Genetics of fertility restoration in Sunflower (*Helianthus annuus* L.)

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ABSTRACT

For employing diverse CMS lines belonging to different cytosterile sources in breeding programmes, knowledge on genetics of fertility restoration is pre requisite. With this objective an investigation was carried out to study the genetics on fertility restoration Fourteen crosses were made for studying the genetics of fertility restoration, five possessing PET1, three possessing PET 2, four with GIG1 and two with ARG cytoplasmic sources. Among these fourteen fertile crosses studied, F_2 segregation of 11 crosses revealed 3 : 1 ratio for fertile and sterile plants, respectively indicating the presence of a single dominant gene for fertility restoration. In the remaining three crosses, F_2 generation segregated in the ratio of 9 fertile and:7 sterile, suggesting the presence of two complementary genes for the fertility restoration.

KEYWORDS: Fertility restoration, inheritance, segregation, sunflower

INTRODUCTION

Sunflower is an important edible oilseed crop. Identification of cytoplasmic male sterility and fertility restorer genes ushered the way to develop number of commercial hybrids. Majority of the hybrids cultivated in the world are dependent on a single source of cytoplasm *i.e.,* PET 1. Dependence on a single source of cytoplasm may pose a threat to the cultivation of hybrids. If any new disease or insect pest becomes virulent on the cytoplasm. Diversification of cytoplasmic base helps in avoiding such a problem and in the development of heterotic hybrids. An attempt was made to study the genetics of fertility restoration for diverse CMS sources for their utilization in hybrid development.

MATERIALS AND METHODS

To study the inheritance of fertility restoration, fourteen fertile F_1s of six CMS lines belonging to different source of cytoplasm were selected. These hybrids were selfed to obtain the seed for inheritance studies. The heads of F_1 plants were covered with cloth bags to avoid cross pollination. The flower heads rubbed with hand to avoid improper seed filling, till the disc florets completed opening. The F_2 seed of each cross was planted in forty rows of 4.5m length with spacing of 60cm x 30cm during *rabi*, 2008-09. F_2 plants were classified into male sterile and male fertile based on dehiscence exertion and amount of pollen shed. Pollen fertility was confirmed in the laboratory using 1% Acetocaramine staining pattern (Chaudhary *et al*., 1981). For chi-square analysis, fully fertile and partially fertile plants were united into one class of male fertile plants clearly distinguished from male sterile plants that did not shed any pollen.

The F_2 data were subjected to chi-square test of goodness of fit.

Chi-square was calculated using the following formula

$$\chi^2 = \frac{\Sigma(O-E)^2}{E}$$

Where, O = Observed frequency of a class

E = Expected frequency of a class

The calculated chi-square value is compared with chi-square table value at 0.05 probability against the appropriate degrees of freedom to test the significance.

Experimental material:

S. No.	Hybrid	S. No	Hybrid
1.	CMS 234A x DRS 52	9.	DCMS 15 x DRS 45
2.	CMS 234A x DRS 19	10.	DCMS 15 x DRS 52
3.	CMS 234A x RHA 340	11.	DCMS 1 x RHA 348
4.	CMS 7-1A x DRS 55	12.	DCMS 1 x DRS 27
5.	CMS 7-1A x DRS 28	13.	DCMS 36 x DRS 27
6.	DCMS 6 x RHA 348	14.	DCMS 36 x RHA-340
7.	DCMS 6 x DRS 9		
8.	DCMS 6 x DRS 55		

RESULTS AND DISCUSSION

In the present investigation, inheritance of fertility restoration was studied in fourteen crosses using six CMS lines belonging to four diverse CMS sources of cytoplasm *i.e.,* PET-1, PET-2, GIG1 and ARG sources.

Based on their male sterile / fertile reaction during *kharif,* 2008 fourteen F_1 crosses consisting of two each based on DCMS 15, DCMS 1, CMS 7-1A and DCMS 36 and three based on DCMS 6 and CMS 234A (PET 1) showing complete fertile reaction were carried to next generation to know the nature of inheritance of fertility restoration based on the F_2 segregation ratio. The F_2 segregation data were subjected to chi-square test of goodness of fit.

Genetics of fertility restoration in CMS 234A (PET-1 cytoplasm)

Three hybrids (CMS 234A x DRS 52, CMS 234A x DRS 19 and CMS 234A x RHA 340) based on the CMS 234A were fully fertile in F_1 generation. The F_2 segregation ratios showed 3 : 1 fertile and sterile plants, respectively indicating the presence of single dominant gene for the fertility restoration. The results obtained are in consonance with that of Guo and Meng (1989), Seiler and Jan (1994), Trinadh Kumar (1999) and Rukmini Devi (2002).

