EXPRESSION OF MEDIUM-CHAIN ACYL-(ACP) THIOESTERASES IN TRANSGENIC RAPESEED

N. MARTINI, J. SCHELL and R. TÖPFER

Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné Weg 10, D-50829 Köln, Germany

ABSTRACT

The fatty acid composition of seed oils of *Brassica napus* has been altered by expression of genes encoding medium-chain specific acyl-(ACP) thioesterases from *Cuphea lanceolata* under the control of their own promoters. In mature T2 seeds obtained from independant transgenic rapeseed lines accumulation of either C_{8}/C_{10} or C_{14}/C_{16} fatty acids has been detected.

INTRODUCTION

During the past decades success in breeding of rapeseed for high quality resulted in an area of rapeseed cultivation of 1.1 Million hactare (Mio ha) in Germany in 1993. However, the recent *General Agreement on Tariffs and Trade* (*GATT*) require a reduction of this area to 0.79 Mio ha of overall oilseed cultivation used for human consumption. Additionally, in the European Union an area of 0.8-0.9 Mio ha is guaranteed for non-food oilseed production. Consequently the use of rapeseed oil as a renewable resource, e.g as biofuel or as rawmaterial for the oleochemical industry, has gained increased interest. In order to open up novel applications of rapeseed oils in the oleochemical industry a big effort in rapeseed breeding including gene technology is required. Conventional breeding of canola (high oleate versus high erucate) demonstrated the potential for significant changes in oil quality without negatively effecting yield. Already in 1992 Calgene Inc. (California) announced two examples of the successful use of genetic engineering to alter the fatty acid profile of rapeseed towards either 40% $C_{18:0}$ stearate (Knutzon *et al.*, 1992) or 50% $C_{12:0}$ laurate (Voelker *et al.*, 1992 and Voelker, pers. communication, 1994).

Our particular focus is to develop rapeseed as a temperate crop containing fatty acids with designed chain lengths of 10 to 14 carbon

atoms in their seed oils, which may serve as rawmaterial for laundry detergent, shampoo and other surfactants. These types of seed oils are naturally found in palmkernel, coconut or wild species like those of the genus *Cuphea*. *Cuphea lanceolata*, for instance, predominantly accumulates up to 83% of C10:0 caprate in its storage triacylglycerols. Therefore, genes encoding acyl-(ACP) thioesterases responsible for the traits of interest were identified in *C. lanceolata* (Töpfer and Martini, 1994) and transferred into rapeseed lines. Here we report on the results obtained so far applying this approach.

EXPERIMENTAL

Cloning of acyl-(ACP) thioesterase genes from *Cuphea lanceolata*

The cloning and identification of type II acyl-(ACP) thioesterase genes from *C. lanceolata* have been described previously (Martini *et al.*, 1994). One complete (*Cl*TE13, Töpfer and Martini, 1994) and several truncated cDNAs were isolated. Including the PCR amplified cDNA 3′ ends a gene family consisting of at least four distinct classes could be distinguished. The subsequent isolation of 23 lambda genomic clones, 11 of which were thoroughly mapped by restriction and Southern analyses, confirmed four to five classes of type II acyl-(ACP) thioesterase genes in *C. lanceolata*. One representative of each class (*Cl*TEg100, *Cl*TEg200, *Cl*TEg600, and *Cl*TEg700) was sequenced and found to correspond to the respective cDNA sequence (Martini, N., Müller, A., Eckstein, L., and Töpfer, R., unpublished 1994), indicating that these genes are expressed *in planta*.

Expression of acyl-(ACP) thioesterase genes in transgenic rapeseed

The cDNA *Cl*TE13 and the genes *Cl*TEg100 and *Cl*TEg200 were transferred into rapeseed, cultivar DRAKKAR, via *Agrobacterium* mediated transformation. Interestingly the transfer of the chimaeric *Cl*TE13 gene construct driven by a seed-expressed ACP promoter of rapeseed resulted only in small amounts of C10:0 capric acid in immature T2 seeds, while in mature ones hardly any capric acid was detectable (Martini *et al.*, 1994). It is assumed, that the timing of expression of *Cl*TE13 in these plants was not optimal. Thus optimal timing and strength are significant requirements of a promoter for the expression of this trait, which were not met by the ACP promoter.

However, the gene transfer of either *Cl*TEg100 or *Cl*TEg200 including their own promoters into rapeseed resulted in altered fatty acid profiles in mature T2 seeds as shown in Table 1.

TABLE 1. Fatty acid profile (mol%) in transgenic rapeseed lines

Fatty acids	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
DRAKKAR					3	2	77	10	6
Cl TEg100	**1**	**3**			**3**	2	75	8	7
Cl TEg200				**7**	**15**	2	56	13	7

The transferred gene *Cl*TEg100 gave rise to 1% and 3% caprylic and capric acid, respectively, whereas transformation of rapeseed with *Cl*TEg200 resulted in 7% myristic acid and 15% palmitic acid in T2 seeds. These two genes, although derived from a species which predominantly accumulates capric acid, seem to encode two distinct traits in transgenic rapeseed lines, one specific for C8/C10 fatty acids, the other for C14/C16 fatty acids. Both these genes are specifically expressed in embryos of *C. lanceolata* (Martini, N., unpublished, 1994). These initial results of only a few transformants each are encouraging with respect to higher percentages of medium chain fatty acids in modified rapeseed oils.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge J. Bautor, C. Bonnesen and E. Filsak for excellent technical assistance. Our research was supported by the BMFT (grant number 0310528D) including financial support from Deutsche Saatveredelung, Hoechst AG, Kleinwanzlebener Saatzucht AG and Norddeutsche Pflanzenzucht GmbH.

REFERENCES

Knutzon, D.S., Thompson, G.A., Radke, S.E., Johnson, W.B., Knauf, V.C. and Kridl, J.C. (1992). Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. *Proceedings of the National Academy of Sciences USA* **89**, 2624-2628.

Martini, N., Schell, J. and Töpfer, R. (1994). Expression of acyl-(ACP) thioesterase in *Cuphea lanceolata* and in transgenic rapeseed. In *The Proceedings of the Eleventh International Symposium on Plant Lipids*. Eds. J.C. Kader and P. Mazliak, Amsterdam, Kluwer, in press

Töpfer, R. and Martini, N. (1994). Molecular cloning of cDNAs or genes encoding proteins involved in *de novo* fatty acid biosynthesis in plants. *Journal of Plant Physiology* **143**, 416-425.

Voelker, T.A., Worrell, A.C., Anderson, L. Fan, C., Hawkins, D.J., Radke, S.E. and Davies, H.M. (1992). Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. *Science* **257**, 72-74.