Improvement of Cauliflower Male Sterile Lines with *Brassica Nigra* Cytoplasm, Phenotypic Expression and Possibility of Practical Application

P. Kamiński, B. Dyki & A. A. Stępowska

Department of Genetics, Breeding and Biotechnology of Vegetable Crops

Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Tel: 48-46-833-4193 E-mail: pkaminsk@iwarz.pl

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Abstract

Improvement of the male sterile cauliflower genotypes with *Brassica nigra* cytoplasm *(CMS)* according to ability for generative propagation and quality of agronomical traits was performed in former Research Institute of Vegetable Crops, presently Institute of Horticulture, Skierniewice, Poland in the years of 2002-2010. Cauliflower *CMS* lines and their fertile maintainers were back-crossed with good quality cauliflower breeding lines followed by the stabilization of sterility/or *rf, rf* nuclear genes in consecutive generations. The presence of untypically developed plants with chimeral generative stacks or partially fertile flowers among segregating test-cross progeny of improved maintainers may suggest the presence of some non-allelic genetic factors modifying the fertility/sterility character for a part of the plants. As a result *CMS* genotypes with *B. nigra* cytoplasm and their maintainers with improved quality, significantly higher seeding index, and lower level of flower deformations were obtained.

Keywords: Cauliflower, *CMS*, *Brassica nigra* cytoplasm, Breeding, Quality, Seeds

1. Introduction

Male sterility described as inability of the plant to produce fertile pollen is widespread in angiosperms and provides one of the most efficient means of directed pollination for the large-scale production of hybrid seeds in crops (Prakash et al., 2009). Male sterility manifests itself usually in floral development as an incompatibility of nuclear-mitochondrial interaction in alloplasmic lines derived from wide hybridization, which carry nuclear and mitochondrial genomes from different species. *CMS* may have a spontaneous character or may arise following intraspecific, interspecific or intergeneric crosses (Kaul, 1988). Maternally inherited cytoplasmic male sterility encoded in mitochondrial genes can be utilized more effectively by breeders than genic male sterility. Another set of nuclear genes – restorers of fertility (*Rf*) – overcomes the effect of the *CMS* genes, restoring the hermaphrodite condition. *CMS* can be also considered as a genetic system with two genetic determinants with different modes of inheritance. It involves variations (i) at the cytoplasmic level, with at least a sterility-inducing cytoplasm (s) and a cytoplasm with no sterility effect (N) and (i, i) at the nuclear level, with a dominant restorer allele (*Rf*) that enables plants with the S cytoplasm to produce pollen and a recessive allele (*rf*), also called a maintainer of sterility, maintaining the male sterile phenotype induced by the S cytoplasm (Budar et al., 2006). Maternally inherited male sterility is an ideal solution as crosses between a male sterile plant and a hermaphrodite plant homozygous for maintainer alleles give rise to 100% female plants. Cytoplasmic male sterility arising in interspecific crosses is often linked to the interaction of the cytoplasm of one species with the nuclear genome of the other parent (Bannerot et al., 1977). Cytoplasmic male sterility among *Brassica oleracea* was broadly investigated in order to implement it as a low-cost, efficient and reliable system for the production of F_1 hybrids that could be easily utilized by breeders. Male sterile plants were obtained from intervarietal crosses with other *Brassic*a species as *B. napus* (Shiga and Baba, 1973), also spontaneous male sterile plants were isolated by Rawat and Anand (1979) among *Brassica juncea* with the cytoplasm of related wild species *B. tournefortii* (Pradhan et al., 1991). However, the most extensively investigated *CMS* system among crop plants started with the discovery of male sterility in Japanese radish *(Raphanus sativus)* (Ogura, 1968). After introgression through conventional breeding to *Brassica oleracea (*Bannerot et al*.,* 1974) several developmental and floral abnormalities were commonly observed among *CMS* plants that affected their agronomic and breeding

value. These include varying degrees of leaf chlorosis or poor production of seeds. In *B. oleracea* abnormalities of floral parts includes petaloid and carpeloid stamens petaloid anthers, crooked style and reduced nectaries (McCollum 1981). Improvement of female fertility and cold tolerance via protoplast fusion (Pelletier et al. 1983; Yarrow et al. 1986; Jourdan et al., 1989) made the *CMS*-Ogura system the most popular one used for the modern breeding of broccoli, cauliflower and other cabbage F_1 hybrids. Generally, the cytoplasm donor species from which the sterility originates also provides the nuclear restorers (Heyn, 1976; Pellan-Delourme and Renard, 1988; Delourme et al., 1991, 1995; Prakash et al., 1998). The Ogura sterility in all *B. oleracea* species does not have any fertility-restorer genes, while all fertile forms act as maintainers (Dickson G.R. 2007, Prakash et al. 2009).

