLG7* was further analyzed using transgenic plants expressing the β-glucuronidase (GUS) reporter gene under the control of *SLG7* promoter. A strong GUS staining was detected in the spikelet hulls of transgenic lines, and the GUS activities in the spikelet hulls from young inflorescences were much higher than those from developed inflorescences (Figure 6C). These data suggested that the expression of *SLG7* decreased with the development of inflorescences and grains. To investigate the subcellular localization of SLG7, we constructed an SLG7-GFP fusion the expression of which was driven by the CaMV 35S promoter. The expression of this fusion protein in rice protoplasts clearly suggested that SLG7 is a plasma membrane protein (Figure 6D).

Sequence diversities of SLG7 in germplasms

It was detected that allelic variances of *SLG7* among 50 rice germplasms from a wide geographic range in Asia and America. The *SLG7* alleles were classified into five types, namely Azucena, 9311, Nipponbare, Guangluai 4, and Dular (Table S1). The Nipponbare and Guangluai four haplotypes were exclusively found in *japonica* and *indica* subspecies, respectively, while the other three haplotypes were detected in both subspecies. The Azucena *SLG7* alleles were identified in 13 germplasms from the United States, the Philippines, Indonesia, and southern China, suggesting that *SLG7* is not a rare allele and has been collected during rice breeding. In addition, the varieties carrying the Azucena-type *SLG7* allele are

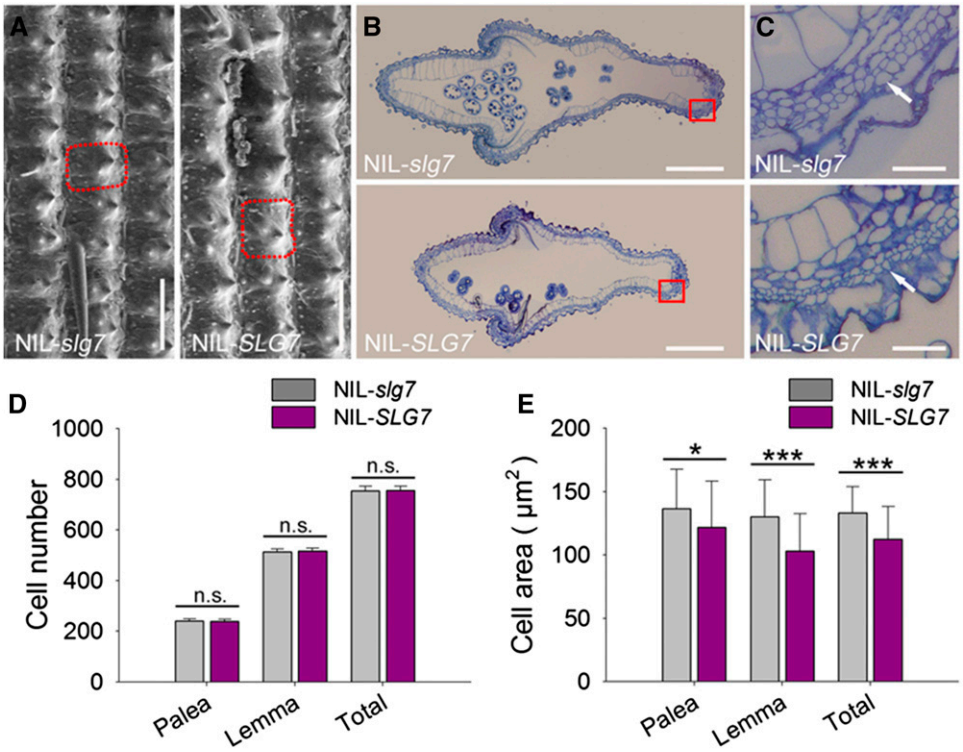

Figure 5 The effect of *SLG7* on cell number and area in lemma/palea. (A) Scanning electron microscope photos of outer glume surfaces. One of the outer glume cells in NIL-*slg7* and NIL-*SLG7* hulls, respectively, is boxed by the red dotted line. Bars, 100 μm. (B) Cross section of middle spikelet hulls in NIL-*slg7* and NIL-*SLG7* plants. Bars, 500 μm. (C) Magnified photos of spikelet hull cross section boxed in B. White arrow shows the outer parenchyma cell layers. Bars, 50 μm. (D) Comparisons of cell number in the cross sections of the outer parenchyma cell layer of NIL-*slg7* and NIL-*SLG7* hulls. Data are given as mean ± SE (n = 4). n.s., not significant. (E) Comparisons of cell area in the cross sections of the outer parenchyma cell layer of NIL-*slg7* and NIL-*SLG7* hulls. Data are given as mean ± SE (n = 80). Significant at ***0.1% and *5%.

significantly higher than those having the other four *SLG7* alleles in grain length (Figure 7). This result indicated that *SLG7* is a positive factor for grain length and plays a dominant role in shaping extremely long rice grains.

