Review Article Cytoplasmic Male Sterility (CMS): Reliable approach for commercialization of hybrids in cauliflower

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ABSTRACT

Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) is an economically important vegetable crop that manifests heterosis. To exploit such heterosis, cytoplasmic male sterility (CMS) system is gaining popularity among public as well as private sector as a most reliable option. CMS is a maternally inherited trait encoded in the mitochondrial genome, and the male sterile phenotype arises as the result of interaction of a mitochondrial CMS gene and a nuclear fertility restoring (*Rf*) gene. The use of CMS lines to produce hybrid seed can overcome the danger of sibs and can achieve 100% purity in hybrids at low cost. Moreover, in cauliflower, the end product is harvested as a vegetative produce (curd stage) and fertility restoration is not needed, this makes CMS more acceptable and valuable hybridizing system in this crop. Some problems like poor seed set, floral abnormalities etc., related to CMS have been overcome through interspecific hybridization and other biotechnological interventions. The various aspects related to CMS including history, its effect on normal growth of the crop, problems, advantages, its practical implications and future prospects in cauliflower breeding have been discussed in this review.

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10 Keywords: Cauliflower, cytoplasmic male sterility, F₁ hybrid, heterosis, Rf gene

11 1. INTRODUCTION

12 CAULIFLOWER (BRASSICA OLERACEA L. VAR. BOTRYTIS L.) IS ONE OF THE MOST IMPORTANT CRUCIFEROUS VEGETABLE CROPS THAT MANIFESTS HETEROSIS [1,2]. 13 14 HETEROSIS IN CAULIFLOWER HAS BEEN REPORTED FOR IMPORTANT CHARACTERS LIKE EARLINESS [3.4] AND YIELD RELATED TRAITS [5,6,7]. DESPITE SUCH REPORTS OF 15 16 HETEROSIS IN CAULIFLOWER, ITS COMMERCIAL EXPLOITATION IS LAGGING BEHIND 17 MAINLY DUE TO THE HIGH COST OF HYBRID SEED PRODUCTION. TO OVERCOME THIS 18 LIMITATION, CMS COULD BE THE MOST VIABLE OPTION [8,9,10]. CMS IS BEING PHASED IN 19 FOR HYBRID SEED PRODUCTION SINCE IT IS MORE RELIABLE IN PROTECTING THE PROPRIETARY OF INBRED LINES TO BE USED AS PARENTS [11] AND FOR ACHIEVING 20 21 HYBRIDIZATION RATES OF UP TO 100% [12]. CMS-BASED F1 HYBRIDS ARE WIDELY GROWN 22 IN THE WORLD, ESPECIALLY IN CHINA [13]. PRIVATE SECTOR IS LEADING IN THIS 23 PERSPECTIVE. REPORTS OF USING CMS SINCE LATE 1990S ARE THERE VIZ., TESTING OF 24 IMPROVED 'OGURA' CYTOPLASMS [14] AND UTILIZATION OF CYBRID CMS LINES 25 CAULIFLOWER BY SEED COMPANIES IN FRANCE [15]. FURTHER, THERE ARE EXAMPLES 26 OF CMS BASED HYBRIDS RELEASED BY SEED COMPANIES, FOR INSTANCE, FREEDOM CAULIFLOWER ((HTTP://JORDANSEEDS.COM/CAULIFLOWER-HYBRID/) BY JORDAN SEEDS 27 28 INC. WOODBURY, MN 55125, USA.

29 Most of the Indian private sector seed companies are importing cauliflower hybrid seeds in bulk (10-20 tons/year) from overseas i.e., from Japan, Taiwan, Korea and China [16]. However, some of these 30 31 companies are using CMS system for hybrid development in India, now a days [17]. For instance, 32 Seminis Vegetable Seeds Inc. obtained patent (US Patent 6046383, April 4, 2000) on "Cytoplasmic 33 male sterile Brassica oleracea plants and the method of producing such plants" through protoplast 34 fusion and conventional back-crossing methods [18]. In past years, only few hybrids (with 35 mechanisms other than CMS) have been developed by public sector in cauliflower (Pusa Kartik 36 Sankar and Pusa Hybrid-2, through the use of Self-incompatibility (SI) system)) and were the only 37 hybrids made available to farmers [19]. Limited research related to hybrids could be attributed to high 38 cost of seed production and instability of mechanisms used. This provides the opportunity to the 39 private sector to sell their imported hybrid seeds at high cost to the farmers, which greatly affects the 40 farmer's wellbeing through the increase in cost of production. At this juncture, it becomes the responsibility of public institutions to narrow such gaps of hybrid seed production to make these seeds
 available to farmers at low cost.

