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Development of high oleic acid oilseed rape

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Introduction

Oilseed rape*, Brassica napus* L., is the most important oilseed crop of temperate regions. On a per hectare basis it follows winter wheat and winter barley as the third most important crop in Germany. In 2001/2002 around 1.3 Mio hectares of winter oilseed rape are grown. From these around 330.000 ha are cultivated for Non-Food purposes, i.e. mainly for the production of Biodiesel. On 98% of the total acreage 'Double Low' ('00') or 'Canola'-type oilseed rape is grown. The '00'-cultivars are characterized by their low glucosinolate content in the seed (<25 µmol/g) and their low content of erucic acid in the seed oil (< 2% C22:1). On 2% of the total acreage high erucic acid oilseed rape (HEAR) is grown on a contract basis and is used as a renewable resource in industrial applications (Piazza and Foglia 2001, Möllers 2002).

Most of the rapeseed oil is produced for human consumption in form of margarine, fried and backed products, and salad oil. Attempts have been made by transgenic approaches and plant breeding to modify the fatty acid composition to make the oil more suitable for specific Food- and Non-Food-applications. Among the different approaches, the development of high oleic acid rapeseed (HOAR) oil is important for improved applications for both, Food- and Non-Food-purposes. Currently, most of the produced high oleic vegetable oil is still used for human consumption as salad oil and as a replacement for partially hydrogenated oils for frying and deep-frying purposes. However, a recently published study of Narocon (2001) summarizes impressively the various oleochemical applications of high oleic vegetable oils (see also Piazza and Foglia 2001). It is therefore expected that the market for high oleic acid vegetable oils will increase considerably during the coming years. Modification of the fatty acid composition in rapeseed beyond the natural occuring variation can be achieved by genetic engineering and by induced mutations. Since the transgenic approach is at present not accepted in the EU, the utilization of induced mutations in breeding cultivars with an increased oleic acid content in the seed oil has currently better chances of being realized. Hence, the following paragraphs concentrate on the progess achieved using induced mutations and other classical plant breeding techniques in developing high oleic acid oilseed rape.

Modification of the fatty acid composition of the seed oil

The oil of the standard '00'-cultivars contains oleic acid as the major fatty acid, followed by linoleic and linolenic acid (Tab. 1). From a nutritional point of view the rapeseed oil has an excellent quality because of its high content of essential ω6-linoleic acid and ω3-linolenic acid and because of its, among vegetable oils lowest content of saturated fatty acids. However, due to the high contents of polyunsaturated C18:2 and C18:3 the oil has only a low oxidative stability, which is a particular drawback when the oil is used for frying purposes. Based on induced mutants of the linoleic acid desaturation (see Rücker and Röbbelen 1996) attempts have been made during the past decades to breed oilseed rape cultivars low in linolenic acid. This has resulted in the release of low linolenic ('LL') spring and winter rapeseed cultivars with less than 3% linolenic acid in the seed oil (Tab. 1). However, at present there is no cultivation of 'LL'-oilseed rape in Europe and in Canada/USA it is only of very limited importance.

Tab.1: Fatty acid composition of different oilseed rape quality types (% of total fatty acids)

Oil quality (fatty acids)	Saturated*	Oleic C18:1	Linoleic C18:2	Linolenic C18:3	Erucic C22:1
'00' bzw. Canola	7	60	20	10	<2
High-Erucic (HEAR)	6	15	13	9	58**
Low-Linolenic (LL)	7	60	30	2	<2
High-Oleic (HOAR)	5	86	4	4	<2
High-Oleic/low-Linolenic (HOLL)	5	85	6	2	<2

* mainly Palmitic acid (3-4% C16:0) and Stearic acid (1-2% C18:0)
** includes ≈8% Eicosenoic acid (C20:1)

Development of high oleic acid oilseed rape (HOAR)

Since some years high oleic (HO)-sunflower, HO-soybean and HO-safflower cultivars are available and are grown to some extent in Europe, Canada/USA and Australia. More recently, high oleic acid oilseed rape (HOAR) forms are being developed for cultivation in the more temperate regions around the world.

