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Characteristics and Molecular Genetics of Lipid Biosynthesis in Tea-oil Tree Seed

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ABSTRACT

Tea-oil tree (*Camellia oleifera*), whose seed has an oil-yield rate of about 55%, is an edible oil tree growing specifically in China. Recent research has suggested that the oiliness of tea-oil is far better than that of palm, rape, bean and peanut oil, and could even exceed that of olive oil. More than 90% of tea-oil consists of unsaturated fatty acids including 82% oleic acid, and 8.6% of both linoleic acid and linolenic acid. They are very healthy for humans. Moreover, tea-oil is abundant in vitamins A and E, and lacks the unhealthy compounds sinapic acid and falvacin. Tea-oil exists in the form of oil bodies in seed, and the oleosins and their number determine the quantity and characteristics of the oil product. The biosynthesis process of tea-oil is very complicated and involved many proteins and enzymes. The dynamic of synthesis and the concentration of malonyl coenzyme A decide how soon the saturated fatty acids are made in *C. oleifera* seed. The key enzymes in the monounsaturated fatty acid formation pathway are stearoyl-ACP desaturase (SAD), which controls the quantity of unsaturated fatty acids, while the fatty acid desaturases (FADs) are important for the synthesis of polyunsaturated fatty acids in *C. oleifera* seeds. ESTs of many genes relative to the biosynthesis of fatty acids in *C. oleifera* seed have been identified. However, the acquirement of the complete genetic information of all genes in the fatty acid pathway is essential for gene functional studies with approaches such as prokaryotic overexpression, transgenic plants or RNA interference (RNAi). These will eventually lead to an understanding of the molecular genetics of lipid biosynthesis in *C. oleifera*.

Keywords: *Camellia oleifera*, fatty acids, lipid synthesis, regulatory genes

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INTRODUCTION

As a world-famous edible oil tree, tea-oil tree (*Camellia oleifera*) is regarded as one of the four primary edible oil tree plants together with oil palm, olive, and coconut. *C. oleifera* originated in China and has developed into the main non-wood forest tree distributed (3.5 million hm^2) mainly south of the Yangtze River valley, especially in Hunan and Jiangxi provinces. *C. oleifera* has been the main tree of forestation in the red-soil upland area of south China for its wide adaptability and endurance to the arid and barren environments (Lei *et al*. 2003).

The oil obtained from *C. oleifera* seeds is named tea-oil, which is the elite edible oil in Asia and profoundly favored by people, especially Chinese and Japanese, and is characterized by its unique flavor, durable storage, and ease of absorption into the body. Recent research has suggested that the oiliness of tea-oil, especially from China as the Chinese specialty, might exceed that of olive oil. It was found that at least 90% of tea-oil is unsaturated fatty acids including oleic, linoleic and linolenic acid, which are very healthy for humans, and tea-oil does not contain erucic acid unhealthy to the body (Hu *et al*. 2006). Besides its high edible merit, tea-oil possesses good medicinal characteristics such as preventing cardiovascular cirrhosis, reducing cholesterol, and decreasing blood pressure (Xiao *et al*. 2006). Other uses are advances in cosmetics and raw materials for the spice industry by deep processing (Wu *et al*. 2005). As a byproduct, *C. oleifera* cake (dregs of tea-oil tree seeds) can be used to distill the residual oil, to extract tea saponin, and to produce polishing powder that enormously increase the economic benefit of comprehensive utilization of *C. oleifera* seed. Therefore, *C. oleifera* is considered as the Oriental olive.

WHERE AND HOW WE CAN GET *C. OLEIFERA* SEEDS?

