



## Identification of Fertility Restorers with Desirable General Combining Ability for Oleic Acid Content, Seed Yield and Oil content in Sunflower (*Helianthus annuus* L.)

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**ABSTRACT:** During kharif 2020, study was conducted to examine the fertility restoration behaviour of 40 new high oleic inbred lines on six CMS lines and to evaluate the effects of general combining ability by mating the parents in a Line-Tester setup. In rabi 2020, the resulting 240 hybrids were assessed using an alpha lattice design. The male fertility or sterility response of all the  $F_1$ 's was visually inspected, and the resultant fertile hybrids were then validated once more using the acetocarmine staining procedure. Six CMS lines and 28 high oleic testers were found to be common restorers, while six were found to be common maintainers, out of the 40 high oleic testers assessed for sterility maintenance and restoration activity. The oleic acid content, seed yield plant<sup>-1</sup>, and oil content of the fertile testers F-20, K-11, L-3-1, G-5, and B-29-2 were found to be good general combiners based on the *gca* effects. The study's findings made it abundantly clear that the high oleic fertile testers with good general combining ability effects could pass on genes with additive effects to the hybrid progenies in the desired direction; as a result, it would be advantageous to use them as parents in breeding programmes to produce high oleic hybrids with high seed yield and oil content.

**Keywords:** Sunflower, oleic acid content, fertility restorers, CMS lines, *gca* effects.

### INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crop in the world as the nutritional quality of its edible oil ranks among the best vegetable oils in cultivation. It can easily be adjusted in existing crop rotations of wheat, cotton, rice and sugarcane and therefore sufficient area for sunflower could be made available in India without disturbing major crops. Sunflower hybrids having higher genetic potential for achene yield and oil content are required to fulfil the gap between supply and demand of vegetable oil all over the world. Like other cross pollinated crops sunflower hybrids are preferred over open pollinated varieties due to high yield and uniformity (Tyagi *et al.*, 2020).

The significant landmark in sunflower hybrid breeding was started economically after the finding of cytoplasmic male sterility (CMS) by Leclercq (1969) and identification of the gene for the fertility restoration by Kinman (1970), which brought the interest from population breeding to heterosis breeding (Asif *et al.*, 2013). The development of heterosis lead to the recognition of pollination control system like cytoplasmic- nuclear genetic male sterility system (CGMS). First ever CGMS system was obtained from an interspecific cross among *Helianthus petiolaris* Nutt and cultivated sunflower *H. annuus* (Leclercq, 1969) which is now generally known as PET 1 CGMS system. In India the first CGMS-based sunflower hybrid *viz.*, BSH-1 was developed by AICRP on sunflower,

Bangalore and released for the commercial production during 1980 (Seetharam, 1980).

The development of higher frequency of heterotic CMS-based hybrids for commercial development depends on the availability of large quantity of fertility-restorer lines (R-lines). The restorer lines are selected based on their capability to produce large number of pollens and anthers, which are worthy for proper pollen dispersal and also these are able to restore fertility to the hybrid progenies developed from CMS lines, as they have nuclear restorer genes *i.e.*,  $Rf_1$  and  $Rf_2$ . Restorer line with branching (Multi head) is advantageous, if there is synchronization problem with CMS lines, the pollens from side branches can be used which has multi heads. Among the identified restorer lines, it is worthy to utilize only those R-lines with significant general combining ability (*gca*) effect as is being used as dependable criteria for selection of parents for use in developing and identifying heterotic hybrids. Hence, the knowledge on the combining ability is crucial for the selection of desirable parents, for hybridization and identification of promising hybrids in sunflower breeding programme (Machikowa *et al.*, 2011).

The analysis of the Line  $\times$  Tester method (Kempthorne, 1957) is the one which is simplest, efficient and most amply used design for assessing the large number of inbreds as well as parents for their combining ability, providing knowledge on the respective principal of its *gca* effects, for elucidating the genetic basis of crucial plant characters. Therefore, the selection of parents

with good combining ability is very essential to produce the superior hybrids for seed yield, oil content and oleic acid content (Jarwar *et al.*, 2017). With this background the present study was conducted with an aim of identifying high oleic restorer lines with desirable general combining ability across different CMS lines of sunflower.

