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A NEW SOURCE OF CYTOPLASMIC MALE STERILITY IN SUNFLOWER ORIGINATING FROM *Helianthus argophyllus*

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SUMMARY

The current sunflower hybrid production is realized only on the base of cytoplasmic male sterility (CMS) of "petiolaris" type. This cytoplasmic uniformity is a potential risk for the yield of sunflower and is a reason for a series of studies aimed at discovering new CMS sources. Large diversity of wild species and cultivated varieties is a premise for successful breeding of hybrids with high yielding potential.

In 1984 and 1987, new sources of CMS, marked CMS-ARG-1 and ARG-3, were produced by interspecific hybridization of the wild annual species *H.argophyllus*, used as the female parent, and the cultivated sunflower, used as the male parent. The new cytoplasmic sterility is maintained by a large number of varieties and self-pollinated lines. Some lines known as restorers for CMS "petiolaris" type appear also to be restorers for the new CMS sources. This shows that these sources can be used for development of sunflower hybrids. The hybrids developed on the base of the two new CMS sources substentiate the above statement.

The conventional CMS and the new CMS sources, ARG-1 and ARG-3, are similar in some points and different in others. Some lines known as maintainers for CMS "petiolaris" type possess the ability to restore, to a certain degree, the fertility of plants produced on the base of the new CMS. A study undertaken to clarify the differences is in progress.

INTRODUCTION

An important premise for yield increase in sunflower is the development of modern hybrids having seed production from 3500 to 4000 kg/ha and the oil content in seed equal to the variety Peredovik (about 50%). The heterotic effect in sunflowers has been known for a long time, but its wide practical utilization began after L e c l e r c q (1969) discovered the first source of cytoplasmic male sterility in *Helianthus petiolaris*. The finding of efficient restorers for this type of sterility is a key stage in the process of development of hybrids (K i n m a n , 1970; E n n s et al., 1970; L e c l e r c q, 1971; etc.).

All currently grown sunflower hybrids are developed on CMS *H.petiolaris* type. This cytoplasmic uniformity represents a potential risk for the practical realization of hybrids, as proved by a trial with maize. A work is in progress on developing new genetic mechanisms for male sterility (MS) and discovering new CMS sources and fertility restoration in all crops possessing hybrids. In this respect, the sunflower is highly perspective on account of the diversity of species and varieties.

In 1973 L e c l e r c q found a new CMS source, again from a cross between *H.petiolaris* and *H.annuus*. At the time A n a s h c h e n k o (1974) discovered another CMS source from a cross of *H.annuus ssp. lenticularis* and *H.annuus*.

W h e l a n (1980,1981); W h e l a n and D e d i o (1980), reported of new CMS sources produced from the cytoplasms of *H.petiolaris, H.giganteus* and *H.maximiliani.*

H e i s e r (1982) found a new source of CMS in a cross between *H.annuus ssp. lenticularis* and *H.annuus*. V r a n c e a n u et al. (1986) reported of discovering a new CMS source in H.annuus ssp. texanus.

S e r i e y s and V i n c o u r t (1987) reported of a large group of new sources produced by crossing *H.petiolaris ssp. falax, H.bolanderi, H.exilis, H.annuus* (wild forms) with the cultivated sunflower.

New sources of CMS have also been produced by interspecific hybridization among the annual sunflower species from the collection of the Wheat and Sunflower Institute, General Toshevo, Bulgaria. In this paper we present the results of a research on possibilities of producing CMS from the annual species *H.argophyllus*. Till now this species has not been determined as a source of CMS, which adds value to our research effort.

MATERIAL AND METHOD

The crossing of *H.argophyllus* as the female parent with *H.annuus* as the male and the production of F_1 hybrid plants was done according to the conventional technique without application of special treatments of plants or pollen. *H.argophyllus*, sample E-006 (Buckland 1317), was used. It was crossed several times with the varieties used as the male perent: Peredovik, Peredovik-improved, No.8931, No.6540, Progress, Start, Skorospelii, Trudovik, Voronyeskii-272, Record, Hemus, Vihren, Stadion, Balkan, Kutuger; and self-pollinated lines: No.32, No.57, No.63/2, No.130, No.1607, No.1721, No.2969, No.3004, No.3840, No.4007, No.4064, No.5977, No. HA-89.

