A CONTRIBUTION TO THE DIVERSIFICATION OF CYTOPLASMIC MALE STERILITY SOURCES IN SUNFLOWER

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INTRODUCTION

The first commercial sunflower hybrids were obtained on the basis of genetic male sterility with anthocyanin pigmented male fertile plants (Leclercq, 1966 ; Vrânceanu and Stoenescu, 1969 ; Vrânceanu 1970). Production of hybrid seed by this system has been however quite difficult, due to the necessity of removing the fertile plants from the female rows to obtain complete hybridization with the male parent. The discovery by Leclercq (1969) of the first cytoplasmic male sterility source, originated from a *H. petiolaris* Nutt. x cultivated *H. annuus* cross, and the subsequent identification of genes for pollen fertility restoration (Kinman, 1970 ; Enns *et al.*, 1970 ; Leclercq, 1971 ; Vrânceanu and Stoenescu, 1971, 1978; Fick *et al.*, 1974) resulted in a rapid wide spread production and cultivation of sunflower hybrids.

The use of only one *cms* source on a large scale may however lead to a genetic variability reduction of the breeding material, and thus to the increase of genetic similarity of hybrids, and therefore to their genetic vulnerability to diseases or other pests, a convincing example beeing the maize hybrids of Texas *cms* with susceptibility to southern corn leaf blight (Tatum, 1971).

Later on, new *cms* sources have been put in evidence, but they are still under studies and not in hybrid seed production because of either the difficulty of total sterile offspring maintenance or incomplete anther and pollen atrophy.

In comparison with the French or *petiolaris cms* most of the new sources have shown to be more or less influenced by different environmental conditions, especially when phenotypic manifestation of sterility is concerned.

This paper presents the genetic study of a new *cms* source discovered at Fundulea recently. Phenotypical and cytological expression of this male sterile form is described, as well as its response to crosses with different normal and restorer inbred lines.

MATERIALS AND METHODS

Beside the *petiolaris cms*, the following new sources have been studied at Fundulea :

— Kuban 1-70, and VIR-126 M, discovered by Anaschenko in 1974 (Mileyeva and Anaschenko, 1976 ; Anaschenko, 1977), and originated from a cross of *H. lenticularis* (wild subspecies of *H. annuus* L.) with the cultivated sunflower.

— CMG-1, CMG-2 and CMG-3, reported by Whelan (1980) and Whelan and Dedio (1980). They are open pollinated composites of partial interspecific substitution of the nucleus of cultivated sunflower into the cytoplasms of the annual species *H. petiolaris* Nutt., and the perennial species *H. giganteus* L. and *H. maximiliani* Schrad.

— Indiana-1, obtained by Heiser (1982) crossing a single male sterile plant in a population of wild sunflower *H. annuus* ssp. *lenticularis* Ckll. with the cultivated sunflower. The most important sterility maintainer has been the inbred RHA 265, which is known as a good pollen fertility restorer of the *petiolaris cms*.

The new Romanian *cms* source was identified in the nursery of sunflower intra and interspecific hybrids at Fundulea in 1983. It came from the open pollination of a *H. annuus* L. ssp. *texanus* accession. The respective hybrid male sterile plant, 2—2.5 m tall, with an intense anthocyanin pigmentation, had well developed branches with inflorescences on the whole stem. Some heads were crossed to 3 inbreds of the cultivated sunflower (Fig. 1). The F_1 offsprings consisted mostly of sterile plants. Some of them, as well as certain fertile plants were used in hybridological studies and as sources for obtaining male sterile or restorer inbreds.

Morphological and cytological studies concerning the anther development of the sterile heads were carried out.

