Fine mapping and candidate gene identification of a recessive genic male sterility gene (*Bnms3*) in rapeseed

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ABSTRACT

On the basis of the previous mapping study for male sterility gene *Bnms3, we* further narrowed *it* from a genomic region of 470kb to 93.7kb, using a large population consisting of 1339 fertile and 1151 sterile plants, and locus specific markers derived from *Brassica rapa* of A10. Seventeen open reading frames (ORFs) were predicated in the target region. Of them, two most likely candidate genes were proposed. Five co-dominant markers flanking *Bnms3* with 0.00cM to 0.15cM in distance were developed, which are available to distinguish three genotypes of *Ms3Ms3, Ms3ms3* and *ms3ms3*. Allelic distribution of *Bnms3* locus among 96 genetically diverse cultivars/lines showed all the materials carrying homozygous *Ms3* allele (*Ms3Ms3*) in *Bnms3* locus, which might explain the phenomenon for why REGMS could be restored by almost all the rapeseed cultivars/lines.

Key words: *Brassica napus*, *Bnms3*, candidate gene, co-dominant markers

INTRODUCTION

Recessive epistatic genic male sterility (REGMS) in *Brassica napus* attracts great attention to rapeseed breeders due to its prominent advantages in stable and complete male sterility, extensive distribution of restorers and diverse cytoplasmic sources (He *et al*. 2008). Previous genetic analysis suggested that the sterility of REGMS is generally controlled by two recessive genes (*Bnms3* and *Bnms4*) interacting with a recessive epistatic suppressor gene (*Bnrf*) (Chen *et al*. 1998). However in more recent, it was reported that the *BnMs4* and *BnRf* might be multiple alleles (Zu *et al* 2010, Dong *et al* 2010) in one locus. Since its relatively complicated genetic control system, developing new sterile lines often meet difficulties without marker assistant selection. So far, a number of markers linked to the sterile gene *Bnms3* have been reported (Huang *et al.* 2007, He *et al.* 2008) and the estimated physical distance between flanking markers (AR23 and AR48) had been fined to 470kb (He *et al*. 2008). However, for cloning this sterile gene, a further fine mapping is still necessary. In addition, so far the most of published markers showed dominant in nature and failed to distinguish the three genotypes of *Ms3Ms3*, *Ms3ms3* and *ms3ms3,* therefore, limited their utilization for marker assistant selection. In this study, we aim to further fine mapping *Bnms3*, search for candidate genes and develop a set of co-dominant markers for assistant selection in breeding programs.

MATERIALS AND METHODS

Plant materials and population construction

A REGMS two-type line HYZ1AB consisting of 221 male sterile and 243 fertile plants was used for initial *Bnms*3 localization. Further a large population of 1896 plants including 336 sterile and 1096 fertile plants obtained from selfing fertile plants of HYZ1B and 464 from HYZ1AB was adopted for fine mapping and co-dominant marker selection. Further, another 594 male sterile individuals from HYZ1AB were used to check the markers, which showed no recombination event in 1896 plants (Table 1).

Ninety six genetic diversiform cultivars/lines were randomly selected to detect the allelic distribution of *Bnms3* locus, of them, 28 and 35 are Chinese cultivars/lines with double low and double high quality, 22 of spring and 21 for winter rapeseed varieties were originated from Europe, respectively.

Table 1. Segregation of mapping populations for *Bnms3*

Population	Total	Fertile	Sterile	χ^2*		Applications
				1:1	3:1	
HYZ1AB	464	243	221	1.05		Primary and fine mapping
HYZ1AB-B□	1432	1096	336		1.84	Fine mapping
HYZ1AB-A	594	——	594	——	——	Further fine mapping
Sum	2490	1339	1151	——	——	

*$\chi^2(0.05,1)=3.84$

Development of locus specific markers and linkage analysis

Locus specific markers were developed based on BAC or scaffold sequences of *Brassica rapa* A10 at the region syntenic to the DNA fragment around the *Bnms3* locus. Specific PCR primers were designed by software PRIMER PREMIER 5.0 and the polymorphic markers were examined in mapping populations.

The phenotypes of individual plant and their marker genotypes linked to *Bnms3* were analyzed with the software MAPMAKER/EXP 3.0. Map distance was calculated using Kosambi's mapping function.

Candidate gene annotation

The online system FGENESH (http://www.softberry.com) was used to identify predicted ORF in a delimited gene region and their putative functions was bioinformaticly analyzed by Blast.