Discussion

Rice grain shape is a determinant of evolutionary fitness and is also an important agronomic trait for yield production and appearance quality. Several genes for this trait have been characterized recently (Zuo and Li 2014). However, the molecular components and regulatory network have not been fully illustrated.

To reveal more genes for rice grain size or shape, we developed a set of backcrossed introgression lines using 9311 as the recipient and Azucena as the donor. The first *indica* rice to have its whole-genome sequenced is 9311; it forms long grains because of a nonsense mutation in the second exon of the *GS3* gene in comparison with short-grain varieties including Nipponbare (Fan *et al.* 2006; Takano-Kai *et al.* 2009; Wang *et al.* 2011). Azucena produces longer and thinner grains than 9311. A map-based cloning strategy was employed to isolate the target gene, *SLG7*, responsible for the slender grains of Azucena. The data from NILs and transgenic lines showed that the *SLG7* allele from Azucena promotes spikelet elongation and produces slender grains under both 9311 and Nipponbare backgrounds. Surprisingly, *SLG7* has no effect on grain weight, probably because of the reduced grain width that accompanies the increased grain length (Figure 4). These results imply that *SLG7* is different from the other grain size-related QTL that directly modify grain weight and yield. Varieties with long and slender grains are preferred by consumers and cultivars in the United States, China, and most Asian countries. By contrast, short and round grain varieties are popular in Japan, South Korea, and Sri Lanka (Juliano and Villareal 1993). Therefore, *SLG7* can be used to develop varieties with diverse grain shapes to meet different market demands.

The BLASTP analysis revealed that *SLG7* shares numerous putative homologs (Figure 3), including two functionally characterized genes, *LNG1* (At5g15580) and *LNG2* (At3g02170), both of which function in the elongation of leaves, siliques, and seeds in *Arabidopsis* (Lee *et al.* 2006). *LNG2* (also named *TON1 Recruiting Motif 1*) is identified as a microtubule-associated protein, which is able to target TON1 to cortical microtubules (Drevensek *et al.* 2012). We found that *SLG7* homologs are restricted to the land plants and are found within several important crops including barley (*Hordeum vulgare*), millet (*Setaria italica*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*) (Figure 3). In view of the results in rice and *Arabidopsis*, *SLG7* homologs represent key components controlling grain and organ size and could be employed to improve agronomic traits in crop breeding.

Organ size or shape is determined by cell proliferation and cell expansion, two processes that are coordinated and compensated (Orozco-Arroyo *et al.* 2015). Morphological and cellular analyses indicated that *SLG7* has a function in regulating cell elongation in spikelets (Figure 5). Similar to our data, *LNG1* and *LNG2* were also reported to play a positive role in increasing leaf and silique length through longitudinal cell elongation in *Arabidopsis* (Lee *et al.* 2006). These results suggested that the functions of *SLG7* family members are probably conserved across monocots and dicots.

