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THE INHERITANCE OF MALE FERTILITY RESTORATION OF THE PET2, GIG1 AND MAXI SUNFLOWER CYTOPLASMIC MALE STERILE SOURCES

Aynur KURAL **Jery F. MILLER**

Southeastern Anatolia Agricultural Research Institute Diyarbakır, TURKEY and Dept. of Crop Weed Sciences, Nort Dakota state University, and USDA-ARS, Northern Crop Science Laboratory, Fargo, ND.

SUMMARY

Present production of hybrid sunflower (*Helianthus annus* L.) is exlusively based on one male sterility inducing cytoplasm of *H. petiolaris* Nutt. (PET1) (1). This single cytoplasm source provides a very narrow germplasm base and makes sunflower production vulnerable to unexpected disease and environmental hazards. The purpose of this investigation was to study new sources of cytoplasmic male sterility and their fertility restoration. Three cytoplasmic male sterile sources, CMS PET2, CMS GIG1, and CMS MAX1, were crossed with their respective restorers, RPET2, RGIG1, and RMAX1 (2). The analysis of F_2 and BC_1F_1 progenies indicated that two dominant genes in RPET2, one dominant gene in RGIG1, and two independent, complementary, dominant genes in RMAX1 appear necessary to restore male fertility to their respective cytoplasms.

The study of F_2 and BC_1F_1 progenies between CMS HA 89 (PET1 cytoplasm) and three male fertility restoring lines, RPET2, GIG1, and RMAX1, indicated that all three sources carry a single, dominant gene to restore male fertility of this cytoplasm. RHA 274 (Rf_1 restoration gene) was not effective for restoring the PET2 and GIG1 cytoplasms; however, it was effective for the MAX1 cytoplasm.

INTRODUCTION

At the present time, production of hybrid sunflower is exclusively based on the PETI cytoplasmic male sterility from *H. petiolaris*. A single cytoplasm source provides a very narrow genetic base and makes sunflower production vulnerable to unexpected disease and environmental hazards. Therefore, it is desirable to widen the cytoplasmic base of sunflower by developing additional and diverse sources of cytoplasms, and their restoration systems.

The purpose of this investigation was to study new sources of cytoplasmic male sterility and their fertility restoration. The objectives were (1) to determine the number of genes and type of gene action conditioning fertility restoration in the sources RPET2, RGIG1, and RMAX1 to their respective CMS sources and CMS PET1, and (2) to determine if the Rf1 gene is effective in restoring the PET2, GIG1, and MAX1 cytoplasms.

MATERIAL AND METHODS

The materials used in this study were; (1) three male-sterile sources, CMS PET2, CMS GIG1, CMS MAX1, and their respective restorers RPET2, RGIG1, RMAX1, (2) cytoplasmic male sterile line, CMS HA 89 (PET1 cytoplasm), (3) fertility restorer line, RHA 274.

These materials were supplied by the USDA Oilseed Research Unit, Fargo, ND. Crosses were made between CMS PET2 and RPET2, CMS GIG1 and RGIG1, and CMS MAX1 and RMAX1. Also, crosses were made between the CMS HA 89 (PET1) line and RPET2, RGIG1, and RMAX1. RHA 274, possesing the Rf1 restorer gene, was crossed to CMS PET2, CMS GIG1, and CMS MAX1.

Male-fertility observations on the F_1 plants at the plant stage R5.3 (3) were made and a backcross of fertile plants to their respective CMS parent was made to develop the BC_1F_1 generation. Also, the F_1 plants were self-pollinated to obtain seed for producing F_2 progeny. F_1 plants of the CMS PET2/RPET2, CMS GIG1/RGIG1 and CMS MAX1/RMAX1, and CMS HA 89 (PET1)/RPET2, RGIG1, RMAX1 were fertile. The BC_1F_1 and F_2 progenies were grown in the field nursey in the summer 1989 at Fargo, ND.

F_1 plants of the CMS PET2/RHA 274 and CMS GIG1/RHA 274 crosses were male sterile. F_1 plants of the cross between CMS MAX1 and RHA 274 were fertile and were self-pollinated to produce F2 seeds. Some populations could not be evaluated due to drought stress in the summer field nursery. F_1 plants of the cross between CMS MAX1 and RMAX1, and F_1 plants of the cross CMS GIG1/RGIG1 were planted in the 1989 fall greenhouse. The BC_1F_1 and F_2 progenies were grown in the 1990 spring greenhouse.

