Theor Appl Genet (2012) 124:1193–1200
DOI 10.1007/s00122-011-1779-1

REVIEW

Mapping of *BnMs4* and *BnRf* to a common microsyntenic region of *Arabidopsis thaliana* chromosome 3 using intron polymorphism markers

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Received: 16 May 2011 / Accepted: 22 December 2011 / Published online: 14 January 2012
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Abstract A recessive epistatic genic male sterile two-type line, 7365AB (*Bnms3ms3ms4msRrfRf/BnMs3ms3ms4ms4RfRf*), combined with the fertile interim-maintainer 7365C (*Bnms3ms3ms4ms4rfrf*) is an effective pollination control system in hybrid rapeseed production. We report an effective strategy used to fine map *BnMs4* and *BnRf*. The two genes were both defined to a common microsyntenic region with *Arabidopsis* chromosome 3 using intron polymorphism (IP) markers developed according to *Arabidopsis* genome information and published genome organization of the A genome. The near-isogenic lines 7365AC (*Bnms3ms3ms4ms4Rfrf/Bnms3ms3ms4ms4rfrf*) of *BnRf* and 736512AB (*Bnms3ms3Ms4ms4RfRf/Bnms3ms3ms4ms4RfRf*) of *BnMs4* were constructed to screen developed markers and create genetic linkage maps. Nine polymorphic IP markers (P1–P9) were identified. Of these, P2, P3, P4, and P6 were linked to both *BnMs4* and *BnRf* with genetic distances <0.6 cM. Three simple sequence repeat markers, SR2, SR3, and SR5, were also identified by using public information. Subsequently, all markers linked to the two genes were used to compare the micro-collinearity of the regions flanking the two genes with *Brassica rapa* and *Arabidopsis*. The flanking regions showed rearrangements and inversion with fragments of different *Arabidopsis* chromosomes, but a high collinearity with *B. rapa*. This collinearity provided extremely valuable reference for map-based cloning in polyploid *Brassica* species. These IP markers could be exploited for comparative genomic studies within and between *Brassica* species, providing an economically feasible approach for molecular marker-assisted selection breeding, accelerating the process of gene cloning, and providing more direct evidence for the presence of multiple alleles between *BnMs4* and *BnRf*.

Communicated by H. Becker.

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Introduction

Cytoplasmic male sterility (CMS) and genic male sterility (GMS), as main approaches for heterosis utilization, are widely used in hybrid rapeseed production. Comparatively, GMS has more advantages, including stable and complete male sterility, rich sources of cytoplasm, and ease of transfer of male sterility genes. Specifically, recessive epistatic genic male sterile (REGMS) can produce a 100% sterile population and is widely used in three-line hybrid system (Chen et al. 1998; Sun et al. 2002; Wang et al. 2004). The procedure of three-line hybrid seed production by using 7365AB is as follows: sterile line (*Bnms3ms3ms4ms4RfRf*) and interim maintainer (*Bnms3ms3ms4ms4rfrf*), both with identical genetic backgrounds are crossed to produce a 100% sterile population. Subsequently, the 100% sterile population was extensively tested with a variety of restorers to produce hybrid seed. Supplementing with marker-assisted selection (MAS), this can be a very economical method for using heterosis. However, due to the self-complexity and instability of molecular markers technology, the identification of specific genotypes in MAS remains expensive

and inaccurate. Co-dominant, economical, more stable, and closer markers are desirable in a breeding program.

REGMS was derived from a spontaneous mutant (Chen et al. 1993). Genetic analysis indicated that the sterility of REGMS line 9012AB is controlled by two pairs of recessive genes (*ms1* and *ms2*) and one pair of recessive epistatic genes (*rf*). The genotype of *rfrf* inhibits homozygous *ms1ms1ms2ms2* and produces a male-fertile phenotype (Chen et al. 1993, 1998). Sterile genes controlling 7365A and 9012A were allelic, named as *Bnms3*, *Bnms4*, and *BnRf* in 7365A, and *BnRf* was mapped to the N7 linkage group (Xiao et al. 2008). However, Zu et al. (2010) preliminarily mapped *BnMs4* to the top of the N7 linkage group in the same region, where *BnRf* had previously been located by six flanking common markers. They further supplied additional classical genetic evidence to support that *BnMs4* and *BnRf* may be multiple alleles.