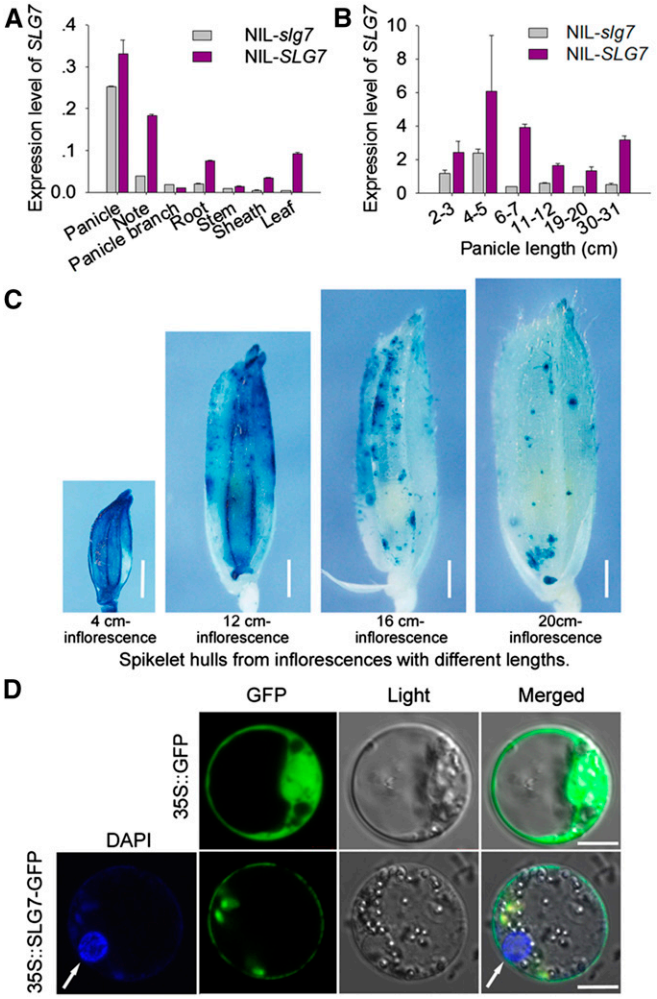

Figure 6 Expression pattern of *SLG7* and subcellular localization of SLG7 protein. (A) Relative expression levels of *SLG7* in different tissues analyzed by qRT-PCR. (B) Quantitative expression analysis of *SLG7* in 2- to 3-, 4- to 5-, 6- to 7-, 11- to 12-, 19- to 20-, and 30- to 31-cm stages of NIL-*slg7* and NIL-*SLG7* panicles. The expression level of the rice *Actin* gene was amplified as a control. Values are means ± SE of three independent experiments. (C) GUS activities in the spikelet hulls from inflorescences with different lengths. Bars, 1 mm. (D) Subcellular localization of SLG7 protein. Localization of 35S::GFP and 35S::SLG7-GFP in rice protoplast cells is shown. The photos were taken in an optic field to examine cell morphology (light), in a dark field to localize green fluorescence (GFP), and in combination (merged). White arrow shows the nucleus stained by DAPI. Bars, 5 μm.

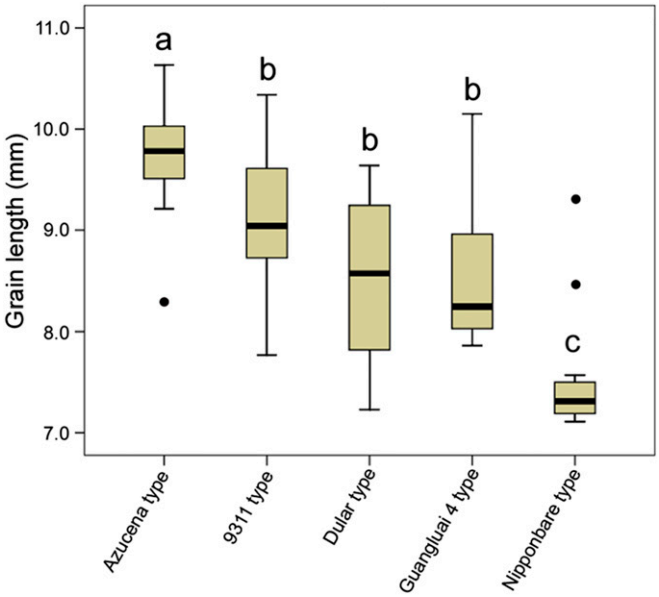

Figure 7 Comparison of the grain length in the five *SLG7* allelic groups. The box plot was generated by the SPSS 10.0 software.

