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ORIGINAL PAPER

SSR and SCAR mapping of a multiple-allele male-sterile gene in Chinese cabbage (*Brassica rapa* L.)

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Abstract The genic multiple-allele inherited male-sterile gene *Ms* in Chinese cabbage (*Brassica rapa* L.) was identified as a spontaneous mutation. Applying this gene to hybrid seed production, several *B. rapa* cultivars have been successfully bred in China. A BC_1 population (244 plants) was constructed for mapping the *Ms* gene. Screening 268 simple sequence repeat (SSR) markers which cover the entire genome of Chinese cabbage was performed with bulked segregant analysis (BSA). On the basis of linkage analysis, the *Ms* gene was located on linkage group R07. In addition, through the amplified fragment length polymorphism (AFLP) and the sequence-characterized amplified region (SCAR) techniques combining BSA, two SCAR markers which were converted from corresponding AFLP markers flanked the *Ms* gene. Finally, a genetic map of the *Ms* gene was constructed covering a total interval of 9.0 cM. Two SCAR markers, syau_scr01 and syau_scr04, flanked the *Ms* gene at distances of 0.8 and 2.5 cM, respectively. All the SSR markers (cnu_m273, cnu_m030, cnu_m295, and syau_m13) were mapped on the same side of the gene as syau_scr04, the nearest one of which, syau_m13, was mapped at a distance of 3.3 cM. These SSR and SCAR markers may be useful in marker-assisted selection and map-based cloning.

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Introduction

Chinese cabbage (*Brassica rapa* L.) is a typically allogamous plant with bisexual flowers and obvious heterosis. The utilization of male-sterile lines is an economical and stable way to improve the cross-breeding of Chinese cabbage. 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. In order to obtain such a high quality male-sterile line, it is crucial to search the male sterility source and reveal the underlying genetic mechanism.

The male-sterile materials of Chinese cabbage can be divided into genic male sterility (GMS) and cytoplasmic male sterility (CMS) (van der Meer 1987). GMS has more obvious advantages, such as stable and complete sterility performance, extensive distribution of restorers, and no negative cytoplasmic effects. However, the known GMS in Chinese cabbage is considered to be mainly controlled by monogenic recessive or dominant genes. If testcrosses are employed in the selection of maintaining lines of the male sterility, a maximum of 50% of the plants in these lines will be sterile. In the utilization of such male-sterile resources, a pronounced drawback is the required manual removal of the fertile plants from the female parent line (van der Meer 1987).

Feng et al. (1995, 1996) obtained four stable hereditary lines comprising 100% male-sterile plants in Chinese cabbage and proposed a genetic hypothesis of a genic

multiple-allele male-sterile gene. Following the genetic model in this hypothesis, the male-sterile allele could easily be transferred into normal fertile lines of Chinese cabbage (Wang et al. 2005; Feng et al. 2007). The model considers a single locus with three alleles: the "*Ms*" allele for male sterility, "*ms*" allele for fertility, and "Ms^f" for fertility restoration. The dominant–recessive relationship among these alleles was $Ms^f > Ms > ms$. In order to obtain a male-sterile line with 100% male-sterile plants, a temporary maintainer line is employed to cross with the homozygous sterile line (Fig. 1). Hybrid seed production can subsequently be carried out with the male-sterile line as the female parent. Thus, manual removal of fertile plants in the sterile line, a necessary step in a two-line system, is obviated.

To date, however, there have been no molecular biological studies on this valuable resource in Chinese cabbage, although some molecular studies have been reported for other GMS resources in *Brassica* crops. For Chinese cabbage, Miao et al. (2003) identified four sequence-tagged site (STS) markers tightly linked to a recessive male-sterile gene; Cao et al. (2005) isolated a cytochrome P450 *CYP86MF* gene from floral buds; and Zhang et al. (2008) developed a sequence-characterized amplified region (SCAR) marker linked to a dominant male-sterile gene. Further, a set of molecular markers linked to the GMS has been identified in *B. napus* (Wang et al. 2000, 2006; Lu et al. 2004a, b; Ke et al. 2005; Hong et al. 2006; Song et al. 2006; Yi et al. 2006; Huang et al. 2007; Lei et al. 2007). Wang et al. (2000a, b) identified a group of markers linked to a dominant male-sterility gene in *B. oleracea*. In the present study, we mapped the multiple-allele inherited male-sterile gene "*Ms*" using simple sequence repeat (SSR) and SCAR markers. These markers may be useful in marker-assisted selection (MAS) and map-based cloning.

