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Utilizing Ogura-CMS and doubled haploid lines of cabbage for new-generation hybrid breeding

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Abstract

In this study, we report the development of a uniform hybrid of cabbage by utilizing cytoplasmic male sterile (CMS) and doubled haploid (DH) genotypes. Firstly, 26 cabbage genotypes were subjected to molecular diversity analysis using 52 SSR primers. Most divergent parents representing CMS (6) and DH (5) genotypes were crossed in Line × Tester mating design during the summer of 2018. During winter, 2018-19, all the parents, their 30 F1's, and one standard check cultivar (Pusa Hybrid-81) were evaluated for different horticultural traits. Based on the mean performance, three lines *viz.* KTCB-836A, KTCB-5A, KTCB-6A and two testers *viz.,* KTCB-50-1 and KTCB-51-19 were found superior. Hence, these genotypes can be utilized in future cabbage hybrid breeding programs. While among the 30 DH-based hybrids, six cross combinations *viz.* KTCB-1A × KTCB-50-1 (586.82 q), KTCB-836A × KTCB-51-19 (568.93 q), KTCB-836A × KTCB-51-2 (557.27 q), KTCB-836A × KTCB-50-3 (527.90 q), KTCB-836A × KTCB-51-6 (517.08 q) and KTCB-836A × KTCB-50-1 (513.06 q) were found superior than the Standard check cultivar, Pusa Hybrid-81 (405.93 q). As these hybrids were quite uniform hence, after multilocations testing they can be released for commercial cultivation in India.

Keywords: Cabbage, CMS, doubled haploid, F_1 hybrid, SSR.

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Introduction

Cabbage occupies the most important position among the *Brassica* vegetable crops, which are cultivated in temperate to tropical climatic conditions throughout the world, including India (Singh *et al.* 2013). It is a foremost commercial crop in hilly regions of India and can be grown throughout the year in open filed conditions (Kumar *et al.* 2019) using different agronomical management practices (Kurmanchali *et al*. 2020, Sindhu *et al*. 2021, Bahadur *et al*. 2021). It constitutes an integral part of traditional cuisine in several countries (Dey *et al.* 2017, Parkash *et al.* 2019) and is consumed as raw, shredded in salads, cooked, boiled or braised in soups and different culinary dishes (Kiber *et al.* 2014). It also possesses medicinal properties especially anticarcinogenic properties due to the presence of glucosinolates along with vitamins A, B, C and fibers (Sarikamis *et al.* 2009). Today, because of rising demand and the outbreak of new pests and diseases, developing new high-yielding quality varieties and/or hybrids in this crop is obligatory. F1 hybrids are advantageous in cabbage since they are very early with uniform maturity and yield better quality heads. They also resist many biotic stresses and tolerate unfavorable weather conditions (Kucera *et al.* 2006). Generally, two pollination control mechanisms, self-incompatibility (SI) and CMS, are widely used to produce F_1 hybrid seed in brassica vegetables (Parkash 2008, Dey *et al.*

2014, Singh *et al.* 2019). So far, the majority of hybrid cultivars in cole crops have been developed through SI system, but the main problem with the SI system is that it breaks at high temperatures and leads to the production of sibs in hybrid seeds besides difficulty in its maintenance and multiplication (Parkash *et al.* 2018).

In such situations, CMS system is the most stable genetic mechanism that offers a good alternative for the production of F_1 hybrid seeds (Kucera *et al.* 2006, Dey *et al.* 2013, Dey *et al.* 2014). Further, selecting suitable genetically diverse parental lines in cabbage is important for the expression of heterosis in a desirable direction (Parkash *et al.* 2018). The estimates of genetic diversity are useful for germplasm characterization and help to identify suitable parents for hybrid breeding in cabbage (Parkash *et al.* 2017). In any plant breeding programme, morphological traits have immense value for the selection of parents with maximum variation. But morphological traits are highly influenced by environmental factors; hence molecular DNA markers principally SSRs and SNPs are preferred for cultivars identification as these are devoid of the environment's perplexing effect. Hence, marker-assisted breeding aids to the selection of breeding material in conventional breeding programs. The breeding of brassica vegetables is mainly focused on developing F1 hybrids, necessitating the constant requirement of uniform parental lines. Since cabbage is highly heterozygous and exhibits higher inbreeding depression, it is difficult to get 100% pure homozygous parental lines with conventional breeding methods. With these constraints, DH technology based on isolated microspore *in-vitro* culture is widely used to produce complete homozygous lines of *Brassica* crops (Bhatia *et al.* 2016). This method allows us to rapidly develop homozygous lines, in contrast to time-consuming traditional breeding for heterosis in cross-pollinating crops which takes about seven to ten years (Pivovarov *et al.* 2017). To date, very meager information is available in the literature on genetic diversity analysis using CMS and DH lines of cabbage and their exploitation for uniform hybrid development in cabbage. Hence, at ICAR-IARI Regional Station, Katrain, we developed and maintained several cytoplasmic male sterile (Parkash *et al.* 2019) and doubled haploid lines of cabbage (Bhatia *et al.* 2018), out of which 26 promising lines were used for molecular diversity analysis and their further use in the development of next-generation hybrid of cabbage.

