Hybrid breeding of winter oilseed rape by utilization of self-incompatibility and cytoplasmic male sterility systems

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Abstract

Winter oilseed rape is the major oilseed crop in the Czech Republic. Several foreign hybrids based on male sterility systems MSL originated from Germany and Ogu-INRA from France is registered in our country at present. Three self-sterility systems including self-incompatibility (SI), cytoplasmic male sterility (CMS) Ogu-INRA and Shaan 2A are used for breeding of domestic rapeseed hybrids. The original self-incompatible lines developed from older European cultivars of rapeseed were characterized by a high glucosinolate (GSL) and erucic acid content. SI lines with improved seed quality have been developed by crossing of original SI materials with donors of double zero quality and subsequent deriving of doubled haploids (DH). Molecular markers based on PCR analyse of SLG gene have been developed for screening of self-incompatible genotypes. Comparison of seed test and molecular analysis of microspore regenerants for detection of SI and SC genotypes showed the possibility to discard majority of self-compatible plants in the early stage of development. Two different CMS systems are also used in breeding of hybrid cultivars. In Ogu-INRA CMS initial material obtained from France and Shaan 2A CMS from China, male sterile lines with improved seed quality were achieved by repeated backcrossing with DH donors of 00 character. The relevant fertility restorers (Rf) were also improved by means of crossing with 00 quality donors and subsequent development of doubled haploids.

Key words: oilseed rape, doubled haploids, hybrid breeding, self-incompatibility, CMS Ogu-INRA, CMS Shaan 2A

Introduction

Production of hybrid cultivars in oilseed rape is mostly based on the utilisation of cytoplasmic male sterility (CMS) or sporophytic self-incompatibility (SI). Although at the present time use of CMS appears to be more successful, some rapeseed hybrids created by means of SI have been released recently. Several foreign hybrids based on male sterility systems MSL originated from Germany and Ogu-INRA from France is registered in our country at present. No original Czech hybrid cultivar has been released yet. Breeding of hybrid cultivars based on self-incompatibility, CMS Ogu-INRA and Shaan 2A is being developed in the Czech Republic (Kucera, Vyvadilova 2001).

Natural occurrence of SI plants in winter oilseed rape is very low, about 0.1 %, modern cultivars are entirely self-pollinating lines. Active S alleles conditioning the high and stable degree of SI could be transferred to the *Brassica napus* genome by means of resynthesis from the original species *Brassica oleracea* and *B. rapa*. Much easier way is detection of SI plants in older oilseed rape cultivars or breeding materials. However, SI lines still produced by means of traditional breeding procedures have been characterized by high glucosinolate (GSL) and erucic acid content. During successive generations of selection for quality improvement after crossing of these lines with 00 quality donors SI gradually vanished (Havel 1996).

Doubled haploid (DH) system has been used to produce SI homozygous lines with desired 00 seed quality (Kucera et al. 1999, 2002). This procedure could be complicated by gametic selection in microspore cultures that may cause lower frequency of SI genotypes. The occurrence of reliable SI regenerants ranged from 10 – 20% (Kucera et al. 1999). Previously obtained SI DH lines showed insufficient GSL and erucic acid content in next generations of reproduction. Some SI lines with desired traits were obtained from one SI genotype by means of repeated cycles of crossing with donors of quality combined with deriving of DH (Koprna et al. 2005). However, for practical hybrid breeding and seed production of rapeseed the development of SI lines with different S genotypes is necessary. Detection of SI plants within a large number of regenerants by seed test is laborious and time consuming. Molecular markers have been developed for screening of self-incompatible genotypes in early stage of plant development (Curn et al. 2003).

Initial Ogu-INRA and Shaan 2A CMS and particularly their fertility restorers (Rf) also showed a high GSL content. CMS lines with improved seed quality have been produced by means of repeated crossing with various 00 quality donors. To develop Rf lines with decreased GSL and erucic acid content for both CMS systems repeated backcrossing with 00 quality donors combined with DH techniques have been used. By the use of these methods, some CMS and Rf lines from both male sterility systems were obtained. Molecular markers have been also verified for the detection of plants containing Rf genes.

The aim of this work was to verify the possibility to use doubled haploid system and molecular markers for creating new self-incompatible, CMS and Rf lines with desired seed quality suitable for breeding rapeseed hybrids.

Material and methods

Self-incompatibility

The initial self-pollinated SI lines with recessive determined self-incompatibility selected from older rapeseed cultivars (Havel 1996) were characterized by unsatisfactory seed quality in term of GSL and erucic acid content. Four F_1 hybrids of SI lines with different S genotypes and self-compatible (SC) donor of double low quality were used to develop DH lines with combination of SI and 00 quality. SI degree and stability of individual DH regenerants were tested using seed-set test, i.e. by repeated self-pollination in flowers and buds during all the period of flowering. Average number of seeds per developed pod after self-pollination in opened flowers (SF) and buds (SB) were determined. Plants with SF from 0,0 to 3,0 were considered as SI, with SF from 3,1 to 5,0 as partially SI and SF above 5 as completely self-compatible (SC).

Molecular markers for screening of self-incompatible genotypes by PCR analyse of SLG gene have been used. Genomic DNA of DH regenerants was extracted from young leaves of flowering plants by means of the DNeasy Plant Mini Kit (QIAGEN). PCR reaction was performed according to Curn et al. 2003. In the four groups of doubled haploid regenerants containing 115 plants, seed-set test was compared with PCR analyses for SLG gene detection.

