Plant Mol Biol (2012) 78:351–359
DOI 10.1007/s11103-011-9868-8

TH1, a DUF640 domain-like gene controls lemma and palea development in rice

Xiaojiao Li · Lianjun Sun · Lubin Tan · Fengxia Liu · Zuofeng Zhu · Yongcai Fu · Xianyou Sun · Xuewen Sun · Daoxin Xie · Chuanqing Sun

Received: 13 January 2011 / Accepted: 11 December 2011 / Published online: 28 December 2011

Abstract The developmental regulation of grasses lemma and palea and their relationship to the floral organs in dicots had been variously explicated and extensively debated. Here, we characterized a triangular hull mutant *th1-1* from EMS-mutagenized *Oryza sativa* ssp. *indica* cv. 93-11. The *th1-1* mutant exhibited obviously triangular hull with tortuous and slender lemma/palea. Using a map-based cloning strategy, the *TH1* gene was narrowed down to a 60-kb region on the long arm of chromosome 2. Sequence verification revealed that the *th1-1* mutant harbored 1-bp deletion in exon 2 of LOC_Os02g56610 which resulted in a frame-shift mutation. The RNA-interference transgenic plants of LOC_Os02g56610 displayed a similar phenotype to the *th1* mutant. Consequently, LOC_Os02g56610 was identified as the *TH1* gene which encoded 248 amino acids and contained a DUF640 domain. RT-PCR analysis and GUS staining showed that the transcripts of *TH1* mainly accumulated in young inflorescence, lemma and palea of spikelet. These results suggested that *TH1* was an important gene controlling the lemma and palea development in rice.

Keywords Triangular hull · Lemma · Palea · *TH1* · Rice (*Oryza sativa*)

Introduction

Grass (Poaceae) is one of the largest flowering plant families of angiosperms with ~10,000 species, including many important crops. Rice, a kind of grass, is one of most important food crop in the world. Spikelet is a fundamental unit of rice yield, the spikelet development and morphogenesis has profound influence on seed production. A typical spikelet of rice (*Oryza sativa*) consists of a pair of rudimental glumes, two empty glumes, a lemma and a palea, two lodicules, six stamens and one pistil. Among these floral organs, the development of lemma and palea dramatically influenced on the grain shape, grain size, even the grain yield in rice (Fan et al. 2006; Song et al. 2007; Shomura et al. 2008; Weng et al. 2008).

The rice *fon1* and *fon4* mutants (Suzaki et al. 2004; Chu et al. 2006) exhibited enlargement of floral meristems, which ultimately led to extra lodicules or lemma/palea-like organs, or homeotic conversion of organ identity (Suzaki et al. 2004; Chu et al. 2006), indicating that the floral meristem maintenance influenced the development of lemma and palea in rice. Similarly, mutations in the *FON*s homologs, the *CLV1*, *CLV2* and *CLV3* genes in *Arabidopsis*, caused enlargement of floral meristems, as well as inflorescence and shoot apex meristems, which led to increase of floral organs and flower number (Clark et al. 1993; Clark et al. 1995; Kayes and Clark 1998).

Xiaojiao Li and Lianjun Sun contributed equally to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s11103-011-9868-8) contains supplementary material, which is available to authorized users.

X. Li · L. Sun · L. Tan · F. Liu · Z. Zhu · Y. Fu · X. Sun · X. Sun · C. Sun (✉)
State Key Laboratory of Plant Physiology and Biochemistry, Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100193, China
e-mail: suncq@cau.edu.cn

D. Xie
School of Life Sciences, Tsinghua University, Beijing 100084, China

Recently, a few of genes, including *LHS1*, *PAL1*, *DH1*, *REP1 GS3*, *GW2*, *qSW5* and *GW5*, had been identified to control the hull development and grain size in rice (Prasad et al. 2001; Luo et al. 2005; Fan et al. 2006; Song et al. 2007; Li et al. 2008; Shomura et al. 2008; Weng et al. 2008; Yuan et al. 2009). Nevertheless, the genetic regulation of the developmental draft of basic lemma and palea was not established in rice.

In this study, we identified the *TH1* gene that controlled grain shape of rice. First, we isolated two mutants, the *triangular hull1-1* (*th1-1*) and *th1-2*, from the ethyl methane sulphonate (EMS)-mutagenized population of the *indica* cv. 93-11 and the *indica* line CP78, respectively. Both mutants exhibited triangular hull and narrower spikelet hull width traits compared with its wild type. Further observation of the lemma and palea revealed that *th1-1* showed thicker lemma and palea, and more bulges in the epidermis. Using the map-based cloning approach, we identified the *TH1* gene that encoded an unknown functional protein with a DUF640 domain and expressed in young panicle, lemma, palea, gynoecia, ovary and young embryos. This work provided novel insight into lemma and palea development and grain traits in rice.

Materials and methods

Plant materials

All the requisite cultivars, including 93-11, Guichao 2, CP78, C418, IR24, Minghui 63, Zhenshan 97B, Milyang 46, Dianchao 3, Zhonghua 17, Asominori and genetic populations, were grown in the field of the Experimental Station of China Agricultural University.

Identification of the *th1-1* and *th1-2* mutants

About 20,000 seeds of the *indica* cv. 93-11 and CP78, respectively, were EMS (ethyl methane sulphonate)-mutagenized and subsequently grown in the field to generate the EMS-mutagenesis populations according to Wu et al. (2005). The *triangular hull 1-1* (*th1-1*) mutant and *th1-2* were identified from the EMS-mutagenized population of the *indica* cv. 93-11 and the *indica* line CP78, respectively.

Histological analysis

Spikelets from *th1-1* and 93-11 plants just before heading stage were fixed in FAA (70% ethanol, 5% glacial acetic acid, 3.7% formaldehyde), embedded in paraffin. Thin sections were prepared and stained with 1% fast green and the 1% safranine. For scanning electron microscopy (Liu et al. 2004), the lemma and palea of *th1-1* and 93-11 spikelet just before heading stage was viewed.

