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PRODUCTION OF NEW CMS SOURCES IN SUNFLOWER

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SUMMARY

Twenty-one new sources of CMS were produced using interspecific hybridization, experimental mutagenesis and spontaneous expression of male sterility in sunflower. For all CMS sources, fertility restorers have been discovered.

All CMS are a result of cytoplasmic mutation. It is theorized that transformation of the mitochondrial genes controlling the pollen production is envoked by the influence of a single command from the cell nucleus to the mitochondria. The transformation appears to be irreversable. Commands for pollen production could be only accepted by the mitochondrial genes in case of existence of a fertility restoring gene in the cell nucleus of sterile cytoplasm.

Key words: sunflower, CMS, mutation, mitochondrial gene

INTRODUCTION

Male sterility is a widespread phenomenon in the plant kingdom. This is the case where no pollen is produced or, if produced, it does not pollinate for various reasons. Cytoplasmic male sterility (CMS) is a form of male sterility. The sterility is transferred only by the female and is maintained no matter how many crosses are made.

Cytoplasmic male sterility was observed as early as 1904 by Correns in *Satureja hortensis* L. (Krupnov, 1973). Today CMS is present in more than 160 cultivated and wild plant species (Dankov, 1973). Edwardson (1970) also stated that CMS is present in more than 150 plant species, belonging to 27 genera. The number of these species constantly increases due to the discovery of new CMS sources. According to Dankov (1973) there are five ways of obtaining CMS: 1) as a result of intergeneric hybridization (*Triticum* x *Secale*); 2) as a result of interspecific hybridization (*Epilobium roseum* x *Epilobium montanum*); 3) intervarietal hybridization in one and the same species, as it is in onions, sorghum, etc.; 4) spontaneous appearance of sterile plants in varieties and populations - onions, carrot, sugar beet, etc.; and 5) as a result of the treatment with chemical and physical mutagenic factors. Krupnov (1973) points out that most of the CMS sources have a hybrid character (origin). They are experimentally produced by transferring the genome of one species into the cytoplasm of another. Spontaneous or mutagenic CMS has been discovered just in 21 species. Krupnov (1971) also indicated that in self-pollinated crops in most cases CMS is a result of hybridization, while in cross-pollinated crops CMS appears most often spontaneously in varieties and populations. There are many CMS sources in all crops thus confirming this tendency. In sunflower, for example, there are at present more than 40 new CMS sources (Serieys, 1996) following the discovery of the first CMS source by Leclercq (1969).

Many investigators have tried to clarify the genetic nature of the cytoplasmic male sterility since the time of its discovery. Though many problems are already solved, the genetic character of CMS as a whole still remains unresolved (Gotsov, 1980). What is the essence of the agent causing the sterility, and in which part of the cytoplasm is this agent located? Which is the substance or complex of substance controlling the cytoplasmic inheritance /heredity/ (Gentchev, 1966)? Many investigations have been made during the last 20 years and with great attention focused on the organelles of the cytoplasm, especially the mitochondria (Turbin and Palilova, 1975; Leroy *et al.*, 1985; Crouzillat *et al.*, 1987; Makaroff *et al.*, 1989; Singh and Brown, 1991; De la Canal *et al.*, 1991; Spassova *et al.*, 1992; Friedt, 1992; Iwabuchi *et al.*, 1993; Moneger *et al.*, 1994; Abad *et al.*, 1995; Horn *et al.*, 1996; and Friedt, 1996).

