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MOLECULAR CLONING AND EXPRESSION OF TWO GENES ENCODING ACCASE SUBUNITS OF *CAMELLIA OLEIFERA* (THEACEAE)

BAOMING WANG[1,2,3], XIAOFENG TAN[2,3*] , JAO JIANG[2,3] AND LINZHANG[2,3]

[1]College of agriculture & forestry science and technology,Hunan Applied Technology University, Changde, 415000,P. R. China
[2]Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees (Central South University of Forestry and Technology), Ministry of Education, Changsha 410004, P. R. China
[3]"2011"Cooperative Innovation Center of Cultivation and Utilization for Non-Wood Forest Trees of Hunan Province (Central South University of Forestry and Technology),Changsha, 410004, P. R. China
[*]Corresponding author's email: tanxiaofengcn@126.com; Tel: +86 731-85623416

Abstract

The heteromeric acetyl coenzyme A carboxylase (ACCase) in plant seeds catalyzes the formation of malonyl-CoA from acetyl-CoA, which is the rate-limiting step in *de novo* fatty acid biosynthesis. In this study, we cloned biotin carboxylase (BC) and β-subunits of carboxyltransferase (β-CT) genes of ACCase subunits from *Camellia oleifera*, namely *Co-accC* and *Co-accD*, respectively. The full-length *Co-accC* (GeneBank accession no.FJ965288) was 1901 bp encoded 530 aa, and the coding regions of *Co-accD* was 1530 bp encoded 510 aa (accession no. FJ965289), which was in the 2574-bp fragments of *rbcL, rbcL-accD*intergenic spacer and a*ccD*. The comparison of genomic and cDNA sequences showed that they were all intronless. Structural analyses showed that their putative amino acid sequences shared high identity with those of other oil-plants, and that they possessed the ATP-binding site, biotin carboxylation site in the Co-accC protein, and zinc finger motif $CX_2CX_{15}CX_2C$ in the Co-accD protein. Moreover, transcript expressions of the two genes were carried out in cultivars as well as in tissues and fruit development stages of 'Huashuo', the former showed that their expression levels in cultivars were almost correlated with oil contentsof matured seed kernels, the higher expression levels, the higher oil content to some extent, suggesting that they could potentially be as molecular markers for selection of higher oil production cultivars. The latter revealed their expression rules in lipid synthesis and accumulation. Taken together, information from our study indicates that they may be significant in the fatty acid and lipid biosynthesis of seeds, thus be valuable for oil yield of *C. oleifera*.

Key words: *Camellia oleifera;* ACCase*; accC; accD;* Cloning*;*Transcript expression.

Introduction

Camellia oleifera of the family Theaceae is an important native edible oil tree of China. Its seed oil contains not only plenty of unsaturated acids of oleic acid (59.4%-82.5%) and linoleic acid (5.24%-14.89%), but also medicinal compositions of vitamin E, squalene and tocopherol. It has protective effects against cardiovascular diseases and certain cancers and is also beneficial to human health if used long-term in diet (Zhuang, 2008; Ma *et al.*, 2011). *C. oleifera*is distributed in the Southern Tsinling Mountains (Qin Ling) and the Huaihe River covering the area of about $3.30\times10^6 hm^2$. It is now being introduced in the Southeast Asian countries including Vietnam and Thailand. However, the lower seed oil content of 33%-45% is a drawback in the production of this oil (Chen *et al.*, 2015). Increasing seed-oil content would result in a productivity boost. An ideal approach is to study the key enzymes involved in fatty acid and lipid biosynthesis in its seeds, and in turn to clone genes controlling these key enzymes.

ACCase is an important enzyme catalyzing the essential substrate formation of malonyl-CoA for fatty acid synthesis (Konishi& Sasaki, 1994; Sasaki & Nagano, 2004). It firstly involves the ATP dependent carboxylation of a biotin prosthetic group, and then results in carboxyl transfer from carboxybiotin to acetyl-CoA to form malonyl-CoA. The heteromeric ACCase in plastids plays significant roles in malonyl-CoA biosynthesis. It comprises of four subunits:the biotin carboxyl carrier (BCCP), biotin carboxylase (BC), alpha-carboxyltransferase (α-CT) and beta-carboxyltransferase (β-CT), of which the BC subunits is encoded by *accC* in the nuclear DNA while β-CT subunit is encoded by*accD* in the plastid (Choi *et al.*, 1995; Ke *et al.*,1997; Reverdatto *et al.*,1999).

The *in vitro* and *in vivo* examination has indicated that heteromericACCase is the key regulatory enzyme for fatty acid biosynthesis in higher plants like spinach (Post-Beittenmiller *et al.*, 1992), barley, maize (Nikolau & Hawke, 1984; Page *et al*., 1994), wheat (Eastwell & Strmpe, 1983), *Brassica napus* (Elborough *et al.*, 1996), soybean (*Glycine max*) (Reverdatto *et al*., 1999), tobacco (Madoka *et al*., 2002), *Gossypium hirsutum*, *Arachishypogaea* (Li *et al.*, 2010), *Elaeis guineensis* (Nakkaew *et al.*, 2008) and *Jatrophacurcas* (Gu*et al*., 2011). Especially, *accC*and *accD* are crucial to heteromeric ACCase and in turn to the amount of seed oil in *Elaeisguineensis*(Nakkaew *et al.*, 2008).