The first cytosterile B. *oleracea* plants with *B. nigra* cytoplasm developed by Pearson (1972) through sexual hybridization were characterized by the lack of nectaries and abnormal flower development. Low seed production reported by the breeders among cabbage, broccoli and cauliflowers with *B. nigra* cytoplasm was one of the reasons why this system was not used for practical breeding (Hoser-Krauze, 1987, 1989, 1992). Also frequent presence of dominant restorer genes among *B. oleracea* populations was mentioned as a disadvantage in hybrid seed production using *B. nigra* cytoplasm (Kalia, 2009). Male sterile cauliflower lines propagated by the use of their fertile maintainers had poor commercial value, with small, early curds, not suitable for the breeding (Kaminski 2005). Flowers of cauliflower lines with *B. nigra* cytoplasm were characterized by the lack of anthers. Instead of stamina, in the internal whorl there were longitudinal thin and spoon-like structures similar to petals. Reduction of the size and number of petals, undeveloped nectaries and various types of style deformations occurred in all male-sterile lines, which had been investigated. Such changes made the flowers less attractive for pollinator insects. However, the most important reason of poor seed set was disorders in the development of pistils leading to deformations of siliques (Kaminski and Dyki, 2007). Dixon (2006) supposed that problems with failure of seed production observed among *CMS* plants may be the result of the lack of rigorous selection in the early generations following fusion or sexual hybridization. However, Dickson (1987) showed that it was possible to find male sterile broccoli plants with good seed set in a large population pollinated in the open field. Several male sterile plants of cauliflower with *B. nigra* cytoplasm described by Kaminski and Dyki (2007) with higher ability for generative propagation had a similar morphological structure in male fertile lines and did not show deformation of styles, petals and sepals.

The aim of this study was an evaluation of the possibility to improve the male sterile cauliflower genotypes with *Brassica nigra* cytoplasm and their fertile maintainers by the use of classical breeding procedures according to ability for generative propagation and quality of agronomical traits.

2. Materials and Methods

Male sterile lines of broccoli and broccoli-cauliflower with *B. nigra* cytoplasm and their maintainers were obtained from Dr. Dickson and have been investigated in the Research Institute of Vegetable Crops, Skierniewice, Poland. Original *CMS* lines with *B. nigra* cytoplasm taken for the experiment had severe flower and silique deformations such as doubled flowers, multiple and often cracked styles in one flower, additional branches with buds and flowers raised from the inner whorl of the flowers and cracked along the ovary or swirled siliques and were characterized by poor quality, low mass of curds and very weak ability for seed set. Two goals of the study were undertaken: a) the improvement of quality of cauliflower *CMS* B. *nigra* and maintainer lines, according to mass and color of curds, covering by leaves, shape and lack of undesired bracting by internal leaves, b) improvement of ability for generative propagation and stability of male sterile lines in consecutive generations.

2.1 Improvement of CMS Lines with B. nigra Cytoplasm and Their Maintainers

A male sterile cauliflower line with *B. nigra* cytoplasm (A) was crossed with two good quality fertile cauliflower inbreeds (PN73, DT75) followed the selection of male sterile genotypes. From 2002 until 2008 three consecutive breeding cycles and a selection for a stable sterility of cauliflower genotypes with *B. nigra* cytoplasm were made. In each generation the screening for cauliflowers with normal flower structure, larger nectaries and with effective seed formation was performed. For every subsequent generation only *CMS* plants that fulfilled the requirements for sufficient seed formation, without major morphological abnormalities of generative organs and with good quality of curds were selected.

Identification of homozygous, recessive rf, rf genes among maintainers followed by the selection for the quality characters was also performed. In 2002 crosses of maintainer (B) with two good quality cauliflower lines (PN73, DT75) was performed and self pollination of heterozygous (*Rf, rf*) F_1 progeny was made in 2003 (Table 1). In 2004 test-crosses in a segregating F_2 generation were made in order to identify homozygous (*rf, rf*) genotypes. In 2005 five homozygous maintainers were back-crossed with fertile cauliflower lines (PN73, DT75) and 15 genotypes of BC_1 generation were obtained. Plants of BC_1 generation were self pollinated in 2006 in order to obtain a segregating population according to *Rf, rf* genes. In 2007, 67 plants of segregating F_2 (BC_1) generation

were crossed with the fast growing male sterile cauliflowers with *B. nigra* cytoplasm. Each tested genotype was also self-pollinated. In 2008 for each of the F_2 (BC_1) population, plants of tester progeny were sown in the greenhouse and observed according to their fertility or petaloid-type sterility. A number of 10 – 12 tester plants of each genotype was assumed to be sufficient for the selection of the homozygous (*rf, rf*) maintainers as a simple one-gene Mendelian segregation was expected. Generally among 67 tester progenies, 682 plants were evaluated according to the flower morphology for identification of male sterile/fertile genotypes.

