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PARTIAL NUCLEOTIDE SEQUENCE OF AN OLEOSIN cDNA FROM *Elaeis guineensis* Jacq.

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In an attempt to isolate a full length oleosin cDNA from oil palm kernel, we identified a putative clone by colony hybridization of cDNAs from a 20 weeks after anthesis (WAA) kernel library with the oleosin gene (oleM) *from maize* (Zea mays). *The recombinant plasmid, pOP-KT115, has an insert of 550 base pair (bp). The nucleotide sequence of this insert showed high similarities with the oleosin genes from maize, barley* (Hordeum vulgare) *and rice* (Oryza sativa).

In a Northern blot analysis with the insert of pOP-KT115 as probe, positive signals were only detected with total RNA from oil palm kernel tissues at 10, 12, and 14 WAA. No hybridization signal was detected with total RNA from mesocarp tissues, leaf, germinating seedlings, and inflorescences at Frond 10 (F10) and Frond 19 (F19). This result concurs with the findings of previous studies carried out with mesocarp tissues of olive (Olea europea) *and avocado* (Persea americana).

INTRODUCTION

Diverse organisms store lipids in subcellular particles as food reserves. In plants, triacylglycerols (TAG) play an important role as high-energy carbon reserves for germination and postgerminative growth of seedlings. TAG are confined to discrete, spherical organelles called oil bodies, sometimes also known as lipid bodies, oleosomes, or spherosomes. An oil body is 0.5-2.5µm in diameter and consists of a 95% neutral lipid (mainly in the form of TAG) core enveloped by a monolayer of phospholipids and

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embedded with proteins termed oleosins which make up most of the remaining 5% (Huang, 1992).

Oleosins are amphiphatic polypeptides which are specifically associated with the lipid storage bodies of plants, and their expression is regulated by abscisic acid (ABA). They are alkaline, hydrophobic proteins of M_r 15-26 kDa, depending on the species (Qu *et al.*, 1986). There are at least two isoforms of oleosins in seed oil bodies (Tzen *et al.*, 1990; Chuang *et al.*, 1996). However, the precise role played by oleosin *in vivo* is unclear. It has been suggested that certain structural functions of the oleosins prevent contact with cytosolic enzymes and coalescence of the oil bodies. A putative lipase attachment site on the oleosin implicates its involvement in the process of lipolysis (Vance and Huang, 1987).

The first oleosin gene cloned was from maize (*Zea mays* L.) (Vance and Huang, 1987; Qu and Huang, 1990). Since then, genes and cDNAs encoding these proteins have been cloned from several species including *Arabidopsis thaliana* (van Rooijen *et al.*, 1992), oilseed rape (Lee and Huang, 1991; Murphy *et al.*, 1991; Keddie *et al.*, 1992), soyabean (Kalinski *et al.*, 1991), carrot (Hatzopoulos *et al.*, 1990), sunflower (Cummins and Murphy, 1992), *Pinus ponderosa* Laws (Lee *et al.*, 1994), and barley (Aalen, 1995).

Here, we report the cloning and partial characterization of the oleosin cDNA from a 20 WAA kernel cDNA library of oil palm by using a heterologous probe from *Z. mays*.

MATERIALS AND METHODS

Kernel tissue from 20 WAA was harvested from the oil palm *Elaeis guineensis* Jacq. Other tissues harvested from *E. guineensis* Jacq. included: germinating seedlings; leaf; 8, 12, and 17 WAA mesocarp tissues; 10,12, and 14 WAA kernel tissues; and female inflorescences at Frond 10 (F10) and Frond 19 (F19).

Plasmid pL310, harbouring the 750 base pair (bp) oleosin gene from *Z. mays*, was a generous gift from Professor Anthony Huang (University of California, U.S.A.).

Construction of cDNA Library from 20 WAA Kernel Tissue and Preparation of Membranes

Total RNA was isolated from 20 WAA kernel tissue by the method of Cheah *et al.* (1987). A cDNA library was constructed by using the cDNA Synthesis Kit (Pharmacia Biotech) containing the vector pT7T3. Clones containing inserts were inoculated into LB medium in a 96-well microtitre plate. The bacterial colonies were then replicated onto nylon membranes and microwaved as described by Buluwela *et al.* (1989).

