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Maintenance of Male Sterility and Fertility Restoration in Different CMS Sources of Sunflower (*Helianthus annuus* L.)

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Abstract: Fertility restoration in three diverse CMS sources of sunflower was studied using fifty inbreds (testers). While 22 inbreds maintained sterility of CMS PET 1 and CMS ARG 1, 28 inbreds restored their fertility. The third CMS line GIG 1, was maintained by all the inbreds indicating involvement of different gene(s). Most of the commercial sunflower hybrids are been produced using CMS PET 1. Now with the identification of restorers for CMS ARG 1, new more productive commercial hybrids can be produced. Efforts should be made to locate restorers for CMS GIG 1 for its utilization in production of sunflower hybrids.

Key words: CMS sources, inbreds, maintainers, restorers

INTRODUCTION

Hybrid breeding has developed successfully in sunflower over the last 30 years since the identification of cytoplasmic male sterility among progenies of the interspecific cross *Helianthus petiolaris*×*Helianthus annuus* by Leclercq (1969) and the subsequent discovery of pollen fertility restoration genes (Kinman, 1970; Leclercq, 1971; Vranceanu and Stoenescu, 1971). This source (PET 1 cytoplasm), of cytoplasmic male sterility has proved to be very stable and is used almost exclusively in breeding programmes throughout the world since late 1970s, when it replaced the NMS system for producing hybrid seeds that was being used in several European countries till the early 1970s.

Nevertheless, frequent use of the same sterile cytoplasm increases the genetic vulnerability of the present sunflower hybrids to diseases and pests. In order to minimize such a risk, new sources of cytoplasmic male sterility and corresponding fertility restorers are essential to increase the genetic diversity of the commercial hybrids. Inspite of the fact that new CMS sources continue to be discovered (Serieys, 2002), there are hardly any reports of their utilization for commercial hybrid production. The reluctance is presumably due to a lack of superior CMS-restorer combinations, as well as the time consuming conversion programs of CMS and restoration genes into inbred lines (Jan *et al*., 2006).

Success in heterosis breeding is largely dependant on the development of inbreds having broader genetic base. In general, inbreds with high combining ability and *per se* performance are either converted into CMS lines or fertility restorer lines for their future use in hybrid breeding programmes. Keeping this in view superior inbreds were evaluated for their maintainer and restorer behaviour, with the objective of identifying diverse sources of CMS maintainers and restorers. We herein make use of the easy method proposed by Chaudhary *et al*. (1981), for ascertaining the pollen fertility of crosses; leading to the identification of selected superior inbreds as maintainers and restorers of three diverse CMS sources, for the practical use of these inbreds in future sunflower breeding programme to augment the genetic diversity of sunflower hybrids.

MATERIALS AND METHODS

Three diverse cytoplasmic male sterile sources (lines) of sunflower viz., CMS PET 1 (*Helianthus petiolaris*) Leclercq (1969), CMS ARG 1 (*Helianthus argophyllus*) Christov (1992), CMS GIG 1 (*Helianthus giganteus*) (Whelan and Dedio, 1980) and fifty parental inbreds (testers) of diverse genetic background were obtained from Directorate of Oilseeds Research (DOR), Hyderabad.

Twenty rows each of the three cytoplasmic male sterile lines and two rows each of the fifty

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testers(inbreds) were planted in separate blocks during the rabi season, with a spacing of 60×30 cm. A row length of 5 m entry was maintained. Staggered sowings of male parents, twice at weekly interval, was done to synchronize the flowering. Recommended agronomic practices were followed.

The heads of male sterile lines and the inbreds were covered with cloth bags at the ray floret stage i.e., just before the commencement of flower opening. The three different CMS sources were crossed to all the fifty inbreds in a line x tester fashion. Crossing was done by collecting pollen from the inbreds in a petridish with the aid of a small brush which was applied on five florets each of the corresponding CMS lines between 8 to 11 am and the procedure repeated till the opening of all disc florets. Precautions were taken to avoid possible contamination. F_1 seeds from each of the 150 crosses were collected separately at maturity for assessing the fertility restoration of the 50 inbreds on the 3 CMS lines.

