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Santhosh N
Ph.D. Scholar, College of Horticulture, UHS campus, Bangalore, Karnataka, India

Tejaswini
Tejaswini, Principal scientist, Division of ornamental crops, IIHR, Bangalore, Karnataka, India

Shivashankar KS
Shivashankar KS, Principal scientist, Department of Plant physiology and Biochemistry, IIHR, Bengaluru, Karnataka, India

Archana K Gadre
Archana Gadre, Senior research fellow, IIHR, Bengaluru, Karnataka, India

Correspondence
Santhosh N
Ph. D. Scholar, College of Horticulture, UHS campus, Bangalore, Karnataka, India

Exploitation of heterosis for biochemical component in African marigold (*Tagetes erecta*) by using different male sterile types

Santhosh N, Tejaswini, Shivashankar KS and Archana K Gadre

Abstract
Two types of male sterile lines isolated in marigold were evaluated for their effective contribution for heterosis exploitation of biochemical components. Among seven male sterile lines used as seed parents in the study, three are petaloid sterile lines and four are apetaloid sterile lines. Three fertile pure lines, were used as pollen parents evaluated in different cross combination with all the 7 sterile lines. Line versus tester mean squares for combining ability were significant for all the biochemical components. The results of combining ability revealed that, total carotenoids, zeaxanthin and lutein appeared to be governed by non-additive gene action. Estimates of general combining ability effects showed that parent IIHRMO 9-7 was a good general combiner for lutein and total carotenoid. For total carotenoids and lutein, the highest positive values of heterotic effects were recorded in hybrid combination IIHRMO 9-8× IIHRMO 12-12 relative to the better parent.

Keywords: carotenoids, lutein, zeaxanthin, combining ability, gene action, heterosis

Introduction
Marigold (*Tagetes sps.*), is one of the most important flower crop that has proved its potential all around the world as an ornamental flower and has been identified as a plant with pharmaceutical properties. Marigold is widely used as colorant in the food and animal feed industry. Commercially, carotenoid pigment in marigold flowers are used in poultry feed to provide yellow colour to the skin of broilers and yolks of layers (Hojnik *et al*., 2008; Liu *et al*., 2011) [12, 16]. Carotenoids are wide spread colouring pigments in plants and are involved in photosynthesis and photo protection. The application of carotenoids in medicine and cosmetics is well documented (Gau *et al*., 1983; Seddon, 1994) [6, 21]. Plant total carotenoids consist of different carotenes (α, β and γ fractions), xanthophylls (lutein, zeaxanthin) and their esterified forms (Goodwin, 1965; Liu *et al*., 2011) [16]. The principal carotenoid pigment of marigold flower is xanthophyll consisting of zeaxanthin and lutein esters which have been reported to be beneficial to several aspects of human health (Timberlake and Henry, 1986; Hadden *et al*., 1999) [10, 26]. Xanthophylls and lutein offer an alternative to synthetic colours and used as natural food colorant and nutrient supplement (Pratheesh *et al*., 2009).
Marigold flower petals are a significant source of the xanthophyll and have a much higher concentration of this pigment compared to other plant materials (Verghese, 1998a; Verghese, 1998b) [27, 28].The most important source is flower petals of marigold, where lutein is chemically bound to various types of fatty acids such as lauric, mystric and palmitic acids (Khalil *et al.*, 2012) [14]. Upon saponification of the marigold extract, the lutein fatty acid esters are converted to free lutein (Bhattacharya, 2010) [10]. Lutein represented over 95% of the pigments identified in the petals of the marigold (Quackenbush and Miller, 1972) [20]. Besides its use as food colorant, lutein is also used as natural dye for textile coloration (Jothi, 2008).
High dietary intake of lutein has been associated with risk reduction of many chronic diseases, and cardiovascular diseases. Risk of age related macular degeneration, heart disease, lung and skin cancers can be reduced by higher intakes of lutein. (Sowbhagya *et al*., 2004, Wang *et al*., 2006) [23, 29]. The discovery of *Tagetes sps.* as the richest common source of lutein and zeaxanthin changed the entire view regarding the commercial importance of marigold. Hybrid varieties of marigold are being cultivated around the world for ornamental purpose as well for pigment. However, not much information is available on the breeding strategies for improving the biochemical compositions (Sreekala and Raghava, 2003) [24].

