WORKING GROUP

IDENTIFICATION, STUDY AND UTILISATION IN BREEDING PROGRAMS OF NEW CMS SOURCES

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Summary of the past 1991-1994 activities and proposals for the next period.

A more complete synthesis was previously reported at the technical FAO meeting held in Montpellier, on 20-23 June 1994 and published in the Helia Journal (Serieys, 1994).

During the 1991-1994 period, nine countries participated in the Working Group (Bulgaria, France, Germany, Hungary, Italy, Romania, Spain, USA and Yugoslavia). The main achievements are related to:

1 - The identification of 44 CMS sources in the *Helianthus* genus. Most of the CMS originated from wild annual section. The most contributing species to the CMS sources were *H. annuus* (19 sources), *H. petiolaris* (6), *H. argophyllus* (3) and *H. praecox* (3). The data show the existence of considerable inter- and intraspecific cytoplasmic diversity in *Helianthus* genus. A standard code has been attributed to each CMS source according to the FAO codification.

2 - The search for new restorer genes. The results of the working group indicate that restorer genes for CMS-PET1 are frequent in the wild germplasm. One of the main significant achievement was discovery of Rf genes for 39 out of 44 CMS sunflower sources. Particularly important results are reported for the CMS sources lacking Rf genes (ANN1, ANN2, ANN3, ANN4, RIGx), where efficient restorer factors have been isolated. The finding of Rf genes was specially interesting for CMS-RIGx. We have to evaluate their efficiency on the CMS-RIG1 cytoplasm discovered by Vulpe (where no Rf gene was previously registered).

3 - The genetic control of the restoration of the male fertility. The genetic determinism of the male fertility restoration appeared generally controlled by single gene or two independent complementary genes. Therefore, the restoration of some CMS sources remained difficult to explain by a simple hypothesis (case of CMS-BOL1).

4 - The comparison of the CMS sources, using genetic approaches or molecular markers. It was shown that restoration patterns allow discrimination of

most CMS sources. Beside, male sterile lines from CMS-PET1 differed from male fertile analogues by a mitochondrial sequence (OrfH522) close to the Atpa gene. A 16 KDa protein is only expressed in lines carrying the PET1 cytoplasm, this polypeptide may play a role in the CMS expression. Moreover, comparison of 16 cytotypes using the genetic and molecular approaches shows that most of the CMS groups defined according to restoration patterns agree closely with restriction fragments of the mt DNA.

5 - The comparison of the CMS sources using agronomic approaches. The experimentation with alloplasmic hybrids has shown significant positive or negative cytoplasmic effects for most of the agronomic traits studied. The seed yield was increased in BOL1, ANN1 and ANL1 cytoplasmes while oil content was found higher only in the CMS-BOL1. Furthermore, important nucleo-cytoplasmic interacion effects were detected, for all studied traits. More extensive trials are necessary to generalize these results.

The proposals for the next period will be a continuation of the work already undertaken in the WG and will be related to the following areas:

1 - Identification of new CMS in *Helianthus* germplasm. Update of the new CMS sources. To make the CMS sources available in the Network, it is suggested that the coordination center receive the CMS sources (A and B forms), multiply and distribute them to the WG participants.

2 - Comparison of the CMS sources. The utilization of genetic approaches and molecular markers to differentiate the different CMS found in the sunflower germplasm should be continued.

3 - Search for Rf genes. This point should be particulary active for the "hard to restore" CMS. It is also suggested that the original Rf genes sources be multiplied and distributed in the Network.

4 - Studies of the genetic determinism of the restoration and the identification of the Rf genes specific (or common) to the different CMS sources should also be an important research aspect of the further work in the Working Group.

5 - Evaluation of the cytoplasmic effects on main agronomic traits (seed yield, oil content and quality, disease resistance...) should be implemented in the FAO Network. In this aim, it will be necessary to define, first, the existing isogenic-alloplasmic lines available to produce the alloplasmic hybrids and second to evaluate these hybrids in multilocation trials, within the Network.

REPORT ON 1991-1994 ACTIVITIES

The report is an update of the synthesis previously reported at the technical FAO meeting held in Montpellier, on 20-23 June 1994 and published in the Helia bulletin (Serieys, 1994).

