

Harnessing Male Sterility Systems in Marigold (*Tagetes erecta* L.) for Hybrid Breeding Focused on Carotenoid Enrichment and Flower Yield

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Abstract

Marigold (*Tagetes erecta* L.) is a commercially important flower crop, valued both for its ornamental use and as a natural source of carotenoids. With the rising global demand for carotenoids in the food, feed, and nutraceutical industries, there is a growing need for marigold hybrids that combine high carotenoid content with superior yield. Male sterility systems are essential for hybrid seed production, but their specific contributions to carotenoid enrichment and yield related traits remain underexplored. This study evaluated the performance of different male sterile systems cytoplasmic male sterility (CMS) and genic male sterility (GMS; apetaloid and petaloid) along with their hybrids derived from a common pollen parent (*Pusa Narangi Gaiinda*). Seventeen agronomic and floral traits, including carotenoid content, were assessed in parental lines and hybrids. The CMS petaloid sterile hybrids showed superior performance for plant height, number of branches, flower yield per plant, flowering duration, and carotenoid content (1.52 g/100 g dry petal weight), whereas the CMS apetaloid hybrids exhibited earliness and shorter crop duration. CMS petaloid hybrids excelled in flower diameter, number of flowers per plant, shelf life, and number of harvests. Correlation and path coefficient analyses revealed that dry petal weight, flower diameter, and fresh flower weight had strong positive direct effects on carotenoid content, while plant height, shelf life, and dry flower weight had negative direct effects. Despite the higher heterosis for carotenoid content observed in CMS apetaloid hybrids, CMS petaloid hybrids recorded the highest mean carotenoid content and overall yield. These findings underscore the potential of CMS petaloid sterile lines in developing dual-purpose marigold hybrids for both floral and industrial carotenoid extraction markets with enhanced flower yield. The results provide actionable insights for breeding programs targeting industrial pigment production through optimised marigold hybridisation strategies

Introduction

Among ornamental crops, marigold (*Tagetes erecta* L.) has gained considerable importance due to its dual utility as a loose flower in the domestic market and as a commercial source of carotenoids for industrial applications. In India, marigold occupies an area of approximately 105.10 thousand hectares, with a production of 1120.40 thousand metric tonnes of loose flowers (DA & FW, 2024). Beyond its ornamental value, marigold serves as a major natural source of carotenoids, which are widely utilized as food colorants, feed additives, and nutraceuticals (Kusumiyati et al., 2025; Rodrigues et al., 2019). Carotenoids possess strong antioxidant properties and play a significant role in mitigating oxidative stress (Sandmann, 2014), while their dietary intake has been associated with a reduced risk of chronic diseases, including cancer (Rowles and Erdman, 2020).

The global carotenoid market, valued at USD 3.80 billion in 2026, is projected to grow at a compound annual growth rate (CAGR) of 6.7% (BCC Research, 2026). This expansion is largely driven by clean label regulatory shifts in Europe and North America, which favor natural pigments such as marigold derived lutein over synthetic azo dyes. Further momentum is provided by emerging clinical evidence, including findings from the L-ZIP trial (2024) (Addo et al., 2024), which highlight the importance of natural carotenoids in maternal health and functional food applications. Concurrently, the global health and wellness market is witnessing substantial growth, with the wellness supplements segment alone projected to reach approximately USD 313–315 billion by 2026. Reports from The Business Research Company (2026) and Grand View Research indicate that this sector is expanding at a CAGR of 7.2–8.8%, driven by a global shift toward preventive healthcare, wherein consumers increasingly rely on dietary supplements particularly vitamins, minerals, and botanicals for proactive health management.

India represents one of the fastest-growing markets in this sector, with an annual growth rate of 11.3%, underscoring the rising demand for high-quality natural raw materials such as marigold for lutein extraction. This increasing demand necessitates the development of high-yielding marigold hybrids with enhanced carotenoid content. In this context, hybrid breeding assumes critical importance, and the exploitation of male sterility offers an efficient and economical system for hybrid seed production by eliminating the need for manual emasculation.

In marigold, two major types of male sterility systems have been reported, namely apetaloid and petaloid. The apetaloid genic male sterility (GMS) system is governed by a single recessive gene and propagated through seed (Gupta et al., 1999; He et al., 2010; Tejaswini et al., 2016a). Petaloid male sterility exists in two forms: a genic system controlled by a single dominant gene and propagated through seed (Sumalatha et al., 2025), and a cytoplasmic male sterility (CMS) system inherited maternally and maintained through vegetative propagation (Kumar et al., 2017; Tejaswini et al., 2016b).

Previous studies have indicated that hybrids derived from petaloid male sterile lines exhibit superior combining ability, resulting in enhanced flower weight and carotenoid content compared to those derived from apetaloid lines (Santhosh et al., 2018). However, most of the existing research on heterosis in marigold has primarily focused on vegetative, ornamental, and yield related traits (Sukwiwat et al., 2023), with limited emphasis on carotenoid content and its genetic associations. Furthermore, studies comparing different male sterile systems and their relative contributions to combining ability, heterosis, and carotenoid accumulation are scarce.

A comprehensive understanding of the genetic basis of carotenoid content, its association with morphological and yield traits, and the role of different male sterile systems is essential for designing an effective heterosis breeding programme. Development of petaloid sterile hybrids with high carotenoid content would not only enhance market value due to superior flower quality but also provide added economic benefits through pigment extraction.

Therefore, the present study was undertaken to evaluate the performance of different male sterile systems and their hybrids, assess combining ability and heterosis, and identify key traits associated with carotenoid content, with the ultimate objective of developing high-yielding, carotenoid-rich marigold hybrids.

