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TA Narkhede
M.Sc (Agri) Department of Genetics and Plant Breeding, College of Agriculture, Latur. (VNMKV, Parbhani), Maharashtra, India

SP Pole
Junior Breeder, Oilseeds Research Station, Latur. (VNMKV, Parbhani) Maharashtra, India

MV Dhuppe
Oilseeds Specialist, Oilseeds Research Station, Latur. (VNMKV, Parbhani) Maharashtra, India

PS Zade
M.Sc (Agri) Department of Genetics and Plant Breeding, College of Agriculture, Latur. (VNMKV, Parbhani), Maharashtra, India

G Singh
M.Sc (Agri) Department of Genetics and Plant Breeding, College of Agriculture, Latur. (VNMKV, Parbhani), Maharashtra, India

Corresponding Author:
TA Narkhede
M.Sc (Agri) Department of Genetics and Plant Breeding, College of Agriculture, Latur. (VNMKV, Parbhani), Maharashtra, India

Identification of sterility maintenance or fertility restoration behaviour for different CMS lines in Sunflower (*Helianthus Annuus* L.) using inbreds

TA Narkhede, SP Pole, MV Dhuppe, PS Zade and G Singh

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Abstract
A study was conducted to evaluate fertility restoration and sterility maintenance in 32 inbred lines crossed with three CMS lines (CMS-47A, CMS-38A, and CMS-103A), producing 96 F_1 hybrids. These hybrids were classified as restorers, maintainers, or partial restorers based on the proportion of fertile and sterile plants. Among the evaluated crosses, 36 (37.50%) were completely fertile, 29 (30.21%) were sterile, and 31 (32.29%) exhibited partial fertility. Out of the 32 inbred lines, eight (USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, RSLP-34, RSLP-36, and PB-892) consistently restored fertility across all CMS lines, confirming the presence of dominant restorer gene (Rf1). Six inbreds (USDA-109, USDA-122, USDA-160, USDA-173, USDA-192, and USDA-224) acted as maintainers, while six others (USDA-145, NDI-23, GMU-520, GMU-713, EC-198072, and Phule Bhaskar) showed partial fertility restoration. Differential restoration was observed across CMS lines, with several inbreds such as USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, RSLP-34, and PB-892 performing as restorers in multiple CMS backgrounds. The variation in fertility expression suggests the influence of modifying genes, nuclear background of the female parent, and complex interactions between cytoplasmic and nuclear factors. Overall, the study identified promising restorer and maintainer lines essential for new restorer and CMS lines in maintenance breeding and hybrid development programme.

Keywords: CMS Source, sterility maintenance, fertility restoration, Inbreds, Sunflower, *(Helianthus annuus* L.)

Introduction
Sunflower (*Helianthus annuus* L.) belongs to Asteraceae family. It's being a highly cross-pollinated crop ideally suited for exploitation of heterosis. The identification of cytoplasmic male sterility among progenies of the interspecific cross *Helianthus petiolaris* × *Helianthus annuus* by Leclercq (1969) [1] and fertility restoration by (Kinman 1970) [2] provided the required breakthrough in the development of hybrids. Moreover, hybrids are more self-fertile and resistant to diseases, thus resulting in enhanced seed set and seed filling (Seetharam, 1984) [3]. 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. Success in heterosis programme is largely dependent on the development of inbreds of wide genetic base (Giriraj, 1998) [4]. 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 breeding programmes. Development of hybrids with diverse cytoplasmic background has been one of the major priority. 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.

Material and Methods
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 three CMS of PET-1 back ground.

Thirty two germplasm accessions of economic importance of sunflower consisting of eleven USDA lines (USDA-46, USDA-64, USDA-109, USDA-122, USDA-145, USDA-160, USDA-173, USDA-179, USDA-189, USDA-192, USDA-224), ten inbreds from ORS, Latur (GMU-477, GMU-520, GMU-713, CMS-249B, CMS-112B, GMU-481, PB-1212, PB-892, Phule Bhaskar, DRSF-108), six inbreds from AICRP-Sunflower, Nandyal (NDI-18, NDI-21, NDI-23, NDI-26, NDI-33, NDI-47), two inbreds from exotic collection (EC-178168, EC-198072), two inbreds from AICRP-Sunflower, Raichur (RSLP-34, RSLP-36) and sunflower core germplasm lines (SCG-107) were used for crossing with three CMS viz., CMS-47A, CMS-38A and CMS-103 during *kharif* 2024 at Oilseeds Research Station, Latur to obtained F_1s by crossing in Line × Tester mating design. Each CMS line was planted in separate blocks with 20 rows of 4.5 m length, while each test inbred was sown in two rows of the same length, maintaining a row spacing of 60 cm and plant spacing of 30 cm. To prevent open pollination, heads of CMS lines and selected inbred plants were bagged with cloth bags just before ray floret opening (star bud stage). Pollen from each restorer line was collected separately in petri dishes using a camel hairbrush during morning hours (9:00-11:00 am) and used to pollinate the heads of CMS lines. After pollination, heads were re-bagged and labeled to ensure proper identification. Brushes and petri dishes were cleaned with alcohol after each use to avoid contamination. Pollinations were repeated until all disc florets of the female parent had opened. Following anthesis and fertilization, cloth bags were removed and seeds from each cross separately harvested, cleaned, and stored for evaluation.

