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ORIGINAL PAPER

Constructing a dense genetic linkage map and mapping QTL for the traits of flower development in *Brassica carinata*

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Abstract

Key message **An integrated dense genetic linkage map was constructed in a *B. carinata* population and used for comparative genome analysis and QTL identification for flowering time.**

Abstract An integrated dense linkage map of *Brassica carinata* (BBCC) was constructed in a doubled haploid population based on DArT-Seq™ markers. A total of 4,031 markers corresponding to 1,366 unique loci were mapped including 639 bins, covering a genetic distance of 2,048 cM. We identified 136 blocks and islands conserved in Brassicaceae, which showed a feature of hexaploidisation representing the suggested ancestral crucifer karyotype. The B and C genome of *B. carinata* shared 85 % of commonly conserved blocks with the B genome of *B. nigra*/*B. juncea* and 80 % of commonly conserved blocks with the C genome of *B. napus*, and shown frequent structural rearrangements such as insertions and inversions. Up to 24 quantitative trait loci (QTL) for flowering and budding time were identified in the DH population. Of these QTL, one consistent QTL (*qFT.B4-2*) for flowering time was identified in all of the environments in the J block of the B4 linkage group, where a group of genes for flowering time were aligned in *A. thaliana*. Another major QTL for flowering time under a winter-cropped environment was detected in the E block of C6, where the *BnFT-C6* gene was previously localised in *B. napus*. This high-density map would be useful not only to reveal the genetic variation in the species with QTL analysis and genome sequencing, but also for other applications such as marker-assisted selection and genomic selection, for the African mustard improvement.

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Introduction

In the genus *Brassica*, there are three amphidiploid species, *B. napus* L. ($2n = 4\times = 38$, genome AACC), *B. juncea* (L.) Czern ($2n = 4\times = 36$, genome AABB) and *B. carinata* Braun ($2n = 4\times = 34$, genome BBCC) which are cultivated worldwide primarily for their edible oils. These species originated because of multiple spontaneous interspecific hybridisations between three pairs of diploid progenitors: *B. rapa* ($2n = 2\times = 20$, genome AA), *B. oleracea* ($2n = 2\times = 18$, genome CC) and *B. nigra* ($2n = 2\times = 16$, genome BB), composing the famous U's triangle (UN 1935). Genomic differentiation would have occurred

between and among the same basic A, B, and C genomes in different species, which would have resulted not only from the rapid genomic changes from interspecific hybridisation during speciation but also from long-term domestication and selection (Lysak and Lexer 2006; Marhold and Lihova 2006; Pires and Gaeta 2011; Schmidt et al. 2001; Zou et al. 2011). Therefore, the members of *Brassica* included in U's triangle would provide an ideal system to analyse the genome changes that have occurred during the process of evolution, domestication, interspecific hybridisation, and cultivation (Schranz et al. 2007; Snowdon 2007; Suwabe et al. 2008). To investigate such events, comparative genetic and genomic analyses using dense genetic maps are essential (Edwards et al. 2013; Parkin 2011).

Extensive efforts have been made to construct genetic linkage maps in most of the cultivated diploid and amphidiploid *Brassica* species, particularly in *B. napus* (Delourme et al. 2013; Lagercrantz 1998; Long et al. 2007; Panjabi et al. 2008; Parkin et al. 2005; Raman et al. 2013b). As a result, genome-wide comparisons have been accomplished between *B. napus* and its diploid progenitors, i.e., *B. rapa* and *B. oleracea*, and the closely related model species, *Arabidopsis thaliana* (Choi et al. 2007; Gao et al. 2007; Iniguez-Luy and Federico 2011; Lagercrantz and Lydiate 1996; Mun et al. 2008; Parkin 2011; Parkin et al. 2005; Quiros and Farnham 2011; Ramchiary and Lim 2011; Schranz et al. 2002). A single locus in the *Arabidopsis* genome is represented in general by three distinct loci in diploid *Brassica* species, and a high degree of co-linearity and congruence in the *A. thaliana* and *Brassica* genomes has been recognised (Parkin et al. 2005; Schranz et al. 2006; Yang et al. 2006; Ziolkowski et al. 2006). Molecular genetic maps have also been utilised for the identification of hundreds of qualitative and quantitative trait loci (QTL) that control various important traits (Basunanda et al. 2010; Guo et al. 2012; Li et al. 2013; Long et al. 2007; Radoev et al. 2008; Raman et al. 2013b; Smooker et al. 2011; Zhang et al. 2012).

B. carinata, which was derived from a hybridisation between *B. oleracea* and *B. nigra*, is one of the four major oilseed crop species,including *B. rapa*, *B. juncea*, and *B. napus* in U's triangle. *B. carinata* has been cultivated in Ethiopia and neighbouring countries for many centuries and used as a source of edible oil, vegetables, medicines, and condiments (Alemayehu and Becker 2002). *B. carinata* harbours genes controlling valuable agronomic traits, such as resistance to blackleg and pod shattering and tolerance to aluminium, salinity, heat, and drought (Enjalbert et al. 2013; Malik 1900; Pan et al. 2012; Warwick 2011). Significant efforts have been made to transfer the favourable alleles for such traits from *B. carinata* to *B. napus* and to other species, including *B. juncea* (Bansal et al. 2004; Navabi et al. 2010; Saal et al. 2004; Song et al. 1993; Xiao et al. 2010; Zou et al. 2010). However, little information regarding the genetics and genomics of *B. carinata* is available for its genetic improvement and related Brassica crops.

