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Development and characterization of "Ogura" based improved CMS lines of cauliflower (*Brassica oleracea* var. *botrytis* L.)

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Abstract

For the first time, three Ogura based improved cytoplasmic male sterile (CMS) lines of cauliflower (*Brassica oleracea* var. *botrytis* L.) *viz.*, Ogu1A, Ogu2A and Ogu3A were developed following seven generations of backcrossing with snowball group. These lines were evaluated and compared with their respective fertile maintainer (B) line for various agronomic traits like curd yield and size, floral traits like size of petals and style, shape of style and seed setting traits like number of seeds per pod and seed yield per plant. Nuclear-cytoplasmic interaction played an important role in determining various agronomic, floral and seed setting related traits. All the three CMS lines were similar with fertile maintainer lines for agronomic traits like days to curd maturity. The curd yield of Ogu1A and Ogu3A were similar with their respective maintainer lines. However, introgression of alien Ogura cytoplasm in the background of snowball cauliflower significantly reduced the petal size, filament length, style and stamen length. Number of pods per plant was significantly lower in all three CMS lines, when compared with their respective B lines. Number of seeds per pod and seed yield per plant in Ogu3A and its respective B line was at par but reduced significantly in Ogu1A and Ogu2A after introgression of Ogura cytoplasm.

Key words : Snowball cauliflower, CMS, commercial traits, floral traits, hybrid

Introduction

Snowball cauliflower belongs to late group and is cultivated extensively in India. Of late, hybrids are covering most of the area in the country. Genetic mechanisms namely, self-incompatibility (SI) and cytoplasmic male sterility (CMS) have been exploited for lowering the cost of commercial hybrid seed production. Although, majority of crucifer hybrids have been developed by means of SI [1] but this system poses a risk of occurance of selfing in hybrid seeds besides problems of maintenance and multiplication of SI lines [2-4]. In snowball cauliflower, SI system is either weak or not present at all [5-7]. Hence, CMS system offers a suitable alternative for F_1 hybrid seed production [8, 4]. Since, pre-floral inflorescence, commonly referred to as curd is the edible marketable part, therefore, F_1 fertility is immaterial and the fertility restoration is not needed.

Besides leading to CMS trait due to nuclear-cytoplasmic interaction under alloplasmic situation, transfer of alien cytoplasm usually also alters agronomic, floral and seed setting related traits. For example, in earlier effort on introgression of Ogura cytoplasm (initially derived from radish) in cauliflower was associated with several undesirable effects like, petaloid stamen, pinnate, silk-like and carpellate anther, splitted anthers, dysfunction of pistil, closed flower, flower bud falling and no nectary development [9]. Similarly, several floral deformities in broccoli were observed after introgression of Ogura cytoplasm [10]. Although usefulness of Ogura cytoplasm based F_1 hybrids to harness heterosis for various commercial traits have been advocated in cauliflower [11] and cabbage [12-13], extensive work regarding effect of Ogura cytoplasm on floral and seed setting related traits in snowball cauliflower has not been reported. Moreover, no report is available on development "Ogura" based improved CMS lines with normal female fertility, good seed setting capacity and chlorosis free at low temperature. In India also such line was not available. In this study, we report results of our systematic efforts to develop and characterize improved CMS lines in snowball group of cauliflower and results are described in the light of

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practical utility of these CMS lines in commercial hybrid seed production.

