Breeding of a stable CMS line without trace-pollen by means of improving Pol CMS line in *Brassica napus* L.

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Abstract

The occurrence of trace-pollen is a serious problem existing widely in CMS lines of rapeseed. To breed stable ms line without trace-pollen, a breeding program for improving Pol CMS line using recessive genic ms line and thermo-sensitive genic ms line had been carried out since 1991. A new stable CMS line, named as Xiangyou66A, had been bred successfully. The investigations to the fertility, economic traits and quality of Xiangyou66A showed that it was very stable in different years with 100% completely sterile plants and it had fine economic traits and double-lowed quality. The identification trial indicated that Xiangyou66A had the same restoring-maintaining relationship as the existing Pol CMS lines. Seven restorers which can completely restore the fertility of Xiangyou66A were selected out. The combinations using these restorers crossed with Xiamgyou66A displayed different heterosis in the variety comparing trial. Six of them over-yielded with the increasing rate of 2.28%~19.21% compared with the check, among which three combinations had the increasing rate more than 10%.

Key words: *Brassica napus* L, CMS, trace-pollen, TGMS, genetic improvement

Thermo-sensitivity of CMS lines which causes trace-pollen is always a boring problem in the application of CMS three-lined hybrid in rapeseed. Among the existing CMS systems applied in production, such as Pol, Shan 2A and MI CMS lines, most of them are thermo-sensitive. The previous research [1] testifies that the basic reason of causing trace-pollen on Pol CMS lines is the thermo-sensitive genes existing in the nucleus of the maintainers. Other researches [2-4] show that the ms plants with trace-pollens had better economic traits and higher combining ability than those without trace-pollen, and the thermo-sensitive genes had the similar regulating function to that of restoring gene R_f. Therefore, it is thought that eliminating thermo-sensitive genes to breed the stable ms lines through wide test-crossings and systematic selections is not worth [5].

To breed the stable CMS lines without trace-pollen but with finer economic traits and higher combining ability, some improving studies in view of Pol CMS line had been conducted by using recessive genic ms line and thermo-sensitive genic ms line respectively since 1991, and a new CMS line without trace-pollen had been bred after thirteen years and twelve generations.

1. Materials and Methods

1.1 Materials

Pol CMS line 254A, Z04A which had been bred by means of transposing the nucleus of 254A using Zhongshuang 4 as the maintainer, RG ms two-used line 86AB, TG ms two-used line Xiangyou91S and Xiangyou402S, and 15 test-crossing lines are all from Hunan Crops Research institute. Zhongshuang 4 comes from the Oil Crops Research Institute of the National Academy of Agricultural Sciences, and the check Xiangyou 15 used in the variety comparing trial comes from the Oil Crops Research Institute of Hunan Agricultural University.

1.2 Methods

1.2.1 Establishment of new CMS line

Firstly, a middle line with about 50% completely sterile (CS) plants and 50% partially sterile (PS) plants has been bred using the fertile plants of a RG ms two-used line to transpose the nucleus of Pol CMS line through hybridizing and continuous back-crossings. Then, a ms group with 100% completely sterile plants has been bred through hybridizing and continuous back-crossings using the completely sterile plant from above middle line as the maternal and TG ms two-used line as the back-crossing paternal. Based this ms group, a stable ms line with fine comprehensive traits has been bred using other fine TG ms line as the back-crossing parents. In the breeding procedure, the comprehensive traits such as economic trait and quality trait besides the fertility must be paid attention.

1.2.2 Investigating for the fertility of different ms lines

The fertility of different materials containing newly bred CMS line Xiangyou66A and its maintainer Xiangyou66B, Xiangyou402S, Z04A and its maintainer Zhongshuang4, had been continuously investigated in 2002 and 2003 respectively. The investigation had been carried out once two days on the typically single plants of each material from the beginning stage of flowering. The flowers would be taken down after investigation each time to prolong the flowering stage and increase the number of flowers. The statistics had been made once ten days.