In Germany, mutation experiments were started about 10 years ago and first mutants with an increased oleic acid content were identified by Rücker and Röbbelen (1995). Genetic studies showed that in most of these lines the mutation was inherited as a monogenic trait in an additive manner (Schierholt et al. 2001). In a doubled haploid population segregating for oleic acid it was found that the mutation itself caused an increase of 11% oleic acid (from 60% to 71%) compared to the wildtype (Schierholt and Becker 2001). Molecular mapping of the mutated gene (Schierholt et al. 2000) indicated that the oleic acid desaturase was affected (Fig. 1). There are two forms of oleic acid desaturases, one is present at the endoplasmic reticulum in the cytosol (FAD2, Fig. 1) and the other one is present in the chloroplasts (FAD6, Fig. 1).

On the DNA level, two types of fad2-alleles, one from the *B. oleracea* and the other one from the *B. rapa* genome have been cloned from *B. napus*. Cloning and sequencing showed two mutations in the *B. rapa* allele, from which one resulted in a change of the FAD2-amino acid sequence. Expression of the mutated sequence in yeast proved a 90% reduction of the oleic acid desaturase activity of the mutatet allele in comparison to the wild type allele (P. Spiekermann, University Hamburg, pers. comm.). The expression of the fad2-sequence of the *B. oleracea* genome in yeast proved intact function of this sequence in the *in vitro* assay. Hence, it seems likely that the remaining oleic acid desaturase activity found in the mutant lines lines is due to the activity of the *B. oleracea* fad2-allele and the remnant activity of the *B. rapa* allele. The chloroplasts do also contain an C18:1 desaturase (FAD6; Fig. 1), which may provide linoleic acid to some extend to the trigylceride synthesis at the Endoplasmatic Reticulum in the cytosol (Fig.1).