Currently, marigold hybrids are produced by traditional hybridization process. The artificial emasculation of flowers is a highly labor intensive process, particularly in the case of Asteraceae family where multiple individual florets combine to form a single capitulum structure (Ai *et al*., 2014) [1] and emasculation is very difficult considering structure of capitulum where functional anthers are hidden within disc florets in the center (Funk *et al.*, 2009) [5]. Cost effectiveness in hybrid seed production can be realized by utilization of male sterile seed parents. Reports of available male sterile lines are very limited in *Tagetes* and there is no information available on different types of male sterile systems other than degenerated form of flowers (Gupta *et al*., 1999, He *et al*., 2009, Ai *et al*., 2014) [1, 8, 11]. The information available on heterosis in *Tagetes* is focused on floral characters ((Ai *et al*., 2015, Gupta, 2009) [2, 8] and limited to apetaloid male sterile lines (Sreekala and Raghava, 2003) [24]. There is complete lack of information about different male sterile systems available in marigold and their contribution for heterosis breeding. Most of the heterosis studies in *Tagetes* is mostly concentrated on ornamental value (Tang *et al*., 2009, Pan *et al*., 2014, Ai *et al*., 2015) [2, 18, 25]. Strategic heterosis breeding program for biochemical components needs an understanding of genetics and contribution of different male sterile systems.

Present study reports contribution of different male sterile systems for biochemical components of marigold. At the Indian Institute of Horticultural Research, Bengaluru, we have identified several male sterile lines and classified into two different male sterile systems. In this paper, we report the contribution of different male sterile systems on heterosis of biochemical components. We have analysed characters contributing for major biochemical components of marigold and report gene action, combining ability as well as heterosis realized from different male sterile systems. Association of heterosis with parental mean and combining ability has been assessed to decide upon breeding strategy for enhanced production of biochemical components.

Material and Methods

Plant material for the experiment consisted of three pure lines and seven male sterile lines developed at Indian Institute of Horticulture Research, Bangalore, India located at $13^0$58'North latitude and 78^0East latitude at an altitude of 890M. Based on floral morphology, seven male sterile lines were classified into two groups *viz*., petaloid and apetaloid sterile types. In flowers of petaloid male sterile lines, all disc florets are replaced by ray florets with functional gynoecium and there are no androecioum (Fig. 1a). In apetaloid sterile lines, all the florets are degenerated in to filament like structures (Fig. 1b). In both petaloid and apetaloid sterile types, flowers were characterized by absence of androecium but well developed functional gynoecium. Male sterile lines were used as seed parents and fertile lines were used as pollen parents (Fig. 1c). Seven male sterile lines and three fertile pure lines were used in crossing programme in accordance with the Line x Tester mating design (Kempthorne, 1957) [13]. Detailed description of line and testers used in the study are presented in Table 1. For hybridization, selected buds from both seed and pollen parents were covered by butter paper covers before anthesis to avoid contamination by unwanted pollen. Best hybridization time was observed to be between 9 a.m to 3 p.m. At the right stage of stigma receptivity, pollen from the selected pollen parents were dusted on seed parents as per the mating design. Twenty one hybrid combinations along with ten parents were planted in randomized block design with three replications. Plants were grown at a recommended spacing of 40 x 60cm under optimal growing conditions. Observations were recorded on five randomly selected plants of each genotype in each replication for various characters *i.e.* flower diameter, individual flower weight, petal meal per flower, number of flowers per plant and flower weight per plant. Line x tester analysis was worked out by following the method of Singh and Chaudhary (1979) [22]. Petals of individual flower were dried and taken as petal meal per flower. Biochemical components were estimated using petal meal as listed below.

Fig 1a: Petaloid flower

Fig 1b: Apetaloid flower

Fig 1c: Fertile flower

Table 1: Characteristic features of parents and checks

Sl. No.	Parent	Source	Feature
Lines (Seed parent)			
1.	L_1= IIHRMO 9-7	IIHR	Petaloid sterile line, Orange flowers
2.	L_2= IIHRMO 9-8	IIHR	Petaloid sterile line, Orange flowers
3.	L_3= IIHRMO 10-1	IIHR	Petaloid sterile line, Orange flowers
4.	L_4= IIHRMO 7-2	IIHR	Apetaloid sterile line, Orange flowers
5.	L_5= IIHRMO 23-2	IIHR	Apetaloid sterile line, Orange flowers
6.	L_6= IIHRMO 23-5	IIHR	Apetaloid sterile line, Orange flowers
7.	L_7= IIHRMO 23-7	IIHR	Apetaloid sterile line, Orange flowers
Testers (Pollen parent)			
1.	T_1= IIHRMO 12-12	IIHR	Fertile pure lines with orange flowers.
2.	T_2= IIHRMO-3	IIHR	Fertile pure lines with orange flowers.
3.	T_3= Pusa Narangi	IARI	Fertile pure lines with orange flowers.
Checks			
1.	Check 1= Indus deep orange	Indus seeds	Commercial F_1 hybrids with orange flowers
2.	Check 2= Tall orange	Ashoka seeds	Commercial F_1 hybrids with orange flowers

Estimation of Biochemical components

Total carotenoid content were analyzed by spectrophotometric method (Lichtenthaler, 1987) [15]. Fresh flowers were dried and used for estimation of biochemical parameters. Dry petals were extracted by using acetone and further separation was carried out by using hexane. Total carotenoid was estimated using spectrophotometer at 470nm absorbance. Lutein content was estimated by UPLC (Aquity UPLC-H class Waters limited, USA) connected to a PDA detector with quaternary pump and controlled by Mass Lynx software of Waters. The column used was aquity UPLC BEH C18 (50mm x 2.1 mm, 1.7 µm) with security guard column BEH C18 (5mm x 2.1 mm, 1.7 µm). The mobile phase was Acetonitrile: Methanol: Tetra hydro furan (10:60:30) as solvent A and pure methanol as solvent B with a flow rate of 0.1mLmin−1. Wavelength used for lutein was at absorbence of 446 nm.