Very recently, two novel alleles of *SLG7*, *GL7* and *GW7*, were identified (S. Wang *et al.* 2015; Y. Wang *et al.* 2015). *GL7* was characterized as a positive regulator for grain length, and the functional allele produced longer seeds resulting from elongated longitudinal cells (Y. Wang *et al.* 2015). By contrast, *GW7* was reported to influence cell proliferation, rather than cell expansion (S. Wang *et al.* 2015). We compared the expression levels of 25 genes, including 14 putatively involved in the G_1/S and 11 in the G_2/M phase, as shown in a previous report (Li *et al.* 2011). None of them showed more than a twofold change in transcript levels between NIL-*SLG7* and NIL-*slg7* inflorescences (Figure S4A). A similar result was also observed between Nipponbare and the *SLG7* overexpression transgenic line (Figure S4B). It seems that our results are similar to the data from *GL7*. Genes regulating rice grain size or shape on chromosome 7, such as *qGL7*, *qGL7-2*, and *GS7*, have been mapped using several populations (Bai *et al.* 2010; Shao *et al.* 2010, 2012; Qiu *et al.* 2012). These four genes are close to *SLG7*, but none of them have been cloned. Perhaps there is a gene cluster associated with rice grain size or shape on the long arm of chromosome 7.

Nucleotide diversities of *SLG7* were analyzed in 50 accessions (Table S1). The Azucena *SLG7* allele was found in 13 germplasms from the United States, the Philippines, Indonesia, and China (Table S1), suggesting that this allele has been selected in breeding in these areas. It was noted that one variety with the Azucena *SLG7* allele, Zenith, did not produce longer and thinner grains, which is different from the other materials within this group. Thus, the genetic basis for the grain size in this variety was of interest. *GS3* and *GW8* are two widely distributed major determinants for rice grain length, and the mutation *gs3* and *gw8* alleles promote the elongation of spikelets (Fan *et al.* 2006; Takano-Kai *et al.* 2009; Wang *et al.* 2012). Sequence analysis indicated that Zenith has negative alleles at the *GS3* and *GW8* loci, respectively (Table S5). This can be explained by the fact that Zenith lacks the *gs3* and *gw8* alleles, which are able to increase grain length, although it has a functional *SLG7* gene. These data also suggested that *SLG7* could be combined with other genes for grain size and shape to regulate rice grain appearance in breeding.

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Natural Variations in *SLG7* Regulate Grain Shape in Rice

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Table S1 Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.181115/-/DC1/genetics.115.181115-9.xls. (.xlsx, 34 KB)

Supplemental Table S2. Allelic variances of six reported genes in NIL-*slg7* and NIL-*SLG7*.

Genes	NIL(*SLG7*)	NIL(*slg7*)
GS3	*gs3*	*gs3*
GIF1	*GIF1*	*GIF1*
GW2	*GW2*	*GW2*
GW8	*gw8*	*gw8*
GL3	*GL3*	*GL3*
GW6	*GW6*	*GW6*

Supplemental Table S3. New developed molecular markers used for fine mapping in this study.

Markers	Forward (5’-3’)	Reverse (5’-3’)
S7-171	TTGTCAGGAGGAAGGGTG	CGGACTTCAAGACGCAAT
S7-137	TGCGAGTGCAGGTGAGTG	ACCATCCCAAGCCAAATC
S7-134	ACAAGTGGAGGGTGGATA	ATGTGCTAATGACGGGTT
S7-272	TCAGTTTAATCCATTATTCAAG	TCTACGTACGGAGGGAAT
S7-257	ACCTTGACCCTGAACCTGA	GGGGGACGATTTTGTAACT
S7-258	AAGTTAGATTGAACGGCAT	AGAGGAGTTTTCTTGGGAG
S7-265	ATTACCCACCGTACCCTT	GGCAGTTCTTAGCACCTCA
S7-35	GTGCTCGTGCTGGACTT	AGGCATAGCACCGAGTT
S7-180	TTGGTTGAAATTGGAATGA	TGGGACTTACCTTTTGTGC

Supplemental Table S4. Primers used for cloning and function analysis.