2.2 Evaluation of Improved Male Sterile and Maintainer Lines according to Flowers Morphology and Ability for Generative Propagation

In 2008 six improved sterile lines with *B. nigra* cytoplasm (A2P5, A2D1, A1P4, A2D3, A2D4, A2D5) were crossed in the greenhouse with eight maintainers of F2 (BC_1) generation: (BD22, BD27, BD29, BP36, BP417, BP474, BP52, BP54). Selected maintainers used for crossing had both recessive genes (*rf,rf*) as all their test-crosses were sterile. As a result of cross-pollination, 26 male-sterile populations with a sufficient number of seeds were obtained. Consecutive generation of 26 *CMS B. nigra* and maintainer lines were sown at the end of January 2009 in the greenhouse. As a control, two fertile inbred lines of cauliflower (PN73 and DT75) and two male sterile lines with Ogu-INRA cytoplasm (CPN73, CDT75) were also used. Fertilization and plant protection against pests and diseases followed current requirements and recommendations for cauliflower. At the beginning of April, each pair of *CMS* and appropriate maintainer lines were transplanted in the field into 9 m^2 growth-cage covered with transparent material to avoid undesired pollination by insects. Cauliflowers were planted in two rows, 100 cm between rows, and 50 cm between plants in one row; 5 male sterile plants and 5 maintainers for each cage. Flowers and seed stalks were screened during their blooming according to their morphology, especially the ability for the creation of male sterile or fertile flowers. Morphological studies on flowers and siliques were made with use of stereoscopic microscope OLYMPUS SZX16 with digital system for picturing. By the use of scanning electron microscope Jeol JSEM-S1 the surface of stigma and petal was observed. At the second decade of May, when plants started to bloom, about 90 hatched insects of Red Mason Bee (*Osmia rufa* L.) were placed into each of cage to ensure appropriate cross-pollination of male sterile components. From the second decade of August until the beginning of September matured siliques were gradually harvested from each cage separately for male sterile and maintainer lines. For ten selected male sterile genotypes an evaluation according to the mass of seeds, percentage of siliques with seeds/plant and percentage of siliques with deformations/plant was made. Two or three male sterile plants and one male fertile maintainer were evaluated for each of selected genotype. For each of tested plant, three green-premature branches were independently observed. After siliques were dried, seeds were extracted, cleaned and weighed separately. Results were subjected to an analysis of variance (ANOVA). The significance of differences among means was evaluated by Newman-Keul's test at $\alpha = 0.05$. Seed effectiveness of male sterile plants (%) were calculated in comparison to their male fertile maintainers and a number of untypical, cracked and twisted siliques was compared with the total number of siliques/plant.

2.3 Field Evaluation of Male Sterile and Maintainer Lines according to Commercial and Agronomical Traits

In 2010 eight male sterile cauliflower lines and their fertile maintainers that developed a sufficient amount of seeds in previous season, were evaluated at the field at the Research Institute of Vegetable Crops, Skierniewice according to commercial and agronomical characters. Unimproved male sterile cauliflower line (A) with *B. nigra* cytoplasm, fertile maintainer (B) and two male sterile lines with Ogu-INRA cytoplasm (CPN73, CDT75) with fertile complementary lines (PN73, DT75) were used as a control. Plants were raised from seeds in the greenhouse at the beginning of May. One-month-old seedlings were planted at the field (spacing 50 x 50 cm) in a completely randomized block design with three replications. Each plot consisted of ten plants in one row. The soil type was a pseudopodsolic over loamy sand (1.5% organic matter, pH 6.5). Fertilization, pest and disease control followed the current recommendations for cauliflower. Plants were harvested gradually from the beginning of August to the end of September when curds reached maturity. Mass of curds was measured and results were subjected to an analysis of variance (ANOVA). The significance of differences among means was evaluated by Newman-Keul's test at $\alpha = 0.05$. Other morphological characteristics of cauliflower population such as: length of vegetation period from planting to harvest, intraline uniformity, covering of curd by leaves, color and shape of curd and leaf bracting were classified separately for each plot according to a multigrade scale.

3. Results

3.1 Evaluation of Test-crosses of F_2 (BC_1) Maintainers according to Flowers Morphology, Identification of Homozygous (rf, rf) Genotypes

From the total of 682 tested plants, 384 genotypes had fertile flowers with normally developed anthers with pollen and 238 genotypes were sterile with dysfunctions of masculine generative organs characteristic for

petaloid-type sterility of *B. nigra CMS*. Besides of the typical fertile or sterile genotypes, 37 tested plants had untypical chimeral shoots where some branches were developed with petaloid-type sterile flowers while the other were fertile (Figure 1H). Twenty three genotypes were characterized by partially recovered fertility among sterile plants with some number of petals transformed into anthers.

