

Original Research Article

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Restoration Ability of New Inbred and Restorer Lines on Different CMS Sources in Sunflower (*Helianthus annuus* L.)

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ABSTRACT

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The development of commercial sunflower hybrids based on new CMS sources is of special interest for reducing the potential risk of vulnerability to biotic stresses and for increasing genetic diversity. Four CMS lines of sunflower (*Helianthus annuus* L.) viz., FMS 852A (*Helianthus petiolaris* sp. fallax), IMS 852A (*H. annuus* sp. lenticularis) and CMS 302A and CMS 234A (*H. petiolaris*) were crossed with 40 inbreds to identify fertility restorer lines for each CMS source. Only a few inbreds could restore fertility on new CMS sources. Out of 25 new inbreds tested, 10 were found to be restorers for CMS PET1. Only two inbreds (RHA-1-1 and IB-60) could restore fertility on CMS PEF, 8 behaved as partial restorers and the remaining 15 inbreds behaved as maintainers. Similarly two inbreds (RHA-1-1 and NS-15) restored fertility on CMS I. Only one inbred line RHA-1-1 could restore fertility on all the three CMS sources. A few effective restorers were identified for the new CMS sources, which can be exploited in developing hybrids with better heterotic potential.

Introduction

Sunflower being a highly cross pollinated crop is ideally suited for exploitation of heterosis. The discovery of Cytoplasmic Male Sterility by Leclercq (1969) from *Helianthus petiolaris* (PET 1) and fertility restoration by Kinman (1970) provided the required breakthrough in the commercial development of sunflower hybrids. Moreover, hybrids are highly self fertile and resistant to diseases, thus resulting in enhanced seed set and seed

filling (Seetharam, 1981). The hybrids are being cultivated on 80-90 per cent area in India due to their high yield potential, suitability to input intensive agriculture and due to the role played by the private companies. All the sunflower hybrids that are commercially grown have a single source (PET-1) leading to homogeneity and potential risk of becoming susceptible to pest and disease due to the continuous use of PET source. From 1972 until now sunflower hybrid program has relied entirely on single

CMS source *viz.*, PET cytoplasm, exposing it to potential risk of pathogen or insects and restricting the variability of genes inherited. It is evident in case of maize, where, Texas (CMS-T) cytoplasm became susceptible to *Helminthosporium maydis* as well as pearl millet due to its susceptibility to ergot disease. In order to reduce the probable chances of occurrence of similar problems diversification of cytoplasmic male sterility in sunflower is needed. In addition to the continuing search for new cytoplasmic male sterility sources, identification and use of new restorer lines is quite essential to know the fertility restoration on new CMS sources and also to diversify the genetic base of the hybrids for increased hybrid vigour, adaptation and resistance to pest and diseases. Hence, diversification of CMS sources is inevitable in heterosis breeding which will add flexibility and nuclear diversity to breeding programmes. More than 40 CMS sources have been described by Serieys (1996), but lack of appropriate maintainers or restorer lines as well as environmental instability limits their agronomic ability. The newly developed CMS sources for broadening of genetic base of cytoplasm have revealed polymorphisms in the mitochondrial DNA (Crouzillat *et al.*, 1994). But using these diverse CMS sources, hybrids could not be developed because of the non-availability of effective restorers. In view of the limitation, an attempt was made to identify restorers for the newly developed CMS sources.

Materials and Methods

Three diverse CMS sources (lines) *viz.*, FMS 852A from *Helianthus petiolaris* sp. *fallax* (CMS PEF), IMS 852A from *H. annuus* sp. *lenticularis* (CMS I) and CMS-234A and CMS-302A from *H. petiolaris* (CMS PET) and 25 new inbred and restorer lines of diverse genetic background were obtained from Directorate of Oilseeds Research

(DOR), Hyderabad. The 25 male parents and three CMS sources (4 CMS lines) were sown in the field to effect crossing in separate blocks during summer 2015-16 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 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). The three different CMS sources were crossed to all the 25 inbreds in a line x tester method. The pollinations were repeated 3-4 times on alternate days to pollinate all the floret whorls proceeding inwards. Hands and all crossing equipments were sterilized with absolute alcohol before pollination to reduce any chance of contamination. The capitulae 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 labeled mentioning specific cross combination. The heads of all the resultant 100 hybrids were harvested, dried and threshed separately. The well filled seeds from each cross were separated out for hybrid evaluation.

The identification of behavior of new inbred and restorer lines with respect to maintenance and restoration of the CMS sources of sunflower was done during *kharif* 2016 at RARS, Vijayapura. F1 seeds from the 100 crosses were planted with two replications and the plot size for each entry consisted of two rows (0.6 meter) in each replication with a spacing of 60 cm x 30 cm. At anthesis stage, plants were classified as male fertile/male sterile based on anther dehiscence and pollen

shedding and the number of plants with or without pollen shedding in each treatment was recorded to work out per cent fertility. Based on these observations, the crosses were grouped as either sterile or fertile. The pollen parent leading to sterile crosses were classified as maintainers, while those parents leading to fertile crosses were grouped as restorers of the corresponding CMS lines.