Turbin and Palilova (1975) point out that CMS is a proper model for the study of the role of the nucleus and the cytoplasm in inheritance, their relationship in the process of development of properties and characters in the organism. It could be also said that CMS is a unique phenomenon, being not only an abnormality transferred from one generation to another, but also a proven means of production of hybrid seeds from varieties with heterotic effect. Forms with spontaneous CMS discovered in sunflower varieties were reported by Shtube in 1958, Gundaev in 1966 and Volf in 1966 (Krupnov, 1973). The first CMS sources were peculiar in that they lacked a total maintenance of their sterility. Leclercq reported in 1969 the discovery of total male sterility in some BC_4 generations, obtained from a cross made in 1964 between the species *Helianthus petiolaris* as a female parent and the cultivated sunflower line originating from the variety Armavirskii 3497. According to Leclercq, the cytoplasm of *H. petiolaris* influences the genome of the cultivated sunflower and causes sterility. The investigation of the relationship between the nucleus and the sterilizing cytoplasm reveals that almost every sunflower line is a maintainer of this male sterility type. After the discovery of CMS by Leclercq, other CMS sources have been produced *via* interspecific hybridization by Anashchenko (1974), Whelan (1980, 1981), Whelan and Dedio (1980), Serieys and Vincourt (1987), and Christov (1990). New CMS sources have been obtained from *H. petiolaris*, *H. annuus*, *H. argophyllus*, *H. bolanderi*, *H. debilis*, *H. exilis*, *H. giganteus*, *H. maximiliani*, *H. neglectus*, *H. niveus*, *H. praecox*, *H. pauciflorus* (*rigidus*) and *H. strumosus*. The discovery of spontaneous CMS was reported by Serieys and Vincourt in 1987 and new CMS sources *via* utilization of mutagenic agents were reported by Christov in 1993 and 1994.

The search for new CMS sources in sunflower and many other crops continues. Many investigations have been undertaken to study the causes for the occurrence of CMS and the function of the system CMS - fertility restoration.

CMS in sunflower was utilized and studied at the IWS "Dobroudja" since 1970. This report presents results describing the methods for obtaining new CMS sources in sunflower, which we use in our research.

MATERIAL AND METHODS

The investigation was carried out during the period 1983-1996. Sterile plants were sought from plant material produced by interspecific hybridization; from seeds, treated with physical and chemical mutagenic factors; from seeds, obtained by self-pollination of varieties; and from seed of old sunflower varieties and populations.

For interspecific hybridization, 6 annual and 19 perennial species of the genus *Helianthus* and 14 varieties and 12 inbred sunflower lines were used. The inflorescences of the wild plants were emasculated and pollinated with pollen from the cultivated sunflower. The hybrid plants were self-pollinated and backcrossed. The sterile plants were pollinated mainly with pollen of well-known lines, maintainers of CMS PET-1 (obtained by Leclercq, 1969) and sometimes with pollen from Bulgarian and Russian sunflower varieties. When the sterile plants were non-branched, they were pollinated with pollen from one or two lines at the most and the head (capitulum) was divided in two parts. The branched sterile plants were pollinated with pollen from several lines or varieties, and every inflorescence was pollinated by one line or variety. When sterile plants were obtained in the following generation, the pollination was performed mainly with pollen from sunflower inbreds. After full maintenance of sterility, the development of sterile analogues of four inbred lines started together with the search for fertility restorer lines of the new CMS sources among the wild *Helianthus* species or the hybrid material produced by interspecific and intergeneric hybridization, or other IWS sunflower materials.

Seeds from 13 varieties, 2 populations and 9 inbred sunflower lines were treated with physical and chemical mutagenic factors. The treatment were gamma rays in doses from 20 Gy to 500 Gy; ultrasound - from 0.5 W/cm^2 to 3 W/cm^2 for 2-4 min. and a buffered solution of ethyl methyl sulfonate (EMS) - 0.2% and 0.8% for 8 h. The mutant plants produced were isolated and self-pollinated separately. This proceeded for every M generation. The same method used for the sterile plants obtained from interspecific hybridization was applied for the maintenance of sterility, when sterile plants were produced from the mutant material.

Twenty-four Bulgarian and Russian varieties were used for the production of sterile forms from seeds of the self-pollinated plants. An additional 103 entries obtained from old seeds of varieties and populations and reproduced during the period 1965 - 1983 were also used. The capitula of all plants grown from old seeds were isolated in paper bags and self-pollinated. About 30%-40% of the plants originating from self-pollinated plants from varieties and populations were selected and self-pollinated. Selection and self-pollination were also done in the succeeding generations for both groups of plants. The method described above was used for the production of sterile plants.

