A molecular genetic linkage map in recombinant inbred lines of *Brassica napus* L. using SSR and SRAP markers

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Abstract

Brassica napus L. (2n = 38, AACC) Recombination Inbred Lines (RILs) population were derived from a cross between *Zhongyou 821* which was brown seed coat and *GH 06* which was yellow seed coat. They have been recombined self-inbred by single seed descent method for seven years. 441 SSR primer pairs and 4096 SRAP primer combinations were used to screen for polymorphisms between black-seeded and yellow-seeded parents. 2536 of 4096 SRAP primer combinations did not yield distinct amplification bands. 92 SSR primer pairs (20.86%) and 260 SRAP primer combinations (16.67%) yielded 106 and 480 unambiguous polymorphic bands respectively. 423 loci (74 for SSR and 349 for SRAP) were mapped on 23 linkage groups ranging from 39 cM to 140 cM with an average length of 76.96 cM containing 3 to 82 markers in each linkage group by JoinMAP 3.0.. The map covered a total of 1770 cM with the average distance between two adjacent markers was 4.18 cM. In order to gain a molecular genetic map which can cover the entire genome, other molecular marker methods are required, for example AFLP and RFLP. The map has very important use in locating some *Brassica napus* L. quantity trait locus (QTL) and molecular marker assist breeding in the future.

Key words: *Brassica napus* L., SSR, SRAP, mapping

Introduction

Nowadays, the highly developed molecular marker technologies have the potential to revolutionize genetic selection in plant breeding (Tanksley *et al.*, 1989). Using common molecular marker methods, e.g. RFLP, RAPD, SSR and AFLP, high-density genetic maps have been constructed for many crops, such as rice, maize and soybean. In recent years, certain achievements have been made in map construction and molecular marker identification in *B. napus* using these methods. For example, Ferreira *et al.* (1994), Parkin *et al.* (1995) and Sharpe *et al.* (1995) constructed RFLP maps; Lombard and Delourme (2001) constructed a consensus map by isozymes, RAPD, AFLP and RFLP; And Lowe *et al.* (2004) and Piquemal *et al.* (2005) constructed SSR maps of *B. napus*. However, no dense genetic map is available for *B. napus* to carry out fine-mapping and efficient MAS of many important traits of rapeseed.

Sequence-related amplified polymorphism (SRAP) is a new based on simple PCR co-dominant molecular marker technology (Li *et al.*, 2001; Lian *et al.*, 2003). The SRAP primer pairs are designed according to common structural features of open reading frames (ORFs) of various known genes. Since its emergence, it has been successfully used to construct genetic linkage maps and to analyze genetic polymorphisms in many crops (Budak *et al.*, 2004; Lin *et al.*, 2003; Ferriol *et al.*, 2003; McCouch *et al.*, 1997; Yu *et al.*, 2005).

The main objective of this research was to construct a high density linkage genetic map of *B. napus* using SSR and SRAP marker methods which are base on simple PCR and easily operated. The map would lay the base for further MAS breeding of *B. napus*.

Materials and methods

Mapping populations

One black-seeded inbred line from cultivar *Zhongyou 821* as male parents and one yellow-seeded inbred line *GH 06* (one parent of the yellow-seeded cultivar *Yuhuang 1* with a complete dominance yellow-seeded major gene) (Li *et al.*, 2001) as female parent were chosen to construct the linkage genetic map. In each combination, the F_1 plants were selfed to produce the F_2 progenies in 1998, and F_2 progenies were continuously selfed by single seed descent method for seven years. The high-generation RIL was used as the mapping population. In 2004 to 2005, the RIL population was grown in Beibei, Chongqing, China.

Assay of DNA markers

Sequences of public SSR primer pairs were downloaded from Brassica database (http://www.ukcrop.net/perl/ace/search/BrassicaDB), whereas sequences of other SSR primer pairs were copied from Piquemal *et al.* (2005). The PCR reaction was carried out on PTC-200 ThermoCycler with a total volume of 10 µl. The composition of the mixture and the reaction procedure were the same as those reported by Piquemal *et al.* (2005). The SRAP

reaction procedure was performed in terms of a previous description (Ferriol *et al.*, 2003). In total, 64 forward primers and 64 reverse primers were employed, which resulted in 4096 primer combinations. The SSR and SRAP primers were synthesized by Shanghai Sangon. (Shanghai, China). All PCR products were detected using non-denatured polyacrylamide gel electrophoresis and silver staining with a polyacrylamide density of 8% (Zhang *et al.*, 2002).

Map construction

JoinMap 3.0 (van Ooijen *et al.*, 2001) was used to build the genetic linkage maps. A minimum LOD score of 3.0 with a maximum genetic distance of 45 cM was first used to integrate SSR and SRAP loci into initial linkage groups. Genetic distances were calculated according to the Kosambi formula. Confirmation of linkage group designations was achieved by comparison to the map positions of public SSR markers (Bancroft 2006; Lowe *et al.*, 2004; Piquemal *et al.*, 2005). The linkage group orders in the map were processed by Mapchart 2.1 (Voorrips *et al.*, 2002).

Results

Screening polymorphisms between parents

441 SSR primer pairs and 4096 SRAP primer combinations were used to screen for polymorphisms between black-seeded and yellow-seeded parents. 2536 of 4096 SRAP primer combinations did not yield distinct amplification bands. 92 SSR primer pairs (20.86%) and 260 SRAP primer combinations (16.67%) yielded 106 and 480 unambiguous polymorphic bands respectively.

Genetic linkage map construction of B. napus

Among of the 586 markers, 423 loci (74 for SSR and 349 for SRAP) were mapped on 23 linkage groups ranging from 39 cM to 140 cM with an average length of 76.96 cM containing 3 to 82 markers in each linkage group. The map covered a total of 1770 cM with a coverage percentage of about 70.8 % (Lombard and Delourme, 2001), and the average distance between two adjacent markers was 4.18 cM (Fig. 1).