In this study, we aim to (1) clone and identify *Co-accC* and *Co-accD* genes of ACCase from *C. oleifera*. (2) Analyze their transcript expression in six cultivars with different oil contentsof matured seed kernels, in tissues and fruit development to reveal the relationships between these two ACCase genes and oil synthesis, and ultimately confirm whether they could be used as molecular markers to screen high seed-oil cultivars. (3) Identify candidate gene (s) that can be manipulated for a desirable outcome on *C. oleifera* oil content.

Plant materials: The research site was located at the germplasm garden of Central South University of Forestry and Technology, Zhuzhou city, hunan province, China (27°50′00″ N, 113°09′00″ E). Six cultivars 'Huashuo', 'Huajin', 'Huaxin', 'Hengdong 31', 'Hengdong 65' and

'Hengdong 17'(with the oil content of seed kernels 50.71%, 56.00%, 62.95%, 39.77%, 33.74% and 32.21%, respectively) were grown in the field. The matured seeds of these cultivars and leaves, stems, flower buds, flower tissues (stamen, pistil and ovary), 5-month-old fruits, 8-month-old fruits, and matured seeds of 'Huashuo' were collected and stored at -80 °C for usage.

Preparation of total RNA and the first strand cDNA synthesis: Total RNAs of the above frozen samples were extracted by lysed Biospin Plant Total Extraction RNA Kit (Bioer Technology Co. Ltd). The DNase (Promeage, USA) was used to remove DNA from the total RNA extracts. The concentration and purity (i.e. the A260/A280 ratio) of RNA was measured by Nanodrop2000 (Thermo scientific, USA) after checking on agarose gel. 500 ng of total RNA was used to synthesize cDNAs by PrimeScript™ II 1st Strand cDNA synthesis kit (Takara, Japan) in a volume of 20 μl according to their instruction manual.

Cloning of the partial fragments of *Co-accC* and *Co-accD*: The partial cDNA fragments were cloned using the reverse transcription polymerase chain reaction (RT-PCR). The primers were *Co-accCF:* 5'-CGTGGMAT GCGTCTTKCTAA-3', *Co-accC*R: 5'-CGCAGTTTYTCT CCCATWGC-3', *Co-accDF:* 5'-GGTGAAAGCGG AAAKAGTMGTGAA-3', *Co-accDR:* 5'-TGTAGGCAW GGGTTCRGCA-3'. The touch-down PCR was performed in a 50 μl reaction including 1×PCR buffer, 0.5 mMdNTPs, 1.5 mM $MgCl_2$, 0.5 mM of each primer, 1U *Taq* polymerase, and 2.0 μlcDNA first strand of matured seed kernels. Thermal cycle programs consisted of pre-cycle at 94°C for 5 min, then 5 cycles of denaturation at 94°C for 40s, annealing at a temperature gradient of 68°C, 60°C, 58°C, 56°C (*Co-accC*), and 65°C, 64°C, 63°C, 62°C, 61°C (*Co-accD*) for 40 s, respectively, elongation at 72°C for 2 min, followed by 94°C 40s, 55°C for *Co-accC,* and 60°C for *Co-accD* at 40s, 72°C at 2 min, 20 cycles, and the final extension at 72°C for 7 min. The above PCR products were separated, purified, ligated, transformed, and used for DNA sequencing.

Cloning of the full-length cDNAs of *Co-accC* and *Co-accD*: 5',3' rapid amplification of cDNA ends (RACE) was performed using reagents from Invitrogen. The gene specific primers (GSPs) of *Co-accC* were as follows: 5'RACEGSP1:5'-CACCGATGTATCCTATTG-3', 5'RACE GSP2:5'-CAGGTGAGGGTGCTTCTTCCA-3'; 5'RAC EGSP3: 5'-CCTCACTCTTGGCTTGCTGTAAC-3'; 3'RACEGSP1:5'-CGTGGAATGCGTCTTGCTAAAGA-3'; 3'RACEGSP2:5'-AGCAGCGT CAATAGGATACATCGG TG-3'.*accD*F: 5'-TGCTGCTTGTGAGGTAT-3', *accD*R: 5'-TTCAATAGGACCAAGAC-3'; 5',3'RACE of *Co-accD* were as follows: 5'RACEGSP1:5'-TAACTCACT CAGAACGCT-3'; 5'RACEGSP2:5'-CTGTCACCCC ACCAGTAGTAG-3';5'RACEGSP3:5'-CTGGAACT CTCGCTTTCACTACTC-3'; 3'RACEGSP: 5'-AGTAGTG AAAGCGAGAGTTCCAGT-3'.

Genomic DNA cloning and identification of *Co-accC* and *Co-accD:* Total DNA was isolated from leaves of 'Huashuo'. The *Co-accC* genomic sequence was obtained by a 25-μl PCR reaction system containing 12.5μl 2×MightyAmp buffer (including Mg^{2+} 4 mM (2×), dNTP 800 μM (2×), each); 0.3 μM primer, 250 ng of the total DNA, Mighty Amp DNA polymerase 0.625U (1.25U/μl). The primers were: *Co-accCF*: 5'-GAAATGGATT CCGCCTTAAC-3',*Co-accCR*: 5'-GAACCTGCTCCAAT CTTGAA-3';The PCR of *Co-accD*genomic sequence was performed in a reaction system of 20 μl including 1×PCR buffer, 0.5 mMdNTPs, 1.5 mM $MgCl_2$, 0.5 μM primer, LA DNA polymerase (TaKaRa) and 400 ng of the total DNA. The primers were *Co-accDF*: 5'-TGCTGCTTGTGA GGTAT-3', *Co-accDR:* 5'-GATCTTATGCCACCAA-3'. Cycling program consisted of precycling at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 40s and elongation at 72°C for 3 min, and finally extending at 72°C for 7 min.

