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Advancement in CMS Based Hybrid Development in Cauliflower (*Brassica oleracea* var. Botrytis)

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Authors' contributions

This work was carried out in collaboration among all authors. Author Amit Kumar designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author Anjani Kumar managed the analyses of the study. Author CR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Among the different mechanism of male sterility operated in the Brassica group crop. Cytoplasmic male sterility mechanism is most suitable for hybrid development in cauliflower because here the curd (intermediate stage) is an edible part of the cauliflower. Further, there is no requirement of restorer line in this case as required in other seed crop. For the multiplication and maintenance of the different lines (A line and B line), sib mating and selfing is not always desirable. In fact, in such situation doubled haploid production through microspore culture is a more appropriate mechanism. Apart from this, the undesirable effect of integration of male sterile cytoplasm can be mitigated by adopting the repeated back crossing, through chloroplast substitution or somatic hybridization mechanism.

Keywords: Cauliflower; Brassica oleracea var. botrytis; chloroplast substitution; somatic hybridization.

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1. INTRODUCTION

Cauliflower (*Brassica oleracea* var *botrytis*) is an important member of Cole crop. It belongs to the Brassicaceae family. Mostly, it can be grown in all corners of the country during winter season. Due to availability of different temperature tolerant cultivar it can be grown during throughout of the year [1]. It is famous for health promoting chemicals like folic acids, phenolics, carotenoids, selenium, glucosinolates, anthocyanins and vitamins A, C, E and K [2]. Development of F_1 hybrids had been started first time in Maize in 1920s [3]. Since then it was developed in Eggplant (1924), Watermelon (1930), Cucumber (1933), Radish (1935, Tomato (1940) and Cabbage (1942). Hybrid production in cauliflower is very much desirable due to uniform maturity, early and high yield, curd compactness and color, resistance to insect-pests, diseases and unfavorable weather conditions [4]. Development of hybrid seed has been always a cumbersome process, but due to the presence of two different pollination controlled mechanism it became very easy now. These two pollination controlled mechanism are self-incompatibility (SI) and male sterility system in cauliflower [5]. The hybrid variety in cauliflower has been developed by utilizing SI system [6,2]. There are several disadvantages associated with the SI system like chance of sibs in the hybrids and reproduction of SI parents through tiresome bud pollination or treatment by enhanced concentration of CO_2 and NaCl spray [7] and it can be broken out during different environmental conditions like high temperature and moisture stress conditions or drought [8,9]. Identification and maintenance of S-allele homozygote plant is another problem associated with SI system in cole crop [4,5]. Report from different experiment revealed that the lines or genotypes from the early group have strongest SI system followed by group II and group III [10]. Moreover, in snowball groups of cauliflower the SI system is either very weak and does not available [11]. There for male sterility mechanism might be useful mechanism for hybrid production. GMS based hybrid production again leads to the rouging of fertile male parent from the female lines [12]. Keeping such views the CMS based is now a most suitable mechanism for hybrid production in cauliflower [4]. Cytoplasmic male sterility (CMS) is an important outcome of nucleo-cytoplasmic incompatibilities that prevents production of functional pollen grain, with or without impacting female fertility [13]. The maintainer and female line require intensive care to maintain the genetic nature of these lines [14]. The objective of this review was to CMS based hybrid development in cauliflower (*Brassica oleracea* var. *botrytis*).

2. DEVELOPMENT OF INBRED LINE

Development of pureline/inbred line is the prerequisite for any hybrid breeding programme [7]. Due to more out crossing nature of crop like cauliflower and presence of strong SI system in *B. oleracea* crops, the inbred development through traditional self-pollination is a tedious and cumbersome process and was also costlier [2]. The development of inbred line is practically impossible in this crop, cauliflower due to high inbreeding depression [15]. Hence, the production of Doubled Haploid (DH) in Brassica vegetables via anther culture or microscope culture make the process of inbred development becomes very easy and accurate attainment of homozygosity [2,16].

3. ADVANTAGE OF DOUBLED HAPLOID PRODUCTION

1. Drastic reduction in time duration taken in inbred line development, reduced from more than six generations to two successive generation [17].
2. There is only single round of recombination occurs in Doubled haploid production leading to preservation of more advantageous gene combinations of parents than Selfing.
3. DH based mapping populations have been proved instrumental in identification of antioxidant capacity related QTLs in *Brassica oleracea.*
4. Hybrid production is easy and less time consuming.

