

CHARACTERIZATION OF SOME NEW CYTOPLASMIC MALE STERILITY SOURCES FROM *HELIANTHUS* GENUS

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INTRODUCTION

The French cytoplasmic male sterility source (CMS F) discovered by Leclercq (1969) in *H. petiolaris* Nutt. is now largely used in commercial sunflower hybrid production without any problem. However, the use of a single cytoplasm may limit the genetic basis of the parental lines and represents a potential risk if it will become susceptible to a new strain of a disease. This is the reason why breeders are looking for new CMS sources. Up to date, 5 new stable male sterilities have been reported in the following material :

— CMS Kuban (CMS K) originated from *H. lenticularis* (Anaschenko, 1974 ; Leclercq, 1980).

— CMS Indiana 1 (CMS I) discovered in *H. annuus* ssp. *lenticularis* (Heiser, 1982).

— CMS CMG₁ derived from an interspecific cross between *H. petiolaris* Nutt. and the sunflower *H. annuus* (Whelan, 1980).

— CMS CMG₂ discovered in the interspecific cross between *H. giganteus* and *H. annuus* Whelan, 1981).

— CMS CMG₃ found in the cross between *H. maximiliani* and *H. annuus* (Whelan, 1980).

Seven additional male-sterility sources were detected in our laboratory from interspecific crosses between some annual wild *Helianthus* species and the sunflower. The purpose of this study was to characterize the nature of these male sterilities, to compare them with the French CMS by crossing with a sample of B or R lines (B, R related to French CMS) and to propose a model for explanation of genetic interactions in restoration processes of these CMS sources.

MATERIALS AND METHODS

In order to screen geno-cytoplasmic male sterilities, interspecific crosses were realized using the wild parent as female and the sunflower (*H. annuus* L.) as pollen source. So,

male sterile plants were found either in interspecific progenies or directly in the wild species as indicated in Table 1.

Table 1

**Origin of the new male sterilities observed
in wild annual sunflower**

Material	Male sterility first observation	Sele- ction level
1. WILD <i>H. ANNUUS</i> :		
397 <i>H. annuus</i> (PI 413057)	1981 Original pop.	BC ₃
517 <i>H. annuus</i> (PI 413178)	1981 Original pop.	BC ₃
519 <i>H. annuus</i> (PI 413180)	1981 Original pop.	BC ₃
521 <i>H. annuus</i> (PI 406647)	1981 Original pop.	BC ₃
2. INTERSPECIFIC CROSSES :		
<i>H. petiol. fal.</i> (200) x <i>H. annuus</i> L.	1980 F ₁	BC ₅
<i>H. bolanderi</i> (255) x <i>H. annuus</i> L.	1980 BC ₁	BC ₄
<i>H. exilis</i> (130) x <i>H. annuus</i> L.	1982 BC ₁ + S ⁰	BC ₃

(e) Self pollination

The wild species giving male sterility were the following :

— *H. petiolaris* ssp. *fallax*, INRA code : No 200 (USDA code : PET 795) (= CMS PF).

— *H. bolanderi*, INRA code : No 255 (= CMS BOL).

— *H. exilis*, INRA code : No 130 (= CMS EXI).

— *H. annuus*, INRA code : No 397 (= CMS 397). PI = 413057 from California.

— *H. annuus*, INRA code : No 517 (= CMS 517). PI = 413178 from Texas.

— *H. annuus*, INRA code : No 519 (= CMS 519). PI = 413180 from Arizona.

— *H. annuus*, INRA code : No 521 (= CMS 521). PI = 406647 from Australia.

Behaviour of some sunflower lines on different cytoplasm

Cytoplasmic origin	Maintainer genotypes	Restorer lines
CMS F	HA 89, WG, OD 66, LA, CP 3.1, CP 3.2 CM, CX, CY	PAH2, RHA 274 RHA 278, RHA 297, RHA 298, RHA 299, RHA 801
CMS PF	HA 89, WG, OD 66, RHA 274, RHA 297, RHA 801	LA, PAH2, RHA 298, CP 3.1, CP 3.2
CMS BOL	WG, CX, PAH2	HA 89, RHA 274, RHA 297, RHA 298, RHA 801
CMS EXI	CM, CY, RHA 278	HA 89, RHA 274, RHA 297, RHA 298,
CMS 517	All tested lines (38)	None
CMS 519	All tested lines (27)	None
CMS 521	All tested lines (50)	None
CMS 397	All tested lines (37)	None