Analysis of testcrosses for $F_2(BC_1)$ maintainers showed that 15 progenies were fertile, 24 progenies segregated on both fertile and sterile plants and eight progenies (BD22, BD27, BD29, BP36, BP417, BP474, BP52, BP54) with only sterile plants were found as homozygous with desired recessive genes (*rf, rf*). Among 13 segregating progenies, besides of typical fertile and sterile offspring, chimeral plants (4 progenies: BP 415, BP441, BP475, BP441), plants with decreased fertility (5 progenies: BD25, BD28, BP24, BP493, BP510), chimeral plants and with decreased fertility (4 progenies BP425, BP426, BP59, BP84) were also found. Three progenies with fertile plants had also chimeral offspring (BP425, BP426, BP510), one progeny (BD45) had partially sterile plant and three generations (BD31, BP11, BP19) had both chimeral and partially sterile plants.

3.2 Flower Morphology and Ability for Generative Propagation of Improved Male Sterile Lines and Their Maintainers

All observed plants with *B. nigra* cytoplasm obtained from 26 populations and being propagated in the field in isolated growth-cages in 2009 were characterized by the lack of anthers with pollen. Instead of stamina, in the internal whorl there were longitudinal thin and spoon structures similar to petals typical for petaloid-type sterility of cauliflowers. Morphological differences between flowers of male sterile plants and male fertile maintainers appeared in almost all their parts, but the most important for setting seeds were the changes in pistils of *CMS* flowers. Generally cytosterile lines were characterized by diversified morphological abnormalities of flowers with over expression of female features showed as doubled-pistil (Figure 1A), additional, opened carpels with ovules (Figure 1B) and cells similar to stigma papillae on the surface of upper part of opened carpels (Figure 1C). Also cracked pistils observed among male sterile lines leads to drying and deformations of ovules and as result to production of siliques without seeds (Figure 1D, E). Morphological differentiation of flower and seed pods was observed both between tested lines and among single plants of the same genotype. *CMS* progeny with *B. nigra* cytoplasm selected from plants with high seed yield had usually smaller in size, doubled number of buds and flowers in comparison to fertile lines. The level of variation according to ability for the generative propagation by seeds among male sterile lines with *B. nigra* cytoplasm was shown in Table 2. Male sterile cauliflowers with *B. nigra* cytoplasm had smaller average yield of seeds/plant (4.80 g) in comparison to fertile maintainers (37.76 g) and to male sterile lines with *Ogu-INRA* cytoplasm (19.55 g). The highest seed yield (20.3 g/plant) was noticed for the AP52/3 male sterile line (seed effectiveness in comparison to fertile maintainer: 78.7%), three other lines (AP36/2, AP417, AD22/7) set an average 10 g of seeds/plant, while three other lines AD22/1, AD29/5 and AD27/3 set the lowest seed yield (0.8, 0.7 and 0.6 g respectively). Two lines with Ogu-INRA cytoplasm were also diversified according to the ability for generative propagation and set form 31.8 g (CDT75) to 7.3 g (CPN73) of seeds/plant. All 26 fertile maintainers used for the propagation of male sterile genotypes with *B. nigra* cytoplasm had good yield of seeds that ranged from 50.4 g (BD27/1) to 17.1 g (BD22/1).

For ten selected male sterile lines with *B. nigra* cytoplasm the mass of seeds/plant, percentage of siliques filled with seeds and percentage of deformed and untypical siliques was shown separately for single plants at Table 3. The highest differences in seed setting among plants were noticed for AP417 *CMS* line: plant no3 produced more than 44 g while plant no1 had only 3.11 g of seeds. Generally *CMS* plants that generated more seeds had also higher percentage of siliques with seeds and lower number of deformations than genotypes with low number of seeds/plant (Figure 1F, G). Two tested *CMS* plants from the line AP52/3 had a high ability for seed set (32 and 20.3 g/plant respectively) and similar number of deformed siliques (18 and 21%), but they differed significantly according to the percentage of siliques with seeds (56.8% and 33.8%). The lowest ability for propagation by seeds was noticed for two plants from line AP52/4 (less than 0.01g), two plants from AD22 line (0.2-0.4 g) and one plant from AD27 line (0.4 g). The number of deformed siliques for those genotypes was significantly higher than for genotypes that set a higher mass of seeds/plant.

3.3 Agronomical and Commercial Traits of Male Sterile and Maintainer Lines

Cauliflower *CMS* lines with *B. nigra* cytoplasm showed a high variability according to most of agronomical traits observed in the field in 2010. The shortest vegetation from planting to harvest maturity (45 days) was noticed for original *CMS* and maintainer lines (A, B) used as donors for the experiment. Original *CMS* and maintainer lines (A, B) were characterized by the smallest mass of curds (0.09 and 0.11 kg), respectively, without covering by internal leaves, flattened shape, yellow color and bracting by leaves (Table 4). Morphological and commercial characters of unimproved forms with *B. nigra* cytoplasm and their maintainers

