MANIPULATING FATTY ACID COMPOSITION IN *BRASSICA NAPUS*: 1-ACYL sn-GLYCEROL-3-PHOSPHATE ACYL TRANSFERASE FROM *LIMNANTHES DOUGLASSII.*

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ABSTRACT

Two cDNAs potentially encoding acyl-CoA:1-acyl-sn-glycerol-3-phosphate acyltransferase (LPA-AT) enzymes from *Limnanthes douglasii* have been isolated and characterised. Heterologous screening with the maize pMAT1 clone (Brown *et al.*, 1994) and in vivo complementation of the E. coli mutant JC201, which is deficient in LPA-AT activity, were carried out. pLAT1 is highly homologous to the maize clone, but pLAT2 encodes a protein which has no significant homology to any published plant protein sequence. Instead the translated sequence is homologous to published LPA-AT sequences from bacteria and yeast: membranes isolated from JC201 containing this new cDNA have an altered specificity for erucoyl CoA in LPA-AT assays. The sequences have been introduced into *B. napus*.

INTRODUCTION

Modification of naturally occurring triacylglycerols (TAGs) in oilseed crops to provide industrially useful products is currently of great interest. Our aim is the development of a *B. napus* cultivar which is able to incorporate very high levels of erucic acid (22:1 D13) into its seed oil and preferably synthesize large amounts of trierucin (trierucoylglycerol) (Taylor *et al.*, 1994). Currently available high-erucic acid cultivars of *B. napus* contain a maximum of 55% erucic acid in their seed oils. These cultivars are unable to incorporate erucic acid at the sn-2 position of the TAGs (Taylor *et al.*, 1994).

The composition of a seed oil TAG depends on both the relative pool sizes of acyl-CoA thioesters and the acyl-CoA selectivity of three membrane-bound acyltransferase enzymes required for its synthesis (Browse and Somerville, 1991). It is apparent that the exclusion of erucic acid from the sn-2 position in *B. napus* TAGs is a result of strong discrimination against erucoyl-CoA by the acyl-CoA:1-acyl-sn-glycerol-3-phosphate acyltransferase (LPA-AT) enzyme [1, 5].

Transfer to *B. napus* of a gene encoding an LPA-AT which can utilise erucoyl-CoA is a potential way of overcoming the theoretical limit of 66% erucic acid in *B. napus* oil. Microsomes from seeds of members of the genus *Limnanthes* are able to synthesize dierucoyl-phosphatidic acid (Cao *et al.*, 1990; Lohden and Frentzen, 1992) and hence LPA-AT genes from these would be good candidates for the above approach.

EXPERIMENTAL

Complementation cloning and heterologous screening have resulted in the isolation of two different cDNA clones from developing seeds of *L. douglasii* which have similarities to LPA-AT sequences. The plasmid pLAT1 contains a cDNA which is a *Limnanthes* homologue of a maize sequence which complements JC201 (Brown *et al.*, 1994). pLAT1 is unable to complement the mutant itself however. Complementation of JC201 was used to select pLAT2, which encodes a protein which, by database comparison, is most homologous to PlsC of *E. coli*. PlsC is the only protein which has been proved to be an LPA-AT enzyme on the basis of increased activity after protein over-expression (Coleman, J. 1992). Nucleotide and amino acid sequence data showed that the cDNA inserts of pLAT1 and pLAT2 encode different proteins and northern blot analysis clearly demonstrate that the expression patterns in *L. douglasii* differ also. The cDNA in pLAT2 corresponds to a gene whose expression is seed-specific.

The specificities for oleoyl-CoA and erucoyl-CoA of membranes from complemented JC201 have been determined with a microsomal assay system. Since we were interested in identifying an LPA-AT from *Limnanthes* that was capable of making dierucoyl phosphatidic acid, 1-erucoyl-sn-glycerol-3-phosphate was used as an acyl-acceptor. The results clearly showed that the complemented JC201 contain an LPA-AT with a different specificity for erucoyl-CoA than membranes from plsC+ *E. coli*. The observed ratios of amount of PA produced with oleoyl- and erucoyl-CoA thioesters are similar to reported data obtained with microsomes from seeds of *L. douglasii*, where the ratio of acylation rates with erucoyl-CoA and oleoyl-CoA was 1:1.2-1.3 over a range of CoA concentrations (Lohden and Frentzen, 1992).

Rape microsomes were also included in the experiments as a control for the methods developed. The data obtained confirmed previous results (Bernerth and Frentzen, 1990) and showed that the LPA-AT in high erucic acid rape strains discriminates against incorporation of erucoyl-CoA. The activity of PlsC with different CoA species has, to our knowledge, not been reported and the results of these experiments interestingly show that this enzyme is able to synthesize dierucoyl PA, although the rate of incorporation of oleoyl-CoA is much greater than erucoyl-CoA. By far the preferred candidate gene for transfer into rape to produce trierucin is the insert of pLAT2, which encodes an enzyme with little discrimination between erucoyl-CoA and oleoyl-CoA. It is likely that there are higher levels of erucoyl-CoA than oleoyl-CoA in high erucic acid rape cultivars, since the glycerol-3-phosphate- and diacylglycerol-acyltransferases show little

discrimination between CoA species and high levels of erucic acid are present at the sn 1 and 3 positions of their TAGs.

Transfer of both clones into rape varieties is currently in progress and transgenic plants will be analysed for the capablity to synthesize the desired product trierucin in their seed oils.

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