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Combining ability and gene action analysis for various biochemical traits in ornamental kale (*Brassica oleracea* L. var. *acephala* DC.)

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ABSTRACT

The present study investigated of general combing ability of the parents and specific combining ability of hybrids and gene action for biochemical traits of Ornamental kale (Brassica oleracea var. acephala) using three cytoplasmic male sterile (CMS) lines and ten doubled haploid (DH) testers in a Line × Tester mating design to elucidate the genetic control of key biochemical traits related to pigment concentration and antioxidant capacity. Thirty F_1 hybrids, along with parents, were evaluated for chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, phenol, lycopene, ascorbic acid, and antioxidant activity (CUPRAC, FRAP) and ascorbic acid. Analysis of variance revealed highly significant differences among genotypes, indicating substantial genetic variability and strong potential for improvement. Significant line × tester interactions for most traits indicated the predominance of non-additive gene action. Mean performance identified CMS-3 × KTDH-55, CMS-1 × KTDH-56, and CMS-2 × KTDH-13 as superior hybrids for multiple biochemical attributes. GCA effects indicated CMS-1, KTDH-56, and KTDH-13 as promising general combiners for pigment and antioxidant traits, demonstrating the involvement of additive gene action. In contrast, several hybrids such as CMS-1 × KTDH-56, CMS-3 × KTDH-55, CMS-2 × KTDH-13, and CMS-1 × KTDH-29 exhibited high and positive SCA effects, confirming the influence of non-additive gene effects. Overall, the study highlights the effectiveness of CMS- and DH-based heterosis breeding for enhancing pigment concentration, biochemical quality, and antioxidant potential in ornamental kale. These findings provide valuable parental combinations and genetic insights for developing high-quality ornamental kale hybrids with improved aesthetic and nutritional properties.

Keywords: Biochemical parameters, Combining Ability, Cytoplasmic male sterile, Doubled haploid, Gene action, Heterosis, Ornamental kale.

Introduction

Ornamental kale (*Brassica oleracea* L. var. *acephala*), belonging to brassicaceae family. In recent years, this ornamental plant has gained wide popularity as an attractive decorative plant and is used in potted or cut form due to its colourful leaves of various shapes and strong cold resistance [1]. The rosette period is usually the best viewing period because ornamental kale leaves vigorously grow during this period. Ornamental kale is also edible and contains many bioactive compounds, such as glucosinolates, phenolic compounds, and carotenoids. These compounds have long been considered to have strong antioxidant capacities and are beneficial to human health [2, 3, 4 and 5]. One of the most important agronomic characteristics of ornamental kale is the color of its leaves, which can be green, white, pink, purple, or other colors, sometimes arranged in complex patterns [1, 6 and 7]. The colourful leaves not only have ornamental value, but are also beneficial to human health due to

high carotenoid and anthocyanin contents [8].

Various studies in vegetable crops have indicated that selection based on additive and dominance genetic variance for growth and flowering traits at early stages can be effective [9]. Despite extensive application of Line × Tester mating design in vegetable and field crop genetics, few such studies have been conducted on ornamental crops. Besides elucidating the nature and magnitude of gene action involved in the inheritance of these characters, combining ability analysis also provides useful information for selecting high order parents for effective breeding strategy. Line × Tester analysis is one of the approaches to know the nature and magnitude of gene action and also to assess the nicking ability of the parents. Therefore, keeping in view the importance of combining ability of the parents for various plant characters in Ornamental kale, a Line × Tester set of 13 parents involving three genetically *Ogura* cytoplasmic male sterile lines and 10 doubled haploid lines of Ornamental kale was studied for gene action and combining ability estimates.