RESULTS

1. Fine mapping of *Bnms3*

A primary mapping for *Bnms3* was carried out in HYZ1AB population of 464 plants using previously reported thirteen SCAR markers (Huang *et al*. 2007; He *et al*. 2008). As a result, five markers (SC1, SEP4, SEP7, AR23 and AR48) showed tightly linkage with *Bnms3* (Figure 1b) and of them, SEP7 was subsequently mapped to the linkage group A10 of SG-map (Figure 1a).Then 3 markers nearby SEP7 were further examined and two of them (ZAAS133 and PB1) were found also co-segregated with *Bnms3*, indicating that *Bnms3* locus might lie in linkage group 10 (A10) of *Brassica napus.*

To further physically map *Bnms3* in a reduced genomic region, we designed 80 PCR primer pairs based on ten corresponding BAC sequences of *Brassica rapa* A10 between AR23 and AR48. Ten polymorphic markers were screened out by the fertile and sterile DNA bulks and subsequently analyzed in a segregated population of 1896 individual plants. The two closest flanking markers MS3-76 and MS3-32 showed two and one recombinants, respectively, and span a genetic distance of 0.10cM, corresponding to a physical region of 93.7kb (Figure 1c; Table2). In the next step, another 30 primer pairs were further designed flanking this 93.7kb fragment and four polymorphic markers (MS3-89, MS3-93, MS3-81 and MS3-66) between sterile and fertile bulks were further examined with 2490 plants and resulted in no recombination event (Figure 1d).

2. Candidate genes of *Bnms3*

A total of 17 open reading frames (ORFs) were predicted in this 93.7kb target region and 16 of them could best matched with *Arabidopsis* proteins by blastp (Altschul, Madden et al. 1997) with E-value of *1E-30*. Of them, ORF13 and ORF 10 (corresponding to MS3-66 and MS3-89) are the two most likely candidates for *Bnms3*. ORF13 encodes a Myb protein, which belongs to a superfamily of transcription factors that play a well-known role in regulating meristem formation, floral and seed development (Chen *et al.* 2006), and ORF10 encodes a KANADI protein (KAN) functional in organ polarity regulated in *Arabidopsis* (Kerstetter *et al*. 2001). According to our more recent result, a large fragment deletion was observed for ORF13 in sterile plants, indicating that this ORF could be a viable candidate for further investigation.

