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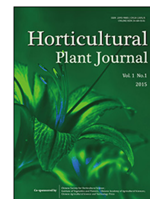

A High Density Linkage Map Facilitates QTL Mapping of Flowering Time in *Brassica rapa*

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Abstract

Flowering is a major developmental transition in the life-history of plants. Flowering time is under complex genetic control. In this study, 172 doubled haploid (DH) lines derived from a cross between two Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) DH lines, Y177 and Y195, were used for a high density linkage map construction and quantitative trait locus (QTL) mapping for flowering time in *B. rapa*. Parents and DH lines were resequenced at depth of 5× and 0.5× coverage, respectively. In total, 22 747 SNPs were called from the resequencing data and combined into 1 708 bins to construct the genetic linkage map. The map length was 958.6 cM, containing 887 bins and the average interval between bin-markers was 1.08 cM. A total of six QTLs controlling flowering time were detected under four different conditions. Potential candidate flowering homologues located near QTLs were identified with the aid of *B. rapa* genome annotation.

Keywords: *Brassica rapa*; linkage map; flowering time; QTL

1. Introduction

Brassica rapa is a diverse and important agricultural species with several morphotypes that are used as oilseed (ssp. *oleifera*, turnip rape; ssp. *dichotoma*, Brown Sarson; ssp. *tricolaris*, Yellow Sarson), leafy vegetables (ssp. *pekinensis*, Chinese cabbage; ssp. *chinensis*, pak choi; ssp. *nipposinica*, mizuna), root vegetables and fodder crop (ssp. *rapa*, turnip) (Guo et al., 2014). Premature flowering is the main problem that affects production of *B. rapa*, especially in leafy vegetables, since the occurrence of premature flowering not only reduces yield, but also affects their quality. Therefore, breeding of the late-flowering varieties has always been one of the important aims in leafy *B. rapa*.

Flowering time is a typical quantitative trait. In the model plant species *Arabidopsis thaliana*, over 180 genes are implicated in flowering time control based on characterization of loss-of-function mutants or analysis of transgenic plants. These genes are categorized into six major pathways that control flowering time, including the photoperiod/circadian clock pathway, vernalization pathway, ambient temperature pathway, age pathway, autonomous pathway, and gibberellin pathway (Fernando and George, 2012). Flowering time is largely affected by temperature and/or day length *B. rapa* species (Young et al., 2013). Genetic analysis of flowering time variation has showed that the genes were involved in vernalization pathway and photoperiod/circadian clock pathway. They usually functioned as floral ingetragor genes. FLC is a flowering repressor that plays an important role in vernalization pathway (Koornneef et al., 1991). In the previous researches, *BrFLC2* has been located on A02 and revealed to play a strong role in flowering time regulation (Zhao and Bonnema, 2010; Wu et al., 2012). *BrFLC1* was identified as a candidate gene affecting transgenerational regulation of flowering time and seed germination (Dechaine et al., 2014). *BrGI* is a newly identified gene that regulates flowering time using a heterogeneous inbred lines population (Xie et al., 2015). Using a recombinant inbred line (RIL) population, *BrFT2* on chromosome A07 was detected as a candidate gene of a major QTL for

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flowering time (Zhang et al., 2015). In previous studies, QTL mapping of flowering time were commonly performed on segregation population derived from crossing between different subspecies or varieties, such as Yellow Sarson (ssp. *tricolaris*), Caixin (ssp. *parachinensis*), Pak choi (ssp. *chinensis*) or rapid-cycling (ssp. *pekinensis*) (Lou et al., 2011; El-Soda et al., 2014; Basnet et al., 2015). However, the identified loci always harbored genes for early flowering, due to their early flowering and short life cycle characteristics of these parental lines. Although the knowledge is contributing to dissecting of flowering time regulation pathway in *B. rapa*, the identified loci could not be used in late-flowering breeding directly.

