Biotechnology in Breeding of Industrial Oil Crops – The Present Status and Future Prospects*

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With increasing "overproduction" of food supplies it is frequently emphasized now that agricultural production of industrial "non-food" raw materials should be intensified. Many adapted crop plants are already available for producing various kinds of natural materials. Particularly the large group of oil-crops could be used even more widely for providing vegetable oils for numerous technical purposes. The example of rape-seed *(Brassica napus)* clearly demonstrates that the composition of vegetable oil can be completely reconstructed according to the wishes of manufacturers or consumers, even by conventional breeding methods. Further, more rapid and efficient breeding is expected by application of modern "biotechnology", i. e. tissue- and cell-culture techniques as well as "genetic engineering". Where variation for a character like oil-quality is limited within a crop plant, a wide range of alien wild species is available for broadening genetic variation of plants like rapeseed, sunflower *(Helianthus annuus)* or linseed (flax, *Linum usitatissimum*). Exploration of such "new" genetic variation is nowadays facilitated by *in vitro* embryo culture or cell (protoplast)-fusion techniques. Such biotechniques can help to overcome crossing barriers between species as shown e. g. in the genus *Brassica*. But also in other important oilcrops like linseed or sunflower, biotechniques can now be applied profitably. For example, it has been demonstrated that protoplasts can be regenerated in *Linum*, so that asexual interspecific hybrids can principally be produced in that way. Alien species of sunflower and linseed show a wide range of variation regarding agronomically important characters, particularly of oil composition and disease resistance. This alien genetic variation can be used for breeding new disease resistant oil-crop cultivars. Other techniques, like the "haploidy-method" can help to accelerate a breeding programme, ultimately leading to a homozygous line or cultivar. For example, haploids are now routinely induced in linseed and various *Brassica* crops. Finally, genetic engineering will allow in the future, to isolate qualitatively highly effective genes from any organism and transfer it functionally into adapted high-yielding crop plants. Therewith, the agronomic and economic value of such crops may be more efficiently and rapidly increased than until now.

Biotechnologie in der Züchtung von Industrie-Ölpflanzen – Gegenwärtiger Stand und Perspektiven für die Zukunft

Bei zunehmender „Überversorgung" mit Nahrungsmitteln wird eine alternative landwirtschaftliche Produktion von nachwachsenden Industrierohstoffen verstärkt diskutiert und gefordert. Zahlreiche heimische Pflanzenarten stehen für eine Erzeugung verschiedenartiger Rohstoffe zur Verfügung. Insbesondere bei den ölliefernden Pflanzen sind sehr gute Chancen einer Gewinnung unterschiedlichster Öle für vielfältige technische Zwecke gegeben. Das „Beispiel Raps" zeigt auf eindrucksvolle Weise, wie die Zusammensetzung pflanzlicher Öle den jeweiligen Wünschen der Verarbeiter bzw. Verbraucher schon mit konventionellen Züchtungsmethoden angepaßt werden kann. Noch raschere und effizientere Züchtungserfolge werden vom Einsatz der „Biotechnologie", d. h. von Gewebe- und Zell-Kulturtechniken sowie der Gentechnik, erwartet. Wo die Merkmals-Variation innerhalb einer Art zu eng ist, stehen zahlreiche Wildformen als Ausgangsmaterial für eine Erweiterung der genetischen Variation zur Verfügung, wie etwa bei den Kultur-Ölpflanzen Raps *(Brassica napus)*, Sonnenblume *(Helianthus annuus)* oder Lein (Flachs, *Linum usitatissimum*). Eine noch intensivere und breitere Nutzung solcher „neuen" genetischen Variation wird heute durch Embryo-Kultur und Zell (Protoplasten)-Fusionstechniken *in vitro* ermöglicht. Mit deren Hilfe können Artgrenzen leichter überwunden werden, wie etwa in der Gattung *Brassica* demonstriert wurde. Aber auch in den Gattungen *Linum* und *Helianthus* sind heute neue, asexuelle interspezifische Hybridisierungen möglich. Verwandte Arten von Sonnenblume und Lein weisen eine große Variation hinsichtlich agronomischer Werteigenschaften auf, insbesondere bzgl. der Ölzusammensetzung und der Krankheitsresistenz. Entsprechende Gene der Wildformen können somit heute für die Züchtung neuer Kultursorten mit veränderter Ölqualität für verschiedene technisch-industrielle Zwecke genutzt werden. Weitere Techniken, wie die „Haploid-Methode" können helfen, den Zuchtgang bis hin zur homozygoten Linie oder Sorte zu beschleunigen. Haploide werden heute etwa bei Lein oder verschiedenen *Brassica*-Arten routinemäßig induziert. Schließlich wird in Zukunft die Gentechnik es erlauben, einzelne qualitativ sehr wirksame Gene aus beliebigen Organismen zu isolieren, in adaptierte Hochleistungspflanzen wirksam zu übertragen und damit deren agronomischen und ökonomischen Wert gezielt und rasch weiter zu verbessern.