Observations were carried out in all hybrid generations. The progenies after F_1 were produced through self-pollination, pollination in a group (sister pollination), or backcrossing. On two occasions, sterile plants were found in the hybrid material. Sterility ARG-1 was obtained in the first case (1981) while in the second one (1987) sterility ARG-3 was produced. The inflorescences of the sterile plants obtained were pollineted by the male variety or self-pollinated line. To produce sterile plants in the following generations, the pollination was done by the same male parent and other fertile sources of *H.annuus*, increasing in that way the selection of varieties and lines used as pollinators. The MS was expressed as stable and complete under different conditions and generations, the last generation being BC_7 for ARG-1 and BC_4 for the source from *H.argophyllus*.

Sterility was determined after isolation before flowering and a check was made for seed set.

Different restorers were tested for restoration of these cytoplasms known as CMS of H.petiolaris type.

RESULTS AND DISCUSSION

Two sterile plants were produced in the first hybrid generations and BC_1 from the crossing of *H.argophyllus* and *H.annuus*. The first sterile plant was obtained in the F_1

generation of the cross *H.argophyllus* x Peredovik in 1984. In 1987, the second sterile plant was produced in the BC_1 generation originating from the cross (*H.argophyllus* x Peredovik) x Peredovik.

1. STUDY OF THE FIRST STERILE PLANT

The plant was produced under greenhouse conditions during the period May - July. It had 7 branches with flower heads developed normally on four of them. The main head which flowered at the end of July was sterile. It was pollinated by pollen of the variety Peredovik. Seed were not obtained from it. At the end of August the plant was preplanted under field conditions. Under these conditions, four flower heads normally developed from the branches, sterile again, and were pollinated by pollen of Peredovik. From this repeated pollination, dated September 6, eleven seeds were obtained. Five of these were planted in a greenhouse in the autumn of 1984, and six under field conditions in 1985. Eleven male-sterile plants were produced from these 11 seeds in BC_1. Some had one or two poorly developed branches appearing after the flowering of the main head. The heads of four plants were isolated before anthesis in pairs and during flowering these were rubbed together every other day. On examination of their productive ability there was no seed set formation.

The varieties Peredovik (BC_2), Progress and Vihren were included to produce the next generation. In the crosses with the variety Vihren, 120 plants were obtained and 98.3% of them were male-sterile. The plants produced with the other two varieties were 100% sterile. The varieties Trudovik, Voronyeskii-272, Start, Skorospelii, Hemus, Stadion, Balkan, Peredovik-improved and self-pollinated lines 140, 1607 and 3004 were also included in further research of the MS produced by *H.agrophyllus*, sample E-006, and for producing subsequent generations. Single fertile plants were obtained in BC_3 and BC_4 with varieties Vihren and Peredovik-improved.

In the fifth and sixth generation, 100% male-sterile plants were produced from all varieties and lines included in the study.

It is known that male sterility is inherited from the female line (H e i s e r, 1969; etc.). In our study, all plants produced in different generations were male-sterile. The reason is that the sterility obtained is the cytoplasmic type. Therefore, all varieties and lines used appear to be sterility maintainers for the cytoplasm of *H.agrophyllus*, recorded as CMS, type ARG-1.

No negative effects were observed on the side of the cytoplasm. The plants produced on the CMS base type ARG-1 developed normally. Seed productivity after pollination of the sterile analogues of type ARG-1 was similar to that of the varieties and the self-pollinated lines. This indicates the normal activity of the female gametophyte. At the same time the seed formation after pollination passed without difficulties. The results are summarized in Table 1.

Table 1. Characteristics of the male-sterile analogues of the type ARG-1 in BC_5

No. of planting 1987	Maintainers and MS analogues	Pollen fertility (%)	No. of tube florets	Seeds produced in free pollination	
				No.	(%)
1	Peredovik	–	1720 – 2056	1474 –2012	85.7 – 97.9
60	SL 1607	–	1207 – 1287	1127 –1167	90.7 – 93.4
77	SL 3004	–	1105 – 1236	705 – 886	63.8 – 71.8
618	MS 3004	0	1860 – 1950	1360 –1600	73.1 – 82.1
626	MS 1607	0	964 – 968	946 – 968	98.1 – 100.0
638	MS Peredovik	0	1425 – 2000	1170 –1800	82.1 – 90.0

In 1988, the male-sterile analogue of the line 3004 and CMS type ARG-1 was crossed with the self-pollinated lines 57m, 63/2, 130, 1721, 3840, 4064, HA-89, NS-26R, NS-71-10 R, RHA-274. It was found that all plants obtained from the crosses with the self-pollinated lines 3840, R 147, NS-26R, NS-71-10 R and RHA-274 were fertile. It is an indication that these lines possess genes for the fertility restoration of CMS type ARG-1.

