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Fine mapping of a male sterility gene *MS-cd1* in *Brassica oleracea*

Xinmei Zhang · Jian Wu · Hui Zhang · Yuan Ma · Aiguang Guo · Xiaowu Wang

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Abstract A dominant male sterility (DGMS) line 79-399-3, developed from a spontaneous mutation in *Brassica oleracea* var. *capitata*, has been widely used in production of hybrid cultivars in China. In this line, male sterility is controlled by a dominant gene *Ms-cd1*. In the present study, fine mapping of *Ms-cd1* was conducted by screening a segregating population Ms79-07 with 2,028 individuals developed by four times backcrossing using a male sterile *Brassica oleracea* var. *italica* line harboring *Ms-cd1* as donor and *Brassica oleracea* var. *alboglabra* as the recipient. Bulked segregation analysis (BSA) was performed for the BC_4 population Ms79-07 using 26,417 SRAP primer SRAPs and 1,300 SSRs regarding of male sterility and fertility. A high-resolution map surrounding *Ms-cd1* was constructed with 14 SRAPs and one SSR. The SSR marker 8C0909 was closely linked to the *MS-cd1* gene with a distance of 2.06 cM. Fourteen SRAPs closely linked to the target gene were identified; the closest ones on each side were 0.18 cM and 2.16 cM from *Ms-cd1*. Three of these SRAPs were successfully converted to dominant SCAR markers with a distance to the *Ms-cd1* gene of 0.18, 0.39 and 4.23 cM, respectively. BLAST analysis with these SCAR marker sequences identified a collinear genomic region about 600 kb in scaffold 000010 on chromosomeA10 in *B. rapa* and on chromosome 5 in *A. thaliana*. These results provide additional information for map-based cloning of the *Ms-cd1* gene and will be helpful for marker-assisted selection (MAS).

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X. Zhang · J. Wu · H. Zhang · Y. Ma · X. Wang (✉)
Institute of Vegetables and Flowers,
Chinese Academy of Agricultural Sciences,
Beijing 100081, China
e-mail: wangxw@mail.caas.net.cn

X. Zhang · A. Guo
College of Life Science, Northwest A & F University,
Yang ling 712100, China

Y. Ma
College of life science, Wuhan University,
Wuhan 430071, China

Introduction

Male sterility in *Brassica* crops has been exploited as an effective and economical pollination control system. Male sterility is defined as the failure of plants to produce functional anthers, pollen, or male gametes, while the female reproduction is normal (Kaul 1988). Based on its inheritance or origin, male sterility may be divided into nuclear male sterility (NMS), also called genic male sterility (GMS), and cytoplasmic male sterility (CMS). CMS systems are convenient for the production of hybrid seeds because of their mode of maintenance and restoration of fertility (Budar and Pelletier 2001). However, several advantages of GMS systems, such as the stable and complete male sterility and lack of any negative cytoplasmic effect on yield, as is sometimes seen with CMS, make it a promising alternative to CMS (Yi et al. 2006). The percentage of male-sterile plants in an ideal male-sterile line should be 100%, and the combining ability of its major economic traits should also be high. Obtaining this type of high-quality male-sterile line requires an extensive search of the male sterility source and knowledge of the underlying genetic mechanism.

A dominant male-sterility gene *Ms-cd1* was identified as a spontaneous mutation in a spring cabbage line 79-399-3 (Fang et al. 1997) and has since been well utilized in

commercial hybrid cabbage seed production. The male sterility controlled by *Ms-cd1* showed abnormal callose degeneration, failure of microspore separation during anther development, and temperature sensitivity (Fang et al. 1997), which allowed creation of a homozygous (*Ms-cd1*/*Ms-cd1*) genotype by selfing heterozygous plants under permissive temperatures. However, identification of the homozygous male-sterile *Ms-cd1*/*Ms-cd1* plants by test crossing with male-fertile testers is a time-consuming, laborious process, especially given biennial nature of cabbage, as male-sterile plants can only be selected when the crop is flowering. Floral initiation requires vernalization by either overwintering or cold treatment during the warm season.