## MATERIAL AND METHODS

**Crossing Programme: kharif 2020.** The high oleic gene pool developed at the University of Agricultural Sciences, Bangalore's AICRP on sunflower served as the basis for the current work. Based on the high oleic acid content, the potential 40 inbred lines (Supplementary Table S1) were chosen from the high oleic gene pool. The six PET 1-based CMS lines (CMS 1103A, CMS 234A, CMS 903A, ARM 249A, CMS 59A, and CMS 103A) and the 40 high oleic lines that were shortlisted were sown in the field during kharif 2020 to accomplish crossing in line-tester mode (Kempthorne, 1957). To ensure that restorer lines and CMS lines for crossing would flower at the same time, all 40 testers were staggered sown twice, three days apart. To prevent unwanted pollination, cotton cloth bags were placed over the capitulum of CMS lines a day before the first ray floret opened. Additionally to preventing pollen mixing from outside pollen/other sources, the capitula of 40 testers were also covered with fabric bags to collect pollen.

A camel hairbrush was used in the early morning to apply, dust, or pollinate pollen from the testers onto the stigma of the female or CMS lines flowers. To guarantee sufficient seed establishment, the pollination was repeated for five to six days (on alternate days) in each combination. All test plants were sib pollinated. The capitula of all the resultant 240 hybrids were harvested, dried and threshed separately at physiological maturity. To evaluate hybrids, the well-filled seeds from each cross were taken out, and the sib-mated seeds of 40 testers were gathered.

**Evaluation of hybrids: rabi 2020.** 240 hybrids were used in the experiment, which was examined during rabi 2020 using an alpha lattice design with two replications to gauge how well fertility was restored and how well sterility was maintained. Using the presence or lack of pollen, anther dehiscence, and pollen shedding at the anthesis stage, all the  $F_1$ 's were visually inspected for male fertility or sterility reaction in order to understand the fertility restoration or sterility maintenance behaviour of inbred lines. They were classified as fertility restorers if all the  $F_1$  plants in the particular entry were fertile and as sterility maintainers if all the plants in  $F_1$ 's were sterile; those that were partially restoring fertility and sterility were regarded as partial restorers. This classification was based on the extent of fertility restoration and sterility maintenance by the respective inbred line in the crosses. These findings led to the classification of  $F_1$ 's as either male sterile, male fertile, or male partially fertile. Additionally, each newly created high oleic inbred line

was categorised with the corresponding CMS lines as a sterility maintainer, fertility restorer, or partial restorer.

**Pollen fertility test.** Morning collection of 10–12 disc florets from each of the five plants chosen at random in a cross combination was done during 50% blossoming. These were prepared with additional smears of 1% acetocarmine dye and evaluated under a light microscope for pollen fertility. Pollen grains that were deeply pigmented and had good exine were thought to be viable. Pollen grain that was badly discoloured and dried out was assumed to be infertile. By utilising a pollen fertility test with 1% acetocarmine, many inbred line reactions were thus confirmed (Chaudhary *et al.*, 1981).

Pollen fertility (%) =

$$\frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

## RESULTS AND DISCUSSION

### Identification of fertility restoration and sterility maintenance behaviour of high oleic inbred lines:

Figs. 1 and 2 demonstrated differential staining of pollen revealing the fertility restoration and sterility maintenance reaction in  $F_1$  hybrids under field conditions, correspondingly, Table 1 details the pollen fertility percentage of the hybrids as well as the degree of fertility restoration and sterility maintenance carried out by the respective inbred in the crosses. The new high oleic inbred lines that produced hybrids with complete fertility were categorised as fertility restorers (R), those that produced hybrids segregating for both sterility and fertility as partial restorers (R), and those that produced hybrids with complete sterility as sterility maintainers (M) (PR). Table 2 lists the specifics of this classification.

