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Molecular cloning and characterisation of an acyl carrier protein thioesterase gene (*CocoFatB1*) expressed in the endosperm of coconut (*Cocos nucifera*) and its heterologous expression in *Nicotiana tabacum* to engineer the accumulation of different fatty acids

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Abstract. Coconut (*Cocos nucifera* L.) contains large amounts of medium chain fatty acids, which mostly recognise acyl-acyl carrier protein (ACP) thioesterases that hydrolyse acyl-ACP into free fatty acids to terminate acyl chain elongation during fatty acid biosynthesis. A full-length cDNA of an acyl-ACP thioesterase, designated *CocoFatB1*, was isolated from cDNA libraries prepared from coconut endosperm during fruit development. The gene contained an open reading frame of 1254 bp, encoding a 417-amino acid protein. The amino acid sequence of the CocoFatB1 protein showed 100% and 95% sequence similarity to CnFatB1 and oil palm (*Elaeis guineensis* Jacq.) acyl-ACP thioesterases, respectively. Real-time fluorescent quantitative PCR analysis indicated that the *CocoFatB1* transcript was most abundant in the endosperm from 8-month-old coconuts; the leaves and endosperm from 15-month-old coconuts had ~80% and ~10% of this level. The *CocoFatB1* coding region was overexpressed in tobacco (*Nicotiana tabacum* L.) under the control of the seed-specific napin promoter following *Agrobacterium tumefaciens*-mediated transformation. *CocoFatB1* transcript expression varied 20-fold between different transgenic plants, with 21 plants exhibiting detectable levels of *CocoFatB1* expression. Analysis of the fatty acid composition of transgenic tobacco seeds showed that the levels of myristic acid (14 : 0), palmitic acid (16 : 0) and stearic acid (18 : 0) were increased by 25%, 34% and 17%, respectively, compared with untransformed plants. These results indicated that *CocoFatB1* acts specifically on 14 : 0-ACP, 16 : 0-ACP and 18 : 0-ACP, and can increase medium chain saturated fatty acids. The gene may valuable for engineering fatty acid metabolism in crop improvement programmes.

Additional keywords: medium chain fatty acids, myristic acid, palmitic acid, stearic acid, transgenic tobacco.

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Introduction

Coconut (*Cocos nucifera* L.), a member of the monocotyledonous family Aracaceae (Palmaceace), is an important oil-yielding plantation crop that is of considerable economic and social importance in the tropics (Rivera *et al.* 1999; Samsudeen *et al.* 2006). The endosperm tissue of coconut stores a substantial amount of oil, which has been used extensively for human consumption and other purposes all over the world. In physicochemical terms, coconut oil differs from other vegetable oils in that it is rich in saturated oil (≈93%), with a high percentage of medium chain fatty acids (MCFA) (≈60%), especially lauric acid (12 : 0) (≈50%) (Ceniza *et al.* 1991; Bhatnagar *et al.* 2009). Compared with long-chain triacylglycerols, medium-chain triacylglycerols that contain caprylic acid (8 : 0), capric acid (10 : 0), lauric acid (12 : 0) and myristic acid (14 : 0) are more soluble in water and have a lower melting point, enabling them to be absorbed and metabolised faster (Jeukendrup and Sarah 2004; Beermann *et al.* 2007). In addition, the unique antiviral, antibacterial and antiprotozoal properties of medium-chain triacylglycerols have found applications in the food industry (Enig 1998), and MCT oils have been used therapeutically since the 1950s. The use of MCT oils as part of a ketogenic diet to treat epilepsy, premature infants and patients infected with human immunodeficiency virus, as well as to prevent fat malabsorption in cystic fibrosis patients, is widely accepted (Ramírez *et al.* 2001; Beermann *et al.* 2007). The potential industrial and medical applications of uncommon seed oils have resulted in rapid advances in efforts to bioengineer their accumulation.