Materials and Methods

The materials used in this study consisted of three *Ogura* CMS lines, namely CMS-1, CMS-2, and CMS-3, and 10 completely homozygous doubled haploid lines of ornamental kale, namely

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KtDH-10, KtDH-55, KtDH-56, KtDH-13, KtDH-19-1, KtDH-19-2, KtDH-52-1, KtDH-26, KtDH-57, and KtDH-29, which were used as testers (pollen parents) for crossing with CMS lines. This genetic material utilized as parents in the line × tester cross was selected from the germplasm purified and maintained at the Sarsai Research farm, ICAR-Indian Agricultural Research Institute, Regional Station, Katrain-Kullu, Himachal Pradesh. Table 1 presents the parental materials. Field trials were conducted during 2022-23 and 2023-24 for the production of F_1 hybrid seeds and their evaluation, respectively, at a research station situated at an altitude of 1688 m above mean sea level at the latitude of 32°10'49" North and 77°11'42" East. Seeds of all parental materials (CMS lines and pollinators) were sown in the nursery beds in August 2023 to raise seedlings. Transplanting of these seedlings was done after one month of seed sowing in the nursery. The crop was maintained with following standard cultural practices. CMS sterile lines were crossed with all 10 testers in 30 cross combinations when their flowers were fully opened to produce F1 hybrid seeds. Hybrid seeds obtained along with parental lines were grown in nursery beds in August 2024. One month after sowing, the seedlings were transplanted in main field in randomized block design with three replications to evaluate F_1 hybrids and their parental lines. Five plants were selected for observation after discarding the border plants at both the ends. Data were recorded on various biochemical traits, viz., chlorophyll estimation (chlorophyll A, chlorophyll B, total chlorophyll), carotenoid content, total phenol content, lycopene, anthocyanin, CUPRAC, FRAP concentration and Ascorbic acid. The line × tester given by [10] was adopted for gene action and combining ability studies. The variances among the parents and hybrids were partitioned further into source attributable to general and specific combining ability components (GCA and SCA).

Table 1. Presents the parental materials

Parent	
Female	
1.	**CMS-1**
2.	**CMS-2**
3.	**CMS-3**
Males	
1.	**KtDH-10**
2.	**KtDH-55**
3.	**KtDH-56**
4.	**KtDH-13,**
5.	**KtDH-19-1**
6.	**KtDH-19-2**
7.	**KtDH-52-1**
8.	**KtDH-26**
9.	**KtDH-57**
10.	**KtDH-29**

Results and Discussion

The analysis of variance (ANOVA) revealed highly significant differences among the genotypes for most of the biochemical traits studied, indicating ample genetic variability among the parents and hybrids. Such variability is essential for the improvement of complex physiological and nutritional traits and aligns with earlier findings in *Brassica oleracea* reporting wide genetic divergence for pigment and antioxidant characteristics [11] and [12]. The Lines × Testers interaction was highly significant (P ≤ 0.001) for most traits such as Chlorophyll A, total chlorophyll, carotenoid content, phenolic content, lycopene content, ascorbic acid, and antioxidant traits (CUPRAC, FRAP, and AA). This indicates the predominant role of non-additive gene action in controlling these traits and suggests substantial scope for heterosis breeding. This suggests that hybrid breeding strategies can effectively exploit heterosis for enhancing pigment biosynthesis and antioxidant potential. Similar dominance-driven inheritance patterns for carotenoids and phenolic content have been observed in cole crops and leafy Brassicas, supporting the suitability of the line × tester design for identifying superior heterotic combinations [13] and [14]. Significant tester effects for carotenoid and phenolic contents further highlight the importance of nuclear genotypes in influencing biochemical accumulation, corroborating previous findings that tester choice strongly affects secondary metabolite expression in Brassica hybrids. The significant line effects for CUPRAC also imply detectable variability among CMS lines, suggesting meaningful cytoplasmic contributions to antioxidant pathways, as reported in CMS-based studies of cole crops [15]. The absence of significant replication effects confirms uniform experimental conditions and reliable phenotypic assessment. Overall, the predominance of non-additive genetic components, together with the observed significant variability among parents and hybrids, indicates that hybrid breeding is a promising approach for the enhancement of biochemical traits in ornamental kale. These results provide a robust foundation for identifying elite cross combinations and developing nutrient-rich ornamental kale hybrids with improved pigment and antioxidant profiles.