were more typical for wild-type *Brassicae* than for cauliflower lines used for the breeding. The longest vegetation period was noticed for CPN73 Ogu-INRA line and the fertile complementary genotype PN73 (100 days after planting). For the lines with *B. nigra* cytoplasm and their maintainers, the length of vegetation showed a wide range of 55 (AD22) to 90 days (AP36). Fifteen from twenty two cauliflower lines were characterized by good internal uniformity of tested traits, six genotypes (AD274, BD274, BD291, BP523, A *nig*, B *nig*) were not uniform according to one of investigated characters and one male sterile line (AD291) was not uniform according to more than one trait. Mass of curds for most of cauliflowers with *B. nigra* cytoplasm and their maintainers ranged from 0.23 kg (BD274) to 0.85 kg (AP36) was comparable with mass of curd for two *CMS* Ogu-INRA lines CDT75 and PN 73 (0.53 kg, 0.26 kg) respectively. Mass of curd for most of male sterile genotypes did not differed significantly from the mass of their fertile maintainers. Only the male sterile line AP36 had a higher mass of curd (0.85 kg) than fertile maintainer BP 36 (0.60 kg), while AP417 (0.39) kg had lower mass of curd than BP417 (0.52 kg). Male sterile and maintainer lines of the same pair were similar to each other according to most commercial traits as covering by leaves, color of curd, tendency for leaf bracting and shape of curd. Three male sterile lines with *B. nigra* cytoplasm (AD22, AD274, AP521) had a higher tendency to leaf bracting than their maintainers and AD291 *CMS* line showed a lower level of interline uniformity than BD291. Generally, *B. nigra* forms and their maintainers had a weaker tendency to covering of curd by leaves than lines with *Ogu-INRA* cytoplasm, with the exception of AP36/BP36 and AP417/BP417 genotypes. However, even uncovered or partially covered *CMS* genotypes such as AD22, AD271, AP521, AP36, AP522 and their maintainers had desired white or creamy – white color of curd. The most desired round shape of curd was noticed for AP417, BP417 lines and both of *CMS Ogu-INRA* genotypes (CDT75, CPN73) with their complementary fertile components (DT75, PN73) while the other genotypes were characterized by a flattened or slightly flattened shape of curds.

4. Discussion

Rectifying of *B. oleracea* genotypes with *B. nigra* cytoplasm from severe morphological malfunctions is very important for their use as an alternative source of male sterility for commercial purposes. Original *CMS* lines with *B. nigra* cytoplasm, presented in this paper, had lower ability for sexual propagation in comparison to fertile cauliflower lines and were characterized by smaller or more significant abnormalities of flowers and morphological structures of seed stalks what is in accordance with a previous report (Kamiński and Dyki, 2007). Generally, *CMS* genotypes used for the breeding should not be associated with deleterious drawbacks for vegetative growth or seed production (Budar et al., 2006). The main reason why *B. oleracea CMS* with *Brassica nigra* cytoplasm were commonly described as unsuitable for the breeding (Hoser-Krauze, 1989,1992; Pearson, 1972; Dickson, 1975, Kamiński and Dyki, 2007; Kalia, 2009) were their abnormalities in flower morphology and lack of nectaries that makes *CMS* plants not attractive for the pollinators.

Selection of *CMS* cauliflowers with *B. nigra* cytoplasm performed in the former Research Institute of Vegetable Crops, Skierniewice, resulted in obtaining genotypes with reasonably higher ability for generative propagation and better quality of commercial characters. Improved *CMS* genotypes that developed majority of generative organs without severe deformations made them more attractive as valuable breeding material. The surplus of flowers among male sterile lines may compensate the smaller number of pollinated ones and in effect their seed set was comparable with fertile maintainers. The other reason of better seed production among selected sterile cauliflowers with *B. nigra* cytoplasm probably resulted from their better flower structure without deformations and well developed functional nectaries. This thesis is supported by author's observations of red mason bee used as pollinator in the field cages. The visitation of bees at *CMS* cauliflowers that set high seed set was comparable to fertile maintainer lines. Improvement of seed productivity and reasonable good stability of this trait in consecutive generations are encouraging for further testing. However, the *B. nigra* system as an alternative male sterility method of propagation among *Brassica oleracea* genotypes seem to need still more studies and selection in order to attain full quality of flowers and siliques. Analysis of morphological and commercial traits showed that *Brassica nigra* cytoplasm had no negative influence on the quality of cauliflowers and therefore constant improvement through traditional breeding was possible. Lower mass of curds in comparison to fertile lines and partial lack of uniformity among several of the tested genotypes may resulted from the insufficient number of back-crosses between unimproved *CMS B. nigra* genotypes and their maintainers with fertile good quality cauliflower lines.