Materials and Methods

Plant Material and Experimental Site

The present study was conducted using marigold (*Tagetes erecta* L.) pre-breeding lines developed under the crop improvement programme at ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru, India (13.14° N latitude, 77.50° E longitude, 890 m above mean sea level). The experimental material comprised one cytoplasmic male sterile (CMS) petaloid line (IIHRMOS-1) and two genic male sterile (GMS) lines, namely apetaloid (IIHRMOAp 129) and petaloid (IIHRMOP 1111), which segregate in a 1:1 ratio (sterile: fertile). The commercial cultivar Pusa Narangi Gaiinda was used as the common pollen parent to generate F₁ hybrids (Fig. 1). These parental lines and their respective hybrids were evaluated to assess the contribution of different male sterility systems in heterosis breeding for carotenoid content. Since the apetaloid sterile line (IIHRMOAp 129) is devoid of petals, its corresponding fertile counterpart was used for carotenoid estimation.

Experimental Design and Trait Recording

The field experiment was laid out in a Randomized Block Design (RBD) with three replications. Each genotype was represented in each replication, and observations were recorded on randomly selected plants following standard procedures. Data were recorded on growth, flowering, yield, and post-harvest traits, including: Plant height (cm), Number of branches (primary and secondary), Days to bud initiation, Days to 50% flowering, Flower diameter (cm), Number of flowers per plant, Fresh flower weight (g), Flower yield per plant (kg), Flowering duration (days), Fresh petal weight (g), Fresh calyx weight (g), Dry flower weight (g), Dry petal weight (g), Dry calyx weight (g), Number of harvests and Shelf life (days).

Carotenoid Estimation

Fully matured flowers were harvested, and petals were separated and dried in a hot air oven maintained at 55°C until constant weight was achieved. The dried petals were ground into a fine powder using a ball mill (Mixer Mill MM 400, RETSCH GmbH, Germany) to obtain uniform particle size. Total carotenoid content was estimated following AOAC method 970.64 (AOAC, 2005) after saponification and expressed as g per 100 g of dry petal meal. Laboratory analysis was carried out using a Completely Randomized Design (CRD) with triplicate samples to ensure analytical precision.

Statistical Analysis

All recorded traits were subjected to analysis of variance (ANOVA). Morphological, flowering, and yield traits were analyzed using two-way ANOVA appropriate for a randomized block design (RBD), while carotenoid content was analyzed using one-way ANOVA under a completely randomized design (CRD). Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at the 5% level of significance using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Genetic variability parameters, including phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and environmental coefficient of variation (ECV), were estimated. Genotypic correlation coefficients and path coefficient analysis based on genotypic variance were computed using R (package: variability). Graphical visualizations were generated using KAU GRAPES (Gopinath et al., 2020). General combining ability (GCA) and heterosis of different hybrid combinations were estimated using Line × Tester (L×T) analysis in TNAU STAT (Manivannan, 2014).

Results and Discussion

Mean Performance of Parents and Hybrids for Yield-Associated Characters

The evaluation of parental lines and their hybrids revealed significant variation across all 17 characters studied (Table 1), indicating substantial genetic diversity and scope for selection in African marigold breeding. This variability provides insights into the genetic potential and combining ability of parental lines for hybrid development.

Table 1
Mean performance of the parents and hybrids resulting from different male sterile lines

S.No	Parents and Hybrids	Segregation ratio observed	PH	NU BR	DTBI	DT 50% FL	FDIA	NFPP	FFW	FDUR	FPW	FCW	DFW	
1	IIHRMOAp 129	1:1	41.73 ^e	10.67 ^c	27.33 ^c	36.80 ^c	2.20 ^f	110.13 ^c	34.47 ^g	84.00 ^e	4.67 ^f	27.00 ^b	6.23 ^f	
2	IIHRMOP 1111	1:1	48.13 ^d	10.13 ^d	30.40 ^a	40.27 ^a	6.73 ^b	114.80 ^b	157.47 ^b	101.00 ^c	119.83 ^b	31.33 ^a	17.59 ^c	
3	IIHRMOS-1	1:0	41.87 ^e	9.53 ^f	29.27 ^b	40.40 ^a	4.43 ^e	35.87 ^g	76.33 ^f	101.00 ^c	52.50 ^d	20.00 ^e	11.83 ^e	
4	PNG	0:1	52.60 ^{cd}	9.87 ^e	28.27 ^{bc}	37.67 ^{bc}	5.50 ^d	50.80 ^f	107.47 ^d	92.00 ^d	74.00 ^c	31.67 ^a	12.07 ^e	
5	IIHRMOAp 129 X PNG	0:1	56.27 ^c	9.53 ^f	28.33 ^{bc}	36.80 ^c	5.93 ^c	150.20 ^a	79.00 ^e	93.00 ^d	38.00 ^e	21.67 ^d	13.19 ^d	
6	IIHRMOP 1111 X PNG	1:1	65.4 ^b	11.40 ^b	28.60 ^b	38.87 ^b	7.63 ^a	103.60 ^d	155.00 ^c	108.67 ^b	120.17 ^b	21.67 ^d	18.82 ^b	
7	IIHRMOS-1 X PNG	1:0	82.00 ^a	12.73 ^a	30.60 ^a	41.00 ^a	5.90 ^c	94.90 ^e	186.00 ^a	112.00 ^a	134.67 ^a	25.67 ^c	20.02 ^a	
	General mean		55.43	10.55	28.99	38.83	5.48	94.33	113.82	98.81	77.69	25.57	12.87	
	SE(m)±		0.62	0.02	0.14	0.17	0.05	0.44	0.19	0.26	0.16	0.11	0.08	
	CD 5%		5.10	0.19	1.12	0.85	1.00	3.55	1.43	2.09	1.31	0.93	0.64	
	CV		5.18	1.04	2.16	1.24	10.33	2.12	0.71	1.19	0.95	2.06	2.76	
PH. Plant height, NUBR. Number of branches, DTBI. Days to bud initiation, DT50%FL. Days to 50% flowering, FDIA. Flower diameter, NFPP. Number of flowers per plant, FDUR. Flowering duration, FPW. Fresh petal weight, FCW. Fresh calyx weight, DFW. Dry flower weight, DPW. Dry petal weight, DCW. Dry calyx weight, H. Number of harvests, Carotenoid														

Plant height ranged from 41.73 to 82.00 cm, with the CMS hybrid IIHRMOS-1 × Pusa Narangi Gaiinda recording the maximum height. As a common pollen parent was used, the observed variation is largely attributable to the differential contribution of the female lines. The number of branches varied from 9.53 to 12.73, with the same CMS hybrid producing significantly more branches than both parents, indicating effective transmission of this trait.

