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NEW TYPE OF CYTOPLASMIC MALE STERILITY IN SUNFLOWER

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SUMMARY

An alteration in the cytoplasm reaction was provoked during the transfer of sunflower line M-C-5 into the cytoplasmic male sterility ARG-3. The new source of CMS was different from CMS ARG-3.

The differences were proved by investigating the fertility restoration in hybrid plants produced with the participation of line M-C-5A in CMS ARG-3 as well as the restorer lines for CMS PET-1 and CMS ARG-3 - lines 147R, 7015R, 7042R and 7045R. Combinations M-C-5A in CMS ARG-3 and lines 7015R and 7045R gave 100% sterile hybrid plants, while combinations M-C-5A in CMS ARG-3 and lines147R and 7042R gave 60.3% and 23.8% sterile plants, respectively. As a result of the combinations between other nine lines in CMS ARG-3 and these four R lines, only fertile hybrid plants were produced. The hybrid plants obtained from combinations between line M-C-5A in CMS DEB-1 and CMS ANN-16 with line 7015R were also fertile. These facts led us to conclude that the mutation has been provoked in the process of transfer of line M-C-5A into CMS ARG-3. Thus a new different CMS source has arisen out of an existing CMS source and it was named CMS ARG-3-M-1. The newly obtained CMS source seems to be a separate type. The way of origination of CMS ARG-3-M-1 was entirely different from the already existing methods for obtaining CMS in sunflower.

Key words: sunflower, cytoplasmic mutation, cytoplasmic male sterility (CMS), restorers of fertility

INTRODUCTION

Cytoplasmic male sterility was established to exist in many plant species but its application was limited to a small number of them. It was due to the level of scientific knowledge in the field of CMS and the available differences between the existent systems of CMS fertility restoration. The reasons for these differences and for the appearance of new CMS have not been clarified yet. To provide answers to these questions, suitable cultivated plants, in which many CMS sources had been identified, have to be investigated. For these cultivated plants it is necessary to find genes which will ensure complete restoration.

Several similar CMS fertility restoration systems have been obtained in the sunflower and they were found to share CMS sources such as CMS PET-1 (Leclerq, 1969), CMS BOL-1, EXI-1, PEF-1 (Serieys and Vincourt, 1987), CMS ARG-3 (Christov, 1990), etc. Their fertility restoration was 100% and it was controlled by a single gene. Some other types of CMS-fertility restoration systems have been identified possessing CMS sources in which the fertility restoration was controlled by more than one gene (Serieys, 1999).

The CMS sources in sunflower were obtained by hybridization - both intraspecific and interspecific (Leclerq, 1969; Whelan, 1980, 1981; Whelan and Dedio, 1980; Serieys and Vincourt, 1987; Christov, 1990, 1993, 1999, etc.), experimental mutagenesis (Christov, 1993, 1994, 1999) and spontaneous occurrence of sterile plants (Christov, 1993, 1999; Jan, 1997).

According to Christov (1999), CMS in sunflower was up to now obtained in three different ways but in all cases it was a result of cytoplasmic mutation. In all CMS sources the mutation was irreversible. The availability of the different types of CMS gave the opportunity to be accepted, that genes responsible for the normal pollen formation were several and influenced by one or in combination of two or more genes.

The aim of this paper was to present a new method for obtaining a new MS source and also partially clarify the system of CMS-fertility restoration.

