PROMISING ROUTES OF OILSEED BREEDING *)

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Summary

The seed oil of different oil crops is basically characterized by specific fatty acid patterns. This is due to the action of specific genes encoding respective enzymes involved in fatty acid biosynthesis. Consequently, fatty acid composition can be further modified by genetic manipulations, i.e., both by classical and modern breeding methods. As an example, erucic acid was eliminated from the seed oil of different cruciferous crops such as rapeseed (Canola, *Brassica sp.*), so that rapeseed provides a prime edible oil for human consumption now. Further examples are sunflower (*Helianthus annuus* L.) cultivars with high oleic acid and oilflax (linseed, *Linum usitatissimum* L.) with extremely low linolenic acid content. Such novel types of vegetable oil are highly appreciated in human and animal diets, e.g., due to their improved stability.

Further breeding progress can be achieved by an application of biotechnology, i.e., cell and tissue culture techniques and molecular methods. Considerable steps toward practical application of such novel techniques have been reported for different oil crops - in particular rapeseed and oilflax. In these species it is possible now to obtain haploid plants reproducibly through microspore or anther culture which principally results in a time gain of several years. Interspecific hybridization is another interesting supplementary technique in plant breeding ; it can help to create new genetic variation. Interspecific crosses have been successfully used, for example in the *Cruciferae* family and in the genus *Helianthus*, by application of the embryo rescue technique. On this basis, related species can be used as gene sources to improve various agronomically important characters.

For species recalcitrant to sexual hybridization, the protoplast fusion technique would be an elegant method to achieve hybrids, provided that the regeneration of intact plants is feasible. Foreign genes can even more elegantly be transferred from one species to another by means of genetic engineering. Necessary prerequisites for an application of gene technology in oilseed breeding are principally available, i.e., efficient vectors and transformation systems have been elaborated and a number of target genes have been isolated meanwhile. Therefore, the transfer of relevant genes, especially for seed oil quality, to cultivated species can be anticipated for the nearer future - provided that entire, reproductive plants can be regenerated from the manipulated cell(s) or tissue. Genetic engineering is recognized as a novel and efficient pathway for creating novel genetic variation for the plant breeder.

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1. Introduction

The world production of vegetable oils presently amounts to about 60 million metric tons.[1] The major source is soybean (27 %), followed by palm (17 %), rapeseed/canola (14 %), sunflower (13 %), peanut (7 %), cottonseed (6 %) and coconut (5 %). Soybean and sunflower oils are basically rich in linoleic acid (50-70 %), whereas canola ('double low' rapeseed), peanut and olive are comparatively high in oleic acid (approx. 50-70 %) ; palm and coconut oils are rich in saturated fatty acids (50 and 90 %, respectively). The bulk of vegetable oils, i.e. more than 90 %, is directed to food uses,[2] predominantly in the form of margarine, shortening, salad oils and for frying purposes. Major concerns with regard to nutritional quality are the impact of dietary oils and fats on blood cholesterol levels, enhancement or inhibition of certain cancers, suppression of auto-immune disease, and obesity.[3]

In addition to this, requests of vegetable oil as basic material for industrial (technical) purposes have been increasing in recent years. Both, in industry and in nutrition, specific fatty acid profiles determining the suitability of vegetable oils for particular applications are needed[2]. The use of oil in nutrition requires a precise mixture of saturated and unsaturated fatty acids with chain lengths of 16-18 carbons, palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic acid (C18:3), respectively.[4,5]

Different oilcrop species like rapeseed, sunflower and linseed are characterized by specific fatty acid compositions. Extensive breeding efforts have been made in the past in order to provide oil crops with an improved seedoil quality, e.g., for nutritional purposes. For example in rapeseed, the removal of erucic acid (C22:1) was one of the most important breeding objectives almost 30 years ago,[6,7] since erucic acid is undesirable in edible oil.[4] Furthermore, the reduction of linolenic acid in the seedoil of flax,[8,9] soybean[10] and rapeseed,[11] just as the development of high oleic sunflower types[12,13] represent successful examples of conventional genetic approaches via mutagenesis and selection. Consequently, a variety of plant species and cultivars with distinct and widely different fatty acid compositions are available now, e.g., low erucic rapeseed (canola) and high oleic safflower and sunflower.[3,14]

