The genetic base of establishing stable ms lines by means of improving Pol cms line in *Brassica napus* L.

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Abstract

The genetic characters of three RG ms two-used lines Xiangyou402AB, 104AB and 127AB, and four TG ms lines Xiangyou402S, 022S, 104S and 1030RS have been studied. The results united with the previous studies on the inheritance of 104AB and 104S show that the ms genes from Xiangyou402A, 104A and 127A are not allele between each other, and Xiangyou402A and 127A have two couples of ms genes respectively, and 104A has a couple of ms gene. Among four TG ms lines, Xiangyou402S, 022S and 1030RS have the same two couples of ms genes and a couple of mainly effective thermo-sensitive genes modified by some minim quantity genes all from Xiangyou91S, while 104S has a couple of ms genes and a couple of mainly effective thermo-sensitive genes modified by some minim quantity genes which are not the allele with that from the former three TG ms lines. Between RG and TG ms lines, Xiangyou402A has the allelic ms genes from Xiangyou402S, 022S and 1030RS, but the ms genes of 127A is not allelic with that of 022S, and 104A has the allelic ms genes from 104S. The results also show the thermo-sensitive genes from several TG ms lines have not allelic relationship with that from Pol cms line Z04A or its maintainer Z04B. Furthermore, all thermo-sensitive genes from different sources can just regulate the corresponding ms genes or sterile cytoplasm but can not regulate other ms genes. Six ms groups have been got by means of improving Z04A using the fertile plants of three RG ms lines and three TG ms lines as the back-crossing parents, among which one group has about 75% partially sterile plants and about 25% completely sterile plants, and two groups have about 50% partially sterile plants and 50% completely sterile plants, and two groups have 100% completely sterile plants and one group has 100% partially sterile plants that has been bred using TG ms line with restoring gene R_f. The genotypes of above ms lines and the gained ms groups are deduced, and a "circular regulation mode" is put forward to explain the genetic mechanism of establishing stable cms lines without trace-pollen at the state of different thermo-sensitive genes existing.

Key words: *Brassica napus*, Pol cms, trace-pollen, genetic improvement, genetic mechanism

Introduction

Cms three-lined method is one of the most important approaches of heterosis utilization in rapeseed. There are many types of cms sources such as Pol cms, Shan2A, nap cms, MI cms and Ogu cms and tour cms, et al, among which Pol cms is the main one applied in production. However, most of Pol cms lines used nowadays in production is unstable with the changing of environmental temperature [1-3]. Trace-pollens will occur when these ms lines under the condition of low temperature, which can greatly influence the purity of hybrid in the process of seed production and decrease the heterosis of F_1[4,5].

The previous study shows that the basic reason of trace-pollen occurrence is the thermo-sensitive (T_s) genes existing in the nucleus of Pol cms lines [6]. One of the ways to solve the problem of trace-pollen is to breed the stable ms lines without thermo-sensitive genes by means of a large amount of test-crossings and systematic selections. Nevertheless, some researchers reported that the ms plants with trace-pollens had better economic traits and higher combining ability than those without trace pollen [7, 8], and the further research showed that T_s genes had the similar regulating function to that of restoring gene R_f [9]. Therefore, breeding the stable ms lines without Ts genes means that the loss overweighs the gain [10]. Yang G S, et al [11] put forward a solution to decrease or eliminate the influence of trace-pollens by means of improving Pol cms lines using recessive genic (RG) or dominant genic (DG) ms materials at the premise that the economic traits and combining ability of ms lines are not influenced, and some stable ms lines which contain Pol cms plants with trace-pollens and completely sterile plants have been bred [12, 13]. Xi D W put forward to breed completely sterile lines through improving Pol cms lines using thermo-sensitive genic (TG) ms materials [14]. A stable ms line without trace-pollen, which is named as Xiangyou66A, has been bred accord to this proposal [15], which indicates that it is feasible to breed the stable completely cms line with thermo-sensitive genes through improving Pol cms line. To make sure the genetic mechanism of establishing stable cms system without trace-pollen, some genetic and breeding studies have been carried out and the results are reported here.