4. MICROSPORE CULTURE TECHNIQUE IN CAULIFLOWER

Microspore methods are the one of method utilize to developed doubled haploid plant in cauliflower [16]. This technique has been utilized in various classes of brassica crops under different environmental conditions. But, its utilization in doubled haploid production in case of cauliflower is very limited. The first successful microspore culture in *Brassica oleracea* crops was reported by [18] with *B. napus*, *B. oleracea* var *botrytis* is considered to be one of the most recalcitrant varieties in terms of embryonic

response. The main difficulties are genotype dependency, a low embryogenesis and germination rate, and unknown ploidy characterization. The success of microspore embryogenesis in Brassicas depends upon several endogenous and exogenous factors. The endogenous factors are donor's plant genotype, growth condition and microspore development stage whereas the exogenous factors are media composition, microspore density in culture and culture incubation conditions. The microspore developmental stage is one of the most crucial aspects of culture process as the developmental stage can greatly affect the yield of microspore derived embryo [16]. The viability of the microspores varied with the bud size and genotypes. Bhatia et al. 2016 reported that in the early (Pusa Kartik Sankar) and mid maturity group cauliflower (Pusa Sharad) a bud size of 4.0-4.5 mm is crucial for highest microspore viability. However a bud size of 4.5-5.0 mm is crucial in case of late maturity group of cauliflower for high microspore viability. It was further correlated that the late uninucleated to early binucleated stages of micropsores were obtained in early group of cauliflower at bud size of 4.0-4.5 mm and same stage in mid-late (Kt-34 variety) and Snow ball type (KT-119). As, the microspore are totipotent in nature during the late uninucleate to early binucleate stage [19]. It has been reported by [20] that binucleate micropores to be antagonistic for microspore embruogenesis. It was also found that mid to late uninucleated stage of microspores in smaller bud size genotypes where as higher percentage of microspores at early binucleated and binucleated stages was found in big size of flower bud. For all group of cauliflower, the bud containing the highest percent of microspore at the late uninucleate to early binucleate stage were observed suitable for efficient microspore embryogenesis. Moreover culture density also plays a significant role in the successful microspore embryogenesis because at optimized bud size the viability of microspore were reported merely 60-65% [16]. The microspore density of 8 × 10^4 per mL produced maximum number of embryos but it was reduced further by increasing the microspore density to 10 × 10^4 per mL in all bud size across the genotypes according to [16]. This might be due to nutrient competition among developing embryos and/ or some toxic substances when old microspores presented in the cultivated medium. Spontaneous chromosome doubling of microspore derived embryo occurs in cauliflower [21]. This spontaneous doubling of chromosome is also favored by [22]. Not all the haploid plant developed into diploid spontaneously rather a fraction of about 50% of regenerated plants were spontaneously doubled haploids, more than 25% were tetraploids, more than 10% were aneuploid and chimera, fewer than 7% were haploid, and about 2% of plantlets were other polyploids such as triploids, pentaploids and hexaploids [21,16].

5. ANTHER CULTURE

Anther culture/ androgensis are one of the haploid inducing techniques used for quick development of inbred line in various crops [22]. But the success of this technique is very less compare to microspore culture because of formation of embryo is affected by various factors like genotype [23], donor plant culture conditions, pre-treatment of buds or anthers, stages of development of the microspores during culture, culture medium composition and incubation conditions of the anthers [24]. [16] agreed that the success of anther culture is being depends upon the nature of genotype. Culture conditions of the donor plants are also very crucial for androgenic capacity of the plants. [25] observed a high frequency of non-viable cells at a temperature of above 25°C or lower than 10°C. it was further observed that the during the winter and spring season the level of embryogenic response for anther culture is high. However autumn and summer season for the anther culture is comparatively less favorable to very low response respectively. The sizable variation for different bud size has been observed between the genotypes, so not possible to fix the size of bud for androgenic success [23]. A strong correlation has been observed between bud size and Petal/anther lengths (P/A length) toward the highest embryonic development. [24] concluded that the embryonic development depends upon the genotype. Some were high responsive at the 1.0 P/A length while some genotypes was responsive at the 1.1-1.3 P/A lengths. In comparison to maltose and glucose, sucrose was found superior source of carbohydrates for inducing androgenic response with an optimal concentration of 140 g/l. A great success for development of androgenic callus in tropical cauliflower has been reported by using medium containing B5 salt+ 100 mg/lt sucrose+ 1 mg/lt 2, 4-D + 1 mg/lt NAA + 1 mg/lt BAP [23].

