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Recent state and trends in breeding of winter rapeseed in the Czech Republic

Obecny stan i kierunki w hodowli rzepaku ozimego w Republice Czeskiej

Słowa kluczowe: rzepak ozimy, *Brassica napus* L., zespół hodowlany, cytoplazmatyczna męska sterylność, CMS, samoniezgodność, SI, podwojone haploidy, odporność na stresy, żółte nasiona, analiza jakościowa, markery molekularne

Key words: winter oilseed rape, *Brassica napus* L., breeding team, doubled haploid, hybrid, cultivar, CMS, cytoplasmic male sterility, SI, selfincompatibility, stress resistance, yellow seed, quality analysis, molecular marker

Aktywność badawcza i hodowlana placówek „Czeski Rzepak" jest ukierunkowana na podniesienie efektywności procedur hodowlanych rzepaku przy zastosowaniu innowacji. Celem jest otrzymanie materiałów wyjściowych cechujących się wysoką jakością, plennością, odpornością na choroby i mróz. Linie, materiały mieszańcowe oraz zaawansowane linie DH z ulepszoną jakością są używane dla otrzymywania genotypów o pożądanych cechach biologicznych i rolniczych. Uzyskano materiały wyjściowe do hodowli mieszańców, np. własne oryginalne samoniezgodne linie DH, linie CMS i restorery Ogu INRA z Francji i linie Shaan 2 z Chin. Żółtonasienny rzepak wytworzony na drodze krzyżowań międzygatunkowych jest używany jako dawca tej cechy. Materiały wyjściowe są doprowadzane do stanu homozygotycznego przy użyciu podwojonych haploidów uzyskiwanych poprzez kultury mikrospor. Innowacje są wprowadzane do analiz jakości nasion w celu opracowania metod selekcji przesiewowej na zawartość glukozynolanów, szczególnie przy użyciu NIRS. Testowanie mrozoodporności roślin rzepaku jest wykonywane w laboratorium oraz opracowaną metodą selekcji in vitro. Testy odporności na choroby *Phoma lingam* i *Sclerotinia sclerotiorum* są przeprowadzane w laboratorium i na polu, jak również na specjalnym polu infekcyjnym. Markery molekularne oparte na metodzie AFLP będą użyte do identyfikacji linii i określania stopnia mieszańcowości.

Activities of research and breeding organizations cooperating in association "Czech Rapeseed" are oriented to increase effectiveness of breeding procedures in winter oilseed rape using innovative methods. The aim is to produce initial breeding materials possessing high parameters of seed quality and seed yield, disease resistance and frost tolerance. Lines, hybrid materials and advanced doubled haploid lines with improved double low quality are used for the development of genotypes with desired biological and agronomic traits. Initial materials for hybrid breeding, i.e. our original self-incompatible doubled haploid lines, Ogu-INRA CMS lines and their male fertility restorer lines from France and CMS lines Shaan 2 from China have been obtained. Yellow-seeded oilseed rape created by means of interspecific hybridization has been used as a donor of specific traits of seed quality. Initial materials are homozygotized using doubled haploids produced by microspore culture. Innovation in seed quality analyses aims at screening for low glucosinolate content, especially by NIRS method. Frost tolerance laboratory tests of rape plants are performed and selection method in vitro is being developed. Disease resistance tests, especially against *Phoma lingam* and *Sclerotinia sclerotiorum* are provided in laboratory and in field conditions and on infection fields. Molecular fingerprinting based on AFLP technique will be used for the identification of lines and determination of hybridity degree.

Introduction

Oilseed rape is the most important oil crop in Czech Republic. Its area is about 300 000 ha per year and it is the third most grown field crop behind winter wheat and spring barley.

Majority of varieties is of winter type. The area of spring rape is about 20 000 ha. Its importance as an alternate crop in case of winter damage of rape plants is rising due to the increasing performance of new cultivars.

Present variety structure of winter oilseed rape in the Czech Republic consists of 27 varieties of double low (00) type in the National List. Most of them are line varieties and only five are foreign hybrids. Three 00 cultivars are of domestic origin (Aglona, Slapská Stela and Odila). Another Czech variety Oáza is of E0 type, with high erucic acid content and low glucosinolate content. It is intended for a special utilization (detergents, lubricants). Recently some doubled haploid lines and hybrids on the basis of self-incompatibility have been included into registration experiments. Spring oilseed rape is represented only by foreign varieties because Czech breeding has been developed only recently.

The research concerning the use of modern methods in practical breeding of rape are developed in the research and breeding institutions and at universities in the Czech Republic. Improved genetic resources of seed quality, CMS and SI lines and some perspective breeding materials have been obtained during the long term cooperation of these institutions on research projects (Havel 1996, Kučera et al. 1996).