Recently, fine mapping of *BnMs3* has been reported. 17 amplified fragment length polymorphism (AFLP) markers linked to the *BnMs3* gene are identified and confirm the presence of two flanking markers with their distance of 0.1 and 0.3 cM using a near-isogenic line (NIL)-derived population consisting of 2,000 plants (Huang et al. 2007). Another NIL-derived population of 4,136 individuals was used to define *BnMs3* gene to a genetical region of 0.56 cM (He et al. 2008). Several AFLP and sequence characterized amplified region (SCAR) markers linked to *BnRf* have been identified (Xiao et al. 2008).To our present knowledge, fine mapping of *BnRf* and *BnMs4* has not been reported yet.

The *Brassica* species are closely related to the model plant *Arabidopsis* (Rana et al. 2004). *Arabidopsis*, as a tool for map-based gene cloning, can provide a ready source of ordered, closely spaced markers for rapid fine mapping in *Brassica* species through exploiting chromosomal synteny. In such an exploiting chromosomal synteny process, intron polymorphism (IP) markers show a powerful force specifically in narrowing the candidate region of a target gene. By using an IP marker strategy (Brunel et al. 1999; Yang et al. 2007), Lei et al. (2007) developed a dominant amplified consensus genetic marker (ACGM, equivalent to IP marker) more closely linked to *BnMs2*, which significantly reduce the candidate chromosome interval. However, such applications of synteny may become challenging by the presence of paralogous chromosome segments and the occurrence of small-scale rearrangements and insertions/deletions within syntenic sequence blocks (Bennetzen 2000; Bennetzen and Ramakrishna 2002).

Extensive comparative genetic mapping studies have been performed on *Brassica* species (Parkin et al. 2005; Lagercrantz and Lydiate 1996; Lagercrantz 1998; Babula et al. 2003). The high efficiency of IP markers in generating comparative relationships between *Arabidopsis* and various *Brassica* species has been established (Panjabi et al. 2008). IP-based comparative genomic study among the three diploid *Brassica* lineages established that the major rearrangements, translocations, and fusions are pivotal to karyotype diversification between the A, B, and C genomes. Especially, the multinational *Brassica* genome project (BrGSP) published the whole genome sequence of *Brassica rapa* (Chiifu-401) in July 2010 (http://brassicadb.org/brad/). The genomic information can allow researchers to conduct comparative genomic, evolutionary, and gene mapping studies by using Chiifu as a model plant.

In the present study, based on information from the *Arabidopsis* loci of previously screened SCAR or simple sequence repeat (SSR) markers and the genome organization comparisons of the A genome and *Arabidopsis*, more closer IP markers were developed to fine map *BnRf* and *BnMs4*. Subsequently, we used all markers linked to *BnMs4* and *BnRf* to study the micro-collinearity of the regions flanking the two genes with *Arabidopsis* and *B. rapa*. This result provides an extremely valuable reference in map-based cloning of interested genes in polyploid crops under the situation of low collinearity between the flanking segments of target genes and sequenced genomes.

Materials and methods

Plant materials and population construction

7365A (*Bnms3ms3ms4ms4RfRf*) and 7365C (*Bnms3ms3 ms4ms4rfrf*) were crossed to produce a 100% sterile F1 population. The F1 were backcrossed with 7365C, and the offspring were repeatedly sib-mated to construct NIL 7365AC (*Bnms3ms3ms4ms4Rfrf*/*Bnms3ms3ms4ms4rfrf*) of *BnRf*. 7365A was crossed with 7-749, a new inbred line of *Brassica napus* (provided by Professor Meng Jinling). The progeny of backcrossing the F1 and recurrent parent 7365A were segregated for fertility. Then co-dominant markers linked to *BnMs3* were used to screen individuals with the genotype *Bnms3ms3Ms4ms4RfRf* for fertile progeny to cross with sterile progeny. Constantly sib-mating was carried to construct NIL 736512AB (*Bnms3ms3Ms4ms4RfRf*/*Bnms3ms3ms4ms4RfRf*) of *BnMs4*.

DNA extraction and bulk segregant analysis (BSA)

Genomic DNA from fertile plants and sterile plants in the two NILs was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). Eight male-sterile bulks and eight male-fertile bulks were prepared to identify polymorphic IP and SSR markers, respectively, each by pooling equivalent amounts of DNA from four plants of the two NILs.