Materials and Methods

The present investigation was carried out at ICAR-IARI Regional Station, Katrain, Kullu Valley, HP. In the present studies, 26 genotypes of cabbage (13 CMS lines and 13 DH lines) were subjected to molecular diversity analysis using 52 SSR markers. For this, isolation, purification and quantification of genomic DNA, PCR amplification, electrophoresis and gel documentation of amplified DNA was done by adopting the standard procedure followed in our previous studies on genetic diversity analysis in cabbage (Parkash *et al.* 2017, Parkash *et al.* 2018). Out of 52 SSR markers only 26 primer pairs showed polymorphic bands in the different genotypes under study (Table 1), which were further used for molecular data analysis. Various genetic diversity estimates such as the observed number of alleles (n_a), the effective number of alleles (n_e), expected heterozygosity (H_e), observed heterozygosity (H_o) and Shannon information index (I) were estimated through POPGENE software (version 1.32). The polymorphism information content (PIC) was computed through Cervus version 3.0 software. UPGMA (unweighted pair group method of arithmetic mean) dendrogram based on principle component analysis was constructed by using NTSYSpc 2.0 software.

Based on the results of molecular characterization, the most divergent genotypes of the cabbage representing both CMS lines (6) and DH genotypes (5) were selected for their use as lines and testers, respectively. In September 2017, selected CMS and DH genotypes were transplanted unpaired in the crossing blocks, according to the Line × Tester mating design as suggested by Kempthorne (1957). The crosses were attempted between six CMS lines and five DH testers in Line × Tester mating design during April-June, 2018. During September 2018, all the parents and their 30 F_1's along with one standard check (Pusa Hybrid-81) were transplanted in the main field in Randomized Complete Block Design (RCBD) with 3 replications in a plot having size of 3.0 x 1.5 m, by maintaining 45 x 45 cm row-row and plant to plant distance, which accommodated 18 plants per plot. During both years, standard cultural practices for raising a healthy crop stand of cabbage cultivation were followed according to ICAR-IARI, Regional Station guidelines (Sharma 2003). Data were periodically recorded on different qualitative and quantitative traits from arbitrarily selected 10 plants from all replications. The data so obtained were subjected to analysis of variance in OPSTAT software by following the procedure of Gomez and Gomez (1984).

Results and Discussion

Molecular Characterization

For molecular characterization, 52 SSR primers were used of which 26 primers were found highly polymorphic and were found useful to differentiate different genotypes under study (Figure 1). SSR markers' usefulness in distinguishing between different genotypes of cabbage has been reported earlier in our studies (Parkash *et al.* 2017, Parkash *et al.* 2018). In overall, 56 alleles were amplified through 26 SSR primers, averaging to 2.15 alleles in each locus (Table 1). This average value agrees with our earlier study (2.20) on genetic diversity analysis in self-incompatible lines of cabbage (Parkash *et al.* 2017), suggesting appreciable allelic frequency

Table 1: Genetic diversity statistics for 26 SSR loci studied in 26 genotypes of cabbage

S. No.	*Locus*	n_a	n_e	*I*	H_o	H_e	*PIC*
1.	BoSF184	2.00	1.57	0.55	0.04	0.37	0.29
2.	BoSF062	3.00	1.45	0.59	0.12	0.32	0.32
3.	BoSF1331	2.00	1.68	0.59	0.08	0.41	0.34
4.	BoSF2612	2.00	1.42	0.47	0.12	0.30	0.25
5.	BoSF1215	2.00	1.27	0.37	0.08	0.22	0.18
6.	BoSF2313	2.00	1.60	0.56	0.00	0.38	0.30
7.	BoSF1167	3.00	1.45	0.60	0.20	0.32	0.33
8.	Na12F03a	2.00	1.89	0.66	0.44	0.48	0.36
9.	BoE783	2.00	1.09	0.17	0.00	0.08	0.07
10.	BrSF422	2.00	1.08	0.17	0.00	0.08	0.07
11.	BoSF2678	2.00	1.37	0.44	0.00	0.27	0.23
12.	BoSF302	2.00	2.00	0.69	0.80	0.51	0.38
13.	BoSF1455	2.00	2.00	0.69	0.80	0.51	0.38
14.	BoSF2345	2.00	2.00	0.69	0.83	0.51	0.38
15.	CB10258	2.00	1.37	0.44	0.00	0.27	0.26
16.	BRAS011	2.00	1.23	0.33	0.21	0.19	0.21
17.	Na12B09	2.00	2.00	0.69	1.00	0.51	0.38
18.	BoE862	2.00	1.68	0.59	0.00	0.41	0.34
19.	BoSF1207	2.00	1.80	0.64	0.48	0.46	0.34
20.	BoSF1846	2.00	1.89	0.66	0.28	0.48	0.36
21.	BoSF2615	2.00	1.72	0.61	0.28	0.43	0.33
22.	BoSF2232	4.00	2.99	1.21	0.38	0.69	0.61
23.	BoSF2860	2.00	1.63	0.57	0.17	0.39	0.31
24.	BoSF1740	2.00	1.23	0.33	0.13	0.19	0.21
25.	BoSF022	2.00	1.30	0.39	0.26	0.23	0.20
26.	BoSF912	2.00	1.46	0.49	0.04	0.32	0.26
	Mean	2.15	1.62	0.55	0.26	0.36	0.30

Where, n_a: observed number of alleles, n_e: effective number of alleles, I: Shannon's Information index, H_o: observed heterozygosity, H_e: expected heterozygosity and PIC: polymorphic information content.