To quickly collect high-quality tea-oil without chemical residuals from *C. oleifera* seed for research purposes, three methods including CO_2-based supercritical fluid extraction (SFE) (Zhong *et al*. 2001), ultrasonic extraction (UE) or microwave-assisted extraction (MAE) (Zeng *et al*. 2005)

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can be employed regardless of their low output. However, it would be difficult to get high yield of tea-oil with great quality (Chen *et al*. 2005). A conventional industry process (Cao *et al*. 2002) that combines physical compression with vegetable fiber filtration does not need to add any chemical and thus maintains the abundant nutrients intact, but the rate of oil-yield is still unsatisfactory. Another industrial approach to isolate tea-oil is the leaching process that starts with a soaking of the seeds with impregnants such as methanol followed by six steps including degreasing, degumming, dehydrating, decolorizing, Deodorizing, and deacidification (Qiang *et al*. 2005). Organic impregnant contamination is a big problem in the leaching process although this method gives high tea-oil yield. An alternative process integrating the two methods has been widely accepted. The *C. oleifera* seeds are first compressed physically to obtain most (80~90%) tea-oil, the leaching process is then used to extract residual oil with recovering percentage of 50~80% (Pan *et al*. 2006). Beside being a source of tea-oil, *C. oleifera* seed can also be processed industrially to produce oleic acid and relative esters by extracting (Hu *et al*. 2006), to manufacture soap and Vaseline by hydrogenation to form stearin (He *et al*. 2004), or even to prepare stearic acid and glycerol by extreme hydrogenation (Hui *et al*. 2006).

After extracting tea-oil from *C. oleifera* seeds, the solid residue, also termed the *C. oleifera* dreg cake, still has great value of comprehensive utilization (Yoshida *et al*. 1994). It contains considerable residual oil (5~10%), abundant amyloses (33.90%), proteins (13.03%), crude fiber (12.50%) and tea saponin (24.06%) (Deng *et al*. 2005). Tea saponin is widely applied to produce shampoo, scour, food additives, clarifiers and epispastics. Generally, the seeds from 0.1 hm^{-2} of *C. oleifera* forest can yield 50 kg tea-oil and one ton of *C. oleifera* dreg cake that can then be used to produce 90 kg tea saponin. Zhang *et al.* (2003) reported that more than 80% of the residual oil can be extracted by impregnant. After the removal of saponin, the remaining proteins and amyloses (40~50%) in *C. oleifera* dreg cake can be developed into vegetable feeds (Hu *et al*. 2006). With its peculiar granular structure, the dreg cake can also be used to produce polishing powder for high-grade lathe (Deng *et al*. 2005). Finally, the *C. oleifera* dreg cake may serve as an organic manure for its considerable amount of nitrogen (1.99%), phosphorus (0.54) and potassium (2.33%) (Zhang *et al*. 2003).

COMPONENTS IN TEA-OIL AND THEIR UTILIZATION

Tea-oil as an elite edible oil contains not only more than 90% unsaturated fatty acids but also vitamins A (5.1 mg/100 g) and E (20.3 mg/100 g). Unlike other vegetable oils, tea-oil lacks harmful erucic acid, gossypol and aflatoxin (Luo *et al*. 2003), as well as behenic acid which is difficult to be adsorbed and digested (Li *et al*. 2005). In the tea-oil from the seeds of some special tea-oil tree cultivars such as 'XiangLin-16', the quantity of vitamin E can reach 49.1 mg/100 g (Hu *et al*. 2006). Even after heating at 100°C for 1 hour or stored for a year, the tea-oil still holds 44.2 mg/100 g or 14.5 mg/100 g of vitamin E and D, respectively (Zen *et al*. 2005). About 82% oleic acid and 8.6% linoleic and linolenic acids account for a total content of more than 90% unsaturated fatty acids in the tea-oil. This number is higher than that of the other three edible oil trees, e.g. oil palm, olive and coconut (**Table 1**).