Further goals are, for example : (1) higher levels of stearic acid to reduce the need for hydrogenation in the manufacture of margarine and frying oil, or (2) reduced level of palmitic acid regarded as the important cardiovascular risk factor in the group of saturated fatty acids, (3) developing commercial source of high oleic canola with more than 80% oleic acid in triglycerides to improve stability of frying oils, (4) decreased linolenic acid in soybean and rapeseed - and even more in linseed - in order to improve stability and reduce the tendency to spoil, (5) development of new commercial sources of gamma-linolenic acid in order to increase supplies for clinical uses, and (6) create a temperate-zone, annual crop as a source of lauric acid.[3,10,15]

Some of these goals (e.g. 1-4) may be at least partially achievable by traditional breeding methods of selection and metagenesis. More rapid progress can possibly be achieved by application of biotechnology as a promising supplementary tool.[3,16-18] However, the last two goals mentioned above (5-6) will definitely not be realized by classical means of plant breeding. In these cases molecular methods will be required, which are based on a better understanding of biochemistry and physiology of seedoil synthesis and storage.[19] In the following, besides conventional genetic approaches new avenues and prospects of biotechnology including cellular and molecular methods will be discussed in more detail.

2. Genetics of oil composition and breeding for modified oil quality

In nearly all important annual oil crops conventional approaches have resulted in new genetic strains with marked alteration of fatty acid composition. These strains or lines were developed as a result of selection of available germplasm of the species (e.g., safflower) or as a result of an artificial induction of genetic variability followed by selection, e.g., chemically induced mutants of sunflower, linseed, soybean and rapeseed. An important requirement for successful selection in all these cases was the development of rapid screening techniques to enable a very large population of seeds to be analyzed for desirable changes in fatty acid composition. Frequently, these genetic changes are inherited in accordance with Mendelian expectations as a result of one or a limited number of genes.[3,20]

In safflower (Carthamus tinctorius) Knowles[14] demonstrated genetic variation in breeding materials ranging from below 10 % up to more than 80 % oleic acid, all of them occurring naturally. Genetic analyses showed that the major levels of linoleic and oleic acid in safflower seedoil are largely under the control of a single dominant locus with three alleles (Ol, ol, ol^1). However, only the standard type with high levels of linoleic acid (OlOl, 70-75 % C18:2) and the high oleic acid type (olol, 75-80 % C18:1) have been grown commercially, so far.

In sunflower oil a strong inverse relationship between oleic and linoleic acid caused by temperature is observed.[13,21] The major fatty acid of the oil of most commonly used sunflower cultivars is linoleic acid (50-70 %), whereas oleic acid represents between 15 and 45 % of the total fatty acids depending on environment. A high oleic mutant was developed by Soldatov[12] derived from dimethyl sulphate induced mutation. After further selection for high oleic acid content in progenies from the original mutant, the open pollinated variety 'Pervenets' with an average oleic acid content of 75 % was released. Individual half-seed analyses in this germplasm showed inter- and intra-plant variation for oleic acid content of individual seeds ranging from 19 to 94 %. Nevertheless, it was possible to select true-breeding progenies from 'Pervenets' with a quite stable, nearly temperature insensitive oleic acid content higher than 83 %.[13] Although the high oleic material used in different studies was derived from the same germplasm, some differences have emerged from examinations of genetic control. Urie[13] suggested, that the high oleic trait was inherited by a single dominant gene (Ol) with embryo genotype control. Miller and coworkers[22] defined a second gene (ml) modifying the oleic acid content determined by the major gene Ol. The high oleic genotype is represented by the recessive ml gene in homozygous condition in combination with the gene Ol. Dominant alleles of the modifier gene (Ml) appear to be present in common sunflower varieties more or less frequently, whereas it is probable that the recessive allele ml is rare. It should not be neglected, that Miller and coworkers[22] also observed the presence of considerable maternal effects on the inheritance of oleic acid content. More recently, Fernández-Martínez et al.[23] confirmed the previous results obtained with similar material[13] indicating a practically complete absence of maternal influence and a high degree of dominance of the factors conditioning high oleic acid content. But the genetic analysis of segregating progeny supported the hypothesis, that the high oleic trait is determined by three dominant complementary genes (Ol_1, Ol_2 and

