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Research Note

Identification of fertility restorers and maintainers in sunflower (*Helianthus annuus* L.) from gene pools and exotic material

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Abstract
Diversification of parental base in any hybrid breeding programme is an important step to sustain the crop. For considering above was taken up to explore the possibilities of finding out good restorers and maintainers of sunflower based on sterility and pollen fertility. Thirty two inbreds of sunflower from gene pool (GP lines) and fourteen exotic collection (EC lines) inbreds of economic importance were crossed with six cytoplasmic male sterile lines *viz.,* CMS-17A, CMS-2A, CMS-10A, CMS-234A, CMS-852A and CMS-7-1A in a Line x Tester fashion to study their maintainer or restorer reaction during *kharif*-2014. The accessions from gene pool, GP_6-1051 acted as restorers for all six CMS lines. GP_6-46 and GP_6-236 were acted as restorers for CMS-10A, CMS-234A, CMS-852A and CMS-7-1A. It was found that GP_6-54 of the restorer for CMS-10A and CMS-852A and maintainer for other CMS lines. From exotic collection EC-601622, EC-601664, EC-601666, EC-601672, EC-601691, EC-601726, EC-601821, EC-601848 and EC-601711-1 were acted as restorers for all six CMS lines. Majority of the gene pool lines and exotic collection were behaved as maintainer for all the CMS. The range of fertility restoration in different cross combinations was between 93.1 and 97.8 per cent. In the present study maximum frequency of pollen fertility restoration was reported for CMS-7-1A (41.3%) followed by CMS-852 (36.95%), CMS-234A (36.95%) and CMS-10A (34.78%). However, lowest frequency of pollen fertility restoration was observed for CMS-17A (28.26%).From the study it was evident that among the six CMS lines, restorers for CMS-17A are rather scarce. Efforts should be made to locate restorers for CMS-17A for its utilization in breeding of more productive sunflower hybrids.

Keywords
Sunflower CMS sources, inbreds, maintainer, restorer

Sunflower (*Helianthus annuus* L.) is an important edibleoilseed crop. Its allogamous nature offers scope for development of hybrids for commercial cultivation. Commercial cultivation of sunflower was started in India during 1972 with the introduction of four open pollinated varieties. In sunflower, hybrids are superior over open-pollinated cultivars in terms of yield, self-fertility and resistance to diseases (Miller, 1987). The first cytoplasmic male sterile source was *Helianthus petiolaris* (PET-1), discovered by Leclercq, (1969) in France and for which fertility restoration genes were subsequently identified by Kinman (1970) in USA and many other researchers. This led to the exploitation of hybrid vigour and commercial use of hybrid sunflower. From, 1972 onwards, many hybrids were developed and released for commercial cultivation. Success in heterosis breeding programme is largely dependents upon the development of inbreds with broad genetic base (Giriraj, 1998) with high combining ability and *per se* performance. The best inbreds identified have to be converted into CMS lines before being used in hybrid development. Those inbreds from maintainer gene pool are used for new CMS lines development and those from restorer gene pools are used as male lines in hybrid breeding programme. Hence, the superior inbreds must be evaluated for maintainer or restorer. The present investigation was taken up to explore the possibilities of finding out good restorers and maintainers based on sterility and fertility reactions in the six CMS of PET-1 back ground.

A total 46 gene pools and exotic collection *viz.*, 14 sunflower exotic collection (EC) lines, 31 gene pool (GP) lines and one inbred line Selection-1000 were used for crossing with six CMS lines *viz.*, CMS-17A, CMS-2A, CMS-10A, CMS-234A, CMS-852A and CMS-7-1A in Line x Tester design during the winter season of 2013. Before flowering (star bud stage) all the heads in the lines (CMS lines) and testers (GP, EC and inbred lines) were covered with cloth bags to prevent open pollination. The pollen from the male lines was collected separately in petri-dishes with the help of camel hair brush, during morning hours (9:00 to 11:00 AM) and pollinated to each of the female lines separately and cloth bags were replaced immediately after pollination. The crossing was repeated (alternate day) till all the disc florets completed their opening. Each test hybrid was grown in a two rows of 3.0 m with 60 x 30 cm row to row and plant to plant distances during the rainy season of 2014 at the Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad in Randomized Block Design with two replications. Recommended

