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Genetic mapping and localization of quantitative trait loci for chlorophyll content in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*)

Yu Ge[a,b], Tao Wang[a], Na Wang[a], Zhe Wang[a], Cui Liang[a], Nirala Ramchiary[c], Su-Ryun Choi[d], Yong Pyo Lim[d], Zhong Yun Piao[a,*]

[a] Department of Horticulture, Shenyang Agricultural University, Shenyang 110-866, China
[b] Department of Horticulture, Northeast Agricultural University, Harbin 150-030, China
[c] Department of Biological Science, Institute of Science and Technology, Gauhati University, Gauhati 781-014, India
[d] Department of Horticulture, Chungnam National University, Daejeon 305-765, Republic of Korea

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ABSTRACT

Chlorophyll a and chlorophyll b present in the chloroplasts of higher plants are the main pigments of leaf photosynthesis. The aim of this study was to localize the genetic factors affecting chlorophyll a and chlorophyll b content in the Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) genome. To achieve this goal, we updated our previous genetic map, which was developed on the basis of an F_2 mapping population derived from a cross between 2 diverse Chinese cabbage lines, '501' and '601' with additional unigene-derived microsatellite markers. Eleven new polymorphic markers along with 227 previously mapped marker loci were used to construct a new updated *B. rapa* map containing 238 marker loci and covering a total length of 926.7 cM, with an average distance of 3.9 cM between markers. Quantitative trait loci (QTL) mapping using the chlorophyll a and chlorophyll b phenotypic data from an $F_{2:3}$ mapping population over 2 years identified 10 QTL in 8 genetic intervals in the Chinese cabbage accounting for 7–17% of the observed phenotypic variation.

1. Introduction

Chlorophyll is one of the major pigments in the chloroplast for photosynthesis, and chlorophyll content has been shown to have a positive relationship with photosynthetic rate (Thomas et al., 2005; Wang et al., 2003). Photosynthetic capacity is positively correlated with crop yield (Rawson and Constable, 1980), and increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield (Wang et al., 2008). Therefore, understanding the genetic mechanism that regulates the chlorophyll content is important for yield improvement in *Brassica rapa*. In addition, most of the previous studies have demonstrated that genes controlling chlorophyll content constituted a multi-allelic system (Guo et al., 2008; Shen et al., 2007; Zhang et al., 2009a).

Close correlations existed among the color values of chlorophyll content at different temperatures, the different values of nitrogen contents in leaves and other environmental factors (Cao et al., 2004; Li et al., 2010). Therefore, the identification of quantitative trait loci (QTL) or genetic factors associated with the chlorophyll a and chlorophyll b contents in the genome under diverse conditions such as environmental factors and cultural practices might reveal a more comprehensive mechanism of gene expression in response to environmental conditions. The chlorophyll content, as a complex physiological trait, might be a "shock" responses indicative of sensitivity under different environmental stresses.

The genus *Brassica* is composed of many species which are of immense economic importance to mankind, providing an array of vegetables, salad, oil, mustard condiments, and fodder crops; these include *B. rapa*, *B. oleracea*, *B. nigra*, *B. carinata*, *B. juncea*, and *B. napus*. *B. rapa* exhibits the most genetic diversity next to *B. oleracea*, with distinct morphological characteristics and forming subspecies that includes Chinese cabbage, pakchoi, flowering Chinese cabbage, turnip, and broccoletti, as well as oilseeds that include yellow and brown sarsons. Chinese cabbage (*B. rapa* ssp. *pekinensis*) is one of the main leafy vegetables that is widely grown in China, Korea, and Japan. QTL analysis of *B. rapa* based on molecular markers has been reported for many complex traits including bolting (Nishioka et al., 2005), flowering time and leaf morphology (Li et al., 2009), hairiness and the color of seed coat (Zhang et al., 2009b) and head morphology (Ge et al., 2011a; Kubo et al., 2010); however, to our knowledge, there is only one published genetic study and QTL analysis for chlorophyll content (Xu et al., 2007).

The objective of this research was to dissect QTL associated with chlorophyll a and chlorophyll b contents so that the identified QTL

* Corresponding author. Tel.: +86 024 8848 7143; fax: +86 024 8848 7144.
E-mail address: zypiao@syau.edu.cn (Z.Y. Piao).

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Table 1
Distribution characteristics of phenotypic traits in $F_{2:3}$ families and parental lines in *B. rapa*.

Trait[a]	Mean and standard deviation			$F_{2:3}$ (range)	$F_{2:3}$ (kurtosis)	$F_{2:3}$ (skewness)
	501	601	$F_{2:3}$			
Chl a (2009)	11.54 ± 0.57	6.27 ± 0.45	9.27 ± 1.41	5.76–13.52	−0.16	0.13
Chl b (2009)	4.24 ± 0.22	2.36 ± 0.14	3.29 ± 0.58	1.96–5.08	−0.13	0.30
Chl a (2010)	8.41 ± 0.50	7.81 ± 0.48	8.73 ± 1.23	4.14–11.57	1.96	−0.54
Chl b (2010)	4.69 ± 0.33	2.96 ± 0.18	4.12 ± 0.93	2.39–6.30	−1.13	−0.02

[a] Chl a (2009), chlorophyll a content measured in 2009; Chl b (2009), chlorophyll b content measured in 2009; Chl a (2010), chlorophyll a content measured in 2010; Chl b (2010), chlorophyll b content measured in 2010.

could be manipulated to breed improved Chinese cabbage cultivars with high chlorophyll content and high yield.