Cytoplasmic male sterility

Initial Ogu-INRA and Shaan 2A CMS and particularly their fertility restorers (Rf) also showed a high GSL content. CMS lines with improved seed quality have been produced by means of repeated crossing with various 00 quality donors. For development of Rf lines with decreased GSL content repeated backcrossing with 00 quality donors and subsequent deriving of DH regenerants are used. Molecular markers have been also verified for the detection of plants containing Rf genes. To this goal more than 500 RAPD markers were tested on the large set of plant samples with known combinations of Rf gene alleles.

Results and discussion

Detection of self-incompatible regenerants

The results of evaluation of four regenerants groups for SI/SC degree by seed test and molecular analysis are presented in Table 1. Identification of S-locus with SLG genes was used to detect self-incompatibility. However, a part of selected plants in the presumed SI group proved to be semi-SC. Seed test confirmed the strength and stability of SI.

Table 1. Comparison of seed test and molecular analysis of regenerants for detection of SI and SC genotypes

Genotype	No. of tested regenerants	No. of SI plants		No. of SC plants	
		Seed test	Molecular analysis	Seed test	Molecular analysis
OP 19	31	0	11	31	20
OP 20	10	5	6	5	4
OP 21	56	8	27	48	29
OP 22	18	7	12	11	6
Total	115	20	56	95	59

None of SC plants detected by molecular analysis showed to be self-incompatible according to seed-set test, but some plants designated as SI turned out to be self-compatible. Concordance rate between molecular analysis and seed test concerning SC plants ranged from 55 to 80%, on average 62 percent. Utilization of this method could facilitate to discard more of SC plants in the early stage of development in contrast to seed-set test that requires growing all plants up to flowering stage. Although the results from the molecular analyses and seed test are not completely in coincidence, PCR amplification of SLG genes appears to be suitable approach for incompatible individuals screening in rapeseed hybrid breeding programmes. At the present time new molecular markers for estimating SI individuals based on SCR gene analysis are being developed.

Table 2. Results of seed quality analyzes (HPLC**) of selected Ogu-INRA CMS and Rf lines**

CMS line	GSL μmol/g seeds	Rf line	GSL μmol/g seeds
189-6	15.30	2097-273	10.48
558-17	10.15	2136-281	15.13
213-29	15.41	2137-285	11.79
13-37	15.03	2141-292	9.95
303-53	16.57		
323-60	13.97		
82-102	16.34		
114-106	14.40		
523-157	19.20		
551-227	13.58		

Development of male sterile and fertility restoring lines

Some CMS lines with decreased GSL and erucic acid content have been created by means of repeated backcrossing with various 00 quality donors. DH lines with stabilized desired traits have been mostly used as donors of quality. Decrease of GSL content in Rf lines in Ogu-INRA system by traditional breeding methods is complicated due to linkage of Rf gene with the rest

of *Raphanus* genome. The improved Rf lines with a high 00 quality for this CMS system were achieved using repeated crossing and subsequent deriving of doubled haploids (Table 2). Using the same methods, some improved CMS and Rf lines for Shaan 2A system have also been obtained (Table 3).

Several RAPD molecular markers was verified for the Ogu-INRA CMS system which are applicable to discriminate of plants carrying Rf genes in segregating progeny after crossing of fertility restoring lines with donors of 00 quality. Although these markers are not able to distinguish Rf gene in heterozygous or homozygous constitution for the present, in case of deriving doubled haploids the homozygous fertility restorers are determined reliably.

Table 3. Results of seed quality analyzes of selected Shaan 2 CMS and Rf lines

(GSL – HPLC, Fatty acids / % / - Gas chromatography)

Genotype	GSL mol/g seeds	C16:0 Palmit.	C18:0 Stear.	C18:1 Oleic.	C18:2 Linoleic.	C18:3 Linolen.	C22:1 Erucic.
S-2 CMS	14.22	3.16	1.08	69.54	18.57	5.59	0.22
S-3 CMS	26.74	3.06	0.81	71.51	16.62	6.27	0.14
S-5 Rf	12.96	3.00	0.85	70.66	17.12	6.02	0.20
S-6 Rf	16.35	3.38	1.04	71.13	16.89	6.25	0.14
S-7 Rf	13.77	2.49	0.86	74.64	16.07	5.08	0.21
S-8 Rf	13.11	2.63	0.92	73.94	16.23	5.34	0.17

References

Curn V., Vyvadilova M., Dolanska L., Kucera V. (2003): Utilisation of molecular markers for screening of self-incompatible *Brassica* napus genotypes. Proceedings of the 11th International Rapeseed Congress, Copenhagen, Denmark, 6. – 10. July 2003, Vol. 1: 97 - 99.

Havel, J. (1996): Production of self-incompatible lines in winter swede rape. Genet. a Slecht., 32: 9-18.

Koprna R., Kucera V., Kolovrat O., Vyvadilova M., Klima M. (2005): Development of Self-incompatible Lines with Improved Seed Quality in Winter Oilseed Rape (*Brassica napus* L.) for Hybrid Breeding. Czech J. Genet. Plant Breed., 41: 105–111.

Kucera V., Vyvadilova M., Tomaskova D. (1999): Development of self-incompatible double low winter oilseed rape lines by means of doubled haploid system. Proceedings of 10th International Rapeseed Congress, Canberra – Australia : CD Rom.

Kucera V., Vyvadilova M. (2001): Recent state and trends in breeding of winter rapeseed in the Czech Republic. Oilseed Crops 22 (1):5-12.

Kucera V., Vyvadilova M., Klima M. (2002): Utilization of doubled haploids in winter oilseed rape (*Brassica* napus L.) breeding. Czech J. Genet. Plant Breed. 38: 50-54.

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