Sequencing fragments of polymorphic markers and PCR walking

Polymorphic fragments were gel purified, re-amplified, and cloned into the pMD18-T vector (Takara Biotech., Dalian, China), as described by Xiao et al. (2008). Four positive clones per transformation were sequenced (Shenzhen Huada Company, Shenzhen, China). The Genome Walker Universal Kit (Clontech, Madison, Wisconsin, USA) was used to isolate flanking sequences by PCR walking (Siebert et al. 1995).

Development and identification of IP markers and SSR markers

IP markers were developed according to information on the corresponding *Arabidopsis* loci of previously screened markers linked to *BnRf* and *BnMs4*, and the published comparative genome organization of the A genome and *Arabidopsis*. Considering the great need to develop polymorphic markers around the target gene in two NILs, a well-designed strategy was adopted: genome survey sequences (GSSs) and cDNAs of *Arabidopsis* genes (regarded as homologous genes tightly flanking target genes) were downloaded from the public *Arabidopsis thaliana* Integrated Database (ATIDB; http://atidb.org/cgi-perl/gbrowse/atibrowse/). The GSSs were blasted in the integrated marker system for oilseed rape breeding (IMSORB) against the published expressed sequence tag (EST) or GSS of *Brassica* crops (http://brassica.bbsrc.ac.uk/IMSORB/). The JAVA application program was used to export the compared results and assembled the corresponding cDNAs to identify the location of introns. Next, Primer 3 (http://www.biowb.sdsc.edu/CGI/BW.cgi) was used to design IP primers within the conserved sequences on both sides of introns. SSR markers were designed using WebSat (http://wsmartins.net/websat/). These IP and SSR primers were amplified using a standard PCR reaction [50 ng template DNA, 1× amplification buffer, 2 mM $MgCl_2$, 150 mM dNTPs, 1 U *Taq* DNA polymerase (Fermentas) and 5 pmol of each special primer] in a total reaction volume of 20 μl. PCR products were separated on a 6% denaturing polyacrylamide gel.

Construction of genetic linkage maps

A total of 1,024 sterile individuals in 736512AB and 493 fertile individuals in 7365AC were used to identify the genetic distances of the polymorphic markers. Data from phenotype survey, IP, and SSR analysis were combined for linkage analysis using MapDraw (Liu and Meng 2003).

Comparative mapping with *A. thaliana* and *B. rapa*

A genome sequence from *B. rapa* (Chiifu-401), which includes sequence scaffolds, was released in July 2010 (http://brassicadb.org/brad/). The predicted *B. rapa* genes were used to conduct comparative mapping between *A. thaliana* and *B. napus*. All polymorphic fragments around *BnMs4* and *BnRf* were sequenced to identify putatively homologous genes using Blastn search against the *Arabidopsis* genome database (The Arabidopsis Information Resource, TAIR; http://www.arabidopsis.org) and the *Brassica* genome database (The Brassica database, BRAD; http://brassicadb.org/brad/).

Results

Sequencing fragments of linked markers and PCR walking

Previously developed SSR and SCAR markers were randomly distributed in the genome and could be located in non-conserved regions; so, we conducted PCR walking to obtain flanking sequences for obtaining reliable blast information. Polymorphic fragments linked to *BnRf* with markers SR1, XSC5, sR4047, CNU063, ENA06, and SC25 were sequenced, extended, and blasted (Table 1). Homologous *Arabidopsis* genes of the six markers were found on three different *Arabidopsis* chromosomes: AT1G29550, AT3G23590, AT2G19670, AT2G19600, AT2G18960, and AT2G15690 (Table 1).

Development and identification of IP and SSR markers

To identify microsyntenic regions between target genes and *Arabidopsis* genes, it was necessary to develop more useful SSR markers. SSR2 and SSR5 were developed from *B. rapa* BAC clones KBrS003D10 and KBrB022C05 (http://www.ncbi.nlm.nih.gov) in the top of the A7 linkage group (http://www.brassica-rapa.org/BGP/NC_brgp.jsp), respectively. We screened previously reported SR3 marker, present on the N7 linkage group (Cheng et al. 2009) and observed that it was linked to *BnMs4*. Around the location of SR1 (AT1G29550), 13 *Arabidopsis* genes with strong homology with the ESTs and GSSs of *Brassica* were selected to develop 39 IP primers. P1 (AT1G30690.1) and P2 (AT1G31160) were identified as polymorphic markers.

