Biotechnology in Sunflower Breeding - Methods, Applicability and Prospects

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Summary

Cultivated, annual lines and hybrids of sunflower (*Helianthus annuus* L.) are still comparatively recalcitrant to an application of biotechnological methods, although substantial progress has been made in recent years. For example, very efficient embryo culture ("rescue") techniques are available for producing new interspecific hybrids which are considered very valuable for broadening genetic variation in sunflower. In particular, interspecific hybrids have been demonstrated to be an unexhausted source for creating "new" traits, like cytoplasmic male sterility (CMS) or resistance against devastating diseases, like *Phomposis* or *Sclerotinia* rot.

For specific genotypes, the successful regeneration of entire plants from cultured somatic tissue or even single cells, i.e. protoplasts, has been demonstrated. Furthermore, the recovery of androgenetic haploid and doubled haploid plants is basically feasible in sunflower, although the success and rate of regeneration strongly depend on both, genotype and "environmental factors". More progress has been made in the field of "genome characterization" by using biochemical and molecular markers. In particular, the chloroplast and mitochondrial genomes have been analyzed and described in detail. Such analyses built the foundation for future "marker based selection" and for the identification and isolation of relevant genes as candidates for transformation experiments ("genetic engineering").

Introduction

The annual sunflower (*Helianthus annuus L.*) is one of the most important oil crops of the world. Its seed oil can be used for human consumption and also as a raw material for oleochemistry. It can also be used as a substitute for mineral oil in various applications, such as a fuel, a lubricant or an oil for hydraulic systems.

The genetic basis of modern hybrids is considered to be comparatively narrow (ARNAUD, 1986). Sunflower cultivation is almost exclusively performed with hybrids based on a single source of cytoplasmic male sterility (CMS), discovered by LECLERCQ (1969) in a cross between *Helianthus petiolaris* and *H. annuus*. Due to the exclusive usage of female lines with this cytoplasm for hybrid seed production, all hybrids cultivated world-wide are closely related - at least with regard to their cytoplasm. Therefore, further expansion of sunflower production may be limited due to epidemic diseases, since sunflower is very sensitive to various fungal pathogens. Consequently, hybrids resistant to the most dangerous pathogens in Central and Northern Europe, e.g. *Sclerotinia sclerotiorum* (Lib.) de

Bary and *Botrytis cinerea* Pers. et Fr., have to be developed for an economic cultivation under temperate climatic conditions. As a basis for this, increased breeding efforts are required in order to broaden the genetic variability of cultivated sunflower. Successful applications of new methods of "biotechnology" can contribute to overcoming the problems of a narrow genetic base, and at the same time accelerate the breeding process. In this field, various techniques have been shown to be relevant for sunflower breeding, e.g. embryo culture ("embryo rescue"), meristem culture, anther and microspore culture, protoplast culture and cell fusion, and molecular techniques including gene transfer ("genetic engineering", cf. Fig. 1; see also FRIEDT, 1992).

Sexual interspecific hybridization via embryo rescue

Interspecific hybridization is one possibility of transferring genes, e.g. for disease resistance or for improved quality, from one species to another, for example from wild species to cultivated lines (cf. SKORIC, this vol.). It is also a means of developing new sources of CMS (cf. HAHN & FRIEDT, this vol.).

