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Identification of Fertility Restoration Status in Sunflower (*Helianthus annuus* L.) Hybrids using New Inbreds

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Abstract

In the present investigation, 42 hybrids were developed by attempting hybridization between six CMS lines and seven inbred lines in Line x Tester mating design to identify or characterize the fertility status among the hybrids. Among 42 hybrids studied, 13 hybrids restored complete fertility while other 29 recognized as completely sterile. Further two inbred lines RHA GPR-96 and GMU 736 were found to be common restorers of four CMS lines *viz*., CMS 107A, CMS-17A, CMS-110A and ARM 249A and none of the inbred line was identified as completely restorer on CMS 108A and IMS 265A sterile lines. This variable behaviour of these inbred lines on different CMS lines could be attributable to their wider genetic diversity as they are interspecific derived lines. The identified restorers for the new CMS sources can be exploited in developing hybrids with good heterotic potential.

Key words : *Restorer lines, sunflower.*

Introduction

Sunflower (*Helianthus annuus*) is one of the globally important oilseed crops attributing for high oil content and its versatility. It belongs to the genus *Helianthus* of the family Asteraceae, tribe *Heliantheae*, sub tribe *Helianthinae*, which comprises 20 genera with 400 species. The top producing nations of sunflower worldwide include Ukraine, Russia, Argentina, Romania, China. In India, during 2021-2022, total production of sunflower is 0.25 m tonnes, which is cultivated in total area of 0.28 m ha, with productivity of 905 kg ha^{-1} (1). In India, top six sunflower producing states are Karnataka, Telangana, Odisha, Haryana, Maharashtra and Andhra Pradesh. Besides, Punjab, Haryana, West Bengal and Uttar Pradesh are promising spring sunflower growing states. Karnataka ranks first in sunflower production with total production of 0.14 m tonnes, cultivated in total area of 0.16 m ha with productivity of 842 kg ha^{-1}. Andhra Pradesh stands at 6th position, with total production of 0.01 m tonnes (which contributes to 4.77% of total sunflower production in India), cultivated in total area of 0.02 m ha (7.17% of total cultivated land under sunflower cultivation in India) with productivity of 602 kg ha^{-1} (2). In Andhra Pradesh, Kadapa district tops in area under cultivation and production (3).

The crop is more convenient for exploitation of heterosis as a consequence of highly cross pollination nature. The discovery of Cytoplasmic Male Sterility by Leclerq (1969) from *Helianthus petiolaris* (PET 1) and fertility restoration by (4) provided the great opportunity for commercial development of sunflower hybrids. Hybrid breeding programs play a pivotal role in sunflower production and fertility restoration is a key factor for the successful utilization of hybrid vigour. The development of interspecific hybrids involves the incorporation of desirable traits from wild sunflower relatives. However, these hybrids often suffer from reduced fertility or complete sterile due to break-down of cytoplasmic male sterility (CMS) systems (5, 6).

The use the restorer lines which are capable of restoring fertility with restorer genes in hybrids can overcome the limitation of reduced fertility. Hence those lines possess high fertility restoration capacity with better pollen viability can hold promise for their potential integration into sunflower hybrid breeding programs, as they offer enhanced fertility restoration capabilities. The main objective of the present study is to identify fertility status information of new inbred lines in isolation of potential hybrids with increased yield and improved fertility.

Materials and Methods

In the present investigation, seven diverse restorer lines comprising of accessions *viz*., AKSFI-78, OPH 74, RHA GPR-96, GMU 736, RHA 1055, NDSI-3, GMU-1181 obtained from Directorate of Oilseeds Research, Hyderabad were crossed with six Cytoplasmic Male Sterile (CMS) lines (CMS 17A, CMS 107A, CMS 108A, CMS 110A, IMS-265A and ARM-249A) during *kharif*, 2022 in Line x Tester mating fashion to obtain 42 hybrids with a spacing of 60 x 30 cm staggered sowings of male parents, twice at weekly interval, was done to synchronize the flowering and recommended agronomic practices were

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followed. Before flower initiation, heads of the CMS lines and restorers were bagged with a cloth bag a day prior to anthesis in order to avoid natural crossing. At anthesis, the pollen from already bagged male parents collected in different Petri-plates separately with the help of a camel hair brush during morning hours (9:00 am to 11:00 am) when pollen is viable at anther tips and high humidity. The pollinations were repeated 3-4 times on alternate days to pollinate all the floret whirls proceeding inwards. The heads of each of the CMS lines were pollinated with known pollen parents and then covered with cloth bags to avoid cross contamination and individual plants were labelled mentioning specific cross combination. The heads of all the resultant 42 hybrids were harvested, dried and threshed separately.