Compared to other plant oils, tea-oil has similar physiccal and chemical properties as olive oil including a high smoking point of 220°C, a low freezing point below 0°C, good stability at high temperatures, even up to 150°C, as well as resistance to oxidization due to its low iodine value contributed by the univalent unsaturated oleic acid (Zhong *et al*. 2006). All these allow the tea-oil to be labeled as a liquid with less cooking fume, no harmful substances such as benzopyrene derived from oil degradation, and long-term storage (Lee *et al*. 2006).

The proportion of linoleic acid in most daily edible lipids is either overly high or overly low (Li *et al*. 2005). Tea-oil possesses an appropriate percentage of linoleic acid that meets the daily dose necessity for patients suffering cardiopathy, vascular sclerosis and hypertension (Wu *et al*. 2006). Tea-oil can also provide hepatoprotection against CCl(4)-induced oxidative damage in rats (Lee *et al*. 2007). Moreover, trace terpenoids in tea-oil can be used to make a faint scent (Wu *et al*. 2005). Compared to other plant oils, tea-oil contains much less tocopherol and behenic acid, which are difficult to be adsorbed and digested (Zhong *et al*. 2006).

LIPID FORMATION AND TRANSFORMATION IN *C. OLEIFERA* SEED

The first step of lipid formation (**Fig. 1**) is that acetyl-CoA is catalyzed into malonyl-CoA by acetyl-CoA carboxylase. Then malonyl-CoA is transformed into palmitic acid by six steps including priming, loading, condensation, reduction, dehydration, and second reduction. This process is catalyzed by fatty acid synthetase with ACP as the backbone. Palmitic acid is posteriorly transformed into stearic acid, which can be catalyzed into oleic acid by *C. oleifera* SAD during the maturation of seeds (Tan *et al*. 2005b). Oleic acid can be further transformed into linoleic and linolenic acid by FADs, and this process can be initiated at 70 days before maturation (**Table 2**). All fatty acids including stearic acid and unsaturated fatty acids are transformed into triglyceride, which is transferred into oil bodies for storage. Oleosin type and number determine the quantity and characteristics of oil bodies, and hence also directly determine the product of tea-oil.

Along with the maturation of fruits, the amount of lipids in the seeds increases while the acid value decreases. Thus, tea-oil from young *C. oleifera* seeds is generally of low quality and yield. Interestingly, the same pattern of change happens to the unsaturated and saturated fatty acids. With the deep maturing of *C. oleifera* seeds, the content of unsaturated fatty acids such as oleic acid and linoleic acid will increase, but the content of saturated fatty acids will de-

Table 1 Fatty acid composition of tea-oil and main edible oils.

Fatty acid	Tea-oil	Rapeseed oil	Peanut oil	Olive oil	Palm oil	Coconut oil
Caprylic acid (C8:0)						9.30
Decanoic acid (C10:0)						9.60
Lauric acid (C12:0)						39.40
Myristic acid (C14:0)					2.35	19.50
Palmitic acid (C16:0)	8.03	8.89	11.68	13.00	40.55	10.60
Stearic acid (C18:0)	1.05	3.19	3.69	1.90	4.85	29.00
Arachidic acid (C20:0)		1.69	0.68	1.65		
Sum of saturated fatty acid	9.08	13.77	16.00	16.55	47.75	91.30
Oleic acid (C18:1)	81.91	43.44	48.00	72.70	38.35	6.20
Linoleic acid (C18:2)	8.05	25.46	32.30	6.95	12.60	2.50
Linolenic acid (C18:3)	0.51	7.22	1.45	4.10	0.30	
Erucic acid (C22:1)		10.12				
Sum of unsaturated fatty acid	90.47	86.24	81.75	83.78	51.25	8.70