The mechanisms by which coconut endosperm accumulates unusual fatty acids are still unknown. Fatty acid biosynthesis in higher plants occurs predominantly in the plastids by a *de novo* iterative 'polymerisation' process, which is commonly primed with the acetyl moiety from acetyl-CoA and proceeds via iterative chain extension through reaction with malonyl- acyl carrier protein (ACP). The synthesis of 16- and 18-carbon (C16 and C18) fatty acids is terminated by the acyl-ACP thioesterase, which catalyses acyl-ACP thioester bond hydrolysis, the terminal reaction of fatty acid biosynthesis that releases a free fatty acid and ACP (Voelker 1996; Jing *et al*. 2011). Therefore, the specificities of thioesterases largely determine the chain lengths of most plant fatty acids (Stumpf 1987).

Acyl-ACP thioesterases are nuclear-encoded, plastid-targeted globular proteins (Voelker *et al*. 1992). Based on amino acid sequence alignments, these enzymes have been functionally characterised and classified into two general families, termed FatA and FatB (Jones *et al*. 1995). All FatAs are orthologous in different species, with the highest activities towards oleoyl–ACP ($18:1^{\triangle 9}$-ACP) (Hitr and Yadav 1992; Sánchez-García *et al*. 2010). In contrast with the high level of conservation in the specificity of FatAs, FatBs primarily hydrolyse saturated acyl-ACPs with chain lengths that contain between 8 and 18 carbons (Voelker and Davies 1994; Jones *et al*. 1995; Jha *et al*. 2010). The first *FatB* gene was isolated from the developing seeds of California Bay Tree (*Umbellularia californica*), and the strong preference of the enzyme for 12 : 0-ACP was verified in *Arabidopsis thaliana* (L.) Heynh. (Davies *et al*. 1991; Voelker *et al*. 1992). This work demonstrated, for the first time, the role of FatB in determing the chain lengths of fatty acids, which spurred efforts to isolate similar enzymes from other plant species with unusual fatty acid phenotypes. Such enzymes included the MCFA-specific thioesterases from *Cuphea* (Dehesh *et al*. 1996; Leonard *et al*. 1997), American elm (*Ulmus americana* L.; Voelker *et al*. 1997) and coconut (Jing *et al*. 2011). Because of the potential applications of special seed oils, several studies have focussed on engineering plant thioesterases with medium-chain-specificities. Three acyl-ACP thioesterases (*CnFatB1* (JF338903), *CnFatB2* (JF338904), *CnFatB3* (JF338905)) from coconut have been isolated and characterised (Jing *et al*. 2011). However, their *in vivo* activities and substrate specificities were only shown in *Escherichia coli*, with no function analyses performed using plants. In the present work, a full-length cDNA of an acyl-ACP thioesterase (*CocoFatB1*: JX275886) was isolated from cDNA libraries prepared from coconut endosperm during fruit development (Li and Fan 2009). The *CocoFatB1* gene was heterologously expressed in transgenic tobacco (*Nicotiana tabacum* L.) under the control of the seed-specific napin promoter (Kridl *et al*. 1991; Zheng *et al*. 2007). Our results provide new insights into the function of *CocoFatB1* and how it might be used to impact on the composition of plant oils.

Materials and methods

Plant materials

Coconut (*Cocos nucifera* L.) fruits (i.e. coconuts), including immature coconuts (8 months old) and ripe coconuts (15 months old), and coconut leaves were obtained from the Coconut Research Institute, Chinese Agricultural Academy of Tropical Crops, Hainan, China. The fruits and leaves were harvested at random, and endosperm tissues were physically isolated and immediately frozen in liquid nitrogen to form three sample pools. The samples were then analysed in duplicate for RNA concentration, cDNA synthesis, gene amplification and differential expression analysis. *Escherichia coli* strain DH5α (Clontech Palo Alto, CA, USA), which was grown in Lysogeny broth (LB) medium (Sangon, Shanghai, China) supplemented with 50 mg L^{-1} ampicilin or kanamycin at 37°C,was used for bacterial cloning. The *pCAMBIA1300S* vector was donated by Yongjun Lin (Professor of Huazhong Agricultural University). All chemicals, endonucleases and other required enzymes were obtained from Sigma (St. Louis, MO, USA) or TaKaRa (Dalian, China), unless otherwise stated.