The generated 42 hybrids were examined during the *kharif* season 16th July of 2022 at Regional Agricultural Research Station, Nandyal (15.46º latitude and 78.48º longitude), Andhra Pradesh for identification of fertility restoration in the CMS lines studied. All the hybrids were studied for maintainer/ restorer behaviour of the inbreds. The hybrids from the 42 crosses were planted in an unreplicated trial in which each entry was sown in two rows of 3 m length with a spacing of 60 cm x 30 cm. The fertility restoration behaviour of F_1 hybrids was assessed by visual observation after emergence of 50% flowering on all the plants in the two rows. The plants were classified as male fertile or sterile based on anther exertion and pollen production during flowering. In case of no pollen production, conspicuous stigma projection coupled with pale yellow disc florets, the lines were classified as restorer or maintainers for the different CMS sources. Identification of effective fertility restorers to these cytoplasmic male sterile lines helps in utilizing them for developing hybrids with diverse cytoplasm and the identified maintainers with good combining ability along with suitable genetic background can be converted into new CMS lines for further exploitation in developing new hybrids.

Results and Discussion

In sunflower, with the eminence of cytoplasmic male sterility system, the chance of improving sunflower crop is possible in all aspects through heterosis breeding. Since from the start of hybrid development programme, the PET-1 cytoplasmic (petiolaris) male sterile source has been utilized extensively in commercial production of hybrids leading to narrow genetic base and limited progress. Henceforth, sunflower breeders uplifted their efforts to develop diverse sources of cytoplasmic male sterility to widen the genetic base and reduce the genetic vulnerability of sunflower hybrids. Besides developing new sources of male sterility, it is better to identify new restorer lines which will enhance the genetic diversity.

In the present study, the hybrids showing maintainer/restorer reaction of different inbred lines in the background of six CMS lines is presented in Table-1. Among the 42 hybrids examined for fertility restoration status, fertility was completely restored in only 13 hybrids (Table-2) and rest of the 29 hybrids were found to be completely sterile. Similar kind of categorization was also performed by (7, 8).

The pattern of fertility restoration by different inbred lines on the CMS-sources is furnished in Table-3. The results revealed that fertility restoration on CMS 107A was found to be completely restored by the lines OPH 74, RHA GPR-96 and GMU 736 except that were found as completely maintainers. Similarly, the fertility restoration on CMS 17A was identified to be completely restored in only two lines OPH 74 and RHA GPR-96 though the rest were recognized as maintainers. It is clear that higher number of maintainers were identified when compared to restorers will often offers scope for CMS conversion programme and identified few restorers would be used for CMS diversification and isolation of potential hybrids. The obtained results were in agreement with the investigations carried out by (9).

None of the lines were behaved as complete restorers in the background of CMS 108A and IMS 265 A where all the lines were found as maintainers. The report of lack of fertility restorers on varied CMS background is in conformity with the findings of (10). Surprisingly, single line RHA GPR-6 behaved as complete restorer on CMS 110A background but rest of all lines behaved as maintainers. All these inbreds can be tried with other CMS lines with different CMS backgrounds to confirm its restoration behavior. The differential behaviour of pollen parents on different CMS sources reconciling significant diversity among cytoplasmic source and lines for fertility restoration as reported by earlier studies of (7).

Interestingly, single tester RHA GPR-96 is identified as common restorer on CMS107A, CMS-17A, CMS-110A and ARM 249A CMS background (Table-3). Such kind of behaviour of male line infers the diverse origin of the line and it would be useful in locating R genes for restoration. The data clearly indicate that most of the tested hybrids found as completely restorers for varied CMS sources. Similar kind of differences in fertility restoration in different CMS background was also confirmed with the results of (7, 10, 11).

This study indicates that the restorer genes for different CMS sources are different. The inbreds which restored fertility on one source of CMS, behaved as

Table-1 : List of hybrids showing maintainer/restorer reaction of different inbred lines in the background of six CMS lines.