1 Materials and Methods

1.1 Materials

Pol cms line Z04A which is thermo-sensitive and has a lot of many trace pollens under the condition of low temperature, and its maintainer Z04B and its restorer 1030R; RG ms two-used line Xiangyou402AB, 104AB and 127AB; TG ms line Xiangyou402S, 022S which has been bred from the separating offspring of combination [(Xiangyou91S×127B)×127B], 104S

and 1030RS with restoring gene R_f which has been bred from the separating offspring of combination [(Xiangyou91S×1030R)×1030R], are all from Crops Research Institute of Hunan Academy of Agricultural Sciences.

1.2 Methods

1.2.1 Analysing for the inheritance of different recessive ms genes and thermo-sensitive genes

In another research [16], the genetic characters of 104S were studied. The results showed that the fertility of 104S was jointly controlled by a couple of recessive ms genes and a couple of mainly effective thermo-sensitive genes modified by some minim genes, and its ms genes and thermo-sensitive genes were not the allele of those of Xiangyou402S. To make sure the inheritance of other genic ms lines, the fertility of F_1 and F_2 generations of the following test-crossing combinations was investigated in 2004 and 2005 respectively: (Xiangyou402A×Z04B), (127A×Z04B), (Xiangyou402S×Z04B), (022S×Z04B) and (1030RS×Z04B). The fertility was identified according to the methods in reference [17]. Average fertility index (AFI) is used as the fertility index of plant group. AFI=(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 AFI more than 5.0 are fertile (F), and the plants with AFI from 1.0 to 5.0 are partially sterile (PS) and the plants with AFI less than 1.0 are completely sterile (CS).

1.2.2 Analysing for the allelism of different recessive ms genes and thermo-sensitive genes

From the breeding procedure of different ms lines, it can be sure that 022S and 1030RS and Xiangyou402S [18] have the same source of thermo-sensitive genes from Xiangyou91S. Meanwhile, Z04A and Z04B also have the same thermo-sensitive genes causing the trace-pollen in Z04A. The allelism of thermo-sensitive genes between Z04B and those TG ms lines can defined based on the studies of item 1.2.1. To make sure the relationship of recessive ms genes and thermo-sensitive genes from different sources, furthermore mutual-test-crossings between different ms lines were undertaken in 2004: (Xiangyou402A×127B), (127A×104B), (Xiang402A×022S), (127A×Xiangyou402S), (104A×104S), (127A×104S), (104A×022S), (127A×022S), (127A×1030RS), (104A×1030RS), (022S×1030RS), (XY402A×XY402S), (Xiangyou402S×1030RS) and (022S×104S). The fertility of all test-crossing F_1 generations was investigated in 2005.

1.2.3 Improving Pol cms line using different genic ms lines

The nucleus of Pol cms line was transposed by three RG ms lines and three TG ms lines respectively. The hybridizations and continuous back-crossings were conducted using Z04A as the maternal and the following materials as the back-crossing fathers: Xiangyou402B, 127B, 104B, 022S, 104S and 1030RS. In the procedure of breeding, once completely sterile plants were found, they would be selected as the back-crossing mothers, and so would be done in next each generation.

2 Results

2.1 Inheritance of different genic ms lines

Z04B is a stable inbreeding line with fine economic traits and double-lowed quality. It has been determined that Z04B has thermo-sensitive gene T_s leading to a lot of trace-pollens in the breeding procedure of Z04A. In the F_1 generations of the test-crossing combinations with RG and TG ms lines using Z04B as the paternal, no sterile plant has been found (Table 1). In the F_2 generations of all combinations, the plants with different fertility displayed a distributing proportion of 15 fertile plants to 1 sterile plants, which indicates that Xiangyou402A, 127A, Xiangyou402S, 022S and 1030RS all have two couples of recessive ms genes based on the analysis of χ^2. No partially sterile plant was found among the offspring of combination (XY402A×Z04B) and (127A×Z04B), that means the thermo-sensitive genes of Z04B can not influence the sterility of Xiangyou402A and 127A. On the other hand, the sterile plants from the combinations with several TG ms lines displayed a distributing proportion of 3 PS plants to 1 CS plants, which means that TG ms lines Xiangyou402S, 022S and 1030RS have a couple of thermo-sensitive genes.