Results

In the study material, total carotenoids per plant ranged from 24.69mg to 888.20mg, whereas lutein per plant was 19.55 to 713.9mg, and zeaxanthin per plant ranged between 0.05 to 2.42mg. Significant variation was observed in mean values of various characters among lines, testers and resulting hybrids (Table 2). Wide variation for mean values was observed among petaloid and apetaloid sterile lines as well as testers for all the characters studied. Per plant lutein content was maximum in petaloid sterile line IIHRMO 9-7 (155.95mg/plant) and hybrid derived from petaloid sterile line IIHRMO 9-8 (713.90mg/plant). Per plant yield of biochemical component differed from percentage of content. For instance lutein content per 100gm of petal meal ranged between 307.90 to 1115.82 mg among parents whereas per plant yield of lutein ranged between 19.55 to 155.95 mg in parents. Similarly, lutein content in hybrids ranged between 330.18 to 1246.18mg per 100 gm of petal meal whereas per plant yield of lutein ranged between 45.82 to 713.90mg. In general, content of biochemical components per 100gm of petal meal were higher in apetaloid sterile lines but yield of biochemical components per plant was higher in petaloid male sterile lines. Among sterile lines used in the study, maximum carotenoid content was in case of apetaloid sterile line IIHRMO 23-7 (1083.20mg/100 gm dry petal meal) but when calculated per plant yield of carotenoid, it was maximum in petaloid sterile line IIHRMO 9-7 (217.97mg/plant). Hybrids derived from petaloid sterile line contained maximum carotenoid content per 100gm of petal meal (1571.18mg) as well as carotenoid yield per plant (888.20 mg). Petal meal per flower was significantly higher in petaloid male sterile lines compared to apetaloid male sterile lines as well as fertile lines. In case of hybrids, total caroteonoid, zea xanthin and lutein per plant was maximum in hybrid combinations resulting from petaloid sterile lines. Petal meal per flower was also maximum in case of hybrids resulting from petaloid sterile. Among the three testers used in the study, biochemical components were maximum in Pusa Narangi.

Table 2: Mean performance of flower characters and biochemical parameters

Parents and Hybrids	FD	FWF	PMF	FNP	FWP	LC	ZC	TC	LCP	ZCP	TCP
Petaloid male sterile lines											
IIHRMO 9-7	4.87	2.44	0.25	116.07	278.07	539.46	1.74	754.98	155.95	0.50	217.97
IIHRMO 9-8	4.70	2.17	0.13	91.05	190.74	307.90	2.14	548.24	37.50	0.26	66.76
IIHRMO 10-1	4.87	3.11	0.27	103.27	322.87	527.02	2.89	667.01	146.99	0.81	186.06
Apetaloid male sterile lines											
IIHRMO 7-2	5.43	1.56	0.05	178.69	212.29	328.12	2.06	534.68	31.77	0.20	51.79
IIHRMO 23-2	5.73	1.84	0.03	170.03	204.24	400.03	3.80	511.23	22.33	0.21	28.61
IIHRMO 23-5	5.87	1.98	0.02	105.44	179.17	835.73	2.21	1052.15	19.55	0.05	24.69
IIHRMO 23-7	5.83	2.86	0.06	70.83	188.12	900.85	3.00	1083.20	40.29	0.13	48.26
Testers											
IIHRMO 12-12	5.07	3.16	0.08	89.10	299.48	429.03	1.75	596.72	30.69	0.12	42.57
IIHRMO-3	5.13	2.40	0.06	112.73	282.67	399.73	4.68	550.26	25.48	0.30	35.03
Pusa Narangi	5.07	2.44	0.07	98.10	266.92	1115.82	7.18	1370.61	76.53	0.49	93.99
Hybrids resulting from petaloid male sterile lines											
IIHRMO 9-7 X IIHRMO 12-12	6.10	4.20	0.25	130.11	574.00	1034.63	2.48	1240.10	342.65	0.82	410.74
IIHRMO 9-7 X IIHRMO-3	5.71	2.67	0.07	90.10	238.62	1246.18	5.96	1464.53	45.82	0.13	57.44
IIHRMO 9-7 X Pusa Narangi	6.37	4.50	0.35	155.89	717.83	549.96	1.74	716.45	301.63	0.95	393.05
IIHRMO 9-8 X IIHRMO 12-12	5.80	3.84	0.34	165.11	691.64	1262.70	1.68	1571.18	713.90	0.95	888.20