among the genotypes studied, but it was considerably lesser than that as reported by Mohamed *et al.* (2016) in different botanical varieties of *Brassica oleracea* (3.92). This might be due to the use of genotypes belonging to same botanical variety of *B. oleracea*. Among all the SSR markers, a maximum number of alleles were amplified by the primer BoSF2232 (4) followed by BoSF062 (3) and BoSF1167 (3). The remaining primers were able to amplify only two alleles per locus among the tested genotypes. The maximum value of Shannon's Information Index (I) was exhibited by the primer BoSF2232 (1.21), while it was observed minimum (0.17) in two primers *viz.* BoE783 and BrSF422. In our study, mean value of 'I' was recorded as 0.55, which is greater than as observed earlier by Paulauskas *et al.* (2013) in *B. napus* (0.12). The expected heterozygosity (0.36) had higher mean values than the observed heterozygosity (0.26). Parkash *et al.* (2017 and 2018) had also observed higher mean values of expected heterozygosity than observed heterozygosity. The highest (1.00) observed heterozygosity (H_o) was reported in the primer Na12B09, while the lowest value (0.00) was recorded for six SSR primers *viz.* BoSF2313, BoE783, BrSF422, BoSF2678, CB10258 and BoE862. The mean value of observed heterozygosity in this study was found greater than that of expected heterozygosity as reported by Pascher *et al.* (2010) in different varieties of *B. napus* (0.23). The expected heterozygosity (H_e) was recorded maximum (0.69) in the primer BoSF2232, while a minimum (0.08) was observed with the primer pairs BoE783 and BrSF422. In line with our study, Parkash *et al.* (2017) and Parkash *et al.* (2018) had also reported similar values of expected heterozygosity in different genotypes of cabbage. Polymorphic information content (PIC) was used to estimate allelic frequency and diversity among different genotypes of cabbage. PIC with a population mean of 0.30 was recorded highest in the primer BoSF2232 (0.61) and the lowest value (0.07) was observed for the primers BoE783 and BrSF422. Parkash *et al.* (2018) had also reported varied values of PIC (0.22–0.38), with a mean value of 0.34 by using SSR markers in different genotypes of cabbage. In the present study, different parameters of diversity exhibited high mean values, signifying allelic abundance in the different genotypes of cabbage. This allelic abundance might be attributed to cross-pollinating behavior of cabbage. The mode of pollination significantly affects the abundance and diversity of alleles within and across different plant species.

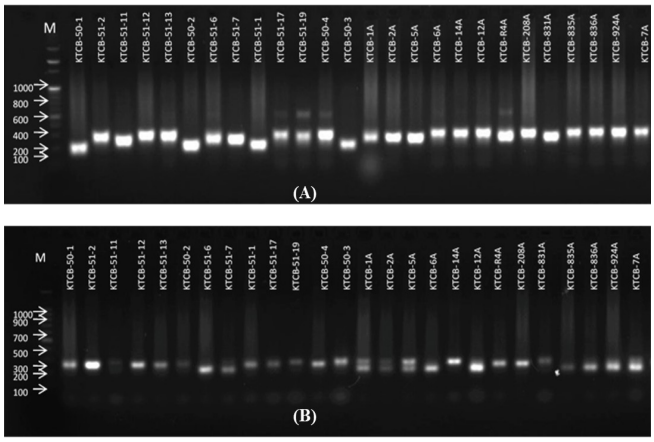


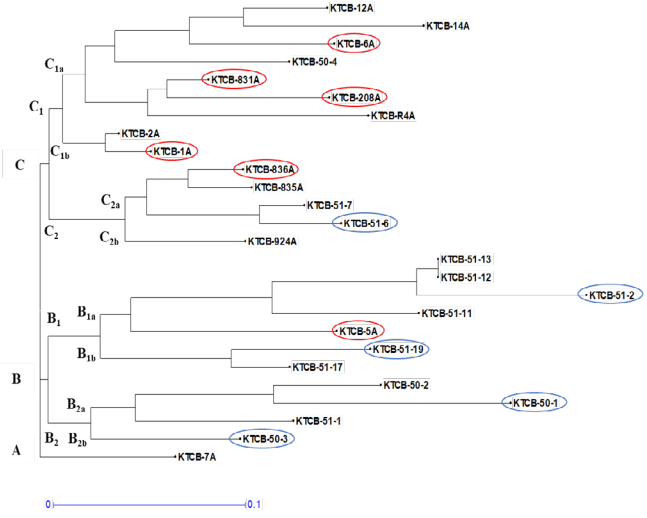
Figure 1: PCR amplification profile of 26 genotypes of cabbage using SSR primer (A) BoSF062 and (B) BoSF1167; where, M = Molecular size marker (1 Kb ladder). Molecular sizes (in bp) are given on left

Dendrogram constructed through the un-weighted neighbor joining method divided the 26 genotypes (CMS and DH lines) of cabbage into three major groups i.e., A B & C (Figure 2). Group A had only one genotype i.e., KTCB-7A, while group B and C accommodated 11 and 14 genotypes, respectively. It is apparent from the results that most of the CMS lines and DH genotypes were clustered separately in group C and B, respectively. Hence, we selected six diverse CMS lines (5 from Group C and one from Group B) and five distinct testers (4 from Group B and one from Group C) for making F_1 crosses in Line × Tester design. Crossing these genotypes was expected to yield heterotic F_1 hybrids in cabbage. Mohamed *et al.* (2016), Parkash *et al.* (2017) and Parkash *et al.* (2018) based on SSR data, had also clustered different genotypes of *B. oleracea* into different groups, indicating considerable level of genetic variations among different *Brassica spp*.

