Regulation of key enzymes involved in the fatty acid biosynthesis in *Crambe abyssinica*

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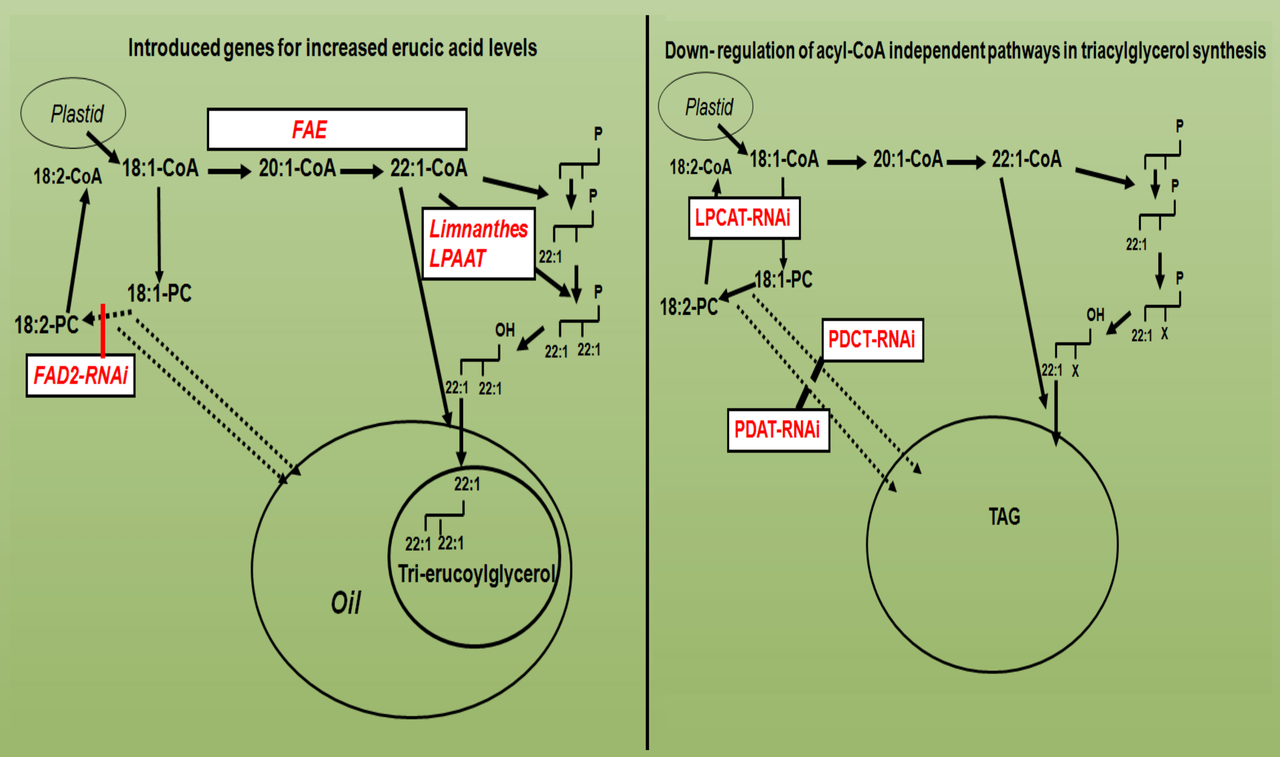
Introduction

Erucic acid (22:1) is a major feedstock for the oleochemical industry. *Crambe abyssinica* is a novel oilseed crop containing ca. 60% of 22:1, but further increasing 22:1 will be of great commercial interest. Through manipulating three genes involved in the fatty acid biosynthesis, we have developed transgenic crambe lines with 22:1 of **73%** in seed oil. The aim of this study is, by modifying another three key genes involved in the fatty acid biosynthesis, to further increase the 18:1-CoA, the precursor of 22:1 biosynthesis.



Alteration of oil composition by manipulating PDAT and LPCAT & PDCT

The preliminary results have showed the trend that the Inhibition of PDAT (phospholipid:diacylglycerol acyltransferase) had led to a drastic increase of 18:2 in PC and moderate increase of 18:2 in TAG, while no clear changes in the levels of other fatty acids. Lines with RNAi targeting LPCATs (lysophosphatidylcholine acyltransferase) and PDCT (phosphatidylcholine:diacylglycerol cholinephosphotransferase) led to significant increase in 18:1, 16:0 and 20:1 and a decrease in 18:2 and 22:1 in TAG. In PC there was a moderate increase in 18:2 and a drastic increase in 18:3 (Fig 1).

