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ORIGINAL PAPER

Fine mapping of a dominant thermo-sensitive genic male sterility gene (*BntsMs*) in rapeseed (*Brassica napus*) with AFLP- and *Brassica rapa*-derived PCR markers

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Abstract
Key message **A new thermo-sensitive dominant genic male sterility (TSDGMS) line of *Brassica napus* was found and mapped in this paper. Our result will greatly accelerate the map-based cloning of the *BntsMs* gene.**
Abstract TE5A is a thermo-sensitive dominant genic male sterility line originating from spontaneous mutation of the inbred line TE5 in *Brassica napus* and provides a promising system for the development of hybrid cultivars. Genetic analysis has revealed that the *BntsMs* mutant is controlled by a single, dominant gene. Here, we describe the fine mapping of *BntsMs* using amplified fragment length polymorphism (AFLP) and intron polymorphism (IP) methodologies. We screened 1,024 primer combinations and then identified five AFLP markers linked to the *BntsMs* gene, two of which were successfully converted into sequence-characterised amplified region (SCAR) markers. The linkage of the markers was identified by analysing a large BC_2 population of 700 recessive-fertility individuals. Two SCAR markers were found in the flanking region of the *BntsMs* gene at distance of 3.5 and 4.8 cm. Based on sequence information from the previously screened AFLP markers and on genome organisation comparisons of the A genome of *Brassica rapa* and Arabidopsis, seven IP markers linked to the *BntsMs* gene were developed. By analysing the 700 recessive-fertility individuals, two IP markers, IP004 and IP470, were localised to the flanking region of the *BntsMs* gene at a distance of 0.3 and 0.2 cm, respectively. A comparison of the *B. rapa* and *Arabidopsis* genomes revealed 27 genes of *B. rapa* in the flanking region of these two IP markers. It is likely that the molecular markers developed from these investigations will greatly accelerate the positional cloning of the *BntsMs* gene.

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Introduction

Hybrid cultivars have been successfully used worldwide to increase seed yield in *Brassica napus* (oilseed rape). Pollination control systems are crucial for hybrid seed production, and four main pollination control systems have been developed for the commercial production of hybrid seed in China: cytoplasmic male sterility (CMS), genic male sterility (GMS), self-incompatibility (SI) and ecotypic male sterility (EMS) systems (Fu and Tu 2002). At present, the most effective and economical pollination control system that facilitates hybrid seed production is rapeseed male sterility. Various GMS mutants have been studied in *B. napus* (Takagi 1970; Mathias 1985; Li et al. 1988; Hou et al. 1990; Chen et al. 1998; Hu et al. 2000; Wang et al. 2001; Song et al. 2005), and *B. napus* GMS systems are classified into many types according to their inheritance. Three GMS systems are currently used in commercial hybrid seed production in China: a recessive genic male-sterile line with two duplicate recessive genes [recessive epistatic genic male sterility (REGMS)] (Pan et al. 1988; Hou et al. 1990; Tu et al. 1997), the control of sterility in the REGMS line by interacting

recessive genes (Chen et al. 1998) and a GMS line with inheritance as a dominant gene [dominant genic male sterility (DGMS)] (Li et al. 1985, 1990, 1995; Song et al. 2005).

To date, two DGMS lines (Yi3A and 609A) have been studied in *B. napus*. Genetic analyses of Yi3A indicated that one DGMS (Ms) gene alone causes sterility; a restorer gene (Rf) suppresses the expression of the Ms gene, restoring male fertility. Song has reported a newly bred DGMS line, 609AB, from a spontaneous mutant, and classical genetic analyses of 609A indicated that one gene with multiple alleles-controlled sterility and its inhibition (or restoration) (Song et al. 2005). This hypothesis was proven by further molecular marker analyses (Song et al. 2006). However, it remains unclear whether the mutant genes controlling the sterility of 609A and Yi3A are actually allelic (Hong et al. 2008).