Table 2. Analysis of variance (ANNOVA) of combining ability for biochemical traits in Ornamental traits

Source of variance	df	Ch a	Ch b	Total Ch	CC	PC	LC	AC	CUPRAC	FRAP	AA
Replications	**2**	8.69*	4.02	3.44	0.01	879.66	0.00	0.00	0.16	0.08	0.04
Lines	**2**	314.41	206.60	175.35	0.05	4312256.73	11.24	15.83	43.90**	3.51	2.01
Testers	**9**	202.59	98.23	83.83	23.34	2484802.38	1.37	4.19	7.08	1.60	13.42
Lines×Testers	**18**	179.89***	97.98	83.38***	12.84***	1785338.58***	1.66***	6.83***	6.40***	0.99***	15.85
Error	**84**	2.60	4.00	3.28	0.01	6389.96	0.00	0.00	0.40	0.06	0.04

***, **, * Significant at 0.1, 1 and 5 % probability respectively

Mean performance

The mean performance of the parents and hybrids for biochemical traits (Table 2) revealed substantial variability across all biochemical traits, reinforcing the presence of considerable genetic diversity within the breeding material. Such variability is essential for meaningful selection and hybrid development in *Brassica oleracea*, and similar trends have been reported in leafy Brassicas for pigment and antioxidant traits [11] and [13]. CMS-1 and KTDH-10 exhibited the highest chlorophyll a, chlorophyll b, and total chlorophyll contents, indicating their potential as strong general combiners for enhancing photosynthetic pigments. High chlorophyll-yielding parents have previously been associated with improved hybrid biomass and ornamental foliage quality. Among hybrids, CMS-3 × KTDH-55 and CMS-1 × KTDH-56 showed superior chlorophyll accumulation, suggesting heterotic enhancement of chlorophyll biosynthesis, consistent with earlier observations in cabbage and kale heterosis studies [16]. Carotenoid content showed wide variation, with CMS-3 performing best among parents, while CMS-3 × KTDH-56 and CMS-2 × KTDH-56 excelled among hybrids. Elevated carotenoid accumulation in hybrids reflects enhanced antioxidant potential, aligning with previous findings that non-additive gene action strongly influences carotenoid pathways in Brassica hybrids [14]. Phenolic content was highest in KTDH-29 and KTDH-26, while hybrids CMS-1 × KTDH-10 and

CMS-2 × KTDH-26 outperformed others, indicating the importance of dominance and epistatic interactions in phenolic synthesis. This is supported by earlier studies reporting strong heterosis and hybrid vigour for phenolic compounds in cole crops [17]. Lycopene content was highest in CMS-1 and KTDH-10 among parents, while hybrids CMS-1 × KTDH-26 and CMS-3 × KTDH-19-1 achieved maximum levels, showcasing effective nuclear–cytoplasmic and nuclear–nuclear interactions for carotenoid accumulation [18]. Ascorbic acid levels were highest in CMS-1 among parents, with hybrids CMS-2 × KTDH-13 and CMS-1 × KTDH-13 recording superior performance. High ascorbic acid in Brassica hybrids is well documented and is often attributed to non-additive gene action and enhanced stress-protective metabolism [19]. The same hybrids CMS-2 × KTDH-13 and CMS-2 × KTDH-19-1—also recorded maximum CUPRAC and FRAP activities, reflecting strong associations between antioxidant potential and phenolic/pigment content. Overall, hybrids such as CMS-3 × KTDH-55, CMS-1 × KTDH-56, and CMS-2 × KTDH-13 consistently excelled across multiple biochemical traits, demonstrating substantial heterosis and the predominance of non-additive gene action in determining pigment and antioxidant attributes. These results support the effectiveness of hybrid breeding for improving the biochemical quality of ornamental kale.