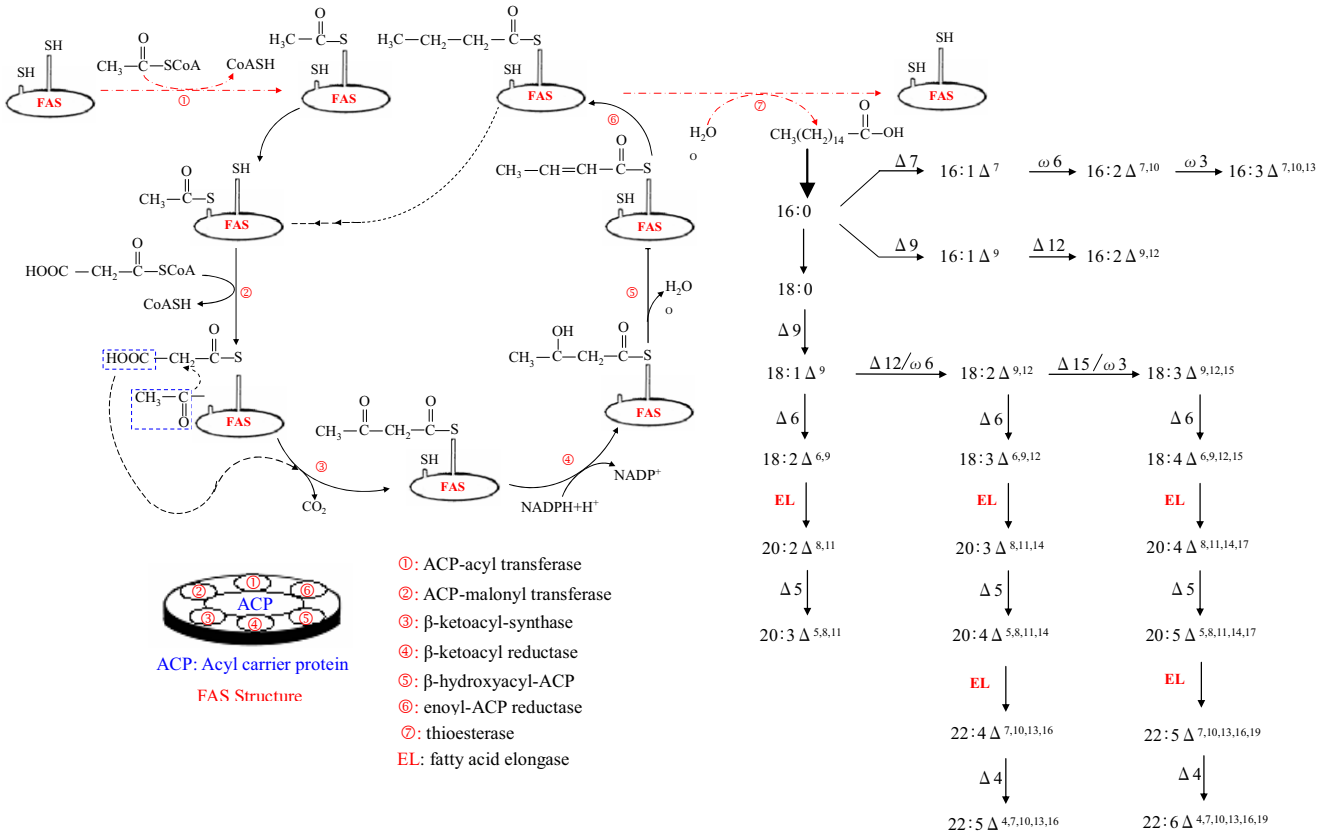

Fig. 1 Lipid biosynthetic pathway.

Table 2 Variation of compound content in seed of cultivar *C. oleifera* Abel during the picking period.

Date of picking	Kernel rate (%)	Oil content in kernel (%)	Acid value	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)
70 days before maturation	57.27	6.25	18.53	11.93	6.16	28.78	0.63
60 days before maturation	59.06	13.98	11.81	19.20	5.74	36.94	3.86
50 days before maturation	5940	17.63	7.52	17.72	5.25	40.02	14.93
40 days before maturation	58.61	29.06	5.45	11.23	4.02	54.00	11.13
30 days before maturation	60.00	39.12	3.03	16.72	2.73	69.29	9.31
20 days before maturation	61.60	51.07	2.44	8.52	3.18	70.76	12.33
10 days before maturation	61.05	53.71	1.70	11.05	2.89	73.85	19.31
Fully matured	62.07	59.99	1.16	7.13	2.71	75.49	24.81

crease as demonstrated in **Table 2**.