43 Thinking on the same line, research work based on economical method i.e. CMS viz., development, characterization [20], in vitro maintenance [10], evaluation [21,2,22] and molecular-agronomic 44 45 characterization [23] of CMS lines has been inititated at Indian Agricultural Research Institute (IARI), 46 Regional Station, Katrain, Kullu Valley, Himachal Pradesh. Similarly, CMS based early and mid-47 maturity cauliflower lines were taken up for combining ability studies by Indian Agricultural Research 48 Institute IARI, New Delhi [24]. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur also 49 developed CMS lines in mid-late and late group which are under investigation and these might play 50 an important role for economical hybrid seed production in cauliflower and could benefit the farmers.

51 2. HISTORY OF CMS

52 Cytoplasmic male sterility is not apparently found in cauliflower or other cole crops but has been 53 introduced from other sources [18]. In cole vegetables, efforts were made by many workers to transfer 54 B. oleracea CMS lines from B. nigra [25], Raphanus sativus, Ogura type [26], B. napus L. (pol 55 cytoplasm) [27], B. rapa spp. pekinensis [28] in their respective studies. But here CMS system confers 56 poor stability as well as poor agronomic traits which could be attributed to functional incompatibility 57 between the B. napus nucleus and R. sativus chloroplasts. To overcome this problem, [29,30 and 31] made a successful attempt and isolated stable CMS lines from different sources viz., Raphanus 58 59 sativus, B. tournefortii and Diplotaxis muralis, respectively. The plants/species derived from these 60 types of cytoplasms had chlorotic leaves and floral deformities. Further attempts were made to minimize these problems through by understanding the molecular biology using biotechnological 61 62 interventions like somatic hybridization [32,15], plant regeneration from cultured mesophyll cells [33], 63 molecular cloning of CMS gene (orf 138) [34], Rf gene (orf 687) [35], interspecific hybridization and 64 embryo culture [36], and in-vitro maintainence of CMS lines [10]. Interspecific hybridization and 65 embryo culture might create an opportunity for developing biotic and abiotic resistant CMS lines 66 besides diversifying CMS sources [37].

67 3. DEVELOPING CMS LINES IN CAULIFLOWER

68 CMS identified in Japanese radish by [28] was introduced to broccoli [29,38] which was then 69 transferred to cauliflower [39,40]. Later on Ogu-CMS was introduced to heat tolerant Indian 70 cauliflower from kale (MS-91, MS-51, MS-11, MS-110) and broccoli (MS-01, MS-04, MS-05, MS-09, 71 MS-10) through repeated backcrosses and are in pipeline for heterosis breeding [18]. Another report 72 of CMS being introduced to cauliflower [41] from cabbage (already introgressed with male sterility 73 from *B. napus*), [42] is also there.

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75 **4. BASIC STEPS IN THE USE OF CMS**

76 Three lines play a major role in the use of CMS system. First one is A line (with good floral and agronomic traits) and is used as female parent, It is basically a line of desired genetic background 77 78 (genetically Smsms) where, S stands for sterile cytoplasm and ms for recessive male sterility gene. 79 To maintain A line, an isogenic line with the genetic background of A line known as B line (maintainer) 80 is required. To utilize this system for hybrid seed production, third line commonly known as C line (male parent) is there which also acts as the best specific combiner with A-line. Genetically this could 81 82 be N/S-Ms/- (fertility restoration) or Nmsms since the economic product in cauliflower is not the true 83 seed. To carry out further hybridization works, maintenance of A, B and C lines is the next important 84 step. For hybrid seed production, female to male ratios generally preferred are 2:1; 3:1; 4:1 depending 85 on varietal characters.

86 5. ADVANTAGES OF CMS OVER OTHER SYSTEMS

In cauliflower, F_1 hybrids are advantageous on the account of uniform maturity, high early and total yield, better curd quality with respect to curd compactness and colour, resistance to insect-pests, diseases and unfavorable weather conditions [43]. An efficient, reliable and stable method of F_1 seed production without contamination by self-fertilized seeds from each parent is vital [44]. Manual

91 emasculation and pollination method in cauliflower is of no importance at commercial level due to 92 unsuitable flower size and structure. So far hybrids in cauliflower have been developed using self-93 incompatibility (SI) system [22,45]. Several reports regarding self-incompatibility in Indian cauliflower 94 which revealed that inbreds/lines of maturity group I have strongest self-incompatibility followed by 95 maturity group II and group III [46-49] and in case of snowball cauliflower group, self incompatibility 96 system is either very weak or not present at all [50,51]. Moreover, SI system poses a risk of 97 occurrence of selfing in hybrid seeds besides problems of maintenance, multiplication of SI lines 98 through tedious bud pollination or CO2 and NaCl spray [52,53] and breaking down of SI under 99 different environmental conditions like high temperature and drought [54]. Even in case of genic male 100 sterility (GMS) system, roguing of fertile individuals from the female line at the time of anthesis poses major problem [21]. To overcome such problems, CMS system offers a better alternative [43,53,55]. 101 102 Usefulness of CMS based hybrids to exploit heterosis has been demonstrated by [56]. In the recent 103 years, significant heterosis has also been reported in hybrids developed using CMS system for yield, 104 yield related [21,24] and guality traits [2].

