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ISOLATION AND SEQUENCING OF cDNA CLONES CODING FOR OIL PALM (Elaeis guineensis) ACY L CARRIER PROTEIN (ACP)

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Five cDNA clones encoding the oil palm acyl carrier protein (ACP) were isolated and characterized. Four of the clones, pACP1, pACP3, pACP4 and pACP9, were completely sequenced and the sizes of inserts shown to be 529, 701, 505 and 370bp, respectively. The clones could be divided into two classes, which probably represent two different isoforms of ACP. pACP1, which was classed separately from the others, is about 92% identical to the other three clones. The clones also had a high similarity to ACPs in other plants – 73%. 72% and 70% identical to barley ACPI, castor bean ACP and barley ACPIII, respectively. The similarity of the transitpeptide was lower at about 45% to 52%. Based on the comparison, we predict that pACP3 contains the full length insert of oil palm ACP with an open reading frame of 432bp which starts at nucleotide 47 from the 5' end and ends at nucleotide 478. This comprises 144 amino acid residues including 65 residues of a transit peptide.

INTRODUCTION

Acyl carrier protein (ACP) is a small acidic molecule of about 9 to 11kDa. It plays an essential cofactor role in fatty acid biosynthesis (Ohlrogge, 1987). In plants and bacteria, the ACPs are highly soluble proteins whose amino acid sequences are well conserved. This was evidenced by 40% homology between the amino acid sequences and their interchangeability in several enzymatic reactions (Kuo and Ohlrogge, 1984; Simoni *et al.,* 1967).

Recent studies on protein purification, western blot and mapping have revealed the complex nature of the protein. There are at least three isoforms of the protein (ACPI, II and III) encoded by a multigene family consisting

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of about 35 genes per haploid genome (de Silva et al., 19901. ACPI and II are localized in the chloroplast while ACPIII is believed to be achloroplastic (Hansen, 1987). These observations have been correlated to a differential tissue and temporal-specific expression (Ohlrogge and Kuo, 1985; Hlousek-radojcic et al., 19921. This was evidenced by differential expression studies in several plants including *Brassica napus* (de Silva et al., 1990) and *Spinacia oleracea* (Battey and Ohlrogge, 1990; Schmid and Ohlrogge, 1990). de Silva et al. (1992) showed that GUS activity was increased 100-fold in the developing seed of transgenic tobacco when the gene was fused to the *B.napus* ACP promoter. Similar observations were also made by Bearson et *al.* (1994; 1997) using *A. thaliana* ACP promoters (*Acl1.2 and Ac11.3).* Studies of ACP levels in developing seeds of both soyabean and rapeseed showed that ACP activities appeared just prior to the onset of storage lipid biosynthesis (Slabas and Fawcett, 1992).

cDNA clones and genes have been isolated and sequenced from several plant species including S. *oleracea* (Scherer and Knauf, 1987), *Hordeum vulgare* (Hansen, 1987; Hansen and Kauppinan, 1991), B. *napus* (de Silva *et al.*, 19901, *Oryza sativa* (Uchimiya *et al.*, 1992), *Zea mays* (Souciet and Weil, 1992) and *Arabidopsis thaliana* (Lammpa and Jacks, 1991; Post-Beittenmiller *et al.*, 1989a). The gene loci, *Acl1* and *Acl3*, have been mapped in *H. vulgare* (Hansen and Wettstein-Knowles, 1991) and *Triticum aestivum* (Devos *et* al., 1991) while *Acl2* was mapped in *H. vulgare* (Hansen and Kauppinen, 1991). *Acl1* and *Acl2* are known to be linked in *H. vulgare* but *Acl1* and *Acl3* are not.

In oil palm, knowledge of the fatty acid biosynthetic pathway in general is still limited. However, efforts have recently been made to obtain a better understanding on this pathway (Cheah *et* al., 1995; Shah, 1994; Parveez *et al.*, 1994). This includes the isolation of stearoyl-ACP desaturase (Rashid and Shah, 1996; Abdullab *et* al., 1997) and β-ketoacyl-ACP synthase I (Shah and Rashid, 1997). Several other proteins in the pathway including acyl-ACP thioesterases (Othman *et* al., 1992) and β-ketoacyl synthase II (Ramli and Sambanthamurthi, 1996) have been purified. This work was aimed to isolate and sequence the genes that code for oil palm ACP. Here, we report the isolation and sequencing of oil palm ACP cDNA clones.

