Classification of NCa CMS in *B. napus* and the inheritance of fertility restoration

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Abstract

The extremely low seedset of NCa CMS after self-pollination indicated that NCa CMS was a good system for hybrid seed production. Test-cross using 50 *B. napus* varieties revealed that Nca CMS had different restorer-maintainer relationships with Pol CMS and Nap CMS. The inheritance study of restorer genes in NCa CMS implied the restoration was controlled by a single dominant nuclear gene.

Key Words: *B. napus* L.; Male sterility; inheritance; Restorer-maintainer relationship

INTRODUCTION

Breeding for novel CMS is one of the key subjects in the application of oilseed rape heterosis. Most of oilseed rape hybrid varieties used in China at present were produced using male-sterility pollination system. Among these, only a small part was produced using genic male sterility (GMS), whereas the majority was derived from cytoplasmic male sterility due to comparatively low cost and established procedure for seed production. Pol CMS was first discovered in China and widely used for oilseed rape production [(1).] The extensive application of Pol CMS in oilseed hybrid breeding in China has made the cytoplasm of Chinese oilseed hybrid varieties monotone, which increase risk for epidemic disease as the maize T-CMS [(2).] Exploitation of novel CMS is important for a sustainable oilseed breeding process in China.

Restorer-maintainer relationship has been used for the classification of cytoplasmic male sterility in major crops such as maize and rice. In *B. napus*, differentiation of CMS system has also based on restorer-maintainer relationship. For example, Pol CMS and another widely used cytoplasmic male sterility source Shan 2A were classified as bearing the same cytoplasm due to similar restorer-maintainer relationships. In this study, we reported the sterility performance of a novel CMS derived from a interspecific cross and its restorer-maintainer relationships compared to Pol CMS and nap CMS, as well as the inheritance of fertility restoration[3]. In addition, the potentiality of NCa CMS in oilseed breeding and its classification as a male sterile cytoplasm were discussed.

MATERIALS AND METHODS

Materials

NCa CMS and related maintainer were from the breeding group on oilseed rape in the Institute o f Oil Crops Research, CAAS. The maintainers were F_{11} and F_{12}, and the sterility lines were BC_7 and BC_8. Polima CMS were also from the Institute of Oil Crops Research. Nap CMS introduced from Jiangsu Academy of Agricultural Sciences. Materials used in the restorer-maintainer relationship test were provided by rapeseed genetic resources group in the Institute of Oil Crops Research, and self-pollinated over three generations.

Experimental methods

Plants for seedset test were grown in two-row plats, inter-planted by two rows of male sterile plants with two rows of male fertile plants. Self-pollination was ensured by bag isolation of inflorescences before flowering. Testcross for restorer-maintainer relationship determination was conducted using five inflorescences simultaneously on NCa CMS, Polima CMS and Nap CMS with pollen from the same single plant. Each of the F1 plants were grown in the next year in three rows. Fertility of the F1 progenies was investigated on March 5, March 15, and March 25 respectively. Plants that were completely sterile at lest at one timepoint were classified as sterile, plants that were not sterile at any timepoint but fertility was not completely

restored were classified as semi-fertile, and those were completely restored were classified as fertile.

RESULTS

Seedset of the NCa CMS

For the self-pollination on 28 principal inflorescences of seven NCa CMS lines, the results were shown in Table 1. The ratio of average seedset per flower of NCa male sterile plants upon self-pollination and open-pollination of the same line was 1393, which indicated that very few seedset of Nca CMS produced without external pollen.

Table 1 seedset of NCa CMS upon self-pollination and open pollination

	Line1		Line2		Line3		Line4		Line5		Line6		Line7		Average	
	S[1]	O[2]	S	O	S	O	S	O	S	O	S	O	S	O	S	O
No. of flowers(A)	212	521	265	491	209	502	235	483	258	560	198	541	285	529	237	518
No. of pods (B)	0	451	7	441	0	431	0	449	4	509	0	438	0	413	1.6	447
No. of seeds (C)	0	6007	7	6186	0	5431	0	6061	7	7686	0	5477	0	5570	2	6059
B/A	0	0.866	0.026	0.898	0	0.859	0	0.929	0.016	0.909	0	0.814	0	0.781	0.007	0.863
C/A	0	11.5	0.03	12.6	0	10.8	0	12.5	0.027	13.7	0	10.1	0	10.5	0.008	11.7

1. S: Self-pollination treatment, 4 principal inflorescences for each CMS line.
2. O: Open-pollinated plants of the same line as the self-pollination treatment. The data were collected from randomly selected 7 principal inflorescences.

Restorer- maintainer relationships comparison of NCa CMS, Polima CMS and Nap CMS

Restorer-maintainer relationship test was conducted on Polima CMS, Nap CMS and NCa CMS with *B. napus* lines selected from different countries. The results were shown in Table 2.