Morphological Characterization

Qualitative traits

Morphological characterization of 11 parents and their 30 hybrids and a check were done using 13 traits (Table 2). The results depicted that all the parental genotypes were semi-erect except the genotype KTCB-831A which is erect while among the 30 crosses, the cross combinations KTCB-836A × KTCB-50-1 and KTCB-836A × KTCB-50-3 exhibited erect leaf altitude while remaining 28 cross combinations and the check cultivar, Pusa Hybrid-81 were semi-erect in nature. With respect to outer leaf profile, most of the parental lines had concave outer leaf profiles, except KTCB-831A, KTCB-50-1, and KTCB-50-3, which had flat outer leaves, while KTCB-51-2 had convex outer leaves. Similarly, among the different crosses, 15 exhibited flat-shaped outer leaf profiles along with check cultivar and four other crosses had convex outer leaf profile. In contrast, rest of 11 crosses exhibited a concave outer leaf profile. Most of the parental genotypes exhibited bluish-green-colored outer leaves but the outer leaves of genotypes KTCB-1A and KTCB-836A were of normal green in color and KTCB-5A, KTCB-50-1, KTCB-50-3, KTCB-51-2 parental genotypes were dark green in color. Among the crosses, 22 combinations had dark green outer leaves, the cross combination KTCB-5A × KTCB-51-6 had green outer leaves, four crosses had pinkish green leaves and the remaining hybrids had bluish green outer leaves and the outer leaf margin of most of the parental lines were serrated except in the CMS line KTCB-1A and among the hybrids half of cross combinations had non-serrated leaves while remaining half were serrated. Though most of the parental genotypes had either weak or medium waxiness of outer leaves, segregation of this character into strong leaf waxiness was observed in the crosses KTCB-6A × KTCB-51-19, KTCB-836A × KTCB-51-2 and KTCB-836A × KTCB-51-6. Head shape of most of the parental genotypes' heads were round, but the lines KTCB-208A and KTCB-6A produced flat heads while the hybrids were of mixed composition of round, flat, oval and conical-shaped heads. Head base was leveled in most of the parents except parental genotypes like KTCB-208A and KTCB-50-3 and the cross KTCB-208A × KTCB-50-3, which had round base. Parental genotypes *viz.*, KTCB-50-1, KTCB-50-3, KTCB-51-2 and KTCB-5A the heads were partly covered by inner leaves at maturity, except in the CMS lines KTCB-1A, KTCB-6A, KTCB-208A and KTCB-836A in which heads were fully covered by inner leaves at maturity which helps to protect the heads from attaining yellow color. While, inner leaves fully covered the heads at maturity in the hybrids KTCB-836A × KTCB-51-2, KTCB-6A × KTCB-51-6, KTCB-6A × KTCB-51-2, KTCB-6A × KTCB-50-1 and KTCB-5A × KTCB-50-1. Anthocyanin pigmentation was observed on cover leaf at head maturity in all the parents and crosses but it was absent in the check cultivar. The internal color of head in most of the parents and crosses were yellowish-white except parental lines KTCB-208A and KTCB-6A which exhibited pinkish-white internal head color. Only five parental lines were affected by black rot, whereas remaining parental genotypes were free from black rot incidence. The incidence of black rot was noticed in only six cross combinations. However, the remaining hybrids were free from black rot incidence. Hence, wide variations among parents and their hybrids were observed for different qualitative traits under study. This offers the chance to select suitable parental genotypes for future hybrid breeding and choose a suitable F_1 hybrid depending upon the consumer's preference and market demand. Earlier workers *viz.* Kibar *et al.* (2016); Parkash *et al.* (2017) and Parkash *et al.* (2018) had also recorded wide variations in leaf colour, head shape and outer leaf waxiness of cabbage.



*Selected CMS lines-encircled red; selected DH testers-encircled blue

Figure 2: Dendrogram constructed using Un-Weighted neighbor-joining method showing clustering pattern of 26 genotypes of cabbage.

Table 2: Characterization of parental lines and their hybrids for different morphological traits of cabbage

S. No.	Parents/ Hybrids	Leaf altitude	Leaf shape	Outer leaf profile	Outer leaf margin	Outer leaf colour	Outer leaf waxiness	Undulated outer leaf margin	Head shape	Base shape	Head cover	Anthocy aninness	Head internal colour	Black rot
1	KTCB-1A	Semierect	Round	Concave	Non-serrated	Green	Weak	Absent	Round	Leveled	Covered	Present	Yellowish white	Present
2	KTCB-5A	Semierect	Elongated	Concave	Serrated	Dark green	Medium	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
3	KTCB-6A	Semierect	Round	Concave	Serrated	Bluish green	Medium	Strong	Flat	Leveled	Covered	Present	Pinkish white	Absent
4	KTCB-208A	Semierect	Round	Concave	Serrated	Bluish green	Medium	Strong	Flat	Round	Covered	Present	Pinkish white	Absent
5	KTCB-831A	Erect	Round	Flat	Serrated	Bluish green	Weak	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Present
6	KTCB-836A	Semierect	Round	Concave	Serrated	Green	Medium	Absent	BE	Leveled	Covered	Present	Yellowish white	Absent
7	KTCB-50-1	Semierect	Round	Flat	Serrated	Dark green	Medium	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
8	KTCB-50-3	Semierect	Round	Flat	Serrated	Dark green	Medium	Absent	Round	Round	PC	Present	Yellowish white	Absent
9	KTCB-51-2	Semierect	Round	Convex	Serrated	Dark green	Medium	Strong	BE	Leveled	PC	Present	Yellowish white	Present
10	KTCB-51-19	Semierect	Round	Concave	Non-serrated	Bluish green	Weak	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Present
11	KTCB-51-6	Semierect	Round	Concave	Non-serrated	Bluish green	Weak	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Present
12	KTCB-1A × KTCB-50-1	Semierect	Round	Concave	Non-serrated	Dark green	Weak	Medium	Round	Leveled	Uncovered	Present	Yellowish white	Absent
13	KTCB-1A × KTCB-50-3	Semierect	Round	Concave	Non-serrated	Dark green	Weak	Medium	Round	Leveled	Uncovered	Present	Yellowish white	Absent
14	KTCB-1A × KTCB-51-2	Semierect	Round	Flat	Serrated	Dark green	Weak	Absent	conical	Leveled	PC	Present	Yellowish white	Absent
15	KTCB-1A × KTCB-51-6	Semierect	Round	Concave	Serrated	Dark green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Present
16	KTCB-1A × KTCB-51-19	Semierect	Round	Convex	Serrated	Dark green	Weak	Absent	conical	Leveled	PC	Present	Yellowish white	Absent
17	KTCB-5A × KTCB-50-1	Semierect	Round	Convex	Non-serrated	Dark green	Weak	Absent	Round	Leveled	Covered	Present	Yellowish white	Absent
18	KTCB-5A × KTCB-50-3	Semierect	Round	Flat	Serrated	Dark green	Weak	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Absent
19	KTCB-5A × KTCB-51-2	Semierect	Round	Concave	Serrated	Dark green	Medium	Strong	Round	Leveled	Uncovered	Present	Yellowish white	Absent
20	KTCB-5A × KTCB-51-6	Semierect	Round	Flat	Serrated	Green	Medium	Absent	conical	Leveled	PC	Present	Yellowish white	Absent

