Identification of Δ5-Desaturase from *Mortierella alpina* by Heterologous Expression in Bakers’ Yeast and Canola*

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A DNA fragment with homology to Δ6-desaturases from borage and cyanobacteria was isolated after polymerase chain reaction amplification of *Mortierella alpina* cDNA with oligonucleotide primers corresponding to the conserved regions of known Δ6-desaturase genes. This fragment was used as a probe to isolate a cDNA clone with an open reading frame encoding 446 amino acids from a *M. alpina* library. Expression of this open reading frame from an inducible promoter in Saccharomyces cerevisiae in the presence of various substrates revealed that the recombinant product had Δ5-desaturase activity. The effects of growth and induction conditions as well as host strain on activity of the recombinant Δ5-desaturase in *S. cerevisiae* were evaluated. Expression of the *M. alpina* Δ5-desaturase cDNA in transgenic canola seeds resulted in the production of taxoleic acid (Δ5,9–18:2) and pinolenic acid (Δ5,9,12–18:3), which are the Δ5-desaturation products of oleic and linoleic acids, respectively.

The long-chain polyunsaturated fatty acids (LC-PUFA) play important roles in adult as well as infant nutrition because they serve as precursors of eicosanoids including prostaglandins and leukotrienes. They are also necessary for membrane structure and function, regulation of cholesterol metabolism and infant brain development (1). There are two main families of LC-PUFAs, the n-6 and n-3 families, which are synthesized through an alternating series of desaturations and elongations beginning with either linoleic acid (LA, Δ9,12–18:2) or α-linolenic acid (ALA, Δ9,12,15–18:3), respectively (Fig. 1). The major end point of the n-6 pathway in mammals is arachidonic acid (AA, Δ5,8,11,14–20:4) and major end points of the n-3 pathway are eicosapentaenoic acid (Δ5,8,11,14,17–20:5) and docosahexaenoic acid (Δ4,7,10,13,16,19–22:6). Although in most cases animals contain the enzymatic activity to convert LA and ALA to LC-PUFAs, they lack the Δ12- and Δ15-desaturase activities necessary to convert oleic acid (Δ9–18:1) to LA and ALA. Thus, LA and ALA are considered essential fatty acids in the human diet. There are instances, however where the rate of conversion of LA and ALA to LC-PUFAs is limiting (1) and thus there is an interest in dietary supplementation with some or all of these LC-PUFAs.

LC-PUFAs are widely distributed in nature (1, 2). γ-Linolenic acid (Δ6,9,12–18:3) can be found in seeds of plants such as borage, evening primrose, and blackcurrant as well as fungi such as *Mucor* and *Mortierella* spp., and blue-green algae (Spirulina, Synechocystis). AA can be found in significant amounts in animal liver and adrenal gland, and is also produced in microorganisms such as *Mortierella alpina* and *Porphyridium cruentum* (2). Eicosapentaenoic acid and docosahexaenoic acid can be found in fish oil and other marine organisms. None of these sources is particularly well suited for use in human nutrition, however. The species of plants that contain γ-linolenic acid are not agronomically suitable for large scale cultivation; growth of LC-PUFA-containing microorganisms is not economically attractive. The ability to produce these valuable LC-PUFAs in an easily cultured, edible microorganism would be of considerable interest. Alternatively, since plant oils are currently the largest source of polyunsaturated fatty acids in the human diet, modification of the fatty acid biosynthetic pathway to produce LC-PUFAs in an oilseed crop would also provide an economical, renewable source of these important fatty acids.

Production of polyunsaturated fatty acids in plants is catalyzed by membrane-bound desaturases that act on glycerolipid substrates in both the plastid and endoplasmic reticulum (3). Commercial oilseed crops such as canola contain only Δ12- and Δ15-desaturases; no Δ6- or Δ5-desaturation-derived PUFA are present in the oil. Genetic techniques have led to the identification of the genes encoding the Δ12- and Δ15-desaturases from *Arabidopsis thaliana* (4, 5). Based on sequence similarities, several examples of cDNAs and genes encoding these enzymes have been cloned from other higher plant species. An ω-3-desaturase has recently been cloned from *Caenorhabditis elegans* (6). Δ6-Desaturase clones have been identified in cyanobacteria (7, 8), borage (9), and *C. elegans* (11). A human gene with homology to membrane fatty acid desaturases has been identified (12), although desaturase activity has not yet been demonstrated for this clone. Based on analogy to the higher plant enzymes, these desaturases are believed to be membrane-bound and require cytochrome δs and cytochrome b5 reductase for activity. The most striking feature of these desaturase amino acid sequences is the presence of three histidine-rich regions that are conserved both in sequence and spacing relative to one another (8, 9). Although various Δ12-, Δ15-, and Δ6-desaturases needed for synthesis of LC-PUFAs have been cloned, a gene encoding the Δ5-desaturase activity necessary for AA production has never been isolated. As the last step in the production of AA, the Δ5-desaturase is also
critical for the production of the eicosanoids derived from AA, which play roles in regulating nearly all physiological activities.