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agronomic practices (fertilization application, earthing up, irrigation, insecticide spraying) were followed to raise a successful experimental crop. At the time of flowering, individual plants in each cross were observed for anther exertion and pollen shedding at anthesis stage and the crosses were categorised into male fertile, male sterile and partially fertile which correspond to restorer, sterile and partially fertile behaviours of gene pool and exotic collection lines, respectively. Pollen fertility percentage was calculated by classifying pollen grains as sterile or fertile (Chaudhary *et al.*, 1981). The pollen fertility was calculated as the ratio between the number of fertile (round and darkly stained) and sterile (yellow, sheveal, partial stained or unstained) pollen grain in the microscopic field (Table-1). Plants were classified into different fertility-sterility groups based on proportion of stained-round pollen grains, as the number of fertile grains/total number observed x 100.

The data indicated that majority of the lines being tested, behaved as maintainers for all CMS lines (Table-2). Frequency of tested material as maintainers/restorer lines based on percent fertility restoration over different CMS sources is presented in Table-3. The restorer for one CMS line behaved as maintainer for other CMS line and vice versa, confirming the diversity among the CMS lines. In this study we found that only 12 out of 46 lines tested behaved as restorers for all six CMS lines and produced fertile hybrids. Similar results were also reported by Reddy *et al.* (2002); Reddy *et al.* (2008); Neelima and Ashok Kumar, (2011) and Satish Chander *et al.* (2011).

From the gene pool, only one accession GP_6-1051 behaved as restorer for all the six CMS lines. While, out of 14 sunflower exotic lines, 11 lines (EC-601622, EC-601664, EC-601666, EC-601669, EC-601672, EC-601691, EC-601704, EC-601726, EC-601821, EC-601848 and EC-601711-1) could restore fertility for all the CMS lines. However, one inbred (Selection-1000) behaved as maintainer for all the tested CMS lines. It was found that GP_6-46 and GP_6-236 behaved as restorers of CMS-10A, CMS-234A and CMS-7-1A while, behaved as maintainers of the other CMS lines. GP_6-54 behaved as restorer of CMS-10A and CMS-852A while, behaved as maintainer of other tested CMS lines. Rukminidevi *et al.* (2006); Wankhade *et al.* (2004) and Sujatha and Vishnuvardhan Reddy, (2008) also reported lack of fertility restorers other than PET-1. Recently, Meena *et al.* (2013) also reported lack of fertility restorers in PET-1 background.

GP_6-135 line acted as maintainer for most of the CMS lines while acted as partial restorer for CMS-234A and CMS-7-1A. GP_6-349 behaved as maintainer for CMS-2A, CMS-10A and CMS-234A while acted as partial restorer for CMS-17A, CMS-852A and CMS-7-1A. Another gene pool line, GP_6-534 behaved as restorer for CMS-234A and CMS-852A and maintainer for CMS-17A and CMS-2A while acted as partial restorer for CMS-10A. GP_6-1436 and GP_4-1217 behaved as maintainers for most of the CMS tested while acted as partial restorers for CMS-17A and CMS-7-1A respectively. A few crosses showed segregation with one or two fertile/sterile plants in their progeny. This was attributed either to contamination of foreign pollen or the heterozygosity of the lines to restorer genes (Virupakashappa *et al.*, 1991) or a possible contamination with the unknown pollen (Yogesh *et al.* 2007) or may be due to modifying effects of genes (Dominguez-Gimenez and Fick, 1975).

Regarding maintainer behaviour of gene pools and exotic collection lines, most of the lines acted as maintainers for all CMS lines (Table-2). A few restorers were also identified for six CMS lines. The differential behaviour of the lines for fertility/sterility reaction may be attributed to genetic architecture especially the number of genes controlling and their interactions with cytoplasm in restoring fertility (Meena and Sujatha, 2013). Thus the present study helped in identification of a few effective restorers for the available CMS lines. Large numbers of maintainers are available when compared to restorers in the experimental material indicating that there is wide choice for CMS conversion programme. The newly identified restorers for the different CMS sources will be useful for exploiting heterosis and sources of male sterility can be converted into new CMS lines, which will serve the purpose of broadening the genetic base of cultivated sunflower.

The restore for one CMS source behaved as maintainer for other CMS sources and *vice versa*, confirming the diversity among the CMS sources. This study indicates limited availability of effective good restorers for different CMS sources. The new restorers identified in the present investigation will help in exploiting new CMS sources in hybrid development by ensuring better heterosis and diversity of cytoplasm in sunflower. The newly identified maintainers, after testing for *per se* performance, combining ability and agronomic performance, will be converted into new CMS lines for utilization in hybrid breeding programs for developing diverse hybrids with better heterosis and resistance to diseases and insect pests.