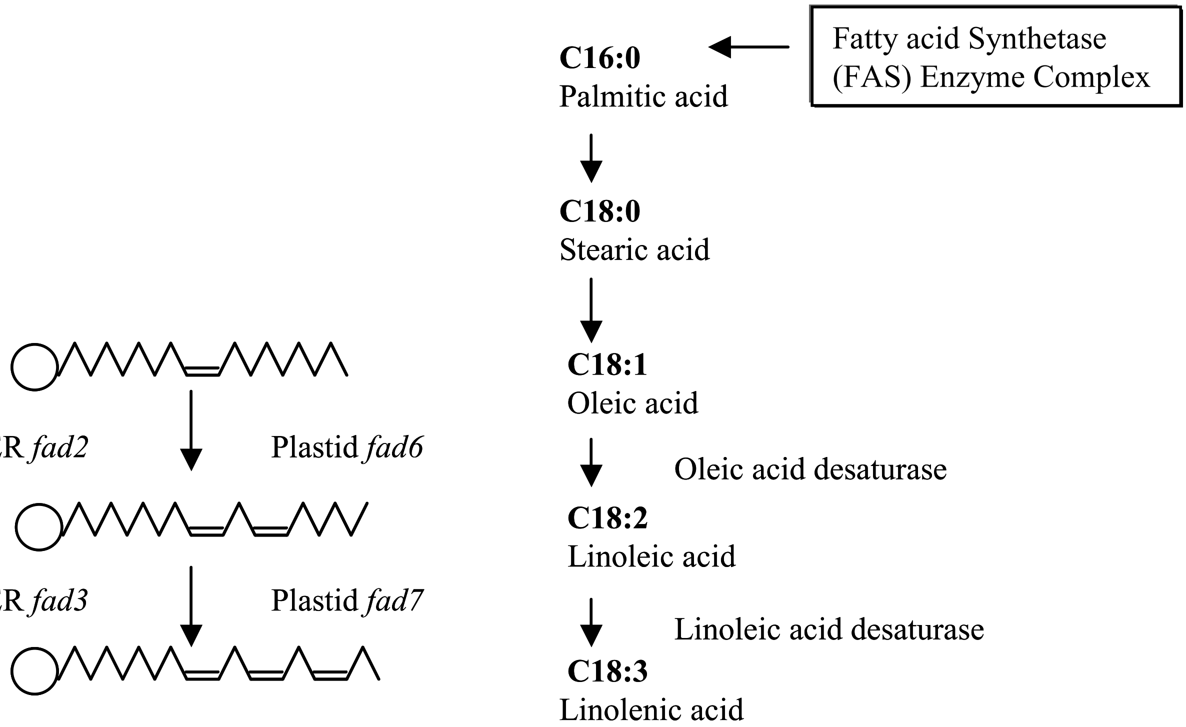

Fig. 1: Schematic drawing of the fatty acid biosynthesis

Further selection in the mutant lines, crossing with other high oleic acid genotypes and continued selection for a high oleic acid content led to the development of genotypes that had 86% oleic acid in the seed oil, as determined after growing the material in the field (Tab. 1; Schierholt and Becker 2000). These experiments have shown that beside one major gene affecting the oleic acid content, three or more minor genes need to be considered, if oleic acid contents of around 85% are to be achieved. This complicates breeding of HO-oilseed rape. Fortunately, Schierholt and Becker (2001) found only minor genotype x environment interactions for the HO-trait in a doubled haploid population segregation for oleic acid content (56%-75%), which was tested in 5 environments (2 years in 2-3 locations). The observed high heritability of h^2=0.99 indicate that selection for high oleic acid genotypes is very efficient. Environmental effects were significant but small, showing that environmental conditions should not influence the oleic acid content to a larger extent.

In further experiments a positive correlation between oleic acid and oil content (Schierholt and Becker 2001, Möllers and Schierholt 2002), and a negative correlation between oleic acid and yield was found. The increase in oil content (0.6 – 1%) was not sufficient to compensate for the lower yield (-5%) when the oil yield was calculated (Schierholt and Becker, pers. comm.). At present, it is unclear whether the yield reduction is a pleiotropic effect of the HO-mutation or whether it is due to linked genes, which are associated with yield reductions. The observed yield reduction of the high oleic acid genotypes was not evident in all tested crosses. Hence, it should be possible to compensate for the yield disadvantage by an increased breeding intensity.

Development of high oleic acid/low linolenic oilseed rape (HOLL)
To further increase the oleic acid content, crosses were made between HO- and LL-rapeseed. Field testing of segregating F3-plants and analysis of F4-seeds showed that the combination of the two traits did not lead to an increase in oleic acid content (Schierholt and Becker, pers. comm.). From one HOxLL-cross a large number of doubled haploid lines (DHs) were produced via microspore culture and tested in 2000/2001 in the field at Reinshof/Göttingen. The results shown in Fig. 2 demonstrate that the traits HO and LL can be combined, but that this does not lead to an increase in oleic acid content. The results show that from this cross only a few genotypes combine the desired traits HO and LL, again indicating the large number of genes involved in the inheritance of this trait. Of the 479 DH-lines tested only 31 contained more than 82% oleic acid and less than 10% polyunsaturated fatty acids (data not shown).