MATERIALS AND METHODS

A cDNA library constructed from mesocarp of 15-week old *Elaeis guineensis var. tenera* fruits (Abdullah *et al.*, 1994) was used in the screening. A total of 5 $\times 10^5$ plaque forming units (pfu) were plated and transferred onto nylon membranes. The membranes were hybridized to a cDNA probe encoding a partial sequence of the *rice ACP gene* (clone C 1555 was a gift from the Rice Genome Research Programme, Japan). The probe used was labelled with α-^{32}P dCTP using a Megaprime DNA Labelling System Kit (Amersham) according to the manufacturer's instructions. Hybridization was carried out at 62°C in 5x Denhardt's solution (0.02% ficoll, 0.02% BSA, 0.02% PVP), 5x SSPE (180mM NaCl, 10mM $NaPO_4$ pH7.5, 1mM EDTA), 0.5% SDS, 100μg ml^{-1} single stranded DNA and 5% dextran. The phagemids of the positive plaques were excised in *vivo* using *ExAssist Helper Phage* and *SOLR* cells (STRATAGENE). Plasmid DNA isolation was carried out using QIAGEN Plasmid Mini Kit (QIAGEN). The size of the insert was determined by digestion with *Eco*RI and *Xho*I, followed by agarose gel electrophoresis. DNA sequencing was performed using AlfExpress automated DNA sequencer. Analysis of DNA sequences was carried out using DNAsis (Hitachi) and DNA homology search performed with BLAST 2.0 (GenBank).

RESULTS AND DISCUSSION

Screening of the cDNA library using Cl555 containing a partial sequence of the ACP gene of rice resulted in the identification of five putative clones. These clones were designated pACP1, pACP3, pACP4, pACP6 and pACP9. The sizes of the inserts were estimated by *Eco*RI and *Xho*I digestion of the plasmid DNAs. Agarose gel electrophoresis showed that the insert sizes ranged between 0.3 to 0.7kb (Figure *1)* with pACP3 containing the largest insert of almost 0.7kb, and pACP1 and pACP4

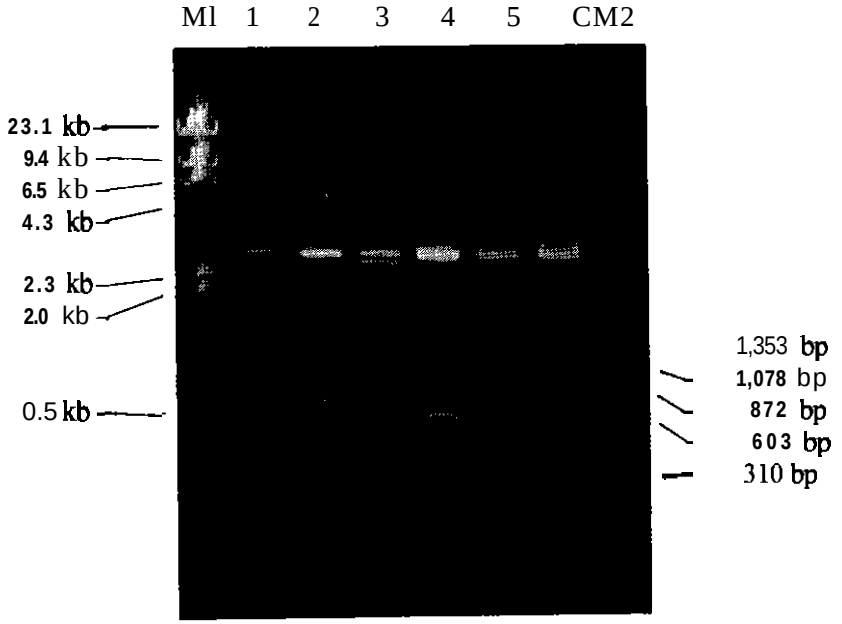

Figure I. Agarose gel electrophoresis Of EcoRI/XhoI digested pACP clones. Lanes I to 5 show clone pACP1, pACP3, pACP4, pACP6 and pACP9, respectively. M1 is HindIII-digested Lambda DNA and M2 is HaeIII-digested θ74DNA. C is the EcoRI/XhoI digested pBluescript vector.

inserts of similar size (0.5kb).

Nucleotide sequencing of these clones showed pACP1, pACP3, pACP4 and pACP9 carried inserts of 529, 701, 505, and 370bp, respectively. The complete sequence of pACP6 could not be obtained even after several attempts. This was probably due to changes that occurred within the insert. Based on comparison to other published ACP sequences, the possible coding sequences for all clones were identified. The translated amino acid sequences showed that among the four clones, only pACP3 coded for the full length ACP. The ORF for pACP3 is 432bp which starts at nucleotide 47 from the 5'-end and ends at nucleotide 478. It comprises 144 amino acids including 65 residues of transit peptide. Therefore, the mature protein of oil palm ACP encoded by pACP3 is 83 amino acid residues. Clones pACP1 and pACP4, on the other hand, contain full length sequences for the mature proteins and partial sequences of the transit peptides, whereas pACP9 contains only a partial sequence for its mature protein. Further sequence comparison and analysis therefore excluded pACP9 and were performed only on the coding and 3' non-coding regions of the other three clones.