The methods for obtaining new CMS sources in all cases are the same for the production of new sunflower forms - interspecific hybridization, experimental mutagenesis, selection and some other techniques of the scientific work (Christov, 1990; Christov, 1991; Christov, 1992; Christov, 1993; Christov, 1996; Christov and Nikolova, 1996; and Christov *et al.*, 1996).

RESULTS AND DISCUSSION

The search for new CMS sources is an integral part of the IWS "Dobroudja" program for development of new sunflower forms with increased genetic potential. There are three ways of obtaining CMS sources:

1. *Via* interspecific hybridization - hybridization between *Helianthus* species and the cultivated sunflower;
2. *Via* experimental mutagenesis - seed treatment with physical mutagenic factors (gamma rays and ultrasound);
3. Spontaneous occurrence of sterile plants in material - produced from old seeds of varieties and populations, stored in room temperature conditions.

Sources of CMS produced by hybridization between wild species of *Helianthus* and the cultivated sunflower

Male sterile plants were obtained in different hybrid generations - F_1, F_2, F_3, ..., BC_1, BC_1F_1, BC_1F_2, BC_1F_3, ..., BC_2F_2. The first male sterile plant was obtained in 1984 in an F_1 cross of *H. argophyllus* E-006 x v. Peredovik (Christov, 1990). Later, sterile plants were obtained from hybrid material produced by using *H. argophyllus* E-007, *H. annuus* E-067, and again *H. argophyllus* E-006, *H. praecox* E-029, *H. debilis* E-010, and again with *H. argophyllus* E-007, *H. annuus* E-058, *H. annuus* E-046, *H. praecox* E-027, *H. argophyllus* E-091, *H. debilis* E-014, *H. annuus* E-002, *H. petiolaris* E-034, *H. pauciflorus* (*rigidus*) M-028, *H. strumosus* M-056, *H. hirsutus* M-007, *H. decapetalus* M-043 and many others. Some of the sterile plants had branched stems. Most of the sterile inflorescences produced seeds after being pollinated with cultivated sunflower pollen, but not all of them produced sterile plants. No seeds were produced from a branched sterile plant which was a cross of *H. annuus* E-004 x *H. annuus* - cultivated sunflower, though

34 of the 48 inflorescences were pollinated with the pollen of 7 varieties and 10 inbred lines. There were crosses where 100% of the plants were male sterile, some produced no male sterile plants, and some produced two groups of plants - sterile and fertile. Sterility was maintained in 13 cases (Table 1). It has been shown to be the cytoplasmic type, i.e., sterility was transferred only through the female line. One of the male sterile sources (ARG-2) effected changes in the size and form of the tube florets in the sterile analogue of the maintainer lines, but it did not exert a negative influence on the seed set. These morphological changes disappeared in the F_1 plants after pollination with R line pollen. Almost all new CMS fertility restorer sources were found in hybrid material with *Rf* genes transferred from wild *Helianthus* species, as well as R lines from the IWS collection.

Table 1: Sources of CMS produced by interspecific hybridization in the period 1984 - 1996

Origin	PI number	Obtained in generation	Year obs.	Year report	IWS code	F.A.O. code
H. annuus E - 067		F_1	1985	1992	AN-67	ANN-10
H. annuus E - 058		F_6	1988	1994	AN-58	ANN-11
H. annuus E - 002		F_5	1991	1991	AN-2-1	ANN-12
H. annuus E - 002		F_6	1992	1992	AN-2-2	ANN-13
H. argophyllus E - 006		F_1	1984	1990	ARG-1	ARG-1
H. argophyllus E - 006		BC_1	1987	1990	ARG-3	ARG-3
H. argophyllus E - 007		F_1	1985	1992	ARG-2	ARG-2
H. debilis E - 010		F_2	1990	1994	DV-10	DEB-1
H. petiolaris E - 034		BC_1F_6	1991	1991	PET-34	PET-4
H. praecox E - 027		F_2	1990	1990	PHIR-27	PRH-1
H. praecox E - 029		F_4	1989	1989	PRUN-29	PRR-1
H. rigidus M - 028		BC_1F_2	1991	1991	RIG-28	RIG-2
H. strumosus M - 056		BC_1F_5	1991	1996	STRUM-56	STR-1

