Characterisation of acyl-ACP desaturases from *Macadamia integrifolia* Maiden & Betche and *Nerium oleander* L.

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Abstract

The seed oil in *Macadamia integrifolia* contains about 30% palmitoleic acid (16:1\(\Delta^9\)) and *Nerium oleander* about 12% isoricinoleic acid (\(\Delta^9\)-hydroxy-18:1\(\Delta^{12}\)). It has been shown that palmitoleic acid can be produced by acyl-acyl carrier protein (ACP) desaturases and it has also been shown that fatty acid hydroxylation can occur via direct substitution of a hydrogen atom. Therefore it seemed possible that the enzymes responsible for the making of these unusual fatty acids in *M*. *integrifolia* and *N*. *oleander* were of acyl-ACP desaturase type. Extracts from developing *M*. *integrifolia* developing seeds showed a relative ratio of 16:0-ACP to 18:0-ACP desaturation that was about 13 times higher than in sunflower seeds. *N*. *oleander* seed extracts catalysed conversion of 18:0-ACP to 18:1\(\Delta^9\) but only trace amounts of \(\Delta^9\)-hydroxy fatty acids were formed. A total of four cDNAs were isolated from developing seeds, of both species, using a fragment isolated with PCR amplification. The *M*. *integrifolia* acyl-ACP desaturase cDNA was expressed in *Escherichia coli*. A partly purified fraction of the enzyme showed a 16:0-ACP to 18:0-ACP desaturation ratio about 90-fold less than that in the *Macadamia* extracts. Expressed *N*. *oleander* acyl-ACP desaturase cDNAs showed predominantly 18:0-ACP desaturase activity and no hydroxylase activity. Thus it is not likely that any of the four acyl-ACP desaturases cloned from *M*. *integrifolia* or *N*. *oleander* is involved in the production of unusual fatty acids. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Acyl-ACP desaturase; *Macadamia integrifolia*; *Nerium oleander*

1. Introduction

Many plant enzymes that are involved in the modification of fatty acids, have been characterised and the genes encoding them cloned. Among these enzymes are the acyl-acyl carrier protein (ACP) desaturases. These typically introduce the first double bond into a saturated fatty acid that is linked to ACP. The most common of these enzymes is the \(\Delta^9\)-stearoyl-ACP desaturase which catalyses the formation of a double bond between the ninth and tenth carbon in the fatty acid chain. The first \(\Delta^9\)-stearoyl-ACP desaturase, that was cloned and expressed in transgenic cells, was the enzyme from castor bean (*Ricinus communis* L.) [1]. The three-dimensional structure of the \(\Delta^9\)-stearoyl-ACP desaturase from castor bean has been determined [2] (http://www.pdb.bnl.gov accession 1AFR). As little as eight amino acids have been identified that can determine the chain length specificity of the substrate accepted by this protein [3].

Most of the characterised acyl-ACP desaturase genes have a high level of sequence homology to the castor bean gene. This includes a number of acyl-ACP desaturases that catalyse the formation of unusual monoenes, such as 18:1\(\Delta^6\), 16:1\(\Delta^9\), 16:1\(\Delta^4\) and 14:1\(\Delta^9\), using different substrates and/or having differing regio-specificity [4–7] than the castor bean gene.
Macadamia integrifolia and Nerium oleander have seed oils containing unusual monoene fatty acids [8]. The oil from M. integrifolia seeds contains approximately 30% of 16:1\(^{\Delta 9}\) (palmitoleic acid) and N. oleander seeds contains about 12% of \(\Delta 9\)-hydroxy-18:1\(^{\Delta 12}\) (isoricinoleic acid).

Because \(\Delta 12\) hydroxylation of plant fatty acids can occur via direct substitution of a hydrogen atom, catalysed by \(\Delta 12\) desaturase-like enzymes [9,10], the possibility was considered that N. oleander contained a \(\Delta 9\)-acyl-ACP desaturase-like hydroxylase catalysing the conversion of stearic acid into \(\Delta 9\)-hydroxy-18:0. Although \(\Delta 9\)-hydroxy-18:0 is a very minor component in N. oleander seeds (less than 1% of all fatty acids), the main hydroxy fatty acid, \(\Delta 9\)-hydroxy-18:1\(^{\Delta 12}\) (isoricinoleic acid) has been shown to be synthesised efficiently in seeds by \(\Delta 12\) desaturation of \(\Delta 9\)-hydroxy-18:0 [11].