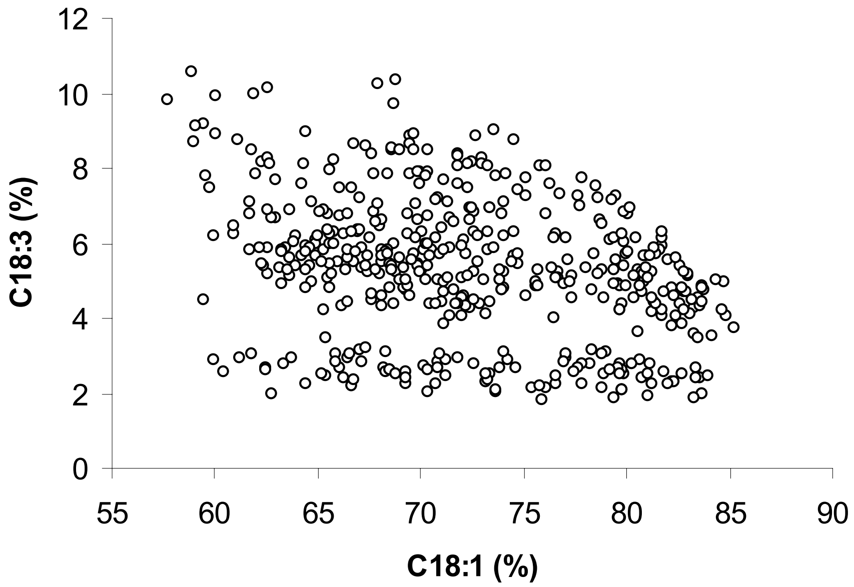

Fig. 1: Linolenic (C18:3) and oleic acid (C18:1) content of a segregating DH population (n=479) of a cross HO x LL

Comparison of high oleic sunflower (HOSF) and high oleic rapeseed (HOAR)
Winter oilseed rape has in Germany a yield advantage of more than 10dt/ha (www.saaten-union.de, Hektar-Spiegel) compared to Sunflower. Furthermore, oilseed rape has a slightly higher oil content (45-48% versus 42-46%). Of the 25.000 ha sunflower cultivated in 2001 around 9.000 ha were of HO-quality. In sunflower two different HO qualities can be distinguished: 80+ and 90plus® (Dr. Frische GmbH) with more than 80% and more than 90% oleic acid, respectively, in the seed oil. In Tab. 2 the fatty acid composition of the DH-line with the highest oleic acid content obtained from the above mentioned population is compared with the fatty acid composition of the 80+ and 90plus® sunflower. The HO-rapeseed quality is superior to the HOSF 80+ type, but there is still some difference to the HOSF 90plus® Type. A particular difference to sunflower oil is the presence of linolenic acid which can be reduced by breeding to around 2%, but which will be difficult to remove completely. Linolenic acid is an important fatty acid of the chloroplast membrane of the growing green seed of oilseed rape. In comparision to the normal quality the HO- types have a lower content

of saturated fatty acids. The HO-rapeseed shown in Tab. 2 has even a lower saturated fatty acid content than the 90plus® sunflower quality.

Tab. 2: Fatty acid composition of a high oleic acid rapeseed line (HOAR) and of two established high oleic acid sunflower qualities (HOSF)

Fatty acid (in %)		**HO - oilseed rape**	**HOSF 80 + Type**	**HOSF 90plus® Type**
Palmitic acid	C16:0	2.7	4.0 – 4.5	3.5 – 4.0
Stearic acid	C18:0	2.0	2.5 – 3.0	1.0 – 1.5
Oleic acid	C18:1	**85**	**81 – 82**	**90 – 91**
Linoleic acid	C18:2	3.4	9-11	2.8 – 3.0
Linolenic acid	C18:3	3.8	0	0
Others	---		1-2	0.5 – 0.8

90plus® is a registered trade mark of the Dr. Frische GmbH, Alzenau.

Conclusions and outlook

The obtained results indicate that HO-rapeseed cultivars with more than 80% oleic acid in the seed oil can be developed. Breeding is complicated by a large number of involved genes and an observed yield penalty of the HO-types. However, the yield penalty was not observed in all crosses and selection of HO-genoytpes in segregating offsprings is efficient because of a high heritability. The combination of HO with LL does not lead to an increase in the oleic acid content.

Further increases in the oleic acid content can be expected from crossing the present HO-plant material with genetically divergent HO-genotypes. Furthermore, the search for new mutants which are defective in the *B. oleracea* fad2-gene looks promising. The present HO-mutants can also be crossed to transgenic HO-material to select genoytpes with a superior oleic acid content among the recombinants.

At present, only HO-sunflower is cultivated on a larger sclae in the USA, France and Argentina (Narocon 2001). Narocon (2001) optimistically prognoses in their study a cultivation potential of about 100.000 ha for HO-sunflower in Germany. It seems that HO-oilseed rape could take a substantial share of this in the future.

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