In general, interspecific crossability of cultivated *H. annuus* and most annual species is fairly high. However, earlier extensive hybridization programs revealed that almost complete incompatibility exists between cultivated sunflower and diploid (2n = 2x =34) perennial species, e.g. *H. angustifolius* L., *H. divaricatus* L., *H. giganteus* L., *H. nuttallii* T. & G. and *H. mollis* Lam. Partial compatibility with *H. annuus* was found for some tetraploids (2n = 4x = 68), e.g. *H. decapetalus* L., *H. hirsutus* Raf., and hexaploids (2n = 6x = 102), e.g. *H. resinosus* Small, *H. tuberosus* L. (GEORGIEVA-TODOROVA, 1984). In these cases, postzygotic incompatibility between the embryo and the endosperm was believed to prevent efficient and direct recovery of interspecific hybrids. Wide hybridizations between cultivated sunflower and *Helianthus* species have been accomplished utilizing the embryo rescue technique (CHANDLER & BEARD, 1983; ESPINASSE *et al.*, 1985; KRÄUTER & FRIEDT, 1989). For example, new hybrids of *H. annuus X H. hirsutus* and *H. scaberimus* (= *H. pauciflorus* Nutt.) X *H. annuus* crosses were successfully grown on a modified White's medium by BOHOROVA *et al.* (1985). Later, WITRZENS *et al.* (1988) described a method for the culture and regeneration of plants from callus of cultivated sunflower and an interspecific hybrid (*H. annuus X H. tuberosus*), where immature embryos were the only explant which consistently gave regenerable callus in the genotypes tested. Difficulties often found in such experiments were the premature initiation of flowering of regenerating shoots and the frequent occurrence of "vitreous" plantlets which could not be successfully grown into mature plants. Culture improvements were achieved by partially replacing inorganic nitrogen with amino acids and by adding 10, 30, and 100 μM phloridzin, esculin, and naringin to the medium (WITRZENS *et al.*, 1988).

Average success of interspecific hybridization, i.e. recovery of hybrid plants, have been reported by KRÄUTER *et al.* (1991). In this hybridization program, a total of 34 different

