

# Isolation of the endosperm-specific *LPAAT* gene promoter from coconut (*Cocos nucifera* L.) and its functional analysis in transgenic rice plants

Li Xu · Rongjian Ye · Yusheng Zheng ·  
Zhekui Wang · Peng Zhou · Yongjun Lin ·  
Dongdong Li

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**Abstract** As one of the key tropical crops, coconut (*Cocos nucifera* L.) is a member of the monocotyledonous family Aracaceae (Palmaceae). In this study, we amplified the upstream region of an endosperm-specific expression gene, Lysophosphatidyl acyltransferase (*LPAAT*), from the coconut genomic DNA by chromosome walking. In this sequence, we found several types of promoter-related elements including TATA-box, CAAT-box and Skn1-motif. In order to further examine its function, three different 5'-deletion fragments were inserted into pBI101.3, a plant

expression vector harboring the *LPAAT* upstream sequence, leading to pBI101.3-L1, pBI101.3-L2 and pBI101.3-L3, respectively. We obtained transgenic plants of rice by *Agrobacterium*-mediated callus transformation and plant regeneration and detected the expression of *gus* gene by histochemical staining and fluorometric determination. We found that *gus* gene driven by the three deletion fragments was specifically expressed in the endosperm of rice seeds, but not in the empty vector of pBI101.3 and other tissues. The highest expression level of GUS was at 15 DAF in pBI101.3-L3 and pBI101.3-L2 transgenic lines, while the same level was detected at 10 DAF in pBI101.3-L1. The expression driven by the whole fragment was up to 1.76- and 2.8-fold higher than those driven by the -817 bp and -453 bp upstream fragments, and 10.7-fold higher than that driven by the vector without the promoter. Taken together, our results strongly suggest that these promoter fragments from coconut have a significant potential in genetically improving endosperm in main crops.

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L. Xu and R. Ye contributed equally to the work.

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L. Xu  
Key Laboratory of Tropical Crops Germplasm Resources  
Utilization of Ministry of Agriculture, Tropical Crops Genetic  
Resources Institute, Chinese Academy of Tropical Agricultural  
Sciences, 571737 Danzhou, Hainan, China

P. Zhou  
Key Laboratory of Tropical Crop Biotechnology,  
Institute of Tropical Bioscience and Biotechnology,  
Ministry of Agriculture, Chinese Academy of Tropical  
Agricultural Sciences, 571737 Danzhou, Hainan, China

Y. Zheng · Z. Wang · D. Li (✉)  
Key Laboratory of Tropic Biological Resources of Ministry  
of Education, Hainan University, 570228 Haikou, Hainan, China  
e-mail: liddfym@hotmail.com

R. Ye · Y. Lin (✉)  
National Key Laboratory of Crop Genetic Improvement  
and National Centre of Plant Gene Research,  
Huazhong Agricultural University, 430070 Wuhan, China  
e-mail: yongjunlin@mail.hzau.edu.cn

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## Abbreviations

CTAB	Cetyltriethylammonium Bromide
GUS	$\beta$ -glucuronidase
LPA	Lysophosphatidic acid
LPAAT	(1-acyl-sn-glycerol-3-acyltransferase)
MU	4-Methylumbelliferone
MUG	4-Methylumbelliferyl- $\beta$ -D-glucuronide
PA	Phosphatidic acid
TGA	Triacylglycerol
X-Gluc	5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide

## Introduction

Endosperm is the storage organ of starch and proteins in cereal crops. It represents 60% of the food supply of the world and provides the major source of calories and proteins for humans. Improving endosperm composition and quality via genetic modification has attracted much attention, and significant progress has been made in this aspect (Bajaj and Mohanty 2005). Compared with other expression systems, endosperm has many advantages as a heterogeneous expression platform, such as cost saving, ease of controlling the production scale, large storage ability, and high safety due to recombinant protein storage and absence of animal-derived pathogens (Takaiwa et al. 2007; Wu et al. 2007). However, only a few strong endosperm-specific expression promoters are known to drive recombinant protein expression in cereal endosperm, which presents a major limitation in improving transgenic mediated crops in the last decade (Furtado et al. 2008). Therefore, it is highly valuable to identify and characterize new endosperm-specific promoters in cereal crops. Foreign protein genes driven by an endosperm-specific promoter are more efficient than those driven by constitutive promoters (Choi et al. 2003).