RNA extraction and cDNA library construction

Total RNA from the pulp of coconuts was extracted using the cetyltrimethylammonium bromide-based methods described by Li and Fan (2007). The quantity and quality of isolated total RNA was examined using spectrophotometry and gel electrophoresis, respectively. Construction of the cDNA library prepared from RNA isolated from coconut endosperm have been reported previously (Li and Fan 2009).

Cloning of the CocoFatB1 gene and bioinformatic analysis

EST sequence information was obtained for 1000 clones that were randomly selected from the cDNA library. All clones were sequenced by Oebiotech Co. (Shanghai, China). A homology search was conducted based on BLAST searches using the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov/BLAST). Among the EST sequences obtained from the library, a partial clone was discovered as being 91% identical to the palmitoyl-acyl carrier protein thioesterase gene from oil palm (GenBank accession: AF147879.2). Complete sequencing of the clone was performed using the primers M13+ (5′-GTAAAACGACGGCCAGT-3′) and M13– (5′-AACAGCTATGACCATGTTCA-3′) followed by primer-walking until complete overlapping sequence data were obtained from both sides.

Fluorescence quantitative reverse transcription–PCR analysis

Total RNA from coconut leaves and endosperm tissues were isolated separately from immature coconuts (8 months old) and ripe coconuts (15 months old). First-strand cDNA was synthesised from 2 µg of total RNA using the TIANScript OneStep RT-PCR Kit (TIANGEN, Beijing, China). Reverse transcription was performed at 42°C for 60 min, with a final denaturation at 70°C for 15 min. The cDNA was then subjected to real-time fluorescent quantitative reverse transcription–PCR (RT-PCR) using standard methods (Marone *et al*. 2001). The RT-PCR primers for *CocoFatB1* were designed using the Primer3 program based on the cDNA sequence. The β-actin gene was used as an internal control for expression. The primers used in this study were:

RTActin-F: 5′-TTACTCTGAAATACCCCATTGAGC-3′,
RTActin-R: 5′-CTCTCTGTTAGCCTTGGGGTTG-3′,

RTFatBF: 5′-ACACTTCTTGATTGGAAACCACG-3′, and *RTFatBR*: 5′-GCGTTTCTATAGAAGCCGTCC-3′.

The RT-PCR amplification step was performed using the SYBR Premix Ex Taq II (TaKaRa) and a RT-PCR detector (TaKaRa Smart Cycler II system) by using the SYBR Green I chimeric fluorescence method according to the manufacturer's instructions. Expression was quantified in terms of comparative threshold cycle (Ct) using the $2^{-\triangle\triangle Ct}$ method, and the results were expressed as the binary logarithm of the relative quantity of the transcript used to normalise gene expression (Livak and Schmittgen 2001). Reactions were performed in triplicate, including the 'no template' and 'no reverse transcriptase' controls, and were monitored using an Applied Biosystems (Foster City, CA, USA) 7500 RT-PCR instrumentation outfitted with SDS software ver. 1.3.1 (Applied Biosystems).

Construction of expression vectors

The *CocoFatB1* coding sequence was cloned from cDNA prepared from coconut endosperm, and *Kpn*I and *Bam*HI sites were added at the 5′ and 3′ ends, respectively. The primers used for the PCR amplification were: *FatBF* 5′-TATGGTACCATGG TTGCTTCAGTTGCCGCTT-3′ (forward) and *FatBR* 5′-TAT GGATCCTCAAGCACTTCCAGCTGAAGTGG-3′ (reverse). The conditions for PCR amplification were 94°C for 3 min, 30 cycles of 94°C for 45 s, 47°C for 45 s, 72°C for 1 min and extension at 72°C for 8 min. The PCR product was cloned into trhe *pMD18-T* vector (TaKaRa) and the recombinant plasmid was transformed into the *Escherichia coli* strain DH5α. To generate the plant overexpression construct, the coding region of *CocoFatB1* was subcloned into the binary *pCAMBIA1300S* vector (*Kpn*I or *Bam*HI site) under the control of the seed-specific napin promoter (Kridl *et al*. 1991; Fig. 1).