The fertility identification of single flower was conduced according to the methods in reference [6]. Fertility index (FI) is used to show the fertility state of plant group. FI=(1×flower number of grade one+2×flower number of grade two+3×flower number of grade three+4×flower number of grade four+5×flower number of grade five+6×flower number of grade six)/total number of all flowers. The fertility index of plant group is bigger, the sterile degree is lower. It is defined here that the plants with FI more than 5.0 are fertile (F), and the plants with FI from 1.0 to 5.0 are partially sterile (PS) and the plants with FI less than 1.0 are completely sterile (CS).

1.2.3 Identifying for the restoring-maintaining relationship of the new CMS line and evaluating its heterosis

Fifteen source materials were selected to test-cross with Xiangyou66A and Z04A in the spring of 2002. The test-crossing F_1 generations and their corresponding parents were planted in the autumn of this year. The fertility of above F_1 generations was identified in the spring of 2003 to make sure the relationship of xiangyou66A. Some materials with 100% restoring plant ratio to Xiangyou66A in the identification trial were selected out to continue to test-cross with Xiangyou66A in the spring of 2004. The F_1 generations were planted for the variety comparing trial in the autumn. Three repeats were adopted and Xiangyou 15 was used as the check in the trial with each plot of 20 m^2. The planting density is about 180000 plants / hm^2. Fifteen plants in each plot were selected out at the ripe stage for testing their economic traits. The pods of each plot were harvested after they were ripe, and their seeds were dried and weighed in single.

2. The breeding procedure of Xiangyou66A and its main traits

2.1The breeding procedure

The breeding procedure of Xiangyou66A is shown in Fig. 1. From Fig.1, it can be found that there are three stages in the procedure. In the first stage from 1991 to 1994, a middle group "Hecheng A" with about 50% completely sterile plants and 50% partially sterile plants had been gained through hybridization and back-crossings using the fertile plants of 86AB as the back-crossing parent to cross with Pol CMS line 254A. In the second stage from 1994 to 1999, a stable CMS line "Xiangxin A" with 100% completely sterile plants had been got through hybridization and continuous back-crossings using TG ms line Xiangyou91S as the back-crossing parent to cross with the completely sterile plants selected out from "Hecheng A". In the third stage from 2000 to 2003, Xiangyou66A had been bred using another TG ms line Xiangyou66B as the back-crossing parent to cross with "Xiangxin A".

It is worth to point out that Xiangyou66B is the sister line of Xiangyou402S which had been bred by means of hybridizing and systematic breeding from the combination [(Xiangyou91S×Zhongshuang4)×Zhongshuang4] [7]. The difference between them is just that Xiangyou66B has higher critical transforming temperature than Xiangyou402S. In the breeding procedures of two TG ms lines, the processes of early generations from F_1 to F_3 were the same. The selecting goal for the fertility of single plants in high generations after F_4 was different between two TG ms lines. The transforming stage of Xiangyou402S was emphasized the moderateness with transforming critical temperature 14~19°C, while the transforming stage of Xiangyou66B should be late with higher transforming critical temperature. A stable TG ms line had been gained in 2000, which was named as Xiangyou66A because its line number was 99066 in 1999. At the same time for breeding Xiangyou66A, some of its plants had been selected out to cross and back-cross with "Xiangxin A" since 1999, and a stable CMS line without trace-pollen was got in 2003, which was named as Xiangyou66A.

Fig. 1 The breeding procedure of Xiangyou66A

Year	Crossing mode	Generation	Fertility of offspring
1991	254A×86B		PS
1992	F_1×86B	F_1	PS
1993	PS plant×86B	F_1BC_1	PS+CS
1994	CS plant×Xiangyou91S	F_1BC_2 (Hecheng A)	PS+CS
1995	CS plant×Xiangyou91S	$F_1BC_2D_1$	PS+CS
1996	CS plant×Xiangyou91S	$F_1BC_2D_2$	CS
1997	CS plant×Xiangyou91S	$F_1BC_2D_3$	CS
1998	CS plant×Xiangyou91S	$F_1BC_2D_4$	CS
1999	CS plant×Xiangyou66B	$F_1BC_2D_5$ (Xiangxin A)	CS
2000	CS plant×Xiangyou66B	$F_1BC_2D_5$ E_1	CS
2001	CS plant×Xiangyou66B	$F_1BC_2D_5$ E_2	CS
2002	CS plant×Xiangyou66B	$F_1BC_2D_5$ E_3	CS
2003	CS plant×Xiangyou66B	$F_1BC_2D_5$ E_4 (Xiangyou66A)	CS

