Hybrid breeding of cauliflower using self-incompatibility and cytoplasmic male sterility

V. Kučera, V. Chytilová, M. Vyvadilová, M. Klíma

Research Institute of Crop Production Prague-Ruzyně, Czech Republic

ABSTRACT: Two self-sterility systems, self-incompatibility (SI) and cytoplasmic male sterility (CMS) were used to verify their suitability for hybrid breeding of cauliflower. The possibility of reproduction of SI and CMS lines in isolation cages using insect pollinators were proved. The best results in reproduction of SI lines derived from the cultivar Montano were achieved by spraying with 3% NaCl solution in the evening and using bumblebees as pollinators. The mean weight of seeds per plant attained approximately 5 g. Two CMS lines bred from cultivars Brilant and Fortuna achieved seed set per plant after honeybee pollination with their fertile analogues 0.8 and 2.0 g, respectively. The yield of F1 seeds in hybridization experiment based on SI was 1.8 grams per plant of SI mother line. In hybridization based on CMS, the yield of F1 seeds per CMS plant was 2.3 grams. The F1 hybrid of SI line Montano × self-pollinating line from cv. Fortuna showed to be the best combination in a preliminary field trial.

Keywords: cauliflower; hybrid breeding; self-incompatibility (SI); CMS; reproduction of lines; hybrid seed production

In cauliflower, F1 hybrids proved advantages especially in uniform maturity, high early and total yield, better curd quality with respect to compactness and colour, resistance to insect pests, diseases and unfavourable weather conditions. There are two pollination mechanisms for hybrid seed production in cauliflower such as self-incompatibility (SI) and male sterility, which are used in the commercial hybrid seed production of the crop.

So far majority of cruciferous hybrid cultivars have been created by means of SI (Watanabe, Hinata 1999). The advantage of this system is the possibility to produce hybrid seed using two SI lines homozygous for different S alleles as parental components. Following disadvantages can be considered: less reliable SI, which results in an undesirable number of "sibs" in hybrid seeds; some difficulties connected with reproduction of SI lines by means of high CO_2 concentration (Jiřík 1985) or spraying with NaCl solution (Kučera 1990).

In contrast to other cruciferous vegetables, cauliflower is predominantly self-compatible; in older European open pollinating cultivars SI was not found at all. Possible genetic resources of active S alleles were at first some winter and tropical types of

cauliflower, later the first European hybrid cultivars (Kučera 1985). We have derived S homozygous self-incompatible lines from Montano F1, the first hybrid of cauliflower registered in the Czech Republic (Kučera, Černý 1992).

Two types of male sterility are known in Brassica oleracea, which can be possibly used for the production of hybrid seeds (Delourme, Budar 1999). In most of the cases the genic male sterility is inherited as a monogenic recessive character. Its practical use is limited because of its frequent instability and because male fertile plants must be discarded just before flowering in the course of hybrid seed production. Many cytoplasmic male sterility (CMS) systems have been elaborated in the cultivated Brassica species. Up to now, the most widespread system used in Brassica oleracea hybrid breeding is the improved Ogura CMS (Pelletier et al. 1983). Another CMS material induced by interspecific cross B. oleracea with rutabaga – B. napus (CHIANG, CRETE 1985, 1987) for the purpose of transfer resistance against Plasmodiophora brassicae has been studied in our institute for several years. We have created the male sterile cauliflower lines using the original donor of this CMS system. The aim of this work was to verify the possibility of utiliz-

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ing created self-incompatible and male sterile lines for practical cauliflower hybrid breeding.

MATERIAL AND METHODS

Plant material

Self-incompatible S homozygous sublines were derived from the cauliflower hybrid cv. Montano (MT) by means of inbreeding and subsequent selection of SI plants. The SI character of these lines is highly reliable; so far they have been maintained and reproduced by bud pollination. The reproduction by means of NaCl solution treatment was successfully checked on a small scale (Kučera 1990). The male sterile analogues of two cauliflower cultivars Brilant and Fortuna were produced using repeated backcrossing with the original CMS material that was characterized by an inferior agronomic quality. The initial CMS cauliflower line described by CHI-ANG and CRETE (1985) was obtained from Canada. After six generations of backcrosses, the CMS lines of desired traits and reliable male sterility were obtained. The open pollinating stocks of cv. Brilant (BR) and Fortuna (FT) were used as maintainers of CMS lines. The trials were carried out at the Department of Gene Bank - Olomouc workplace.

