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Cloning and characterization of a β-ketoacyl-acyl carrier protein synthase II from *Jatropha curcas*

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ABSTRACT

A cDNA clone encoding a putative β-ketoacyl-acyl carrier protein (ACP) synthase II (KASII), a key enzyme in fatty acid biosynthesis, was isolated from *Jatropha curcas* L., a woody oil plant. The isolated cDNA clone of *JcKASII* contained a 1722-bp open reading frame coding for 573 amino acids with a predicted molecular mass of about 60.98 kDa and the conserved Cys^{324} residues that has been proposed as the active site of KASII proteins. The deduced amino acid sequence of the cDNA clone had about 70–84% identity with the KASII from other plants. The transcript of *JcKASII* was detected in all tissues examined and increased during seed maturation. Expression of *JcKASII* in the *Arabidopsis KASII* mutant (*fab1*) could complement the fatty acid composition of the mutant. Overexpression of *JcKASII* cDNA under the cauliflower mosaic virus 35S promoter in *Arabidopsis* resulted in decreasing 16-carbon fatty acids and increasing 18-carbon fatty acids in leaves and seeds. Taken together, these results show that *JcKASII* could function in 18-carbon fatty acids accumulation in plant and may be useful in the genetic engineering of *J. curcas*.

Introduction

In higher plants, fatty acid biosynthesis occurs along a plastid-localized pathway and is catalyzed by two sets of enzymes, acetyl-coenzyme A (CoA) carboxylase (ACCase) and fatty acid synthase (FAS) (Sasaki and Nagano, 2004). ACCase catalyzes the first step from acetyl-CoA to malonyl-CoA (Konishi and Sasaki, 1994). Malonyl is transferred from malonyl-CoA to form malonyl-ACP by malonyl-CoA:ACP transacylase (Ohlrogge and Browse, 1995). FAS transfers the malonyl moiety to ACP and catalyzes the extension of the growing acyl chain with malonyl-ACP (Ohlroggee and Jaworski, 1997). The β-ketoacyl-acyl carrier protein synthase III (KASIII) catalyzes the first FAS reaction, from acetyl-CoA and malonyl-ACP to 3-ketobutyryl-ACP (Jackowski and Rock, 1987). Before a subsequent cycle of fatty acid synthesis begins, the β-ketoacyl-ACP intermediate is reduced to the saturated acyl-ACP in the remaining FAS reactions, catalyzed sequentially by the β-ketoacyl-ACP reductase (KAR), β-hydroxyacyl-ACP dehydrase (HAD), and the enoyl-ACP reductase (ER) (Ohlroggee and Jaworski, 1997). And KASI and KASII are the condensing enzymes for the elongation of the carbon chain from C4 to C18 (Wu and Xue, 2010). KASI catalyzes the substrate C4:0-ACP to produce C16:0-ACP by 6 reiterative cycles of C2 condensation step (Shimakata and Stumpf, 1982). The final elongation in plastid from 16:0-ACP to 18:0-ACP is conducted by KASII (Harwood and Stumpf, 1971). C16 and C18 fatty acids, the main compounds of elongation, can be further desaturated both in the plastid membrane and the endoplasmic reticulum to produce unsaturated fatty acids (Li et al., 2007).

KASII genes have been cloned and characterized from several plants, such as *Arabidopsis thaliana* (Carlsson et al., 2002), *Arachis hypogaea* (Li et al., 2009), *Perilla frutescens* (Hwang et al., 2000), *Betula pendula* (Martz et al., 2006), *Cuphea lanceolata* (Klein et al., 1992), *Cuphea wrightii* (Leonard et al., 1998) and *Jessenia bataua* (Teh and Ramli, 2011). The altered expression levels of *KASII* result in a change of fatty acid composition in some species. The *fab1* mutant of *Arabidopsis*, a single nucleotide change in *Arabidopsis KASII* by EMS (James and Dooner, 1990), exhibits an increased amount of 16:0 and decreased amounts of 18-carbon fatty acids. In particular, for the major chloroplast phospholipid, phosphatidylglycerol, 40.1 mol% of the total fatty acid content is 16:0 (Wu et al.,

Abbreviations: ACP, β-ketoacyl-acyl carrier protein; CoA, coenzyme A; ACCase, acetyl-CoA carboxylase; FAS, fatty acid synthase; KAS, β-ketoacyl-ACP synthase; KAR, β-ketoacyl-ACP reductase; HAD, β-hydroxyacyl-ACP dehydrase; ER, enoyl-ACP reductase; RACE, rapid amplification of cDNA ends; FA, fatty acid; ORF, open reading frame; DAG, day after germination; DAF, day after flowering.