Preparation of the Probe, Prehybridization and Hybridization

QIAGEN-tip 100 (Qiagen, Germany) was used to isolate pL310 and the method used was as described in the manufacturer's manual. The insert bearing *oleM*, was excised with *Eco*RI, gel-purified, and labelled by using the Megaprime™ DNA Labelling System (Amersham, U.K.), and used as probe.

The nylon membranes were prehybridized/hybridized under the following conditions: 6X SSPC, 5% (w/v) SDS, 100μg ml^{-1} denatured salmon sperm DNA, 40%(v/v) formamide, and 5x Denhardt's solution (Sambrook *et al.*, 1989) at 42°C. After hybridization, the filters were removed and subjected to stringency washing in 0.1X SSC, 0.1% (w/v) SDS. The highest stringency wash was performed at 60°C. The membranes were subsequently exposed to x-ray films for 48hr at -80°C.

Southern Hybridization

Recombinant plasmids extracted from bacterial clones were digested with *Eco*RI and subjected to 1% (w/v) agarose gel electrophoresis in 0.5X TBE. After electrophoresis, the gel was submerged in depurination solution (0.2 N HCl) for 10min, followed by denaturation solution (1.5M NaCl, 0.5M NaOH) for 15-20min. Following this, the gel was rinsed again before it was left to soak in neutralization solution (1M Tris-HCl, pH 7.5, 1.5M NaCl) for 30min. The denatured DNA was transferred to a piece of nylon membrane (GeneScreen, Dupont)

through capillary transfer overnight as described by Sambrook *et al.* (1989).

Northern Blot Analysis

Total RNA was extracted from various tissue samples from *E. guineensis* Jacq. by the method of Cheah *et al.* (1987). The integrity of the total RNA was analysed under denaturing conditions by 1.8% (w/v) formaldehyde agarose gel electrophoresis. The formaldehyde agarose gel was prepared in 1X MOPS buffer (40mM morpholinopropanesulforic acid, pH 7.0, 10mM sodium acetate, 1mM EDTA, pH 8.0) and 1.1% (v/v) formaldehyde. Samples (up to 10µg) were denatured by mixing with three volumes of 67% (v/v) formamide, 20% (v/v) formaldehyde solution, and 13% (v/v) 10X MOPS and heating at 65°C for 15min and then chilling on ice. Before loading, 0.1 volume of treated formaldehyde gel loading buffer was added to the chilled mixture. 1X MOPS buffer was used as the reservoir buffer. The RNA was electrophoresed for 2hr at 65V, followed by capillary blotting onto a nylon membrane (GeneScreen, Dupont, U.S.A). The method used was as described in Sambrook *et al.* (1989).

DNA Sequencing

Plasmid pOP-KT115 was purified by using QIAGEN-Tip 100 (Qiagen, Germany). Sequencing reactions were carried out by using the T_7 Sequenase™ Quick-Denature plasmid sequencing kit (Amersham, Life Science, U.K.) and the M13 universal or reverse primer. The sequencing was carried out with an ALFexpress DNA Sequencer (Pharmacia Biotech). The nucleotide sequences obtained were analysed by BLAST search of similar sequences from the GenBank database.

RESULTS AND DISCUSSIONS

Screening for Oleosin cDNA from Oil Palm

When the oleosin gene from maize was used to probe the membranes, 27 clones gave a strong hybridization signal. Plasmids were extracted from these clones and digested with *Eco*RI to release the inserts, which ranged from 300 to 1000bp (*Figure 1*). However, only one insert gave a distinct signal after Southern hybridization (lane 20, *Figure 2*). The insert was about 550bp. The recombinant plasmid harbouring this insert was designated pOP-KT115.