2.2 The fertility performance of different ms lines

The results of fertility investigation (Table 1) show that the sterility of Xiangyou66A is stable with 100% sterile plant ratio and 100% sterile degree in different years. Its pistils, sepals and honey-body grow normally, but its petals become narrow bars which bases are separate and its stamens become small trigons without pollen. Zhongshuang 4 is completely fertile in whole flowering stage. The sterile plant rates of the plant group of Xiangyou402S, Xiangyou66B and Z04A are all 100%, but their fertilities display different characters with the growing process. At the beginning stage of flowering before 5 March, all flowers of Z04A were partially sterile with FI more than 4.0, and most of the flowers of Xiangyou402S and Xiangyou66B

were partially sterile and a small number of flowers of them were completely sterile with FI more than 3.0 but less than 4.0. At the transition between the beginning stage of flowering and the full blooming stage from 5 March to 25 March, most of the flowers of the three ms lines were partially sterile with a lot of trace-pollens and their FIs were about or more than 4.0. At the full blooming stage from 25 March to 4 April, the trace-pollen of the three ms lines decreased with the temperature increasing, especially that of Z04A decreased apparently and its FI of two years counted in 25 March were both less than 2.0 and became 0 till 4 April, which means that its flowers blooming after 25 March were completely sterile. While Xiangyou66B and Xiangyou402S still kept the state of partially sterility for a long time but the trace-pollens of Xiangyou66B were more and lasted longer time than that of Xiangyou402S. The flowers blooming on Xiangyou402S after 4 April and those blooming on Xiangyou66B after 14 April all became completely sterile.

In terms of sterile degree, that of Xiangyou66A was lowest with FI less than 1.0 in two years, and that of Xiangyou402S and Z04A was lower with average FI of 1.45 and 1.50 respectively, and that of Xiangyou66B was highest with average FI of 2.33.

Table 1 Fertility change of the observed materials in different years (Changsha)

Year	Material	Number of plants	FI							Average FI	Sterile plant ratio in group
			03-05	03-15	03-25	04-04	04-14	04-24	05-04		
2002	Xiangyou66A	5	0.37	0.24	0	0	0	0	0	0.09	100
	Xiangyou66B	5	3.76	4.74	3.83	2.67	1.81	0	0	2.40	100
	Xiangyou402S	5	3.13	4.25	1.34	0.84	0	0	0	1.37	100
	Z04A	5	4.68	4.18	1.77	0	0	0	0	1.52	100
	Zhongshuang4	2	5.54	5.69	5.72	5.53	5.39	5.17	5.33	5.48	0
2003	Xiangyou66A	5	0.41	0.38	0	0	0	0	0	0.11	100
	Xiangyou66B	5	4.05	4.64	3.47	2.19	1.46	0	0	2.26	100
	Xiangyou402S	5	3.47	3.95	2.12	1.17	0	0	0	1.53	100
	Z04A	5	4.27	4.30	1.81	0	0	0	0	1.48	100
	Zhongshuang4	2	5.76	5.47	5.72	5.39	5.24	5.36	5.07	5.43	0

Notes: The sterile plants contain CS and PS plants, and sterile plant ratio is the percent of sterile plants in plant group.

2.2 The economic traits and quality of Xiangyou66A

Xiangyou66A is half-wintered middle-ripe rapeseed, which total growth duration is about 220d when it is planted in the southern region of China in autumn. Its main economic traits tested on 20 plants taken from the isolating field randomly are listed in Table 2. Compared with its maintainer Xiangyou66B, its plant height and branch height are higher and its 1000-seed weight is weightier, but its first branches, pods, seeds per pod and yield of single plant are less than those of the latter.