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1994). Overexpression of *CwKASII* in *Arabidopsis* reduces the content of 16:0, and promotes the content of 18:0, 18:1, and 18:2 (Leonard et al., 1998). Strong reductions of *AtKASII* expression by seed-specific hairpin-RNAi result in up to 53% of 16:0, approximately a seven-fold increase over wild-type levels (Pidkowich et al., 2007). Transformation of *fab1* plants with *BnKASII* could complement the fatty acid composition of the mutant (Carlsson et al., 2002).

J. curcas is a multipurpose bush/small tree belonging to the family of *Euphorbiaceae* (Openshaw, 2000). It can grow well under such adverse climatic conditions because of its low moisture demands, fertility requirements, and tolerance to high temperature (Augustus et al., 2002). *J. curcas* is an important oil-rich plant. It has received much attention for its high oil content of kernel and its high yield of oil per unit land area, which is second only to oil palm (Openshaw, 2000; Fairless, 2007). Oil from *Jatropha* is regarded as a potential fuel substitute (Kumar and Sharma, 2008). The seed oil contains high levels of the C18 fatty acids, of which the oleic acid and linoleic acid contents are approximately 40% and 30%, respectively. The total C16 fatty acid contents are about 15%. In recent years, a number of research groups have explored techniques for the extraction of oil from *J. curcas* (Haas and Mittelbach, 2000; Shah et al., 2004; Achten et al., 2008; Karaj and Muller, 2011). Some genes involved in fatty acid synthesis in *J. curcas* have also been cloned (Tong et al., 2006; Li et al., 2008a; Wu et al., 2009; Gu et al., 2011). Recently, the whole genome sequence of *Jatropha* is published (Sato et al., 2011), which could promote the research of *Jatropha*.

Here we report the cloning and characterization of cDNA from *J. curcas* encoding a putative β-ketoacyl-ACP synthase involved in the carbon-chain elongation, designated *JcKASII*. The function of this enzyme was investigated by overexpressing this gene in the *fab1* mutant of *Arabidopsis* and in the wild-type plant under the 35S promoter. Transformation of the *fab1* plant with *JcKASII* complements the fatty acid composition of the mutant. Overexpression of *JcKASII* in *Arabidopsis* affects the fatty acid composition of leaves and seeds.

Materials and methods

Plant materials

Matured seeds of *Jatropha curcas* were collected from Guizhou province, PR China, and planted in farmland in Guangzhou, China. Different kinds of tissues and the developing seeds at various stages of *J. curcas* were harvested from two-year-old trees in autumn. Wild type and the *fab1* mutant of *Arabidopsis thaliana* were germinated and grown at 22 °C, 16 h light/8 h dark.

Cloning and sequencing of the KASII gene

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. The first-strand cDNA was synthesized from 3 μg of total RNA by using Superscript II (Invitrogen, Carlsbad, CA, USA). The specific fragment of *JcKASII* was amplified using a primer pair of KAS21 and KAS22 (Table 1), which were designed based on the conserved regions of the corresponding genes from Viridiplantae. The PCR amplification for *JcKASII* was performed as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 50 °C for 1 min and 72 °C for 2 min; with a final extension step of 72 °C for 5 min. The PCR fragments were gel-purified with an Agarose Gel DNA Purification Kit, and were ligated into the pMD18-T Vector (TaKaRa, Otsu, Japan). Sequence analysis was performed by Invitrogen bio-company in Shanghai. Specific primers of KAS23 together with adaptor-dT and adaptor (Table 1) were used for *JcKASII* 3′RACE-PCR and the 5′ end was obtained using BD SMART™ RACE cDNA Amplification Kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions, with the specific primers KAS24 and KAS25 (Table 1).