Ol_3). The dominant gene Ol_1 probably is the result of mutation in the Russian 'Pervenets' material. The different alleles of Ol_2 and Ol_3 were present in the genetic background of the respective sunflower lines used as cross parent.[23,24] With regard to biochemical mechanisms it is interesting to note that the high oleic character is expressed exclusively in the tissue of the developing seed. The block in oleate synthesis is confined to the ER (endoplasmic reticulum), and due to reduced oleoyl-PC(phosphatidylcholine) denaturation both membrane lipids and triacylglycerol composition of the seed is affected.[25,26]

Linseed normally produces a seedoil rich in linolenic acid (approx. 40-65 %), which makes the oil highly susceptible to autoxidation. Since natural variability of fatty acid composition of oilflax (Linumusitatissimum) is comparatively limited, mutation breeding seems to be the most promising approach for reducing the linoleate content in linseed oil, so far.[9] An exceptionally successful example in this respect was the metagenesis program of Green and Marshall.[27] Following ethyl-methanesulfonate (EMS) treatment of seeds of the high linolenic linseed cultivar 'Glenelg', the two mutants M1589 and M1722 with about 50 % of wild-type linoleate level were isolated. By recombining the two mutants, the line 'Zero' with less than 2 % linolenic acid in the seedoil was obtained. Genetic analysis demonstrated that the low linolenic trait is determined by two genes (*Ln1* and *Ln2*) located on separate chromosomes and acting additively.[8] Biochemically, the mutation events display a genetic block in the conversion of linoleate to linoleate in the developing seed, due to a reduced activity of linoleoyl-PC denaturation.[28] As a consequence, the oleic acid content is nearly the same as in cv. 'Glenelg', but the level of the nutritionally desirable linoleic acid in the low linolenic genotype 'Zero' is greatly increased.

Recently, the potential of breeding for improving soybean oil quality has been reviewed.[10] With respect to modification of fatty acid composition the following breeding objectives are mentioned : (1) decreasing linoleate (C18:3) to improve oil flavour and stability, (2) reducing palmitate in soybean oil as a result of nutritional concerns about excessive proportions of saturated fats in the human diet, and (3) increasing stearic acid - provided that this oil will have the processing qualities needed by the food industry to substitute palmitate rich tropical fats. Several sources of low linolenic acid germplasm with approx. 3-4 % C18:3, i.e., with a 50 % reduction in linoleate content compared to traditional soybean cultivars, have been identified.[10,29] With regard to the inheritance of the low linolenic trait in such lines, e.g., the chemically induced mutants 'C1640' and 'A5', considerable differences have been established. In 'C1640' the low C18:3 level is controlled by a recessive allele at the Fan locus ;[30] the decrease in C18:3 is accompanied by a corresponding increase in linoleate (C18:2). In contrast to the fan mutant, the 'A5' mutant has decreased levels of both C18:2 and C18:3 suggesting a defect in oleate denaturation. The genetic basis for the low linolenic, high oleic phenotype of 'A5' could not be attributed to a single nuclear mutation. The trait rather behaves like a quantitative character with partial maternal effects.[31] In experiments with extreme temperature regimes low linolenic lines with the fan allele were relatively stable, while the other low linolenic lines were quite sensitive to environmental variation.[10,29]

With regard to the breeding objective to lower palmitic acid in soybean oil, several lines with reduced levels have been reported.[10] By recombining two different mutagen-derived lines Fehr et al.[32] recently produced a new genotype, which had approx. 4 % palmitic acid, vs. 10-12 % in commonly grown cultivars. The segregation data revealed that the low level of palmitic acid is controlled by different alleles (fap1 and fapx) at two independent loci.

at two independent loci. Further more, the alleles at each locus exhibit additive gen action. The oil of chemically induced high stearic mutants ranged from approx. 15 % to 30 % as compared to 4 % for most standard cultivars. Genetic studies indicated that the high stearic trait was determined by different recessive alleles at the same Fas locus.[33]