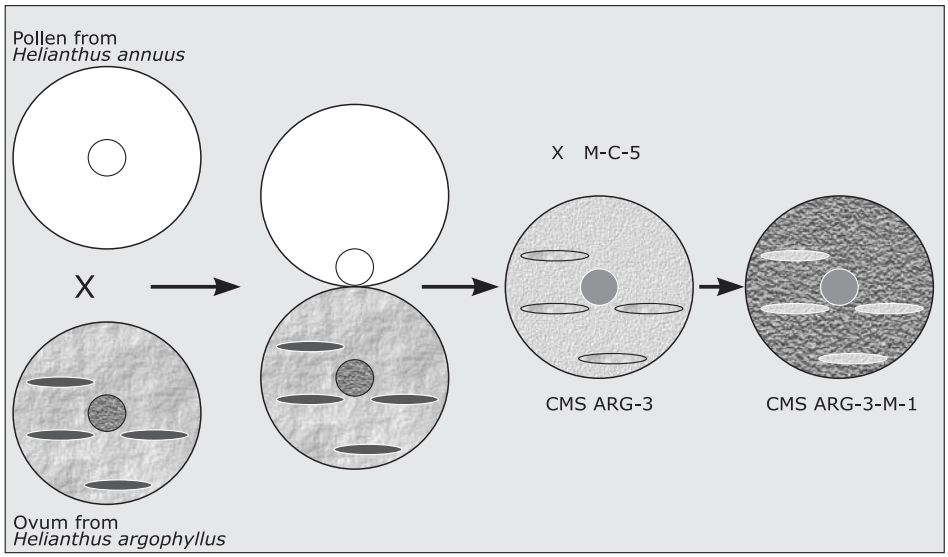

Figure 1: Shematic representation of the occurrence of CMS ARG-3 and CMS ARG-3-M-1
Note: *CMS ARG-3 was obtained in an F_1 plant from a cross Helianthus argophyllus × Helianthus annuus and CMS ARG-3-M-1 was obtained during the formation of the sterile analogue of line M-C-5 in CMS-ARG-3. The large circle and the small circle inside the large one together with the elongate figures shows the sexual plant cells with their nuclei and mitochondria. The color changes in the cells correspond to the changes of cytoplasm from one type to another.*

Table 1: List of hybrid combinations and results of the check for fertile and sterile plants

CMS source	Hybrid combination (A x R)	Fertile plants (%)	Sterile plants (%)
PET-1	2607A x 147R	100	0
ARG-3	2607A x 147R	100	0
PET-1	1607A x 147R	100	0
ARG-3	1607A x 147R	100	0
PET-1	3004A x 147R	100	0
ARG-3	3004A x 147R	100	0
PET-1	6046A x 147R	100	0
ARG-3	6046A x 147R	100	0
PET-1	62-P-4A x 147R	100	0
ARG-3	62-3-4A x 147R	100	0
PET-1	M-B-4A x 147R	100	0
ARG-3	M-B-4A x 147R	100	0
PET-1	M-C-5A x 147R	100	0
ARG-3	M-C-5A x 147R	39.7	60.3
DEB-1	M-C-5A x 147R	100	0
PET-1	2607A x 7015R	100	0
ARG-3	2607A x 7015R	100	0
PET-1	1607A x 7015R	100	0
ARG-3	1607A x 7015R	100	0
PET-1	3004A x 7015R	100	0
ARG-2	3004A x 7015R	100	0
ARG-3	3004A x 7015R	100	0
PET-1	6046A x 7015R	100	0
ARG-3	6046A x 7015R	100	0
PET-1	6054A x 7015R	100	0
ARG-3	6054A x 7015R	100	0
PET-1	HA 341A x 7015R	100	0
ARG-3	HA 341A x 7015R	100	0
PET-1	M-C-5A x 7015R	100	0
ARG-3	M-C-5A x 7015R	0	100
ANN-16	M-C-5A x 7015R	100	0
DEB-1	M-C-5A x 7015R	100	0
PET-1	M-C-5A x 7042R	100	0
ARG-3	M-C-5A x 7042R	76.2	23.8
PET-1	6046A x 7042R	100	0
ARG-3	6046A x 7042R	100	0
PET-1	2607A x 7045R	100	0
PET-1	1234A x 7045R	100	0
ARG-3	1234A x 7045R	100	0
PET-1	FS 924A x 7045R	100	0
PET-1	M-B-4A x 7045R	100	0
ARG-3	M-B-4A x 7045R	100	0
MUT-1	1607A x 7045R	100	0
MUT-2	2607A x 7045R	100	0
ARG-3	M-C-5A x 7045R	0	100
PRH-1	HA 821 x 7045R	100	0

