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Identification of Restorers with Desirable General Combining Ability from among New Inbred Lines of Sunflower (*Helianthus annuus* L.)

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A B S T R A C T

The present investigation was carried out in order to study the sterility maintenance and fertility restoration behavior of 47 new inbred lines on three CMS lines and also to estimate the combining ability and heterosis by crossing them in a L × T mating design. The resultant 141 hybrids along with their parents and two checks *viz.*, KBSH-44 and KBSH-53 were evaluated in simple lattice design. Out of 47 inbred lines studied, 10 inbred lines *viz*., DOR-P-40 (oleic), GMU-456, GMU-716, GMU-746, GMU-759, GMU-773, GMU-785, GMU-1092, GP_6-952 and GP_6- 990 were identified as common fertility restorers for all the three CMS lines. Common fertility restorers for all the three CMS lines revealed that, even though the CMS lines differed in nuclear background, the fertility restoration genes could be same. It was evident from the present investigation that few inbred lines behaved differentially in the three CMS lines with respect to sterility maintenance and fertility restoration behavior, suggesting the presence of modifying genes, which could influence the fertility restoration thus, resulting in partial fertility. The identified 10 fertility restorer lines with high *gca* effect could be directly used as male parent in heterosis breeding programme for the development of superior hybrids.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crop in the world belonging to family Asteraceae with 2n=2x=34 chromosomes. It is primarily grown for edible oil which has high concentration of polyunsaturated fatty acids *viz.*, linoleic and oleic acids and hence considered as good quality oil from the health point of view. It is also a good source of calcium, phosphorus, nicotinic acid and vitamin E (Aslam *et al.*, 2010). In India, commercial cultivation of sunflower started during 1972 with the introduction of four Russian varieties *viz.*, EC 68413 (Vniimk), EC 68414 (Perdovik), EC 68415 (Armavirskii) and EC 69874 (Armaverts). In the initial year of its introduction, sunflower cultivation suffered a setback due to poor seed set and other related problems in the open pollinated varieties. Commercial exploitation of heterosis in sunflower began after the discovery of a stable source of cytoplasmic male sterility CMS PET 1 by Leclercq (1969) in the progeny of cross between *Helianthus petiolaris* Nutt and cultivated sunflower (cv. Armavirskii 9345) and subsequent identification of fertility restoration genes by Kinman (1970) and Vranceanu and Stoenescu (1971). The discovery of CMS-restorer system led to the shift from population breeding to heterosis breeding (Asif *et al.,* 2013).

The ideal method to develop new restorer lines (R line) is by crossing R × R lines from restorer gene pools, which are already identified for fertility restoration in sunflower. In order to identify new restorer lines in sunflower the superior inbred line is crossed with the CMS line and the performance of the male fertility or sterility data of the F1 plants is checked for restorer/maintainer reaction. The inbred which restorers 100% fertility restoration in CMS line is designated as new restorer line for the CMS line. The superior inbred line is produced either through systematic breeding programme or by selection from agronomically superior germplasm and selfing for 6-7 generations. (Dudhe *et al.,* 2009)

Efficiency of developing high frequency of heterotic CMS-based hybrids for commercial exploitation depends (among others) on the availability of large numbers of fertility restorer lines (R-lines). The R-lines should possess effective restorer genes for complete restoration of fertility in the hybrids developed from CMS lines. From among the identified R-lines, it is desirable to use only those R-lines with significant *gca* effects. The *gca* effect is being used as a dependable criterion for selection of parents for use in developing and identifying heterotic hybrids. The *gca* effect of a genotype also provide useful clues about the mode of action of genes controlling target traits. It is also useful in predicting the performance of untested hybrids in the absence of *sca* effects. The support for the use of gca as one of the criteria for choosing parents comes from theoretical results that indicates higher heterosis in the hybrids derived from the parents differing in their gene frequency. Parents differ in their gca due to differences in frequency of genes with additive effects. Under these premises, the present study was carried out with the objective to identify sterility maintainers and fertility restorers of new inbred lines. (Jan *et al.,* 2007).

Materials and Methods

The material used for the study consisted of three cytoplasmic male sterile lines *viz.*, CMS 234A, NDCMS 2A and CMS 911A, 47 new inbred lines as testers and two standard check hybrids KBSH 44 and KBSH 53. The CMS lines and the testers were collected from AICRP on sunflower, ZARS, UAS, GKVK, Bengaluru.