Several strains of filamentous fungi of the genus Mortierella produce significant amounts of AA (13). Large scale fermentation and culture conditions for \textit{M. alpina} have been optimized, with AA content reaching 30–50% of total fatty acids (14). \textit{M. alpina} mutants for \(\Delta 12\)-, \(\Delta 6\)-, and \(\Delta 5\)-desaturases have been isolated and characterized (15). Hence, this microbe was selected as the source of \(\Delta 5\)-desaturase. Bakers’ yeast (\textit{Saccharomyces cerevisiae}), which is incapable of producing LC-PUFA endogenously because of the absence of \(\Delta 12\)-, \(\Delta 15\)-, and \(\Delta 6\)- and \(\Delta 5\)-desaturases, was used as the host for expression of the fungal \(\Delta 5\)-desaturase cDNA. In this work we describe the isolation of a desaturase-like sequence from \textit{M. alpina} and demonstrate its ability to produce \(\Delta 5\)-unsaturated fatty acids in bakers’ yeast and transgenic canola seeds.

**EXPERIMENTAL PROCEDURES**

**Strains and Growth Conditions**—\textit{M. alpina} ATCC22221 was grown in PD medium (Difco). Cultures were inoculated on PDA plates and grown at room temperature. When plates were completely covered, the fungal growth was peeled off and cut into 1–2-cm squares. These squares were used to inoculate 1 liter of potato dextrose broth and fungal growth was peeled off and cut into 1–2-cm squares. These

**PCR Amplification and Cloning of \textit{M. alpina} Desaturase Sequence**—

Degenerate oligonucleotides were designed for use as PCR primers. The forward primer contained all possible codons corresponding to the conserved amino acid sequence HHTYTN and had the following sequence: 5’-CAUAUCACAUACUACUCAUCAT/CT/CA/CAT/CACU/TTAC/GAAACACAT-AT-3’. The reverse primer contained all possible codons corresponding to the conserved amino acid sequence HHLFP and had the following sequence: 5’-CAUAUCACAUACUACUCAUCAT/CT/CA/CAT/CACU/TTAC/GAAACACAT-AT-3’. Template for PCR was generated by reverse transcription of 5 µg of total \textit{M. alpina} RNA using Superscript reverse transcriptase (Life Technologies, Inc.) and the primer 5’-CAACAGCTTCTGCGAGGAGCTCGTCTTTTTTTTTTTTCC-3’. PCR amplification was conducted in a PTC-200 thermal cycler (MJ Research) in 50 mM Tris-Cl, pH 8.5, 15 mM (NH₄)₂SO₄, 2 mM MgCl₂. Samples were subjected to an initial denaturation step of 95 °C for 5 min and then held at 75 °C, while 0.2 unit of AmpliTaq DNA polymerase (Perkin-Elmer) was added. Amplification took place using cycles of 1 min at 94 °C, 1.5 min at 45 °C, and 2 min at 72 °C. Following amplification, PCR products were subcloned into pAMPl using the CLONEMAP system from Life Technologies, Inc. The PCR clones were used as probes with \textit{M. alpina} cDNA library to identify full-length cDNA clones.