Introduction

Production of traditional agricultural crops, particularly of cereals, has been progressively increased during the last decades due to an improvement of both, yield potential and agrotechnique. Increased productivity has recently led to growing surpluses of these crops in developed countries, e. g. in Europe, where about one sixth of the world's cereal grain is produced (Table 1).

At the other hand, demand of plant products, like vegetable oils, for industrial "non-food" purposes is increasing. Only 13 % of the oils-and-fats world-production are presently used for industrial "non-food" products (Fig. 1, from Lit. 8). Therefore, cultivation of respective "alternative crops" is one possibility of reducing cereal production. Many plant species from which different types of starch, sugar, protein, oil or fat can be extracted, are available. Particularly the large group of oilcrops, including e. g. rapeseed, mustards, linseed, sunflower and soybean, can be used for multiple industrial purposes[1].

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The present status

Adapted oil crops

The EC already plays an important role in the production of oilcrops, mainly for nutritional purposes; more than one quarter of the world's production of sunflower kernels and more than one third that of rapeseed is produced in the EC. For other crops, like soybean and linseed, European production is less important (Table 1).

Table 1

Cultivation and production of important world crops

		World 1984	Europe (%)* 1984	Germany F. R. 1984	1986	1987
Rapeseed	a)	13606**	2717 (20.0)	254	307	430
	b)	16368	5802 (35.4)	662	956	?
Linseed		4928	236 (4.8)	–		
		2574	130 (5.1)	–		
Sunflower		13431	2915 (21.7)	–		
		15941	4385 (27.5)	–		
Soybean		52056	577 (1.1)	–		
		89893	946 (1.1)	–		
for comparison Cereals		730011	69867 (8.6)	4993		
		1801684	306952 (17.0)	26064		

*percent of world production; **a) 1000 ha, b) 1000 t

Cultivation of oilcrops could even be extended if alternative industrial utilizations of vegetable oils would be exploited. This is certainly feasible, since each plant oil is characterized by its own specific fatty acid composition (Table 2). Therefore, individual industrial usages could be envisaged for each particular crop species[1].

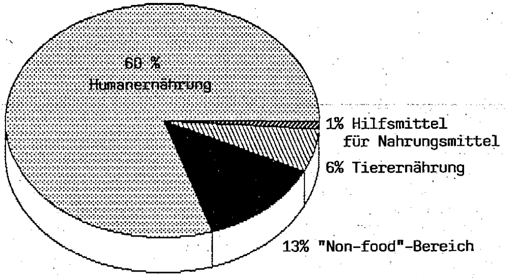

Fig. 1. Utilization of world fat production (after Lit. 8)

Table 2

Average fatty acid composition of various types of seed-oil (% total fat)

Fatty acid	Rapeseed old	Rapeseed new	Mustard (*S. alba*)	Sunflower nut.	Sunflower ind.*	Soy-bean	Lin-seed
$C_{14:0}$ Myristic	1	<1	–	t	t**	t	–
$C_{16:0}$ Palmitic	2	4	3	6	3	8	5
$C_{18:0}$ Stearic	1	1	<1	4	3	4	4
$C_{18:1}$ Oleic	15	**60**	20	20	**90**	25	15
$C_{18:2}$ Linoleic	15	20	9	**70**	4	**55**	20
$C_{18:3}$ Linolenic	7	9	10	t	t	6	**60**
$C_{20:0}$ Arachidic	1	<1	<1	<1	<1	<1	t
$C_{20:1}$ Eicosenic	7	2	9	–	–	–	–
$C_{22:0}$ Behenic	1	1	<1	<1	<1	–	–
$C_{22:1}$ Erucic	**50**	2	**>50**	–	–	–	–

* nut. = nutritional, ind. = industrial type; ** t = traces

For example, soybean- and sunflower-oils are characterized by high linoleic acid contents, while the main component of linseed is linolenic acid. Genetic manipulation of the fatty acid composition is possible not only by "modern" but also by conventional breeding methods, as many examples demonstrate. One impressive example is the elimination of erucic acid from rapeseed oil, which made it possible to use this particular vegetable oil for human consumption. Recently, new rapeseed- and sunflower genotypes with high oleic acid ($C_{18:1}$) content, i. e. more than 80% (or even 90 %) of the total fatty acids, became available for breeding new industrial crop cultivars. The substantial demand of chemical industries for this specific type of high-oleic oil can therefore be satisfied now. However, further genetic manipulations of fatty acid composition by breeding, depending on future demands, are still feasible.