IIHRMO 9-8 X IIHRMO-3	6.10	4.30	0.28	146.17	626.16	767.13	2.13	961.66	510.05	2.42	598.56
IIHRMO 9-8 X Pusa Narangi	6.57	4.83	0.35	193.83	723.00	330.18	0.83	498.40	345.32	0.86	521.16
IIHRMO 10-1 X IIHRMO 12-12	5.63	3.53	0.20	189.44	681.78	741.34	3.48	1028.91	279.22	1.31	387.52
IIHRMO 10-1 X IIHRMO-3	5.91	3.71	0.12	148.67	629.05	659.10	3.14	832.16	119.60	0.57	151.00
IIHRMO 10-1 X Pusa Narangi	6.53	3.85	0.54	163.67	623.78	401.85	1.42	519.27	299.63	1.06	387.24
Hybrids resulting from apetaloid male sterile lines											
IIHRMO 7-2 X IIHRMO 12-12	6.37	3.67	0.12	111.07	421.23	637.95	1.71	877.84	82.81	0.22	113.58
IIHRMO 7-2 X IIHRMO-3	5.77	3.19	0.06	108.57	359.48	413.29	5.47	512.92	28.07	0.37	34.87
IIHRMO 7-2 X Pusa Narangi	5.46	3.27	0.08	103.65	333.97	1227.17	2.30	1420.72	106.53	0.20	123.41
IIHRMO 23-2 X IIHRMO 12-12	5.50	2.63	0.08	106.97	257.15	642.32	3.29	780.48	56.68	0.29	68.89
IIHRMO 23-2 X IIHRMO-3	6.07	3.38	0.05	99.23	315.87	1042.34	4.11	1304.90	54.01	0.21	67.63
IIHRMO 23-2 X Pusa Narangi	5.80	3.55	0.11	96.82	366.78	756.27	2.20	961.51	83.58	0.24	106.30
IIHRMO 23-5 X IIHRMO 12-12	5.63	3.11	0.17	115.80	346.47	473.92	3.14	675.29	93.19	0.62	132.75
IIHRMO 23-5 X IIHRMO-3	5.40	3.74	0.08	90.60	319.80	1163.85	5.55	1349.09	85.17	0.40	98.70
IIHRMO 23-5 X Pusa Narangi	6.23	3.88	0.17	123.02	472.72	608.35	3.20	827.64	126.97	0.67	172.77
IIHRMO 23-7 X IIHRMO 12-12	6.45	3.24	0.09	84.07	280.15	985.40	4.77	1224.78	74.38	0.36	92.45
IIHRMO 23-7 X IIHRMO-3	5.67	3.18	0.13	98.74	313.07	456.50	3.48	580.39	58.32	0.44	74.15
IIHRMO 23-7 X Pusa Narangi	5.67	3.33	0.10	127.10	352.67	927.05	2.94	1147.08	112.83	0.36	139.83
S. Em±	0.07	0.13	0.01	7.22	22.24	11.81	0.13	8.36	20.20	0.06	24.94
CD (5%)	0.20	0.37	0.04	20.39	62.83	33.35	0.38	23.60	57.10	0.18	70.50
CV (%)	2.09	6.96	15.64	10.46	9.93	2.88	7.67	1.60	24.60	20.84	23.02

FD flower diameter (cm), FWF fresh weight per flower (gm), PMF petal meal per flower (gm), FNP flower number per plant, FWP flower weight per plant (gm), LC lutein (mg/100 g dry petal meal), ZC zeaxanthin (mg/100 g dry petal meal), TC total carotenoid (mg/100 g dry petal meal), LCP lutein (mg/plant), ZCP zeaxanthin (mg/plant) and TCP total carotenoid (mg/plant).

Genotypic correlations were found to be similar to phenotypic correlations in all the characters studied (Table 3). Association between biochemical components indicated significant and positive correlation among carotenoids, lutein and zea xanthin content. Carotenoids, lutein and zea xanthin content were found to be positive and significantly correlated with flower weight, petal meal per flower, number of flowers per plant as well as flower weight per plant. Total carotenoid was not significantly correlated with flower diameter. Similarly, correlation between fresh weight per flower and number of flowers per plant was not significant. All the characters studied were positively correlated with each other.

