APPLICATION OF GAMMA-IRRADIATED POLLEN AND *IN VITRO* TECHNICS FOR TRANSFER OF GENETIC INFORMATION

J. GEORGIEVA-TODOROVA
and R. VASSILEVA
Institute of Genetics, Sofia, Bulgaria

P a n d e y (1975) first began to study the ability to use gene engineering methods in plants, thus showing that limited gene transfer from the donor to the recipient is possible by irradiating pollen with gamma rays. He pointed that fact in his studies on interspecific hybridization of tobacco and he called it "egg transformation". In its essence it is a variety of transgenosis which in our case can be called "sex transgenosis". Its essence is as follows : recipient plants are pollinated with donor's pollen and the irradiation is done with high doses (50 up to 100 krad). Pseudofertilization from this pollen leads to incomplete cycle of cell division of the egg and formation of a pseudozygote which develops parthenogenetically. Such kind of development is described for all types of Nicotiana. There is no natural pollination but pseudopollination stimulates the egg in phase G_1 from the cell cycle of DNA replication. During the replication, separate chromatine fragments of irradiated pollen are included in the egg genome. In this way the gamet content in the transformed chromosomes is balanced in the second meiotic division and in M_2 the plants are phenotypically identical with mother's organism with the exception of the donor's traits transferred by irradiated pollen. G r a n d et al. (1980) established by cytological investigation on irradiated pollen that the natural division of generative nucleus is discontinued due to chromatine spoil as a result of irradiation and the chromosomes can't find their way in the metaphase plate. The generative nucleus is a concentrated mass of different chromatine fragments which can be transferred on the egg (*Fig. 1*).

A transformation of three marker genes was obtained as a result of investigations carried out with Nicotiana species. The plants-transformants (T_1) were with highly fertile pollen (from 70 to 85%) ; they were characterized by complete identity with mother's organism, the only exception being the traits transferred by irradiated pollen. Ordinary sex hybrids differed from both parents and were with absolutely sterile pollen (P a n d e y, 1980 ; 1981). The perspective of Pandey's techniques for gene transfer increase causes a number of differences connected with the unefficient study on parthenogenetic development in many plants. What is more, the quantity (number) of the "transformed" viable seeds obtained by pollination with irradiated pollen is very low. It is a restricting factor not only for representatives of Nicotiana species with high

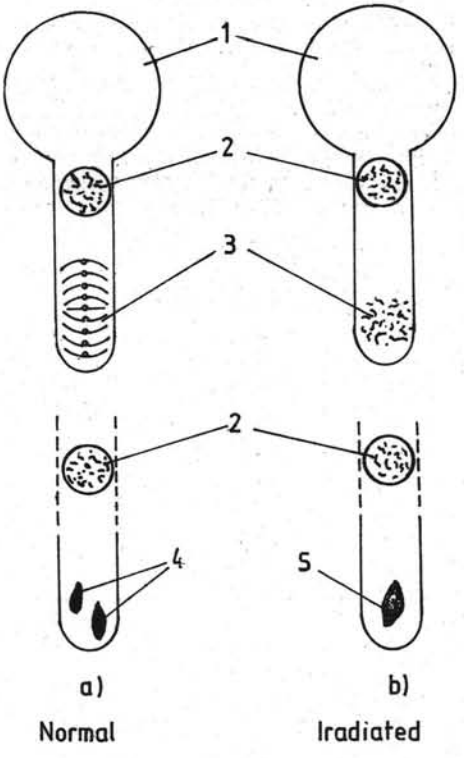

Fig. 1. — "Division" events in normal and irradiated pollen tubes (based on results of Grant et al. 1980)
1) pollen ; 2) pollen nucleus ; 3) mitotic division of the generative nucleus ; 4) spermium ; 5) fragments of chromatine

percentage of seed formation, but also for the plants with higher percentage than that of the cultivated ones, with only one or two seed buds in their fruitset. It is necessary to seek efficacious conditions for increasing the fruitsets.

Investigation on the abilities to transfer genetic information with the help of irradiated pollen, though with contradictory results, found its way in the improving work in a number of important agricultural plants. In the last few years results from investigations on this problem appeared in the literature dealing with barley (Powell et al., 1983), wheat (Snape et al. 1983), tomatoes (Drianovska, 1983), pepper (Daskaloff, 1984), pea, maize (Vassileva, 1985) etc.

In sunflower no published data are known, but having in mind the fact that the incompatibility barriers are great in cultivated sunflower and in many of the Helianthus species carriers of genes which determine important traits, we have tested in the last 2—3 years a scheme of variants in hybridization using the method of irradiated pollen.

As there are no concrete data at the moment about irradiation doses of the pollen for many cultures including sunflower, we have tested different doses of irradiation and different sources. We have tested gamma radiation and laser. Pollen from wild species was irradiated with doses of 50 krad and 100 krad. Laser duration was 15 and 25 minutes. For the moment it is difficult to say whether this method for transfer of genetic material from the genome of wild species to the genome of cultivated ones will be effective.