6. CYTOPLASMIC MALE STERILITY

Cytoplasmic male sterility (CMS) is an important outcome of nucleo-cytoplasmic incompatibilities

that prevents production of functional pollen grain, with or without impacting female fertility [13]. Based on CMS system, Hybrid development requires three lines namely A, B and R line called female, male and restorer line respectively. [1] observed a similar phenotype between CMS (A line) and their respective B line or maintainer line in terms of floral traits like, petal color, style shape, type of ovary and presence of functional nectaries. However, other floral traits like, flower size, length of style and stamens were reduced significantly after introgression of Ogura cytoplasm. These traits were guided by ogura cytoplasm and are not responsive to selection through backcrossing. [1] also observed that the only male traits were affected by introgression of ogura cytoplasm however female traits were free from this introgression. A vigorous annual selection before flowering is required during maintenance of CMS lines (A line) and Maintainer line (B line) [26]. Seed is not the edible part of cauliflower; however the pre floral meristem i.e. curds is the edible part. Henceforth there is no need of fertility restoration in this crop, as require in other seed crop [1]. Therefore, hybrid seed production can be possible without restorer gene. It has noticed certain adverse effect of self-pollination, during maintenance of the B lines like reduced plant vigour, small curd size and deformed curd due to inbreeding depression [26]. Maintenance of B lines through sib-mating is again problematic, it could disturbed or decline the uniformity of these lines due to heterozygosity after two to three generations. The curd produced in the hybrid developed by Ogura CMS line based parent is of lower quality compared to SI lineages based hybrid [5]. This defect solved by the [27] but not concluded how they refined the problem. It was further corrected through chloroplast substitution by [28].

7. SOURCE OF CMS IN BRASSICA FAMILY

Presently, Ogura cybrid cytoplasm from Japanese radish is the only source of CMS which is being used commercially in hybrid seed production of vegetable brassicas [28]. It was first discovered in Japanese radish (*R. sativus*) of an unknown cultivar [29]. Which is now popularly famous by his name i.e ogura cms, and is used worldwide in F_1 breeding of all cole crops [9]. Due to absence of *Rf* gene in Japanese radish it could not be possible to utilize it further in hybrid radish but utilized in European radish as having *rf* gene in it [30]. The ogura cytoplasm can be easily transferred to the brassica species through intergeneric hybridization. Another source of cms in brassica is Polima (pol) CMS of *B. napus*, a temperature sensitive source of CMS. Hence its practical utility in F1 hybrid breeding is limited [9]. There is absence of restorer gene for *hau* CMS in *B. juncea* made again difficult to utilize it in hybrid breeding [31].

Table 1. Different types of CMS

S.N	Source of CMS	Remarks
1	*ogu* CMS	[29]
2	*pol* CMS	[32]
3	*nap* CMS	[33]
4	*nig* CMS	[34]
5	*hau CMS*	[31]

8. CONTROL OF UNDESIRABLE EFFECT OF OGURA CYTOPLASM INTROGRESSION

The introgression of ogura cytoplasm in the background of brassica species lead to expression of some undesirable characteristics feature even induced by male sterile cytoplasm [5]. Different experimental results suggested that it was due to incongruity between the nucleus and cytoplasm. The undesirable effect of introgression of ogura cytoplasm in brassica crop is the discoloration of tissues, leaf chlorosis [35] at low temperature, and yellowing at low temperature (below 15°C) [36,28], poor development of nectaries [37], small flower size, low female fertility [38] unopened and partially opened flowers, rudimentary ovaries [39]. The discolouration of tissue or chlorosis causes due to delayed development of chlorophyll in male sterile plants [34]. This effect was observed due to incompatibility of Japanese radish chloroplast in the nuclear back ground of *B. oleracea* [28].

This can be controlled by adopting protoplast fusion between male sterile lines and breeding line having protoplast regenerability [35]. [5] observed that most of the deformity found in flower can be resolve by successive backcrossing with high selection pressure. [40] were also supported this conclusion.

9. CONCLUSION

Among the different mechanism of male sterility operated in the Brassica group crop, Cytoplasmic male sterility mechanism is most suitable for

hybrid development in cauliflower because here the curd (intermediate stage) is an edible part of the cauliflower. Further, there is no requirement of restorer line in this case as required in other seed crop. For the multiplication and maintenance of the different lines (A line and B line) sib mating and selfing is not always desirable. In fact, in such situation double haploid production through microspore culture is more appropriate mechanism should be adopted. Apart from this the undesirable effect of introgression of male sterile cytoplasm can be mitigated by adopting the repeated back crossing, through chloroplast substitution or somatic hybridization mechanism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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