105 6. PROBLEMS RELATED WITH CMS

106 Development of superior "Ogura" based CMS system with normal female fertility, good seed setting 107 capacity and free from chlorosis at low temperature and their successful utilization in heterosis 108 breeding of snowball cauliflower is rare. Introgression of sterile Ogura cytoplasm in cauliflower nuclear 109 background poses undesirable effects like reduced nectarines, less honey bee visit [2], reduced 110 flower size, small curved style, unopened and partially opened flowers, almost rudimentary ovaries 111 [21,57], petaloid stamen, pinnate, silk-like and carpellate anther, splitted anthers, dysfunction of pistil, 112 closed flower, flower bud falling and no nectar development in cauliflower [58]. Similar reports were 113 also been there in case of B. juncea [59,60]. High degree of self-compatibility and subsequent selfing has lead to narrow genetic base of Indian snowball cauliflower [50,51] which poses difficulty in 114 115 achieving maximum heterosis for various characters [61,21]. The loss incurred in the form of above 116 discussed demerits like slightly low seed set etc., could be easily compensated by high price of F1 117 hybrid seeds which is almost 5 times as compared to open pollinated seed [21].

118 **7. UTILIZATION OF CMS**

119 In vegetable crops, exploitation of CMS for hybrid breeding and seed production is one of the major 120 research priorities. On the same line, various reports regarding the use of CMS in different vegetable 121 crops are listed in Table 1. In cauliflower also, various CMS lines have been reported with good 122 agronomic, commercial and floral traits by different workers in their respective studies (Table 2). 123 These evaluated CMS lines could offer better options for developing hybrids with desired characteristics. Some promising hybrids developed using these lines (KTH-27, KTH-52, and KTH-51) 124 125 are in pipeline along with one (KTCBH-84) being under evaluation in AICRP (VC) trials 126 (www.iari.res.in).

127 Table 1 Use of CMS in different vegetable crops

Vegetables	Reference
Onion	[62]
Cabbage	[57,63]
Radish	[64]
Broccoli	[65-67]

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129 Table 2 Reported CMS lines in cauliflower

CMS line	Scope for Heterosis	Reference
Ogu1A, Ogu2A, Ogu3A	Earliness and yield related traits	[21]

Ogu14A, Ogu15A, Ogu33A	Good commercial and floral traits	[22]
Ogu13A, Ogu14A, Ogu33A	Different quality traits	[2]
8410-22, 8498-2	Earliness and yield traits	[24]
KTH-27, KTH-52	Very high yielding hybrids	http://ztmbpd.iari.res.in/? g=cauliflower

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131 8. FUTURE PROSPECTS

132 In addition to better understanding and better control of cms system in cauliflower, extension of this 133 system to crops (presently having no cms systems) can be suggested. Further improvement of floral 134 and agronomic traits through interspecific hybridization and protoplast fusion is needed. Stable CMS 135 lines along with resistance to major diseases and pests must be identified for future use in hybrid 136 seed production programmes. Molecular genetics work will help to explicate molecular and functional 137 basis of cms. Biotechnological aspects related to various genes conferring cms in Brassicaceae crops 138 have been discussed exhaustively in various reviews [44, 68, 69]. Such studies would also contribute 139 in establishing efficient f1 breeding systems in Brassicaceae crops.

141 9. CONCLUSION

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143 From the above literature, CMS system can be considered as "Reliable approach for commercial 144 hybrid seed production". It has proved to be advantageous over traditionally used 'self-incompatibility' 145 and 'GMS' systems on the account of stability under varying environmental conditions and 100% pure 146 hybrid seeds. Significant work has been reported in northern belt of India in this context and development and evaluation of potential CMS based hybrids is in pipeline. This could help the farmers 147 148 in two-way approach; reducing cost of production (availability of hybrid seeds at cheaper rates than 149 private sector) and increasing turnover (uniform and high quality produce, yield almost double than 150 OP vars) from same piece of land.

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