Table 2 The economic traits of Xiangyou 66A and Xiangyou 66B(Changsha, 2004)

Material	Plant height /cm	Branch height /cm	1st branch No.	Pods of single plant	seeds per pod	1000-seed weight/ g	Yield of single plant/g
Xiangyou66A	194.53	67.70	10.76	374.70	19.65	3.73	17.24
Xiangyou66B	188.40	55.15	12.50	414.35	20.40	3.64	20.51

In the procedure of breeding Xiangyou66B and Xiangyou66A especially in early generations for selecting the single plants, the qualities as well as the fertility and the economic traits had been taken into account and those plants with erucic acid in oil more than 1% and glucosinolate in cake more than 30μmol/g had all been eliminated. According to the analysis results tested the Center of Determination for Oil Crops and Products Quality of Agricultural Administration in 2004, the erucic acid content in seeds of Xiangyou66A taken from the isolating reproduction field was 0.05% and the glucosinolate content was 17.99μmol/g cake.

3. The restoring-maintaining relationship of Xiangyou66A and evaluation for its heterosis

The investigating results for the fertility of test-crossing F_1 generations (Table 3) in 2003 show that, in addition to the known Pol CMS restorers 1030R and Zhong 2R, other four test-crossing lines 9011, 9016, 9024 and 9044 have the completely restoring action to the fertility of Xiangyou66A and Z04A with 100% restoring plant ratio in their F_1 generations, and 9018 has half restoring action to two ms lines with about 50% restoring plant ratio, which means that its restoring genotype is not pure yet. 9022 can completely restore the fertility of Xiangyou66A with 100% restoring plant ratio but can just partially restore that of Z04A with 93.87% restoring plant ratio, which may be caused by pollution in the procedure of pollinating to Z04A. All F_1 generations of other test-crossing lines such as 9007, 9015, 9019, 9021, 9025, 9027 and 9031 with two ms lines are partially sterile, which indicates that these sources are all the maintainers of two ms lines. From the above results, it can be sure that Xiangyou66A and Z04A have the same restorers and the same maintainers.

The results of the variety comparing trial for testing the heterosis of seven combinations with 100% restoring plant ratio in identification trial showed that they had different yield heterosis (Table 4). The analysis of variance indicated that the difference was significant with 1% level between varieties but insignificant between repeats. There are 6 combinations over-yielding compared with the check by 2.28%~19.21%, among which three combinations over-yielded by more than 10%.

Table 3 The fertility of F_1 generations of test-crossing combinations (Changsha, 2003)

Combination	Restoring plant ratio /%	Combination	Restoring plant ratio /%	Combination	Restoring plant ratio /%
XY66A×1030R	100	XY66A×9016	100	XY66A×9024	100
Z04A×1030	100	Z04A×9016	100	Z04A×9024	100
XY66A×Zhong2R	100	XY66A×9018	42.7	XY66A×9025	0
Z04A×Zhong2R	100	Z04A×9018	54.2	Z04A×9025	0
XY66A×9007	0	XY66A×9019	0	XY66A×9027	0
Z04A×9007	0	Z04A×9019	0	Z04A×9027	0
XY66A×9011	100	XY66A×9021	0	XY66A×9031	0
Z04A×9011	100	Z04A×9021	0	Z04A×9031	0
XY66A×9015	0	XY66A×9022	100	XY66A×9044	100
Z04A×9015	0	Z04A×9022	93.87	Z04A×9044	100

Note: XY66A is the abbreviation of Xiangyou66A.

Table 4 The yield of combinations with Xiangyou66A in variety comparing trial (Changsha, 2005).

Combination	Yield in each plot /g			Average yield /g	Average yield In unit/kg.hm^2	Increasing (or decreasing) rate/%	Difference significance	
	I	II	III				0.05	0.01
XY66A×1030R	6027.5	5837.8	5885.4	5916.9	2958.45	14.55	b	AB
XY66A×中 2R	5488.1	5712.4	5641.6	5614.0	2806.95	8.68	cd	C
XY66A×9011	5369.4	5197.8	5281.5	5282.9	2641.50	2.28	e	DE
XY66A×9016	6093.6	6237.5	6142.3	6157.8	3078.90	19.21	a	A
XY66A×9022	5496.7	5638.5	5433.4	5522.9	2761.95	6.94	d	CD
XY66A×9024	5114.5	5094.6	4841.7	5016.9	2508.45	-2.87	f	E
XY66A×9044	5836.2	5787.9	5757.4	5793.8	2896.95	12.17	bc	BC
Xiangyou15(CK)	4983.5	5214.8	5165.3	5165.3	2582.70	0	ef	E