21	KTCB-5A × KTCB-51-19	Semierect	Round	Flat	Non-serrated	Dark green	Absent	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Absent
22	KTCB-6A × KTCB-50-1	Semierect	Round	Concave	Serrated	Dark green	Weak	Absent	Round	Leveled	Covered	Present	Yellowish white	Absent
23	KTCB-6A × KTCB-50-3	Semierect	Round	Flat	Serrated	Dark green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
24	KTCB-6A × KTCB-51-2	Semierect	Round	Flat	Serrated	Dark green	Medium	Absent	Round	Leveled	Covered	Present	Yellowish white	Absent
25	KTCB-6A × KTCB-51-6	Semierect	Round	Flat	Non-serrated	Dark green	Medium	Absent	Round	Leveled	Covered	Present	Yellowish white	Absent
26	KTCB-6A × KTCB-51-19	Semierect	Round	Convex	Non-serrated	Bluish green	Strong	Absent	Round	Leveled	PC	Present	Yellowish white	Present
27	KTCB-208A × KTCB-50-1	Semierect	Round	Flat	Serrated	Dark green	Medium	Strong	Flat	Leveled	PC	Present	Yellowish white	Present
28	KTCB-208A × KTCB-50-3	Semierect	Round	Flat	Serrated	Pinkish green	Weak	Medium	Flat	Round	PC	Present	Yellowish white	Absent
29	KTCB-208A × KTCB-51-2	Semierect	Round	Concave	Serrated	Bluish green	Medium	Medium	Flat	Leveled	Uncovered	Present	Yellowish white	Absent
30	KTCB-208A × KTCB -51-6	Semierect	Round	Flat	Non-serrated	Pinkish green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
31	KTCB-208A × KTCB-51-19	Semierect	Round	Flat	Non-serrated	Pinkish green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
32	KTCB-831A × KTCB-50-1	Semierect	Round	Concave	Non-serrated	Dark green	Weak	Absent	Oval	Leveled	PC	Present	Yellowish white	Present
33	KTCB-831A × KTCB-50-3	Semierect	Round	Flat	Non-serrated	Dark green	Weak	Absent	Flat	Leveled	Uncovered	Present	Yellowish white	Absent
34	KTCB-831A × KTCB-51-2	Semierect	Round	Concave	Non-serrated	Dark green	Medium	Absent	Flat	Leveled	Uncovered	Present	Yellowish white	Absent
35	KTCB-831A × KTCB-51-6	Semierect	Round	Concave	Non-serrated	Bluish green	Medium	Absent	Round	Leveled	Uncovered	Absent	Yellowish white	Absent
36	KTCB-831A × KTCB-51-19	Semierect	Round	Flat	Serrated	Dark green	Medium	Absent	Round	Leveled	PC	Present	Yellowish white	Present

37	KTCB-836A × KTCB-50-1	Erect	Round	Flat	Non-serrated	Dark green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Present
38	KTCB-836A × KTCB-50-3	Semierect	Round	Concave	Serrated	Bluish green	Weak	Absent	Round	Leveled	PC	Present	Yellowish white	Absent
39	KTCB-836A × KTCB-51-2	Erect	Round	Flat	Non-serrated	Bluish green	Strong	Absent	Oval	Leveled	Covered	Present	Yellowish white	Absent
40	KTCB-836A × KTCB-51-6	Semierect	Round	Concave	Non-serrated	Bluish green	Strong	Absent	Round	Leveled	Uncovered	Present	Yellowish white	Absent
41	KTCB-836A × KTCB-51-19	Semierect	Round	Convex	Serrated	Pinkish green	Weak	Medium	Flat	Leveled	PC	Present	Yellowish white	Absent
42	Pusa Hybrid -81 (C)	Semierect	Round	Flat	Non-serrated	Green	Weak	Absent	Round	Leveled	PC	Absent	Yellowish white	Absent

BE: Broad elliptic; PC: Partially covered

Quantitative traits

Significant differences were perceived among the parents and hybrids for all the quantitative traits under study (Table 3). For the traits related to plant stature such as plant height, plant spread and number of non-wrapper leaves marked variations were detected amongst the parents and hybrids *viz.* plant height (16.00–22.33 and 17.37–21.97, respectively), plant spread (46.67–58.97 and 43.93–57.13, respectively) and number of non-wrapper leaves (12.97–18.67 and 10.60–16.03, respectively). The parental genotype KTCB-50-3, KTCB-831A and KTCB-51-6 exhibited maximum plant height (22.33 cm), minimum plant spread (46.67 cm) and number of non-wrapper leaves (12.97), respectively, while among the hybrids KTCB-836A × KTCB-51-2 exhibited maximum plant height (21.97 cm) and two cross combinations, KTCB-5A × KTCB-51-19 and KTCB-1A × KTCB-51-2 had minimum plant spread (43.93 cm) and non-wrapper leaves (10.60) which were considered as best with respect to plant stature. Wide variations concerning these traits were also reported by Kumar *et al.* (2013) and Parkash *et al.* (2017 and 2018) in different cabbage genotypes.