All the three CMS lines and 47 inbred lines were sown in the field to effect crossing in a Line × Tester fashion in order to obtain F_1's. Staggered sowing of all CMS lines were carried out three times at an interval of two days to ensure synchronized flowering with inbred lines for crossing. A day prior to opening of first ray floret all the heads of CMS lines and testers were covered with cloth bags in order to prevent undesirable pollination. Pollen from the inbred lines was collected separately in petri dishes with the help of camel hair brush and applied to the flowers of female lines using brushes during morning hours. The pollination was repeated for five to six days in each of the combination to ensure sufficient seed set and simultaneously all inbreds were sib pollinated. For investigation on sterility maintenance or fertility restoration reaction and gca of inbreds hybrids were evaluated with two replications along with parents during *kharif* – 2016.

Experiment was laid out in simple lattice design (14 × 14 involving hybrids, parents and checks). Each genotype was sown in a single row of three-meter length with a row spacing of 60 cm and 30 cm between plants within a row.

All the F_1 progenies at flowering were visually screened for complete male fertility or sterility reaction to know the fertility restoration or sterility maintenance behavior of inbred lines based on the presence or absence of pollen, anther dehiscence and pollen shedding at the anthesis stage in all the plants.

Based on the extent of fertility restored and sterility maintained by the respective inbreds in the crosses, inbreds used in the study were classified as fertility restorers, if all the F_1 plants were fertile, as sterility maintainers, if all the plants in F_1 were sterile and those which were segregating for fertility and sterility were considered as partial restorers. Based on this data, F_1s were grouped as male sterile or male fertile or partially fertile. Further each inbred lines were classified as sterility maintainers or fertility restorers or partial restorer types for the respective CMS lines.

Pollen fertility test

During flowering, in each of five randomly selected plants, 10-12 disc florets were collected in the morning hours. These were examined for pollen fertility by preparing anther smears in one *per cent* acetocarmine stain. Deeply stained pollen grains with good exine were considered as fertile. While, poorly stained and shriveled pollen grains were taken as sterile. By this way different types of reactions of inbred lines were confirmed by pollen fertility test using one *per cent* acetocarmine (Chaudhary *et al.*, 1981).

Results and Discussion

The extent of fertility restored and sterility maintained by the respective inbreds in the crosses is presented in Table 1. The new inbred lines which resulted in hybrids with complete fertility were classified as fertility restorers, with completely sterile plants as sterility maintainers and those which resulted in hybrids segregating for both fertility and sterility were considered as partial restorers.

Fertility restoration and sterility maintenance reaction of three CMS lines with 47 inbred lines are presented in Table 2a. F_1 hybrids showing fertility restoration and sterility maintenance reaction is depicted in Figure 1. Differential staining of pollen showing fertile / sterile reaction is depicted in Figure 2.

Out of the 47 pollen parents tested for fertility restoration on three CMS lines, some turned out to be sterility maintainers, some acted as fertility restorers and some as partial restorers. In general out of 141 crosses 40 F_1's (29.78 %) were fertile, 82 cross combinations (58.15 %) were sterile and 19 cross combinations (13.47 %) resulted in restoring partial fertility for three CMS lines *viz*., CMS 234A, NDCMS 2A and CMS 911A. It is evident from the data that the probability of identifying the restorers from out of new inbred lines screened with three different CMS lines, is almost same 27.65 per *cent* for CMS 234A, 25.53 *per cent* for NDCMS 2A and 31.93 *per cent* for CMS 911A. The frequency of sterility maintainer / fertility restorer behavior (%) of inbred lines for three CMS lines is given in the Table 2b.

Table.1 Per cent of male fertility and sterility as indicated by pollen fertility test (%) in the F_1 generation of the crosses between three CMS lines and 47 inbred lines