Crosses for genetic studies were performed in the field in 1984. The F_1 generation was obtained in the greenhouse over the winter 1984—1985, and the F_2 generation resulted by

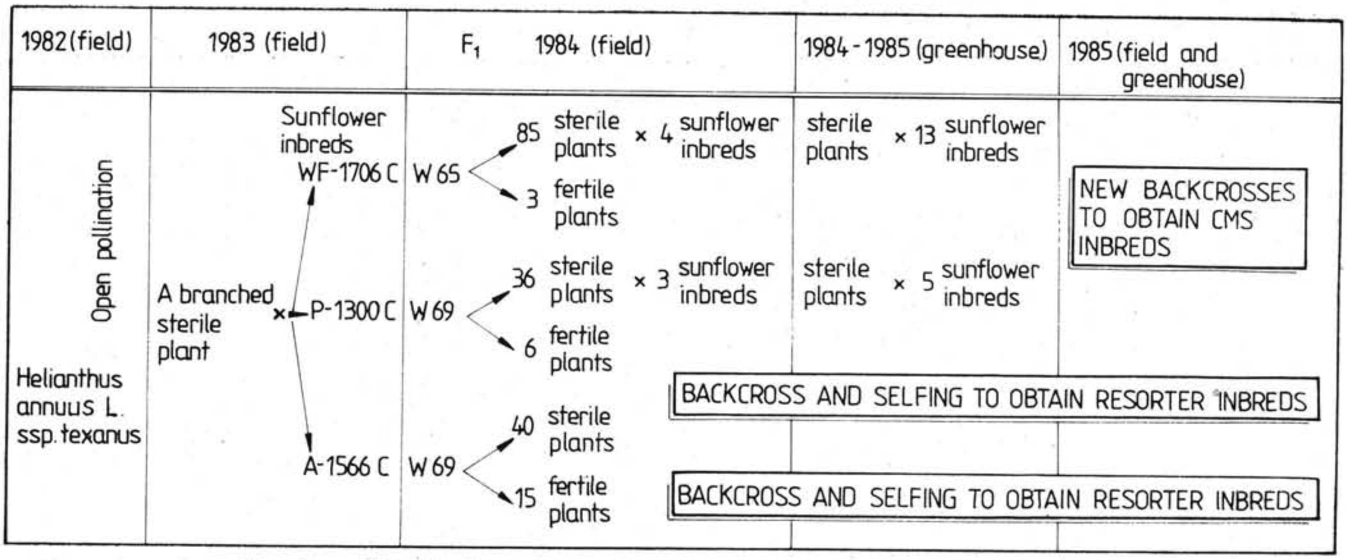

Fig. 1 — Identification and hybridological study of Fundulea-1 *cms* source

selfing F_1 fertile plants in the field, in 1985.

Selfings and crosses were performed under cotton and paper bags and emasculations by hand, with tweezers.

RESULTS AND DISCUSSION

The *cms* sources have been studied in the field as well as in the greenhouse. Morphological expression, microsporogenesis and behaviour of their hybrid offsprings have been evaluated.

The *cms* sources Kuban-1-70 and VIR-126 M provided complete sterile plants in different ratios, up to 60%, but no entire sterile progenies were obtained when crossed to a multitude of sunflower inbreds.

Complete sterile offsprings have been recorded in different backcross generations of the *cms* sources CMG-1, CMG-2 and CMG-3, with certain sunflower inbreds, but the male sterility expression varied greatly even inside of the same offspring. Most plants showed almost normal developed anthers with few aceto-carmin unstainable pollen grains, but quite frequent were also the plants with vestigial anthers, which contained little atrophied or no pollen.

All F_1 progenies of the *cms* Indiana-1 crossed to 37 different sunflower inbreds were entire sterile with drastic atrophied anthers. Later on, only the backcrosses of three inbreds were composed entirely by complete sterile plants. In other cases, different ratios of partial fertile plants were noted, especially in BC_1 and BC_2 generations. Similar remarks were noted by G e d g e (1985).

As concerns the recent Romanian *cms* source, sterile and fertile plants of the segregating offsprings W 65 and W 69 were crossed to two cultivated sunflower inbreds. The composition of F_1 and F_2 generations of the direct and reciprocal crosses (Table 1) has made evident the cytoplasmic inheritance. So, when the *cms* source was used as a female parent, sterile plants were noted in F_1 generation, but when the fertile plants of this source were used as male parents, all offsprings were entirely fertile.

