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CMS *POLIMA*

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Gene-cytoplasmic male sterility *polima* type — CMS*pol* was found in population of rapeseed spring variety Polima in China (Fu, 1981). It is one of CMS forms which can be used for creation of hybrid varieties of oilseed rape and because of that it is the object of investigations in a lot of research centers in the world.

The factor limiting the possibility of utilization of CMS*pol* for hybrid seed production is the instability of male sterility expression as well as low frequency of maintaining and restoring genotypes in *Brassica napus* species.

The level of heterosis effect gained in hybrids with sterile cytoplasm originating from Polima is also discussed.

The most important problem is the instability of male sterility expression under the various environmental conditions. Because of that the heterotic gain in performance of F_1 hybrid varieties may not be sufficient to balance for the additional cost of hybrid seed production. In addition European regulations for hybrid seed production stipulate 100 per cent of hybridity. In Canada according to regulations there must be at least 75 per cent of hybrid seeds in the seed lot. Also in China 100 per cent of hybridity is not required, but the level of hybridity must be rather high to reach good yielding level.

In order to ensure the required level of hybridity using CMS*pol* system for hybrid seed production many investigations have been undertaken. At first factors responsible for male sterility breakdown in CMS*pol* lines were investigated.

CMS *polima* according to Kaul classification (1988) belongs to the structural type of male sterility. Mechanism of this type of male sterility consists to the lack of male archespor development or to the delay of its differentiation. In consequence anthers of male sterile plants CMS*pol* are small, arrow-shaped and white (Bartkowiak-Broda, 1991; Tai and McVetty, 1988). The action of male sterility gene appears during anther development as well as male archespor differentiation and simultanously undergoes strong environmental influences. The main factor influencing male sterility expression is the temperature. On the basis of realised investigations it is possible to put forward hipothesis that male sterility breakdown results from additive action of modifier genes which expression appears at the moment of action of particular temperature in critical phase of microsporogenesis (Bartkowiak-Broda, 1991; Gurjeet, Banga, 1995). This temperature must operate on buds at the stage before archespor differentiation to promote the revertion to the normal development of the anthers.

Investigations carried out by different authors on male sterile lines CMS*pol* with different nuclear background indicate various critical temperature giving revertion of male sterile forms to partially male fertile. For example Fan and Stefansson (1986) observed in male sterile lines CMS*pol* with genotype of summer rapeseed varieties Karat and Regent the revertion to partially male fertility after the plant treatment by the day/night temperature 30°C/24°C. Bartkowiak-Broda (1991) observed male sterile lines CMS*pol* with different genotype of winter oilseed rape double low lines. Some of them started to produce traces of pollen grain already after the treatment of day/night temperature 25°C/20°C. Li, Zhang *et al.*. (1995) found that the critical temperature for changing of fertility level is mean day temperature 7-9°C in the flower bud differentiation stage.

Taking into consideration these results it is possible to state that the stability of CMS*pol* lines depends on the genotype of maintaining lines as well as that in the population of CMS*pol* plants may occur:

— plants which are male sterile only in low temperature,
— plants which are male sterile only in high temperature,
— stable male sterile plants in either temperature .

Also there are observed plants which are partially male sterile during whole flowering period.

In unstable male sterile plants there are not stated the male archespor differentiation in each anther cells, most often only in one or two of them. Pollen in favourable temperature conditions spills from dehiscent, partially developed anthers. The percentage of viable pollen grain is only a little lower than in fertile plants but it is sufficient for selfpollination of this plants (Bett, Seguin-Swartz, 1995). It is the cause of impurity of sowing material of hybrid progeny F_1 by seeds of male sterile plants.

Fertilization is another factor which can modify the expression of male sterility. High nitrogen fertilization causes instability of CMS*pol* lines. Moderate fertilization with phosphorus, potassium and boron leads to significantly positive effect on the stability of male sterility. In general too high fertilization is unfavourable for the production of CMS*pol* lines (Li, Zhang *et al.* 1995).

Many investigations were carried out to find the method of selection of maintaining lines. Few maintaining lines have been selected up to now by different methods.

In India Sodhi *et al.* (1993) tested CMS*pol* lines maintained during several generations by three different *Brassica napus* genotypes under a course of temperature and photoperiod conditions. Only in CMS*pol* maintained by line ISN 706 no breakdown in sterility was observed. This *B. napus* genotype was developed as a result from cross *B. campestris* ssp. *oleifera* var. brown sarson with *B. oleracea* var. *botrytis* cv. Pusa Katki.

Also in India Gurjeet and Banga (1995) have recently selected nine new *Brassica napus* maintaining lines for CMS*pol* system. This selection was made on plants treated by high day/night temperature (32°C/26°C) just at bolting stage.

In Poland the aim of investigations is the obtention of a system for winter rapeseed hybrid seed production with the use of CMS*pol*. Because of that the searches of maintaining genotypes are conducted among winter oilseed rape lines and varieties.