Days to bud initiation ranged from 27.33 to 30.60 days. Apetaloid sterile lines and their hybrids exhibited earlier bud initiation, whereas the taller CMS hybrid showed delayed initiation, indicating an association between dwarfness and earliness. Days to 50% flowering ranged from 36.80 to 41.00 days, with the earliest flowering observed in IIHRMOAp 129 and its hybrid, confirming strong parental influence on flowering behaviour.

Flower diameter varied from 2.20 to 7.63 cm, with the hybrid IIHRMOP 1111 × Pusa Narangi Gaiinda producing the largest flowers. In contrast, the number of flowers per plant ranged from 35.87 to 150.20, with the apetaloid hybrid IIHRMOAp 129 × Pusa Narangi Gaiinda recording the maximum. An inverse relationship between flower size and flower number was evident, likely due to resource allocation constraints.

Fresh flower weight ranged from 34.47 to 186.00 g, with the CMS hybrid recording the highest value. This superiority is attributed to complete petaloid expression, resulting in increased petal biomass. Similar trends were observed for dry flower weight (6.23–20.02 g), fresh petal weight (0.40–15.40 g), and fresh petal weight of ten flowers (4.67–134.67 g), indicating the advantage of CMS hybrids in biomass accumulation.

Flowering duration ranged from 84 to 112 days. Apetaloid lines exhibited shorter duration due to early bud initiation, whereas petaloid lines showed extended flowering, making them suitable for prolonged harvesting. Fresh calyx weight ranged from 20.00 to 31.67 g, with CMS hybrids showing higher values, suggesting pollen parent influence. In contrast, dry calyx weight (2.93–6.47 g) was largely governed by the female parent, indicating scope for selection towards reduced calyx proportion.

The number of harvests ranged from 6 to 9, with the CMS hybrid recording the highest value, associated with its extended flowering duration. Shelf life varied from 3.00 to 5.70 days, with petaloid lines and their hybrids showing superior performance, indicating improved post-harvest longevity.

Flower yield per plant ranged from 179.33 to 722.00 g, with the CMS hybrid IIHRMOS-1 × Pusa Narangi Gaiinda recording the highest yield. Carotenoid content ranged from 0.44 to 1.78 g per 100 g of dry petal meal, with higher values observed in petaloid hybrids.

Overall, GMS petaloid hybrids excelled in flower quality traits such as flower diameter, dry petal weight, number of flowers per plant, number of harvests, and shelf life. In contrast, CMS hybrids were superior for growth and yield attributes, including plant height, number of branches, fresh and dry biomass, flowering duration, and flower yield per plant. Apetaloid hybrids were characterized by earliness in bud initiation and flowering.

Although the GMS petaloid sterile line exhibited relatively higher carotenoid content, its commercial utility is limited due to segregation leading to non-marketable flowers. Therefore, CMS-based hybrids are more suitable for achieving higher yield and carotenoid production, whereas GMS petaloid lines may be utilized in breeding programs targeting carotenoid enhancement in seed-propagated systems.

The overall trends observed for growth, flowering, yield, and carotenoid traits are consistent with earlier reports in African marigold (Santhosh et al., 2018; Gulia et al., 2017; Poornachandragowda et al., 2016; Thirumalmurugan et al., 2020; Steberl et al., 2020).

Correlation Coefficient Analysis

Correlation analysis revealed that carotenoid content was positively associated with fresh petal weight, dry petal weight, fresh flower weight, flower diameter, and shelf life, indicating that selection for these traits would enhance carotenoid yield. In contrast, plant height, number of branches, number of flowers per plant, and calyx-related traits exhibited negative associations, suggesting a trade-off between vegetative growth and pigment accumulation.(Table 2, Fig. 2). These results indicate that emphasis on positively associated traits can facilitate simultaneous improvement in carotenoid content, while negatively associated traits may require restricted selection.

Table 2
Phenotypic and Genotypic relation between carotenoid and contributing characters

S.No		Carotenoid	PH	DBI	NB	FPW	DPW	FCW	DCW	FFW	DFW	NFPP	FDIA	SI
1	Carotenoid	1	0.238	0.348*	-0.084	0.447**	0.595**	-0.027	-0.480**	0.450**	0.534**	-0.025	0.641**	0.
2	PH	0.251	1	0.572**	0.291	0.619**	0.546**	-0.016	0.172	0.622**	0.644**	-0.014	0.720**	0.
3	DBI	0.362	0.635 *	1	0.073	0.684**	0.549**	-0.005	-0.296	0.690**	0.526**	-0.303	0.648**	0.
4	NB	-0.087	0.308	0.071	1	-0.030	-0.028	0.116	0.024	-0.018	-0.018	0.108	0.079	0.
5	FPW	0.454*	0.644*	0.761**	-0.030	1	0.929**	-0.150	-0.511**	0.995**	0.891**	-0.080	0.871**	0.
6	DPW	0.607 *	0.586 *	0.625*	-0.037	0.938**	1	-0.177	-0.554**	0.925**	0.970**	0.007	0.869**	0.
7	FCW	-0.026	-0.013	-0.004	0.120	-0.152	-0.179	1	0.188	-0.148	-0.147	-0.133	-0.188	0.
8	DCW	-0.493*	0.180	-0.330	0.025	-0.518	-0.561*	0.190	1	-0.520**	-0.351*	0.303	-0.335*	-0
9	FFW	0.456*	0.649*	0.769**	-0.020	0.995**	0.931**	-0.149	-0.526	1	0.892**	-0.094	0.862**	0.
10	DFW	0.550*	0.709**	0.614 *	-0.034	0.917**	0.974**	-0.15	-0.362	0.912**	1	0.084	0.871**	0.
11	NFPP	-0.014	-0.018	-0.336	0.112	-0.080	0.012	-0.15	0.309	-0.093	0.097	1	0.020	-0
12	FD	0.651 *	0.749**	0.707**	0.079	0.872**	0.878**	-0.191	-0.340	0.863**	0.890**	0.022	1	0.
13	SL	0.626 *	0.433	0.531*	0.153	0.670**	0.778**	0.132	-0.519	0.689 **	0.736**	-0.092	0.774**	1
Carotenoid, PH- Plant Height,DBI- Days to Bud Initiation, NB- Number of Branches, FPW- Fresh Petal weight DPW- Dry Petal weight, FCW- Fresh calyx weight, Dry calyx weight, FFW- Fresh Flower Weight, DFW- Dry Flower Weight, NFPP- Number of Flowers per Plant, FDIA- Flower Diameter, SL- Shelf life.														
**, * Significant at 5% and 1% level, respectively; Note: Phenotypic above the diagonal and Genotypic below the diagonal														

