Discovery and application of a new kind of genic male sterile material Mian 7AB-4-2 in *Brassica napus* L.

YUAN Daibin, MENG Daqing, PU Dingfu, LI Zhifan, XU Lan, GUO Zirong, TANG Tianze, HE Qichuan

Mianyang Institute Of Agricultural Sciences, Mianyang 621002, China. Email: youcai@scghseed.com

Abstract

We discovered a new genic male sterile material Mian 7AB-4-2 in the selection of a genic male sterile line Mian 7AB-4 in *Brassica napus* in 1998. The rate of sterile plants from the selfing progeny of fertile plants was over 60%. We made test crosses and back crosses with a sib line Mian 7AB-4-1 and 9 other male sterile materials of different sources. In test crosses, 80% of the materials produced over 90% of sterile rate. In backcrosses, the rate of sterile plants was also over 80%. The results were evidently different from other recessive genic male sterile lines in *Brassica napus*. In present paper, we discussed the possibility of seed production with three-line genic male sterility system in hybrid rapeseed production.

Key words: *Brassica napus* L., recessive genic male sterility, fertile plant selfing; testcross, backcross

Introduction

In recent years, the study of heterosis utilization in *Brassica napus* in our country developed strikingly. Single or double-low hybrids took a great proportion of rapeseed production in acreage. Because of the shortcomings in the CMS three line and GMS two line systems, large areas of rapeseed production meet certain problems. Many rapeseed breeders have been seeking technical routes to overcome the disadvantages of CMS three line and GMS two line systems in present time. For example, Li Shu-Ling and Chen Feng-Xiang have developed genic male sterile lines, which are controlled by dominant epistasy and recessive epistasy, respectively, and have established corresponding three line systems. Since the middle of 1990's, we have studied the fertility segregation rules of part of our genic male sterile lines, and developed the recessive genic male sterile lines Mian 7AB-4-1 and Mian 7AB-4-2, and their maintainer lines and restorer lines. Meanwhile, a GMS three-line hybrid Mianyou 12 has been registered by the state authority. This hybrid has been released to crop production. A new GMS three-line hybrid Mianyou 17 was also released to production.

1 Materials and Methods

A number of genic male sterile lines, Mian 1AB-1 (double-high), Mian 1AB-3 (double-high), Mian 9AB-1 (double-high), Mian 4AB-3 (low erucic acid), and Mian 7AB-4 (double-low), have been developed in our institutes. A few sterile materials, such as Shu 6A-1, Rong 4A-1, Youyan 9A-1, Guizaxuan 1A-1, Deyou 4A-1, Pin 87-161-134, and Pin 93-496(originated from Crop institute of Sichuan Agriculture academy), were obtained from some experimental combinations in parts of breeding places in Sichuan and Guizhou. We used these materials to catty out test-crosses for transfer of fertility by backcross. The experiments were carried out in our institute. Furthermore, we did fertility observations in Ma'erkang. Erucic acid content was measured by gas chromatography, glucocinolates content was measured by liquid chromatography, and oil content was measured by nuclear magnetic resonance.

2. Results and Analysis

1.1 Discovery and breeding of genic male sterile three lines

In 1991, we used a double-high recessive GMS line Mian 1AB-1 bred in our institute as the maternal parent, and Pin 87-161-134 as the paternal parent to transfer fertility. Sterile plants in F_2 were sib-mated in pairs. Through multi-generation selection, recessive GMS line Mian 7AB-4 (double low) was bred. During the selection for advanced lines, segregation ratios of fertility in some sib-mated families and the self progenies of fertile plants were distinctly different from the theoretical values of 1:1(or 3:1) and 3:1 (or 5:1). In 1995, we used 6 sterile plants to sib-mate with 10 fertile plants in Mian 7AB-4 with a incomplete diallel design, and the fertility of sib matings and the selfed progenies of 10 fertile plants was investigated. Based on the statistic panalysis, we found that the sterility rate of the sterile plants was significant positively correlated to that of the selfed progenies of the fertile plants. Through regression analysis, we could preliminarily concluded that sterility rate of corresponding sterile plants could get to over 70% when sterility rate of selfed progenies in fertile plants reached 40%~50%. Under the theory conducting, we took sterile characteristic as the selecting objective, and segregated and purified two kind of different sterile stains, named Mian 7AB-4-1 and Mian

7AB-4-2, respectively. Sterility rate of sibmating and selfing of fertile plants in the former, were about 50% and 25%, respectively. But that in the latter were over 80% and 60%, respectively. There were no distinct differences in morphological characters between them. The breeding program of Mian 7AB-4-1 and Mian 7AB-4-2 was shown in Figure 2.