2. STUDY OF THE SECOND STERILE PLANT

The plant was produced under field conditions in the BC_1 generation. It had eight branches which developed normally on the upper part of the stem. Three flower heads were pollinated by pollen from the self-pollinated lines 3004 and 130; 564 seeds were obtained in the backcrossing with the line 3004. The head pollinated by pollen from the line 130 failed to produce seeds. All heads left to flower freely yielded a considerable number of seeds.

The 320 seeds produced from the cross with the line 3004 were planted to produce the next generation; 299 plants were obtained from these seeds and all of them had male sterile single heads which developed normally. The heads of 30 plants were isolated before the beginning of anthesis to prove the sterility. Ten of these were put in an isolator individually, the rest in pairs. The heads isolated in pairs were rubbed together every other day. At the end of the vegetation period all the heads were examined for seed-set formation which did not occur. The inability of the atrophied anthers to emit pollen was proved also by microscope tests. The size of the seeds produced on the pollinated heads and their husk color were different.

In the further study of this male sterility source, the following varieties and self-pollinated lines were included:

Varieties - Peredovik, Peredovik-improved, Progress, No.8931, No.6540, Vihren, Start, Stadion, Kutuger, Record;

Lines - 57M, 63/2, 2969, 3004, 5977, HA-89, R-147, NS-26R, NS-71-10R, RHA-274.

The larger part of the materials in BC_3 were grown under field conditions. The plants produced in the backcrossing with all varieties, as well as with the self-pollinated lines 57M, 63/2, 2969 and 3004, showed a complete maintainance of sterility (100%). Fertile plants were obtained with the line HA-89 - 3.44% and with the lines R-147, 5977,

NS-26R, NS-71-10R and RHA-274 (100%). The BC_3 plants from the crosses with the lines 3004, 2968 and HA-89 were grown under greenhouse conditions. The maintainance of MS was 100%. The new backcrosses were produced again with the same lines, while pollination was done in pairs. The seeds produced were planted in the field.

The BC_4 plants had similar phenotypes with those of the self-pollinated lines, but in two crosses with the line HA-89, fertile plants have been produced, totaling 14.3%.

The study of the sterile material obtained from the second sterile plant, shows that this sterility is inherited from the female line and it is of cytoplasmic type. It is recorded as CMS type ARG-3.

No negative effects were observed on the side of CMS type ARG-3. All plants produced on the CMS base type ARG-3 developed normally. The seed productivity of the sterile analogues with CMS-ARG-3 was equal to that of the varieties and self-pollinated lines. There were slight differences in husk thickness and color. Summarized results for BC_4 of CMS type ARG-3 with the self-pollinated lines 3004 and HA-89 are presented in Table 2.

Table 2. Characteristics of the male sterile analogues of the type ARG-3 in BC_4.

No. of planting 1987	Maintainers and MS analogues	Pollen fertility (%)	No. of tube florets	Seeds produced in free pollination	
				No.	(%)
42	SL 3004	–	1028 – 1361	595 – 684	50.26 – 57.88
48	HA–89	–	1447 – 1590	723 – 1132	49.97 – 71.19
1067	MS HA–89	0	1398 – 1476	787 – 1053	56.29 – 71.54
1069	MS 3004	0	1364 – 1481	819 – 1156	60.04 – 77.72
1389	MS 3004	0	1177 – 1464	1077 – 1102	75.27 – 91.50

The observations conducted on the newly developed forms of CMS, types ARG-1 and ARG-3, show that they can be used as a male-sterile base for development of sunflower hybrids. Together with the development of sterile analogues, several restorers were also discovered.

The results given in Table 3 indicate that the restorers for CMS type H.petiolaris R-147, NS-26R, NS-71-10R and RHA-274 also appear to be restorers for CMS types ARG-1 and ARG-3. Several maintainers of CMS type H.petiolaris showed the capacity for restoring fertility in the materials with CMS from H.argophyllus.

These facts show that a similarity exists between the conventional CMS and the new one. At the same time there were also differences between them. Furthermore it is assumed that the two CMS sources, ARG-1 and ARG-3, are mutually different. Their distinctions and similarities cannot be explained by systematic considerations regarding the characteristics of the two species, and show the individual features of two cytoplasms. Direct evidence of differences in the mitochondrial genome structure could confirm or refute this assumption.

Such study has already started in our laboratory for genetic engineering.