Fang et al. (1997) reported abnormal pollen development in 79-933. Light microscopy studies of paraffin sections revealed that meiotic division stopped at the tetrad stage (Fang et al. 1997). Little is known at the molecular level regarding how the *Ms-cd1* gene halts meiotic division. Isolation of the *Ms-cd1* gene is therefore regarded as an important effort, not only for developing specific PCR markers for marker-assisted selection (MAS) but also for understanding the mode of action of male sterility genes. Previous molecular markers linked to this gene have included a RAPD marker with a linkage distance of 8.0 cM (Wang et al. 1998), and this RAPD was converted to an ERPAR marker (Wang et al. 2000a), and a SCAR marker (Wang et al. 2000b). Some genes that are differentially expressed during bud development have been detected by cDNA-AFLP, which suggested that the dominant *MS-cd1* gene may disrupt proper separation of pollen from the tetrads and lead to the suppressed expression of a number of genes (Lou et al. 2007). Microarray analysis identified some differentially expressed genes between fertile and sterile individuals that corresponded to anther developmental pathways and especially those expressed in tapetum cells (Kang et al. 2008). Wang et al. (2005a, b) indirectly mapped *MS-cd1* onto linkage group O9, corresponding to chromosome 3 of *B. oleracea*. This region corresponds to the top of chromosome 5 in *Arabidopsis thaliana* through the comparative genomics. Because traditional breeding is relatively long procedure, the availability of inexpensive, rapid, and reliable PCR-based markers for the *Ms-cd1* locus would greatly improve the efficiency of breeding programs using the *Ms-cd1* allele. However, the genetic distance between these markers and *MS-cd1*was not sufficiently close to allow practical utilization in marker-assisted selection. Hence, the need remains for simple PCR-based markers, such as sequence characterized amplified region (SCAR) markers, for practical use in MAS or fine mapping.

The members of the Brassicaceae are particularly important in the field of plant genomics, without doubt due to the establishment of *A. thaliana* as a model plant in numerous disciplines of plant sciences. The sequencing of the *A. thaliana* genome has produced a wealth of genomic data and resources which are utilized in comparative studies across *Brassicaceae* (Lysak and Lexer 2006). Comparative mapping between model plants and major crops provides a new strategy for exploiting the public information resource of model plants. *Brassica* and *A. thaliana* share a recent common ancestry (Yang et al. 1999). As increasingly greater number of *B. rapa, B. oleracea*, and *B. napus* sequence data are available on public websites, especially with the completion of *B. rapa* genome sequencing (http://brassicadb.org/brad/), these can then be comparatively mapped in *A. thaliana* genome and thus provide gene location in *B. oleracea*. He et al. (2008) reported fine mapping results for the *BnMs3* gene in 9012AB using AFLP-derived SCAR markers and making use of the collinearity between *B. napus* and *A. thaliana*. Lei et al. (2007) mapped a recessive male-sterile gene (*BnMs2*) in *B. napus* L. and identified a collinear region containing 68 *A. thaliana* genes using syntenic region identification based on the *A. thaliana* genome sequences, in which the orthologue of *BnMs2* might be included.

In this study, we screened a large-scale BC_4 population segregating in male fertility with SRAP and SSR markers in order to identify markers closely linked to *MS-cd1*. The identified markers were converted into dominant SCAR markers and were used to identify the collinearity between *B. oleracea* and *B. rapa*. The synteny region was further mapped to *A. thaliana* chromosome 5 to find the candidate gene. This strategy may be useful for marker-assisted selection in breeding programs and map-based cloning.

Materials and methods

Plant materials and population

The *Ms-cd1* gene originated from the cabbage male sterile mutant 79-399-3. A BC_4 population Ms79-07 consisting of 2,028 individuals,using *Brassica oleracea* var. *alboglabra* as a recurrent parent and a male sterile *B. oleracea* var. *italica* line harboring *Ms-cd1* as donor. Plants were grown in the open field and greenhouse and management was conducted normally. During the flowering period, fertility was characterized based on the criterion of the observation of pollen grains in anthers of opening flowers at least three times (Fang et al. 1997; Wang et al. 2000a). Sterile plants were those with non-viable pollen, abortive stamens, and degenerate stamen. The χ^2 test was performed on two grouped data to check the goodness of fit of the segregating populations to the expected Mendelian phenotypic segregation ratio.

DNA extraction and bulked segregant analysis

Genomic DNA was extracted from freeze-dried leaf tissue of individuals from the Ms79-07 population according to the CTAB method (Doyle and Doyle 1990). Bulked segregant analysis (BSA, Michelmore et al. 1991) was employed to identify SRAP and SSR markers linked to the *Ms-cd1* gene. Four bulks were established: two fertile DNA bulks (BF) and two male sterile DNA bulks (BS), each of them containing DNA from 20 individuals.