Materials and Method

Development superior CMS lines

In the past we successfully transferred sterile Ogura cytoplasm into the background of snowball cauliflower (var. Snowball-1, Snowball-16). However, these CMS lines were not suitable for commercial utilization because of associated undesirable traits, low female fertility and very poor seed set (Fig. 1). Ten elite recurrent parental lines (Pusa Snowball K-1, Pusa Snowball K-25, Pusa Snowball-1, Kt-13-01, Kt-22, Kt-15, DB-187, EC-162587, Kt-13-85 and Kt-20) of snowball cauliflower were crossed with previously developed CMS lines and since 1998 F_1s were successively back crossed with all the recurrent parents in order to transfer sterile cytoplasm. Very high selection pressure was practiced to recover plants free from floral deformities and with high seed set. In each backcross generation 10 plants with desirable traits were selected from a population of 250 plants. In 2005, after seven generations of backcrossing (BC_7) few alloplasmic lines of three recurrent parents (Pusa Snowball K-1, Pusa Snowball K-25 and Pusa Snowball-1) with high seed setting capacity were finally selected as they were free from floral deformities and had normal female fertility (Fig. 1). Morphological characteristics of three CMS lines *viz.* Ogu1A, Ogu2A and Ogu3A were compared with their respective recurrent parental lines.

Fig 1. Flowers male sterile (CMS) cauliflower (a) initial defective system (b) corrected system after BC_7

Evaluation of CMS lines

Three CMS lines along with their maintainers were evaluated for various commercial, floral and seed setting traits during 2008 and 2009. Six commercial traits, *viz.* (i) days to curd maturity, (ii) net curd weight (g), (iii) net curd yield (t/ha), (iv) curd length (cm), (v) curd width (cm), (vi) curd depth (cm) were taken for the present study. They were also compared with their respective maintainers for various floral traits like, (i) petal colour (ii) shape of style (iii) type of ovary (iv) presence of functional nectaries (v) petal length (mm) (vi) petal width (mm) (vii) length of long (bearing functional anthers) and short (bearing non-functional anther) filament (mm) and (viii) style length. The observations were recorded on freshly opened flowers. The ratio between length and width of petal was calculated to estimate the change in petal size. Similarly, the ratio between length of long filaments and the style was calculated to determine the relative position of stigma to anthers. Presence/absence of pollen grains in anther was determined on the basis of visual observation. Pollen viability was estimated on the basis of staining pollen with 2% acetocarmine and viewing under light microscope at 10× and 40× magnification. Data were recorded from ten randomly selected plants from three plots (36plants/plots). The plots of male sterile and maintainer lines were adjacent to each other in the same contour. One plot of male sterile lines was followed by one plot of maintainer lines. Data recorded on single plant basis for of 2 successive years were pooled for analysis and significance was tested by paired't' test. Mean values of each trait of CMS lines and their corresponding maintainers were compared to establish difference between A and B lines.

Seed setting capacity was assessed by keeping two rows of CMS lines (6 plants/row) after six rows of its fertile maintainer line and repeated nine times. The experiment was conducted within the cage with one honeybee colony kept inside for pollination. Observations were recorded from ten randomly selected plants in both CMS lines and their respective maintainers. Data were recorded for seed yield related characters like, (i) number of primary branches, (ii) number of secondary branches, (iii) number of pods/ plant, (iv) number of seeds /pod, (v) seed yield /plant and (vi)1000-seed weight. Data gathered after 2 years were pooled for analysis and significance was tested by paired 't' test.

Result and discussion

Development and evaluation of CMS lines

Literature regarding effect of Ogura cytoplasm on floral, agronomic and seed set related traits in snowball cauliflower is inadequate. Introgression of Ogura cytoplasm resulted in reduced female fertility and floral abnormalities in the CMS cauliflower lines. They had very small curved style, reduced nectaries, unopened and partially opened flowers and with almost rudimentary ovaries. Similar deformities after introgression of Ogura cytoplasm had been reported in broccoli [10] and cauliflower [9]. Large scale use of these lines will be possible only after proper modifications.

