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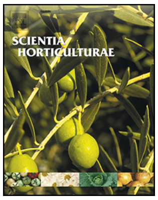

Genetic analysis and QTL mapping of traits related to head shape in cabbage (*Brassica oleracea* var. *capitata* L.)

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ABSTRACT

Traits related to head shape, including Hvd (head vertical diameter), Htd (head transverse diameter), and Hsi (head shape index, the ratio of Hvd/Htd), are very important agronomic traits associated with both yield and quality in cabbage (*Brassica oleracea* var. *capitata* L.). However, reports of inheritance analysis and quantitative trait locus (QTL) mapping of these traits remain rare. In this study, a double haploid (DH) population with 130 lines constructed from a cross between 24-5 (inbred line, oblate head) × 01-88 (inbred line, round head) was used to analyze inheritance and to detect QTLs related to Htd and Hsi using major gene plus polygene mixed inheritance analysis and inclusive composite interval mapping (ICIM). The results indicated that Htd was controlled by two independent major genes and polygenes with recessive-epistatic effects. Hsi was controlled by two linkage major genes and polygenes with cumulative effects. A genetic linkage map with 48 insertions or deletions (InDel) and 149 simple sequence repeat (SSR) markers was constructed based on the DH population, with a total length of 866.2 cM and an average interval length of 4.40 cM. Fourteen QTLs for Htd and Hsi were identified on six chromosomes based on two years of phenotypic data with ICIM. Ten of the QTLs explained greater than 10.0% of the phenotypic variance, and five QTLs could be repeatedly detected in two years. For Htd, two major QTLs, *Htd* 3.1 and *Htd* 8.1, explained 19.16–24.56% and 11.25–21.55% of the phenotypic variation in the two years, respectively. For Hsi, two major QTLs, *Hsi 7.1* and *Hsi 7.2*, explained 22.30–24.93% and 14.85–16.79% of phenotypic variation in the two years, respectively. The results from QTL mapping and genetic analysis in both years were partially consistent and complemented each other. Our results provide a foundation for further research on genetic regulation, gene cloning and molecular marker-assisted selection (MAS) for head shape in cabbage.

1. Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is an economically important vegetable crop that is cultivated and consumed worldwide. Leafy heads of cabbage are harvested as commodities and edible organs. The head shape is an important appraisal index for market grading, classification and quality evaluation as well as an important component of the quality of cabbage. Cabbage has many types of head shape, including oblate, highly oblate, round, highly round, blunt-pointed, and pointed. Requirements for cabbage head shape vary in different areas with different cultivation patterns and consumption habits. Although it is simple and intuitive, shape description is easily affected by subjective judgment. Khambanonda (1950) first put forward the ‘fruit shape index’ (ratio of length and width) used to describe the fruit shape of red pepper. Compared with the traditional phenotypic description, the fruit shape index was a breakthrough because it was based on quantitative analysis (Qiao et al., 2011). At present, studies of fruit shape have focused on solanaceous vegetables, including tomato (Van der Knaap and Tanksley, 2003; Cong et al., 2008; Grandillo et al., 2015), eggplant (Nunome et al., 2001; Doganlar et al., 2002; Qiao et al., 2012), pepper (Chaim et al., 2001; Zygier et al., 2005; Barchi et al., 2009) and cucurbitaceous vegetables, including cucumber (Wenzel et al., 1995; Fazio et al., 2003; Yuan et al., 2008). Many quantitative trait loci (QTLs) underlying fruit shape-related traits have been mapped in these vegetables. For *Brassica rapa*, some QTLs for head height-to-diameter ratio (HHD) and head diameter

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(HD) were detected repeatedly (Yu et al., 2013). Mao et al. (2014) found that the expression of PCF transcription factor 4 (*BrpTCP4*) was associated with head shape in *Brassica rapa*.