Observation and quantitation of bulges

Bulges (a rounded projection and a bent or protruding part originated from the cuticular thickening on the epidermal cells) of the lemma and palea were observed by SEM (Prasad et al. 2005). Their numbers were quantified manually. Scan areas studied were 40,000 μm^2 from proximal, middle and distal regions of the lemma and palea.

Map-based cloning of *TH1*

110 F_2 progenies derived from the cross between the *th1-1* mutant and an *indica* cv. Guichao 2, was used to preliminarily map the *TH1* gene. To further map the *TH1* gene, we generated a large F_2 population with more than 15,000 progenies from the cross between *th1-1* and a *japonica* cv. C418. 2,400 progenies from this F_2 population showed triangular hulls at the heading stage, which were chosen for fine mapping of *TH1*.

RM3774 and RM3248 (McCouch et al. 2002), two SSR markers flanking the *TH1* gene, were used to screen the recombinants. Three new polymorphic markers, including a CAPS (cleaved amplified polymorphic sequence) marker C1 and two SNP (single nucleotide polymorphism) markers C2 and C3 (Table S1), were developed based on the reference sequence (Nipponbare) to fine-map *TH1*. Fine mapping with these markers placed *th1* within a 60-kb region between C1 and C2 markers. To find the discrepancy of genomic sequence between 93-11 and the *th1* mutants, we sequenced the 60-kb region by PCR amplification using more than 40 pairs of primers (Data not shown).

Among these primers, HX1 (5′-gtactggcaaagcaagatgg-3; 5′-atcgggaagcagattcatcc-3′) were used to amplify the ORF sequence of LOC_Os02g56610 from *th1-1*, 93-11 and other cultivars. Moreover, to analyze the sequence of *th1-2*, another pair of primers HX2 (5′-catgtgtgtggcactgttgc-3; 5′-cactctagctacatagtgtgc-3′) were used to amplify the genomic sequence of LOC_Os02g56610 from *th1-2* and CP78.

Vector construction and plant transformation

To generate the hairpin p*TH1-RNAi* construct, we cloned two copies of a 355-bp fragment (from +1 to +355 bases) of *TH1* cDNA at inverted repeats, which was driven by the ubiquitin promoter, into the pJL1460 vector (Wang et al. 2004).

The p*TH1*::*GUS* construct was generated by cloning a 2,225-bp fragment harboring the endogenous *TH1* promoter into the p*CAMBIA1381* vector, which was amplified with the primers (5′-agcGAATTCctgccacaagatatgttcgg-3′ and 5′-acaGTCGACgcaacctgaatcacaagaagc-3′).

Both p*TH1-RNA*i and p*TH1*::*GUS* constructs were transformed into a *japonica* cv. Zhonghua 17 through the

method of Microprojectile Bombardment (PDS-1000/He, BIORAD).

To generate the p*Act1*::*TH1-GFP* construct, we fused the *TH1* coding region, the 744-bp fragment amplified by the primers (5′-acaCCCGGGaatggatcgtcaccatcacc-3′ and 5′-accTCTAGAcgggatgatgaactgcggc-3′), in-frame with *GFP*, which was driven by the *Act1* promoter. The p*Act1*::*TH1-GFP* construct was introduced into onion epidermal cells through Microprojectile Bombardment (PDS-1000/He, BIORAD). The fluorescence of GFP was inspected under a confocal fluorescence microscope after incubation for 16–24 h at 26°C.

RNA isolation and RT-PCR

Total RNA was prepared from the inflorescences, the lemma, pelea, stamen, pistil, culm, flag leaf, rachis and uppermost node and young embryo with an RNAprep pure Plant Kit [TIANGEN BIOTECH (BEIJING) CO.LTD]. The first strand of cDNA was synthesized with Superscript II RT kit (INVITROGEN, USA) from total RNA. RT-PCR was carried out to amplify the *TH1* (5′-ccatcatccactgtt-catcc-3′; 5′-gatctcctcctccactgctc-3′) transcripts with 30 PCR cycles, using the first-strand cDNA as a template. *Actin* (5′-tccatcttggcatctctcag-3′; 5′-gtaccctcatcaggcatctg-3′) was also amplified as the control.

Histochemical GUS assays

The histochemical GUS assay was processed as previously described (Sieburth and Meyerowitz 1997). Samples for GUS staining were gently fixed by incubation in icy 90% acetone on ice for 20–30 min, then rinsed in 50 mM Na_3PO_4, pH7.2, 0.5 mM $K_3Fe[CN]_6$, and 0.5 mM $K_4Fe[CN]_6$. The samples were then placed in staining solution (50 mM Na_3PO_4, pH 7.2, 1 mM X-gluc, 0.5 mM $K_3Fe[CN]_6$, and 0.5 mM $K_4Fe[CN]_6$), vacuum infiltrated for 10 min, and incubated at 37°C overnight. Then the staining buffer was removed, and the samples were incubated in H_2O for 5 min, and discolored in solution of 90% ethanol and 10% acetic acid.

Results

Identification of the *th1* mutants

To understand the genetic regulation of lemma and palea development in rice, we identified a mutant that displayed triangular hull with a tortuous lemma and palea (Fig. 1A, B) from the ethyl methane sulphonate (EMS)-mutagenized population of an *indica* cv. 93-11. F_1 plants, which were derived from the cross between this mutant and wild-type 93-11, showed the wild-type-like hull. 58 plants from the 240 F_2 populations showed triangular hull. The segregation rate of wild-type (WT) and mutant plants was fit for the ratio of 3:1 and $X_C^2 < X_{0.05,1}^2 = 3.84$. These results indicated that the triangular hull was controlled by a single recessive gene, referred to as *triangular hull 1*(*th1*).