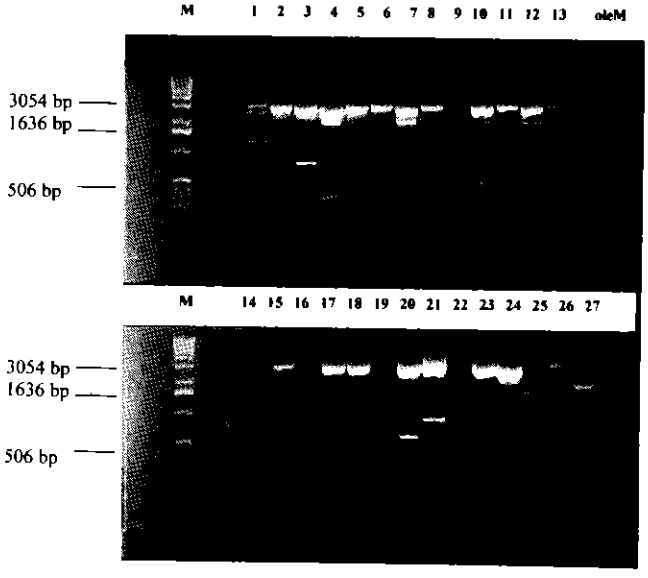

Figure 1. Ethidium bromide stained 0.8% (w/v) agarose gel of 27 recombinant clones digested with Eco*RI. 1-27: recombinant clones, M: 1kb ladder (Gibco, BRL).*

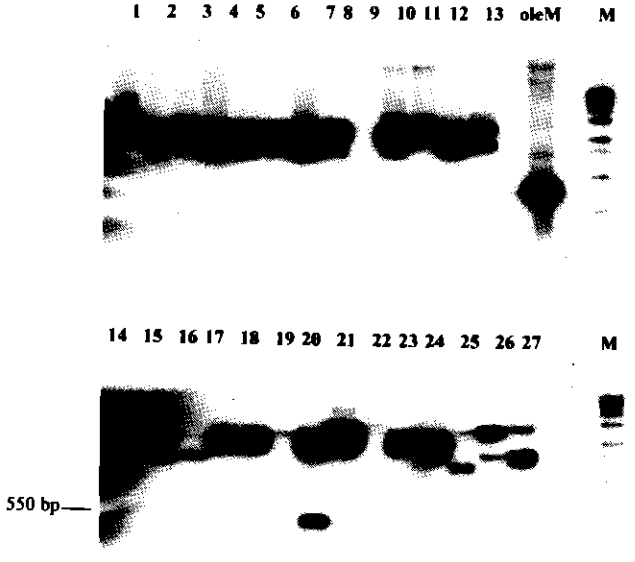

Figure 2. Results of Southern hybridization of the gel in Figure 1. *The probe used was* oleM. M: *The 1kb marker was labelled and 5ng of marker was loaded.* oleM: *5ng of probe was loaded.*

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Zm16  LVIFSPVLVPAAIALALMAAGFVTSGGLGVAALSVFSWMY
ZmL3  LVIFSPVLVPAAIALALMAAGFVTSGGLGVAALSVFSWMY
Hv    LVIFSPVLVPAAIALALMSAGFVTSGGLGVAALSVFSWMY
Os    LVIFSPVLVPAAIALALMAAGFVTSGGLGVAALSVFSWMY
pOP   LVIFSPVLVPAVIAVFLLFTGFVTSGGSGVAALSVLSWLY

Zm16  KYLTGKHPPGADQLDHAKARLASKARDIKDAAQHRIDQAQ
ZmL3  KYLTGKHPPGADQLDHAKARLASKARDIKDAAQHRIDQAQ
Hv    KYLTGKHPPGADQLDHAKARLASKARDIKDAAQTRIDQAQ
Os    KYLTGKHPPGADQLDHAKARLASKARDIKEAAQHRIDQAQ
pOP   KYLTGKRLPGSDQLDQARARTASKARDIKESAQHRSEQAQ
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Figure 3. Amino acid sequence alignment of the conserved hydrophobic region of oleosins from maize, barley, rice, and oil palm. Zm16, (Lee and Huang, 1994); ZmL3, (Vance and Huang, 1987); Hv, (Aalen, 1995); Os, (Chen et al., *1996); and pOP, amino acid sequence encoded by the insert, in plasmid pOP-KT115.*

Analysis of DNA Sequence

Nucleotide sequences of the two strands of the insert in pOP-KT115 were then determined and were deposited into the GenBank. The Accession Number is AF147758. The nucleotide sequences were subsequently compared with other oleosin gene sequences in the GenBank database by using BLAST search. The nucleotide sequences of the insert of pOP-KT115 exhibited high similarities with the corresponding regions of the oleosin genes from rice (*Oryza sativa*), barley (*Hordeum vulgare*), and maize. *Figure 3* shows the alignment of the amino acid sequence encoded by the insert of pOP-KT115 with the amino acid sequences from rice, barley, and maize.