SRAP analysis

Eight-hundred SRAP primers from published papers (Li and Quiros 2001; Budak et al. 2004; Wang et al. 2005a, b; Riaz et al. 2001; Lin et al. 2003) were screened for polymorphism between BFs and BSs. Primer combinations with consistent amplifications and clear banding patterns were selected and used to screen 226 randomly selected individuals of the BC_4 population to confirm polymorphism. The confirmed primer pairs were run for the other 1,802 individuals of BC_4 population. The SRAP procedure was performed as described by Li and Quiros (2001). The located markers were named after primer combination, for instance, AB represents the combination of primers A and B. In order to expand the resources of SRAP primers, random primers of SRAP, AFLP primers, and SSR primers were combined as new SRAP primers in addition to standard SRAP primers.

SSR analysis

Three-hundred SSRs derived from *B. rapa* (Suwabe et al. 2002; Choi et al. 2007) were used for SSR analysis. A set of SSRs designed from published EST sequence of *B. oleracea* in NCBI (Dr. Mu Zhuang, personal communication) were screened for polymorphisms between fertile and sterile bulks. SSR amplification was performed as described by Lowe et al. (2002), and the amplified products were resolved on 8% polyacrylamide gel.

Conversion of SRAP markers into SCAR markers

Putative SRAP fragments were excised from the dried polyacrylamide gels and the DNA was purified following Cho et al. (1996). Purification products were cloned into the pMD18-T vector (TaKaRa) and sequenced. Specific primers were designed with the software Primer 5.0 and amplified in sterile and fertile individuals. Finally, the PCR products were fractionated on 2.5% agarose gel to confirm polymorphism between sterile and fertile individuals. PCR was carried out in a total volume of 20 µl containing 200 ng of genomic DNA, 0.2 mM dNTPs, 0.3 µM of each primer, 1 × PCR reaction buffer and 1 U *Taq* polymerase. The PCR running program was 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min.

Linkage analysis

In total, 2,028 BC_4 individuals were used for fine mapping of the *Ms-cd1* gene. 226 individuals were initially used for mapping the *Ms-cd1* gene and a rough map was constructed. Based on this primary mapping, we obtained the two flanking markers and determined the recombinants plants in 2,028 plants through the flanking markers. Increasingly greater numbers of new SRAP primer and random primer combinations were screened with bulked segregant analysis (BSA) to get more polymorphic markers. To determine the relative distance between the polymorphic markers and the target gene, we genotyped 1,802 individuals of the Ms79-07 mapping population using the two markers most far away from the target gene. The individuals showing recombinant events were selected and subsequently used to analyze all of the new markers obtained though flanking marker analysis (Dixon et al. 1995). Once an *Ms-cd1* linked marker was identified, all recombinant individuals of the BC_4 population were screened for the marker to verify the linkage between the marker and *Ms-cd1*. The presence or absence of each SRAP fragment was coded as "1" and "0", where "1" indicated the presence of a specific allele, and "0" indicated its absence, with the letter "u" designating missing. Only unambiguous bands were scored and analyzed. Data from phenotype survey and SRAP, SCAR, and SSR analyses were combined for linkage analysis using the JoinMap® 4.0 (http://www.kyazma.nl). Map order was based on maximum-likelihood estimates. Map distances were estimated by the Kosambi function (Kosambi 1944).

Sequence similarity analysis with the *B. rapa* and *A. thaliana* genome

After genetic mapping of the Ms79-07 population, sequences of the three SCAR markers were used to identify putatively homologous sequences in the *B. rapa* genome (http://brassicadb.org/brad/) using BLAST searches (http://www.ncbi.nlm.nih.gov). The linear arrangement relationship of these markers in *B. oleracea* genome was then compared with that of their homologues in the *B. rapa* genome to decide whether the synteny exists around *Ms-cd1* between these two related species. Once the syntenic region around the *Ms-cd1* gene in the *B. rapa* genome was identified, we BLAST the sequence of this region to the genome