Reproduction of SI lines

The possibility to reproduce SI lines in a large scale by means of spraying with 3% NaCl solution in the open flower stage using insect as pollinators was tested. In the first trial six SI MT sublines were planted into isolation cages of 5×3 m size (plant spacing 50×50 cm) and grown until flowering. The application of insecticides and fungicides during the period of the curd formation provided a protection against virus and fungal diseases. Honeybees were used as pollinators from the beginning of the flowering. The lines were treated during the flowering period with 3% NaCl solution spraying every morning before bees flying out. The second trial was arranged in

the same way but the NaCl treatment was applied every other evening and bumblebees were used as pollinators (Fig. 1). After harvesting, the mean weight of seeds per mature plant was counted for every subline.

Reproduction of CMS lines

The trials were situated into two isolation cages and were arranged under the same conditions as mentioned above. CMS lines of Brilant and Fortuna were grown with their fertile analogues in alternate rows (2 rows of CMS and 1 row of fertile lines). Honeybees were used as pollinators (Figs. 2 and 3). After harvesting, the mean weight of seeds per one CMS and fertile plant was counted.

Production of hybrid seeds based on SI and CMS lines

The possibility of hybrid seed production in isolation cages with insect pollinators were verified using SI (MT) and CMS (BR) lines as mother parents and the fertile stock from cv. Fortuna as a donor of pollen. SI and CMS lines were grown in separate isolation cages in alternate rows at the rate of two rows of SI or CMS line and one row of the fertile parent FT 13. The arrangement and treatment of the trials was the same as in the case of reproduction of SI and CMS lines. Bumblebees were used as pollinators. The mean weight of seeds per mature SI/CMS and fertile plant was counted.

Preliminary evaluation of F1 hybrids

F1 hybrids based on SI and CMS were evaluated in the preliminary field trial with 30 plants per plot in two replicates at the Research Institute of Crop Production Prague-Ruzyně. Seeds were sown during the last week of May. The seedlings were planted in the field at the end of June in plant spacing 50×50 cm. Treatment and harvesting of the trial were arranged in accordance with the state variety trials.

Table 1. Reproduction of SI MT lines by means of NaCl spraying

SI subline	Number of grown plants	Number of harvested plants	Weight of harvested seeds (g)	Mean weight of seeds per plant (g)
3/1	6	6	39	6.5
4/1	6	5	30	6.0
4/2	6	3	15	5.0
5/1	6	4	18	4.5
6/1	6	4	16	4.0
8/1	6	4	19	4.8

Table 2. Reproduction of CMS lines by means of pollination with fertile analogues

CMS/fertile line	Number of grown plants	Number of harvested plants	Weight of harvested seeds (g)	Mean weight of seeds per plant (g)
BR CMS	24	20	15	0.8
BR 19	16	16	90	5.6
FT CMS	24	22	43	2.0
FT 13	16	16	157	9.8

RESULTS AND DISCUSSION

The results of the first trial concerning SI lines reproduction by means of NaCl solution spraying were unsatisfactory. When using the NaCl treatment in the morning, honeybees were not willing to pollinate the flowers and the seed set in a few developed pods was minimal. In the second trial (NaCl treatment every other evening, bumblebees as pollinators), the results were much better. Mean weight of seeds in all SI MT sublines reached from 4.0 to 6.5 g per harvested plant (Table 1). The mean weight of seeds per plant from all sublines was 5.1 g.

Similar results were achieved by Jiřík (1985) who used CO_2 for overcoming SI in cabbage lines. Some results concerning SI lines reproduction by means of NaCl treatment were published before (Kučera 1990); most of them deal with the NaCl treatment on individual inflorescence before or after hand pollination and counting number of seeds in selected pods. Our results obtained in much larger measure from the plant material could be considered as satisfactory for the practical SI lines reproduction. Some differences between seed set in individual sublines are in accordance with our previous results (Kučera 1990).