Table 3. Mean performance biochemical parameters

Parents	Chl a	Chl b	(T) Chl	CC	PC	LC	AC	CUPRAC	FRAP	AA
Lines										
CMS-1	28.43	21.69	20.02	6.80	3225.00	1.64	3.36	25.33	9.93	6.79
CMS-2	4.93	5.19	4.76	2.88	2840.63	1.31	0.14	25.79	9.29	**5.47**
CMS-3	**2.57**	**1.98**	**1.83**	8.78	**2555.63**	1.56	0.86	24.94	9.15	6.16
SE(lines)	0.7	0.25	0.15	0.24	123.41	0.09	0.03	1.88	0.5	0.19
Testers										
KTDH-10	28.43	21.69	20.02	6.80	3225.00	1.64	3.36	25.33	9.93	6.79
KTDH-55	4.93	5.19	4.76	2.88	2840.63	1.31	0.14	25.79	9.29	**5.47**
KTDH-56	2.57	1.98	1.83	8.78	**2555.63**	1.56	0.86	24.94	9.15	6.16
KTDH-13	0.7	0.25	0.15	0.24	123.41	0.09	0.03	1.88	0.5	0.19
KTDH-19-1	8.63	6.93	6.39	1.99	3806.25	0.73	3.73	25.11	9.02	10.38
KTDH-19-2	9.24	8.58	7.89	4.58	3187.50	1.06	**0.07**	25.53	**7.16**	**12.16**
KTDH-52-1	14.80	11.85	10.93	2.33	3766.88	1.21	3.56	**23.95**	8.46	7.72
KTDH-26	17.76	15.18	13.98	1.80	4468.13	1.29	3.80	25.52	**10.95**	5.97
KTDH-57	25.53	20.01	18.46	3.73	4243.13	1.34	3.56	26.21	9.82	7.79
KTDH-29	9.60	9.28	8.53	**1.03**	**5092.50**	**0.24**	**4.07**	24.35	8.38	7.08
SE(testers)	0.37	0.36	1.081	0.8	35.409	0.018	0.037	0.325	0.176	0.116
Hybrids										
CMS-1*KTDH-10	10.60	4.69	4.39	5.13	4443.75	1.38	3.74	22.99	9.67	10.26
CMS-1*KTDH-55	13.78	9.78	9.04	1.08	3948.75	0.83	4.02	24.11	9.06	8.10
CMS-1*KTDH-56	27.44	15.56	14.47	2.33	3706.88	1.02	3.60	24.19	9.13	11.88
CMS-1*KTDH-13	7.38	6.68	6.14	1.86	2634.38	0.92	4.07	26.13	9.72	5.94
CMS-1*KTDH-19-1	5.27	4.28	3.95	2.87	3013.13	2.03	3.75	22.33	9.40	9.18
CMS-1*KTDH-19-2	15.13	10.87	10.05	1.22	4216.88	0.73	3.20	24.28	9.76	8.64
CMS-1*KTDH-52-1	6.58	4.14	3.84	1.52	2726.25	0.95	2.60	23.16	9.46	5.94
CMS-1*KTDH-26	5.32	2.60	2.43	4.19	3907.50	2.79	3.70	24.18	9.32	5.94
CMS-1*KTDH-57	8.46	6.29	5.81	2.21	2782.50	1.43	2.56	25.46	8.87	7.56
CMS-1*KTDH-29	20.53	14.07	13.01	3.31	3890.63	2.18	3.89	25.45	8.49	7.02
CMS-2*KTDH-10	7.68	4.17	3.88	1.05	3453.75	0.15	0.69	24.05	9.34	5.40
CMS-2*KTDH-55	8.31	2.85	2.70	0.33	2131.88	0.08	1.03	26.46	7.97	5.94
CMS-2*KTDH-56	**1.27**	2.57	2.34	8.14	2296.88	0.56	0.63	26.63	10.34	10.80
CMS-2*KTDH-13	13.89	10.13	9.36	3.52	3847.50	3.48	4.23	28.66	10.72	12.42
CMS-2*KTDH-19-1	6.25	5.85	5.38	4.53	3888.75	0.40	3.72	28.78	8.14	7.56
CMS-2*KTDH-19-2	6.91	3.88	3.61	1.74	3073.13	0.12	2.32	28.06	10.26	5.40
CMS-3*KTDH-52-1	3.54	4.31	3.94	1.01	2146.88	0.18	3.57	24.55	9.89	5.94
CMS-2*KTDH-26	7.01	**1.09**	**1.08**	2.22	4443.75	1.38	3.74	22.99	9.67	10.26
CMS-2*KTDH-57	3.09	3.25	2.98	1.15	3948.75	0.83	4.02	24.11	9.06	8.10
CMS-2*KTDH-29	6.39	2.49	2.34	2.23	3706.88	1.02	3.60	24.19	9.13	11.88
CMS-3*KTDH-10	6.37	4.06	3.76	1.62	2634.38	0.92	4.07	26.13	9.72	5.94
CMS-3*KTDH-55	**31.30**	**26.99**	**24.85**	4.76	3013.13	2.03	3.75	22.33	9.40	9.18
CMS-3*KTDH-56	27.42	19.30	17.84	**10.05**	4216.88	0.73	3.20	24.28	9.76	8.64
CMS-3*KTDH-13	5.86	4.29	3.97	3.59	2726.25	0.95	2.60	23.16	9.46	5.94