The most general conclusion for the time being is that higher percentage of seed set was obtained when pollination was performed with treated pollen in F_0 thus enabling us to deal with treater number of plants in F_1. We are not entitled to say that individual plants in F_1 in some combination as *H. mollis* × *H. annuus, H. rigidus* × *H. hirsutus, H. rigidus* × *H. annuus* obtained namely by pollination with irradiated pollen are under the influence of irradiation.

Summarizing the data published on investigating the abilities for transfer of genetic material with the help of irradiated pollen, it should be underlined that similar results were obtained in different places. This points to the general biological character and necessity to use this technique for transfer of some hereditary traits. The study of the mechanisms of this process and the conditions for its efficiency will have not only theoretical but also practical importance. That is we recommend this method to find its place in investigations of sunflower and interspecific hybrization in Helianthus species.

Another method we would like to draw your attention to is the utilization of *in vitro* technics in sunflower.

Methodologies are being elaborated in our labs for the successful of organogenesis, androgenesis, embryocultures and recently, for isoltion, cultivation and callus regeneration of protoplasts in wild Helianthus species and in sunflower.

Several Helianthus species and F_1 hybrids were used for experiments on organogenesis, androgenesis and embryo culture. The type of development observed was dependent on genotype, source of explant and composition of the culture medium. When cultured on agar-solidified medium of Murashige and Skoog enriched with 1 mg/l naphtalenacetic acid, 0.1 mg/l 6-benzylaminopurine, 0.01 mg/l gibberellic acid and 40 mg/l adenine, pith parenchyma and shoot apical explants from *H. annuus* × *H. decapetalus* hybrids and shoot apeces from *H. annuus* × × *H. hirsutus* and *H. annuus* × *H. tomentosus* hybrids underwent shoot organogenesis. One mg/l 2.4 dichlorophenoxyacetic acid and 0.2 mg/l kinetin stimulated the production of anther-derived callus in most hybrid combinations. Direct shoot formation was observed in anthers of *H. divaricatus* × *H. annuus* and *H. annuus* × × *H. decapetalus* cultured in the presence of 5 mg/l zeatin. New hybrids were obtained from *H. annuus* × *H. hirsutus* and *H. scaberimus* × *H. annuus* combinations by culturing embryos on a modified White's medium. Depending on the origin of the regenerated shoots, different degree and intensities of rooting on White's medium were observed. Shoots could be successfully propagated on shoot-including medium supplemented with 800 mg/l L-glutamine — 800 mg/l L-asparagine (Bohorova et al., 1986).

Protoplasts were also isolated from seedling roots, hypocotyls, and cotyledons of four cultivars of *Helianthus annuus* and from leaves of axenic shoot cultures of the wild species *H. praecox, H. scaberimus* and *H. rigidus*. Optimal culture conditions were established for the respective protoplast systems, using the agarose bead method of culture. Protoplast division was induced for all the species examined. In the case of the cultivars of *H. annuus* hypocotil and cotyledon protoplast division was sustained leading to callus formation, which in turn, could be induced to produce roots and organized meristematic regions in the presence of L-naphthalene acetic acid and 6-benzylaminopurine.

Suitable *in vitro* method will be applied having in mind the use of biotechnologies in different scientific fields as well as the importance of the utilization of incompatibility by species determined by different barriers according to their character.

REFERENCES

Bohorova N. E., Cocking E. C. and Power J. B., 1986. *Insolation, culture and callus regeneration of protoplasts of wild and cultivated Helianthus species.* Plant Cell Reports, 5 : 256 –258.

Dryanovska O. A., 1983 *Transfer of genetic information through irradiated pollen.* Dokl. Bolg. AN, vol. 36, N8, pp. 1105—1106.

Grant J. E., Pandey K. K., Williams E. G.,

1980. *Pollen nuclei after ionizing irradiation for egg transformation in Nicotiana.* N.Z.J.Bot., vol. 18 (3), pp. 339—341.

Pandey K. K., 1975. *Sexual transfer of specific genes without gametic fusion.* Nature, vol. 256, pp. 310—313.

Pandey K. K., 1980. *Further evidence for egg transformation in Nicotiana.* Heredity, vol. 45(1), pp. 15—29.

Pandey K. K., 1981. *Egg transformation and parthenogenetic diploidy induced through the aid of ionizing radiation. Theory and practice.* In : Induced mutations. a tool in plant research. Proc. of a symposium, Vienna, pp. 49—65.

Powell W., Caligari P. D. S. and Hayter A. M 1983. *The use of pollen irradiation in barley breeding.* Theor. Appl. Genet., vol. 65, pp. 586—588.

Snape J. W., Parker B. B., Simpson E., Ainsworth C. C., Payne P. I. and Law C. N., 1983. *The use of irradiated pollen for differential gene transfer in wheat.* Theor. Appl. Genet, vol. 65, pp. 103—110.

Vassilieva R.D., 1985. *Nasledtvennaja izmenchivost, inducirovannaja ecsogennoi DNK u kukuruzi.* Avtoreff. kand. diss. (in Russian)