In genetic transformation, the repetitive use of the same promoter is one major reason for transgenic silencing (Bhullar et al. 2003; Charrier et al. 2000). To achieve the multiple purposes of gene expression in plants, there is a great need for promoters with high and regulated expression of transgenes in monocot seeds (Hiroi and Takaiwa 2006; Furtado et al. 2008). In previous studies, several endosperm-specific expression promoters have been isolated and characterized from rice (*Oryza sativa* L.) (Rasmussen and Donaldson 2006; Qu and Takaiwa 2004), wheat (*Triticum aestivum* L.) (Lamacchia et al. 2001; Wiley et al. 2007), maize (*Zea mays* L.) (Russell and Fromm 1997) and barley (*Hordeum vulgare* L.) (Choi et al. 2003). However, few promoters with endosperm-specific expression have been available in plant genetic transformation research because of unsatisfied expression level and pattern (Li et al. 2008; Qu et al. 2008).

As one of the key tropical crops, coconut (*Cocos nucifera* L.) is a member of the monocotyledonous family Aracaceae (Palmaceae). It is the only species of the genus *Cocos* and belongs to the subfamily Cocoideae that includes 27 genera and 600 species (Rivera et al. 1999, Daniel et al. 2005). The food and industrial products of coconut (such as coconut oil, copra and desiccated coconut) play an important role in the economy of many developing countries. As a special plant in tropics, few genes in coconut have been cloned and characterized. Thus, a large number of unidentified coconut genes provide a great resource for exploring new gene and function (Li and Fan 2008).

*LPAAT* (1-acyl-sn-glycerol-3-P acyltransferase) is an enzyme, which catalyzes acylation of sn-2 position of Lysophosphatidic acid (LPA) to yield phosphatidic acid (PA) in the Kennedy pathway (Kim and Huang 2004). The PA is dephosphorylated to produce diacylglycerol. Diacylglycerol acyltransferase catalyzes the transfer of a third acyl group to the sn-3 position of the glycerol backbone, which can produce triacylglycerol (TGA) (Okazaki et al. 2006). The acyltransferases, especially *LPAAT*, have been shown to have a nonrandom distribution (Coleman and Lee 2004; Nath et al. 2009), and they are specifically expressed in the endosperm of coconut. The coding region of *LPAAT* has been cloned and sequenced from coconut (Knutzon et al. 1995), but the promoter of this gene has not been described in detail. As a gene specifically expressed in the endosperm, the upstream sequence of *LPAAT* may help us to find a new endosperm-specific promoter.

In this study, we cloned and sequenced the *LPAAT* promoter from coconut. Then, with an *Agrobacterium*-mediated transformation of embryogenic callus and plant regeneration, we investigated its function by examining the stable expression of *gus* gene in the endosperm of rice. Our results provide a novel promoter for producing valuable recombinant proteins and multigene transformation in rice and other cereal crops.

## Materials and methods

### Materials

Coconut (*Cocos nucifera* L) leaves were obtained from Institute of Coconut, Chinese Agricultural Academy of Tropical Crops (Wenchang, Hainan, China). *Escherichia coli* strain *DH5 $\alpha$*  was used for bacterial cloning. The strains were grown at 37°C in LB medium supplemented with kanamycin (50 mg/L) or ampicillin (50 mg/L) according to the plasmid type. *Agrobacterium tumefaciens* strain *EHA105* and plant expression vector pBI101.3 were kindly provided by Professor Lin YJ (National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan, China). All chemicals, restriction endonucleases, and other required enzymes were obtained from Sigma (China) or Sangon Biological Engineering T&S Company (Shanghai), unless stated otherwise.