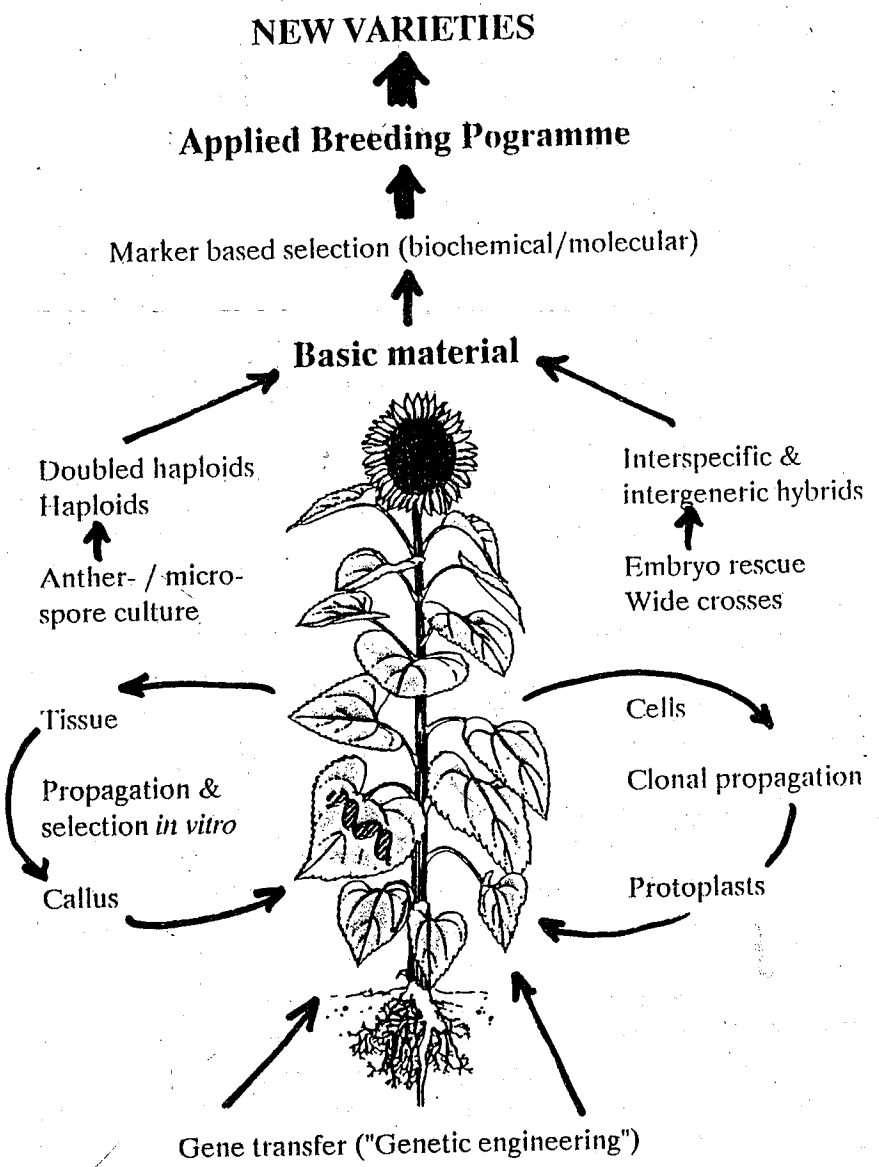

Fig. 1. Possible applications of biotechnology, i.e. cell- and tissue culture techniques, biochemical and molecular methods including genetic transformation, in sunflower for creating novel genetic variability and acceleration of a breeding program.

interspecific cross combinations were successfully raised via *in vitro* embryo culture by using a solid culture medium (9 g/l agar, pH 5.6) containing the usual macro- and micronutrients, supplemented by myo-inositol (100 mg/l) and with 10 g/l sucrose. Potentially useful cross combinations including perennial polyploid species have been repeated in order to create broad genetic variation for disease resistances (DAHLHOFF *et al.*, 1991, this vol.).

The recovery of further interspecific hybrids by using *in ovulo* embryo culture has been reported by ESPINASSE *et al.* (1991). In particular, several rare hybrids of sunflower (female) and *H. maximiliani* were obtained. Such hybrids have also been reported by KRÄUTER *et al.* (1991). Other highly interesting new hybrids are those with *H. nuttallii* (NUT 1517 and NUT 2000) reported by VASILJEVIC *et al.* (1991). The latter authors also attempted to recover many other interspecific combinations which so far have been shown to be more difficult to obtain.

Many interspecific F_1 hybrids are partially or even completely sterile. In these cases, chromosome doubling using colchicine can facilitate selfing and/or backcrossing. For example, dipping *H. annuus X H. bolanderi* hybrid plantlets for 5h into a colchicine solution of 150 or 250 mg/l, supplemented by 0.02 mg/l DMSO (pH 5.4) in the dark, without moistening the roots resulted in about 30% doubled sectors and highly increased fertility of the amphiploids derived (JAN & CHANDLER, 1989). However, further studies with respect to the appropriate stage of colchicine treatment and the optimum experimental conditions must be carried on in order to improve ease of application of this method. Alternatively, backcrossing of interspecific hybrids can also be facilitated by storing pollen in liquid nitrogen without loss of pollen viability as reported by ROATH *et al.* (1988). Altogether, interspecific hybridization using *in vitro* and *in ovulo* embryo rescue is an applicable and highly efficient method, for obtaining many wide hybrids in the genus *Helianthus*. Furthermore, this technique can be utilized to accelerate a sunflower breeding program, by obtaining four to five generations in one year (e.g. AZPIROZ *et al.*, 1987).

Protoplast culture and asexual hybridization via protoplast fusion

The establishment of protoplast culture in *Helianthus* and the regeneration of entire plants are essential for somatic cell fusion. Basic studies on protoplast culture of wild and cultivated *Helianthus* species have been carried out, for example by BOHOROVA *et al.* (1986), and LENEE & CHUPEAU (1986). GUILLEY & HAHNE (1989) determined the conditions which allow the repeated regeneration of green, nodular, vigorously growing calli from isolated sunflower mesophyll protoplasts. Use of CAYLA cellulase and pectinase was found to be the best for protoplast isolation. Although cell divisions were achieved, the method has failed to regenerate intact plants so far.

More recently, the successful regeneration of plants from hypocotyl-derived protoplasts, capable of being grown in soil, has been reported for the first time in *Helianthus* (BURRUS *et al.*, 1991; CHANABE *et al.*, 1991). Protoplasts were produced from two sunflower cultivars and three wild species. After inclusion in agarose droplets and culture on a TL-medium with 2,4D, loose colonies and "embryoids" were obtained. Finally after two transfers, shoot formation and subsequent rooting was only obtained from calli derived from *H. petiolaris* itself or from cross progeny of this species (for a comparison of methods cf. FRIEDT, 1992). High protoplast "regeneration potential" has also been reported for *H. nuttallii* and *H. divaricatus* by BOHOROVA (1991). Recently, KRASNYANSKI *et al.* (1992) have obtained rooted plants after preparation of protoplasts from *in vitro* grown plants, induction of embryogenesis and culture of somatic embryos in agarose droplets in a medium containing BA and NAA. Intact plants were finally obtained on media with reduced auxin content.