All the parents and hybrids also showed remarkable variations with respect to yield and yield contributing traits *viz.* head polar diameter (10.63–13.87 and 11.60–17.37 cm, respectively), head equatorial diameter (11.67–14.33 and 14.80–24.60 cm, respectively), head size (129.30–183.71 and 117.35–317.88 cm, respectively), days to head maturity (76.33–87.00 and 69.33–91.67 days, respectively), gross head weight (1.19–1.66 and 1.07–2.77 kg, respectively), net head weight (0.49–0.76 and 0.60–1.49 kg, respectively), harvest index (36.72–52.74 and 42.41–66.56%, respectively) and yield per hectare (193.38–299.84 and 237.24–586.82 q, respectively). The genotype, KTCB-836A (13.87 cm) followed by KTCB-208A, KTCB-831A and KTCB-50-1 recorded maximum head polar diameter, while among the hybrids KTCB-1A × KTCB-50-1 (17.37 cm) followed by four other combinations exhibited maximum head polar diameter. Maximum head equatorial diameter (14.33 cm) and head size (183.71 cm^2) were observed in genotype KTCB-51-2 and a cross combination, KTCB-836A × KTCB-50-3 (18.59 and 317.88 cm^2, respectively). Minimum days to attain marketable head maturity were observed in the genotype KTCB-50-1 (76.33), while among the hybrids, KTCB-5A × KTCB-50-1 (69.33) recorded minimum duration for head maturity and it was found at par with 24 other cross combinations. Highest gross head weight was observed in the genotype KTCB-51-2 (1.66 kg) and amongst the crosses, KTCB-836A × KTCB-50-3 (2.77 kg) followed by four other combinations recorded highest gross head weight. Amongst the parents, KTCB-51-2 and KTCB-5A exhibited highest net head weight 0.76 kg) and within the crosses, KTCB-1A × KTCB-50-1 (1.49 kg) followed by three other combinations revealed highest net head weight. Among the parents, highest harvest index

Table 3: Mean performance of parents and hybrids for different quantitative traits of cabbage

S. No.	Hybrids	Plant height (cm)	Plant spread (cm)	No. of non-wrapper leaves	Head polar diameter (cm)	Head equatorial diameter (cm)	Head size (cm^2)	Head compactness (%)	Head core length (cm)	Stalk length (cm)	Days to head maturity	Gross head weight (kg)	Net head weight (kg)	Harvest Index (%)	Yield (q/ha)
	Lines (CMS)														
1.	KTCB-1A	17.67	51.00	14.00	12.83	11.67	151.33	36.62	4.20	1.49	87.00	1.38	0.68	48.74	266.90
2.	KTCB-5A	20.17	50.00	14.33	12.63	14.00	177.78	32.65	4.35	1.15	80.00	1.60	0.76	47.79	298.97
3.	KTCB-6A	16.00	51.67	13.67	12.07	13.53	163.32	35.28	3.33	1.05	87.00	1.47	0.74	50.19	292.28
4.	KTCB-208A	18.33	57.07	18.67	11.17	12.83	143.67	28.81	5.17	1.28	77.67	1.35	0.50	36.72	195.67
5.	KTCB-831A	19.00	46.67	17.87	11.03	12.30	135.75	30.75	4.97	1.25	82.00	1.28	0.49	38.18	193.38
6.	KTCB-836A	20.47	58.97	17.37	13.87	11.83	164.01	27.92	4.57	1.38	82.00	1.51	0.59	39.20	234.23
	Testers (DH)														
1.	KTCB-50-1	20.10	53.87	13.20	11.40	14.00	160.20	36.44	5.00	1.15	76.33	1.45	0.74	51.52	293.78
2.	KTCB-50-3	22.33	53.67	16.00	12.17	13.00	158.33	31.08	5.40	0.84	82.00	1.45	0.62	42.61	244.46
3.	KTCB-51-2	20.50	53.50	15.00	12.82	14.33	183.71	30.36	4.87	1.04	81.67	1.66	0.76	45.88	299.84
4.	KTCB-51-6	19.00	54.33	12.97	10.63	12.17	129.30	42.12	4.47	1.21	82.00	1.19	0.63	52.74	248.31
5.	KTCB-51-19	16.40	51.33	13.67	12.43	12.87	157.84	35.45	5.80	1.14	81.00	1.43	0.72	50.19	282.56
	Hybrids														
1.	KTCB-1A × KTCB-50-1	21.40	51.31	12.03	17.37	17.47	303.27	28.11	5.65	1.04	70.00	2.63	1.49	56.53	586.82
2.	KTCB-1A × KTCB-50-3	17.47	48.90	15.13	11.73	12.97	152.06	33.50	4.04	0.90	71.33	1.39	0.63	45.36	249.80
3.	KTCB-1A × KTCB-51-2	18.47	48.17	10.60	12.67	12.97	164.08	46.07	3.52	0.85	91.67	1.46	0.96	66.56	379.73
4.	KTCB-1A × KTCB-51-6	18.13	47.40	12.63	12.03	13.60	163.78	37.65	4.22	0.77	81.67	1.47	0.79	53.85	312.47
5.	KTCB-1A × KTCB-51-19	17.41	44.47	13.00	12.57	12.82	161.49	37.24	3.53	1.00	89.00	1.45	0.77	52.42	302.56
6.	KTCB-5A × KTCB-50-1	20.47	53.40	12.67	13.50	14.80	199.82	33.76	4.95	0.81	69.33	1.77	0.96	54.08	378.70
7.	KTCB-5A × KTCB-50-3	20.03	49.90	12.57	13.27	14.50	192.75	34.73	5.38	0.89	72.00	1.71	0.92	54.19	365.09
8.	KTCB-5A × KTCB-51-2	19.63	47.87	12.33	13.00	14.23	185.34	35.98	4.30	0.92	72.33	1.65	0.91	55.16	359.48