MATERIALS AND METHODS

This investigation included 8 CMS sources: PET-1, AGR-2, ARG-3, DEB-1, PRH-1, MUT-1, MUT-2 and ANN-16; sterile analogues of 12 lines (B lines) of CMS PET-1 -1234, 1607, 2607, 3004, 6046, 6054, HA-341, HA-821, FS-924, 62-P-4, M-C-5, M-B-4; and 4 fertility restorers (R lines) for CMS PET-1 and CMS ARG-3-147R, 7015R, 7042R, 7045R (Table 1).

The sources of cytoplasmic male sterility were obtained in different ways: PET-1 (Leclercq, 1969), ARG-2, ARG-3, DEB-1, PRH-1 - from interspecific hybridization; MUT-1, MUT-2 – by experimental mutagenesis and ANN-16 from a spontaneous sterile plant (Christov, 1999).

The lines 1234, 1607, 2607, 3004, 6046, 6054, 62-P-4, M-C-5, M-B-4 were developed in DAI – General Toshevo; lines HA 341 and HA 821 were developed in USDA-ARS, Fargo, ND; line FS-924 was developed by Asgrow, France. The sterile analogues of lines M-C-5 and M-B-4 were sixth generation and the remaining 10 sterile analogues were obtained using more than 10 backcross progenies.

The Rf genes of lines 7015R, 7042R and 7045R were transferred into the cultivated sunflower from the wild species *Helianthus debilis* ssp. *debilis*, *H.rigidus* ssp. *rigidus* and *H.petiolaris* ssp. *petiolaris*, respectively. The origin of Rf genes in line 147 R was not established.

Forty-six combinations made were (Table 1). The seeds from all combinations together with the parental lines (A and R) were planted in the field. Phenological observations and measurements were made during the vegetation period. Ten plants from each combination or line were investigated. The numbers of fertile and sterile plants were counted during the stage of flowering.

RESULTS AND DISCUSSION

As a result of our previous investigations connected with CMS, we established that the new CMS in sunflower had been obtained through the application of interspecific hybridization, experimental mutagenesis and by spontaneous occurrence of sterile plants in cultivars and lines with normal cytoplasm (Christov, 1999). The number of the new CMS sources was twenty-four.

Now we are presenting a new CMS obtained in a way absolutely different from the ways mentioned in the previous paragraph. The appearance of the new CMS, which was named ARG-3-M-1, was observed in 1997.

It was observed that a part of the plants of the previously developed sterile analogue of line M-C-5 with ARG-3 cytoplasm underwent changes in leaf form and the color of tube florets.

The new CMS was observed for the second time in 1998 when new male sterile plants were obtained from the cross between line M-C-5A with ARG-3 cytoplasm and line 7015R. In this cross, line 7015 R could not demonstrate its restoration

ability although it was a successful restorer for several sterile sources, including CMS ARG-3.

In view of the above, we made the hybrid combinations listed in Table 1, expecting that a comparative investigation of the hybrid material should reveal the reason for the observed changes in the sterile plants in CMS ARG-3 and in the hybrid plants obtained from crosses with line 7015R.

The check of the obtained hybrid plants made during the period of flowering showed that in all of the crosses where line M-C-5 was included in cytoplasm AGR-3 only sterile plants were obtained. The crosses of line M-C-5A in CMS ARG-3 with R lines 7015R and 7045R produced 100% sterile plants. In the crosses with lines 147R and 7042R, the percentages of sterile plants were 60.3% and 23.8%, respectively (Table 1).

