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General Combining Ability Effects of New Inbred Lines and Identification of Best Specific Combiners to Exploit Heterosis for Seed yield and Oil content in Sunflower (*Helianthus annuus* L.)

M.S. Umar Farooq[1*], M. S. Uma[2], S.D. Nehru[2] and C.P. Manjula[2]
[1]Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore-560065, (Karnataka), India.
[2]AICRP on Sunflower, University of Agricultural Sciences, GKVK, Bangalore, (Karnataka), India.

(Corresponding author: M.S. Umar Farooq)*
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ABSTRACT: Successful selection of genotypes with a high genetic potential for seed yield and oil content requires all the possession of the appropriate genetic variability in the initial material. In order to achieve this, it is necessary to have information on the mode of inheritance and the manifestation of heterotic effect for trait of interest as well as to identify superior genotypes based on combining abilities, with this background an investigation was carried out on 200 sunflower hybrids produced through line x tester mating design using 5 CMS lines and 40 newly developed inbred lines (testers) to generate information on effects due to combining ability in respect of seed yield and oil content in sunflower. Analysis of variance revealed that the mean sum of squares due to the testers was significant for the two traits under study. Estimation of general combining ability (GCA) effects of testers indicated that, the testers *viz.*, F-20, L-3-1 and B-29-2 exhibited significant GCA effects for seed yield per plant while K-19, D-11 and L-17 were the testers considered as good general combiners for high oil content. High significant L × T interaction towards total variance emphasized the predominance of non-additive gene action in exploitation of heterosis. Ten best specific combiners were selected based on their *per se* performance which also exhibited high significant SCA effects. As in sunflower the hybrids possessing both high seed yield plant^{-1} and oil content are greatly preferred, the study also emphasized on the identification of five promising hybrids surpassing the standard checks *viz.*, ARM249A × D-11, CMS234A × L-3-1, ARM249 × M-25, CMS234A × D-11 and CMS234A × K-3 possessing both high seed yield plant^{-1} and high oil content in combination and these resultant hybrids could be tested in large scale yield trials over locations and seasons to confirm their potentiality for commercial cultivation.

Keywords: GCA, Heterosis, line × tester analysis, Oil content, Seed yield and SCA.

INTRODUCTION

The main objective of sunflower (*Helianthus annuus* L.) breeding is to increase seed yield and oil content. Yield as a complex trait requires the most effort in breeding process due to its polygenic character and great influence of the environment, characterized by low heritability (Nasreen *et al*. 2011). Successful selection of genotypes with a high genetic potential for seed yield and oil content requires all the possession of the appropriate genetic variability in the initial material. In order to achieve this, it is necessary to have information on the mode of inheritance and the manifestation of heterotic effect for trait of interest as well as to identify superior genotypes based on combining abilities (Gjorgjieva *et al*., 2015).

The success of the hybridization is largely dependent on the correct selection of parents. The combining ability effects of parents gives useful information on the choice of parents in terms of expected performance of their progenies (Reddy *et al*., 2021). The general combining ability (GCA) effects are important indicators of the value of inbred lines in hybrid combinations. The high GCA effect for a particular trait of a parent indicates the additive gene effect for the trait governed by the genes in the parent concerned and it is fixable. Differences in general combining ability effects have been attributed to additive, additive × additive and higher-order additive interactions (Kang *et al*., 2013).

The specific combining ability effects (SCA) take into account the relative performance of a specific cross combination. The SCA variance denotes non-additive or dominance portion of variance and generally non fixable on selfing but can be exploited in hybrid combination. The ratio of these variances indicates the nature of gene actions governing the traits, which is important to decide upon the breeding methods to be adopted, parents and crosses to be selected for exploiting the type of gene actions in a crop for selection of superior genotypes and for eventual success (Radanović *et al*., 2018).

The line × tester analysis (Kempthorne, 1957) is one of the simplest and efficient method of evaluating large number of inbreds for combining ability and *per se* performance, also it is a powerful tool to discriminate the good as well as poor combiners for choosing appropriate parental material in successful hybrid breeding program. However, the success of hybrid sunflower program depends upon the magnitude of heterosis which also helps in the identification of potential cross combinations to be used in the conventional breeding program to create wide array of variability in the segregating generations. Therefore, the present investigation has been conducted to determine the combining ability effects for seed yield and oil content using line × tester mating design to find out the best combiners among the newly evolved Inbred lines and also to identify hybrids surpassing the best checks.

