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Identification of quantitative trait loci for yellow inner leaves in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) based on SSR and SRAP markers

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ABSTRACT

Yellow inner leaves are a valuable agronomic trait in *Brassica rapa* L. ssp. *pekinensis* (*B. rapa*) due to its higher nutritional content and huge economic value. The objective of the present study is to determine the heritability of yellow inner leaves, to construct a genetic linkage map, and to map the quantitative trait loci (QTLs) controlling this trait. An $F_{2:3}$ population was generated from a cross derived from 07A237 and Chiifu, which are excellent inbred lines having yellow and white inner leaves, respectively. A linkage map was developed with 71 SSR and 145 SRAP markers, covering a total genetic distance of 819.4 cm and an average map distance of 3.8 cm. With the anchor SSR markers, the present map could be aligned to previously reported genetic maps, and the corresponding chromosome of the *B. rapa* A genome. Three QTLs controlling yellow inner leaves, accounting for 40% of the phenotype variance, were identified with composite interval mapping analysis. The present study could be a reference for QTL studies of yellow inner leaves in *B. rapa*. Our findings can be used to identify markers linking to the QTLs for marker-assisted selection in the practical breeding program.

1. Introduction

Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) is one of the most popular vegetables, has the largest cultivation acreage in China, and is gaining acceptance by the people all over the world. In contrast to external leaves which appear green, the colors of inner leaves of Chinese cabbage can be white, orange, or yellow. Chinese cabbage with colored inner leaves has a brighter appearance and higher nutritional values than that with white inner leaves. In recent years, the yellow leaf cultivars are becoming the most preferred Chinese cabbage due to its higher nutritional values and significant economic benefit. In Asia, many countries have already replaced the outdated cultivars with yellow inner leaf varieties.

The leaf pigments of Chinese cabbage are reported to consist mainly of chlorophyll, carotenoid, and anthocyanin (Wang et al., 2008). Of the three pigments, chlorophyll and anthocyanin can be easily found in the external leaves exposed to visible sunlight, which is essential for the synthesis of both pigments. In contrast, these two pigments are relatively low in the inner leaves that are not exposed to sunlight. Thus, carotenoid becomes the main pigment found in the inner leaves and results in a yellow color of the inner leaves. The carotenoid contents of the yellow inner leaf cultivar of Chinese cabbage are obviously higher than that of common white leaf cultivars (Chen, 2008). Almost all carotenoids have benefits to human health by converting into vitamin A (Olson, 1989), resisting oxidation (Di Mascio et al., 1989; Fraser and Bramley, 2004), preventing angiocardiopathy, and cancers (Yie et al., 2008; Molnar et al., 2004; Giovannucci et al., 1995; Clinton, 1998). Increasing interest in pursuing healthy lifestyles has resulted in increased demand for vegetable cultivars with health benefits, which receive more attention from plant breeders.

To develop new cultivars of Chinese cabbage, many valuable traits showing quantitative trait inheritance are breeding objectives, but the relatively long breeding cycle of traditional technology sets a slow pace. Quantitative traits are affected by environmental conditions, making the selection of these traits imprecise when breeders screen these traits by their experience alone. The development of molecular biology techniques, especially the availability of molecular markers, makes it possible to study single quantitative trait loci (QTL), and to develop linked markers for marker-assisted selection (MAS). The QTL studies of disease resistance and stress resistance have been widely conducted in crops (Li et al., 2009; Han et al., 2008; Ochiai et al., 2008; Santra et al., 2008; Qiao et al., 2005). In *B. rapa*, the studies were focused on disease resistance (Soengas et al., 2007; Piao et al., 2004; Sakamoto et al., 2008; Saito et al., 2006; Yu et al., 2009), as it is important to use these QTL in practice to improve yield. In terms of morphological characteristics, Lu et al. (2002) identified QTLs of leaf length, leaf

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number, leaf width and leaf lobes, and Yu et al. (2004) mapped several QTLs for head weight, head diameter, head length, and days to harvest.

Xu et al. (2007) carried out studies on exterior leaf color, confirmed that it was controlled by multiple genes as a typical of quantitative trait, and identified several QTLs controlling the exterior leaf color. The inner leaves of Chinese cabbage are the main edible part. Inner leaves with orange color have been studied as a type of qualitative trait and several molecular markers linking to the known *or* gene have been identified (Liu et al., 2003; Matsumoto et al., 1998; Wang et al., 2007; Zhang et al., 2008). Yellow inner leaves have been accepted widely in Asian countries such as Japan and Korea as another kind of inner leaf of *B. rapa*. Furthermore, the traditional white inner leaf cultivars have been largely replaced by these new yellow ones. Since this inner leaf trait has been rarely reported, it is necessary to carry out studies on this important characteristic in Chinese cabbage.

The aims of this study were to determine the inheritance of the yellow inner leaves of Chinese cabbage, and to map the QTLs controlling this trait based on a linkage map constructed with SRAP markers and anchor SSR markers. The identified markers linking to the QTLs may be used for MAS for practical breeding programs in the future.