The full-length cDNA from \textit{M. alpina} encoding desaturase-like gene in pZL1 (Life Technologies, Inc.) was amplified using PCR with the forward primer RO-145 (5’-ATCTGAGCTCTTGGAAACGACAAA-GGAAAAACCTTCT-3’) and the reverse primer RO-146 (5’-TTCAAGAG-ATTCTACTCTCTTCTGGGAGGATCCAG-3’) homologous to the beginning and end, respectively, of the open reading frame in the longest cDNA clone (see "Results"). The HindIII site (underlined) in the forward primer was placed just upstream of the translational initiation codon (shown in boldface), and the EcoRI site (underlined) was placed immediately downstream of the termination codon (complementary bases shown in boldface) of this open reading frame. The HindIII/EcoRI gene fragment was cloned in yeast expression vector pYES2 (Invitrogen, San Diego, CA) downstream of the GAL1 promoter, and the clone was designated pCGR4. Transformation of \textit{S. cerevisiae} was done using a PEG/LiAc protocol (19). Transformants were selected by plating on synthetic medium plates lacking uracil (16) and grown overnight in minimal medium containing 2% glucose and lacking uracil at 30 °C. Two to four ml of the overnight culture were used to inoculate 100 ml of minimal medium containing 2% glucose and dihomo-\(\gamma\)-linolenic acid (DGLA, \(\Delta 8,11,14\)-20:3) or other fatty acid substrates but lacking uracil. Each culture was grown at 30 °C and induced with 2% galactose at 15 °C for 48–52 h (unless otherwise indicated) and subsequently harvested by centrifugation. Cell pellets were washed once with sterile doubled-distilled H₂O to remove the medium and used for fatty acid analysis. The host strain transformed with vector alone was used as a negative control in all experiments.

For cloning in plant expression vectors, the coding region of the \textit{M. alpina} desaturase gene was PCR-amplified using the following primers: 5’-ATCTGAGCTCTTGGAAACGACAAA-GGAAAAACCTTCT-3’ and 5’-CAUAUCACAUACUACUACUACAT/CT/CA/CAT/CACU/TTAC/GAAACACAT-AT-3’. These primers introduced XhoI cloning sites (underlined) upstream and downstream of the start and stop codons (in boldface), respectively. The PCR product was subcloned into pAMPl (Life Technologies, Inc.), and the open reading frame was verified by sequencing of both strands. The XhoI fragment containing the desaturase coding region was inserted into the napin expression cassette, pCGN2223 (20), that had been linearized with SalI. The HindIII fragment of the resulting plasmid containing the napin 5’-regulatory region, \textit{M. alpina} desaturase coding region, and napin 3’- regulatory region was inserted into the HindIII site of pCGN1557 (21) to create pCGN5530. The plasmid pCGN5530 was digested with HindIII and religated at a high DNA concentration to produce a tandem expression construct, pCGN5531. This construct contains two copies of the napin/\textit{M. alpina} desaturase gene fusion oriented in the same direction with respect to transcription of the nptII selection gene. pCGN5531 was transferred to Agrobacterium tumefaciens EHA105 and used to transform Brassica napus cv. LP004 as described by Radke et al. (22).

**Fatty Acid Analysis**—Fatty acid contents of yeast cells expressing \textit{M. alpina} desaturase gene, in the presence of various substrates, were analyzed. The rate of conversion of substrate to product was considered to be directly proportional to the expression/activity of a given desaturase in the yeast cell assay system. Washed yeast pellets were vortexed with 15 ml of methanol. After addition of approximately 100 µg of tridecanoin and 29 ml of chloroform, the mixture was incubated for at least 1 h at room temperature or at 4 °C overnight. The mixture was transferred to a 250-ml separatory funnel. The ethyl ester layer was rinsed twice with 5 ml of water to recover residual material and added to the separatory funnel. The lipids segregated to the lower chloroform layer were filtered through a Whatman filter with 1 go of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated to dryness at 40 °C under a stream of nitrogen. Triglycerides and total phospholipids were separated by thin-layer chromatography.
regions containing histidine residues (His boxes) conserved among acyl-lipid desaturases and hydroxylases are overlined. *M. alpina* of N terminus of the deduced amino acid sequence of *D*. Conserved amino acids are boxed.