### Genomic DNA isolation and upstream region isolation of the *LPAAT* gene

Coconut high-molecular-weight DNA was isolated from young leaves by the improved CTAB (Cetyltriethylammonium Bromide) method (Liu et al. 2008). The upstream region of *LPAAT* gene was amplified according to the user

manual of Universal Genome Walker Kit (Clontech, Palo Alto, CA, USA). Genomic DNA of coconut was digested with four blunt-end restriction endonucleases (*EcoRV*, *PvuII*, *DraI* and *StuI*), respectively. The digested products were purified and then ligated to GenomeWalker adaptors. The ligated products were used as template for the first-round nest PCR. The primers (CEP-1: *ttcggttgggacatcccgaagctcgctt*, and AP1: *gtaatacagactcactatagggc*) were designed according to the related adaptor and *LPAAT* sequence. Briefly, the primary PCR amplification was carried out by a two-step cycle: seven cycles at 94°C for 25 s, 72°C for 3 min; 32 cycles at 94°C for 25 s, 67°C for 3 min, and an additional 7 min at 67°C after the final cycle. The primary PCR products were used as template in the secondary PCR amplification with the same program, and the nested primers (CEP-2: *gaacttgcccctgaagcatcataggac*, and AP2: *actatagggcagcgcgtggt*) were used to detect CEP-1 and AP1, respectively. The product was recovered and ligated to linear vector pMD18-T (TaKaRa, Dalian, China). The sequence analysis was carried out by TaKaRa Biotechnology Company (Dalian, China).

#### Sequence analysis

In order to predict the function of the upstream region of *LPAAT* gene, the sequence was analyzed by PlantCARE, a database of plant *cis*-acting regulatory elements, enhancers, and repressors (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). Based on the motif location, different 5'-deletion fragments were selected and amplified for expression vector construction.

#### Plant expression vector construction and promoter deletion analysis

To construct plant expression vectors harboring *LPAAT* upstream region, three different 5'-deletions were inserted into pBI101.3, leading to pBI101.3-L1, pBI101.3-L2 and pBI101.3-L3, respectively. In order to amplify 5' *LPAAT* upstream and its deletions, we synthesized 18–20 bp upstream primers corresponding to the sequence beginning at –453, –817, and –896. The 5' sequence of each primer was extended 8 bp to generate a *HindIII* site (underlined). These related primer sequences were as follows: p101-3L: 5'-ataagcttggcagcgcgtggtcgacgg-3', p101-2L: 5'-ataagcttctcgatctattacggtga-3', p101-1L: 5'-ataagcttcctcttcttgaccgac-3'. A *BamHI* site (underlined) was added to the 5' end of the reverse primers 5'-atggatccaggactatataccccactccc-3'. Three amplified products were digested with *HindIII* and *BamHI*, and then they were ligated into the likewise digested vector pBI101.3. The related molecular procedures, such as fragment purification, ligation and transformation, were performed as described previously (Sambrook

et al. 1989). The pBI101.3 was used as a promoterless control. Plasmid construction and manipulation were carried out according to the previously described standard methods. The constructed vectors were verified by PCR and sequencing (Promoter 101a: 5'-*ggacactttatgcttccggctc*-3', and Promoter 101b: 5'-*attccacagtttccgatcc*-3').