Table 1
Primer sequences used in the experiments.

Primer	Sequence (5′–3′)
KAS21	AG(A/G)ATTGC(T/C)GG(A/T)GA(G/A)ATCAA
KAS22	TT(A/G)TGNCC(A/G/C)CC(A/G)AA(A/T/C)CC(A/G)AA
KAS23	GATGCATATCACATGACTGA
KAS24	CAGAGCCAATCAAAACTCCA
KAS25	GCATGAACTTATCCATTCTC
KAS26	ACGTCGAATTTCTTGGAGGAAGC
KAS27	TCGAGCATTTGTATCCACGCCTTCG
KAS28	TGCTCTAGATTTCTCGATTGACATTTTCT
KAS29	TGCGAGCTCGTGAGTATTTCCCTCTTGAT
KAS210	GCCAAACTATTCTATTTCAACTGCTT
KAS211	CAACAAAACCTCCCAACCCTAT
KAS212	CTAAATGTGGAGTTTTGATTGGCT
KAS213	ACAGGCAGTGGAGATTGAATAGTTT
Adaptor	GACTCGAGTCGACATCGA
Adaptor-dT	GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT
Actin-F	ATGAGCTTCGAGTTGCACCA
Actin-R	AGCATCAGTGAGATCACGAC
β-Tubulin-F	GAGCCTTACAACGCTACTCTGTCTGTC
β-Tubulin-R	ACACCAGACATAGTAGCAGAAATCAAG

Protein sequence comparisons and phylogenetic analysis

Comparative and bioinformatics analyses of *JcKASII* were carried out online. The nucleotide sequence, deduced amino acid sequence, and ORF were analyzed and the sequence comparisons were conducted through a database search using the BLAST program (http://www.ncbi.nlm.nih.gov). To build a phylogenetic tree, the selected sequences were submitted for alignment with ClustalX v1.83 (Thompson et al., 1997). The maximum-parsimony algorithm was executed with Phylip 3.69 (Felsenstein, 2009). Additionally, 1000 bootstrap replicates were used to test the robustness of the phylogenetic tree. Accession numbers of these amino acid sequences for protein sequence comparisons and phylogenetic analysis are as follows: *Arachis hypogaea* (KASI, ACZ06069; KASII, ACJ07142); *A. thaliana* (KASI, NP_199441; KASII, NP_565097); *Cuphea hookeriana* (KASII, AAC68861); *Cuphea lanceolata* (KASII, CAC59946); *Cuphea pulcherrima* (KASI, ABA07910; KASII, AAC68860); *Cuphea wrightii* (KASII, AAB37271); *Elaeis guineensis* (KASII, AAF26738); *Escherichia coli* (KASI, 71042786; KASII, YP_002382969); *Glycine max* (KASI, AAF61730; KASII, AAW88762); *Helianthus annuus* (KASI, ABM53471; KASII, ABI18155); *Hordeum vulgare* (KASI, BAJ86549; KASII, CAA84023); *J. curcas* (KASI, ABJ90468; KASII, ABJ90469); *Oryza sativa* (KASI, NP_001057053; KASII, NP_001060274); *Perilla frutescens* (KASI, AAC04691; KASII, AAC04692); *Populus trichocarpa* (KASI, XP_002316735; KASII, XP_002326139); *Ricinus communis* (KASI, XP_002519707; KASII, XP_002516228); *Sorghum bicolor* (KASI, XP_002438009); *Zea mays* (KASI, NP_001149639; KASII, NP_001132041).

Determination of Jatropha seed oil content

According to the method of the lipid extraction (Hara and Radin, 1978; Li et al., 2006), the oil contents of *Jatropha* seeds at four stages of development (23 days after flowering (DAF), 26 DAF, 29 DAF and 32 DAF) were measured.

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from different tissues and seeds at various development stages (Li et al., 2008a) (Fig. 3B) of *J. curcas*. The cDNA was reverse-transcribed from 2 μg of total RNA. The