After all, plant regeneration from cultivated sunflower protoplasts remains very difficult (G. HAHNE, 1991, pers.comm.). Many basic problems, for example "genotypic effects", vitrification and rooting, need to be solved before the regeneration of entire plants from protoplasts becomes routine. The use of protoplasts for genetic transformation will be discussed later.

Tissue culture for rapid propagation and screening *in vitro*

There are several reports of shoot regeneration from cultured hypocotyls, cotyledons, or leaf pieces with or without callus formation (PATERSON & EVERETT, 1985; GRECO *et al.*, 1984; KRÄUTER & FRIEDT, 1991). In particular, BOHOROVA *et al.*, (1985) obtained shoot organogenesis from pith parenchyma and shoot apical explants of the interspecific hybrids *H. annuus X H. decapetalus*; shoot apices from *H. annuus X H. hirsutus* and *H. annuus X H. tomentosus* (= *H. resinosus*) hybrid plants underwent shoot organogenesis. However, other authors were not able to regenerate plants from cotyledon- and hypocotyl-derived embryoids (e.g. PIUBELLO & CASO, 1986). More recently, PUGLIESI *et al.* (1991) regenerated "somaclonal variants" from callus-derived adventitious buds induced from *in vitro* culture of cotyledons of seven sunflower genotypes. Both, well-known and "new" genetic variation - mainly for morpho-physiological traits - were described (see also PUGLIESI *et al.*, this vol.). Furthermore, CHRAIBI *et al.* (1992) have recently shown, that even genotypes previously described as being recalcitrant can be induced to regenerate shoots in high frequency. They obtained rooted, fertile plants after culturing mature cotyledon tissue of three sunflower genotypes in a liquid medium with comparably high concentrations of NAA and BAP.

Direct somatic embryogenesis and plant regeneration from immature embryos of the hybrid 'cmsHA401' X 'RHA699'(Restorer) has been obtained by FINER (1987) after growing immature zygotic embryos on a high sucrose (12%) medium. Plants matured and were harvested from regenerated plants. Furthermore, PELISSIER *et al.* (1990) developed a method to obtain somatic embryos from hypocotyl epidermis and parenchyma cell layers.

Somatic embryos were used to produce secondary embryos, which were regenerated into plants. PRADO & BERVILLE (1990) were able to induce somatic embryogenesis out of suspension cultures from hypocotyl- and cotyledon-derived calluses. Whereas direct organogenesis was obtained from the original explants, no regeneration was achieved from somatic embryos. In this context, a remarkably promising technique has been established at Pisa, where PUGLIESI & BARONCELLI (pers. comm.) were able to improve the frequency of shoot regeneration via direct embryogenesis from cultured leafpieces.
Another technique which requires the ability to regenerate plants from cultured somatic tissue via callus is the screening for disease resistance *in vitro*. In this case, an extension of time in culture would probably be beneficial, since this is known to increase genetic variation within the cultured calluses and among the plants regenerated from them. Two promising methods for screening *in vitro* have been developed: the first is the application of fungal filtrates to callus cultures in screening for resistance to *Phomopsis/Diaporthe* Munt.-Cvet. (MASIREVEC *et al.*, 1988) or to *Phoma macdonaldi* Boerma (HARTMAN *et al.*, 1988); the second is the use of oxalic acid in order to screen for resistance to *Sclerotinia sclerotiorum* (HARTMAN *et al.*, 1988). Nevertheless, it remains finally necessary to demonstrate that screening *in vitro* is a reliable method which shows a high correlation with the reaction of the crop in the relevant field environment. This is an indispensable precondition for practical application of screening for resistance *in vitro*. However, such correlations are lacking so far, both for *Phomopsis* and *Sclerotinia*. In the latter case, even results of different tests applied to entire plants are often contradictory due to the complex nature of plant-pathogen interactions (cf. TOURVIEILLE DE LABROUHE & VEAR, 1984).

