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CMS-Rf SYSTEM FOR SUNFLOWER BREEDING

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SUMMARY

Restoration behavior of 20 CMS sources in combination with 11 restorer lines has been studied. Genetic mechanism of fertility restoration was determined for all investigated CMS sources. Three groups of CMS sources were distinguished in dependence of the exhibited differences in restoration response from CMS-Rf PET1 system. The group of alternative CMS sources that exhibited regular restoration effect in crosses with different fertility restorers but completely failed to restore fertility in crosses with PET1 restorer lines represents material of practical value. When discovered, these new CMS sources will permit to use new CMS-Rf systems in sunflower breeding.

Key words: **sunflower, cytoplasmic male sterility (CMS), fertility restoration, hybrid, gene**

INTRODUCTION

At present, the entire sunflower production in the world is based on a single source of cytoplasmic male sterility, *Helianthus petiolaris* (CMS) PET1. That has led to a wide distribution of hybrids with related type of cytoplasm, which makes them highly susceptible to new virulent races of many obligate parasites. In order to prevent such negative results, recent research programs have attempted to find new CMS sources and fertility restorer genes for them (Series, 1996, 1999). Introduction of different cytoplasmic backgrounds into hybrid production will permit to increase the general variability of the cultivated sunflower and to avoid problems that occur with CMS-PET1. In this connection, the aim of this research was to investigate CMS sources that possess restoration systems different from CMS-PET1. The search for alternative CMS-Rf systems and their utilization in breeding programs seems to have good prospective.

MATERIAL AND METHODS

This investigation was conducted under field conditions in the period 1999-2002. Twenty CMS sources from the Institute's sunflower genetic collection were

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used: *Helianthus petiolaris* (PET1), *H.argophyllus* (ARG1, ARG3), *H.giganteus* (GIG1/141, GIG1/477 GIG1/647), *H.debilis* (DEB1), *H.texanus* (ANT1/479, ANT1/645), *H.praecox* (PRR1, PRH1), *H.fallax* (PEF1), *H.annuus* (wild) (ANN5), *H.rigidus* (RIG1, RIG2, RIGL). Non-identified CMS sources DCS1, DCS2, DCS3 and DCS4 (double-color sterile 1-4) obtained from ornamental sunflower and the sterile cultivated sunflower line ZL-809A with unknown cytoplasm type were also used in the experiment.

Eleven fertile lines carrying Rf genes for different germplasms were used as fertility restorers: RHA-274, SH-416/5, RJUG-1, RFT-1, DCF-1, DCF-2, DCF-3, DCF-4, RCMG-2, RPEF-1 and RARG-1. Three of them, RHA-274, RJUG-1 and RFT-1, were received from VNIIMK (Russia), the others were obtained from another source (Chepurnaya and Pershin, 2001).

The hybrid analysis by full top-cross scheme was used as a main research method. In order to determine the mechanism of fertility restoration, fertile plants of the obtained hybrid combinations were self-pollinated. Progenies of each plant were grown in a separate plot and individually observed. The segregation ratio in the tested generations was evaluated by chi square statistical method.

RESULTS AND DISCUSSION

The main objective of the experiment was to compare different CMS-Rf systems against the classical CMS-PET1 used as a control in order to establish essential differences.

The analysis of the test-hybrids obtained by crossing CMS-PET1 with fertility restorers showed the presence of fertility restoration in all combinations except PET1 x RJUG-1. In all cases two dominant complementary genes controlled the fertility restoration of CMS-PET1 with the dominant allele of one of them in the female plant.

The analysis of the obtained data permitted to classify the investigated CMS sources in three groups according to the exhibited differences in restoration behavior in relation to CMS-PET1:

1. PET1-like CMS sources;
2. alternative CMS sources;
3. hard-to-restore CMS sources.

First of all the ranging was performed on fact of fertility restoration presence in crosses with CMS-PET1 restorers RHA-274 and SH-416/5. The summarized results are presented in Table 1.

Fertility restoration of PET1-like CMS sources

The CMS sources with fertility restoration system similar to CMS-PET1 were placed in a separate group of PET1-like CMS sources (ARG1, ARG3, DEB1, PRR1, PRH1, RIG2, DCS2 and DCS3). Their test-hybrids with the restorer RHA-274 were completely fertile. Fertility restoration of PET1-like CMS was invariably controlled by two dominant complementary genes and one of the Rf genes was in the female plant genome. The genetic analysis indicated a similarity among these CMS sources.

The PET1-like CMS group determination according to results of genetic analysis is confirmed by molecular studies R. Horn and W. Friedt (Horn and Friedt, 1999). Wishing to explain CMS mechanisms and to compare mitochondrial genome organization of different CMS sources, they performed an extensive molecular study of 28 CMS sources and determined 5 CMS groups. Those CMS, present in our investigation are included to the same groups. Thus the similar restoration responses of the PET1-like CMS sources are based on similar mutations in the mitochondrial genome. Our analysis at the phenotypic level permitted to indicate objectively the same principles, what confirmed its efficiency.