Genetics of fertility restoration in CMS 7-1A (PET-1 cytoplasm)

With regard to CMS 7-1A, two fertile crosses were studied. In the F_2

generation of CMS 7-1A x DRS 55, the plants segregated in the ratio of 3 fertile : 1 sterile indicating the presence of a single dominant gene for the fertility restoration. In another cross *i.e*., CMS 7-1A x DRS 28, plants in F_2 generation segregated in the ratio of 9 fertile : 7 sterile, suggesting the presence of two complementary genes for the fertility restoration. Seilar and Jan (1994) reported that two complementary genes were restoring the fertility.

Genetics of fertility restoration in DCMS 6 (PET 2)

The crosses DCMS 6 x RHA 348, DCMS 6 x DRS 9, DCMS 6 x DRS 55 showed complete fertile reaction in F_1 generation showing the dominance of fertility over sterility. In the F_2 generation of DCMS 6 x RHA 341, DCMS 6 x DRS 9 and DCMS 6 x DRS 55, 372 fertile and 132 sterile plants; 309 fertile and 110 sterile plants, and 272 fertile and 101 sterile plants were observed, respectively (Table 2). Segregation of fertile and sterile plants in the F_2 generation involving these crosses were fitted to 3:1 ratio in both the crosses and confirmed χ^2 test. This suggested that a single dominant gene controlled the fertility restoration in PET 2 cytoplasm.

The results obtained are in consonance with that of Horn and Friedt (1997) and Madhavi Latha (2002), who also reported single dominant gene control of fertility restoration in the hybrids based on PET 2 cytoplasm whereas Kural and Miller (1992), Megale *et al.* (1992) and Trinadh Kumar (1999), reported that two complementary genes were essential for fertility restoration of PET 2 cytoplasm.

Genetics of fertility restoration in DCMS 1 (GIG-1)

Against the DCMS 1 also two restorers were utilized to study the genetics of fertility restoration (Table 3). The cross involving DCMS 1 showed the dominance of fertility over sterility by producing all fertile hybrids in F_1 generation. In the F_2, the cross DCMS 1 x RHA 348 showed 415 fertile and 147 sterile plants out of 562 plants thus fitting into segregation ratio of 3 fertile : 1 sterile plants and indicated the presence of a single dominant gene for the fertility restoration. On contrary the restorer DRS 27 with DCMS 1, in F_2 segregated into 228 fertile and 170 sterile plants thus showing 9:7 ratio of male fertile and sterile plants, respectively. The F_2 ratio of this cross suggested the presence of two complementary genes for fertility restoration. The control of inheritance of fertility restoration by two complementary genes in CMS GIG1 cytoplasm was also reported by Anashchenko and Duka (1985).

Genetics of fertility restoration in DCMS 15 (GIG1 Source)

In the crosses involving DCMS 15 (CMS GIG1), the inbreds used were DRS 45 and DRS 52 (Table 3). The F_1s of all the crosses were fully fertile indicating dominance of fertility over sterility.

It was observed that out of 592 plants of F_2 of DCMS 15 x DRS 45 segregated in a ratio of 452 fertile to 140 sterile plants indicating the presence of single dominant gene for the control of fertility restoration. In DCMS 15 x DRS 52, plants segregated into 216 fertile : 193 sterile (9 : 7) of the total 409 plants suggesting that two complementary genes were operating the fertility restoration (Table 3).

These results are in agreement with those obtained by Leclercq (1971), Vranceanu and Stoenescu (1973), Dominguez-Gimenez and Fick (1975), Guo and Meng (1989), Seiler and Jan (1994) and Trinadh Kumar (1999).

Genetics of fertility restoration in DCMS 36 (ARG)

In the F_2 generation of two fertile hybrids based on DCMS 36, plants segregated in the ratio of 3 fertile : 1 sterile, suggesting the presence of a single dominant gene for the fertility restoration. The results obtained are in agreement with that of Guo and Meng (1989), Horn and Friedt (1997) and Rukmini Devi (2002).

Vranceanu and Stoenescu (1978) reported that in three cases, fertility restoration was conditioned by three complementary genes and in one case by cumulative action of two non-allelic dominant genes. Dominguez-Gimenez and Fick (1975) and Whelan (1980) also reported the case in which fertility restoration was controlled by four non-allelic genes. However, in this study, no case was found in which three or more genes are responsible for pollen fertility restoration. Thus, it can be concluded from the present study that the fertility restoration ability was controlled by a single or two dominant genes with different interaction effects depending on the restorers involved and CMS lines possessing four different cytoplasmic sources.