A first genetic linkage map of *B. carinata* was constructed using a DH population derived from Yellow-BcDH64/White-BcDH76 (YW), using 151 simple sequence repeat (SSR), 44 amplified fragment length polymorphism (AFLP), 12 sequence-related amplified polymorphism (SRAP), and five intron-based polymorphism (IBP) markers (Guo et al. 2012). This linkage map was subsequently utilised for the identification of (1) four QTL controlling seed colour and (2) a few ancestral blocks of Brassicaceae. However, it is valuable for the Brassica research community having a high-density map of *B. carinata* constructed with high-throughput markers with known sequence information. These markers would provide a high resolution of loci associated with traits of interest especially linked with quantitative traits having low heritability. High-density molecular maps have been accomplished in several crops including *B. napus* (Bus et al. 2012; Delourme et al. 2013) and utilised to perform a range of genomic analyses, such as genomic comparisons with other *Brassica* species carrying B/C genomes. Traditional Diversity Array Technology (DArT) markers have been developed in *B. napus* and subsequently used for the identification of loci associated with the components of flowering time and resistance to blackleg disease and genetic diversity analysis (Raman et al. 2012, 2013a, b). In this study, a new approach based on traditional DArT and next-generation sequencing technique, called as DArT-Seq™ (Raman et al. 2014), was used to analyse genome of *B. carinata*.

We report (1) the construction of an integrated dense genetic linkage map based upon DArT-Seq™ based markers produced by genotyping-by-sequencing technology as well as traditional PCR-based markers (2) the comparative genomic analyses of *B. carinata*, *A. thaliana*, and of other *Brassica* species carrying B or C genomes, and (3) the identification of QTL associated with budding time and flowering time, which are two important agronomic traits concerning the reproductive development phase of the plant, under different environments. The results will provide valuable resources for the study of the *Brassica* genomics and for the genetic improvement of *Brassica* crops.

Materials and methods

Plant materials and phenotype evaluation

A genetically mapped doubled haploid (DH) population called the YW DH population, which was derived from a F_1 between two DH lines of *B. carinata*, Y-BcDH64, and

W-BcDH76 (Guo et al. 2012), was used in this study. The population consisting of 185 individual lines was re-genotyped for genetic mapping using DArT-Seq markers and for distinguishing the ancestral blocks of Brassicaceae in the B/C genome.

The YW DH population along with two parents was grown in both spring-cropped and winter-cropped environments in China and phenotyped for the identification of QTL controlling the budding time and flowering time. The field trials in winter-cropped environments were conducted in the city of Wuhan for 2 years, as described previously (Guo et al. 2012). The field trials in spring-cropped environments were performed in two locations of northwest China: one in Xining, Qinghai province and one in Hezheng, Gansu province (the meteorological data are provided in Supplementary Table 1; Supplementary Fig. 1).

A randomised complete block design was used with three replicates for all of the environments except for the experiment conducted in the second year in Wuhan with two replicates. Each plot measured 3.0 m^2 and contained three rows with a distance of 40 cm between rows, 30 plants per plot and with 25 cm gap between individual plants.

The phenotypic variation in the flowering time was measured for all environments, whereas the budding time was only measured for the spring environments. The budding time and flowering time were measured as the number of days between the date of sowing to the date when the first bud and flower appeared, respectively, on 50 % of the plants in each plot.

Genotyping and map construction

Genomic DNA was isolated from fresh and young leaves of plants using a DNeasy Plant Mini Kit according to the manufacturer's protocol (QIAGEN, China). The DNA concentration of each sample was adjusted to 50 ng/μl.

Whole-genome profiling was performed using the DArT-Seq™ technology at Diversity Arrays Technology Pty Ltd, Australia. Essentially, DArT-Seq™ technology relies on a complexity reduction method to enrich genomic representations with single copy sequences and subsequently perform next-generation sequencing using HiSeq2000 (Illumina, USA). DArT-Seq™ detects both SNPs and presence–absence sequence variants, collectively referred to as DArT-Seq™ markers (Raman et al. 2014). We used the presence-absence sequence variants in this study. The DArT-Seq™ technology (abbreviated as DArT-Seq) was optimised for *Brassica* by selecting the most appropriate complexity reduction method (*Pst*I-*Mse*I restriction enzymes). DNA fragments digested with restriction enzymes were ligated with *Pst*I adaptors and unique barcodes, and then amplified following PCR. All amplicons were pooled and sequenced in a single lane on HiSeq2000. DArT P/L analysed all sequences and provided scores of markers originated from the sequence polymorphism data as present/absent type (present = 1 vs. absent = 0). Sequences of DArT-Seq markers were aligned with *B. rapa* reference genome sequence (Phytogene). Reference C genome of *B. oleracea* was accessed by Dr. Raman (Courtesy Dr. Isobel Parkin, AAFC, Ag Canada). The parameters used for the DArT marker assaying pipeline for quality control (Kilian et al. 2012), such as the overall call rate (percentage of valid scores in all possible scores for a marker) over 95 %, and the *Q* value (the logarithm of the minimum false discovery rate at which the test may be called significant) above 2.0, was used for selecting high-quality DArT-Seq markers for genetic mapping. The average reproducibility and call rate of the markers were 99.41 and 95.29 %, respectively. The maximum and average *Q* values could reach 12.13 and 3.28, respectively. The DArT-Seq markers with high-quality scores were merged with the segregation data of 214 markers (151 SSR markers; 44 AFLP makers; five IBP markers; 12 SRAP markers; and two morphological markers based on anther colour and seed colour) that were scored in a previous study (Guo et al. 2012). In addition, a gene-specific marker, HG-FT-C6a, based on the sequence of the *FT* gene underlying *BnC6.FTa*, which is the QTL for flowering time in the E block of C6 in *B. napus* (Wang et al. 2009), was also used.