Analysis of the sequences showed that these clones can be divided into two classes, I and II. pACP4 showed a high similarity to pACP3 and both can be grouped together in class I. The coding sequences of these clones were 100% identical over 248bp (*Figure* 2). A difference appeared in the 3' non-coding region where a single base change occurred at position 580. In addition, the 3' non-coding region of pACP4 was shorter than that of pACP3 and terminated at nucleotide 652. The differences could have resulted from cloning artifacts. The coding region of pACP1, on the other hand, was only 92% similar to those of the other two and could represent another class of ACP. Major differences were found within their 3' non-coding regions, in which pACP1 only showed 55% similarity to pACP3 as compared with 99% with pACP4.

These results suggest that we have successfully isolated two different members of the oil palm ACP gene family. This is well in line with observations in other plants where ACP is

*Figure 2. The complete nucleotide sequences of pACP1, pACP4 and pACP3 which are 529, 505 and 701bp, respectively. Similar bases are denoted by *. The start (ATG) and stop (TAG) codons are in bold. The start codons of the mature protein are in bold and italics. The sequence coding for a transit peptide in pACP3 is underlined.*

encoded by a gene family (Hoj and Swendsen, 1983; de Silva et al., 1990; Hansen and Wettstein-Knowles, 1991). Moreover, our observation that the differences were found not only in the coding region but more in the 3' non-coding region also concurs with findings in other plant ACP genes. For example, the coding regions of the two ACP genes from rapeseed are 94% similar (de Silva et al., 1990). A lower level of similarity (71%) occurs between the coding regions of the two barley ACP isoforms (Hansen, 1987). The results strongly suggest that the mature sequence of oil palm ACP is highly conserved. In subsequent analysis, the three clones were only considered as two classes in which pACP3 was chosen to represent class I and pACP1 for class II.

Compared to other published ACP sequences, such as those from barley, spinach, rapeseed, and maize, both clones had a very strong similarity. Their DNA sequence coding for mature protein was 68% to 73% similar (*Table 1*). Both clones showed a strong similarity to barley ACPI with 73.1% and 72.7% for pACP3 and pACP1, respectively. They were least similar to *Escherichia coli* ACP. The sequence coding for the transit peptide had a lower similarity. The transit sequences of both clones were 45% to 53% similar compared with the transit sequences of other published ACP clones. Hlousek-Radojcic et al. (1992) showed that when the region from several plant species was compared, they showed similarities of between 30% to 50%.

TABLE 1. SIMILARITY BETWEEN THE DNA SEQUENCES CODING FOR TRANSIT PEPTIDE (TP) AND MATURE PROTEIN (MP) OF pACP1 AND pACP3 TO OTHER PUBLISHED ACP SEQUENCES

	pACP3		pACP1
	TP	MP	MP
Barley ACP I	51.4	73.1	72.7
Castor bean ACP	52.8	72.1	71.4
Barley ACP III	51.6	71.5	69.1
Cotton ACP I	52.2	71.5	71.3
Barley ACP II	51.1	70.1	71.7
Maize ACP	53.5	69.6	66.5
Rape ACP II	45.3	68.7	69.4
E. *coli* ACP		53.3	54.1

At the protein level, the deduced amino acid sequences of the two classes were 96.4% similar (Figure 3). There were nine variant residues of which three were located within the mature protein sequence. Two of them were due to a single base substitution and the other to a double base change. Within the transit peptide however, 6 out of 14 residues were varied, indicating less conservation in this region. A similar observation was also reported in barley ACPs in which no similarity was found between the transit peptide of barley ACP or to that of other plant ACPs. This implies that the isoforms are encoded by genes with different transit peptide sequences, and this probably can give rise to temporal and spatial regulation (Hansen, 1987). Compared to other translated amino acid sequences of ACPs, oil palm ACPs are 68% to 78% similar.