The identification of zero-erucic mutants in rapeseed (*Brassicanapus, B.campestris*) was indeed the first discovery opening the era of mutant-derived quality improvement in oil crops.[6,7,20] Traditional varieties of rapeseed and mustard species typically contain high levels (up to 65 %) of eicosenoic (C20:1) and erucic acid (C22:1). Canola quality rapeseed almost lacking these two fatty acids meets all requirements on a prime edible oil.[5,34] However, the nutritional quality of the oil can still be improved by decreasing the linolenate content from average 10 % to less than 3 % accompanied by increasing the level of the dietary valuable linoleic acid to 30 % and even more.[11,34-36] In the last two decades improvements of the C18 fatty acid composition especially were achieved by inducing altered linoleic/linolenic genotypes via experimental mutagenesis.[11,20,37,38] In 1988, the spring rapeseed cultivar 'Stellar', which produces an oil containing less than 3 % linolenate, was released for commercial production in Canada, although its agronomic performance is not satisfactory.[39] Recently, Kräling and Röbbelen[36] showed the possibility to transfer the desirable alleles displaying the low linolenic trait into winter rapeseed by different breeding strategies.

Due to strong environmental and marked maternal influences, only low correlations have been found between the contents of polyenoic fatty acids determined in half-seeds and their progenies.[35] One most important factor influencing the biogenesis of the unsaturated fatty acids is temperature prevailing during seed development. Also maternal effects have been reported with regard to the inheritance of oleic, linoleic and linolenic acid.[40,41]

Only recently, research on mutants with reduced levels of polyunsaturated fatty acids (PUFA) by blocking oleic acid desaturation was carried out or reported. Following gamma radiation treatment of 'Regent' spring rape Wong and coworkers[42] identified a single M_4 family, FA677, with substantially increased oleate content, viz., 79 %. By combining the high oleic with low linolenic trait through crossing and subsequent progeny selection recombinant genotypes with very high oleate (> 85 %) and low linolenate levels (< 3 %) were found.[43] Auld et al.[44] used chemical mutagenesis via EMS-treatment for the same objective. Starting materials in this case were the spring HEAR cultivar 'R-500' (B. *campestris*) and 'Cascade' winter rapeseed (B. *napus*). In B. *napus*, 'X-82' was identified as the most promising mutant displaying 84.4 % C18:1 and 6.6 % PUFA in the M_2 generation, vs. 26.7 % PUFA in 'Cascade'. The best M_3 selections of 'X-82' had oils with > 86 % C18:1, < 3 % C18:2 and < 3 % C18:3. Furthermore, it was possible to select four M_4 lines with good seed yield producing approx. 80 % oleic acid.

3. Alternative biotechnological approaches

3.1. General

The term 'biotechnology' comprises a wide range of different cell and tissue culture techniques and molecular methods. Cellandtissue culture techniques aim in the regeneration of plants out of tissue explants or isolated plant cells in vitro, i.e., meristems, immature embryos, anthers, microspores, protoplasts, etc. Molecular methods include techniques for an identification, isolation and transfer of genes. Since manipulated cells and tissue must be regenerated to entire plants, efficient cell and tissue culture methods are important prerequisites even for genetic engineering. New genotypes produced that way are anything else but cultivars ; they rather represent basic material for further selection and testing in applied breeding programs.

3.2. The use of haploids

Rapeseed and linseed are currently among the crop species most amenable to improvement through biotechnology. For instance, it is possible in these species to obtain haploid and subsequently doubled haploid plants reproducibly through anther and/or microspore culture. For sunflower, such techniques are currently developed.[18] The principle advantage of breeding procedures using haploids, i.e., individual cells and organisms with the single, gametic chromosome number, is the rapid fixation of segregating genotypes - particularly those which occur in low frequency.[45] Following doubling the haploid chromosome complement, so-called doubled haploids - i.e., completely homozygous diploid individuals - are immediately obtained. Consequently, recessive genes coding for specific traits are readily expressed since they are not masked by dominant alleles. Thus, the use of microspore culture principally allows a substantial abbreviation of a breeding cycle.[46] At the same time sufficient variability in other desirable traits is usually available due to genetic recombination, even if haploids are produced from F_1 donor plants.[47] Rapeseed (*Brassica napus*) is known to be well responsive to haploidy techniques, suitable preculture conditions and microspore culture methods have been identified which usually lead to high frequencies of embryogenic microspores, which produce haploid rapeseed shoots.[48,49] The efficiency of this procedure may even be increased in the future by the possibility to select for specific fatty acid composition among individual androgenic embryos.[50,51] Due to the generally high response of B. *napus* genotypes, many oilseed rape breeders are actually testing doubled haploids (DH-lines) in their breeding programs and a large number of lines are already in advanced stages of field testing, thus some of them may soon be released as licensed cultivars.