The software JoinMap (Van Ooijen 2006), version 4.0, was used for the map construction, and the threshold for the goodness of fit was set to $\leq$5.0, with a recombination frequency of <0.4. Linkage groups were separated using an LOD score of $\geq$3.0. Markers with a mean Chi Squared value of recombination frequency >3.0 were discarded. The maximum likelihood mapping algorithm, which was optimised for constructing dense genetic maps using this software (Jansen et al. 2001), was first used for grouping all of the polymorphic markers. Then, the method of regression mapping (Haley and Knott 1992) was used for map construction with approximately 1,000 markers with appropriate genetic distance and the marker position, and the order of markers for three rounds to merge the tightly adjacent markers into bins. The markers in adjacent loci with genetic distance below 0.2 cM were classified into a bin during the first two rounds of mapping. Moreover, one marker with sequence information and with the least missing genotype from each bin was chosen as a "bin representative" for the next round of genetic mapping. For the last (the third) round of mapping, the makers in adjacent loci pairs with genetic distances below 0.1 cM were classified into a bin to avoid incorrect classification when the markers were decreased in the map. The software MapDisto.V.1.7.5 (Lorieux 2012) was used to adjust genotypes that showed unreliable double crossing errors (181 possible double crossing errors were found) possibly because of missing

data and recording errors and to generate a new dataset before each round of map construction. The Kosambi mapping function (Kosambi 1943) was used to convert recombination frequencies into map distances, and only "Map 1" was used for further analysis. The linkage groups were assigned according to the published PCR-based genetic map of the YW DH population (Guo et al. 2012).

Assigning segments in the linkage map to the ancestral blocks of Brassicaceae

All of the markers located in the genetic map with known sequence information were employed to perform a map alignment between *B. carinata* and *A. thaliana* according to the method reported by Long et al. (2007). The sequences associated with each set of genetic markers were used as queries in homology searches against the *Arabidopsis* pseudo-chromosomes, which were discriminated as 24 ancestral blocks of Brassicaceae (Schranz et al. 2006). The full-genome sequence of *A. thaliana*, which was provided by TAIR (TAIR9 Genome Release, ftp://ftp.arabidopsis.org/home/tair/Genes/TAIR9_genome_release/TAIR9_chr_all.fas) was used as a reference for homology searches following the default parameters in BLAST, which was conducted using a Linux system. Because the DArT-Seq markers had only 69 bp of sequence information, the short sequence resulted in many homology searches. To identify the appropriate alignment for each locus, the highest *E* value was not the exclusive standard; we also used the homology searches with an *E* value <0.1 for selection. The length, *E* value, and the consistency with the adjacent loci were synchronously used as requirements to select a final alignment match. When the "bin representative" marker was not informative enough for the alignment, then another marker(s) from the bin showing the highest consistency with other markers in the bin or with the adjacent loci was chosen for the alignment. The result of the homology searches was used to construct the synteny block or insertion island (small block), which corresponded to 24 ancestral blocks of Brassicaceae between *Arabidopsis* chromosomes and YW linkage groups. At least three consecutive homologous loci were required to define a synteny block, and only one or two closest homologous loci were considered as an island with an expanding boundary calculated according to the method described by Long et al. (2007). The chromosome constitution of *B. carinata* was compared with that of other *Brassica* species carrying the B/C genome, based on the constitution and arrangement of the 24 ancestral blocks of Brassicaceae (Schranz et al. 2006). According to the constitution of the 24 ancestral blocks in the eight suggested ancestral crucifer karyotypes (ACK) of Brassicaceae (Schranz et al. 2006; Warwick and Al-Shehbaz 2006), we deduced the chromosomal constitution of each compared B and C genome originated from ACK. We ignored the segment without block information in each of the species. We used the block information of the B genome, which was integrated by Parkin (2011), according to the available conserved block constitution of *B. nigra* (Lagercrantz 1998) and *B. juncea* (Panjabi et al. 2008) as shown in Supplementary Table 2. The chromosome constitution of the C genome of *B. carinata* was compared with that of the C genome of *B. napus* (Delourme et al. 2013; Parkin 2011; Parkin et al. 2005), as summarised in Supplementary Table 2.

Statistical analysis and QTL identification

The PROC GLM procedure was used to estimate the variance for individual traits/environments using the SAS software version 8.1 (SAS Institute 1999). Genotypes were considered fixed effects, whereas environments were considered random effects. The mean values of each trait were calculated and then used for the genetic analysis.

The model of composite interval mapping in the software WinQTL cartographer 2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) was used for QTL identification (Wang et al. 2010). The walking speed, window size, and number of control markers were set to 1 cM, 10 cM, and 5, respectively. The default genetic distance (5 cM) was used to define a QTL in a specific experiment. LOD thresholds were conducted to determine the significance of a QTL, and the thresholds were established with 1,000 permutations. (Churchill and Doerge 1994). LOD values corresponding to $P = 0.05$ were used as the threshold LOD score for identifying a "significant" QTL. The "consistent" QTL, which refers to the same trait detected in different environments, was integrated by a meta-analysis using the software BioMercator 2.1. The average R^2 variation and the LOD value of the integrated QTL were used as the final values of the meta-QTL (Arcade et al. 2004). The genes underneath a QTL were aligned by comparing the conserved block of *Arabidopsis* according to the method described by Long et al. (2007).

Results

Construction of a dense genetic linkage map of *B. carinata*

We identified a total of 5,389 high-quality DArT-Seq markers that segregated in a 1:1 ratio in the YW DH population. However, 4,281 markers with *Q* values above 2.0 and <9 % missing data were selected for further analysis (Supplementary Table 3). These high-quality DArT-Seq markers were merged along with a set of 215 markers as described under "Materials and methods" section. This combined dataset was used for map construction, QTL identification, and comparative genomic analyses.

