

NEW SOURCES OF MALE STERILITY AND OPPORTUNITIES FOR THEIR UTILIZATION IN SUNFLOWER HYBRID BREEDING

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SUMMARY

Results are presented in this report indicating studies of new sources of CMS and MS produced at IWS "Dobroudja", General Toshevo. Sterile analogues are developed through multiple crossing on the base of CMS ARG-1, ARG-3, AN-67 and MS ARG-2 of some fertile lines and varieties known as maintainers for CMS F. It is found also that some R lines for CMS F completely restore the fertility of sterile forms produced on the base of new CMS sources ARG-1, ARG-3 and AN-67 and MS ARG-2. Hybrid varieties are developed on the base of the sterile analogues with CMS ARG-1, ARG-3, AN-67 and MS ARG-2 and R lines determined for these CMS. These varieties are similar in qualities with those obtained by the participation of CMS F. These results show that the new sources of CMS could have a practical utilization in sunflower production.

Key words: sunflower, male sterility sources, breeding.

INTRODUCTION

Several sources of cytoplasmic male sterility in sunflower have been produced through application of interspecific hybridization (Leclercq, 1969; Anashchenko, 1974; Whelan, 1980 and 1981; Heiser, 1982; Vranceanu et al., 1986; Serieys and Vincourt, 1987; Christov, 1990; and etc.), but still only that one produced on the base of *Helianthus petiolaris* (Leclercq, 1969) is used for hybrid seed production.

CMS from *H. petiolaris* is inherited only through the mother line, but it can be overcome by nuclear factors of some lines or varieties of sunflower which makes it usable in breeding. This is the main purpose in our activity on the study of the new sources of CMS produced at IWS "Dobroudja", General Toshevo.

Together with the solution of the problems for similarity and difference between the new CMS produced by us or with those developed by other authors, a work is in progress for creation of sterile analogues on the base of new CMS of some promising lines with ability for maintenance of CMS F and for determination or development of new restorers of fertility. Hybrids were made on this base to check the ability of the new systems CMS - Rf genes and to find opportunities for producing hybrid varieties by the use of new CMS.

MATERIAL AND METHODS

The following materials were used for the study:

1. MS sunflower forms on the base of cytoplasmic male sterility (CMS) F, produced by *H. petiolaris* CMS ARG-1 and CMS ARG-3, produced from *H. argophyllus*-

- R-006; CMS AN-67 obtained from *H. annuus*-E-067 and MS ARG-2 - from *H. argophyllus*-E-007.
2. Sunflower lines and varieties with normal cytoplasm, known as maintainers for CMS F.
 - a) Lines: 623, 1234, 1607, 3004, 4064, HA 89.
 - b) Varieties: Hemus, Peredovik, Peredovik-improved, Skorospelii, Stadion, Start, Trudovik, Vihren and VNIIMK 8931.
 3. Lines known as restorers of fertility for CMS F: R 47, R 147, R 3840, RHA 274, NS 26 R, NS 71-10 R.
 4. Hybrid varieties: Super Start, Dobritch.

The plants were grown under field conditions. The backcross method was used for development of sterile analogues. Each male sterile source was crossed with all lines and varieties with a normal cytoplasm included in the study. In finding that a certain line maintainer for CMS F is also a maintainer for some of the new CMS, the backcrossing with it continued for development of a sterile analogues.

If in crossing with a certain line there is a complete fertility restoring the testing is repeated and after confirmation of the restoring ability this line is determined as a R line for the respective CMS.

The detection of finished R lines for the new sources of CMS and MS, as well as a study of the similarity and divergence between CMS F, CMS ARG-1, CMS ARG-3, CMS AN-67 and MS ARG-2 was conducted by crossing male sterile forms of the CMS and MS indicated with all R lines included in the study.

The hybrids created by crossing sterile analogues on the base of the new CMS and MS with the R lines determined for these are investigated and compared with identical hybrids included in the cytoplasm - CMS F. Thirty plants each were studied in two replications.

RESULTS AND DISCUSSION

I. Cytoplasm with sterile effect.

1. Cytoplasm *Helianthus argophyllus*-E-006. Its sterilizing ability was found while conducting hybridization between it and sunflower (Christov, 1990). In two different generations, from two single crosses, two male sterile plants among the hybrid ones were produced. The sterility of the plants was preserved after their pollination by the variety Peredovik and the line 3004 - maintainers for CMS F. Two new sources of CMS were produced from these two male sterile plants, marked as ARG-1 and ARG-3.