Cytoplasmic male sterility (CMS) and associated restorer genes (Rf) have been the major promoters for the development of commercial hybrids in the

world. Now, more than 90% of the hybrids are made on the basis of the unique CMS-PET1 cytoplasm discovered by Leclerq in the progeny of a cross between *H. petiolaris* an the sunflower. The recent research programs have attempted to find new CMS sources in order to broaden the genetic base and to reduce the vulnerability of the cultivated crop to environmental stress and diseases. The diversification of CMS sources may also be useful to optimise the utilisation of genetic resources in breeding programs by "changing" the restorer status of an inbred line (i.e., a restorer genotype of one cytoplasm may be a male sterility maintainer of a second one). Another application is linked to the "agronomic" value of the new CMS sources which offered to the breeder a way to improve the hybrid performance by transferring the nuclear genotype onto a more updated cytoplasmic background. Finally, the availability of different CMS sources constitutes a powerful tool for genetic studies and molecular biology to understand the basic mechanisms of the CMS.

During the 1987-1990 an important structured work was achieved in the CMS working group. The main goals were to: (1) identify all the existing new CMS sources, so as to define a catalogue and propose a codification for all the CMS, (2) compare 13 CMS sources by crossing with a sample of 18 inbred sunflower lines (provided by the coordinating center of Montpellier), (3) search for restorer genes through F_1 combinations, (4) study the stability of the CMS in different environments, and (5) study the genetic determinism of the restoration of the CMS. This important work, involving cooperation between 9 countries, lead to interesting results on the stability and the comparison of the CMS. They were presented in a detailed report at the 7th FAO consultation in Pisa, 1991.

The work performed in the more recent period (1991-1994) was a continuation of the studies initiated in 1988. The following aspects were particulary developed:

- identification of new CMS
- search for new restorer (Rf) genes
- studies on the genetics determinism of the restoration
- preparation of allelism tests to identify the Rf genes involved in some CMS
- comparison of the CMS sources by genetic and molecular approaches.

Several CMS have been genetically and moleculary differentiated, but separation is not still clearly established for all of them. So, this topic requires additional studies to classify the CMS sources within homogeneous groups.

IDENTIFICATION OF NEW CMS SOURCES IN THE WORKING GROUP

The number of new cytoplasmic male sterilities (CMS) reported up to date, increased significantly. More than 40 sources were described in the literature.

For all of them, demonstration has been made that origin of the androsterility results from nucleocytoplasmic interactions.

Table 1.: List of the known CMS sources.

Common denomination	Species	Acc_code	Year obs.	Author report	FAO code
KOUBAN	*H. annuus lenticularis*			ANASCHENKO, 1974	ANL1
INDIANA 1	*H. annuus lenticularis*			HEISER, 1982	ANL2
VIR 126	*H. lenticularis*			ANASCHENKO, 1974	ANL3
397	*H. annuus* wild	INRA-397	81	SERIEYS, 1984	ANN1
517	*H. annuus* wild	INRA-517	81	SERIEYS, 1984	ANN2
519	*H. annuus* wild	INRA-519	81	SERIEYS, 1984	ANN3
521	*H. annuus* wild	INRA-521	81	SERIEYS, 1984	ANN4
NS-ANN-81	*H. annuus* wild			MARINKOVIĆ, 1986	ANN5
NS-ANN-2	*H. annuus* wild			ŠKORIĆ, 1987	ANN6
	H. annuus wild	PI 413024	88	JAN, 1988	ANN7
	H. annuus wild	PI 413043	88	JAN, 1988	ANN8
	H. annuus wild	PI 413158	88	JAN, 1994	ANN9
FUNDULEA 1	*H. annuus texanus*			VRANCEANU, 1986	ANT1
AN-67	*H. annuus*	E-067	86	CHRISTOV, 1992	ANN10
AN-58	*H. annuus*	E-058	88	CHRISTOV, 1994	ANN11
AN-2-91	H. annuus	E-002	91	CHRISTOV, 1991	ANN12
AN-2-92	*H. annuus*	E-002	92	CHRISTOV, 1992	ANN13
HEMUS	*H. annuus*	Cms H	91	CHRISTOV, 1993	MUT1
PEREDOVICK	*H. annuus*	Cms P	92	CHRISTOV, 1993	MUT2
ANOMALUS	*H. anomalus*	INRA-525	87	SERIEYS, 1994	ANO1
ARGOPHYLLUS	*H. argophyllus*	E-006	84	CHRISTOV, 1990	ARG1
ARGOPHYLLUS	*H. argophyllus*	E-007	87	CHRISTOV, 1990	ARG2
ARGOPHYLLUS	*H. argophyllus*	E-006	85	CHRISTOV, 1993	ARG3
BOLANDERI	*H. bolanderi*	INRA-255	80	SERIEYS, 1984	BOL1
DV-10	*H. debilis*	E-010	90	CHRISTOV, 1994	DEB1
EXILIS	*H. exilis*	INRA-130	82	SERIEYS, 1984	EXI1
EXI2	*H. exilis*	INRA-331	88	SERIEYS, 1984	EXI2
CMG2	*H. giganteus*			WHELAN, 1981	GIG1
CMG3	*H. maximiliani*			WHELAN, 1980	MAX1
	H. maximiliani		83	JAN, 1994	MAX2
NEGLECTUS	*H. neglectus*	INRA-201	83	SERIEYS, 1994	NEG1
CANESCENS	*H. niveus canescens*	INRA-197	82	SERIEYS, 1987	NIC1