The association „Czech Oilseed Rape“

Team cooperation is necessary for maintaining and further successful development of Czech rapeseed breeding and for creating competitive cultivars adapted for domestic growing conditions. For this reason the voluntary association of research and breeding organizations Oseva PRO Ltd. (Research Institute of Oilseed Crop — RIOC Opava), Sempra Praha Joint Stock Co. (Breeding station Slapy u Tábora), Selgen Joint Stock Co. (Breeding station Chlumec nad Cidlinou), AGRITEC Šumperk Ltd. and Research Institute of Crop Production Praha-Ruzyně (RICP), called „Czech Oilseed Rape“ has been established. The main aim of this activity was to create a counterbalance to breeding firms from abroad.

The evidence of this intensive competition is an increasing number of foreign new bred materials in State registration experiments every year.

Individual workstations in the association „Czech Oilseed Rape“ share the work in special fields of activities to ensure the connection between research and breeding and optimal utilization of their possibilities and abilities. The rules of cooperation and mutual responsibilities have been negotiated. There is some cooperation with other organizations, mainly with agricultural universities.

Doubled haploid production

Problems of doubled haploid utilization for breeding of line and hybrid varieties have been solved in The Research Institute of Crop Production Prague-Ruzyně in cooperation with RIOC Opava and breeding station Slapy. The method of production of DH regenerants by means of microspore culture has been optimised. Some perspective DH lines derived from different hybrid combinations have been obtained (Table 1).

Table 1

Yield of selected DH lines in field trials 1999/2000
Plon wybranych linii DH w doświadczeniach polowych 1999/2000

Genotype *Genotyp*	Generation *Pokolenie*	Yield [t per ha] *Plon [t/ha]*	Yield of standard *Plon wzorca*	%
OP 1043	F9	6.28	5.26	119
OP 1120	F8	6.05	5.26	115
OP 1032	F7	6.57	5.26	125
OP 1011	DH	6.27	5.26	119
OP 1014	DH	7.45	5.26	142
OP 1018	DH	6.32	5.26	120
OP 1021	DH	6.41	5.26	122
OP 482	DH	4.32	2.91	148
OP 528	F5	3.47	2.11	164
OP 571	F5	3.8	2.54	150
OP 595	F5	3.46	2.65	131
OP 664	F5	5.28	3.45	153

Hybrid breeding

Further development is directed to gradual change from line to hybrid cultivars. Various genetic mechanisms of self-sterility are utilised for hybrid seed production, as CMS, GMS a SI. Majority of foreign hybrids grown in CR are based on German MSL (Male Sterility Lembke) and French CMS Ogu-INRA systems (Renard et al. 1998).

Another way to develop hybrids is the utilization of self-incompatibility (Kott 1995). The main problem of this system is time consuming and difficult procedure of maintaining and reproduction of parental lines by spraying with NaCl solution or CO_2 application. The possibility to grow parental lines blended together on hybridisation field is an advantage in comparison with growing lines in strips in case of CMS system.

The use of SI for hybrid breeding

The use of SI for hybrid breeding have been studied in RICP Praha and RIOC Opava. Some DH lines from original genetic resources of SI have been developed up to now. But they have not reached the desired quality yet. The aim of our work is to obtain homozygous SI lines with improved seed quality by means of development of DH lines derived from F1 hybrids of SI and 00 quality donors (Kučera et al. 1996, 1999) Table 2.

Table 2

Evaluation of selected SI regenerants of rapeseed genotype OP 23 derived from F_1 hybrids of SI Tandem 6/85 × quality donor 2051 — *Ocena wybranych regenerantów SI genotypu rzepaku OP 23 uzyskanych z mieszańców F_1 SI Tandem 6/85 × dawca jakości 2051*

Plant No. *Roślina*	Number of seeds per pod *Liczba nasion w łuszczynie*		Rs	Chromosome no. in PMC *Liczba chromosomów*	Notice *Uwagi*
	SF	SF NaCl			
OP 23/2	0.33	3.47	9.51	15, 19	
OP 23/3	0	11.36	0.00	19	
OP 23/4	0.5	6.88	7.27	11, 12, 15, 17	aneuploid
OP 23/5	2	15.44	12.95	15, 19	
OP 23/6	0.56	7.45	7.52	19	
OP 23/8	1	7.8	12.82	19, 15, 17	
OP 23/10	0.1	2.47	4.05	19	
OP 23/11	0.5	2.8	17.86	not determined	
OP 23/13	1.24	2.16	57.41	15, 19	low seed set
OP 23/18	1.06	5.65	18.76	15, 19	
OP 23/22	0	10.67	0.00	not determined	

The use of CMS in hybrid breeding

Ogu INRA type CMS lines and fertility restorers have been obtained by licence agreement from France. The original lines were not of desired quality parameters particularly regarding GSL content. Many CMS lines with improved quality have been developed by repeated backcrossing in breeding station Chlumec nad Cidlinou. Workstations RICP and Agritec are engaged in improving quality traits of Rf lines by means of DH techniques and biochemical markers (Delourme et al. 1992) Table 3.

