Breeding of a homozygous two-type line with orange flower of dominant genic male sterility in *Brassica napus* L.

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Abstract

A cross between a sterile plant of the heterozygous two-type line Huyou15AB and the restorer line HF03 of dominant genic male sterility was made in *Brassica napus* L.. All plants of F_1 were fertile. In 6 lines of F_3 generation, fertility segregation was observed in five selfing populations. The progeny of 04-4307 had total 131 plants: 70 fertile plants with yellow flowers, 30 fertile plants with orange flowers, 22 sterile plants with yellow flowers, and 9 sterile plants with orange flowers. This F_3 progeny segregated in a 3:1 ratio for fertile and sterile plants and in the same ratio for yellow and orange flower plants. The homozygous two-type line with orange flower is developed from sib-mating in this progeny among which the sterile and fertile plants have the genotypes MsMsrfrf$y_1y_1y_2y_2$ and MsMsRfrf$y_1y_1y_2y_2$, respectively.

Key words: *Brassica napus* L., dominant genic male sterility, orange flower, homozygous two-type line, restorer line, temporary maintainer line

Introduction

The inheritance of dominant genic male sterility (GMS) in *Brassica napus* L. was demonstrated (Li et al., 1985; 1986; 1987; 1988). A three-line method was established to produce hybrid seeds (Li et al., 1995). After improvement of the homozygous two-type line, a double low hybrid variety Heza 7 was developed by the three-line method in *Brassica napus* L. (Zhou et al., 2006). It was the first dominant GMS hybrid variety that was released by the National Crop Registration Committee.

Because there is no distinct morphologic difference between sterile and fertile plants of the homozygous two-type line, fertile plants can completely not be rogued in the field of production for the full sterile line by use of the temporary maintainer line as male parent. The sterile plants can be pollinated by male parent and the residual fertile plants of female parent. The purity of the full sterile line may drop to 95% or lower. Therefore, the indicative character must be used to firmly preserve the sterile rate of the full sterile line about 100%.

Generally, the color of rapeseed flowers is yellow, but also orange, clear yellow, white and so on (Liu, 1985). The yellow flower is dominance to the orange flower, controlled by two pairs of duplicate recessive genes (Y_1 and Y_2) (Li et al., 1994). The plants are orange-flowered for genotype $y_1y_1y_2y_2$, and yellow-flowered for other genotypes. The character of orange flower may be used as an indicative character to control the purity of the full sterile line.

In the present paper, we described developing of the homozygous two-type line with orange flowers in *Brassica napus* L.

Materials and methods

Huyou15, a double low rapeseed variety, is a temporary maintainer line (msmsrfrf) of dominant GMS. Huyou15AB is a heterozygous two-type line. It was obtained through a back cross using Huyou15 as recurrent parent. In this line, 50% plants are male sterile (Msmsrfrf) and the rest is male fertile (msmsrdrd). HF03 is a restorer line with genotype of RfRf.

The sterile plant of Huyou15AB was crossed with the restorer line HF03. During the florescence, the characters of fertility and flower color were investigated in the progeny of cross Huyou15A×HF03. Fertile plants were self-pollinated in F_2 and F_3 progenies. The progeny which segregated in a 3:1 ratio for male fertile and male sterile plants was identified by the percentage of sterile plants and by the results of test-crosses between sterile plants and the temporary maintainer line Huyou15. In the F_3 progeny that fertile and sterile plants segregate in a 3:1 ratio, sterile plants were pollinated by fertile plants to obtain a homozygous two-type line with orange flower if the progeny of sib-mating segregates in a 1:1 ratio.

Results

All of the 165 plants were fertile in F_1 population from which two plants with free pollination were selected. In F_2 generation, one progeny segregated for fertile and sterile plants, but another was full fertile (Table 1).

Six plants were self-pollinated in the F_2 progeny 03-4217 which segregated for fertile and sterile plants. In the F_3 progeny, five selfing-populations segregated for the two phenotypes, the percentage of sterile plants varied from 18.7% to 28.7% (Table 2).

In the F_3 population of 04-4307, there were 70 fertile plants with yellow flower, 22 sterile plants with yellow flower, 30 fertile plants with orange flower and 9 sterile plants with orange flower. The percentage of sterile plants was 23.7%, and the percentage of orange flower plants was 29.8%. This population segregated in a 3:1 ratio for fertile and sterile plants

($\chi^2_{3:1}$=0.0636), and in the same ratio for yellow and orange flower plants ($\chi^2_{3:1}$=1.0256).