All 96 experimental test hybrids developed during *kharif* 2024 were evaluated in the *rabi* season of 2024-25. Each hybrid was planted in a single row, 4.5 meters long, with a spacing of 60 cm between rows and 30 cm between plants. The crop was managed using the recommended package of practices, including all necessary agronomical cultural practices and plant protection measures, to ensure healthy growth. All F_1 plants were examined at anthesis to assess fertility or sterility reactions by observing pollen presence and anther dehiscence. The percentage of fertile and sterile plants was calculated for each hybrid by counting the respective plants. Based on their fertility restoration ability, inbred lines were classified as fertility restorers when all F_1 plants were fertile, sterility maintainers when all F_1 plants were sterile, and partial restorers when segregation for fertility and sterility was observed. Accordingly, F_1 hybrids were categorized as male fertile, male sterile, or partially fertile, and each inbred line was grouped with the respective CMS line.

Results and Discussion

Thirty-two new inbred lines were crossed with three CMS viz., CMS-47A, CMS-38A and CMS-103A. The 96 crosses thus obtained were evaluated for fertility restoration and sterility maintenance reaction by counting the number of sterile or fertile plants in the respective crosses. The extent of fertility restoration and sterility maintenance by the respective inbred lines in the crosses was presented in table 1. The new inbred lines which resulted in hybrids with complete sterility were classified as sterility maintainers and with completely fertile plants as fertility restorers and those which resulted in hybrids segregating for both fertility and sterility were considered as partial restorers was presented in table 2. Out of the 32 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. Based on the presence of number of completely sterile or fertile plants in the respective crosses. Out of 96 crosses evaluated 36 F1s (36.50 %) were fertile, 29 cross combinations (30.20 %) were sterile and 31 cross combinations (32.29 %) were found to be partially fertile. The frequency of sterility maintainer or fertility restorer behavior (%) of inbred lines with respected to three CMS lines are given in the table 3. Similar results were obtained in the study of Satish Chandra *et al*. (2011); Meena and Sujatha (2013); Meena and prabakaran (2016) [5, 7, 6, 12].

Out of the 32 inbred lines evaluated, eight lines (USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, RSLP-34, RSLP-36, and PB-892) restored fertility in the F1 hybrids across all three CMS lines. These acted as restorers of the PET-1 cytoplasmic source, indicating that they carry the dominant restorer gene (Rf1) in the nucleus with male fertile pollen. Six inbred lines (USDA-109, USDA-122, USDA-160, USDA-173, USDA-192, and USDA-224) functioned as maintainers, suggesting the absence of the fertility restoration gene or the presence of the gene in recessive form. Another six lines (USDA-145, NDI-23, GMU-520, GMU-713, EC-198072, and Phule Bhaskar) showed partial fertility restoration, likely being heterozygous for the fertility gene in all three CMS lines. The common fertility restorers and sterility maintainers identified across the three CMS lines are presented in table 4.

Additionally, results revealed that USDA-46, USDA-64, USDA-189, NDI-18, NDI-21, NDI-33, NDI-47, RSLP-34, RSLP-36, GMU-477, GMU-481, PB-1212, PB-892, and DRSF-108 restored fertility in CMS-47A, while sterility was maintained by nine inbreds (USDA-109, USDA-122, USDA-160, USDA-173, USDA-192, USDA-224, NDI-26, SCG-107, and EC-178168). Furthermore, nine inbreds (GMU-520, GMU-713, CMS-249B, CMS-112B, EC-198072, Phule Bhaskar, USDA-145, USDA-179, and NDI-23) exhibited partial fertility restoration.

