Fine genetic mapping and physical delimitation of the recessive genic male sterile gene *Bnms1* to a 21-kb DNA segment in *Brassica napus* L.

YI Bin, CHEN Yuning, LEI Shaolin, MA Chaozhi, TU Jinxing, FU Tingdong

National Key Laboratory of Crop Genetic Improvement, National Sub-center of Rapeseed Improvement in Wuhan, Huazhong Agricultural University, Wuhan 430070, China Email: yibin324@yahoo.com.cn

Abstract

A recessive genic male sterility (RGMS) system, S45 AB, has been developed from spontaneous mutation in *Brassica napus* canola variety Oro, and is being used for hybrid cultivar development in China. The male sterility of S45 was controlled by two duplicated recessive genes, named as *Bnms1* and *Bnms2*. To better understand the molecular basis underlying recessive genic male sterility, a map-based cloning strategy has been employed to isolate *Bnms1*. Seven *Bnms1*-linked AFLP markers were developed and four of them were converted to sequence-characterized amplified region (SCAR) marker. A high-resolution genetic map was developed using a NIL population consisting of 4,132 individuals, in which the recessive allele was homozygous at the second locus. Recombination suppression was observed in the vicinity of *Bnms1*. Three molecular markers tightly linked to *Bnms1* were identified and used to screen a BAC library. A contig spanning the *Bnms1* locus was constructed and physical mapping delimited *Bnms1* to a 21-kb DNA segment within a single BAC clone. These results provide the essential information for the final isolation of this important gene in rapeseed microsporogenesis.

Keywords: Recessive genic male-sterility,*Brassica napus*,S45 AB,Genetic mapping , Physical mapping ,BAC contig

Introduction

Hybrid cultivars have been successfully used to increase double-zero rapeseed production worldwide. Male sterility has been applied in this crop as an effective and economical pollination control system. Plant male sterility has been generally classified into cytoplasmic male sterility (CMS) and genic male sterility (GMS). The advantage of using CMS system is that it can generate a complete male sterile population economically. However, this system involves the development of three lines: male sterile line (A), maintainer (B), and restorer (R), and it usually take years to develop A and R lines since most CMS systems have stringent restoring-maintaining relationships. Moreover, some CMS have negative effects on F1 yields, or produce pollen grains under stress environments. In contrast, recessive GMS have many advantages. Firstly, GMS involves only two lines and is transferred feasibly among parental lines, which may result in a shortened breeding cycle. Secondly, for recessive GMS, most breeding lines are restorers, so it is easy to combine any elite lines to produce strong heterosis. Thirdly, GMS does not have the negative cytoplasmic effect on yield as CMS might do. But GMS system has its limitation of being difficult to derive a complete male sterile population. About 50% male fertile plants must be removed from the female lines during hybrid seed production. Development of a complete genic male sterile population with a temporary maintainer B-line of GMS was a breakthrough to overcome the previous limitation (Chen et al. 1993, 1998). Consequently, several GMS-based hybrids have been released commercially in China.

S45 AB is a recessive genic male sterile line derived from a natural mutant found in the *Brassica napus* canola variety Oro. Genetic analysis indicated that two duplicate recessive genes, named as *Bnms1* and *Bnms2*, controlled the male sterility in S45 populations (Pan et al. 1988). S45 AB line is composed of 50% male sterile (ms) plants (i.e. S45 A, Genotype: *Bnms1ms1ms2ms2*) and 50% male fertile (mf) plants (i.e. S45 B, Genotype: *BnMs1ms1ms2ms2*), in which the recessive allele was homozygous at the second locus (Li et al. 1993). S45 AB is maintained by harvesting the progeny from S45 A plants through sib-mating (i.e. S45 A ms plants pollinated by S45 B mf plants). Although the recessive genic male sterility (RGMS) system is being used for hybrid cultivar development in China, detailed information regarding the RGMS gene is still unknown. The fine mapping of the *Bnms1* gene will be helpful for hybrid breeding and understanding the molecular mechanism of genic male sterility. Recently, *Bnms1* was mapped on linkage group N7 of the *B. napus* map (Yi et al. 2006). In the study reported in this paper, we further localized the *Bnms1* locus to a genomic DNA fragment of 21 kb in length, thus achieving a major step toward our ultimate goal of isolating this gene.