Observation of the lemma and palea of *th1-1* using scanning electron microscopy (SEM) revealed that the *th1-1* had narrower lemma and palea (Fig. 1C, D). Just before heading, we viewed the epidermis of lemma and palea using SEM, and found that the bulges in the *th1-1* mutant were more than that of wild type (Fig. 1E, F). Further examination on transverse sections of lemma and palea showed that the number of fibrous sclerenchyma cells was increased in the *th1-1* mutant (Fig. 1G, H). Quantitative analysis showed that the bulge densities on lemma and palea epidermis in the *th1-1* mutant were increased by 43.57 and 49.52%, respectively (Fig. 1I, J). The width of the spikelet hull in *th1-1* was markedly narrower (24.4%) than that of wild type (Fig. 1K), whereas the spikelet hull thickness of transverse sections became thicker (76.16%) than that of wild type (Fig. 1L). In addition, we also found that the seed set rate of *th1-1* were obviously less (22.5%) than that of wild type (Fig. 1M).

Isolation of the *TH1* gene

In order to map the *TH1* gene, we generated a population of 110 F_2 plants from a cross between *th1-1* and an *indica* cv. Guichao 2. Analysis of genetic linkage between 72 simple sequence repeat (SSR) markers and triangular hull phenotype showed that the *TH1* gene was linked with the SSR marker RM8024 (Fig. 2A) on the long arm of chromosome 2.

To further map the *TH1* gene, we generated a large F_2 population with more than 15,000 progenies from the cross between *th1-1* and a *japonica* cv. C418. RM3774 and RM3248, two SSR markers flanking the *TH1* gene, were used to screen the recombinants from this F_2 population, with 65 recombinants for RM3774 and 142 for RM3248 (Fig. 2A).

To fine-map *TH1*, we developed three new polymorphic markers, including a CAPS (cleaved amplified polymorphic sequence) marker C1 and two SNP (single nucleotide polymorphism) markers C2 and C3 (Table S1), based on the reference sequence (Nipponbare). *TH1* was finally narrowed down within a 60-kb region between C1 and C2 markers in the BAC clone AP004081 (Fig. 2B; Table S1).

Within this 60-kb region, there were six predicted genes based on the genomic sequence of Nipponbare (the TIGR Rice Genome Annotation Database) (Fig. 2B). Comparing the genomic sequence of the mapping region (~60-kb) between the 93-11 and *th1-1*, we found 1-bp deletion in exon 2 of LOC_Os02g56610, which resulted in a frame-shift mutation (Fig. 2C, D).