Common denomination	Species	Acc_code	Year obs.	Author report	FAO code
FALAX	*H. petiolaris fallax*	INRA-200	80	SERIEYS, 1984	PEF1
PET/PET	*H. petiolaris petiol.*	INRA-737	87	SERIEYS, 1994	PEP1
CLASSICAL CMS	*H. petiolaris Nutt.*			LECLERCQ, 1969	PET1
CMG1	*H. petiolaris Nutt.*			WHELAN, 1980	PET2
PETIOLARIS BIS	*H. petiolaris Nutt.*			LECLERCQ, 1983	PET3
PET34	*H. petiolaris*	E-034	91	CHRISTOV, 1991	PET4
PHIR-27	*H. praecox*	E-027	90	CHRISTOV, 1990	PRH1
PRACEOX	*H. praecox praecox*	INRA-678	88	SERIEYS, 1994	PRP1
RUN-29	*H. praecox*	E-029	89	CHRISTOV, 1989	PRR1
RIG.RUSSIAN	*H. rigidus*			JAN, 1994	RIGx
VULPE	*H. rigidus*			VULPE, 1972	RIG1
RIG-M-28	*H. rigidus*	M-002	91	CHRISTOV, 1991	RIG2

The list of the known CMS, with indication of author and the species from which they originated, has been reported before. The denomination according to FAO codification is also proposed for spontaneous or CMS issued from interspecific crosses. Since several CMS were issued from mutagenesis (gamma irradiation, chemical or physical treatments) or might result from protoplast fusion programs, their codification was discussed at the last technical FAO meeting. It was decided to attribute the prefix "MUT" to the CMS coming from mutagenesis and the prefix "PFU" to CMS originated from protoplast fusion.

Twenty-one additional CMS sources have been discovered recently:

- nine in the *H. annuus* species: cms-ANN7, ANN8, ANN9 (Jan 1984, 1988); cms-ANN10, ANN11, ANN12, ANN13 (Christov 1991, 1992, 1994) and cms-MUT1, MUT2 (Christov, 1993.)
- three in the *H. praecox* ssp: cms-PRR1, cms-PRH1 (Christov, 1990) and cms-PRP1 (Serieys, 1994)
- two in *H. rigidus*: cms-RIG2 (Christov, 1994) and cms-RIGx (Russian source, reported by Jan 1994). In fact, this latter CMS could be similar to cms-RIG1 previously reported by Vulpe (the new restores of RIGx have to be tested nextly on cms-RIG1).
- two in *H. petiolaris* ssp: cms PET3 (Christov, 1991) and cms PEP1 (Serieys, 1994).
- the other five CMS were detected in different *Helianthus* species: cms-MAX2 (Jan, 1994);
- cms-DEB1 (Christov, 1994); and cms-ANO1, EXI2, and cms-NEG1 (Serieys, 1994).

If we consider the 44 cms sources listed above, they come from 12 different *Helianthus* species, most of them (36) being issued from *Helianthus* (ex *annuus*)

section. The highest frequency for CMS cytotypes were found in the wild *H. annuus* (19 CMS), *H. petiolaris* species (6), *H. agrophyllus* (3); *H. praecox* species (3 CMS), *H. exilis* (2 CMS) and *H. maximiliani* (2 CMS).

These results suggest the existence of considerable inter- and intraspecific cytoplasmic diversity.

2) SEARCH FOR CMS RESTORERS GENES

Important results have been obtained in the working group on isolation of efficient Rf restorer genes. Screening for Rf genes among wild *Helianthus* for sunflower PET1 male sterile cytoplasm indicate that Rf genes are common in the wild germplasm (Seiler, 1994). Twentu wild perennial and 6 annual *Helianthus* species proved to carry fertility restoration factors (Christov, 1992). In most of the reported CMS sources, Rf genes have been found either in the wild donor-parents of the CMS, within interspecific progenies or cultivated inbred lines.