Panjabi et al. (2008) reported comparative mapping between Brassicaceae and *A. thaliana* using IP markers to analyze genome-wide synteny and showed an *Arabidopsis* linear region (AT3G23290–AT3G24800) at the top of the linkage group A7. XSC5 was corresponded directly to AT3G23590, so 66 IP markers from 39 *Arabidopsis* genes between AT3G23290 and AT3G24800 were designed. 7 polymorphic IP markers: P3 (AT3G23610), P4 (AT3G23700), P5 (AT3G23810), P6 (AT3G23910), P7 (AT3G24030), P8 (AT3G24550), and P9 (AT3G24550) were identified (Table 1). P3, P4 and P6 were co-dominant polymorphic

Table 1 Characterization of IP, SSR, SCAR markers linked to *BnRf* and *BnMs4*

Markers	Sequences(5′–3′)	Original size (bp)	SSR motifs	Extended length (bp)	Homologous *Arabidopsis* gene and *E* value	Homologous *B. rapa* gene and *E* value
SR1(SSR1)	CTCGATACGGATAAGGTTCG TTTTGCTTCGGTTGTGTATG	234	(GA)11	888	AT1G29550, 5.6e−27	Bra030147, e−141
SR2	TGTGTCTTCAATCTCAACCA TTTCTTCTCCTCGGAATGTA	442	(CAAAA)5	970	AT1G30650, 1.1e−84	Bra026228, 0.0
SR3	GATTTGTGAACCCAAACAGTGA CAGTACCAAGTCAAAGCCTCAA	393	(CGACTT)4, (CTT)6	763	AT1G30420.1, 2.3e−48	Bra014879, 0.0
P1	TGTGAAGCCTGGATCATCTGAA CGTCCCAAACCAATGTTCCT	169	–	–	AT1G30690.1, 2.0 e−3	Bra026229, 2e−18
P2	CATCCTTCAGCACCGTTGTT CTTGACCACGCTTGGCAAG	460	–	–	AT1G31160.1, 2.6e−17	Bra014904, 9e−63
SC1(XSC5)	CTCATGATGGCTCGTCTGA TGCAGGTCCTTGGTGAATT	215	–	519	AT3G23590.1, 2.0e−62	Bra014963, e−166
P3	GTTCGCCATCTCTTCCAATG TAGGCACGAGCGACTTTGAT	267	–	–	AT3G23610.2, 1.9e−10	Bra014966, 1e−32
P4	AACTTCTCTCCTCTGCGTCG CCAGCTTCCAATCCGTAGAC	208	–	–	AT3G23700.1, 4.8e−4	Bra014973, 7e−50
P5	ACCAGGTGATTGCCCAGC ACAACCCCTTATTCATACCAAT	307	–	–	AT3G23810.1, 7.0e−54	Bra014984, e−119
P6	GTCG CAATCGTCTTTCTTCTCT TTCTCCAGATTCGTCTATGCAA	264	–	–	AT3G23910, 2.10 e−2	No hit gene, abutting Bra014992
P7	TTGAAGGCGTGCTTGGAG TGACTTGGCAGCTTCTACGG	258	–	–	AT3G24030.1, 1.1e−11,	Bra015014, 5e−54
P8	GTCGTGCCTGTTTCCACCAT GTCGTCCCGTTGATGCAAAC	156	–	–	AT3G24550.1, 2.1e−05	Bra015053, 3e−23
P9	CACCAGATTGAAGATTGCTC TGCGTGTTTGTATCAGAAGC	338	–	–	AT3G24550.1, 9.7e−16	Bra015053, 3e−34
SR4(sR4047)	AGAGAGGGTGTTGTTCCCAG ATTCTTTGAATGGCCAGCAC	270	(CT)6	535	AT2G19670.1, 1.5e−05	Bra039010, e−25
SR5	TCTTGTCTCCAAAACCCTAA ACATCCTTCAACATTTCCTG	416	(CT)12	963	AT2G19670.1, 1.5e−64	Bra039010, 1e−85
SR6(CNU063)	GAGAGAAGAAGAAGAAGGA AGCAGAA GATCTCGCGTGTGGCAACT	238	(AG)10	708	AT2G19600.1, 1.0e−19	Bra031091, 4e−41
SR7(ENA06)	CTCGTCTTCTTCACCTACAAC CTGACATCTTTCTCACCCAC	117	(CT)9	615	AT2G18960.1, 2.5e−11	Scaffold000153, no hit gene
SC2(SC25)	CATCCGAGAGAAGAGTAGAG TACACAGGAGGTGATCTGAC	230		1,773	AT2G15690.1, 1.9e−44	Bra015230, e−145