Development of androgenetic doubled haploids for an accelerated production of inbred lines

An application of the "haploidy-technique" which produces either haploids or spontaneously doubled haploids, should allow the breeder to accelerate the breeding process. In addition, the haploidy-technique facilitates the selection for characters controlled by recessive genes or genes incorporated from alien species. Several publications reported extensive callusing induced from anthers of various interspecific hybrids cultured *in vitro* (BOHOROVA *et al.*, 1985; MEZZAROBBA & JONARD, 1986; 1988; VASILJEVIC *et al.*, 1991). Successful regeneration of shoots was also reported by MEZZAROBBA & JONARD (1988), and by GÜREL *et al.* (1991a). In the latter case, M2 a medium with half-strength MS macronutrients and complete MS micronutrients, supplemented by amino acids and with high sucrose content (120 g/l) proved to yield the largest number of regenerated shoots in most of the genotypes tested.
In particular, BOHOROVA *et al.* (1985) described direct shoot formation from anthers of *H. divaricatus* and a *H. annuus X H. decapetalus* hybrid cultured on a medium with 5 mg/l Zeatin. Other hormone combinations, e.g. kinetin (0.2 mg/l) and 2,4D (1.0 mg/l), tended to promote callus formation but failed to induce shoot regeneration. In addition, secondary callus and shoot formation was achieved from culturing stem explants on a medium with 2 mg/l BA, 0.2 mg/l IAA and 20 mg/l adenine. Shoots developed roots on White's medium, even after several transfers and subcultures. However, with repeated subculturing, an increase in chromosome number (2n = 45-51, 68 and 102) was observed, deviating from the diploid (2n = 34) number (BOHOROVA *et al.*, 1985).
The regeneration of six androgenetic plants from anthers of a French variety ('Inra') was reported by MIX (1985); two of them were shown to be haploid. Another haploid plant was recovered from anthers of cv. 'Luciole'. ALISSA *et al.* (1985) regenerated haploid, polyploid and aneuploid plants from four hexaploid and one diploid wild *Helianthus* species and their interspecific hybrids with different sunflower lines. Best results were observed for a *H. annuus X H. resinosus* hybrid, where 53% of anthers produced plants; the most effective medium proved to be the one supplemented with 0.5 mg/l NAA and 0.5 mg/l BAP. However, no plants could be regenerated from a *H. annuus X H. tuberosus* hybrid.
Initial steps to establish a suitable culture method for isolated microspores in our laboratory yielded embryoid formation in a very low frequency only, and no organogenesis or regeneration has been achieved up till now (GÜREL *et al.*, 1991b). It appears to be very important in future experiments to give priority to an optimization of the growing conditions of the donor plants ("physiological effects") and to elucidate the nature of "genotypic effects" on androgenetic response.

Application of biochemical and molecular methods in *Helianthus*

Biochemical and/or molecular methods have already been demonstrated to be highly useful for genetic analyses in many crop species, where a large number of investigations on isozymes have demonstrated their utility in basic and applied research (e.g. GOODMAN & STUBER, 1980; TANKSLEY & ORTON, 1983; COOKE, 1984). Today, these methods can also serve the sunflower breeder as a tool for early identification of important agronomic traits, quality characteristics, disease resistances, or stress tolerance. Starchgel-electrophoresis is a comparatively simple, fast and inexpensive method applicable for the identification of *Helianthus* species, sunflower lines, and hybrids. For example, RISEBERG & SEILER (1990) used this technique for an evolutionary analysis of cultivated sunflower in relation to wild *H. annuus*, and they concluded that domesticated sunflower must have evolved from a very limited gene pool.
Contrary to a statement of GEORGIEVA-TODOROVA (1984), interspecific F_1 hybrids do not always resemble the wild parent(s) morphologically. Instead, it often remains difficult to distinguish a hybrid from either parent. However, starch-gel isozyme-electrophoresis can be successfully used to identify *Helianthus* species, their interspecific crosses, and progenies (cf. DAHLHOFF *et al.*, this vol.).