In Table 2, the restoration response is reported for 39 CMS sources. The restoration status was measured on the flowering F_1 plants issued from crosses between the male-sterile parent and male-fertile pollinator. Due to the activity of the working group, restorer genes have been reported either because these CMS were not still studied, or because the Rf genes are very rare in Helianthus germplasm.

Interesting results were reported by Jan (1994) who found restorer genes related to three CMS (for which not any efficient Rf gene was detected): cms-ANN2 (restored by P21, RMAX1 and the wild parent), cms-ANN3 (restored by P21, RHA280, RPET2, RHA801, and the wild parent), and cms-ANN4 (restored by P21, RHA280, and the wild parent). For this last source, INRA Montpellier (1994 report) has also fixed an inbred restorer line, R-ANN4, in a progeny involving the cytoplasm-donor wild parent. Similarly, M. Iouras has reported three efficient restorer genes for cms-ANT1, in the wild *H. annuus ssp lenticularis* and two other wild species.

Another attractive result is the discovery of Rf genes for the cms-RIGx sterility. Indeed, Miller (1991) found Rf genes in the following genotypes (Luch and RPET2). Since cms-RIGx and RIG1 are suspected to have a common origin (Russian *H. rigidus* species, difficult to restore Cms), complementary crosses and molecular studies are necessary to check the hypothesis of similarity.

Table 2.: Restoration response of some sunflower inbred lines (or populations) of different cytoplasmic backgrounds.

Common denomination of the CMS	FAO code	% of restorer lines among: CMS-PET1 maint. genotypes	% of restorer lines among: Cms PET1 restorer genotypes	Restoration sources
KOUBAN	ANL1	61.5 % (26)[1]	40.00 % (20)	HA89, HA99, HA291, RCMG3
INDIANA 1	ANL2	55.6 % (18)	27.7 % (18)	PAH3, RHA273, HA291, RCMG3
397	ANN1	3.3 %* (30)	0 % (19)	HA291*, PAH2*, HA822*, Lyra*
517	ANN2	3.7 % (27)	0 % (21)	P21, RMAX1, PI 413178, P5.231*
519	ANN3	14.2 % (21)	0 % (19)	P21, RHA280, RPET2, RHA801, PI 413180
521	ANN4	3.2 % (31)	3.2 % (31)	P21, RHA280, PI 406647, R-ANN4
NS-ANN-81	ANN5	0 % (2)	0 % (2)	
NS-ANN-2	ANN6	-	-	
PI 413024	ANN7	-	-	P21, RHA280, PI 413024
PI 413043	ANN8	-	-	HA89, RHA266, RHA274, RHA294, PI 413043
PI 413158	ANN9	-	-	P21, PI 413058
AN-67	ANN10	0 % (5)	100 % (5)	RHA274, R3880, NS26R, R147
AN-58	ANN11	-	-	
AN-2-91	ANN12	-	-	
AN-2-92	ANN13	-	-	
ANOMALUS	ANO1	77 % (13)	66.7 % (9)	HAB, PAH3, RHA265, PAH2
FUNDULEA 1	ANT1	0 % (4)	8.3 % (12)	RANT1, RCMG2, Rf_t, Rf339
ARG1	ARG1	0 % (5)	100 % (6)	R147, R3840, RHA274, RHA280, NS26R
ARG2	ARG2	33.3% (3)	-	85B3, D34, R147, RHA274, NS26R
ARG3	ARG3	0 % (6)	25.0 % (4)	R147, R3840, NS26R
BOLANDERI	BOLI	69.2 % (39)	82.1 % (28)	HA291, HA89, RHA266, RHA279, RHA801
DV-10	DEBI	-	-	
EXILIS	EXI1	63.3 % (30)	57.1 % (21)	HA89, LA, RHA276, RHA298, RHA299
EXI2	EXI2	33.3 % (3)	100 % (4)	RHA274, RHA801, PAH3, PW1
CMG2	GIG1	8.0 % (18)	25.0 % (24)	RHA280, RHA801, PAH3, BZA2, RHA294

