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# Linkage map construction using SSR markers and QTL analyses of stem expansion traits in *Brassica juncea*

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ABSTRACT

Tuber mustard (*Brassica juncea* (L.) Czern. et Coss. var. *tumida* Tsen et Lee) is an economically important vegetable crop, valued for its expanded stem. Quantitative trait loci (QTL) analyses of the traits related to stem expansion provide a basis for fine mapping, gene cloning, and marker assisted selective breeding. However, the genetic bases of stem expansion traits remain unclear. In this study, high levels of phenotypic variation were observed for stem expansion traits in an $F_2$ segregation population derived from the cross between tuber mustard and leaf mustard, which exhibit significantly differing stem traits. A linkage map was constructed containing 116 simple sequence repeat (SSR) markers using 200 randomly selected $F_2$ individuals. The genetic map consisted of 17 linkage groups (LGs) covering a total of length of 2061.0 cM with an average interval of 17.92 cM. A total of 5 QTLs were identified for the stem expansion traits, stem weight (SW) and stem diameter (SD). Among these, 4 QTLs for SW were detected, SW1 and SW2 were located on LG3 with the remaining located on LG13. And each QTL explained 26.46%–28.18% of the phenotypic variance, and their additive effects were all negative. The QTL SD1 controlling SD explained 6.54% of the phenotypic variance and was located on LG1. Additionally, it possessed a negative additive effect and the shortest distance with flanking markers (BjSSR1811b) displaying 0.99 cM. These data demonstrate the genetic basis of stem expansion and will facilitate the marker-assisted selective breeding of tuber mustard.



## 1. Introduction

Tuber mustard (*Brassica juncea* (L.) Czern. et Coss. var. *tumida* Tsen et Lee, 2n = 4 × = 36, genome AABB) is one of the most commercially important vegetables of the Cruciferae family. Stem of tuber mustard can expand to more than 20 cm in diameter (Shi et al., 2012), and expanded stems are used as raw material for pickled products, which are very popular for their special flavour and nutritional value (Liu, 1996). According to previous studies, quantitative stem expansion traits generally pertain to stem weight (SW), stem diameter (SD) and stem length (SL), and these traits are reportedly controlled by both genetic factors and environment conditions (Liu, 1996; Shi et al., 2012). Moreover, Zhang et al. (2014) fitted the stem expansion traits SW and SD to an E-1 model that refers to two additive-dominance-epitasis major genes plus additive-dominance polygene model and fitted SL to an additive-dominance polygene model, indicating that more than four major genes might control stem expansion. The studies on quantitative trait loci (QTL) analyses of stem expansion traits will facilitate the identification of associated genes in tuber mustard.

Genetic linkage mapping has proven to be a useful tool for positioning QTLs and cloning genes that control target agronomic traits. Accordingly, extensive efforts have been made to construct and use genetic linkage maps to identify QTLs associated with important agronomic traits in *B. juncea*. A linkage map was constructed with a doubled haploid (DH) population derived from J90-2733 (high seed oil containing)/J90-4317, using 343 restriction fragment length polymorphism (RFLP) markers (Cheung et al., 1997). With the application of a linkage map and QTL analyses, Lionneton et al. (2004) discovered that oil content and fatty acid composition was tightly connected to the color of the seed coat. Pradhan et al. (2003) constructed a linkage map with 1029 amplified fragment length polymorphism (AFLP) and RFLP markers, currently consisting of the largest number and highest density of markers among linkage

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maps in *B. juncea*. Most previous studies of *B. juncea* have focused on the oil content or oil component (Sharma et al., 2002; Mahmood et al., 2003; Lionneton et al., 2002, 2004), and seed coat color-related traits (Negi et al., 2000; Lionneton et al., 2004; Padmaja et al., 2005; Xu et al., 2012; Huang et al., 2012). However, little attention has been paid to construct a linkage map for exploration of stem expansion traits, and the associated QTL patterns remain unclear.

RFLP and AFLP molecular markers are the most frequently used in previously constructed linkage maps of *B. juncea* (Cheung et al., 1997; Axelsson et al., 2000; Lionneton et al., 2002; Mahmood et al., 2003; Pradhan et al., 2003), and RAPD and SSR markers were also used to construct a linkage map (Sharma et al., 2002; Kaur and Banga, 2015). However, the applications of RFLP and AFLP technologies in genetic research and marker-assisted breeding are limited due to the labor intensiveness and high cost. Hence, simple sequence repeat (SSR) markers have been used as an alternative to construct high density linkage maps for various important crops. However, SSR markers of *B. juncea* were insufficient due to limited EST sequences information except sixteen SSR markers of *B. juncea* reported by Hopkins et al. (2007), and prior to the present study, SSR markers have rarely been used in the linkage map construction of *B. juncea* (Kaur and Banga, 2015).

