Genetic engineering and breeding of Long-Chain Polyunsaturated Fatty Acids (LCPUFA) in Rapeseed

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Abstract

Polyunsaturated fatty acids (C18, C20 and C22) are essential fatty acids that represent important components in the human diet. Numerous studies demonstrated their health benefits, thus a more regular consumption and an accordingly sustainable source of these compounds is highly recommended. With the aim to produce C20-polyunsaturated fatty acids in plants, we engineered their biosynthesis in transgenic rapeseed and linseed by expressing fatty acyl-desaturases and elongase that have activities not present in agronomically important plants. As a result, we observed significant proportions of C20 polyunsaturated fatty acids including arachidonic and eicosapentaenoic acid.

Key words: Long chain polyunsaturated fatty acids, *Brassica napus*, *Linum usitatissimum,* Desaturase, Elongase

Introduction

It is now well established, that Long Chain Polyunsaturated Fatty Acids (LCPUFA) are health beneficial and important components in the human diet. The nutritionally most important LCPUFA are arachidonic (ARA, ω6-20:4), eicosapentaenoic (EPA, ω3-20:5) and docosahexaenoic acid (DHA, ω3-22:6). Human physiology depends in various ways on these LCPUFA, either as components of membrane phospholipids in specific tissues or as precursors for the synthesis of the different groups of eicosanoid effectors (e.g. prostaglandins, etc) (Jump, 2002). LCPUFA are not only required for the development of the fetal neuronal system, but also contribute via a multiplicity of beneficial roles to the maintenance of health with increasing development and age, particularly by reducing the incidence of cardiovascular heart diseases (Demaison and Moreau, 2002). A constant supply of LCPUFA as part of the human diet is considered beneficial. These ω6- and ω3-fatty acids are not present in reserve triacylglycerols of angiosperm plants and, therefore, enter the human diet mainly in the form of marine and freshwater fish. In view of the increasing world population and the problem of overfishing marine resources, transgenic rapeseed might constitute a sustainable source of LCPUFAs. In addition, in contrast to the very high levels to which industrial fatty acids have to be enriched in plant oils for competitive use as chemical feedstocks, much lower percentages of LCPUFA in edible plant oils would satisfy nutritional requirements (Trautwein, 2001). ARA and EPA are synthesized from 18:2 and 18:3, respectively by the successive action of a Δ6-desaturase, a Δ6-elongase and finally a Δ5-desaturase. On the other hand, biosynthesis of DHA requires two additional enzymes, a Δ5-elongase and a Δ4-desaturase (Abbadi et al., 2001; Domergue et al. 2005; Sayanova and Napier 2004). Genes for the implementation of LCPUFA biosynthesis by genetic engineering of rapeseed have already been identified in lower plants, yeasts, fungi, and animals and first results of the feasibility of this strategy have been reported (Abbadi et al., 2004; Qi et . al., 2004; Wu et al., 2005). The challenge now is to produce these valuable fatty acids in rapeseed for commercial utilization. Here we present results from two integrated projects (NAPUS2000 and OLeRa) supported by the Federal Ministry of Research (BMBF) in Germany.

Materials and Methods

Here we report experiments on the production of ARA and EPA in seeds of transgenic rapeseed and linseed. For this purpose plant transformation binary vectors carrying two different desaturases (Δ6 and Δ5) from the algae *Phaeodactylum tricornutum*, and a Δ6-elongase from the moss *Physcomitrella patens* (Abbadi et al., 2004), each under the control of the USP seed-specific promoter and the OCS terminator, were used for transformation of *Brassica napus (L.),* variety "Lisora" and *Linum usitatissimum,* variety "Solin" (rich in 18:2). Plant transformation was accomplished *via Agrobacterium tumefaciens* using an improved protocol for *B. napus* (Orsini, unpublished) and as described previously for linseed (Drexler et al., 2003). After regeneration of transgenic plants, seeds were collected at maturity for fatty acid analysis by gas chromatography coupled to mass spectrometry (GC/MS). Moreover, seeds from *B. napus* primary transformants (T_2-seeds) showing a synthesis of ARA and EPA were selected for generation of T_3-seeds. The latter were analyzed by gas chromatography.