Here the possibility that the production of palmitoleic acid in M. integrifolia, and \(\Delta 9\)-hydroxy-18:1\(^{\Delta 12}\) in N. oleander were due to variant acyl-ACP desaturases was investigated.

2. Materials and methods

2.1. Plant material

All seeds used in the experiments were collected at a stage where about half the lipids found in the mature seeds had been synthesised and therefore could be expected to be close to a stage of maximum lipid synthesis. The M. integrifolia Maiden & Betch seeds (provided by Dr Cameron McConchie, Canberra, Australia) had their husks removed and were sliced immediately and frozen in liquid nitrogen. The N. oleander L. seeds (provided by Dr Manuel Mancha, Sevilla, Spain) were collected from wild plants and frozen in liquid nitrogen. N. oleander seeds with about 9% of iso-ricinoleic acid were used for the experiments. Sunflower seeds (Helianthus annus L. var. Avante) were collected from plants grown in the greenhouse. The seeds were immediately frozen in liquid nitrogen.

2.2. Chemicals

\([1-\text{14C}]\text{Palmitic (hexadecanoic) acid (specific activity 1.85–2.29 GBq/mmol) and [1-\text{14C}]oleic (9-octadecenoic) acid (specific activity 1.85–2.29 GBq/mmol) were obtained from Amersham (Buckinghamshire, UK). ACP (Escherichia coli), ferredoxin (spinach) and ferredoxin reductase (spinach) were purchased from the Sigma (St. Louis, MO). [1-\text{14C}]Acyl-ACP was synthesised enzymatically from free acid and ACP using E. coli acyl-ACP synthetase (provided by Dr John Ohlrogge, East Lansing, USA) according to Ref. [12].

2.3. Isolation of acyl-ACP desaturase cDNAs from M. integrifolia and N. oleander

cDNA libraries were constructed in Eco R1 pre-digested and dephosphorylated Lambda ZAP II vector (Stratagene) with poly(A) + RNA isolated from frozen developing seeds from M. integrifolia and N. oleander. Two degenerate primers, based on the conserved amino acid sequences in all acyl-ACP desaturases so far published (5’-GGN GAY ATG ATH ACN GAR GA-3’ corresponding to the amino acid sequence GDMITE(E/D) and 5’-ARR TAT GTY TTY TC-3’ corresponding to the amino acid sequence EKTIQY), were used to PCR amplify a portion of a M. integrifolia acyl-ACP desaturase gene using cDNA from developing M. integrifolia seeds as template. The amplified fragment was gel purified and radio-labelled by random priming with \([\text{32P}]\text{dCTP. This probe was used to screen the M. integrifolia library by hybridisation at 60°C, and washing at 60°C. Ten different clones were isolated. One of these (mac6) was used further in this study. The N. oleander library was screened with the randomly primed M. integrifolia cDNA clone insert (mac6) by hybridisation at 50°C, and the filters were then washed in 2 × SSC, 0.1% SDS, at 50°C. Three different N. oleander clones, named N1, N2 and N10, were obtained. pBlue-script phagemids were excised according to the manufacturer’s protocol and double stranded DNA plasmid produced. Both strands of the cDNAs were sequenced by fluorescent dideoxy chain termination with an ABI Prism 377 automated sequencer from PE Biosystems (Foster City, CA).

2.4. Northern blot hybridisation

Each lane was loaded with 20 µg of total RNA from leaves or developing seeds. After electrophoresis RNA was transferred onto a nylon
membrane (Hybond-N+, Amersham, Buckinghamshire, UK) by capillary blotting. DNA probes were obtained by PCR amplification of the 3' untranslated regions of each N. oleander clone. PCR fragments were purified and labelled with \([\alpha-\text{32P}]\text{dCTP}\) (Amersham, Buckinghamshire, UK) by random priming. Prehybridisation and hybridisation were done in 0.5 M NaHPO4 (pH 7.2), 7% SDS and 1 mM EDTA at 65°C. After hybridisation overnight, blots were washed in 2× SSC, 0.1% SDS for 2× 5 min and then in 0.1× SSC, 0.1% SDS at 65°C for 2× 10 min before exposure to X-ray film.