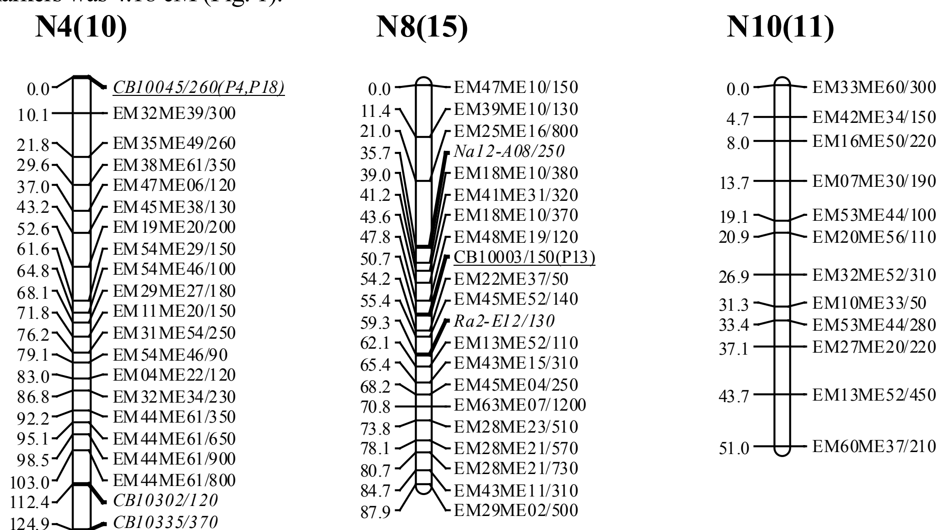

Fig. 1 Three linkage groupsof the genetic linkage map based on 20 SRAP and SSR linkage groups, which span 1770 cM with a total of 423 loci (74 for SSR, 349 for SRAP). The numbers in the bracket after linkage group shows numbers using JoinMap 3.0 to construct linkage group. Among the italic-lettered markers located within the same linkage groups reported in previous SSR maps, the underlined letters "P", "L" or "B" in the parentheses following the marker names mean unanimous with the markers of Piquemal *et al.* (2005), Lowe *et al.* (2004), or Bancroft (2006), respectively.

Nomenclature of the linkage groups

The linkage groups were designated using the standard N1-N19 nomenclature system for *B. napus* (Bancroft 2006; Lowe *et al.*, 2004; Piquemal *et al.*, 2005). For lack of consensus markers, linkage groups LG 5, LG 11, LG 14, LG 18 and LG 20 were not assigned with the N1-N19 nomenclature system. Based on consensus markers of the other population (not data shown), they were tentatively and temporally designated as N3, N10, N12, N18, N20. In our results, the major markers constituting the linkage map were SRAP markers (81.91%), and SSR markers distribute among the SRAP markers.

Discussion

SRAP is a new molecular marker system based on simple PCR and has some advantages over other marker systems, such as RFLP, RAPD, AFLP and SSR. At firstly, the 17 or 18 nucleotides long primers are designed according to open reading frames (ORFs) and consist of the following elements. Core sequences, which are 13 to 14 bases long, where the first 10 or 11 bases starting at the 5′ end, are sequences of no specific constitution ("filler" sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer. The core is followed by three selective nucleotides at the 3′ end. The filler sequences of the forward and reverse primers must be different from each other and would be 10 or 11 bases long.

The three selective bases at the 3′end of SRAP primers could be altered according to primer design rules, that is to say, more primer pairs could be created by combining forward and reverse primers, which can reduce the expense of synthesizing primers greatly and enhance primer-using efficiency largely. Secondly, a simple and convenient silver staining method was used to detect bands in this study. In previous researches, the PCR products were used denaturalization gel electrophoresis by using high voltage (Li and Quiros, 2001; Lin *et al.*, 2005). Thirdly, The PCR reaction system in this assay combinates general characters of PCR and AFLP, and a good amplification result could be obtained using the system and electrophoresis parameters mentioned in this paper(Li and Quiros, 2001; Lin *et al.*, 2005).

Above-mentioned, SRAP is adapted to popularize on the molecular assisting selection (MAS) of the crop breeding. RFLP has the advantage of producing mostly co-dominant markers, good repetition and consistence, but the operation is too complicated to realize automatization. Moreover, high-quality and a large amount of DNA are required, and it is very expensive to analyze big population. RAPD is a simple method to fingerprint genomic DNA, but poor consistency and low output limit its application. SSR is a good marker system for its simplicity and mostly co-dominant information; however, there are limited SSR primers in *B. napus* and their development is considerably expensive and time-consuming. AFLP is now widely used for a variety of applications due to its high multiplex ratio, but the operation is very complicated. SRAP is a comparatively better marker system, but it may have less amplification for centromere and telomere regions because SRAP mainly targets coding sequences in the genome. If SRAP combines with SSR that could amplify repeat regions, the linkage map may cover the whole genome. So SRAP would be a powerful tool in MAS of the crop breeding. It is a new molecular marker method and has been used to construct some crops' genetic linkage maps(Li and Quiros, 2001; Lin *et al.*, 2005; Lin *et al.*, 2003; Wang *et al.*,2005) and to study the genetic relationships(Fufa *et al.*, 2005; Ferriol *et al.*, 2003; Vollmann *et al.*, 2005; Yu *et al.*, 2005). In previous researches, the PCR products were used denaturalization gel electrophoresis by using high voltage (Li and Quiros, 2001; Lin *et al.*, 2005; Ferriol *et al.*, 2003).

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