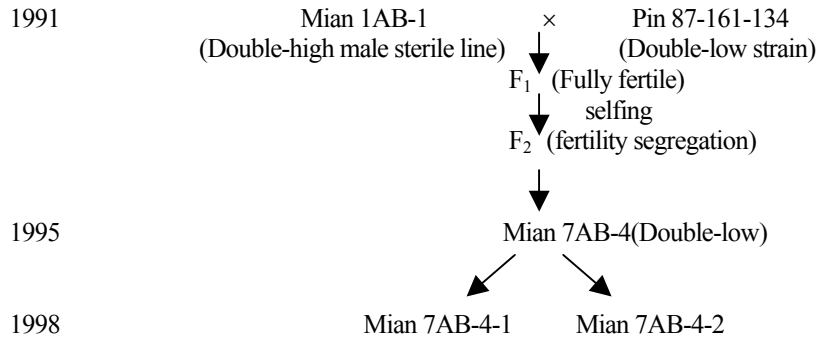

Fig. 2 The breeding procedure of Mian 7AB-4-1 and Mian 7AB-4-2

2.2 The main traits of Mian 7AB-4-1 and Mian 7AB-4-2

Mian 7AB-4-1 and Mian 7AB-4-2 were planted to a middle level fertility rice field in September. We cultivated with a density of 110 thousand plants per ha. Compared to Mian 7AB-4-1, plant height of Mian 7AB-4-2 was decreased by 7 cm, pod number per plant increased by 35, seed number per pod increased by 0.4, 1000-seed weight dropped by 0.36 gram, oil content increased by 0.56%, erucic acid content increased by 0.28%, glucosinolate content increased by 9μmol/g, and whole growth duration increased by 2 days(Table 1). Other characters were not obviously different between the two lines. For example, branches were symmetric; leaves were dark green; leaf margin was sinuate with little saw-teeth; leaves bore wax but no seta; petals were middle sized and yellow; seed was ball-shaped and in red brown.

2.3 Fertility performance in sibmating between Mian 7AB-4-1 and Mian 7AB-4-2 and testcrossing between the sterile plants of Mian 7AB-4-1 and the fertile plants of Mian 7AB-4-2

From 1998 to 2006, we had investigated the flower morphology and the fertility performance of sibs between Mian 7AB-4-1 and Mian 7AB-4-2 and the progeny of Mian 7AB-4-1(sterile plants) and Mian 7AB-4-2(fertile plants). There were no distinct differences in flower morphology between them, filament was long, anther shape was wrap-pined, no pollen in shrunken anther, the color was light yellow to light brown. The cross-pollination habit was good. The setting of seed also was normal. The sterile plant rates in the two lines were not obviously different among years, showing a good stable fertility. In March and July, 2000, we invited some experts to make field technical inspection and indoor achievement evaluation for Mian 7AB-4-1 and Mian 7AB-4-2. The evaluation was presided by the Sichuan Provincial Department of Science and Technology. The evaluation conclusions were that the project objective was distinct, the pertinence was strong, the technical routes were innovative, the breeding procedure was clear, the fertility was stable, the maintainer lines and the restorer lines of the GMS lines' have been developed. It created a new pathway in rapeseed breeding in Sichuan.

2.4 Discovery and introduction of exotic male sterile materials

In Spring of 2001, we observed male sterile plants in Shuza 6, Youyan 9, Zaxuan 1, Deyou 4 and Rongyou 4 from the Sichuan Provincial regional trials. According to the flower morphology, all male sterile plants, except those from Rongyou 4, a CMS hybrid, belong to GMS. We also introduced GMS 97AB from Deyang Kele seed Co.,LTD.

2.5 Fertility performance in testcrosses and backcross between many sterile plants and the fertile plants in Mian 7AB-4-2

From 2002 to 2006, we made testcrosses between the sterile plants in Shu 6A-1, Youyan9A-1,Zaxuan 1A-1,Deyou 4A-1, Rongyou 4A-1,Mian 1AB-3, Mian 4AB-3, Mian 7AB-4-1 and Mian 9AB-1,De 97A-1 and the fertile plants in Mian 7AB-4-2,. Also we made backcrosses with male sterile plants of F_1 progeny and fertile plants of Mian 7AB-4-2. In table 3, Mian 7AB-4-2 could remain very high sterility in most of GMS materials, but could completely recover the fertility of De 97AB. This will be further studied. The testcross progenies between Mian 7AB-4-2 and Rongyou 4 were half-sterile plants. It was shown that we could breed the corresponding maintainer line and restorer line for Mian 7AB-4-2 and many same-type GMS line, furthermore, Mian 7AB-4-2, different from the other current maintainer line, could separate its fertility, and sterility rate could reach over 80% by backcross. Therefore, we thought that Mian 7AB-4-2 was a new type GMS material of *brassica* napus.