2.5. Expression of acyl-ACP cDNAs in E. coli

The mature protein coding region of the N. oleander acyl-ACP desaturase cDNA clone N1 (corresponding to amino acids 26–384) was inserted into the Nde I-Xho I site of the expression vector pET21 c (+) (Novagen). The mature protein coding regions of the clones N2 and N10 (corresponding to amino acids 34 through 386 and 32 through 394, respectively) were inserted in the Neo I-Sal I site of the expression vector pET21 d (+) (Novagen). The plasmids were transformed into the E. coli strain BL21 (DE3) (Novagen).

The mature protein coding region of the Δ9-stearoyl-ACP desaturase cDNA from castor bean (provided by Dr John Shanklin, New York, NY) (corresponding to amino acids 34–396) and the coding region of the acyl-ACP desaturase from M. integrifolia (corresponding to amino acid 34 through 396) were inserted in the Neo I-Hind III site of the expression vector pTrc 99a. These plasmids were introduced into the E. coli strain JM 105.

Transformed E. coli cells were grown at 37°C to an \(A_{600} = 0.6\) whereafter isopropyl thio-β-galactoside (Saveen, S2140) was added to a concentration of 1 mM and the incubation continued for an additional 4 h.

2.6. Enzyme preparation

Tissue (0.3 g) from M. integrifolia, N. oleander and sunflower developing seed were homogenised with Ultra-turrax T25 (Janke & Kunkel, Staufen, Germany) in 3 ml of ice-cold medium (0.1 M Tris–HCl, pH 7.5, 1 mM dithiothreitol, 1 mM ascorbate, 10% glycerol and 1.5% polyvinyl-polypyrrolidone). The homogenate was centrifuged for 20 min at 10 000 × g, the supernatant between the fat layer and the pellet was removed, and used immediately in enzyme assays or stored in −80°C until used.

Transformed E. coli cells were harvested by centrifugation, washed in 50 ml of 40 mM Tris–HCl, pH 8.0, resuspended in 30 mM Tris–HCl, pH 8.0, 20% glycerol, 10 mM EDTA (80 ml/g wet weight) and treated with 0.5 mg lysozyme/ml suspension. After centrifugation, the pellet was resuspended in distilled water (10 ml/g of pellet) and sonicated for 4×15 s on ice. Deoxyribonuclease I (250 μg/g of original pellet) and Tris–HCl, pH 8.0 were added to a final concentration of 40 mM. The sample was centrifuged at 200 000 × g for 30 min and the supernatant loaded onto a DEAE-Sephacel column (Pharmacia, Uppsala, Sweden) that was equilibrated with 40 mM Tris–HCl, pH 8.0 at a run rate of 1 ml/min. The enzyme was eluted with a NaCl-gradient (0–1 M in 40 mM Tris–HCl, pH 8.0) at a rate of 0.01 M/min using a FPLC system (Pharmacia, Uppsala, Sweden). The desaturases eluted between 35 and 85 mM of NaCl. These fractions were pooled and used in the enzyme assays.

2.7. Acyl-ACP desaturase assays

Acyl-ACP desaturation assays in the seed extracts were performed in a total volume of 150 μl with 33 mM PIPES, pH 6.0, 3 mM ascorbate, 0.1 mg bovine serum albumin, 1 mM dithiothreitol, 1.2 mM NADPH, 25 milliunits spinach ferredoxin-NADP+ reductase, 15 μg spinach ferredoxin and 3000 U of bovine liver catalase. [1-\(^{14}\text{C}\)] acyl-ACPs were added at concentrations as indicated in Table 2. The assay, run at room temperature, was initiated by the addition of the enzyme and terminated by the addition of 0.5 ml of 2.35 M NaOH.

2.8. Analytical procedures

The lipids in the assay mixture were saponified at 90°C for 60 min. The resulting free fatty acids were extracted into chloroform by acidification of the mixture with 0.5 ml 3 M HCl followed by addition of 2.25 ml CHCl₃/MeOH/HAc (50:50:1 v/v/v). The CHCl₃-phase was removed and evaporated under \(N_2\). Fatty acids were then methylated in 2 ml of 4% (v/v) methanolic HCl for 60 min at
Table 1
Fatty acid composition of mature macadamia and nerium seeds

<table>
<thead>
<tr>
<th>Acyl group</th>
<th>Fatty acid distribution (wt.%)</th>
<th>Macadamia seeds</th>
<th>Nerium seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.8</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>9.4</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>16:1</td>
<td>30.8</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>2.2</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>18:1[^{1}]</td>
<td>49.6</td>
<td>29.2</td>
<td></td>
</tr>
<tr>
<td>(\Delta 9)-hydroxy-18:1[^{1}]</td>
<td>–</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>2.5</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>0.1</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>1.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>20:1</td>
<td>1.8</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>–</td>
<td>2.9</td>
<td></td>
</tr>
</tbody>
</table>