Analysis of sequence properties: Sequence data were analyzed with the NCBI server (http://www.ncbi.nlm.nih.gov), ProtParam Tool (http:// www.expasy.ch/tools/protparam. html). The secondary structure was analyzed using SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/ NPSA/ npsa_sopma.html). Subcellular location and cleavage sites were conducted using the TargetP service (http://www.cbs.dtu.dk/services/TargetP/) and ChloroP 1.1 Prediction Server (http://www.cbs.dtu.dk/ services/ ChloroP/). Multiple sequences were aligned by Vector NTI10.0 and then displayed by GeneDoc.

Expression analyses of *Co-accC* and *Co-accD*: Samples mentioned in Materials and Methods were used for multiplex RT-PCR analysis. Total RNA and generated cDNA was performed as described above. *Glyceraldehyde-3-phosphate dehydrogenase enzyme (GAPDH*) from *C. oleifera* was used as the reference gene. The primers of reference and target genes were designed by Primer Premier 5.0. Their sequences were *Co-accCF:* 5'-TGTTACAGC-AAGCCAAGAGT-3', *Co-accC*R: 5'-GAT GGCAAGTACGCTGTTAT-3'.*Co-accDF:*5'-GTGAAAG CGAGAGTTCCAGTAT-3',*Co-accDR:*ACCCCCAGAA GCACACACTA-3'.*Co-GAPDHF:*5'-CTACTGGAGTTTT CACCGA-3',*GAPDHR:*TAAGACCCTCAACAATGCC-3'.For *Co-accC*, the single-tip amplification was performed in a 20-μl reaction system containing 2.0 μl 10×PCR buffer, 1.5 mM $MgCl_2$, 0.6 mMdNTP, 10 μM*Co-GAPDH* pair 1.2 μl, 10 μM*Co-accC* primer pair 0.4 μl, 1.0 U Taq DNA polymerase (TaKaRa). The PCR of *Co-accD* was performed in the similar reaction system with exception for 10 μM*Co-accD* primers 0.6 μl each, 10 μM*Co-GAPDH* primers 1.4 μl each. Cycling of the two genes were pre-cycling at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 2 min, and a final elongation at 72°C for 7 min. The PCR products were checked by 1.2 % (w/v) agarose gel. Three replicates of each reaction were performed, then the optical densities of strips were calculate by Quantity One 4.4 to quantitatively analyze the differences of gene expression.

Results

Cloning and characterization of *Co-accC* and *Co-accD*: The partial sequence of *Co-accC,* obtained via degenerate PCR was 470 bp (Fig.1A). This sequence shared the highest identity (88%) to *Ricinuscommunisacc C*(GenBank accession no.XM_002519765). Further, the fragments of 5' and 3' RACE were obtained by touch-down PCR, they are 838 bp and 840 bp, respectively (Fig. 1BC). The full-length cDNA of *Co-accC*gene was 1901 bp (GenBank accession no.FJ965288), and its coding region was 1599 bp encoded 533 aa.

The degenerate fragment of *Co-accD* was 733 bp by touch-down PCR (Fig.2A), possessing the highest identity (94%) to *Ehretiaacuminataacc D* (GenBank accession no.ADD31593). Fragments of 5',3'RACE were approximately 700 and 1500 bp, respectively (Fig. 2BC).The coding regions of *Co-accD* was 1530 bp (GenBank accession no. FJ965289), encoding 510 amino acids. In addition, a737-bp fragment of the 5' -flanking region was obtained (Fig. 2D), which had a consensus sequence from the conserved regions of *rbcLs* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) and *accD*s. This part included a 58-bp fragment of *rbcL* gene, and a 529-bp *rbcL-accD* intergenic spacer. The overall cDNA sequence was 2574 bp.

On the basis of the above obtained information of cDNAs, The *Co-accC* genomic and its corresponding cDNA sequences were obtained, and they were all 1638 bp (Fig3A). Similarly, the isolated genomic sequence and corresponding cDNA sequence of *rbcL*, *rbcL-accD* intergenic and *Co-accD* gene were all 2222 bp (Fig3B). Thus, both genomic sequences were intronless.