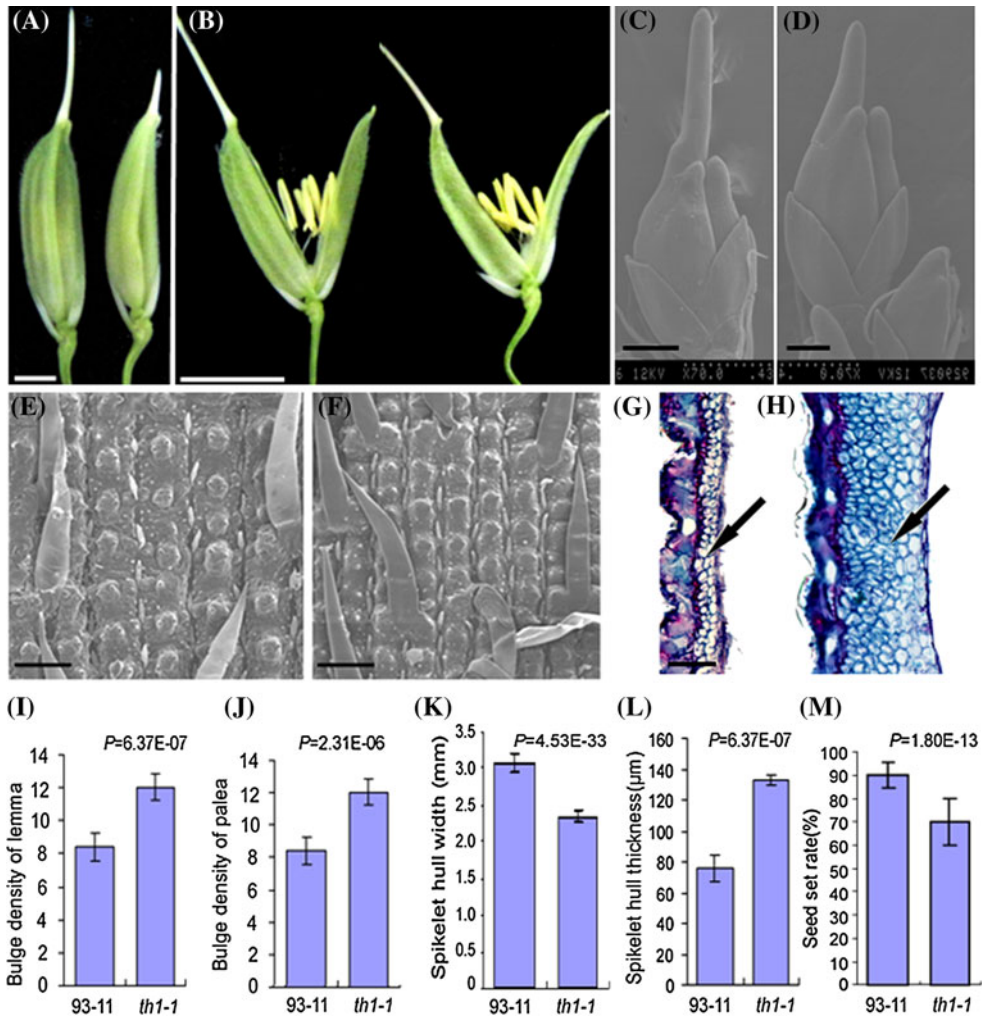

Fig. 1 Phenotypes of the *th1-1* mutant. **A**, **B** Spikelets in wild-type 93-11 (*left*) and *th1-1* (*right*) just before heading. The *th1-1* mutant exhibited triangular hull trait without other floral organs variation. *Scale bar* is 2 mm in **A** and 5 mm in **B**. **C**–**F** Scanning electron microscope (SEM) images of lemma and palea in 93-11 (**C**, **E**) and the *th1-1* mutant (**D**, **F**). **C**, **D** SEM images of the spikelet morphology in the stage of the elongation of empty glumes, lemma and palea. *Scale bar* is 0.214 mm. **E**, **F** SEM images of the lemma/palea epidermis. The *th1-1* mutant showed narrower spikelet hull width in **D** and more bulges in **F**. *Scale bar* is 75 μm. **G**, **H** Lemma and palea transverse sections of 93-11 (**G**) and *th1-1* plants (**H**). *Arrow* points to fibrous Sclerenchyma. *Scale bar* is 80 μm. **I**, **J** Bulge density of lemma (**I**) and palea (**J**) in 93-11 and *th1-1* was calculated by mean the number of bulges in a scan area of 40,000 μm^2 ($n = 5$ area). **K** Spikelet hull width in 93-11 and *th1-1* just before heading ($n = 30$ spikelets). **L** Comparison of spikelet hull thickness of transverse sections in 93-11 and *th1-1* just before heading ($n = 5$ spikelets). **M** Seed set rate in 93-11 and *th1-1* ($n = 30$ panicles). All data are given as mean ± SD. A Student's *t* test is used to generate the *P* values in **I**–**M**

Alignment of the genomic sequence of six *indica* cultivars (Teqing, Guichao 2, IR24, Minghui 63, Zhenshan 97B and Milyang 46) and four *japonica* cultivars (Dianchao 3, C418, Zhonghua 17 and Asominori) further confirmed that the 1-bp deletion of LOC_02g56610 occurred only in the *th1-1* mutant (Data not shown).

Sequence analysis revealed that the LOC_Os02g56610 gene was consisted of 2,100 bp, and its full-length cDNA (AK111446) contained three exons and two introns in 93-11 (Fig. 2C). The CDS located in the second exon and encoded a 248 amino-acid protein with a DUF640 domain according to prediction by NCBI and TIGR Web site. Among DUF640 protein family, there were ten predicted LIGHT SENSITIVE HYPOCOTYLS (LSH) proteins in *Arabidopsis thaliana* and six homologs in rice, which had 50–65% and 50–60% amino acids sequence identity, respectively. Most of the homologs were hypothetical or predicted proteins in rice.

In addition to *th1-1*, we identified another triangular hull mutant from EMS-mutagenized an *indica* line CP78, referred as *th1-2* (Fig. 3A, B). The *th1-2* displayed similar triangular hull as *th1-1*, and the width of the spikelet hull of *th1-2* was markedly narrower (22.4%) than that of wild type (Fig. 3C). The F_1 plants derived from the cross of *th1-1* with *th1-2* displayed triangular hull phenotypes which

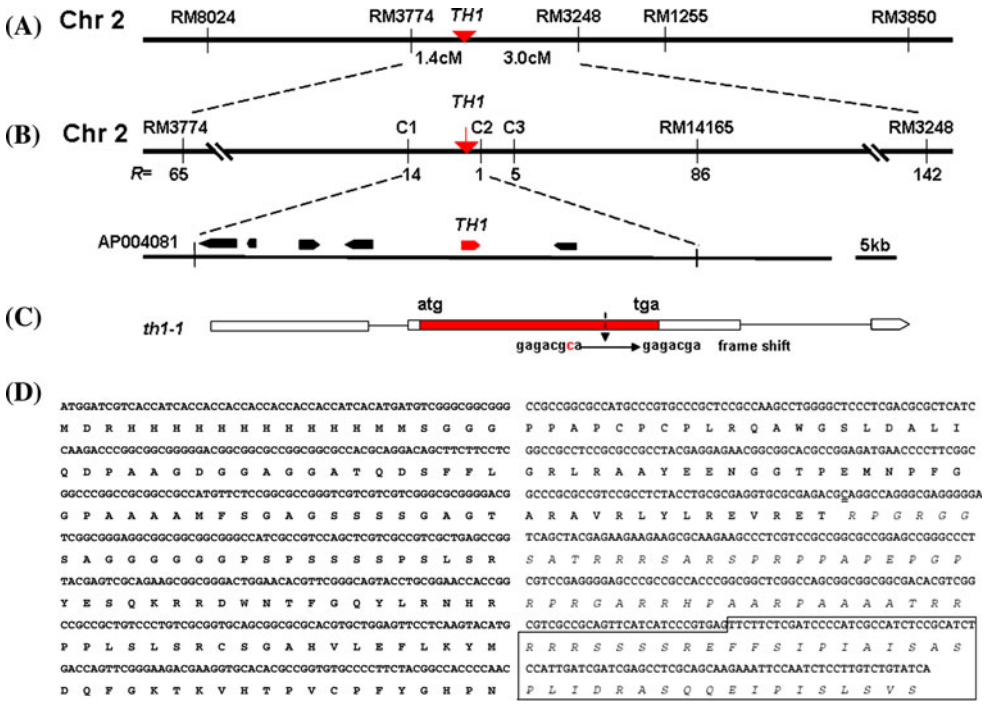

Fig. 2 Map-based cloning of *TH1*. **A** Genetic mapping located *th1* between marker RM3774 and RM3248; cM, centimorgan. **B** Fine mapping placed *th1* in the region of 60-kb, between the a CAPS (cleavable amplified polymorphic sequence) marker C1 and a SNP (single nucleotide polymorphism) marker in the BAC AP004081; Within this 60-kb region, there were six predicted genes based on the genomic sequence of Nipponbare, the fifth gene (LOC_Os02g56610) corresponded to *th1* by genetic complement tests; r, recombinant; scale bar is 5 kb. **C** Schematic structure of candidate gene and mutation site in *th1-1*. Three exons and two introns were shown. A 1-bp deletion (ΔC583) was observed within exon 2 of LOC_Os02g56610 in the *th1-1* mutant, which resulted in reading frame shift. *Empty box* exon; *thin line* intron; *filled box* ORF; *atg* start code; *tga* stop code. **D** The 93-11 *TH1* gene predicted a 1,586 bp transcript including a 747-bp CDS sequence encoding a 248-amino-acid expression protein, while *th1-1* predicted a 1,673-bp transcript including a 834 bp coding DNA sequence because of a 1-bp deletion, from gagacgca to gagacga (the letter *c with double underlines*), which led to reading fame shift and amino acid changing (additional coding nucleotides and Italic amino acids in the frame)

were similar to the *th1-1* and *th1-2* mutants (Fig. 3D), suggesting that these two mutants were allelic.