Out of the 40 pollen parents tested for fertility restoration on six CMS lines, some turned out to be sterility maintainers, some acted as fertility restorers and some as partial restorers. In general, out of 240 crosses 181  $F_1$ 's (75.4 %) was fertile, 36 cross combinations (15 %) were sterile and 23 cross combinations (9.5 %) were found to be partially sterile. The pollen fertility in the fertile hybrids ranged from 83.2 to 100 *per cent*. The inbred lines L-1-1, M-19-1 and L-3-1 yielded hybrids with highest pollen fertility of 100 *per cent* amongst all crosses attempted using all the 6 CMS lines under the study (Table 1).

An attempt was made to identify common high oleic restorers and maintainers for all six CMS lines. As a result of it, out of 40 high oleic inbred lines studied, 28 inbred lines restored the fertility in the  $F_1$ 's for all the six CMS lines and behaved as common restorers (Table 3).

These inbreds were restorers of PET 1 cytoplasmic source thereby suggesting that these lines have male fertile pollen carrying dominant restorer gene ( $Rf_1$ ) in the nucleus. Similar results were obtained by Meena and Sujatha (2013), Neelima *et al.* (2016), Satish and Sudheer (2011); while six inbred lines *viz.*, M-9, C-25, D-40-1, B-32-1, G-21-1, M-1 (Table 3) behaved as common maintainers in  $F_1$ 's by exhibiting sterile

reaction for all the six CMS lines, indicating the absence of fertility restoration gene in these lines or might be due to the presence of fertility restorer gene in recessive form. Sujatha *et al.* (2011); Meena and Sujatha (2013); Markin *et al.* (2017) also reported lack of fertility restorers in PET-1 background.

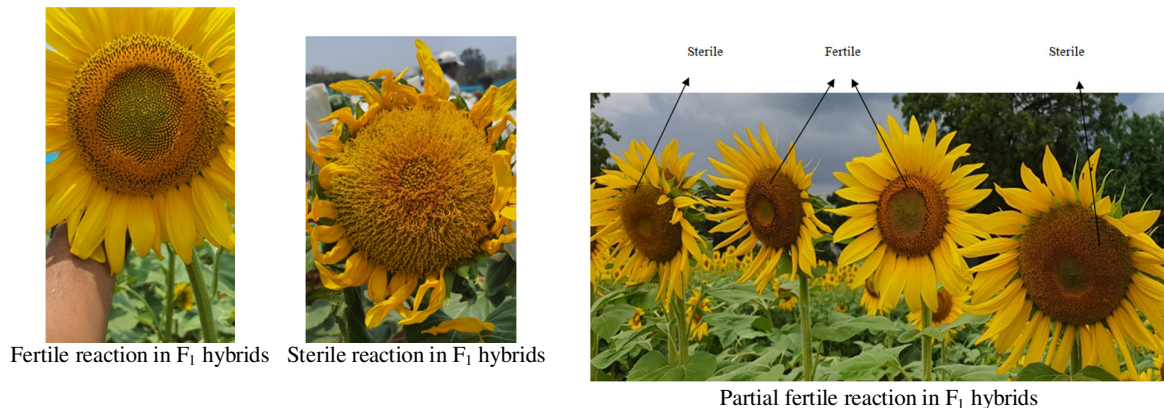
It was evident from present investigation that few inbreds behaved differently with the six CMS lines with respect to fertility restoration reaction, suggesting the influence of modifying genes on fertility restoration or it might also be suggesting that the nuclear background of the female parent also 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. The inbreds H-30, K-8, B-30-2, I-15, B-33, M-13-1 (Table 3) were partial restorers, indicating the presence of fertility restoration gene in heterozygous condition or a possible contamination with the unknown pollen or may be due to modifying effects of genes. Similar findings in sunflower fertility restoration studies were reported by Yogeesh *et al.* (2007); Meena and Prabhakaran (2017); Nehru *et al.* (2020).