We consider as occurrence of a stable cytoplasmic male sterility when at least two successive backcross-progenies are fully male sterile in the same maternal filiation. On the other hand, it is possible to differentiate two cytoplasmic male sterility sources by the responses of different restorer and maintainer lines on that cytoplasm. If restorer genes of a first cytoplasm cannot restore a second cytoplasm, this suggests that they could be different. Nevertheless, this method is usable only if nuclear genic structure for restoration of the females is well known or identical (alloplasmic lines). Indeed, in the case of a determinism with two or more complementary restoration genes, fertility responses may differ according to the genetic configuration of the female parent when the pollinator is used. To this purpose, the different cytoplasmic male sterile sources were crossed by a large sample of B or R lines for the French source (between 30 and 50 lines were used according to the source). The restoration response of the lines was recorded and then used to compare the new cms to the French source.

Moreover, the restoration response of sunflower populations through F_1 hybrids on CMS F, CMS HPF, CMS K may give information both on similarities between the studied cytoplasm and on "genetic distance" between these populations. An appropriate analysis was used to this purpose.

RESULTS

1. Characterization of the nucleic-cytoplasmic nature of the new male sterilities (Table 2)

— CMS *petiolaris fallax* (CMS PF).

Most of sunflower lines tested (87%) behaved on that cytoplasm as maintainers as for instance HA 89, RHA 278, RHA 297, RHA 274. Conversion of these lines on CMS HPF cytoplasm is in progress (BC_6 for RHA 274). Up to date, this sterility has proved to be stable in all combinations examined. The results observed show clearly that this source is of genocyttoplasmic nature. Among sunflower lines, restorer lines for CMS PF are found at lower frequency (13%), for instance: LA (from VNIIMK 8883), PAH 2, RHA 298. Additional restorer genotypes must be isolated in cultivated sunflower populations (Table 3) or searched in the male progenies of the initial crosses. Examination of segregating progenies reveals that restoration of male fertility is governed by two to three independent complementary dominant genes designated as: *Rpf*₁, *Rpf*₂, *Rpf*₃.

— CMS *bolanderi* (CMS BOL)

Male sterile plants from *H. bolanderi* source crossed by a sample of 48 different sunflower lines revealed that 12% of the tested lines maintain the sterility of the female plants, indicating that this sterility is of a cytoplasmic type. For instance, the maintainer lines WG, CX or the restorer line PAH 2 are both maintainers for the CMS BOL cytoplasm. During three successive backcrosses on that cytoplasm with the latter lines, the progenies were completely sterile, indicating the stabilization of the cytoplasm. Nevertheless, several families seem difficult to stabilize for male sterility, even after 4 or 5 backcrosses. The inheritance of this restoration fertility is not clear.

— CMS *exilis* (CMS EXI).

Female plants from *H. exilis* source have been crossed by 34 different sunflower lines (18 B + 16 R). Thirteen of the tested lines behaved as maintainers on this source and revealed the cytoplasmic nature of this sterility. For instance, the maintainer line CM and the restorer line RHA 278 are maintainers for the CMS *exilis*. Up to date, three successive backcrosses with the later sunflower lines have given fully male sterile progenies, underlining the stability of this source.

Table 3

The genetics of the restoration is governed in 90% combinations by one dominant gene and in the others by two dominant complementary genes.

— CMS from wild *H. annuus* (CMS 517, 519, 521, 397)

In these male sterility sources, the male sterile plants were found directly in the original ecotypes. After one or two backcrosses by different B or R lines, fully male sterile progenies were got. Up to date, these lines tested on the 4 sources are unable to restore male fertility. Female fertility appears quite normal in all combinations. CMS 517, 519, 521, 397 may be considered as cytoplasmic and stable (BC₃ to BC₄ level). Since all tested lines react as maintainers, restoration factors should be searched in the original male fertile ecotypes.

2. Comparison of the new cytoplasmic with the CMS French cytoplasm

Using a set of restorers from the French cytoplasm, we observed that for each studied source some of these restorers behaved as maintainers. Otherwise, restorer genes efficient on CMS F become ineffective on other cytoplasmic backgrounds. So, for all new observed sources, we may conclude that they are different from the classical French CMS.

— CMS PF is maintained by the CMS F restorers RHA 274, RHA 278, RHA 298.

— CMS BOL is maintained by the CMS F restorer PAH 2.

— CMS EXI is maintained by the CMS F restorer RHA 278.

— CMS 517, 519, 521, 397 are maintained by all the B or R lines tested: RHA 274, RHA 278, RHA 298, RHA 299 etc.