Molecular marker technology has been extensively used in modern agricultural biotechnology. Various types of markers have been developed to map important traits in *B. napus*, and many molecular markers associated with various GMS genes have been identified in *B. napus*. A total of 17 AFLP markers linked to the BnMs3 gene in RGMS7-7365 (Huang et al. 2007), seven AFLP markers associated with the BnMs1 gene in RGMS S45A (Yi et al. 2006) and eight AFLP markers associated with the Ms gene in DGMS 609A (Song et al. 2006) have been identified. Some of these markers have been converted into sequence-characterised amplified region (SCAR) markers. Such molecular markers that are linked to the target gene are useful for gene cloning and molecular-assisted selection (MAS).

It is well known that *Arabidopsis thaliana* and *Brassica* species share a recent common ancestry, and *Arabidopsis* and *B. napus* share up to 86 % sequence identity (Cavell et al. 1998). Comparative genome analyses between *Arabidopsis* and *Brassica* species provide a ready source of ordered, closely spaced markers that can be used for rapid, fine mapping in *Brassica* species. Furthermore, the entire genome sequence of *Brassica rapa* (Chiifu-401) was published in July 2011 (http://brassicadb.org/brad/) (Wang et al. 2011). Based on the genome sequence information of *Arabidopsis* and *B. rapa*, intron polymorphism (IP) markers could be developed for *B. napus*. IP markers are a powerful tool, particularly with regard to fine mapping and narrowing the candidate region of a target gene. The BnMS1 and BnMS2 genes have been successfully cloned using the IP marker strategy (Yi et al. 2010), and Dun successfully cloned the *BnaC.Tic40* gene using IP markers to limit a candidate region that included four genes (Dun et al. 2011).

In this study, we report the identification and mapping of the TDGMS mutant gene in *B. napus*. We performed a genetic analysis of the TDGMS mutant, identified many AFLP and IP markers linked to *BntsMs* and narrowed the candidate region to one region comprising 27 genes. Our results will greatly accelerate map-based cloning of the *BntsMs* gene in *B. napus*.

Materials and methods

Plant materials

TE5A is a newly bred DGMS line, which originated from a spontaneous mutant of the inbred line TE5 which exhibits ecotypic sensitivity, the fertility of TE5A is normal at low temperature, and it will transform to completely sterility when temperature is higher than 20 °C during florescence. However, the sterility of Yi3A and 609A is very stable and is unaffected by environment (Song et al. 2005). TE5A is completely male sterile in Xining (a spring oilseed rape area in Qinghai Province, Northwest China) in which temperature is higher than 20 °C during florescence. However, when TE5A is grown in Wuhan (a winter oilseed rape area, Hubei Province), the fertility of TE5A is normal at low temperature, but it will transform to completely sterile when temperature increases to 20 °C (Fig. 1c). Fertile plants exhibited yellow, well-developed anthers, whereas sterile plants were characterised by a normal pistil and withered anthers with no pollen (Fig. 2a). A normal fertile-breeding line (TE1) was used to generate populations that were used to determine the genetic model and fine mapping of the *BntsMs* gene.

Genetic analysis

One hybrid population (F_1) was obtained by crossing the mutant TE5A line with TE1. The F_1 plants were grown at the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences, Wuhan, Hubei Province. An F_2 population was derived from self-pollination of the F_1 plants. The F_1 and F_2 plants were grown at the Qinghai University, Xining, Qinghai Province. A BC_1 line was derived by backcrossing of F_1 to TE1. The F_1 phenotype and the segregation ratios of the F_2 and BC_1 populations were used to detect the genetic pattern of the TE5A mutant. The mapping population BC_2 was derived by the subsequent backcrossing of sterile plants of the BC_1 population to TE1. The BC_2 population was planted at the Qinghai University, Xining, Qinghai Province. The fertility of BC_2 individuals was identified directly in the field.

Data from both experiments were analysed using the SAS system (SAS 8.1). The measurements in the experiments were analysed as a completely random design.

DNA extraction and marker analysis

Young leaves were removed at the seedling stage, and the genomic DNA was extracted using the CTAB method, with some modifications (Doyle and Doyle 1990). The DNA concentration was measured using a NanoDrop

Fig. 1 Flower morphology of TSDGMS line TE5 AB. **a** male-sterile bud of TE5 A. **b** male-fertile bud of TE5 B. **c** Opening flower of a male-sterile plant when grown in Wuhan, *red arrow* is male-sterile buds of TE5 A, *black arrow* is male-fertile buds of TE5 A (colour figure online)

2000 spectrophotometer (Thermo, USA), and adjusted to 50 ng/l in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0).