Common denomination of the CMS	FAO code	% of restorer lines among: CMS-PET1 maint. genotypes		Cms PET1 restorer genotypes		Restoration sources
CMG3	MAX1	25.0 %	(20)	11.1 %	(18)	HA291, PAH3, RHA801, XH
	MAX2	-		-		Hopi Dye, Seneca, RHA294, RHA266
NEGLECTUS	NEGI	26.3 %	(19)	94.1 %	(17)	WG, FJ, HAB, RHA265, RHA266, RHA274
CANESCENS	NIC1(**)	30.4 %	(23)	11.7 %	(17)	RHA265, RHA274, CAC, D34
FALLAX	PEF1	15.2 %	(46)	16.6 %	(36)	CP3.1, LA, PAH3, RHA298
PET/PET	PEP1	27.3 %	(11)	20.0 %	(10)	CP3.1, LA, PAH2, PAH3
CMG1	PET2	8.0 %	(25)	28.0 %	(25)	RCMG1, CP3.1, RHA280, 82HR38, RHA294
PET34	PET4	-		-		
PHIR-27	PRH1	0 %	(2)	-		
PRACEOX	PRP1	0 %	(7)	100 %	(7)	PAH3, RHA278, RHA274
RUN-29	PRR1	0 %	(2)			
RIG.RUSSIAN	RIGx					Luch, RPET2
VULPE	RIG1	0 %	(3)	0 %	(2)	none
RIG-M-28	RIG2					

- (1) number of tested lines
- (*) partial restoration
- (**) NIC1: incomplete male - sterility
- underlined restorer lines = 100% restoration in the progeny
- PAH2, PAH3, 85B3, D34, PW1, FJ, CP3, CP3.1, B2A2, XH, WG, FJ, HAB, CAC, LA 82HR38, R-ANN4 = INRA inbred lines
- R3880, NS26RM, R147 are I.W.S. inbred lines
- RANT1, RF339 are lines from ICCPT, the other lines are from USDA

The stability of the cytoplasmic male sterility sources has been estimated through the phenotypic expression of the male fertility restoration, in different locations and years. The data underline some unsteady cytoplasmic backgrounds. We can explain these results by stronger CMS* location interactions.

This variability makes difficult a global comparison between the CMS sources. In spite of these difficulties, we found stable restores for most of the CMS sources. Examples of such genotypes are listed (underlined) in Table 2..)

Table 3 Frequency of RF genes in the Helianthus germ plasm, related to CMS sources (Expressed as % of genotypes containing RF genes)

High >50%	Intermediate 25-50%	Low <25%
ANL1, ANL2, BOL1, EXI1, EXI2, NEG1, PET1	ANN10, ANO1, ARG1, ARG2, NIC1, PRP1	ANN1, ANN2, ANN3, ANN4, ANN5, ANT1, ARG3, GIG1, MAX1, PEF1, PEP1, PRH1, RIG, RIG1

The CMS-sources may be classified in three groups according to the frequency of restorer genes found in the *Helianthus* germplasm (Table 3).

GENETIC DETERMINISM OF THE RESTORATION

During the last four years, interesting results were reported in the Network. Jan (1994) studied the heredity of the restoration in cms-ANN2 and ANN3. He indicates that the restoration is controlled respectively by a single dominant Rf gene and 2 dominant complementary genes. The variation in pollen stainability, in some crosses, suggests the presence of modifier genes. Studies were also performed on the genetics of the Rf genes in the cms-RIGx source. The data agree with the hypothesis of two dominant complementary Rf genes involved in the restoration of this CMS source.

Another important study was performed at I.F.V.C. Novi Sad, where multiple nuclear combinations (involving 11 inbred lines) were studied on cms-ANN2, ANN3, ANN4 and BOL1.

The results exhibit a lack of restorer genes in the inbred lines tested with cms-ANN2, ANN3 and MAX1. Interesting results are found in cms-BOL1, for which frequency of good maintainer lines is low. The following nuclear combinations produced either complete male-sterile hybrids (RCMG1 x RCMG2, RCMG1 x RHA276, RHA271 x RHA278, RHA271 x RCMG3). In other respects, most of the F2 segregations already studied on cms-BOL1 agreed with the hypothesis of the male-sterility controlled by two independent recessive Rf genes. The explanation of the restoration responses observed in this study is complex and probably involves more than 2 Rf genes.

Table 4: Genetic determinism of the male-fertility restoration.

