

from *Jatropha curcas* and *Ricinus communis* on protein level. The 3 FAD2 EST clones were not full-length cDNA by aligning with FAD genes from other plants in GenBank, DBJ and EMBL. Based on the constructed EST library of *C. oleifera*, a full-length cDNA of FAD2 gene was obtained by methods of 5'RACE and overlap extension PCR with total RNA extracted from *C. oleifera* 'Yanggulao No.1' seeds. The gene was 1682 bp in length and contained an ORF encoding 382 amino acids, which formed typical conserved domains of FAD2 and showed high homology with those of other plant species. The results of 3D structure prediction indicated that the Co-SAD and Co-FAD2 were much more advantageous than other SAD and FAD2 in lipids biosynthesis. These results could explain the higher content of oleic acid and linoleic acid in *C. oleifera* than that of other oil plants theoretically and could be applied for breeding high yield teaoil cultivars.

(315) Identification of Two Calmodulin cDNA Genes for *Camellia oleifera* Abel

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Camellia oleifera Abel is a woody shrub or small tree that has been widely cultivated for its edible oil production in China. After establishing the cDNA library using matured seeds of *C. oleifera* 'Yanggulao No.1', the two full-length cDNA genes, 953 base pairs (bp) and 1024 bp, were cloned and identified as the calmodulin genes. They were named *CaM1* and *CaM2* because of their high similarity in nucleotides in the encoding regions of CaMs between the two cDNAs and other higher plant CaM from GenBank (*Prunus avium* and *Actinidia kolomikta*). Both genes contained two opening reading frames (ORFs) of 450 bp with 25 nucleotide substitutions, encoding the identical protein of 149 amino acids (predicted Mw of 16.83 kDa). The characteristic is consistent with the hypothesis theory "multigenes possess identical amino acid sequence." The two amino acid sequences of the putative CoCaM protein were highly homologous and conservative while comparing with those of other higher plants. The protein was comprised of 19 amino acids with pI (theoretical isoelectric point) of 4.10 and should be classified into a hydrophilic and acidic protein. It possessed the structure domains including four EF-hand domains and contained enzyme-binding sites. The putative protein had some degree of identity among its hydrophilicity, flexible regions and antigenicity, and shows the high flexibility. The Blast analysis indicated that the protein shared more than 94% amino acid sequence identity with those of other plant CaMs. The two genes, severed as the transducer of Ca²⁺-dependent signals, were expressed during the peak of lipid biosynthesis, which may be crucial during *Camellia oleifera* physiological processes of the immune responses to bacterial pathogens, gene expression, protein synthesis, cell proliferation and apoptosis.

(316) Cloning Cyclophilin cDNA Gene from *Camellia oleifera* Abel

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Camellia oleifera Abel (Teaoil *Camellia*) is a most important woody plant for edible oil production in China. Its oil possesses higher nutritive and medicinal values. Based on the cDNA library generated from mature seeds of 'Yanggulao No. 1', a full-length cDNA sequence of a gene, which consisted of 975 base pair (bp) and an open reading frame (ORF) of 621bp gene, was cloned. The gene encoded a protein (id: ACJ06541, predicted Mr of 22.24 kDa) of 207 amino acids. The gene was identified as cyclophilin gene because its nucleotide sequence shared the similarity of 96% (843/877) with that of *Camellia sinensis* cyclophilin. The putative protein possesses peptidyl-prolyl cis-trans isomerase (PPIase, EC 5.2.18.) activity and belongs to CyPB protein. Homology analysis indicated that its putative amino acid sequences shared high identity with those of other higher plant CyPs (e.g., 86% and 82% identity of those of *Nicotiana tabacum* cyclophilin-like protein and *Arabidopsis thaliana* cyclophilin protein). It also shows nearly 100% conservativeness in the four residues (Arg-96, Phe-101, His-167 and Tyr-162) of binding cyclosporin A (CsA) and catalysis. Prediction of structure showed that it mainly contains an N-terminal signal sequence, beta sheet accompanying with alpha helices, beta turns and numerous random coils. The protein possesses an array of enzyme-binding sites, cyclophilin-type peptidyl-prolyl cis-trans isomerase signature, and a unique seven amino acid sequence KSGKPLH in numerous plant cyclophilins. The gene was submitted to GenBank (GenBank access number: FJ377540) and was termed as co-cyp1 gene. The gene expression during the peak of lipid biosynthesis might protect cell against reactive oxygen species (ROS) damage. Therefore, it may be crucial during both development and stress responsiveness.

(317) Fertility Restoration of *Buddleia* Species by In Vitro Chromosome Doubling

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Mitotic chromosome doubling using colchicines has been successful for many crop species. It was involved in the production of triploid watermelons, tetraploid grapes, and some autopolyploid ornamental species. Induced autopolyploids could enhance the crossability of two species, particularly if both are diploids. Amphiploids could be used to restore some fertility of a totally sterile F₁ hybrids thus to facilitate further backcrossing and introgression. In this study, chromosome doubling of three *Buddleia* sterile lines, *B. marrubifolia* × (*B. davidii* × *B. crispa*), *B. marrubifolia* × *B. crispa*, and *B. marrubifolia* × *B. alternifolia*, was carried out to restore their fertility. Based on the field observation, these three lines are sterile. In vitro shoot tips and nodes were treated with colchicine solution at 0, 0.01, 0.1, and 1 mM for 1, 2, and 3 d. After the treatment, the explants were washed in sterile distilled water and transferred into the Petri dishes containing MS medium plus 20 g l⁻¹ sucrose with 0.5–2.5 μM BA for shoot recovery. Results showed that