To identify of molecular markers linked to the *BntsMs* gene, ten plants of each phenotypic class (sterile and normal fertile) from the BC_2 population were randomly selected to construct three groups of SB (sterile bulk) seed and three groups of FB (fertile bulk) seed. SB and FB DNA samples were adjusted to 50 ng/μl with TE buffer and detected using an AFLP survey. The SB and FB DNA samples were digested separately with *Eco*RI and *Mse*I enzyme combinations in 12.5-μl volume. The digested restriction fragment ends were then ligated with specific double-stranded adapters using T4 DNA ligase. The adapter-ligated DNA was diluted fivefold and then pre-amplified with AFLP primers (EA/MC, EA/MG, EC/MC and EC/MG) in a 25-μl volume. The pre-amplified products were analysed by 1.0 % agarose gel electrophoresis and diluted 10-to 30-fold for selective amplification (Negi et al. 2000). The selective amplification products were separated on a 6 % polyacrylamide denaturing sequencing gel and visualised by silver staining (Lu et al. 2004, with some modifications).

Converting AFLP markers into SCAR markers

AFLP fragments exhibiting polymorphism were cloned and sequenced as described previously by Ke et al. (2004) and Yi et al. (2006). The sequences of markers linked to the *BntsMs* gene were extended using BlastN searches at http://brassica.bbsrc.ac.uk/IMSORB/. Based on these sequences, specific primers were designed using Primer3 (Rozen and Skaletsky 1999). These primers were used to detect polymorphisms in the SB and FB seeds.

Genetic map of the *BntsMs* gene

We used the BC_2 population comprising of 30 recessive normal fertile individuals to construct a rough flanking map linked to the *BntsMs* gene. A larger population of BC_2 with 700 recessive normal fertile individuals was subsequently selected and analysed to fine map the *BntsMs* gene. Data of these markers and individual phenotypes were analysed with the MAPMAKER/EXP 3.0 programme (Lander et al. 1987; Lincoln et al. 1992). The map order was estimated by the maximum likelihood method.