Further sequence analysis of the *th1-2* mutant and the corresponding wild-type CP78 showed that the *th1-2* mutant contained a section of 1,166-bp nucleotide deletion covering the whole ORF in the LOC_Os02g56610 gene (Fig. 3E).

RNAi-mediated knockdown of *TH1*

To verify whether triangular hull phenotype was caused by a loss-of-function mutation of the LOC_Os02g56610 gene, we generated a hairpin RNAi construct by cloning two copies of a 355-bp fragment (from +1 to +355 bases) of *TH1* cDNA at inverted repeats, which was driven by the ubiquitin promoter, into the pJL1460 vector (referred to as p*TH1-RNAi*) (Wang et al. 2004). The construct was introduced into a *japonica* cv. Zhonghua 17 and the transgenic lines were thoroughly surveyed.

Seven out of 16 lines similarly displayed *th1*-like phenotypes. The transgenic lines L1 and L2 were representatively shown in Fig. 4. L1 and L2 exhibited triangular hull phenotype with a tortuous lemma and palea (Fig. 4A). Further examination at the histological level showed that the density of bulges in lemma and palea epidermis in the transgenic plants was increased (Fig. 4B–E). To confirm whether the phenotype found in the transgenic plants were in virtue of down-regulation of the LOC_Os02g56610 expression, we detected the endogenous expression level of LOC_Os02g56610 by RT-PCR method. As shown in Fig. 4, the transcripts in the lemma and palea of the transgenic plants were distinctly decreased in comparison with the control plants (Zhonghua 17 with an empty plasmid).

The remaining nine lines, including CP1 and CP2, exhibited wildtype-like phenotype. Further RT-PCR analysis showed that the expression of endogenesis LOC_Os02g56610 in these lines was similar to the control plant (Fig. 4F). The expression level of the LOC_Os02g56610 gene in the CP1 and CP2 lines were representatively shown in Fig. 4.

Taken together, these results demonstrated that down-regulation of the LOC_Os02g56610 gene caused the phenotypes which were similar to the *th1* mutants, suggesting that the LOC_Os02g56610 gene corresponded to *TH1* and

