Genetic Analysis and Improvement of a Recessive Genic Male Sterility in *Brassica napus*

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Abstract

To utilize heterosis in *Brassica napus*, both the pollination control system and genetic distance are major factors. Recessive epistatic genic male sterile (REGMS) three-line system 7365ABC were widely applied to hybrid seed production because it can produce 100% sterile population by crossing with the temporary line7365C. Previous study has illustrated the system related to three genes model: *Bnms3*, *Bnms4* and *BnRf*. Near-isogenic lines 736512AB and 7365AC correspond to the *BnMs4* and *BnRf* were respectively constructed for mapping. Comparison of mapping results, six markers were linked to both *BnRf* and *BnMs4*. Both genes were located in the top of the N7 linkage group, and might be multiple alleles. We propose a new method of reproducing the male sterile line using self-pollinated seeds generated by heat shock. The seeds were crossed with the maintainer line to drive a 100% sterile population for hybrid seed production. We further study the relation between genetic difference of two parents and F1 yield. We transfered the target genes into two subgenomic ($A^rA^rC^cC^c$) materials, and bred new A and B lines in REGMS system. The combining ability analysis of yield and yield-related characters were done in the crosses from the new A lines and 10 restorers. The results demonstrated that the improved sterile line showed positive effects of value in the general combining ability (GCA).

Key words: *Brassica napus*; recessive epistatic genic male sterile; multiple alleles; MAS; heterosis

Introduction

Genic male sterility (GMS) as an effective pollination control system has more advantages compared to cytoplasm male sterility (CMS). Specifically, genetic male sterile suppressor gene can make produce a 100% male sterile population. GMS lines 9012A, 20118A and ZWA were reported contain this suppressor gene with the same genetic model (Chen et al., 1998; Sun et al., 2002; Wang et al., 2004). Xiao et al. (2008) reported that sterile genes of 7365A and 9012A were allelic, designed as *Bnms3*, *Bnms4* and *BnRf*. When breeders used a 1:1 mixed population of 7365AB to reproduce the completely male sterile line, they have to remove the 50% male fertile plants. We therefore propose a

new method of reproducing the male sterile line using self-pollinated seeds generated by heat shock for hybrid seed production.

Genetic difference between two parents is another considerable aspect of heterosis utilization. Breeding practices have shown that the appropriate genetic difference between parents is a positive correlation with the F1 yield. Li et al. (2005) reported some differences existed between 749 and conventional *B. napus* and therefore the hybrids of these lines had strong intersubgenomic heterosis. Qian et al. (2005) also found that there is subgenomic heterosis between A^r genomes from the new *B. napus* ($A^rA^rC^nC^n$) and A^n genomes from the general *B. napus* ($A^nA^nC^nC^n$) for the subgenomic hybrids ($A^rA^nC^nC^n$) because of the interaction of the two A genomes.

Material and Methods

Material

Near-isogenic lines 736512AB and 7365AC were used for mapping of *BnMs4* gene and *BnRf* gene, respectively. 7365A was used to engage heat shock experiments. We used the methods of crossing, backcrossing, and selfing to improve the 7365A line (The donor line, 7365A *(Bnms3ms3RfRf)*, was a RGMS line in *B. napus*). The two new *B. napus* lines, 749 ($A^rA^rC^cC^c$) and 750 ($A^rA^rC^nC^n$), were the receptors and were synthesized by Li et al. (2005) and Qian et al. (2005), respectively. Ten materials, including three pol CMS restorers, one pol CMS maintainer, three open pollination cultivars, and two subgenomic lines, were used to test the combining ability of the new GMS lines.

Mapping of BnMs4

We used the published B. napus genetic linkage map Tapidor × Ningyou7 (Qiu et al., 2006) and Quantum × No212 17 (Chen et al., 2007) to conduct the mapping of *BnMs4*. AFLP markers linked to *BnMs4* were used to detect the polymorphism between parents of the two DH (double haploid). Polymorphic markers were analyzed DH populations to map *BnMs4*.

Temperature treatments

7365A plants were exposed to 35–45℃ in an artificial climate box for 16 h with light and for 8 h in the dark, then placed the treatment plants into natural environment for normal growth.