MATERIAL AND METHODS

The material used for the study consisted of five cytoplasmic male sterile lines *viz.*, CMS 1103A, CMS 903A, CMS 234, ARM 249A and CMS 59A whereas 40 new inbred lines as testers and four standard check hybrids *viz.*, KBSH 44, KBSH 53, KBSH 78 and RSFH 1887. The CMS lines and the testers were collected from AICRP on sunflower, ZARS, UAS, GKVK, Bengaluru.

Crossing programme. All the five CMS lines and 40 inbred lines were sown in the field to effect crossing in a Line × Tester fashion in order to obtain F_1's. Staggered sowing of all CMS lines were carried out three times at an interval of two days to ensure synchronized flowering with inbred lines for crossing. A day prior to opening of first ray floret all the heads of CMS lines and testers were covered with cloth bags in order to prevent undesirable pollination. Pollen from the inbred lines was collected separately in petri dishes with the help of camel hair brush and applied to the flowers of female lines using brushes during morning hours. The pollination was repeated for five to six days in each of the combination to ensure sufficient seed set and simultaneously all inbreds were sib pollinated. The capitulum of all the resultant 200 hybrids were harvested, dried and threshed separately at physiological maturity. The well filled seeds from each cross were separated out for hybrid evaluation and sib mated seeds of 40 inbred lines were collected for future evaluation.

Evaluation of hybrids: The resultant 200 experimental hybrids with four standard checks were evaluated in two replications along with parents. Experiment was laid out in simple lattice design. Each genotype was sown in a single row of three-meter length with a row spacing of 60 cm and 30 cm between plants within a row. Observations were recorded in each entry on randomly selected five plants for seed yield per plant and oil content.

Combining ability analysis: The mean values computed for each character were subjected to combining ability analysis as suggested by (Kempthorne, 1957).

General combining ability effects were calculated using the expression:

$g_i = (X_i. /tr) - (X.../ltr)$

Specific combining ability effects were calculated using the expression:

$s_i = (X_{ij}/r) - (X_i.../tr) - (X._j./lr) + (X.../ltr)$

l = number of lines

t = number of treatments

r = number of replications

Estimation of Heterosis:

Heterosis over Standard Checks (SC) was computed by the method suggested by Turner (1953).

Heterosis *per cent* over standard check (%)

$$= \frac{F_1 - SC}{SC} \times 100$$

RESULTS AND DISCUSSION

Analysis of variance for seed yield and oil content: Analysis of variance for combining ability (Table 1) revealed that variance due to lines was significant for seed yield per plant and oil content. Testers and Line × tester interaction variance was highly significant for the traits under study which indicated the significance of dominance variance. The significance due to line x tester variance indicated the presence of heterosis for the respective traits. Similar results were also noticed by earlier workers (Pavani *et al*., 2006; Shankar *et al*., 2007 and Meena *et al*., 2013).

Table 1: Analysis of variance for Line × Tester and combining ability.

		Mean sum of squares	
Source of variation	**Df**	**Seed yield plant^{-1} (g)**	**Oil content (%)**
Replication	1	14.21	1.96
Hybrids	95	155.01 ***	7.14***
Line Effect	5	152.52*	9.14*
Tester Effect	15	378.26***	12.71 *
Line × Tester Effect	75	110.52 ***	5.89***
Parents vs Hybrids	1	38081.74***	12.760***
Error	95	9.01	0.07
Total	191	81.65	3.60

*Significance at P = 0.05 level   ** Significance at P= 0.01 level   ***Significance at P= 0.001 level

General combining ability effects: The breeding value expressed as deviation from the population mean is nothing but *gca* effect, has proved to be very useful tool in identifying the potential parents for hybrid cultivar development. The estimates of *gca* effects provide a relative measure of additive variance, which aids in the selection of superior genotypes for breeding programme. The estimates of general combining ability (GCA) effects due to 5 lines and 40 testers for seed yield per plant and oil content are presented in Fig. 1a and 1b, respectively.