Table 1 The number of markers, bins, and marker coverage within linkage groups of the genetic map of the YW DH population of *B. carinata*

Linkage group[a]	No. loci[b]	No. total mapped markers[c]	No. integrated markers from previously mapped markers[d]	Coverage (cM)	Marker interval (cM)	
					Average	Maximum
B1	84 (43)	405 (364)	10 (4)	92.4	1.1	7.2
B2	114 (56)	375 (317)	9 (5)	148.2	1.3	10.0
B3	120 (67)	429 (376)	8 (3)	148.7	1.3	9.4
B4	96 (47)	279 (230)	8 (6)	133.3	1.4	8.7
B5	120 (58)	400 (338)	13 (8)	111.6	0.9	5.5
B6	86 (42)	281 (237)	11 (5)	102.0	1.2	9.3
B7	105 (55)	374 (324)	13 (8)	114.4	1.1	9.4
B8	122 (66)	378 (322)	14 (7)	141.0	1.2	10.2
Subtotal	847 (434)	2,921 (2,508)	86 (46)	991.6	1.2	8.7
C1	48 (23)	114 (89)	11 (10)	120.7	2.6	11.6
C2	41 (15)	114 (88)	10 (7)	121.7	3.0	14.9
C3	104 (44)	222 (162)	19 (9)	168.0	1.6	11.4
C4	51 (14)	80 (42)	7 (7)	145.3	2.9	19.4
C5	43 (12)	84 (53)	9 (9)	123.9	3.0	8.8
C6	59 (23)	157 (121)	11 (8)	104.9	1.8	16.2
C7	62 (24)	107 (69)	10 (9)	102.3	1.7	5.3
C8	58 (25)	126 (93)	13 (9)	90.3	1.6	9.5
C9	53 (25)	107 (79)	9 (7)	79.7	1.5	7.1
Subtotal	518 (205)	1,111 (796)	99 (75)	1,056.8	2.2	11.5
Total	1,366 (639)	4,032 (3,304)	185 (121)	2,048.4		
Mean	80 (38)	237 (206)	11 (7)	120.4	1.7	10.2

[a] The linkage groups were assigned in accordance with the previous genetic map of *B. carinata* based on common markers (Guo et al. 2012)

[b] The numerals shown in the bracket are the number of loci present as bins in the linkage groups

[c] The numerals shown in the bracket are the total number of markers integrated into bins in the linkage groups

[d] The numerals shown in the bracket are the number of previously mapped markers integrated into bins in the linkage groups

In total, 17 linkage groups were identified which corresponded to the previously published genetic linkage groups (Guo et al. 2012). The newly constructed integrated genetic linkage map of the YW DH population contained 1,366 discrete loci with 4,031 markers, of which 3,304 markers were assigned into 639 bins and the rest of 727 markers each represent an individual locus, covering 2,048.4 cM (Table 1; Fig. 1). The average interval between marker loci was 1.7 cM. However, four linkage groups, B2, B3, B5, and B8, had a high density of markers, and an apparent higher density was observed in the B genome than in the C genome (Table 1).

Discrimination of ancestral blocks of Brassicaceae from the dense genetic map of *B. carinata* and a comparison with its relatives

In total, 1,937 marker sequences representing the 731 loci on the genetic map of the YW DH population were aligned with the sequences of *A. thaliana* (Supplementary Table 4). Of the 24 conserved blocks, which were common in species of Brassicaceae and suggested to be composed of eight chromosomes in ancestral crucifer karyotypes (ACK) (Schranz et al. 2006; Warwick and Al-Shehbaz 2006), 22 (except of P block) were identified in the dense genetic map of the YW DH population, and block M was detected as an 'island' (small block). We identified a total of 136 blocks including 36 'islands' which were further used to trace genomic signatures of the B and C genomes of *B. carinata* (Fig. 1). The number of the blocks varied from two to ten copies. However, six blocks, i.e., A, E, F, J, R, and U, each with an average of 8.3 copies and often with large genomic regions (e.g., a large U block at the C1 linkage group) recognised from the *Arabidopsis* model, were prevalent in the genomes of *B. carinata*.

The chromosome constitution of *B. carinata* was compared with the conserved blocks identified in the linkage groups of the B genome in *B. nigra* and *B. juncea*, and of the C genome in *B. napus*, which were revealed from previously published papers (Delourme et al. 2013; Lagercrantz 1998; Panjabi et al. 2008; Parkin 2011; Parkin et al. 2005). Our results showed that *B. carinata* shared 85 % of