The identification of inbred behaviour, with respect to maintenance and restoration of the cytoplasmic male sterile sources of sunflower involved in the present study, was conducted during the spring season. F_1 seeds from the 150 crosses were planted in a replicated experiment with 3 replications. Three rows of 5 m for each F_1 entry were planted maintaining a row to row distance of 60 cm and a plant to plant distance of 30 cm. Pollen fertility percentage was calculated by classifying pollen grains as sterile or fertile following (Chaudhary *et al*., 1981). Based on these observations, the crosses were grouped as either sterile or fertile. The pollen parents leading to sterile crosses were classified as maintainers, while those that gave fertile crosses were classified as restorers of the corresponding CMS lines.

RESULTS AND DISCUSSION

It can be shown from Table 1 that twenty two inbreds namely; RHA 348, 7-1 B, 234 B, 302 B, 378 B, 851 B, 852 B, HA 341, HA 380, GP 290, GP 2008, GP 2111, GP 761, GP 898, M 307-2, M 1008, M 1015, M 1026, DRM 34-2, DRM 70-1, NDOL 87 and LTRR 1 produced sterile F_1s on the CMS PET 1 and CMS ARG 1 sources. Further, all fifty inbreds produced sterile F_1s on CMS GIG 1 as well. Though a minute fraction of aborted pollens (sterile pollens) was also observed, it can be seen from the Table 1 that twenty eight (RHA 271, RHA 273, RHA 274, RHA 297, RHA 298, RHA 341, RHA 344, RHA 345, RHA 346, RHA 356R, RHA 587, RHA 859, RHA 6D-1, HAM 161, HAM 174, HAM 175, HAM 180, SF 206, SF 207, SF 208, SF 211, SF 216, BLC P6, PARRUN 1329, RES 834-1, RCR 8297, R 83 R6 and NDLR-1) out of fifty inbreds produced sufficient fertile F_1s with CMS PET 1 and CMS ARG 1. The inbreds which produced sterile F_1s were classified as maintainers, while the ones that produced fertile F_1s were classed as restorers of the respective CMS sources (Table 2).

The present findings agree with the conclusion of Spirova (1990), regarding the infrequency observed for fertility restoration. This is more obvious in case of CMS GIG 1; whose fertility was not at all restored by any of the 50 inbreds evaluated in the present study. Moreover it is known that the restorer of one CMS source may act as a maintainer of other CMS types. Furthermore, the observation that while all 50 inbreds acted as maintainers of the cytoplasmic male sterility of GIG 1, 28 common inbreds acted as restorers of both CMS lines (PET 1 and ARG 1), suggests that while ARG 1 is similar to the French CMS source; PET 1, the cytoplasm of GIG 1 is different. Petrov and Nenov (1992), drew similar conclusions regarding the differences of three new CMS sources with the French CMS source PET 1. That the two CMS sources PET 1 and ARG 1 had different reactions than that of GIG 1; the third CMS source in the present study, to the inbreds, further indicates a distinct mechanism of cytoplasmic male sterility operating in CMS GIG 1 (Jan, 2000).

The results also revealed that RHA 274 is able to fully restore the fertility of CMS PET 1 and CMS ARG 1, whereas it failed to restore the fertility of CMS GIG 1. These results are in agreement with those of Havekes *et al*. (1991). RHA 274, (*H. petiolaris* restorer line) restorer with higher oil percentage, has also been found to completely restore the fertility of mutant CMS HA 89 lines produced by treating maintainer line HA 89 with mitomycin C and streptomycin (Jan and Rutger, 1988). Likewise RHA 274 has also been observed to restore the fertility of CMS PI 432513 (Jan and Vick, 1997, 1998, 2007). The restoration of CMS PET 1 and ARG 1 by RHA 274 observed in the present investigations, along with the above reports by various workers, suggests that RHA 274 is a useful restorer and, this inbred which likely carries Rf_1; the dominant *H. petiolaris* restorer gene (Jan and Vick, 2007), should be used in hybrid sunflower breeding programme, especially for higher oil content.

