EFFECTS OF GENETIC BACKGROUNDS ON THE EXPRESSION OF THE DOMINANT MALE STERILE GENE IN BRASSICA NAPUS

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INTRODUCTION

The male sterility governed by dominant gene(s) has interested crop breeders greatly in recent years because of its convenience in population improvement of self pollenated crops (Kaul 1988). However, it is difficult to transfer a dominant male sterile gene into genotypes with various cytoplasm in ordinary cases, for no pollen can be produced by the individuals with dominant male sterile gene (Sorrels and Friz 1982). The sterile plants, therefore, have to be as a pollen receiver in the transfer of male sterile lins in existing projects in which the dominant male sterility is used (Deng and Ji 1983). In this situation, there is a potential crisis caused by monotonous cytoplasm.

Fortunately this status could be changed in <u>Brassica</u> napus due to the discovery of a new type of male sterile material which contains a dominant inhibition gene, Rf, as well as a dominant male sterile gene, Ms (Li et al 1985). The Rf gene can inhibit the expression of Ms gene. As a result, the individuals with the genotype of Ms_Rf_ can produce normal pollen and by using them as pollen donors the Ms gene can be transferred to different cytoplasmic backgrounds. In this study we proved the feasibility of the procedure and eximinated the effects of genetic background and environment on the expression of the Ms gene during the development of male sterile lines with various cytoplasmic types.

MATERIALS AND METHODS

Plant materials

GMS3, a strain of <u>Brassica napus</u> with the Ms gene, was kindly provided by Prof. Li Shulin, Shanghai Academy of Agricultural Sciences. There are two kinds of genotypes within GMS3. One is MsMsRf_ and another MsMsrfrf. The two genotypes can be kept through the sib-mating within the strain (Li et al 1986).

Selections of <u>Brassica napus</u> having various target characteristics were chosen as mother parents with different cytoplasmic origin.

The Principle of transfering the Ms gene

Suppose the genotypt of mother parents is msmsrfrf (ms and rf are corresponding alleles of Ms and Rf, respectively). The transferring principle can be showed as follows.

P msmsrfrf × MsMsRfrf (recurrent parent) (Ms gene donor)

F1 1 msmsrfrf : 1 Msmsrfrf (male fertile) (male sterile)

Therefore the Ms gene can be introduced into different cytoplasmic background by manual emasculating only one time. The sterile lines with various origin can be obtained by pollinating from recurrent parents or any other seletions to the male sterile plants in following generations. These sterile lines could be used conveniently to produce random mating populations in <u>Brassica napus</u>.

Transfer of Ms gene and observing methods

The transferring experiment was carried out both in Wuhan (autumn sowing, main season) and in Xilin (summer sowing).

Fifteen selections with eleven types of cytoplasm were chosen to accept the pollen from GMS3 in Wuhan in the spring of 1989. Then half of the hybrid seeds of each crosses was grown in Xilin in the summer, and segregation of fertility vs. sterility was observed during the anthesis period. Some recurrent parents with better comprehensive traits were backcrossed with corresponding hybrid and some new selections were crossed with other combinations to broaden the genetic compositions of the combinations. All the crosses which were made in two seasons were grown and investigated for their fertility performance in the next main growing season. X² test is used to examinate the fitness of segregating ratio.

RESULTS

Sterility performance controlled by Ms gene

It was found in the observation to male sterile plants from all the crosses obtained that anthers of sterile flowers are shriveled without pollen. Yet the stigma is normal and projects over the four overlapped petals apparently, which is useful to fertilize by outbreeding. No significant differences in plant morphology were found between sterile plants and fertile ones.

Although the male sterility governed by Ms gene is complete in most sterile plants, a few flowers on the some sterile plants may produce certain amount of pollen. Three different types have been observed among these plants with a few fertile flowers. (1) The anthers only in few flowers can be developed to a certain extent and might produce little pollen in a plant and most of other flowers at the same plant are male sterile completely. (2) Flowers anthesising at early stage of anthesis period can form some pollen but flowers anthesising at late anthesis period can not, or vice versa. (3) There is a chimera of sterile flowers and fertile flowers at a same plant, or at some branches of a plant. The ratio of sterile flowers vs. fertile flowers at a chimera plant may vary from plant to plant. Among the three kinds of individuals, first two kinds account for a predominant numbers. We call all the three types described above partial sterile plants, and put them into the group of sterile plants when

making fitness test because of their limited numbers. The investigation on what has resulted in the formation of the partial sterile plants is undergoing.

Fertility segregation of the crosses containing Ms gene in different growing seasons and locations

Datumsgiven in table 1 come from the observation of F1 hybrids in two growing seasons at two locations. According to them, we could conclude following three points. (1) The fact that most combinations (90%) have a segregation ratio of 1 fertile: 1 sterile varifies that the transferring procedure used in the investigation is reliable. (2) There are no sterile plants in three combinations. This implicates that inhibition gene(s) of Ms could exist in the mother parents of the three crosses. (3) Genetic backgrounds and environmental factors could influence the expression of Ms gene to a certain extent. On one side a same combination has a different segregating in different locations. All plants from the combination with mother parent of FP03, for example, were partial sterile at Xilin in summer, and neither complete sterile nor complete fertile. When the same combination was grown at Wuhan in main growing season, however, the segregation of fertile plants vs. sterile plants appeared. In addition there were a few partial sterile plants in some combinations in the the spring growing season, but there were not at all in summer. On the other side, when all the materials were grown at same location and in same season, whether partial sterile indiviuals are formed could be depended on the differences of genetic background in combinations. Obviously genetic backgrounds play a part in the expression degree of Ms gene. But the effects are limited in terms of the segregating performance of all the combinations.