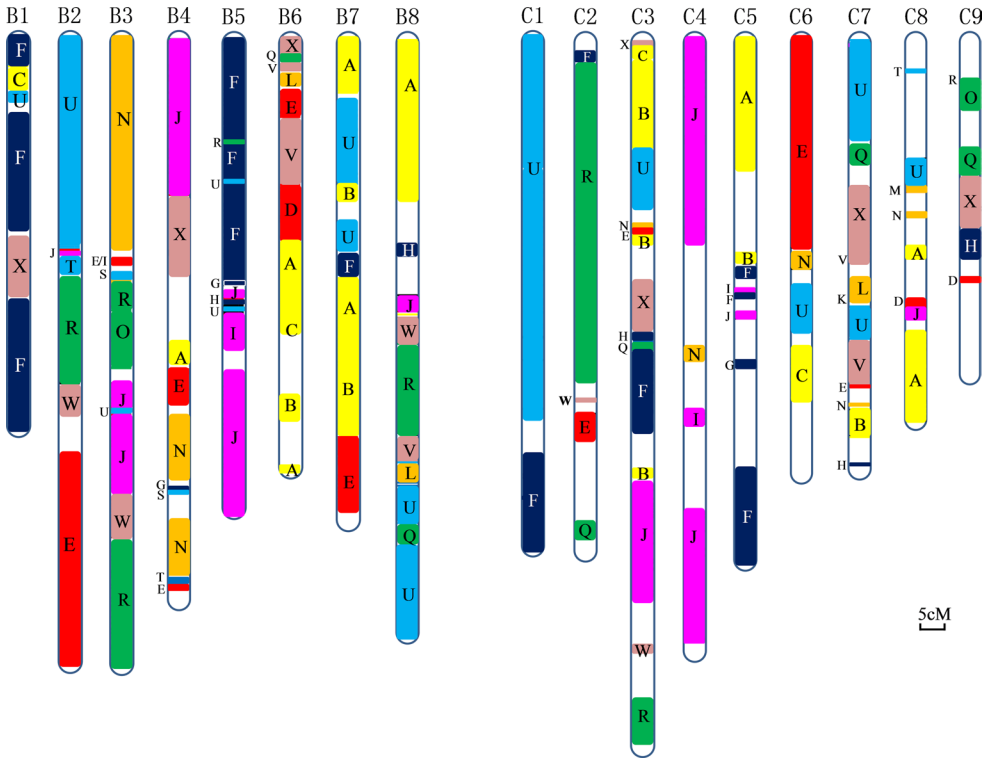

Fig. 1 The genetic linkage map of *B. carinata* showing the ancestral conserved blocks of Brassicaceae. The letters from A to X represent the 24 conserved blocks that were assumed to build eight chromosomes (shown with *eight different colours*) of ancestral species of Brassicaceae (Schranz et al. 2006). Twenty-three of the 24 conserved blocks have been identified in the genetic map, except for the P block. The Roman and small letters appearing inside the columns and to the *left* of the *columns* represent the blocks and islands (small blocks), respectively

ancestral blocks with the B genome of *B. nigra* and of *B. juncea*, and approximately 80 % of the ancestral blocks of the C genome with *B. napus* (Table 2). The conserved blocks in chromosome B2 were completely co-linear with *B. juncea* and with *B. nigra*. Some chromosomes, e.g., B6 and B7, shared the most commonly conserved blocks but showed variation in block arrangement. This variation was frequently observed for conserved block(s) inserted into co-linear block chains, e.g., in the middle of the block chain of U-Q-V-L on chromosome C7, blocks X and S were inserted in *B. carinata* and in *B. napus*, respectively.

To trace the chromosomal origin of the B and C genomes in *B. carinata* and in other *Brassica* species, we investigated the synteny to the suggested ACK of Brassicaceae with the conserved blocks. It was shown that each of *Brassica* B and C chromosome was originated as a result of rearrangement events involving 2–6 ancestral chromosomes of Brassicaceae (Table 2). Half of the chromosomes, i.e. B2, B5, B6, C1, C3, C5, C6, C7, and C9, of *B. carinata* showed the same chromosome origin with its relatives in *Brassica*, i.e., chromosomes in the B genomes of *B. nigra*/*B. juncea*, and in the C genome of *B. napus*.

Identification of QTL controlling the flower development traits in *B. carinata*

Both budding time and flowerting time showed continuous variation in the YW DH population under various growing environments (Fig. 2; Supplemental Table 5). Transgressive segregation for both traits was clearly evident in the DH lines. Both budding and flowering time were highly correlated when the YW DH population was grown under the spring-cropped environments (genetic correlation coefficient $r = 0.83$, $P < 0.001$). The budding time had a high (75 %) broad-sense heritability. Comparatively, the flowering time had a lower broad-sense heritability with the value of 65 % and 69 % under spring- and winter-cropped environments, respectively.

In total, 24 QTL controlling flower development, 6 for the budding time and 18 for the flowering time, were identified in the mapping population grown in different environments (Fig. 3; Supplementary Table 6). Half of the QTL for the budding time overlapped with the QTL for the flowering time, which coincided with the high genetic correlation between the two traits.

QTL for the flowering time were found to be highly environment-specific. For example, six of these QTL were only detected in winter-cropped environments and 11 of these QTL were only detected in spring-cropped environments (Fig. 3; Supplementary Table 6). However, one consistent QTL, *qFT.B4-2*, was identified in all of the investigated environments with high LOD values, which varied from 6.1 to 15.2, explaining for 5.3–21.7 % of the phenotypic variation (Fig. 4a). Twelve flowering time QTL were frequently identified from the conserved blocks (four in the F block, three in the J block, three in the U block, and two

Table 2 The constitution of chromosomes in *B. carinata* and in other *Brassica* species carrying B and C genomes illustrated with ancestral blocks and karyotypes of Brassicaceae

Linkage groups[a]	Identified ancestral conserved blocks[b]	Supposed chromosomal constitution originated from ancestral karyotypes[c]
B1	F C U F ***X*** F	***AK1*** AK3–***AK8***
B1	F Q F	—AK3***AK6***—
B2	U T R W E	AK2 AK6 AK7 AK8
B2	U T R W E	AK2 AK6 AK7 AK8
B3	N R O J W R	—AK4 ***AK5*** AK6 AK8
B3	F P O J W R	***AK3*** AK4–AK6 AK8
B4	J X A E N	AK1 AK2 AK4 AK5–***AK8***
B4	J S A E N T	AK1 AK2 AK4 AK5 ***AK7***—
B5	F J I J	AK3 AK4
B5	F J	AK3 AK4
B6	X L E V D A C B A	AK1 AK2 AK5 AK8
B6	K L V D C B A	AK1 AK2 AK5 AK8
B7	A U B U F A B E	AK1 AK2 ***AK3***–AK7
B7	A U B A E K	AK1 AK2–***AK5*** AK7
B8	A H J W R V L U Q U	AK1 AK3 AK6 AK7—
B8	A G H I W R U Q	AK1 AK3 AK6 AK7 ***AK8***
C1	U F	AK3 AK7
C1	U T F	AK3 AK7
C2	F R W E Q	AK2–AK6 AK8
C2	R W E V L Q X	AK2 ***AK5*** AK6 AK8
C3	C B U B X F B J W R	AK1 AK3 AK4 AK6 AK7 AK8
C3	C U B F P O J W R	AK1 AK3 AK4 AK6 AK7 AK8
C4	J N I J	AK4 AK5—
C4	J I N S I J	AK4 AK5 ***AK7***
C5	A B F	AK1 AK3
C5	A B F	AK1 AK3
C6	E N U C	AK1 AK2 AK5 AK7
C6	E N C	AK1 AK2 AK5 AK7
C7	U Q X V L K U V B H	AK1 AK3 AK5 AK6 AK7 AK8
C7	U Q S V L V B H G K	AK1 AK3 AK5 AK6 AK7 AK8
C8	U A J A	AK1–AK7 ***AK8***
C8	C U A B N A	AK1 ***AK5*** AK7—
C9	O Q X H D	AK2 AK3 AK6 AK8
C9	O Q X H D V W R	AK2 AK3 AK6 AK8