In contrast to the response of rapeseed, microspores or anthers of linseed (*Linum usitatissimum*) mainly produce callus. However, it has been demonstrated that shoots can be regenerated out of this undifferentiated tissue in comparatively high frequencies, depending on genotype.[52] On the contrary, in sunflower the application of anther- and microspore- culture is rather difficult and the response is very low. Nevertheless, the production of haploid sunflower plants derived from embryogenic microspores is principally possible.[18,53] In general, the efficiency of haploidy methods depends on a sufficient competence of callus and tissue and, therefore, on numerous variables ; the following factors are particularly important : a) genotype of donor plant, b) stage of microspore development, c) temperature during culture induction, d) duration of application of induction phase temperature, e) physiological condition of donor plants, f) composition of the culture medium.[54]

3.3. Sexual interspecific hybridization

Genetic variability for important traits is sometimes narrow in cultivated crop species. For example, actual sunflower cultivars seem to possess a limited genetic basis for various agronomic traits ; most of the current varieties - which are single cross hybrids - are very sensitive to various fungal pathogens - such as *Sclerotinia sclerotiorum*. Therefore, wild *Helianthus* species are of considerable interest as new sources of genetic variation in sunflower. However, it is often difficult to obtain interspecific hybrids by conventional crossing - due to the incompatibility mechanisms leading to an abortion of hybrid embryos. The rescue of such hybrid embryos or complete ovules and their cultivation on artificial media in vitro can help to circumvent such barriers and has been greatly successful in a couple of species including sunflower.[18] An improvement of recovery rates of interspecific hybrid embryos represents a prerequisite to obtain sufficient numbers of offspring, i.e., interspecific hybrid plants and sexual progeny.[55]

In comparison to sunflower and *Helianthus* species, linseed and *Linum* species are much more recalcitrant to an application of embryo rescue technique. Nevertheless, hybridizations of L. *usitatissimum* to low linolenic *Linum* sp. were successful and appear to open new possibilities for reducing linolenic acid content of linseed.[16]

Oilseed rape (*Brassica napus*) is an amphidiploid (AACC) species derived from spontaneous hybridization between B. *campestris* (A-genome) and B. *oleracea* (C-genome).[56] Due to its origin, the gene pool of B. *napus* only contains the genetic variability of those sub-species or accessions which were involved in the original cross(es). Thus, genetic variation seems to be rather limited in B. *napus*. Accordingly, intrageneric-interspecific gene transfer might be a means of increasing genetic variability.[57,58] A summary of successful interspecific crosses in the genus *Brassica* was presented by Nishiyama and coworkers.[59] A particularly impressive strategy in order to broaden the genetic base of B. *napus* is 'resynthesis' by crossing its original ancestors, i.e., B. *oleracea* and B. *campestris*. Several techniques of embryo culture[60-63] have been used to circumvent incompatibility in such hybridizations, and some resynthesized rape forms have already been used in rapeseed breeding.[64] Recently, crosses between B. *campestris* ssp. *trilocularis* ('Yellow sarson') and several selected cauliflower genotypes (B. *oleracea* convar. *botrytis* var. *botrytis*) have been carried out in our laboratory[54] in order to obtain rapeseed lines with modified seedoil quality ; the offspring display desirable variation in their fatty acid composition.

3.4. Asexual or somatic hybridization

In many cases sexual hybridization is not possible - even by embryo rescue - due to the genetic distance between species. In such cases prezygotic incompatibility mechanisms prevent an undisturbed growth of pollen tubes through foreign pistil tissue, so that fertilization cannot be accomplished. In this situation somatic hybridization by protoplast fusion can be a practical avenue for producing respective interspecific hybrids. Basic requirements for this kind of method(s) are the recovery of a sufficient number of intact protoplasts and a satisfactory regeneration capacity of these single "naked" cells.

