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RESEARCH PAPER

Cloning and Expression Analysis of Lysophosphatidic Acid Acyltransferase (LPAT) Encoding Gene in Peanut

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Abstract: Lysophosphatidic acid acyltransferase (LPAT) is one of the key enzymes in biosynthesis pathway of triacylglycerol (TAG) in plant. A full-length cDNA library of peanut (*Arachis hypogaea* L.) was constructed from seed by means of a large number of sequences of expressed sequence tag (EST) and gene functional annotation. A lysophosphatidic acid acyltransferase gene, designated *AhLPAT*, and its genomic DNA sequence was isolated from peanut. The sequence of *AhLPAT* cDNA was 1753 bp, and its genomic sequence was 5331 bp. Bioinformatic analysis showed that *AhLPAT* was composed of 11 exons and 10 introns with typical GT-AG sequence at the splice site. A peptide of 387 amino acid residues was deduced from *AhLPAT*, with molecular weight of 43.2 kD and isoelectric point (pI) of 9.42. Conserved domain prediction indicated that AhLPAT comprised a typical conserved acyltransferase domain and a conserved lysophospholipid acyltransferase domain. The deduced amino acid had a high sequence similarity with the LPAT proteins from other species. Similarities for amino acid sequence of LPAT protein between peanut and *Tropaeolum majus*, *Brassica napus*, *Crambe hispanica* subsp. *abyssinica*, *Ricinus communis*, and *Arabidopsis thaliana* were 90%, 89%, 89%, 88%, and 87%, respectively. The phylogenetic tree suggested that AhLPAT and AtLPAT2 derived from *A. thaliana* were grouped into the same class, and both of them were endoplasmic reticulum type LPATs. The result of quantitative RT-PCR assay indicated that *AhLPAT* was ubiquitously expressed in root, stem, leaf, flower, gynophore, and seed of peanut with the highest level in gynophore and seed. The peak expression was in the period of 50–60 d after flowering. Correlation between *AhLPAT* expression and oil accumulation was significant ($r = 0.63$, $P < 0.05$). These results suggest that *AhLPAT* plays an important role in peanut TAG biosynthesis.

Keywords: peanut; lysophosphatidic acid acyltransferase; cloning; expression analysis; triacylglycerol synthesis

Vegetable oil is mostly composed of triacylglycerol (TAG), the major form of energy and carbon resource existing in plant seeds. In higher plants, fatty acids are *de novo* synthesized predominantly from acetyl-coenzyme A (CoA) present in plastid and transported to endoplasmic reticulum (ER) or cytoplasm [1]. Subsequently, TAG is synthesized by bio-assembly of free fatty acids and glycerol through the classical Kennedy pathway. The Kennedy pathway involves the sequential incorporation of the fatty acyl groups acylation of *sn*-glycerol-3-phosphate catalyzed by glycerol-3-phosphate acyltransferase (GPAT, EC 2.3.1.15), lysophosphatidic acid acyltransferase (LPAT, EC 2.3.1.51), and diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) [2], respectively. In plants, the TAG biosynthesis occurs primarily in the ER [3]; accordingly, the 3 acyltransferases are thought to be located in the ER. LPAT is the major enzyme responsible for acylating the *sn-2* hydroxyl group of lysophosphatidic acid (LPA) to form phosphatidic acid (PA). The PA is then dephosphorylated to diacylglycerol, or used to produce phosphatidylglycerol as an essential component of all cellular membranes [4]. So LPAT plays a critical role in the regulation of many physiological processes. Up to now, multiple homologous *LPAT* genes have