The main problem facing breeders trying to use interspecific *CMS* is the limited number of good maintainer genotypes (Delourme and Budar, 1999, Laughnan and Gabay-Laughman, 1983). Commercial utilization of *CMS* cauliflowers with *B. nigra* cytoplasm could be successful only if an effective method of improvement maintainer lines will be applied. The only source of maintainers for the propagation of sterile forms with *B. nigra* cytoplasm were obtained from Pearson's genotypes (1972) that were constantly improved by breeders (Dickson, 1975;

Hoser-Krauze, 1987; Kaminski and Dyki, 2007). The frequent presence of restorer genes (*Rf*) among *B. oleracea* populations (Kalia, 2009) and lack of known molecular or morphological markers useful for the early identification of (r*f, rf*) genotypes among fertile plants are the reasons why improvement of maintainers for *B. oleracea* genotypes with *B. nigra* cytoplasm is very laborious. As no other maintainer source among commercially used *B. oleracea* genotypes are being found, the test-cross method of segregating BC plants with sterile line with *B. nigra* cytoplasm, presented in this paper, was the only available method of improvement of Pearson's maintainers that allowed the identification of desired (*rf*) genotypes. The major disadvantages of the testcross method are additional space, labor and time for the checking of their results. Maintainers with desired morphological characters crossed with sterile *CMS* plants should improve sterile lines after several consecutive generations. According to the author's experience, results of testcrosses should be evaluated very carefully at several stages of floral development for each tested plant during the blooming season, to spot phenotypes with chimeral or partially sterile generative shots different than the expected type of flowers. The results obtained from the testcross generations among improved maintainers may suggest that one-gene segregation as a simple model of inheritance could not fully elucidate the presence of different fertile/sterile phenotypes. Chimeral and partial sterile phenotypes of cauliflower with *B. nigra* cytoplasm may result from segregation of plasmotypes within a single tested plant (Szklarczyk et al., 2008). Also the presence of other non-allelic genetic factors transferred from good quality cauliflower lines used for the back-crosses could modify the phenotype of test-cross progeny. Nevertheless, evaluation of test-crosses, for which all tested progeny were sterile, allowed the selection of improved maintainers with recessive (r*f, rf*) genes.

5. Conclusions

Obtained results showed an extended diversity among male sterile genotypes with *B. nigra* cytoplasm according to seed set. *CMS* cauliflowers with *B. nigra* cytoplasm and their maintainers at the BC_1 stage were characterized by incomplete internal uniformity of morphological characters. Most *CMS* genotypes had lower ability for generative propagation in comparison to Ogu-INRA *CMS* and fertile maintainers.

Improvement of morphological traits and ability for generative propagation of *CMS B. nigra* cauliflowers and their maintainers by classical breeding methods, resulted in the identification of genotypes with good seed set and quality comparable with fertile breeding lines.

Flower structure of improved cauliflowers with *B. nigra CMS* was without major deformations affecting the ability for seed set. Most of petaloid-type flowers of selected lines were attractive for the pollinator insects, probably due to the present and functional nectaries.

Analysis of test-crosses for improved maintainers suggests the presence of another, non-allelic genetic factor that could modify the fertility/sterility effect of segregating testing progeny.

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Table 1. Breeding scheme of cauliflower maintainer lines for the quality improvement and maintenance of *rf* genes

Year of evaluation	Generation	Maintainer status	Action	Crossed genotype
2002	P_1 x P_2	Maintainer line–B nig (*rf/rf*)	Cross pollination	Fertile cauliflower lines C (PN73, DT70)
2003	F_1/F_2	B nig x C (*Rf/rf*)	Self pollination	
2004	F_2 x T	B nig x C segregating population	Cross pollination	Tester line (T) with *B. nigra* cytoplasm and *rf/rf* genotype
2005	BC_1	B nig x C segregating population	Identification of maintainers (*rf/rf*); Cross pollination	Fertile cauliflower lines C (PN73, DT70)
2006	F_2 / BC_1	(B nig x C) x C	Self pollination	
2007	$F_2(BC_1)$xT	(BxC)xC segregating population	Cross pollination	Tester line (T) with *B. nigra* cytoplasm and *rf/rf* genotype
2008	F_2 (BC_1)	(BxC)xC segregating population	Identification of maintainers (*rf/rf*);	

Table 2. Seed set of cauliflower male sterile lines with *B. nigra* cytoplasm in comparison with their fertile maintainers and Ogu-INRA *CMS*. Skierniewice, 2009