Table 3. Fertility restorers for CMS types ARG-1 and ARG-3

Fertility restorers	Degree of restoring ability – %		
	for ARG–1	for ARG–3	for CMS type H.petiol.
R–147	100	100	100
NS–26R	100	100	100
NS–71–10R	100	100	100
RHA–274	100	100	100
SL 3840	100	–	–
SL 5977	–	100	0
HA–89	0	0 – 14.3	0

For the time being, some results could be presented about the performances of the hybrids developed on the base of CMS ARG-1 and CMS ARG-3 (Table 4).

Table 4. First hybrids produced on the basis of the new cytoplasm and the restorers of fertility

Hybrid No.	Cross type	1000–seed wgt/g	Oil in (%)	Yield kg/ha
894	CMS/H. petiolaris/ MS 3004 x R–147	57.2	47.4	247.5
958	CMS/ARG–1/MS 3004 x R–147	51.6	46.5	220.0
1075	CMS/ARG–3/ MS 3004 x R–147	54.0	44.5	257.7
1078	MS 3004xNS–71–10R	48.6	41.0	174.5

CONCLUSION

The CMS sources ARG-1 and ARG-3 have been produced by interspecific hybridization of the annual species Helianthus argophyllus used as the female parent and tha cultivated sunflower used as the male parent. The cytoplasmic sterility has been maintained by a large number of varieties and self-pollinated lines. This indicates that the new type of sterility can be utilized as a CMS source in developing sunflower hybrids. Some lines have been identified as possible restorers of the two CMS types.

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UNE NOUVELLE SOURCE DE STERILITE MALE CYTOPLASMIQUE ISSUE DE *Helianthus argophyllus*

Michail CHRISTOV

La production d'hybrides de tournesol est réalisée uniquement à partir de la stérilité mâle cytoplasmique (CMS) de type "petiolaris". L'uniformité de cette source de stérilité présente un risque potentiel pour le rendement du tournesol, et pour cette raison des recherches ont été mises en oeuvre pour découvrir de nouvelles sources de CMS. Les fortes potentialités du tournesol, representées par le diversité de ses especés et de ses variétés, sont autant d'atouts pour la réussite de ce type d'étude.

Entre 1984 et 1987 de nouvelles sources de CMS, notées CMS-ARG-1 et CMS-ARG-3, ont été produites à partir d'hybridisations interspécifiques par croisement entre *H.argophyllus* utilisé comme parent maternel, et un tournesol cultivé comme parent paternel. Ce caractère a été maitenu à travers un large nombre de variètes et de lignées auto-fécondées. Certaines lignées connues comme restaureur de fertilité pour la CMS de type "petiolaris" se sont révèlées être également restoreur pour cette nouvelle source de CMS prouvant que ces sources de stérilité peuvent être utilisées comme CMS dans le développement d'hybrides. Cela a été confirmé par la production d'hybrides développés sur la base de ces deux nouvelles source de CMS.

S'il existe un certain nombre de similitudes entre la CMS classique et les nouvelles CMS-ARG-1 et CMS-ARG-3, nous avons noté des différences. Certaines lignées mainteneuses pour la CMS de type "petiolaris" sont apparues capables de restaurer jusqu'a un certain degré la fertilité de plantes produites sur la base de ces nouvelles CMS. Des études sont en cours pour clarifier ce fait.

UNA NUEVA FUENTE DE ANDROESTERILIDAD CITOPLASMICA EN GIRASOL DERIVADA DE *Helianthus argophyllus*

Michail CHRISTOV

La producción de girasol híbrido se hace en base a un solo citoplasma proveniente de *H.petiolaris*. Esta única fuente puede ser un riesgo potencial; por lo que la búsqueda de nuevos citoplasmas es siempre de grán interés.

En 1984 y 1987 dos nuevas fuentes de androesterilidad citoplásmica identificadas con CMS-ARG-1 y CMS-ARG-3 fueron producidas mediante la hibridación interespecífica entre *H.argophyllus* y girasol cultivado.

El citoplasma esterilizante es mantenido por una gran cantidad de variedades y líneas puras. Algunas líneas restauradoras de la fertilidad del citoplasma petiolaris tambien restauran estas nuevas fuentes de androesterilidad, por lo que estas pueden ser utilizadas directamente en la producción de híbridos. Sin embargo lineas mantenedoras del citoplasma petiolaris son restauradoras del citoplasma petiolaris son restauradoras de este nuevo citoplasma.

En la actualidad se siguen haciendo estudios sobre las similaridades de los dos citoplasmas.