Table 2 Restorer- maintainer relationship comparison of NCa CMS, Polima CMS and Nap CMS

B.napus lines	Source	PolCMS	NapCMS	NcaCMS	*B.napus* lines	Source l	PolCMS	NapCMS	NcaCMS
Wesmooner	E[1]	S[3]	-	F[4]	Rongyou No.3	A	-	S-F	S-F
Marmnor	E	S	F	F	4483	A	-	S	F
Paster	E	-	F	F	4500	A	-	F	S
Vagvard	E	S	-	S	Cp015	A	F	S	S
2493	E	S	F	F	4610	A	S	F	S
Ruma	E	S	-	S	783	A	-	F	S
Grinmit	E	S	F	S	4780	A	-	F	S
Pubta-sR	E	S	F	F	4725	A	-	S	S
Hettat	E	S-F	F	S	XinDo No.4	A	-	F	F
Sel-w	E	-	S-F[5]	F	5899	A	S	F	S
Yellow seed Sweden	E	F	S	S	ED34	A	S	S	F
8290	A[2]	S	F	F	B1	A	S	F	F
Zhongshuang No.2	A	S	S-F	F	B2	A	S	F	F
Zhongshuang No.3		S	-	F	R1	A	F	F	F
L-5	A	S	F	F	R2	A	F	F	F
Hunanhue	A	F	S-F	F	R3	A	F	F	F
Zhongyou 119	A	S	-	F	R4	A	F	F	F
HL-2	A	F	F	F	R5	A	F	-	S-F
HL-9	A	S	S	F	R6	A	F	-	S-F
HL-10	A	S	-	F	ED47	A	S	-	F
N21		S-F	F	F	227	A	F	F	F
8937-13	A	-	S	S	Zheyou NO.2	A	S	S	
88-2051	A	S	S	S	Huaye	A		S	F
Hanzhong No.3	A	S	S-f	S	Zhong 27	A	F	S	F
Wanyou No.10	A	S	F	F	N9	A	S	F	F

1. E: Materials from Europe; 2. A: Materials from Asia; 3. S: F1 of the testcross was sterile; 4. F: F1 of the testcross was fertile; 5. S-F: F1 of the testcross was semi-fertile.

Among 9 European *B.napus* lines used for testcross, 6 lines demonstrated different performance on fertility restoration between Pol CMS and NCa CMS, which means 66.7% of the tested lines had different restorer-maintainer relation to the two CMS systems. Similarly, using 8 European *B.napus* lines to test the restorer-maintainer relationship between nap CMS and NCa CMS, 3 lines showed differences, accounting for 37.5% of the tested lines. In comparison, among the Asian lines used for testing, the ratio of different performance on fertility restorer-maintenance was 41.7% between NCa CMS and Pol CMS, 59.4% between NCa CMS and nap CMS.

Inheritance of restore genes of NCa CMS

The inheritance of restoration of NCa CMS was studied using three populations. The result was shown in Table 3. The ratio of fertile plants to sterile plants in F2 generation was 3:1, and the ratio in the backcross generations was 1:1. So we concluded that the restoration of NCa CMS was controlled by a single dominant nuclear gene according to Mendelian law.

Table 3 Analyze of Inheritance of restore genes of NCa CMS

Populations	Years	Plant No. Of F or S		E.V	$X^2_{,C}$	P
		No. of male sterile plant	No. of male fertile plant			
a^1	1997	121	43	3:1	0.1641	0.5~0.75
	1998	148	52	3:1	0.4050	0.5~0.75
b^2	1997	64	58	1:1	0.2049	0.5~0.75
	1998	61	56	1:1	0.1367	0.5~0.75
c^3	1997	51	53	1:1	0.0865	0.75~0.90
	1998	76	68	1:1	0.0278	0.75~0.90

a: F2 of NCa CMS restorer; b: NCa CMS (NCa CMS restorer);
c: (NCa CMS restorer) maintainer

DISCUSSION

Although the seedset of rapeseed was affected by bag isolation in self-pollination, the result might not be completely the same as in open pollinated plants but without external pollen. However, the low seedset of NCa CMS after self-pollination compared to that of open-pollination strongly suggested NCa CMS is suitable for hybrid seed production.

The restorer-maintainer relationship determination using *B.napus* lines indicated NCa CMS is different from Pol CMS and nap CMS. Since it was derived from an interspecific cross with *B.napus* as female parent and *B.carinata* as male parent, the cytoplasm should mainly come from *B.napus.* However, recombination of both parental genomes might occur during hybridization and the subsequent sorting. Investigation on the origin of NCa cytoplasm on molecular level is needed to confirm our classification in this study.

ACKNOWLEGEMENTS

The authors are grateful to Prof. Cai Ming for the development of NCa CMS.

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