markers linked to both *BnMs4* and *BnRf*. Precisely, P3 and P4 could distinguish *BnMs4*, *BnRf*, and *Bnrf* (Fig. 1). P5 was linked only to *BnMs4*, whereas P7 was linked only to *BnRf*. P8 and P9 were developed from the same gene AT3G24550.

Genetic mapping of *BnMs4* and *BnRf*

A total of 1,024 sterile plants in 736512AB and 493 fertile plants in 7365AC were used to construct genetic linkage maps of *BnMs4* and *BnRf*, respectively (Fig. 2). SR1, P2, P3, P4, P6, SR4, SR5, SR6, and SC2 markers exhibited polymorphisms in both 736512AB and 7365AC. These common markers were distributed on both sides of the two genes (Fig. 2). P6 was co-segregated in the two populations (Fig. 2). P4 and P7 were the nearest flanking markers of *BnRf* and had a genetic distance of 0.6 cM between them (Fig. 2). P5 and P8 were the nearest flanking markers of *BnMs4* and contained a genetic distance of 0.8 cM (Fig. 2).

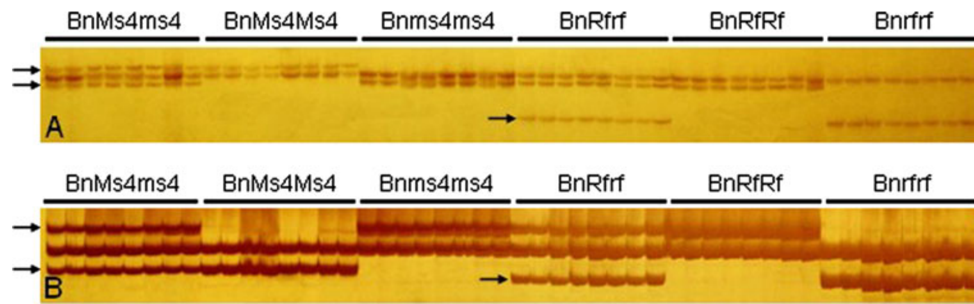

Fig. 1 Polyacrylamide gel electrophoresis of amplification results of different genotypes in two NILs by common IP markers. **a** Amplification of diverse genotypes using common marker P3. **b** Amplification of diverse genotypes using common marker P4. *Black arrows* indicate polymorphic amplified bands which could distinguish *BnMs4*, *BnRf* and *Bnrf*