Table 1 The fertility performance of the offspring from different combinations (Changsha)

	F_1 (2004)			F_2 (2005)									
Combination	F	PS	CS	F	PS	CS	F: (PS+CS)	Expected proportion	χ_c^2	$\chi^2_{0.05,1}$	PS:CS	Expected proportion	χ_c^2	$\chi^2_{0.05,1}$
XY402A×Z04B	116	0	0	574	0	31	574:31	15:1	1.1241	3.84	0	0		
127A×Z04B	123	0	0	565	0	28	565:28	15:1	2.1101	3.84	0	0		
XY402S×Z04B	105	0	0	1147	44	17	1147:61	15:1	2.7691	3.84	44:17	3:1	0.1366	3.84
022S×Z04B	127	0	0	1135	45	16	1135:61	15:1	2.5052	3.84	45:16	3:1	0.0055	3.84
1030RS×Z04B	94	0	0	1093	42	17	1093:59	15:1	2.3148	3.84	42:17	3:1	0.2768	3.84

Notes: XY402A and XY402S is the abbreviation of Xiangyou402A and Xiangyou402S respectively.

Furthermore, various types of plants with different sterile degree were found among PS plants of three combinations with three TG ms lines. They can be classified as three types according to their transforming stage from fertility to sterility: 1) early type: turning before 20 March with AFI more than 1.0 but less than 2.0; 2) middle type: turning at the stage from 20 March to 10 April with AFI from 2.0 to 3.0; 3) late type: turning after 10 April with AFI more than 3.0 but less than 4.0. The distribution of PS plants of different types in three combinations displays the normal distribution, in which the proportion of middle type of plants is highest. This result indicates that these thermo-sensitive genes are modified by some quantitatively minim genes.

Table 2 Distribution of PS plants with different transforming stage in F_2 generations of combinations with TG ms lines

Combination	Turning early			Middle type			Turning late		
	number	rate%	AFI	number	rate%	AFI	number	rate%	AFI
XY402S×Z04B	7	15.91	1.14	26	59.09	2.37	11	25.0	3.71
022S×Z04B	11	24.44	1.87	25	55.56	2.92	9	20.0	3.65
1030RS×Z04B	5	11.90	1.52	20	47.62	2.54	12	28.57	3.47

2.2 Allelism between different recessive ms genes and thermo-sensitive genes

Table 3 shows the fertility of F_1 generations of mutual-test-crossing combinations with several RG and TG ms lines. Partially sterile plants were found in five combinations: (XY402A×022S), (104A×104S), (022S×1030RS), (XY402A×XY402S), (XY402S×1030RS), which means that Xiangyou402A and 022S have the allelic ms genes, and Xingyou402S and 022S and 1030RS have the allelic ms genes and allelic thermo-sensitive genes originating from Xiangyou91S. Meanwhile, only fertile plants were found in other combinations, which presents the following points: (1) There is no allelic relationship between the ms genes of Xiangyou402A and 127A and 104A respectively. (2) The ms genes of 127A and 022S are not allele and the thermo-sensitive genes of 022S can not regulate the fertility of 127A. (3) The thermo-sensitive genes of 104S are not the allele of that from 022S and 1030RS. Therefore, the thermo-sensitive genes of 104S can not regulate the fertility of 127A and the thermo-sensitive genes of 022S and 1030RS can not regulate the fertility of 104A.

Table 3 The fertility performance of mutual-test-crossing F_1 generations (Changsha, 2005)

Combination	Fertile plants	PS plants	CS plants	Combination	Fertile plants	PS plants	CS plants
XY402A×127B	127	0	0	127A×022S	115	0	0
127A×104B	104	0	0	127A×1030RS	122	0	0
XY402A×022S	0	116	0	104A×1030RS	106	0	0
127A×XY402S	113	0	0	022S×1030RS	0	132	0
104A×104S	0	121	0	Y402A×XY402S	0	114	0
127A×104S	121	0	0	XY402S×1030RS	0	117	0
104A×022S	126	0	0	022S×104S	117	0	0

2.3 The breeding progress of improving Pol cms line using different genic ms materials

2.3.1 The effect of improving Pol cms line using RG ms two-used lines

The fertile plants from three RG ms two-used lines had been selected out as the back-crossing parents to improve Pol cms line since 1997, 1998 and 2001 respectively. Three ms groups with different fertility were gained. It can be found from Table 4 that, it took different time to get completely ms plants and relatively stable ms groups through continuous back-crossings in three programs (Table 4).