Among 40 testers around 18 testers have exhibited significant *gca* effects in desirable direction for seed yield per plant whereas 15 testers exhibited significant *gca* effects in desirable direction for oil content. Highest positive GCA effect for seed yield per plant was recorded by the tester F-20 (17.78) followed by L-3-1 (15.67) and B-29-2 (11.15) whereas highest positive GCA effect for oil content was recorded by the tester K-19 (2.80) followed by D-11 (2.75) and L-17 (2.73). The results were in agreement with the findings of (Cvejić *et al.*, 2017; Jocković *et al.*, 2018 and Sujatha *et al.*, 2009). For simultaneous improvement of seed yield and oil content in the hybrids, either the male or female parent should possess good *gca* for these two traits together or the parents involved in the cross combinations should have good *gca* for one of the traits and able to nick well in the hybrids to produce superior heterosis and high *sca*. From this study the identified testers with high significant positive GCA effects for the above traits implicates their capacity to transmit additive genes in desirable direction and also suggests that it would be productive to exploit them as parents in hybridization to derive the hybrids with high seed yield and oil content.

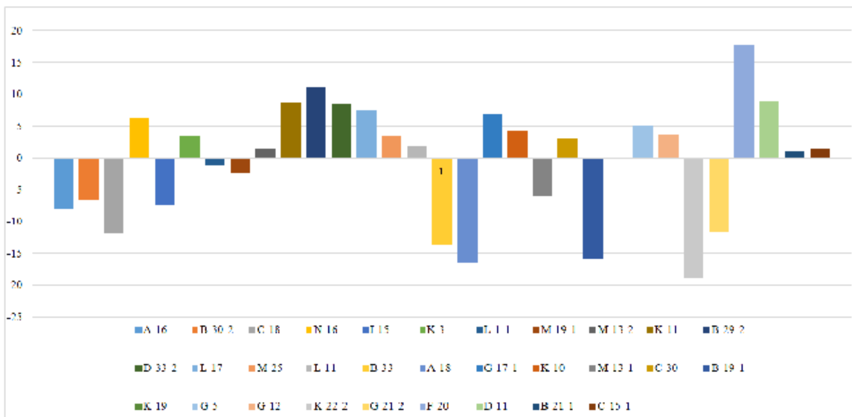

Fig. 1a. Estimates of general combining ability effects of Testers for seed yield per plant.

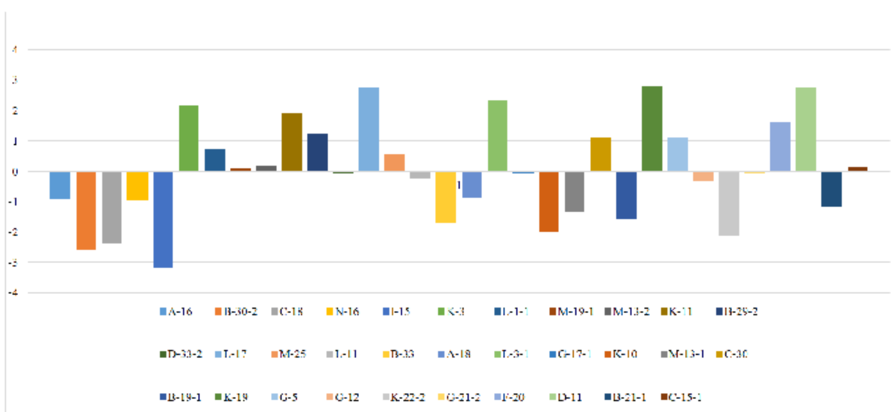

Fig. 1b. Estimates of general combining ability effects of Testers for oil content.

Proportional contribution of CMS lines, restorer testers and their interaction to total variances: The relative contribution of line, tester and their interaction to total variances for seed yield per plant and oil content are presented in Fig. 2. The proportion contributed by CMS lines to total variances was very low with only 3.03 per cent of contribution towards seed yield per plant while 5.33 per cent for oil content. However, testers showed relatively higher contribution as evident for seed yield per plant (58.79%) and oil content (56.54%). It is clear from Fig. 2. that testers contribution was more for both the traits under study indicating that the new testers used in the study contributed more positive alleles for these two traits having predominance for additive gene action.

