

UTILIZATION OF CYTOPLASMIC MALE STERILITY SHAAN 2A IN A HYBRID BREEDING OF WINTER OILSEED RAPE

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Abstract

The possibility of using Shaan 2A cytoplasmic male sterility (CMS) system for breeding domestic rapeseed hybrids was verified. Three reliable male sterile lines with low glucosinolate (GSL) content were derived from the original high GSL Chinese materials by six times repeated backcrossing with donors of 00 characters. Four relevant fertility restorers (Rf) were also improved by means of crossing with quality donors and additional development of doubled haploids (DH). The experimental three-way hybrids were produced using CMS lines crossed with the cultivar Californium as the sterile mother components and subsequently with DH Rf lines. The yield and agronomic performance of the five hybrids tested in three-location trials were comparable with high performance line and hybrid cultivars.

Key words: oilseed rape, hybrid breeding, CMS Shaan 2A, doubled haploids, experimental hybrids

INTRODUCTION

Hybrid winter oilseed rape (*Brassica napus*) cultivars enjoy a 15 to 20% yield advantage over conventional varieties, thanks to the expression of hybrid vigour (Brandle and McVetty, 1989). In oilseed rape, a hybrid seed production is mostly based on cytoplasmic (CMS) or genic (GMS) male sterility and rarely on sporophytic self-incompatibility (SI). No original Czech hybrid cultivar has been released yet. At present several foreign hybrids based on male sterility systems MSL originated from Germany and Ogu-INRA from France are registered in our country. Three self-sterility systems including SI, CMS Ogu-INRA obtained from France and Shaan 2A from China are verified for breeding of domestic rapeseed hybrids (Kučera et al., 2007). CMS Shaan 2A has been considered to be related to Polima CMS system, which is characterized as instable under variable environmental conditions. The temperature is the most important factor influencing male sterility expression (Bartkowiak-Broda, 1995). Polima and Shaan 2A are two main rapeseed CMS systems that have been used for hybrid seed production in China (Zhou and Fu, 2007). Sequence analysis of the gene correlated with CMS indicated that Polima and Shaan 2A were not the same CMS lines (Wang et al., 2002). As far as it is known, no hybrid cultivar has been bred based on Shaan 2A CMS in Europe. The Shaan 2A CMS lines possessing reliable

male sterility in glasshouse as well as in field conditions were created by means of repeated selection (Kučera et al., 2007). All domestic breeding materials used showed to be maintainers of sterility.

Original Shaan 2A CMS lines and their fertility restorers (Rf) obtained from China initially showed a high GSL content. To develop CMS and Rf lines with decreased GSL content repeated backcrossing with 00 quality donors combined with DH techniques have been used. By means of these methods, some CMS and Rf lines with doubled zero quality were obtained. The aim of this work was to verify the possibility of using Shaan 2A CMS system for breeding domestic rapeseed hybrids.

MATERIAL AND METHODS

Plant material

Initial Shaan 2A CMS lines and relevant fertility restorers (Rf) originated from College of Agronomy, Northwest A&F University, Yangling, Shaanxi, P. R. China. We have bred CMS and Rf lines with decreased GSL content by means of six times repeated backcrossing with doubled haploid (DH) donors of 00 characters. To achieve homozygous Rf lines subsequent deriving of DH regenerants by means of microspore culture has been used.

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Seed quality analyses

Standard methods of seed quality analyses of selected CMS and Rf lines were used: for GSL – High Performance Liquid Chromatography (HPLC), for fatty acids – Gas chromatography (GC). Seed quality of the final three-way hybrids was evaluated by NIRS, for comparison NMR method was also used for oil content and GC for GSL content assessment.

Development of experimental three-way hybrids and field trials

Three CMS lines were crossed with cultivar Californium in isolation cages using bees as pollinators. Obtained F1 hybrids that turned out to be completely male sterile were subsequently crossed with three doubled haploid Rf lines as described above. The seed quality analyses of each hybrid combination were carried out by means of standard methods.