Table 4 The breeding procedure through RG ms lines (Changsha)

Program I			Program II			Program III		
Year	Generation	Fertility	Year	Generation	Fertility	Year	Generation	Fertility
1998	Z04A×XY402B	100%PS	1997	Z04A×127B	100%PS	2001	Z04A×104B	100%PS
1999	F_1×XY402B	100%PS	1998	F_1×127B	100%PS	2002	F_1×104B	100%PS
2000	F_1BC_1×XY402B	100%PS	1999	F_1BC_1×127B	78.3%PS+21.7%CS	2003	BC_1×104B	76.2%PS+23.8%CS
2001	F_1BC_2×XY402B	100%PS	2000	F_1BC_2×127B	55.8%PS+44.2%CS	2004	F_1BC_2×104B	54.7%PS+45.3%CS
2002	F_1BC_3×XY402B	97.4%PS+2.6%CS	2001	F_1BC_3×127B	53.5%PS+46.5%CS	2005	Group III	52.4%PS+47.6%CS
2003	F_1BC_4×XY402B	78.7%PS+21.3%CS	2002	F_1BC_4×127B	57.6%PS+42.4%CS		PS: CS	52.4: 47.6
2004	F_1BC_5×XY402B	73.2%PS+26.8%CS	2003	F_1BC_5×127B	55.9%PS+44.15%CS		Expected proportion	1: 1
2005	Group I	76.9%PS+23.1%CS	2004	F_1BC_6×127B	52.3%PS+47.7%CS		χ_c^2	0.038
	PS: CS	76.9: 23.1	2005	Group II	46.8%PS+53.2%CS		$\chi^2_{0.05,1}$	3.84
	Expected proportion	3: 1		PS: CS	46.8: 53.2			
	χ_c^2	0.1045		Expected proportion	1: 1			
	$\chi^2_{0.05,1}$	3.84		χ_c^2	0.054			
				$\chi^2_{0.05,1}$	3.84			

In program I, it had taken five years and four generations to get CS plants, and eight years and seven generations to get group I with 76.9% PS plants and 23.1% CS plants. χ^2 test shows this distributing proportion in groupIaccords with three to one. According to this point and the genetic characters of Xiangyou402A analyzed in 2.1, the genotype of Xiangyou402B can be deduced as: N ($M_{s1}M_{s1}m_{s1}m_{s1}$) From the breeding procedure, it can be found that the ms group with about 75% PS plants and 25% CS plants had been obtained after five generations upon to 2003. The similar proportion was kept along with the next back-crossing generations.

In programII, it had taken three years and two generations to get CS plants, and nine years and eight generations to get groupIIwith 46.8% PS plants and 53.2% CS plants. χ^2 test shows the proportion in groupIIaccords with one to one. According

to the genetic characters of 127A, the genotype of 127B can be deduced as: N ($M_{s2}m_{s2}m_{s2}m_{s2}$) The ms group with about 50% PS plants and 50% CS plants was obtained after three generations in 2000.

In programIII, it had taken three years and two generations to get CS plants, and five years and four generations to get groupIII with 52.4% PS plants and 47.6% CS plants. χ^2 test shows this proportion in groupIII also accords with one to one. According to the genetic characters of 104A, the genotype of 104B can be deduced as: N ($M_{s3}m_{s3}$). The ms group with about 50% PS plants and 50% CS plants was obtained after three generations in 2004.

2.3.2 The effect of improving Pol cms line using TG ms two-used lines

In program IV, V and VI, three TG ms lines had been used to improve Pol cms line respectively since 2000 and 2001. Three stable ms groups were gained, among which both of group IV and V were completely sterile, and group VI was partially sterile with trace-pollens, which means that CS plant can not be got when the restoring gene R_f exists. In programIV, 22.6% CS plants were found in F_1BC_1, but a small number of PS plants were still found in next back-crossing generations and the CS group was not got until F_1BC_4 in 2005. In programV, 41.9% CS plants were found in F_1BC_1, and the CS group was got just in F_1BC_2. This presents that programV is more effective than programIV. This result maybe has something to do with the number of ms genes from 022S (with two couples of ms genes) and 104S (with one couple of ms genes). On the other hand, the fertility performance of each back-crossing offspring in program IV and V also indicates that 022S and 104S have not R_f genes.