The developed CMS (A) lines were similar to their respective fertile maintainer (B) for floral traits like, petal colour, style shape, type of ovary and presence of functional nectaries (Table 1 & Fig. 2) after 7 generations of backcrossing. None of the CMS lines produced viable pollen. However, other floral traits like, flower size, length of style and stamens were reduced significantly after introgression of Ogura cytoplasm (Table 2; Figs. 2&3). It was obvious from the measurement that petal length and width had drastically reduced in all three A lines as compared with their respective B lines (Table 2). Narrowing of petal when Ogura cytoplasm was introgressed in the nuclear background of *Brassica juncea* was reported from various countries [14, 15]. Among CMS lines, highest petal length was in Ogu3A and least in Ogu2A while petal width was highest in Ogu1A and least in Ogu3A. Ratio between petal length: width in CMS lines was 2.59-3.10 as compared to 2.01-2.24 in the fertile maintainer lines. Long and short filament length and stamen length were reduced significantly in all the sterile (A) lines. Similarly, there was reduction in style length. The ratio of stamen: style in the Table 2 depicts stamen and stigma were almost at the same level in fertile B lines whereas, stamen was reduced to much lower level in sterile A lines after introgression of Ogura cytoplasm. Stamen: style ratio was highest in Ogu1A and least in Ogu3A. Therefore, it may be concluded that these traits are associated with Ogura cytoplasm and are not responsive to selection through backcrossing. The length of long and short filament as well as stamen and style length were also reduced significantly as compared to the maintainer lines irrespective of nuclear background. It was evident from the ratio between style and stamen length that the relative position of stamen was changed significantly due to introgression of Ogura cytoplasm. In all the CMS lines, reduction in length was more drastic in stamen as compared to style. The study also revealed that the reduction was more severe in case of functional long stamen than non-functional short stamen. Thus, it may be concluded that Ogura cytoplasm affected all the traits related with male sex expression without much alteration in female sex expression.

Table 1. Floral characteristics of the developed CMS lines (A) and their respective maintainers (B)

S.No.	Traits	Ogu1A	Kt-1B	Ogu2A	Kt-2B	Ogu3A	Kt-3B
1	Petal color	Yellow	Yellow	Yellow	Yellow	Cream	Cream
2	Shape of style	Straight to slightly curved	Straight to slightly curved	Straight	Straight	Straight	Straight
3	Type of ovary	Normal	Normal	Normal	Normal	Normal	Normal
4	Presence of functional nectaries	Present	Present	Present	Present	Present	Present
5	Presence of viable pollen grains	Absent	Present	Absent	Present	Absent	Present

Table 2. Floral characteristics of the developed CMS lines (A) and their respective maintainers (B)

Lines	Petal size (mm)		L: W	Filament length (mm)		Style length (mm)	Stamen: Style	Stamen length (mm)	
	Length	Width		Long	Short			Long	Short
Ogu1A	14.3**	5.5**	2.6**	3.7**	3.1**	7.5**	0.8**	5.7**	4.9**
Kt-1B	19.6	8.8	2.2	8.7	7.3	10.8	1.1	11.5	9.5
Ogu2A	13.1**	4.8**	2.8**	2.9**	2.4**	6.9**	0.7**	4.5**	4.1**
Kt-2B	15.2	7.6	2.1	6.8	5.5	10.5	0.9	9.50	8.2
Ogu3A	14.5**	4.7**	3.1**	3.5**	3.1**	9.7**	0.5**	5.3**	4.5**
Kt-3B	15.7	7.7	2.1	8.4	5.9	10.2	1.0	9.9	8.1

