Breeding and agronomic development of linseed and sunflower for technical markets

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21.1 INTRODUCTION

In recent years, the interest in vegetable oils and fats as basic materials for industrial usage has been increasing. Specific demands require specific fatty acid profiles which determine the suitability of vegetable oil for different applications. Whereas food use requires an optimum mixture of saturated and unsaturated fatty acids with chain lengths of 16–18 carbon atoms, industrial applications need an oil composition with a predominance of a single desirable fatty acid. The wide range of industrially valuable fatty acids is the basis for a number of different breeding targets. Such targets are, for example, maximum linolenic content in linseed oil and maximum oleic acid content in sunflower oil (e.g. Pryde and Rothfus, 1989; Eierdanz and Hirsinger, 1990). The seed oil of sunflower (*Helianthus annuus* L.) is normally rich in linoleic acid (C18:2, about 70%), whereas the oil of linseed (*Linum usitatissimum* L.) genotypes contains comparatively high levels of linolenic acid (C18:3, 55–60%). Extensive breeding efforts have been made in the past to provide oil crops with optimum oil quality, i.e. with a maximum content of a single fatty acid.

Both sunflower and linseed are crop species very well adapted to relevant European agricultural production areas. Whereas sunflower cultivation is limited to more favourable climates (i.e. in southern regions with elevated temperatures), cultivation of linseed is almost unrestricted by climatic conditions. Highly efficient agricultural production systems have been developed for both species, so that they can be beneficially used for producing different types of renewable vegetable oil.

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Table 21.1 Fatty acid composition of prominent genotypes of major oil crops

Rapeseed (*Brassica napus*)	
Original oil type:	45–50% erucic acid
'Canola'	≤1% erucic, 55–65% oleic
HEAR oil	≥60% erucic acid
HOAR oil	≥80% oleic acid
Linseed (*Linum usitatissimum*)	
Original oil	55–60% linolenic acid
'Linola'	≤3% linolenic acid
Sunflower (*Helianthus annuus*)	
Conventional type	70% linoleic acid
High oleic type	≥85% oleic acid

HEAR, high erucic acid rapeseed; HOAR, high oleic acid rapeseed.

21.2 CLASSICAL BREEDING METHODS

Because of great interest in linseed and sunflower as sources for new types of vegetable oil, a variety of valuable lines and cultivars have been developed by conventional breeding methods, which allow the recovery of custom-tailored oil (Baumann *et al.*, 1988; Pryde and Rothfus, 1989; Scowcroft, 1990). For example, the removal of erucic acid (C22:1) from rapeseed oil was one of the most important breeding objectives in rapeseed 20 years ago, since erucic acid is undesirable in edible oil and fat (Stefansson and Hougen, 1964). Also, the almost complete elimination of linolenic acid from linseed oil (Green, 1986) and the development of high oleic sunflower types (Soldatov, 1976; for more details cf. Friedt, 1992) are examples of successful conventional genetic approaches via mutagenesis and selection (Table 21.1).

Linseed is a self-fertilizing species where breeding via pedigree selection is a rather straightforward process, leading to homozygous breeding lines and cultivars (Table 21.2). New breeding lines have recently been developed after mutagenic treatment by ethyl methane sulphonate (EMS) (Nichterlein *et al.*, 1989a) and repeated cycles of recombination and selection (Table 21.3).

In contrast to linseed, sunflower is an outcrossing species. Breeding activities nowadays focus on the development of inbred lines with high

Table 21.2 Phases of breeding of auto- and allogamous species

Phase	*Self-pollinator*	*Outbreeder*
Creation of variation:	Hand cross (recombination)	Open pollination, specific crosses
Selection:	Performance *per se*	Performance *per se* plus combining ability
Testing and maintenance:	Replicated multilocational field trials	
	Control of variety type	Isolation and control of type

Table 21.3 Oil flax mutants with extreme oil compositions (%) (Nickel, personal communication)