Cage No	Line	Type of sterility	Aver. mass of seeds/plant (g)		Seed effectiveness of male sterile lines in comparison to fertile maintainers (%)
			Male sterile	Fertile maintainer	
1	AP52/3	*B. nigra*	20,30	25,80	78,68
2	AP36/2	*B. nigra*	10,59	38,60	27,44
3	AP417	*B. nigra*	9,70	27,30	35,50
6	AD 22/7	*B. nigra*	9,40	54,20	17,38
7	AD 22/4	*B. nigra*	6,40	50,00	12,78
8	AD 27/2	*B. nigra*	6,40	48,80	13,60
9	AP36/1	*B. nigra*	5,20	38,60	13,47
10	AP52/2	*B. nigra*	5,20	29,80	17,45
11	AD 22/6	*B. nigra*	4,40	45,70	9,68
12	AD29/1	*B. nigra*	4,04	44,00	9,18
13	AD 22/5	*B. nigra*	4,00	17,20	23,22
14	AD 29/4	*B. nigra*	4,00	49,00	8,14
15	AD 27/4	*B. nigra*	3,80	42,70	8,85
16	AD 29/3	*B. nigra*	2,50	58,10	4,24
17	AP52/1	*B. nigra*	2,49	21,10	11,81
18	AD29/2	*B. nigra*	2,44	42,66	5,73
19	AD27/1	*B. nigra*	2,22	50,40	4,40
20	AP474	*B. nigra*	1,99	45,28	4,39
21	AD22/2	*B. nigra*	1,70	45,60	3,72
22	AD22/3	*B. nigra*	1,54	31,86	4,83
23	AD22/1	*B. nigra*	0,81	17,10	4,74
24	AD 29/5	*B. nigra*	0,70	26,20	2,67
25	AD 27/3	*B. nigra*	0,60	18,50	3,24
26	AP52/4	*B. nigra*	0,10	18,30	0,55
Aver. value for *B. nigra CMS*			4,80	37,76	14,14
27	CDT75	Ogu-INRA	31,80	34,00	94,00
28	CPN73	Ogu-INRA	7,30	41,00	17,80
Aver. value for Ogu-INRA *CMS*			19,55	37,50	55,90

Table 3. Characteristics of selected male sterile plants with *B. nigra* cytoplasm according to ability for generative propagation and siliques morphology in 2009

Male sterile line	CMS plant No	Mass of seeds/plant (g)	Siliques with seeds/plant (%)		Deformed siliques/plant (%)	
AP417	1	3,11	2,59	p-r	51,20	h-j
	2	25,12	88,70	a	18,80	a-c
	3	44,43	67,53	c	30,52	c-e
AP36	1	3,65	11,56	n-p	21,20	a-c
	2	10,59	26,67	j-l	14,30	a-b
	3	6,67	15,68	m-o	21,83	a-c
AP52/3	1	32,00	56,80	d-e	18,18	a-c
	2	20,30	33,85	i-j	20,96	a-c
AP54	1	5,20	11,62	n-p	42,99	f-j
	2	4,92	32,65	i-k	47,50	f-j
AD27	1	0,40	8,67	m-r	57,10	j
	2	8,00	41,60	f-h	54,03	h-j
AD22	1	0,20	17,86	m-n	77,53	k
	2	2,20	35,91	h-j	51,20	h-j
	3	0,40	22,32	l-m	71,23	k
AP52/1	1	1,75	27,65	j-l	44,17	f-j
	2	1,97	27,98	j-l	47,75	f-j
AP474	1	2,60	22,80	k-m	43,00	f-i
	2	7,34	87,30	a	15,67	a-b
AD29	1	3,10	48,50	f-g	30,52	c-e
	2	4,78	34,00	i-j	44,30	f-j
AP52/4	1	0,01	0,00	r	47,30	f-j
	2	0,00	0,00	r	50,33	h-j

Means followed by the same letter are not significantly different at α=0,05

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Table 4. Morphological and commercial characters of male sterile cauliflower lines with B. nigra cytoplasm, Ogu-INRA cytoplasm and their fertile components

Line	Sterility/ fertility	Earliness (days from planting)	Mass of curd (kg)	Covering by leaves[*1]	Color of curd[*2]	Leaf bracting [*3]	Shape of curds[*4]	Uniformity [*5]
AD22	*CMS B. nigra*	55	0,41 df	3	1	0-1	3	1
BD22	Fertile maintainer	55	0,36 be	3	1	0	3	1
AD271	*CMS B. nigra*	60	0,35 be	1	1	0	3	1
BD271	Fertile maintainer	60	0,30 bd	1	1	0	3	1
AD274	*CMS B. nigra*	60	0,26 BC	1	1	2	5	2
BD274	Fertile maintainer	60	0,23 b	1	1	1	5	2
AD291	*CMS B. nigra*	70	0,46 ef	3	1	1	5	3
BD291	Fertile maintainer	70	0,53 fg	3	1	0	5	2
AP523	*CMS B. nigra*	60	0,33 be	1	0	2	3	1
BP523	Fertile maintainer	60	0,32 be	1	0	1	3	2
AP36	*CMS B. nigra*	90	0,85 h	5	0	3	3	1
BP36	Fertile maintainer	90	0,60 g	5	0	3	3	1
AP522	*CMS B. nigra*	70	0,45 df	3	1	3	3	1
BP522	Fertile maintainer	70	0,41 cf	3	1	3	3	1
AP417	*CMS B. nigra*	70	0,39 ce	5	0	3	1	1
BP417	Fertile maintainer	70	0,52 fg	5	0	3	1	1
A	*CMS B. nigra*	45	0,09 a	1	3	3	5	2
B	Fertile maintainer	45	0,11 a	1	3	3	5	2
CDT75	*CMS* Ogu-INRA	80	0,53 fg	5	0	1	1	1
DT75	Fertile maintainer	90	0,60 g	5	1	1	1	1
CPN73	*CMS* Ogu-INRA	100	0,26 BC	5	0	3	1	1
PN73	Fertile maintainer	100	0,33 be	5	0	3	1	1