Table 3: Genotypic and phenotypic correlation for biochemical and its contributing characters

Characters[a]		**FD**	**FWF**	**PMF**	**FNP**	**FWP**	**LC**	**ZC**	**TC**
FD	VG	1.00	0.62**	0.39**	0.32**	0.56**	0.87**	0.33**	0.1
	VP		0.57**	0.37**	0.29**	0.53**	0.78**	0.31**	0.1
FWF	VG		1.00	0.66**	0.12	0.77**	0.66**	0.57**	0.32**
	VP			0.62**	0.13	0.73**	0.60**	0.55**	0.25**
PMF	VG			1.00	0.55**	0.79**	0.84**	0.36**	0.49**
	VP				0.50**	0.75**	0.78**	0.34**	0.48**
FNP	VG				1.00	0.67**	0.61**	0.21*	0.26**
	VP					0.63**	0.58**	0.20*	0.26**
FWP	VG					1.00	0.75**	0.20*	0.79**
	VP						0.72**	0.18*	0.59**
LC	VG						1.00	0.21*	0.24**
	VP							0.20*	0.23*
ZC	VG							1.00	0.40**
	VP								0.39**
TC	VG								1.00
	VP								

*, ** Denote significant differences at 5 and 1% probability levels, respectively

[a] See footnote in Table 2

Analysis of variance for combining ability (Table 4) shows that line versus tester mean squares were highly significant for total carotenoids, zeaxanthin and lutein indicating significant interaction effect between lines and testers. Line versus tester mean squares for combining ability were significant for all the characters studied. Variance due to line and due to testers were significant only in case of petal meal per flower and flower number per plant.

Table 4: Analysis of variance (ANOVA) of combining ability for biochemical and its contributing characters

Source	**df**	**Mean squares**							
Characters [a]		**FD**	**FWF**	**PMF**	**FNP**	**FWP**	**LC**	**ZC**	**TC**
Replication	2	0.05*	0.007	0.002	395.72	6339.13	88.51	0.01	107.95
Lines	6	0.19	1.41	0.11**	7848.75**	212926.23**	92271.62	5.74	97963.61
Testers	2	0.43	1.35	0.10*	3669.51*	67093.93	132438.7	25.19**	192889.21
Lines vs. testers	12	0.48**	0.64**	0.01**	843.80**	27294.07**	384655.84**	3.05**	483418.60**
Error	40	0.01	0.06	0.0009	146.2	1940.91	386.87	0.01	166.81

[a]See footnote in Table 2

Our study indicates SCA being predominant for total carotenoids, zeaxanthin and lutein content. General combining ability (GCA) to specific combining ability (SCA) ratio was less than unity in all the characters except for number of flowers per plant and petal meal per flower (Table 5).

Table 5: Variance due to GCA and SCA effects

Source	Random effect		
Characters	**Var. GCA**	**Var. SCA**	**Var. GCA: Var. SCA**
Flower diameter	-0.011	0.15	-0.07
Fresh weight per flower	0.04	0.19	0.21
Petal meal per flower	0.0062	0.0057	1.08
Flower number per plant	327.68	232.53	1.4
Flower weight per plant	7514.4	8451.05	0.88
Lutein (mg/100 g dry petal meal)	-18153.4	128089.7	-0.14
Zeaxanthin (mg/100 g dry petal meal)	0.8276	1.0137	0.81
Total carotenoids (mg/100 g dry petal meal)	-22532.8	161083.9	-0.13

For total carotenoids, among testers, IIHRMO 12-12 (80.97) and IIHRMO-3 (24.84) showed significant positive GCA effect where as it was negative in case of Pusa Narangi (-105.81). Among petaloid male sterile lines, IIHRMO 9-7 (164.39) and IIHRMO 9-8 (34.44) exhibited significant positive GCA effect (Table 6). Among apetaloid male sterile lines, IIHRMO 23-2 (39.66) exhibited significant positive GCA effect. Among hybrids resulting from petaloid male sterile lines, IIHRMO 9-7 X IIHRMO 12-12 (18.76), IIHRMO 9-7 X IIHRMO-3 (299.33), IIHRMO 9-8 X IIHRMO 12-12 (479.79) and IIHRMO 10-1 X IIHRMO 12-12 (154.49) exhibited significant positive SCA effect. Among hybrids resulting from apetaloid male sterile lines, IIHRMO 7-2 X Pusa Narangi (589.37), IIHRMO 23-2 X IIHRMO-3 (264.43), IIHRMO 23-2 X Pusa Narangi (51.69), IIHRMO 23-5 X IIHRMO-3 (373.57), IIHRMO 23-7 X IIHRMO 12-12 (159.72) and IIHRMO 23-7 X Pusa Narangi (268.81) exhibited significant positive SCA effect.