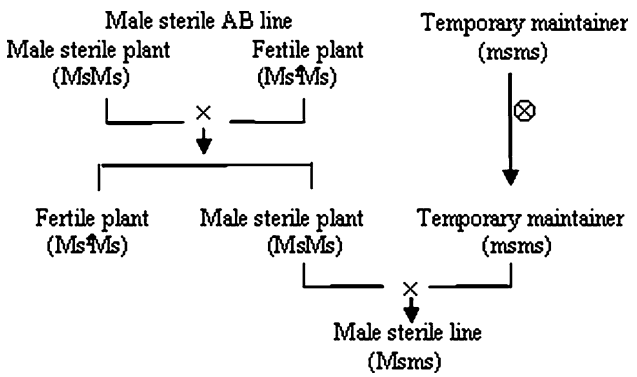

Fig. 1 Genetic model of the genic multiple-allele inherited male-sterile line in Chinese cabbage

Materials and methods

Plant materials

Chiifu (provided by Chungnam National University, Korea) is the model material of the *B. rapa* Genome Sequencing Program (BrGSP). It is a fertile inbred line in Chinese cabbage and was used as the male parent of the mapping population. AB01-1, which was used as the female parent of the mapping population, is the male-sterile plant of a previously bred male-sterile AB line in which sterile plants (AB01-1, *MsMs*) and fertile plants (AB01-2, Ms^fMs) segregated in a 1:1 ratio. It is a complete and stable male-sterile material, with withered anthers and normal pistil (Fig. 2).

For the genotyping of Chiifu, sterile plants "AB02-1" (*MsMs*) and fertile plants "AB02-2" (Ms^fMs) of an AB line, a genic male-sterile line "MS-11" (*Msms*), and a temporary maintainer line "ms-05" (*msms*), were employed in a testcross.

Development of the mapping population

A BC_1 population was developed for the SSR and SCAR mapping of the genic multiple-allele inherited male-sterile gene *Ms*. One AB01-1 plant was crossed with Chiifu, resulting in an F_1 generation with 100% male-sterile plants. A segregating population was obtained by backcrossing a sterile F_1 plant with Chiifu. All the seeds were orderly programmed in the seed plot, and the seedlings with four to five leaves were transplanted to the cropland in Shenyang Agricultural University. Plants were examined at flowering for male sterility/fertility through visual examination.

DNA isolation and BSA design

Genomic DNA was isolated from fresh leaves of the parents and the BC_1 plants following the procedure of Pierre and Marechal-Drouard (1992), with minor modifications. Fertile and sterile bulks were prepared by pooling an equal quantity of DNA from ten fertile and ten sterile individuals, respectively. The DNA of the two parents and the two bulks were used for bulked segregant analysis (BSA) (Michelmore et al. 1991) combined with the SSR and amplified fragment length polymorphism (AFLP) techniques.

SSR analysis

Two hundred and sixty-eight SSR primer pairs were used to screen for polymorphism between the parents. PCR amplification was carried out in a volume of 20 μl, containing 1 U *Taq* polymerase, 0.5 μM primers, 200 μM

Fig. 2 Open flower of a normal fertile plant "Chiifu" (**a**) and a male-sterile plant "AB01-1" (**b**) with the multiple-allele inherited male-sterile gene

dNTPs, 1.5–2.0 mM $MgCl_2$, 1 × PCR buffer and 40 ng genomic DNA as the template. The PCR profile was as follows: 95°C for 5 min, followed by 30–35 cycles at 94°C for 30 s, certain appropriate annealing temperature for 45 s, 72°C for 60 s, and ended by extension at 72°C for 7 min. PCR was carried out in a BIO-RAD iCycler thermocyler and the products were separated on a 6% denaturing polyacrylamide gel.

Additionally, in order to identify SSR markers more closely linked to the gene of interest, ten SSR primer pairs were designed with the sequence of BAC clones positioned on linkage group R07 (http://www.brassica-rapa.org) using Primer3 software (Rozen and Skaletsky 1996).