Gene Expression

To study the expression of oleosin gene during oil palm development with respect to the insert in pOP-KT115, we carried out hybridization with total RNA isolated from various tissues harvested at different stages after anthesis. *Figure 4* shows the integrity of total RNA used for Northern hybridization. The insert of pOP-KT115 was used as probe. The insert was excised with *Eco*RI, gel-purified, and labelled. *Figure 5* shows that the probe hybridized to a 780bp mRNA only in kernel tissues

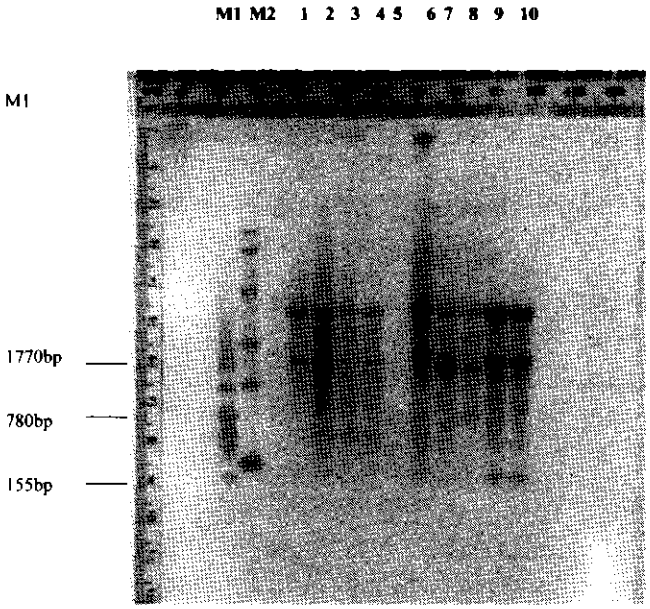

Figure 4. Ethidium bromide stained 1.8% (w/v) formaldehyde agarose gel of total RNA from various tissues of oil palm. Equal amount of RNA (10μg) was loaded in each lane. M1: RNA ladder 0.16-1.77kb (BRL), M2: RNA ladder 0.24-9.5kb (BRL), Lane 1: Germinating seedlings, 2: Leaf, 3: 8 WAA mesocarp, 4: 12 WAA mesocarp, 5: 17 WAA mesocarp, 6:10 WAA kernel, 7: 12 WAA kernel, 8: 14 WAA kernel, 9: Inflorescence F10, and 10: Inflorescence F19.

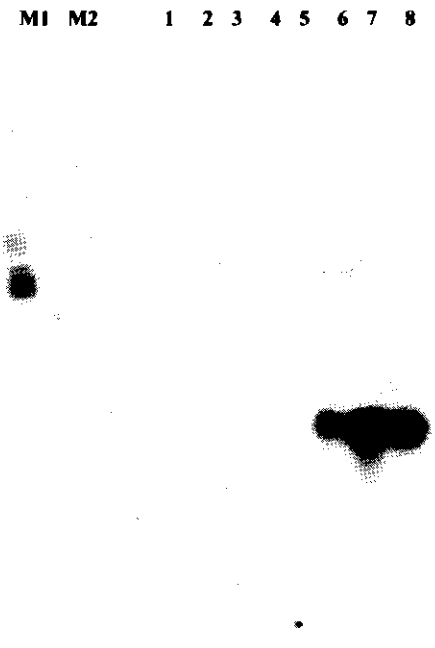

Figure 5. Results of Northern blot analysis of the gel in Figure 4. The probe used was oleM.

10,12, and 14 WAA (lanes 6 to 8). No signal was detected with total RNA accumulated in the mesocarp or any other tissue. This result corresponds well to the fact that oleosins are found only in tissues that undergo dehydration. Previous studies of oil bodies from mesocarp tissues in avocado (*Persea americana*) and olive (*Olea europaea*) did not show the presence of this protein (Ross *et al.*, 1993).

From our results, we conclude that the expression of the oleosin gene from oil palm is tissue-specific, and that the mature mRNA encoded by the gene is 780bp. This is only a partial sequence of the oleosin cDNA from oil palm. However, part of the sequence showed high similarities with sequences from maize, barley, and rice. This region corresponds to the hydrophobic domain of oleosin and is strongly conserved among all oleosins.

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