2.9. Transformation of Arabidopsis thaliana

Binary vector constructs were made in the host plasmid pGPTV-Kan [14]. The full length cDNA from *M. integrifolia* was placed downstream of a 309 bp fragment of the napin promoter [15]. This construct was transformed into *Agrobacterium tumefaciens* (pGV2260) and these bacteria used to transform *A. thaliana* Columbia (C-24) [16].

3. Results and discussion

The fatty acid composition of *M. integrifolia* and *N. oleander* seeds was determined. Fatty acid analysis of *N. oleander* seeds showed that iso-ricinoleic acid increased, from levels that were just detectable at an early stage of development to about 12% of total fatty acids in mature seeds. The oil from mature *M. integrifolia* seeds consisted of 31% of palmitoleic acid (Table 1).

To identify the enzymes responsible for the production of palmitoleic acid in *M. integrifolia*, and the hydroxy fatty acids in *N. oleander*, the desaturation of acyl-ACP substrates in extracts of developing seeds of *M. integrifolia* and *N. oleander* were studied. Extracts from developing seeds of sunflower, a tissue that do not contain any unusual fatty acid, were used as a comparison. The desaturation of 18:0-ACP, based on activity per mg of tissue, was an order of magnitude higher in developing sunflower seed extracts than in *M. integrifolia* seed extracts (Table 2). The relative ratio of 16:0–18:0 desaturation was about 13 times higher in *M. integrifolia* than in sunflower (Table 2). No desaturase activity was detected.

90°C. The methyl esters were extracted into hexane and analysed with TLC using silver nitrate impregnated TLC plates (Merck, silica 60) with hexane/diethylether/acetic acid (85:15:1 v/v/v). The relative distribution of radioactivity in saturated and monoene fatty acid methyl esters was determined by monitoring the TLC plates with an electronic radioactivity imager (Instant Imager, Packard, Meriden, USA). *R*\(_f\) values for saturated methyl esters, monoene and \(\Delta 9\)-hydroxy-18:0 were 0.52, 0.45 and 0.10, respectively.

For GLC analysis, lipids were extracted from seed material into chloroform according to Bligh and Dyer [13] and methylated in 4% (v/v) methanolic HCl. The methyl esters were separated by GLC on a CP-Wax58-CB fused-silica column (Chrompack, Middelburg, The Netherlands).

Table 2
Reaction rates of the acyl-acyl carrier protein (ACP) desaturases in macadamia and sunflower seed extract and *E. coli* (JM 105) transformed with the acyl-ACP desaturase cDNAs from *M. integrifolia* and *R. communis*

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Substrate</th>
<th>16:0-ACP (desaturation rate)</th>
<th>18:0-ACP (desaturation rate)</th>
<th>Ratio (16:0/18:0 desaturation rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>67 nM</td>
<td>267 nM</td>
<td>67 nM</td>
<td>267 nM</td>
</tr>
<tr>
<td>Sunflower extract</td>
<td>0.033(^[a])</td>
<td>0.076(^[a])</td>
<td>0.495(^[a])</td>
<td>2.08(^[a])</td>
</tr>
<tr>
<td>Macadamia extract</td>
<td>0.037(^[a])</td>
<td>0.089(^[a])</td>
<td>0.041(^[a])</td>
<td>0.165(^[a])</td>
</tr>
<tr>
<td>Macadamia desaturase</td>
<td>0.913(^[b])</td>
<td>1.925(^[b])</td>
<td>93.75(^[b])</td>
<td>281.59(^[b])</td>
</tr>
<tr>
<td>Ricinus desaturase</td>
<td>0.324(^[b])</td>
<td>1.188(^[b])</td>
<td>100.67(^[b])</td>
<td>368.45(^[b])</td>
</tr>
</tbody>
</table>

\(^[a]\) Units: pmol/min per mg tissue.

\(^[b]\) Units: pmol/min per mg protein.
with 14:0-ACP as substrate. Extracts from frozen *N. oleander* developing seeds could readily desaturate \(^{[14C]}18:0\)-ACP, but only traces of radioactivity resided in compounds migrating as \(\Delta 9\)-hydroxy-18:0 and \(\Delta 9\)-hydroxy-18:1\(^{12}\) on silver nitrate impregnated thin layer chromatography plates (data not shown).