Materials and methods

Plant materials

RGMS two-type line, S45 AB, was used as materials. S45 AB line has been maintained by full sib-mating (S45 A × S45 B) for 25 generations. The ms plant S45A and the mf plant S45B are therefore near isogenic lines (NIL) that differ only by the ms/mf trait. The fertile plants had yellow, well-developed anthers whereas the sterile plants were characterized by declining anthers with no pollen. A NIL population including 4,132 plants was used for molecular mapping of the *Bnms1* gene. The male fertility was visually scored at flowering time.

DNA preparation and marker assay

Fresh leaves were harvested from field-grown plants and genomic DNA was extracted by use of the CTAB method (Dyole and Dyole 1990) and used for PCR-based marker genotyping. DNA concentration and purity was measured by a Beckman spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA) at a wavelength of 260 nm vs. 280 nm. Final DNA concentration was 25 ng/µl in TE buffer (10 mM Tris, 1 mM EDTA pH 8.0). PCR reactions for SCAR markers were performed as described previously (Yi et al. 2006). Primers were designed from the shotgun sequences of bacterial artificial chromosome (BAC) using the OLIGO program (Rychlik and Rhoads 1990).

B. napus *BAC library screening*

A BAC library of the *Brassica napus* cultivar Tapidor from John Innes Centre was used to construct a physical map encompassing the *Bnms1* region. The BAC clones were screened by hybridization of probes to doubled-spotted macro-arrayed clones as described in Rana et al. (2004). The BAC clone was sequenced using the shotgun sequencing strategy (Birren et al. 1997).

Results

Localization of the Bnms1 *locus to a 21-kb DNA fragment*

The special fragments of SCAR markers SC1, SC6 and SC7, were labeled with $\alpha-^{32}P$, and directly used as probe to screen high-density BAC filters. 41 positive BAC clone were identified from the BAC library. Then all 41 BAC clone, along with two parents, a mixture of genomic DNA from 12 ms plants, and a mixture of genomic DNA from 12 mf plants , were subjected to screening with three SCAR markers. Two flanking SCAR markers, SC1 and SC7 identified a common BAC clone, which obviously traversed the *Bnms1* locus. The BAC clone was sequenced using the shotgun sequencing strategy. To narrow down the *Bnms1*-containing region further, 35 primer pairs were designed based on the shotgun sequence of the BAC. DNA samples from the ms and mf plants were assayed with each of the primer pairs. Polymorphisms were detected with 6 of the primer pairs, which were subsequently used to assay the recombinant plants identified by SC1 and SC7. Two SCAR markers (SC8 and SC11) flank 21 kb, which detected two and six recombinants with the *Bnms1* locus, respectively, were identified as the nearest markers on both sides. In addition, two SCAR markers, SC9 and SC10, were shown to co-segregate with the *Bnms1* locus. Thus, the *Bnms1* locus was delimited to a 21-kb DNA fragment (Fig. 1).

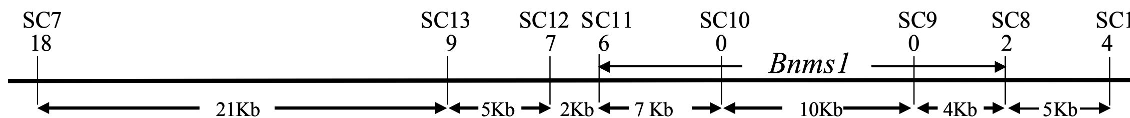

Fig.1 Physical map of the region encompassing the *Bnms1* locus. The SC1 and SC7 are from a published linkage map (Yi et al. 2006). The SC8, SC9, SC10, SC11, SC12 and SC13 are SCAR markers obtained during the course of this work. The numbers under the markers gave recombinants between markers and the *Bnms1* locus from 4,132 NILs. *Bnms1* is located in the region between the two markers, SC8 and SC11.