Line no.	*16:0*	*18:0*	*18:1*	*18:2*	*18:3*
1974/1/9[a]	5.0	4.2	65.2	5.8	19.7
615/1/10[a]	4.0	4.0	21.7	39.7	30.5
1951/1/16[a]	5.0	2.9	9.8	37.3	45.0
515/2/33[a]	4.2	3.2	9.4	8.5	74.7
cv. Antares[b]	4.6	4.1	18.5	16.6	56.2
cv. Atalante[b]	6.1	4.0	17.3	15.8	56.8
cv. Hella[b]	5.4	4.5	16.1	15.2	58.8

[a] F_2 halfseeds; [b] average of three samples of 20 seeds, each.

combining ability (Table 21.2). Crosses between pairs of such lines lead to high-yielding single cross hybrids (Fig. 21.1). Commercial production of hybrid seed in an hermaphrodite plant like sunflower requires a system for controlled pollination. For this purpose, sunflower cytoplasmic male sterility (CMS) can be used efficiently. However, at the present time a unique source of CMS, first reported by Leclercq (1969) in the progeny of a cross between *Helianthus petiolaris* (female) and a cultivated sunflower line (male), is used for hybrid seed production worldwide. This narrow cytoplasmic background of the cultivated sunflower hybrids implies the potential danger of disease epidemics due to the occurrence of pathogens compatible with this cytoplasm. Therefore, broadening of the cytoplasmic–genetic background of sunflower is needed urgently. Related breeding activities have been initiated (Horn *et al.*, 1991; Friedt, 1992).

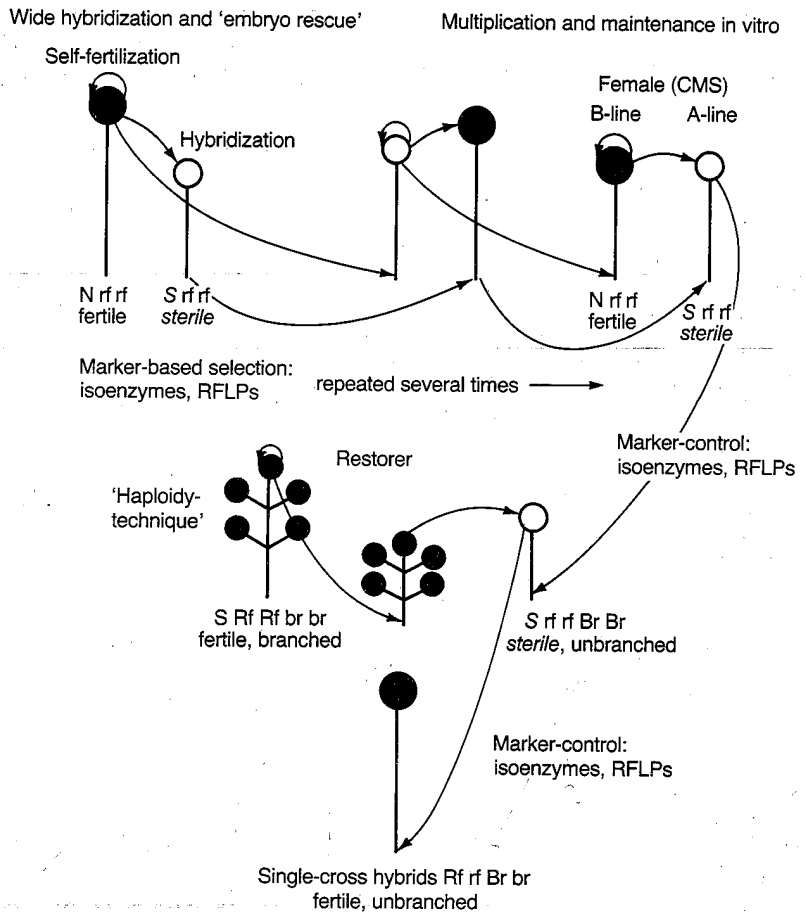

Figure 21.1 Possible applications of biotechnology in sunflower breeding. CMS, cytoplasmic male sterility; A-line, male sterile female; B-line, fertile analogon (maintainer). Gene symbols: N, 'normal' cytoplasm; S, sterility-inducing cytoplasm; Rf/rf, restorer/non-restorer gene; Br/br, unbranched/branched growth habit; RFLPs, restriction fragment length polymorphisms.

