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AtDGAT. In addition, the DGAT sequences share another invariant domain IERXLKL at Ile-352 of AtDGAT and a cluster of three to four consecutive arginines within the hydrophilic beginning of the sequence [12].

A phylogenetic tree was calculated from these aligned protein sequences (Figure 2), illustrating the consistent position of CeDGAT between the vertebrate and the plant DGATs, with the highest similarity to the vertebrate DGAT family, then the plant DGAT family, then a putative DGAT from the protozoan Plasmodium falciparum. The presence of a DGAT gene in yeast is also very likely since an acyl-CoA-dependent DGAT activity is clearly measurable in this organism (Figure 1) [8,12]. Thus the DGAT gene appears to be widespread among living organisms. The yeast and animal ACATs are also included in Figure 2, which shows that ACATs and DGATs are encoded by two distinct but related groups of genes. The fact that they present highly homologous domains [12] suggests that ACAT and DGAT genes might derive from a common ancestor.

References

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Differential expression of diacylglycerol acyltransferase (DGAT) genes in olive tissues

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Abstract
Fatty acids are accumulated in triacylglycerols (TAGs), in specialized organelles of seeds named oil bodies. The major site of TAG accumulation is detected in developing seed and mesocarp of certain species. We have isolated two cDNAs encoding DGAT enzymes from olives. The deduced polypeptides differ by 26 amino acids in size. However, they have high homology and almost identical hydropathy profiles. The DGAT gene is expressed in all tissues that synthesize TAGs. However, higher levels of DGAT transcripts have been detected in seed tissues of developing olive drupe. DGAT expression and mRNA accumulation in drupe tissues is developmentally regulated. Each DGAT transcript shows a distinct profile of accumulation. The existence of two different DGAT transcripts might reflect two different enzymes with discrete function and/or localization.

Introduction
Plant seed-oil biosynthesis and accumulation is a developmentally regulated process. Fatty acids are accumulated in triacylglycerols (TAGs) in specialized seed organelles named oil bodies. The major site of TAG accumulation is detected in the developing seed and mesocarp of certain species.

Key words: embryo, mesocarp, oil biosynthesis, Olea
Abbreviations used: TAG, triacylglycerol; DGAT, diacylglycerol acyltransferase.
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Fatty acids are synthesized in the plastids of the seed and mesocarp. However, TAG biosynthesis occurs in the endoplasmic reticulum and is channelled to the growing oil body. There are three acyltransferases involved in the production of TAGs in a stepwise acylation of the glycerol backbone. The acylation of sn-1,2-diacylglycerol by diacylglycerol acyltransferase (DGAT) is the final step of the Kennedy pathway [1].

We have isolated a number of genes from olive involved in the biosynthesis and storage of fatty acids [2,3]. In order to determine factors involved in regulating the process of TAG biosynthesis and storage in the developing olive seed and mesocarp, we have isolated the DGAT gene that encodes the enzyme that catalyses the final step in the Kennedy pathway. In this paper we describe the isolation of DGAT genes from olive as well the expression and mRNA accumulation during development. The DGAT gene is expressed in all tissues that synthesize TAGs. However, high levels of DGAT transcript have been detected in seed tissues of developing olive drupe.

**Results and discussion**

Using degenerate primers based on the *Arabidopsis* DGAT sequence [4] and RNA from olive seed tissue we isolated a fragment of 333 bp and another of 258 bp. Sequence analysis showed that these fragments have high homology to DGAT genes. In order to isolate the full-length cDNA, we designed primers for 5'- and 3'-RACE (rapid amplification of cDNA ends) PCR. Two full-length cDNAs were isolated. These two cDNAs differ by about 78 bp in the middle of the sequence. The longer cDNA is 2138 bp long and contains an open reading frame of 1599 bp encoding a protein of 532 amino acids with a molecular mass of 60799 Da and a pI 7.5. The shorter cDNA is 2060 bp long and contains an open reading frame of 1527 bp encoding a protein of 509 amino acids having molecular mass of 58067 Da and a pI 6.94. The difference between the two deduced DGAT polypeptides is 26 amino acids in the middle of the gene. The genomic gene is about 10 kb, showing a large number of introns with almost 8 kb of intervening sequences. Similar organization has been observed for *Arabidopsis* and tobacco genes [4,5]. Two DGAT genes are present in the olive genome. The deduced polypeptides show considerable similarity to published sequences from plants. A putative diacylglycerol/phorbol ester-binding motif, HKWXXRHXYXP, is observed at position 425. The deduced polypeptide has multiple hydrophobic domains and an analysis by the PC GENE program predicted that the protein has six possible transmembrane regions [6].