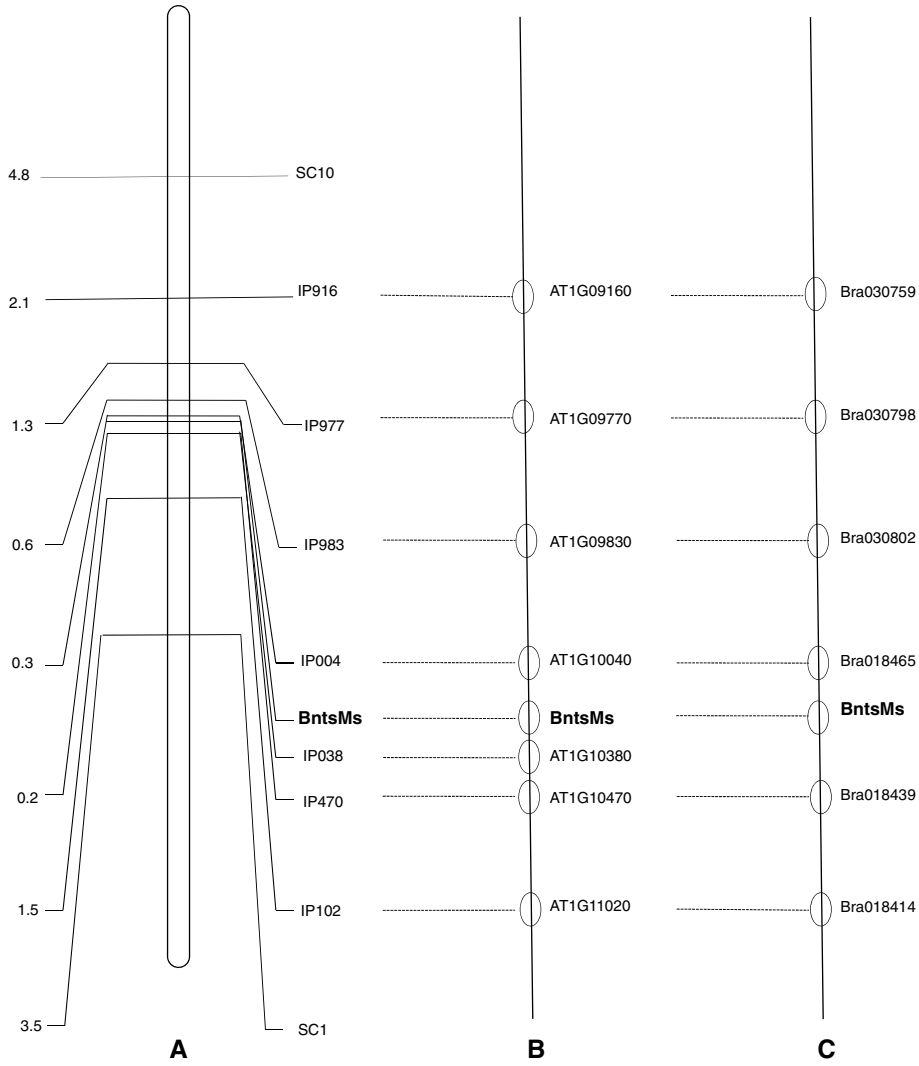

Fig. 2 Partial linkage maps of *B. napus* indicated the relative location of the *BntsMs* gene. **a** The genetic linkage map of the *BntsMs* gene. **b** A partial physical map of chromosome 1 of *Arabidopsis thaliana* shows the homologues of mapped marker sequences. **c** A partial physical map of A8 of *B. rapa*

IP markers and comparative mapping with *Arabidopsis*

To develop markers that are tightly linked to the *BntsMs* gene, the sequences of the AFLP markers P16-MG12 and EC12-MG16 were extended using BlastN searches at http://www.geboc.org/ and http://brassicadb.org/brad/index.php. To identify a putative syntenic region around the *BntsMs* gene in the *Arabidopsis* genome, extended sequences of the molecular markers in the *B. napus* genome were compared with those of their homologues in the *Arabidopsis* (*Arabidopsis thaliana* Integrated Database(ATIDB), http://atidb.org/) and *B. rapa* (http://brassicadb.org/brad/index.php; http://www.brassica-rapa.org/BGP/NC_brgp.jsp) genomes. Specific primers were developed based on the *B. rapa* gene information available at (http://brassicadb.org/brad/index.php) using sequences that were homologous to genes in the syntenic region of the *Arabidopsis* genome [available from The Arabidopsis Information Resource (TAIR), http://www.arabidopsis.org/] using the Primer3 programme (Rozen and Skaletsky 1999). The *Arabidopsis*-derived specific primers that exhibited polymorphism among the samples were used as SCAR markers for the fine mapping of the *BntsMs* locus. The sequences of these markers in the *B. napus* genome were compared with those of their homologues in the *B. rapa* genome (http://brassicadb.org/brad/index.php; http://www.brassica-rapa.org/BGP/NC_brgp.jsp).

Results

Genetic analysis of the *BntsMs* mutants

The phenotypic expression of the filial generations obtained by crossing the *BntsMs* mutant and the normal parent TE1 were thoroughly investigated. The F_1 plants exhibited

normal fertility when grown in Wuhan (a winter oilseed rape area, Hubei Province) under conditions of low temperature, but the normal fertile pollen transform to complete sterility at much higher temperatures. The *BntsMs* mutant is completely male sterile in Xining (a spring oilseed rape area, Qinghai Province in northwestern China), indicating that the *BntsMs* mutant exhibits ecotypic sensitivity (mainly to temperature). The segregating F_2 population in Xining comprised 551 individuals, of which 399 were male sterile and 152 were normal fertile plants. The segregation ratio of the F_2 population showed the expected Mendelian segregation ratio of 3:1 ($\chi^2 = 1.83$, $P > 0.05$). The BC_1 population comprised 217 individuals, of which 105 were male sterile and 112 were normal fertile plants. The segregation showed the expected Mendelian inheritance ratio of 1:1 ($\chi^2 = 0.17$, $P > 0.05$). These data indicate that the phenotype of the *BntsMs* mutant was controlled by a single, temperature-responsive dominant gene.