21.3 BIOTECHNOLOGY AS A SUPPLEMENTARY TOOL

In addition to traditional breeding methods, further progress can be achieved through the application of biotechnology as a promising supplementary tool (Friedt *et al.*, 1989, 1991; Friedt, 1992; Thierfelder *et al.*, 1992). Plant biotechnology includes a wide range of different cell and tissue culture techniques and molecular methods. Cell and

tissue culture techniques aim at the regeneration of intact plants out of isolated plant cells or tissue *in vitro*, i.e. meristems, immature embryos, anthers, microspores, protoplasts, etc. Molecular methods are used, for example, for identification of genomes or genes and the isolation and transfer of individual genes. Since manipulated cells and tissue must be regenerated to entire plants, efficient cell and tissue culture methods are important requirements even for genetic engineering. New genotypes produced in this way are anything else but cultivars; however, they may represent valuable basic materials for further applied breeding.

Linseed can now be considered as one of the crop species most amenable to improvements through these techniques. For instance, it is possible in this species to obtain haploid and doubled haploid plants reproducibly through anther or microspore culture and protoplast regeneration. In sunflower, initial results have also been obtained. However, success rates are still very low and substantial improvements of these techniques are still required.

21.4 HAPLOIDY

The principle advantage of haploidy, i.e. the presence of a single set of chromosomes, is the possibility of rapid fixation of rarely segregating genotypes, in which recessive genes coding for specific traits are combined in homozygous condition. Thus, the use of microspore culture allows a substantial reduction in a breeding cycle. Genetic variation in desirable traits, such as oil quality, is usually sufficient as a result of genetic recombination, even if haploids are produced from F_1 donor plants (cf. Umbach and Friedt, 1991). Microspores of linseed, either cultured within the anthers or as isolated single cells, mainly produce callus. However, it has been demonstrated by Nichterlein *et al.* (1989b, 1992) that shoots can be regenerated out of this undifferentiated tissue in comparatively high frequencies, depending on genotype (Table 21.4).

Contrary to linseed, the application of anther and microspore culture is rather difficult in sunflower although the regeneration of haploid sunflower plants derived from embryogenic microspores has been demonstrated in a few cases (Alissa *et al.*, 1985; Bohorova *et al.*, 1985; Mix, 1985; Mezzarobba and Jonard, 1986, 1988). However, the response is comparatively low and depends on numerous factors, such as genotype, culture medium and interactions between genotype and medium (Gürel *et al.*, 1991, cf. Tables 21.4 and 21.5).

In general, the efficiency of haploidy methods depends on the sufficient competence of cells, callus and tissue and, therefore, on numerous variables. The following factors are particularly important.

Table 21.4 Effect of culture media on callus formation and shoot regeneration from anthers of linseed (*Linum usitatissimum*; Nichterlein *et al.*, 1992)

Genotype	*Anthers producing callus (%)*[a]		*Calli forming shoots (%)*[b]	
	A22	*G23*	*RL20*	*P20*
Atalante	39.1 (129/330)		19.7 (12/61)	80.0 (52/65)
		17.4 (59/339)	0 (0/24)	45.7 (16/35)
Hella	38.9 (109/280)		6.3 (3/48)	11.5 (6/52)
		36.7 (117/319)	0 (0/49)	13.0 (7/54)
Midas	6.7 (22/330)		0 (0/6)	0 (0/15)
		10.5 (41/389)	0 (0/17)	20.8 (5/24)
AR2	11.8 (33/280)		5.3 (1/19)	0 (0/13)
		9.1 (29/319)	0 (0/11)	0 (0/17)

Figures in parentheses indicate: [a] number of callusing anthers/total number of plated anthers, [b] number of shooting calli/total number of calli. A22, G23, RL20, P20, culture media.

Table 21.5 Average response of sunflower anthers cultured on different media (modified after Mezzarobba and Jonard, 1988) in the tetrad stage of microspores (adapted from Gürel *et al.*, 1991)

Genotype	*A*	*C*	*C(%A)*	*S(%C)*	*S(%A)*
B11 A3	786	454	57.8	2.2	1.5
DO 131	435	251	57.7	3.2	1.9
Frankasol	393	94	23.9	0	0
MH 1-2	428	124	29.0	3.0	0.7
Sunbred 262	450	96	21.3	0	0

A, number of anthers cultured; C, number of anthers forming callus; C(%A) % anthers with callus; S(%C), % calluses with shoots; S(%A), % anthers showing shoot formation.