Common denomination	FAO code	Genetic control of the restoration	Reference
KOUBAN	ANL1	2 complementary dominant genes	Leclercq 1984; FAO report 1991
INDIANA 1	ANL2	no clear cut segregation. Possible 2 complementary dominant Rf genes	FAO report 1991
397	ANN1	-	
517, PI	ANN2	one Rf dominant gene + modifiers	Jan, 1994
519, PI	ANN3	2 complementary dominant genes+modifiers	Jan 1994
521, PI	ANN4	-	
NS-ANN-81	ANN5	-	
NS-ANN-2	ANN6	-	
	ANN7		
	ANN8		
	ANN9		
AN-67	ANN10		
AN-58	ANN11		
AN-2-91	ANN12		
AN-2-92	ANN13		
ANOMALUS	ANO1	single dominant Rf gene	Serieys, 1994
FUNDULEA 1	ANT1	complex. Al least two complementary dominant genes	Iuoras, 1991, 1994
ARGOPHYLLUS	ARG1	-	
ARGOPHYLLUS	ARG2	-	
ARGOPHYLLUS	ARG3	-	
BOLANDERI	BOL1	complex. 2 independent dominant Rf genes explain many segregations.	Serieys, 1991 (FAO report)
DV-10	DEB1		
EXILIS	EXI1	2 complementary dominant Rf genes	Serieys, 1987, 1994
EXI2	EXI2		
CMG2	GIG1		
CMG3	MAX1	2 of more complementary Rf genes	
	MAX2	no clearcut segregations	
NEGLECTUS	NEG1	one dominant Rf gene	Serieys, 1994

Common denomination	FAO code	Genetic control of the restoration	Reference
CANESCENS	NIC1		
FALLAX	PEF1	2 (or rare 3) complementary dominant independent genes	Serieys 1987 1991
PET/PET	PEP1	2 independent complementary Rf genes	Serieys 1994
CLASICAL CMS	PET1	1 or 2 complementary dominant independent genes	Kinman, 1970; Fick, 1974
CMG1	PET2	one dominant Rf gene	FAO report, 1991
PRAECOX	PRP1	one dominant Rf gene	Serieys, 1994
RUN-29	PRR1		
RIG.RUSSIAN	RIGx	2 complementary dominant genes	Jan, 1994
VULPE	RIG1		
RIG-M-28	RIG2		

The genetics of the restoration was analysed at Montpellier for cms-NEG1, ANO1, PRP1, EXI1 and PEP1 sources. The F_2 segregations studied clearly indicate that the restoration of the three first CMS were governed by a single Rf gene and that two complementary dominant Rf genes were involved in ECI1 and PEP1 sources.

The data shown in Table 4, indicate that restoration in sunflower is generally controlled by single Rf genes or series of 2 independent complementary genes. Therefore, the restoration of some sources (particularly Bol1) remain difficult to explain by a simple hypothesis.

An important work is now in progress at Fargo (allelism tests between restorer lines), to identify the RF genes involved in cms-BOL1 and cms-ANL1 sources.

A summary of the inheritance studies for the restoration of the different CMS is reported in Table 4.

COMPARISON OF THE CMS SOURCES

Genetic and molecular approaches.

Both genetic and molecular studies involving mitochondrial DNA RFLP and transcripts products were undertaken on several CMS sources. The objectives were to compare the CMS and to explain the mechanisms of the cytoplasmic male-sterility.

Interesting work was performed at Giessen on the comparison of cms-PET1 and normal fertile cytoplasms (Horn, 1990; Kohler, 1991). Cytoplasmic male-sterile lines cms-PET1-89 and cms-PET1-Baso differ from the male fertile analogue lines in a mitochondrial sequence (open reading frame *orfH522*) in the vicinity of the *atpA* gene. The transcriptional pattern of the *atpA* is changed in male-sterile lines carrying the *H. petiolaris* (PET1) cytoplasm, as well as in the restored male fertile lines. This protein is not detectable in the wild *H. petiolaris* species. It is suggested that this polypeptide may play a role in the CMS.

Another work is reported by M. Iuoras (1992). The plasmide P1t (1.45 Kb) is a good probe to distinguish the cytoplasms from the cmd-PET1, since it is present in fertile maintainer lines, but not in cms-PET1. No hybridization signal was detected between the plasmide P1t and the total DNA from cms-ANT1 or the wild *H. annuus ssp texanus*.

Besides, Spassova (1992) studied 6 new Bulgarian CMS, including PET1 and ANT1. She used *atpA* gene and showed that cms-ANT1 differed for mt genomic structure, from both the fertile genotypes and the 6 new CMS.

