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Research Paper

Molecular-agronomic characterization and genetic study reveals usefulness of refined *Ogura* cytoplasm based CMS lines in hybrid breeding of cauliflower (Brassica oleracea var. botrytis L.)

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ABSTRACT

Cytoploasmic male sterility (CMS) is the most widely used system for hybrid seed production in vegetable brassicas. Information of morphological and molecular divergence in combination with combining ability is instrumental in selecting suitable parent in hybrid development. Twenty five CMS lines developed after more than 9 generations of backcrossing were analysed for different agronomic and floral traits. Besides, they were also evaluated for combining ability to reveal their breeding potential. Two CMS lines, Ogu402-6A and Ogu76-4A were distantly placed from rest of the CMS genotypes based on morphological characterization. Molecular analysis through SSR primers also revealed the genetic distance of the CMS lines Ogu402-6A from rest of the CMS pool. Three genotypes (Ogu402-6A, Ogu76-4A and Ogu119-2A) with early maturity were identified for their use in development of short duration hybrids. Similarly, 3 CMS lines Ogu118-2A, OguHL-3A and Ogu126-1A were identified with yield potential of more than 60 t/ha. Based on 13 polymorphic SSR markers 25 CMS lines were grouped into 4 major clusters. Molecular diversity in combination with agronomic characterization will be very useful in diverse parental lines. Introgression of Ogura cytoplasm into cauliflower nuclear background caused variety of flower deformities. However, these deformities were genotype specific. General combining ability (GCA) of the CMS lines revealed their potential for use in hybrid breeding. Few genotypes were identified based on combining ability for their use in development of short duration and high yielding F_1 hybrids.

1. Introduction

Vegetable brassicas (*Brassica oleracea* L.) are one of the important groups of *Brassicaceae* grown throughout world. They are becoming popular and form an integral part of human diet mainly because of their nutritional value. Vegetable *brassicas* are now considered one of the important groups of vegetables with anti-carcinogenic properties (Podsedek, 2007; Jahangir et al., 2009). This anti-carcinogenic property is attributed to the presence of several sulphur-related compounds (glucosinolates) and plant vitamins and pigments (Cartea et al., 2011). Cauliflower is the most important member of brassica vegetables and consumed as cooked, streamed and raw vegetable in different parts (Nieuwhof, 1963). Cultivation of F_1 hybrids is very popular in all members of *B. oleracea* including cauliflower mainly because of uniformity, better quality and tolerance to biotic and abiotic stresses (Kucera et al., 2006). It is well documented that *brassica* vegetables (*B. oleracea*) show very wide range of heterosis and very high heterosis has been reported in cabbage, cauliflower and broccoli (Tanaka and Niikura 2006; Dey et al., 2013a; Parkash et al., 2015). Breeding for development of F_1 hybrids is one of the major research focuses in this group of *Brassica* crops. Because of the flower structure, size and few seeds/pollination, it is not practical to produce hybrid seed through manual emasculation and pollination. It is most important to have an efficient, reliable and stable method of F_1 seed production without any possible self-fertilized seeds from each parent is vital in successful hybrid breeding (Yamagishi and Bhat, 2014). The self-incompatibility system as reviewed by Kitashiba and Nasrallah (2014) was developed mainly by Japanese breeders and has been effective in F_1 breeding of vegetable *Brassicaceae* in the last decade. However, self-incompatibility (SI) is not always stable. In the present scenario of climate changes and increasing temperature the SI alleles are not stable. Thus, SI lines are more prone to incompatibility breakdown and sibbing. Under this scenario,

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cytoplasmic male sterile (CMS) system is the most attractive and widely used mechanisms in hybrid seed production of *B. oleracea* vegetables (Yamagishi and Bhat, 2014).

Presently, *Ogura* cytoplasm from Japanese radish (Ogura, 1968) is the only sterile cytoplasm available in the nuclear background of *B. oleracea*. This sterile cytoplasm is the only source of CMS used in commercial hybrid seed production. CMS is very important trait for successful F_1 seed production because of its stability in all brassica crops. This trait is maternally inherited and controlled by a mitochondrial gene. Pollen production in CMS lines is highly impaired and the sterile lines generally possess shriveled and non viable pollen or no pollen at all. Nuclear fertility restorer (*Rf*) genes can suppress the expression of the mitochondrial CMS gene allowing the plant to produce functional viable pollen. A combination of a CMS-inducing mitochondrial genome and a nuclear genome devoid of *Rf* genes is required for expression of CMS system with no functional pollen grains (Yamagishi and Bhat, 2014). Sterile *Ogura* cytoplasm always results in male sterility as there are no restorer genes available *in B. oleracea*. Moreover, sterility is not dependent on growing condition of the plants. However, the female fertility of the genotypes with sterile cytoplasm depends in nuclear-cytoplasmic interaction and in several cases female fertility is not affected or least affected (Budar and Berthomé, 2007; Chase, 2007; Prakash et al., 2009; Yamagishi and Bhat, 2014).