1. Genotype of donor plants: the embryogenic or callusing capacity of microspores varies greatly amongst species and cultivars (cf. Tables 21.4, 21.5); however, corresponding variation is even found between individuals of the same genotype, due to unknown environmental effects, as affected, for example, by the factors below.
2. The stage of microspore development: the uninucleate stage is usually most suitable for inoculation.
3. The temperature during the culture induction period.
4. The duration of application of the induction phase temperature.
5. The physiological state of donor plants: optimum growing conditions of donor plants depend on species and genotype.

6. The composition of the culture medium: high levels of sucrose (i.e. 8–13%) are essential for the induction of embryogenesis in anther culture; specific concentrations and compositions of plant growth regulators (i.e. auxins and cytokinins) are particularly beneficial for some species.

Owing to the promising response of linseed genotypes, breeders have started the testing of doubled haploids (DH-lines) in their breeding programmes. Some DH-lines are in the advanced stages of field testing, so that the release of a new cultivar derived via the haploidy breeding technique may be anticipated. The successful application of a haploid technique results in the development of homozygous breeding lines having the inherent genetic variability created by the crosses from which they are derived so that the selection of improved transgressive lines and the production of cultivars in autogamous and allogamous species may be feasible.

21.5 INTERSPECIFIC CHROMOSOME OR GENE TRANSFER

Genetic variation for important traits is occasionally limited in cultivated crop species. Thus, in the sunflower cultivars presently available the genetic basis for various agronomic traits seems to be rather narrow (Arnaud, 1986), probably due to the derivation of the cultivated sunflower from a limited gene pool (e.g. Rieseberg and Seiler, 1990). Most of the present varieties are very sensitive to fungal pathogens such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

Furthermore, most of the commercial sunflower cultivars represent single cross hybrids and their production is solely based on the unique source of CMS mentioned above. In general, wild *Helianthus* species are of considerable interest as a source of genetic variation for economically important characters, such as CMS (Whelan, 1980, 1981; Anaschenko, 1981; Heiser, 1982) and disease resistance (Rogers *et al.*, 1982, 1987; Lipps and Herr, 1986). Therefore, wide hybridization can principally help to create new genetic variation.

However, it has been difficult to obtain interspecific hybrids by sexual crossing due to incompatibility mechanisms. For instance, the failure of the endosperm to develop normally represents a 'post-fertilization barrier' and leads to the abortion of hybrid embryos. The rescue of these embryos or the complete ovules and their cultivation on artificial media *in vitro* can help to circumvent such barriers and has been very successful in a couple of species including sunflower (e.g. Chandler and Beard, 1983; Espinasse *et al.*, 1985, 1991). An improvement in recovery rates of interspecific hybrids is a prerequisite to obtain the required large numbers of progeny. These conditions were optimized in our institute and led to a series of sunflower hybrids (Kräuter *et al.*, 1991) which have already proved to be highly valuable in breeding both disease resistant and male sterile female lines (cf. Friedt, 1992).

In comparison to sunflower and *Helianthus* species, linseed and *Linum* species are more recalcitrant to an application of the embryo rescue method, due to 'prefertilization barriers'. Nevertheless, hybridizations of *L. usitatissimum* with other high-linolenic *Linum* spp. were successful and appear to open new possibilities for increasing the linolenic content of linseed (Nichterlein *et al.*, 1989b).

21.6 ASEXUAL HYBRIDIZATION VIA PROTOPLAST FUSION

Many interspecific sexual hybridizations are not feasible, even by embryo rescue, due to the genetic distance between the respective species. In such cases, 'prezygotic incompatibility' mechanisms prevent undisturbed growth of pollen tubes through foreign pistil tissue; thus, fertilization cannot be accomplished. In this situation somatic hybridization, i.e. cell or protoplast fusion, can be a practical avenue for producing respective interspecific hybrids. In such a way, various combinations of the cytoplasmic and the nuclear genomes of different species can be obtained. Basic requirements for this kind of method(s) are the feasibility of protoplast isolation and the subsequent regeneration capacity of isolated protoplasts.