Path Coefficient Analysis

Path coefficient analysis indicated that dry petal weight, fresh flower weight, flower diameter, and days to bud initiation exerted strong positive direct effects on carotenoid content, identifying them as key selection criteria. In contrast, plant height, fresh petal weight, dry flower weight, and shelf life exhibited negative direct effects. Although shelf life showed a positive correlation with carotenoid content, its negative direct effect suggests that the association is indirect via other traits. The residual effect (0.08) indicates that most of the variability in carotenoid content was explained by the traits included in the analysis(Table 3, Fig. 3). These findings highlight the importance of prioritizing traits with strong direct effects for improving carotenoid content, in agreement with earlier reports (Sahu et al., 2018).

Table 3
Direct (bold/diagonal) and indirect effects of correlated characters to carotenoid content as assessed by path coefficient analysis

S.No.		1	2	3	4	5	6	7	8	9	
	Effects	PH	DBI	FPW	DPW	DCW	FFW	DFW	FDIA	SL	Gen_corr with Carotenoid
1	PH	-0.186	0.178	-5.59	11.606	0.651	3.887	-11.372	1.535	-0.458	0.252
2	DBI	-0.118	0.28	-6.607	12.371	-1.193	4.6	-9.861	1.45	-0.563	0.362
3	FPW	-0.12	0.213	-8.672	18.574	-1.871	5.957	-14.709	1.788	-0.71	0.454
4	DPW	-0.109	0.175	-8.14	19.788	-2.027	5.572	-15.632	1.799	-0.824	0.607
5	DCW	-0.033	-0.092	4.496	-11.106	3.609	-3.027	5.812	-0.698	0.55	-0.493
6	FFW	-0.121	0.215	-8.636	18.431	-1.826	5.982	-14.633	1.769	-0.73	0.456
7	DFW	-0.132	0.172	-7.955	19.289	-1.308	5.458	-16.036	1.837	-0.78	0.551
8	FD	-0.139	0.198	-7.57	17.376	-1.229	5.168	-14.385	2.048	-0.82	0.651
9	SL	-0.08	0.149	-5.819	15.407	-1.876	4.126	-11.814	1.587	-1.058	0.627
PH-plant height, DBI-days to bud initiation, FPW-fresh petal weight, DPW-dry petal weight, DCW-dry calyx weight, FFW-fresh flower weight, DFW-dry flower weight, FSIA-flower diameter, SL-shelf life											

General Combining Ability (GCA)

The GMS petaloid sterile line exhibited significant positive general combining ability (GCA) effects for flower diameter, number of harvests, and shelf life, whereas the GMS apetaloid line showed positive GCA for dry calyx weight and number of flowers per plant. The CMS petaloid sterile line exhibited significant positive GCA effects for most growth, flowering, and yield traits, including plant height, number of branches, flowering traits, biomass accumulation, and flower yield per plant. Carotenoid content was comparable among petaloid lines irrespective of propagation method. These results indicate that CMS lines are more suitable for hybrid development when the objective is to improve yield and associated traits (Table 4). These findings are consistent with earlier reports in marigold and other ornamentals (Santhosh et al., 2018; Tejaswini et al., 2016a).

Table 4
Contribution of different male sterile system in terms of GCA for heterosis

S.No	Genotypes	PH	NO BR	DTBI	DT 50% FL	FDIA	NFPP	FFW	FDIA	FPW	FCW	DFW	DPW	DCW	H	SL	FYPP
1	IIHRMOAp 129	-11.62	-1.69	-0.84	-2.09	-0.56	33.97	-61.33	-11.56	-59.61	-1.33	-12	-5.49	1.33	-1	-0.9	-142
2	IIHRMOP 1111	-2.49	0.18	-0.58	-0.02	1.14	-12.63	15.47	4.11	22.56	-1.33	0.6	2.28	-0.79	1	0.1	12.6
3	IIHRMOS- 1	14.11	1.51	1.42	2.11	-0.59	-21.33	45.87	7.44	37.06	2.67	5.8	3.21	-0.54	0	0.8	129.1
	GCA of ms lines	3.75	0.09	0.48	0.33	0.5	1	0.15	0.65	0.53	0.33	3.2	0.17	0.06	0	0	2.05
	CD 5%	10.43	0.35	1.91	1.31	1.98	3.96	0.61	2.5	2.1	1.31	9.87	0.69	0.25	0	0	8.07
PH. Plant height, NUBR. Number of branches, DTBI. Days to bud initiation, DT50%FL. Days to 50% flowering, FDIA. Flower diameter, NFPP. Number of flowers per plant, FFW. Fresh flower weight, FDUR. Flowering duration, FPW. Fresh petal weight, FCW. Fresh calyx weight, DFW. Dry flower weight, DPW. Dry petal weight, DCW. Dry calyx weight, H. Number of harvests, SL. Shelflife, FYPP. Flower yield per plant, C. Carotenoid																	