4. Discussion and summary

Successful breeding of Xiangyou66A means that it is feasible to breed stable CMS line without trace-pollen by means of improving Pol CMS line using thermo-sensitive genic ms lines, which is based on the following points: 1) The thermo-sensitive genes existing in the nucleus of Pol CMS lines and thermo-sensitive genic ms lines are all regulating genes whose functions are realized through regulating the fertility of ms lines, and above two kinds of thermo-sensitive genes are not allele. The furthermore studies have testified that the thermo-sensitive genes in Pol CMS lines can not regulate the recessive ms genes. 2) Stable completely sterile group can be gained through combining the cytoplasm of Pol CMS line and recessive ms genes. 3) Polima sterile cytoplasm can restrain the expression of thermo-sensitive genes in thermo-sensitive genic ms lines. Therefore, the improved groups with the Polima cytoplasm and the nucleus of thermo-sensitive genic ms lines are completely sterile. However, this restraining action of sterile cytoplasm can be released by the restoring gene R_f, which causes the expression of thermo-sensitive genes again, thus, the improved CMS lines by using those thermo-sensitive genic ms lines with R_f genes. Above three points will be reported in other studies.

From the breeding procedure of Xiangyou66A, it can be found that the thermo-sensitive maintainer is the key for establishing the stable CMS system without trace-pollen. In the procedure of breeding new thermo-sensitive maintainer, the selection of parent is very important. The combining ability of parent should be the emphasis to be taken account except its economic traits and qualities. According to the character of new CMS system which has the same restoring-maintaining relationship as Pol CMS lines, the parents has better to be selected among the maintainers of Pol CMS lines especially those which can cause a lot of trace-pollens. The restorers should be avoided to be selected lest trace-pollen occur in new lines. Meanwhile, those thermo-sensitive single plants transforming late from fertility to sterility should be selected in early generations which may be beneficial to the reproduction of new ms lines. The breeding method for the restorers of new CMS system is the same as that for Pol CMS restorers, and the practical technologies such as reproduction of parents and hybrid seed production are also the same as those of Pol CMS system.

The results of the variety comparing trial showed that the combinations with Xiangyou66A had fine yield performance. Six of seven combinations over-yielded compared with the check, among which the increasing rates of three combinations were more than 10%. Therefore, Xiangyou66A is a fine ms line with high practical value.

References

[1] Yang G S, Fu T D, Yang×N, et al. Studies on the ecotypical male sterile line of *Brassica napus* L.I.Inheritance of the ecotypical male sterile line [J]. Acta Agronomica Sinica, 1995, 21(2): 129-135.

[2] Chen S Y, Guan C Y, Tian S L, et al. Relationship of trace pollen with agronomic characters in a cytoplasmic male sterile line of rapeseed (*Brassica napus*). Proc of 11th Intern Rapeseed Cong., Copenhagen-Denmark, 2003.

[3] Zhu G.. Effect of Shan 2A (CMS) with micropollen on vegetative growth and agronomic characters of its plants [J]. Guizhou Agricultural Sciences, 1999, 27 (5): 19-23.

[4] Yang G S, Fu T D. Development of super-hybrid by ecotypical cytoplasmic male sterility in *Brassica napus* L. Proc of 11th Intern Rapeseed Cong., Copenhagen-Denmark, 2003.

[5] Fu T D. Acceptance lecture GCIRC superior scientific award. Proc of 8th Intern Rapeseed Cong, 1991, 1:6-11.
[6] Yang G S, Fu T D. A preliminary study on the restoring-maintaining relationship of cytoplasmic male sterility in rapeseed (*Brassica napus and Brassica campestris*)[J]. Acta Agronomica Sinica, 1991, 17(2):151-156.
[7] Wu×M, Xi D W, Ning Z L, et al. Breeding of thermo-sensitive genic ms line Xiangyou402S in *Brassica napus*[J]. Chinese Journal of Oil Crop Science. 2005, 27(4): 1-6.