The high oleic inbreds which were grouped as common restorers were further tested in the current study for combining ability of high oleic acid and high yield. The identified high oleic common maintainers from this

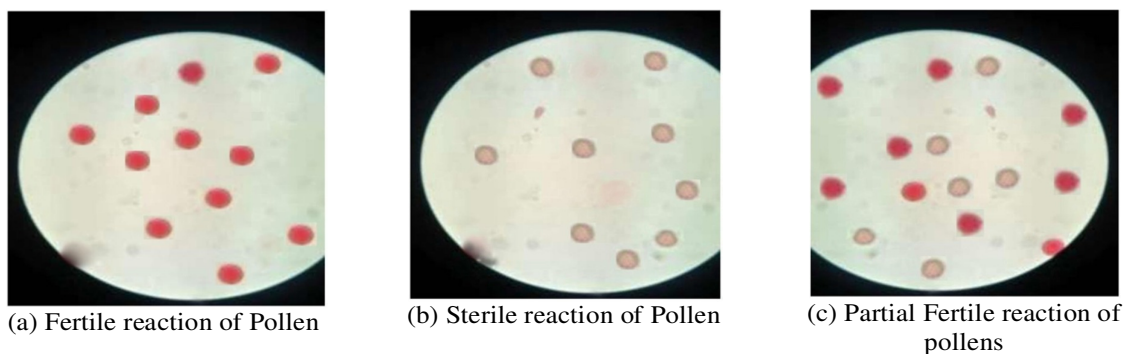
study could be converted into new high oleic CMS lines through backcrossing, which would serve the purpose of broadening the genetic base of CMS source and it might serve as useful CMS line for exploiting heterosis for oleic acid content in sunflower.

**General combining ability effects.** Among 28 high oleic fertile testers, 16 testers exhibited *gca* effects towards positive direction while 9 testers exhibited *gca* effects towards negative direction for oleic acid content Fig. 3a. The testers F-20 (13.53) followed by K-10 (12.08) recorded significantly highest *gca* effects for oleic acid content among all the testers. The eleven testers and three CMS lines with significant positive *gca* effect for oleic acid content identified in this study had the potential to produce high oleic hybrids.

The effects concerning general combining ability of lines and testers showed that none of the parents were good general combiner for every trait in the study. Though, good testers with desirable general combining ability for combination of three important traits in the study *viz.*, oleic acid content along with seed yield plant<sup>-1</sup> and oil content were identified and presented in Fig. 3 and Table 4. Among the testers exploited in the present study, there were five testers *viz.*, F-20, K-11, L-3-1, G-5 and B-29-2, that appeared to be good general combiners for oleic acid content in combination with seed yield plant<sup>-1</sup> and oil content (Table 4).



**Fig. 1.** Visual screening of the F<sub>1</sub>'s in field for male fertility or sterility reaction based on the presence or absence of pollen.



**Fig. 2.** Differential staining of pollen showing fertile and sterile reaction.

**Table 1: Per cent of male fertility as indicated by pollen fertility test (%) in the F<sub>1</sub> generation of the crosses between six CMS lines and 40 high oleic inbred lines.**