Table 3

Evaluation of Rf lines and experimental F_1 hybrids CMS × Rf seed quality
Ocena linii Rf i eksperymentalnych mieszańców F_1 CMS × Rf jakość nasion
(NIRS RICP 2000)

Genotype *Genotyp*	GSL µmol/g seeds *µmol/g nasion*	Oil *Tłuszcz* %	Acid — *Kwas*				Dry matter *Sucha masa*
			erucic *erukowy*	linolenic *linolenowy*	linolic *linolowy*	oleic *oleinowy*	
Rf 8/3 × 6	51.02	40.48	0.24	9.54	18.89	62.35	93.41
Rf 9/4 × 5	48.38	40.37	0.32	10.33	19.85	60.90	93.84
Rf 10/5 × 5	46.85	40.86	0.25	10.31	20.63	60.42	93.16
Rf 11/3 × 4	56.45	39.04	0.19	10.29	20.82	59.91	93.55
Rf 11/5 × 4	40.50	40.61	0.28	10.32	20.81	60.07	93.68
Rf 12/3 × 4	56.15	37.76	0.15	10.10	19.67	61.11	93.44
Rf 13/5 × 3	49.79	41.82	0.12	10.09	19.58	61.75	93.17
CMS × Rf 8/3	52.15	46.53	0.17	9.42	19.21	62.78	93.47
CMS × Rf 9/4	41.71	43.86	0.25	10.29	19.83	61.69	93.19
CMS × Rf 10/1	46.88	44.30	0.18	9.97	20.35	61.41	93.84
CMS × Rf 11/3	41.48	43.98	0.19	10.06	20.21	60.98	93.74
CMS × Rf 12/3	55.41	38.61	0.17	10.31	20.67	58.62	94.52
CMS × Rf 17/3	47.53	44.16	0.11	10.41	21.02	59.62	93.53

Another source of CMS Shaan 2 type originating from China has been investigated recently. Reliability and stability of two different CMS lines in our climatic conditions have been verified and first backcrosses were carried out to improve seed quality.

Breeding for stress resistance

Breeding for disease resistance in particular against *Phoma lingam* and *Sclerotinia sclerotiorum* is performed in Agritec, RIOC Opava and breeding station Slapy. Artificial infection have been applied more and more. Besides conventional methods of testing disease resistance in field conditions and in infection field methods of selection in vitro and molecular markers techniques will be included.

Laboratory techniques of frost hardiness tests in controlled conditions are utilized in RICP and a method of in vitro selection is being developed.

Breeding for yellow-seed

Initial materials for yellow-seeded rape created in Agricultural University of Prague by means of interspecific hybridisation of *Brassica oleracea* and *B. rapa* (Bechyně 1995) have been maintained in breeding station Slapy. Because of a failure of attempts to stabilize the seed colour and quality by traditional breeding procedures (Meng 1995, Tang et al. 1997) utilization of doubled haploid method has been verified as well (Vyvadilová et al. 1999). This method has been proved to be not fully successful until now for stabilization of desired seed colour and ploidy level in DH regenerants

Methods of molecular genetics

Problems of characterization and identification of different rape genotypes and development of molecular markers for specific traits (Somers et al. 1998) have been solved as well. In RICP the research is aimed at disease resistance and in South Bohemian University at characterization of SI genotypes. Molecular fingerprinting based on AFLP technique will be used for identification of lines and determination of hybridity degree.

Methods of quality analyses

Innovated rapid and non-destructive methods are used for screening of GSL and oil content mainly Near-Infrared Spectroscopy (NIRS) in RICP. For the assessment of individual GSL content HPLC method is used in RIOC where the influence of some GSL on the disease resistance will be studied. The screening methods of GSL content determination used in individual workstations are compared with standard methods such as gas chromatography for verifying their reliability (Table 4).

Conclusion

The joint project supported by Ministry of Agriculture of the Czech Republic aimed at innovation of breeding procedures in winter oilseed rape by the use of improved initial materials and biotechnological methods started in 2000. The goal of the project that includes all of the mentioned activities is to produce initial breeding materials possessing high parameters of seed quality, yield and biotic and abiotic stress resistance appropriate for breeding of new cultivars, mainly hybrids.