The reverse curves begin from the germination of the *C. oleifera* seed where the lipid content decreases gradually but where the acid value increase proportionally. The lipid in the seed during germination is firstly transformed into carbohydrates such as saccharide and amylum, serving as energy for seed germination and seedling growth (Hu *et al*. 2006). This implies that seed germination should be inhibited by dry preservation during storage to protect aganst the loss of tea-oil.

IDENTIFIED *C. OLEIFERA* GENES IN LIPID SYNTHESIS PATHWAY

Several cDNA libraries have been constructed with the mature seeds of cv. 'XiangLin-1', which is widely planted in China (Hu *et al*. 2004). To date, two thousand clones have been sequenced by one-passing from the 5′ terminal of cDNA clones (Tan *et al*. 2006). A search of public databases for ESTs suggested fourteen genes may be involved in the lipid biosynthesis pathway of *C. oleifera*. Among them, six are for saturated fatty acids or related genes, including those for acyl carrier protein (ACP), very-long-chain fatty acid condensing enzyme (very-long-chain FACE), palmitoyl-acyl carrier protein thioesterase (FatB1), acetyl-CoA acetyltransferase (ACAA), enoyl-CoA hydratase (ECAH) and propionyl-CoA synthase (PCAS), and two unsaturated fatty acids-related genes including stearoyl-ACP desaturase (SAD) and fatty acid desaturase (FAD) (Tan *et al*. 2004).

ACP gene. ACP originally controls the synthesis of long-chain saturated fatty acids by catalyzing palmitic acid (16:0) to stearic acid (18:0) or to very-long-chain saturated fatty acids (Millar *et al*. 1997). There are several ACP isoenzymes (ACP-1, ACP-2, ACP-3, etc.) that have been identified in different plants (Ohlrogge *et al*. 1985). Currently, one ACP gene sequence has been noticed in our *C. oleifera* ESTs databse (Tan *et al*. 2006) which shows more than 80% identity to the ACP genes of eight plants as demonstrated in **Table 3**.

Very-long-chain FACE gene. Very-long-chain FACE decides the acyl-chain length of long-chain fatty acid (Millar *et al*. 1998), and is the first one of four active enzymes termed as the microsome fatty acid extending enzymes that are associated with the biosynthesis of long-chain fatty acids. One sequence of the very-long-chain FACE gene from *C. oleifera* ESTs was found. The BLAST result revealed that it has 81% identity to its *A. thaliana* ortholog with a score of 123 and an e-value of 5e-25 (Zhang *et al*. 2006a). Given that very-long-chain FACE is expressed with a special pattern in different plants in the process of long-chain fatty acids biosynthesis (James *et al*. 1995), a complete picture of the gene function might be critical for understanding the molecular mechanism of long-chain fatty acid biosynthesis.

Table 3 BLAST results of the ACP gene.

Nucleic acid library	Accession Number	Species	Gene	Score	Expected Value	Identities
EMBL	Y10994	*Casuarina glauca*	ACP	170	2e-39	152/174 (87%)
GenBank	BT012821	*Lycopersicon esculentum*	ACP	109	5e-21	130/155 (83%)
GenBank	AF127796	*Capsicum chinens*	ACP	109	4e-21	130/155 (83%)
GenBank	AF428256	*Olea europaea*	ACP	99.6	5e-18	140/170 (82%)
EMBL	AJ584698	*Cicer arietinum*	ACP	91.7	1e-15	55/58 (94%)
GenBank	AF083950	*Coriandrum sativum*	ACP	89.7	5e-15	78/89 (87%)
EMBL	AJ001446	*Fragaria vesca*	ACP	83.8	3e-13	129/158 (81%)
GenBank	AY088071	*Arabidopsis thaliana*	ACP	71.9	1e-09	57/64 (89%)

Table 4 Identities of SAD genes between *Camellia oleifera* and others species.