[a] For each pairs of chromosomes, the first line with grey colour background represents the information for *B. carinata*, and the second line with white colour background represents the information for the published B genome of *B. nigra*/*B. juncea* (Lagercrantz 1998; Panjabi et al. 2008) and the C genome of *B. napus* (Delourme et al. 2013; Parkin 2011; Parkin et al. 2005)

[b] The names of all of the blocks are arranged according to the order in the linkage groups. The islands in the genome of *B. carinata* have not been listed, except for three islands shown by underlines showing consistency with the compared reference genome

[c] The ancestral karyotypes are ordered from AK1 to AK8, and the divergent karyotypes between *B. carinata* and other *Brassica* species are highlighted in italics and in bold

in the E block) which were prevalent in the genome of *B. carinata* (Supplementary Table 6).

A group of genes encoding transcriptional factors that regulate floral transition in *Arabidopsis*, such as *FPA* (*FLOWERING BY PREVENTING THE ACCUMULATION OF mRNA ENCODING THE FLORAL REPRESSOR*), *AGL6* (*AGAMOUS-LIKE 6*), and *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO1*) were localised in the *qFT.B4-2* (in the J block) (Fig. 4a; Supplementary Table 7). Another major QTL, *qFT.C6-2*, with the largest genetic effect that accounted for 39–56 % of the variation for the flowering time in winter-cropped environments was

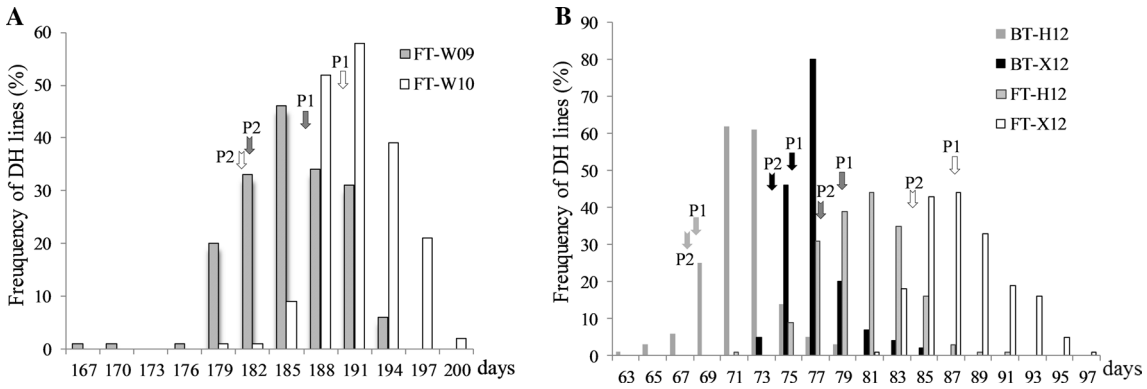

Fig. 2 The frequency distribution of the budding time (BT) and the flowering time (FT) in the YW DH population. **a** DH lines grown under the spring-cropped environment. **b** DH grown under the winter-cropped environment. *Arrows* with *different shapes* show the budding time or the flowering time for the two parents under different environments (Y-BcDH64 with a flat tail denotes as P1, and W-BcDH76 with a fork tail denotes as P2) in the corresponding environment. Transgressive segregation was observed in the population from all of the environments

identified in the E block on linkage group C6 (Fig 4b). A gene-specific marker for *FT*, HG-FT-C6a, was mapped near the peak position (37 cM) of the QTL (*qFT.C6-2)*, and the *FT* gene, along with other flowering time genes, e.g., *AP1* (*APETALA 1*) and *FKF1* (*FLAVIN-BINDING KELCH DOMAIN F BOX PROTEIN 1*), underlying *qFT.C6-2,* were identified by aligning the markers to the genome of *A. thaliana* (Supplementary Table 7).

Validation of QTL controlling seed colour using the dense genetic map

Using the integrated dense genetic linkage map, we re-detected QTL controlling seed colour with the phenotypic data that were scored in a previous study (Guo et al. 2012). Compared to the three major and one micro-real QTL detected previously in this population, the LOD value, additive genetic effects, and confident interval of the QTL had been obviously improved in this study when the dense genetic linkage map was employed for the analysis with this trait. For example, a major QTL was identified on the linkage group C3 which had an increased additive genetic effect (from 1.17 to 1.67 for colour degree) and explained greater phenotypic variation (R^2, from 0.32 to 0.66) with much higher LOD value (from 19.22 to 57.24) (Fig. 3; Supplementary Table 6). The micro-real QTL detected previously on the linkage group C4 (Guo et al. 2012) was re-detected at a significant level (LOD value from 2.58 to 10.87) with an increased additive effect (from 0.45 to 0.55 for colour degree) and with decreased confidence interval (from 18.2 to 9.2 cM). Two small adjacent QTL previously detected on the C9 linkage group were merged into a single QTL having large allelic effect. Besides, we detected a new QTL delimited within 1.7 cM between the marker 100,076,711 and bin510 on C3 linkage group accounting for small (2 %) of the trait variation (Supplementary Table 8).