#### Plant transformation

The obtained four expression vectors (pBI101.3-L3, pBI101.3-L2, pBI101.3-L1 and pBI101.3-L0) were sequenced to verify the gene orientation and transferred into *Agrobacterium tumefaciens* *EHA105* by a freeze–thaw method (Huang et al. 2001). Then, all the constructs were introduced into Zhonghua11 (*Oryza sativa* L ssp. *japonica*) by *Agrobacterium*-mediated transformation. The callus culture and transformation procedures were carried out as in Hiei et al. (1994). Scutellum-derived embryonic calli were co-cultured with the *Agrobacterium* strain *EHA105* with the expression vector for 3 days. Then the calli were transferred to the selection medium containing 50 mg/L G418 and 400 mg/L Carbenicillin. After 8–9 weeks, survived calli on the selection medium were transferred to the pre-regeneration medium containing 50 mg/L G418 and 200 mg/L Carbenicillin. After 7 days, the resistant calli were transferred to the regeneration medium without antibiotic, to regenerate transformed plantlets. The original roots were cut off, and the plantlets were transferred to the rooting medium. After 7 days, the root was washed, and the plantlets were transferred with the vigorous root in the greenhouse.

#### PCR analysis and Southern blot analysis

PCR and Southern blot analyses were used to determine the presence of *gus* gene and the copy number in transformed plants. PCR analysis was performed with the primers *gus-F* (5'-*gggcgaacagtctctgatta*-3') and *gus-R* (5'-*aacgtatccagccgtattc*-3'). A 20 µl mixture of 30 ng of template DNA, 1× buffer (50 mM KCl, 10 mM Tris–HCl and 0.1% Triton X-100), 0.15 mM dNTPs, 1.875 mM MgCl<sub>2</sub>, 0.1 µM of each primer and 1 U of *Taq* DNA polymerase was prepared for the PCR assay. Initially, the PCR reaction was performed at 94°C for 5 min, then the amplification was carried out with 30 cycles at a melting temperature of 94°C for 1 min, an annealing temperature of 57°C for 1 min, and an extension temperature of 72°C for 1 min, followed by an additional extension at 72°C for 5 min. The PCR products were then analyzed by gel electrophoresis.

For Southern blot analysis, 5 µg of genomic DNA from each sample was digested with *HindIII* and separated on a 0.8% agarose gel. Subsequently, the purified PCR product was transferred onto a nylon membrane. The probe was

prepared from a PCR-amplified *gus* fragment. All procedures for the hybridization were performed as described previously (Lin and Zhang 2005).

#### Histochemical $\beta$ -glucuronidase (GUS) assays

Histochemical localization of GUS activity in transgenic plants was carried out essentially as described previously (Jefferson et al. 1987). In 2008, T<sub>0</sub> transgenic plant (transgenic-positive transformants) tissue was incubated in 50 mM sodium phosphate pH 7.0, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 10 mM Na<sub>2</sub>-EDTA, 0.1% w/v Triton X-100 and 1 mg/mL 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide (X-Gluc) at 37°C for 4–7 h. After staining, the samples were fixed in 70% ethanol, and photographs were taken under a dissecting microscope. More than 10 seeds from each transgenic plant were analyzed. For each construct, five families with high expression level of GUS were selected based on staining levels.

#### Quantitative GUS activity

In 2009, the selected T<sub>1</sub> transgenic plant families were grown on the experimental farm of the Huazhong Agricultural University in Wuhan, China. For each construct, developing endosperm tissues used in fluorometric determination were obtained at 10, 15 and 20 DAF (days after flowering), respectively. Then, the endosperm (at the same developmental stage, but from different families) was mixed as one sample. The samples were grinded in a mortar with liquid nitrogen. Then, 1 mL extraction buffer (0.05 M NaH<sub>2</sub>PO<sub>4</sub> at pH 7.0, 0.01 M EDTA at pH 8.0, 0.1% SDS, 0.1% Triton X-100, and 0.01 M  $\beta$ -mercaptoethanol) was added to ~100 mg of frozen tissue powder, and homogenized by vortexer. The homogenate was centrifuged at 13,000 rpm at 4°C for 10 min, and the supernatant was used for protein quantification and fluorometric assay (Bradford 1976). The fluorometric analysis of GUS activity was performed according to the method described by Jefferson et al. (1987). The substrate 4-methylumbelliferyl  $\beta$ -D-glucuronide (MUG) was added to the supernatant, and then the mixture was incubated at 37°C for 20 min. GUS activity was analyzed in a Microfluor fluorometer (Hitachi) with an emission at 455 nm and an excitation at 365 nm. The fluorometer was calibrated with freshly prepared MU standards in the same buffer prior to the assay. GUS enzyme activity was expressed as pmol of 4-methylumbelliferone (MU) produced per minute per milligram of protein.