Table 6: GCA and SCA effects of biochemical parameters

Lines (females)	SCA effects of hybrids							
	IIHRMO 9-7	**IIHRMO 9-8**	**IIHRMO 10-1**	**IIHRMO 7-2**	**IIHRMO 23-2**	**IIHRMO 23-5**	**IIHRMO 23-7**	**GCA effect of testers**
	Petaloid male sterile lines			**Apetaloid male sterile lines**				
Testers (pollen parents)								
Lutein (mg/100 g dry petal meal)								
IIHRMO 12-12	43.07**	428.06**	92.60**	-169.48**	-219.28**	-322.75**	147.78**	47.96**
IIHRMO-3	258.88**	-63.24**	14.64	-389.88**	184.99**	371.44**	-376.84**	43.69**
Pusa Narangi	-301.96**	-364.82**	-107.24**	559.36**	34.29**	-48.68**	229.06**	-91.66**
GCA effect of lines	166.09**	9.17	-176.36**	-18.03**	36.14 **	-28.79**	12.15	
Zeaxanthin (mg/100 g dry petal meal)								
IIHRMO 12-12	-0.752**	0.297**	0.960**	-1.290**	0.247**	-0.662**	1.200**	-0.160**
IIHRMO-3	1.397**	-0.585**	-0.706**	1.144**	-0.255**	0.422**	-1.416**	1.166**
Pusa Narangi	-0.645**	0.288**	-0.254**	0.146*	0.008	0.240**	0.216**	-1.006**
GCA effect of lines	0.298**	-1.550**	-0.414**	0.061	0.105**	0.868**	0.631**	
Carotenoid (mg/100 g dry petal meal)								
IIHRMO 12-12	18.76*	479.79**	154.49**	-140.29**	-316.12**	-356.35**	159.72**	80.97**
IIHRMO-3	299.33**	-73.59**	13.87	-449.08**	264.43**	373.57**	-428.53**	24.84**
Pusa Narangi	-318.09**	-406.19**	-168.36**	589.37**	51.69**	-17.22**	268.81**	-105.81**
GCA effect of lines	164.39**	34.44**	-182.51**	-38.80**	39.66**	-25.29**	8.11	

*, **Denote significant differences at 5 and 1% probability levels, respectively

Among hybrids resulting from petaloid male sterile lines, hybrids IIHRMO 9-7 X IIHRMO 12-12 (64.26), IIHRMO 9-7 X IIHRMO-3 (93.98), IIHRMO 9-8 X IIHRMO 12-12 (163.60), IIHRMO 10-1 X IIHRMO 12-12 (54.26) and IIHRMO 10-1 X IIHRMO 12-12 (24.76) exhibited significant positive heterosis over the better parent for total carotenoid (Table 7). Among hybrids resulting from apetaloid sterile lines, hybrids IIHRMO 7-2 X IIHRMO 12-12 (47.11), IIHRMO 7-2 X Pusa Narangi (3.66), IIHRMO 23-2 X IIHRMO 12-12 (30.80), IIHRMO 23-2 X IIHRMO-3 (137.14), IIHRMO 23-5 X IIHRMO-3 (28.22) and IIHRMO 23-7 X IIHRMO 12-12 (13.07) exhibited significant positive heterosis over the better parent for total carotenoid.

Table 7: Heterosis for biochemical characters in marigold

Crosses	Lutien (mg/100 g dry petal meal)		Zeaxanthin (mg/100 g dry petal meal)		Total carotenoid (mg/100 g dry petal meal)	
	Heterosis% over		Heterosis% over		Heterosis% over	
	MP	**BP**	**MP**	**BP**	**MP**	**BP**
Hybrids resulting from petaloid sterile lines						
IIHRMO 9-7 X IIHRMO 12-12	113.66**	91.79**	42.32**	42.12**	83.49**	64.26**
IIHRMO 9-7 X IIHRMO-3	165.37**	131.00**	85.66**	27.38**	124.41**	93.98**
IIHRMO 9-7 X PUSA NARANGI	-33.55 **	-50.71 **	-60.96**	-75.75 **	-32.59**	-47.73 **
IIHRMO 9-8 X IIHRMO 12-12	242.69**	194.32**	-13.40**	-21.31 **	174.45**	163.30**
IIHRMO 9-8 X IIHRMO-3	116.82**	91.91**	-37.59**	-54.55 **	75.09**	74.77**
IIHRMO 9-8 X PUSA NARANGI	-53.62 **	-70.41 **	-82.28**	-88.50 **	-48.05**	-63.64 **