Sources of CMS produced by application of experimental mutagenesis

More than 30 male sterile plants were found in different M generations after treating seeds of sunflower varieties and lines with gamma rays and ultrasound. The largest number of male sterile plants were obtained from line 1721 (9 plants) and from the variety Peredovik (8 plants). In the group of plants obtained from seeds treated with gamma rays, 31 sterile plants were found, whereas only three sterile plants were produced after treatment with ultrasound. Only 5 sources of male sterility were maintained - 4 of them were produced using gamma rays and one using ultrasound (Table 2). The five sterile sources are of the cytoplasmic sterility type. They provide the basis for creating sterile analogues of certain B lines found in the BC_5 to BC_{10} generations. Fertility restorers were discovered for three of the sources. Up till now no sterile plants have been produced in the mutant materials obtained after seed treatment with EMS.

Table 2: Sources of CMS induced by gamma rays and ultrasound in the period 1984 - 1996

Origin	Mutagen		Produced in gen.	Year obs.	Year report	IWS code	F.A.O. code
	Gamma rays Gy	Ultra sound W/cm^2-min					
v. Hemus	150	-	M_1	1986	1993	H	MUT1
Stadion	70	-	M_1	1984	1993	S	MUT3
Peredovik	70	-	M_9	1991	1993	P-114	MUT4
Peredovik	250	-	M_6	1992	1996	P-92	MUT5
Peredovik	-	(2-2) + (2-4)	M_3	1987	1993	P-UZ	MUT2

Sources of CMS produced spontaneously in materials of the cultivated sunflower

From seeds of 103 old cultivated sunflower varietes, plants were produced from only 37 of them. In the progenies of these 37 varieties, five cases of spontaneous occurrence of sterile plants were observed. These plants originated from seeds obtained in 1965, 1967, 1978, 1980 and 1983. Two sterile plants produced from 1965 and 1967 seeds were pollinated with pollen of the varieties Peredovik and Vihren. No seeds were obtained in either case. The results of the three remaining cases have been described by Christov in 1993 as new CMS sources (Table 3).

Table 3: CMS sources spontaneously produced from seeds of cultivated sunflower

Source	Generation	Year obs.	Year report	IWS code	F.A.O. code
v. Gigant	0	1986	1993	CMS G	ANN15
p. DP-[1]08	I_1	1987	1993	CMS DP	ANN16
p.[1]638/4	I_2	1990	1993	CMS VL	ANN17

A sterile plant denoted as (CMS G) was produced from 1980 seeds of the cultivar Gigant planted in a greenhouse. Completely sterile plants were produced after pollinating with pollen from the same cultivar, but from a different seed lot (1986). Sterility was also maintained by other cultivars and lines.

The CMS source CMS DP originated from peeled kernels of N 8 - material from the population-breeding program of D. Petrov (1978). The seeds were stored under normal room conditions and planted in the field in 1986. Two sterile plants were produced from the materials in 1987. Completely sterile plants were produced by crossing with L 3853. The sterility was maintained also by other lines such as 1607, 2607, 3004 and HA-89.

The CMS source CMS VL originated from a hybrid cross (L 275 x L 1418) made in 1975 and planted in the field in 1988. Two sterile plants were produced after double self-pollination in 1990. Sterility was maintained by lines 1721, 3004 and HA-89.

In all three cases male sterility was the cytoplasmic type. The analogues created could be found in the BC_5 - BC_8 generations. Fertility restorers have been discovered for all three sources. Sterile plants from the materials produced from seeds of self-pollinated plants from 24 Bulgarian and Russian cultivars have not been obtained.

During the period 1984 - 1996, 21 new CMS sources were produced. No negative effects on the morphology and productivity of the plants of these new CMS sources have been observed. This shows that the 21 new sources of CMS could be of interest for heterotic breeding of sunflower.