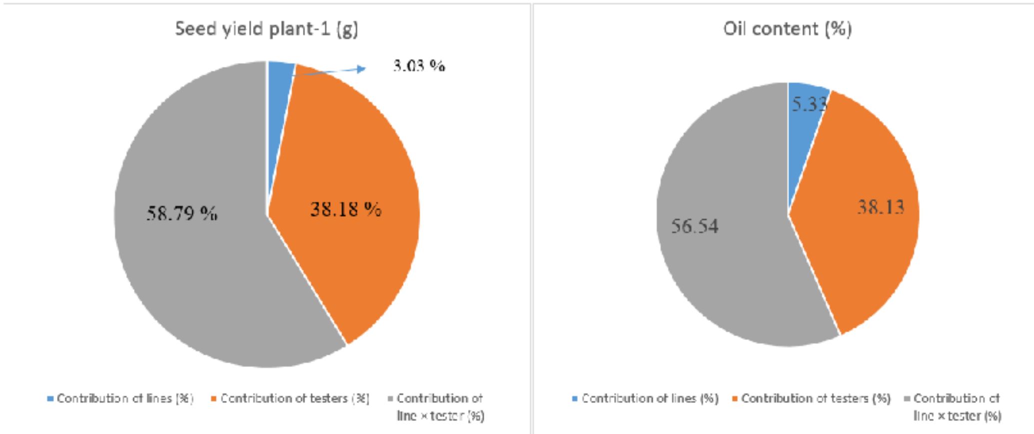

Fig. 2. Proportional contribution of CMS lines, testers and line × tester interaction to the total variances among the hybrids.

Specific combining ability effects: The term "specific combining ability" is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the lines involved (Baloch *et al*., 2018). Specific combining ability effects of top ten best performing hybrids with respect to seed yield per plant is presented in Table 2a. while with respect to oil content is presented in Table 2b. All the ten hybrid combination showed highly significant SCA effect. It is clear from Table 2. that the hybrid combination ARM249 × M-25 exhibited highest seed yield per plant (70.92g) and as well oil content (40.96 %) with high significant SCA effects of 25.83 and 2.57 respectively indicating that it is the best specific combiner among all the hybrids under this study bearing high significant SCA effects for both seed yield per plant and oil content in combination and also justifying the presence of high degree of dominance and non-additive gene action for these traits in this particular cross combination.

Table 2a: Specific Combining Ability Effects of top ten best performing hybrids with respect to seed yield plant^{-1}.

Seed yield plant^{-1}			
Rank	**Hybrid Combination**	**Mean seed yield per plant (g)**	***sca* effects**
1	ARM249A × D-11	77	26.305***
2	CMS234A × L-3-1	75.30	14.860***
3	CMS1103A × F-20	72.61	16.520***
4	ARM249 × M-25	70.92	25.833***
5	CMS234A × G-12	70.42	22.05***
6	CMS903A × M-13-2	70.33	29.286***
7	CMS234A × F-20	68	5.443***
8	CMS903A × L-3-1	67.30	12.043***
9	CMS234A × D-11	66.85	13.135***
10	CMS234A × K-3	66.70	18.542***

*Significance at P = 0.05 level ** Significance at P= 0.01 level ***Significance at P= 0.001 level

Table 2b: Specific Combining Ability Effects of top ten best performing hybrids with respect to Oil Content.

Oil content			
Rank	**Hybrid Combination**	**Mean Oil Content (%)**	***sca* effects**
1	CMS1103A × K-19	42.20	3.957***
2	CMS234A × L-17	41.63	2.042***
3	ARM 249A × M-25	40.96	2.574***
4	ARM249A × K-10	40.61	1.603**
5	CMS903A × K-3	40.19	1.612**
6	ARM249A × D-11	39.80	1.005*
7	CMS234A × D-11	39.70	0.099*
8	CMS234A × L-3-1	39.61	2.046***
9	CMS234A × G-5	39.40	1.414*
10	CMS234A × K-3	39.31	5.273***

*Significance at P = 0.05 level ** Significance at P= 0.01 level ***Significance at P= 0.001 level

Estimation of Heterosis: Heterosis is the increase or decrease in vigour of F_1 over its mid or better parental value. One of the objectives of present study was to estimate the extent of heterosis and to isolate promising hybrids over standard check hybrids for seed yield and oil content for commercial exploitation. The maximum utilization of heterosis is possible when the variance due to both additive and non-additive gene actions are fully exploited since they play a significant role in determining the magnitude of expression of yield and its component characters.

