

Development of Doubled Haploids (DHs) and their Utilization in Hybrid Breeding to Enhance Productivity and Quality in Cauliflower (*Brassica oleracea* var. *botrytis* L.)

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Abstract—The heterosis has been proved miraculous tool for the development of F_1 hybrids with better nutritional traits and high productivity in Brassica vegetables. Development of doubled haploid lines through microspore culture enables to derive inbreds within a very short period of times. Besides, DHs lines are also instrumental in developing F_1 hybrids with uniform performance for various traits. In this context the present study conducted using 20 genetically diverse Ogu CMS lines and 6 doubled haploid male fertile inbred to estimate combining ability, heterosis and nature of gene action for different agronomic and antioxidant traits. The genetic diversity in parental lines was also assessed by SSR markers. Then variation in mitochondrial genome was studied using mitochondria specific and mt-SSR markers. The parental CMS lines and DH testers were crossed to develop 120 F_1 hybrids in line \times tester mating design. The resulting 120 test cross progenies plus 26 parents along with 4 standard checks were evaluated in 10×15 alpha lattice design with three replications. The variance analysis revealed that all the studied traits were found to be under the genetic control of both additive and non-additive gene effects. The proportions of σ^2_A/D and $\sigma^2_{gca}/\sigma^2_{sca}$ ratios were less than unity in all the cases indicating preponderance of non-additive gene action in the genetic control of all the traits. The CMS lines Ogu118-6A, Ogu33A, Ogu34-1A and Ogu33-1A were good combiner for developing hybrids with early maturity. The CMS lines Ogu122-5A, Ogu33A, OguKt-2-6A, Ogu1-6A and Ogu126-1A could be used as female parent for developing high yielding hybrids. The CMA lines CMS lines Ogu122-5A and Ogu115-33A were found good combiner for majority of antioxidant traits. The SCA analysis revealed that most of the heterotic hybrids were associated with high positive SCA effects. Highest number of heterotic hybrids with SCA effects in desired positive direction was recorded for ascorbic acid content and phenolic content followed by total carotenoid content. The crosses Ogu34-1A \times 53-1, Ogu2A \times DH-53-1, Ogu2A \times DH-53-9 and Ogu33A \times DH-53-9 can be put into further evaluation trial for developing hybrids with early maturity. The crosses, Ogu126-1A \times DH-18-8-3, Ogu122-5A \times DH-53-10 and Ogu307-33A \times DH-18-8-3, were found best heterotic crosses for yield and its contributing traits. The heterotic crosses Ogu125-8A \times DH-53-1, Ogu125-8A \times DH-53-6 and Ogu34-1A \times DH-53-9 found best for accumulation of ascorbic acid content. The molecular analysis revealed that, among 350 microsatellite markers, 87 primers showed high polymorphism among the parental lines. The PIC content ranged from 0.24 to 0.80 and genetic distance was varied from 0.44 to 0.98. The significant association of genetic distance based on polymorphic genomic-SSR and EST-SSRs with heterosis for commercial traits indicated the utility of genetic distances in prediction of

*heterosis in cauliflower. The F_1 hybrids with better combining ability and better per se performance could be useful in accumulation of favorable allele for enhancing antioxidant traits and total marketable yield in cauliflower. The ratio of petal length to petal width was >2 in majority of the CMS lines indicating the great reduction in petal width relative to petal length with the Ogura cytoplasm. The highest quantity of nectar was found in the CMS line Ogu1-8A followed by Ogu308-6A and Ogu2-6A. The results revealed that in majority of the cases the relative position of stamen was reduced to lower level as compared to style. In all the CMS lines under study, the test weight of > 3 g was recorded. PCR-amplification of all the 76 CMS lines with respect to mitochondrial-DNA specific primers revealed that all the CMS lines were derived from the Ogura cybrid cytoplasm, although there are differences among the CMS lines with respect to nucleotide sequences. Mitochondria specific markers can be effectively utilized for identification of CMS types, assessment of cytoplasmic diversity and variability in mitochondrial genome of different male sterility specific cytoplasmic sources in *Brassica oleracea* vegetables and enhancing heterosis breeding programmes based on cytoplasmic male sterility.*