Table 1

Composition of F_1 and F_2 generations resulted from direct and reciprocal crosses between segregating generations (FP and SP) * of the *cms* source Fundulea-1 and two normal male fertile sunflower inbreds

Segregation generations of Fundulea-1 source		Normal male fertile inbreds		Generations			
	Plant used in crosses		Plant used in crosses	F_1		F_2 (resulted from selfed F_1 plants)	
				FP	SP	FP	SP
W 65	SP/1 (♀)	A-1566	FP/1 ♀ ()	0	32	—	—
	FP/1 (♂)		FP/2 (♂) (emasculated)	26	0	62	0
W 69	SP/2 (♀)	O-7240	FP/1 (♀)	0	41	—	—
	FP/2 (♂)		FP/2 (♂)	25	0	68	0

* FP = fertile plants ; SP = sterile plants

Table 2

Offsprings * of the crosses between Fundulea-1 *cms* source and 5 sunflower inbreds carrying different genes for pollen fertility restoration of the *petiolaris cms*

Inbred (recurrent partner in the crosses with *cms* sources)	Rf genes for *petiolaris cms*		*Petiolaris cms*		Fundulea-1 *cms*					
	Symbol	Originated from **	F_1		F_1		BC_1		BC_2	
			FP	SP	FP	SP	FP	SP	FP	SP
T-66006-2-1	Rf_1	Complex composite	22	0	0	85	0	24	0	20
F-1020 C-1	Rf_1	RHA-274	20	0	4	26	0	22	0	20
S-1358 Rf-SL	Rf_2	*Slovenska siva*	18	0	0	32	0	64	0	22
P-1380 Rf Sint. 11	Rf-	*H. tuberosus* × cultivated sun.	21	0	6	36	6	40	0	94
V-1633 Rf Sint. 11	Rf-	*H. tuberosus* × cultivated sun.	20	0	6	55	0	74	0	22

* FP = fertile plants ; SP = sterile plants.
** Kinman, 1970 ; Vrânceanu and Stoenescu, 1978.

The observations and determinations performed in different years, both in the field and greenhouse, have proved that this new *cms* source which one could refer to as Fundulea-1, is very stable under various environmental conditions.

Disk flowers on the whole male sterile head have a normal development till their opening when stigmas appear without anthers being visible. The style grows and the stigma branches roll in the absence of pollen. The atrophied anthers could be observed in the longitudinal section of the disk flower as dry filaments.

Cytologic analyses of the pollen mother cells (PMC) carried out in the stage of bud formation revealed the complete absence of meiosis. So, the PMC degeneration took place in a premeiosis stage.

"Fundulea-1" *cms* plants were crossed to genotypes carrying homozygous genes for pollen fertility restoration of *petiolaris cms*. The results are presented in Table 2. In two cases (recurrent partner : T-66006-2-1 and S-1358-Rf-SL), completely sterile F_1, BC_1 and BC_2 generations were obtained. A reduced number of fertile plants in F_1 or in F_1 and BC_1 generations, but completely sterile BC_2 generations were recorded in the other three cases. The existence of fertile plants in F_1 and BC_1, could be explained by the action of two or more complementary Rf genes. The example presented in Fig. 2 shows the way of getting rid of the Rf alleles by crossing consecutively sterile plants from BC_1 and BC_2 to a single heterozygous recurrent male partner, obtaining thus completely sterile offsprings in the next generations.

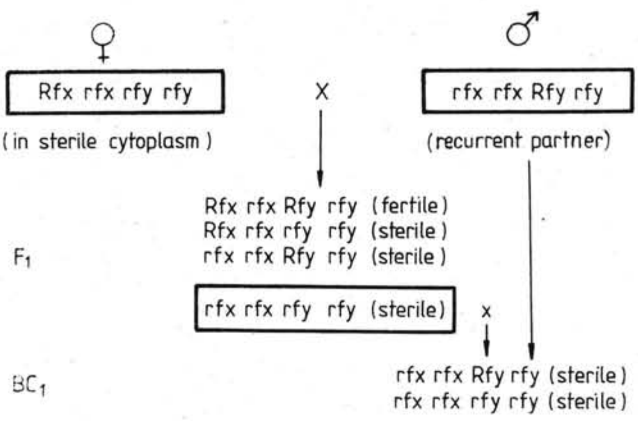

Fig. 2 — Breeding procedure for obtaining complete *cms* offsprings

All these results prove clearly that Fundulea-1 and *petiolaris* are two differently inherited *cms* sources.