Nucleic acid library	Accession Number	Species	Gene	Score	Expected Value	Identities
GenBank	M59857	*Ricinus communis*	SAD	383	e-103	418/493 (84%)
DBJ	D49832	*Sesamum indicum*	SAD	317	1e-83	310/360 (86%)
GenBank	U07552	*Thunbergia alata*	Δ-9 SAD	307	1e-80	305/355 (85%)
GenBank	AF395441	*A. thaliana*	SAD	246	4e-62	289/344 (84%)
GenBank	L34346	*Glycine max*	SAD	222	6e-55	388/480 (80%)
EMBL	X97325	*Brassia napus*	SAD	216	4e-53	292/353 (82%)
GenBank	U58141	*Olea europaea*	SAD	208	9e-51	300/365 (82%)
GenBank	M59858	*Cucumis sativus*	SAD	204	1e-49	301/367 (82%)
GenBank	AF172728	*Arachis hypogae*	SAD	198	9e-48	247/296 (83%)
GenBank	AF153420	*Brassica juncea*	Δ-9 SAD	198	9e-48	295/360 (81%)

Table 5 Identities of FAD2 gene between *Camellia oleifera* and others species.

Nucleic acid library	Accession Number	Species	Gene	Score	Expected Value	Identities
GenBank	AF192486	*Sesamum indicum*	FAD2	176	4e-41	188/221 (85%)
GenBank	L43920	*Glycine max*	FAD2	161	2e-36	222/269 (82%)
GenBank	AY083163	*Olea europaea*	FAD2	137	3e-29	165/197 (83%)
GenBank	AF188264	*Vernonia galamensis*	FAD2	131	2e-27	171/206 (83%)
GenBank	U86072	*Petroselinum crispum*	FAD2	127	3e-26	157/188 (83%)
GenBank	AF331163	*Gossypium hirsutum*	FAD2	123	5e-25	152/182 (83%)
GenBank	AF272951	*Arachis duranensis*	FAD2	119	8e-24	165/200 (82%)
GenBank	AY525163	*Cucurbita pepo*	FAD2	119	8e-24	165/200 (82%)
GenBank	AY489049	*Capsicum annuum*	FAD2	117	3e-23	104/119 (87%)

FatB1 gene. FatB1 is one of the key enzymes catalyzing the synthesis of fatty acids by terminating the chain extension (Heath *et al*. 1995). The fact that three sequences of *C. oleifera* FatB1 (CoFatB1) have been identified in the *C. oleifera* ESTs indicated that the FatB1 gene is relatively abundant. BLAST searching the GenBank, DBJ, and EMBL data-bases with the longest version showed that CoFatB1 displays 81% identity to that of *A. thaliana* with a score of 128 and an e-value of e-67.

ACAA gene. ACAA, a member of the thiolase family and important for the synthesis of the secondary metabolite iosprenoid, initiates the synthesis of steroid by catalyzing two CoA molecules to form acetoacetyl-CoA (Petersen *et al*. 1991). Three ESTs in the *C. oleifera* cDNA library have the homologies to ACAA when searching GenBank, DBJ, and EMBL data-bases, and been named CoACAA. CoACAA appears to be 88% identical to the ACAA gene of *Hevea brasiliensis* with a score of 494 and an e-value of e-136.

ECAH gene. ECAH can catalyze the hydration of *trans*-2-crotonyl-CoA to form 3'-hydroxyl butyryl-CoA (Gautam *et al*. 2003). Our laboratory has obtained one *C. oleifera* ECAH gene sequence. The BLAST searching indicated that 92% of the CoECAH sequence is identical to ECAH of *Cicer arietinum* with a score of 109 and an e-value of 7e-21.