In the genus Brassica progress in protoplast culture and plant regeneration has led to an extensive application of somatic hybridization.[65] Contrary to rapeseed, protoplast culture of linseed and other *Linum* species is still in an initial stage of development. Nevertheless, entire plants have already been regenerated from protoplasts of different *Linum* species and genotypes.[66-68] In Helianthus big efforts have been invested with regard to protoplast culture in sunflower, but many basic problems need to be solved in this genus before the regeneration of entire plants from protoplasts becomes routine.[18] Among those, pronounced genotypic effects, vitrification, premature flowering, and the difficulty of rooting regenerated shoots represent the most prominent ones.[69] Chanabe et al.[70] regenerated plants from H. *petiolaris* protoplast for the first time, whereas Burrus et al.[71] succeeded in regenerating fertile plants from protoplasts of cultivated sunflower derived from an interspecific hybrid to H. *petiolaris*. Recently, Krasnyanski et al.[72] obtained plants from protoplasts of H. *giganteus*. Although the efficiencies of several steps in the protocols are still low, results encourage continued efforts to develop a system suitable for the transfer of agronomically useful genes into sunflower.

3.5. State and prospects of genetic engineering for modification of oil quality

Efficient transformation systems[73] have already been described to be effective in different crop plants. Alterations to fundamental aspects of plant growth - and especially quality and amount of high-valuable storage products - are in principle possible now, and numerous projects are under way.[74,75] However, extensive field trials are indispensible before the anticipated commercial introduction of the transgenic 'designer' crops and products.[76]

The most effective method of transferring foreign DNA into a host plant is via the Ti-plasmid of Agrobacterium sp., which inserts part of its DNA into host plants. For instance, in rapeseed,[77,78] linseed,[79,80] and *Helianthus*[81,82] successful transformations by transfection using *Agrobacterium tumefaciens* were principally achieved. Other major routes by which transgenic tissue can be obtained are : "gene gun approach",[83] where tiny metal beads coated with DNA are fired at high velocity into living plant tissue, and DNA uptake by protoplasts either directly[84] or after electroporation.[85,86] The usefulness of protoplast technology in the production of transgenics is limited by the ability to regenerate plants from protoplasts, which is confined to a comparatively small number of species, for which suitable tissue culture methods have been worked out.[73] Recently, Bidney et al.[87] have demonstrated that the transformation frequency by *Agrobacterium tumefaciens* in plants recovered from sunflower apical explants was substantially increased, when the meristems were wounded first by particle bombardment. The authors concluded that *Agrobacterium* mediation of stable transformation is more efficient than the analogous particle/plasmid protocol alone.

Basically, all the enzymes determining specific fatty acid biosynthesis and controlling the triacylglycerol composition are targets for genetic engineering approaches.[15,88,89] However, it has to be kept in mind that this technology requires detailed knowledge of lipid biochemistry in order to isolate the specific genes ; this information is still rather limited.[19,26,88,90]

Fatty acid and glycerolipid biosynthesis in plants is a complex process, intracellularly compartimentalized and regulated in a tissue-specific manner.