## Results

#### Isolation and sequence analysis of the *LPAAT* up-stream fragment

We amplified a 1.2 kb DNA fragment that contains the upstream sequence of the *LPAAT* gene from coconut genomic DNA by the two-cycle PCR. Then we identified the product with agarose electrophoresis and sequenced the fragment. The sequence analysis showed that a 96 bp sequence of this clone was identical to the *LPAAT* cDNA sequence, confirming that the correct fragment of upstream sequence of *LPAAT* was amplified and cloned. As shown in Fig. 1, a 910 bp upstream sequence from the ATG start codon was assigned as the candidate *LPAAT* promoter region, and it was submitted to PlantCARE to detect putative endosperm-specific elements involved in the regulation of gene expression. We found several types of motifs in this promoter: one putative TATA box (TAATA) (–11), four CAAT boxes (CACAAAT and CAAT) (–872, –838, –352 and +18) and four 5'UTR *Py*-rich stretches (TTTCTTCTCT) (–170, –216, –276 and –326). Moreover, the active role of the CAAT boxes was reinforced by the absence of the GC box. In addition, we also detected one *cis*-acting regulatory element, *Skn*-1 motif (–784), which is required for endosperm expression.

#### Construction of expression vectors for function analysis (*gus* chimeric genes)

In order to assess the expression pattern of the *LPAAT* promoter in different tissues, we performed *Agrobacterium*-mediated transformation with the constructed vectors, which contained different 5'-deletion fragments of *LPAAT* promoters fused into a *gus* gene. In order to determine whether the promoter directs the endosperm-specific expression of the target gene, the promoter fragments extending to –896, –817 and –453 were fused to the *gus* reporter gene, respectively (Fig. 2). These deletions were designed based on the locations of putative elements in the *LPAAT* upstream region. The constructions were confirmed by PCR and subsequent sequence analysis.

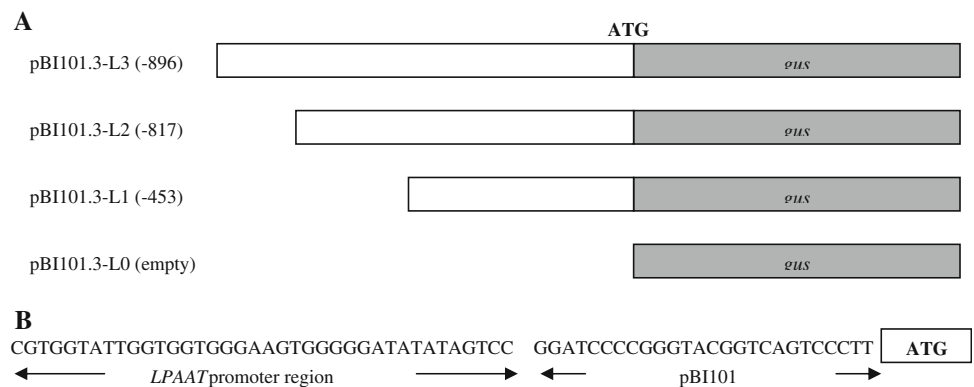
#### Generation of transgenic plants and DNA assay

After the transformation, 37, 42, 39 and 33 transformants were obtained from the constructions pBI101.3-L3, pBI101.3-L2, pBI101.3-L1 and pBI101.3-L0, respectively. Subsequently, we identified 30, 32, 33 and 25 transgene-positive transformants by PCR analysis, respectively. Figure 3 shows that the copy number of the transgenic plants varied from 1 to 4 in the Southern blot analysis. Our

**Fig. 1** Sequence analysis of the upstream region of the *LPAAT*. The code region of *LPAAT* gene was *underlined*. Different types of elements related to endosperm-specific promoter are shown in *different kinds of lines*. The deletion site of promoter is marker by *arrows*



**Fig. 2 a** Schematic representation of the expression vector of *LPAAT* gene promoter and *gus* gene constructs. **b** Details of the translational and transcriptional fusions



data indicated that various *LPAAT* promoter fragments/*GUS* fusions were stable inserts in the rice genome.