Molecular methods will certainly play an important role in sunflower breeding in the future. For example, the analysis of Restriction Fragment Length Polymorphisms (RFLPs) is a promising method for the comparison of genotypes. It has been already used for genotype identification in various plant species representing different families and genera, such as *Brassica, Hordeum, Solanum* or *Zea*. In sunflower, the identification of valuable characters such as CMS, stress tolerances, and disease resistances utilizing RFLPs would facilitate selection at early growth stages and at the very beginning of a breeding program. RISEBERG & SEILER (1990) used the RFLP technique for comparing cpDNAs of wild *H. annuus* and cultivated germplasm. They concluded that the chloroplast genome of wild and domesticated forms is almost identical in size and sequence. Earlier restriction analysis of cpDNA by JANSEN and PALMER (1987) revealed that a 22-kb-inversion marks an evolutionary split in the sunflower family (*Asteraceae*). This inversion corresponds to the 23-kbp inverted repeat described in circular cpDNA of sunflower by HEYRAUD *et al.* (1987).

Species and genotypes in *Helianthus* can be efficiently discriminated by restriction analysis of mtDNA, as demonstrated, e.g., by HEYRAUD *et al.* (1987), SERROR *et al.* (1990) and CROUZILLAT *et al.* (1987; 1991). For example, molecular relationships were revealed between some CMS lines and the species from which they were derived, e.g. CMS-I/*H. annuus* ssp. *lenticularis* and CMS-F/*H. petiolaris fallax*. On the basis of restriction fragment patterns, a phylogenetic tree was proposed illustrating molecular polymorphism in the mitochondrial genome of *Helianthus*.

CHOUMANE & HEIZMANN (1988) showed that restriction analysis of nuclear ribosomal genes (rDNA) of *H. annuus* using EcoRI and BamHI can also be efficiently used to differentiate *Helianthus* species and populations of *H. annuus*. However, cultivated sunflower lines proved to be identical on the basis of the physical properties of their ribosomal DNA. Also, KRÄUTER *et al.* (1991) used an rDNA probe for the differentation of sunflower genotypes, wild species and their interspecific hybrids by RFLP. Recently, DEHMER & FRIEDT (1992, this vol.) have shown, that *Helianthus* species, populations, interspecific hybrids and even related lines can be efficiently discriminated by PCR fingerprinting techniques (RAPD, AP-PCR). Progress in this field is expected to be rapid with extensive results available in the near future.

After analyzing mtDNA, cpDNA, and dsRNA patterns, BROWN *et al.* (1986) speculated that fertile cytoplasm ('CM400' fertile) was associated with a 1.45-kb-plasmid. However, PEREZ *et al.* (1986) could not detect low molecular weight mtDNA-molecules in cytoplasmic male sterile sunflower lines ("LECLERCQ's cytoplasm"), while such molecules were present in all of the sunflower lines studied and in the subspecies *H. petiolaris petiolaris* and *H. petiolaris fallax*. "LECLERCQ's cytoplasm" is supposed to be derived from *H. petiolaris* and it leads to a breakdown of microsporogenesis due to unknown earlier physiological events (cf. LAVEAU *et al.*, 1989). Further investigations showed that there is no strict correlation between CMS and the presence of a specific plasmid. CROUZILLAT *et al.* (1989) reported that the 1.45kb plasmid is present in many *H. annuus* materials, in some ecotypes of *H. petiolaris*, but not in mitochondria of germplasm with the "LECLERCQ-cytoplasm". However, PEREZ *et al.* (1988) were able to detect the plasmid in total cellular DNA of male-sterile sunflower with a comparatively low copy number, and they also described the nucleotide sequence of this plasmid.

Furthermore, no differences were detected between cpDNAs of *H. annuus* and the "LECLERCQ-cytoplasm" after digestion with different restriction enzymes. On the other hand, restriction fragment length polymorphisms of the major mtDNA were shown to correspond with cytoplasmic male sterility of sunflower (CROUZILLAT *et al.*, 1987). Later, SICULELLA & PALMER (1988) unequivocally demonstrated that sterility caused by the "LECLERCQ-cytoplasm" is associated with a 12-kb-inversion and a 5-kb-insertion/deletion near the *atp*A gene in the mtDNA. These findings were later investigated in more detail and confirmed by KÖHLER *et al.* (1991). According to their results, the "LECLERCQ-CMS" is correlated with the co-transcription of a new open reading frame ('orf 522') with the *atp*A gene in the mitochondrial genome, as also has been confirmed by LAVER *et al.* (1991). This 'orf' seems to be responsible for an additional mitochondrially encoded polypeptide in CMS sunflower lines (HORN *et al.*, 1991). The 5-kb-insertion has already been partially sequenced, and it can further be used for the construction of selectable markers aiming in an early identification of male sterile lines in a breeding program.