(1) First of all, atmospheric carbon dioxide is fixed and converted to organic carbon, finally in the form of soluble sucrose which serves as substrate for starch, protein and oil as major storage metabolites, equally.
(2) Carbon supply is an important prerequisite for fat metabolism; it begins with the import of sucrose from the apoplast, and ends with the conversion of acetyl-CoA to manoyl-CoA, the step immediately preceding acylcarrierprotein-dependent de novo fatty acid synthesis.[19]
(3) Fatty acid metabolism starts with the priming of the acyl carrier protein (ACP) with an acetyl group from acetyl-CoA and ends with the final triacylglycerol (TAG) assembly. The whole process is driven by a number of enzymes or enzyme systems; at least 50 different metabolic enzymes must be anticipated involved in the conversion of carbon dioxide to fats containing a mixture of saturated and unsaturated fatty acids of different chain length - indicating numerous points of regulation at both the metabolic and genetic level.[19]
(4) The primary enzymes controlling de novo fatty acid biosynthesis - confined solely to the chloroplast or proplastid - are the acetyl-CoA carboxylase (ACC) and the fatty acid synthetase (FAS) system; the former is an ancillary enzyme responsible for the ATP-dependent conversion of acetyl-CoA to manoyl-CoA, which is an essential substrate for FAS.
(5) The FAS system of plants is composed of the acyl carrier protein (ACP) as a structural component and 7 individual enzymes catalyzing the partial reactions of fatty acid synthesis, viz., acetyl-CoA transferase (AT), malonyl-CoA transacylase (MT), ß-ketoacyl-ACP synthetaseI (KASI), ß-ketoacyl-ACP reductase (KR), ß-hydroxyacyl--ACP dehydratase (HD), enoyl-ACP reductase (ER) and acyl-ACP thioesterase (TE). Following successive elongation of the acyl chain by two-carbon units the product of plant FAS is usually palmitoyl-CoA. This acyl-CoA with 16 carbon is turned to stearoyl-CoA through subsequent elongation by probably another elongation system-[91,92] differing from FAS by the specific condensing enzyme KASII.
(6) However, there are certain vegetable oils, e.g., from coconut and palm kernel, containing up to 90% short- and medium-chain fatty acids (C8:0 to C14:0). With regard to FAS regulation several mechanisms of chain length determination have been discussed.[19,91,93] Recently, the discovery of a third isoform of ß-ketoacyl-ACP synthetaseIII (KASIII), which appears to be responsible for the first condensation of acetate with manoyl-ACP, reveals a further possibility of FAS regulation.[93,94]
(7) Stearoyl-ACP rapidly undergoes desaturation to oleoyl-ACP and subsequent hydrolysis to free oleic acid as the main product of fatty acid synthesis in the plastid of common oil plants. The free fatty acids, viz., palmitate, stearate and oleate, are then transported to the cytosolic compartment, particularly the endoplasmic reticulum (ER) membranes for further modification. Therefore, special CoA-dependent elongation and phosphatidylcholine (PC)-dependent desaturation systems as well as specific acyltransferases (AT) are involved in the assembly of triacylglycerols typical for each kind of oil plants.[51,90,93,95,96]

Triacylglycerols occur predominantly in the seed of oil plants, e.g., rape (*Brassica napus*) and soybean (*Glycine max*), formed in the cotyledons of the embryo tissue during maturation, stored in subcellular organelles, i.e., oleosomes or oil bodies, and again 'mobilized' as a source of energy for the growth and early metabolism of the embryo during germination.[51,97]

Great efforts are currently invested in order to isolate the genes encoding various desirable enzymes of lipid biosynthesis, and transfer them from undomesticated or even wild species to rapeseed[98-100] or other important annual oilseed crops. Within the last few years, substantial progress has been reported in several areas of research. For example, attempts to isolate the ACC - as a possibly rate-limiting step of fatty acid biosynthesis - of rapeseed are based on available amino acid sequences of the ACC of other organisms (bacteria and mammals). Slabas and coworkers purified ACC and demonstrated that, in rape (B. *napus*), both the seed and leaf form of the enzyme is composed of a single high molecular weight 220 kDa polypeptide.[92]

ACP is regarded as a key component involved in the regulation of fatty acid synthesis in plants. For its stability and relatively easy purification it is at once the best characterized protein in lipid metabolism. The occurrence of multiple ACP isoforms in a diverse group of bacteria and plants was examined by Battey and Ohlrogge[101], as well as the tissue-specificity and the effect of light on the expression of ACP isoforms. Various plant genes encoding for ACPs have already been isolated and cDNA-clones prepared, e.g., from B. *napus*[102] and from B. *campestris*.[103] Structurally distinct gene sets seem to be responsible for ACPs in the seed and in the leaves. Based on quantitative Southern analysis, it has been estimated that the seed-specific expressed gene family in rapeseed (B. *napus*) is made up by about 35 genes.[104]