As compared with other species, such as rapeseed, the protoplast culture technique of linseed and other *Linum* species is still in an initial stage. Nevertheless, entire plants have already been regenerated (Barakat and Cocking, 1983, 1985; Ling and Binding, 1987). Recently, similar experiments have also been successful in our institute (Bergmann, personal communication). Although major efforts have already been invested in protoplast culture of sunflower, many basic problems need to be solved in this genus. Among these, pronounced genotypic effects, vitrification and the difficulty of rooting regenerated shoots are prominent (Bohorova *et al.*, 1986; Lenée and Chupeau, 1986; Guilley and Hahne, 1989; Burrus *et al.*, 1991; Chanabe *et al.*, 1991).

21.7 PROSPECTS OF GENETIC ENGINEERING FOR MODIFICATION OF OIL QUALITY

After necessary transformation systems have been elaborated, genetic engineering is principally applicable to both linseed (e.g. Basiran *et al.*, 1987; Zhan *et al.*, 1988) and sunflower (cf. Schrammeijer *et al.*, 1990). However, constraints of the successful application of gene transfer in applied breeding programmes are not solely due to the difficulty of

Table 21.6 Structure of seed yield of linseed and sunflower compared with winter-rapeseed

	Rapeseed	*Linseed*	*Sunflower*
No. of plants per m^2	50	400	7
No. of seeds per plant	2400	130	1200
1000-seed-weight (g)	5	8	65
Seed yield (t/ha)			
Potential	6.0	4.2	5.5
Realized	3.2	2.0	3.0

regenerating intact plants from transformed cells or tissue. There may also be basic problems restricting breeding by genetic engineering.

Fatty acid biosynthesis, from the initial acetyl-CoA and leading to final triacylglycerol (TAG) assembly, is a complex process which is controlled by a large number of enzymes or enzyme systems. More than 30 different metabolic enzymes may control synthesis of a seed oil (e.g. of rapeseed) containing saturated and unsaturated fatty acids of different chain length from C16 to C22 (Gurr, 1980; Stumpf, 1988). Besides the primary fatty acid synthase (FAS) system, elongases, desaturases and specific acyltransferases are involved in the assembly of triacylglycerols, at least in crucifers (Stumpf and Pollard, 1983; Mukherjee, 1985; Fehling *et al.*, 1990; Taylor *et al.*, 1991). However, the systems of other plant species are of a similarly complex nature. Finally, even if the attempt to modify a specific target reaction is feasible and successful, it must be taken into account that the expression of a transformed gene may affect normal seed development, e.g. disruptive cell membrane function caused by unusual polar lipids may be the consequence (Stumpf, 1988; Ohlrogge, 1988; Battey and Ohlrogge, 1989).

21.8 ASPECTS OF AGRONOMY AND PLANT PRODUCTION

Optimum plant production systems are complex and they do not only require the provision of new, improved cultivars. Successful plant production also requires sophisticated agronomic techniques. The yield potential of modern sunflower hybrids is estimated to be about 5–6 t/ha; linseed cultivars may yield 4 t/ha under optimum growing conditions (Table 21.6). This potential is usually not fully exploited under 'normal' agricultural conditions, owing to the difficulty of optimizing all of the relevant yield components. Furthermore, the complete exploitation of the maximum yield potential is limited by both economic and ecological constraints. Economic constraints are, for example, the necessity of reducing inputs (i.e. fertilizer application and chemical treatments) owing to falling prices for agricultural products. Ecological obstacles result from activities aimed at environmental preservation, e.g. the reduction of chemicals and biocides in crop production. As a result, in the future new cultivars will be needed which only require reduced amounts of fertilizer and less treatment with fungicides, insecticides, etc. Such varieties must be more disease or pest resistant and able to use available nutrients efficiently. Therefore, breeding for disease resistance and stress tolerance will be major goals in the future. Genetic variation for both trait complexes is partially available in cultivated linseed and sunflower. Further variability can be created by means of biotechnology, e.g. by interspecific hybridization. Gene technology will only make significant contributions to the improvement of such complex characters after the genetic basis of these complexes has been investigated and understood in much more detail than has been available to date.

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