Crossing the sterile analogues of the other lines in CMS ARG-3 with the four R lines produced only fertile plants. Crossing the sterile analogues of line M-C-5 in CMS DEB-1 with line 7015 R also produced only fertile plants. Also, 100% fertile plants were obtained from the crosses between these four R lines with all other sterile analogues in the other CMS sources listed in Table 1.

The results of each hybrid combination were analyzed and compared. On this basis we arrived at the conclusion that during the transfer of line M-C-5 into CMS ARG-3 a change in cytoplasm ARG-3 occurred. This change may be interpreted as obtaining a new type of CMS which was distinguished from CMS ARG-3.

The new CMS source obtained in a new way enriches the knowledge of the origin of and differences between the CMS sources and casts light on the whole system of CMS fertility restoration.

The discovery of the new type of CMS was connected with another very important result. The different reactions of some R lines were expressed in accordance with the origin of Rf genes they possessed. It was an indication that there exist different Rf genes in the *Helianthus* genus and their different combinations could help the fertility restoration of some CMS types.

The facts mentioned above confirm the discovery of a new type of CMS which was designated as CMS ARG-3-M-1.

Our idea how CMS ARG-3 was formed and how it was transformed into CMS ARG-3-M-1 under the influence of line M-C-5 is shown figuratively in Scheme 1. The color changes down the diagram show that a change occurred in the normal cytoplasm of *Helianthus argophyllus* to become the sterile cytoplasm of CMS ARG-3 and then the sterile cytoplasm of CMS-ARG-3-M-1.

The fact that a new CMS was obtained from another CMS, different from the first one, was very interesting not only because of the transformation itself but because the transformation took place as a result of our interference. What was the reason and what is the explanation for the change in CMS? The reason for the occurrence of CMS ARG-3-M-1 was obviously line M-C-5. This line was obtained

from the Russian cultivar Start. Several seeds of this cultivar were irradiated with gamma rays at the dose of 70 Gy. Self-pollination of plants was made in the M-generations. Line M-C-5 was selected from several mutant forms and it was distinguished for interesting plant morphology and biochemical characters of seeds. The development of sterile analogues of the line with several CMS had already begun. The "incidental" transfer of this line into CMS ARG-3 gave an opportunity to obtain not only the new CMS but the new type of CMS too. The new CMS was obtained in a new way absolutely different from the ways already known for obtaining of CMS in sunflower.

The difference between the new CMS and the previously developed ones was that when including CMS ARG-3 in the line M-C-5 a mutation occurred during the transfer and this mutation was reflected in some cytoplasmic genes. The new mutation was different from the mutation which occurred during the development of CMS ARG-3.

Several hypotheses were made about the occurrence of mutation in CMS ARG-3. The first one was that the gene that mutated to produce CMS ARG-3 mutated again. In this case the later mutation was more complicated than the previous one. It affected a much larger number of mutation sites (units) of the gene. An entirely new "map" was obtained in intragenic recombination between mutated and non-mutated sites and thus a new heredity was obtained too. The second hypothesis was that another gene or several genes responsible for pollen reproduction underwent mutation. The investigation on CMS ARG-3-M-1 and other CMS sources is still in due course and new information in this field are expected.

CONCLUSION

A new type of CMS in sunflower was discovered while making a sterile analogue of line M-C in CMS-ARG-3.

This entirely new type of CMS was obtained using a novel procedure different from the already known procedures for obtaining CMS.

During the transfer of line M-C-5 in CMS ARG-3 an alteration was provoked in CMS ARG-3, which was reflected on some cytoplasmic genes responsible for pollen reproduction.

The new mutation was entirely different from the mutation that occurred during the discovery of CMS ARG-3. What is most interesting is the fact that one type of CMS was transformed into the other type of CMS. The new type of CMS was designated as CMS ARG-3-M-1.

The discovery of CMS ARG-3-M-1 was confirmed during the check of fertility restoration of the obtained hybrid plants. These plants were obtained from crosses between the sterile line M-C-5A with CMS PET-1, ARG-3, DEB-1 and ANN-16 on

one side and restorer lines for CMS PET-1 and ARG-3 - 147R, 7015R, 7042R and 7045R on the other.