Table 1. The fertility of F_1 and F_2 progenies of the cross Huyou15A×HF03

code	Generation	No. of fertile plants	No. of sterile plants
02-4247	F_1	165	0
03-4216	F_2	138	0
03-4217	F_2	149	20

Table 2. Fertility and flower color segregation in the F_3 progeny of the cross Huyou15A×HF03

code	No. of yellow flower plants		No. of orange flower plants		% of sterile plants
	Fertile	Sterile	Fertile	Sterile	
04-4307	70	22	30	9	23.7
04-4308	134	25	0	0	18.7
04-4309	166	45	0	0	27.1
04-4310	154	0	0	0	0.0
04-4311	129	37	0	0	28.7
04-4312	140	36	0	0	25.7

In this F_3 population, three sterile plants with orange flower were crossed by the temporary maintainer line Huyou15. All plants were sterile with yellow flower in the progeny of these three test-crosses (Table 3).

Table 3. The behavior of test-crosses

code	No. of sterile plants	No. of fertile plants	No. of plants with yellow flower
05-4357	35	0	35
05-4358	58	0	58
05-4359	28	0	28

Nine fertile plants with orange flower were self-pollinated from the F_3 progeny of 04-4307. The flower color was orange for all plants in the F_4 progeny. Five selfing-populations segregated in a 3:1 ratio for fertile and sterile plants. The percentage of sterile plants varied from 22.1% to 29.8% (Table 4).

Table 4 Fertility and flower color segregation in F_4 and F_3SM_1 progenies of the cross Huyou15A×HF03

code	Generation	code of last year	Total No. of plants	No. of plants with orange flower	No. of fertile plants	No. of sterile plants
05-4347	F_4	04-4307B1	121	121	121	0
05-4348	F_4	04-4307B2	98	98	75	23
05-4349	F_4	04-4307B3	136	136	136	0
05-4350	F_4	04-4307B4	121	121	85	36
05-4351	F_4	04-4307B6	115	115	115	0
05-4352	F_4	04-4307B8	154	154	120	34
05-4353	F_4	04-4307B9	153	153	153	0
05-4354	F_4	04-4307B5	37	37	28	9
05-4355	F_4	04-4307B7	45	45	33	12
05-4356	F_3SM_1	04-4307A1×B4	34	34	18	16

A sib-mating cross (05-4356) between sterile and fertile plant with orange flower was made in the selfing population of 04-4307. The flower color was orange for all of the 34 plants in the F_3SM_1 generation. There were eighteen fertile plants and sixteen sterile plants in this population (Table 4). This cross segregated in a 1:1 ratio for fertile and sterile plants ($\chi^2_{1:1}$=0.0294). Its male parent (05-4350) segregated in a 3:1 ratio for the two phenotypes ($\chi^2_{3:1}$=1.2149).

Conclusion and Discussion

The fertility and flower color segregation indicated that the genotypes were MsMsrfrf for sterile plants, MsMsRf- for fertile plants; $y_1y_1y_2y_2$ for orange flower plants, and $Y_{1-1}y_2y_2$ or $y_1y_1Y_{2-2}$ for yellow flower plants in the F_3 population of 04-4307. The results of test-crosses verified that the genotype of all sterile plants in this F_3 population was homozygous MsMsrfrf.

The fertility behavior of the progenies by sib-mating from this population has two kinds: one is 1MsMsrfrf:1MsMsRfrf (homozygous two-type line), another is MsMsRfrf (fertile) if the genotype of fertile plant is MsMsRfrf and MsMsRfRf, respectively. The flower color of all the sterile and fertile plants is orange because we selected only orange flower plants for selfing and sib-mating. So the cross 05-4356 by sib-mating is a homozygous two-type line with orange flower. The genotype of the sterile and fertile plants is MsMsrfrf$y_1y_1y_2y_2$ and MsMsRfrf$y_1y_1y_2y_2$, respectively. The test-cross between sterile plants of this two-type line and Huyou15 (genotype msmsrfrf) produces only sterile plants with yellow flower.

When we use the homozygous two-type line with orange flower as female parent, the temporary maintainer line with yellow flower as male parent, we can obtain the full sterile line with yellow flower. The residual fertile plants in the two-type

line will produce some fertile individuals in the full sterile line. But the flower color of these fertile plants is orange, they can be easily distinguished and pulled out during the florescence. The purity of the full sterile line is also guaranteed.

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