Concepts of CMS origination and of the appearance of sterile plants

The uniqueness of CMS and especially of the CMS working system is that the fertility restorer enables adequate usage of heterosis in breeding of cultivated plants.

The experimental approach to obtaining new CMS sources in sunflower appears to be a very laborious task which takes a lot of time to create material suitable for breeding. The increased number of new CMS sources during recent years indicates that this process has significantly improved. Different ways of producing CMS have been used. Obtaining new CMS sources adds to our knowledge about the causes inducing the occurrence of male sterility, its maintenance, and the fertility restoration of the sterile material.

Our search for new CMS sources supports the concept of creating a greater diversity through new CMS sources, and should permit us to add knowledge about the system and present new ideas about the occurrence of CMS. Twenty-one new CMS sources were obtained in three seemingly different ways, i.e., by interspecific hybridization, physical mutagenic factors, and the spontaneous occurrence of sterile plants in the cultivated sunflower. The largest number of new CMS sources (13) was obtained through interspecific hybridization. Experimental mutagenesis resulted in five CMS sources, whereas three CMS sources were due to the spontaneous appearance of sterile plants. Sterile plants were separately pollinated with pollen from different varieties and inbred lines. Sterility was maintained in a part of the sterile plants obtained. They were pollinated with pollen from varieties and lines that were obtained from one species only (*H. annuus*) and originated from Russian breeding material. The cultivated sunflower of the same origin was used in the interspecific hybrids (wild species x cultivated sunflower) from which sterile plants were obtained. The same schemes for sterility maintainance and for fertility restoration were used for the different sterile sources. These facts support the assumption that by using the three methods, the same change was effected in the cytoplasm of the female cell.

Sterile plants occurred in different generations, i.e., F_1, F_2, ..., BC_1, BC_1F_1, .., BC_2, ..., M_1, M_2, ... M_9. In most cases there was only one plant for each origin. The probable cause for the occurrence of a single sterile plant in the different generations is that the changes which occurred in the cytoplasm either occupied small "territories" at the beginning or they had a low frequency. In the process of cell division, all changes in the cytoplasm could appear in a larger proportion in only one of the newly formed cells. Even if this proportion of cytoplasmic changes is not large enough in this generation, it could reach 100 percent in the following generations. In this case the induced sterility could manifest itself. The newly obtained CMS sources could be expressed only when the genes controlling the pollen reproduction in the nucleus of the cell with sterile cytoplasm were in the homozygous recessive state.

Our results and observations during the investigation as well as the conclusions obtained by other researchers support the opinion that CMS is the result of cytoplasmic mutation.

CMS occurrence caused by interspecific hybrids

During the fertilization process the nuclear material from cultivated sunflower pollen combines with the nucleus of the female gamete belonging to the wild species and forms a zygote. The embryo develops from the zygote, and forms the hybrid plant. Combining of the two nuclear materials is a single non-reversable reaction which causes constant change in the cytoplasm.

According to Sager (1972), a larger part of the mitochondrial proteins are coded by nuclear genes and are also synthesized in the cytoplasm. The nuclear genome and also the genomes of these organelles participate in the biogenesis of mitochondria. This indicates that there is a significant correlation between the two kinds of genomes. This correlation enables the information reproduced a single time and obtained by merging of the nuclear material from the two gametes to be transferred into the mitochondria and also to cause change in some of the genes controlling the pollen production. In this case, the gene affected by the mitochondria mutates because the change remains permanent. What is the reason for this? How does it happen? Most probably, during the single "reaction" resulting from the fusion of the two nuclei, one of the gene-regulators is transformed into another gene and sets off a kind of product that "strikes", suppresses the functioning of the mitochondrial genes connected with it (being subordinate to it) which control the pollen production, or it causes a kind of inversion of those genes which, being in this inverted state, are not able to provide the information suitable for the pollen production.

