(312) Full Length cDNA Sequence of Pear (*Pyrus bretschneideri* Rehd.) S29-RNase and S29-Allele Identification

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Chinese white pear (*Pyrus bretschneideri* Rehd.) belongs to Rosaceae that exhibits characteristic gametophytic self-incompatibility. This type of self-incompatibility is controlled by S-locus which carries a series of multi-allelic alleles encoding S-RNases. To elucidate the function of S-allele and the possible molecular mechanism of gametophytic self-incompatibility in Chinese white pear, full length cDNA encoding S_{29}-RNase was cloned by rapid amplification of cDNA ends (RACE) approach from cultivars 'Mili' ($S_{19}S_{29}$) and 'Zaomi' ($S_{19}S_{29}$). The S_{29}-RNase gene contained an open reading frame of 684 nucleotides encoding 228 amino acid residues. S_{29}-RNase displayed typical sequence features of rosaceous S-RNases, i.e. five conserved regions (C1, C2, C3, RC4 and C5) and a hypervariable (HV) region. At the deduced amino acid level, S_{29}-RNase showed 30% to 92% similarities with other rosaceous S-RNases. Phylogenetic analysis revealed that rosaceous S-RNases occurred before divergence of species, but after divergence of subfamilies Maloideae and Amygdaloideae. Genomic PCR amplification with primer combination FTQQYQ and anti-(I/T)IWPNV followed by digestion with the restriction enzyme AccII allowed effectively distinguishing S_{29}-allele from other pear S-alleles.

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(313) Analysis of Seed-Expressed Sequence Tags in *Vernicia fordii*

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Tung-oil tree (*Vernicia fordii*) is one of the high-value industrial oil trees in China, whose seed has 52% to 64% oil-yields content. The tung oil is widely used in painting and chemical industry. Its principal component is eleostearic acid (9,11,13-octadecatrienoic acid) that is a conjugated trienoic fatty acid. It is readily oxidized when exposed to air, which resulted in formation of the unique polymer. To isolate the genes related to tung oil synthesis and examine their expression patterns in *V. fordii*, we constructed a cDNA library using the developing seed tissues of *V. fordii* 'duiniantong' and picked out 3,107 clones randomly for sequencing on the 5'-end to construct the EST library. We then performed homology comparison with nucleic acid NR database. The results indicated that 2,205 cDNA sequences were highly homological to these sequences of other plant species in database of NCBI. A total of 482 different genes had been identified preliminarily and 342 clones possibly represented genes with unknown function. The sequences related to tung oil synthesis had delta 12 oleic acid desaturase (FAD2), omega-3 fatty acid desaturase, 3-ketoacyl-CoA thiolase, beta-ketoacyl-ACP synthase I, enoyl-acyl-carrier-protein reductase, esterase/lipase/thioesterase family protein, polyunsaturated fatty acid synthase subunit B, enoyl-CoA hydratase/isomerase family protein, and etc. Seed storage protein had caleosin, oleosin, legumin, albumin, and etc. There are lots of ribosomal RNA and pollen allergen genes. Many sequences corresponded to transfer protein, gene expression, development regulation, resistance, growth substances and embryogenesis, and etc., while the sequences of 338 genes related to universal substances biochemical metabolism were expressed only once. The numbers and trends of expressed genes were at the phase of *V. fordii* seeds near to late embryogenesis. Establishing the EST library of *V. fordii* and researching the genes related to the tung oil synthesis have great significance to producing high-quality tung oil.

(314) SAD and FAD2 cDNA Genes Cloned from *Camellia oleifera* Abel

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Seeds of *Camellia oleifera* Abel produced high-quality edible oil, important cosmetic ingredients, and bio-fuel, such as poly unsaturated fatty acids (about 90%). Oleic acid transformed from saturated fatty acid in the process of grease biosynthesis catalyzed by stearoyl-ACP desaturase (SAD), then transformed into linoleic acid and other poly unsaturated fatty acids catalyzed by fatty acid desaturase (FAD) family gradually. So the cloning SAD and FAD genes from *C. oleifera* are very important for revealing the lipids biosynthesis patterns and achieving molecular-aided breeding in *C. oleifera*. We constructed cDNA and EST libraries of *C. oleifera* and 3 copies of SAD gene and 3 copies of FAD gene were obtained from the EST library. One of three SAD EST clones was confirmed to be the full-length cDNA and named as *co-sad*, and 3 FAD2 EST clones were not full-length cDNA by BLAST with SAD and FAD genes from other plants via GenBank, DBJ and EMBL. Bioinformatics analysis showed that *co-sad* was 1579 bp in length and contained an 1188 bp open reading frame (ORF) encoding 396 amino acids. However, there was no obvious signal peptide and trans-membrane structure in the deduced protein sequence. BLAST results suggested that *co-sad* shared high homology with SAD genes

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 Ca^{2+}-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 nutrient 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 nucleotied 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 cyclosporn A (CsA) and catalysis. Prediction of structure showed that it mainly contains an N-terminal signal sequence, beta sheet accompanying with alpha helixes, 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 F_1 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