When the normal B-lines which maintain the *petiolaris cms* were crossed to Fundulea-1 sterile plants (Table 3) most of the F_1 generations were completely sterile, suggesting thus that the frequency of the major genes controlling fertility restoration in Fundulea-1 *cms* is very low within the cultivated sunflower ge-

Table 3

Offsprings * of the crosses of Fundulea-1 sterile plants to different *petiolaris cms* maintainer inbreds

Number of inbreds	Origin	Total plants					
		F_1		BC_1		BC_2	
		FP	SP	FP	SP	FP	SP
7	VNIIMK 8931	1	65	0	64	0	22
5	Smena	0	22	0	44	0	44
6	Peredovik	7	79	2	20	0	22
5	Armavir 3497	0	22	0	65	0	7
5	Saliut	2	45	0	44	0	17
3	Record **	0	22	0	44		
11	Orizont **	2	175	0	39	0	18
2	Select **	0	20	0	22		
8	Single hybrids	3	108	2	37	0	22
4	Synthetic populations **	2	20	0	49		
1	Early synthetic population	0	22				
3	3 Gene-pool populations **	3	41	0	8		

* FP = fertile plants ; SP = sterile plants.
** Open pollinated varieties or synthetic populations bred at Fundulea.

notypes. As in the cases included in Table 2, the reduced number of fertile plants in F_1 and BC_1 are the result of the action of two or more complementary dominant alleles for fertility restoration. In the successive backcrosses the respective dominant alleles become insufficient for recovering fertility, thus resulting only entirely sterile offsprings.

CONCLUSIONS

The *cms* source Fundulea-1, identified as a male sterile hybrid plant resulted from the open pollination of the wild species *H. annuus* ssp. *texanus*, is characterized by a complete anther and pollen atrophy and has proved very stable under various environmental conditions.

The hybridological analyses have shown a different reaction of its crosses to the classical restorers commonly used in petiolaris *cms* system, suggesting thus the existence of a new type of cytoplasmic male sterility.

Major pollen fertility restorer genes have not yet been found in the cultivated sunflower genotypes but they are supposed to be identified in composite populations originating from crosses of the cultivated sunflower to wild annual sunflower species. A large testing programme has been organized in this respect and we are confident of being able to use the new *cms* source in hybrid sunflower breeding.

REFERENCES

Anaschenko A. V., 1977, *Some economically useful donors in the sunflower collection.* Trudy pe Prinkladnoi Botanike, Genetike i Selektsii, 59 (3), 130—136, Leningrad.

Enns H., Dorrell D. G., Hoes J. A. and Chubb W. O., 1970, *Sunflower research, a progress report.* Proc. 4th Sunfl. Conf., Memphis, Tenn. 162—167.

Fick G. N., Zimmer D. E., Dominguez Gimenez J. and Rehder D. A., 1974, *Fertility restoration and variability for plant and seed characteristics in wild sunflower.* Proc. 6th Intern. Sunfl. Conf., Bucharest, Romania, 333—338.

Gedge L. D., 1985, *Our experience with Heiser cytoplasm.* Proc. Sunfl. Research Workshop, Dec. 9, p. 12.

Heiser C. B., 1982, *Registration of Indiana-1* cms *sunflower germplasm.* Crop. Science 22, 1089.

Kinman M. L., 1970, *New development in the USDA and State Experiment Station sunflower breeding programs.* Proc. 4th Intern. Sunfl. Conf. Nemphis, Tenn., 181—183.

Leclercq P., 1966, *Une stérilité mâle utilisable pour la production d'hybrides simples de tournesol* Ann. Amél. Plantes, 16, 2, 135—144.

Leclercq P., 1969, *Une stérilité mâle cytoplasmique chez le tournesol.* Ann. Amél. Plantes, 19, 99—1969, 99—106.

Leclercq P., 1971, *La stérilité mâle cytoplasmique du tournesol. I. Premières études sur la restauration de la fertilité.* Ann. Amél. Plantes, 21, 45—54.

Mileyeva T. V., Anaschenko A. V., 1976, *Reliable* cms *and* Rf *source.* Proc. 7th Intern. Sunfl. Conf., Krasnodar, 358—360.