Obtaining different types of CMS supports the assumption that the number of genes controlling the pollen production in mitochondria is at least 3. By inverting one of the genes, a certain type of CMS appears, whereas by inverting another gene - a different type of CMS can occur. Reversion to the initial state even for the time being could be done by transferring one dominant gene (restorer) controlling the pollen production into the nucleus of the sterile CMS. By transferring this dominant gene restorer, the gene regulator for the inverted gene from the mitochondria releases the inverted one that "sets straight" and afterwards the information about the pollen production could be provided. This "setting straight" is possible only in the cases where the dominant gene - restorer is present in the nucleus of the sterile cytoplasm. When the dominant gene restorer leaves the nucleus of the sterile cytoplasm, the "struck" gene from the mitochondria takes its inverted state.

When two mitochondrial genes controlling the pollen production invert simultaneously, the cytoplasms that appear are assumed to have no fertility restoration. If two suitable restorer genes could be revealed and combined in one and the same place and if they could be transferred into the nucleus of this type of sterile cytoplasm, the fertility is likely to be restored.

Obtaining CMS under the influence of physical mutagenic factors

Mutation has been induced in the cytoplasm of the cultivated sunflower. Mutating can appear in two ways. The first one is when the mitochondrial genes controlling the pollen production are directly affected. The second one is when in the female gamete nuclear pollen material affected by the mutagene influence is transferred during the self-pollination of mutant plants. A reaction takes place which is similar to that obtained in the interspecific hybridization. From the nucleus of the zygote, new information is transmitted only once, which exerts its effect on the mitochondrial genes. The code in the chromosomes changes in the place where the genes controlling the development of the male gametophythe are present. At a certain stage of plant development, transmission of suitable information may break down or no information are transmitted at all, thus bringing to a stop the development of elements belonging to the reproductive system, as well as the production of viable pollen or no pollen is produced at all.

Spontaneous occurrence of sterile plants

This is also a kind of induced mutation caused by unknown sources. Probably, this is to a great extent the result of the influence of internal biological factors. The process and results of sterility appear the same as these causing the appearance of sterile plants obtained by interspecific hybridization and experimental mutagenesis.

CONCLUSIONS

Twenty-one new CMS sources were produced using interspecific hybridization, experimental mutagenesis and spontaneous expression of male sterility in sunflower. The CMS sources were maintained by a number of lines and cultivars. For all CMS sources, fertility restorers have been found. The new CMS sources can be used when they are needed in the heterotic breeding of sunflower.

In all cases, the obtained CMS was a result of cytoplasmic mutation. The most reliable proved to be the concept stating that the transformation of the mitochondrial genes controlling the pollen production is accomplished under the influence of information set off only once from the cell nucleus to the mitochondria. The transformation produced appears to be non-reversible. The inverted mitochondrial genes are not able to transmit suitable information about pollen reproduction. Pollen production seems to be possible only by transferring a dominant gene - restorer into the nucleus of the sterile cytoplasm. When the dominant gene - restorer is available, the inverted mitochondrial gene reverses and transmits information about pollen reproduction. When the dominant gene - restorer leaves the nucleus of the sterile cytoplasm, the mitochondrial gene takes its inverted position.

REFERENCES

Abad, A.R., Mehrtens, B.J. and Mackenzie, S.A., 1995. Specific expression in reproductive tissues and fate of a mitochondrial sterility-associated protein in cytoplasmic male-sterile bean. The Plant Cell, Vol. 7, pp. 271-285.

Anashchenko, A.V., 1974. Cytoplasmic forms of male sterility in sunflower. Reports of VASHNIL, 4, pp. 11-12 (in Russian).

Christov, M., 1990a. A new source of cytoplasmic male sterility in sunflower originating from *Helianthus argophyllus*. Helia, 13: 55-61.

Christov, M., 1990b. Study of wild species of the genus *Helianthus* in view of their use in sunflower breeding. Dissertation. Sofia, SA. (in Bulgarian).

Christov, M., 1991. Possibilities and problems in the hybridization of cultivated sunflower with species of the genus *Helianthus* L. Helia, 14(15): 35-40.

Christov, M., 1992. New sources of male sterility and opportunities for their utilization in sunflower hybrid breeding. Helia 15(16): 41-48.