Table 1 Markers linked to the *MS-cd1* gene and their characteristics

Primer combination	Primer sequence (5′–3′)	Primer type	App. size marker (bp)
CoEm17R-E37	GACTGCGTACGAATTCCA	SRAP	210
	GACTGCGTACCAATTCACG	AFLP	
OD3R-Ni2E12F	CCAAAACCTAAAACCAGGA	SRAP	153
	TTATCTGCTTGTCTTGGGGC	SSR	
BMe12F-BC64F	TGAGTCCAAACCGGAGA	SRAP	248
	TTCCGTCCCTTCCCTAAACA	SSR	
ENA14F-CoEm7R	CTTACGGTGGAAATGCTG	SSR	141
	GACTGCGTACGAATTATG	SRAP	
ENA20R-rem2	TCTGAACTACCAAAGCCAAC	SSR	144
	GACTGCGTACGAATTTGC	SRAP	
nssr016F-nssr037F	TTCATCCACTTTGTTCAATACAAGA	SSR	223
	GCGGTTAATAGGTTCCGGTT	SSR	
8c00909	GTTTTTGTTCTTTTGCCATCACT	SSR	190
	ATAAACTCCGTCTTCCACCATC	SSR	
FITO57R-nssr017F	AGTAGGTTGATTGTATTGACCAG	SSR	150
	GGTTAAGCAGACGATGGAAGTAA	SSR	
M83-BEm10R	GATGAGTCCTGAGTAATCA	AFLP	140
	GACTGCGTACGAATTCAT	SRAP	
FITO109L-BEm14R	CAAATCAACCCAAGAGGAC	SSR	170
	GACTGCGTACGAATTCTT	SRAP	
FITO163L-CoEm11R	GTGCTGTTGAATGTAGTCCTG	SSR	140
	GACTGCGTACGAATTTCG	SRAP	
nssr014F-nssr051F	CATGATACTTTCCCAAAACCAAA	SSR	183
	AAATCCCGCATTTTTACACG	SSR	
nssr030R-nssr052F	TGCTCTCCTCAAGTGAATCAAA	SSR	140
	AGTTGGATTCTCGTTGGGAA	SSR	
nssr016F-nssr039F	TTCATCCACTTTGTTCAATACAAGA	SSR	348
	GCATATGACCAAAGCAAGAGAA	SSR	
FITO7.1R-CoEm16R	TAAAATTTAATCGCAGCGCG	SSR	105
	GACTGCGTACGAATTCGG	SRAP	

App. Size marker approximate size of the marker

sequence of *A. thaliana* (http://www.arabidopsis.org/) to find the syntenic region.

Results

Fertility segregation in BC_4 population

A total of 2,028 individuals were obtained from the Ms79-07 population; 1,064 individuals were male-fertile and 964 were male-sterile. The χ^2 test showed that the segregation ratio of fertility and sterility was consistent with the expected ration of 1:1 (χ_c^2 =2.4 and $P > 0.05$), confirming the deduction that only one male sterile gene segregates in the BC_4 population (Fang et al. 1997).

Screening SRAP markers linked to the *Ms-cd1* gene

Out of 26,417 SRAP primer pairs, 14 markers showed polymorphism between fertile bulks (BF) and sterile bulks (BS); both BFs and BSs were consistent in amplification. All of these 14 polymorphic markers amplified single clear polymorphic band (Table 1), and all were dominant markers. These markers were then verified by amplification in 80 individuals comprising the bulks and 226 randomly selected BC_4 individuals.

Screening SSR markers linked to the *Ms-cd1* gene

In total, 1,300 SSR primers were screened to the four bulks, resulting in one dominant SSR marker 8C0909 (Fig. 1)

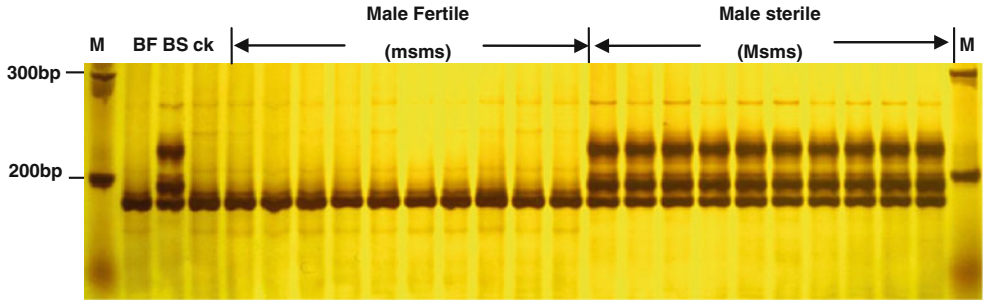

Fig. 1 PCR products of SSR marker 8C0909 in the individuals segregating in male fertility. *BF* male fertile bulk, *BS* male sterile bulk, *ck* fertile parent. *M* 50 bp DNA ladder. The genotype of male fertile plants is msms, while it is Msms for male sterile plants