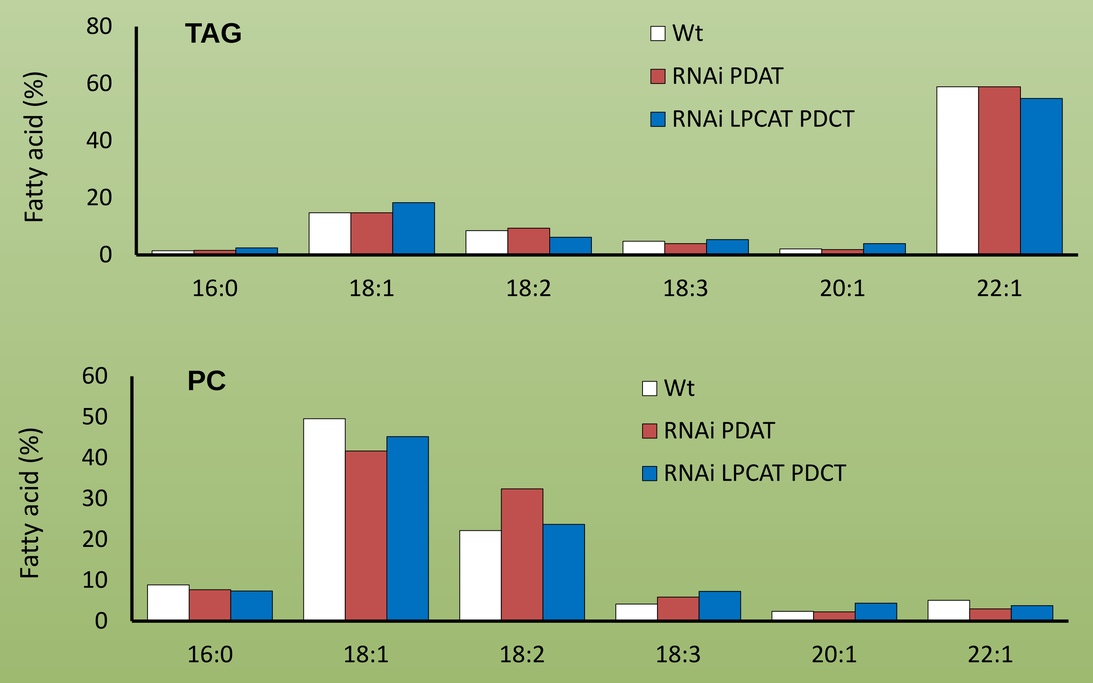


Fig 1. Fatty acid compostion of TAG and PC from pooled seeds of the transgenic lines and WT.

Demonstration of relative activities of enzymes in the Kennedy Pathway in microsomes from the high erucic acid transgenic crambe lines

Previously introduced *LdLPAAT* gene enabled efficient acylation of 22:1-CoA to the *sn*-2 position of 22:1-LPA in microsomal preparation from transgenic seeds, resulting in accumulation of PA, DAG and TAG (Fig 2A). In WT microsomes, the LPA substrate was hardly acylated, but was partially metabolised to monoacylglycerols (MAG).(Fig 2B)

