Review Article

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

.

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.

Keywords: Cauliflower, cytoplasmic male sterility, F_1 hybrid, heterosis, Rf gene

1. INTRODUCTION

CAULIFLOWER (*BRASSICA OLERACEA* L. VAR. *BOTRYTIS* L.) IS ONE OF THE MOST IMPORTANT CRUCIFEROUS VEGETABLE CROPS THAT MANIFESTS HETEROSIS [1,2]. HETEROSIS IN CAULIFLOWER HAS BEEN REPORTED FOR IMPORTANT CHARACTERS LIKE EARLINESS [3.4] AND YIELD RELATED TRAITS [5,6,7]. DESPITE SUCH REPORTS OF HETEROSIS IN CAULIFLOWER, ITS COMMERCIAL EXPLOITATION IS LAGGING ~~BEHIND~~ MAINLY DUE TO THE HIGH COST OF HYBRID SEED PRODUCTION. TO OVERCOME THIS LIMITATION, CMS COULD BE THE MOST VIABLE OPTION [8,9,10]. CMS IS BEING PHASED IN 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 HYBRIDIZATION RATES OF UP TO 100% [12]. CMS-BASED F_1 HYBRIDS ARE WIDELY GROWN IN THE WORLD, ESPECIALLY IN CHINA [13]. PRIVATE SECTOR IS LEADING IN THIS PERSPECTIVE. REPORTS OF USING CMS SINCE LATE 1990S ARE THERE *VIZ.,* TESTING OF IMPROVED 'OGURA' CYTOPLASMS [14] AND UTILIZATION OF CYBRID CMS LINES CAULIFLOWER BY SEED COMPANIES IN FRANCE [15]. FURTHER, THERE ARE EXAMPLES OF CMS BASED HYBRIDS RELEASED BY SEED COMPANIES, FOR INSTANCE, FREEDOM CAULIFLOWER ((HTTP://JORDANSEEDS.COM/CAULIFLOWER-HYBRID/) BY JORDAN SEEDS INC. WOODBURY, MN 55125, USA.

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 companies are using CMS system for hybrid development in India, ~~now a days~~ [17]. For instance, Seminis Vegetable Seeds Inc. obtained patent (US Patent 6046383, April 4, 2000) ~~on~~ "Cytoplasmic male sterile *Brassica oleracea* plants and the method of producing such plants" through protoplast fusion and conventional back-crossing methods [18]. In past years, only few hybrids (with mechanisms other than CMS) have been developed by public sector ~~in~~ cauliflower (Pusa Kartik Sankar and Pusa Hybrid-2, through the use of Self-incompatibility (SI) system)) and were the only hybrids made available to farmers [19]. Limited research related to hybrids could be attributed to high cost of seed production and instability of mechanisms used. This provides the opportunity to the private sector to sell their imported hybrid seeds at high cost to ~~the~~ farmers, which greatly affects the 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.

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 characterization [23] of CMS lines has been inititated at Indian Agricultural Research Institute (IARI), Regional Station, Katrain, Kullu Valley, Himachal Pradesh. Similarly, CMS based early and mid-maturity cauliflower lines were taken up for combining ability studies by Indian Agricultural Research Institute IARI, New Delhi [24]. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur also developed CMS lines in mid-late and late group which are under investigation and these might play an important role for economical hybrid seed production in cauliflower and could benefit the farmers.

2. HISTORY OF CMS

Cytoplasmic male sterility is not apparently found in cauliflower or other cole crops but has been introduced from other sources [18]. In cole vegetables, efforts were made by many workers to transfer *B. oleracea* CMS lines from *B. nigra* [25], *Raphanus sativus*, Ogura type [26], *B. napus* L. (pol cytoplasm) [27], *B. rapa* spp. *pekinensis* [28] in ~~their respective~~ studies. But ~~here~~ CMS system confer~~s~~ poor stability as well as poor agronomic traits which could be attributed to functional incompatibility 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 sativus*, *B. tournefortii* and *Diplotaxis muralis*, respectively. The plants/species derived from these types of cytoplasms had chlorotic leaves and floral deformities. Further attempts were made to minimize these problems through ~~by~~ understanding the molecular biology usi~~ng~~ biotechnological interventions like somatic hybridization [32,15], plant regeneration from cultured mesophyll cells [33], molecular cloning of CMS gene (orf 138) [34], Rf gene (orf 687) [35], interspecific hybridization and embryo culture [36], and in-vitro maintainence of CMS lines [10]. Interspecific hybridization and embryo culture might create an opportunity for developing biotic and abiotic resistant CMS lines besides diversifying CMS sources [37].