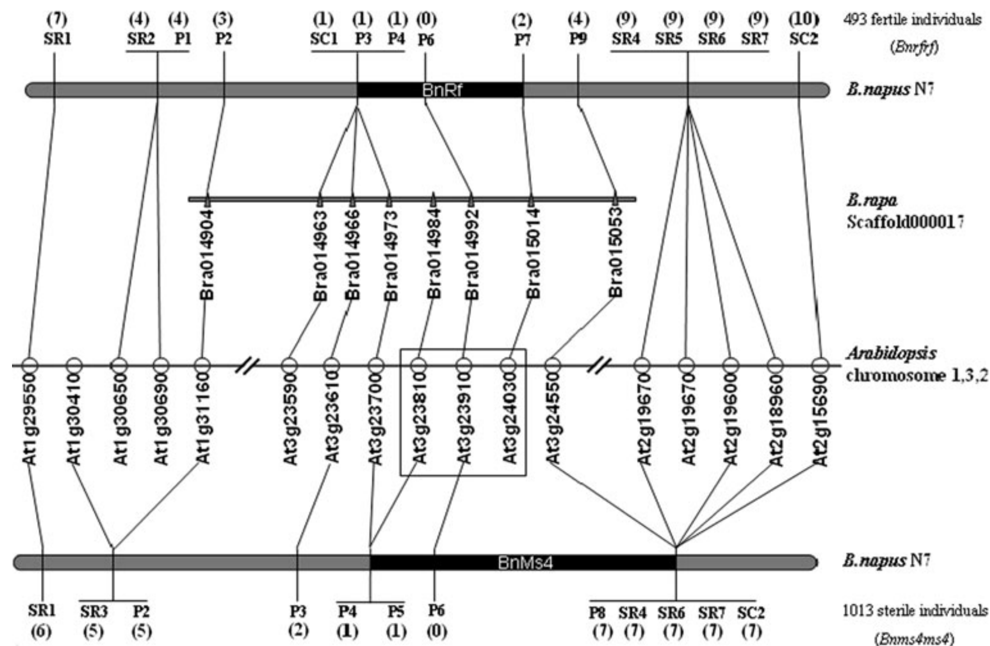

Fig. 2 Genetic and comparative mapping of *BnMs4* and *BnRf*. *Brackets* show numbers of recombinant plants, *square box* shows the common physical distance of 304 kb defined by IP markers

Comparative mapping between *A. thaliana* and *B. rapa*

Compared with the *Arabidopsis* genome, the region around the two genes contained three linear sections corresponding to AT C1 (represents *Arabidopsis* chromosome 1 subsequently followed by chromosome number 2, 3, 4, and 5), AT C3 and AT C2 (Fig. 2). After successful identification of the flanking IP markers, we preliminarily identified a relatively small microsyntenic region between AT C3 and the flanking sequence of the *BnRf* and *BnMs4*. *BnRf* was located between AT3G23700 and AT3G24030, whereas *BnMs4* was between AT3G23810 and AT3G24550. Most of the markers were riveted to Scaffold000017 of *B. rapa* by BLASTN (The Brassica database, BRAD; http://brassicadb.org/brad/), and showed a strong linear relationship with the *B. rapa* genome. By comparative mapping with Scaffold000017, the closest markers flanking *BnRf*, P4 and P7, had a physical distance 380 kb, whereas P5 and P8 flanking *BnMs4* had a physical distance 668 kb showing a common region of 304 kb.

Comparative analysis of the flanking regions of *BnRf* and *BnMs4*

The region flanking the two target genes mainly contained three separate segments from AT C1, AT C3, and AT C2. These observations might be the result of cross-hybridization between the three segments of the *Arabidopsis* genome. Additionally, the flanking region had a strong linear relationship with *B. rapa* after comparatively analyzing the sort order of those makers within Scaffold000017. Similarly, it was observed that the *B. rapa* sequence scaffold

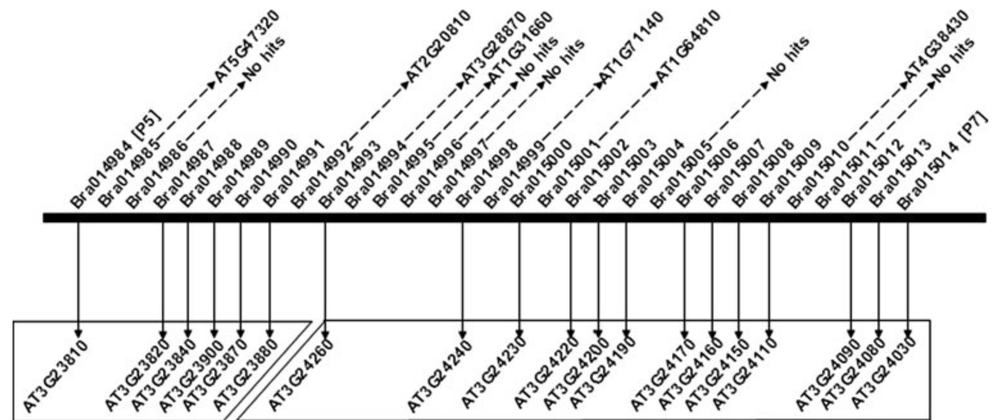

Fig. 3 Comparative analysis of the common target section showing *Brassica rapa* genes and *Arabidopsis* genes. Two *trapezoids* show an inversion of AT C3 in the 304 kb region

showed extensive breakdown of collinearity relative to the genome of *Arabidopsis*. However, the detailed mapping of the two genes in *B. napus* shows that the arrangement of the *B. rapa* genome sequence in this region is actually conserved in *B. napus*.