9.	KTCB-5A × KTCB-51-6	17.97	44.53	12.00	12.77	12.93	165.06	39.45	3.44	0.69	74.67	1.48	0.84	56.68	330.12
10.	KTCB-5A × KTCB-51-19	17.37	43.93	11.13	12.03	9.50	117.35	47.24	4.45	0.70	71.33	1.07	0.64	61.26	254.35
11.	KTCB-6A × KTCB-50-1	18.63	51.63	13.83	12.40	13.83	171.57	33.66	3.37	1.04	71.33	1.55	0.76	49.17	300.25
12.	KTCB-6A × KTCB-50-3	18.33	52.60	16.03	11.84	14.17	167.73	29.54	3.03	1.23	75.67	1.53	0.65	42.41	256.68
13.	KTCB-6A × KTCB-51-2	18.53	47.20	15.17	12.07	11.93	143.88	34.68	3.50	1.05	73.67	1.33	0.60	45.29	237.24
14.	KTCB-6A × KTCB-51-6	19.63	51.80	12.43	13.70	14.13	193.63	34.86	3.33	0.97	74.33	1.72	0.94	54.73	371.44
15.	KTCB-6A × KTCB-51-19	18.60	50.77	12.37	12.60	13.70	172.59	37.33	4.60	1.16	69.67	1.54	0.85	55.05	334.98
16.	KTCB-208A × KTCB-50-1	20.73	54.73	12.67	12.87	14.73	189.58	34.81	3.97	1.10	77.67	1.69	0.91	54.19	359.42
17.	KTCB-208A × KTCB-50-3	20.43	56.33	14.28	12.03	14.70	176.86	31.82	4.62	1.25	70.00	1.59	0.76	47.72	300.41
18.	KTCB-208A × KTCB-51-2	18.40	51.93	14.15	11.60	13.07	151.53	35.91	3.97	1.24	77.33	1.38	0.67	48.81	265.57
19.	KTCB-208A × KTCB-51-6	20.20	53.50	13.67	12.23	14.47	177.11	33.29	4.80	1.20	82.00	1.59	0.79	49.82	313.01
20.	KTCB-208A × KTCB-51-19	17.63	46.70	13.20	12.90	14.80	190.94	33.13	4.47	1.13	72.67	1.70	0.88	51.78	348.42
21.	KTCB-831A × KTCB-50-1	21.87	54.93	15.13	13.77	14.47	199.17	28.54	4.02	0.96	71.33	1.79	0.80	44.95	316.98
22.	KTCB-831A × KTCB-50-3	20.62	50.32	14.53	13.43	14.72	197.83	29.71	4.52	1.19	72.67	1.77	0.83	46.80	327.26
23.	KTCB-831A × KTCB-51-2	19.63	54.53	11.80	13.03	15.03	196.08	36.16	3.83	1.27	89.00	1.73	1.00	57.65	394.11
24.	KTCB-831A × KTCB-51-6	21.23	51.37	14.10	12.57	14.07	176.91	32.53	4.60	1.22	71.00	1.59	0.77	48.23	303.18
25.	KTCB-831A × KTCB-51-19	20.58	49.88	12.40	12.97	13.90	180.32	36.59	4.33	0.81	70.67	1.61	0.89	55.36	352.42
26.	KTCB-836A × KTCB-50-1	21.93	52.90	14.33	16.47	17.62	290.32	26.21	4.87	1.01	71.00	2.54	1.30	51.16	513.06
27.	KTCB-836A × KTCB-50-3	21.77	57.13	14.62	17.10	18.59	317.88	23.49	5.87	1.14	72.67	2.77	1.34	48.20	527.90

28.	KTCB-836A × KTCB-51-2	21.97	51.33	12.55	16.50	18.17	299.74	27.08	4.07	1.30	71.00	2.60	1.41	54.20	557.27
29.	KTCB-836A × KTCB-51-6	21.57	56.57	11.87	13.90	16.50	229.37	37.35	4.53	0.70	69.67	2.24	1.31	58.44	517.08
30.	KTCB-836A × KTCB-51-19	21.67	56.53	12.00	16.40	17.83	292.33	28.73	4.47	0.67	70.00	2.54	1.44	56.82	568.93
31	Pusa Hybrid-81(C)	19.43	57.17	14.50	14.17	15.37	217.99	32.13	5.10	1.09	80.00	1.94	1.03	53.27	405.93
32	± SE(d)	0.87	2.42	0.85	0.52	1.04	15.97	2.91	0.44	0.15	4.86	0.13	0.09	3.37	33.51
33	$CD_{(0.05)}$	1.74	4.83	1.70	1.04	2.08	31.83	5.8	0.87	0.29	9.68	0.27	0.17	6.72	66.78
34	CV (%)	5.46	5.74	7.62	4.89	9.02	10.45	10.55	12.13	17.08	7.77	9.75	12.11	8.13	12.10

CMS: Cytoplasmic male sterile, DH: Doubled haploid, ±SE(d): Standard error of difference

was observed in the genotype KTCB-51-6 (52.74%) and among crosses, KTCB-1A × KTCB-51-2 (66.56%) followed by KTCB-5A × KTCB-51-19 exhibited highest harvest index. Among the parents, highest yield per hectare was observed in the genotype KTCB-51-2 (299.84 q), while among crosses KTCB-1A × KTCB-50-1 (586.82 q) followed by KTCB-836A × KTCB-51-19 (568.93 q), KTCB-836A × KTCB-51-2 (557.27 q) and KTCB-836A × KTCB-50-3 (527.90 q) exhibited highest yield per hectare. The results conform with Rai and Singh (2010), who reported high variability for gross head weight, net head weight, and number of non-wrapper leaves in cabbage. Similar results were also reported by Kumar *et al.* (2013).