CMS-3*KTDH-19-1	2.24	3.39	3.10	0.36	3907.50	2.79	3.70	24.18	9.32	5.94
CMS-3*KTDH-19-2	2.43	2.01	1.85	1.65	2782.50	1.43	2.56	25.46	8.87	7.56
CMS-3*KTDH-52-1	3.64	2.62	2.42	1.82	3890.63	2.18	3.89	25.45	8.49	7.02
CMS-3*KTDH-26	18.61	12.05	11.16	0.36	3453.75	0.15	0.69	24.05	9.34	5.40
CMS-3*KTDH-57	18.92	12.46	11.54	**0.08**	2131.88	0.08	1.03	26.46	7.97	5.94
CMS-3*KTDH-29	3.51	3.60	3.31	0.85	2296.88	0.56	0.63	26.63	10.34	10.80
SE(m)	0.64	0.31	0.23	0.08	110.06	0.06	0.14	0.96	0.32	0.29

***, **, * Significant at 0.1, 1 and 5 % probability respectively

General combining ability of the parental lines and testers

The general combining ability analysis for biochemical traits revealed substantial variation among the parents, confirming the involvement of both additive and non-additive gene actions, consistent with earlier reports in *Brassica oleracea* and other cole crops [13] and [16]. Among the CMS lines, CMS-1 emerged as the best general combiner, showing significant positive GCA effects for chlorophyll pigments, phenolics, lycopene, and ascorbic acid. Such multi-trait superiority aligns with earlier findings where CMS lines contributed strongly to pigment and antioxidant enhancement in Brassica hybrids [20]. CMS-3 also exhibited positive GCA effects for chlorophyll components but negative effects for carotenoid and phenolic traits, indicating trait-specific combining ability, a common trend in leafy Brassicas where parents rarely excel uniformly across biochemical traits CMS-2 performed poorly for most pigment traits, suggesting limited additive gene contribution. Among testers, KTDH-56 showed the highest positive GCA effects for chlorophylls, carotenoids, and antioxidant activity (CUPRAC, FRAP, AA), identifying it as a superior general combiner for improving biochemical quality. Similarly, KTDH-13 showed strong positive GCA effects for ascorbic acid and antioxidant traits, supporting its use in enhancing health-promoting compounds through additive gene action—consistent with earlier observations in cabbage and kale breeding [21]. KTDH-55 and KTDH-26 were also favourable for ascorbic acid and phenolics, respectively. Parents such as KTDH-19-1, KTDH-52-1, KTDH-57, and KTDH-29 recorded predominantly negative GCA effects, indicating poor combining ability for most biochemical traits. Overall, CMS-1, KTDH-56, and KTDH-13 are identified as the most promising general combiners, and their use in hybridization programmes could effectively enhance pigment and antioxidant attributes in ornamental kale. These results corroborate the importance of selecting trait-specific combiners to achieve targeted nutritional improvement in Brassica hybrids (Kumar et al., 2021).[22].