Comparative responses of B and R lines on different cytoplasmic backgrounds is instructive (Fig. 1). Similar ratios of CMS PF restorer factors are found both in the B and R lines (respectively 15 and 11% of the lines). The same observation can be made for CMS BOL and CMS EXI where the restorer line percent is clearly higher: (71 to 85%). For all these three sources, restorer lines are equally distributed between B and R lines suggesting no close relation with CMS F restorer genes.

To the CMS K, the restorer factors are much more frequent in the CMS F restorer lines (70%) than in the maintainer lines (55%).

3. Comparative responses of some sunflower populations on three CMS F, PF, K cytoplasmic (Table 3)

Restoration ability, in terms of % fertile plants, for the three cytoplasmic differs according to the considered populations. Most populations appear maintainers of the CMS F or the CMS K and partly restore the CMS PF cytoplasm. Since we know that the female line

Fertility restoration :
Comparative responses of some populations
on 3 cytoplasmic CMS F, CMS PF, CMS K *

Population	CMS F (125)	CMS PF (RHA 274)	CMS K (WG)
V.B.F.G.	0	42	—
Pop. s. Canada	0	71	0
Sunrise	0	94	—
Record	0	54	—
Zaria	0	70	0
Nain Rouge	21	45	19
Stepniak	—	26	0
Argentine 35	—	71	0
Olea	1	60	6
Saratov	0	63	0
Mennonite	0	70	0
H. Hopi	51	1	49
R. Scott	88	7	89
B. Scott	0	0	0

* Male plant percent

RHA 274 used in the crosses carries one Rpf restoration gene, the observed data indicate that the second Rpf gene is largely distributed in the sunflower populations examined. Note that the B. SCOTT population behaves as sterility maintainer for the three CMS F, PF and K sources.

Corresponding analysis may be used to estimate a genetic distance between the sunflower groups or populations (Fig. 2) by measurement of their restoration ability on different cytoplasmic backgrounds. Obviously, accuracy of this method will be improved by the number of different cytoplasmic sources involved.

4. Explanatory attempt of the fertility restoration in five cytoplasmic sources (Table 4)

Table 4

Expression of male fertility on some lines explained by complementation between cytoplasmic and nuclear status (Complementation model)

Nuclear fact. → cyto. fact. ↓	+++ HA 89	+++ RHA 274	+++ WG	+++ RHA 266	+++ CM	+++ RHA 298	+++ D 34
+++ CMS F	—	+	—	+	—	+	—
+++ CMS PF	—	—	—	—	—	+	—
+++ CMS IND	+	—	(—)	—	(—)	(—)	(—)
+++ CMS K	+	+	—	—	(—)	(+)	(+)
+++ CMS 397	—	—	—	—	—	—	—

o Similar response for CMS 517, 519, 521.

+ Male phenotype

— Female phenotype

* If female is of HA 89 or D 34 type.