Suggesting the influence of modifying genes on fertility restoration or it might also be suggesting that the nuclear background of the female parent also had the profound influence on the expression of the fertility in these concerned inbred lines or also might be due to the genetic architecture, especially the number of genes controlling and their interactions with cytoplasm in restoring fertility. Similar results were also reported by Gouri Shankar *et al*. (2006); Nandini *et al*. (2018); Biradar *et al*. (2019); Dudhe *et al*. (2019) and Mohan *et al*. (2022) [8, 9, 10, 11, 12].

It was found that inbreds USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, RSLP-34, RSLP-36, GMU-477, PB-892, and CMS-112B restored fertility with CMS-38A, while twelve inbreds NDI-26, NDI-47, GMU-520, GMU-713, GMU-481, EC-198072, EC-178198, PB-1212, USDA-145, USDA-189, Phule Bhaskar, and NDI-23 partially restored fertility. In contrast, USDA-109, USDA-122, USDA-160, USDA-173, USDA-179, USDA-192, USDA-224, SCG-107, CMS-249B, and DRSF-108 acted as sterility maintainers for CMS-38A.

Similarly, USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, NDI-47, RSLP-34, RSLP-36, GMU-481, EC-178168, PB-1212, and PB-892 restored fertility with CMS-103A, whereas USDA-109, USDA-122, USDA-160, USDA-173,

USDA-192, USDA-224, USDA-179, USDA-189, CMS-249B, and DRSF-108 behaved as maintainers. Partial fertility restoration with CMS-103A was observed in USDA-145, NDI-23, NDI-26, GMU-477, GMU-520, GMU-713, SCG-107, CMS-112B, EC-198072, and Phule Bhaskar. The study further indicated that NDI-47, GMU-481, and PB-1212 restored fertility in both CMS-47A and CMS-103A. Conversely, USDA-179, CMS-249B, and DRSF-108 maintained sterility in CMS-38A and CMS-103A, while GMU-477 acted as a restorer and SCG-107 as a sterility maintainer for CMS-47A and CMS-38A, respectively. In conclusion, local inbreds had more frequency of maintainers than restorer lines. Thus present study helped to identify a few effective restorers for available CMS lines, which can be exploited in future hybrid development or may be utilized in the development of new restorer lines. Newly developed CMS lines used in heterosis breeding programme for developing diverse hybrids with better heterosis and resistance to disease and insect pests.

Table 1: *Per cent* of male fertility and sterility as indicated by pollen fertility test (%) in the F1 generation obtained by crossing of 32 inbred with three CMS lines

Sr. No.	Inbred lines	CMS-47 A		Status	CMS-38 A		Status	CMS-103 A		Status
		Fertility	Sterility		Fertility	Sterility		Fertility	Sterility	
1	USDA-46	100.00	0.00	Fertile	100.00	0.00	Fertile	100	0.00	Fertile
2	USDA-64	100	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
3	USDA-109	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
4	USDA-122	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
5	USDA-145	23.08	76.92	Partial Fertile	45.45	54.55	Partial Fertile	50.00	50.00	Partial Fertile
6	USDA-160	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
7	USDA-173	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
8	USDA-179	64.07	35.93	Partial Fertile	0.00	100.00	Sterile	0.00	100.00	sterile
9	USDA-189	100.00	0.00	Fertile	33.33	66.64	Partial Fertile	0.00	100.00	Sterile
10	USDA-192	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
11	USDA-224	0.00	100.00	Sterile	0.00	100.00	Sterile	0.00	100.00	Sterile
12	NDI-18	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
13	NDI-21	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
14	NDI-23	66.67	33.33	Partial Fertile	58.33	41.67	Partial Fertile	53.33	46.67	Partial Fertile
15	NDI-26	0.00	100.00	Sterile	30.00	60.00	Partial Fertile	6.67	93.33	Partial Fertile
16	NDI-33	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
17	NDI-47	100.00	0.00	Fertile	90.00	10.00	Partial Fertile	100.00	0.00	Fertile
18	RSLP-34	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
19	RSLP-36	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
20	GMU-477	100.00	0.00	Fertile	100.00	0.00	Fertile	71.43	28.57	Partial Fertile
21	GMU-520	28.57	71.43	Partial Fertile	20.00	80.00	Partial Fertile	42.86	57.14	Partial Fertile
22	GMU-713	64.28	35.72	Partial Fertile	27.27	72.73	Partial Fertile	23.08	76.92	Partial Fertile
23	SCG-107	0.00	100.00	Sterile	100.00	0.00	Sterile	60.00	40.00	Partial Fertile
24	CMS-249B	11.11	88.89	Partial Fertile	0.00	100.00	Sterile	0.00	100.00	Sterile
25	CMS-112B	42.86	57.14	Partial Fertile	100.00	0.00	Fertile	86.67	13.33	Partial Fertile
26	GMU-481	100.00	0.00	Fertile	66.67	33.33	Partial Fertile	100.00	0.00	Fertile
27	EC-198072	65.00	35.00	Partial Fertile	70.00	30.00	Partial Fertile	33.33	66.67	Partial Fertile
28	EC-178168	100.00	0.00	Sterile	81.82	18.18	Partial Fertile	100.00	0.00	Fertile
29	PB-1212	100.00	0.00	Fertile	90.25	9.75	Partial Fertile	100.00	0.00	Fertile
30	PB-892	100.00	0.00	Fertile	100.00	0.00	Fertile	100.00	0.00	Fertile
31	Phule Bhaskar(C)	50.00	50.00	Partial Fertile	62.50	37.50	Partial Fertile	41.67	58.33	Partial Fertile
32	DRSF-108(C)	100.00	0.00	Fertile	0.00	100.00	Sterile	0.00	100.00	Sterile