Results and Discussion

The maintainer/restorer reaction of the inbreds for different CMS sources has been presented in table 1. Results indicated that out of 25 test inbreds, 10 were found to be restorers for the traditional PET-1 cytoplasm CMS 234A, 12 behaved as partial restorers, while remaining behaved as maintainers. However, out of 25 inbreds, 10 were found to be restorers for CMS-302A, 7 showed partial fertility restoration and remaining 8 behaved as maintainers.

Two inbreds *viz.*, RHA-1-1 and IB-60 acted as restorers for CMS PEF (FMS 852A) cytoplasm, 8 inbreds behaved as partial restorers and 15 inbreds behaved as maintainers. For CMS I (IMS 852A) cytoplasm, 2 inbreds *i.e.*, RHA-1-1 and NS-15 were found to be restorers whereas 8 inbreds showed segregation and the remaining 15 behaved as maintainers. Such inbred lines that turned out as maintainers for FMS 852A were also behaving as maintainers on IMS 852A background. Only two inbred lines namely RHA-1-1 and IB-60 on FMS 852A and RHA-1-1 and NS-15 on IMS 852A acted as complete restorers. This indicates that the genetic constitution and interaction of FMS 852A and IMS 852A are different from that of the PET source and necessitates identification of restorer lines having R genes for fertility restoration. Similar results of differences in fertility restoring genes for different CMS backgrounds have been

reported by Reddy *et al.*, (2008) and Dudhe *et al.*, (2009).

Only one elite inbred RHA-1-1 restored fertility in all the four CMS lines and acted as common restorer, this indicated that though CMS lines were different by cytoplasmic background, the fertility restoring gene could be same. While 15 inbreds acted as common maintainers for two new CMS sources, suggesting the absence of fertility restoration genes in these inbreds. Kukosh (1981) reported that inbreds were found to carry Rf genes and can restore fertility with CMS lines developed with diverse cytoplasmic background. The inbred lines restoring fertility to different forms of CMS sources were found to be most useful in practical breeding programmes.

Ten inbreds *viz.*, GP-5, GP-9, NS-15, DSR-107, DSR-35, IB-03, DSR-37 and IB-104 acted as partial restorer for CMS PEF cytoplasm and behaved as restorers for PET-1 cytoplasm. It is evident from present investigation that few inbreds behaved differently with the three cytoplasmic backgrounds in respect of maintainer and restorer behaviour suggesting the presence of modifying genes influencing the fertility restoration, resulting in partial fertility (Rukmini Devi *et al.*, 2006; Dudhe *et al.*, 2009). The inbred lines *viz.*, GP-5, GP-9, DSR-107, DSR-35, IB-60, IB-03, DSR-37 and IB-104 behaved as partial restorer on CMS I cytoplasm, acted as fertility restorer for PET-1 cytoplasm. Higher number of maintainers was identified compared to restorers offering the more scope for CMS conversion programme and few restorers identified for new CMS sources suggested that new CMS sources could be used for CMS diversification as well as development of potential hybrids. Similar results were obtained in the study of Sujatha and Reddy *et al.*, (2008).

Table.1 Maintainer/restorer reaction of different inbred and restorer lines in the background of four CMS lines (three sources)

Inbreds	PET 1		CMS PEF	CMS I
	CMS 302A	CMS 234A	FMS 852A	IMS 852A
RHA-1-1	R	R	R	R
GP-9	R	R	R	R
GP-5	R	R	R	R
5 RI	M	S	M	M
IB-03	R	R	R	R
DSR-107	R	R	S	S
DSR-35	R	R	S	S
IB-60	R	R	R	R
DSR-37	R	R	S	S
NS-15	R	R	R	R
DSR-133	M	S	M	M
IB-51	S	S	M	M
NR P1	M	S	M	M
IB-104	R	R	R	R
ID-2089	S	S	M	M
14R	M	S	M	M
NS-8	S	S	M	M
GP6-1418	S	S	M	M
DSRI-411	S	S	M	M
B4 L-21	M	M	M	M
B2 L-13	S	S	M	M
B5 L-16	M	S	M	M
B4 L-6	M	M	M	M
B4 L-18	M	S	M	M
NS-3	S	S	M	M

The restorers identified for new CMS sources will help in their exploitation for hybrid development with better heterosis and diversity of cytoplasm in sunflower. The new CMS lines can be safely included in the breeding programme to broaden the genetic base of cytoplasmic male sterility in sunflower to avoid the possible risk of susceptibility. The identified maintainers after testing for their combining ability will be converted into new cytoplasmic male sterile lines and may be used in sunflower breeding programmes for developing diverse hybrids

with better heterosis and resistance to disease and insect pests.

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