PCAS gene. PCAS is one of the key enzymes of the 3'-hydroxyl propionate cycle during the fixation of CO_2 in autotrophs by catalyzing 3'-hydroxyl propionate to form propionyl-coenzyme A. PCAS is a large native binding protein (201 kDa) consisting of CoA ligase, enoyl-CoA hydratase and enoyl-CoA reductive in the form of homological trimer or homological quatrimer. At present, two motifs of PCAS have been identified from the genomic DNA of *Chloroflexus aurantiacus* (Birgit *et al*. 2002). In our *C. oleifera* EST sequences, one PCAS gene was recognized as being completely identical (100%) to that of PCAS of *C. aurantiacus*.

IDENTIFIED *C. OLEIFERA* GENES FOR UNSATURATED FATTY ACIDS SYNTHESIS

SAD gene. By dehydrogenating stearic acid to form oleic acid (18:1) (Yukawa *et al*. 1996), SAD plays a critical role in the formation of unsaturated fatty acids. Three *C. oleifera* SAD (CoSAD) sequences were present in our *C. oleifera* ESTs. The longest sequence was selected to perform a BLAST search of GenBank, DBJ and EMBL data-bases. CoSAD resulted in nine hits, including *Ricinus communis*, *Sesamum indicum*, *Thunbergia alata*, *Arabidopsis thaliana*, *Glycine max*, *Brassia napus*, *Olea europaea*, and *Cucumis sativus* with more than 80% identities (**Table 4**).

FAD gene. With the help from NADP+ (Coenzyme II), FAD catalyzes oleic acid to form polyunsaturated fatty acids including linoleic acid (cis 9,12-18:2) and linolenic acid (*cis* 9,12,15-18:3) (Zhang *et al*. 2006b). The FAD genes have been cloned from several important herbaceous crops such as peanut and rape. In *C. oleifera*, several FAD (CoFAD) genes have been isolated and classified as FAD2 ω-6, FAD2 δ-12 and FAD8 ω-3. As the longest cDNA, FAD2 ω-6 was selected to perform BLAST analysis using GenBank. The result showed that CoFAD has the highest homology to that of *S. indicum* with a score of 176, an identity of 85% and an e-value of 4e-41. The homologies between CoFAD and the FAD of other species are ranked from high to low as *S. indicum*, *G. max*, *O. europaea*, *Vernonia galamensis*, *Petroselinum crispum*, *Gossypium hirsutum*, *Arachis duranensis*, *Cucurbita pepo* and *C. annuum* (**Table 5**).

OTHER IDENTIFIED *C. OLEIFREA* GENES RELATED TO LIPID SYNTHESIS

There are several other genes that were proved to be important in lipid synthesis and tea-oil storage such as glyceral-

dehyde-3-phosphate dehydrogenase (GAPDH), oil body proteins and storage proteins (Tan *et al*. 2006).

Using NADH+ as coenzyme, GAPDH can transform dihydroxyacetonephosphate (DHAP) from the glycolytic pathway into 3-glycerophosphate, which plays roles in lipid synthesis (Figge *et al*. 1999). Very conservative during evolution, GAPDH contains a coding sequence (CDS) of about 1300 bp for 322~333 residues which consists of a NAD+ binding motif at both the N-end and the C-end. A 386 bp EST with GAPDH homology from *C. oleifrea* cDNA library was sequenced. Work to ascertain the full-length cDNA is in progress (Tan *et al*. 2006).