Table 2 SCAR markers developed from SRAP fragments linked to the *MS-cd1* gene

SCAR marker	Primer sequence (5′–3′)	Size (bp)	Character
ENA20R-rem2SC	F: GTACGAATTTGCAGGACTAGG	135	Dominant
	R:GAACTACCAAAGCCAACAAAC		
ENA14F-CoEm7RSC	F: TTACGGTGGAAATGCTGGTGA	138	Dominant
	R: CTGCGTACGAATTATGAGTC		
OD3R-Ni2E12FSC	F:CTAAAACCAGGACCGCCAACTAA	128	Dominant
	R: GCCACCCGCTTTCCTTCCTC		

F forward primer and *R* reverse primer

which distinguished the phenotype of male sterility and male fertility. This marker was further mapped on the linkage map of *Ms-cd1* gene.

Conversion of SRAP markers to SCAR markers

To facilitate marker-assisted selection, the identified SRAP markers were converted into SCAR markers. Ten SRAP markers were sequenced. Based on the sequence data of these ten markers, primers were designed for direct amplification from genomic DNA. Three of the ten SRAP markers (ENA14F-CoEm7RSC, ENA20R-rem2SC and CoEm17R-E37SC) were successfully converted into SCAR markers (Table 2), locating at the positions of 0.18, 0.39 and 4.23 cM to *Ms-cd1*, respectively. The amplified band of these three SCAR markers was present almost exclusively in sterile individuals and absent in fertile individuals. All of the three markers were dominant.

Genetic mapping of *Ms-cd1*

Polymorphic bands that appeared in BS, but not in BF, were potentially linked to the gene *Ms-cd1*. Therefore, 226 individuals in the Ms79-07 population were screened for primary linkage analysis (data not shown). According to the primary analysis, two flanking markers (OD3R-Ni2E12FSC and FITO7.1R-CoEm16R) were identified as the outside markers flanking *Ms-cd1*, at distances of 4.23 and 2.61 cM from the *Ms-cd1* gene, respectively. For the fine mapping of *Ms-cd1*, the two flanking markers were assayed first in the extra1,802 BC_4 individuals to identify recombinants, In total 139 recombinants were identified, including 47 individuals displayed recombination between the *Ms-cd1* gene and OD3R-Ni2E12FSC, and 92 recombinants of the *Ms-cd1* gene and FITO7.1R-CoEm16R. The polymorphic markers were then analyzed using only the 139 recombinants. Linkage analysis was performed with the 11 SRAP markers, one SSR marker, and three SCAR markers and revealed that all these markers mapped to a 6.88 cM region around the *Ms-cd1* gene. The three SCAR markers distributed at both sides of *Ms-cd1* with a distance of 0.18, 0.39, and 4.23 cM, respectively, and the SCAR marker located 0.18 cM to *Ms-cd1* was the closest one. BMe12F-BC64F was the most closely linked one on the other side with a distance of 2.16 cM. The SSR marker 8C00909 was located at the distance of 2.06 cM to *Ms-cd1*.

Ms-cd1 linked markers match *B. rapa* genome

We BLAST the sequences of the three SCAR markers to the sequence assembly of *B. rapa* (http://brassicadb.org/brad/) and found that two of them having highly conserved homologues on Scaffold000010. This scaffold was assigned on *B. rapa* chromosome A10. The corresponding region was on *A. thaliana* chromosome 5, containing 108 predicted genes of which 54 genes had homologues in *B. rapa* genome. Through the Genevestigator, we found At5 g 22940 in this region, which was reported to be expressed