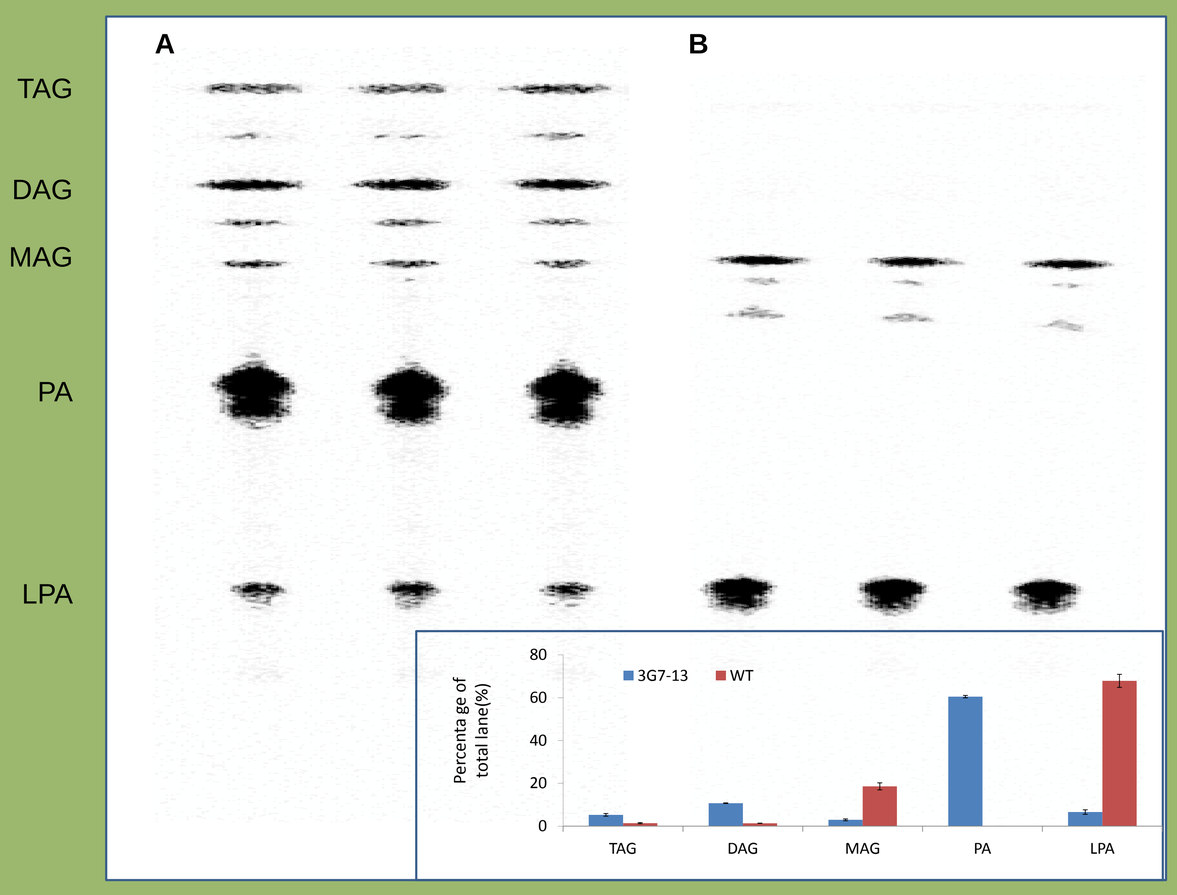


Fig 2. Autoradiogram of ^{14}C-lipid separation by TLC showing the different utilization of [^{14}C] 22:1-LPA in microsomes prepared from developing seeds of high erucic acid transgenic line 3G7-13 (A) and WT (B) when using 22:1-CoA as acyl donor. Incubation time was 80 min.

Comparison of the relative activities of enzymes in the Kennedy Pathway in microsomes from WT crambe and safflower

The DAG-PC equilibration as measured in the microsomes of developing seeds from WT crambe has low activity and resulted in very little amount of PC accumulation from [^{14}C] glycerol-3-phosphate, whereas safflower microsomes catalysed a major flow of glycerol backbone into PC (Fig 3).

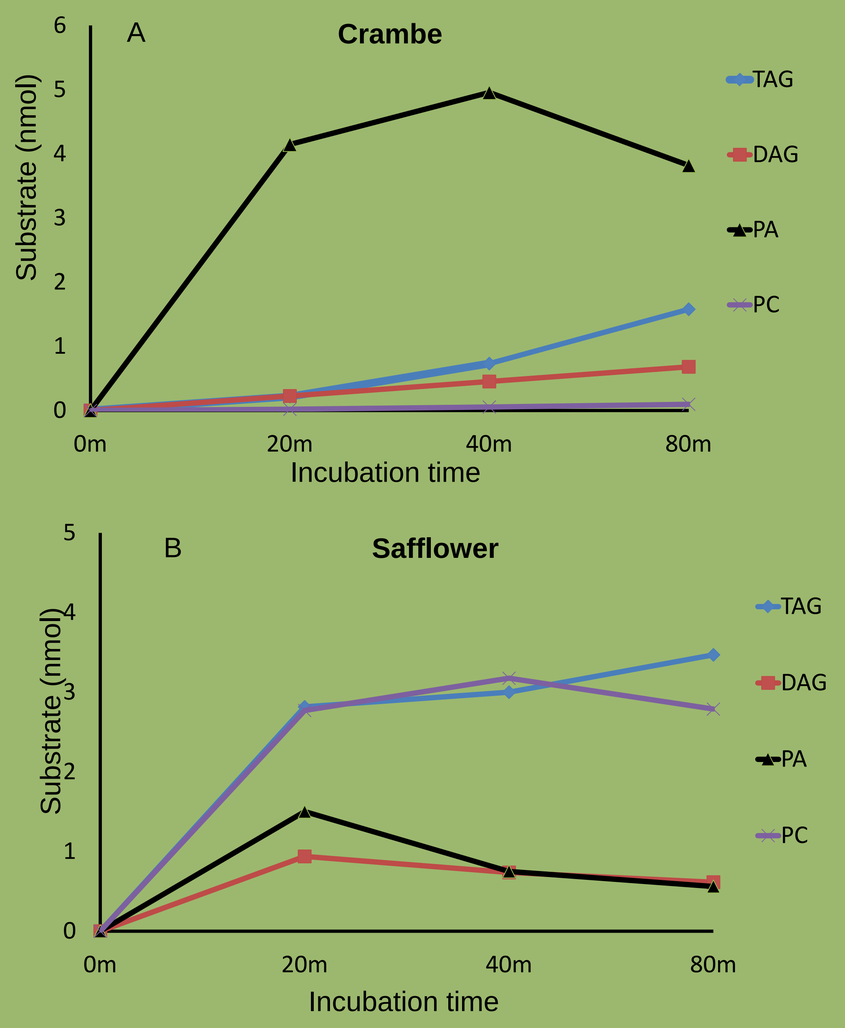


Fig 3. The formation of ^{14}C-lipids from 18:1-CoA and [^{14}C] glycerol-3-phosphate in WT crambe (A) and safflower micromsomes (B).

Acknowledgements: Financial support to this study from FORMAS, EU-ICON and Vinnova is highly acknowledged