Table 4. General combing ability effects of parents for different characters in Ornamental kale

Parents	Chl a	Chl b	(T) Chl	CC	PC	LC	AC	CUPRAC	FRAP	AA
					Lines					
CMS-1	1.88***	0.89*	0.83*	0.01	416.31***	0.68***	0.78***	-0.53***	0.18***	0.01
CMS-2	-3.74***	-2.95***	-2.72***	0.03	-90.88***	-0.18***	-0.13	1.38***	0.21***	-0.26**
CMS-3	1.86***	2.07***	1.90***	-0.04	-325.44***	-0.50***	-0.65	-0.85***	-0.40***	0.26*
SE(lines)	0.29	0.37	0.33	0.02	14.60	0.01	0.01	0.12	0.05	0.04
					Testers					
KTDH-10	-1.95***	-2.71***	-2.48***	0.04	1059.25***	-0.08***	0.31***	-1.51***	0.04	0.24***
KTDH-55	7.63***	6.20***	5.71***	-0.51***	-286.38***	-0.42***	0.31***	0.21	-0.58***	0.17*
KTDH-56	8.54***	5.47***	5.07***	4.28***	-168.25***	-0.19***	-0.87***	0.63**	0.41***	2.10***
KTDH-13	-1.13*	0.02	0.01	0.43***	-197.00***	0.86***	0.96***	1.60***	0.51***	2.13***
KTDH-19-1	-5.58***	-2.50***	-2.34***	0.03	316.75***	0.13***	0.82***	-0.31	-0.45***	-0.49***
KTDH-19-2	-2.02***	-1.42*	-1.32*	-1.02***	-32.00	-0.38***	-0.18***	0.61**	0.56***	-0.28***
KTDH-52-1	-5.59***	-3.32***	-3.08***	-1.11***	-828.25***	-0.29***	-0.14***	-0.65**	0.17	-1.18***
KTDH-26	0.15	-1.77*	-1.60*	-0.30***	276.75***	0.35***	0.25***	0.45*	0.10	-0.48***
KTDH-57	-0.02**	0.32	0.29	-1.42***	-482.63***	-0.12***	-0.25***	-0.20	-0.42***	-1.17***
KTDH-29	-0.03**	-0.29	-0.26	-0.43***	341.75***	0.16***	-1.21***	-0.82***	-0.34***	-1.04***
SE (tester)	0.54	0.67	0.60	0.03	26.65	0.01	0.02	0.21	0.08	0.07

***, **, * Significant at 0.1, 1 and 5 % probability respectively

Specific combining ability Effects

The specific combining ability (SCA) analysis for biochemical traits showed significant variation across hybrids, highlighting the predominance of non-additive gene action in the inheritance of pigments and antioxidant attributes. Such strong SCA effects indicate the importance of specific parental interactions, consistent with earlier findings in cole crops and legumes where dominance and epistasis predominantly govern biochemical traits [20]. Hybrids CMS-1 × KTDH-56 and CMS-3 × KTDH-55 recorded the highest positive SCA effects for chlorophyll a, total chlorophyll, and carotenoid content, confirming their potential for enhanced pigment biosynthesis. Crosses such as CMS-1 × KTDH-29 and CMS-2 × KTDH-13 also expressed favourable SCA effects for chlorophylls and antioxidant traits, demonstrating complementary allelic interactions and strong dominance effects. For phenolic content and ascorbic acid, the crosses CMS-2 × KTDH-13, CMS-1 × KTDH-56, and CMS-3 × KTDH-55 showed high positive SCA effects.