More than 90 percent pollen fertility was observed in majority of the hybrids and the highest pollen fertility (97.8%) was observed in CMS-2A x EC-601622 and CMS-17A x EC-601672 followed by CMS-7-1A x EC-60122 and CMS-7-1A x EC-601664 (97.7%) and CMS-852A x EC-601669(97.5%). In contrast, less than 1 per cent or no pollen fertility was observed in most of the F_1 hybrids of the crosses involving the genotypes, GP_6-564, GP_6-623,GP_6-699, GP_6-952, GP_6-969, GP_6-976, GP_6-1153, GP_6-1217, GP_6-1423 GP_6-1433 GP_6-1436 GP_6-1470 GP_6-1518 GP_6-1521 GP_6-1594 GP_4-363 GP_4-548 GP_4-1217 GP_4-2679 and Selection-1000. Similar type of results reported by Meena and Prabhakaran, (2014) in two diverse CMS sources (Unpublished).

Frequency of tested material as maintainer, restorer and segregating types based on percent pollen fertility restoration were presented in Table-3.In the present study 28.26 to 41.3% frequency of pollen fertility was reported for all the six CMS. The maximum percent pollen fertility (41.3 %) was observed for CMS-7-1A followed by CMS-852A (36.9%), CMS-234A (36.9%), CMS-10A (34.7%), CMS-2A (32.6%) and the minimum per cent pollen fertility (28.2%) was observed for CMS-17A. Maximum frequency (67.3%) of tested material as maintainer was recorded for CMS-17A and CMS-2A. Maximum per cent (6.52%) frequency of partial restorer was observed for CMS-7-1A and minimum for CMS-10A. This finding is in agreement with Hu, (1983). Many more authors from India and abroad were reported very low frequency of fertility restoration genes for different CMS sources (Gouri Shankar *et al.* 2007; Virupakshappa *et al.* 1991; Meena and Sujatha, 2013). They concluded that hybrids could not be developed because of the non-availability of effective restorers for these new CMS sources.

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Table 1. Frequency of pollen fertility percent in F_1 after crossing with six PET-1 CMS with 46 inbred testers

Genotypes	CMS-17A	CMS-2A	CMS-10A	CMS-234A	CMS-852A	CMS-7-1A
	Pollen Fertility (%)	Pollen Fertility (%)	Pollen Fertility (%)	Pollen Fertility (%)	Pollen Fertility (%)	Pollen Fertility (%)
GP_6-18	0	0	0	0	0	98.1
GP_6-46	0	0	96.7	96.5	97.3	96.5
GP_6-54	0	0	95.8	0	96.7	0
GP_6-59	0	0	0	0	0	0
GP_6-135	0	0	96.3	0	93.7	0
GP_6-219	0	0	0	0	0	0
GP_6-236	0	0	93.7	93.7	95.7	96.5
GP_6-346	0	0	0	0	0	97.2
GP_6-349	0	0	0	0	0	0
GP_6-534	0	0	0	94.4	0	0
GP_6-564	0	0	0	0	0	0
GP_6-623	0	0	0	0	0	0
GP_6-699	0	0	0	0	0	0
GP_6-872	0	0	0	0	0	95.5
GP_6-952	0	0	0	0	0	0
GP_6-969	0	0	0	0	0	0
GP_6-976	0	0	0	0	0	0
GP_6-1051	96.9	96.4	95.3	96.9	95.6	0
GP_6-1153	0	0	0	0	0	0
GP_6-1217	0	0	0	0	0	0
GP_6-1423	0	0	0	0	0	0
GP_6-1433	0	0	0	0	0	0
GP_6-1436	0	0	0	0	0	0
GP_6-1470	0	0	0	0	0	0
GP_6-1518	0	0	0	0	0	0
GP_6-1521	0	0	0	0	0	0
GP_6-1594	0	0	0	0	0	0
GP_4-363	0	0	0	0	0	0
GP_4-548	0	0	0	0	0	0
GP_4-1217	0	0	0	0	0	0
GP_4-2679	0	0	0	0	0	0
EC-601622	95.7	97.8	97.1	97.6	97.2	97.7
EC-601664	95.2	95.6	95.8	97.2	95.3	97.7
EC-601666	95.5	95.7	97.1	95.6	97.2	96.7
EC-601669	97.1	96.6	96.3	93.1	97.5	96.8
EC-601672	97.8	96.3	95.4	97.5	94.4	96.4
EC-601691	97.4	97.1	97.8	96.8	96.8	96.7
EC-601704	96.9	95.9	96.5	96.9	0	96.1
EC-601705	0	97.2	0	96.4	0	96.4
EC-601721	0	94.6	0	0	0	0
EC-601726	95.8	97.6	97.1	95.3	97.0	96.2
EC-601821	96.4	97.0	97.1	97.5	96.9	97.0
EC-601848	95.4	97.3	97.3	97.1	95.9	95.8
EC-602006	96.5	97.3		97.1	97.5	95.8
EC-601711-1	98.1	95.8	93.8	97.4	96.5	95.6
Selection-1000	0	0	0	0	0	0