Transformation, selection and regeneration of Nicotiana tabacum

The *CocoFatB1* gene in the *pCAMBIA1300S* expression vector was transformed into *Agrobacterium tumefaciens* LBA4404/ EHA105 by electroporation, as described by Hoekema *et al*. (1983). Young leaves from wild-type tobacco (*Nicotiana tabacum* L.) were cut into small square discs ($0.5 \times 0.5\ cm^2$) and immersed in 10× diluted cultures of *Agrobacterium tumefaciens* for 15 min. The leaf discs were first transferred to sterile filter paper to remove excess *Agrobacterium*, and then transferred onto solidified cocultivation MS medium (Sigma; Murashige and Skoog 1962). After cocultivation, the transformants were selected on MS medium containing 500 mg L^{-1} carbenicillin (Sigma) and 10 mg L^{-1} hygromycin B (Sigma). Regenerated tobacco plants propagated *in vitro* were transferred to soil and grown to maturity in a greenhouse in a 16-h-light; 8-h-darkness photoperiod with a PPFD of 200–900 µmol m^{-2} s^{-1}. Positive transformants were identified by PCR screening of genomic DNA. Primary transformants were self-fertilised and the seeds were collected.

Analysis of CocoFatB1 *expression in transgenic tobacco using fluorescence quantitative RT-PCR*

To detect the introduced transgene with PCR in the putative regenerated transgenic plants, DNA samples were extracted from leaf tissue using the DNAeasy Mini-kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The *FatBF–FatBR* primer pair was used for PCR. The PCR conditions were identical to those described above, and the PCR products were then electrophoresed on a 1.0% (w/v) agarose gel and visualised under ultraviolet light. Positive transformants that yielded a single PCR product (~1200 bp) were selected for future investigation.

Total RNA from each of the mature tobacco seeds was isolated as described above. First-strand cDNA synthesis and fluorescent quantitative RT-PCR were carried out as described above using the *RTFatB-F* (5′-GTAGCCAAACCCACCTCT-3′) and *RTFatB-R* (5′-TTTCAGCCCAACCTTCG-3′) primers, which were used to detect the expression level of *CocoFatB1* in the seeds of transgenic plants. Transcripts of the *18S* gene, which was used as an internal control for expression, were amplified using the *RT18S-F* (5′-GCAACAAACCCCGACTTCT-3′) and *RT18S-R* (5′-GCGATCCGTCGAGTTATCAT-3′) primers.

Fatty acid methyl ester analysis by GC

Total lipids were extracted in triplicate using dichloromethane : methanol (2 : 1) from mature seeds from single plantlets of the transgenic and wild-type tobacco plants. The fatty acid methyl esters were recovered using *N*-hexane. Analysis of fatty acid methyl esters was performed using GC, with methyl heptadecanoate (17 : 0) (Sigma) as an internal standard. All GC analysis was performed using a HP5890 GC instrument equipped with a BPX-70 (30 m × 0.25 mm) chromatography column (SGE, Melbourne, Vic., Australia). The initial column temperature (90°C) was held for 10 min and then raised at 4°C min^{-1} until it reached 240°C, after which it was held at this temperature for another 10 min (Beermann *et al*. 2007; Chi *et al*. 2011).

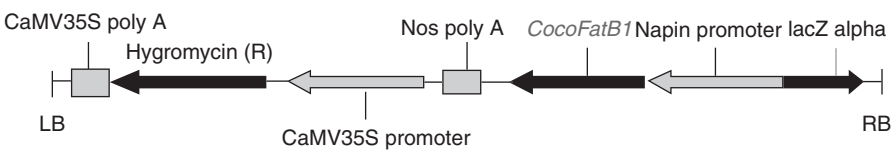

Fig. 1. The T-DNA region of the construct used to transform *Nicotiana tabacum* plants. Sequences of functional importance are the left border (LB), the right border (RB), cauliflower mosaic virus 35S (*CaMV35S*) promoter and polyA addition sequences and the nopaline synthase (Nos) polyA addition sequence. The seed-specific expression cassette consists of a napin promoter fragment and *CocoFatB1* from coconut.

