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Spatial and temporal expressions of two distinct oleate desaturases from olive (*Olea europaea* L.)

Georgios Banilas, Anastassios Moressis[1], Nikos Nikoloudakis, Polydefkis Hatzopoulos*

Laboratory of Molecular Biology, Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece

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Abstract

Two cDNAs, *OeFAD2* and *OeFAD6*, encoding ω-6 fatty acid desaturases, the key enzymes for the conversion of oleic into linoleic acid, were isolated from olive. OeFAD2 contains a C-terminal ER retrieval motif, whereas OeFAD6 possess a putative N-terminal plastidial signal peptide. The deduced amino acid sequence of *OeFAD2* showed higher similarities to other plant microsomal ω-6 desasurases than to OeFAD6 or plastidial orthologues, and vice versa. Southern analysis indicated that the *OeFAD2* gene is represented by one or two copies and *OeFAD6* by a single copy gene. Expression analysis by RT-PCR showed that both genes are expressed in all tissues of olive tree tested, but higher levels of mRNA accumulation were detected in reproductive organs and cells that proliferate rapidly or store lipids. The two genes exhibited distinct patterns of mRNA accumulation during olive drupe growth. *OeFAD2* was constitutively expressed, with maximum transcript accumulation in mesocarps, whereas *OeFAD6* was developmentally regulated in both endosperms and embryos. The expression patterns observed reflect the discrete roles of these genes in membrane synthesis for cell division, thylakoid development, and lipid storage or in the biosynthetic pathway for the production of signaling molecules that influence plant development or defense.

Keywords: FAD2; FAD6; Fatty acid desaturase; Olive; Spatial expression; Temporal expression

1. Introduction

Fatty acids have many and diverse roles in plants. They are major structural components of membrane lipids, provide a substantial reserve of free energy and serve as key precursors for the biosynthesis of messengers in signal transduction mechanisms that influence plant growth, development and responses to various environmental cues [1–5]. A major fraction of the fatty acids in plants are the polyunsaturated fatty acids linoleic ($18{:}2^{\Delta 9,12}$) and α-linolenic acid ($18{:}3^{\Delta 9,12,15}$). Besides their important physiological roles, these fatty acids are also crucial for human health and nutrition since they cannot be synthesized in the body and hence must be provided by the diet. Consequently, the desaturation of fatty acids is an important aspect in oil biochemistry since it determines the level of unsaturation and therefore the economic value of oils [6,7].

During lipid biosynthesis pathway, the formation of the first double bond in stearic acid (18:0) to produce the monounsaturated oleic acid ($18{:}1^{\Delta 9}$) is catalyzed by the soluble plastidial stearoyl-ACP Δ9 desaturase [8]. cDNAs corresponding to this enzyme have been isolated from many plant species, including olive [9]. Subsequently, oleic acid is incorporated into the glycerolipids either in plastids or in the endoplasmic reticulum (ER) membranes before being further desaturated by the membrane-bound ω-6 (Δ12) desaturases. In Arabidopsis two genes have been isolated, namely *FAD2* and *FAD6*, encoding the ER (microsomal) and the plastidial ω-6 desaturases, respectively [10,11]. Since then, cDNAs encoding for microsomal ω-6 desaturases have been isolated from several plant species, such as soybean [12], parsley [13], rape [14], peanut [15], sesame [16],

* Corresponding author. Tel.: +30 210 529 4321; fax: +30 210 529 4321.
E-mail address: phat@aua.gr (P. Hatzopoulos).
[1] Present address: Laboratory of Molecular Neurobiology, Institute of Molecular Biology and Genetics, BSRC "Alexander Fleming", 34 Al.Fleming Street, 166 72 Vari, Greece.

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sunflower [17], cotton [18], tung [19] and spinach [20]. cDNAs encoding for plastidial ω-6 desaturases have also been isolated from different species, including rape [21], soybean [21] and spinach [22]. These two distinct oleate desaturases also have different electron donor systems, i.e. NADH, NADH:cytochrome b_5 reductase and cytochrome b_5 for FAD2, and NAD(P)H, ferredoxin:NAD(P) reductase and ferredoxin for FAD6.