In the present investigations, sufficient restorers were observed for PET 1 and ARG 1. Since no restorers for CMS GIG 1 could be identified, the cytoplasm of CMS GIG 1 is indicated to be distinct from those of commercially used PET 1 cytoplasm as well as that of ARG 1. Hence it is safe to conclude that, while PET 1 can continue to be utilized for the production of commercial sunflower hybrids, promising hybrids with a different cytoplasm of ARG 1 can also be obtained. Similarly chances do exist for

Table 1: Frequency of F_1 sterile pollens (SP) and fertile pollens (FP) from anthers of *H. annuus* plants after crossing CMS PET 1, CMS ARG 1 and CMS GIG 1 with 50 inbred testers

	CMS line											
	PET 1				ARG 1				GIG 1			
Inbred	PF%	SP	FP	TP	PF%	SP	FP	TP	PF%	SP	FP	TP
RHA 271	96	2	48	50	98	1	54	55	0	0	0	0
RHA 273	97	2	53	55	94	3	49	52	0	0	0	0
RHA 274	96	2	50	52	95	3	49	52	0	0	0	0
RHA 297	95	3	48	51	93	3	45	48	0	0	0	0
RHA 298	96	2	50	52	96	2	49	51	0	0	0	0
RHA 341	93	4	49	53	96	2	51	53	0	0	0	0
RHA 344	96	2	52	54	97	2	51	53	0	0	0	0
RHA 345	95	3	50	53	94	3	46	49	0	0	0	0
RHA 346	82	9	43	52	98	1	53	54	0	0	0	0
RHA 348	0	0	0	0	0	0	0	0	0	0	0	0
RHA 356R	97	2	52	54	94	3	44	47	0	0	0	0
RHA 587	92	4	48	52	95	3	47	50	0	0	0	0
RHA 859	95	3	51	54	96	2	46	48	0	0	0	0
RHA 6D-1	97	2	53	55	98	1	51	52	0	0	0	0
7-1B	0	0	0	0	0	0	0	0	0	0	0	0
234B	0	0	0	0	0	0	0	0	0	0	0	0
302B	0	0	0	0	0	0	0	0	0	0	0	0
378B	0	0	0	0	0	0	0	0	0	0	0	0
851B	0	0	0	0	0	0	0	0	0	0	0	0
852B	0	0	0	0	0	0	0	0	0	0	0	0
HAM 161	96	2	51	53	96	2	47	49	0	0	0	0
HAM 174	97	2	52	54	95	3	48	50	0	0	0	0
HAM 175	96	2	53	55	97	2	48	50	0	0	0	0
HAM 180	97	2	54	56	96	2	50	52	0	0	0	0
HA 341	0	0	0	0	0	0	0	0	0	0	0	0
HA 380	0	0	0	0	0	0	0	0	0	0	0	0
GP 290	0	0	0	0	0	0	0	0	0	0	0	0
GP 2008	0	0	0	0	0	0	0	0	0	0	0	0
GP 2111	0	0	0	0	0	0	0	0	0	0	0	0
GP 761	0	0	0	0	0	0	0	0	0	0	0	0
GP 898	0	0	0	0	0	0	0	0	0	0	0	0
SF 206	92	4	47	51	98	1	52	53	0	0	0	0
SF 207	82	9	43	52	96	2	42	44	0	0	0	0
SF 208	91	5	46	51	95	2	42	44	0	0	0	0
SF 211	85	8	45	53	94	3	47	50	0	0	0	0
SF 216	97	2	52	54	96	2	53	55	0	0	0	0
M 307-2	0	0	0	0	0	0	0	0	0	0	0	0
M 1008	0	0	0	0	0	0	0	0	0	0	0	0
M 1015	0	0	0	0	0	0	0	0	0	0	0	0
M1026	0	0	0	0	0	0	0	0	0	0	0	0
DRM 34-2	0	0	0	0	0	0	0	0	0	0	0	0
DRM 70-1	0	0	0	0	0	0	0	0	0	0	0	0
NDOL 87	0	0	0	0	0	0	0	0	0	0	0	0
BLC P 6	97	2	49	50	96	2	51	53	0	0	0	0
PARRUN 1329	82	10	43	53	98	1	48	50	0	0	0	0
RES 834-1	82	9	43	52	97	2	51	53	0	0	0	0
RCR 8297	91	5	48	53	95	3	51	54	0	0	0	0
R 83 R6	97	2	50	52	97	2	51	53	0	0	0	0
NDLR 1	97	2	53	55	97	2	49	51	0	0	0	0
LTRR 1	0	0	0	0	0	0	0	0	0	0	0	0

