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Molecular cloning and characterization of two β-ketoacyl-acyl carrier protein synthase I genes from *Jatropha curcas* L

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ABSTRACT

The β-ketoacyl-acyl carrier protein synthase I (KASI) is involved in de novo fatty acid biosynthesis in many organisms. Two putative *KASI* genes, *JcKASI-1* and *JcKASI-2*, were isolated from *Jatropha curcas*. The deduced amino acid sequences of JcKASI-1 and JcKASI-2 exhibit around 83.8% and 72.5% sequence identities with AtKASI, respectively, and both contain conserved Cys-His-Lys-His-Phe catalytic active sites. Phylogenetic analysis indicated that JcKASI-2 belongs to a clade with several KASI proteins from dicotyledonous plants. Both *JcKASI* genes were expressed in multiple tissues, most strongly in filling stage seeds of *J. curcas*. Additionally, the JcKASI-1 and JcKASI-2 proteins were both localized to the plastids. Expressing *JcKASI-1* in the *Arabidopsis kasI* mutant rescued the mutant's phenotype and restored the fatty acid composition and oil content in seeds to wild-type, but expressing *JcKASI-2* in the *Arabidopsis kasI* mutant resulted in only partial rescue. This implies that *JcKASI-1* and *JcKASI-2* exhibit partial functional redundancy and *KASI* genes play a universal role in regulating fatty acid biosynthesis, growth, and development in plants.

1. Introduction

In plants, fatty acid (FA) synthesis is the source for membrane lipids, which play vital roles in cell growth and plant development. In addition, FA synthesis produces the triacylglycerols that are deposited in seeds. *De novo* FA biosynthesis is catalyzed by acetyl-coenzyme A carboxylase (ACCase) and fatty acid synthases (Yasuno et al., 2004). The process is initiated by ACCase, which catalyzes the conversion of acetyl-CoA into malonyl-CoA. The nascent fatty acid chain is then extended by β-ketoacyl-acyl carrier protein synthases (KAS), which catalyze chain-elongation condensation steps (Slabaugh et al., 1998; White et al., 2005). KAS enzymes can be classified into three categories (KASI, KASII, and KASIII) based on their substrate specificity. KASIII enzymes initiate the elongation process by combining acetyl-coenzyme A (CoA) and malonyl-ACP to generate 3-ketobutyryl-ACP (Jackowski and Rock, 1987; Jackowski et al., 1989; Gulliver and Slabas, 1994). KASI enzymes then catalyze six further condensation steps, generating C16:0-ACP (Shimakata and Stumpf, 1982). Finally, KASII enzymes catalyze the conversion of C16:0-ACP into C18:0-ACP (Harwood and Stumpf, 1971). Acyl chain elongation is terminated by thioesterases; C16 and C18 fatty acids may subsequently undergo desaturation reactions catalyzed by desaturases in the plastid membrane and the endoplasmic reticulum in higher plants (Wu et al., 2013). Many storage oils in plant seeds are enriched in triacylglycerols incorporating polyunsaturated fatty acids formed in this way.

The functions of *KASI* genes have been characterized in several plants. The *kasI* mutant in *Arabidopsis* produces seeds with significantly lower oil content than wild-type plants (Wu and Xue, 2010). Seedlings of the *AtkasI* mutant exhibit semidwarfism, with chlorotic and curly rosette leaves whose chloroplasts are larger than those in wild-type plants but fewer in number. The *AtkasI* mutant also exhibits significantly reduced fertility; many of its seeds are shriveled and cannot develop into normal seedlings (Wu and Xue, 2010). A *KASI* mutation in rice (*OskasI*) also exhibited reduced seed fertility and FA contents (Ding et al., 2015). Additionally, the roots of the *OskasI* mutant were significantly shorter than wild type (Ding et al., 2015). Two KASI homologs, *NtKASI-1* and *NtKASI-2*, were isolated from tobacco. Double silencing of both homologs by RNAi produced plants with mildly