For genetic analysis of head shape in cabbage, previous studies indicate that the inheritance of a pointed head was dominant to round head, and many genetic factors were involved in determining the shapes of heads (Yarnell, 1956). Lei et al. (1994) found that the inheritance of head vertical diameter and head transverse diameter of cabbage were super-dominant and controlled by multiple genes. Fang et al. (2011) discovered that head transverse diameter and head shape index had high heritabilities of 95.43% and 89.84%, respectively, and head vertical diameter had low heritability of only 22.52%. Lv et al. (2014) mapped eight QTLs for head vertical diameter on chromosomes 1–3, 5, and 6, and these QTLs explained 38.5%, 39.3%, and 28.3% of the phenotypic variation in three seasons. However, no QTL studies for head transverse diameter and head shape index in cabbage have been reported to date.

In this study, a DH population that consists of 130 lines from a cross between 24-5 and 01-88 was developed and further be used to perform genetic analysis according to mixed gene and poly-gene genetic models (Gai et al., 2003). We also designed simple sequence repeat (SSR) and insertion or deletion (InDel) markers to construct a linkage map using the DH population. Based on the map and two years of phenotypic data, we sought to identify significant QTLs associated with head shape traits, which would be helpful for better understanding the genetic basis of these traits and MAS for cabbage breeders.

2. Materials and methods

2.1. Plant materials and field experiments

The female parent 24-5 (P_1) was from a high-generation inbred line with an oblate head, originating from the landrace ‘huang miao’ of Northern China. The male parent 01-88 (P_2) was a cabbage from a high-generation inbred line with a round head, originating from a Canadian spring-early-maturing cultivar (Fig. 1). Then, 24-5 was crossed with 01-88 to generate F_1 plants. The DH population consisted of 130 DH lines derived from the F_1 plants through isolated micro-spores cultures (Yuan et al., 2011, 2012).

All generations were cultivated in the experimental field of Changping District (Beijing, China) in the autumns of 2013 and 2014. The parental, F_1 and RF_1 (01–88 × 24-5) lines were planted in a randomized complete block design with three replicates, and each replicate contained 48 plants. For the DH population, a block in replication design was adopted with three replicates (Su et al., 2015) for two years. Each replicate consisted of 16 plants.

2.2. Phenotypic evaluation and statistical analysis

In each replicate, five plants with consistent growth potential were selected for phenotypic evaluation at maturity. The head-shape traits were evaluated according to the following standards (Li and Fang, 2007): head vertical diameter (Hvd), which is the height from the base to the top of a mature head; head transverse diameter (Htd), which is the widest length of the mature head; and head shape index (Hsi, the ratio of Hvd to Htd). The data were analyzed with SAS8.1 and SPSS12.0 (SPSS Inc., Chicago, IL, USA).

2.3. Mixed major gene plus polygene inheritance analysis

Genetic analysis of the DH population and parental lines was performed according to mixed major gene plus polygene inheritance analysis (Gai et al., 2003). The distribution parameters were estimated using the maximum likelihood method based on the iterated expectation conditional maximization (IECM) algorithm. The optimal model was determined according to the Akaike information criterion (AIC) and a series of fitness tests. Then, the first-order and second-order genetic parameters were estimated by using a least-squares method in the optimal model.

2.4. DNA extraction and molecular marker analysis

A modified CTAB method (Murray and Thompson, 1980) was used to extract genomic DNA from all DH lines and parental lines. The DNA concentrations were determined using a spectrophotometer (BioDrop, UK) and diluted to a working concentration of 40–50 ng/μL.

Briefly, 2170 pairs of SSR primers designed from the sequence scaffolds in the genomic database for *Brassica oleracea* (http://brassicadb.org) (Wang et al., 2012; Liu et al., 2014) and 149 pairs of InDel primers based on re-sequencing data from cabbage lines (Su et al., 2015) were used to scan for polymorphisms between the two parents.

Each 15-μL PCR reaction mixture contained 2 μL of DNA template (40–50 ng/μL), 1.5 μL of 10 × PCR buffer (Mg^{2+} included), 1.2 μL of dNTP (2.5 μM), 0.5 μL of the forward primer (10 μM), 0.5 μL of the reverse primer (10 μM), 0.15 μL of Taq DNA polymerase (5 U/μL) and 9.15 μL ddH_2O. Reactions were performed in a thermal cycler as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s; and finally, 72 °C for 7 min. The PCR

Fig. 1. The difference between parents in head shape traits. (A) The female parent 24-5 with oblate heads; (B) The male parent 01-88 with round heads. Bar = 5 cm.