Means followed by the same letter are not significantly different at $\alpha = 0.05$

[1] Covering by leaves: 1 – uncovered curds, 3 – partially covered, 5 - covered
[2] Color of curd: 0 - white, 1 – creamy-white, 3 - yellow
[3] Leaf bracting: 0 – absent, 1 – weak, 2 – medium 3 - strong
[4] Shape of curd: 1 – round, 3 – slightly flattened, 5 - flattened
[5] Uniformity: 1- totally, 2- partial, 3 – lack of uniformity

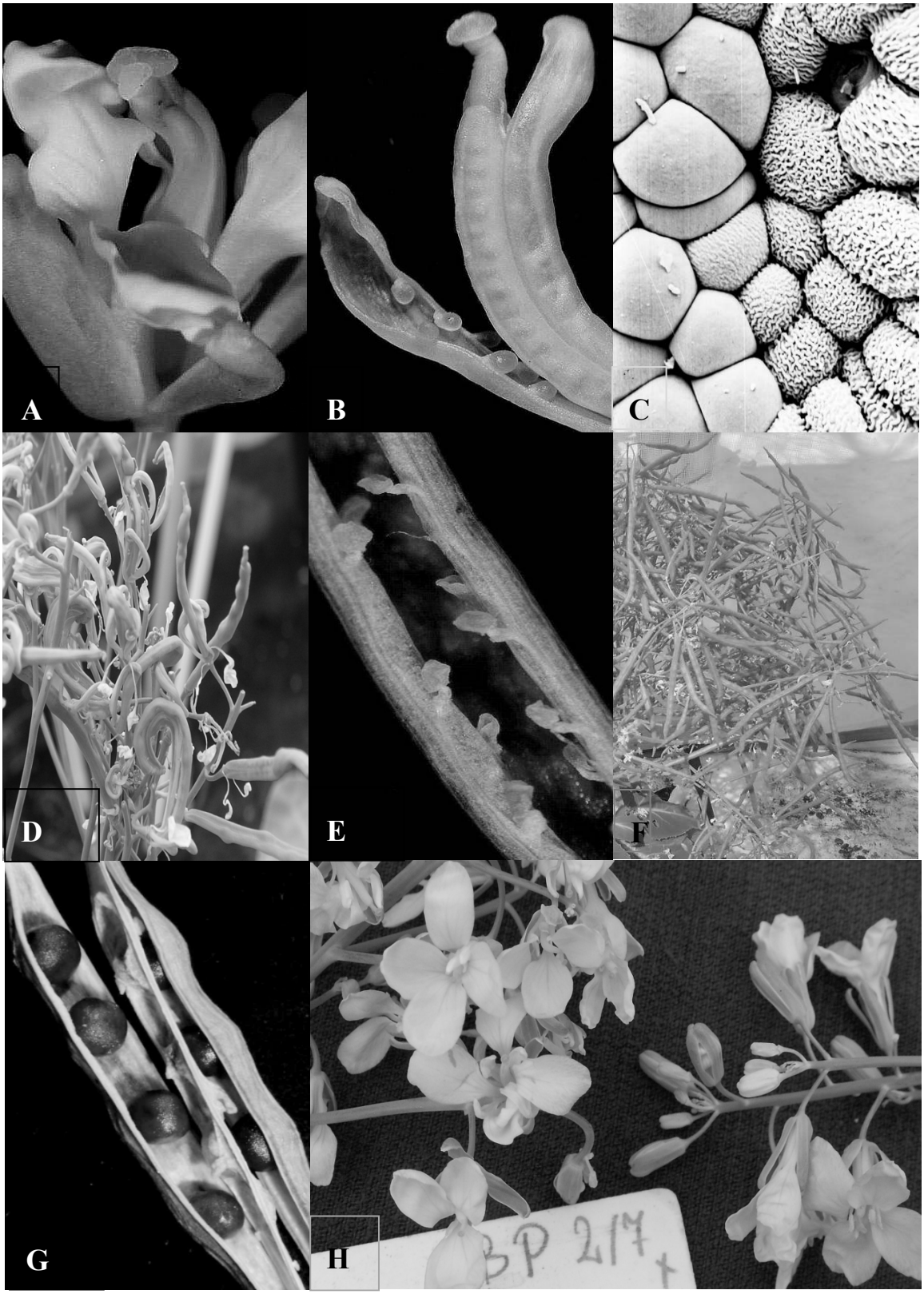

Figure 1. Generative organs of male sterile cauliflower with *B. nigra* cytoplasm; A) Flower with two conjoined pistils; B) Pistil with additional, opened carpels and ovules; C) Surface of tissues on the top of opened carpel; D,E) Siliques with deformed ovules; F,G) Siliques with normal seeds; H) Chimeral flower shots

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