Tea-oil seed lipid is mainly stored in an oil body with a liquid triglyceride inner and an exterior membrane. The exterior is composed of a phospholipid bilayer and mosaic proteins termed the oil body proteins such as oleosin, caleosin and steroleosin. As an abundantly expressed gene, the ESTs of eighty four *C. oleifera* oleosin genes have been found and clustered into 5 classes according to their lengths, namely 447 bp, 426 bp, 423 bp, 465 bp and 474 bp (Hu *et al*. 2005). The aligning of these cDNAs and their corresponding genomic DNA shows that all *C. oleifera* oleosin genes do not contain any intron.

To date, 44 ESTs of *C. oleifera* storage proteins have been sequenced and represent legumin (36 ESTs), albumin (15 ESTs) and glutelin (2 ESTs). Storage proteins are small molecules with a conservative center region and two hypervariable regions that are located at both ends. The high expression levels of storage protein and oleosin genes result in lipid formation and transformation in *C. oleifera* seeds.

The appropriate expression balance among the genes that directly or indirectly relate to lipid biosynthesis in *C. oleifera* seed is critical for the presence of high-grade tea-oil in the seed. Extensive studies on the discovery and functions of these genes will eventually lead to a complete understanding of lipid formation, transformation and storage in *C. oleifera* seeds.

WHAT'S NEXT?

Most recent research on *C. oleifera* have focused on conventional planting, breeding and characterization of its products and components (Onodera *et al*. 2006), and very few on the illustration of molecular mechanisms for high-grade tea-oil formation (Tan *et al*. 2005a). The identification of genes involved in lipid formation in seeds is essential for this purpose. Sequencing tea-oil seed cDNA libraries is a quick and efficient way for gene discovery, and also provides basic information for the cloning of full-length cDNAs and genomic DNAs of related genes. Hereafter, the available gene sequences can be used for loci determination, protein overexpression in prokaryotic and other eukaryotic systems, functional analyses by site-specific mutagenesis and RNAi, and engineering plants for advantageous traits.

Currently, the ESTs of several *C. oleifera* lipid genes have been recognized although they are only partial sequences. The complete cDNA sequences of many related genes are expected to be done soon with methods such as RACE, reverse PCR, RT-PCR and full-length cDNA library screening (Lei *et al*. 2006). For those whose full-length cDNA sequences have been elucidated, work on cloning genomic DNAs are in progress with techniques such as PCR with gene-specific primers or direct screening of BAC or Cosmid libraries of *C. oleifera*. The information obtained from these studies will be useful for determining the promoter region, for gene structure analysis and for mapping the evolutionary status of *C. oleifera* in the plant kingdom.

Some lipid genes such as FAD, oleosin and storage proteins genes are gene families. Their sequences, copy number and distribution patterns at different chromosomes vary between cultivars (Dai *et al*. 2007). The location and identification of genes by Fluorescence *in situ* hybridization (FISH) will add more data in terms of the characteristics of lipid genes and their molecular evolution.

One of the main goals of the research is to finally engineer a tea-oil tree with advantageous traits to increase its economic value. Appropriate mutants of model plant *A. thaliana* can be used as material to confirm the functions of *C. oleifera* trait genes by function compensation. Meanwhile, the development of a tea-oil tree transformation technology is also an area into which great effort should be placed. With the availability of a *C. oleifera* transformation technique, RNAi can be used to directly knock down genes of interest. It was expected that the transformation of trait genes, especially CoSAD and CoFAD, into low-grade oil plants might enhance the content of oleic or linoleic acid (Tan *et al*. 2006). Moreover, the expressed CoSAD and CoFAD can also simultaneously be changed to obtain different kinds of tea-oil with different proportions of each fatty acid by interfering with their expression by RNAi and controlling the interference intensity. It has been reported that the existence of CoFAD can improve the resistance of plants against low temperature (Dai *et al*. 2007).

Studies on molecular genetics and cellular biology of lipid biosynthesis in tea-oil tree seed are very necessary, and the information obtained can be used for marker-assisted breeding, genetic diversity analysis and plant genetic engineering. All these will lead to the improvement of tea-oil yield and quality.

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