AFLP analysis

In order to narrow down the *Ms* locus, AFLP analysis was performed. The AFLP technique followed that of Vos et al. (1995), with minor modifications. Genomic DNA (500 ng) was digested with *Pst*I and *Mse*I in a total volume of 25 µl at 37°C overnight. After the inactivation of restriction enzymes at 75°C for 15 min, adapters were ligated in a total volume of 50 µl at 37°C for 3 h. A 5-µl aliquot of a tenfold dilution of the restriction/ligation mixture was used as the template DNA in a pre-amplification reaction containing 2.5 mM dNTP, 1 × PCR buffer (15 mM $MgCl_2$), 27 ng each of *Pst*I + G/*Mse*I + C primers, and 1 U *Taq* DNA polymerase. The amplicon was diluted 50-fold and used as the template DNA in the selective PCR. In order to identify markers linked to the *Ms* locus, 256 primer combinations derived from 16 *Pst*I + GNN and 16 *Mse*I + CNN primers were employed. The selective PCR was performed with 1 × PCR buffer (15 mM $MgCl_2$), 2.5 mM dNTP, 30 ng *Mse*I + CNN primer, 15 ng *Pst*I + GNN primer, and 0.4 U of *Taq* DNA polymerase. All amplifications were performed in a BIO-RAD iCycler using the PCR conditions described by Vos et al. (1995). PCR products were mixed with an equal volume of loading dye (98% formamide, 10 mM EDTA, and 0.001% each of xylene cyanol and bromphenol blue). Samples were denatured at 94°C for 5 min and separated on a 6% denaturing polyacrylamide gel at 85 W for 2.5 h. After electrophoresis, the gel was developed using a silver staining kit (Bioneer, Daejeon, Korea).

Conversion of AFLP markers

Gel pieces containing the AFLP markers were sliced and boiled for 5 min in 100-µl sterile water. After centrifugation, a 5-µl aliquot of the supernatant was amplified with the corresponding selective primer combinations and the same PCR conditions used in the selective amplification. The amplicons were separated on a 1.0% agarose gel, followed by elution using a gel extraction kit (Tiangen, Beijing, China). The eluted AFLP fragments were cloned into the pGEM-T Easy Vector system I (Promega). The AFLP clones were sequenced from both ends using an ABI PRISM 377 automated sequencer. Primer pairs for developing SCAR markers were designed based on the two terminal sequences using the Primer3 program. PCR conditions were optimized with pre-amplification products of the two parents and the two bulks as templates. Subsequently, these conditions were used to amplify genomic DNA from the 20 BC_1 plants that constituted the two bulks. PCR products were separated on a 2% agarose gel or a 6% denaturing polyacrylamide gel. If the fragment amplified with the primer pairs and under the optimized condition showed the same polymorphism between fertile and sterile plants as in the AFLP analysis, then the SCAR markers were considered to be successfully developed. The primer sequences and PCR conditions used here are listed in Table 3.

Linkage analysis

The SSR and SCAR markers were used to survey 244 BC_1 plants. Linkage analysis was performed with MAPMAKER/EXP3.0, with a minimum LOD score of 3

(Lander et al. 1987). Map distances in centimorgans (cM) were calculated using the Kosambi mapping function (Kosambi 1944).

Results

The genotyping of Chiifu

A plant of Chiifu was testcrossed with four identified genotypes. The F_1 plants from crosses between Chiifu and two homozygous materials, AB02-1 (*MsMs*) and ms-05 (*msms*), were all sterile and fertile, respectively. A 1:1 ratio for fertility:sterility was detected in the F_1 generations obtained from crosses between Chiifu and two heterozygous materials, AB02-2 (*Ms*^{f}*Ms*) and MS-11 (*Msms*). All the results confirmed the multiple-allelic inheritance of the male sterility in Chinese cabbage. On the basis of the testcross results, the genotype of Chiifu at the target locus was determined as "*msms*" (Table 1).

Construction of the mapping population

A BC_1 mapping population comprising 244 individuals was developed between AB01-1 (*MsMs*) as the female parent and Chiifu (*msms*) as the recurrent parent. Among the 244 plants, 130 were fertile and 114 were male-sterile, consistent with a 1:1 segregation ($\chi^2 = 0.922$; $\chi^2_{0.05,1} = 3.84$).