2. Cytoplasm *H. argophyllus*-E-007. This cytoplasm is different from cytoplasm *H. argophyllus*-E-006 and cytoplasm *H. petiolaris*, from which the first CMS in sunflower (CMS F) was produced. This male sterility was obtained from the hybridization between *H. argophyllus*-E-007 and sunflower, which is still maintained only by line 3004-33. It is marked as MS ARG-2 (Christov, 1990 a). Sterile and fertile plants were produced in pollination with the other varieties and lines included in the study. The percentage of sterile and fertile plants varied in different crosses. These results give ground to suppose that MS ARG-2 does not represent a cytoplasmic type and that the character of sterilizing ability of this cytoplasm is rather different from those established till now. It

is typical for the sterile plants with MS ARG-2 that the size of the tubular florets is enlarged. These are two times longer and wider as compared with the florets of the maintaining line. In pollination by line R 147 the F₁ plants produced are fertile and with normal floret size.

3. Cytoplasm *H. annuus-E-067*. In the selection of inflorescences for conducting hybridization between *H. annuus-E-067* and lines and varieties of sunflower, two inflorescences were detected with florets excreting no pollen. After the castration of the remaining florets and pollination by pollen from line 1607 there were 52 F₁ plants of which 51 with male sterile inflorescences. Only male sterile plants were obtained in BC₂ and BC₃ after a separate backcrossing with several lines and varieties. Since the sterility is inherited through the mother line, it was marked as CMS AN-67 (Christov, 1990 a).

4. Cytoplasm *H. petiolaris*. The first source of CMS in sunflower was produced from *H. petiolaris* (Leclercq, 1969) and is labelled as CMS F (Serieys and Vincourt, 1987). It is used as a standard in the study for comparison and evaluation of the newly produced CMS and MS.

II. Development of sterile analogues.

Sterile analogues were developed according to Jones and Clarke /1943/. The transfer of the pollinators nuclear factors was made into the sterilizing cytoplasm by multiple crossing and selection. The genome translocation of the CMS source begins by a pollination of the sterile plant produced originally by a fertile analogue of some of the well-known sterile lines with CMS F or by a fertile line or a variety possessing certain genetic factors of the type Nr₁rf. The cultivars Peredovik, Hemus, Stadion, Start and VNIIMK 8931 are also very suitable for this purpose. If sterile plants are produced in the next generation after pollination of the sterile plant obtained initially by pollen from sunflower, part of these plants are pollinated by the same variety or line, while the rest by other lines or varieties of sunflower which are supposed to be maintainers and carriers of valuable economic qualities. The development of sterile analogues on the base of the new CMS and MS was initiated in this way. For the time being we can say that good phenotypic uniformity was obtained with the fertile analogues already in BC₅ - BC₆. The sterile analogues developed are with 100% male sterile plants and their quantitative indexes are almost equal to those of their fertile analogues or the sterile ones of the same fertile lines in the cytoplasm CMS F (Table 1).

Negative effects of the new cytoplasm were not observed. The plants produced on the base of CMS ARG-1, ARG-3 and AN-67 developed normally. A variation in the tubular florets shape and size of the plants from the sterile analogue 3004-33 is observed only in MS ARG-2. This, however, did not affect plant growth. The seed productivity of the sterile analogues on the base of the new CMS and MS is equal to that of the varieties and lines used as maintainers. This indicates that the female gametophyte performs normally and the seed formation is not prone to complications after pollination.

In the development of sterile analogues on the base of both the new CMS and MS and of CMS F it was found that the line 3840 was a maintainer of CMS ARG-3, but it restored the fertility of the sterile forms developed on the base of CMS F. In the present study it was labelled as a restorer (R) line.

Table 1. Characteristics of some lines and cultivars and their sterile analogues in cytoplasm F, ARG-1, ARG-3 and AN-67