The five final experimental hybrids (H1–H5) were evaluated in one-year field trials in three locations. Each trial consisted of three complete randomised blocks with 10 m² plots. The trial locations were situated in areas of Humpolec and Slapy u Tábora (potato growing regions)

and Hněvčoves (beet growing region). Seed yield of individual hybrids corrected to 12 % moisture content was compared with the mean of standard cultivars, registered in the National Cultivar List. Each trial was analysed individually by the method of multiple comparison from ANOVA with the assessment the least significant differences (LSD). Resistance to *Phoma lingam*, *Sclerotinia sclerotiorum* and *Alternaria brassicae* and lodging resistance were scored using a 1–9 scale, with 9 indicating the most favourable score. The thousand seed weight (TSW) was determined from two 500-seed samples.

RESULTS AND DISCUSSION

The results of seed quality analyzes of selected CMS and Rf lines after four backcrosses with quality donors are summarized in Table 1. In all materials considerable reduction of GSL content have been achieved, in case of Rf lines even below the limit of 18 µmol/g required by the Czech Variety Office for “low glucosinolate” cultivars. Seed of three way hybrids (CMS line × Californium) × Rf line showed additional reduction of GSL content (Table 2). It appears from these results that Rf gene of Shaan 2A CMS is not linked to genes controlling

Tab. 1: Results of seed quality analyzes of selected Shaan 2A CMS and Rf lines (GSL – HPLC, Fatty acids %/ – Gas chromatography)

Genotype	GSL µmol/g seeds	C16:0 Palmitic (%)	C18:0 Stearic (%)	C18:1 Oleic (%)	C18:2 Linoleic (%)	C18:3 Linolenic (%)	C22:1 Erucic (%)
S-1 CMS	26.99	2.5	1.1	69.2	17.6	8.1	0.2
S-2 CMS	14.22	3.2	1.1	69.5	18.6	5.6	0.2
S-3 CMS	26.74	3.1	0.8	71.5	16.6	6.3	0.1
S-5 Rf	12.96	3.0	0.9	70.7	17.1	6.0	0.2
S-6 Rf	16.35	3.4	1.0	71.1	16.9	6.3	0.1
S-7 Rf	13.77	2.5	0.9	74.6	16.1	5.1	0.2
S-8 Rf	13.11	2.6	0.9	73.9	16.2	5.3	0.2

Tab. 2: GSL (µmol/g seeds) and fatty acids (%) content in seed of five three-way experimental hybrids (GSL – HPLC, FA – GC)

Hybrid No. (combination)	GSL µmol/g seeds	C16:0 Palmitic (%)	C18:0 Stearic (%)	C18:1 Oleic (%)	C18:2 Linoleic (%)	C18:3 Linolenic (%)	C22:1 Erucic (%)
H 1 (S1 × Cal × S6)	11.18	3.8	1.1	66.7	18.0	8.4	0.3
H 2 (S2 × Cal × S6)	10.43	4.1	1.2	64.8	19.0	9.0	0.1
H 3 (S3 × Cal × S6)	9.33	4.1	1.2	67.3	16.9	8.6	0.2
H 4 (S1 × Cal × S8)	10.68	4.1	1.3	66.3	18.7	7.7	0.1
H 5 (S3 × Cal × S8)	10.39	4.1	1.2	67.7	17.1	8.0	0.3

Tab. 3: Yield of experimental hybrids compared with standard cultivars from three location trials

Slapy				Humpolec				Hněvčeves			
Hybrid/ Standard	yield t/ha	relative yield %	*	Hybrid / Standard	yield t/ha	relative yield %	*	Hybrid / Standard	yield t/ha	relative yield %	*
H4	5.02	115.14	a	H5	5.18	109.75	a	H4	4.36	112.95	a
H5	4.88	111.93	ab	Labrador	5.14	108.90	a	Baldur	4.15	107.51	ab
H1	4.70	107.80	ab	H4	5.04	106.78	a	H5	3.66	94.82	abc
Sitro	4.37	100.23	bc	H2	5.03	106.57	a	Exagone	3.56	92.23	bc
Baldur	4.34	99.54	bc	H1	4.91	104.03	ab	H2	3.55	91.97	bc
H3	4.33	99.31	bc	Ladoga	4.84	102.54	ab	H3	3.32	86.01	c
H2	3.77	86.47	c	H3	4.58	97.03	b	H1	3.17	82.12	c
				Californium	4.18	88.56	c				
Mean of standards	4.36			Mean of standards	4.72			Mean of standards	3.86		

*Letters a–c indicate homogeneous subgroups (LSD; P = 0.05)

seed GSL content as in the case of Ogu-INRA system (Delourme et al., 1998).