The inheritance of the fertility restoration for the traditional source of cytoplasmic male sterility PET 1 is controlled by a single dominant gene and in some cases two independent, complementary dominant genes. This was reported earlier by Leclercq (1971), Enns (1972), Vranceanu and Stoenescu (1973), Stoyanova and Velkov (1974), Dominguez-Gimenez and Fick (1975), Mileeva (1981), Miller (1996) and Horn and Friedt (1997). Four loci with effective genes were identified, of which one is apparently present in the cytoplasmic male sterile parent (Seiler and Jan, 1994). The gene in the CMS parent is termed as fertility factor gene rather than restoration gene. Partial restoration of fertility has been observed in many sources indicating the presence of modifying genes which are often influenced by the environment, making their inheritance difficult to determine (Seiler and Jan, 1994).

Two types of genetic control for one CMS source may be due to differential response in different test inbreds under study. Regarding fertility restoration the differences in the results with the CMS lines might be due to the differences in the inbred lines used in the investigation. In this study, the specificity displayed by several inbred lines for fertility restoration further indicated that loci of restorer genes in an inbred line might vary from each other, which revealed the capacity to restore fertility. All the six CMS lines utilized in the experiment showed the diversity among themselves, thus broadening the genetic base of CMS lines which could be safely included in breeding programmes thereby mitigating the vulnerability of the lines to various pests and diseases.

Table 1: Segregation pattern of male fertile and male sterile plants in F_2 population of crosses with PET-1 cytoplasm

Cross	Total plants	Fertile reaction				Ratio tested	Calculated χ^2 value	Table χ^2 value
		Observed		Expected				
		Fertile	Sterile	Fertile	Sterile			
CMS 234A x DRS 52	580	449	131	435	145	3:1	1.8022	3.84
CMS 234A x DRS 19	495	365	130	371	124	3:1	0.4208	3.84
CMS 234A x RHA 340	450	329	121	338	112	3:1	0.8563	3.84
Cross	**Total Plants**	**Fertile reaction**				**Ratio tested**	**Calculated χ^2 value**	**Table χ^2 value**
		Observed		**Expected**				
CMS 7-1A x DRS 55	373	272	101	279.75	93.25	3:1	0.8588	3.84
CMS 7-1A x DRS 28	536	316	220	301.50	234.50	9:7	1.5939	3.84

Table 2: Segregation pattern of male fertile and male sterile plants in F_2 population of crosses with PET-2 cytoplasm

Cross	Total plants	Fertile reaction				Ratio tested	Calculated χ^2 value	Table χ^2 value
		Observed		Expected				
		Fertile	Sterile	Fertile	Sterile			
DCMS 6 x RHA 348	504	372	132	378	126	3:1	0.38	3.84
DCMS 6 x DRS 9	419	309	110	314	105	3:1	0.35	3.84
DCMS 6 x DRS 55	373	272	101	280	93	3:1	0.85	3.84

Table 3: Segregation of male fertile and male sterile plants in F_2 population of crosses with ARG Cytoplasm

Cross	Total plants	Fertile reaction				Ratio tested	Calculated χ^2 value	Table χ^2 value
		Observed		Expected				
		Fertile	Sterile	Fertile	Sterile			
DCMS 36 x DRS 27	562	408	154	421.50	140.50	3:1	1.7295	3.84
DCMS 36 x RHA 340	464	337	127	348.00	116.00	3:1	1.3908	3.84

Table 4: Segregation pattern of male fertile and male sterile plants in F_2 population of crosses with GIG-1 Cytoplasm

Cross	Total plants	Fertile reaction				Ratio tested	Calculated χ^2 value	Table χ^2 value
		Observed		Expected				
		Fertile	Sterile	Fertile	Sterile			
DCMS 15 x DRS 45	592	452	140	444	148	3:1	0.57	3.84
DCMS 15 x DRS 52	409	216	193	230	179	9:7	1.96	3.84
Cross	**Total plants**	**Fertile reaction**				**Ratio tested**	**Calculated χ^2 value**	**Table χ^2 value**
		Observed		**Expected**				
		Fertile	**Sterile**	**Fertile**	**Sterile**			
DCMS 1 x RHA 348	562	415	147	422	140	3:1	0.46	3.84
DCMS 1 x DRS 27	398	228	170	224	174	9:7	0.16	3.84

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