Sl. No.	Inbred lines	CMS 234A		Status	NDCMS 2A		Status	CMS 911A		Status
		Fertility	Sterility		Fertility	Sterility		Fertility	Sterility	
1	AKSFI-78	100.00	0.00	Fertile	33.33	66.66	Partially sterile	73.33	26.66	**Partially sterile**
2	CGP-10	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
3	DOR-P-40	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
4	DRSI-106	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
5	DRSI-530	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
6	DRSI-550	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
7	GMU-266	31.57	68.42	Partially sterile	12.50	87.25	Partially sterile	33.33	66.66	**Partially sterile**
8	GMU-302	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
9	GMU-324	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
10	GMU-325	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
11	GMU-355	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
12	GMU-420	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
13	GMU-452	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
14	GMU-456	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
15	GMU-469	21.06	78.94	Partially sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
16	GMU-485	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
17	GMU-489	80.00	20.00	Partially sterile	33.33	66.66	Partially sterile	100	0.00	**Fertile**
18	GMU-502	0.00	100.00	Sterile	00.00	100.00	Sterile	0.00	100.00	**Sterile**
19	GMU-563	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
20	GMU-575	0.00	100.00	Sterile	0.00	100.00	Sterile	20.00	80.00	**Partially sterile**
21	GMU-711	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
22	GMU-716	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
23	GMU-719	50.00	50.00	Partially sterile	77.77	22.22	Partially sterile	88.88	11.11	**Partially sterile**
24	GMU-746	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**

25	GMU-759	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
26	GMU-773	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
27	GMU-774	52.64	47.36	Partially sterile	68.75	31.25	Partially sterile	90.00	10.00	**Fertile**
28	GMU-785	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
29	GMU-1058	100.00	0.00	Fertile	100.00	0.00	Fertile	0.00	100.00	**Sterile**
30	GMU-1060-I	0.00	100	Sterile	0.00	100.00	Sterile	100.00	0.00	**Fertile**
31	GMU-1075	35.29	64.70	Partially sterile	60.00	40.00	Partially sterile	0.00	100.00	**Sterile**
32	GMU-1092	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
33	GP6-18	0.00	100.00	Sterile	0.00	100.00	Sterile	100.00	0.00	**Fertile**
34	GP6-131	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
35	GP6-135	100.00	0.00	Fertile	100.00	0.00	Fertile	0.00	100.00	**Sterile**
36	GP6-217	0.00	100.00	Sterile	0.00	100.00	Sterile	100.00	0.00	**Fertile**
37	GP6-236	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
38	GP6-792	72.22	27.77	Partially sterile	85.00	15.00	Partially sterile	0.00	100.00	**Sterile**
39	GP6-794	0.00	100.00	Sterile	0.00	100.00	Sterile	33.33	66.66	**Partially sterile**
40	GP6-799	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
41	GP6-819	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
42	GP6-952	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
43	GP6-990	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	**Fertile**
44	IB-55	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
45	IB-107	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
46	PSECO-65	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	**Sterile**
47	**PSCCO-87**	**0.00**	**100.00**	**Sterile**	**0.00**	**100.00**	**Sterile**	**0.00**	**100.00**	**Sterile**

Table.2a Male fertility restoration / male sterility maintainer reaction of new inbred lines under three nuclear backgrounds of PET 1 CMS cytoplasm

Sl. No.	INBRED LINES	CMS234A	NDCMS 2A	CMS 911A
1	AKSFI-78	Restorer	Partial restorer	partial restorer
2	CGP-10	Maintainer	Maintainer	Maintainer
3	DOR-P-40	Restorer	Restorer	Restorer
4	DRSI-106	Maintainer	Maintainer	Maintainer
5	DRSI-530	Maintainer	Maintainer	Maintainer
6	DRSI-550	Maintainer	Maintainer	Maintainer
7	GMU-266	Partial restorer	Partial restorer	Partial restorer
8	GMU-302	Maintainer	Maintainer	Maintainer
9	GMU-324	Maintainer	Maintainer	Maintainer
10	GMU-325	Maintainer	Maintainer	Maintainer
11	GMU-355	Maintainer	Maintainer	Maintainer
12	GMU-420	Maintainer	Maintainer	Maintainer
13	GMU-452	Maintainer	Maintainer	Maintainer
14	GMU-456	Restorer	Restorer	Restorer
15	GMU-469	Partial restorer	Maintainer	Maintainer
16	GMU-485	Maintainer	Maintainer	Maintainer
17	GMU-489	Partial restorer	Partial restorer	Restorer
18	GMU-502	Maintainer	Maintainer	Maintainer
19	GMU-563	Maintainer	Maintainer	Maintainer
20	GMU-575	Maintainer	Maintainer	Partial restorer
21	GMU-711	Maintainer	Maintainer	Maintainer
22	GMU-716	Restorer	Restorer	Restorer
23	GMU-719	Partial restorer	Partial restorer	Partial restorer
24	GMU-746	Restorer	Restorer	Restorer
25	GMU-759	Restorer	Restorer	Restorer
26	GMU-773	Restorer	Restorer	Restorer
27	GMU-774	Partial restorer	Partial restorer	Restorer
28	GMU-785	Restorer	Restorer	Restorer
29	GMU-1058	Restorer	Restorer	Maintainer
30	GMU-1060-I	Maintainer	Maintainer	Restorer
31	GMU-1075	Partial restorer	Partial restorer	Maintainer
32	GMU-1092	Restorer	Restorer	Restorer
33	GP6-18	Maintainer	Maintainer	Restorer
34	GP6-131	Maintainer	Maintainer	Maintainer
35	GP6-135	Restorer	Restorer	Maintainer
36	GP6-217	Maintainer	Maintainer	Restorer
37	GP6-236	Maintainer	Maintainer	Maintainer
38	GP6-792	Partial restorer	Partial restorer	Maintainer
39	GP6-794	Maintainer	Maintainer	Partial restorer
40	GP6-799	Maintainer	Maintainer	Maintainer
41	GP6-819	Maintainer	Maintainer	Maintainer
42	GP6-952	Restorer	Restorer	Restorer
43	GP6-990	Restorer	Restorer	Restorer
44	IB-55	Maintainer	Maintainer	Maintainer
45	IB-107	Maintainer	Maintainer	Maintainer
46	PSECO-65	Maintainer	Maintainer	Maintainer
47	**PSCCO-87**	**Maintainer**	**Maintainer**	Maintainer