The practical use of CMS has been explained in detail by several earlier reviews (Delourme and Budar, 1999; Budar and Berthomé, 2007; Chase, 2007; Gabay-Laughnan and Newton, 2012; Prakash et al., 2009) in different plant species. In our earlier studies we have found that *Ogura* cytoplasm drastically reduce flower size in *B. oleracea* however, female fertility can be restored after careful backcrossing for several generations (Dey et al., 2011, 2013a). Moreover, usefulness of CMS lines in hybrid breeding of cauliflower and cabbage has also been reported by our group (Dey et al., 2013b; Parkash et al., 2015). Presently, we have developed large number of CMS lines through protoplast fusion followed by recurrent backcrossing with the desirable nuclear background (Bhatia et al. 2015). These lines have varied floral and agronomic traits. However, morphological and molecular characterization of these lines is urgently needed for evaluating the usefulness of these lines. Moreover, it is also necessary to select the diverse lines in breeding programme. Using the CMS lines with high degree of similarity may reduce the chance of getting heterotic hybrids. Studying the combining ability in general and general combining ability (GCA) in particular will reveal the potential of the lines in hybrid breeding. Lines with high GCA for different traits are necessary for developing hybrids with specific desirable traits. This study was conducted to select the potential CMS lines for their ready use in hybrid development based on molecular-genetic characterization and combining ability analysis. For the first time, we have also studied the different kinds of deformities results from the introgression of *Ogura* cytoplasm. Recording the different kinds of floral deformities are fundamentals in better understanding of these resultant deformities. This documentation will be the starting point in understanding the molecular genetic basis of floral deformities as a result of alien cytoplasm introgression.

2. Materials and methods

The study was conducted at Baragram Farm, ICAR-Indian Agricultural Research Institute (IARI), Regional Station, Katrain, Kullu Valley, Himachal Pradesh, India. The Farm is located at 32.12N latitude and 77.13 E longitudes with an altitude of 1560 m above the mean sea level. The Farm received 950–1000 mm rainfall along with 1000–1100 mm snowfall annually. Crops were grown according to IARI, Regional Station guidelines followed for cauliflower cultivation to have better phenotypic and morphological expression (Sharma, 2003). The data on these traits were collected from 2 consecutive years from 10 randomly selected plants from each line. The average data was used for analysis.

2.1. Agronomic characterization of the CMS lines

Among the 70 CMS lines, 25 lines were selected based on their agronomic performance. These 25 lines had no difference with their male fertile maintainers for various agronomic traits (data not presented). These lines were at advance stage of backcrossing. These lines were characterized for different vegetative and commercial traits viz. i) days to 50% curd initiation ii) days to 50% curd maturity iii) plant height (cm) iv) number of leaves v) leaf length (cm) vi) leaf diameter (cm) vii) gross plant weight (kg) viii) marketable curd weight (kg) ix) net curd weight (kg) x) curd length (cm) xi) curd diameter (cm) xii) core length (cm) xiii) total yield (t/ha) xiv) harvest index (%). Days to 50% curd initiation and maturity were calculated based on the duration when 50% plants of single plot had visible curd and 50% plants of plot become ready for harvesting, respectively.

2.2. Characterization of CMS lines based on floral traits

The same set of 25 CMS lines characterized for important floral traits *viz*. i) petal length (cm) ii) petal width (cm) iii) long stamen length (cm) iv) short stamen length (cm) v) long filament length (cm) vi) short filament length (cm) and vii) style length (cm). Data were collected from 2 consecutive years from 10 randomly selected plants. Five flowers from each plant were taken for data recording and average data was used for analysis.

2.3. Suitability of CMS lines in hybrid breeding

All 25 CMS lines were crossed with 3 open pollinated inbred lines in line × tester fashion. In this way 75 hybrids were made in the year 2014–15. The CMS lines and the testers used for hybridization were maintained for the next season. The CMS lines were pollinated with the mixed pollen of the respective maintainers for maintenance. Three tester lines were sibbed for their maintenance. The hybrids along with the testers were evaluated in the next year (2015–16) for different vegetative and agronomic traits to test the combining ability of the CMS lines.

2.4. Statistical analysis for different morphological and floral traits

All the CMS lines were clustered into different groups using DARwin 6 software. These lines were classified through principal component analysis and neighbor joining hierarchical cluster using UPGMA methods. The CMS lines were grouped both for agronomic and floral traits. Statistical analysis was done based on average data of 2 seasons. For evaluation of agronomic and floral traits 10 plants among 36 plants were selected randomly for data recording. Flower data were recorded from 5 flowers from each of the 10 plants. Then average data per plants were calculated. Finally, average pooled data of two years were taken for analysis.

2.5. Molecular characterization of the CMS lines

All the CMS lines were grown in a mixture of cocopeat, vermiculite and perlite (3:1:1) under glass house condition. Genomic DNA was extracted from the young expanding leaves using a modified sodium dodecyl sulphate method (Doyle and Doyle, 1990). In our experiments we had isolated DNA using a small quantity (500 mg) of young leaf samples using 2 ml centrifuge tube. The PCR reaction mixture contained 20 µl reaction with 1 µl of each forward and reverse primers, 1 µl genomic DNA (50 ng) and 10 µl of 2× PCR master mix (GoTaq DNA polymerase; Promega, USA). The PCR master mix supplied in 2× Green GoTaq master mix. The PCR cycling programme consisted of an initial denaturation at 94 °C for 5 min followed by 30 cycles of 45 s at 94 °C, 45 s at annealing temperature of the primes and 1 min at 72 °C with a final extension cycle of 72 °C for 5 min. The PCR products were