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The construction of a genetic linkage map of non-heading Chinese cabbage (*Brassica campestris* ssp. *chinensis* Makino)

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Abstract

Non-heading Chinese cabbage (*Brassica campestris* ssp. *chinensis* Makino) is one of the most important vegetables in eastern China. A genetic linkage map was constructed using 127 doubled haploid (DH) lines, and the DH population was derived from a commercial hybrid "Hanxiao" (lines SW-13 × L-118). Out of the 614 polymorphic markers, 43.49% were not assigned to any of the linkage groups (LGs). Chi-square tests showed that 42.67% markers were distorted from expected Mendelian segregation ratios, and the direction of distorted segregation was mainly toward the paternal parent L-118. After sequentially removing the markers that had an interval distance smaller than 1 cM from the upper marker, the overall quality of the linkage map was increased. Two hundred and sixty-eight molecular markers were mapped into 10 LGs, which were anchored to the corresponding chromosome of the *B*. *rapa* reference map based on common simple sequence repeat (SSR) markers. The map covers 973.38 cM of the genome and the average interval distance between markers was 3.63 cM. The number of markers on each LG ranged from 18 (R08) to 64 (R07), with an average interval distance within a single LG from 1.70 cM (R07) to 6.71 cM (R06). Among these mapped markers, 169 were sequence-related amplified polymorphism (SRAP) molecular markers, 50 were SSR markers and 49 were random amplification polymorphic DNA (RAPD) markers. With further saturation to the LG, the current map offers a genetic tool for loci analysis for important agronomic traits.

Keywords: non-heading Chinese cabbage; genetic linkage map; SRAP; SSR; RAPD

Introduction

Brassica rapa includes many important vegetables, such as Chinese cabbage [*Brassica campestris* ssp. *pekinensis* (Lour.) Hanelt], non-heading Chinese cabbage (*B*. *campestris* ssp. *chinensis* Makino), and rape seed (*B*. *rapa* L. subsp. *rapa*). Non-heading Chinese cabbage, which originated from China, is an important leafy vegetable in eastern Asia.

Molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR), are good platforms for genetic fingerprinting and constructing of linkage maps because they are randomly distributed across the genome and can be used without prior knowledge of the gene sequence information. Sequence-related amplified polymorphism (SRAP), a PCR-based marker system, has been reported by Li and Quiros (2001). SRAP markers are arbitrarily designed to contain AT- and GC-rich motifs that anneal to introns and exons, respectively. Sequenced SRAP amplicons from *Cucurbita moschata* revealed sig-

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nificant similarities to gene sequences deposited in GenBank database (Ferriol et al., 2003). SRAP markers have been integrated into genetic linkage maps of *Brassica* (Li and Quiros, 2001).

Assigning molecular markers to the linkage groups and constructing genetic maps are important in analyzing genomes of crop species. The maps provide a better insight into genome organization, evolution of the crop species, and synteny with related species. In addition, the maps are useful for tagging and cloning genes of economically important traits, marker assisted breeding and gene pyramiding (Beavis and Grant, 1991).

Molecular mapping of *B. rapa* was initiated by Song et al. (1991) using RFLP markers and a segregating F_2 population. Subsequently, several additional maps were constructed based on different molecular markers using F_2, recombinant inbred lines (RIL) or DH populations (Chyi et al., 1992; Teutonico and Osborn, 1994; Kole et al., 1997; Kim et al., 2006; Suwabe et al., 2006; Choi et al., 2007; Geng et al., 2007; Wu et al., 2008). The recent development of *Brassica* SSR markers has facilitated the anchoring of linkage groups (LGs) to one common reference map and allows comparison of map positions in the studies involving different populations (Suwabe et al., 2006; Choi et al., 2007). Furthermore, the syntenic relationship with the related genus *Arabidopsis* is well established (Schranz et al., 2006) and allows comparison of map positions between *Brassica* and *Arabidopsis*.

In non-heading Chinese cabbage, the first genetic map was previously produced by Geng et al. (2007) using Shulv DH population. One hundred and thirty-eight molecular markers were mapped into 14 LGs. The total length of the linkage map was 1,923.75 cM, with an average marker spacing of 15.52 cM. However, the number of LGs on the map does not match the 10 expected chromosomes in the genome of *B. campestris* ssp. *chinensis* Makino. To integrate the linkage groups into the 10 chromosomes, further research needs to be done through adding more individuals in the DH population, saturating the map, and using different types of mapping populations, e.g., F_2, RIL, and backcrosses.

The objective of this study was to construct a molecular linkage map of non-heading Chinese cabbage using SRAP markers in conjunction with SSR and RAPD markers. The map was based on a commercial cultivar Hanxiao, which originated from a cross between two high inbred lines SW-13 and L-118. The framework linkage map will facilitate selective breeding and mapping of qualitative trait loci (QTL).

Materials and methods

Production of DH lines for the mapping population

Before the mapping population, high inbred lines have been developed from both of the parental genotypes. The maternal parent SW-13, a self-incompatible line, was developed from cultivar Aijiaohuang by molecular assisted selection; the paternal parent L-118, a high inbred line, was derived from cultivar Liangbaiye. There are a number of different traits between the two parents, including leaf shape, peduncle color, disease resistance, hot tolerance, cold hardiness, and quality. F_1 plants were produced by crossing high inbred line L-118 with SW-13. Several F_1 plants originating from one crossing bag were then used to generate a DH population by microspore culture. A total of 127 DH lines, named as the Hanxiao DH population, were generated in 2005.

DNA extraction

About 0.3 g fresh flower buds were used to extract genomic DNA using the cetyl-trimethyl-ammonium bromide (CTAB) method. An Eppendorf protein and nucleic determine instrument was used for determining DNA concentration.

SRAP analysis

The sequences of SRAP primers were provided by G. Li of the University of Manitoba, Canada (Supplemental Table 1). Reactions were performed in a 10 μL volume containing 30 ng of template genomic DNA, 0.5 μmol/L forward and reverse primers, 0.2 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, and 0.75 U *Taq* DNA polymerase. The protocol of PCR reaction was conducted at 94°C for 3 min, then 94°C for 90 s, 35°C for 90 s, 72°C for 90 s for five cycles; 94°C for 90 s, 50°C for 90 s, 72°C for 90 s for 33 cycles; and finally 72°C for 7 min. PCR products were separated on a 5% vertical polyacrylamide gel, which was run at a 150 V constant voltage for 1.5 to 2 h when xylencyanol reached at the bottom of the gel. After electrophoresis, the gel was stained by $AgNO_3$ solution.

SSR analysis

Primer sequences for SSR markers obtained from various sources (Suwabe et al., 2002, 2004, 2006; Lowe et al., 2004; Kim et al., 2006; Choi et al., 2007; Cui et al., 2008)