Although the biochemical evidence was not conclusive for the involvement of acyl-ACP desaturase type enzymes in unusual monoene fatty acid biosynthesis in *M. integrifolia* and *N. oleander*, it was decided to clone acyl-ACP desaturase genes from these plants in order to better characterise the individual enzymes. cDNA libraries were prepared from developing seeds. The *M. integrifolia* library was screened by hybridisation with a cloned PCR fragment (Section 2), that encoded a portion of an acyl-ACP desaturase from *M. integrifolia*. Amongst those clones analysed, a clone encoding an acyl-ACP desaturase was isolated and fully sequenced. Partial DNA sequencing of ten different clones suggested that clones with this sequence were the only or predominant mRNAs, that encoded acyl-ACP desaturase, expressed in developing *M. integrifolia* seeds. However, the possibility that other acyl-ACP genes are expressed, that have a low level of sequence homology to the commonly observed acyl-ACP desaturase, or that are expressed at a much lower level, in this tissue cannot be excluded. The homology expressed as sequence identity between the open reading frame of the *M. integrifolia* cDNA and castor bean 18:0-ACP desaturase was 88% (Fig. 1). This demonstrates that the acyl-ACP desaturases are highly conserved although *M. integrifolia* belongs to the Proteaceae family, one of the world’s most primitive flowering plant families [17].

The *M. integrifolia* acyl-ACP desaturase cDNA was expressed in *E. coli* and a total extract was assayed with 16:0-ACP and 18:0-ACP as substrate. A high level of desaturation activity could be demonstrated with \(^{[14C]}18:0\)-ACP, but desaturation of \(^{[14C]}16:0\)-ACP was only observed when high concentrations of 16:0-ACP were used. The addition of extract, from non-transformed *E. coli*, to seed extracts of *M. integrifolia* substantially decreased desaturation of added 16:0-ACP, but did not effect the desaturation of \(^{[14C]}18:0\)-ACP (data not shown). Since it is known that *E. coli* contains appreciable amounts of 16:0-ACP, but little 18:0-ACP [18], it is likely that dilution of the radioactive 16:0-ACP was responsible for the low activity toward the \(^{[14C]}16:0\)-ACP substrate in *E. coli* extracts. Therefore the acyl-ACP desaturase activity was partially purified from the recombinant *E. coli* extract. The purified enzyme had about a 100-fold higher activity with 18:0-ACP than with 16:0-ACP desaturase (Table 2). This corresponded to a 3-fold higher ratio of 16:0–18:0 desaturation when compared to the partially purified recombinant castor bean acyl-ACP, but about a 90-fold decreased ratio when compared to the *M. integrifolia* seed extract (Table 2).

The structure of the *M. integrifolia* acyl-ACP desaturase was computer modelled. This modelling was based on the crystal structure of the castor bean acyl-ACP desaturase [2]. The resultant model did not suggest that the enzyme would have unusual substrate specificity, although a slightly wider ‘pocket’ for the acyl chain was predicted compared to the castor enzyme (data not shown). The *M. integrifolia* gene was transformed into *A. thaliana* under the control of the napin promoter. T\(_1\) seeds (derived from the primary transformant line) were analysed for altered fatty acid composition. No elevated levels of 16:1\(^\alpha\) or 18:1\(^\alpha\) were observed in any of the pooled seed samples, although some plants had seeds which showed much elevated levels of 18:0 and 20:0, an effect likely to be due to co-suppression of the endogenous acyl-ACP desaturase gene. It is concluded from these experiments that the high levels of palmitoleic acid observed in *M. integrifolia* seeds is probably not due to the activity of the acyl-ACP desaturase gene which have been cloned.