Foreground selection and background selection of improved materials

In the two BC_2F_4 populations, the markers linked to *BnMs3* (Huang et al. 2007) and *BnMs4* were used to select sterile individuals and their sib-mates. Twenty AFLP primer combinations were used to screen the F_2, BC_1F_3, and BC_2F_4 populations. We selected two or three individuals with the highest index of subgenomic components (ISG). The ISG (X^y) was used to estimate the ratio of introgression of foreign subgenomes within the corresponding A or C genome in the new *B. napus*, which was visualized by molecular markers. (Huang, et al., 2011; Li et al. 2006).

Field test

The two improved RGMS lines and the 7365A line were crossed with ten restorers. The 30 hybrid combinations and one check were evaluated by a randomized block design with three replications in Wuhan (2007), Xiangfan (2008), and Ezhou (2008). In the field experiments, each treatment used plots with 5 rows and 20 plants in each row. The rows were 3.6 m long with 25 cm between rows. All plants of each plot were harvested to measure seed yield (Huang, et al., 2011).

Results

Mapping of BnMs4 and identification common markers with BnRf

Five of thirteen AFLP markers linked to *BnMs4* were converted into SCAR markers: SCH3, SC98, SC113, SC129, and SC25 (Fig. 1). Six markers, SC25, CNU063, SR4047, ENA06, SC916, and SSR1 were identified as common polymorphic markers both linked to *BnMs4* and *BnRf* (Fig. 1). Finally, we mapped *BnMs4* in the top of N7 linkage map with the same section of *BnRf* by markers XM1 (Xiao et al., 2008), EC09MG12, CNU063, P16MC08, and sR4047 (Fig. 1). Therefore, they were not independent genetic genes.

The fertility of 7365A can be restored by temperature Treatment

We observed that placing plants in the natural environment after heat shock restored the fertility of very few sterile 7365A. The stamens produced a few viable pollens and the filament elongation was restored to the level in 7365A. This result suggests that heat shock can partially restore male fertility in 7365A (Zhu et al., 2010).

Foreground, background selection and field trials

In the BC_2F_4 generation from the two crosses,1,194 individuals with 70 sterile plants in the first cross (7365A× 749) and 1,194 individuals with 64 sterile plants in the second cross (7365A× 750) were selected according to agronomic traits. As a result, 65 and 57 individuals from the first and second crosses, respectively, were identified as having the *BnMs3ms3RfRf* genotype. In the BC_2F_4 populations, one fertile plant and one sterile plant that had similar ISGs in every BC_2F_4 population were selected to construct the sterile lines through successive sib-mating twice.

We analyzed the seed yield of every combination from the three sterile lines hybridized with ten restorers under three environments (Table.1). The differences of the various effects of the female parents in all circumstances were significant (P=0.0001). The interactions between locations and females were not significant (P = 0.18). In the three environments, The improved sterile lines exhibited significant and positive GCAs under different environments compared to original line 7365A(Table.1)

Discussion

The recessive epistatic genic male sterile (RGMS) line 9012A, 20118A, and ZWA were controlled by three independent genes, designed as *Bnms3*, *Bnms4* and *BnRf* (Chen et al., 1998; Sun et al., 2002; Wang et al., 2004). We further concluded that *BnMs4* and *BnRf* were not independent genetic genes by the mapping results. Through field genetic design and linkage disequilibrium analysis involved in the two genes, Zu et al (2010) concluded that *BnMs4* and *BnRf* might be multiple alleles. Both *BnRf* and *Bnms4* were the same sterile genes but different names. It was difficult to distinguish similar fertility segregation ratio in genetic model research of *B. napus* RGMS according to classical genetics method. Here, we proposed that *BnMs4* and *BnRf* were two multiple alleles, there exited three multiple alleles at the locus (fertility restorer gene *BnMs*, sterile gene *Bnms* and recessive suppressor *Bnrf*), their explicit-implicit relationship was: *BnMs4>Bnms4=BnRf>Bnrf*, *Bnrfrf* could suppress *Bnms3ms3* and result the fertile genotype by genotype *Bnms3ms3rfrf.*