S. No.	Crosses	F_1 plants scored	No. of plants fertile	No. of plants sterile	% fertility	Status of hybrid	Status of male plant
1.	CMS 107 A × AKSFI 78	10	0	10	0	S	Maintainer
2.	CMS 108 A × AKSFI 78	10	0	10	0	S	Maintainer
3.	CMS 110 A × AKSFI 78	10	0	10	0	S	Maintainer
4.	IMS 265 A × AKSFI 78	10	0	10	0	S	Maintainer
5.	ARM 249 A × AKSFI 78	10	0	10	0	S	Maintainer
6.	CMS 17 A × AKSFI 78	10	0	10	0	S	Maintainer
7.	CMS 107 A × OPH 74	10	10	0	100	F	Restorer
8.	CMS 108 A × OPH 74	10	10	0	100	F	Restorer
9.	CMS 110 A × OPH 74	10	0	10	0	S	Maintainer
10.	IMS 265 A × OPH 74	10	0	10	0	S	Maintainer
11.	ARM 249 A × OPH 74	10	0	10	0	S	Maintainer
12.	CMS 17 A × OPH 74	10	10	0	100	F	Restorer
13.	CMS 107 A × GMU 1181	10	0	10	0	S	Maintainer
14.	CMS 108 A × GMU 1181	10	0	10	0	S	Maintainer
15.	CMS 110 A × GMU 1181	10	0	10	0	S	Maintainer
16.	IMS 265 A × GMU 1181	10	0	10	0	S	Maintainer
17.	ARM 249 A × GMU 1181	10	0	10	0	S	Maintainer
18.	CMS 17 A × GMU 1181	10	0	10	0	S	Maintainer
19.	CMS 107 A × RHA GP6-96	10	10	0	100	F	Restorer
20.	CMS 108 A × RHA GP6-96	10	10	0	100	F	Restorer
21.	CMS 110 A× RHA GP6-96	10	10	0	100	F	Restorer
22.	IMS 265 A × RHA GP6-96	10	0	10	0	S	Maintainer
23.	ARM 249 A × RHA GP6-96	10	10	0	100	F	Restorer
24.	CMS 17 A × RHA GP6-96	10	10	0	100	F	Restorer
25.	CMS 107 A × RHA 1055	10	0	10	0	S	Maintainer
26.	CMS 108 A × RHA 1055	10	0	10	0	S	Maintainer
27.	CMS 110 A × RHA 1055	10	0	10	0	S	Maintainer
28.	IMS 265 A × RHA 1055	10	0	10	0	S	Maintainer
29.	ARM 249 A × RHA 1055	10	0	10	0	S	Maintainer
30.	CMS 17 A × RHA 1055	10	0	10	0	S	Maintainer
31.	CMS 107 A × NDSI 3	10	0	10	0	S	Maintainer
32.	CMS 108 A × NDSI 3	10	0	10	0	S	Maintainer
33.	CMS 110 A × NDSI 3	10	0	10	0	S	Maintainer
34.	IMS 265 A × NDSI 3	10	0	10	0	S	Maintainer
35.	ARM 249 A × NDSI 3	10	0	10	0	S	Maintainer
36.	CMS 17 A × NDSI 3	10	0	10	0	S	Maintainer
37.	CMS 107 A × GMU 736	10	10	0	100	F	Restorer
38.	CMS 108 A × GMU 736	10	10	0	100	F	Restorer
39.	CMS 110 A × GMU 736	10	10	0	100	F	Restorer
40.	IMS 265 A × GMU 736	10	0	10	0	S	Maintainer
41.	ARM 249 A × GMU 736	10	10	0	100	F	Restorer
42.	CMS 17 A × GMU 736	10	10	0	100	F	Restorer

Table-2 : List of completely fertile hybrids.

S. No.	Crosses
1.	CMS 107 A×OPH 74
2.	CMS 108 A×OPH 74
3.	CMS 17 A×OPH 74
4.	CMS 107 A×RHA GP6-96
5.	CMS 108 A×RHA GP6-96
6.	CMS 110 A×RHA GP6-96
7.	ARM 249 A×RHA GP6-96
8.	CMS 17 A×RHA GP6-96
9.	CMS 107 A×GMU 736
10.	CMS 108 A×GMU 736
11.	CMS 110 A×GMU 736
12.	ARM 249 A×GMU 736
13.	CMS 17 A×GMU 736

maintainers on other sources of CMS and vice versa. All the six CMS sources utilized in the experiment showed diversity among themselves, thus broadening the genetic base of the CMS lines, which could be safely included in breeding programmes thereby mitigating the vulnerability of the lines to various insect pests and diseases.

Conclusion

The identified maintainers (AKSFI-78, RHA 1055, NDSI-3 and GMU-1181) after testing for their combining ability and agronomic performance will be converted into new cytoplasmic male sterile lines and may be used in sunflower breeding programmes for isolating diverse hybrids together with better heterosis. These CMS lines can be safely included in the breeding programme to

Table-3 : Pattern of fertility restoration by different inbred lines on the CMS-sources.

S. No.	CMS-sources	Group	Maintainers	Restorers
1.	CMS 107A	*Helianthus petiolaris*	AKSFI-78, RHA 1055, NDSI-3, GMU-1181	OPH 74, RHA GPR-96, GMU 736
2.	CMS-17A	*Helianthus petiolaris*	AKSFI-78, GMU 736, RHA 1055, NDSI-3, GMU-1181	OPH 74, RHA GPR-96
3.	CMS 108 A	*Helianthus petiolaris*	AKSFI-78, OPH 74, RHA GPR-96, GMU 736, RHA 1055, NDSI-3, GMU-1181	None
4.	CMS-110 A	*Helianthus petiolaris*	AKSFI-78, OPH 74, GMU 736, RHA 1055, NDSI-3, GMU-1181	RHA GPR-96-6
5.	ARM 249A	*Helianthus petiolaris Nutt. sp. petiolaris*	AKSFI-78, OPH 74, RHA 1055, NDSI-3, GMU-1181	GMU 736, RHA GPR-96-6
6.	IMS 265A	*H.annuus ssp. lenticularis*	AKSFI-78, OPH 74, RHA GPR-96, GMU 736, RHA 1055, NDSI-3, GMU-1181	None

broaden the genetic base of cytoplasmic male sterility in sunflower to avoid the possible risk of susceptibility. By the identification of effective restorers (OPH 74, RHA GPR-96-6 and GMU-736) in the present investigation as alternate sources of cytoplasm, a significant improvement towards diversifying the parental base, especially with respect to the cytoplasm can be achieved and further leads to yield improvement and also help in their exploitation for hybrid development with better heterosis and diversity of cytoplasm in sunflower.

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