Tatum L. A., 1971, *The southern corn leaf blight epidemic.* Science, 171, 1113—1116.

Vrânceanu A. V., Stoenescu F. M., 1969, *Sterilitatea masculă la floarea-soarelui şi perspectiva utilizării ei în producerea seminţelor hibride.* Analele I.C.C.P.T. Fundulea, 35, Seria C, 560—571.

Vrânceanu A. V., 1970, *Obţinerea de hibrizi de floarea-soarelui pe bază de androsterilitate genică.* Analele I.C.C.U.T. Fundulea, 36, Seria C, 281—290.

Vrânceanu A. V., Stoenescu F. M., 1971, *Pollen fertility restorer gene from cultivated sunflower.* Euphytica 20, 536—541.

Vrânceanu A. V., Stoenescu F. M., 1978, *Genes for pollen fertility restoration in sunflower.* Euphytica 27, 617—627.

Whelan E. D. P., 1980, *A new source of cytoplasmic male sterility in sunflower.* Euphytica 29, 33—46.

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

UNE CONTRIBUTION À LA DIVERSIFICATION DES SOURCES DE STÉRILITÉ-MÂLE CYTOPLASMIQUE CHEZ LE TOURNESOL

Résumé

Les sources de stérilité-mâle cytoplasmique découvertes jusqu'à présent chez le tournesol sont présentées. Parmi celles-ci, seulement la source du type *„petiolaris"* est utilisée avec succès dans la production des semences hybrides de tournesol. On apprécie que l'emploi d'une source cytoplasmique stérile unique, à une èchelle si large pourra entraîner une réduction de la variabilité génétique des hybrides de tournesol, celle-ci provoquant la diminution de la stabilité de la récolte dans certaines zones et l'augmentation de la vulnérabilité génétique potentielle.

En 1983, une plante mâle-sterile a été identifiée à Fundulea, dans une population résultée par la pollinisation libre de l'espèce sauvage *H. annuus* ssp. *texanus*. Celle-ci a été utilisée dans une série d'hybridations aux formes de tournesol cultivées et soumise aux études génétiques et cytologiques. La stérilité-mâle a été caractérisée par une atrophie totale des anthères et du pollen et s'est avérée très stable en conditions différentes de milieu. Les analyses hybridologiques ont mis en évidence une hérédité de nature cytoplasmique. La nouvelle source d'androstérilité a été dénommée „Fundulea-1" et s'est montrée différente de la source du type *„petiolaris"*. Des nombreux croisements avec des lignées autofécondées de tournesol ont démontré que les gènes majeurs pour la restauration de la fertilité du pollen chez la nouvelle source *cms* ont une fréquence faible dans le cadre de ces génotypes.

UNA CONTRIBUCION A LA DIVERSIFICACION DE LAS FUENTES DE ANDROESTERILIDAD CITOPLASMATICA DEL GIRASOL

Resúmen

Están presentadas las fuentes de androesterilidad citoplasmática descubiertas en el girasol hasta el presente. De éstas, solamente la fuente tipo *petio-*

laris llamada clasica, se emplea con éxito en la producción de semillas híbridas de girasol. Se estima que el empleo de un solo citoplasma estéril en una escala tan grande determina una reducción de la variabilidad genética de los híbridos de girasol, lo que causa la reducción de la estabilidad de la cosecha en algunas zonas y el aumento de la vulnerabilidad genética potencial.

En el año 1983, en Fundulea, se identificó una planta androestéril dentro de una población resultada de la polinización libre de una especie salvaje *H. annuus* ssp. *texanus*.

Ésta fue empleada en una serie de hibridaciones con formas de girasol cultivado y sometida a estudios genéticos y citológicos. La androesterilidad se caracterizó por una atrofia total de las anteres y del polén y se mostró muy estable en diferentes condiciones de medio. Los análisis hibridológicos evidenciaron una heredidad de natura citoplasmática. La nueva fuente de androesterilidad fue llamada Fundulea-1 y se mostró distinta de la fuente de tipo „*petiolaris*" (clasica).

Numerosos cruces con líneas consanquíneas de girasol demonstraron que los genes mayores para la restauración de la fertilidad del polen en la nueva fuente citoplasmática tienen una frecuencia reducida dentro de estos genotipos.