The cabbage genotypes with short core length are desirable for having compact head. Among the parents and hybrids core length varied form 3.03 to 5.87 cm. The shortest core length was recorded in genotype KTCB-6A (3.33 cm) followed by KTCB-1A and subsequently, these lines can be utilized for hybrid breeding of cabbage to develop compact heads. Among the hybrids, minimum core length (3.03 cm) was observed in the cross combination KTCB-6A × KTCB-50-3 and it was found at par with nine other hybrid combinations. A similar variation for core length in cabbage was also reported earlier by Kumar *et al.* (2013). Furthermore, shorter stem length is preferred for cabbage cultivation as it can withhold higher head weight. Stalk length ranged from 0.67-1.49 cm among the parents and hybrids. The minimum stalk length was observed in parental genotypes KTCB-50-3 (0.84 cm) followed KTCB-1A and among different crosses, KTCB-836A × KTCB-51-19 (0.67 cm) exhibited minimum stalk length and it was found at par with 11 other hybrids. Significant differences for stalk length were also reported by Kumar *et al.* (2013), Sharma *et al.* (2018), Parkash *et al.* (2017) and Parkash *et al.* (2018) in cabbage.

Conclusion

The present studies concluded that SSR markers were useful for selecting diverse parental lines for heterotic F_1 hybrid development in cabbage. Three lines *viz.* KTCB-836A, KTCB-5A, KTCB-6A and two testers *viz.* KTCB-50-1 and KTCB-51-19 were found superior based on mean performance for different traits under study, hence these genotypes can be utilized in future heterosis breeding programs for yield improvement in cabbage. While among the 30 DH based hybrids, six cross combinations *viz.* KTCB-1A × KTCB-50-1 (586.82 q), KTCB-836A × KTCB-51-19 (568.93 q), KTCB-836A × KTCB-51-2 (557.27 q) and KTCB-836A × KTCB-50-3 (527.90 q), KTCB-836A × KTCB-51-6 (517.08 q) and KTCB-836A × KTCB-50-1 (513.06 q) were found superior than the check cultivar Pusa Hybrid-81 (405.93 q) with respect to yield per hectare. Hence, these hybrid combinations can further be tested at multilocations before releasing as a substitute for existing hybrid cabbage varieties in different parts of India.

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सारांश

वर्तमान अध्ययन में कोषकीय नर बन्ध्यता (सी.एम.एस.) और डबल हैपलॉइड (डी.एच.) प्रभेदों का उपयोग कर गोभी के समरूप संकर विकसित किये जाने की सूचना दी जा रही है। गोभी के 26 प्रभेदों पर 52 एस.एस.आर. प्राइमर्स का उपयोग कर आण्विक विविधता विश्लेषण किया गया और कोषकीय नर बन्ध्यता (6) और डी.एच. (5) के प्रभेदों का प्रतिनिधित्व करने वाले अधिकांश जनकों को वर्ष 2018 (ग्रीष्मकाल) के दौरान लाइन ग टेस्टर मेटिंग डिजाइन द्वारा संकरित किया गया। वर्ष 2018-19 (शीतकालीन) के दौरान, सभी जनकों और उनके 30 संकरों का एक मानक किस्म (पूसा हाइब्रिड-81) को नियंत्रक के रूप में समाहित कर विभिन्न औद्यानिक गुणों के लिए मूल्यांकित किया गया। औसत प्रदर्शन के आधार पर तीन लाइनों जैसे-के.टी.सी.बी.-836ए, के.टी.सी.बी.-5ए, के.टी.सी.बी.-6ए और दो परीक्षकों जैसे- के.टी.सी.बी.-50-1 और के.टी.सी.बी.-51-19 सर्वोत्तम पाया गया। इसलिए इन प्रभेदों का उपयोग भविष्य में गोभी संकर प्रजनन कार्यक्रमों में किया जा सकता है। हालांकि, 30 डबल्स हैपलॉइड्स (डी.एच.) आधारित संकरों में, छः संकर संयोजकों जैसे-के.टी.सी.बी.-1ए ग के.टी.सी.बी.-50-1 (586.82 कु.), के.टी.सी.बी.-836 ए ग के.टी.सी.बी.-51-19 (568.93 कु.), के.टी.सी.बी.-836 ए ग के.टी.सी.बी.-51-2 (557.27 कु.), के.टी.सी.बी.-836 ए ग के.टी.सी.बी.-50-3 (527.90 कु.), के.टी.सी.बी.-836 ए ग के.टी.सी.बी.-51-6 (517.08 कु.) और के.टी.सी.बी.-836 ए ग के.टी.सी.बी.-50-1 (513.06 कु.) मानक किस्म पूसा हाइब्रिड-81 (405.93 कु.) से उत्कृष्ट पाया गया। ये संकर बहुत समरूप हैं इसलिए बहुस्थानीय परीक्षण के बाद उन्हें भारत में व्यावसायिक खेती के लिए विमोचित किया जा सकता है।