RESULTS

GENETIC STUDY OF CMS PET2/RPET2, CMS GIGI, AND CMS MAX1/RMAX1 CROSSES

Analysis of the cross between CMS PET2 and RPET2

All F_1 plants had fully developed pollen and were fertile, indicating dominant gene action for fertility restoration. The F_2 and BC_1F_1 progenies were grown in the summer 1989 field nursery. Fertile plants had normal anthers and fully developed, yellow pollen grains. Sterile plants did not develop anthers or pollen. The probability values for the F_2 plants of the two families indicated a good fit to a 15:1 (fertile:sterile) ratio. Also the probability value of the BC_1F_1 progenies gave a good fit to a 3:1 (fertile:sterile) ratio (Table 1).

Analysis of the cross between CMS GIGI and RGIGI

The F_2 and BC_1F_1 populations grown in the field in 1989 were severely affected by drought stress. Data on male fertility could not be taken. Therefore, this study was repeated in the 1989-1990 greenhouse.

In the winter greenhouse (1989-90), all F_1 plants were fertile and had fully developed pollen, indicating dominant gene action. The F_2 and BC_1F_1 progenies were grown. in the spring 1990 greenhouse. The fertile plants had normal anthers and fully developed pollen. The sterile plants developed an anther without or small amount of white, pale yellow, inviable pollen. The one F_2 family had a probability value that indicated a good fit to the expected 3:1 (fertile:sterile) ratio. The segregation of BC_1F_1 progenies was in agreement with the one dominant gene hypothesis with a 1:1 ratio of fertile to sterile plants (Table 1).

Analysis of the cross between CMS MAX1 and RMAX1

All F_1 plants of CMS MAX1/RMAX1 were fertile indicating dominant gene action. However, the plants had split anthers intstead of the normal five, fused anthers. Split anthers are usually associated with less pollen fertility and considered undesirable for hybrid seed production. Pollen production, however, was profuse and the pollen had a normal, yellow color.

In the summer field nursery, the BC_1F_1 plants were adversely affected by the hot, dry weather due to location of planting in the field. Most plants were sterile and fertility of pollen could not be determined for the BC_1F_1 progeny. One F_2 family was not affected severely by the field location. The probability value for the F_2 population indicated that segregation for male fertilty and sterility was in agreement with a 9:7 (fertile:sterile) phenotypic ratio. Due to the uncertainty of the data the field studies, this cross was repeated in the greenhouse. The probability value for the F_2 populations indicated a good fit to a 9:7 (fertile:sterile) ratio. The BC_1F_1 Chi-square value confirmed this hypothesis with a good fit to a 1:3 (fertile:sterile) phenotypic ratio.

CMS HA (PET1) STUDY

Analysisof the cross between CMS HA 89 (PET1) and RPET2, RGIG1, and RMAX1

All F_1 plants of the three crosses were fertile. The F_2 and BC_1F_1 progenies were grown in the summer 1989 field nursery and it appeared that restoration of male fertility was not affected by the dry and hot weather. The probability values for the F_2 progenies of the crosses indicated a good fit to a 3:1 phenotypic ratio of fertile to sterile plants. The segregation of BC_1F_1 progenies was in agreement whit a 1:1 ratio of fertile to sterile plants. These segregation suggest that all three sources cary a single dominant gene to restore male fertility of CMS HA 89 (PET1). Since RHA 274, which posses the Rf1 gene, did not restore the CMS PET2 and GIG1 cytoplasms, it is unlikely that the single dominant gene involved in this study is Rf1. The single dominant gene in RMAX1 is probably not Rf1, since male fertility of the CMS MAX1 was conditioned by two independent, complementary dominant genes, however RHA 274 fully restored CMS MAX1 cytoplasm (Table 1).

RHA 274 STUDY

Analysis of the cross between CMS PET2, CMS GIG1 and CMS2 MAX1 and RHA 274.

All F_1 progenies of the crosses CMS PET1/RHA 274 and CMS GIG1/RHA 274 were completely sterile. The cross between CMS MAX1 and RHA 274 had fully developed pollen and was fertile. The probability values for the F_2 progenies were in agreement with a 3:1 phenotypic ratio of fertile to sterile plants. The F_2 results were verified by the segregation of the BC_1F_1 prognies (Table 1).

