

# Genetic analysis and applied studies on three recessive GMS lines in *Brassica napus* L.

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## Abstract

In recent years, studies were undertaken on three recessive GMS lines for use in the breeding of double low hybrid rapeseed, whose fertility was controlled by the interaction of two pairs of recessive duplicate sterile genes and one pair of recessive epistatic inhibitor genes. The result showed that: The sterility of the three recessive GMS lines was very thorough, and was not affected by environment. Hybrids were very pure, and hybrid production from this kind of GMS was easy. The selection of the homozygous two-type line was the foundation, and the selection of the temporary maintainer was key. In order to quicken the breeding process, the best way is to screen and analyze the genotypes by investigating segregation ratios of the crossed and selfed generations. Three recessive GMS lines had wide restoring resource, which allowed strong heterotic combinations to be selected in broad range. The identification of more temporary maintainer line may enrich the genetic basis of the sterile line. The three recessive GMS lines are very valuable for the development of hybrid varieties in *B.napus* because their sterility was very complete, their restores were very wide and hybrid seed production was easy.

**Key words:** *B.napus* L.—three recessive GMS—recessive epistasis—temporary maintainer line—all sterile line

## INTRODUCTION

Because the problem of high yield and high quality of rapeseed had been solved by heterosis utilization, the area of double low hybrid rapeseed were rapidly expanded in China(Fu,1995). At present approaches of heterosis utilization in rapeseed has CMS, double dominant GMS and double recessive GMS. Though they themselves had some advantages and disadvantages, they were released with large area in production(Zhang,et al,2002). It was worth paying attention that the three recessive GMS (TRGMS) whose fertility was controlled by interaction of two pairs of recessive duplicate sterile genes and one pair of recessive epistatic inhibitor genes was discovered in the early of the 1990s in China(Chen et al,1994,1998). TRGMS had thorough sterility and rich restorers, and may depend on temporary maintainer line to realize the three-line model in hybrids production. It was a very good approach for heterosis utilization. Our research progress in TRGMS will be introduced in this paper.

## MATERIALS AND METHODS

**Materials** Original TRGMS materials for the trial were an all-sterile line 4258A, two hybrids C022 and 5C21, which were from Mr. Fengxiang Chen(1996), and test-crossed materials were double-low hybrid, conventional varieties and stable self-line from our institute.

**Methods** Through investigating fertility of the cross segregation generations in blooming stage and doing  $\chi^2$ -test and analyzing the genotype of the parent materials, homozygous two-type line, temporary maintainer, all-sterile line and restorer were selected in order to screen cross combinations with strong heterosis.

## RESULTS

### Selection of double-low homozygous two-type line (HTL)

From the analysis of TRGMS genetic development, if there was not ready-made homozygous two-type line, all-sterile line 4258A would be as female parent and double-low rapeseed varieties with good agronomical characters or self line as male parent to get combinations. Self-cross of single plant was made to new hybrids and original hybrids C022 and 5C21 in big group plants, and their progenies fertility were investigated and segregation ratio was done  $\chi^2$ -test and the strains whose segregation ratio was 3:1 between fertile plants and sterile plants were selected, and 6~10 fertile plants were bagged for self-crossing, at the same

time pairs test-crosses were made(GMS plant × fertile plant) in the line. Next year, the selfing plants and corresponding test-crossed combinations were sown. The fertility of selfing plants was investigated in blooming stage, if progeny of selfing plant was complete fertile plant, the selfing plant and corresponding test-crossed combination would be eliminated, if it was still 3:1 segregation ratio, the corresponding test-crossed combinations would be investigated, if its fertile segregation ratio was 1:1, the test-crossed combination maybe was homozygous two-type line(HTL), and then, sibling crosses were made to the combination, the truth of HTL would be verified again by test-cross with temporary maintainer as male parent. When the stable HTL were gotten, the quality and agronomic traits of fertile plants were selected in the line.

**Optimum method for the temporary maintainer line(TML)**

Screening TML was the key for TRGMS utilization, but from a large number of test-crossed combinations, TML were not yet discovered in other rapeseed materials, so the mutant gene "d" in all-sterile line 4258A was used to get TML from test-crossed progenies or selfed progenies of hybrids. According to gene segregation development, there were various theoretical probability 0, 1/64, 1/16, 1/8 and 1/4 etc. for getting TML. Three suitable models (Fig.1) for TML were selected, in them model1 was optimum. In 2001, 11 TMLs were selected, in them, 4 TMLs were gotten by model 1, 3 TMLs by model 2, 2 TMLs by model 3 and 2 TMLs by other models for proving the correctness and practicality of the three kinds of models. It is very difficult in selecting TML for using TRGMS, so it was the first important problem to solve.