2. Materials and methods

2.1. Plant materials

An inbred line, 07A237, which has a deep yellow inner leaf, was used as the female parent. Chiifu, an inbred line with a white inner leaf, was used as the male parent. The materials were provided by the College of Horticulture, Shenyang Agricultural University, China. An F_2 population with 140 individuals was produced from cross 07A237 × Chiifu by bud selfing an individual F_1 plant. The F_3 families, obtained by selfing each individual of the F_2 population, were used to score the phenotype value of yellow inner leaves. These scores were used as the phenotype data of each F_2 individual mentioned above. SPSS statistical software was used to analyze the heritability of yellow inner leaves in Chinese cabbage.

The parent plants, F_1, F_2, and F_3 families were planted in plastic tunnels in field in Shenyang, Liaoning Province, China. The plant samples of each F_2 individual were frozen for construction of a genetic linkage map. The 12th leaf from the outer side of the plant was selected to estimate the phenotypic scores using two methods. One method was to estimate the color of the inner leaf by visual observation, with the color scored in five levels of color including 1 (white), 2 (light yellow), 3 (medium yellow), 4 (yellow) and 5 (deep yellow) (Fig. 1). The second method was to use a portable colorimeter CR-400 (Konica Minolta, Japan), which is a very sensitive apparatus to discriminate the color range from blue to yellow (−A to +A), to estimate the phenotypic value of the inner leaf.

2.2. DNA marker analysis

Leaf samples were taken at the seedling period and preserved at −80 °C. Genomic DNA was isolated from the parents and all F_2 individuals following the procedure described by Murray and Thompson (1980) with minor modifications.

Primer sequences of SSR markers were obtained from the *B. rapa* Genome Sequencing Program (BrGSP). PCR amplification was carried out in a total volume of 10 μL containing 25 ng template DNA, 0.8 μL of 2.5 mM dNTP, 1.0 μL of 10× Buffer (containing Mg^{2+}), 1 μL of 0.5 μM primer, and 1 U Taq polymerase. The amplification was performed on a BIO-RAD iCycler thermocycler using the following program: PCR was initiated at 95 °C for 3 min; followed by 30 cycles at 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; and ended with extension at 72 °C for 5 min.

The sequences of the forward and reverse SRAP primers used in this study were obtained from previous studies (Li and Quiros, 2001; Li et al., 2003; Ferriol et al., 2003). The PCR reaction was carried out in a total volume of 10 μL containing 30 ng template DNA, 0.8 μL of 2.5 mM dNTP, 1.0 μL of 10× Buffer (containing Mg^{2+}), 1 μL of 0.5 μM primer, and 0.5 U Taq polymerase. The PCR amplification was performed on a BIO-RAD iCycler thermocycler using the following program: PCR was initiated at 95 °C for 3 min; followed by 8 cycles at 95 °C for 30 s, 35 °C for 45 s, and 72 °C for 1 min; followed by 35 cycles at 95 °C for 30 s, 50 °C for 45 s, and 72 °C for 1 min; then extension at 72 °C for 5 min. The PCR products were separated on a 6% denaturing polyacrylamide gel, and were stained with $AgNO_3$.

2.3. Linkage map construction

Linkage analysis was performed with JoinMap 3.0 (Van Ooijen and Voorrips, 2001) to assign molecular markers to each linkage group (LG), and to calculate their order of probability and distance in each LG. Individuals with too many missing genotypic data were excluded from contributing to the construction of the genetic map. The LGs were generated at a minimum logarithm of odds (LOD) threshold of 3.0. The Kosambi mapping function (Kosambi, 1944) was employed to calculate genetic distances between markers at a recombination fraction of <0.4. Markers showing segregation distortion from the expected Mendelian ratio (1:2:1 for co-dominant markers, 1:3 for dominant markers) were also included in the final map. The linkage map was generated using software MapChart 2.0 (Voorrips, 2002).

2.4. QTL analysis

Composite interval mapping (CIM) was applied to detect the presence of QTL controlling yellow inner leaves using the Windows QTL Cartographer 2.5 software (Wang et al., 2005). CIM was performed at 2.0 cm walk speed to identify potential positions of QTL. The threshold value of LOD for a presence of QTL was 2.5, as estimated by running 1000 permutation tests at a significance level of 0.05 (Churchill and Doerge, 1994; Doerge and Churchill, 1996).

3. Results

3.1. Phenotype analysis

In this study, we used visual testing and portable colorimeter testing to score the color in the inner leaves of Chinese cabbage. The mean scores of parents were 1.0 (Chiifu) and 4.9 (07A237) using visual observations. The mean score of F_1 was 1.74 and the data of the F_2 progeny showed continuous variation with values ranging from 1.2 to 5.0.

The mean scores of parents were 25.5 (Chiifu) and 58.1 (07A237) when color was estimated using the Portable colorimeter CR-400. The mean score of F_1 was 40.3 and data of F_2 progeny had continuous color variation with values ranging from 34.6 to 57.5.

There was a significant difference in the phenotypic data between the parental lines, making them suitable to conduct QTL studies. Using SPSS software to analyze the distribution of the yellow inner leaf trait in the F_2 population, we found that both the parameters of kurtosis (−0.534 by visual observation; −0.634 by colorimeter testing) and skewness (−0.331 by visual observation; −0.502 by colorimeter testing) were less than 2 and a histogram was created (Fig. 2).

To confirm the correlation between the data obtained with two methods, we performed a correlation analysis using SPSS software. According to the results, the correlation coefficient is 0.931 at the