Putative genes in the 21 kb region

Four ORFs were found in the 21 kb region by use of GenScan1.0 (http://www.genes.mit.edu) (Fig. 2). A BLAST search of the 21-kb sequence against the EST database in GenBank found five homologic expressed sequences (Fig.3). Except for ORF1, other three ORFs correspond with the full length cDNAs or ESTs in GenBank. The functional identification of one of the putative genes for the recessive genic male sterility is being investigated.

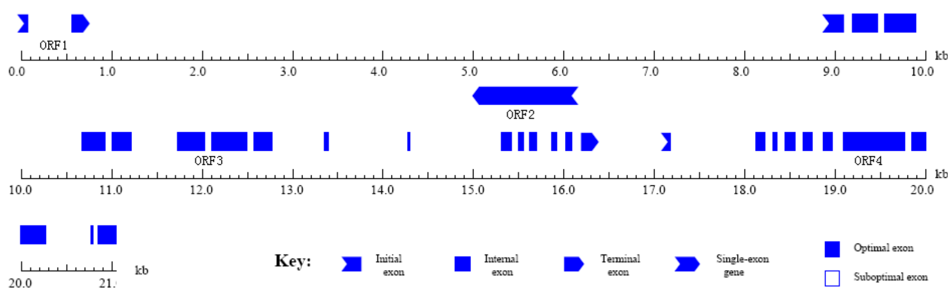

Fig.2 Four putative ORFs of the 21 kb region identified by GenScan1.0 (http://www.genes.mit.edu)

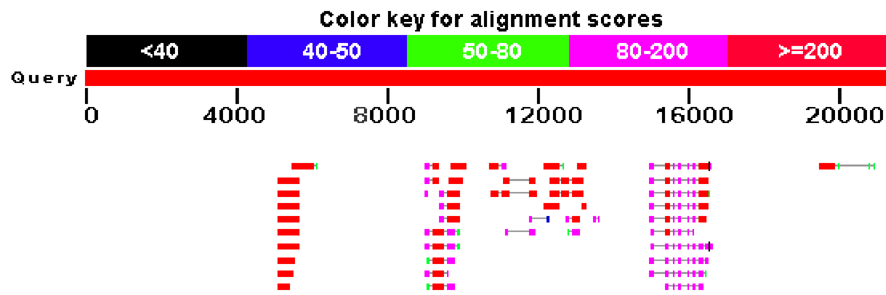

Fig.3 BLAST analysis of the 21-kb sequence against the EST database in GenBank.

Discussion

Even though the GMS system is an effective alternative hybrid system in *Brassica napus*, very little is known regarding the molecular basis of the GMS trait, which limits its wide application in hybrid breeding. Cloning, isolation, and characterization of GMS genes in the future will improve its application in hybrid breeding. Cigan and Albertsen (1998) introduced an interesting strategy to multiply ms line by using an inducible promoter. In this strategy, the native promoter is taken off from the male fertility gene and replaced with an inducible promoter responding to chemical stimulation. The ms line remains ms (for use in hybrid production) without the chemical treatment, but is reverted to be mf (for multiplication) with the chemical application. Cloning of the *BnMs1* gene is the first crucial step if we are to apply this in GMS system. Map-based cloning is generally regarded time-consuming, laborious and expensive. This strategy is further complicated by the amphidiploid nature of the *B. napus* genome. In the present study, fine-scale mapping of the gene in a large segregating population and identification of tightly linked flanking markers led to chromosome landing on a BAC clone carrying the *Bnms1* gene. This is, to our knowledge, the first report of localizing the *Bnms1* locus to a DNA fragment approximately 21 kb in length, which is an important step toward map-based cloning of this gene.

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