Isolation of a *M. alpina* Desaturase Sequence—In order to isolate various *M. alpina* desaturase sequences we designed degenerate oligonucleotide primers for use in PCR. Alignment of the amino acid sequences of known acyl lipid desaturases reveals three main regions of similarity, including 8 conserved histidine residues (Fig. 2A) (10). PCR amplification of *M. alpina* cDNA using degenerate primers corresponding to the sequences HHTYTN found in the second His box of cyanobacterial Δ6-desaturases (amino acid residues 127–132 of the *Synechocystis* Δ6-desaturase (Fig. 2A) (7, 8)) and HHLPF found in the 3rd His box of many desaturases (amino acid residues 306–310 of the *Synechocystis* Δ6-desaturase and 308–312 of the *Arabidopsis* Δ15-desaturase (Fig. 2A) (10) resulted in the production of a 550-base pair fragment. Comparison of the deduced amino acid sequence of this fragment to protein sequence data banks indicated homology to the Δ6-desaturases from *Synechocystis* spp. and *Spirulina* spp. The PCR fragment was used as a probe to isolate corresponding cDNA clones from a library. The longest cDNA isolated, designated pCGN5521, was 1481 base pairs and contained an open reading frame encoding 446 amino acids. The sequence of the PCR-derived probe was entirely contained within pCGN5521. BLAST analysis of the deduced amino acid sequence of pCGN5521 revealed significant matches to the cyanobacterial Δ6-desaturases, however the sequence can be aligned with other membrane-bound desaturases as well (Fig. 2A). The *M. alpina* sequence contains the three His boxes found in other membrane-bound desaturases, but the third HXXHH motif is changed to QXXHH in this clone. This change to Q residue in the motif is also found in Δ6-desaturases from *Synechocystis* spp. and borage. Fig. 2B shows the alignment of the N-terminal sequences of the *M. alpina* and borage desaturases with those of cytochrome b₅ proteins from a variety of species. Similar to the borage clone, this desaturase-like sequence from *M. alpina* contains an N-terminal cytochrome b₅ domain not found in cyanobacterial Δ6-desaturase sequences. Analysis of the hydrophobicity profile of the protein encoded by pCGN5521 using TopPred II (24) predicts multiple transmembrane stretches as would be expected for an integral membrane protein.

**FIG. 2. Alignment of deduced amino acid sequence of *M. alpina* Δ5-desaturase with other membrane-bound desaturase sequences.** Conserved amino acids are boxed; identical residues are shaded in black. A, sequence alignment of regions of the deduced *M. alpina* Δ5-desaturase with Δ6-desaturases from *Synechocystis* PCC6803 (7) and borage (9), Δ12-desaturase (4), and Δ15-desaturase (5) from *A. thaliana*. The three regions containing histidine residues (His boxes) conserved among acyl-lipid desaturases and hydroxylases are overlined. B, sequence alignment of N terminus of the deduced amino acid sequence of *M. alpina* Δ5-desaturase to regions of the borage Δ6-desaturase (9), and cytochrome b₅ sequences from yeast (31), tobacco (32), and human (33).
determine \( \Delta 5 \)-desaturase activity. With linoleic acid as the added substrate, conversions to \( \gamma \)-linolenic acid and ALA were monitored to indicate \( \Delta 6 \)-desaturase and \( \Delta 15 \)-desaturase activities, respectively. AA was also used as a substrate to monitor conversion to eicosapentaenoic acid to determine if the clone exhibited the \( \Delta 17 \)-desaturase activity. In addition, conversion of endogenous oleic acid in this strain to LA in the absence of any added substrate was monitored as an indication of \( \Delta 12 \)-desaturase activity. The results in Fig. 3 show the accumulation of a new fatty acid in the lipid fraction of yeast containing pCGR4 grown in the presence of exogenous DGLA, but not in yeast strain containing the vector pYES2 (Fig. 3, panels A and B). The retention time (22.32 min) of this new fatty acid was identical to that of authentic AA. The GC-MS analysis indicates that this compound has a mass peak at \( m/z = 318 \) (the expected molecular ion for 20:4), and a fragmentation pattern identical to that of the authentic \( \Delta 5,8,11,14-20:4 \) standard (Fig. 4). When incubated with other exogenous fatty acid substrates, SC334(pCGR4) strain did not exhibit any \( \Delta 6 \), \( \Delta 12 \), \( \Delta 15 \), or \( \Delta 17 \)-desaturase activity (data not shown). Therefore, it was concluded that the cloned fragment of cDNA from \( M. \) alpina expressed a \( \Delta 5 \)-desaturase activity in yeast.