3. DEVELOPING CMS LINES IN CAULIFLOWER

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

4. BASIC STEPS IN THE USE OF CMS

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 (genetically Smsms) where, S stands for sterile cytoplasm and ms for recessive male sterility gene. To maintain A line, an isogenic line with the genetic background of A line known as B line (maintainer) 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 be N/S-Ms/- (fertility restoration) or Nmsms since the economic product in cauliflower is not the true seed. To carry out further hybridization work~~s~~, maintenance of A, B and C lines is the next important step. For hybrid seed production, female to male ratios generally preferred are 2:1; 3:1; 4:1 depending on varietal characters.

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

emasculation and pollination method in cauliflower is ~~of no importance~~ at commercial level due to unsuitable flower size and structure. So far hybrids in cauliflower have been developed using self-incompatibility (SI) system [22,45]. Several reports regarding self-incompatibility in Indian cauliflower which revealed that inbreds/lines of maturity group I have strongest self-incompatibility followed by maturity group II and group III [46-49] ~~and in~~ case of snowball cauliflower group, self incompatibility ~~system~~ is either very weak or not present at all [50,51]. Moreover, SI system poses a risk of occurrence of selfing in hybrid seeds besides problems of maintenance, multiplication of SI lines through tedious bud pollination or CO_2 and NaCl spray [52,53] and breaking down of SI under different environmental conditions like high temperature and drought [54]. Even in case of genic male 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]. Usefulness of CMS based hybrids to exploit heterosis has been demonstrated by [56]. In ~~the~~ recent years, significant heterosis has also been reported in hybrids developed using CMS system for yield, yield related [21,24] and quality traits [2].

6. PROBLEMS RELATED WITH CMS

Development of superior "Ogura" based CMS system with normal female fertility, good seed setting capacity ~~and~~ free from chlorosis at low temperature ~~and their successful~~ utilization in heterosis breeding of snowball cauliflower is rare. Introgression of sterile Ogura cytoplasm in cauliflower nuclear background ~~poses~~ undesirable effects like reduced nectarines, less honey bee visit [2], reduced flower size, small curved style, unopened and partially opened flowers, ~~almost~~ rudimentary ovaries [21,57], petaloid stamen, pinnate, silk-like and carpellate anther, splitted anthers, dysfunction ~~of~~ pistil, closed flower, flower bud ~~falling~~ and ~~no~~ nectar development in cauliflower [58]. Similar reports ~~were 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 achieving maximum heterosis for various characters [61,21]. ~~The loss~~ incurred in the form of above discussed demerits ~~like slightly low seed set etc.,~~ could be easily compensated by high price of F1 hybrid seeds which is almost 5 times ~~as compared to~~ open pollinated seed [21].

7. UTILIZATION OF CMS

In vegetable crops, exploitation of CMS for hybrid breeding and seed production ~~is one of the~~ major research priorities. ~~On the same line, various reports~~ regarding the use of CMS in different vegetable crops are listed in Table 1. In cauliflower also, various CMS lines have been reported with good agronomic, commercial and floral traits by different workers in their respective studies (Table 2). 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) are in pipeline along with one (KTCBH-84) being under evaluation in AICRP (VC) trials (www.iari.res.in).

Table 1 Use of CMS in different vegetable crops

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

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/?q=cauliflower

8. FUTURE PROSPECTS

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

9. CONCLUSION

From the above literature, CMS system can be considered as "Reliable approach for commercial hybrid seed production". It has proved to be advantageous over traditionally used 'self-incompatibility' and 'GMS' systems on the account of stability under varying environmental conditions and 100% pure 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 in two-way approach; reducing cost of production (availability of hybrid seeds at cheaper rates than private sector) and increasing turnover (uniform and high quality produce, yield almost double than OP vars) from same piece of land.

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