We further investigated the distribution of genes in the core section of 304 kb. The region from AT3G23810 to AT3G24030 was a common section defined by the nearest IP markers of the two genes. The corresponding section of *B. rapa* was from Bra014984 to Bra015014 (Fig. 3). Obviously, there was an inversion of AT C3 in this microsyntenic region. Meanwhile, there were several insertions by other *Arabidopsis* chromosome genes, such as AT5G47320 and AT2G20810. Bra014986, Bra014996, Bra014997, Bra015005, and Bra015011 showed no hit genes in *Arabidopsis*. Mainly, this 304 kb microsyntenic region in *B. rapa* could have a complex evolutionary history with inversion and insertion of homologous *Arabidopsis* genes, which in turn lead to the emergence of new genes.

Discussion

Inheritance of 7365A

BnMs4 was located in the top of the N7 linkage group (Zu et al. 2010) within the same region of the *BnRf* gene (Xiao et al. 2008). Furthermore, field experiments between two NILs were conducted to qualitatively differentiate between two genetic models: a recessive epistatic model of three loci and a two loci multiple allele model. Linkage disequilibrium analysis using molecular markers linked to *BnMs4* and *BnRf* confirmed that *BnMs4* and *BnRf* could be multiple alleles. In our study, we identified three closer common molecular markers (P3, P4, and P6), and defined *BnMs4* and *BnRf* into a common physical interval of 304 kb according to the *B. rapa* genome sequence. These results give further evidence of multiple alleles between *BnMs4* and *BnRf*. A survey of literature show more support for multiple allele model of 7365AB: fertility is controlled by two loci, one recessive mutant locus (*BnMs3/Bnms3*) and one multiple allele locus (*BnMs/Bnms/Bnrf*). *Bnms3* was the recessive mutant sterile gene, *BnMs3* was the wild-type fertile gene, *BnMs* was the fertility restoration gene, *Bnms* was the mutant sterile gene, and *Bnrf* was the wild-type fertile gene. The explicit–implicit relationship of these alleles was *BnMs* > *Bnms* > *Bnrf*.

Map-based cloning strategy in *Brassica* species

Throughout our entire mapping process, it was recognized that map-based cloning strategy of *Brassica* species genes mainly contains three crucial aspects. Firstly, genome-wide molecular markers linked to target genes, such as AFLP and SSR, should be sufficiently identified: they better show strong colinearity with sequenced plant like *Arabidopsis* or *B. rapa*. However, we identified only one marker XSC5 in the core section corresponding to AT C3 previously. Secondly, accurate information of genetic map, comparative genomics or the released *Brassica* sequence associated with molecular marker linked to target genes should be excavated. In our study, XSC5 was corresponded exactly to AT3G23590 which was present in *Arabidopsis* linear region (AT3G23290-AT3G24800) located on the top of the A7 linkage group (Panjabi et al. 2008), so we considered that our target genes were in this linear region. The whole genome sequence of *B. rapa* (Chiifu-401) was published by the multinational *Brassica* genome project (BrGSP) in July 2010. This could facilitate fine mapping of *Brassica* genes. Thirdly, an effective molecular marker strategy could gradually be applied to close the distance of the target gene. Our study confirmed that IP markers were efficient and convenient for map-based cloning. 39 and 66 pairs of IP primers were designed in AT C1 and AT C3, respectively and ultimately 2 and 7 were polymorphic IP markers. The percentages of polymorphic markers were 5.1 and 10.6%, respectively, they successfully defined the target genes in a micro-collinear region with AT C3. Previously, IP markers