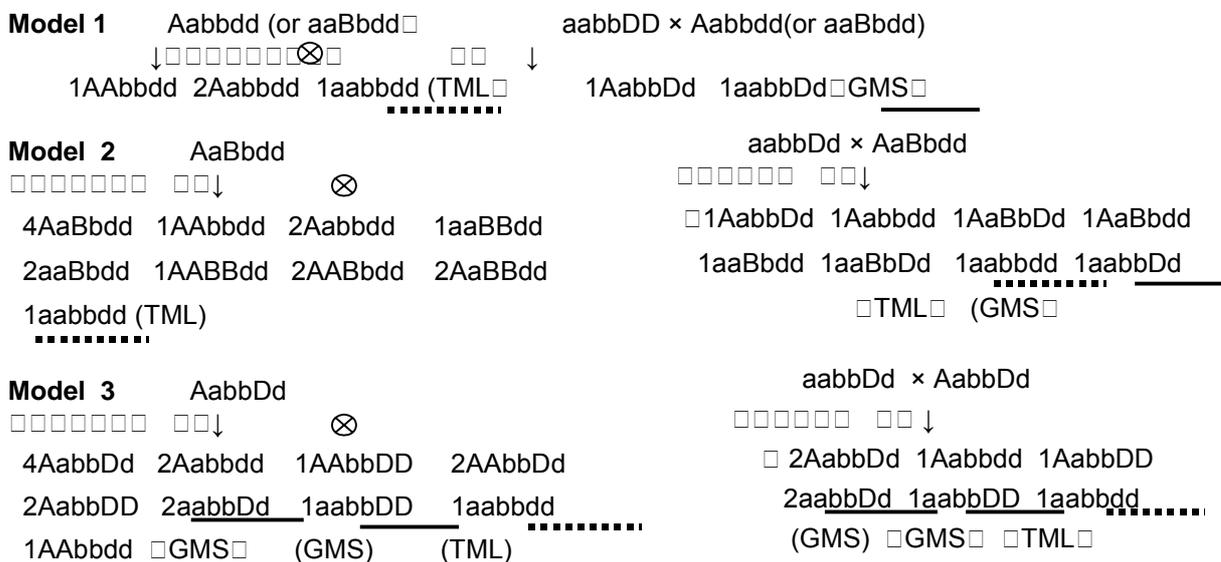


Fig. 1 Optimum genetic model of selecting temporary maintainer line for TRGMS

**Selection of the double low all-sterile line**

Double low all-sterile line (aabbDD X aabbdd → aabbDd) was gotten through crossing sterile plants of double low HTL and double-low TML. Because agronomic characters and combining ability of all-sterile line affected directly heterosis, it will be important to screen all-sterile line with good agronomic characters and high combining ability.

**Selection of TRGMS restorer.**

From the genetic analysis, TRGMS has 15 all-restoring genotypes that can restore fertility completely. When all-sterile line was gotten, a large number of test-crossing combinations were made. In 2001, 59 *B.napus* of different types were tested for 126 combinations (table 1). The results of fertility investigation indicated that 96.8% combinations were restored fully, this showed that corresponding test-crossed male parents were restorer. 51 of 59 test-crossed male parents were from stable double-low rapeseed varieties in *B.napus* at home and abroad, and other 8 from CMS hybrids of China, so restoring resources of TRGMS were very wide, and its hybrids production was very easy, and the demand of the isolation condition was lower than that of CMS, therefor, the risk of hybrid production was reduced greatly.

Table 1 Fertility expression of test-crossed combinations for TRGMS

Type of test-crossed male parent	No. of Test-crossed male parent	No. of test-crossed combinations	No. of full restoring combinations	Percentage of full restoring combinations
Restorer of CMS	18	32	32	100
Maintainer of CMS	7	28	27	96.4
Restorer of GMS	14	28	28	100
Maintainer of GMS	12	22	20	90.9
Hybrid of CMS	8	16	15	93.8
Total or mean	59	126	122	96.8

Notes: The percentage of full restoring combinations were from No. of full restoring combinations divided by No. of all combinations. full restoring combinations were these combinations whose restoring ratio were over 95%.

### Screening TRGMS combinations with strong heterosis

In spring 2001, test-crosses were made between 32 double- low varieties and all-sterile lines ( 20T197, 20T216 and 20T 399) with good agronomic characters, and 96 combinations were gotten. The result of trial showed that most of combinations have obvious heterosis in vegetation growth, for example, leaves of these plants were broad and thick with dark green and its stems were thick and hard. Yield of single plant and No. of the first branches and pods and grains of pod all had stronger ultra-parent and ultra-norm heterosis. Height and branch location of plants lowered distinctly than that of CK QinyouNo.7(CMS hybrids), and No. of the first branches and pods and grains of pod increased markedly, and parts of combinations had outstanding heterosis in yield. The strong heterosis combinations of TRGMS will be gotten through improving agronomic characters of all-sterile lines and determining its combining ability, as well as screening a mass of restorers.

### DISCUSSION

CMS and GMS were two main approaches in rapeseed heterosis utilization. CMS system was simple and easy for hybrids production, but sterile lines were affected easily by environment to cause trace pollen and reduced purity of hybrids. Double recessive GMS had stable sterility, but the techniques for hybrids production were complex. TRGMS combined advantages of CMS and double recessive GMS perfectly, and overcame the problem of trace pollen of CMS and the disadvantage that 50% fertile plants must be removed in producing hybrids by double recessive GMS, so it is thought that TRGMS has broad applied prospect in rapeseed heterosis utilization(Li et al,2001).

TRGMS was an important discovery in the study of rapeseed male sterility. It enriched the type of the plant male sterility in theory and contributes to the male sterile mechanism and the expression regulation of the sterile genes. Three-line hybrids production model can be realized by TRGMS with stable sterile line and high purity hybrids. If the molecular marker technique was applied in breeding, the efficiency of screening TML will be raised and can widen the source of TML and enrich gene composition of all-sterile line, so breeding progress will be speeded greatly. The key that TRGMS will be applied in greater range in the future is to combine molecular biology technique with conventional breeding methods.

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