Besides oil quality, i. e. composition of the oil, the economic output, i. e. seed and oil yield, has to be considered. It needs to be finally competitive with that of other high yielding crops, such as cereals or sugarbeet. At the present time, this is particularly the case for rapeseed (*Brassica napus*), because of an extensive improvement of its seedyield in recent years. However even higher rapeseed yields can be expected for the future (Table 3).

For other *Cruciferae*, such as yellow mustard (*Sinapis alba*), an openpollinating crop with high erucic acid ($C_{22:1}$) content (Table 2), higher yields can also be obtained through intensified breeding programmes. This is also the case for linseed (*Linum usitatissimum*), whereas sunflower (*Helianthus annuus*) can already be considered as a highly performing crop even for areas with moderate climates in Europe (Table 3).

Table 3

Cultivation and utilization of important oilcrops

Species	Suitability for European Climate	Agriculture	Utilization	Yield (dt/ha)
Rapeseed	++	++	++	25⟶50
Mustard species	++	+	++	10⟶25
Linseed	++	+	++	10⟶20
Sunflower	+	+	++	15⟶40
Soybean	0	0	++	20⟶30

++ = very good, + = good, 0 = needs improvement

Possible future crops

Beyond that, other potential oil crops including the world's most important oil- and protein-crop, soybean (*Glycine max*), will need many more breeding activities to improve their adaptation to areas in central Europe including Germany (Table 3). Other even more exotic species will also be available as crop plants in the future. For example, evening primrose (*Oenothera biennis*), is considered to be an interesting source of medicinal products. Its oil contains approximately 10 % γ-linolenic acid, that is a metabolic compound in the biosynthesis of prostaglandines, which are important in medicine and cosmetics. More intensive breeding is still necessary, however, to improve seed weight and yield of this species, just like that of many other medicinal plants.

Prospects for the future

Cell- and tissue culture

Further progress by breeding can certainly be achieved through an application of tissue- and cell-culture and molecular techniques[6,17,26,27] (Fig. 2). Tissue- and cell-culture techniques are already supplementary aids in plant genetics and breeding. For example, meristem culture is now routinely used for virus elimination and rapid propagation of agricultural species like potato and ornamentals like tulips or Gerbera. A time gain of up to two years can be achieved with this system[17].

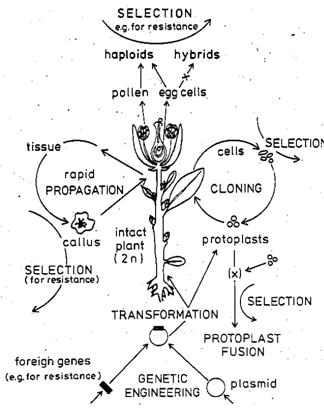

Fig. 2. Biotechnology – Overview of cell- and tissue-culture techniques and genetic engineering (modified from Lit. 26)

Culture systems for single cells like microspores or protoplasts are one of the most essential requirements to incorporate "genetic engineering" into plant breeding procedures. Considerable progress in this field has been reported for rapeseed and other members of the genus *Brassica*[5,12,20,24], but also for *Linum* species[2,3,18]. In these species as well as in *Solanaceae* and others, it is possible to obtain "haploid" plants reproducibly through microspore- or anther-culture[17,26,27]. Haploid plants carry a single set of chromosomes in their somatic cells. By doubling this chromosome set artificially, e. g. by colchicine, homozygous diploid, i. e. doubled haploid, lines are obtained. Such inbred lines are an unalterable prerequisite for breeding F_1-hybrids, particularly in outcrossing species like maize or sunflower.

For selfpollinating species like wheat, barley, linseed (flax) or soybean, inbred lines are the ultimate product of a long-term breeding and selection process. Therefore, with the aid of the above mentioned "haploid-techniques", a gain of several years can be achieved in a breeding programme[17].

Interspecific gene transfer

Breeding of "wide crosses" via interspecific hybridization is another interesting supplementary technique for plant genetics and breeding. It can help to create new genetic variability and, finally, select new genotypes, e. g. disease resistant plants or lines with modified fatty acid composition. Interspecific crosses have been successfully used, for example in the *Cruciferae* (e. g. Lit. 11). This led to a better understanding of the relationship between the species and, consequently, provided the basis to use related species as "gene sources" for an improvement of various agronomically important characters.

