Review Article

Cytoplasmic Male Sterility (CMS) in Cauliflower Breeding: A Review

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ABSTRACT

Cauliflower (*Brassica oleracea* L. var. *botrytis* L.), a member of family *Brassicaceae*, is one of the principal vegetable crops cultivated throughout the world. To elevate the productivity and enhance the quality, hybrid breeding of cauliflower is the most reliable option. Hybrid cauliflower seed may be produced without costly, labour intensive emasculation pollination systems and climate affected self-incompatibility systems, by the utilization of cytoplasmic male sterility (CMS). CMS has gained popularity among public as well as private sector as a most trustworthy breeding opportunity. Interaction of a mitochondrial CMS gene and a nuclear fertility restoring (*Rf*) genes resulted in the production of non-functional pollen which helped to achieve 100% purity in hybrids. CMS emerged as the more adequate and valuable system for cauliflower hybrid breeding, as fertility restoration is not desired, crop being harvested as a vegetative produce (curd stage). Biotechnological tools nowadays have been explored to improve the available CMS germplasm. In this review, we provide insights into the history, effects of cytoplasm on growth and flower characteristics, utilization of biotechnological tools along with the advantages, practical implications and future prospects of CMS for cauliflower hybrid breeding.

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]. Moreover, it has gained popularity among vegetable breeders [13]. The private sector is leading in this perspective. Reports of using CMS since the late 1990s are there *viz.,* testing of improved 'Ogura' cytoplasms [14] and utilization of hybrid 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 now using CMS system for hybrid development in India [17]. For instance, Seminis Vegetable Seeds inc. obtained patent (us patent 6046383, April 4, 2000) for "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 a few hybrids (with mechanisms other

than CMS) have been developed by public sector cauliflower breeders (Pusa Kartik Sankar and Pusa Hybrid-2, through the use of a Self-incompatibility (SI) system) and were the only hybrids made available to farmers [19]. Limited research related to hybrids could be attributed to the high cost of seed production and instability of the mechanisms used. This provides the opportunity to the private sector to sell their imported hybrid seeds at relatively high cost to farmers, which greatly affects the farmer's wellbeing through the increase in the 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 establishing an ECONOMICAL METHOD I.E., CMS *viz.,* development and characterization [20], in vitro maintenance [10], evaluation [21,2,22] and molecular-agronomic characterization [23] of CMS lines has been initiated 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]. Indian CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P.) also developed CMS lines in mid-late and late groups 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 Brassicas, 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 the referenced studies. But those CMS systems confer 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, further research was carried out, where [29,30 and 31] made a successful attempt and isolated the 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. Improved Ogura based system was exploited in case of cauliflower where CMS lines were developed through repeated backcrossing. Furthermore attempts were made to minimize the related problems through understanding of molecular biology via the use of biotechnological interventions like somatic hybridization [32,15], plant regeneration from cultured mesophyll cells [33], molecular cloning of CMS gene (orf 138) [34] and 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]. IMPROVED OGURA SYSTEMS HAVE BEEN EXPLOITED WORLD OVER FOR HYBRID BREEDING OF CAULIFLOWER nowadays [13].

3. DEVELOPING CMS LINES IN CAULIFLOWER

CMS identified in Japanese radish by [26] WAS INTRODUCED TO BROCCOLI [29,38] WHICH WAS THEN TRANSFERRED TO CAULIFLOWER [39,40]. LATER ON, OGU-CMS WAS INTRODUCED TO HEAT TOLERANT INDIAN CAULIFLOWER from kale male sterile (MS) lines (MS-91, MS-51, MS-11, MS-110) and broccoli MS lines (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 available.

4. BASIC STEPS IN THE USE OF CMS

Three lines play a major role in the use of CMS system. First is, A line (with good floral and agronomic traits) used as female parent, It is basically a line of desired genetic background (genetically Srfrf) WHERE S STANDS FOR STERILE CYTOPLASM AND rf for a 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, the 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-Rf/- (fertility restoration) or Nrfrf since the economic product in cauliflower is not the true seed.

To carry out further hybridization work, MAINTENANCE OF A, B AND C LINE IS THE NEXT IMPORTANT STEP. FOR HYBRID SEED PRODUCTION, FEMALE TO MALE RATIOS GENERALLY PREFERRED ARE 2:1; 3:1 or 4:1 depending on varietal characters.

5. ADVANTAGES OF CMS OVER OTHER SYSTEMS

In cauliflower, F_1 hybrids are advantageous due to uniform maturity, high early and total yield, better curd quality with respect to curd compactness and colour as well as for 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 not useful 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]. In the case of the SNOWBALL CAULIFLOWER GROUP, SELF INCOMPATIBILITY 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), roguing of fertile individuals from the female line at the time of anthesis poses a 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 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 to CMS

Development of a superior “Ogura” based CMS system with normal female fertility, good seed setting capacity, freedom from chlorosis at low temperature suitable for utilization in heterosis breeding of snowball cauliflower is rare. Introgression of sterile Ogura cytoplasm in cauliflower nuclear background risks undesirable effects like reduced nectarines, less honey bee visits [2], reduced flower size, small curved styles, unopened and partially opened flowers, rudimentary ovaries [21,57], petaloid stamen, pinnate, silk-like and carpellate anthers, split anthers, dysfunctional pistils, closed flowers, flower bud excision and lack of nectar development in cauliflower [58]. Similar reports have also appeared for *B. juncea* [59,60]. HIGH DEGREE OF SELF-COMPATIBILITY AND SUBSEQUENT SELFING HAS to LEAD TO a narrow genetic base of Indian snowball cauliflower [50,51] which poses difficulty in achieving maximum heterosis for various characters [61,21]. Losses incurred in the form of the above discussed demerits could be easily compensated by high price of F1 hybrid seeds which is almost 5 times the price of open pollinated seed [21].

7. UTILIZATION OF CMS

In vegetable crops, exploitation of CMS for hybrid breeding and seed production has been a major research priority. Reports regarding the use of CMS in different vegetable crops are listed in Table 1. Now in cauliflower also, various CMS lines have been developed using improved Ogura systems possessing 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 superior CMS lines/ Hybrids 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 (Pusa cauliflower hybrid 1)	Very high yielding hybrid (Identified for NCR region, New Delhi, India)	http://www.iari.res.in/files/LatestNews/Directors_Report2016-06022016.pdf

8. FUTURE PROSPECTS

In addition to better understanding and better control of CMS system in cauliflower, further improvement of floral and agronomic traits through interspecific hybridization and perhaps protoplast fusion is needed. Stable CMS lines with superior yield, yield contributing and market quality traits [13] along with the 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 may also contribute in establishing efficient F_1 breeding systems in *Brassicaceae* crops.

9. CONCLUSION

From the above literature, CMS system can be considered a "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 the northern belt of India in this context and development and evaluation of potential CMS based hybrids is in the pipeline. This could help the farmers in two-way approach; reducing the 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 the same piece of land.

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