Maintainer and MS analogue	Plant height cm	Head diameter cm	Seed formation %	1000 seed weight g	Oil in seed %
Peredovik	190	19.5	92.8	73.0	48.3
L 1607	100	16.5	82.1	60.2	42.5
L 3004	110	13.5	78.8	55.8	39.8
L HA 89	85	17.0	86.3	49.2	48.6
<i>CMS F</i>					
MS Peredovik	185	20.0	86.2	72.3	46.2
MS 1607	105	17.0	79.2	62.1	42.9
MS 3004	110	14.0	71.5	56.3	40.0
MS HA 89	85	17.0	80.2	48.4	48.0
<i>CMS ARG-1</i>					
MS Peredovik	205	21.0	86.0	71.7	45.4
MS 1607	110	16.5	89.0	60.0	43.5
MS 3004	115	14.0	73.4	55.3	40.1
<i>CMS ARG-3</i>					
MS Peredovik	195	18.5	90.1	73.8	45.2
MS 3004	120	14.5	75.3	56.0	39.1
MS HA 89	100	17.5	79.2	49.8	47.7
<i>CMS AN-67</i>					
MS Peredovik	190	21.0	89.8	71.6	48.3
MS 3004	110	13.5	70.9	54.4	39.6
MS 1607	105	17.0	90.6	62.2	43.0
MS HA 89	90	16.5	82.7	49.0	49.1

III. Restorers of fertility.

It was found from the crossing of male sterile forms developed on the base of CMS ARG-1, ARG-3 and AN-67 and MS ARG-2, with several Bulgarian and foreign R lines for CMS F, that the larger part of these R lines restore 100% the fertility of the sterile forms produced on the base of the new sources of CMS and MS ARG-2 (Table 2). This result shows that these R lines could be included directly in the heterotic breeding if the new CMS are used. Besides, the determination of the line 3840 as a restorer for CMS F and a maintainer for CMS ARG-3 indicates that there is a difference between these two sources of CMS. The use of this line in the study shows also that more lines both maintainers and such known as R lines for CMS F have to be included for a complete clarification of the similarities and differences between CMS F and the new CMS.

Table 2. Fertility restorers determined against CMS F as restorers for the cytoplasm ARG-1, ARG-3, AN-67 and MS ARG-2

Restorer	Degree of restoration %				
	for CMS F	for ARG-1	for ARG-3	for AN-67	for ARG-2
R 147	100	100	100	100	100
RHA 274	100	100	100	100	100
NS 26 R	100	100	100	100	100
R 3840	100	0-65	0	100	-

Single or many fertile plants were obtained from the crossing of male sterile forms possessing CMS ARG-1, ARG-3, AN-67, with maintainers for CMS F or with some sunflower varieties. Some of these plants were self-pollinated and at the same time male-sterile forms of these CMS were pollinated by pollen taken from the plants. The fertile plants were checked for their restoring ability. Thus, it was found that forms with Rf genes for the new CMS were produced from the cultivar Peredovik-improved, cv. Vihren and some other cultivars and lines. A work is in progress for producing R lines for the new CMS through self-pollination, selection and control of the restoring ability of the material.

IV. Development, study and evaluation of hybrid varieties through the use of CMS F, ARG-1, ARG-3, AN-67, MS ARG-2.

The check of the possibilities for development of hybrid varieties begins after three- or four-fold backcrossing of CMS forms with a certain line or variety.

In a complete fertility restoring of F₁ produced by crossing the developed sterile analogues with R lines, an activity starts for producing a larger quantity of hybrid seeds and a study of the hybrids developed. The hybrids obtained with the new CMS and MS were compared with hybrids with CMS F, developed under the same conditions by us, and the hybrids Super Start and Dobrich used as standards. The comparison and the evaluation of the hybrids were made on the most important biomorphological characters. The study shows that the hybrids obtained with different cms sources and one and the same female and male lines are similar in phenotype. Although most of the sterile analogues included in the hybridization are still in BC₅ - BC₆, these hybrids are equal or have slight differences in the expression of the most important quantitative characters (Table 3). The data obtained for the hybrid 1607 ms X R 147 developed on the base of CMS F, ARG-1, AN-67, are in support of this. The data are the same also for the other hybrids developed according to the identical patterns.

The results summarized in Table 3 show that hybrid varieties could be created on the base of the new CMS sources. These varieties have the same quality as the hybrids developed by the same parental lines on the base of CMS F. Therefore, these CMS sources can replace CMS F without serious effects on sunflower hybrid production.