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Table 2. Maintainer and restorer behaviour of sunflower inbreds in different CMS background

S. No.	Genotypes	Different CMS of PET-1 Background					
		CMS-17A	CMS-2A	CMS-10A	CMS-234A	CMS-852A	CMS-7-1A
1	GP_6-18	M	M	M	M	M	R
2	GP_6-46	M	M	R	R	R	R
3	GP_6-54	M	M	R	M	R	M
4	GP_6-59	M	M	M	M	M	M
5	GP_6-135	M	M	R	S	R	S
6	GP_6-219	M	M	M	M	M	M
7	GP_6-236	M	M	R	R	R	R
8	GP_6-346	M	M	M	M	M	R
9	GP_6-349	S	M	M	M	S	S
10	GP_6-534	M	M	S	R	R	M
11	GP_6-564	M	M	M	M	M	M
12	GP_6-623	M	M	M	M	M	M
13	GP_6-699	M	M	M	M	M	M
14	GP_6-872	M	M	M	M	M	R
15	GP_6-952	M	M	M	M	M	M
16	GP_6-969	M	M	M	M	M	M
17	GP_6-976	M	M	M	M	M	M
18	GP_6-1051	R	R	R	R	R	R
19	GP_6-1153	M	M	M	M	M	M
20	GP_6-1217	M	M	M	M	M	M
21	GP_6-1423	M	M	M	M	M	M
22	GP_6-1433	M	M	M	M	M	M
23	GP_6-1436	S	M	M	M	M	M
24	GP_6-1470	M	M	M	M	M	M
25	GP_6-1518	M	M	M	M	M	M
26	GP_6-1521	M	M	M	M	M	M
27	GP_6-1594	M	M	M	M	M	M
28	GP_4-363	M	M	M	M	M	M
29	GP_4-548	M	M	M	M	M	M
30	GP_4-1217	M	M	M	M	M	S
31	GP_4-2679	M	M	M	M	M	M
32	EC-601622	R	R	R	R	R	R
33	EC-601664	R	R	R	R	R	R
34	EC-601666	R	R	R	R	R	R
35	EC-601669	R	R	R	R	R	R
36	EC-601672	R	R	R	R	R	R
37	EC-601691	R	R	R	R	R	R
38	EC-601704	R	R	R	R	M	R
39	EC-601705	M	R	M	R	M	R
40	EC-601721	M	R	M	M	M	M
41	EC-601726	R	R	R	R	R	R
42	EC-601821	R	R	R	R	R	R
43	EC-601848	R	R	R	R	R	R
44	EC-602006	R	R	M	R	R	R
45	EC-601711-1	R	R	R	R	R	R
46	Selection-1000	M	M	M	M	M	M

M = Maintainer R= Restorer S= Segregating type

Table 3. Frequency of tested material as maintainer, complete restorer, partial restorer and segregating types based on percent pollen fertility restoration over different CMS sources

CMS line	Tested genotypes	M	Percentage (%)	R	Percentage (%)	S	Percentage (%)
COSF-17A	46	31	67.39	13	28.26	2	4.34
COSF-2A (PET-1)	46	31	67.39	13	32.60	-	-
CMS-10A (PET-1)	46	29	63.04	16	34.78	1	2.17
CMS-234A (PET-1)	46	28	60.86	17	36.95	1	2.17
CMS-852A (PET-1)	46	28	60.86	17	36.95	1	2.17
CMS-7-1A (PET-1)	46	24	52.17	19	41.30	3	6.52

M = Maintainer R = Restorer S = Segregating type or partial restorer (P)