Identification of SSR markers linked to the *Ms* gene

In order to map the *Ms* gene, 268 SSR primers covering the entire genome of Chinese cabbage were employed to identify polymorphisms between the parents, AB01-1 and Chiifu. Of these, 182 were polymorphic, and three, named cnu_m295, cnu_m030, and cnu_m273 (Table 2) on linkage group R07 were identified as being linked to the *Ms* gene based on the BSA analysis. From BAC clones positioned on R07 (http://www.brassica-rapa.org), 10 SSR primers were designed to identify SSR markers more tightly linked to the *Ms* gene. One SSR marker, syau_m13 (Table 2), showed linkage to the *Ms* gene, whereas the other nine were monomorphic (Supplementary material S1).

Identification of AFLP markers and conversion to SCAR markers

BSA was carried out with 256 (16 *Pst*I + GNN × 16 *Mse*I + CNN) AFLP primer combinations. The *Pst*I + GTA

Table 1 Genotyping results of Chiifu at the *Ms* locus

Tested line	Testcross lines	Number of fertile plants in the F_1 generation	Number of sterile plants in the F_1 generation	Segregation ratio	χ^{2*}
Chiifu	AB02-1 (*MsMs*)	0	55	All sterile	–
	AB02-2 (*Ms*^{f}*Ms*)	32	25	1:1	0.632
	ms-05 (*msms*)	52	0	All sterile	–
	MS-11 (*Msms*)	31	25	1:1	0.446

* Significant at the 0.05 level of probability, $\chi^2_{0.05,1} = 3.84$

Table 2 SSR markers linked to the *Ms* gene

SSR markers	Accession number of the clone	Primers (5′–3′)[a]	Motif and repeat	Size (*Ms*/*ms*)	PCR conditions[b]
cnu_m273	AC189445	ATAAGGGCATCGCCTCAACA and TGCACGCATCCACATAAACA	$(AG)_{23}$	271 bp/273 bp	58°C, 45 s 72°C, 45 s
cnu_m030	AC189617	GAAACAAATTATTTAAAAATCAGACCA and TGGAACAATCCGTAAAACTATGC	$(AT)_{16}$	205 bp/199 bp	55°C, 45 s 72°C, 45 s
cnu_m295	–	GCTGCCTAATAGGGTGCTTG and AGAGCGCATTCAAGTCTGGT	$(CT)_{12}$	193 bp/197 bp	59°C, 45 s 72°C, 45 s
syau_m13	AC189476	TGTTCCTGACTGGAAACTAGTGT and GTCAAAATGAGTCGTAAAGAAAGC	$(GA)_{45}$	296 bp/300 bp	60°C, 45 s 72°C, 45 s

[a] Forward and reverse primers are listed in that order

[b] PCR conditions for all primers were 5 min at 94°C, 35 cycles of 30 s at 94°C

and *Pst*I + GGG primers in combination with any of the 16 *Mse*I + CNN primers failed to yield any product. Each of the remaining 224 primer combinations resulted in the amplification of an average of 42 fragments. The frequency of polymorphism between the two parents was approximately 24%, with a range of 2–32%. Of the 2,288 polymorphic loci detected between the two parents, 18 displayed polymorphism with respect to two bulks. After verification of these candidate markers in the BC_1 individuals derived from the two bulks, markers P01 and P04 showed the closest linkage to the gene.

Several features of the AFLP technique limit its application in large-scale screening for map-based cloning or MAS. In order to overcome this problem, the AFLP polymorphic fragments of P01 and P04 were cloned and sequenced, and the corresponding AFLP primers were found at the end of the fragments as expected. With removal of the AFLP adapter sequences, the exact sizes of the cloned fragments were determined to be 378 bp for P01 and 204 bp for P04. Specific primers were designed using Primer3 software (Table 3). Finally, two SCAR markers (syau_scr01 and syau_scr04) were derived from the two AFLP markers, P01 and P04, respectively. Amplification with these markers in the two parents and BC_1 individuals indicated that the two AFLP markers were converted successfully (Supplementary material S2). Of these two markers, syau_scr01 was dominant and syau_scr04 was co-dominant (see Table 4).

