GENES FOR THE IMPROVEMENT OF THE STORAGE OIL CONTENT OF *BRASSICA NAPUS*

R. TÖPFER*, L. HAUSMANN, M. VOETZ, J. WALEK, J. SCHELL and N. MARTINI

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

ABSTRACT

Genetic engineering techniques have successivly been used to alter the storage oil composition, thus opening new dimensions for plant breeders. In order to apply these techniques we have isolated and characterized various genes from *Cuphea lanceolata* encoding proteins involved in *de novo* fatty acid biosynthesis as well as genes coding for glycerol-3-phosphate dehydrogenase. Some of these genes can be used in order to engineer the storage oil composition in rapeseed and the promoter elements are useful for the expression of genes of interest in transgenic plants.

INTRODUCTION

Oils and fats, chemically glycerol esters of fatty acids (triacylglycerols), play a major role in human nutrition due to their high energy content. 90 % of vegetable oil production is used for human consumption, predominantly for margarines, shortenings, salad oils and for cooking. The remaining 10 % of vegetable oils are used as sources for non-food applications such as lubricants, hydraulic oil, biofuel, or oleochemicals for coatings, plasticizer, soaps and detergents (Lühs and Friedt 1994). For both food and non-food uses breeding efforts have been made including gene technology to change the degree of desaturation and to reduce or increase the chain length of fatty acids (for reviews see Kinney 1994, Ohlrogge1994). Tissue specific promoter elements derived from seed storage protein genes have been used to direct gene expression to the desired storage tissue and thus avoid possible deleterious effects of the expressed transgene throughout the plant. Here we present alternative tissue specific promoters from genes coding for enzymes of the lipid metabolism which might be of value in order to engineer this pathway.

* present address: Bundesanstalt für Züchtungsforschung, Institut für Züchtung landwirtschaftlicher Kulturpflanzen, Institutsplatz 1, D-18190 Groß Lüsewitz, Germany.

EXPERIMENTAL

Isolation and characterization of genes

cDNAs and genes form *Cuphea lanceolata* encoding acetyl-CoA carboxylase (ACCase), acyl carrier protein (ACP), β-ketoacyl-[ACP] synthase I (KAS I), β-ketoacyl-[ACP] reductase (KAR), enoyl-[ACP] reductase (EAR) and acyl-[ACP] thioesterase (TE) have been isolated using homologous probes generated by PCR-technology as described previously (Töpfer and Martini 1994, Schulte et al. 1994, Voetz et al. 1994, Martini et al. 1995a). A cDNA encoding glycerol-3-phosphate dehydrogenase (GPDH) was isolated by functional complementation in *Escherichia coli* (Hausmann et al. 1995). All these genes showed multiple bands in Southern blot hybridisation analyses with genomic DNA of *C. lanceolata* indicating that they are organized in multigene families (Table 1). Gene specific-probes applied in these analyses showed that the different multigene families were divided into several subclasses which is in agreement with restriction mapping and sequence data of the corresponding isolated genomic clones.

Using gene-specific probes in Northern blot analyses with RNA from roots, leafs, flowers, and embryos of *C. lanceolata* KAR was expressed in all tissues almost equally (Klein et al. 1992) while all members of the ACP and EAR accumulated transcripts in all tested tissues with a preferred expression in embryos. In contrast, members of the gene families for TE and GPDH showed tissue specific expression. Two out of four TE genes were expressed in embryos only and two predominantly in flowers. Similarly two GPDH genes were expressed in embryos and one gene predominantly in flowers. It is tempting to speculate whether tissue-specific gene expression of GPDH points to a regulatory role of this enzyme for the formation of glycerol-3-phosphate at the junction of two metabolic pathways (glycolysis and fatty acid synthesis) leading to storage oil formation.

Table 1: Organisation of various gene families of *Cuphea lanceolata* encoding proteins involved in lipid biosynthesis.

	minimal No. of genes	minimal No. of gene subclasses	specificity of gene expression
acetyl-CoA carboylase	2	2	not tested
acyl carrier protein	6	3	predominantly embryo
β-ketoacyl-[ACP] synthase I	4	4	not tested
β-ketoacyl-[ACP] reductase	3	3	no specificity found
enoyl-[ACP] reductase	5	3	1 predominantly embryo and leaf, 1 predominantly flower and root
acyl-[ACP] thioesterase	4	2	2 embryo, 2 predominantly flower
glycerol-3-phosphate dehydrogenase	4	3	2 embryo, 1 predominantly flower

It has been previously shown that a thioesterase from *Umbellularia californica* expressed under the control of a napin promoter was the key determinant for the formation of medium chain fatty acids in transgenic *Arabidopsis* and rapeseed (Voelker et al. 1992). Similarly two thioesterase genes from *C. lanceolata* proved responsible for medium chain fatty acid formation in transgenic rapeseed (Martini et al. 1995b). These genes were controlled by there own embryo specific TE gene promoters of *C. lanceolata* (Table 1) indicating that promoters from genes encoding enzymes in the lipid pathway are useful tools for crop improvement.

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