These results align with previous reports showing that antioxidant traits in Brassica hybrids are strongly influenced by non-additive gene action and parent-specific interactions [21]. Similarly, crosses such as CMS-1 × KTDH-29, CMS-2 × KTDH-19-1, and CMS-3 × KTDH-52-1 exhibited desirable SCA effects for CUPRAC and FRAP, while CMS-1 × KTDH-10 and CMS-2 × KTDH-26 performed best for ascorbic acid accumulation. These results further support evidence that antioxidant assays show high heterotic responsiveness due to dominant complementary gene effects (Singh et al., 2019).[26]. No hybrid was superior for all traits, confirming trait-specific heterotic expression, as commonly reported in Brassica and chickpea breeding programmes [22] and [20]. Overall, the crosses CMS-1 × KTDH-56, CMS-1 × KTDH-29, CMS-2 × KTDH-13, and CMS-3 × KTDH-55 emerged as promising combinations for simultaneous improvement of pigment concentration and antioxidant capacity in ornamental kale. These findings strongly support the potential of heterosis breeding for enhancing biochemical quality.

Table 5. Specific combining ability effects of crosses for different characters in Ornamental kale

Hybrids	Chl a	Chl b	(T) Chl	CC	PC	LC	AC	CUPRAC	FRAP	AA
CMS-1*KTDH-10	0.51	-0.50	-0.45	2.52***	-142.56	0.03	-0.08	0.27	0.34	1.97***
CMS-1*KTDH-55	-5.89***	-4.31***	-3.98***	-0.99***	708.06***	-0.18**	0.20	-0.33	0.35	-0.12
CMS-1*KTDH-56	6.86***	2.20	2.09***	-4.52***	348.06**	-0.21***	0.95***	-0.67	-0.57	1.74***
CMS-1*KTDH-13	-3.54***	-1.24	-1.17**	-1.14***	-695.69***	-1.36***	-0.41**	0.30	-0.08	-4.24***
CMS-1*KTDH-19-1	-1.20	-1.11	-1.02*	0.27	-830.69***	0.47***	-0.58***	-1.59	0.56	1.62***
CMS-1*KTDH-19-2	5.09***	4.40	4.05***	-0.33*	721.81***	-0.31***	-0.14	-0.56	-0.09	0.87**
CMS-1*KTDH-52-1	0.11	-0.44	-0.39	0.06	27.44	-0.19**	-0.78***	-0.42	0.00	-0.92**
CMS-1*KTDH-26	-6.87***	-3.53	-3.29***	1.92***	103.69	1.02***	-0.06	-0.49	-0.07	-1.63***
CMS-1*KTDH-57	-3.57***	-1.93	-1.79***	1.05***	-261.94*	**0.13***	-0.70***	1.43	0.00	0.69*
CMS-1*KTDH-29	8.51***	6.46	5.97***	1.16***	21.81	0.60***	1.59***	2.05	-0.46	0.01
CMS-2*KTDH-10	3.20***	2.82	2.60***	-1.58***	-625.38***	-0.33***	-2.21***	-0.59	-0.01	-2.62***
CMS-2*KTDH-55	-5.75***	-7.41	**-6.78***	-1.76***	-601.63***	-0.05	-1.87***	0.11	**-0.77***	-2.01***
CMS-2*KTDH-56	**-13.71***	-6.95	-6.49***	1.27***	-554.75***	0.19***	-1.10***	-0.13	0.63	0.93**
CMS-2*KTDH-13	8.59***	6.05	5.59***	0.50***	1024.63***	2.06	0.67***	0.92	**0.90***	2.51***
CMS-2*KTDH-19-1	5.41***	4.29	3.96***	1.91***	552.13***	-0.29***	0.30*	**2.95***	-0.72*	0.27
CMS-2*KTDH-19-2	2.49***	1.24	1.16**	0.17	85.25	-0.06	-0.10	1.31	0.38	-2.10***
CMS-2*KTDH-52-1	2.69***	3.57	3.27***	-0.47**	-44.75	-0.10	1.11***	-1.34	-0.40	-0.65*
CMS-2*KTDH-26	0.43	-1.21	-1.08*	-0.07	999.00***	**-0.71***	2.03***	-1.34	-0.40	3.50***
CMS-2*KTDH-57	-3.33***	-1.13	-1.07*	-0.03	207.75	-0.29***	2.16***	-1.35	-0.47	0.42
CMS-2*KTDH-29	-0.02	-1.28	-1.16**	0.07	-1042.25***	-0.42***	-1.00***	-0.93	0.06	-0.26
CMS-3*KTDH-10	-3.71***	-2.32	-2.15***	-0.94***	767.94***	0.30***	2.29***	0.32	-0.34	0.65*
CMS-3*KTDH-55	**11.64***	**11.72**	10.76***	2.75***	-106.44	0.23***	1.67***	0.22	0.42	**2.12***
CMS-3*KTDH-56	6.85***	4.75	4.40***	**3.25***	206.69	0.02	0.15	0.80	-0.06	-2.67***
CMS-3*KTDH-13	-5.04***	-4.81	-4.42***	0.65***	-328.94**	-0.69***	-0.26	-1.22	-0.82*	1.73***
CMS-3*KTDH-19-1	-4.21***	-3.18	-2.94***	**-2.18***	278.56*	-0.19**	0.28*	-1.37	0.16	-1.89***
CMS-3*KTDH-19-2	-7.59***	-5.64	-5.21***	0.16	-807.06***	0.37***	0.23	-0.75	-0.29	1.23***
CMS-3*KTDH-52-1	-2.80***	-3.13	-2.87***	0.41**	17.31	0.29***	-0.34*	1.36	-0.41	1.58***
CMS-3*KTDH-26	6.44***	4.74	4.38***	-1.85***	-1102.69***	-0.30***	-1.97***	1.83	0.46	-1.88***
CMS-3*KTDH-57	6.91***	3.06	2.87***	-1.02***	54.19	0.16**	-1.46***	-0.08	0.47	-1.10***
CMS-3*KTDH-29	-8.49***	-5.19	-4.81***	-1.23***	1020.44***	-0.18**	-0.59***	-1.12	0.40	0.25
SE (hybrids)	0.931	1.155	1.046	0.057	46.152	0.017	0.038	0.367	0.146	0.119