"Genetic engineering" of sunflower

Basic research for the incorporation of foreign genes into the *Helianthus annuus* genome has already been accomplished. MOYNE *et al.* (1989) succeeded in the direct transformation of sunflower protoplasts as demonstrated by the presence of the NPTII marker gene in kanamycin resistant calli. However, no plants could be regenerated from these calli. In general, the recovery of intact transformed plants by manipulating protoplasts from cultivated sunflower remains the major bottle-neck for an application of "genetic engineering" via protoplasts in sunflower. Further improvements will be necessary for the application of this method in sunflower breeding.

Using disarmed *Agrobacterium tumefaciens* plasmids as vectors, genetically transformed calli have been obtained by KEMPF and HALL (1981), MATZKE *et al.* (1984), and HELMER *et al.* (1984), while EVERETT *et al.* (1987) obtained transgenic plants out of hypocotyl callus. SCHRAMMEIJER *et al.* (1990) attempted the transformation of sunflower cv. 'Zebulon' via co-cultivation of dissected shoot apical meristems from seeds with a disarmed *Agrobacterium tumefaciens* strain harbouring a binary vector carrying genes encoding GUS- and NPTII-activity. Chimeric expression of the two genes was observed in transformed plants, and integration of the foreign DNA into the sunflower genome was

confirmed by PCR. However, transformation of shoot meristem cells occurred at low frequencies. Recently, BIDNEY *et al.* (1992) have demonstrated that the transformation frequency by *Agrobacterium tumefaciens* in plants recovered from sunflower apical explants was substantially increased, when the meristems were wounded by particle bombardment first. The authors concluded that *Agrobacterium* mediation of stable transformation is more efficient than the analogous particle/plasmid protocol alone. In the meantime, stable recombinant secondary transgenic plants could be derived from seeds of the primary chimeric transformant (cf. BIDNEY *et al.*, this vol.).

Conclusions and prospects

The cultivated sunflower has long been considered a species very recalcitrant to biotechnology. However, substantial progress has been made in recent years. Highly efficient embryo culture techniques are available for creating numerous wide crosses which can be very valuable for broadening genetic variation in sunflower. Regeneration of entire plants from cultured somatic tissue or even single cells (protoplasts) has been demonstrated for specific genotypes. Further, the recovery of haploid and doubled haploid plants is basically feasible in sunflower, although strongly depending on genotype, too. Particular progress has been made in the field of "genome characterization" both, by using biochemical and molecular markers. This builts the foundation for future "marker based selection" and for the identification and isolation of specific genes as candidates for "genetic engineering". Based on the present state of knowledge, future research activities must focus on the following objectives, in order to provide the basis for an application of respective biotechniques in a sunflower hybrid breeding program (cf. FRIEDT, 1992):

- Improvement of the efficiency of somatic tissue culture techniques for rapid propagation of any kind of sunflower breeding materials - i.e. independent of the respective genotype; this can lead to a gain of time during the breeding procedure and it may help to facilitate the screening for stress tolerance and/or disease resistance *in vitro*.
- Establishment of methods for the reproducible regeneration of androgenetic haploids as a basis for an accelerated development of doubled haploids, i.e. inbred breeding lines.
- Development of improved techniques of chromosome doubling for an easier use of doubled haploids and interspecific hybrids in a sunflower breeding program.
- Detailed characterization and description of sunflower lines as well as *Helianthus* species and subspecies, aided by biochemical and molecular markers in order to create a better basis for selecting appropriate, improved breeding materials.
- Last but not least, applicable techniques for the successful and stable genetic transformation of sunflower must be developed for the future improvement of specific characters which could not be improved by conventional methods, e.g. resistances to *Botrytis* and *Sclerotinia*.

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