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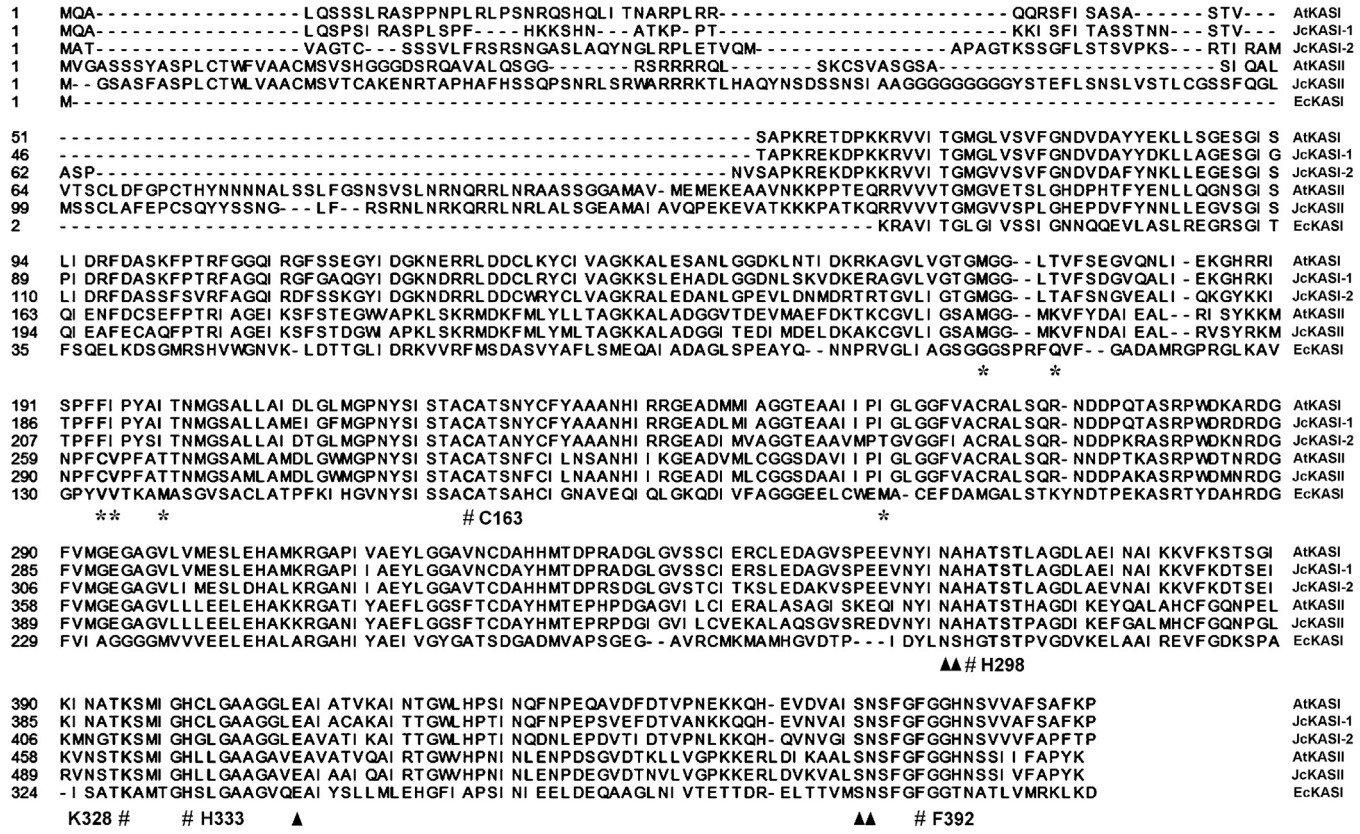

Fig. 1. Sequence alignments of JcKASI and KASI proteins from other organisms, together with KASII proteins from *Arabidopsis* and *J. curcas*. Active site residues are shown with a grey background and labeled with letters. ▲ indicates residues of the cation site; # indicates active site residues; *indicates residues in the substrate-binding pocket residues.Accession numbers of the proteins in the alignment: *Arabidopsis thaliana* (AtKASI, At5G46290; AtKASII, At1G74960), *Escherichia coli* (EcKASI, WP_024224009), *Jatropha curcas* (JcKASI-1, KDP37953; JcKASI-2, KDP25270; JcKASII, KDP33964).

variegated leaves during the early stage of development. As their development progressed, the double-silenced plants exhibited more serious growth defects, such as loss of apical dominance, highly stunted stems, and curled leaves with few chloroplasts. Plants in which only *NtKASI-1* was silenced exhibited mildly variegated leaves and stunted stems relative to the wild-type plants. Conversely, silencing of *NtKASI-2* alone had no apparent effect on growth and development, and later observation showed that plants overexpressing *NtKASI-2* had chlorotic leaves with fewer chloroplasts than the wild type, like those of plants in which *NtKASI-1* or both *NtKASI-1* and *NtKASI-2* were silenced (Yang et al., 2016).

Jatropha curcas is an important oil-rich plant of the Euphorbiaceae family (Fairless, 2007). Several lipid metabolism related genes from *J. curcas* have been cloned and characterized (Li et al., 2008; Wu et al., 2009; Wei et al., 2012; Wu et al., 2013; Kim et al., 2014). Here we report the isolation of two *KASI* genes from *J. curcas*, designated as *JcKASI-1* and *JcKASI-2*, which were both expressed strongly in filling stage seeds. To elucidate their functions, we expressed them in an *Arabidopsis kasI* mutant, revealing that *JcKASI-1* expression completely recovered the mutant's phenotypes, whereas *JcKASI-2* expression partially complemented the *Arabidopsis kasI* mutant.

2. Materials and methods

2.1. Plant materials

J. curcas trees were grown in a natural environment (N23°11′, E113°21′, a humid subtropical climate) in the South China Botanical Garden from seeds collected from trees growing naturally in Guizhou Province, China. Different tissues and seeds at various stages of development were harvested from five-year-old trees in the autumn. *A. thaliana* (Columbia) plants were germinated and grown at 22 °C under 16 h light/8 h dark photoperiods.

2.2. Sequence alignments and phylogenetic analysis

Selected KASI sequences from *Glycine max*, *Oryza sativa*, and *Zea mays* were downloaded from Phytozome (https://phytozome.jgi.doe.gov/) for comparative and bioinformatic analyses. KAS protein sequences from *Arabidopsis*, *Escherichia coli*, *J. curcas*, *Nicotiana tabacum*, *Ricinus communis*, and *Vitis vinifera* were downloaded from the NCBI (http://www.ncbi.nlm.nih.gov). Sequence alignments were generated with Clustal_X (Thompson et al., 1997). A phylogenetic tree was built by the neighbor-joining method with MEGA 5.05 (Tamura et al., 2011), using 1000 bootstrap replicates. A 60% bootstrap cutoff was applied.

2.3. RNA isolation

Total RNA was extracted from roots, leaves, and flowers of *J. curcas* using the modified CTAB method (Xiong et al., 2013). Total RNA from stems was extracted by a modified SDS method (Ainsworth, 1994). In brief, stem cortices were ground with liquid nitrogen and then transferred to tubes containing 600 μL of extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 500 mM NaCl, 1% SDS). The