Results

In a first approach to produce ARA without EPA we transformed the linseed type "Solin". This type is rich in linoleic acid (18:2 up to 70%) in its seed oil. Figure 1 shows the fatty acid profiles of T_2-seed from wild type (A) and transgenic linseed (B). In contrast to the fatty acid profile of the wild type, additional fatty acids appear in the fatty acid composition of the oil from transgenic linseed. Indeed among the new fatty acids produced in transgenic seeds, γ-18:3$^{\Delta 6,9,12}$ is the most abundant, whereas

18:4$^{\Delta6,9,12,15}$ was the lowest fatty acid. However, despite the low amount of α-linolenic acid (α-18:3, up to 3%) in the wild type plants, the presence of both γ-18:3$^{\Delta6,9,12}$ and 18:4$^{\Delta6,9,12,15}$ indicates that the Δ6-desaturase from *P. tricornutum* used both 18:2$^{\Delta9,12}$ and α-18:3$^{\Delta9,12,15}$ as substrates in plants. On the other hand, the various C20- PUFA fatty acids totalled up to 18%. 20:3$^{\Delta8,11,14}$, the elongation product of the Δ6-elongase, accumulated up to 8%. Following the sequence of LCPUFA biosynthesis, 20:3$^{\Delta8,11,14}$ in turn has been the substrate of the subsequent Δ5-desaturase giving raise up to 10% ARA (20:4$^{\Delta5,8,11,14}$) in the seed oil and a Δ5-desaturation rate of 60%.

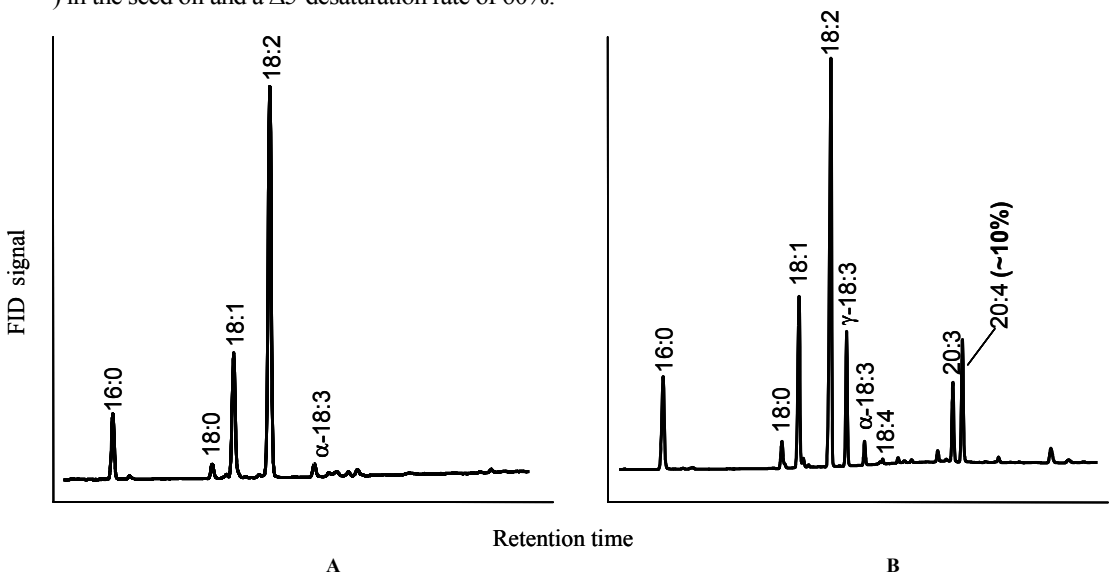

Fig. 1: Fatty acid profile of wild type (A) and transgenic linseed Solin (B)