Table 1: CMS-Rf systems in sunflower

CMS-Rf	RHA-274	SH-416/5	RFT-1	DCF-2	DCF-3	DCF-1	DCF-4	RARG-1	RPEF-1	RCMG-2	RJUG-1
PET1	+	+	+	+	+	+		+	+	+	-
DCS2	+	+	+	+	+	+	+		+	+	+
ARG1	+	+	+	+	+	+	-	+	+	+	-
PRR1	+	+	+	+	+	+	+	+	+	-	-
RIG2	+	+	+	+	+	+	+	+	+	-	-
DCS3	+	+	+	+	+	+	+	+	+	-	-
DEB1	+	+	+	+	+	+	+	+	-	-	-
ARG3	+	+	+	+	+	+	+	+	-	-	-
PRH1	+	+	-	+	+	+	+	+	-	+	-
PEF1	-	-	-	+	+	+	-	+	+	-	+
GIG1/141	-	-	-	-	+	-	-	-	-	+	+
GIG1/477	-	-	-	+	-	-	-	+	+	+	+
GIG1/647	-	-	-	+	+	-	-	-	-	+	+
ANT1/645	-	-	-	+	+	+		+	-	-	+
ANT1/479	-	-	+	-	+	-	-	+	+	+	+
ANN5	-	-	+	-	-	-	+	-	+	-	+
RIGL	-	-	-	-	-	+	+	-	-	-	-
RIG1	-	-	-	-	-	-	+	-	-	-	-
DCS1	-	-	-	-	-	+	-	-	-	-	-
DCS4	-	-	-	-	-	-	-	-	-	-	-
ZL-809A	-	-	-	-	-	-	-	-	-	-	-

+: fertility restoration in the cross; -: unrestored combination

Group of alternative CMS sources

The restoration response of the other investigated CMS sources differed principally from the control. The response of complete sterility in the crosses with RHA-274 and SH-416/5 indicated the incompatibility of these cytoplasmic backgrounds with the standard Rf_{PET1} gene complex (Chepurnaya and Pershin, 2000).

The sources of CMS that completely failed to restore with lines – donors of Rf_{PET1} genes but demonstrated fertility restoration in combinations with the other fertile lines comprised the group of alternative CMS sources. The CMS sources GIG1, ANT1, PEF1 and ANN5 were included to this group (Table 2).

Table 2: Genetic restoration mechanisms of CMS sunflower sources

CMS	F_2 segregation (F:S)	Mechanism
PET1-like CMS sources		
DCS-2	9:7	two dominant complementary genes
DCS-3		
ARG-1		
ARG-3		
PRR-1		
PRH-1		
RIG-2		
DEB-1		
Alternative CMS sources		
GIG-1	3:1	one dominant gene
ANT-1	9:7	two dominant complementary genes
PEF-1	9:7	two dominant complementary genes
ANN-5	7:9	two recessive complementary genes
Hard-to-restore CMS sources		
DCS-1	3:1	one dominant gene
RIG-L	1:3	recessive epistasis

A single dominant gene governed the restoration of CMS GIG1 and two dominant complementary genes were involved in CMS ANT1 and PEF1 restoration. These results correspond with those from other reports (Kural and Miller, 1992; Miller, 1996; Series, 1999).

The CMS ANN5 was the most hardly restored in this group. The test-hybrids with few restorer lines only appeared to be fertile (Table 3). In all F_2 segregations studied, two recessive complementary genes probably controlled the fertility restoration of CMS ANN5 (Chepurnaya, 2001).

Thus, the group of alternative CMS sources has the CMS-Rf system distinguished from PET1. Different restoration response within this CMS group indicates the existence of complex mechanisms of "cytoplasm – Rf genes" interaction.

Hard-to-restore CMS sources

The CMS sources RIG1, RIGL, DCS1 and DCS4 and the sterile cultivated sunflower line ZL-809A with unknown cytoplasmic background possessed an extremely low fertility restoration reaction (Table 1).

The single efficient restorer for CMS DCS1 was the fertile line DCF-1. The segregation ratio in the F_2 generation clearly fits the ratio 3:1 ($\chi^2=0$, $P=1.0$). All other hybrid combinations were sterile. All eleven Rf lines failed to restore the CMS DCS-4 and sterile line ZL-809A.