Statistical analysis

All experiments were performed in triplicate, and the data provided are means ± s.d. Intergroup comparisons between the two tested groups were performed using a paired *t*-test, using Statview ver. 6.0 software (SAS Institute, Cary, NC, USA) and Microsoft Office Excel ver. 2007 (Microsoft Corporation, Richmond, WA, USA). A *P*-value of <0.05 was regarded as indicating a statistically significant difference.

Results

Complementary DNA cloning and conservation analysis of the CocoFatB1 *sequence*

The full-length cDNA sequence (1858 bp) of an acyl-ACP thioesterase was isolated (termed *CocoFatB1;* GenBank accession: JX275886) from total RNA from the coconut endosperm using EST sequences and RT-PCR. Sequence analysis revealed that the *CocoFatB1* sequence was homologous to other acyl-ACP thioesterases and the predicted protein has similar properties to previously identified orthologues and is homologous across its entire length. CocoFatB1 has 73% amino acid identity with the rice enzyme (Os06 g0143400).

Expression of CocoFatB1 *in different tissues and at different development stages*

To reveal the expression of *CocoFatB1* genes during the development of coconut pulp, fluorescence quantitative RT-PCR was used to analyse *CocoFatB1* expression during two different developmental stages: 8-month-old and 15-month-old coconut fruits, with the abundance of β-actin transcripts providing an internal control. The *CocoFatB1* transcript was most abundant in the endosperm from 8-month-old coconuts, whereas the leaves and endosperm from 15-month-old coconuts had ~80% and ~10% of this level.

Generation of transgenic tobacco plants expressing the CocoFatB1 *gene under the control of the napin promoter*

To further determine the function of *CocoFatB1* and to establish whether its expression can change the fatty acid profile in plants, we investigated the effects of *CocoFatB1* expression in transgenic tobacco. Analysis of the transgenic plants' genomic DNA using PCR indicated the presence of the *CocoFatB1* coding sequence in the tobacco genome. Following transformation and selection in the presence of hygromycin, 32 independent transgenic plants were obtained. *CocoFatB1* transcript expression varied 20-fold between different transgenic plants, with 21 plants exhibiting detectable levels of *CocoFatB1* expression (Fig. 2). Four transformant lines (7, 8, 9 and 2) that showed different levels of *CocoFatB1* transcript were selected for further analysis.

Analysis of fatty acid composition

As an acyl-ACP thioesterase, CocoFatB1 was expected to increase the medium chain saturated fatty acid composition of tissues in which it is expressed. To further confirm its function *in vivo*, the fatty acid composition of transgenic and untransformed tobacco plants were determined and compared. Expression of transgenic CocoFatB1 in tobacco seed increased levels of myristic acid (14 : 0), palmitic acid (16 : 0) and stearic acid (18 : 0) by 10.2%, 3.9%, 8.4% and 4.3% in Plants 7, 8, 11 and 15 respectively. Meanwhile, there was no obvious difference in the levels of other fatty acids when the seeds of transgenic and untransformed tobacco plants were compared (Table 1).

Discussion

Plant fatty acids are synthesised in the stroma of the plastids of both leaves and developing seeds (Weaire and Kekwick 1975;

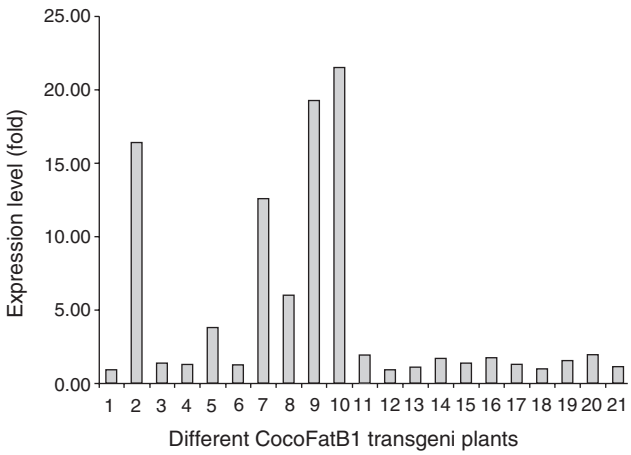

Fig. 2. Expression levels of the *CocoFatB1* gene in *CocoFatB1* transgenic plants. Expression is quantitated as the fold increase compared with plants expressing the lowest *CocoFatB1*.