In this study, we crossed tuber mustard with leaf mustard to construct $F_2$ mapping population. Two parents showed significantly different stem traits, of which stem of leaf mustard is less than 5 cm in diameter. Subsequently, 112 pairs of polymorphic SSR primers, developed from the transcriptome of tuber mustard, were used to analyze the segregation patterns in randomly selected $F_2$ individuals for the construction of a genetic map. Based on this new genetic map, 5 QTLs associated with SW and SD were detected using the composite interval mapping (CIM) method. The present data may facilitate genetic improvement of stem expansion and can be used as a basis for future studies of stem expansion-related genes.

## 2. Materials and methods

### 2.1. Plant materials

Two varieties of *Bassica juncea* with significantly different stem traits were used to construct an $F_2$ segregation population. The female parent was tuber mustard (*B. juncea* (L.) Czern. et Coss. var. *tumida* Tsen et Lee) designated by $P_1$ with expanded stem approximately 10–20 cm in diameter. The male parent was leaf mustard (*B. juncea* var. *multiceps* Tsen et Lee) designated by $P_2$ with a stem diameter < 5 cm. The two parents were inbred lines obtained in our laboratory. Subsequently, 200 individuals of the $F_2$ generation were randomly selected to develop the mapping population. The two parents and $F_1$ generations were planted in a randomised complete block design with three replications, with 20 plants in each replication. A total of 200 $F_2$ plants were planted beside the blocks. All plants were grown in a greenhouse at Huajiachi campus of Zhejiang University (Zhejiang Province, China). The spacing between plants was 50 cm between rows and 90 cm between columns. Organic fertilizer with phosphorus and potassium were used as basal fertilizer. Subsequently, Nitrogen as side dressing was added once before stem expansion. Irrigation was carried out when water was needed.

### 2.2. Measurement of stem expansion traits

All plants were harvested by hand during the middle of March when the tuber mustard plants reached stem harvest maturity (about 175 days from sowing to harvest). Stem weights, stem diameters and stem lengths (SW, SD and SL) of the populations of the two parents, the $F_1$ generation and the 200 $F_2$ individuals were measured. SW was measured using the whole harvested stem. The maximum SD refers to the widest part of the expanded stem and maximum SL refers to the distance from stem base to point at which internode elongation starts. The SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software was used to analyze the variance of stem traits among generations of parents and the $F_1$ generation and to calculate the frequency distributions of the $F_2$ population for the three stem traits.

### 2.3. DNA extraction and EST-SSR marker analysis

Genomic DNA from the $F_2$ population and parents were extracted from frozen young leaves according to the modified CTAB method (Liu et al., 2003). DNA quality and concentration were determined using 1.2% agarose gel electrophoresis and the NanoDrop Spectrophotometer 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). DNA was then diluted to a final concentration of 100 ng/μL and stored at −20 °C.

A total of 520 pairs of SSR primers developed from the transcriptome of tuber mustard were selected to amplify within the two parents and the $F_1$ generation using two rounds of screening for the identification of polymorphic markers. The tuber mustard transcriptome data from our laboratary were unpublished and all sequencing reads were deposited in the Short Read Archive (SRA) database with the accession number SRR2089765. SSR amplification was performed within a 20 μl mixture containing 1.0 μl DNA template (100 ng/μl), 1.0 μl forward primer (10 μM), 1.0 μl reverse primer (10 μM), 0.2 μl *Taq* DNA polymerase (5 U/μl), 2.5 μl PCR Buffer (10 ×, $Mg^{2+}$ included), 2 μl dNTP (2.5 mM each) and 12.3 μl $ddH_2O$. The thermal cycling consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 7 min. The PCR products were isolated on 12% non-denaturing polyacrylamide gel and visualised by silver staining (Brant et al., 1991; Byun et al., 2009). The fragment sizes of the PCR products were estimated using 50 bp DNA ladder.

### 2.4. Construction of the linkage map

Primers were further analyzed in the $F_2$ population. The segregation distortion of each marker locus was evaluated using the $\chi^2$ test against the expected 3:1 (dominant) or 1:2:1 (codominant) Mendelian segregation ratio at $P < 0.05$. The genetic linkage map was constructed using JoinMap 4.0 with the minimum logarithm of odds (LOD) threshold of 3.0, and the Kosambi mapping function was applied to calculate the genetic distances between markers.

### 2.5. QTL analysis

QTL analysis for SW, SD and SL were performed separately. QTL analyses were performed using Windows QTL Cartographer 2.5 with a composite interval mapping (CIM) procedure. Initially, the thresholds were established with 1000 permutations to determine QTL significance. Then, based on the permutation results, CIM analyses were performed using model 6, and the number of control markers, walk space and window size were set to 5, 1 and 10 cM, respectively. Loci with the highest LOD scores were assigned as QTLs, and QTLs were named with the abbreviation of the stem trait and numeral.