The modern *B. napus* cultivars have a comparatively narrower genetic basis limiting their potential for improving seed yield and heterosis (Becker et al. 1995). Therefore, importing other Brassica genomes to *B. napus* will significantly broaden the genetic basis of *B. napus*. In our study, we transferred the sterile genes to the new *B. napus* lines by MAS to improve the original sterile line, 7365A. The two improved sterile lines had A^r genomes from *B. rapa* or C^c genomes from *B. carinata*. The genetic distance between the sterile lines and the conventional *B. napus* was increased. The significant GCA differences between 7365A and the new sterile lines indicated that the introgression of

genomic components from the other Brassica species improved the heterosis of sterile lines and conventional-typed rapeseed. In addition, by utilization of our material properties, we could make improved sterile lines inbred to produce seeds by heat shock treatment, derived 100% sterile improved population.

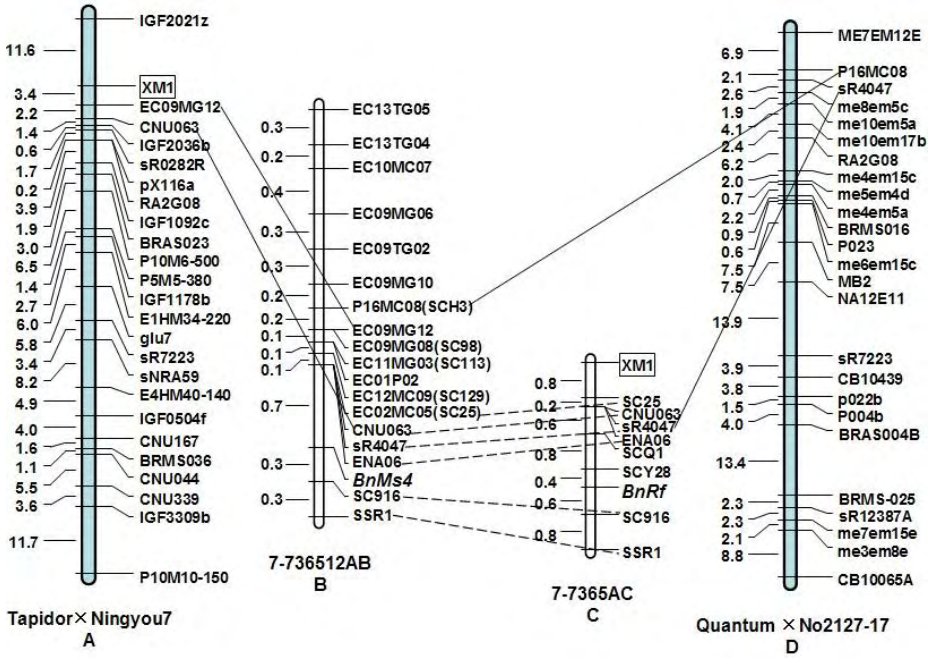

Fig. 1 A: A linkage map of N7 developed from Tapidor×Ningyou 7; B: A linkage map of the region encompassing the *BnMs4* gene from 736512AB; C: A linkage map of the region encompassing the *BnRf* gene from 7365AC; D: A linkage map of N7 developed from Quantum×No212 17. The dotted line showed six identical markers between *BnMs4* and *BnRf*

Table 1. Values and comparison of GCAs for seed yield in three environments

Environments	Sterile lines	GCA	Range		LSD0.05	LSD0.01
Wuhan	7-749A	0.031				
	7-750A	0.022	0.009			
	7-7365A	-0.053	0.084*	0.075*	0.067	0.089
Xiangfan	7-750A	0.083				
	7-749A	0.003	0.080			
	7-7365A	-0.086	0.169**	0.089*	0.082	0.109
Ezhou	7-750A	0.070				
	7-749A	0.066	0.004			
	7-7365A	-0.137	0.207**	0.203**	0.088	0.117
Mean	7-750A	0.059				
	7-749A	0.034	0.026			
	7-7365A	-0.093	0.152**	0.126**	0.059	0.079

* significant at p = 0.05, and ** significant at p = 0.01