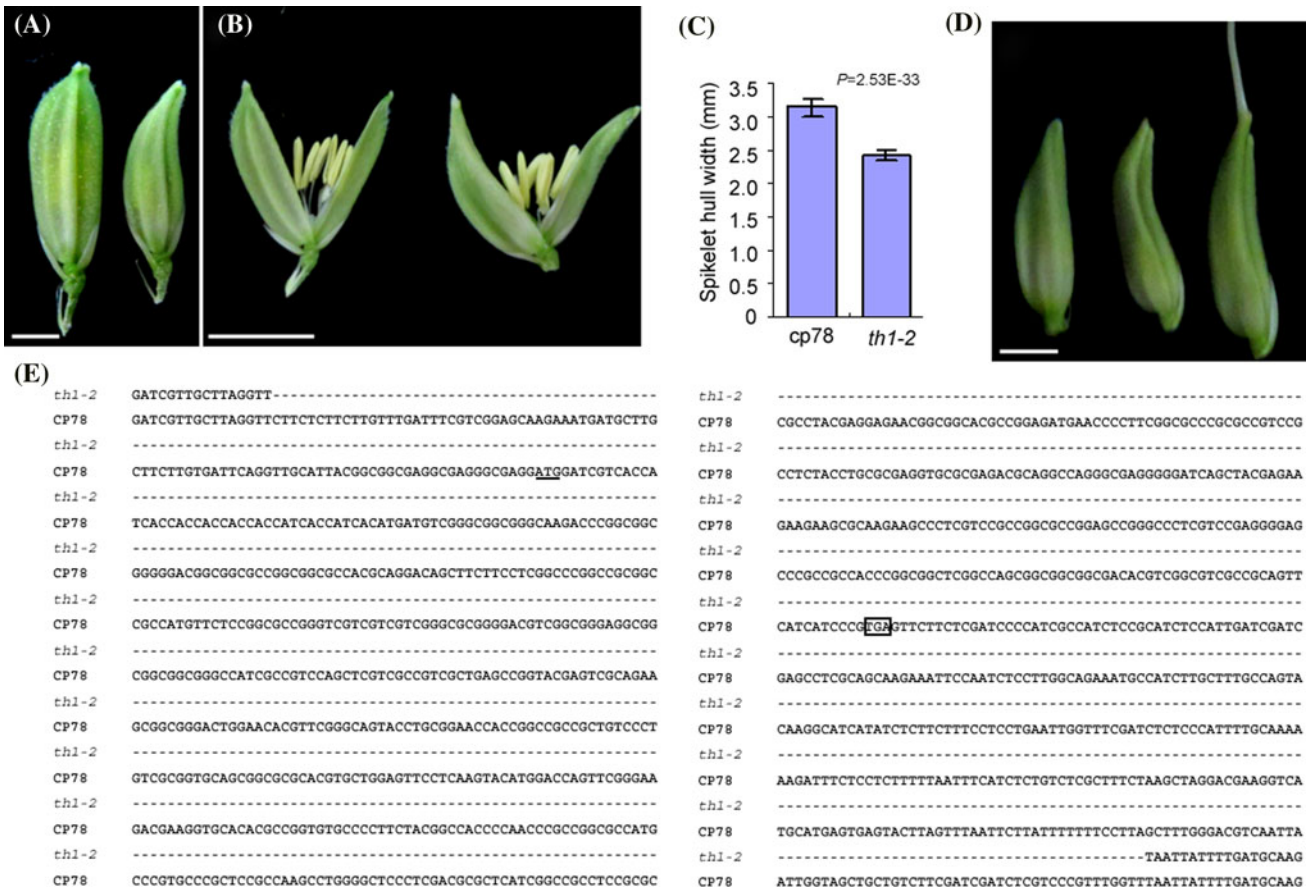

Fig. 3 Phenotypes and sequence of the *th1-2* mutant. **A**, **B** Spikelet of the *th1-2* mutant plant (*right*) and wild-type CP78 plant (*left*). The *th1-2* mutant exhibited triangular hull trait without other floral organs variation. *Scale bar* is 2 mm in **A** and 5 mm in **B**. **C** Spikelet hull width in CP78 and *th1-2* just before heading ($n = 30$ spikelets). **D** Allelic test of the *th1-1* (*right*) and the *th1-2* (*left*). The spikelet hull of the F_1 plants (*middle*) derived from the cross of *th1-1* with *th1-2* displayed triangular hull phenotypes which were similar to the *th1-1* and *th1-2* mutants. *Scale bar* is 2 mm. **E** Sequence analysis of *th1-2* and CP78. The *underlined* nucleotides is initiator codon, the nucleotides in the *frame* is stop codon. All data are given as mean ± SD. A Student's *t* test is used to generate the *P* values in **C**

that mutations in LOC_Os02g56610 were responsible for the triangular hull phenotypes in the *th1* mutants.

Subcellular localization and expression patterns of *TH1*

To investigate the subcellular localization of *TH1*, we constructed a vector of the *TH1-GFP* fusion gene driven by the *Act1* promoter. Transient expression of the construct in the epidermal cells of onion showed that the TH1-GFP fusion protein localized to the nucleus (Fig. 5A–C), indicating that the TH1 was a nuclear protein.

To study the tissue specificity of the *TH1* expression, we introduced the construct consisting of the *TH1* promoter regions fused to the *GUS* reporter gene into the *japonica* cv. Zhonghua 17. The GUS expression was detected in the lemma and palea (Fig. 5D–F), not in stamen, rachis branches and flag leaf (Data not shown), indicating that *TH1* was mainly accumulated in lemma and palea.

Further analysis of the expression patterns of *TH1* by RT-PCR showed that the *TH1* transcript was strongly accumulated in reproductive organs including inflorescence primordium (rachis length <0.5 cm), young panicle (rachis length from 0.5 to 6 cm), lemma, palea, uppermost node, gynoecia combined ovary and young embryo (10–15 days after pollination) (Fig. 5G). However, no *TH1* transcript was detected in stamens, rachis and flag leaf (Fig. 5H). These results were consistent with the expression patterns observed by GUS staining. Taken together, we concluded that the *TH1* gene expressed temporally on the periods of inflorescence development and spatially on the lemma, palea, pistil and young embryo.

Discussion

TH1 regulates lemma and palea development

In recent years, interest in genetic regulation of inflorescence and flower development in grasses has been increased (Bommert et al. 2005). However, only a few

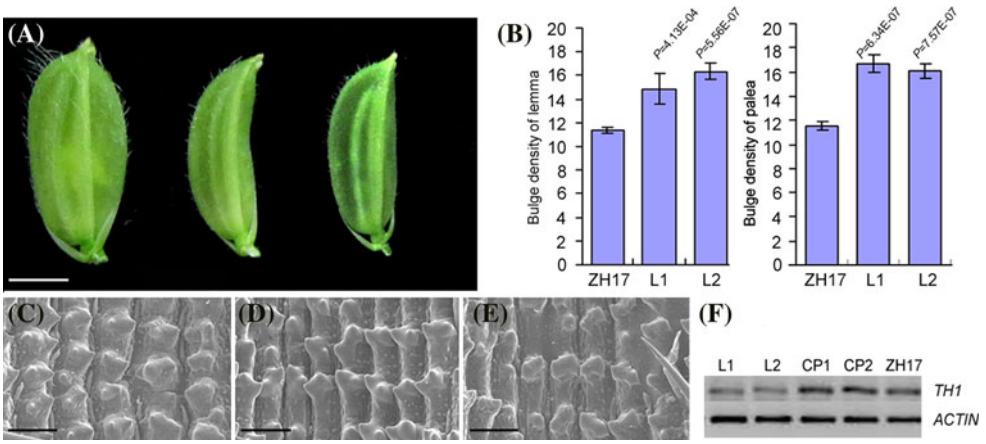

Fig. 4 Functional complementation for the *TH1* gene. **A** Spikelets morphology of a transgenic plant with p*TH1-RNAi*. Spikelet of a Zhonghua 17 plant harboring an empty plasmid (ZH17) was on the *left*. Spikelet of two transgenic plant lines harboring with the construct p*TH1-RNAi* (L1 and L2) were in the *middle* and on the *right*, respectively. *Scale bar* is 2.5 mm. **B** Bulge density of lemma (*left*) and palea (*right*) in 93-11 and *th1-1* was calculated by mean the number of bulges in a scan area of 40,000 μm^2 ($n = 5$ area). **C**–**E** SEM images of the lemma and palea epidermis from ZH17 **C**, L1 **D** and L2 **E**. *Scale bar* is 50 μm. **F** RT-PCR analysis in the lemma and palea of transgenic plants L1, L2, CP1, CP2 and ZH17. Reduction of the endogenous *TH1* was detected in the L1 and L2 that exhibited *th1*-like phenotype, but not in CP1 and CP2 that exhibited wild-type phenotype. All data are given as mean ± SD. A Student's *t* test is used to generate the *P* values in **B**