IIHRMO 10-1 X IIHRMO 12-12	55.08**	40.67**	50.32**	20.62**	62.84**	54.26**
IIHRMO 10-1 X IIHRMO-3	42.24**	25.06**	-16.93**	-32.83 **	36.73**	24.76**
IIHRMO 10-1 X PUSA NARANGI	-51.08 **	-63.99 **	-71.77**	-80.21 **	-49.03**	-62.11 **
Hybrids resulting from apetaloid sterile lines						
IIHRMO 7-2 X IIHRMO 12-12	68.52**	48.70**	-10.26*	-17.03 **	55.18**	47.11**
IIHRMO 7-2X IIHRMO-3	13.56**	3.39	62.41**	16.90**	-5.45**	-6.79 **
IIHRMO 7-2 X PUSA NARANGI	69.98**	9.98**	-50.27**	-68.01 **	49.13**	3.66**
IIHRMO 23-2 X IIHRMO 12-12	54.95**	49.72**	18.59**	-13.44 **	40.89**	30.80**
IIHRMO 23-2 X IIHRMO-3	160.66**	160.56**	-2.95	-12.09 **	145.86**	137.14**
IIHRMO 23-2 X PUSA NARANGI	-0.22	-32.22 **	-59.89**	-69.34 **	2.19*	-29.85 **
IIHRMO 23-5 X IIHRMO 12-12	-25.06 **	-43.29 **	58.99**	42.40**	-18.09**	-35.82 **
IIHRMO 23-5 X IIHRMO-3	88.41**	39.26**	61.34**	18.72**	68.38**	28.22**
IIHRMO 23-5 X PUSA NARANGI	-37.65 **	-45.48 **	-31.88**	-55.47 **	-31.68**	-39.62 **
IIHRMO 23-7 X IIHRMO 12-12	48.19**	9.39**	101.05**	59.10**	45.81**	13.07**
IIHRMO 23-7 X IIHRMO-3	-29.80 **	-49.33 **	-9.39**	-25.67 **	-28.94**	-46.42 **
IIHRMO 23-7 X PUSA NARANGI	-8.06 **	-16.92 **	-42.28**	-59.09 **	-6.51**	-16.31 **
SEm±	13.9	16.05	0.08	0.09	9.13	10.54
CD at 5%	28.1	32.45	0.16	0.18	18.45	21.31
CD at 1%	37.61	43.43	0.21	0.25	24.69	28.52

For lutein, among testers, IIHRMO 12-12 (47.96) and IIHRMO-3 (43.69) showed significant positive GCA effect whereas PUSA NARANGI exhibited negative (-91.66). Among petaloid male sterile lines, IIHRMO 9-7 (166.09) exhibited significant positive GCA effect (Table 6). Among apetaloid male sterile lines, IIHRMO 23-2 (36.14) exhibited significant positive GCA effect. Among hybrids resulting from petaloid male sterile lines, IIHRMO 9-7 X IIHRMO 12-12 (43.07), IIHRMO 9-7 X IIHRMO-3 (258.88), IIHRMO 9-8 X IIHRMO 12-12 (428.06) and IIHRMO 10-1 X IIHRMO 12-12 (92.60) exhibited significant positive SCA effect. Among hybrids resulting from apetaloid male sterile lines, IIHRMO 7-2 X Pusa Narangi (559.36), IIHRMO 23-2 X IIHRMO-3 (184.99), IIHRMO 23-2 X Pusa Narangi (34.29), IIHRMO 23-5 X IIHRMO-3 (371.44), IIHRMO 23-7 X IIHRMO 12-12 (147.78) and IIHRMO 23-7 X Pusa Narangi (229.06) exhibited significant positive SCA effect.

Among hybrids resulting from petaloid male sterile lines, hybrids IIHRMO 9-7 X IIHRMO 12-12 (91.79), IIHRMO 9-7 X IIHRMO-3 (131.00), IIHRMO 9-8 X IIHRMO 12-12 (194.32), IIHRMO 9-8 X IIHRMO-3 (91.91), IIHRMO 10-1 X IIHRMO 12-12 (40.67) and IIHRMO 10-1 X IIHRMO-3 (25.06) exhibited significant positive heterosis over the better parent for lutein content. Among hybrids resulting from apetaloid sterile lines, hybrids IIHRMO 7-2 X IIHRMO 12-12 (48.70), IIHRMO 7-2 X Pusa Narangi (9.98), IIHRMO 23-2 X IIHRMO 12-12 (48.70), IIHRMO 23-2 X IIHRMO-3 (160.56), IIHRMO 23-5 X IIHRMO-3 (39.26) and IIHRMO 23-7 X IIHRMO 12-12 (9.39) exhibited significant positive heterosis over the better parent for lutein content (Table 7).

For zeaxanthin among the testers only IIHRMO-3 exhibited positive GCA (1.16), and among petaloid sterile lines, only IIHRMO 9-7 had positive GCA (0.29). Contrary to this, all apetaloid sterile lines exhibited positive and significant GCA except in case of IIHRMO 7-2 where it was non-significant.

Maximum heterosis was realized with petaloid sterile line IIHRMO 9-8 in combination with IIHRMO 12-12 both for carotenoid as well lutein. For both carotenoid and lutein, IIHRMO 12-12 gave significantly higher heterosis with petaloid sterile line IIHRMO 9-8 while IIHRMO-3 gave significantly higher heterosis with apetaloid sterile line IIHRMO 23-2. On the contrary, Pusa Narangi resulted in negative heterosis in most of the cross combinations attempted.

