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SILENCING OF FERTILITY RESTORATION GENES IN SUNFLOWER

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SUMMARY

Single dominant genes for fertility restoration of cytoplasmic male-sterile *cms*ANN2 have been identified in PI 413178, P21, and RCMG3, while PI 413180, P21, RHA801, RCMG1, and RHA280 were identified as restoration sources for *cms*ANN3. However, some *cms* plants were observed in progenies of homozygous restoration lines crossed with HA89, RHA266, and RHA274. It is believed that HA89, RHA266, and RHA274 possess gene(s) which suppress, or silence the expression of some *Rf* genes. HA821 does not have silencing gene(s), thus producing all male-fertile progenies after crossing with the restoration lines. F_2 progenies of the half-diallel crosses among the three restoration lines for *cms*ANN2 and among the five restoration lines for *cms*ANN3 were nearly all male-fertile, suggesting a single *Rf* gene for each of the *cms* sources. However, testcross progeny families from crosses of half-diallel F_1 with respective *cms*ANN2 and *cms*ANN3 were not all male-fertile, with most families having all male-sterile plants or segregating for both male-sterile and male-fertile plants. This could happen if *cms*ANN2(HA89) and *cms*ANN3(HA89) contain silencing gene(s) obtained from backcrossing with HA89. Restoration lines containing silencing genes can be identified and the silencing genes eliminated. Hidden silencing gene(s) in *cms* lines is a problem, and lines without these genes, such as HA821, should be used to develop maintainer lines. Gene silencing appears to be simply inherited. Molecular marker development would assist in identifying lines without silencing genes.

Key words: **sunflower, *Helianthus annuus* L., cytoplasmic male sterility, fertility restoration, gene silencing**

INTRODUCTION

Single dominant fertility restoration genes for cytoplasmic male-sterile *cms*ANN2 (*cms*PI 413178) have been identified in PI 413178, P21, while RCMG3, and PI 413180, P21, RHA801, RCMG1, and RHA280 restore fertility in hybrids

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with *cms*ANN3 (*cms*PI 413180) (Jan, 2000). Two complementary dominant genes in RCMG1 and Luch restored fertility in *cms*RIGX (Jan *et al.*, 2002). The male sterility characteristic of these three groups were of the typical *cms*PET1 type and the restoration genes provided full male fertility in the heterozygous condition.

Lines homozygous for the *Rf* genes in the respective *cms* cytoplasms in HA89 background were developed. However, following attempts to convert these restoration lines into an RHA 274 background with Rf_1 and the new *Rf* genes, a high frequency of male-sterile plants in progenies heterozygous for the *Rf* genes was observed. Similarly, there were also occasional male-sterile plants among the progenies of homozygous restoration lines crossed with HA89. However, introducing the Rf_1 gene into the two fertility restoration lines for the *cms*RIGX with a RHA274 background did not produce any male-sterile progeny, and had an expected 1F : 3S segregation ratio in backcross progenies.

This unusual interaction of the *cms*ANN2 and *cms*ANN3 restoration genes after crossing with cultivated lines has not been reported in sunflower or other major crops. Therefore, an experiment was initiated to study this specific gene interaction and to establish methods to effectively utilize these *cms* sources and fertility restoration genes in a breeding program.

MATERIALS AND METHODS

The homozygous fertility restoration lines were crossed with HA89, RHA266, RHA274, and HA821 in the greenhouse in 2001, and progenies observed for segregation of male-fertile and male-sterile plants in the greenhouse in 2002 (Table 1).

Half-diallel crosses among the three restoration lines for *cms*ANN2 and among the five restoration lines for *cms*ANN3 were produced in 2001 in the greenhouse. Testcrosses were made in 2002 by crossing half-diallel F_1's with *cms*ANN2 or *cms*ANN3, maintained by backcrossing with HA89 (Tables 2 and 3). The half-diallel F_2 and the testcross F_1 generations were planted in the field in 2002 for fertility evaluation. These crosses were initially made to evaluate allelic relationships among the fertility restoration genes, but also provided information on gene silencing.

The inheritance of the silencing gene(s) will be studied. Male-sterile progeny, obtained from crossing homozygous restoration lines with HA89, RHA266, and RHA274, were backcrossed with pollen of homozygous restoration lines. Their progeny are expected to segregate for male sterility based on the silencing gene(s), and not the *Rf* gene.

RESULTS AND DISCUSSION

Segregation ratios of F_1 male-fertile (F) and male-sterile (S) plants from crosses of homozygous restoration lines in the male-sterile cytoplasms of PI 413178

(*cms*ANN2) and PI 413180 (*cms*ANN3) with four USDA lines are shown in Table 1. Single dominant fertility restoration genes for cytoplasmic male-sterile *cms*ANN2 have been identified in PI 413178, P21, and RCMG3, while PI 413180, P21, RHA801, RCMG1, and RHA280 were identified as restoration sources for *cms*ANN3 (Jan, 2000). Therefore, these progenies, heterozygous for the *Rf* gene, should be all male-fertile provided there are no unusual gene interactions. Male-sterile plants were observed in progenies involving HA89, RHA266, and RHA274, but not in crosses with HA821. This indicated that HA89, RHA266, and RHA274 possess silencing gene(s) which suppress the expression of the respective *Rf* genes. HA821 produced all male-fertile progenies, indicating that it does not contain any silencing gene(s).