Table 5 The breeding procedure of improving Pol cms line using TG ms lines (Changsha)

ProgramIV			ProgramV			ProgramVI		
Year	Generation	Fertility	Year	Generation	Fertility	Year	Generation	Fertility
2000	Z04A×022S	100%PS	2002	Z04A×104S	100%PS	2000	Z04A×1030RS	100%PS
2001	F_1×022S	100%PS	2003	F_1×104S	100%PS	2001	F_1×1030RS	F
2002	F_1BC_1×022S	77.4%PS+22.6%CS	2004	F_1BC_1×104S	58.1%PS+41.9%CS	2002	F_1BC_1×1030RS	53.4%F+46.6%PS
2003	F_1BC_2×022S	7.3%PS+92.7%CS	2005	Group V	100%CS	2003	F_1BC_2×1030RS	100%PS
2004	F_1BC_3×022S	2.1%PS+97.9%CS				2004	F_1BC_3×1030RS	100%PS
2005	Group IV	100%CS				2005	Group VI	100%PS

According to the fertility performance of each back-crossing offspring in six programs, it can also be determined that all the back-crossing fathers have T_s of Z04A and Z04B .

3 Summary and discussion

3.1 About the inheritance and allelism of recessive ms genes and thermo-sensitive genes from different sources.

According to the above genetic analysis to several RG ms lines and TG ms lines and the previous studies on the genetic base of 104S and 104AB [16], it can be concluded as the following points:

(1) All of the ms genes from Xiangyou402A, 127A and 104A are not allele. The ms genes from 127A and 022S are not allele too, which means that the ms genes from 127A have been eliminated through selection in the procedure of breeding 022S. The sterility of Xiangyou402A and 127A is controlled by two couples of recessive ms genes respectively, and the sterility of 104A is controlled by a couple of recessive ms genes.

(2) The ms genes and thermo-sensitive genes of Xiangyou402S, 022S and 1030RS are allele originated from Xiangyou91S. The thermo-sensitive genes from Xiangyou402S and 104S are not allele, and they all contain a couple of mainly effective genes modified by some quantitatively minim genes. These thermo-sensitive genes can just regulate the fertility of their corresponding ms lines but can not regulate that of others. Meanwhile, there is no allelic relationship between these genes and the thermo-sensitive gene T_s from Z04A and Z04B. Furthermore, the above several TG ms lines also have T_s besides their own thermo-sensitive genes.

(3) Presuming the thermo-sensitive gene from Z04A as T_{s1} and that from Xiangyou402S and 104S as T_{s2} and T_{s3}, and their corresponding recessive genes as t_{s1}, t_{s2} and t_{s3} respectively, the genotypes of several ms lines used in this study can be deduced as follows:

Z04A: S ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}t_{s3}t_{s3}\ldots$);

Xiangyou402A: N ($m_{s1}m_sm_{s1}m_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}r_fr_f\ T_{s1}T_{s1}t_{s2}t_{s2}\ t_{s3}t_{s3}\ldots$);

127A: N ($M_{s1}M_{s1}\ M_{s1}M_{s1}m_{s2}m_{s2}m_{s2}m_{s2}M_{s3}M_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}\ t_{s3}t_{s3}\ldots$);

104A: N ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}m_{s3}m_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}\ t_{s3}t_{s33}\ldots$);

Xiangyou402S and 022S: N ($m_{s1}m_{s1}m_{s1}m_{s1}M_{s1}M_{s1}M_{s1}M_{s1}M_{s1}M_{s1}M_{s1}r_fr_fT_{s1}T_{s1}T_{s2}T_{s2}\ t_{s3}t_{s3}\ldots$);

1030RS: N ($m_{s1}m_{s1}m_{s1}m_{s1}M_{s2}M_{s2}M_{s1}M_{s2}M_{s2}M_{s3}M_{s3}R_fR_fT_{s1}T_{s1}T_{s2}T_{s2}\ t_{s3}t_{s3}\ldots$);

104S: N ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}m_{s3}m_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}T_{s3}T_{s3}\ldots.$).