AFLP markers screening and SCAR markers conversion

For our AFLP analysis, previously described assays involving two commonly used enzymes (*Eco*RIand *Mse*I) and a total of 1,024 pairs of primer combinations (EA/MC, EA/MG, EC/MC and EC/MG) were used to identify putative markers linked to *BntsMs*. Five AFLP markers linked to the *BntsMs* gene were developed using an AFLP assay combined with BSA (Bulk Segregant Analysis) (Table 1), and two of these AFLP markers were converted to SCAR markers (Table 2). The two SCAR markers exhibited polymorphism between the SB and FB seeds, and we focused only on these two SCAR markers in the ensuing analysis. These SCAR markers were subsequently analysed in a population of 30 recessive individuals from the BC_2 population, and the results indicated that the two SCAR markers were located on either side of the *BntsMs* gene.

The IP markers linked to the *BntsMs* gene

To identify a collinear region between *B. napus* and *B. rapa* for the *BntsMS* region, the sequences of the two SCAR markers (SC1 and SC10) were submitted to the BRAD website (The *Brassica* database, BRAD; http://brassicadb.org/brad/) for analysis using the BlastN programme. The SCAR markers SC1 and SC10 were riveted to Scaffold000020 and Scaffold000072 of *B. rapa,* respectively. These two scaffolds were located on *B. rapa* chromosome 8. BlastN searches were performed at the *Arabidopsis* database (http://atidb.org/cgi-perl/blast) using a score value of 82 or higher as the cut-off for putative orthology (Lukens et al. 2003). Homologues for the marker sequences were found on Arabidopsis chromosome 1, and the region from AT1G07140 to AT1G12850 was commonly

Table 1 AFLP markers associated with the *BntsMs* gene and their sizes

AFLP marker designation	Approximate size of marker (bp)	Primer combination
P16-MG12	130	P0-ACT/MG-CG
EA05-MG11	600	EA-TA/MG-CC
EA10-MG13	200	EA-CT/MG-GA
EA09-MC08	400	EA-CA/MC-TG
EC12-MG16	100	EC-CG/MG-GG

defined using these two SCAR markers. The corresponding section of *B. rapa* was used to develop IP markers, and primer pairs was designed based on the *Arabidopsis* gene sequences from this region of chromosome 1 and the *B. rapa* sequences homologous to this region. In total, 25 primer pairs were designed and tested, and seven showed polymorphism among the bulked DNA samples from the BC_2 population (Table 3).

Genetic mapping of the *BntsMs* gene

Seven hundred fertile plants from the BC_2 population were used to construct genetic linkage maps of *BntsMs*. The SCAR markers SC1 and SC10 were analysed in the population of 700 fertile individuals that were pooled into 233 groups of FB seed. Twenty-three recombination events were detected between the *BntsMs* gene and SC1, and 34 recombination events were detected between the *BntsMs* gene and SC10. We restricted our study to these recombinants, which were then used to identify other molecular markers.

The IP markers exhibiting polymorphism among the bulked DNA samples were then used to screen for recombinants in the 700 plants of the BC_2 population, and the IP markers were distributed on either sides of the BntsMs gene (Fig. 2a). IP004 and IP470 were the nearest flanking markers of *BntsMs* and had a genetic distance of 0.5 cm between them (Fig. 2a). IP038 co-segregated in the BC_2 populations (Fig. 2a). From this analysis, we concluded that *BntsMs* was genetically delimited to a region of 0.5 cm between the markers IP004 and IP470.

Comparative analysis of the flanking region of *BntsMs*

To identify a collinear region between *B. napus* and *B. rapa* for the *BntsMS* region, the sequences of all the IP markers (Table 3) shown in Fig. 2a are submitted to the BRAD website (The Brassica database, BRAD; http://brassicadb.org/brad/) for analysis using the BlastN programme. The markers IP916, IP977 and IP983 were riveted to Scaffold000072 (which is located on linkage group A8) of *B.*

Table 2 SCAR markers developed from AFLP fragments linked to the *BntsMs* gene

SCAR names	AFLP primer combinations	Sequences of primer pairs 5′–3′	Annealing temperature (°C)	Product length
SC1	P16-MG12	F:ACCTTGTCGATCTTCTGCAG R:CCGGGTGGCAGGTTAAG	55	130
SC10	EC12-MG16	F: GATTCTTTTGGTTCTTGAATTC R: TTCGTTTCTCCAACAGTTAA	57	100