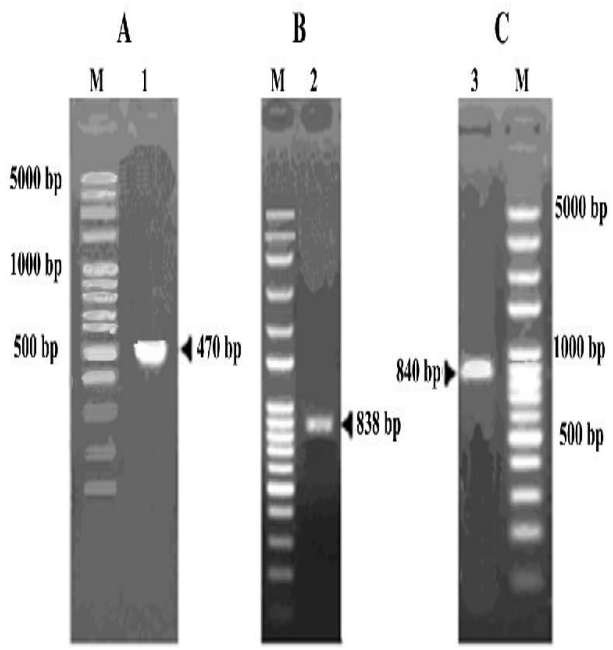

Fig. 1 The PCR results of the cloning of *Co-accC* encoded BC subunit of *C. oleifera* ACCase
(panel A): lane 1 indicates the degenerate PCR amplification fragment; (panel B): lane 2 presents PCR result of *accC* 5'RACER; (panel C): lane 3 is indicative of the 3'RACE result of *Co-accC*. M: 100 bp plus DNA ladder.

Sequence analyses, cleavage and subcellular localization of*Co-accC* and *Co-accD*: The analysis result using ProtParam (http://www.expasy.ch/tools/protparam.html) SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma. html) showed that the BC subunit encoded by *Co-accC* includes 38.46 % alpha helix, 18.76 % extended strand, 8.63% beta turn, and 34.15% random coil. The predicted molecular mass of the precursor and mature BC subunit was 58.51 kDa and 53.52 kDa, respectively. Their corresponding calculated PIs (isoelectric point) were 6.88 and 6.08, respectively. The subcellular localization of BC subunit was in plastid, and its processing site occurred between Arg^{46} and Val^{47} using the TargetP service (http://www.cbs.dtu.dk/services/TargetP/) and ChloroP 1.1 Prediction Server (http://www.cbs.dtu.dk/ services/ ChloroP/).The amino acid alignment results of *accC*s showed that there were no significant regions of homology evident in the N-terminal domain (Fig. 4). The amino acid sequence of *Co-accC* from tea-oil shared the identity of 89%, 88% and 86% with those *accCs* from *G. hirsutum* (ABP98813), *J. curcas* (ACR61637) and *R. communis* (XP_002519811), respectively; and of 84% with those of *accCs* from *G.max*(AAF80468) and *B. napus* (ADI79335), and of 52% with that of *accC* from *E. coli* (ACI77105).

The analysis result by ProtParam (http://www.expasy.ch/tools/protparam.html) and SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl? page=/NPSA/npsa_sopma.html) showed that *C. oleifera* β-CT subunit of ACCase included 35.29 % alpha helix, 20.78% extended strand, 7.06% beta turn and 36.86% random coil. Its molecular mass of the precursor protein was 58.08 kDa with a theoretical pI of 5.17. The subcellular localization of the β-CT subunit was in plastid with no chloroplast transit peptide using the TargetP service (http://www.cbs.dtu.dk/services/TargetP/) and ChloroP 1.1 Prediction Server (http://www.cbs.dtu.dk/services/ChloroP/). As shown in Fig.5, there were no significant regions of homology in the N terminal of *accD* from tea-oil and from other higher plant *accDs*. There was a highly conserved part and the zinc-binding region ($CXXCX_{15}CXXC$), locating at the central region of *accDs*. GSMGSVVG and PLIIVCASGGARMQE are present in the *accDs* of higher plants and *E. coli*. The binding sites for Acetyl-CoA were located at amino acid residues 332-346(Val-332, Gly-335, Leu-339, Gly-341, Pro-343, Val-344 and Ile-346), and for carboxybiotin at residues 351-368(Phe-351, Gly-355, Gly-356, Ser-357 and Arg-368). These were followed by the putative catalytic site of carboxyltransferase at residues 379-392 [Pro(P)-378, Leu (L-380), Ile(I)-382, Ser(S)-386, Gly(G)-387, Gly(G)-388, Arg(R)-390 and Gln (Q)-392)] (Lee *et al.*, 2004). The amino acid sequence of *Co-accD* from tea-oil shared approximately 80% identity with *accDs* from oil plants *Oleaeuropaea* (YP_004376430), *G. hirsutum* (ABC73637), *E. guineensis* (AAY86362), *A. hypogaea* (ACX69849) and *G. max* (ABC25134).

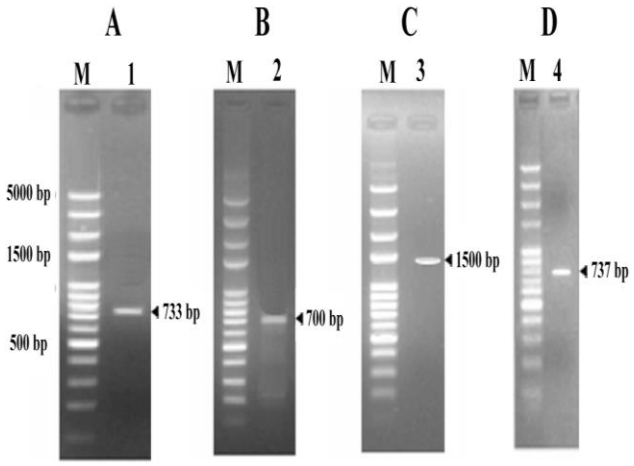

Fig. 2. The PCR results of *Co-accD* encoded β-CT subunit of *C. oleifera* ACCase
(panel A): lane 1 indicates the degenerate PCR amplification result of *Co-accD* fragment; (panel B):lane 2 indicates the 5' RACE result of *Co-accD*; (panel C):lane 3 is indicative of the3'RACE result of *Co-accD;* (panel D): lanes 4 presents the PCR amplification results of the 5-flanking region of *Co-accD*, respectively. M: 100 bp plus DNA ladder.