Primers	Forward (5’-3’)	Reverse (5’-3’)	Purpose
07g41200_ox	AAATCTAGAATGCCTCCGGCGAGGGTGCTCGGC	AAAGTCGACGCTTGTACTACTAAATGACAGCTGCCTCT	Overexprssion for *SLG7*
41200HB_Pro	AAAGGTACCCCCTGTTACTTGGTTAACCGCCCTTGTATG	AAAGTCGACCTCCTCCTCCGACTCCGACTCCTCC	Complementary test for *SLG7*
41200_CDS	AAAGTCGACATGCCTCCGGCGAGGGTGCTCGG	AAACCATGGTCAGCTTGTACTACTAAATGACAGCTGCCT	Complementary test for *SLG7*
07g41200GFP	AAAGTCGACATGCCTCCGGCGAGGGTGCTCGGCG	AAACCATGGTGCTTGTACTACTAAATGACAGCTGCCTC	Subcellular Localization of SLG7 protein
07g41210HB	AAAGGTACCTAGGCTCGCTGTCACTTC	AAAAAGCTTCGTCGTTCCCTCACTCTT	Complementary test for LOC_Os07g41210
07g41210OX	AAACCCGGGATGGACGACCACCCCAAGTTCACG	AAATCTAGAGTTGCAGATGGATCCGCGGATAGCC	Overexprssion for LOC_Os07g41210
07g41200GUS	AAAGGATCCAACCGCCCTTGTATGTCACACTCCAGC	AAACCATGGCTCCTCCTCCGACTCCGACTCCTC	Promoter-GUS staining for *SLG7*
Real_Actin	GATGACCCAGATCATGTTTG	GGGCGATGTAGGAAAGC	Expression analysis for *SLG7*
Real_41200	CCCAAGCAAGAAGTCCAG	TGAAGCAAGAACTGAAGGA	Expression analysis for *SLG7*
41200 cx-1	TACCTCTGCTTGCTCCTT	TGTGCTGCCATATTACATT	Sequencing for LOC_Os07g41200
41200 cx-2	AACAGATGGCTTACAGTAGTTG	AGCTCAGGGTTTCTTTCC	Sequencing for LOC_Os07g41200
41200 cx-3	AAGGAGGAATGGGCAGTA	GGTGAGACAGGTGGACGA	Sequencing for LOC_Os07g41200
41200 cx-4	GGTCCATTCTCGCATTTC	GTCATTGGCACGCTTACA	Sequencing for LOC_Os07g41200
41200 cx-5	CCCCTCTACAAATACACCT	ACTTCCTATCCCTAACTAATG	Sequencing for LOC_Os07g41200
41200 cx-6	AGTATGATTCAGTTGATCCGTAG	GGCCACTTCCTATCCCTAA	Sequencing for LOC_Os07g41200
41200 cx-PRO	GTCTGCTTTTGTTCCG	CACCCCATCTGCCTCT	Sequencing for LOC_Os07g41210
41210seq-1	CAGTGGGCGTGCCTATGGT	CCGTTGCCGTCGTGAAAG	Sequencing for LOC_Os07g41220
41220-seq1	TCACTCATTAGATGGACTGA	CACCGCAGCCGCGTCGCCAT	Sequencing for LOC_Os07g41220
41220-seq2	CTCAGTATGAAGAATGCCAAAT	CGCTTTCTTACCTCGCTAT	Sequencing for LOC_Os07g41220
41220-seq3	CTCAGTATGAAGAATGCCAAAT	TTTGTGGCGTCGGTTTCG	Sequencing for LOC_Os07g41220
41220-seq4	AATGAGTTTAAGTCGGCTGGAT	TTTGTGGCGTCGGTTTCG	Sequencing for LOC_Os07g41220
41220-seq5	GCTGGCTGTTGGTGAGAA	CAAGATAAGGGACAAATGGAAA	Sequencing for LOC_Os07g41220
41220-seq6	TTGTTCGGTTTGCTTCCT	ACGGCAAGATAAGGGACA	Sequencing for LOC_Os07g41220

41220-seq7	TTGTTCGGTTTGCTTCCT	AGATTTCCGATACTGCCTTC	Sequencing for LOC_Os07g41220
41230-seq1	CAACACTAACCACCAGCAAC	TCAGGTAGACGGTAACCAAAT	Sequencing for LOC_Os07g41230
41230-seq2	CAGTTAGTTGATGAAGGCGTAT	CAGGCTAAAGGGTGCTAC	Sequencing for LOC_Os07g41230
41230-seq3	ATTCTAAGGACTTGGGTGTT	GAAACCTGAAGGGAGAAAC	Sequencing for LOC_Os07g41230

Supplemental Table S5. Allelic variances of *GS3*, *GW8* and *SLG7* in 9311, Azucena and Zenith.

Varieties	Alleles		
Azucena	*gs3*	*gw8*	*SLG7*
9311	*gs3*	*GW8*	*slg7*
Zenith	*GS3*	*GW8*	*SLG7*