Table 1: Segregation of F_1 male-fertile (F) and male-sterile (S) plants after crossing homozygous restoration lines in the *cms* cytoplasms of PI 413178 (*cms*ANN2) and PI 413180 (*cms*ANN3) with four USDA lines

Restoration lines	HA89		HA821		RHA266		RHA274	
	F	S	F	S	F	S	F	S
PI 413178/HA89//PI 413178/**P21**	0	4	4	0	0	4	0	4
PI 413178/4*HA89/3/PI 413178/RPET2//**RCMG3**	4	0	-	-	0	4	0	4
PI 413178/HA89//**PI 413178**	0	4	4	0	1	3	2	2
PI 413180/2*HA89//**PI413180**/3/HA89	4	0	4	0	1	3	3	1
PI 413180/2*HA89//**P21**/3/HA89	4	0	4	0	0	4	0	4
PI 413180/4*HA89/3/PI 413180/2*HA89//**RHA801**	4	0	4	0	3	1	4	0
PI 413180/4*HA89/3/PI 413180/RPET2//**RCMG1**	2	2	4	0	0	4	0	4
PI 413180/HA89//PI 413180/**RHA280**	1	3	4	0	0	3	0	4

Male-fertile and male-sterile plants in F_2 of the half diallel crosses among homozygous restoration lines and in testcross progenies of *cms*ANN2 (HA89) × half-diallel cross F_1's are shown in Table 2.

Table 2: Male-fertile (F) and male-sterile (S) plants in F_2 of half-diallel crosses among homozygous restoration lines (top) and in testcross progenies (bottom) of *cms*ANN2 (HA89) × half-diallel cross F_1

& %	PI 413178		RCMG3	
	F	S	F	S
RCMG3	26	4	-	-
P21	18	o	24	9

& %	PI 413178		RCMG3	
	F	S	F	S
RCMG3	1	35	-	-
P21	0	38	5	30

The F_2 segregation ratios suggested that the *Rf* genes derived from P21 and PI 413178 are at the same locus, and the gene from RCMG1 could be different. The testcross progeny ratio of 0F : 38S for P21 × PI 413178 suggested the presence of

homozygous dominant hidden silencing gene(s) in *cms*ANN2 plants used for the testcross. The F and S testcross progeny segregation ratio of the other two intercrosses could be the result of segregation of both the silencing gene(s) and the *Rf* gene(s).

Male-fertile and male-sterile F_2 plants from the half-diallel crosses among homozygous restoration lines and in testcross progenies of *cms*ANN3 (HA89) × half-diallel cross F_1's are shown in Table 3. F_2 progenies of the half-diallel crosses were all male-fertile, suggesting a common *Rf* gene for the five restoration sources. However, testcross progeny families were not all male-fertile, with most of the families segregating for male-sterile and male-fertile plants. This can be explained if *cms*ANN3(HA89) contains silencing gene(s) accumulated from the backcrossing with HA89. The all male-fertile testcross progeny indicated the absence of silencing gene(s) in the *cms*ANN3 plants used for the cross. The differing F:S segregation ratios in testcross progeny likely reflect the silencing gene dosage of individual *cms*ANN3 plants used as parents.

Table 3: Male-fertile (F) and male-sterile (S) plants in F_2 of half-diallel crosses among homozygous restoration lines (top) and in testcross progenies (bottom) of *cms*ANN3 (HA89) × half-diallel cross F_1

& %	R801		RCMG1		PI 413180		P21	
	F	S	F	S	F	S	F	S
RCMG1	59	0	-	-	-	-	-	-
PI 413180	38	0	53	0	-	-	-	-
P21	70	0	58	0	71	0	-	-
R280	54	o	59	0	60	0	38	0

& %	R801		RCMG1		PI 413180		P21	
	F	S	F	S	F	S	F	S
RCMG1	11	29	-	-	-	-	-	-
PI 413180	43	0	22	19	-	-	-	-
P21	14	35	5	35	36	0	-	-
R280	40	6	24	18	45	0	12	29

To study the inheritance of the silencing gene(s) from HA89, RHA266, and RHA274, male-sterile progeny obtained from crosses with the homozygous restoration lines were backcrossed with pollen from respective homozygous restoration lines. These backcross progenies will be evaluated during the winter of 2003, and are expected to segregate for male sterility based solely on silencing gene(s).