Mean of parents were found to be having negative significant influence on heterosis for all the biochemical components. GCA was found to be positive and significantly assoiciated with heterosis realized for carotenoid, lutein as well as zea xanthin (Table 8).

Table 8: Mean and GCA correlation with mid parent heterosis for biochemical characters

Characters	Lutein (mg/100 g dry petal meal)	Zeaxanthin (mg/100 g dry petal meal)	Total carotenoid (mg/100 g dry petal meal)
Mean with heterosis	-0.4761**	-0.3756**	-0.4699**
GCA with heterosis	0.3984**	0.4909**	0.4018**

*, ** Denote significant differences at 5 and 1a probability levels, respectively

Discussion

Mean values of carotenoid, lutein and zeaxanthin indicated lutein being major component of caroenoid in marigold compared to zeaxanthin. Petals of marigold were reported to be rich in lutein and reported to constitute 90% of the pigments identified in this plant (Quackenbush, 1973). Wide variation available in percentage of biochemical components among the genotypes used as parents and the resulting hybrids indicated the potential for improving the character by breeding program. Biochemical content was maximum in hybrids derived from petaloid sterile line indicated the importance of these sterile lines in hybrid development.

Variation in total yield of carotenoid, lutein and zeaxanthin per plant compared to content of the respective components suggest the necessities of breeding program to focus on the necessity of increasing the yield in addition to percentage. Identification of contributing characters was taken up by correlation studies between the characters and with biochemical components.

Significant correlation of number of flowers per plant and flower weight per plant with biochemical content indicates the breeding program to focus on these characters for realizing higher yield of biochemical. Result of our study was in accordance with earlier studies that had reported correlation

between xanthophyll content and flower yield. Flower yield were reported to be related with flower diameter and number of flowers per plant.

Significant interaction between line x tester for combining ability suggested the necessity of identifying right combination of parents for development of hybrids. Predominance of SCA for biochemical components indicated non-additive gene action prevailing for the characters and the prospects of hybrid development. Similar results of no additive gene action prevailing for carotenoids, lutein was reported by Sreekala and Raghava (2003) [24]. The SCA variance was higher than the GCA variance for all the biochemical components. Higher SCA variance over GCA variance indicates the presence of non-additive or dominance variance. Hence heterosis can be exploited for such characters with non-additive gene action. Sreekala and Raghava (2003) [24] reported that exploitation of heterosis should be useful in breeding for increased carotenoid esters and lutein. Higher GCA values observed in case of flower number per plant and petal meal per plant indicated additive gene action prevailing for these characters and the improvement is possible by selection. As number of flowers per plant and petal meal per plant was found to be correlated with biochemical components, selection approach for these characters can be followed during development of pure lines a prerequisite for heterosis breeding.

Results indicated that, IIHRMO 9-7 and IIHRMO 9-8 are best general combiners among petaloid male sterile lines, IIHRMO 23-2 is the best among apetaloid sterile lines and IIHRMO 12-12 and IIHRMO-3 are best general combiners among testers for total carotenoids. For lutein and zeaxanthin among petaloid male sterile lines, IIHRMO 9-7 is the best general combiner. IIHRMO 23-2 among apetaloid sterile lines and IIHRMO 12-12 and IIHRMO-3 among testers are best general combiners for lutein. Best combiners varied with biochemical component suggesting selection of appropriate male sterile lines depending upon objective of breeding program.

Mean value of Pusa Narangi was maximum however GCA was negative for all the biochemical component estimated. It clearly indicates parental selection based on mean values will not result in good hybrids. Assoication of mean with heterosis, helps in selection of parents. However, predicting the performance of hybrid based on mean performance is expected to be severely hampered by masking non-additive effects (Ortis *et al*., 2005) [17]. Mean values of parents was not associated with mean values realized in their hybrids. This observation was in agreement with earlier workers on heterosis for biochemical components (Sreekala and Raghava, 2003) [24]. Heterosis was negatively associated with mean whereas GCA had positive and significant association. The results are in confirmation with basic theory of heterosis where in mean is not taken as indicator of combining ability of parents (Dabholkar, 1992) [4].

Conclusion

The production of F_1 hybrids for higher total carotenoids holds promise in the improvement of all the commercially important carotenoid fractions. Identification of multiple male sterile lines and two distinct male sterile systems are of significant importance considering their contribution for hybrid development. This study showed that IIHRMO 9-7 and IIHRMO 9-8 are best general combiners among petaloid male sterile lines, IIHRMO 23-2 is the best among apetaloid sterile lines and IIHRMO 12-12 and IIHRMO-3 are best general combiners among testers for development of F_1 hybrids with enhanced total carotenoids. For development of F1 hybrids with high lutein and zea xanthin, IIHRMO 9-7 is the best general combiner among petaloid male sterile lines, IIHRMO 23-2 among apetaloid sterile lines. IIHRMO 12-12 and IIHRMO-3 are best general combiners among testers for lutein.

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