2. Materials and methods

2.1. Plant materials and DNA extraction

The individuals from the 117 F_2 population, a subpopulation of a previously used mapping population derived by crossing 2 diverse Chinese cabbage lines '501' (a microspore culture-derived double-haploid line) and '601' (an inbred line) was used (Ge et al., 2011b) for the development of an updated map. Young leaves from the 117 F_2 mapping population, F_1 and 2 parental lines were collected from 3 week old seedlings. The leaves were stored at −80 °C after freezing in liquid nitrogen until DNA extractions. DNA was isolated according to the procedure described by Guillemaut and Laurence (1992), with minor modifications.

2.2. Field trials

The seeds of the 117 $F_{2:3}$ families harvested from each F_2 plant of the mapping population along with the seeds of F_1 and parental lines were sown in multi-cell trays (5 cm × 5 cm × 5 cm) and the seedlings were cultivated in an unheated greenhouse from August 2009 to November 2009 (2009 trial) and from March 2010 to June 2010 (2010 trial), respectively, in Shenyang, China (41°80′N, 123°38′E). The average temperature of each month in Shenyang is listed in supplementary materials 1. The experiment was a randomized complete block design with 2 replications. Twelve plants per replication with a distance of 0.4 m between plants were grown in each row. Field management was followed according to the standardized agronomic procedures.

2.3. Analysis of chlorophyll a and chlorophyll b contents

Six plants from each F_3 family, F_1, and parental lines were randomly selected from the middle of the row (boundary plants were not considered for data scoring) for phenotypic measurements in 45 days after transplanting. Three leaf discs of approximately 0.2 g each in the middle of the fourth leaf were punched from each plant. Chlorophylls were extracted using ether and acetone (v:v = 1:1) solvent in dark for 24 h, and then the absorbance was measured at 645 and 663 nm using a UV-1601 spectrophotometer (Shanghai, China). Finally, the amounts of chlorophyll a and chlorophyll b were calculated (mg g^{-1} FW) using the method for chlorophyll analysis described by Arnon (1949). Each sample was measured repeatedly 3 times. The mean value for each sample was used in data analysis.

2.4. Marker genotyping

A total of 128 new primer pairs, including 96 newly developed unigene-derived microsatellite (UGMS) markers and 32 bacterial artificial chromosome derived simple sequence repeats (BAC-SSR) markers, were screened for polymorphisms between the parental lines '501' and '601'. The 96 new UGMS markers were developed according to the method described by Ge et al. (2011b), and detailed information on these newly developed UGMS markers is provided in supplemental materials 2. Primer sequence information for the BAC-SSRs was kindly provided by Prof. Yong Pyo Lim, Chungnam National University, Daejeon, Korea. PCR amplification was carried out in 10 μL reaction volumes, containing 0.5 U of Taq polymerase, 1 × Taq buffer, 250 μM of each dNTPs, 5 pmol of each primer, 2.0 mM of $MgCl_2$, and 15 ng of template DNA. Amplification was carried out using the following PCR conditions: 5 min at 94 °C, followed by 30–35 cycles of DNA denaturation at 94 °C for 30 s, annealing at the appropriate temperature based on the melting temperature of the primers for 30–45 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min in a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA, USA). Amplified fragments were separated on 6% denaturing polyacrylamide gels. After electrophoresis, the gels were stained as previously described (Sanguinetti et al., 1994).

2.5. Map construction and statistical analysis

The genetic map was constructed by JoinMap version 4 (Stam, 1993; Van Ooijen and Voorrips, 2001). LOD scores of 4.0–5.0 were used to assign the markers into linkage groups (LGs), and Kosambi (1944) mapping function was used to convert the recombination value into the map distance. Thresholds was set to ≤5.0 for goodness-of-fit, <0.4 for a recombination frequency and 2.0 for minimum logarithm of odds scores. The map was drawn using MapChart 2.1 (Voorrips, 2002).

Statistical analyses of the correlations between traits were performed using the Statistical Package for the Social Sciences, version 17.0 (SPSS, Inc., Chicago, IL, USA). QTL mapping was performed using the composite interval mapping function provided in Windows QTL Cartographer version 2.5 (Wang et al., 2007). Tests for the presence of QTL were performed at 2 cM intervals using a 10 cM window and 5 background cofactors (Model 6). To declare the presence of a QTL, genome-wide threshold values ($P = 0.05$) were estimated from 1000 permutations of trait data across all genetic intervals (Deorge and Churchill, 1996). A LOD value of 2.5 was used as the significant threshold for the presence of a candidate QTL. The gene action mode was determined according to the criterion (Stuber et al., 1987).

3. Results

3.1. Phenotypic trait analysis

The 2 Chinese cabbage parental lines, '601' and '501', differed in chlorophyll a and chlorophyll b content. '501' had higher chlorophyll a and chlorophyll b content values than did '601' in 2 growing seasons based on statistical analysis (Table 1). Analysis of the 2 traits in the $F_{2:3}$ mapping population showed a normal distribution curve, suggesting that the 2 traits might be governed by many genes (Fig. 1). The chlorophyll b content trait revealed