***, **, * Significant at 0.1, 1 and 5 % probability respectively

Conclusion

The study demonstrated significant genetic variability among CMS lines, DH testers, and their hybrids for all biochemical traits in ornamental kale. Non-additive gene action played a major role, indicating strong potential for heterosis breeding. Hybrids such as CMS-3 × KTDH-55, CMS-1 × KTDH-56, and CMS-2 × KTDH-13 showed superior pigment and antioxidant profiles. CMS-1, KTDH-56, and KTDH-13 emerged as valuable general combiners for quality improvement. Overall, integrating CMS and DH systems proved effective for developing high-quality ornamental kale hybrids with enhanced biochemical traits.

Authors' contributions

All authors contributed significantly to this field study. Fieldwork, data collection and analysis was done by Deachen Dolma**.** All authors were involved in the conception, design of the experiment and provided critical revisions for finalization of experiment.

Future Scope

The promising CMS lines, DH testers, and superior hybrids identified in this study can be further evaluated across multiple locations and seasons to confirm stability of biochemical traits. Molecular marker–assisted selection can be incorporated to identify QTLs linked to pigments and antioxidant pathways. The best hybrids may be advanced for large-scale trials and commercial hybrid development in ornamental kale. Additional biochemical profiling, including flavonoids and glucosinolates, can broaden the understanding of quality traits. Future breeding programmes can integrate CMS, DH, and genomic tools to accelerate hybrid development and improve both ornamental and nutritional value.

Conflict of interest

The author do not have any conflict of interest.

Abbreviations

Short name	Full form
Ch a	Chlorophyll a
Ch b	Chlorophyll b
CC	Carotenoid content
PC	Phenol content
LC	Lycopene content
AC	anthocyanin content
CUPRAC	Cupric Reducing Antioxidant Capacity
FRAP	Ferric Reducing Antioxidant Capacity
AA	Ascorbic acid
CMS	Cytoplasmic male sterility
DH	Doubled haploid
GCA	General combining ability
SCA	Specific combining ability

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