We transformed in parallel *B. napus* with the same construct as for linseed and after regeneration of the transgenic plants we analyzed the seeds for their fatty acid composition. It should be noted, that the fatty acid profile of wild type *B. napus*, variety "Lisora" used for transformation, a spring rapeseed type, is of canola quality and dominated by oleic acid (18:1, up to 65%), followed by linoleic acid (18:2 up to 20%) and Linolenic acid (α-18:3, up to 12%), thus very negligible amounts (0-0.2%) of fatty acid longer than C18 are present in the seed oil, but not interfering with LCPUFA. The analysis of T_2-Progeny indicated that ARA and EPA were synthesized in the seed oil of rapeseed. The amounts of ARA and EPA accounted in certain lines for 1% and 0.4%, respectively, whereas γ-18:3$^{\Delta6,9,12}$ accumulated from 2 to 10%. Different lines were then selected and propagated to generate T_3-seeds. The proportions of LCPUFA in the T_3 seeds were comparable or even higher to those found in the T_2 progeny, indicating that a stable phenotype was transferred to the next generation. Figure 2 shows the fatty acid composition of C18 and C20-fatty acids found in the different T_3-seeds from control and selected T_2 transgenic plants. In contrast to control plants where newly synthesized LCPUFA are not found, the T_3-seeds are segregating for the trait and in some cases accumulated large amounts of γ-18:3$^{\Delta6,9,12}$ (up to 14%). 18:4$^{\Delta6,9,12,15}$ was also found in the seeds of the T_3-progeny. On the other hand, ARA now accumulated up to 1.2% and EPA up to 0.6%. Thus these lines represent a good starting point for breeding new rapeseed varieties with LCPUFA in the seed oil.

Discussion

The production of LCPUFA in transgenic plants requires the heterologous reconstitution of the biosynthetic pathway in the new host. To this end transgenic linseed and *B. napus* lines expressing the two desaturases from *P. tricornutum* and the elongase from *P. patens* were regenerated and analyzed with regards to their fatty acid composition in the seed oil. The use of the USP promoter permitted a strong and restricted expression of the genes in the seed. As results we observed the accumulation of ARA alone or ARA and EPA in linseed or rapeseed, respectively. These data demonstrate again, that oilseeds can in fact be transformed to produce LCPUFA and that the proportions we observed in rapeseed are already close to the nutritionally relevant levels recommended by the different internationally recognized nutrition organizations. The separate production of ARA (free of EPA) in the linseed line "Solin" would represent a first alternative source for the production of infant formulae enriched of this fatty acid.

The higher amounts of PUFA found in the T_3-seed of transgenic *B. napus* could be attributed to homozygous plants after segregation. In addition, the bottleneck observed in the previous study using the same genes (Abbadi et al., 2004) is apparently depending on the host used for the reconstitution of LCPUFA biosynthesis and the biochemical pathway of triacylglycerols (TAG) accumulation in these hosts. Indeed we observed in both rapeseed and linseed low accumulation of C18-Δ6-desaturated products and high elongation in comparison to the previous study carried out with linseed rich in α-18:3 (Abbadi et al. 2004). This in turn raises additional interest in using different strategies to enhance the level of LCPUFA in TAGs of seed oil crops. Further efforts will be directed toward increasing the channelling of C20-PUFA into TAG by using the genetic diversity of different desaturases, elongases and acyltransferases found in different LCPUFA producing organisms.