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NUEVO TIPO DE ESTIRILIDAD CITOPLÁSMICA MASCULINA EN GIRASOL

RESUMEN

En ocasión de traslación de la línea de girasol M-C-5 en la forma estéril citoplásmica masculina (CMS) ARG-3, se ha provocado el cambio de reacción citoplásmica, con lo cual se ha creado una nueva fuente de CMS, diferente de la línea CMS ARG-3.

Dichas diferencias se confirmaron por la investigación de restauración de fertilidad de las plantas híbridas, engendradas con la participación de la línea M-C-5A en la línea CMS ARG-3 tanto como de las líneas 147R, 7015R, 7042R y 7045R, que son restauradores de las líneas CMS PET-1 y CMS ARG-3. Las combinaciones de la línea M-C-5A del CMS ARG-3 y de las líneas 7015R y 7045R, dieron las plantas híbridas 100% estériles, mientras que en la combinación de la línea M-C-5A del CMS ARG-3 y de las líneas 147R y 7042R los porcentajes eran 60.3% y 23.8% respectivamente. Las combinaciones entre las demás nueve líneas del CMS ARG-3 y de estas cuatro líneas R, han generado solamente las plantas fértiles híbridas. También eran fértiles las plantas híbridas obtenidas por medio de las combinaciones entre las líneas M-C-5A del CMS DEB-1 y CMS ANN-16, y la línea 7015R. Estos resultados han conducido hasta la conclusión de que se ha presentado la provocación de mutación en ocasión de traslación de la línea M-C-5A en CMS ARG-3. Con esto, de la fuente existente de CMS, se ha obtenido una fuente de CMS, nueva y diferente, llamada CMS ARG-3-M-1. La nueva fuente de CMS obtenida, pertenece al otro

tipo. El modo en que se ha obtenido el CMS ARG-3-M-1 se difiere completamente de los métodos existentes de obtención de CMS en girasol.

NOUVEAU TYPE DE STÉRILITÉ CYTOPLASMIQUE MÂLE DANS LE TOURNESOL

RÉSUMÉ

Au cours du passage de la ligne de tournesol M-C-5 à la forme stérile mâle cytoplasmique (CMS) ARG-3, un changement de réaction cytoplasmique a été provoqué et une nouvelle source de CMS différente de la ligne CMS ARG-3 a été créée.

Ces différences ont été confirmées par l'examen de la restauration de la fertilité des plantes hybrides apparues avec la participation de la ligne M-C-5A dans la ligne CMS ARG-3 ainsi que des lignes 147R, 7015R, 7042R et 7045R qui sont restaurateurs des lignes CMS PET-1 et CMS ARG-3. La combinaison de la ligne M-C-5A en CMS ARG-3 et des lignes 7015R et 7045R a donné des plantes hybrides stériles à cent pour cent alors que le pourcentage était de 60,3% et de 23,8% dans la combinaison des lignes M-C-5A de CMS ARG-3 et des lignes 147R et 7042R. Les combinaisons entre les neuf lignes de CMS ARG-3 restantes et ces quatre lignes R n'ont donné que des plantes hybrides fertiles. Les plantes hybrides issues des combinaisons entre les lignes M-C-5A en CMS DEB-1 et CMS ANN-16 et les lignes 7015R étaient elles aussi fertiles. Ces résultats indiquent qu'une mutation a été causée au cours du passage de la ligne M-C-5A en CMS ARG-3. Ainsi, une nouvelle source différente CMS, nommée CMS ARG-3-M-1 a-t-elle été obtenue de la source CMS existante. La nouvelle source CMS obtenue appartient à un autre type. La manière dont la CMS ARG-3-M-1 a été obtenue est tout à fait différente des méthodes d'obtention de CMS déjà existantes.