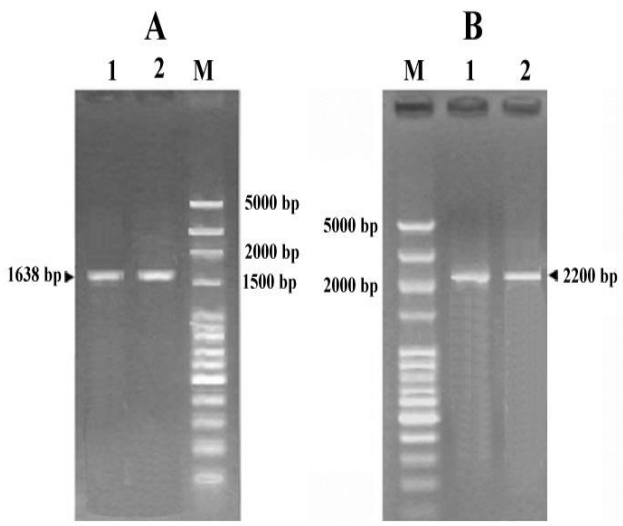

Fig. 3. (panel A): lanes 1 and 2 present the PCR amplification results of genomic DNA and cDNA of *Co-accC*, respectively. (panel B):lanes 1 and 2 indicate the PCR amplification results of genomic DNA and cDNA of *Co-accD*, respectively.

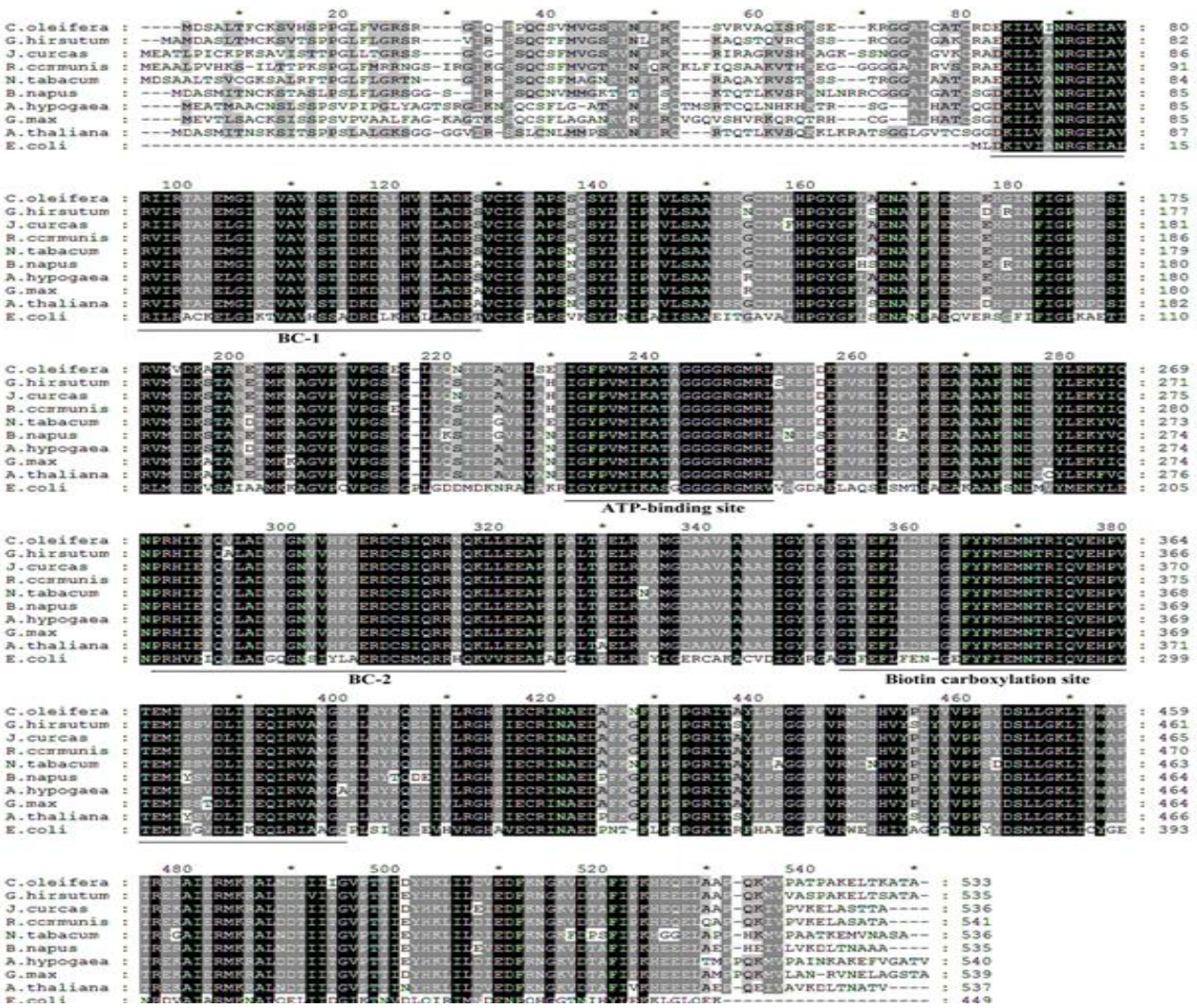

Fig. 4. Multiple sequence alignment of *accCs* from *C. oleifera*, *Arachishypogaea* (GenBank access number: ACO53616), *B. napus* (AAK60339), *Gossypiumhirsutum* (ABP98813), *Glycine max* (AAC02267), *Arabidopsis thaliana* (AAC09008), *Nicotianatabacum* (AAC41659), and *E. coli* (ACI77105). Identical and conserved residues are shaded black and grey, respectively. Four conserved motifs BC-1, ATP-binding site, BC-2 and Biotin carbxylation site, are underlined (Li *et al*., 2010; Sun *et al*., 1997).