Christov, M., 1993. Sources of cytoplasmic male sterility produced at IWS "Dobroudja". Biotechnol. & Biotechnol. Eq., 7, No. 4, pp. 132-135.

Christov, M., 1996a. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. In: P.D.S. Caligari & D.J.N. Hind (eds). *Compositae*: Biology & Utilization. Proceedings of the International *Compositae* Conference, Kew, 1994. (D.J. N. Hind, Editor-in- Chief), Royal Botanic Gardens, Kew, Vol. 2, pp. 547-570.

Christov, M., 1996b. Hybridization of cultivated sunflower and wild *Helianthus* species. In: P.D.S. Caligari & D.J.N. Hind (eds). *Compositae*: Biology & Utilization. Proceedings of the International *Compositae* Conference, Kew, 1994. (D. J. N. Hind, Editor-in- Chief), Royal Botanic Gardens,Kew, Vol. 2, pp. 603-615.

Christov, M., and Nikolova, V., 1996. Increasing of the sunflower genetic diversity by mutagenesis. Proceedings of 14th International Sunflower Conference. Beijing/Shenyang, pp. 19-30.

Christov, M., Shindrova, P. and Entceva, V., 1996. Transfer of new characters from wild *Helianthus* species to cultivated sunflower. Genet. a Slecht., 32 (4), Praha, pp. 275-286.

Crouzillat, D., Leroy, P., Perrault, A. and Ledoigt, G.,1987. Molecular analysis of the mitochondrial genome of *Helianthus annuus* in relation to cytoplasmic male sterility and phylogeny. Theor. Appl. Genet., 74, pp. 773-780.

Dankov, T., 1973. Tsitoplazmatichnata muzhka sterilnost pri kulturnite rastenya i znachenieto za hibridnoto semeproizvodstvo. Agricultural Science, Vol. XII, No. 2, Sofia.

De La Canal, I., Crouzillat, D., Flamand, M.C., Perrault, A., Boutry, M. and Ledoigt, G., 1991. Nucleoitide sequence and transcriptional analysis of a mitochondrial plasmid from a cytoplasmic male-sterile line of sunflower. Theor. Appl. Genet., 81, pp. 812-818.

Edwardson, J., 1970. Cytoplasmic male sterility. Bot. Rev., Vol. 36, pp. 341-420.

Friedt, W., 1992. Present state and future prospects of biotechnology in sunflower breeding. Field Crops Research, 30, pp. 425-442.

Friedt, W., 1996. Use of biotechnology in sunflower breeding. Helia 19, Special issue, pp. 161-179.

Genchev, G., 1966. Suvremenni problemi na genetikata. Zemizdat, Sofia, pp. 191-207.

Gotsov, K., 1980. Tsitoplazmena muzhka sterilnost pri pshenitsata i neinoto izpolzvane v selektsiyata. Zemizdat, Sofia.

Heiser, C., 1982. Registration of Indiana - 1 CMS sunflower germplasm. Crop Science, 22, p. 1089.

Horn, R., Hustedt, J.E.G., Hahnen, J., Zetsche, K. and Friedt, W., 1995. The molecular basis of cytoplasmic male sterility (CMS) in sunflower. The F.A.O. European Research Network on Sunflower, Bucharest 25-28 July.

Iwabuchi, M., Kyozuka, J. and Shimamoto, K., 1993. Processing followed by complete editing of an altered mitochondrial *atp6* RNA restorers fertility of cytoplasmic male sterile rice. The EMBO Journal, Vol. 12, No. 4, pp. 1437-1446.

Krupnov, V.A., 1971. Istochniki tsitoplazmaticheskoy muzhskoy sterilnosti u rasteniy. Genetika t. 7, No. 3, pp. 159-174.

Krupnov, V.A., 1973. Gennaya i tsitoplazmaticheskaya muzhskaya sterilnost rasteniy. Moskva, "Kolos".

Leclercq, P., 1969. Une sterilite male cytoplasmique chez le tournesol. Ann. Amel. Plantes, 10, pp. 99-106.