Lots of genetic maps have been constructed for *B. rapa* using different molecular markers, including RAPD (Random Amplified Polymorphic DNA) (Tanhuanpää et al., 1996), AFLP (Amplified Fragment Length Polymorphism) (Wu et al., 2008), RFLP (Restriction Fragment Length Polymorphism) (Teutonico and Osborn, 1995), and SSR (Simple Sequence Repeats) markers (Suwabe et al., 2006). There are mainly two disadvantages of using traditional markers to construct linkage map. First, the number of these markers is always limited and their distribution on genomes is also asymmetry, which made the subsequent lineage map low-density. Second, massive time and energy are needed for genotyping, which affects the efficiency of mapping. In the latest ten years, with the development of high-throughput sequencing and genome assembly technologies, genome resequencing has provided great opportunity to develop genome-scope and sequence-based markers with high-confidence, such as SNP markers (Zou et al., 2012) and InDel markers (Liu et al., 2013a, 2013b). Compared to the traditional markers, these markers are more reliable and always with more steady DNA polymorphisms, which is helpful to construct high-density linkage map. In 2009, a high-density lineage map for 150 recombinant inbred lines with 2 334 SNP markers was constructed firstly by genome resequencing in rice (Huang et al., 2009). A sliding window approach was designed to collectively examine genome-wide single nucleotide polymorphisms for genotype calling and recombination breakpoint determination. Using this method, the genotype calling accuracy of the map was as high as 99.94% and the average of recombination breakpoints was 40 kb. Genetic linkage maps based on SNP markers were also constructed in Brassicaceae species, such as *B. oleracea* (Wang et al., 2012), *B. napus* (Liu et al., 2013a, 2013b) and *Raphanus sativus* (Mun et al., 2015). Overall, developing SNP markers by sequencing is an efficient approach to construct high-density linkage map.

In this study, we identified QTLs controlling flowering time using a doubled haploid (DH) population derived from a cross between two Chinese cabbage DH lines ‘Y195’ and ‘Y177’ (Wu et al., 2008). They show differences in flowering time, but both are relatively late flowering compared to early flowering types, such as rapid-cycling, Yellow Sarsons or Caixins. We expect that the loci identified from this study will offer a direct route for molecular breeding of Chinese cabbage varieties with resistance to premature bolting.

2. Materials and methods

2.1. Plant material

The *B. rapa* segregating population consisting of 172 DH lines, produced through microspore culture from F_1 hybrids of two Chinese cabbage DH lines ‘Y195’ and ‘Y177’ (Double Hapoild) (Wu et al., 2008), was used for QTL mapping of flowering time variation. ‘Y177’ originated from a spring type Japanese cultivar ‘Jianchun’ and ‘Y195’ is derived from a summer type Japanese cultivar ‘Xiayang’.

2.2. Field trial and trait investigation

Flowering time was investigated in two growing seasons, spring and autumn in the number four glasshouse Chinese Academy of Agricultural Sciences in 2015. In the spring trail, we investigated flowering time for the vernalized plants under different day-lengths, long day (LD) or short day (SD), while in the autumn trail flowering time was scored for plants with or without vernalization under LD condition.

In the spring sowing trail, ten germinated seeds from each DH line and parental lines were sown into hole plates in a greenhouse without climate control in Beijing in 1 February 2015. The temperature during 1 February to 2 March 2015 varied between −3 °C to 7 °C; therefore the plants were grown under a condition for being naturally vernalized (Table 1). In 3 March 2015, all plants were transplanted to pots and moved to a warm condition (2 °C–14 °C) with LD (14 h/10 h, day/night) or SD (10 h/14 h, day/night). Three individuals from each DH line and parental lines were used for each treatment. Light pollution was prevented by drawing black curtains. Due to the limited seeds, there were 120 and 115 DH lines investigated for LD and SD, respectively.

In the autumn sowing trail, three germinated seeds from each line were treated at 4 °C for 25 d to be vernalized (VL) before being sown in 24 August, 2015 in the same greenhouse. Three

Table 1 Average monthly temperatures during the two growing seasons in 2015 and 2016

	Spring sowing				Autumn sowing					
	Feb 2015	Mar 2015	Apr 2015	May 2015	Aug 2015	Sep 2015	Oct 2015	Nov 2015	Dec 2015	Jan 2016
HT/°C	7	14	22	28	32	26	20	7	16	18
LT/°C	−3	2	9	16	22	16	9	1	5	10

Note: HT: average high temperature during a day; LT: average low temperature during a day.

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