Table 1. Male-fertile and male-sterile plants on F_2 and BC_1F_1 progenies and Chi-square analysis.

Cross	Generation	Plants (no) Fertile	Sterile	Expected ratio	P
CMS PET2/RPET2	F_2	246	20	15:1	.30-.50
	BC_1F_1	78	29	3:1	.50-.70
CMS GIG1/RGIG1	F_2	47	13	3:1	.50-.70
	BC_1F_1	58	58	1:1	1.00
CMS MAX1/RMAX1	F_2	76	60	9:7	.90-.95
	F_2	61	48	9:7	.90-.95
	BC_1F_1	39	111	1:3	.80-.90
CMS HA89/RPET2	F_2	389	132	3:1	.80-.90
	BC_1F_1	86	79	1:1	.50-.70
CMS HA89/RGIG1	F_2	200	66	3:1	.90-.95
	BC_1F_1	54	54	1:1	1.00
CMS HA89/RMAX1	F_2	91	24	3:1	.30-.50
	BC_1F_1	119	105	1:1	.30-.50
CMS MAX1/RHA274	F_2	232	87	3:1	.90-.95
	BC_1F_1	102	87	1:1	.20-.30

DISUSSION AND CONCLUSION

Present production of hybrid sunflower (*Helianthus annuus* L.) is exclusively based on one cytoplasm (PET1). The purpose of this investigation was to study new sources of cytoplasmic male sterility and their fertility restoration. The objectives were (1) to determine the number of genes and type of gene action conditioning fertility restoration in the sources RPET1, RGIG1 and RMAX1 to their respective CMS sources and CMS PET1, and (2) to determine if the Rf1 gene is effective in restoring the PET2, GIG1, and MAX1 cytoplasm.

Three cytoplasmic male sterile sources, CMS PET2, CMS GIG1, and CMS MAX1 were crossed with their respective restorer lines, RPET2, RGIG2, RGIG1, and RMAX1. Male-fertility observations on the F_1 plants were made and a backross of fertile plants to their respective CMS parents was made to develop the BC_1F_1 generation. At the same time, F_1 plants were self-pollinated to produce F_2 seeds. The F_2 and BC_1F_1 populations were grown in the field in 1989 and greenhouse 1989-1990.

The analysis for the F_2 and BC_1F_1 generations involving the RPET2 restorer line and the PET2 cytoplasm indicated that two dominant genes

were conditioning restoration of male fertility of CMS PET2. The RPET2 restorer line is homozygous dominant for the two alleles conferring male fertility restoration of the PET2 cytoplasm. These results disagree with Whelan's initial suggestion that two complementary, dominant genes were conditioning of this cytoplasm (4).

The analysis of F_2 and BC_1F_1 generations involving the RGIG1 restorer line and the GIG1 cytoplasm indicated that a single, dominant gene was conditioning restoration of male fertility of CMS GIG1. The fertile F_1 plants and the F_2 and BC_1F_1 ratios suggest complete dominant gene action. The RGIG1 restorer line is homozygous dominant for the alleles conditioning male fertility restoration of the GIG1 cytoplasm.

Restoration of male fertility of the MAX1 cytoplasm by RMAX1 was conditioned by two independent, complementary dominant genes. A dominant allele must be present at both loci for complete restoration.

A 3:1 (fertile:sterile) ratio was obtained on F_2 plants of the crosses between CMS HA 98 (PET1) and RPET2, RGIG1, and RMAX1. All three sources carry a single dominant gene to restore male fertility of CMS HA 89 (PET1). Further Studies are necessary to verify whether these genes are $\underline{Rf_1}$ or are different genes restoring male fertility of the PET1 cytoplasm.

RHA 274, which has the $\underline{Rf_1}$ fertility restoration gene . was only effective in restoring fertility of the MAX1 cytoplasm. The $\underline{Rf_1}$ gene does not appear to be effective for restoring the PET2 and GIG1 cytoplasms. Since the $\underline{Rf_1}$ gene is as effective as the two genes in RMAX1 in conditioning restoration of male fertility of the MAX1 ctoplasm, lines possessing the $\underline{Rf_1}$ gene could be utilized in addition to RMAX1 to produce hybrid seed with CMS MAX1. The results of this study also verify that the CMS MAX1 cytoplasm is different from CMS PET1, CMS PET2 and CMS GIG1.

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