Molecular comparison of a large sample of CMS was also realised by Crouzillat (1991, 1994). The genetics of the male fertility restoration and the RFLP of the mitochondrial DNA were studied for 16 sunflower cytoplasms. Male fertility restoration/male sterility maintainers patterns distinguished 12 cytotypes. Four cytoplasms completely unrestored were not analysed and permitted the distinction of 13 cytotypes. It was shown that mt DNA diversity occurs both between and within the *Helianthus* species.

For genetic and mitochondrial RFLP studies, phenograms built according to similarity indexes show that most of the CMS groups defined by restoration patterns correspond to a restriction fragment pattern of the mt DNA. One exception occurred for cms-ANN4, but recently restorer genes were found in this CMS (Jan 1994, Serieys 1994) which now made possible the genetic separation from the other three CMS: (ANN1, ANN2 and ANN3).

Agronomic approach

Field trials were undertaken at INRA Montpellier to compare the agronomic effects of the CMS sources. The classical cms-PET1 was compared to the other 9 sunflower CMS (PEF1, BOL1, EXI1, PEP1, GIG1, PET2, ANL2, ANO1, NEG1) through isogenic alloplasmic hybrids. Results show significant, positive or negative, cytoplasmic effects for all the studied characters. Seed yield was increased with BOL1, ANO1, ANL1, flowering date was delayed with BOL1 and ANL1, plant height increased with ANO1, NEG1, ANL1 and oil content increased with BOL1, but lowered with ANL2, PEF1, PEP1 cytoplasmic backgrounds. Some cytoplasmic effects were also suggested for *Phomopsis* tolerance, but they have to be confirmed in further experiments.

CONCLUSIONS

Due to particularly active work developed in the CMS working group by most of the participants, interesting results have been obtained. The most significant achievements were:

- the identification of many new CMS sources.
- the discovery of restorer genes for "difficult to restore" CMS.
- the analysis of the genetic determinism of the fertility restoration in new CMS.
- the comparison of the CMS through genetic, molecular or agronomic ways.

During the previous work the stability of thirteen CMS sources was investigated. Some CMS lacking stability or leading to frequent fertility segregation's were identified; they cannot be easily used in breeding programs. Nevertheless, complete sets of efficient female, maintainer and restorer genotypes (A, B, R components) have been found for several CMS sources and are now available. These new CMS can be practically used for hybrid production and, if necessary, could easily substitute the cms-PET1.

Therefore, it is evident that much study is still necessary to differentiate the CMS sources. It is likely that, some of the 44 CMS sources reported may be identical. So, it is clear that complementary genetic studies have to be performed. Molecular studies on Mt DNA proved to be powerful tools and fast methods, either to separate the CMS or to explain the mechanisms of the cytoplasmic male sterility.

Another aspect to be studied is the identification of the specific Rf genes in the different CMS. Several participants have already started crosses between restorer genotypes to prepare allelism tests.

The agronomic value of the CMS sources is a component particulary important for breeding purposes. This aspect has been already addressed in the previous step and significant cytoplasmic effects were detected. Nevertheless, a more complete evaluation should be performed at a lager scale (international experimental design) to test the multilocal effects on the cytoplasmic backgrounds. Such an experimentation should provide support for further generalisation about the agronomic value of the cytoplasms.

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Program of work for the period 1995-1998

The plan of work involves: (1) the pursuit of general activities already undertaken in the working group:

- Identification of new CMS sources is sunflower,
- Comparison of the CMS sources using genetic and molecular approaches,
- Identification of the Rf genes involved in each CMS and characterization of the genetic determinism of the restoration,
- Evaluation of the agronomic value for the most stable CMS sources.

IDENTIFICATION OF THE NEW SUNFLOWER CMS SOURCES, SPONTANEOUS OR DERIVED FROM INTERSPECIFIC CROSSES.

This topic represents a permanent activity in this Working Group. Facing the increasing number of reported CMS sources, it is of primary importance, for the scientific community, (1) to establish an exhaustive list on the CMS sources found in the *Helianthus* genus (2) to codify them, according to FAO standards and (3) to gather information on the origin and behavior of the new CMS sources.

To make the CMS sources available in the network, it is suggested that the Coordination Center (1) receives the different CMS sources reported in the WG (A and B components), (2) multiplies and (3) distributes them to all the participants in the WG for further investigations.

COMPARISON OF CMS SOURCES

Comparison of CMS sources using genetic approach.