The standard heterosis of top ten best performing hybrids with respect to mean seed yield per plant are presented in Table 3a. While for oil content is presented in Table 3b. Test hybrids showed highly significant standard heterosis over commercial check hybrids. Similar results were reported by (Hladni *et al*., 2018; Tyagi *et al*., 2020; Haddadan *et al*., 2020). One of the prime objectives of present study was to estimate the extent of heterosis and to isolate promising hybrids over standard checks bearing both high seed yield and oil content in combination for commercial exploitation. Hence from the Table 3a and 3b the hybrids surpassing the standard checks for both seed yield per plant and oil content were selected *viz*., ARM249A × D-11 (77g and 39.80%), CMS234A × L-3-1 (75.30g and 39.61%), ARM249 × M-25(70.92g and 40.96%), CMS234A × D-11 (66.85g and 39.70%) and CMS234A × K-3 (66.70g and 39.31). The heterosis for seed yield and oil content is utilized in maximum to select the best possible hybrids in combination of these two traits and it could be possible as the variance due to both additive and non-additive gene actions were fully exploited since they play a significant role in determining the magnitude of expression of yield and oil content.

Table 3a: Standard Heterosis of top ten best performing hybrids with respect to seed yield plant^{-1}.

Rank	Hybrid Combination	Mean seed yield per plant (g)	Standard Heterosis (%)			
			KBSH-44	KBSH-53	KBSH-78	RSFH-1887
1	ARM249A × D-11	77	67.85**	88.36**	38.74**	100.70**
2	CMS234A × L-3-1	75.30	64.14**	84.20**	35.68**	96.27**
3	CMS1103A × F-20	72.61	58.27**	77.61**	30.82**	89.25**
4	ARM249 × M-25	70.92	54.58**	73.47**	27.77**	84.84**
5	CMS234A × G-12	70.42	53.49**	72.25**	26.87**	83.54**
6	CMS903A × M-13-2	70.33	53.31**	72.04**	26.72**	83.32**
7	CMS234A × F-20	68	48.23**	66.34**	22.52**	77.24**
8	CMS903A × L-3-1	67.30	46.70**	64.63**	21.26**	75.42**
9	CMS234A × D-11	66.85	45.72**	63.53**	20.45**	74.25**
10	CMS234A × K-3	66.70	45.40**	63.16**	20.18**	73.86**
C-1	KBSH 44	45.87				
C-2	KBSH 53	40.88				
C-3	KBSH 78	55.5				
C-4	RSFH-1887	38.36				

*Significance at P = 0.05 level ** Significance at P= 0.01 level ***Significance at P= 0.001 level

Table 3b: Standard Heterosis of top ten best performing hybrids with respect to Oil Content.

Rank	Hybrid Combination	Mean Oil Content (%)	Standard Heterosis (%)			
			KBSH-44	KBSH-53	KBSH-78	RSFH-1887
1	CMS1103A × K-19	42.20	21.53**	6.84**	5.11*	17.71**
2	CMS234A × L-17	41.63	19.88**	5.39**	3.69*	16.12**
3	ARM 249A × M-25	40.96	17.96**	3.70*	2.02*	14.25**
4	ARM249A × K-10	40.61	16.95**	2.81*	1.15*	13.28**
5	CMS903A × K-3	40.19	15.75**	1.76*	0.11	12.12**
6	ARM249A × D-11	39.80	14.61**	0.76	-0.87	11.02**
7	CMS234A × D-11	39.70	14.33**	0.51	-1.12	10.74**
8	CMS234A × L-3-1	39.61	14.08**	0.29	-1.33	10.50**
9	CMS234A × G-5	39.40	13.46**	-0.25	-1.87	9.90**
10	CMS234A × K-3	39.31	13.20**	-0.48	-2.09	9.65**
C-1	KBSH 44	34.72				
C-2	KBSH 53	39.5				
C-3	KBSH 78	40.15				
C-4	RSFH-1887	35.85				

*Significance at P = 0.05 level ** Significance at P= 0.01 level ***Significance at P= 0.001 level

CONCLUSION

Identification of superior hybrids using empirical estimates of *gca* and *sca* originated by Line × Teter is fundamental strategy in evaluation of breeding value of genotypes involved in breeding program. Combining ability analysis indicated that both genetic components, additive and non-additive, were important in expression of investigated traits. GCA effects showed that the inbred lines F-20, L-3-1 and B-29-2 can be useful for improving seed yield per plant while the inbred lines K-19, D-11 and L-17 can be distinguished as a best general combiner for improving oil content in sunflower. The present study also resulted in the identification of five promising hybrids *viz.,* ARM249A × D-11, CMS234A × L-3-1, ARM249 × M-25, CMS234A × D-11 and CMS234A × K-3 with high seed yield plant^{-1} and oil content in combination and these resultant hybrids could be tested in large scale yield trials over locations and seasons to confirm their potentiality for commercial cultivation.

Conflict of Interest. None.

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