Discussion

A number of genetic linkage maps have been generated over the past 20 years in *Brassica* crops. These molecular maps have greatly enhanced our understanding in genome organization and evolution, as well as facilitated genetic dissection of loci associated with traits of interest (summarised by Parkin 2011; Snowdon and Luy 2012). Recent developments in high-throughput resequencing and genotyping-by-sequencing technologies have enabled reseachers to construct high-density linkage maps of *Brassica napus* and other crops, which are valuable for various modern genomic approaches, such as genome-wide association studies, genomic selection and so on, for plant breeding-related purpose in the genomics era (Bus et al. 2012; Delourme et al. 2013; Foolad and Panthee 2012; Joukhadar et al. 2013; Li et al. 2012; Liu et al. 2013; Raman et al. 2013b, 2014; Randhawa et al. 2013; Snowdon and Luy 2012; Zhang et al. 2013; Xu 2013). For the first time, we have constructed a high-density integrated genetic map of *B. carinata* containing 4,031 markers mostly with known sequence information and integrated all genotypic data generated in a previous study (Guo et al. 2012).

This integrated linkage map proved to be very useful in (1) dissection of loci associated with seed and flower colour in the YW DH population and (2) comparative mapping of *B. carinata* and other *Brassica* species carrying the B/C genome. The present map distinguished 136 conserved blocks and islands in the genome of *B. carinata*, which revealed a polyploidy origin similar to other polyploid *Brassica* species, e.g., *B. napus* (Iniguez-Luy and Federico

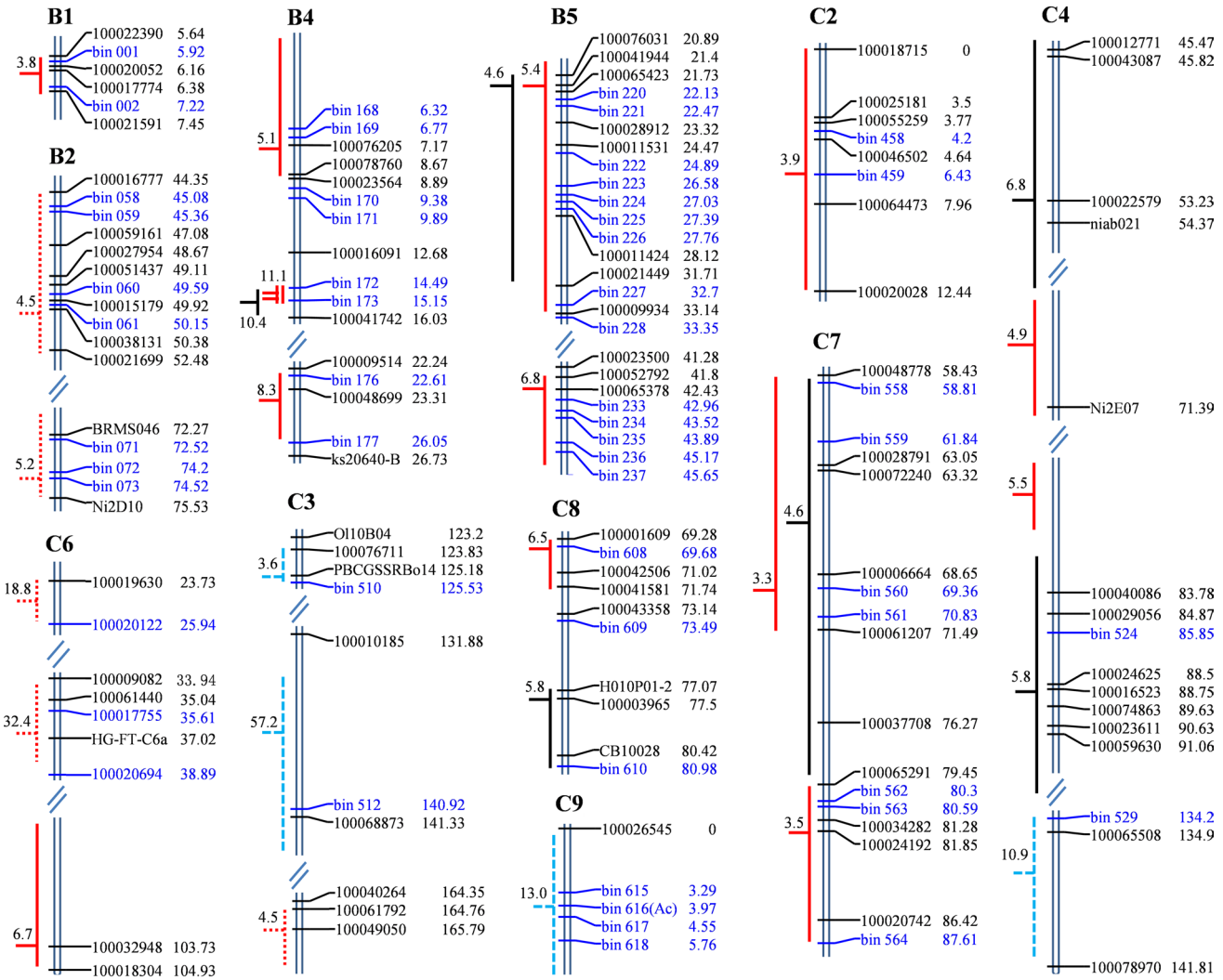

Fig. 3 Dense genetic linkage map showing the localisation of the QTL controlling the flowering time, budding time and seed colour in the YW DH population. The *numbers* on the *right* of each linkage group indicate the genetic distance (cM). All of the QTL are arranged to the left of each linkage group with *vertical lines* representing confidence interval, and the peak position of QTL is shown with a *horizontal line*, above which the numerals indicate the LOD value of the QTL. The *lines* with *black*, *red* and *bright blue colour* represent the QTL referring to the budding time, the flowering time, and the seed colour, respectively. The *solid lines* and *broken lines* represent the QTL appearing in the spring-cropped and winter-cropped environments, respectively. One consistent QTL related to the flowering time was detected across all investigated environments on chromosome B4 and is indicated with *double lines*