Most of the work hitherto on the isolation and characterization of genes involved in oil synthesis has been focused on seeds of oilseed crops, and relatively little is known about the respective molecular regulation in plant species bearing lipid-storing fruits, such as olive. Although the lipid content of olive oil and its nutritional or nutraceutical values have been well documented, the molecular basis of lipid biosynthesis regulation in olive fruits has only partially been explored [23,24]. Here we report on the cloning and characterization of two olive (*Olea europaea*) cDNAs encoding a microsomal and a plastidial ω-6 desaturase. Fatty acid desaturases in all organisms are subject to several types of regulation, depending on their localization or function, and there may be requirement for different or specific desaturase activities during certain developmental processes in various tissues [25]. Therefore, in order to elucidate expression patterns of these desaturases in olive, transcript accumulation levels of both genes were investigated during olive fruit development and also in different tissues of the olive tree.

2. Materials and methods

2.1. Plant materials

Floral buds, flowers and drupes at different developmental stages were harvested from self-pollinated olive trees (*O. europaea* L., cv. Koroneiki) grown in a natural environment in Agricultural University of Athens. Young expanding leaves, mature leaves, young stems, shoot tips and roots were harvested from 4-month-old seedlings of the same cultivar, grown in a growth chamber at 23 °C under 16-h photoperiod. All samples were harvested at about midday, immediately frozen in liquid nitrogen and stored at −80 °C for later RNA extraction.

2.2. RNA extraction and RT reactions

Total RNA was isolated from different olive tissues using a phenol–chloroform extraction procedure [9]. RNA concentration was determined spectrophotometrically and verified by ethidium bromide staining of agarose gel. Total RNA was then treated with Rnase-free DNAse I (Invitrogen) and about 2 μg were used as template in first strand cDNA synthesis using Superscript™ II RNase H-Reverse Transcriptase (Invitrogen), according to the manufacturer's protocol. Unless otherwise stated, the first-strand cDNA was primed off by the poly A tail with the reverse transcription primer T17XHO (5′-GTCGACCTCGAGTT-TTTTTTTTTTTTTTT-3′).

2.3. PCR amplification and cloning of olive ω-6 desaturase cDNA fragments

To amplify central cDNA fragments corresponding to olive microsomal (designated as *OeFAD2*) and plastidial (designated as *OeFAD6*) ω-6 desaturases, degenerate oligonucleotide primers were designed based on reverse translation of conserved peptide sequences of FAD2 or FAD6 from Arabidopsis and other microsomal or plastidial orthologues, respectively, and used in RT-PCR reactions. The first-strand cDNA used was synthesized from total RNA isolated from young expanding olive leaves. For *OeFAD2* gene two oligos with sense and antisense directions were used, i.e. AFAD2 (5′-GA A/G TG C/T GG C/T CA C/T CA C/T GC A/G/C/T-3′) and cDFAD2 (5′-A/G/T AT C/T GC C/T TT A/G/C/T GT A/G/C/T GC C/T TCCAT A/G/C/T GC-3′), respectively. PCR was conducted with an initial denaturation step at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 150 s at various temperatures between 55 and 63 °C, and 72 °C for 45 s. For isolating a central cDNA fragment of *OeFAD6* gene we used the forward primer FAD6A (5′-TGGTATCT G/T CT A/T CC A/G/C/T TT A/G GC A/G/C/T TGG-3′) with the reverse cFAD6C (5′-G/T GT A/G AA A/C/T GT A/G CTCATCCA A/G AA A/G TG-3′). PCR was conducted with an initial denaturation step at 94 °C for 2 min and 30 cycles of 94 °C for 30 s, 90 s at various temperatures between 52 and 58 °C, and 72 °C for 45 s. cDNA amount corresponding to 200 ng total RNA was used as template, together with 200 μM of each dNTP, 20 pmol of each oligo, 2 U of DNA polymerase (Expand™ High Fidelity, Boehringer, Mannheim) and 1× PCR buffer (provided by the manufacturer of the enzyme) in a 50 μl reaction volume. All amplifications were achieved in a PTC-200 Peltier thermal cycler (M.J. Research). PCR products were isolated from agarose gel and purified using QIAquick® Gel Extraction Kit (Qiagen), before cloned into pGEMt-easy vector (Promega) according to the manufacturers' instructions. The resultant clones were send to MWG Biotech AG (Ebersberg, Germany) for sequencing on both strands from plasmids using T7/SP6 primers.

2.4. Rapid amplification of cDNA ends (RACE)

For amplification of the unknown 3′- and 5′-ends of the above cDNA fragments, the 3′ and 5′ RACE methods were used according to protocols described elsewhere [26]. For 3′ RACE, the first-strand cDNA was primed with the T17XHO primer, as above. Based on sequence information of the central cDNA fragments specific forward primers were designed. For *OeFAD2* we used the primer 3FAD2 (5′-