2.6 Fertility performance of the GMS two-line and three-line hybrids

In 2005, we developed 4 combinations of two line and three line hybrids with the two-line and the three-line system from Mian 7AB-4-2 and tested their yield performance in a varietal trial. It is shown in table 4 that the plant height and 1000-seed weight of the three-line hybrids were lower than that of the two-line hybrid. The seed number per pod and yield per plant were higher than the two-line hybrid, but the differences were not big. The results were similar to those of Houshumin's.

3 Conclusion and discussion

As many well known GMS lines such as S45AB, 117AB and Mian 9AB, Mian 7AB-4-1 and Mian 7AB-4-2, belonged to double-recessive GMS lines. Their sterility was iso-allelic, but not iso-allelic with De97AB-1. The sterility was complete and the restoring sources were wide. Mian 7AB-4-2 was a good maintainer line for many GMS lines of the same type. The rate of sterility was over 90%. It was very important in the application of GMS lines. The features different from the temporary

maintainer line 9012AB reported by Chen Fengxiang include: Mian 7AB-4-2 showed segregation in fertility, and the sterility rate reached about 70%. According to the genetic theories, we could primarily deduced that there may be one or many pairs of gene to control the sterility in rapeseed. These genes may link to dominant genes. Because Mian 7AB-4-2 had about 70% sterile plants, and fertile plants were few and pollen amount were not enough, it is difficult to use Mian 7AB-4-2 as a maintainer line in seed propagation of the three-line GMS lines. We are applying biotechnology to multiply the fertile plants in Mian 7AB-4-2 to solve the problem of propagation of the three GMS lines. It is affirmed that the method is applicable after a few years of practice.

Table 1 The main agronomic traits of Mian 7AB-4-1 and Mian 7AB-4-2

Material	Plant height (cm)	Pod number per plant	Seed number per pod	1000-seed weight(gram)	Oil content(%)	Erucic acid content(%)	Glucosinolate content umoi/g	Whole growth period(d)
Mian7AB-4-1	191.7	616	11.8	3.32	39.15	0.38	29.5	225
Mian 7AB-4-2	184.6	645	12.3	2.98	40.78	0.56	34.1	227

Table 2 Fertility performance of Mian 7AB-4-1 and Mian 7AB-4-2

Year / Material		1998-2004		2005		2006	
		population(plants)	Sterility rate(%)	population(plants)	Sterility rate(%)	population(plants)	Sterility rate(%)
Mian 7AB-4-1	sibmating	1534	51.1	1742	49.1	3346	52.7
	selfing	1237	25.4	807	27.1	120	28.5
Mian 7AB-4-2	sibmating	1422	73.3	1583	83.1	516	81.6
	selfing	1696	61.0	10288	79.8	877	71.7

Table 3 Fertility performance of the test crosses and backcrosses between other male sterile materials and fertile plants in Mian 7AB-4-2

Year / Material	2002(testcross)		2003(backcross)		2005(testcross)		2006 (backcross)	
	population(plants)	Sterility rate(%)	population(plants)	Sterility rate(%)	population(plants)	Sterility rate(%)	population(plants)	Sterility rate(%)
Shu6A-1	136	97.2	134	91.9				
De4A-1	172	94.2	135	82.2				
Zaxuan1A-1	87	95.4	67	89.6				
Mian1A-3	85	96.5	69	95.7				
Mian 4A-3	88	96.6	69	84.1				
Mian 9A-1	89	94.4	68	92.6				
Rong4A-1	88	2.3						
De97A-1					60	0.0		
Youyan9A-1					60	93.3	60	85.4
Mian7AB-4-1	2153	96.1	4421	92.3	2473	93.4	687	88.1

Table 4 The main agronomic traits of the two-line and the three-line hybrids of Mian 7AB-4-1

Hybrid		Plant height (cm)	Seed number per pod	1000-seed weight(gram)	Yield		Oil content(%)	Erucic acid content(%)	Glucosinolate content umoi/g
					Kg/hm^2	Three-line to two-line±%			
Mianyou11	Three-line	196.8	13.37	3.09	2890.05	+4.90	43.09	16.61	78.98
	Two-line	199.1	12.74	3.14	2755.05		43.75	36.79	162.95
Mianyou 12	Three-line	205.7	16.47	3.09	3214.95	+5.06	38.93	0.42	23.3
	Two-line	218.0	13.59	3.42	3060.00		39.71	0.44	31.88
Mianza99-13	Three-line	210.1	12.65	3.35	2824.95	+4.24	38.23	0.17	29.90
	Two-line	210.1	1037	3.46	2710.05		41.32	0.21	23.60
Mianza99-67	Three-line	212.1	12.49	3.25	2880.05	+4.73	40.96	0.10	15.05
	Two-line	212.6	11.75	3.35	2749.95		41.48	0.16	15.25

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