Mapping of the *Ms* locus with SSR and SCAR markers

A total of 244 BC_1 plants were screened with the SSR and SCAR markers. Sterility-specific markers were present in most male-sterile plants but absent from most fertile plants (Supplementary material S1 and S2). The dominant marker, syau_scr01, and the co-dominant marker, syau_scr04, closely flanked the *Ms* gene at 0.8 and 2.5 cM, respectively. All other markers (cnu_m273, cnu_m030, and cnu_m295, and syau_m13) were found located on the same side of the gene as syau_scr04, although mapped at father distances (Fig. 3).

Discussion

For mapping the *Ms* gene, Chiifu was used as the male parent of the mapping population. Using Chiifu as model material, the BrGSP has developed a number of genomic resources, including two genetic mapping populations, two BAC libraries, 22 cDNA libraries, 107,280 BAC-end sequences, and 104,914 ESTs (Trick et al. 2007) and has constructed a genetic (Choi et al. 2007) and a physical map (Mun et al. 2008) of *B. rapa*. Employing position-specific SSR markers, as well as the information of contigs and BAC clones developed by the BrGSP, we identified four SSR markers (cnu_m273, cnu_m030, cnu_m295, and syau_m13) closely linked to the gene on the same side. The primers of all the four markers were designed in the BAC clones positioned on linkage group R07. Of the four markers, syau_m13, as the nearest one linked to the gene, was positioned at the 26.9 cM locus on R07 (http://www.brassica-rapa.org). Subsequently, the gene was mapped on R07, at a distance of 3.3-cM to syau_m13. Furthermore, there is extensive genome collinearity between *Arabidopsis* and *B. rapa* (Choi et al. 2007). In our work, cnu_m273,

Table 4 Segregation of the *Ms* locus, four SSR markers and two SCAR markers in the BC_1 population

Traits or markers	Number of BC_1 plants with the markers[a]		Expected ratio	χ^{2b}
	aa	Aa		
Ms	130	114	1:1	0.922
cnu_m273	130	114	1:1	0.922
cnu_m030	128	116	1:1	0.496
cnu_m295	130	114	1:1	0.922
syau_m13	134	110	1:1	2.168
syau_scr01	130	114	1:1	0.922
syau_scr04	136	108	1:1	2.988

[a] The number of BC_1 plants showing homozygous and heterozygous bands: *aa* fertile homozygous, *Aa* male-sterile heterozygous

[b] Significant at the 0.05 level of probability, $\chi^2_{0.05,1} = 3.84$

Table 3 AFLP markers and converted SCAR markers linked to the *Ms* gene

AFLP markers	AFLP primers	SCAR markers		Marker type	Size	Accession number	PCR conditions[b]
		Name	Primers (5′–3′)[a]				
P01	Pggc/Mctt	syau_scr01	GCAAATTTGTCAAACTTCACC and TCCACCACATTACTTCCCAA	Dominant	378 bp (*Ms*)	FJ775532	58°C, 45 s 72°C, 60 s
P04	Pgca/Mcac	syau_scr04	AGGATATATCTTGGCTCACGAG and CATCAATAGTGGCGTATGTCTG	Co-dominant	204 bp/208 bp (*Ms/ms*)	FJ775534/ FJ775531	58°C, 45 s 72°C, 60 s

[a] Forward and reverse primers are listed in that order

[b] PCR conditions for all primers were 5 min at 94°C, 35 cycles of 30 s at 94°C

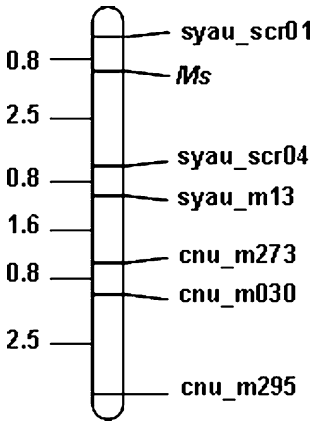

Fig. 3 Genetic linkage map of the *Ms* locus on R07, generated in the BC_1 population (244 individuals) derived from a Chiifu/AB01-1 cross. Genetic distance in cM was calculated using the Kosambi function

cnu_m030, and cnu_m295 have their own aligned regions in the range 20,145,282–22,784,532 bp on chromosome 3 of the *Arabidopsis* genome. On the basis of the present results, the increasing number of sequences for the *Brassica* A genome, and information on alignment with the *Arabidopsis* genome, the fine mapping and map-based cloning of the *Ms* gene may be completed within a short time.