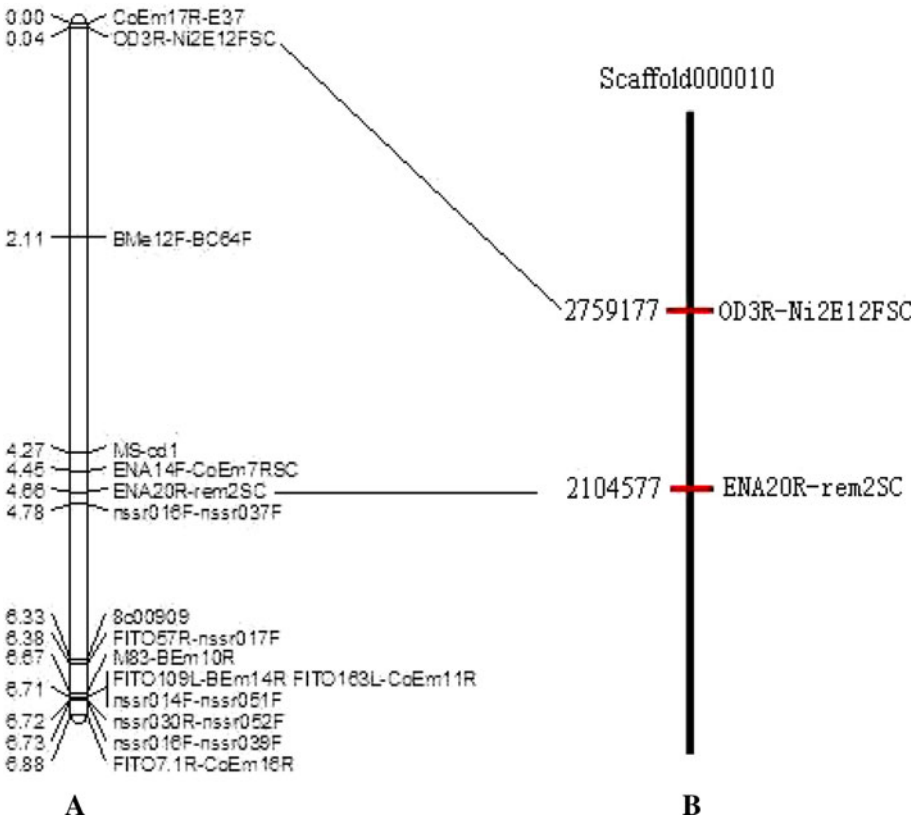

Fig. 2 **a** The genetic linkage map of the *Ms-cd1* gene and associated molecular markers. Recombination distances are indicated on the *left* side of each linkage group in centimorgans (cM), and the loci names are shown on the *right* side. **b** The distribution of homologous DNA segments on *B. rapa* chromosome A10

Table 3 SCAR markers of *B. oleracea* being aligned to *B. rapa* genome sequence

Marker	Scaf. ID	Marker size (bp)	Aligned length (bp)	Identity (%)	Start position (bp)	End position (bp)
ENA20R-rem2SC	Scaf000010	153	120	89	2,759,177	2,759,296
OD3R-Ni2E12FSC	Scaf000010	144	122	92	2,104,457	2,104,577

Scaf. scaffold

largely in stamen and pollen, but less in other organs (Lee et al. 2009). The order of the molecular markers linked to *Ms-cd1* was consistent with the physical distance of their homologues in *B. rapa* (Fig. 2a, b; Table 3). This suggests that the syntenic region was perfectly collinear between *B. oleracea* and *B. rapa*. Accordingly, we concluded that SCAR markers that are tightly linked to *Ms-cd1* gene in *B. oleracea* should match this 600 kb genomic region on chromosome 10 in *B. rapa* and that some specific PCR primers from this region are expected to map the *Ms-cd1* gene more closely.

Discussion

In the present study, SRAP and SSR technology were used in combination with BSA to identify markers linked to the *Ms-cd1* gene. We screened 800 SRAP primers, 25,617 SRAP combined random primers, and 1300 SSR primers and identified 14 SRAP markers and one SSR marker linked to the *Ms-cd1* gene. We constructed a high-resolution map around the *Ms-cd1* gene and found there were 16 markers around the *Ms-cd1* gene region. In previous studies by Wang et al. (2000a, b) and Lou et al. (2007), RAPD, SCAR, and AFLP markers were identified as being linked to the *Ms-cd1* gene using a small population and located on one side. In the present study, the closest SCAR and SRAP markers flanking the *MS-cd1* gene on each side were at distances of 0.18 and 2.16 cM, respectively, which are much closer than the previously reported markers. The resulting high-density genetic map would be useful for map-based cloning of the male sterility gene *Ms-cd1*. We used a large BC_4 population to allow fine mapping, which not only provided information on some available SCAR and dominant SSR markers, but also allowed us to locate the *Ms-cd1* gene on chromosome A10 in *B. rapa*, a very close relative of *B. oleracea*. Fine mapping and cloning of the *Ms-cd1* gene in *B. oleracea* will definitely promote the molecular-based study of the DGMS.