Table 4

Comparison of results of rape seed quality analyses by means of different methods
Porównanie analizy jakości nasion rzepaku oznaczanej różnymi metodami

Workstation/Method *Stacja badawcza/metoda*	Sample — *Doświadczenie*					
	Opava 1	Opava 2	Opava 3	Slapy 1	Slapy 2	Slapy 3
Glucosinolate content — *Zawartość glukozynolanów* [µmol/g of dry seeds — *µmol/g nasion*]						
Slapy/gas chromatography	16.23	9.65	196.63	9.66	57.03	160.95
RICP/NIRS I.	11.02	9.06	107.36	12.02	52.83	101.49
RICP/NIRS II.	10.65	6.86	110.6	11.18	54.41	104.15
RIOC/HPLC	19.68	11.82	210.6	14.71	84.4	162.74
Oleic acid $C_{18:1}$ content — *Zawartość kwasu oleinowego* [%]						
Slapy/gas chromatography	66.5	67.5	22.6	62.6	63.6	14.5
RICP/NIRS I.	64.34	62.17	67.19	62.88	60.89	72.7
RIOC/gas chromatography	63.73	63.43	18.48	61.3	61.91	13.04
Linolic acid content $C_{18:2}$ — *Zawartość kwasu linolowego* [%]						
Slapy/gas chromatography	16.8	17.7	14.1	19.8	19.3	12.4
RICP/NIRS I.	19.6	20.46	13.53	20.81	21.27	10.24
RIOC/gas chromatography	17.51	19.11	13.16	20.51	18.99	11.72
Linolenic acid content $C_{18:3}$ — *Zawartość kwasu linolenowego* [%]						
Slapy/gas chromatography	9.5	7.6	7.8	8.2	9.3	6.9
RICP/NIRS I.	9.37	11.06	6.09	10.18	9.7	4.19
RIOC/gas chromatography	9.68	8.71	8.94	9.1	9.88	6.51
Erucic acid content $C_{22:1}$ — *Zawartość kwasu erukowego* [%]						
Slapy/gas chromatography	0.1	stopy	38.2	trace	trace	50.21
RICP/NIRS I.	0.26	0.3	47	0.25	0.14	46
RIOC/gas chromatography	0.14	0.17	42.46	0.14	0.35	53.34
Oil content — *Zawartość tłuszczu* [%]						
Slapy/NMR	42.7	44.7	43.3	47.4	45.7	48.1
RICP/NIRS I.	47.06	46.29	43.5	48.36	46.98	45.94
RIOC/extraction	45.68	47.28	42.72	48.18	47.26	45.93

References

Bechyně M. 1995. Development of four valved yellow seeded rapeseed. Proc. 9th Int. Rapeseed Congress Cambridge, 4-7 July 1995: 1147-1149.

Delourme R. Eber, F. 1992. Linkage between an isozyme marker and a restorer gene in radish cytoplasmic male sterility of rapeseed (*Brassica napus* L.). Theor. Appl. Genet. 85: 222-228.

Havel J. 1996. Získávání autoinkompatibilních linií řepky ozimé. Genet. a Šlecht. 32: 9-18.

Kott L.S. 1995. Hybrid production systems based on self-incompatibility in oilseed *Brassica* Proc. 9th Int. Rapeseed Congress Cambridge, 4-7 July 1995: 73-78.

Kučera V., Vyvadilová M., Tomášková D., Havel J. 1996. Tvorba autoinkompatibilních linií řepky ozimé pomocí dihaploidního systému. Genet. a Šlecht. 32 (1): 1-8.

Kučera V., Vyvadilová M., Tomášková D. 1999. Development of self-incompatible double low winter oilseed rape lines by means of doubled haploid system. Proc. of 10th International Rapeseed Congress, Canberra – Australia, 26-29 September 1999.

Meng J. 1995. Interspecific hybridization and seed coat quality. Proc. 9th Int. Rapeseed Congress Cambridge, 4-7 July 1995: 1128-1133.

Renard M., Delourme R., Vallée P., Pierre, J. 1998. Hybrid rapeseed breeding and production. Acta Horticulturae 459: 291-298.

Somers D.J., Friesen K.R.D., Rakow G. 1998. Marker assisted selection of low linolenic acid in oilseed species. Acta Horticulturae 459: 157-163.

Tang Z.L., Li J.N., Zhang X.K., Chen L., Wang R. 1997. Genetic variation of yellow-seeded rapeseed lines (*Brassica napus* L.) from different genetic sources. Plant Breeding 116: 471-474.

Vyvadilová M., Tomášková D., Bechyně M., Kučera V. 1999. The use of doubled haploids to stabilize yellow-seedness in oilseed rape (*Brassica napus*). Czech J. Genet. Plant Breed. 35 (1): 7-9.