**Histological expression analysis**

In 2008, all of the T<sub>0</sub> transgenic plant (transgenic-positive transformants) tissues were assayed by histochemical staining, respectively. Our data indicated that *LPAAT* promoter-directed expression of GUS (initiation codon ATG is provided by GUS) was absent in the floral, stalk, root and leaf sheath tissue (Fig. 4e–i) at different developmental stages (data not shown). In contrast, the three *LPAAT* promoter fragments could generate *gus* expression

in mature seeds, while no GUS expression was observed in the seeds of plants transformed with a promoterless reference plasmid (pBI101.3, Fig. 4a). In particular, GUS expression was not observed in the embryo, but was observed in the endosperm (Fig. 4b–d). For each construct, most of the positive transformants follow the same expression pattern. The ratios are 26/30, 27/32, 25/33, respectively. We found that the expression levels of *gus* gene directed by different 5'-deletions were different, suggesting the existence of some functional regulatory elements in these fragments. In addition, the transgenic plants with pBI101.3-L3 showed the highest GUS expression level. These findings indicated that *gus* gene directed

by *LPAAT* promoter was stably expressed in an endosperm-specific manner. Based on the histochemical assay, for each construct, five families with high expression level of GUS were selected for quantitative analysis.

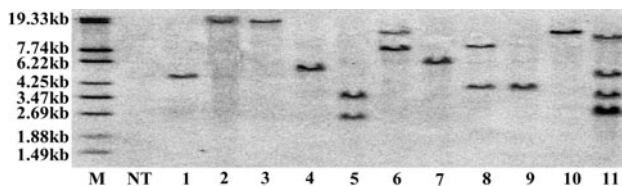
#### Quantitative developmental analysis

In 2009, the selected  $T_1$  transgenic plant families were grown on the experimental farm of the Huazhong Agricultural University in Wuhan, China. We obtained developing endosperm tissues in transgenic plants containing different *LPAAT* promoter fragments (or a promoterless vector) at 10, 15 and 20 DAF, respectively, and then measured the GUS activity by fluorometric determination. In general, the average GUS expression of LD3 (*pBI101.3-L3*), LD2 (*pBI101.3-L2*), and LD1 (*pBI101.3-L1*) transgenes in endosperm was 8.2-, 4.8- and 3.2-fold higher than that in promoterless controls (Fig. 5). The GUS expression

of the three different transgenic lines was different during this developmental period. The highest expression level of GUS was at 15 DAF in LD3 and LD2, while the same level was at 10 DAF in LD1. Removal of approximate 443 bp between *pBI101.3-L3* and *pBI101.3-L1* resulted in a two third loss of GUS activity, suggesting the existence of positive elements driving the expression in this region. Figure 5 shows that the expression driven by the whole fragment was up to 1.76- and 2.8-fold higher than those driven by the  $-817$  bp and  $-453$  bp upstream fragments, and the expression was 10.7-fold higher than that of the promoterless vector.