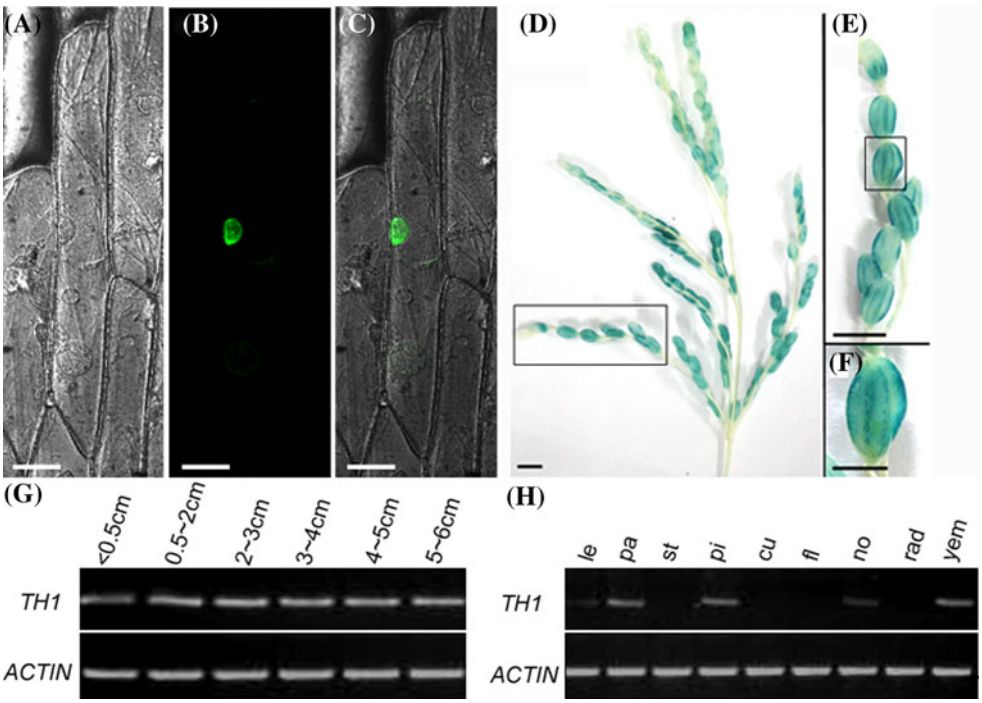

Fig. 5 The expression pattern of the *TH1* gene. **A**–**C** The TH1-GFP fusion protein was localized to the nucleus. The photographs were taken in dark field for green fluorescence **B**, in bright light for the morphology of the cell **A**, and in combination of the cells **C**. *Scale bar* is 1 μm. **D**–**F** The expression of *TH1* was detected to express in the lemma and palea. *Scale bar* is 1.0 cm in **D** and **E**, 0.5 cm in **F**. **G**, **H** RT-PCR analysis in inflorescences at various developmental stages indicated by rachis length **G** and RT-PCR analysis in various organs. **H** *le* lemma; *pa* palea; *st* stamen; *pi* pistil; *cu* culm; *fl* flag leaf; *no* uppermost node; *rad* rachis without spikelets; *yem* young embryo

mutants and genes related to the lemma and palea development were identified in rice (Yoshimura et al. 1997; Prasad et al. 2001; Luo et al. 2005; Li et al. 2008; Yuan et al. 2009). In this study, we characterized two triangular hull mutants, *th1-1* and *th1-2*. The allelic test demonstrated that the *th1-1* and *th1-2* were allelic. Observation at a histological level showed that the number of fibrous sclerenchyma cells of lemma and palea was increased in the mutants. Using a map-based cloning strategy, we identified the *TH1* gene that regulated the shape and development of the lemma and palea. Sequence verification revealed that the *th1-1* mutant harbored 1-bp deletion in exon 2 which resulted in a frame-shift mutation, and *th1-2* mutant harbored a section of 1,166-bp nucleotide deletion covering the whole ORF of the *TH1* gene, which caused triangular hull mutation. These results demonstrated that *TH1* was a novel gene controlling lemma and palea development in rice.

TH1 is a new gene encoded the protein with a DUF 640 domain

The lemma and palea of cereals, corresponded to sepals of dicotyledon, are important components of grass floret, which determine the grain shape and protect the kernel from the attacking of pathogens and insect (Bowman 1997; Ambrose et al. 2000; Jeon et al. 2000; Prasad et al. 2001; Abebe et al. 2004). Up to date, four mutants (*lhs1*, *pal1*, *dh1* and *rep1*) of lemma and palea were identified in rice (Prasad et al. 2001; Luo et al. 2005; Li et al. 2008; Yuan et al. 2009). The *lhs* mutant exhibited an elongated leafy palea and lemma, an open hull, decreased stamen number, and increased the number of carpel (Jeon et al. 2000; Prasad et al. 2001). The *pal1* mutant showed paleless spikelets, and palea was substituted by two leaf-like organs (Luo et al. 2005). The *dh1* florets showed a degenerated hull with naked stamens and pistils which replaced by velum-like or filamentous organs (Li et al. 2008). The development of palea in the *rep1* mutant was markedly retarded (Yuan et al. 2009). *LHS1* belonged to MADS-box gene family (Prasad et al. 2001), *PAL1* was a DNA-binding protein gene (Luo et al. 2005), the *DH1* gene was a member of *LOB* genes family and expressed only at axillary bud and young ear (Li et al. 2008), the *REP1* transcript was a TCP family transcription factor and mainly accumulated in 0.5–4 cm young inflorescence (Yuan et al. 2009).

The *th1* mutants identified in this study displayed tortuous and slender lemma/palea, and did not show floral organs degeneration or homeotic transformation. The *TH1* gene encoded a 248 amino-acid protein with a DUF640 domain. The *TH1* gene transcript was mainly accumulated in 0.5–6 cm young inflorescence, lemma and palea of the spikelets, not in stamen, rachis branches and flag leaf. Taken together, we inferred that *TH1* was a new gene which had the function on regulating the lemma and palea development in rice.