High Oleic Inbred Lines	CMS 234A		CMS 1103A		CMS 903A		ARM 249A		CMS 59A		CMS 103A	
	PF (%)	Class	PF (%)	Class	PF (%)	Class	PF (%)	Class	PF (%)	Class	PF (%)	Class
A-16	92.7	R	95.6	R	97.7	R	90.2	R	88.6	R	94.7	R
B-30-2	66.5	PR	88.3	R	47.2	PR	60.56	PR	49.8	PR	89.2	R
C-18	84.5	R	85.6	R	85.6	R	88.2	R	0	M	90.4	R
N-16	100	R	99.2	R	100	R	91.5	R	89.7	R	95.5	R
I-15	95.6	R	66.5	PR	87.2	R	86.5	R	67.7	PR	89.5	R
K-3	100	R	98.5	R	97.6	R	94.5	R	95.2	R	96.6	R
L-1-1	100	R	100	R	100	R	100	R	100	R	100	R
M-19-1	100	R	100	R	100	R	100	R	100	R	100	R
M-13-2	94.5	R	95.6	R	98.2	R	97.4	R	90.2	R	93.6	R
M-9	0	M	0	M	0	M	0	M	0	M	0	M
M-1	0	M	0	M	0	M	0	M	0	M	0	M
K-11	100	R	100	R	100	R	97.5	R	98.6	R	100	R
B-29-2	97.6	R	98.5	R	93.4	R	91.2	R	89.6	R	88.5	R
D-33-2	95.6	R	93.1	R	95.5	R	96.2	R	90.7	R	99.5	R
L-17	98.2	R	97.5	R	95.6	R	91.7	R	90.2	R	95.6	R
M-25	98.4	R	91.5	R	98.6	R	92.5	R	90.6	R	95.2	R
L-11	95.2	R	96.4	R	97.5	R	94.6	R	92.4	R	93.4	R
C-25	0	M	0	M	0	M	0	M	0	M	0	M
B-33	65.3	PR	77.6	PR	83.6	R	85.6	R	74.3	PR	88.6	R
A-18	88.7	R	88.2	R	88.9	R	89.2	R	83.2	R	88.9	R
L-3-1	100	R	100	R	100	R	100	R	100	R	100	R
K-8	56.6	PR	65.2	PR	61.1	PR	70.2	PR	55.6	PR	53.1	PR
G-17-1	95.6	R	95.2	R	90.2	R	95.3	R	90.1	R	95.6	R
K-10	96.4	R	92.4	R	90.1	R	92.5	R	89.6	R	90.4	R
M-13-1	84.8	R	88.6	R	83.2	R	88.2	R	0	M	90.4	R
C-30	98.2	R	95.6	R	97.4	R	93.2	R	90.6	R	94.2	R
B-19-1	97.8	R	98.3	R	97.2	R	94.1	R	90.2	R	95.1	R
K-19	96.2	R	96.4	R	90.7	R	94.3	R	98.7	R	96.3	R
D-40-1	0	M	0	M	0	M	0	M	0	M	0	M
G-5	96.7	R	89.2	R	95.2	R	88.9	R	87.6	R	92.6	R
G-12	97.5	R	98.6	R	97.2	R	94.1	R	90.2	R	95.1	R
H-30	65.5	PR	59.6	PR	68.2	PR	70.4	PR	78.5	PR	71.2	PR
K-22-2	98.2	R	97.5	R	95.6	R	91.7	R	90.2	R	95.6	R
G-21-2	98.4	R	91.5	R	98.6	R	92.5	R	90.6	R	95.2	R
F-20	100	R	100	R	98.2	R	98.6	R	95.6	R	100	R
D-11	98.8	R	99.5	R	98.2	R	97.6	R	94.3	R	99.1	R
B-32-1	0	M	0	M	0	M	0	M	0	M	0	M
B-21-1	90.5	R	97.6	R	89.1	R	88.9	R	87.6	R	90.2	R
G-21-1	0	M	0	M	0	M	0	M	0	M	0	M
C-15-1	97.6	R	98.5	R	93.4	R	91.2	R	89.6	R	88.5	R

PF- Pollen Fertility (%); R- Fertility Restoration; M- Sterility Maintenance; PR- Partial Fertility Restoration

**Table 2: Fertility restoration or sterility maintenance reaction of inbred lines.**

Sr. No.	HO Inbred lines	CMS 234A	CMS 1103A	CMS 903A	ARM 249A	CMS 59A	CMS 103A
1.	A-16	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
2.	B-30-2	Partial restorer	Restorer	Partial restorer	Partial restorer	Partial restorer	Restorer
3.	C-18	Restorer	Restorer	Restorer	Restorer	Maintainer	Restorer
4.	N-16	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
5.	I-15	Restorer	Partial restorer	Restorer	Restorer	Partial restorer	Restorer
6.	K-3	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
7.	L-1-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
8.	M-19-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
9.	M-13-2	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
10.	M-9	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
11.	M-1	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
12.	K-11	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
13.	B-29-2	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
14.	D-33-2	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
15.	L-17	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
16.	M-25	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
17.	L-11	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
18.	C-25	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
19.	B-33	Partial restorer	Partial restorer	Restorer	Restorer	Partial restorer	Restorer
20.	A-18	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
21.	L-3-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer

22.	K-8	Partial restorer	Partial restorer	Partial restorer	Partial restorer	Partial restorer	Partial restorer
23.	G-17-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
24.	K-10	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
25.	M-13-1	Restorer	Restorer	Restorer	Restorer	Maintainer	Restorer
26.	C-30	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
27.	B-19-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
28.	K-19	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
29.	D-40-1	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
30.	G-5	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
31.	G-12	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
32.	H-30	Partial restorer	Partial restorer	Partial restorer	Partial restorer	Partial restorer	Partial restorer
33.	K-22-2	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
34.	G-21-2	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
35.	F-20	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
36.	D-11	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
37.	B-32-1	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
38.	B-21-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer
39.	G-21-1	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer	Maintainer
40.	C-15-1	Restorer	Restorer	Restorer	Restorer	Restorer	Restorer

**Table 3: Common sterility maintainer and fertility restorer inbred lines for all the six CMS lines.**

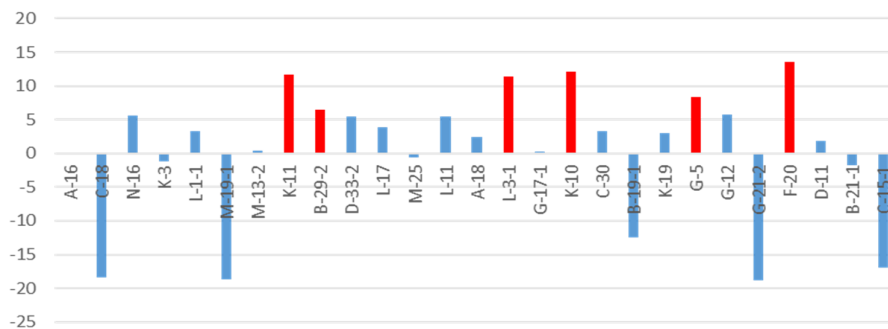
	Inbred lines	Total
Common restorers	A-16, A-18, B-19-1, B-21-1, B-29-2, C-15-1, C-18, C-30, D-11, D-33-2, F-20, G-12, G-17-1, G-21-2, G-5, K-10, K-11, K-19, K-22-2, K-3, L-11, L-1-1, L-17, L-3-1, M-13-2, M-19-1, M-25, N-16	28
Common maintainers	M-9, C-25, D-40-1, B-32-1, G-21-1, M-1	06
Partial Restorers	H-30, K-8, B-30-2, I-15, B-33, M-13-1	06

**Table 4: Best testers for oleic acid content along with seed yield plant<sup>-1</sup> and oil content based on *gca* effect.**

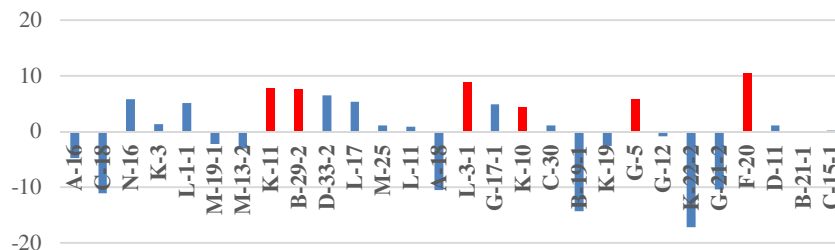
General combining ability effects ( <i>gca</i> effects)			
Testers	Oleic acid content (%)	Seed yield plant <sup>-1</sup> (g)	Oil content (%)
F-20	13.52**	10.49**	1.00**
K-11	11.61**	7.67**	1.59**
L-3-1	11.44**	8.75**	1.51**
G-5	8.30**	5.753**	0.55**
B-29-2	6.47**	7.54**	0.55**
K-10	12.07**	4.20**	-2.06*