The *M. integrifolia* acyl-ACP desaturase was used as a probe to screen a cDNA library prepared from developing seeds of *N. oleander*. Three different cDNA clones (N1, N2 and N10) which encoded putative acyl-ACP desaturases were isolated (Fig. 1). Northern blot analysis indicates that of these three genes the N2 gene was the most highly expressed in developing *N. oleander* seeds (Fig. 2). The N1 and N2 clones were truncated in the 5' end. The N1 and N10 sequences had a high degree of sequence identity to the castor bean acyl-ACP desaturase (86 and 83%, respectively). The clone N2 had only 63% identity with the castor bean enzyme, but had 76% identity with the acyl-ACP desaturase from milkweed (*Asclepias syriaca* L.). Computer modelling of the three di-
Fig. 1. Comparison of the amino acid sequences of the castor, cat’s claw, milkweed, macadamia and the three nerium acyl-acyl carrier protein (ACP) desaturase-like enzymes. Identical residues are shaded. The cleavage sites for expression of the genes in *E. coli* are indicated by arrows. These represent the cleavage sites for the plastid transit peptide, based on similarities with other acyl-ACP desaturases except for the nerium 2 and milkweed enzymes. The sequences of these two enzymes are too divergent, in this area, for such alignment. The two degenerate primers used for PCR amplification of a portion of *M. integrifolia* acyl-ACP gene are marked with a thick line.

The dimensional structure of the *N. oleander* desaturases did not suggest any major divergence in structure from the castor bean desaturase; suggesting that they all had similar substrate specificity and catalytic functions. When the three *N. oleander* acyl-ACP desaturase clones were expressed in *E. coli* and the total extracts from these assayed, they showed activities similar to the castor bean enzyme, i.e. they were predominantly 18:0-ACP desaturases with no hydroxylase activity (data not shown). Integral membrane-bound desaturase-like enzymes in plants have been shown to catalyse a variety of catalytic functions, such as hydroxylation [9] epoxidation and acetylenation [20]. The soluble acyl-ACP desaturase and membrane-bound desaturases share biochemical similarities and the membrane bound-desaturases are, like the soluble acyl-ACP desaturases likely to use a diiron centre for catalysis [21]. It is not likely that any of the three *N. oleander* acyl-ACP desaturases cloned in this study are involved in the production of Δ9-hydroxy fatty acids. Thus, the existence of soluble acyl-ACP desaturase-like enzymes with catalytic functions other than desaturation have yet to be shown.
The seeds of cat’s claw (Doxantha unguis-cati L.) and milkweed are rich in palmitoleic acid (16:1\[^{A9}\]) and 18:1\[^{A11}\]. The latter fatty acid is likely to be formed by elongation of 16:1\[^{A9}\]. A cDNA encoding an acyl-ACP desaturase was cloned from cat’s claw, and shown to be 85% identical in amino acid sequence to the castor bean \(\Delta^9\)-18:0-ACP desaturase [5]. This desaturase, when expressed in \(E.\ coli\), had higher catalytic activity towards 16:0-ACP than 18:0-ACP and was considered to be the main enzyme responsible for the production of palmitoleic acid and 18:1\[^{A11}\] in this plant. A single amino acid change could account for the different substrate specificity between the cat’s claw desaturase and the castor bean \(\Delta^9\)-18:0-ACP desaturase [5]. Thus, in contrast to \(M.\ integrifolia\), the accumulation of palmitoleic acid and 18:1\[^{A11}\] in cat’s claw is likely due to an acyl-ACP desaturase that is very homologous to the castor bean desaturase. An acyl-ACP cDNA, cloned from milkweed [19], was shown to encode a divergent acyl-ACP desaturase (61% identity with the castor bean desaturase) which, when produced in \(E.\ coli\), had a 10-fold higher specificity for 18:0-ACP than 16:0-ACP. However, this milkweed enzyme, like the \(M.\ integrifolia\) acyl-ACP desaturase, had a ratio of 16:0–18:0 desaturation that was higher than that of the castor bean enzyme. Thus, there is compelling evidence that the high 16:0-ACP desaturase activity of the acyl-ACP desaturase in cat’s claw is responsible for the production of 16:1\[^{A9}\] in this plant [5]. However, it is less likely that the acyl-ACP desaturases identified in milkweed [19] and \(M.\ integrifolia\) are directly responsible for the production of palmitoleic acid in the seeds of these plants. If a \(\Delta^9\)-16:0-ACP-specific acyl-ACP desaturase is primarily responsible for palmitoleic acid production in \(M.\ integrifolia\) seeds, its structure is perhaps considerably divergent from known acyl-ACP desaturases and was not identified using our cloning strategy.

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**References**


Fig. 2. Northern blot analysis of total RNA from \(N.\ olerander\) leaves (L) and developing seeds (S) hybridised with 3’ untranslated probes from nerium acyl-acyl carrier protein (ACP) desaturase cDNAs N1, N2 and N10.