Table 1. Comparison of the fatty acid composition of untransformed and transgenic tobacco seed oil

Mature seeds from single plants were used for the analysis and extracted in triplicate. Data are means from three measurements, with s.d. Asterisks indicate statistically significant differences compared with the control (Student's *t* test: *, $P<0.05$; **, $P<0.01$). 14 : 0, myristic acid; 14 : 1, myristoleic acid; 16 : 0, palmitic acid; 16 : 1, palmitoleic acid; 18 : 0, stearic acid; 18 : 1, oleic acid; 18 : 2, linoleic acid; 18 : 3, linolenic acid

Fatty acid	Untransformed tobacco	*CocoFatB1* transgenic line 7	8	9	2
14 : 0	0.08 ± 0.001	0.10 ± 0.002**	0.11 ± 0.008**	0.06 ± 0.006**	0.18 ± 0.005**
16 : 0	8.98 ± 0.003	18.15 ± 0.010**	12.32 ± 0.010**	16.89 ± 0.136**	12.06 ± 0.033**
16 : 1	0.12 ± 0.007	0.10 ± 0.096**	0.09 ± 0.003**	0.13 ± 0.004	0.24 ± 0.008**
18 : 0	2.85 ± 0.013	3.84 ± 0.0048**	3.37 ± 0.108**	3.36 ± 0.148**	3.98 ± 0.005**
18 : 1	12.53 ± 0.025	12.40 ± 0.125	10.9 ± 0.010**	11.89 ± 0.231*	11.16 ± 0.011**
18 : 2	69.97 ± 0.066	62.53 ± 0.007**	68.20 ± 0.055**	64.77 ± 0.004**	67.66 ± 0.012**
18 : 3	0.95 ± 0.005	1.22 ± 0.008**	1.32 ± 0.043**	1.08 ± 0.070*	1.90 ± 0.075**

Ohlrogge *et al*. 1979). Accordingly, *CocoFatB1* transcripts are detected not only in the endosperm, but also in the leaves of coconut plants. Thioesterases play a pivotal role in fatty acid synthesis owing to their role in catalysing the terminal reaction of fatty acid biosynthesis, which regulates the fatty acid composition of storage lipids, especially in plant seeds (Brown *et al*. 2010; Jing *et al*. 2011). The expression of thioesterase genes displayed the highest levels in expanding tissues that are typically very active in lipid biosynthesis, such as developing seed endosperm and young expanding leaves (Oo and Stumpf 1979; Sánchez-García *et al*. 2010).

To confirm the activity and substrate specificity of CocoFatB1 in plants, we analysed the effects of its expression in transgenic tobacco. This result indicated that *CocoFatB1* showed specificity towards 14 : 0-ACP, 16 : 0-ACP and 18 : 0-ACP. Compared with the results of function analysis of *CnFatB1*, *CocoFatB1* is specific not only towards 14 : 0-ACP and 16 : 0-ACP, which have been demonstrated in *E. coli* by Jing *et al*. (2011), but also showed specificity to 18 : 0-ACP in plant. These results are similar to those previously reported for *FatB* thioesterases from other plants, such as *Elaeis guineensis* (Othman *et al*. 2000), *Jatropha curcas* L. (Wu *et al*. 2009), *Cuphea hookeriana* (Jones *et al*. 1995), *Diploknema (Madhuca) butyracea* (Jha *et al*. 2006) and Indian mustard (*Brassica juncea* L. Czern.; Jha *et al*. 2010). All of these enzymes displayed a high level of activity towards 16 : 0-ACP; *BjFatBs* from *B. juncea* were also specific to 18 : 0-ACP. Moreover, like other *FatB* thioesterases, which preferably hydrolyse acyl-ACPs with saturated fatty acid chains (Jones *et al*. 1995), *CocoFatB1* showed a preference for saturated acyl-ACPs, especially palmitoyl-ACP.