\* Significant @ P=0.05; \*\*Significant @ P=0.01

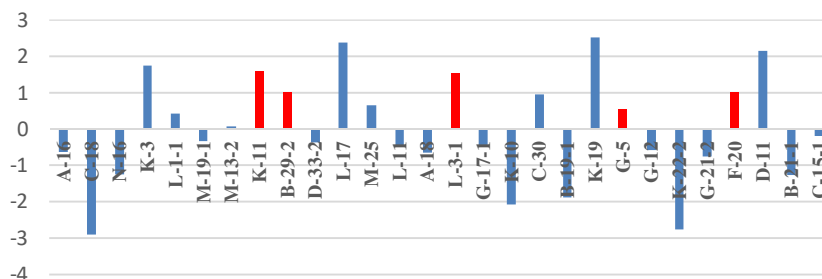
**a) Oleic acid content**



**b) Seed yield plant<sup>-1</sup>**



### c) Oil content



\*The testers indicated in red exhibited significant positive *gca* effects for oleic acid content along with seed yield plant<sup>-1</sup> and oil content

**Fig. 3.** Estimates of general combining ability effects of testers for a) Oleic acid content b) Seed yield plant<sup>-1</sup> c) Oil content.

**Supplementary Table S1: Per se performance of selected 40 high oleic inbred lines for yield, oil content and oleic acid content.**

Sr. No.	High Oleic Inbred Lines	Oleic acid content (%)	Seed yield plant <sup>-1</sup> (g)	Oil content (%)
1.	A-16	70.75	14.90	37.85
2.	B-30-2	74.75	12.95	36.28
3.	C-18	67.05	18.46	34.44
4.	N-16	75.85	17.87	38.24
5.	I-15	73.50	14.19	35.33
6.	K-3	65.80	19.10	35.00
7.	L-1-1	70.78	16.00	36.69
8.	M-19-1	68.65	17.52	40.49
9.	M-13-2	67.25	12.80	35.45
10.	M-9	77.00	12.67	35.54
11.	M-1	72.50	12.40	35.50
12.	K-11	81.60	14.17	35.27
13.	B-29-2	78.28	14.52	37.11
14.	D-33-2	76.40	12.93	35.44
15.	L-17	78.85	12.63	35.50
16.	M-25	70.25	13.88	35.44
17.	L-11	65.50	12.28	35.73
18.	C-25	69.00	12.72	38.08
19.	B-33	70.00	12.75	35.40
20.	A-18	65.00	12.74	35.39
21.	L-3-1	83.20	12.73	35.55
22.	K-8	74.00	12.55	35.25
23.	G-17-1	80.26	18.40	37.50
24.	K-10	87.50	12.45	36.10
25.	M-13-1	74.50	12.61	35.73
26.	C-30	74.10	17.54	38.19
27.	B-19-1	67.15	14.39	38.36
28.	K-19	66.00	12.55	37.79
29.	D-40-1	65.50	12.77	37.90
30.	G-5	81.05	17.01	36.05
31.	G-12	76.75	15.71	35.40
32.	H-30	67.50	16.19	36.41
33.	K-22-2	65.35	12.61	36.42
34.	G-21-2	65.30	14.20	35.25
35.	F-20	84.40	12.75	36.05
36.	D-11	75.75	13.30	38.27
37.	B-32-1	73.50	12.60	35.55
38.	B-21-1	65.25	12.35	40.11
39.	G-21-1	77.50	12.59	35.25
40.	C-15-1	70.75	12.43	35.63
	<b>Mean</b>	72.90	14.12	36.42
	<b>SE(m)</b>	0.52	0.24	0.33
	<b>CV</b>	1.02	2.40	1.29
	<b>CD @ 5%</b>	1.50	0.69	0.95

## CONCLUSIONS

The results from the study clearly suggested that the identified high oleic fertile testers with good general combining ability effects for oleic acid content in combination with seed and oil yield could transmit genes with additive effects to the hybrid progenies in the desirable direction thereby, it would be valuable to utilize them as parents in breeding programme to derive high oleic hybrids along with high seed yield and oil content.

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