Table 3 Characterization of IP markers linked to the *BntsMs* gene

IP names	Sequences of primer pairs 5′–3′	Homologous Arabidopsis gene	Product length
IP916	F: GCTTCAGCTGGAGATTGGTC R: TTATCTGCTGAGCTTGCTGC	AT1G09160	600
IP977	F: ATCCTTCACGCAATCATCG R: AAGGAGTCAAGAACCGTGCT	AT1G09770	420
IP983	F: TTCCTGCGGCTCTGTGTTAT R: CACGATCGGTACACCTTGCT	AT1G09830	720
IP004	F: TGGAGGTGGAAGAAGATAGC R: GACCCATAACATCAACTCCAT	AT1G10040	650
IP038	F: GGAAGCGAGAACAAGAGGAT R: ACTAATGTGAACGCCGTCAG	AT1G10380	400
IP470	F: TGTCGATTCTGGTCAACACG R: AGCCTCGTTGATCGGATTG	AT1G10470	650
IP102	F: TGGTGTTCGTAGCTGTGCAG R: CAGCAGTTCTGACACCCGT	AT1G11020	250

rapa and corresponded directly to Bra030759, Bra030798 and Bra030802 respectively. The markers IP004, IP047 and IP102 were riveted to Scaffold000025 (which is located on linkage group A5) of *B. rapa* and corresponded directly to Bra018465, Bra018439 and Bra018414 respectively. IP038 showed no hit genes in *B. rapa*. A *B. rapa* sequenced BAC clone KBrB111O21 (GenBank code: CU695277) was searched (http://www.ncbi.nlm.nih.gov) using the sequences of IP004, IP047 and IP102 as queries.

KBrB111O21 was derived from a BAC library of 'Chiifu', the same cultivar used for *B. rapa* genome sequencing. Moreover, KBrB111O21 contains an identical sequence to the fragment of Scaffold000025 (located on linkage group A5 of *B. rapa*) of *B. rapa*. However, KBrB111O21 was wholly mapped to the *B. rapa* A8 chromosome by linkage mapping (http://www.brassica-rapa.org/BGP/NC_brgp.jsp). This suggests that the sequence of KBrB111O21 was probably misassembled to linkage group A5 of *B. rapa*. Therefore, the closest flanking markers (IP004 and IP470) cover 0.5 cm in the genetic map and delimit only a 168-kb fragment comprising 47 predicted *Arabidopsis* genes (from At1g10040 to At1g10470, Fig. 2b). By comparative mapping with *B. rapa*, the closest markers flanking IP004 and IP470, had a physical distance of 103-kb comprising 27 predicted genes of *B. rapa* (from Bra018465 to Bra018439, Fig. 2c).

Discussion

The genomes of *Arabidopsis* and *B. napus* have extensive colinearity (Parkin et al. 2005), and the *B. rapa* genome is highly homologous with the A genome of *B. napus*. The entire genome sequence of *B. rapa* (Chiifu-401) was published in July 2011. Based on the genomic information of *Arabidopsis*, we developed markers, cloned genes using map-based cloning, and identified candidate genes for *Brassica* crop breeding (Schmidt 2002; Snowdon and Friedt 2004). Indeed, the genomic information of *B. rapa* could facilitate such progress. Xia has reported a map-based cloning strategy for use with *Brassica* genes (Xia et al. 2012). First, genome-wide molecular markers, such as AFLP and SSR, which are linked to target genes, should be sufficiently identified because they are widely distributed at random across entire genomes (McCouch et al. 1997; Blair et al. 2003; Semagn et al. 2006). In the present study, we identified AFLP markers that are tightly linked to the *BntsMs* gene, and two SCAR markers that were located on *Arabidopsis* chromosome 1 using homologous sequence analysis. Second, useful information associated with molecular markers linked to target genes, such as accurate information regarding genetic maps, comparative genomics or the released *Brassica* sequence, should be excavated. In our study, SC1 and SC10 corresponded exactly

to AT1G07140 and AT1G12850, respectively, which are present in an *Arabidopsis* linear region (AT1G07140-AT1G12850) located on the A8 linkage group; accordingly, we determined that our target genes were in this linear region. Third, an effective molecular marker strategy should be applied to delineate the target gene of interest. Previous studies have shown that IP markers are efficient and convenient for map-based cloning in *B. napus* (Xia et al. 2012; Dun et al. 2011). In our study, 25 pairs of IP primers were designed based on *A. thaliana* chromosome 1, and seven of these ultimately showed polymorphism. The percentage of polymorphic markers was 28 %.