Several components of the FAS system have already been isolated. For example, KASI has been purified from developing rape seeds.[92,105] Furthermore, reports of isolation of cDNA clones of KASI from barley,[106] *Ricinus communis* (castor) and rapeseed (Töpfer, pers. comm.) may open the possibility of developing plants with medium-chain fatty acids due to the role of KASI in chain length determination. Recently, another mechanism for medium-chain fatty acid synthesis in plants has been demonstrated from scientists at Calgene Inc., Davis, California.[100] A lauroyl-ACP specific thioesterase (TE), a probably essentiell enzyme in the formation of lauric acid in plants, has been isolated from the seeds of the undomesticated California bay tree (Umbellulariacalifornica), which contains considerable amounts of caprate (C10:0) and laurate (C12:0). The trait has been introduced into the common wall cress (Arabidopsis thaliana), a weedy crucifer which normally produces only long-chain fatty acids with 16 to 22 carbons. The transferred gene encodes a thioesterase hydrolyzing the growing fatty acid chains from ACP, thus terminating them at medium chain length. In different transgenic Arabidopsis plants, laurate content ranged from 10 to 25% of total fatty acid produced, at the expense of long-chain fatty acids the plant usually generates. By the same procedure, the Calgene group has also engineered laurate production into rapeseed (canola),[100,107] although the production of novel medium-chain fatty acids seems to occur in low frequency. Nevertheless, this example demonstrates that such an approach producing an economically important fatty acid in a transgenic oil crop is principally feasible.

After all, the usefulness of genetic engineering concerning target reactions of lipid biosynthesis might be limited by the fact, that the expression of chimaeric genes may affect normal seed development; for example, disruptive cell membrane function caused by unusual fatty acids in the polar lipids may be a consequence. Furthermore, the frequency, stability and functionality of foreign genes in progeny of transformants is not always satisfactory. This is partially due to the fact, that engineered plants will likely require seed specific promoter sequences, which regulate the expression of the structural genes, e.g., coding for enzymes of lipid biosynthesis.[19,90,108]

Finally, the transgenic plants obtained will represent basic material which still needs selection and testing by plant breeders, rather than highly performing varieties which can readily be released as licensed cultivars.

4. Conclusions

The specific fatty acid composition of different seed oils is determined by a number of major genes. In several plant species one or a few genes have been described as being responsible for high or low content of individual fatty acids, like oleic, linoleic, linolenic or erucic acid. Due to its comparatively high degree of heritability, the composition of seed oil can be efficiently modified - even by "classical" breeding procedures.

Biotechnology methods have recently been optimized for practical application in breeding programs. In particular, haploidy techniques have already been integrated in breeding cycles of rapeseed. Nevertheless, further improvements of culture conditions and donor plant preculture are still necessary since they represent undesirable selection factors, which may narrow genetic variation. Also in linseed, microspore and anther culture could meanwhile be further developed and improved. However, further investigations will be necessary to identify factors which determine the frequency of embryogenic microspores and to develop conditions which prevent callus formation affecting normal shoot development. Big efforts have been invested in order to establish an efficient haploidy technique in sunflower. Until now, it is possible to induce shoot regeneration out of callus tissue, but the production of entire plants which can be transferred to soil and grown to maturity still remains difficult.

Furthermore, interspecific hybridization has already a tradition in the genus *Brassica* for the introduction of desirable genes into rapeseed germplasm. Of particular interest is the development of resynthesized rapeseed, e.g., in order to modify fatty acid composition in rapeseed oil. With regard to this approach, the application of embryo culture techniques represents a supplementary tool which allows to increase the yield of progeny plants from such crosses. A large number of valuable interspecific hybrids were also realized in the genus *Helianthus* with the aid of the embryo rescue technique. The latter technique has also led to a number of interspecific *Linum* hybrids and thus has principally proven its usefulness. Whereas techniques of protoplast culture in rapeseed are ready for practical application, this technique is still in an initial stage in linseed and even more in sunflower.

In the near future genetic engineering will certainly reach importance in breeding of vegetable oil crops with custom-tailored oil profiles. The intensive efforts aiming in an isolation and transfer of genes responsible for specific enzymes involved in the triacylglycerol biosynthesis - for example in rapeseed - stimulate this expectation. Therefore, genetic engineering is seen as a powerful tool for the modification of oilseed crops. However, it has to be kept in mind that this technology requires detailed knowledge of lipid biochemistry in order to isolate the specific genes ; this information is still rather limited. Furthermore, efficient and reproducible transformation protocols are required. Finally, the transgenic plants obtained will represent basic material which still needs selection and testing by plant breeders, rather than highly performing varieties which can readily be released as licensed cultivars.