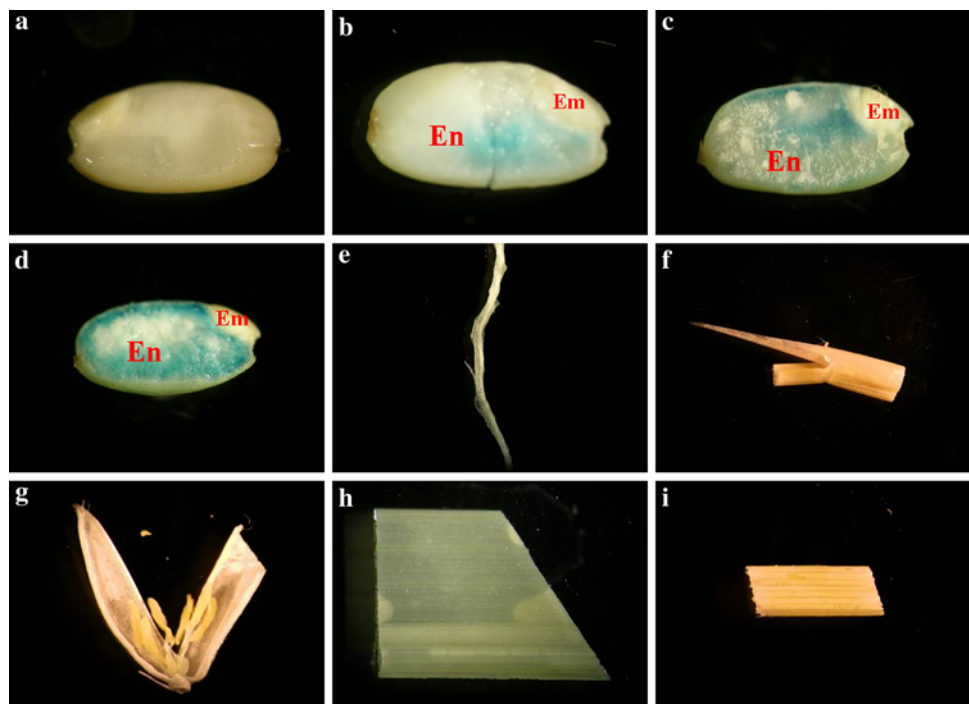
#### Discussion

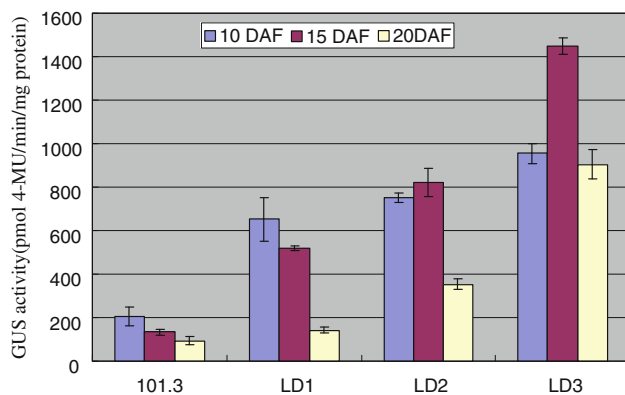
In this study, we cloned the *LPAAT* promoter in coconut and investigated its expression pattern based on stable genetic transformation in transgenic rice plantlets. Using histochemical staining of *gus* gene expression driven by *LPAAT* promoter, we found that the stable GUS expression was specially localized in the endosperm of transgenic rice seeds, while no GUS expression was detected in floral, stalk, root and leaf sheath tissue. To our knowledge, this is the first promoter sequence from a palmaceous plant species that was characterized and used for cereal genetic modification. A previous study shows that a promoter from a monocotyledonous species has different expression patterns when it is tested in other monocotyledonous species (Furtado et al. 2008). In contrast, the effective expression



**Fig. 3** Southern blot analysis of total genomic DNA of  $T_0$  transgenic plants. The DNA samples were digested with *Hind*III and hybridized with the prepared radioactive probe. The probe size was 0.7 kb and was a fragment of *gus* gene. *M* DNA marker, *NT* Zhonghua 11, and lanes *1–11* transgenic plants