Table 3. Characteristics of F₁ hybrids produced on the base of sterile cytoplasm F, ARG-1, ARG-3, AN-67 and MS ARG-2, tested in 1990

F ₁ hybrid	Plant height cm	Head diameter cm	Vegetation period days	1000 seed weight g	Oil in seed %	Seed yield kg/ha
F-cytoplasm						
1607 ms X R 147	170	19.0	120	57.6	48.4	2970
3004 ms X R 147	180	18.5	123	55.4	46.2	2650
3004 ms X RHA 274	170	17.5	121	53.4	43.0	2690
HA 89 ms X R 147	165	19.0	123	53.2	46.1	3065
ARG-1 - cytoplasm						
1607 ms X R 147	170	19.0	122	55.8	47.8	2905
HA 89 ms X R 147	165	19.5	126	61.0	47.6	2990
ARG-3 - cytoplasm						
623 ms X R 147	185	18.0	126	56.2	47.4	2900
3004 ms X R 147	180	19.0	123	67.8	44.4	2805
3004 ms X NS 71-10 R	180	18.0	123	55.4	43.2	2770
3004 ms X RHA 274	170	18.0	120	52.2	42.1	2750
HA 89 ms X R 147	170	20.0	125	54.0	46.5	2820
AN-67 - cytoplasm						
1607 ms X R 147	170	18.0	121	57.4	47.3	2860
4064 ms X NS 71-10 R	175	17.0	126	61.0	39.7	2280
HA 89 ms X R 147	175	18.0	125	54.4	44.8	2710
HA 89 ms X RHA 274	170	18.0	123	54.0	47.1	2240
ARG-2 - cytoplasm						
3004-33 ms X R 147	180	18.5	126	62.0	46.8	3670
3004-33 ms X RHA 274	175	18.0	121	52.6	41.9	2640
Standard hybrids						
Super Start	170	19.5	120	62.2	47.0	3040
Dobrich	170	19.0	119	61.0	47.5	2960

CONCLUSION

As a result of this study the conclusion is that sterile analogues can be developed by including lines known as maintainers for CMS F in sterile cytoplasm ARG-1, ARG-3 and AN-67. Besides, it is established that some R lines for CMS F restore completely the fertility of the sterile forms produced on the base of the new CMS sources ARG-1, ARG-3, AN-67 and MS ARG-2. Thus, on the base of the sterile analogues with CMS ARG-1, ARG-3, AN-67, and MS ARG-2 and R lines found for these CMS, hybrid varieties can be developed similar to those based on CMS F. Therefore, the new CMS sources can have a practical application for the sunflower hybrid seed production.

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NUEVAS FUENTES DE ANDROESTERILIDAD Y OPORTUNIDADES PARA SU UTILIZACION EN MEJORA PARA OBTENCION DE HIBRIDOS

RESUMEN

Se presentan resultados en este trabajo mostrando estudios de nuevas fuentes de CMS y MS producidas en IWS "Dobroudja", General Toshevo. Los isogénicos estériles son desarrollados a través de cruzamiento múltiples sobre la base de CMS, ARG-1, ARG-3, AN-67 y MS ARG-2 de algunas líneas y variedades fértiles conocidas como CMS F. Se ha encontrado también que algunas líneas R para CMS F restauran completamente la fertilidad de las formas estériles producidas sobre la base de nuevas fuentes CMS, ARG-1, ARG-3 y AN-67 y MS ARG-2. Las variedades híbridas son desarrolladas sobre la base de isogénicos estériles con CMS ARG-1, ARG-3, AN-67 y MS ARG-2 y líneas R determinadas para éstas CMS. Estas variedades son similares en calidad con las obtenidas por la participación de CMS F. Estos resultados muestran que las nuevas fuentes de CMS podrían tener una utilización práctica para la producción de girasol.

NOUVELLES SOURCES DE STÉRILITÉ MÂLE ET LA POSSIBILITÉ DE LEURS UTILISATIONS DANS LA SÉLECTION D'HYBRIDES DE TOURNESOL.

RÉSUMÉ:

Les résultats que nous présentons portent sur l'étude de nouvelles sources de CMS et de MS produites à l'Institut du Blé et du Tournesol "Dobroudja", Général Toshevo. Des lignées analogues stériles ont été développées grâce à de nombreux croisements fondés sur les CMS ARG-1, ARG-3, AN-67 et MS ARG-2 de certaines lignées fertiles et de variétés connues comme mainteneurs pour la CMS F. Il a été trouvé également que certaines lignées R pour la CMS F restaurent complètement la fertilité de formes stériles produites sur la base des nouvelles sources CMS ARG-1, ARG-3, AN-67 et MS ARG-2 et de lignées R déterminées pour ces CMS. Ces variétés ont des qualités similaires aux variétés obtenues à partir de la CMS F. Ces résultats montrent que ces nouvelles sources de CMS peuvent avoir une utilisation pratique pour la production de tournesol.