Production of $22:2^{D5,13}$ and $20:1^{D5}$ in *Brassica carinata* and soybean breeding lines via introduction of *Limnanthes* genes

Ashok Jadhav¹, Elizabeth-France Marillia¹, Vivijan Babic¹, E. Michael Giblin¹, Edgar B. Cahoon², Anthony J. Kinney³, Elzbieta Mietkiewska¹, Jennifer M. Brost¹ and David C. Taylor¹,*

¹National Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, Saskatchewan, Canada S7N OW9; ²USDA-ARS, Plant Genetics Research Unit, Donald Danforth Plant Science Center, 975 N. Warson Road, St. Louis, Missouri 63132; ³Dupont Experimental Station, P.O. Box 80402, Wilmington, Delaware 19880-0402; *Author for correspondence (e-mail: David.Taylor@nrc-cnrc.gc.ca; phone: +1-306-975-5268; fax: +1-306-975-4839)

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**Abstract**

Seed oils of meadowfoam (*Limnanthes douglasii*, *L. alba*) contain very long-chain fatty acids of strategic importance for a number of industrial applications. These include the monoene $20:1^{D5}$ and the diene $22:2^{D5,13}$. Engineering of meadowfoam-type oils in other oilseed crops is desirable for the production of these fatty acids as industrial feedstocks. Accordingly, we have targeted *Brassica carinata* and soybean (*Glycine max*) to trigeneically engineer the biosynthesis of these unusual fatty acids. An *L. douglasii* seed-specific cDNA (designated *Lim Des5*) encoding a homolog of acyl-coenzyme A desaturases found in animals, fungi and cyanobacteria was expressed in *B. carinata*, which resulted in the accumulation of up to 10% $22:2^{D5,13}$ in the seed oil. In soybean, co-expression of *Lim Des5* with a cDNA (*Lim FAE1*) encoding an FAE1 (elongase complex condensing enzyme) homolog from *L. douglasii* resulted in the accumulation of $20:1^{D5}$ to approximately 10% of the total fatty acids of seeds. The content of C 20 and C 22 fatty acids was also increased from <0.5% in non-transformed soybean seeds to >25% in seeds co-expressing the *Lim. douglasii Des5* and *FAE1* cDNAs. In contrast, expression of the *Lim Des5* in *Arabidopsis* did not produce the expected $20:2^{D5,11}$ in the seed oil. Cumulatively, these results demonstrate the utility of soybean and *B. carinata* for the production of vegetable oils containing novel C 20 and C 22 fatty acids, and confirm that the preferred substrates of the *Lim Des5* are 20:0 and $22:1^{D13}$, respectively.

**Abbreviations:** *Lim Des5* – *Limnanthes* Acyl-CoA A 5 desaturase; *Lim FAE1* – *Limnanthes* elongase complex condensing enzyme; CoA – Coenzyme A; DEA – Diethylamide; DW – dry weight; ER – endoplasmic reticulum; FAME – fatty acid methyl ester; FFA – free fatty acid; PC – phosphatidylcholine; PE – phosphatidylethanolamine; TAG – triacylglycerol; TLC – thin layer chromatography; VLCFA – very long-chain fatty acid
Introduction

We are investigating possible sources of new genes to engineer unusual fatty acids in Brassicaceae and soybean. One target is the capacity to desaturate very long-chain fatty acids (VLCFAs) at the Δ5 position. A number of plants produce seed oils enriched in unusual fatty acids with a Δ5 functionality, including species of meadowfoam: *Limnanthes douglasii* and *L. alba*. *Limnanthes* seed oils are enriched in Δ5-eicosenoic acid (20:1Δ5) and, to a much lesser extent, an unusual diene, Δ5, Δ13-docosadienoic acid (22:2Δ5,Δ13) (Phillips et al. 1971). Because of their unique double bond positioning, both of these fatty acids are of strategic interest as industrial feedstocks. Its oxidative stability and high content of VLFCAs impart to the seed oil of *Limnanthes* species a number of properties that are desired by the cosmetic, surfactant, and lubricant industries (Hirsinger 1989; Burg and Kleiman 1991; Isbell et al. 1999). The 20:1Δ5 component of this oil can also serve as a chemical precursor of compounds such as estolides and δ-lactones that can be used for a wide range of industrial applications, including lubricants and plasticizers (Erhan et al. 1993; Isbell and Plattner 1997). The relatively high price of meadowfoam oil, however, limits its commercial use to primarily cosmetic applications, and as a result, this plant is currently grown only as a niche crop in the Pacific Northwest of the United States (Hirsinger 1989).

The proposed biosynthetic pathway for 20:1Δ5 in *Limnanthes* species involves three steps (Pollard and Stumpf 1980; Moreau et al. 1981): a flux of palmitic acid (16:0) from the plastid to the ER, followed by microsomal elongation of 16:0 to eicosanoic acid, and the Δ5 desaturation of 20:0 to yield 20:1Δ5.

We have recently confirmed this pathway by identification of cDNAs for a Δ5 desaturase (*Lim Des 5*) and a divergent FAE1 (elongase complex condensing) homolog (*Lim FAE1*) from *L. douglasii* (Cahoon et al. 2000). These cDNAs were co-expressed in soybean somatic embryos to produce 20:1Δ5 (Cahoon et al. 2000).

The small but significant proportion of a unique diene, 22:2Δ5,Δ13 in meadowfoam oil (10–15%; Miller et al. 1964; Phillips et al. 1971) is also of industrial interest. This diene possesses widely spaced, non-conjugated or methylene-interrupted double bonds making it quite stable and not as prone to oxidation. There are niche market applications that have been identified for its use as a feedstock for generating estolides, which can be used to synthesize hydroxy fatty acid feedstocks, and to produce dimer acids, esters and amides for use as lubricants, and slip-promoting anti-block agents in plastic film manufacturing (Burg and Kleiman 1991; Erhan et al. 1993). The proposed pathway for 22:2Δ5,Δ13 biosynthesis is thought to involve Δ5 desaturation of erucic acid, 22:1Δ13.

In the current study, *B. carinata* and soybean were tested as potential ‘transgenic vehicle’ crops, for engineering the production of 22:2Δ5,Δ13 and 20:1Δ5, respectively. An *L. douglasii* seed-specific cDNA (*Lim Des5*) encoding a homolog of acyl-coenzymeA (CoA) desaturases found in animals, fungi and cyanobacteria, was heterologously expressed in *B. carinata*. In addition, this cDNA as well as one (*Lim FAE1*) encoding an (elongase complex condensing enzyme) homolog from *L. douglasii* were co-expressed in somatic soybean embryos.

Materials and methods

General molecular biology techniques and analyses of transgenic plants

Unless otherwise stated, all molecular biological techniques (plasmid preparation, PCR, Southern and northern analyses, etc.) were carried out by methods prescribed by Sambrook et al. (1989) or Ausubel et al. (1995).

Transgenic expression of the *Limnanthes* Des5 in Brassica carinata

The plant transformation vector pSE 129A, already prepared from pRD400 plasmid (Datla et al. 1992), was obtained by introducing a HindIII/XbaI fragment containing the *B. napus* napin promoter and a KpnI/EcoRI fragment containing the *Agrobacterium nos* terminator. The 1.0 kb open-reading frame of the *Lim Des5* (*Limnanthes* Acyl-CoA Δ5 desaturase, GenBank Accession no AF247133) was amplified by PCR designed to contain XbaI and KpnI restriction sites and was ligated into XbaI/KpnI-digested pSE129A in the sense orientation. The sense construct *Lim des5/pSE* was then