Of the 268 SSR primers covering the whole Chinese cabbage genome, 186 were found to be polymorphic between the two parents. The rate of polymorphism was 69.4%. Such a high polymorphism can be attributed mainly to the significant difference in the genetic backgrounds of the two parents: Chiifu is an ovate ecotype originating from the oceanic climate region in China, whereas AB01-1 is a cylindrical ecotype originating from the crossover region of continental–oceanic climates in China. There were obvious differences in leaf color, leaf number, single-leaf weight, and leaf ball between these two materials.

Some molecular markers have been identified to be linked to the GMS in Chinese cabbage, including the SCAR marker named SM264300 linked to a dominant male-sterile gene (Zhang et al. 2008) and four STS markers linked to a recessive male-sterile gene (Miao et al. 2003). These markers were tested for mapping in our segregating population; however, the results revealed that they were monomorphic. The results did not reveal whether the genic multiple-allele inherited male-sterile gene "*Ms*" was identical to these genes.

In the breeding process of a genic male-sterile line, it is very important to select individuals with target genotypes in the segregating generation. Due to the characteristics of multiple-allele inheritance at the locus, six genotypes (*MsMs*, *Msms*, *msms*, Ms^fMs^f, Ms^fMs, and Ms^fms) are possible when transferring the *Ms* allele to fertile materials (*msms* or Ms^fMs^f). Thus, the identification of a desired genotype traditionally requires more plant materials and additional growing seasons. AFLP is a powerful technique for identifying associated markers; however, the relatively long and costly procedure makes it uneconomic for routine application in plant breeding programs (Mienie et al. 2005) or large-scale genotyping. If cheap, fast, and reliable PCR-based markers were to be made available for the *Ms* locus, this would greatly improve the efficiency of breeding programs using the *Ms* allele.

In the present study, two SCAR markers were found to be suitable for MAS programs by virtue of their ability to distinguish the genotypes including *MsMs*, *Msms*, and *msms*. syau_scr01 and syau_scr04, which flank the *Ms* gene, detected only four and six recombinants among 244 BC_1 plants, respectively, suggesting that they could correctly predict the genotype in all but 0.8 and 2.5% of analyses when used separately. However, if these markers are used in combination, the error rate of prediction is decreased to 0.002%.

These two markers were, nevertheless, ineffective when using plants with three other genotypes at the locus, namely Ms^fMs^f, Ms^fMs and Ms^fms. This is partly because these two markers were not developed inside the target gene, but in regions at some distance from the locus. Therefore, molecular markers closely linked to the fertility restoration allele Ms^f should also be developed in order to construct a comprehensive molecular marker system that can distinguish all the genotypes at the genic multiple-allele inherited male-sterility locus. This would include *MsMs*, *Msms*, and *msms*, and also Ms^fMs^f, Ms^fMs, and Ms^fms, if full use of MAS is made in the breeding of male-sterile lines comprising 100% male-sterile plants.

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References

Cao JS, Yu XL, Ye WZ, Lu G, Xiang X (2005) Functional analysis of a novel male fertility *CYP86MF* gene in Chinese cabbage (*Brassica campestris* L. ssp. chinensis makino). Plant Cell Rep 24:715–723

Choi SR, Teakle GR, Plaha P, Kim JH, Allender CJ, Beynon E, Piao ZY, Soengas P, Han TH, King GJ, Barker GC, Hand P, Lydiate DJ, Batley J, Edwards D, Koo DH, Bang JW, Park BS, Lim YP (2007) The reference genetic linkage map for the multinational *Brassica rapa* genome sequencing project. Theor Appl Genet 115:777–792

Feng H, Wei YT, Zhang SN (1995) Inheritance of and utilization model for genic male sterility in Chinese cabbage (*Brassica pekinensis* Rupr.). Acta Hortic 402:133–140

Feng H, Wei YT, Ji SJ, Jin G, Jin JS, Dong WJ (1996) Multiple allele model for genic male sterility in Chinese cabbage. Acta Hortic 467:133–142

Feng H, Xu W, Wang YG (2007) Directive transfer of the genetic male sterile line of milk Chinese cabbage AI023. Acta Hortic Sinica 34:659–664