3.2 About the effectiveness of improving Pol cms line using RG and TG ms lines and the genotype of the gained ms groups.

From the breeding procedure of six programs, it can be found that it is feasible to decrease or even to eliminate trace-pollen using RG and TG ms materials to improve Pol cms line. Comparing above six ways, it seems that the programs of using TG ms lines are more effective than that of using RG ms maintainers considering the fertility performance of the gained ms groups and the time taken in back-crossings. Nevertheless, the result is not optimistic when the TG ms lines with R_f genes such as 1030RS are used as the back-crossing parents because their back-crossing offspring still have trace-pollens. The

original plan of breeding 1030RS and using it to Pol cms line was to breed stable ms lines without trace-pollen but with wider restoring resources like RG or TG ms lines. This proposal seems to be impossible now. Therefore, the restorers of Pol cms line should be avoided as the back-crossing parents in the procedure of breeding the thermo-sensitive maintainers.

According to above deductions for the genotypes of different ms lines, the genotypes of the gained groups can also be deduced:

Group I: 75% PS plants [S (M_{s1}---$M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}t_{s3}t_{s3}\ldots$)] + 25% CS plants [S ($m_{s1}m_{s1}m_{s1}m_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}$ $r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}$ $t_{s3}t_{s3}\ldots$)];

Group II: 50% PS plants [S ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}$---$M_{s3}M_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}$ $t_{s3}t_{s3}\ldots$)] + 50% CS plants [S ($M_{s1}M_{s1}M_{s1}M_{s1}m_{s2}m_{s2}$ $m_{s2}m_{s2}$ $M_{s3}M_{s3}r_fr_f$ $T_{s1}T_{s1}t_{s2}t_{s2}$ $t_{s3}t_{s3}\ldots$)];

Group III: 50% PS plants [S ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}$-$r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}$ $t_{s3}t_{s3}\ldots$)] + 50%CS plants [S ($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}m_{s3}m_{s3}r_fr_fT_{s1}T_{s1}t_{s2}t_{s2}$ $t_{s3}t_{s3}\ldots$)];

Group IV: 100% CS plants [S($m_{s1}m_{s1}m_{s1}m_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}r_fr_f$ $T_{s1}T_{s1}T_{s2}T_{s2}$ $t_{s3}t_{s3}\ldots$)];

Group V: 100% CS plants [S($M_{s1}M_{s1}M_{s1}M_{s1}M_{s2}M_{s2}M_{s2}M_{s2}m_{s3}m_{s3}r_fr_f$ $T_{s1}T_{s1}t_{s2}t_{s2}T_{s3}T_{s3}\ldots$)];

Group VI:100%CS plants[S ($m_{s1}m_{s1}m_{s1}m_{s1}M_{s2}M_{s2}M_{s2}M_{s2}M_{s3}M_{s3}R_fR_fT_{s1}T_{s1}T_{s2}T_{s2}$ $t_{s3}t_{s3}\ldots$)].

3.2 About the genetic mechanism of establishing stable cms lines without trace-pollen.

Two ms groups with 100% completely sterile plants have been gained responding to the breeding of Xiangyou66A. From the deduced genotype of groupIV and groupV, it is known that both of the groups have pure recessive ms genes and at least two types of thermo-sensitive genes. Why can the stable cms lines without trace-pollen be got at the state of thermo-sensitive genes existing and what relationship is between different genes and the cytoplasm? A "circular regulation" mode is put forward to explain the genetic base of cms lines without trace-pollen, of which the main points are listed as follows:

(1) The occurrence of trace-pollens of Pol cms lines is caused by the thermo-sensitive gene "Ts_1" in the nucleus. That is to say, "T_{s1}" can regulate the expression of sterile cytoplasm "S".

(2) "S" and "T_{s1}" can not regulate the expression of recessive ms genes. Therefore, the plants with pure recessive ms genes display complete male-sterility even though they are in the condition with "S" and "T_{s1}".

(3) Thermo-sensitive genes like "T_{s2}" and "T_{s3}" can regulate the expression of their corresponding recessive ms genes to result in the normal or trace pollens in the condition of normal cytoplasm. The regulating function will be restrained by "S" in the condition of sterile cytoplasm. Consequently, the plants with "S" and thermo-sensitive gene "T_{s1}" and "T_{s2}" or "T_{s1}" and "T_{s3}" display complete sterility. However, this restraining action of "S" can be released by restoring gene "R_f", thus trace-pollen occurs again.

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