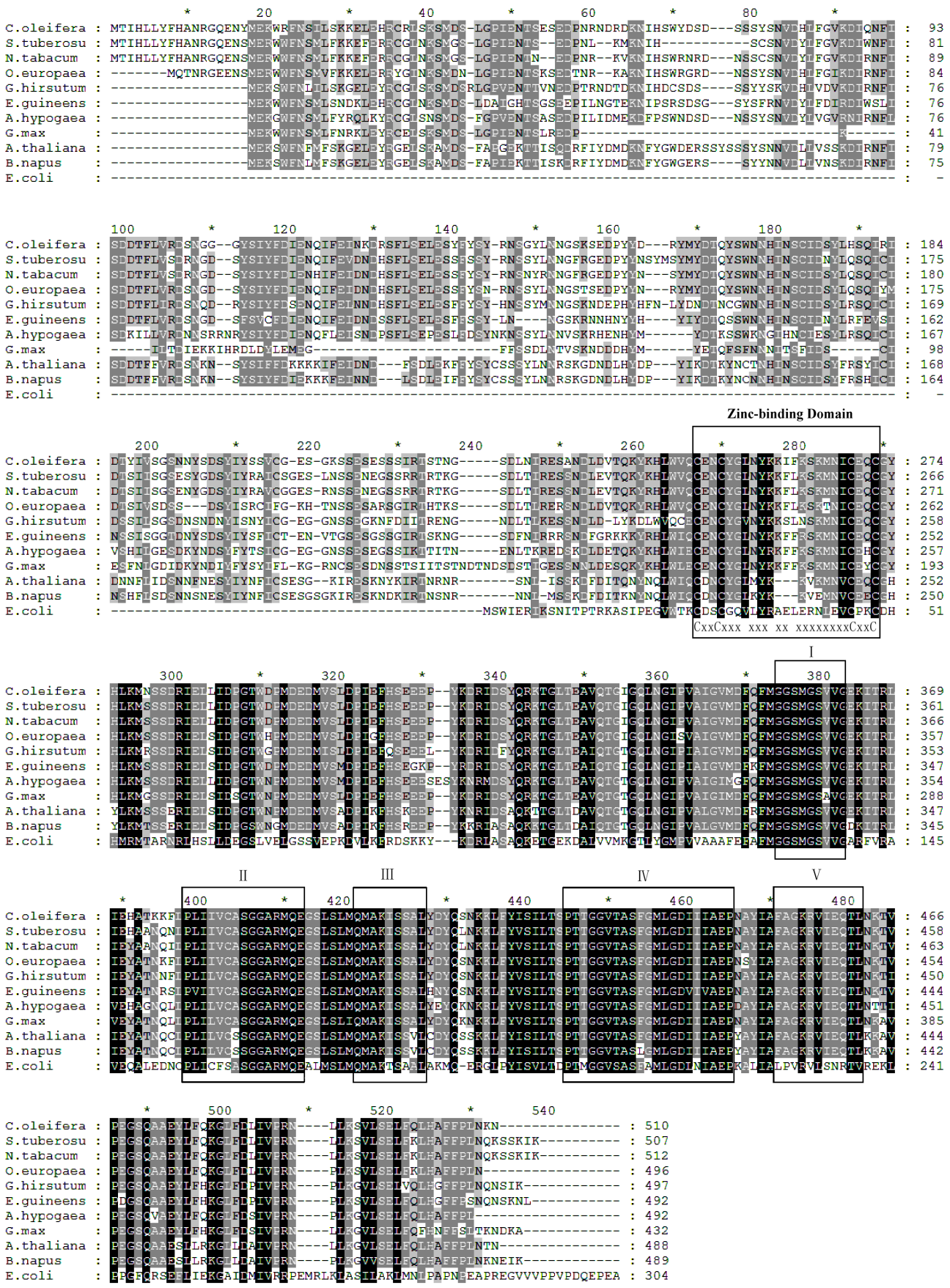

Fig. 5. Multiple sequence alignment of *accD* homologs from *C. oleifera*, *Solanumtuberosum* (AAC23997), *A. hypogaea* (*ACX69849*), *N. tabacum* (CAA77362), *Oleaeuropaea* (YP_004376430), *Gossypiumhirsutum* (ABC73637), *Elaeisguineensis* (AAY86362), *Arachishypogaea* (ACX69849), *Glycine max* (ABC25134), *Arabidopsis thaliana* (BAA84394), *B. napus* (CAA90747), *E. coli* (*AAA23965)*. Identical and conserved residues are shaded black and grey, respectively. Putative zinc finger motif CX2CX15CX2C.