Hong DF, Wan LL, Liu PW, Yang GS, He QB (2006) AFLP and SCAR markers linked to the suppressor gene (*Rf*) of a dominant genetic male sterility in rapeseed (*Brassica napus* L.). Euphytica 151:401–409

Huang Z, Chen YF, Yi B, Xiao L, Ma CZ, Tu JX, Fu TD (2007) Fine mapping of the recessive genic male sterility gene (*Bnms3*) in *Brassica napus* L. Theor Appl Genet 115:113–118

Ke LP, Sun YQ, Hong DF, Liu PW, Yang GS (2005) Identification of AFLP markers linked to one recessive genic male sterility gene in oilseed rape, *Brassica napus*. Plant Breeding 124:367–370

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

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Eewburg L (1987) MAPMARKER: an interactive computer package for constructing primary genetic linkage maps for experimental and natural populations. Genomics 1:174–181

Lei SL, Yao XQ, Yi B, Chen W, Ma CZ, Tu JX, Fu TD (2007) Toward 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

Lu GY, Yang GS, Fu TD (2004a) Identification of AFLP markers linked to the dominant genic male sterility gene in *Brassica napus* L. Acta Agronom Sinica 30:104–107

Lu GY, Yang GS, Fu TD (2004b) Molecular mapping of a dominant genic male sterility gene *Ms* in rapeseed (*Brassica napus* L.). Plant Breed 123:262–265

Miao Y, Dreyer F, Cai DG, Jung C (2003) Molecular markers for genic male sterility in Chinese cabbage. Euphytica 132:227–234

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 using segregating populations. Proc Natl Acad Sci 88:9828–9832

Mienie CMS, Liebenberg MM, Pretorius ZA, Miklas PN (2005) SCAR markers linked to the common bean rust resistance gene *Ur-13*. Theor Appl Genet 111:972–979

Mun JH, Kwon SJ, Yang TJ, Kim HS, Choi BS, Baek SH, Kim JS, Jin M, Kim JA, Lim MH, Lee SI, Kim HI, Kim HT, Lim YP, Park BS (2008) The first generation of a BAC-based physical map of *Brassica rapa*. BMC Genomics 9:280

Pierre G, Marechal-Drouard L (1992) Isolation of plant DNA: a fast, inexpensive, and reliable method. Plant Mol Biol Rep 10:60–65

Rozen S, Skaletsky HJ (1996) Primer3: a software component for picking PCR primers. Source code available at http://www.genome.wi.mit.edu/genome_software/other/primer3.html

Song LQ, Fu TD, Tu JX, Ma CZ, Yang GS (2006) Molecular validation of multiple allele inheritance for dominant genic male sterility gene in *Brassica napus* L. Theor Appl Genet 113:55–62

Trick M, Bancroft I, Lim YP (2007) The *Brassica rapa* genome sequencing initiative. Genes, Genomes Genomics 1:35–39

van der Meer QP (1987) Chromosomal monogenic dominant male sterility in Chinese cabbage (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt). Euphytica 36:927–931

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang JX, Yang GS, Fu TD, Meng JL (2000a) Development of PCR-based markers linked to the fertility restorer gene for the polima cytoplasmic male sterility in rapeseed (*Brassica napus* L.). Acta Genetica Sinica 27:1012–1017

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 27:143–144

Wang XW, Fang ZY, Huang SW, Sun PT, Liu YM, Yang LM, Zhuang M, Qu DY (2000c) 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 YG, Feng H, Lin GR, Xu SF, Yang KY, Zhang NJ (2005) The Transfer of genetic male sterile lines in *Brassica campestris* L. ssp. chinesis (L.) Makino. Acta Hortic Sinica 32:628–631

Wang DJ, Guo AG, Li DR, Tian JH (2006) Molecular markers linked to mono-dominant genic male sterile gene in rapeseed (*Brassica napus* L.). J Plant Physiol Mol Biol 32:513–518

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

Zhang SJ, Li F, Han HP, Zhang SF, Niu XG, Sun RF (2008) Molecular marker linked to a dominant genetic male sterile gene in Chinese cabbage. [*Brassica rapa* L. ssp. *pekinensis* (Lour.) Olsson]. Scientia Agric Sinica 41:2379–2385