Molecular markers linked to numerous agronomically important traits have been reported, and a number of these have now been successfully integrated into oilseed breeding programs (Agarwal et al. 2008). The ability to convert anonymous PCR markers that are closely linked to traits of interest into sequence-characterized amplified region (SCAR) or sequence-tagged site (STS) markers has provided a platform for the development of simple PCR-based markers that meet the requirements and capacity of commercial rapeseed breeders. SRAP technique has been widely used in genetic diversity studies and in construction of a linkage map in *B. napus* (Riaz et al. 2001; Li and Quiros 2001; Sun et al. 2007; Gao et al. 2007). In this study, SRAP primers were randomly combined with AFLP primers or SSR primers, which proved to be a successful strategy for polymorphism screening. This approach is a valuable way to make more widespread use of SRAP.

In China, GMS has become an important hybrid system and several GMS-based hybrids have already been registered in *Brassica* family (Fang et al. 1997). For MAS to be practical in agriculture, simple PCR-based markers are needed, such as those provided by sequence-characterized amplified regions (SCARs). In the present study, three SRAP markers were successfully converted to dominant SCAR markers. In particular, we identified a dominant SSR marker 8C0909 with a distance of 2.06 cM to *Ms-cd1*. Hong et al. (2008) reported that two AFLP markers linked with the *Ms* gene were converted to SCAR markers. The SCAR markers and SSR marker obtained in the present study will greatly facilitate the transfer of the dominant GMS alleles into desirable genetic backgrounds via marker-assisted selection.

Sequence information and genomic resources of *A. thaliana* is recognized for its usefulness in marker development, map-based gene cloning, and candidate gene identification in *Brassica* crops (Snowdon and Friedt 2004). This strategy has been successfully used to develop markers closely linked to recessive genic male sterility gene (*Bnms2*, *Bnms3*) in *B. napus* (Lei et al. 2007, He et al. 2008) as well as to locate the homologous gene of *Ms-cd1* on *A. thaliana* chromosome 5 (Wang et al. 2005a, b). In the present study, we constructed a fine scale map of *Ms-cd1* locus using a combination of BSA, SRAP, SSR, and SCAR methodologies. Through comparative mapping with *B. rapa* and *A. thaliana*, a syntenic region covering 108 genes was identified in which the homologue of *Ms-cd1* might be included. We intend to exploit candidate gene strategy to clone the *Ms-cd1* gene in the future.

Even though the GMS system is an effective alternative hybrid system in *B. oleracea*, very little is known regarding the molecular basis of the GMS trait, which limits its wide application to hybrid breeding. Cloning and characterization of GMS genes in the future will improve its application in hybrid breeding. In our next step, we expect to develop greater numbers of useful markers (including Indel and SNP markers) from the identified region where *Ms-cd1* located in, by sequencing of the monomorphic PCR products between the fertile and sterile plants. The newly developed markers tightly linked with the *Ms-cd1* will be very useful in MAS of male-sterile plants and will facilitate the isolation of the *Ms-cd1* gene by map-based cloning.

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References

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27:617–631

Budak H, Shearman RC, Parmaksiz I, Gaussoin RE, Riordan TP, Dweikat I (2004) Molecular characterization of Buffalograss germplasm using sequence-related amplified polymorphism markers. Theor Appl Genet 108:328–334

Budar F, Pelletier G (2001) Male sterility in plants: occurrence, determinism, significance and use. C R Acad Sci III 324:543–550

Cho YG, Blair WM, Panaud O, McCouch SR (1996) Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphisms (AFLP) from silver stained polyacrylamide gels. Genome 39:373–378

Choi SR, Teakle GR, Plaha P et al (2007) The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. Theor Appl Genet 115:777–792

Dixon MS, Jones DA, Hatzixanthis K, Ganal MW, Tanksley SD, Jones JDG (1995) High-resolution mapping of the physical location of the tomato *cf-2* gene. Mol Plant Microbe Interact 8(2):200–206

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Fang ZY, Sun PT, Liu YM, Yang LM, Wang XW, Hou AF, Bian CS (1997) A male sterile line with dominant gene (Ms) in cabbage (*Brassica oleracea* var. capitata) and its utilization for hybrid seed production. Euphytica 97:265–268

Gao MQ, Li GY, Yang B, Qiu D, Farnham M, Quiros CF (2007) High-density *Brassica oleracea* linkage map: identification of useful new linkages. Theor Appl Genet 115:277–287