Transcript expression patterns of *Co-accC* and *Co-accD*: Previous transcriptional analysis of ACCase genes showed that they were expressed throughout plant development stages (Elborough *et al*., 1996; Nakkaew *et al*., 2008). To measure the transcript expression of the two ACCase genes in *C. oleifera*, multiplex RT-PCR analysis was performed using cDNAs from matured seed kernels of six cultivars, leaves, stems, flower buds, flower tissues (stamen, pistil and ovary) and seed kernels of 5-month-old fruits, 8-month-old fruits and matured seeds of 'Huashuo'. As shown in Fig.6, the highest expression level of *Co-accC* was observed in 'Huashuo', followed by 'huajin' and 'huaxin'. Higher expression levels of *Co-accC* were noted in seed kernels with higher oil content while the lower levels were observed in ones that yielded lower amount of oil to some extent. Thus, its expression levels were more related with oil contents of matured seed kernels of cultivars. The expression level of *Co-accC* was the highest in flower buds, followed by in 8-month-old fruits and in 5-month-old fruits. The stamens, pistils, ovaries and leaves showed the lower expression levels compared with flower buds, developing fruits and matured seeds (Fig. 7).

The highest expression level of *Co-accD* in matured seed kernels was found in 'Huaxin', followed by 'Huashuo', and there was no distinct difference in other four cultivars. Thus, the higher expression levels resulted in higher oil contents of matured seed kernels with exception for that of 'Huajin' (Fig. 6). The highest expression level of *Co-accD* was present in seed kernels of 8 month-old fruits followed by 5 month-old fruits, and the transcript level in matured seeds was slightly lower than those of 8 month-old and 5 month-old fruits, suggesting that the transcript expression of *Co-accD* gradually increased, and reached the top in 8 month-old fruits, then decreased slightly in matured seeds during the stages of seed development. This was positively correlated with oil synthesis and deposition of seed kernels. In addition, the transcript level in leaves was relative higher than those in flower buds, ovary stamens and pistils (Fig. 7).

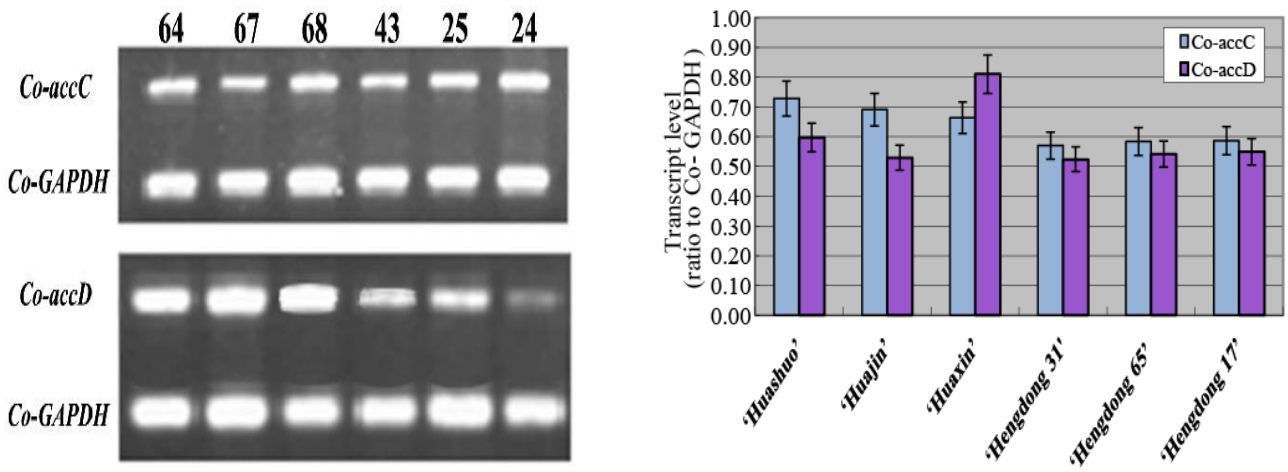

Fig. 6. Expression patterns of *Co-accC* and *Co-accD* in six *C. oleifera* cultivars. The above Figure shows ethidium bromide-stained gels of the PCR amplification using cDNA produced from six species of *C. oleifera* matured seeds 'Huashuo'(64), 'Huajin'(67), 'Huaxin'(68), 'Hengdong 31'(43), 'Hengdong 65'(25) and 'Hengdong 17'(24). The *GAPDH* gene was used as an internal reference to normalize the RNA content of each sample.