Similar efforts have been made in the genera *Linum* and *Helianthus*[4,7,9,10,14,19]. Both include a large number of distinct and valuable wild species, besides the cultivated types[7,19] (Tables 4 and 5). Unfortunately, in many combinations of cultivated and interesting primitive wild species, hybrids have not been obtained yet (Figs. 3 and 4). This can be due to incompatibility during endosperm or embryo development, respectively. In these cases embryo rescue techniques can be applied in order to obtain hybrid plants.

For an application of the embryo culture technique, the initial steps of an ordinary sexual hybridization have to be carried out first; i. e. emasculation and pollination. About 7–10 days after pollination, depending on the stage of development, the young embryo has to be dissected out of the ovarium and plated on a suitable medium, which allows the embryo to grow and form shoots and roots. Therewith, early degeneration of the immature embryo can be avoided. As an example, resistance to light spot (*Phoma lingam*) has been successfully transferred from Indian mustard (*Brassica juncea*) to the cultivated oilseed rape (*B. napus*) with the aid of ovule culture technique[11].

Table 5

*Chromosome-number and linolenic-acid ($C_{18:3}$) content of various wild Linum species**

Species	2n =	$C_{18:3}$ (%)	
L. altum	30	4.7	
L. album	18	5.7	
L. berlandieri	30	20.6	
L. campanulatum	28	16.4	17.0
L. capitatum	28	8.4	9.5
L. catharticum	16	12.1	9.6
L. flavum	30	12.6	>5.8
L. floridanum	36	4.2	
L. imbricatum	30	6.5	
L. maritinum	18	26.1	43.3
L. mysorense	60	16.8	
L. puberulum	30	4.7	
L. rupestre	36	4.3	
L. schiedeanum	36	5.3	
L. striatum	36	8.0	–10.5
L. strictum	18	39.7	
L. sulcatum	30	9.7	–16.9
L. tenuifolium	18	2.4	

* after various authors cited in Lit. 19

However, many related species are extremely recalcitrant to hybridization, because of interspecific incompatibility of pollen and stigma. In these cases, respective interspecific hybrids could possibly be recovered by protoplast fusion techniques[2,3,5,12,20,24] (cf. Fig. 2). For this procedure, the cellular walls of somatic cells of the parents are first removed enzymatically, and the resulting protoplasts are subsequently treated with special enzymes, like PEG (Polyethylenglycol), or by electroshock for attachment and finally fusion of the cells. A successful fusion product will

Table 4

Seed and oil characteristics (range and means) of Helianthus species (after Lit. 7)

Species	No.	Seed wt. (mg)	Hull (%)	Oil (%)	$C_{18:2}$ (%)
H. annuus (wild)	30	5.5–10.3	52–60	18.1–28.6	74.7–82.6
		7.9	54.6	25.0	79.7
H. petiolaris	18	3.8–11.1	36–53	22.8–39.5	75.5–84.3
		6.7	44.4	32.5	79.5
H. maximiliani	17	1.4–2.7	–	19.9–36.0	80.5–87.4
		1.9		29.4	83.4
H. giganteus	15	1.3–2.5	–	18.2–34.3	78.4–84.5
		1.7		27.4	81.7
H. rigidus	12	4.1–5.8	43–46	22.9–30.9	74.5–85.3
		4.8	45.5	26.6	81.5
H. tuberosus	3	5.2–5.3	–	14.0–25.0	77.6–85.5
		5.2		17.8	80.9
H. annuus (cv. 'Saturn')		59.5	27.2	44.4	71.8

include the entire genetic material of the fused cells, i. e. parents (species). This fusion product, the newly formed hybrid protoplast ("heterokaryon"), can be regenerated to a hybrid plant if plated on a suitable culture medium and maintained under appropriate growing conditions. Fusion techniques have been successfully adapted to members of the genus *Brassica*[5,11,24]. For *Linum* species the requirements for fusion and regeneration have recently been established, too[2,3,18].