Two lines of double low winter oilseed rape giving in progeny with CMS*pol* lines about 90 per cent of male sterile stable plants have been selected. Earlier observations of PN 410/88 winter oilseed rape line for ability to maintain CMS*pol* revealed that with the increase of homozygocity by inbreeding, this line with CMS*pol* line gave progeny with higher stability of male sterility expression (Bartkowiak-Broda, 1991). Therefore from this line doubled haploids (DH) were obtained by androgenesis in vitro. Out of investigated DH lines in test hybrids with CMS*pol* from F_1 to BC_3 progeny, four have been selected as complete maintainers. The improvement of male sterility stability after several backcrosses of another CMS*pol* lines with some of obtained DH lines was also stated (Bartkowiak-Broda *et al.* 1995). That means that the maintaining lines containing a set of analogous recessive genes determining male sterility in homozygous stage are indispansable to produce lines with stable male sterility expression.

Fu *et al.* (1990) selected three maintaining lines with summer oilseed rape genotype. However the transfer of this cytoplasm to summmer Canadian *B. napus* cultivars does not give stable CMS*pol* lines. This result indicate that the number of modyfier genes to a large extend responsible for male sterility breakdown in CMS*pol* system is probably very huge.

Another way to solve the problem of instability of CMS*pol* lines has been choosen by Li, Tan *et al.* (1995). This team created a new system which consisted of CMS*pol* line and genic male sterile line, called twin-ms line system. Nevertheless by the use of this system is not ensured total male sterility because of interaction between genotypes of both male sterile forms. Moreover the lack of uniformity of flower shape of plants originating from two different male sterile systems might be unfavourable element for hybrid seed production.

Problem of restoration in CMS*pol* system can be considered to be solved. Several sources of restorer genes have been found. In Canada were discovered restorer genes in genotype of summer oilseed rape variety Italy, in line UM2353 (Fang, McVetty, 1989) and in *B. juncea* Zem variety (Fan, Stefansson and Sernyk, 1986). In China several restoring lines were selected in *B. napus* species (Yung and Fu,1990). In Poland was selected restoring double low winter oilseed rape line PN 5297/86 and forms from partially male sterile plants which occur in CMS*pol* population (Bartkowiak-Broda, 1991). There is limited number of restoring genotypes for CMS*pol* system, but introduction of restorer genes into different *Brassica napus* forms by the use of classical methods of selection is possible.

Some investigations on heterosis effect gained in hybrids obtained on the base of CMS*pol* indicate that the biological cost of sterile cytoplasm *polima* is relatively high. There was stated that *polima* cytoplasm in comparison with *napus* cytoplasm can have negative influence not only on seed yield but in some cases also on oil and protin content in seeds (McVetty, Edie and Scarth, 1990). This negative effect is probably related to the depth of male sterility expressed which is a result of negative nuclear-cytoplasm interactions (McVetty and Pinnisch, 1994). Nevertheless there is possible to select hybrids with *polima* cytoplasm which exhibit superior performance compared to the convetional cultivars (Bartkowiak-Broda, 1995; McVetty, Edie and Scarth, 1990). The best example are first summer rapeseed hybrid varieties Energol and Hybridol developed in France by the use of CMS*pol* (Pinochet, 1995). The yield of these varieties is over 20 per cent higher than the yield of standard varieties. Several varieties of summer rapeseed have been developed also in Canada (Downey, 1994).

In production of CMS*pol* line and hybrid seeds a problem of pollination appears. The pollen of oilseed rape plants is enthomophilous and because of that first of all an insect vector is required to achieve good level of cross pollination between male sterile A-line and male fertile maintaining B-line or restoring R-line. Investigations made in Canada shoved that insect and wind pollination is not enough effective in this case, because seed yield drop rapidly as the distance between the male and female rows increase (Downey, 1994). One of supposition was that the cause of low pollination effect are changes in floral morphology of male sterile plants. CMS*pol* plants are characterized by profound changes in the floral morphology (Fan, Stefansson, 1986; Bartkowiak-Broda, 1991). Petals are small and narrow what allows bees to obtain nectar by approaching the flower from side without touching the stigma. In this case flowers are not pollinated.

Investigations carried out by McVetty, Pinnisch and Scarth (1989) revealed that floral morphology changes and the number of "sideworking" bees did not lead to a decrease in seed yield of the A lines. This suggest that the improvement of floral morphology of male sterile plants is not important to solve the problem of seed production.

Due to the fact that the level of hybridity has important influence on the performance of hybrids (Pinnisch and McVetty, 1992; 1994) and to economical aspect of hybrid seed production Downey (1994) propose to mix 10 to 15 per cent of male parent B-line with good male sterile A-line and sow and harvest the male and female lines together as commercial hybrid seeds.

In conclusion taking into consideration the results obtained during last years it seems that the progress in selection of stable male sterile lines and the possibility of restored hybrid production will allow to develop performant oilseed rape hybrid varieties.

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