Fig. 1. Self-incompatible cauliflower plant cv. Montano after treatment with NaCl solution



Fig. 2. CMS cauliflower plant cv. Brilant pollinated by fertile analogue





Fig. 4. Field trial for evaluation of F1 cauliflower hybrids

Table 3. Hybridization trial based on SI and CMS

Parental genotype	Number of grown plants	Number of harvested plants	Weight of harvested seeds (g)	Mean weight of seeds per plant (g)
AI MT	24	22	40	1.8
FT 13	16	14	91	6.5
BR CMS	24	20	45	2.3
FT 13	16	15	275	18.3

Table 2 shows results obtained in the trials with the reproduction of CMS lines. The mean seed set per harvested plant in Brilant CMS line was 0.8 g and in Fortuna CMS line 2.0 g. In the fertile analogues of Brilant seed set it was 5.6 g and in Fortuna 9.8 g per plant. The low seed set in Brilant CMS line might have been caused by the fact that fertile parent plants finished flowering earlier than the sterile analogue. The CMS plants were also weaker in growth and some of them were partly damaged by fungal diseases. Flowering of both parental components from Fortuna were better time-balanced. The results confirm the possibility to reproduce CMS cauliflower



Fig. 5. Curd of the hybrid of SI line Montano \times self-pollinating line Fortuna FT 13



Fig. 6. Plants of the hybrid of SI line Montano \times self-pollinating line Fortuna FT 13 in the field trial

lines in isolation cages by pollination with their fertile analogues using insect pollinators.

The results of hybridization trials are presented in Table 3. In the hybridization experiment based on self-incompatibility, the homozygous SI MT line was crossed with fertile line FT 13. The mean seed weight per SI plant was 1.8 g and in the fertile parent FT 13 line 6.5 g.

In the experiment based on Chiang CMS, the maternal sterile line BR CMS was also pollinated with the line FT 13. The mean seed weight per plant was 2.3 g in the CMS line and 18.3 g in the FT 13 male line. Lower seed set, particularly in earlier female components could be due to curd reduction caused by putrescence. It was caused by humid and cold weather, which made chemical disease control ineffective.

Markedly lower seed set of CMS plants detected in both reproduction and hybridization trials could be caused by a special behaviour of honeybees. On sterile plants, bees usually learn to collect nectar mainly from the outer side of flowers ("sideworking") and therefore they rarely contact stigma to provide pollination (PIERRE, RENARD 1999).

The F1 hybrid of SI line Montano × self-pollinating line FT 13 turned out to be the best combination in a preliminary field trial (Fig. 4). Good uniformity, high curd quality, good covering of curd by inner leaves and a satisfactory disease resistance characterized



Fig. 7. Curd of the hybrid of CMS line Brilant \times self-pollinating line Fortuna FT 13

this hybrid (Figs. 5 and 6). The other hybrid BR CMS × FT showed to be less uniform forming smaller and lighter curds (Fig. 7).

In general, it can be concluded that both SI and CMS lines are suitable for hybrid breeding of cauliflower. As regards seed production, self-incompatibility appears to be more effective than cytoplasmic male sterility. On the contrary, CMS system provides much more reliable sterility than self-incompatibility.

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Šlechtění hybridů květáku na bázi autoinkompatibility a cytoplazmatické samčí sterility

ABSTRAKT: Pro hybridní šlechtění květáku byly ověřovány dva systémy autosterility, autoinkompatibilita (AI) a cytoplazmatická samčí sterilita (CMS). Byla prokázána možnost reprodukce AI a CMS linií v izolačních klecích s využitím hmyzích opylovačů. Nejlepších výsledků při reprodukci AI linií odvozených z odrůdy Montano bylo dosaženo pomocí postřiku 3% roztokem NaCl ve večerních hodinách při opylování čmeláky. Průměrná hmotnost semen na jednu sklizenou rostlinu dosáhla 5 g. Násada semen u dvou CMS linií vyšlechtěných z odrůd Brilant a Fortuna dosáhla po opylení fertilními analogy pomocí včel 0,8 a 2,0 g semen na rostlinu. V hybridizačním pokusu na bázi autoinkompatibility bylo dosaženo výnosu semen 1,8 g na AI rostlinu. Při hybridizaci na bázi samčí sterility výnos semen na CMS rostlinu činil 2,3 g. V předběžném polním pokusu se jako nejlepší projevil F1 hybrid AI linie Montano × fertilní linie Fortuna.

Klíčová slova: květák; hybridní šlechtění; autoinkompatibilita; CMS; reprodukce linií; produkce hybridních semen

Corresponding author:

Ing. Vratislav Kučera, CSc., Výzkumný ústav rostlinné výroby, Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika

tel.: + 420 233 022 368, fax: + 420 233 022 286, e-mail: kucerav@vurv.cz