**P<0.01, *P<0.05

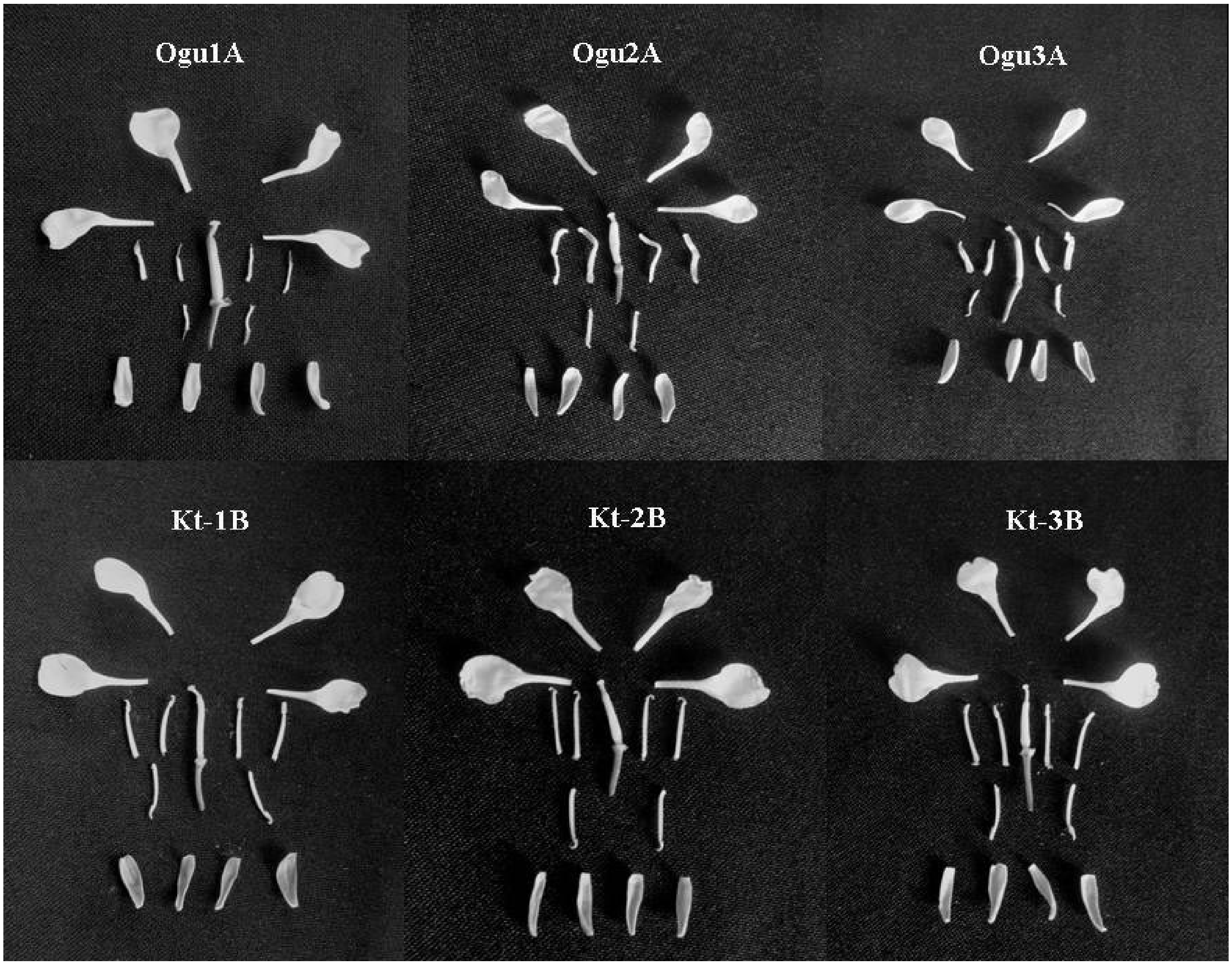

Fig 2. Flowers of three CMS lines of cauliflower *viz*., Ogu1A, Ogu2A and Ogu3A with their respective maintainers showing reduction in size of all floral organs after introgression of Ogura cytoplasm

Cytoplasmic male sterile lines are highly useful in the development of cabbage F_1 hybrids [16, 17]. It is possible to transfer superior Ogura CMS systems in *Brassica oleracea* through successive backcrossing and selection [17]. However, successful utilization of CMS lines in heterosis breeding is possible only when they possess normal female fertility with good seed setting capacity [4]. Seed setting related traits are important for producing F_1 hybrid seeds in commercial scale. Good setting was recorded in all the CMS lines however, difference between A and B lines were evident (Table 3). All three CMS lines had lower number of pods per plant as compared to respective fertile maintainers. This was mainly because of non-fertilization of flowers, due to non-availability of pollinating agents and/or pollen grains when flowers of these lines were receptive. Numbers of pods per plant was highest in Ogu1A while it was lowest in the line Ogu2A. Paired 't' test revealed no significant differences between A and B lines for numbers of primary and secondary branches bearing pods and their ratio. Numbers of seed per pod was highest in CMS line, Ogu1A (9.2) and lowest in Ogu2A (5.2). Nuclear-cytoplasmic interaction was also evident for seed yield per plant as no difference was found between Ogu3A and Kt-3B however, it was reduced in Ogu1A and Ogu2A as compared to their maintainers. Among the three CMS lines, Ogu1A had highest seed yield per plant (30.8g) and Ogu2A had lowest seed yield (19.7g/plant). Seed yield in Ogu1A and Ogu2A were significantly lower as compared to their fertile B lines. Reduced seed yield in the CMS lines were attributed to the lower number of pods per plant and seeds per pod. Kucera *et al*. [8] also reported lower seed yield and pods per plant in the CMS lines of cauliflower and was mainly because of collection of nectar from outer side of the flowers by the bees ("sideworking"), and thereby, less