Heterosis

The CMS petaloid hybrid exhibited positive heterosis for most growth, flowering, and yield traits, including plant height, branching, flowering time, biomass, and flower yield per plant. The GMS apetaloid hybrid showed positive heterosis for number of flowers per plant and dry calyx weight, and also exhibited heterosis over the better parent for carotenoid content. Despite higher heterosis in certain apetaloid hybrids, the mean carotenoid content and yield were higher in CMS petaloid hybrids, indicating their superiority for commercial exploitation. GMS petaloid hybrids also exhibited positive heterosis for flower quality traits such as flower diameter, number of harvests, and shelf life (Table 5). These results suggest that CMS-based hybrids are more suitable for heterosis breeding aimed at improving yield and carotenoid content, while GMS systems may be useful for improving specific quality traits. Similar trends have been reported in marigold and other crops (Choudhary et al., 2020; Gramaje et al., 2020; Zhang et al., 2023).

Table 5
Heterosis for different characters in response to different male sterile systems used as seed parent in yield associated characters

S.No	Parents and Hybrids	PH	NO BR	DTBI	DT 50% FL	FDIA	NFPP	FFW	FDIA	FPW	FCW	DFW	DPW	DCW	H	SL	FYPP
Heterosis over better parent																	
1	IIHRMOAp 129 X PNG	7	-10.6	-0.2	1.9	8.2	36.3	-26.5	1.1	-48.6	-29.8	132.35	-12.6	11.2	0	-14.3	120
2	IIHRMOP 1111 X PNG	24.3	12.5	-5.9	-2.6	13.8	-9.7	-1.1	7.6	0.3	-31.9	45.79	-1.1	-1.1	12.5	-11.8	180
3	IIHRMOS-1 X PNG	55.9	29.1	4.6	1.3	56.2	87.1	73.3	10.9	82	-17	73.83	76.3	4.8	0	21.4	170
Heterosis over mid parent																	
4	IIHRMOAp 129 X PNG	-43.7	-90.5	-71.7	-60.9	-94	50.2	-21	-7	-62	-78	-92.1	-93.3	-93.5	-93	-96	110
5	IIHRMOP 1111 X PNG	-34.6	-88.6	-71.4	-60.1	-92.3	3.7	55.8	8.7	20.2	-78.7	-84.4	-85.5	-95.6	-91	-95	160
6	IIHRMOS-1 X PNG	-18	-87.3	-69.4	-58.3	-92.3	-5	86.2	12	34.7	-74	-81.4	-84.6	-95.4	-92	-94.3	190
Heterosis over better parent																	
7	IIHRMOAp 129 X PNG	7	-3.4	-0.2	1.9	8.2	195.8	-26.5	1.1	-48.6	-29.8	-26.16	-12.6	47.3	0	-14.3	120
8	IIHRMOP 1111 X PNG	24.3	15.5	0.7	3.8	39.3	104.1	45	18.1	62.4	-31.9	45.79	88.7	-1.1	28.6	7.1	220
9	IIHRMOS-1 X PNG	-55.9	-29.1	-7.7	-8.7	-39.2	-87.1	-73.3	-21.7	-82	17	73.83	-100.9	-4.8	-14.3	-21.4	-260
PH. Plant height, NUBR. Number of branches, DTBI. Days to bud initiation, DT50%FL. Days to 50% flowering, FDIA. Flower diameter, NFPP. Number of flowers, FFW. Fresh flower weight, FDUR. Flowering duration, FPW. Fresh petal weight, FCW. Fresh calyx weight, DFW. Dry flower weight, DPW. Dry petal weight, DCW. weight, H. Number of harvests, SL. Shelflife, FYPP. Flower yield per plant, C. Carotenoid																	

Conclusion

The present study demonstrated substantial genetic variability among male sterile lines and their hybrids in African marigold, indicating significant scope for genetic improvement. Among the different systems evaluated, CMS-based petaloid hybrids consistently outperformed others for key growth and yield attributes, including plant height, number of branches, biomass accumulation, flowering duration, and flower yield per plant. In contrast, GMS petaloid hybrids were superior for flower quality traits such as flower diameter, dry petal weight, number of flowers per plant, number of harvests, and shelf life, while apetaloid lines contributed to earliness in flowering.

Carotenoid content was positively associated with traits such as dry petal weight, fresh flower weight, flower diameter, and days to bud initiation, and these traits also exhibited strong positive direct effects, suggesting their importance as reliable selection criteria for improving carotenoid yield. Although GMS petaloid lines exhibited relatively higher carotenoid content, their commercial utility is limited due to segregation leading to a proportion of non-marketable flowers.

Combining ability and heterosis analyses further confirmed the superiority of CMS petaloid lines, which exhibited favourable general combining ability and significant heterosis for most yield-related traits. Despite some hybrids showing higher heterosis for carotenoid content, CMS-based hybrids maintained higher mean performance and overall yield advantage.

Overall, the study indicates that CMS petaloid lines are the most suitable for hybrid development aimed at maximizing flower yield and carotenoid production, whereas GMS petaloid lines can be effectively utilized in breeding programmes targeting carotenoid enrichment under seed-propagated systems. The identified trait associations and selection criteria provide a strong basis for developing high-yielding, carotenoid-rich marigold hybrids with enhanced commercial and industrial value.

Declarations

Data Availability Statement

All data supporting the findings of this study, including field evaluation data, breeding line performance records, and associated statistical analyses, are provided within this article.

Material Availability Statement

All plant materials used in the present study were sourced from the ongoing breeding programme and are maintained at the germplasm repository of ICAR–IIHR. The materials are conserved as part of the institutional breeding resources and are available subject to the policies and guidelines of ICAR–IIHR.