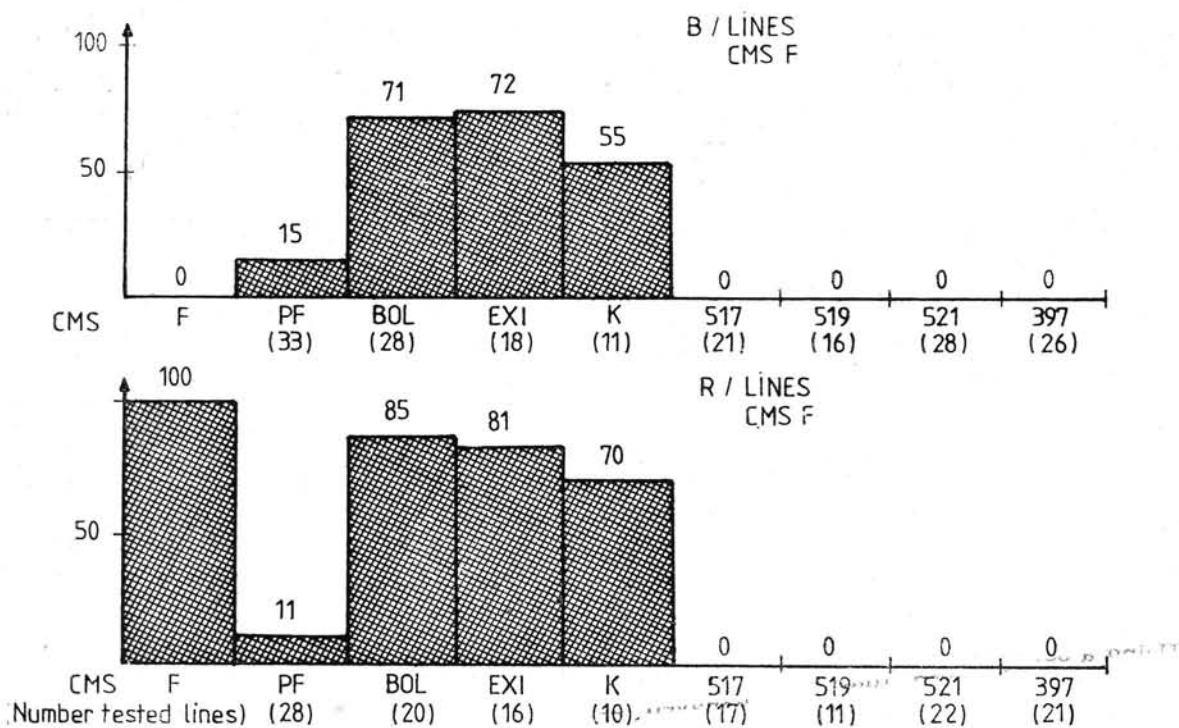


Fig. 1 — Distribution of restorer factors for different CMS sources in B or R sunflower lines

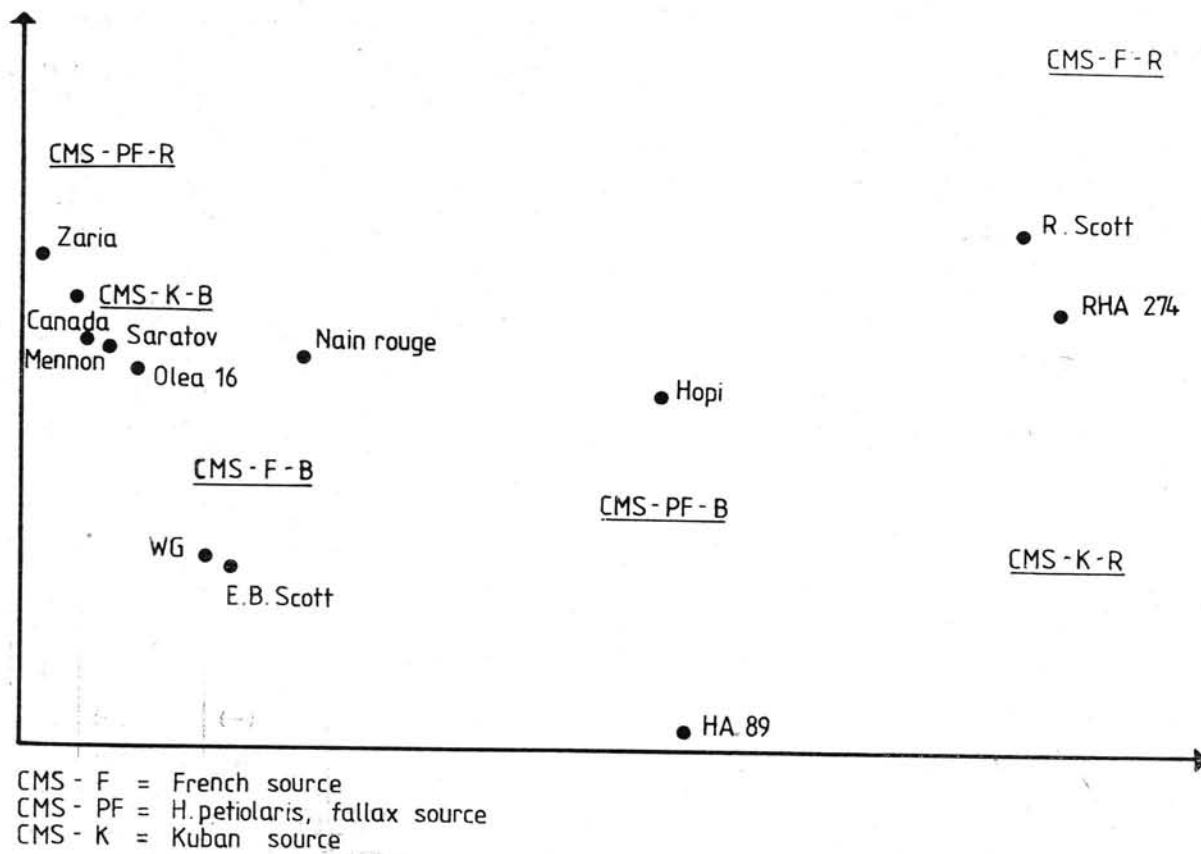


Fig. 2 — Association of genetic background and fertility restoration status on three cytoplasmic male sterility sources

In this model we suppose that both nuclear and cytoplasmic dominant factors contribute to the fertility status of the plant. To each nuclear factor (Rf gene) corresponds a specific cytoplasmic factor. This hypothesis could be supported by the sequence homologies found between nuclear DNA and organelles DNA (Timmis and Scott, 1984). So, plants exhibit male fertility when complementation occurs between different factors (nuclear and/or cytoplasmic). In Table 4, nuclear and cytoplasmic structures for male fertility are proposed and from a four factor model, the deducted responses are confirmed by the experimental data.