Table 3. Comparison of A and B lines for various seed setting and yield related traits

S.No.	Traits	Ogu1A	Kt-1B	Ogu2A	Kt-2B	Ogu3A	Kt-3B
1	Number of pods per plant	1197.2**	1408.9	652.4**	783.7	1007.5**	1418.8
2	Number of primary branches per plant	13.7	14.0	11.9	12.1	9.6	10.2
3	Number of secondary branches per plant	55.4	56.1	68.3	67.4	26.1	26.7
4	Ratio of secondary to primary branches	4.0	3.9	5.7	5.6	2.7	2.6
5	Number of seeds per pod	9.2**	12.3	5.9**	7.5	8.4	9.3
6	Seed yield per plant (g)	30.8**	40.7	19.7**	27.9	26.8	29.7
7	Test weight (g)	4.3	4.2	2.8	2.7	4.7	4.7

**P<0.01, *P<0.05

Table 4. Comparison of A and B lines for various commercial traits

S.No.	Traits	Ogu1A	Kt-1B	Ogu2A	Kt-2B	Ogu3A	Kt-3B
1	Days to curd maturity	133.1	132.6	136.1	134.3	132.8	131.3
2	Net curd weight (kg)	0.8	0.7	0.7**	0.8	0.8	0.8
3	Net curd yield (tonnes/ha)	33.5	33.0	33.0**	36.5	35.5	37.2
4	Curd length (cm)	11.9	11.5	12.1	12.4	11.7*	10.5
5	Curd width (cm)	14.3*	13.6	14.0	13.4	12.9*	11.7
6	Curd depth (cm)	7.2	6.9	6.5	6.7	6.3*	6.6