Author Contributions

Akkalareddy Sumalatha executed the research work, conducted the experiments, and compiled the data. Tejaswini Prakash conceptualized and designed the research programme and provided the germplasm for the study. Arivalagan M co-designed the research programme and contributed to manuscript preparation. D.C. Lakshmana Reddy assisted in manuscript writing and revision. V. Vijaya Bhaskar critically reviewed and corrected the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Funding Declaration

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Declaration of Usage of Generative AI

During the preparation of this manuscript, the authors used generative AI tools (ChatGPT, OpenAI) to improve language clarity and readability. The AI tool was used solely for linguistic editing and did not contribute to the scientific content, data analysis, or interpretation. The authors have reviewed and edited the output generated by the AI tool and take full responsibility for the content of the manuscript.

Consent to Publish

Consent to publish: Not applicable.

Ethics and Consent to Participate

The plant material used in this study comprised established breeding lines of *Tagetes erecta* L. maintained at ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru, India, as part of an ongoing institutional breeding program. All plant materials were cultivated and handled in accordance with applicable institutional and national guidelines. No wild collections were undertaken for this study, and therefore no specific collection permits or ethical approvals were required.

Permissions for Plant Collection/Use

The experimental material was obtained from the maintained breeding stock of ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru. The study did not involve fresh collection of plant material from wild populations, government-owned land, or private farmland. Therefore, no specific permits or licences for plant collection were required. The use of plant material complied with institutional regulations governing research on genetic resources.

References

1. Addo EK, Gorka JE, Allman SJ, Harrison DY, Sharifzadeh M, Hoffman RO, Bernstein PS. Ocular Effects of Prenatal Carotenoid Supplementation in the Mother and Her Child: The Lutein and Zeaxanthin in Pregnancy (L-ZIP) Randomized Trial-Report Number 2. *Ophthalmol Sci.* 2024;4(5). 100537. <https://doi.org/10.1016/j.xops.2024.100537>.
2. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists International. 18th ed. Maryland, USA: AOAC International; 2005.
3. Research BCC. Global carotenoids market: Industry analysis and forecasts. Wellesley, MA, USA: BCC Research; 2026.
4. DA & FW (Department of Agriculture & Farmers Welfare). Horticultural Statistics at a Glance 2024. New Delhi: Ministry of Agriculture & Farmers Welfare, Government of India; 2024.
5. Choudhary RR, Avtar RSR, Kumar D. Heterosis studies based upon Mori CMS system in Brassica juncea L. *J Oilseed Brassica.* 2020;11(2):116–20. <http://srmr.org.in/ojs/index.php/job/article/view/410>.
6. Gopinath PP, Parsad R, Joseph B, Adarsh VS, General R. Shiny Based Analysis Platform Empowered by Statistics.
7. Grand View Research. Health and wellness market size, share and trends analysis report. San Francisco, USA: Grand View Research; 2026.
8. Gramaje LV, Caguiat JD, Enriquez JOS, Dela Cruz QD, Millas RA, Carampatana JE, Tabanao DAA. Heterosis and combining ability analysis in CMS hybrid rice. *Euphytica.* 2020;216:1–22. <https://doi.org/10.1007/s10681-019-2542>.

9. Gulia R, Beniwal BS, Sheoran S, Sandooja JK. Evaluation of marigold genotypes for growth, flowering, yield and essential oil content. *Res Crops*. 2017;18(2):299–304. <https://doi.org/10.5958/2348-7542.2017.00051.1>.
10. Gupta YC, Raghava SPS, Misra RL. Inheritance of male-sterile apetalous inflorescence in African marigold. *J Ornament Horticult*. 1999;2(2):65–6.
11. He YH, Ning GG, Sun YL, Hu Y, Zhao XY, Bao MZ. Cytological and mapping analysis of a novel male sterile type in marigold (*Tagetes erecta* L.). *Mol Breeding*. 2010;26(1):19–29. <https://doi.org/10.1007/s11032-009-9372-x>.
12. Kumar KR, Singh KP, Raju DVS, Panwar S, Bhatia R, Jain PK, Kumar V. Standardization of rapid multiplication protocol in petaloid male sterile lines of African marigold (*Tagetes erecta* L.). *Indian J Agric Sci*. 2017;87:31–8. <https://doi.org/10.56093/ijas.v87i10.74827>.
13. Kusumiyati K, Putri IE, Hadiwijaya Y, Kartika A, Maulana YE, Sutari W. 2025. Quality assurance of total carotenoids and quercetin in marigold flowers (*Tagetes erecta* L.) as edible flowers. *International Journal of Food Science* 2025, 3277288. <https://doi.org/10.1155/2025/3277288>
14. Manivannan N. TNAU STAT: Statistical package. Coimbatore, India: Tamil Nadu Agricultural University; 2014.
15. Patel MA, Chawla SL, Chauhan DA, Sudha P, Chhatrola HN. Character association and path analysis studies in marigold (*Tagetes* spp). *J Pharmacognosy Phytochemistry*. 2018;7(2):3576–80.
16. Poornachandragowda G, Jayanthi R, Mahantesh J. Evaluation of African marigold (*Tagetes erecta* L.) genotypes for growth, yield and xanthophyll content. *Environ Ecol*. 2016;34(2A):807–10.
17. Core Team R. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
18. Rodrigues DB, Mercadante AZ, Mariutti LRB. Marigold carotenoids: Much more than lutein esters. *Food Res Int*. 2019;119:653–64. <https://doi.org/10.1016/j.foodres.2018.10.043>.
19. Rowles JL, Erdman JW. Carotenoids and their role in cancer prevention. *Biochimica et Biophysica Acta (BBA)*. – *Mol Cell Biology Lipids*. 2020;1865(11):158613. <https://doi.org/10.1016/j.bbalip.2020.158613>.
20. Sahu J, Gaurav S, Nair SK, Neelima N. Character association and path coefficient analysis in African marigold (*Tagetes erecta* L.). *Int J Bio-resource Stress Manage*. 2018;9(3):402–7. <https://doi.org/10.5958/2322-0996.2018.00016.9>.
21. Sandmann G. Carotenoids of biotechnological importance. *Biotechnology of Isoprenoids*. Springer; 2014. pp. 449–67. https://doi.org/10.1007/10_2014_277.
22. Santhosh N, Tejaswini SK, Seetharamu GK, Gadre A. Genetic diversity for morphological characters and biochemical components in African marigold. *Int J Chem Stud*. 2018;6(6):624–7.
23. SAS Institute Inc. SAS/STAT® 9.3 User's Guide. Cary, NC, USA: SAS Institute Inc.; 2012.
24. Steberl K, Hartung J, Munz S, Graeff-Hönniger S. Effect of row spacing, sowing density and harvest time on floret yield of safflower. *Agronomy*. 2020;10(5):664. <https://doi.org/10.3390/agronomy10050664>.
25. Sumalatha A, Arivalagan M, Bhaskar VV, Prakash T. Genetic inheritance and identification of molecular markers linked to male sterility in African marigold (*Tagetes erecta* L.). *J Horticult Sci*. 2025;20(1). <https://doi.org/10.24154/jhs.v20i1.2290>.
26. Sukwivat K, Kumchai J, Bundithya W, Potapohn N. Apetaloid and petaloid female performance in F1 marigold hybrids. *SABRAO J Breed Genet*. 2023;55(5):1754–67. <http://doi.org/10.54910/sabao2023.55.5.27>.
27. Tejaswini S, Anuradha S, Archana G, Ghatke M. Characterisation and utilization of male sterile systems in marigold. *Indian J Agric Sci*. 2016a;86(10):1271–5. <https://doi.org/10.56093/ijas.v86i10.62101>.
28. Tejaswini S, Anuradha S, Archana G. IHRMGYP-1 marigold germplasm with petaloid sterility. *Indian J Plant Genetic Resour*. 2016b;29(2):221–2.
29. The Business Research Company. Global wellness supplements market report 2026. London, UK: The Business Research Company; 2026.
30. Thirumalmurugan V, Manivannan K, Nanthakumar S. Genotypic evaluation of African marigold (*Tagetes erecta* L.) for flower yield and xanthophyll content. *Plant Archives*. 2020;20(2):3902–4.
31. Zhang T, Xuan L, Mao Y, Hu Y. Cotton heterosis and hybrid cultivar development. *Theor Appl Genet*. 2023;136(4):89. <https://doi.org/10.1007/s00122-023-04189-2>.