**Fig. 4** Histochemical localization of GUS activity driven by the *LPAAT*-promoter and its deletions in matured seeds of transgenic rice. *En* endosperm, *Em* embryo. **a** Negative control seeds transformed with promoterless *pBI101.3*. **b** Transgenic seeds with *pBI101.3-L1*. **c** Transgenic seeds with *pBI101.3-L2*. **d** Transgenic seeds with *pBI101.3-L3*. **e** Rootlet of transgenic rice with *pBI101.3-L3*. **f** Leaf sheath of transgenic rice with *pBI101.3-L3*. **g** Flower of transgenic rice with *pBI101.3-L3*. **h** Leaves of transgenic rice with *pBI101.3-L3*. **i** Stem of transgenic rice with *pBI101.3-L3*





**Fig. 5** The quantitative analysis of GUS activity in developing endosperm tissues at 10, 15, and 20 DAF (days after flowering), respectively. *101.3* Control plasmid, *LD1* pBI101.3-L1, *LD2* pBI101.3-L2, *LD3* pBI101.3-L3

of *gus* gene in our study indicates that *LPAAT* promoter has acceptable universal properties.

The endosperm-specific expression has been investigated in a number of cereal plants, but most of these studies have mainly focused on the expression of storage proteins such as *zein* (Giovinazzo et al. 1992), *glutenin* (Thomas and Flavell 1990), *glutelin* (Kawakatsu et al. 2008) and *hordein* (Muller and Knudsen 1993). Although the aim in most studies is to increase the carbohydrate and nitrogen content of endosperm (Joersbo et al. 2001), it is also desirable to identify endosperm-specific promoters that respond to the process of fatty acid metabolism (Rasmussen and Donaldson 2006). Most of the known endosperm-specific promoters from monocots are active in the late phases of grain development, and they are mainly expressed in starchy endosperm (Qu and Takaiwa 2004). However, *LPAAT* is a pivotal enzyme controlling the metabolic flow of lysophosphatidic acids into different phosphatidic acids in various tissues. In higher plants, the subcellular compartments of glycerolipid synthesis are mainly in the endoplasmic reticulum (ER). Glycerol-3-phosphate (GP) is sequentially acylated with acyl-CoA to LPA and PA, which is also catalyzed by glycerol-3-phosphate acyltransferase (GPAT) and *LPAAT*, respectively. Therefore, the *LPAAT* promoter is the first tissue-specific promoter responding to fatty acid metabolism in endosperm cells. The special function and subcellular compartment of this *LPAAT* promoter make it a unique promoter candidate in crop endosperm modification. This promoter offers the potential to improve the rice quality by modifying the quality and quantity of nutrient uptake through the endosperm.

Instead of transient expression analysis, we applied *Agrobacterium*-mediated stable transformation to validate the function of *LPAAT* promoter. While transient transformation in specific plant tissues by ballistic transformation is a useful approach to assess the activity of various

endosperm-specific promoters (Ahlandsberg et al. 2005; Hwang et al. 2004), Kluth et al. (2002) showed that the transient expression of some tissue-specific promoters can not completely reflect the expression pattern in stable transformants. Furthermore, transient expression studies in endosperm tissues may help elucidate the required promoter sequences, but few studies have shown tissue specificity, relative promoter strength or developmental program as observed for the endogenous genes in the endosperm (Muller and Knudsen 1993). Frisch et al. (1995) showed that chromosomal integration is critical for proper regulation of seed-active promoter by comparing the transient and stable expression of the seed promoter. Therefore, it is more reliable to examine the stable expression of *gus* gene directed by *LPAAT* promoter after genetic transformation and plant regeneration in rice.

In the present study, we detected different GUS expression patterns controlled by three deletion fragments without quantification. While GCN4, AACA and G-box or endosperm motif (E-motif) are known to be crucial for endosperm-specific expression in rice (Furtado et al. 2008) and transgenic tobacco (Washida et al. 1999), latent key E-motif in the *LPAAT* promoter is still unknown. Further studies should focus on the spatial and temporal control of *LPAAT* promoter and other homologous endosperm-specific promoters in rice. Moreover, in order to completely characterize *LPAAT* promoter, it is essential to determine the cellular and subcellular localizations of GUS expression under this promoter in rice seeds as well as to compare with the expression level under other endosperm-specific promoters.

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