TH1 controls grain shape through regulating the cell division and extension of lemma and palea

The grain shape is a complicated trait which plays an important role in influencing yield and quality of rice. Recently, four key genes or QTLs for grain shape, *GS3* (Fan et al. 2006), *GW2* (Song et al. 2007), *GW5* and *qSW5* (Shomura et al. 2008; Weng et al. 2008), had been identified in rice. *GW2* controlled rice grain width and weight, and encoded an unknown RING-type E3 ubiquitin ligase (Song et al. 2007). *GS3* was a negative regulator and mainly regulated length and weight of grain (Fan et al. 2006). The deletion of *qSW5* resulted in a significant increase in seed size owing to an increase in cell number in the outer glume of the rice flower, while *GW5* that encoded a novel nuclear protein acted in the ubiquitin–proteasome pathway to regulate cell division during seed development (Shomura et al. 2008; Weng et al. 2008). The *TH1* gene identified in this study controlled the grain shape and development of lemma and palea. Loss-function or down-regulation of the *TH1* gene caused tortuous and slender lemma/palea and the decrease of grain weight. Examination at the histological level showed that the *th1-1* mutant exhibited smaller cell (Fig. 1E, F, I, J), closer cell arrangement and more fibrous sclerenchyma cells on the epidermis of the lemma and palea comparison with the wild type 93-11(Fig. 1G, H, L). Therefore, we speculated that the mutation of *TH1* might suppress cell division and extension of lemma and palea, which caused to decrease the width of lemma and palea.

The seed set rate of the *th1-1* mutant was obvious lower than that of the wild-type 93-11. The transgenic plants of p*TH1-RNAi* exhibited *th1*-like phenotypes with triangular hull and no filled seed. These results demonstrated that disruption of *TH1* may severely affect the hull development, causing defect in fertility of rice. Further analysis of the *TH1* function is important for understanding molecular mechanism of the grain development in rice.

Acknowledgments We thank Prof. Zhizhong Gong for his kindest help. This work was supported by Self-Regulated Projects of State Key Laboratory of Plant Physiology and Biochemistry, and by grants from the National Program of Transgenic Variety Development (2008ZX08009-004, 2008ZX08009-008).

References

Abebe T, Skadsen RW, Kaeppler HF (2004) Cloning and identification of highly expressed genes in barley lemma and palea. Crop Sci 44:942–950

Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanofsky MF, Schmidt RF (2000) Molecular and genetic analyses of the *Silky1* gene reveal conservation in floral organ specification between eudicots and monocots. Mol Cell 5:569–579

Bommert P, Nagasawa NS, Jackson D, Hirano HY (2005) Genetics and evolution of inflorescence and flower development in grasses. Plant Cell Physiol 46:69–78

Bowman JL (1997) Evolutionary conservation of angiosperm flower development at the molecular and genetic levels. J Biosci 22:515–527

Chu HW, Qian Q, Liang WQ et al (2006) The *FLORAL ORGAN NUMBER4* gene encoding a putative ortholog of *Arabidopsis* CLAVATA3 regulates apical meristem size in rice. Plant Physiol 142:1039–1052

Clark SE, Running MP, Meyerowitz EM (1993) *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. Development 119:397–418

Clark SE, Running MP, Meyerowitz EM (1995) *CLAVATA3* is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. Development 121:2057–2067

Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Jeon JS, Jang S, Lee S et al (2000) *leafy hull sterile1* is a homeotic mutation in a rice MADS box gene affecting rice flower development. Plant Cell 12:871–884

Kayes JM, Clark SE (1998) *CLAVATA2*, a regulator of meristem and organ development in *Arabidopsis*. Development 125: 3843–3851

Li A, Zhang Y, Wu X et al (2008) DH1, a LOB domain-like protein required for glume formation in rice. Plant Mol Biol 66:491–502

Liu F, Ni WM, Griffith ME et al (2004) The *ASK1* and *ASK2* genes are essential for Arabidopsis early development. Plant Cell 16:5–20

Luo Q, Zhou KD, Zhao XF, Zeng QC, Xia HG, Zhai WX, Xu JC, Wu XJ, Yang HS, Zhu LH (2005) Identification and fine mapping of a mutant gene for palealess spikelet in rice. Planta 221:222–230

McCouch SR, Teytelman L, Xu YB et al (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

Prasad K, Sriram P, Kumar CS, Kushalapa K, Vijayraghavan U (2001) Ectopic expression of rice *OsMADS1* reveals a role in specifying the lemma and palea, grass floral organs analogous to sepals. Dev Genes Evol 211:281–290

Prasad K, Sriram P, Vijayraghavan U (2005) *OsMADS1*, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. Plant J 43:915–928

Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40: 1023–1028

Sieburth LE, Meyerowitz EM (1997) Molecular dissection of the *AGAMOUS* control region shows that *cis* elements for spatial regulation are located intragenically. Plant Cell 9:355–365

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Suzaki T, Sato M, Ashikari M, Miyoshi M, Nagato Y, Hirano H-Y (2004) The gene *FLORAL ORGAN NUMBER1* regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. Development 131:5649–5657

Wang Z, Chen CB, Xu YY, Jiang RX, Han Y, Xu ZH, Chong K (2004) A practical vector for efficient knockdown of gene expression in rice (*Oryza sativa* L.). Plant Mol Biol Rep 22:409–417

Weng JF, Gu SH, Wan XY et al (2008) Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. Cell Res 18:1199–1209

Wu JL, Wu CJ, Lei CL et al (2005) Chemical- and irradiation-induced mutants of *indica* rice IR64 for forward and reverse genetics. Plant Mol Biol 59:85–97

Yoshimura A, Ideta O, Iwata N (1997) Linkage map of phenotype and RFLP markers in rice. Plant Mol Biol 35:49–60

Yuan Z, Gao S, Xue DW, Luo D, Li LT, Ding SY, Yao X, Wilson ZA, Qian Q, Zhang DB (2009) *RETARDED PALEA1* controls palea development and floral zygomorphy in rice. Plant Physiol 149:235–244