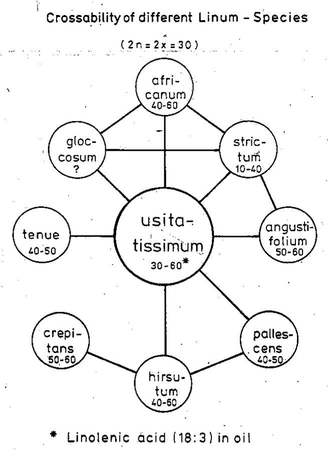

Fig. 3. Possibilities of interspecific hybridization in the genus *Linum*[14,19]

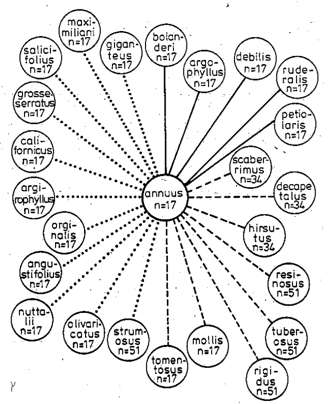

Fig. 4. Possibilities of interspecific hybridization in the genus *Helianthus*[4,10]

Another possible application of the fusion technique is the production of new "cytoplasmically male-sterile" (cms) lines, for example of rapeseed. Since the mitochondria carry the genetic information (mtDNA) for "cms", new plasma combinations through cell fusion are of particular interest. Fusions of cytoplasm and nucleus, so called cybrids[5,11], proved to be very helpful for hybrid breeding programmes in rapeseed, e. g. using the OGURA cms-system derived from *Raphanus sativus*[20].

Application of genetic engineering

Modern gene technologies or "genetic engineering" can be considered as one distinct area of biotechnology, which includes the identification, isolation[23], possible modification, multiplication ("cloning"), and transfer into a foreign "genetic background", i. e. the cell of a related or unrelated plant. For the final success of "genetic transformation", the expression of the respective manipulated DNA-sequences, i. e. gene(s) from a donor, in the receptor plant has to be established.

For an incorporation of genetic engineering into basic and applied plant breeding research, several requirements are already fulfilled. For example, working vector-systems for gene-transfer are available: e. g. the *Agrobacterium tumefaciens* system is an established tool for transferring genetic information into dicotyledoneous plants (e. g. Lit. 15). Other vector systems have been developed, including the direct ("vector-free") transfer of genes into protoplasts[21,22]. Therefore, transferring agronomically important genes, encoding for resistances or quality traits, from wild species to cultivated ones and from one cultivar within a species to another is no utopia any more, provided that entire and fertile plants can be regenerated from the manipulated cell(s).

Summary and Outlook

Conventional plant breeding procedures have already proven to be very well suited for successfully improving the quantity as well as the quality, i. e. fatty acid composition, of oil-yield of our adapted oil-plants, like rapeseed. Even "exotic" plants, like sunflower, could be adapted to our growing conditions (climate etc.) by classical breeding methods.

Modern techniques ("biotechniques") can help to improve the efficiency of breeding, e. g. regarding adaptation to extreme environments. Specific techniques, like "haploid-steps" or genetic engineering can help to accelerate breeding progress through avoidance of long-lasting inbred and/or backcross generations in the near future.

The following bio-techniques relevant to plant breeding can nowadays be summarized (cf. Fig. 2):

1. somatic tissue or cell-culture for rapid propagation of plants from meristems or calluses, e. g. exemplified in potato[27];
2. anther- or microspore-culture for regeneration of haploid plants, as successfully demonstrated e. g. in potato, rapeseed and many other species[27];
3. embryo-culture for production of haploid individuals after wide crosses, as for example of barley with *H. bulbosum*[25];
4. culture of hybrid-embryos out of wide crosses of cultivated species to their wild relatives, for example in the genera *Brassica*, *Linum* (Fig. 3) and *Helianthus* (Fig. 4);
5. culture and regeneration of hybrid-protoplasts for the establishment of "asexual" interspecific or intergeneric hybrids;
6. genetic engineering with isolated plant protoplasts ("direct gene transfer"), particularly in the *Solanaceae* (tabacco, potato) and *Brassicaceae* (Crucifers);
7. application of genetic engineering via specific vector systems (i. e. particularly *Agrobacterium tumefaciens*); up till now restricted to dicotyledoneous plants, particularly *Solanaceae* and *Brassicaceae*, with a few exceptions in monocots[13,16].

Most of the cell- and tissue-culture methods and techniques mentioned above have been introduced into basic or even applied breeding programmes already, where many of these techniques proved to be profitable. For the future, it is expected, that such new ways of breeding will allow to run breeding programmes even more efficiently and rapidly than before[9,26].

For an application of "genetic engineering" in basic and applied plant breeding research, several requirements are given already, like the basic transformation techniques. Others, which are required for a more general application of gene technologies, like the accessibility of any important crop plant (including for example cereals or sunflower) to these techniques, have to be elaborated. However, this is expected to be achievable in the (near) future.

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