In the present study, *BntsMS* was delimited to a genetic interval of 0.5 cm between the markers IP004 and IP470. These markers delimited *BntsMS* to a 103-kb region referring to the syntenic region in the *B. rapa* genome. The corresponding syntenic region in *B. rapa* contains 27 genes, six of which are involved in sporophytic tissue and pollen development. The data suggest that these six genes may be candidate genes of *BntsMS*. To test this hypothesis, the full-length sequence including promoter of the six genes was comparative sequenced between the male-sterile line TE5A and the normal fertile line TE5, which was the original parent of TE5A. No sequence variation was detected between TE5A and TE5 (data not shown). The analysis indicated that these six genes were not candidate genes of *BntsMS*. Two potential methods are available to identify the candidate genes of *BntsMS*. First, fine mapping of the *BntsMS* gene should be further performed in larger population to narrow down the region containing *BntsMS*. Second, comparative sequencing of the remaining genes between the closest flanking markers (IP004 and IP470) should be carried out.

Developing new DGMS lines through conventional backcrossing and selfing is both time and labour intensive. Therefore, a set of markers linked to the *BntsMs* locus might accelerate this process by enabling marker-assistant selection (MAS). Although AFLP and SSR markers have been widely used to map important genes in crops, AFLP markers have not been adapted for the large-scale application of MAS to plant breeding. IP markers designed based on conserved sequences are more suitable for MAS (Xia et al. 2012), and IP markers were likely to be distributed in conserved regions because they exhibit greater stability and conservation than AFLP and SSR markers. Additionally, the polymorphisms of IP markers are more likely to exhibit show co-dominance because of the different lengths of their intron. We screened 1,024 AFLP primer pairs and identified only five AFLP markers that were linked to the *BntsMs* gene; these markers were successfully converted into SCAR markers. Moreover, we developed seven IP markers that are tightly linked to the *BntsMs* gene, markers that will prove valuable for creating novel sterile lines in plant breeding, particularly when used in combination with co-dominant IP markers that are tightly linked to the *BntsMs* gene.

Several temperature-sensitive genic male-sterile systems have been found in *B. napus*, including SP2S (Guo et al. 2012), H50S (Sun et al. 2009), 373S (Yu et al. 2007) and N196S (Liu et al. 2006). However, these genic male-sterile systems have rarely been applied to hybrid seed production due to their genetic complexity and unstable fertility under different temperature conditions. TE5A is completely fertile when the temperature is below 20 °C in Wuhan. The fertility of TE5A is completely male sterile when grown in Xining in which temperature is higher than 20 °C during florescence, and the fertility is very stable. Thus, this plant line can be used in a two-line approach for the large-scale commercial production of rapeseed hybrids. This approach would generate a population of 100 % sterile individuals that can propagate without maintainer lines at the low temperatures (such as those prevailing in Wuhan), and hybrid seeds can be produced by interplanting this line with restorer lines at the high temperatures (such as those prevailing in Xining). Moreover, this strategy could avoid the manual removal of fertile plants in the sterile line when using RGMS and reduce the cost of hybrid seed production. TDGMS was controlled by a single dominant gene; therefore, it would be very easy to develop novel sterile lines using marker-assistant selection (MAS) in this system compared to other male-sterile systems in *B. napus*. Nevertheless, all F_1 plants produced from TDGMS will be sterile when the temperature is higher than 20 °C, and the use of a restorer line is critical for TDGMS application. We screened 34 *B. napus* lines during the two years of this study; of these, two were able to completely restore TDGMS (data not shown) at high temperature. This very useful characteristic could simplify the breeding of *B. napus*.

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Conflict of interest The authors have declared that no conflict of interest exists.

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