Table 3: Fertility restoration of CMS ANN5

Rf line	F_1					F_2				
	Ammount of plants		Segregation ratio	χ^2	P	Ammount of plants		Segregation ratio	χ^2	P
	F	S				F	S			
RHA-274	0	60	0:1	0	1.0	-	-	-	-	-
SH 416/5	0	44	0:1	0	1.0	-	-	-	-	-
RFT-1	4	4	1:1	0	1.0					
	31	0	1:0	0	1.0	7	16	7:9	1.59	0.2
DCF-1	0	98	0:1	0	1.0	-	-	-	-	-
DCF-2	0	18	0:1	0	1.0	-	-	-	-	-
DCF-3	0	40	0:1	0	1.0	-	-	-	-	-
DCF-4	0	53	0:1	0	1.0	-	-	-	-	-
	17	48	1:3	0.08	1.0	-	-			
RARG-1	0	52	1:0	0	1.0	-	-	-	-	-
RPEF-1	12	13	1:1	0.04	1.0	33	56	7:9	1.64	0.2
RCMG-2	0	67	0:1	0	1.0	-	-	-	-	
RJUG-1	12	12	1:1	0	1.0	45	61	7:9	0.04	1.0
						55	78	7:9	0.28	0.6

F - fertile plants, S - sterile plants

Only a few fertile lines restored the CMS sources on the base of *H.rigidus*. In CMS RIG1 crosses with all eleven Rf lines only sterile plants were observed, except the combination RIG1 × DCF-4. The percentage of sterile plants in the F_1 and F_2 generations varied in different years from 30 to 75%. It appeared extremely difficult to detect the genetic mechanism of fertility restoration because of very unsteady restoration effect. The restoration response in combination RIG1 × DCF-4 was probably under the influence of environmental conditions. Because of this we could not perform a correct genetic analysis.

The segregation data for both generations of the cross RIGL × DCF-4 indicated that the Rf gene interaction could have developed according to the recessive epistasis type (Table 4).

Table 4: Fertility restoration in the cross [RIGL x DCF-4]

Cross	F_1					F_2				
	Ammount of plants		Segregation ratio	χ^2	P	Ammount of plants		Segregation ratio	χ^2	P
	F	S				F	S			
[RIGL	22	68	1:3	0.01	1.0	10	30	1:3	0	1.0
x	8	31	1:3	0.54	0.4	10	25	1:3	0.15	1.0
DCF-4]						19	59	1:3	0.02	1.0

F - fertile plants, S - sterile plants

In the F_1 and F_2 generations of the cross RIGL × DCF-1, different segregation types were observed with percentages of fertile plants varying from 5 to 50%. The obtained data could be explained either by heterozygosity of Rf genes in the restored combinations or by the existence of more then two alleles in each locus. Fertility restoration of CMS RIGL is complex and it requires further investigation by realization of extensive additional system of individual crosses.

The hard-to-restore CMS sources probably possess a specific CMS-Rf system with Rf genes absent in fertile lines.

CONCLUSION

The group of alternative CMS sources is of large importance for sunflower breeding and seed production. These CMS sources have the CMS-Rf system distinguished from PET1 and they possess the stable fertility restoration effect. The different restoration response within this CMS group indicates the existence of complex mechanisms of “cytoplasm – Rf genes” interaction. The introduction of the new CMS-Rf system in hybrid sunflower production will allow solving the problems associated with utilization of CMS PET1.

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SISTEMAS CMS-Rf PARA LA MEJORA GENÉTICA DE GIRASOL

RESUMEN

En el trabajo se ha investigado la restauración de fertilidad en 20 fuentes de CMS combinando con 11 líneas restauradoras. Para todas las fuentes de CMS investigadas, se ha determinado el mecanismo genético de restauración de fertilidad. Las fuentes se han dividido en tres grupos, a base de diferentes reacciones al sistema CMS-Rf PET1. El valor práctico lo tiene el grupo de las fuentes de CMS alternativas, en las cuales se presentaron hasta un efecto de restauración normal, en el cruzamiento con diferentes restauradores, los cuales en los cruzamientos con la línea restauradora PET1 han una total ausencia de restauración. La movilización de estas fuentes de CMS permitirá la utilización de nuevos sistemas de CMS-Rf en la mejora genética de girasol.

SYSTÈMES CMS-Rf POUR LA CULTURE DU TOURNESOL

RÉSUMÉ

Ce travail examine la restauration de la fertilité pour 20 sources CMS lors de la combinaison avec 11 lignes restauratrices. Pour toutes les sources CMS examinées, un mécanisme génétique de restauration de la fertilité a été confirmé. Les sources ont été séparées en trois groupes d'après les différentes réactions au système CMS-Rf PET1. Le groupe de sources CMS alternatives chez lequel il y a eu un effet normal de restauration dans les croisements avec différents restaurateurs et qui ont montré une absence totale de restauration dans les croisements avec la ligne restauratrice PET1 a une valeur pratique. La mobilisation de ces sources CMS permettra l'utilisation de nouveaux systèmes CMS-RF dans la culture du tournesol.