If we consider the different cytoplasmic sources, it is possible to range them from easy to hard "restorable".

— The CMS K with three restorer cytoplasmic components appears easier to restore than others (55 to 70% restorer lines).

— The CMS F, CMS I, CMS PF group carrying two dominant cytoplasmic "restorer factors" results in intermediate restoration ability.

— The CMS 397, 517, 519, 521, with only one cytoplasmic restorer factor are difficult to restore (no restorer genotypes in the observed lines).

CONCLUSIONS

Up to date, several cytoplasmic male sterilities have been found in the world. So, about 12 sources have been quoted. In our laboratory we have discovered 7 characterized CMS sources. All these sources appear different from the French CMS, but we have not definitive responses about their differences. In this way, one of our projects is to create alloplasmic lines for comparing the present cytoplasmic sources between them and to evaluate the agronomic value of the different cytoplasmic backgrounds with the same hybrid nucleus. So, seed yield, oil content and disease resistance will be measured in connection with the different sources.

In summary, the diversification of the CMS sources in sunflower breeding will contribute to the following topics :

1. Avoid the risks linked with the use of a single cytoplasm, for instance new strains of disease occurrence.

2. Better utilization of the genetic variability in breeding programmes, by the development of recurrent reciprocal schemes using two different CMS sources.

3. Estimation of the genetic "distance" between combining groups : populations, gene-pools, lines so as to develop better combining ability.

4. Improvement of the agronomical value of the varieties (like seed yield, oil content, adaptation to physical or biological environment) in connection with their cytoplasmic components.

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CARACTÉRISATION DE QUELQUES SOURCES NOUVELLES DE STÉRILITÉ MÂLE CYTOPLASMIQUE DU GENRE HELIANTHUS

Résumé

Sept nouvelles sources de stérilité mâle cytoplasmique ont été découvertes dans les descendance des croisements intra ou interspécifiques entre espèces sauvages annuelles (*H. petiolaris fallax*, *H. bolanderi*, *H. exilis* et *H. annuus* sauvage) et le tournesol cultivé *H. annuus* L. Le croisement de ces différentes sources d'androsterilité avec un échantillonnage de lignées mainteneuses ou restauratrices vis à vis du cytoplasme Français, a permis de différencier les nouvelles sources de cette dernière stérilité.

Pour les stérilités issues d'*H. petiolaris fallax*, *H. bolanderi*, *H. exilis* de nombreuses lignées restauratrices existent ; par contre, la fréquence des génotypes restaurateurs de fertilité est très faible pour les 4 stérilités originaires d'*H. annuus* sauvages.

Un modèle basé sur la complémentation de facteurs nucléaires et cytoplasmiques permettant l'explication de la restauration de fertilité dans les différents contextes cytoplasmiques est proposé.

CARACTERIZACIÓN DE UNAS FUENTES NUEVAS DE ANDROESTERILIDAD CITOPLASMÁTICA DEL GENE HELIANTHUS

Resúmen

Siete fuentes nuevas de androesterilidad citoplasmática se han descubierto en las descendencias de los cruces intra o interespecíficos entre especies salvajes anuales (*H. petiolaris fallax*, *H. bolanderi*, *H. exilis*, y *H. annuus* salvaje). El cruce de estas fuentes diferentes de androesterilidad con un grupo de líneas mantenedoras o restauradoras frente al citoplasma francés, permitió la diferenciación de las fuentes nuevas de este última esterilidad.

Hay numerosas líneas restauradoras para las androesterilidades procedentes de *H. petiolaris fallax*, *H. bolanderi* y *H. exilis*, al contrario, la frecuencia de los genotipos restauradores de fertilidad es muy escasa para los cuatro androesterilidades originarias de *H. annuus* salvaje.

Se ha propuesto un modelo basado en la complementación de los factores nucleares y citoplasmáticos que permite explicar la restauración de la fertilidad en contextos citoplasmáticos diferentes.