THE USE OF CLONED RAPESEED GENES FOR THE CYTOPLAMSIC FATTY ACID DESATURASES AND THE PLASTID ACYL-ACP THIOESTERASES TO ALTER RELATIVE LEVELS OF POLYUNSATURATED AND SATURATED FATTY ACIDS IN RAPESEED OIL

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ABSTRACT

Seed specific alteration of several enzymes in triacylglyceride biosynthesis in developing rapeseeds was achieved by seed specific expression of re-introduction of cDNAs into transgenic rapeseed plants. Reduction of the expression of the cytoplasmic oleate desaturase and the cytoplasmic linoleate desaturase by co-supression using the cDNAs for their respective genes decreased linoleic and linolenic acid level in the seed oil to 5% and 1.2% of the total fatty acids. Over-expression of the oleate preferring acyl-ACP thioesterase increased both the palmitate and stearate content of seed oil such that the saturated fatty acid content was about 20% of the total. Reduction of expression of the stearoyl-ACP desaturase by co-suppression increased stearate content to about 12% while increase expression reduced the stearate content to about 1%.

INTRODUCTION

The quality of vegetable oils and their suitabilty for use in differing applications is in large measure determined by the relative abundance of the fatty acids which comprise the triacylglyceride. As examples, the oxidative stability of oils, especially at high temperature is decreased by the presence of tri-unsaturated acids and to a lesser extent, di-unsaturated acids while the solids content of an oil at a given temperature is controlled by the proportion of saturated fatty acids and the relative proportion of polyunsaturated fatty acids. Currently these parameters are controlled and oils are taylored for specified uses by processing proceedures such as hydrogenation and fractionation. We have investigated ways of manipulating fatty acid biosynthetic pathways operating during storage lipid synthesis in temperate oliseeds to produce oils with fatty acid profiles that are more suitable to specific applications in order to reduce the need for extensive oil processing.

Using seed specific promoter sequences obtained from the genes for seed storage proteins and the cDNAs encoding the enzymes: stearoyl-ACP

desaturase, oleoyl-ACP thioesterase, cytoplasmic oleate desaturase and cytoplasmic linoleate desaturase we have produced stabely transformed rapeseed plants with targeted, altered fatty acid profiles in their seed oils.

EXPERIMENTAL

Transgene constructs and rapeseed transformation

Rapeseed cDNAs encoding the cytoplasmic linoleate (18:2) desaturase were obtained as described (Yadav et. al 1993), cDNAs encoding the cytoplasmic oleate (18:1) desaturase were obtained by screening a cDNA library made from developing rapeseed using the *Arabidopsis* cDNA encoding the same enzyme (Okuley et al 1994) as a heterologous probe. cDNA clones for stearoyl-ACP desaturase and the oleate preferring acyl-ACP thioesterase were obtained from soybean by purification of the encoded proteins from developing soybean seeds followed by direct sequencing and cloning using oligonucleotide probes based on that sequence. Seed specific expression of these cDNAs in transgenic rapeseed was driven by one of three seed storage protein promoters. The promoter regions from napin and cruciferin from *B. napus* or of phaseolin from *P. vulgaris* were used. Rapeseed transformation was done by an *Agrobacterium* proceedure into the cultivar Westar using a binary vector system with the T-DNA border fragments and kanamycin resistance as the selectable marker.

From 60 to 200 transformed plants were produced for each cDNA and promoter combination. The transformed lines were screened for phenotype by analysis of the relative fatty acid contents of bulk seed from the first transformed generation by GC seperation of fatty acid methyl esters. Plant lines which were signifcantly different from control lines in this analysis were grown and selfed to obtain transgene homozygotes.

Fatty acid profiles of mature rapeseeds obtained after transgenic modification

Table 1 shows the relative content of the seven major fatty acids in mature seeds of plants homozygous for the various promoter:cDNA combinations. For three of the genes (stearoyl-ACP desaturase, oleate desaturase and linoleate desaturase) both over expression phenotypes and under expression phenotypes due to co-suppression were observed and both are listed. Analysis was done on bulk seeds from the fourth generation past transformation or on homozygous F3 seeds from transgene containing crosses. The fatty acids which were targeted for change in each transgenic construction are shown in bold.

The results may be interpreted to indicate that both the 18:1 desaturase and 18:2 desaturase activites are limiting for the production of 18:2 and 18:3 respectively; either increased or decreased activity of either enzyme results in a change in product formation. Conversely, the activity of the 18:0-ACP desaturase is present in great excess to the substrate flux and large changes in activity are required to observe even small changes in fatty acid phenotype.

TABLE 1. Fatty acid profiles of seeds of transgenic (canola) rapeseed.

TRANSGENE CONSTRUCTION	FATTY ACID (% OF TOTAL FATTY ACIDS								
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
non-transformed Westar	3.9.	1.8	67.0	19.0	7.5	0.6	0.8	0.6	0.1
napin:FAD 2 (overexpression)	4.5	2.5	33.3	**49.2**	7.2	0.7	0.8	0.7	0.2
napin:FAD 2 (co-suppression)	4.3	1.4	**84.1**	5.2	2.9	0.6	0.9	0.5	0.2
napin:FAD 3 (overexpression)	4.1	1.7	67.0	4.0	**20.9**	0.6	1.0	-	-
napin:FAD 3 (co-suppression)	3.8	1.5	68.5	22.1	**1.2**	0.6	1.1	0.4	0.1
napin:soybean 18:0-ACP desaturase (overexpression)	3.8	**1.1**	72.1	15.9	5.3	0.4	0.8	0.3	0.1
napin:soybean 18:0-ACP desaturase (sense suppression)	3.9	**12.5**	45.0	24.5	10.3	2.8	0.4	0.6	-
phaseolin:18:1-ACP thioesterase (overexpression)	**9.2**	**10.1**	57.5	13.8	4.0	2.5	0.4	0.5	-

References:

Okuley, J, Lightner, J, Feldmann, K, Yadav, N, Browse, J (1994) The Arabidopsis FAD 2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis The Plant Cell 6:147-158

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