**P<0.01, *P<0.05

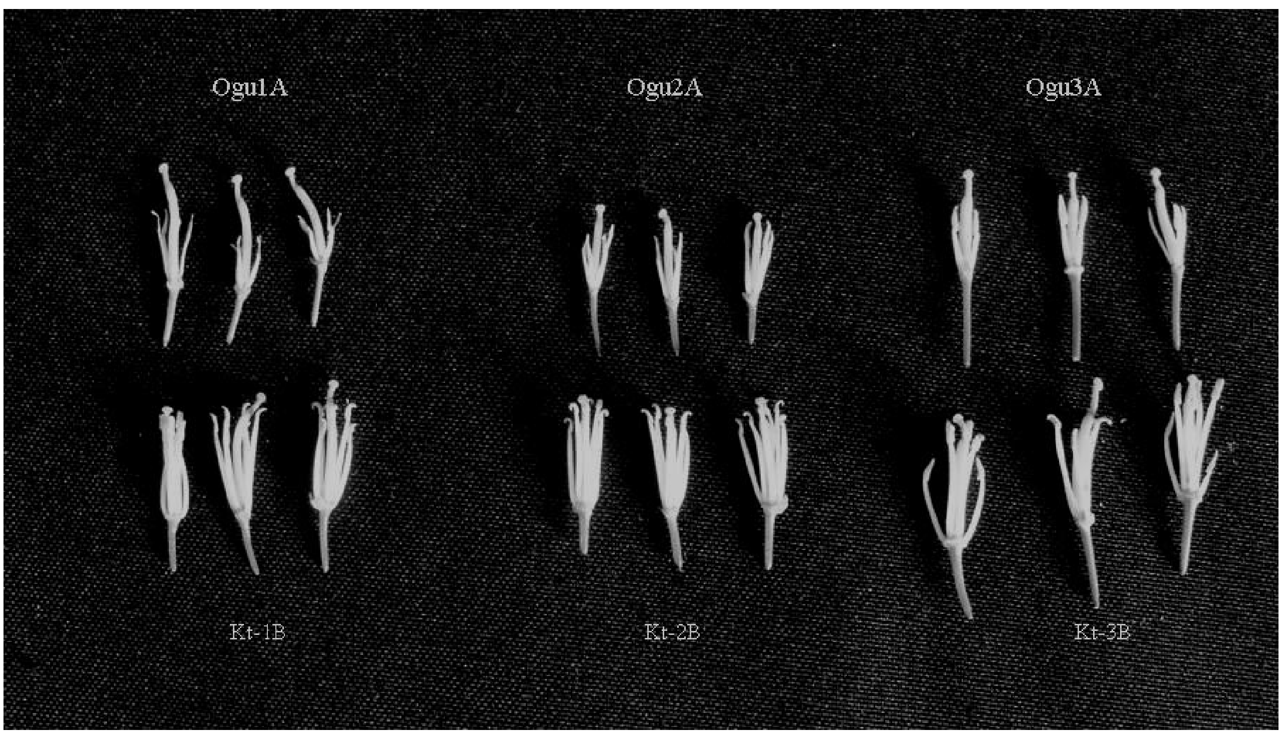

Fig 3. Flowers (without sepals and petals) of three CMS lines of cauliflower *viz*., Ogu1A, Ogu2A and Ogu3A with their respective fertile maintainer (B) lines showing reduction in size of filaments and styles after introgression of Ogura cytoplasm

chance to contact stigma and effective pollination. Test weight (1000-seed weight) of all three A lines were same with their B lines.

Characterization of different CMS lines and their maintainers for commercial traits are presented in Table 4. Good agronomic potential is essential for effective use of the CMS lines in hybrid breeding. Highest curd yield was recorded in Ogu3A (35.51t/ha) and least in Ogu2A (33.00t/ha). Earliness is highly desirable as, it saves time and other resources in obtaining the final produce. Ogura cytoplasm had no effect on days to maturity as none of the lines differed from their maintainers for this trait. Ogu3A was earliest to mature among all the CMS lines and Ogu2A took maximum days to mature. There was no significant difference for traits like days to curd maturity, net curd weight, net curd yield and curd length in Ogu1A line as compared to its maintainer (Kt-1B). However, in this line, curd width of was significantly reduced after introgression of Ogura cytoplasm. Ogura2A differed significantly from its maintainer line (Kt-2B) for net curd weight and net curd yield. However, Ogura2A and Kt-2B lines were at par for days to curd maturity, curd length and curd width. Curd length and curd width of Ogura3A was higher than its maintainer (B) line. Depth of curd was reduced in Ogu3A however, no effect of Ogura cytoplasm was observed in Ogu1A and Ogu2A. Nuclear-cytoplasmic interaction was an important factor in determining net curd yield, curd weight and curd size. Melo and Giordano, 1994 [13] also reported the influence of Ogura cytoplasm in determining head size in cabbage.

Though in all CMS lines flower size was reduced, however, it did not affect seed set drastically. The loss incurred in the form of slightly low seed set in CMS lines could be easily compensated by high price (~ almost 5 times) of F_1 hybrids as compared to the open pollinated cultivars. Based on cluster analysis it was found that these three CMS lines had high inter-cluster distance from many genotypes of snowball cauliflower and would be useful in heterosis breeding [18]. Thus, the developed CMS lines free from any floral deformities and normal female fertility would be highly useful in development of F_1 hybrid seeds of cauliflower after proper selection of parental combinations.

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