Table.2b Frequencies of maintainers, restorers and partial restorer lines for three nuclear backgrounds of PET 1 CMS cytoplasm

CMS lines	Inbred lines evaluated	Number of inbred lines behaved as		
		Restorers	Maintainers	**Partial restorers**
CMS 234A	47	13 (27.65 %)	27 (57.44 %)	**7 (14.89 %)**
NDCMS 2A	47	12 (25.53 %)	28 (59.57 %)	**7 (14.89 %)**
CMS 911A	**47**	**15 (31.91 %)**	**27 (57.44 %)**	**5 (10.63 %)**

Figures in the parenthesis indicate per cent estimates

Table.3 Common sterility maintainer and fertility restorer inbred lines for all the three CMS lines in sunflower

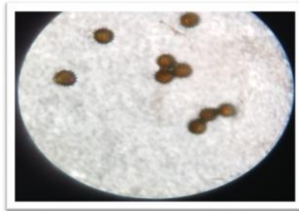
	Inbred lines	Total
Common Restorers	DOR-P-40 (oleic), GMU-456, GMU-716, GMU-746, GMU-759, GMU-773, GMU- 785, GMU-1092, GP_6-952, GP_6-990.	**10**
Common maintainers	**CGP-10, DRSI-106, DRSI-530, DRSI-550, GMU-302, GMU-324, GMU-325, GMU- 355, GMU-420, GMU-452, GMU-485, GMU-502, GMU-563, GMU-711, GP_6-131, GP_6-236, GP_6-799, GP_6-819, IB-55, IB-107, PSECO-65, PSECO-87.**	**22**

Fig.1 Fertile and sterile reaction in F_1 hybrids

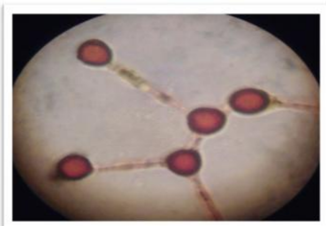
FERTILE REACTION IN F_1 HYBRIDS

STERILE REACTION IN F_1 HYBRIDS

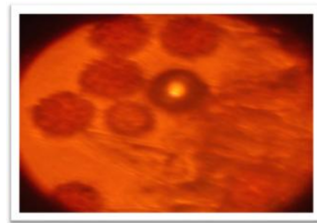
Fig.2 Staining of pollen using acetocarmine depicting fertile and sterile pollen



FERTILE POLLEN



STERILE POLLEN



PARTIAL FERTILE POLLEN

Sunflower breeders have extensively exploited the PET 1 source of male sterility to develop high yielding hybrids in cross combinations. Among the lines CMS 234A, NDCMS 2A and CMS 911A were widely used in present day heterosis breeding programme. Hence, attempts are made to identify new restorer lines to exploit heterosis for same CMS back ground.