He JP, Ke LP, Hong DF, Xie YZ, Wang GC, Liu PW, Yang GS (2008) Fine mapping of a recessive genic male sterility gene (*Bnms3*) in rapeseed (*Brassica napus*) with AFLP- and Arabidopsis-derived PCR markers. Theor Appl Genet 117:11–18

Kang JG, Zhang GY, Bonnema G, Fang ZY, Wang XW (2008) Global analysis of gene expression in flower buds of *Ms-cd1*in *Brassica oleracea* conferring male sterility by using an *Arabidopsis* microarray. Plant Mol Biol 66:177–192

Kaul MLH (1988) Male sterility in higher plants. Springer, Berlin

Kosambi DD (1944) The estimation of map distance from recombination value. Ann Eugen 12:172–175

Lee CH, Teng Q, Huang WL, Zhong RQ, Ye ZH (2009) The F8H Glycosyltransferase is a Functional Paralog of FRA8 Involved in

Glucuronoxylan Biosynthesis in *Arabidopsis*. Plant Cell Physiol 50(4):812–827

Lei SL, Yao XQ, Yi B, Chen W, Ma CZ, Tu JX, Fu TD (2007) Towards map-based cloning: fine mapping of a recessive genic male-sterile gene (*BnMs2*) in *Brassica napus* L. and syntenic region identification based on the *Arabidopsis thaliana* genome sequences. Theor Appl Genet 115:643–651

Li GY, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theor Appl Genet 103:455–461

Lin ZX, Zhang XL, Nie YC, He DH, Wu MQ (2003) Construction of a genetic linkage map for cotton based on SRAP. Chin Sci Bull 48(19):2063–2067

Lou P, Kang JG, Zhang GY, Bonnema G, Fang ZY, Wang XW (2007) Transcript profiling of a dominant male sterile mutant (*Ms-cd1*) in cabbage during flower bud development. Plant Sci 172:111–119

Lowe AJ, Jones AE, Raybould AF, Trick M, Moule CJ, Edwards KJ (2002) Transferability and genome specificity of a new set of microsatellite primers among *Brassica* species of the U triangle. Mol Ecol Notes 2:7–11

Lysak MA, Lexer C (2006) Towards the era of comparative evolutionary genomics in Brassicaceae. Plant Syst Evol 259:175–198

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Riaz A, Li G, Quresh Z, Swati MS, Quiros CF (2001) Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breed 120(5):411–415

Snowdon RJ, Friedt W (2004) Molecular markers in Brassica oilseed breeding: current status and future possibilities. Plant Breed 123:1–8

Sun ZD, Wang ZN, Tu JX, Zhang JF, Yu FQ, McVetty PBE, Li GY (2007) An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. Theor Appl Genet 114:1305–1317

Suwabe K, Iketani H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Wang XW, Fang ZY, Sun PT, Liu YM, Yang LM (1998) Identification of a RAPD marker linked to a dominant male sterile gene in cabbage. Acta Hortic Sinica 25(2):197–198

Wang XW, Fang ZY, Huang SW, Sun PT, Liu YM, Yang LM, Zhuang M, Qu DY (2000a) An extended random primer amplified region (ERPAR) marker linked to a dominant male sterility gene in cabbage (*Brassica oleracea* var. capitata). Euphytica 112:267–273

Wang XW, Fang ZY, Sun PT, Liu YM, Yang LM, Zhuang M (2000b) A SCAR marker applicable in marker assisted selection of a dominant male sterility gene in cabbage. Acta Hortic Sinica 27(2):143–144

Wang XW, Lou P, Bonnema G, Yang BJ, He HJ, Zhang YG, Fang ZY (2005a) Linkage mapping of a dominant male sterility gene *Ms-cd1* in *Brassica oleracea*. Genome 48:848–854

Wang G, Pan JS, Li XZ, He HL, Wu AZ, Cai R (2005b) Construction of a cucumber genetic linkage map with SRAP markers and location of the genes for lateral branch traits. Sci China Ser C Life Sci 48(3):213–220

Yang YW, Lai KN, Tai PY, Li WH (1999) Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between Brassica and other angiosperm lineages. J Mol Evol 48:597–604

Yi B, Chen YN, Lei SL, Tu JX, Fu TD (2006) Fine mapping of the recessive genic male-sterile gene (*Bnms1*) in *Brassica napus* L. Theor Appl Genet 113:643–650