Table I shows that 6% of the total fatty acids in SC334(pCGR4) was AA when DGLA was supplied exogenously under the conditions described above. No accumulation of AA above the background level was observed in the negative control strain, SC334(pYES2). In addition, DGLA was incorporated in significant quantities in the lipid fraction of this recombinant yeast strain. When the distribution of DGLA and AA formed in the major lipid fractions in SC334(pCGR4) grown in the presence of DGLA were examined, approximately 80% of AA produced by \( \Delta 5 \)-desaturase and 30% of incorporated DGLA accumulated in the phospholipid fraction (data not shown).

When grown in the absence of exogenous substrate (i.e. DGLA), a new fatty acid was detected in the GC-FAME profile of SC334(pCGR4) compared with that of SC334(pYES2) (Fig. 5A). GC-MS analysis indicates this fatty acid has a mass peak at \( m/z = 294 \) (the expected molecular ion of 18:2), and a fragmentation pattern very similar to those of \( \Delta 6,9-18:2 \) and \( \Delta 9,12-18:2 \) (Fig. 5B). However, this new fatty acid has a retention time at approximately 18 min (Fig. 5A), which was different from that of \( \Delta 6,9-18:2 \) (16.89 min) and that of \( \Delta 9,12-18:2 \) (17.28 min). These findings suggest that the new fatty acid, which could be detected only in SC334(pCGR4) yeast expressing the \( \Delta 5 \)-desaturase activity, was an isomer of 18:2 and most likely the \( \Delta 5,9-18:2 \) or taxoleic acid.

Expression of \( M. \) alpina \( \Delta 5 \)-Desaturase in Transgenic Canola Seeds—To verify its ability to function as a \( \Delta 5 \)-desaturase in higher plants, the \( M. \) alpina cDNA was introduced into a low linolenic variety of \( B. \) napus under control of the seed-specific napin promoter (20). The fatty acid composition of pools of mature T2 seeds from thirty independent transformation events were analyzed. Fig. 6 shows the GC-FAME profile of the fatty acids from one such transformant. AA, the normal product of \( \Delta 5 \)-desaturase, was not present and was not expected since canola does not contain DGLA, the normal substrate for the desaturase. When compared with the GC-FAME profile of the fatty acids from a pool of control seeds (data not shown), two new peaks, shown by the arrows in Fig. 6, were observed in the profile obtained from the transformant. The new fatty acid, indicated by the solid arrow, had the same retention time as the new peak observed in the recombinant yeast strain (Fig. 5A). Mass spectroscopic analysis (data not shown) indicated that this new peak (solid arrow) has a mass peak at \( m/z = 294 \), and a fragmentation pattern identical to that seen in the new fatty acid observed in the recombinant yeast strain (Fig. 5B). These data suggest that this peak was taxoleic acid (\( \Delta 5,9-18:2 \)). The second new peak, as indicated by the open arrow, appeared to be a molecule, whose methyl ester has a mass peak at \( m/z = 292 \), the expected molecular ion of a 18-carbon fatty acid with three double bonds. The fragmentation pattern of this new fatty acid was very similar to that of the authentic \( \Delta 6,9,12-18:3 \) standard (data not shown); however, the retention time of this peak in GC was somewhat different from the
standard. This peak was tentatively identified as pinolenic acid (D5,9,12–18:3). Both D5,9–18:2 and D5,9,12–18:3 are the D5-desaturation products of oleic and linoleic acid, respectively, the two most abundant fatty acids in canola oil. Of the 30 transgenic plants analyzed, 24 contained detectable levels of taxoleic acid, ranging from 0.9 to 6.21% of the total fatty acids in the seed pools.

**DISCUSSION**

A desaturase-like cDNA was cloned from a fungal species, *M. alpina*, by PCR using degenerate oligonucleotide primers. The predicted amino acid sequence encoded by this cDNA contains the highest homology to previously reported cyanobacterial D6-desaturases, although similarity to other higher plant and animal membrane-bound desaturases is observed as well. The two most abundant fatty acids in canola oil. Of the 30 transgenic plants analyzed, 24 contained detectable levels of taxoleic acid, ranging from 0.9 to 6.21% of the total fatty acids in the seed pools.