Leroy, P., Bazetoux, S., Quetier, F., Delbut, J. and Berville, A., 1985. A comparison between mitochondrial DNA of an isogenic male-sterile and male-fertile couple (HA-89) of sunflower. Curr. Genet., 9, pp. 245-251.

Makaroff, C.A., Apel, I.J. and Palmer, J.D., 1989. The *atp6* coding region has been disrupted and a novel open reading frame generated in the mitochondrial genome of cytoplasmic male-sterile radish. J. Biol. Chem., 264, pp. 11706-11713.

Moneger, F., Smart, C.J. and Leaver, C.J., 1994. Nuclear restoration of cytoplasmic male sterility in sunflower is associated with the tissue-specific regulation of a novel mitochondrial gene. The EMBO Journal, Vol. 13, No. 1, pp. 8-17.

Sager, R., 1972. Cytoplasmic genes and organelles. Academic press New York and London.

Serieys, H., 1996. Identification, Study and Utilisation in Breeding Programs of New CMS Sources. Helia 19, Special issue, pp. 161-179.

Serieys, H. and Vincourt, P., 1987. Characterization of some new CMS sources from *Helianthus* genus. Helia 10, pp. 9-13.

Singh, S.P. and Brown, G.G., 1991. Suppression of cytoplasmic male sterility by nuclear genes alters expression of a novel mitochondrial gene region. Plant Cell, 6, pp. 811-825.

Spassova, M., Christov, M., Bohorova, N., Petrov, P., Dudov, K., Atanassov, A., Nijkamp, H.J.J. and Hille, J., 1992. Molecular analysis of a new cytoplasmic male sterile genotype in sunflower. FEBS, Vol. 297, No. 1,2, pp.159-163.

Turbin, N.V. and Palilova, A.N., 1975. Geneticheskie osnovi tsitoplazmaticheskoy muzhskoy sterilnosti u rasteniy. Izdatelstvo "Nauka i tehnika".

Whelan, E.D.P., 1980. A new source of CMS in sunflower. Euphytica, 29, pp. 33-46.

Whelan, E.D.P., 1981. CMS in *Helianthus giganteus* L. x *H. annuus* L. interspecific hybrids. Crop Science, 21, pp. 855-858.

Whelan, E.D.P., Dedio, W., 1980. Registration of sunflower germplasm composite crosses CMG-1, CMG-2, CMG-3. Crop Science, 20, p. 832.

PRODUCCIÓN DE NUEVAS FUENTES DE CMS EN EL GIRASOL

RESUMEN

Veintiuna nuevas fuentes de CMS fueron producidas por la utilización de la hibridación interspecie, la mutagenesis experimental y la expresion espontanea de esterilidad masculina en el girasol. Para todas fuentes de CMS fueron descubiertos los restauradores de fertilidad.

CMS es el resultado de la mutación citoplásmica. Suponese que la transformación de los genes mitocondriales los cuales controlan la producción de polen es causada por la influencia de un unico mando trasladado del nucleo celular de mitocondria. Parece que ésta transformación es irreversible. Los genes mitocondriales aceptan el mando para empezar a producir polen solo en caso de existencia de los genes restauradores de fertilidad en el nucleo celular del citoplásma esteril.

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CREATION DE NOUVELLES SOURCES CMS DANS LE TOURNESOL

RÉSUMÉ

Vingt et une nouvelles sources CMS ont été produites par hybridation interspecies, mutagenèse expérimentale et apparition spontanée de stérilité mâle dans le tournesol. Des restaurateurs de fertilité ont été trouvés pour toutes les nouvelles sources de CMS.

Le CMS est un résultat de la mutation cytoplasmique. On suppose que la transformation des gènes de mitochondries contrôlant la production du pollen est due à l'influence d'une seule commande transmise du noyau de la cellule aux mitochondries. Il semble que cette mutation soit irréversible. Les gènes de mitochondrie acceptent la commande de production de pollen seulement dans le cas où il existe un gène restaurateur de fertilité dans le noyau de la cellule du cytoplasme stérile.