Fertility segregation of progenies from the combinations of tri-parent crossing or backcrossing

Both backcrossing and multi-parent crossing will result in the change of genetic composition. It is showed that the increase of composition of recurrent parents may slightly cause raise of numbers of partial sterile plants (table 2). It is possible that there are some minor genes affecting Ms gene in those recurrent parents.

In the tri-parent combinations derived from FP01, all the plants are fertile (table 3). This provides further evidence that inhibition gene(s) of Ms in FP01 is stable.

If taking table 1, table 2 and table 3 as a whole, we can find that the appearance of partial sterile plants has some relation with the parents involved in these combinations. For instance, the combinations with FPO2 always produce partial sterile plants no matter in single crossing or back crossing, whereas the combinations containing FPO4. FPO5 or FP13 have no this kind of plant in any cases (table 1 and table 2). Therefore various genetic backgrounds exhibit different roles in modification on the male sterility conditioned by Ms gene.

DISCUSSION

Kaul (1988) speculated that types of cytoplasm could

seldom alter the expression of dominant male sterile gene(s). Yet no direct evidence has been provided because of the difficulty in introduction of a dominant sterile gene into various kinds of cytoplasm until now. The investigation proved positively the assumption first time.

The expression of Ms gene is only slightly modified by nuclear background. It is not observed that cytoplasm origin could change the expression of Ms gene for a detective extent. The formation of partial sterile plants could be an evidence to this point. For example, the combinations with the same female parent of FP02 not always produce partial sterile plants in backcross generation and tri-parent crossing (table 2, table 3). Thus introduction of Ms gene to various types of cytoplasm has no negative effects on the utilization of Ms gene.

The identification of new inhibition gene(s) of Ms gene is significant at least in two aspects. Firstly, the misusing of varieties with inhibition gene(s) in constructing a random mating population can be avoided through the identifying of parents, selections in advance. Secondly, the discovery of new inhibition gene(s) could be helpful for the utilization of male sterility in heterosis of rapeseed. Du and Li (1986) proposed a scheme of making hybrids using genic three lines. Actually the restoring line is the selection with inhibition gene(s) of Ms gene in the three lines. So the inhibitory selections can be used in the development of restoring lines, which can broaden the genetic composition in selecting highly vigorous hybrids. In addition to these, the understanding of the genetic behaviour and origin of the inhibitory gene(s) are also of great help for the studies of mechanism of genic male sterility in higher plants.

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Table 1. Plant numbers of three types of fertility in each cross with the same pollen parent of GMS3

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Female	cytoplasm	Summer				Spring			
parent origin		of 1989				of 1990		90	
		Xilin		,	Wuhan				
		F*	S#	PS§	P(1:1)	F*	S#	PS§	P(1:1)
FP01	H13	30	0	0		36	. 0	0	
FP02	Н8	21	10	0	0.05-0.01	49	13	2	<0.005
FP03	DR	0	0	24		16	29	0	0.05-0.10
FP04	DS 3 1	17	22	0	0.5-0.75	23	20	0	0.75-0.90
FP05	Z821	12	14	0	0.75-0.95	22	17	0	0.50-0.75
FP06	E13	17	13	0	0.5-0.75	21	14	0	0.25-0.50
FP07	Н8	11	12	0	1	47	47	0	1
FP08	Bokow	14	16	0	0.75-0.9	24	19	0	0.25-0.50
FP09	H13	21	0	0		45	0	0	,
FP10	DS31	13	10	0	0.5-0.75	9	9	0	1
FP11	Marnoo	12	13	0	1	24	26	1	0.75-0.90
FP12	H 1 2	17	0	0		44	0	0	ļ
FP13	Marnoo	11	1 4	0	0.50-0.75	17	18	0	1

^{*} F=fertile;

[#] S=sterile;

[§] PS=patially sterile.

Table 2. Plant numbers of three types of fertility in backcross generation

Combination	fertile	sterile	e partially sterile	P(1:1)
955/8750-5//955	33	26	2	0.50-0.75
E821/8705-5//E821	16	7	0	0.05-0.10
FP02/GMS3//FP02	40	32	2	0.25-0.50
FP03/GMS3//FP03	27	23	1	0.75-0.90
FP04/GMS3//FP04	22	20	0	0.75-0.90
FP05/GMS3//FP05	32	2 1	0	0.10-0.25
FP07/GMS3//FP07	16	15	2	1
FP10/GMS3//FP10	48	29	0	0.025-0.05
FP13/GMS3//FP13	13	11	0	0.75-0.90

Table 3. Plant number of three types of fertility in tri-parent crosses

Combination	fertile	sterile	e partially sterile	P(1:1)
FP02/GMS3//86001	12	13	0	1
FP02/GMS3//FP01	14	0	0	
FP06/GMS3//FP13 ·	28	23	0	0.50-0.75
FP06/GMS3//FP01	26	0	0	
FP06/GMS3//821	15	11	0	0.50-0.75
FP06/GMS3//FP04	14	14	0	1
FP15/GMS3//FP08	20	13	1	0.10-0.25
FP15/GMS3//FP05	15	16	0	1
FP15/GMS3//FP01	30	0	0	*
FP08/GMS3//186	44	58	1	0.10-0.25
FP08/GMS3//84001	21	12	0	0.10-0.25
FP11/GMS3//083	29	20	3	0.25-0.50