Figure 3 also shows the presence of a highly conserved region (underlined) surrounding the prosthetic serine residue (amino acid 114) which further proves that these clones code for ACP. The 18 residues of the region are 100% conserved in both classes of the oil palm ACP and are also the cases between barley ACPI and ACPIII (Hansen,1987), spinach ACPI and ACPII (Schmid and Ohlrogge, 1990) and B. *napus* ACP gene family members (Hlousek-Radojcic et al., 1992). The high conservation of the region is well retained across plant species. Only a single residue difference was observed (alanine to glutamic acid at position 127) in this region between oil palm ACP and other plant ACPs, with the exception of barley ACPI. This observation provides further evidence of the crucial role of this region in the formation of a phosphodiester bond between the acyl intermediates and ACP protein in the fatty acid biosynthesis pathway.

In summary, we have successfully isolated two classes of cDNA clones coding for oil palm ACP. This conclusion was based on the strong similarity between these two clones to other published plant ACP sequences. In addition, we have also shown the presence of a conserved sequence within the serine prosthetic group in the genes isolated. Based on sequence comparison, it is believed that these two clones may code for different isoforms of oil palm ACP.

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pACP3                                    LDLSLSPSLFSSDRSMASISGTVTATSVRPRLT   33
Barley1      RAAHDSTYQPASSHRPQILP.S.AGQPMAHCLA.VS.FSPS.VRR.LSSQ              50
Barley2                LPLCCPVAAPTAASTAA.TAHRAGLSPCP.SPMASAAASA              40

pACP3        SRAPWKGFSGLKSVSFSIQRNGFPSVRLQAAPRRLRFQVSCPAKPETVQ               83
pACP1                                                SR.L....V.......        16
Barley1      VANV.SSRS.VSFHSRQMSFVSISSRPSSLRFKICCAAMG.........               100
Barley2      VSFARPVKAICVN.....AL.KDNV.F...PV.Q--..S.C....K...E              48
Barley3                    L.FSGARRGNAF.RLQ.VPM..A.C.S..QD..E                36
Rapeseedl                                                . ..Q...E          8
Rapeseed2                                                .......E           8
Rapeseed3                                                ......IE           8

pACP3        KVCEIVKKQLALSDDTPVSGESKFSTLGADSLDTVEIVMGLEEAFGISVE              133
pACP1        ...D..............T.....................                        66
Barley1      EAQ..K...D...M.......VP.G...TA.....E.......E.N.T.D              150
Barley2      .  D  .  .  ..S........E...S.T.AD.....................          98
Barley3      ...........VPEG.E.C.TT...D.................E.Q.TWQ               86
Rapeseedl    ..S........-SK..QQ.VA.T..VD.................E...QMA             57
Rapeseed2    ..SK...... S.K..QN.VA.T..AD.................E.H.EMA            58
Rapeseed3    ..SK.... ..S.K..QK.VA.T..AD.................E.D.F.MA           58

pACP3        EESAQSITTVQDAADLIEKLVEAKSS*MNKNVQFSSKKE*ECSRVEALSG              183
pACP1        ....................D....*..N...ELKFF.SGTFPLSWFCT              116
Barley1      .T...D.A......N...... TE.TA*APRFIGQL..AKVSSGS.DF.FF             200
Barley2      .S.. .T.A..E...N..DS.. GK*TQF.AIMR.ELKRLLLDHCNGCCWMD            148
Barley3      .T.. .A.A..E...T..D...S.... ●  REVVRNDW.S.ASSPTLG.S.LF          136
Rapeseedl    ..K..K.A..EQ..E...E.MQ..K*                                      82
Rapeseed2    ..K..K.. ..EE..E..DE..Q..K*                                     83
Rapeseed3    ..EKAQKIATVEE.AELIEELVLLKK*                                     84

pACP3        *QTPSVLVLYFVGYVWFLFNCTSLFQIAKGPYQSDIRLGLLVNLFCFFDM              233
pACP1        LSALCQ*DGFI*RVHI*FCFVLWNC*E*VSDILLGF*FN*FCIRVVGLNI              166
Barley1      GRIWGERK*FMDSFAFS*CCVFIAICSFFILSRFCVVAAG.LPKGFINKL              250
Barley2      PF.RARFYRGELVTYVSV.LPVLRYYS.HLS.C.RNYFL.K.D.ISL                 245
Barley3      VIIWW.GDFLRTIVSCCV..DNW.SM..VDHLS..FSC*                         275

pACP3        Q                                                               234
pACP3        PITFYLL*TS                                                      176
Barley1      GPCP*F                                                          256
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*Figure 3. Comparison between deduced amino acid sequences of oil palm **pACP1** and **pACP3** to barley and **rapeseed ACPs** (Hansen and **Kauppinen,** 1991; Hansen, 1987). The **region** of the **serine** prosthetic group **(with** 18 residues) is underlined. Dots indicate identical amino acid residues.*

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