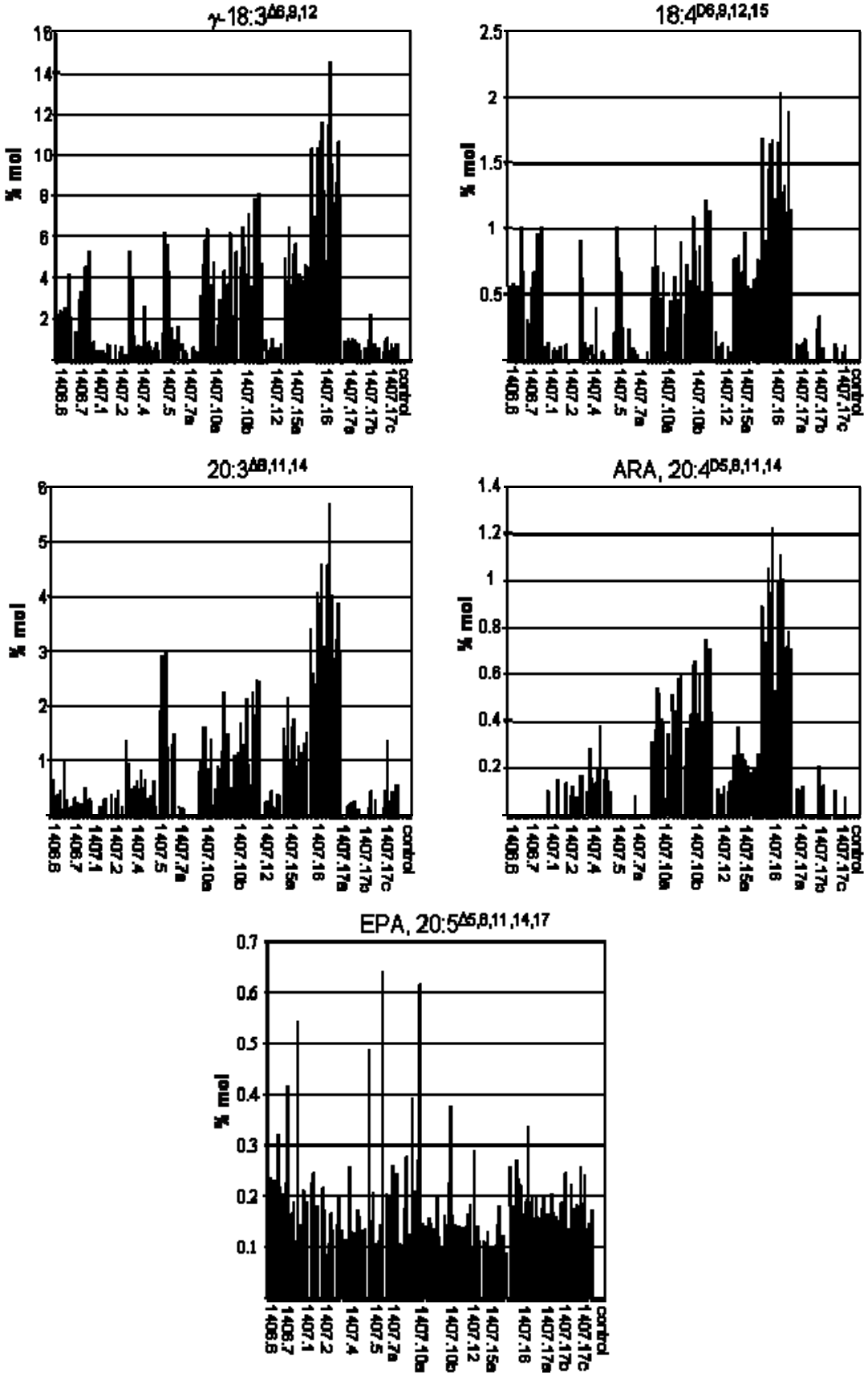

Fig. 2: Fatty acid composition of control and transgenic T3-seed from *B. napus*. Results from individual plants are shown.

Conclusion

Our data clearly demonstrate the feasibility of the production of the nutritionally relevant LCPUFA in transgenic rapeseed and linseed. The availability of such oils enriched in LCPUFA will be of ecological and health socio-economical benefit.

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