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been isolated from different plant species including *Arabidopsis thaliana* [5], oilseed rape (*Brassica napus* L.) [6], coconut (*Cocos nucifera* L.) [7], maize (*Zea mays* L.) [8], and nasturtium (*Tropaeolum majus* L.) [9]. At least 9 distinct genes encoding LPAT-like proteins have been characterized in Arabidopsis [10]. Different members of *LPAT* gene family have similar functions in establishing the acyl composition of lipids. At the same time, there are many differences between LPATs, such as substrate preference, organs-specific expression, and subcellular location [1, 5, 11, 12]. It is now clear that LPAT largely determines the *sn*-2 lipid acyl composition of TAG through its high substrate specificity [13]. The microsomal LPATs from several plant species discriminate strongly against 16:0-CoA, 18:0-CoA, and unsaturated fatty acyl moieties with chain lengths >18 carbons in the substrates. It possesses substrate preference for C18:1-CoA [14]. In major oilseed crops, the *sn*-2 positions of TAGs are dominated by unsaturated C18:1 fatty acid. Therefore, LPAT is of interest for gene engineering of seed oil and fatty acids composition, especially for decreasing the saturated fatty acid in edible oils. Recent studies have shown that the conversion of LPA to PA catalyzed by LPAT is a potential rate-limiting step in the TAG synthesis in oilseeds [2, 15]. Apparently, the increase of LPAT quantity and activity will result in the enhancement of storage lipid sink size. Thus, overexpression of *LPAT* gene leads to a significant increase of the seed oil content. The transgenic overexpression of yeast (*Saccharomyces cerevisiae*) *SLC1-1* gene, encoding a variant LPAT, in the oilseed rape and Arabidopsis was capable of enhancing the overall proportion and content of very long chain fatty acyl at the *sn*-2 position in seed TAG; meanwhile, seed oil content increases from 8% to 48% were also observed in the transgenic plants [16]. The microsomal *LPAT* genes (*BAT1.13* and *BAT1.5*) from oilseed rape were overexpressed in Arabidopsis seeds. The average seed weight and the total fatty acid content of seed storage lipids were increased by 6% and 13% compared with nontransformed plants, respectively [2]. LPAT has already become the research hotspot in modification of the fatty acid composition of TAG and enhancement of oil content in seeds.

Peanut (*Arachis hypogaea* L.) is one of the most important cash crops cultivated worldwide, and it is also an important source of edible oil. Increasing seed oil content and improving oil quality have become the most important aim for peanut breeding and genetic improvement. However, the large size and complicated genome of peanut makes molecular study on peanut generally very limited and far behind many other crops [17]. So far, the sequence characteristics and expression pattern of *LPAT* gene in peanut are still unknown. In this study, we isolated *LPAT* gene from peanut seed, the sequence was analyzed by bioinformatic tools and the expression of *LPAT* gene was investigated in different organs and in different seed developmental stages. The results will facilitate understanding of mechanism of fatty acid and storage lipid synthesis in peanut, which provides a theoretical basis for improvement of peanut oil quality and content with *LPAT* gene.

1 Materials and methods

1.1 Plant materials and treatment

Our laboratory has constructed a peanut seed full-length cDNA library from mixed mRNA of peanut seed. This cDNA library was used to screen *LPAT*. The peanut cultivar 06-4104 with high oil content was used to amplify genomic DNA of *LPAT* and to analyze gene expression. Cultivar 06-4104 was grown in the National Wild Arachis Nursery Garden, Wuchang, China. Root, stem, leaf, flower, and gynophore, and seed were sampled 15, 20, 25, 30, 40, 50, 60, 70, 80, and 90 days after flowering (DAF) for quantitative real-time RT-PCR analysis. Three replications of 2 plants each were sampled and bulked. All fresh organ samples were frozen in liquid nitrogen immediately and stored at –80 °C before RNA extraction. Immature seeds from 10 different seed developmental stages were air-dried separately to analyze seed oil content.

1.2 Determination of seed oil content

We used air-dried seeds from each developmental stage for direct nuclear magnetic resonance (NMR) measurements. The NMR analysis was carried out using a bench-top Minispec mq-20 NMR analyser (Bruker Optik GmbH, Germany) according to the guideline of ISO 10565 (International Organization for Standardization, 1998). For each sample, triplicates were performed and the mean value was used to calculate oil content.

1.3 Reagents and *E. coli* strain

Vector pMD19-T, Premix Ex *Taq* PCR polymerase, DNase I (RNase free), and MiniBEST Plasmid Purification Kit were purchased from TaKaRa Biotechnology Company, Dalian, China. EZNA Gel Extraction Kit was from Omega-Biotech, Doraville, USA. DNA molecular marker was from TransGen Biotech Co., Ltd., Beijing, China. Trizol Reagent for RNA extraction was from Invitrogen, San Diego, CA, USA. RNeasy Plant Mini Kit for total RNA of peanut seed extraction was from Qiagen, Hilden, Germany. First Strand cDNA Synthesis Kit ReverTra Ace –α– and SYBR Green Real-time PCR Master Mix were from TOYOBO Life Science, Osaka, Japan. The primers were synthesized by Invitrogen Biotechnology Co., Ltd., Shanghai, China. Other chemical reagents in analytical purity were made in China. The competent *Escherichia coli* strain DH5α was purchased from TransGen Biotech Co., Ltd., Beijing, China.