The objective of this study is to classify the CMS sources with similar restoration patterns into specific groups, in order to define the really distinguishable sets of cytotypes.

The classical method used will consist on evaluation of the fertility restoration of F_1 hybrids, after crossing the female CMS sources with different sunflower lines. The study initiated in the working group provided interesting data, suggesting many different cytotypes. Now, around 43 CMS sources are reported and we propose to study the new CMS.

Since restoration in *Helianthus* seems governed by series of complementary genes, we recommend to evaluate the restoration responses of the CMS sources on well-defined nuclear genotypes. The original sample of lines used to test restoration needs to be completed by additional genotypes, including specially the recent restorer genotypes (RANT1, RANN4...) of the "hard to restore" cytoplasms.

Comparison of the CMS sources, using molecular markers

In this field, studies are now performed in France, Germany, Romania, Bulgaria. In addition to the genetic approach, the molecular markers involving mt DNA organisation have to be used (1) to differentiate the sunflower CMS sources and (2) for a better understanding of the nucleus / cytoplasm relationships.

Similar approaches have to be carried on for comparison of the whole CMS sources to establish the molecular relationship between cytotypes in sunflower crop. This powerful tool could be used in breeding programs for rapid identification of cytoplasmic backgrounds.

Moreover, molecular markers for specific Rf genes are also promising, to search for restorer genes in *Helianthus* germplasm.

SEARCH, CHARACTERISATION AND IDENTIFICATION OF THE RF GENES INVOLVED IN THE CONTROL OF THE RESTORATION OF THE DIFFERENT CMS

Search for Rf genes in particular CMS sources

Due to the activity of the working group, efficient restoration factors have been identified for most of the reported CMS sources. For some cytotypes (ANN1,ANN5, RIG1...), Rf genes appear rather scarce among cultivated sunflower germplasm. For all the CMS sources and specially the latter ones, the diversification of the Rf genes is important and has to be completed by further screening within wild and cultivated germplasm.

We suggest that the identified restorer genes related to the different CMS sources be also multiplied and distributed to the participants in the WG.

Studies on the genetic determinism of the restoration.

Inheritance studies of the restoration are lacking for several recent CMS sources, (MAX2, ANN4, ARG1, ARG2, ARG3...). The analysis of the F_1 and BC_1 segregation will be carried on these CMS. The objective being to define for each CMS, the number and the genetic relationship between the Rf genes involved.

Identification of the RF genes involved in each CMS

The bibliographical data suggest that several Rf genes (complementary or not) are involved in the control of the restoration in sunflower. We presume that some of them may be common to different CMS sources. So, allelism tests are proposed for more detailed analysis of the relationship between the Rf genes.

- at Novi Sad: back crosses were performed between the F_1 combination between 10 restorer lines and female cms-BOL1 plants.

- at Fargo: allelism tests were undertaken to study Rf genes in cms-BOL1 and cms-ANL1.
- at Montpellier: crosses between restorer lines have been initiatiated to identify the Rf genes in CMS-PET1, CMS-PEF1 and CMS-BOL1 sources. The F_1 were backcrossed on alloplasmic female lines carrying the CMS-PET1, CMS-PEF1 and CMS-BOL1 sources.

These studies have to be extended to include the other CMS sources.

EVALUATION OF CYTOPLASMIC EFFECTS ON THE AGRONOMIC CHARACTERS

This point appeared basically very important for breeders. The cytoplasmic component may be used to improve the performance of the hybrids. In some institutes, the cytoplasmic effects on the agronomic traits have been evaluated according to a comparison of alloplasmic hybrids (same nuclear genotype on different cytoplasmic backgrounds).

New CMS sources have with be compared to the reference cms-PET1 cytoplasm to provide additional information on the agronomic value of the CMS sources. The cytoplasmic effects have to be evaluated for most traits of economic importance such as:

- main agronomic traits: seed yield, plant height, flowering period, earliness
- disease resistance (*Sclerotinia, Phomopsis*, mildew...)
- seed quality (content and quality for oil and seed protein)
- drought tolerance

Experimentation of alloplasmic hybrids in the Network supposes the following steps:

1. availability* of series of isogenic alloplasmic lines in the Network,
2. the creation of alloplasmic hybrids in isolated plots, with enough seed produced to support multilocal and pluriannual experimentation.
3. the evaluation of the agronomic performance in multilocal field trials. A two-year experimentation should be preferable.

(*) each participants has to propose the alloplasmic lines he wants to test in the Network.