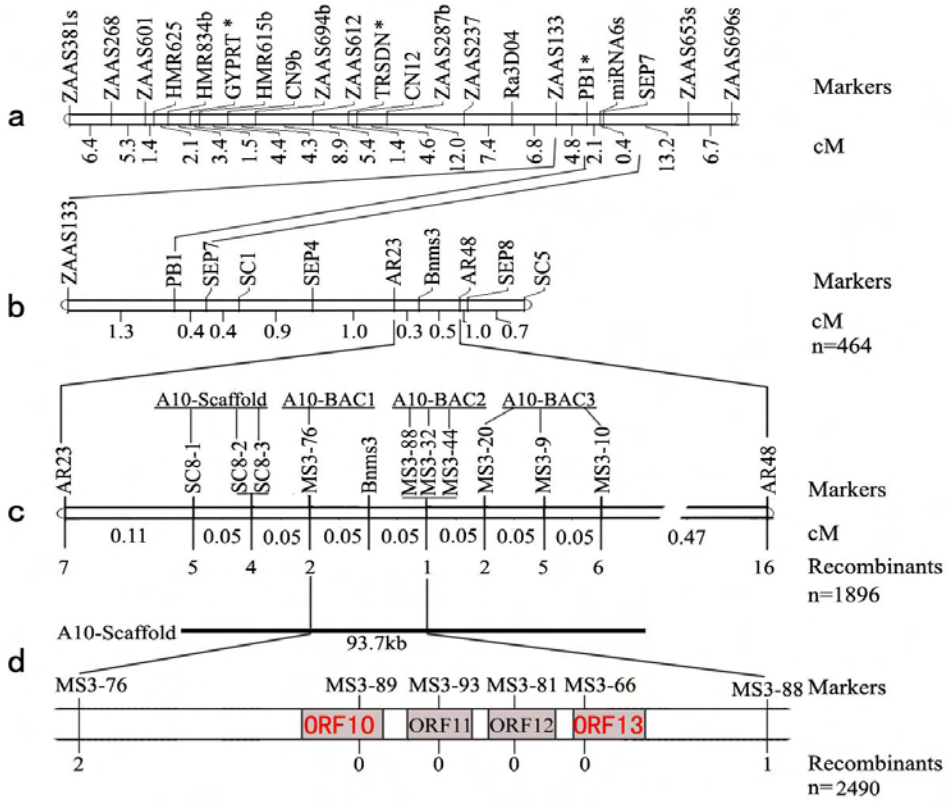

Figure 1. Genetic and physical maps of the *Bnms3* gene and candidate gene analysis. **a.** The linkage group A10 of SG-map. --- were markers mapped on linkage group A10 (Li 2006). **b.** Primary mapping of *Bnms3* using previously reported markers. **c**. Fine mapping of *Bnms3* with newly developed locus soecific markers derived from *Brassica rapa* A10 BAC. **d.** Seventeen ORFs were predicted in the 93.7kb region of one scaffold from *Brassica rapa* A10 and four of them (ORF10, ORF11, ORF12 and ORF13) with 0.00cM in *Bnms3* were observed. ORF10 and ORF13 (red) are most

3. Screening of co-dominant markers linked to *Bnms3*

Of fourteen newly developed markers closely linked to *Bnms3*, five (MS3-9, MS3-32, MS3-89 SC8-2 and SC8-3) exhibited co-dominant in nature, which located on 0.10cM from *Bnms3* in one side (SC8-2 and SC8-3), 0.05/0.15cM (MS3-32 /MS3-9) in another side and 0.0cM for MS3-89 (Figure 2) on either side of *Bnms3* (Figure 1c,d).

4. The genotypes of varieties in *Bnms3* gene locus

Ninety six cultivars/lines were analyzed with marker (MS3-66) developed from ORF13 covering the part of deletion. The result showed that all the materials were *Ms3Ms3* genotype in *Bnms3* locus (Figure 3).

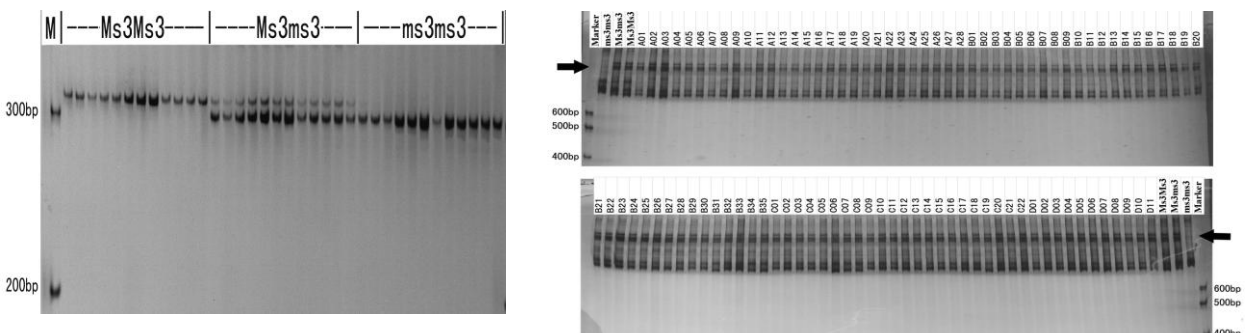

Figure 2. The amplification of marker MS3-89 in three genotypes of *Bnms3* locus in 36 Individuals' plants

Lanes: M: Marker, Ms3Ms3 (1-12), Ms3ms3 (13-24) and ms3ms3(25-36):

Figure 3. The amplification of marker MS3-66 (ORF13) in 96 cultivars/lines

Lanes: Marker, ms3ms3, Ms3ms3, Ms3Ms3, A01-A28: Chinese cultivars/lines with double low quality; B01-B35: Chinese cultivars/lines with double high quality; C01-C22: Spring oilseed rape; D01-D21: Winter oilseed rape. The arrows indicate the DNA fragment of Ms3 allele

CONCLUSION

All the evidences obtained from present study demonstrated that the *Bnms3* might originate from *Brassica rapa* A10. We further narrowed down the flanking region of *Bnms3* from 470kb to 93.7kb and discovered two candidate genes for *Bnms3*. ORF13 is probably the more important candidate for *Bnms3.* Five co-dominant flanking markers with genetic distances of 0.00-0.15cM were developed, which largely facilitate the activities in hybrid breeding of rapeseed. Allelic distribution examination of *Bnms3* locus explained the phenomenon for why REGMS could be restored by almost all the rapeseed cultivars/lines.

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