BIOTECHNOLOGY IN BREEDING OF SUNFLOWER AS AN INDUSTRIAL OIL CROP

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Introduction

Production of traditional agricultural crops, particuarly 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. 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. Therefore, cultivation of respective "alternative" oil-crops, like sunflower, for multiple industrial purposes is one possibility of reducing cereal production.

The present status

The EEC already plays an important role in the production of oilcrops, mainly for nutritional purposes; e.g. more than one quarter of the world's production of sunflower kernels is produced in the EEC.

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. For example, sunflower-oil contains mainly linoleic acid. Genetic manipulation of the fatty acid composition is possible not only by "modern" but also by conventional breeding methods, as many examples demonstrate. Recently, new sunflower genotypes with high oleic (C18:1) 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. Opposite to various other "exotic" oil-plants, sunflower (*Helianthus annuus*) can already be considered as a highly performing crop even for areas with moderate climates in Europe.

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 (11, Fig. 1). Tissue- and cell-culture techniques are already supplementary aids in plant genetics and breeding; e.g. meristem culture is now routinely used for virus elimination and rapid propagation of agricultural species (like potato).

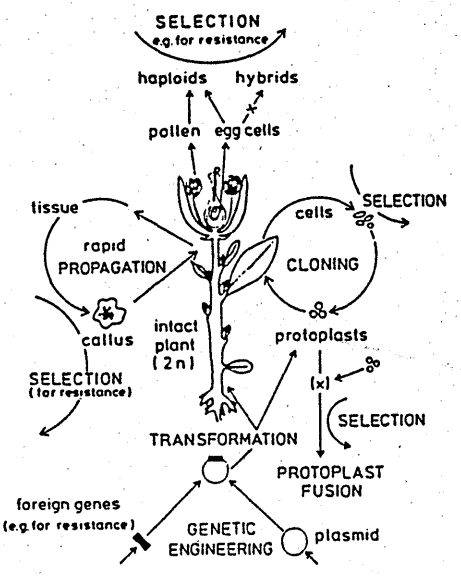

Fig. 1. Biotechnology in plant breeding (modified after WENZEL).

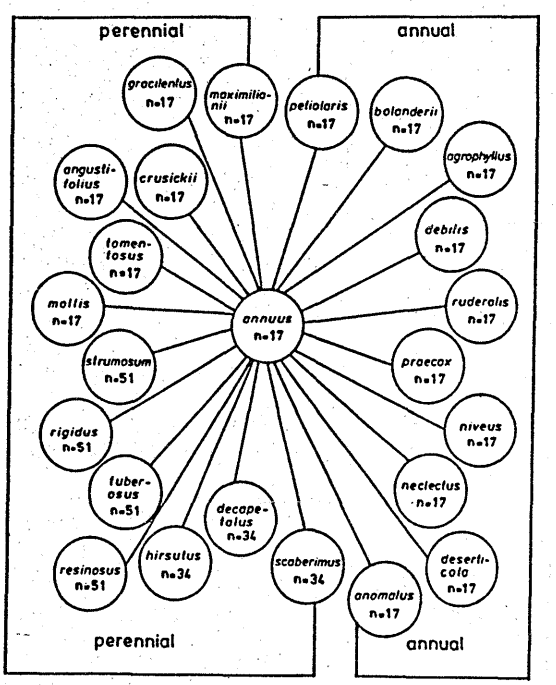

Fig. 2. Possibilities of interspecific hybridization in the genus *Helianthus* (2,6).

Culture systems for single cells like microspores or protoplasts are one of the most essential requirements to incorporate "genetic engineering" into plant breeding procedures. In many species it is possible now to obtain "haploid" plants reproducibly through microspore- or anther-culture (11), while sunflower still remains rather recalcitrant to "haploidy-techniques". Haploid plants carry a single set of chromosomes in their somatic cells. By doubling this chromosome set artificially, e.g. by colchicine, doubled haploid, i.e. homozygous diploid, lines are obtained. Such inbred lines are a prerequisite for breeding F_1-hybrids, particularly in outcrossing species like sunflower.

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. Related species can be used as "gene sources" for an improvement of various agronomically important characters. Such efforts have been made in the genus *Helianthus* (2,5,6), which includes a large number of distinct and valuable wild species, besides the cultivated type (Table 1). Unfortunately, in many combinations of cultivated and interesting primitive wild species, hybrids have not been obtained yet (Fig. 2). This can be due to incompatibility either 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.

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 culture and fusion

techniques (1,9; cf. Fig. 1). For this procedure, protoplasts are treated with special enzymes, like PEG (Polyethylenglycol), or by electroshock for attachment and finally fusion of the cells. A successful fusion product will include the entire genetic material of the fused cells, i.e. parents (species). This 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.

Table 1. Seed and oil characteristics (range and means) of *Helianthus* species (according to 4)

Species	No.	Seed wt. (mg)	Hull (%)	Oil (%)	C18:2 (%)
H.annuus (wild)	30	5.5-10.3 7.9	52 - 60 54.6	18.1-28.6 25.0	74.7-82.6 79.7
H.petiolaris	18	3.8-11.1 6.7	36 - 53 44.4	22.8-39.5 32.5	75.5-84.3 79.5
H.maximiliani	17	1.4-2.7 1.9	- -	19.9-36.0 29.4	80.5-87.4 83.4
H.giganteus	15	1.3-2.5 1.7	- -	18.2-34.3 27.4	78.4-84.5 81.7
H.rigidus	12	4.1-5.8 4.8	43 - 46 45.5	22.9-30.9 26.6	74.5-85.3 81.5
H.tuberosus	3	5.2-5.3 5.2	- 	14.0-25.0 17.8	77.6-85.5 80.9
H.annuus cv. 'Saturn'		59.5	27.2	44.4	71.8

Another possible application of the fusion technique is the production of new "cytoplasmically male-sterile" (cms) lines. Since the mitochondria carry the genetic information ("mtDNA") for "cms", new plasma combinations are of particular interest. Fusions of cytoplasm and nucleus, so called cybrids (3), proved to be very helpful for initial hybrid breeding programmes, e.g. in rapeseed.

Application of genetic engineering

Modern gene technologies or "genetic engineering" can be considered as one distinct area of biotechnology, which includes the identification, isolation, 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, functional vector-systems for gene-transfer are available: e.g the *Agrobacterium tumefaciens* system is an established tool for transferring genetic information into dicotyledoneous plants including sunflower (10). Therefore, transferring agronomically important genes, encoding for resistances or quality traits, from wild *Helianthus* species to cultivated *H.annuus* and from one sunflower cultivar to another is no utopia any more (7,8,10), 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 of oil-yield of major oil-plants, like sunflower.

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

Cell- and tissue-culture methods and techniques have been introduced into basic or even applied breeding programmes already, where they proved to be very profitable. For the future, it is expected also in sunflower, that such new techniques will allow to run breeding programmes even more efficiently and rapidly than before (5).

For an application of "genetic engineering" in basic and ap-

plied sunflower breeding research, several requirements are given already, like the basic transformation techniques (7,8,11). Others have to be elaborated. However, this is expected to be achievable in the (near) future.

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