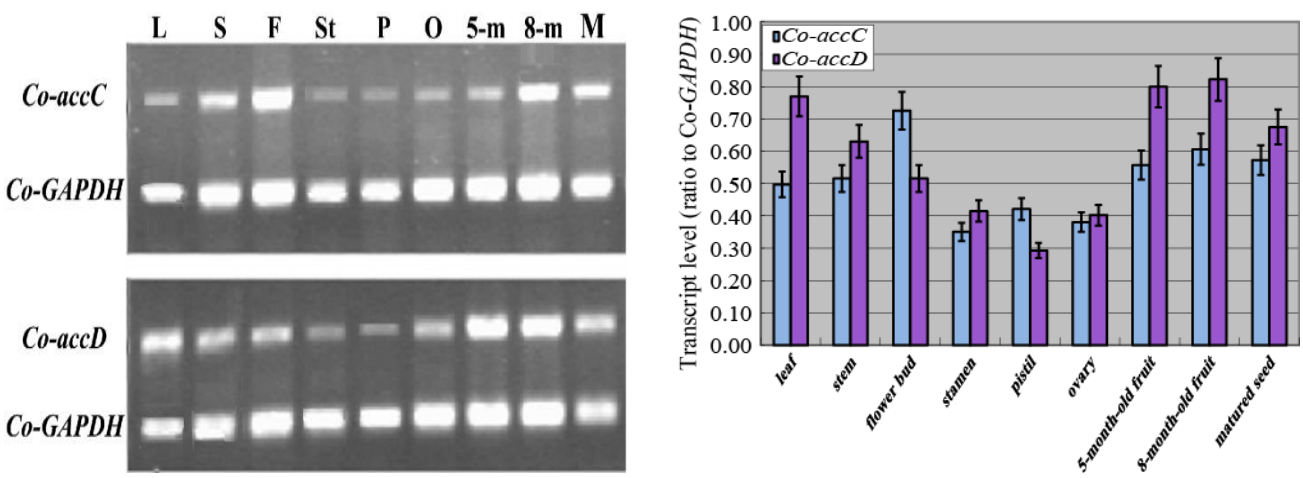

Fig. 7. Expression patterns of *Co-accC* and *Co-accD* in tissues and in stages of seed development of 'Huashuo'. The above Figure shows ethidium bromide-stained gels of the PCR amplification using cDNA produced from leaves(L), stems(S), flower buds(F), and flowers (stamen(St), pistil(P) and ovary(O), 5-month-old fruits(5-m), 8-month-old fruits(8-m), and matured seeds(M) of 'Huashuo'. The *Co-GAPDH* gene was used as an internal reference to normalize the RNA content of each sample.

Discussion

The heteromeric ACCase plays important roles in the biosynthesis of malonyl-CoA for *de novo* fatty acid (FA) biosynthesis in plastids (Konishi & Sasaki, 1994; Sasaki & Nagano, 2004). Moreover, FA producted in seeds could restrict the oil deposition during the lipid synthesis, so improving FA biosynthesis maybe greatly affected the amount of oil (Chapman & Ohlrogge, 2012). In present study, we obtained *Co-accC* and *Co-accD* genes of *C. oleifera* ACCase. The former encoding protein contained four conserved motifs, BC-1, BC-2, ATP-binding site and biotin carboxylation site, but the functions of BC-1 and BC-2 still remains unclear (Li *et al*., 2010; Sun *et al*., 1997). The latter includes five conserved motifs (G/A)SMG(S/C)(V/A)VG(V/L)(I/L)(I/M/L)V(C/S)(A/S)S GGARMQE, QM(A/G)KI(S/A)(S/A)(A/V) (L/S), PT(T/A) GGVTAS(F/L)(G/A)(M/T)LGDIII(A/T)EP and FAGKR (V/I)IE(Q/E)(T/L)L, which were presented in all plant accDs (Fig. 6). This suggests that Co-accD is highly related to the plastid accDs, and has a close genetic relationship (Nakkaew *et al*., 2008). Furthermore, the subcellular localization of the Co-accC protein showed that it contained plastid transit peptides while the Co-accD protein was encoded by a plastid genome (Kozaki *et al*., 2001; Li *et al*., 2010). The features of the two *C. oleifera* ACCase genes indicate that they might be as the key enzyme to involve in malonyl-CoA formation, affect *de novo* FA biosynthesis, and ultimately determine oil content.

Quantitative trait loci (QTL) linkage has showed that the ACCase genes are linked to a QTL linkage group that greatly affected oil amount of oat groats and maize (Alrefai *et al*., 1995; Kianian *et al*., 1999; Yang *et al*., 2010). Moreover, ACCase have the regulating roles in seed oil accumulation (Parker *et al*., 1990; Somers *et al*., 1993). In our study, we have found that the transcript levels of *Co-*accC and *Co-accD* share the almost correlation expression trend, and the higher expression levels present in the cultivars with much higher oil contents of seed kernels (Fig. 6). So we believe it is long-term selection of high oil cultivars that results in improving the transcript levels of ACCase genes in oil biosynthesis, ultimately improving oil yields of seeds in *C. oleifera*, and we thus confirm 'Huashuo', 'Huajin', 'Huaxin' as ideal breeding cultivars. Furthermore, their expression levels gradually increased, reached the top in 8 month-old fruit stages, and then decreased slightly, this was positively correlated with oil synthesis and accumulation during the stages of seed development (Fig. 7). Our results support that *accC* and *accD* transcript levels are correlated with lipid accumulation (Madoka *et al*., 2002).

In summary, we have cloned *Co-accC* and *Co-accD* genes of heteromeric ACCase subunits from *C. oleifera*, and analyzed their structural features and transcript expressions. It was found that they are crucial to the levels of heteromeric ACCase and in turn to the amount of seed oil (Nakkaew *et al.*, 2008). They could potentially be molecular markers to screen high-oil *C. oleifera* cultivars. Our results support the hypothesis that ACCase possesses foremost roles of affecting oil content in oil plants (Roesler *et al.*, 1996; Bouvier-Nave *et al*., 2000; Jako *et al.*, 2001; Nakkaew *et al*., 2008), thus, are helpful for further evaluating the roles of ACCase in fatty acid and lipid synthesis.

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