Table 2: Fertility restoration or sterility maintenance reaction of new inbred lines with three CMS lines

Sr. No.	Inbred lines	Cytoplasmic Male Sterile Lines		
		CMS-47 A	CMS-38 A	CMS-103 A
1	USDA-46	Restorer	Restorer	Restorer
2	USDA-64	Restorer	Restorer	Restorer
3	USDA-109	Maintainer	Maintainer	Maintainer
4	USDA-122	Maintainer	Maintainer	Maintainer
5	USDA-145	Partial restorer	Partial restorer	Partial restorer
6	USDA-160	Maintainer	Maintainer	Maintainer
7	USDA-173	Maintainer	Maintainer	Maintainer
8	USDA-179	Partial restorer	Maintainer	Maintainer
9	USDA-189	Restorer	Partial restorer	Maintainer
10	USDA-192	Maintainer	Maintainer	Maintainer
11	USDA-224	Maintainer	Maintainer	Maintainer
12	NDI-18	Restorer	Restorer	Restorer

13	NDI-21	Restorer	Restorer	Restorer
14	NDI-23	Partial restorer	Partial restorer	Partial restorer
15	NDI-26	Maintainer	Partial restorer	Partial restorer
16	NDI-33	Restorer	Restorer	Restorer
17	NDI-47	Restorer	Partial restorer	Restorer
18	RSLP-34	Restorer	Restorer	Restorer
19	RSLP-36	Restorer	Restorer	Restorer
20	GMU-477	Restorer	Restorer	Partial restorer
21	GMU-520	Partial restorer	Partial restorer	Partial restorer
22	GMU-713	Partial restorer	Partial restorer	Partial restorer
23	SCG-107	Maintainer	Maintainer	Partial restorer
24	CMS-249B	Partial restorer	Maintainer	Maintainer
25	CMS-112B	Partial restorer	Restorer	Partial restorer
26	GMU-481	Restorer	Partial restorer	Restorer
27	EC-198072	Partial restorer	Partial restorer	Partial restorer
28	EC-178168	Maintainer	Partial restorer	Restorer
29	PB-1212	Restorer	Partial restorer	Restorer
30	PB-892	Restorer	Restorer	Restorer
31	Phule Bhaskar(C)	Partial restorer	Partial restorer	Partial restorer
32	DRSF-108(C)	Restorer	Maintainer	Maintainer

Table 3: Frequencies of restorers, maintainers and partial restorers for three CMS lines in sunflower

CMS Line	Total no. of inbred tested	Number and percentage of inbred lines behaved as					
		Restorer	% Restorers	Maintainer	% Maintainers	Partial restorer	% Partial restorers
CMS-47A	32	14	43.75	9	28.13	9	28.13
CMS-38A	32	10	31.25	10	31.25	12	37.50
CMS-103A	32	12	37.50	10	31.25	10	31.25
Total	96	36	37.50	29	30.21	31	32.29

Table 4. Common sterility maintainer and fertility restorer inbred lines for all the three CMS lines in sunflower

Fertility/sterility reaction	Inbred Lines	Total
Common Restorers	USDA-46, USDA-64, NDI-18, NDI-21, NDI-33, RSLP-34, RSLP-36, PB-892	08
Common Maintainers	USDA-109, USDA-122, USDA-160, USDA-173, USDA-192, USDA-224	06

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