Northern-blot analysis showed that the DGAT genes are expressed in olive tissues accumulating TAGs. However, in order to differentiate the two DGAT transcripts and to determine whether both transcripts are present in TAG-producing tissues, reverse-transcriptase PCR analysis was performed. We designed specific primers in order to either produce, after amplification, a doublet reflecting both transcripts, or to amplify specifically the longer or the shorter transcript. Total RNA was extracted from different tissues including leaves, flowers, pollen grains, developing seeds (embryos) or mesocarp. In order to determine an indicative measure of TAG biosynthesis we compared the extent of gene expression and mRNA accumulation of two genes involved in the TAG biosynthetic pathway during drupe development. The first one, enoyl-acyl carrier protein (ACP) reductase (ear), catalyses the reduction of the trans-2,3 double bond to a saturated acyl chain. This is part of the multimeric enzyme, the fatty acid synthase is localized in plastids and catalyses the initial step in fatty acid synthesis. DGAT is the enzyme that catalyses the last step in TAG biosynthesis. Each gene showed a distinct pattern of transcript accumulation. ear gene expression peaks during mid torpedo and thereafter drops to very low levels. ear gene expression in early stages of mesocarp development is medium and as growth proceeds transcripts accumulate to higher levels (Table 1). DGAT gene expression is low in the early and mid torpedo stages of embryo development. However, high levels of transcript are detected in late torpedo stages. Similarly, high levels of DGAT transcript are detected in early and mid stages of mesocarp growth. As growth proceeds, transcript levels drop. The shorter DGAT gene transcript has a different profile of mRNA accumulation to the longer gene transcript; its overall levels are lower than for the longer transcript. During embryo development the transcript level peaks at late torpedo stage but at early and mid torpedo stages the levels are low. During mesocarp growth accumulation of the the shorter transcript has a unique profile. Transcript accumulation is higher during mid mesocarp growth and decreases during early or late mesocarp. Gene expression was also
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Table I

Developmental expression of ear and DGAT genes in vegetative and reproductive tissues

+,- ++ and +++ indicate the extent of gene expression in each tissue. +/- indicates very little expression.

<table>
<thead>
<tr>
<th>Level of expression</th>
<th>ear</th>
<th>DGAT (long transcript)</th>
<th>DGAT (short transcript)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young drupes</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Early torpedo</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mid torpedo</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Late torpedo</td>
<td>+/-</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Early mesocarp</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Mid mesocarp</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Late mesocarp</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anther (young)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Mature pollen</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

detected in young drupes, anthers and mature pollen grains (Table I). Fatty acids produced by the fatty acid synthase in mesocarp and embryo tissues are mostly used by the DGAT enzymatic activity to produce TAGs. Therefore, it is reasonable to detect earlier accumulation of ear gene transcripts than of DGAT gene transcripts in embryos and mesocarp. However, in mesocarps, DGAT gene transcripts are accumulated more rapidly than the ear transcripts. Further, during late mesocarp development ear transcripts accumulate more than DGAT transcripts.

We conclude that the DGAT gene expression in olives correlates with the TAG accumulation during fruit growth. During later stages of embryo development ear gene expression and mRNA accumulation drops to undetectable levels, while DGAT expression is high, indicating that fatty acids are mostly present bound to glycerol as TAGs. Two DGAT genes are expressed that encode two different DGAT polypeptides. It is unknown whether these two proteins have the same catalytic activities or whether their activity depends on other factors. It is known that ear is also expressed in tissues that do not accumulate fatty acids or TAGs. However, in tissues that store TAGs, ear expression correlates with DGAT expression.

References


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