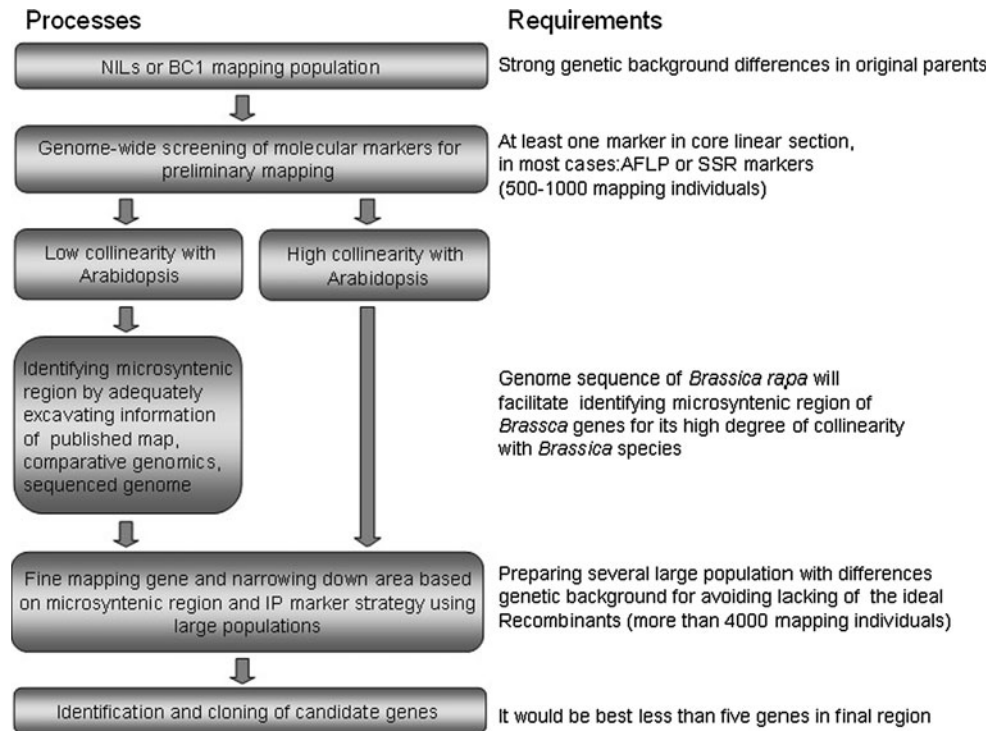

Fig. 4 A map-based cloning procedure in *Brassica* species

were also used in mapping genes of *Brassica* species (Yi et al. 2006; Lei et al. 2007; He et al. 2008; Fig. 4).

IP application in MAS

AFLP and SSR markers have been widely used to map important genes in crops because of their random and wide distribution across whole genomes (McCouch et al. 1997; Blair et al. 2003; Semagn et al. 2006). These markers were likely to be distributed in non-conserved regions, so their stability and conservation were less favorable than the IP primers, which were designed based on conserved sequences. Therefore, IP markers are more suitable for MAS. Additionally, the polymorphism of IP markers is generally due to different intron lengths. As a result, these markers are more likely to show co-dominance. P3 and P4 were able to distinguish the three genes (*BnMs/Bnms/Bnrf*) through different fragment length. They could be strongly used to select a specific genotype (Fig. 1).

Prediction and identification of candidate genes in *B. napus*

Rana et al. (2004) analyzed the microstructure of corresponding segments between the *B. rapa* genome and the *Brassica oleracea* genome. They show that segments in the *B. napus* genome have few or no changes in the microstructure of the analyzed segments in the *Brassica* A and C genomes as a consequence of the hybridization event that formed natural *B. napus*. Suwabe et al. (2008) constructed an integrated *Brassica* A genome linkage map between *B. napus* and *B. rapa*. These results indicated that the *Brassica* A genome had retained a high degree of collinearity between *Brassica* species. Therefore, predicting candidate genes in *B. napus* could be carried out according to the whole genome sequence of *B. rapa*. Our results also showed that the region flanking *BnMs4* and *BnRf* in *B. napus* had a strong collinearity with the *B. rapa* genome. However, the microstructure of target region showed inversion, rearrangement of *Arabidopsis* chromosome segments, and insertion or deletion of an *Arabidopsis* unigene (Fig. 3). These minor changes were similar to the previous findings (Suzuki et al. 1999; Quiros et al. 2001). This analysis provides an extremely valuable reference for prediction and identification of candidate genes contributing to traits of agronomic value in *Brassica* crops.

Acknowledgments We sincerely thank Gautam Mayank for his critical reading of the manuscript. This research was supported by funds from the National Natural Science Foundation of China (31130040) and the National '863' High-Tech Project (2006AA10Z146).

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