Figures

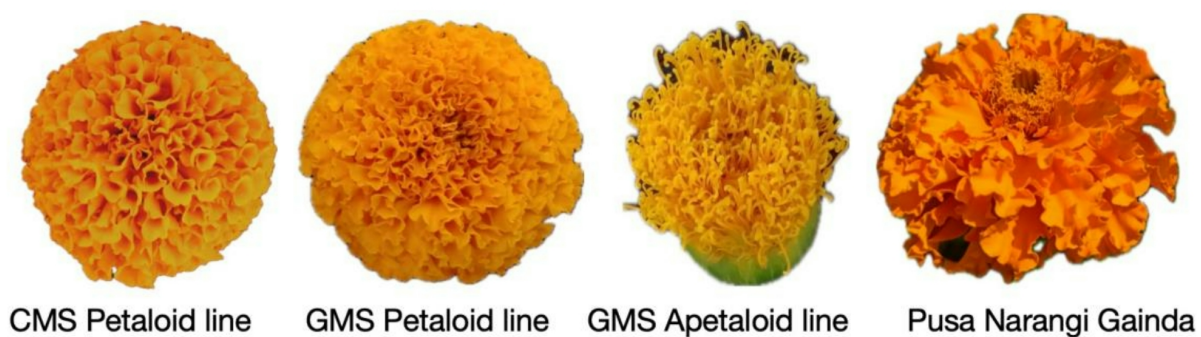


Figure 1

Floral phenotypes of (CMS) and (GMS) and Fertile lines used in the study

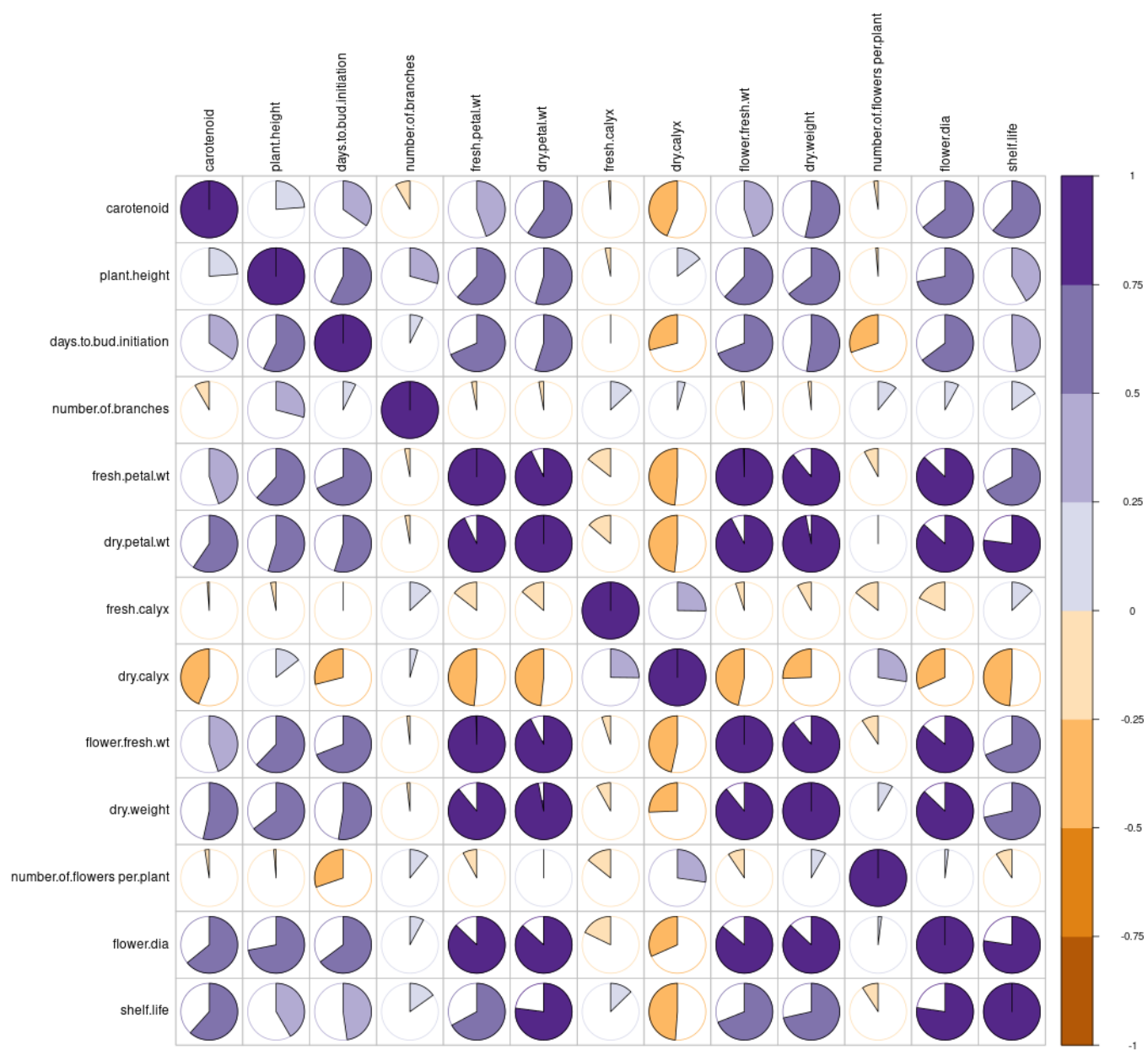


Figure 2

Correlogram of Phenotypic and Genotypic relation between carotenoid and other variables