However, compared with some *FatBs* from other plants that contain rich MCFAs, *CocoFatB1* still displayed some unique characteristics. The *FatBs* from *U. californica* (Pollard *et al*. 1991; Voelker *et al*. 1992), *A. thaliana* (Dormann *et al*. 1995), *U. americana* (Voelker *et al*. 1997) and nutmeg (*Myristica fragrans*; Voelker *et al*. 1997) are specific for 12 : 0-ACP and play a critical role in MCFA production. Unlike these *FatB*s, *CocoFatB1* shows a preference for 14 : 0-ACP, 16 : 0-ACP and 18 : 0-ACP. Recently, *CnFatB3* has been demonstrated to be specific for 12 : 0-ACP, 14 : 0-ACP and 14 : 1-ACP in *E. coli* (Jing *et al*. 2011), which may make a great contribution to fatty acid profiles containing abundant MCFAs in coconut endosperm. More functional analysis is needed to confirm the characterisation of *CnFatB3*, especially in plants. Meanwhile, some crucial enzymes involved in fatty acid synthesis may also play an important role in determining the lengths of fatty acid chains.

Although the specificities of thioesterases determine the chain length of most plant fatty acids to a large extent, the action of specific β-ketoacyl-ACP synthases (KAS) and acyl-ACP acyltransferases shift the synthesis of fatty acids towards molecules with shorter chains (Davies *et al*. 1995; Leonard *et al*. 1998). Of three known classes of plant KAS enzymes, only KASI elongates substrates from 4 : 0-ACP to 14 : 0-ACP (Shimakata and Stumpf 1983). Seeds transformed with *CwKASA* and *CwFatB2* thioesterases in comparison with the seeds transformed with thioesterases only had greatly increased concentrations of 10 : 0 (capric acid) and 12 : 0 (lauric acid). Coexpression of *CwKASA* with California bay *FatB1* in transgenic canola (*Brassica napus* L.) increased amounts of 12 : 0 fatty acids when compared with expression of the *FatB1* only (Leonard *et al*. 1998). Additionally, expression of the *LPAAT* gene of coconut endosperm in *E. coli* and canola indicates that the enzyme displays a marked preference for the transfer of medium-chain CoAs to 12 : 0-lysophosphatidic acid relative to unsaturated long-chain substrates(Wiberg *et al*. 1997). Co-expressed with thioesterase, lysophosphatidyl acyltransferase (LPAAT) expression generated triacylglycerol (TAGs) with high levels of lauric acid (12 : 0) at *sn*-2 (Davies *et al*. 1995; Knutzon *et al*. 1999; Wiberg *et al*. 2000).

The aim of this study was to explore why coconut endosperm is so rich in MCFAs and to identify one of the genes responsible for this phenotype. The *CocoFatB1* gene we have isolated and characterised was highly effective in redirecting plant fatty acid synthases to palmitate and myristate production, and appears to be specific to 14 : 0-ACP, 16 : 0-ACP and 18 : 0-ACP. The ectopic expression of *C. nucifera CocoFatB1* in *N. tobaccum* increased the levels of saturated acids, including myristic acid, palmitic acid and stearic acid, to provide a fatty acid profile distinct from that of other oil crops that produce large amounts of MCFAs, such as *U. californica* (Pollard *et al*. 1991; Voelker *et al*. 1992). Based on the previous studies, more detailed research about the actions of the thioesterases, KAS and LPAAT from coconut endosperm is needed to confirm the function of these crucial enzymes in fatty acid synthesis. Co-expression of special thioesterases with KASI or LPAAT in plants should enable the effects of the various enzymes on fatty acid composition to be separated. The ability to engineer the accumulation of these MCFAs, especially lauric acid (12 : 0), will be beneficial in efforts to improve crops by engineering fatty acid metabolism.

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