**DISCUSSION**

A desaturase-like cDNA was cloned from a fungal species, *M. alpina*, by PCR using degenerate oligonucleotide primers. The predicted amino acid sequence encoded by this cDNA contains the highest homology to previously reported cyanobacterial D6-desaturases, although similarity to other higher plant and animal membrane-bound desaturases is observed as well. Expression of this *M. alpina* cDNA in both recombinant yeast cells and transgenic plants demonstrates its ability to introduce a Δ5 double bond into both 18- and 20-carbon substrates. The *M. alpina* Δ5-desaturase sequence contains two features that distinguish it from the group of Δ12- and Δ15-desaturases and would seem to place it in a newly emerging Δ5/Δ6 class. The first characteristic of this class is the presence of an N-terminal cytochrome b5 domain (Fig. 2B). This domain is similar to that seen in the Δ6-desaturases from borage (9) and *C. elegans* (11) as well as a desaturase-like sequence isolated from sunflower (25). This feature would appear to be of some functional importance due to the role of cytochrome b5 in electron transport of at least some membrane-bound desaturases and related hydroxylases (26, 27). The second distinguishing characteristic of the Δ5/Δ6 class of desaturases is the QXXHH sequence at the third His box (Fig. 2A). This also would appear to be functionally significant, due to the proposed role of these conserved histidines in the ligation of iron atoms (10). The precise biological or functional ramifications of these characteristics remain to be demonstrated, however. These characteristics of the Δ5/Δ6 desaturases could be related to either the form of substrate utilized or the type of reaction carried out. As discussed by Napier et al. (11), both the Δ5- and Δ6-desaturases carry out a so-called “front-end desaturation.”

Expression of the Δ5-desaturase from *M. alpina* in *S. cerevisiae* indicated that a polyunsaturated fatty acid of 20-carbon length could accumulate in significant quantities in bakers’ yeast (Table I) without any major growth problems. In separate experiments with 25 μM exogenous DGLA, accumulation of AA in SC334(pCGR4) reached as high as 22% of the total lipids (data not shown). In addition, the phospholipid fraction of

**TABLE I**

| SC334 containing | Total fatty acid | AA | DGLA |
|------------------|-----------------|----|------|
| pCGR4            | 309.4           | 6.1| 5.1  |
| pYES2            | 287.7           | 0.3| 14.3 |

* The volume of culture used for lipid extraction was 100 ml.
* Exogenous DGLA concentration used was 100 μM.

**Fig. 4.** GC-MS analysis of the new peak in the lipid fraction of the recombinant yeast. Mass spectrum of the fatty acid (AA) identified in Fig. 3 (top panel) compared with that of the authentic Δ5,8,11,14–20:4 standard (bottom panel).
SC334(pCGR4) contained significant proportions of substrate and product PUFAs for Δ5-desaturase activity. Hence, it appears that the substrate is accessible in the phospholipid form to the Δ5-desaturase. Since AA was stored in the phospholipid fraction of yeast, the quantity of AA produced in yeast is limited by the amount that can be stored in the phospholipid fraction. If AA could also be stored in other fractions such as the triacylglycerol fraction in an oleaginous yeast strain, the level of AA produced in the yeast strain might be increased.

The M. alpina Δ5-desaturase was also able to function in seeds of transgenic Brassica plants to produce taxoleic and pinolenic acids in plant oils. Taxoleic acid, the Δ5-desaturation product of oleic acid, reached levels of up to 6% of the total fatty acids in pooled seed samples. This may, in effect, be an underrepresentation of the amount of Δ5-desaturase activity present in these seeds, as the rate of activity of the desaturase on an 18-carbon monounsaturated substrate may be less than that of the authentic Δ8,11,14–20:3 substrate, DGLA. Δ5-Polyunsaturated fatty acids are found in a wide variety of conifer species (28), however, studies of the biological effects of these fatty acids have been limited and few commercial sources exist (29).

It is also significant that the fungal desaturase is able to efficiently interact with the components of Brassica seed triacylglycerol assembly. The M. alpina Δ5-desaturase shows considerable sequence similarity to the Arabidopsis Δ12- and Δ15-desaturases (Fig. 2A), which are known to utilize membrane glycerolipids as substrates (30). This, in addition to the finding that the AA produced in recombinant yeast accumulates primarily in the phospholipid fraction, suggest that the M. alpina Δ5-desaturase is also a glycerolipid desaturase. This raises the possibility that the other desaturases involved in LC-PUFA synthesis in M. alpina would also be compatible with the Brasp.
sica machinery. The ability to use the \textit{M. alpina} \(\Delta 5\)-desaturase in the production of LC-PUFAs such as AA in plant oils, such as canola, would create an economical and agronomically viable source of these valuable fatty acids.

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