* Phenotypic above the diagonal and Genotypic below the diagonal

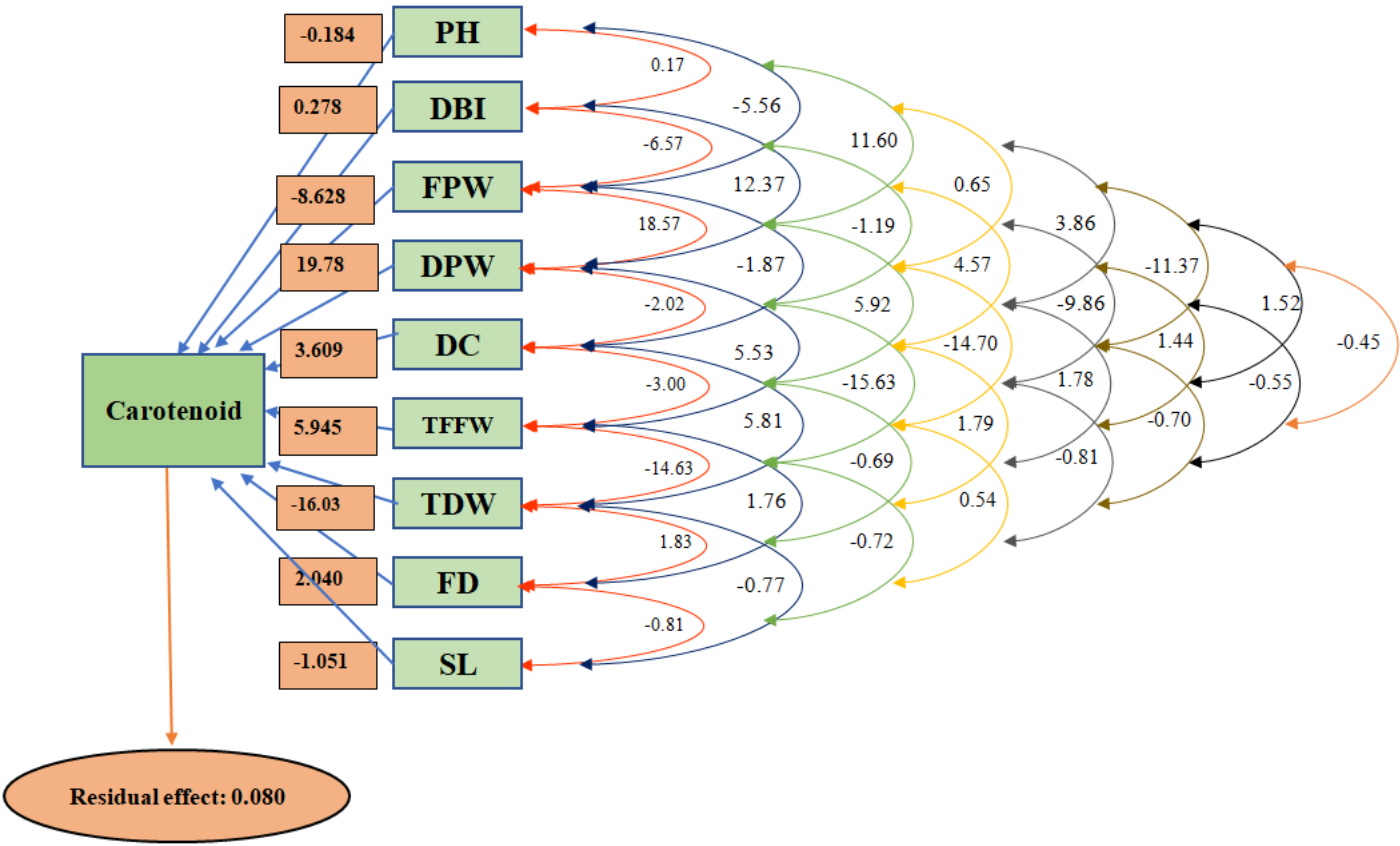


Figure 3

Path diagram of the direct effects and indirect effects of significantly correlated characters with carotenoid