The yield of five experimental hybrids in comparison with relevant standard cultivars is summarized in Table 3. In the Slapy region, the yield of hybrids achieved from 86.47 to 115.14% as compared to the average yield of hybrid cultivars Baldur and Sitro. One of the hybrids (H4) significantly overcame both standards; yield of other hybrids was not significantly different from them. In the Humpolec region, the yield of hybrids was compared with high performance line cultivars Labrador, Ladoga and Californium. It ranged from 97.03 to 109.75% relative to the average yield of standards. The yield of four hybrids was not significantly different from cultivars Labrador and Ladoga, all of them overcame significantly the cultivar Californium.

In Hněvčeves the yield of hybrids ranged from 82.12 to 112.95% compared with average yield of standard hybrid cultivars Baldur and Exagone. The hybrid H 4 over-

came significantly standard cultivar Exagone and only two showed the yield inferior to Baldur. The preliminary performance test of the experimental hybrids indicated the possibility to utilize the Shaan 2A CMS system for creating high yielding hybrid cultivars.

Agronomic and biological traits of experimental hybrids were at average comparable with standard cultivars. Table 4 shows evaluation of some important agronomic traits compared to standard cultivars at Hněvčeves. The hybrids showed a high degree of resistance against *Phoma lingam* and medium against *Sclerotinia sclerotiorum*. The plant height was at average lower compared with standards. Vegetation period of hybrids and standards was almost the same.

Seed quality of final hybrids was estimated by means of NIRS method, the GSL content was simultaneously analysed by GC method and the oil content by nuclear magnetic resonance (NMR). All of experimental hybrids showed seed quality parameters fully satisfying requirements of

Tab. 4: Evaluation of economic traits of hybrids and standards in the field trial at Hněvčeves

Genotype	TSW	Winter resistance	Resistance to disease					Lodging resistance	Plant length	Ripening uniformity	Vegetation period
			Phoma lingam	Perono- spora	Botrytis cinerea	Sclero- tinia	Alternaria brassicae				
	(g)	(%)	(9–1)	(9–1)	(9–1)	(9–1)	(9–1)	(9–1)	cm	(9–1)	(days)
H1	5.72	100.00	8.00	8.00	9.00	2.67	8.00	4.00	191.57	8.00	194.00
H2	6.28	100.00	7.00	8.00	9.00	3.00	7.00	3.00	185.33	7.00	195.00
H3	5.46	100.00	8.00	8.00	9.00	3.00	7.00	3.00	176.57	7.00	196.00
H4	5.74	100.00	8.00	8.00	9.00	5.00	7.00	5.00	179.57	8.00	192.00
H5	5.54	100.00	8.00	8.00	9.00	4.00	8.00	4.00	189.23	7.00	195.00
Baldur	4.86	100.00	6.00	9.00	9.00	2.00	7.00	3.00	188.33	8.00	195.00
Exagone	4.86	100.00	6.00	9.00	9.00	3.00	8.00	5.00	193.10	8.00	194.00

Tab. 5: Evaluation of seed quality of experimental hybrids from the trial at Slapy u Tábora 2008

Hybrid	Oil content NMR (%)	GSL GC ($\mu\text{mol.g}^{-1}$ seed)	NIRS					
			Oil (%)	GSL ($\mu\text{mol.g}^{-1}$ seed)	FA C18:1 Oleic (%)	FA C18:2 Linoleic (%)	FA C18:3 Linolenic (%)	FA C22:1 Erucic (%)
H1	45.40	16.42	47.60	11.01	68.6	20.2	6.5	0.3
H2	46.75	16.58	49.46	11.56	66.9	21.1	6.7	0.1
H3	47.39	9.49	48.45	9.28	66.1	19.9	6.5	0.2
H4	47.11	14.31	48.98	9.11	70.1	19.4	6.0	0.1
H5	46.18	13.52	47.44	7.59	66.9	19.9	7.9	0.3

NMR – Nuclear magnetic resonance, GC – Gas chromatography

standard specifications (Table 5). Next experiments will be focused on creating mother components of hybrids with stable and reliable trait of sterility in variable climatic conditions and verifying their combining ability with improved Rf lines for achievement of a high hybrid vigour.

The present results suggest the possibility of using Shaan 2A CMS and Rf lines for breeding of oilseed rape hybrids with a satisfied yield and seed quality.

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