This specific silencing gene-*Rf* gene interaction is rare and this is the first time such an interaction has been observed in sunflower. The silencing gene(s) is likely to affect *cms* and maintainer line development because of its invisibility in both *cms* and maintainer lines. Restoration lines homozygous or heterozygous for all silencing gene(s) will be male-sterile, and can be easily eliminated. Recent results on the

inheritance of the silencing gene(s) suggest a simple inheritance. Future molecular marker development will simplify selection of lines without silencing genes.

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SILENCIAMIENTO DEL GEN DE RESTAURACIÓN DE FERTILIDAD EN GIRASOL

RESUMEN

Los genes de restauración de fertilidad dominantes particulares de la línea de esterilidad citoplasmática masculina *cms*ANN2, se identificaron en PI413178, P21 y RCMG3, mientras que PI 413180, P21, RCMG1 y RHA280 se identificaron como fuentes de restauración para la línea *cms*ANN3. Pero, en la descendencia de las líneas restauradoras homocigotas cruzadas con HA89, RHA266 y RHA274 fue registrado cierto número de las plantas *cms*. Se cree que HA89, RHA266 y RHA274 poseen genes que impiden, es decir, silencian la expresión de algunos genes *Rf*. HA821 no tiene genes de silenciamiento, y por lo tanto, tras el cruzamiento con dichas líneas restauradoras, produce descendencias que son todas fértiles masculinamente. Las descendencias F_2 de cruzamientos semidialelos (sin combinaciones recíprocas) entre tres restauradores para *cms*ANN2 y cinco restauradores para *cms*ANN3, casi todas eran fértiles masculinamente, lo que sugiere la presencia del gen *Rf* particular, para cada una de las fuentes investigadas *cms*. Pero, las familias de descendencia de las combinaciones del ensayo, de los cruzamientos de las plantas semidialelas F_1 con el correspondiente *cms*ANN2 y *cms*ANN3, no eran todas fértiles masculinamente, sino que la mayoría de las familias tenía o todas las plantas estériles masculinamente o se presentó la segregación en plantas estériles masculinamente y las fértiles masculinamente. Eso puede ocurrir si *cms*ANN2 (HA89) y *cms*ANN3 (HA89) contienen genes de silenciamiento, obtenidos por el cruzamiento reversible con HA89. Las líneas restauradoras con genes de silenciamiento, pueden identificarse y estos genes pueden ser eliminados. Los genes de silenciamiento escondidos en las líneas *cms*, representan problema. Las líneas que no tienen estos genes, como es HA821, deben ser utilizadas para formar líneas de mantenimiento. Es probable que el silenciamiento de genes se hereda simplemente. La formación de los marcadores moleculares, ayudaría en la identificación de las líneas sin genes de silenciamiento.

"ENDORMISSEMENT" DES GÈNES DE RESTAURATION DE FERTILITÉ DU TOURNESOL

RÉSUMÉ

Certains gènes dominants pour la restauration de la fertilité de la ligne cytoplasmique mâle stérile *cms*ANN2 ont été identifiés dans PI 413178, P21 et RCMG3 tandis que PI 413180, P21, RCMG1 et RHA280 ont été identifiés comme sources de restauration pour la ligne *cms*ANN 3. Cependant, dans les descendants des lignes de restauration homozygotes croisées avec HA89, RHA266 et RHA274, un certain nombre de plantes CMS a été enregistré. On croit que HA89, RHA266 et RHA274 possèdent des gênes qui empêchent, en fait endorment, l'expression de certains gènes *Rf*. HA821 n'a pas de gènes d'endormissement et, lors de croisements avec les lignes de restauration mentionnées, produit des descendants qui sont tous mâles fertiles. Les descendants F_2 de croisements demi-diallèles parmi les trois restaurateurs pour *cms*ANN2 et les cinq restaurateurs pour *cms*ANN3 étaient presque tous mâles fertiles, ce qui suggère la présence d'un gène *Rf* unique pour chacune des sources *cms*. Cependant la famille de descendants du test des combinaisons de croisements des plantes demi-diallèles F_1 avec les *cms*ANN2 et *cms*ANN3 respectivement n'étaient pas tous mâles fertiles mais la plupart des familles étaient composées de plantes toutes mâles stériles ou se séparant en plantes mâles stériles et mâles fertiles. Cela pourrait se produire si *cms*ANN2 (HA89) et *cms*ANN3 (HA89) contenaient des gènes d'endormissement obtenus par un croisement rétroactif avec HA89. Les lignes de restauration ayant des gènes d'endormissement peuvent être identifiées et ces gènes peuvent être éliminés. Les gènes d'endormissement cachés dans les lignes *cms* posent problème. Les lignes exemptes de ces gènes comme HA821 doivent être utilisées dans la création de lignes de maintien. L'endormissement des gènes semble être tout simplement hérité. Créer des marqueurs moléculaires aiderait à identifier les lignes exemptes de gènes d'endormissement.