%PF: Percent Pollen Fertility; SP: Sterile Pollen; FP: Fertile Pollen; TP: Total Pollen

the development of hybrids with CMS GIG 1 cytoplasmic background. This surmise is based on the fact that Havekes *et al*. (1991) have already recovered 50, 81 and 100% male fertile F_1 plants derived out of crosses between CMS GIG 1 and the male fertility restorer lines namely; RGIG 1, RPET 2 and RHA 294, respectively.

To be useful for hybrid seed production, a CMS line needs complete male sterility and female fertility. That male sterility of CMS GIG 1 is stable and completely maintained by 50 different inbred testers, is confirmed in the present experiment. Thus further selection, at least for fertility restoration of the CMS source GIG 1 is necessary before it can be used for commercial hybrid production. However since this CMS source (GIG 1), is from *H. giganteus*, a species different from the cultivated sunflower (*H. annuus*), it remains to be seen whether, it

Table 2: Identification of inbred behaviour for maintenance and restoration of diverse CMS sources of sunflower

	CMS line		
Inbred	PET 1	ARG 1	GIG 1
RHA 271	R	R	M
RHA 273	R	R	M
RHA 274	R	R	M
RHA 297	R	R	M
RHA 298	R	R	M
RHA 341	R	R	M
RHA 344	R	R	M
RHA 345	R	R	M
RHA 346	R	R	M
RHA 348	M	M	M
RHA 356R	R	R	M
RHA 587	R	R	M
RHA 859	R	R	M
RHA 6D-1	R	R	M
7-1B	M	M	M
234B	M	M	M
302B	M	M	M
378B	M	M	M
851B	M	M	M
852B	M	M	M
HAM 161	R	R	M
HAM 174	R	R	M
HAM 175	R	R	M
HAM 180	R	R	M
HA 341	M	M	M
HA 380	M	M	M
GP 290	M	M	M
GP 2008	M	M	M
GP 2111	M	M	M
GP 761	M	M	M
GP 898	M	M	M
SF 206	R	R	M
SF 207	R	R	M
SF 208	R	R	M
SF 211	R	R	M
SF 216	R	R	M
M 307-2	M	M	M
M 1008	M	M	M
M 1015	M	M	M
M1026	M	M	M
DRM 34-2	M	M	M
DRM 70-1	M	M	M
NDOL 87	M	M	M
BLC P 6	R	R	M
PARRUN 1329	R	R	M
RES 834-1	R	R	M
RCR 8297	R	R	M
R 83 R6	R	R	M
NDLR 1	R	R	M
LTRR 1	M	M	M

PET 1 (*Helianthus petiolaris*); R: Restorer; ARG (*Helianthus argophyllus*)., M: Maintainer; GIG 1 (*Helianthus giganteus*), Results are pooled observations of F_1s (CMS×inbreds), from a replicated experiment with three replications, for fertility reactions of inbreds

will contribute towards reduction of the genetic vulnerability of world wide sunflower hybrids, by providing an alternative to the CMS PET 1 cytoplasm.

Nevertheless, efforts toward identification of different restorers for CMS GIG-1 are desirable for greater genetic diversity to be used in the development of new restorer inbred lines (Gimenez and Fick, 1975) and the hybrids. The different CMS lines and their concerned maintainer and restorer inbreds of sunflower can be utilized directly in maintenance breeding and hybrid development programme.

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