Out of 47 inbred lines studied, 10 inbred lines *viz*., DOR-P-40 (oleic), GMU-456, GMU-716, GMU-746, GMU-759, GMU-773, GMU-785, GMU-1092, GP_6-952, and GP_6-990 showed fertility restoration in the F_1's derived from three CMS lines. Whereas twenty inbred lines viz., CGP-10, DRSI-106, DRSI-530, DRSI-550, GMU-302, GMU-324, GMU-325, GMU-355, GMU-420, GMU-452, GMU-485, GMU-502, GMU-563, GMU-711, GP_6-131, GP_6-236, GP_6-799, GP_6-819, IB-55, IB-107, PSECO-65 and PSECO-87 were sterility maintainers in F_1's of all three CMS lines. Common sterility maintainer and fertility restorer inbred lines with all the three CMS lines is presented in Table 3. However, majority of the inbreds tested were sterility maintainers of CMS sources suggesting the absence of fertility restoration genes in these inbreds. This indicated that, though CMS lines differed by nuclear background, the fertility restoring genes could be same. Similar results were obtained by Virupakshappa and Jayarame Gowda (1996), Neelima *et al.*, (2011), Meena and Sujatha (2013), Satish Chandra and Sudheer Kumar (2011).The inbreds which were grouped as maintainers after testing for combining ability coupled with good agronomic performance could be used to develop heterotic hybrids (Yogesh *et al.*, 2007) or to develop new CMS lines (Sujatha and Vishnuvardhan Reddy, 2008).

The results revealed that the inbreds GMU-1058 and GP_6-135 were fertility restorers on two CMS background CMS *viz.*, NDCMS 2A and CMS 234A. The inbreds GMU-575, GMU-1060-I, GP_6-18, GP_6-217 and GP_6-794 were maintainers for two CMS lines *viz.*, CMS 234A and NDCMS 2A. However, in case of line CMS 911A, the inbreds GMU-489, GMU-774, GMU-1060-I, GP_6-18 and GP_6-217 were complete fertility restorers, however the inbreds GMU-575 and GP_6-794 were partial restorers. Hence it is evident that with respect to maintenance and restoration of fertility, few inbreds differed in all three CMS background, suggesting the influence of modifying genes on fertility restoration which results in partial fertility (Rukmini Devi *et al.*, 2006; Dudhe *et al.,* 2009). This indicates the presence of partial fertility restorer in a heterozygous condition or a possible contamination with the unknown pollen (Yogesh *et al.,* 2007).

It was observed that GMU-774, GMU-489, GMU-1075, and GP_6-792 showed partially fertility reaction for two lines *viz.,* CMS 234A and NDCMS 2A, whereas the inbreds GMU-774, GMU-489 were fertility restorers and GMU-1075, GP_6-792 were maintainers for CMS 911A background. The inbred AKSFI-78 was found to be partial fertility restorer for NDCMS 2A, CMS 911A and complete fertility restorer for CMS 234A respectively. The inbred GMU-469 was partial fertility restorer for CMS 234A, whereas for NDCMS 2A, CMS 911A was sterility maintainer. Similar reports were made by Wankhade *et al.,* (2004). It is evident from present investigation that few inbreds behaved differently with the three CMS lines with respect to maintainance and restoration of fertility suggesting the influence of modifying genes on fertility restoration, resulting in partial fertility (Rukmini Devi *et al.*, 2006; Dudhe *et al.*, 2009). The inheritance of partial restoration may be complex and highly dependent on environmental condition. These findings are in agreement with those of Rukmini Devi *et al.*, (2006). Based on general combining ability effects of the inbred lines which were identified as promising fertility restorers and sterility maintainers in different nuclear background could be utilized for hybridization programme. Worth converting inbreds into male sterile versions could be identified. These results are similar to those reported by Yogesh *et al.*, (2007) and Sujatha and Vishnuvardhan Reddy (2008).

In conclusion the common fertility restorers for all the three CMS lines revealed that, even though the CMS lines differed in nuclear background, the fertility restoration genes could be same. It was evident from the present investigation that few inbred lines behaved differentially in the three CMS lines with respect to sterility maintenance and fertility restoration behavior, suggesting the presence of modifying genes, which could influence the fertility restoration thus, resulting in partial fertility. The 10 fertility restorer lines with high *gca* effect identified could be directly used as male parent in heterosis breeding programme for the development of superior hybrids. Among 22 sterility maintainer lines, few promising lines with high *gca* effect could be converted into new CMS lines through backcross breeding.

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