2011; Marhold and Lihova 2006; Schranz et al. 2006; Wang et al. 2009; Yang et al. 2006), with approximately six times more blocks, referring to a set of 24 ancestral blocks from suggested ACK. As determined by a comparison based on the conserved blocks of *Arabidopsis* reported in other *Brassica* species, 80–85 % of the genome of *B. carinata* shared the same conserved genomic segments with the B genome of *B. nigra*/*B. juncea* and with the C genome of *B. napus*. Although the ratio of the similarity might increase with more common markers/loci mapped on comparative genetic maps, insertions and inversions at the block level revealed a unique genetic variation in *B. carinata*, which may have originated during or after speciation. Additionally, the six blocks, i.e., A, E, F, J, R, and U, which each had more than six copies, may carry many favourable alleles for important agronomic traits resulting from the domestication and selective breeding of this species. For example, the QTL accounting for the flowering time and the budding time were frequently detected in the conserved blocks (Supplementary Table 6). It is expected that a comprehensive genomic variation would be revealed once the genome of *B. carinata* is fully sequenced, following the genome sequence of *B. napus* and of *B. nigra* (Snowdon and Luy 2012). The high-density genetic linkage map

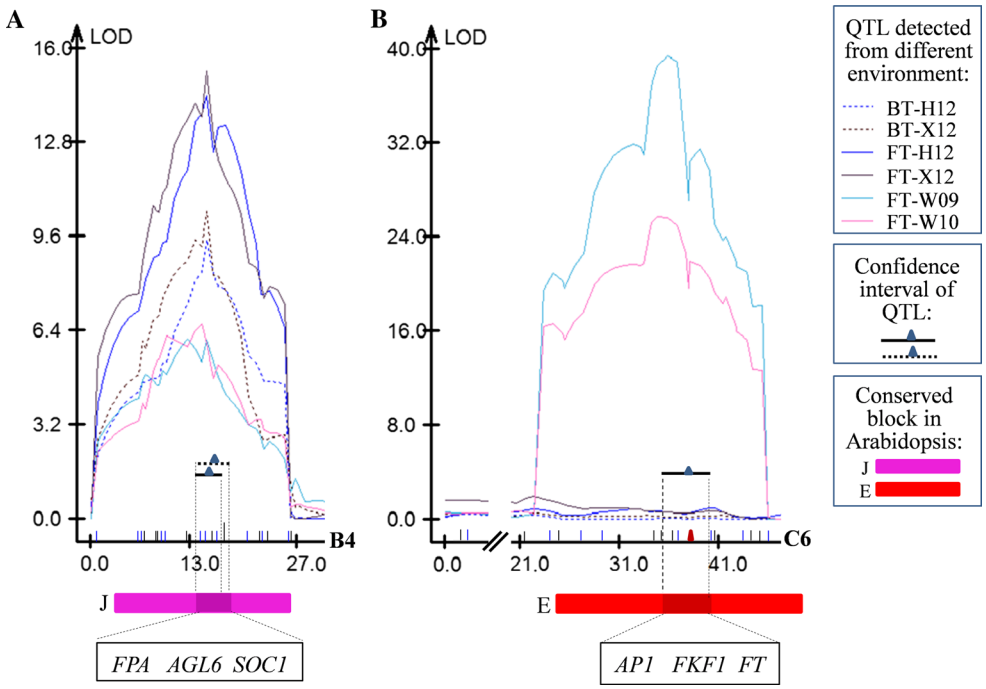

Fig. 4 Graphical representation of three major QTL controlling the traits of flower development on linkage groups B4 and C6 in the YW DH population of *B. carinata*. **a** One QTL controlling the flowering time, *qFT.B4-2*, was detected on the B4 linkage group from both spring- and winter-cropped environments and overlapped with a major QTL controlling the budding time. **b** A major QTL for the flowering time, *qFT.C6-2*, on the C6 linkage group was only detected from winter-cropped environment. Additionally, a genetic marker, HG-FT-C6a, for *FT* is highlighted with a *red closed upper triangle*. Target genes involved in the flowering time in the conserved block(s) of *Arabidopsis* are shown at the bottom: *FPA* (*FLOWERING BY PREVENTING THE ACCUMULATION OF mRNA ENCODING THE FLORAL REPRESSOR*); *AGL6* (*AGAMOUS-LIKE 6*); *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO 1*); *AP1* (*APETALA 1*), *FKF1* (*FLAVIN-BINDING KELCH DOMAIN F BOX PROTEIN 1*); and *FT* (*FLOWERING LOCUS T*). Detailed information concerning the target genes is listed in Supplementary Table 7

published here, may provide a useful tool for assembling the duplicated genome of *B. carinata*.

Compared with the low-density map constructed previously (Guo et al. 2012), the present high-density genetic map proved to be more powerful for identifying precise QTL controlling important agronomic traits and for revealing candidate genes underneath those QTL. For example, a significant QTL for seed colour was redetected on the linkage group C3 with a higher confidence level (LOD value 57.2, and a smaller confidence interval 9.2 cM) in this study. In addition, a new QTL was identified on the linkage group C3 (Fig. 3; Supplementary Table 6). In comparison with the previously reported QTL (refer to Guo et al. 2012) and the latest published data (Liu et al. 2013; Stein et al. 2013), none of the QTL for seed colour has been detected on linkage groups C3 [where only a QTL for cellulose content was mapped by Liu et al. (2013) in *B. napus* with non-comparable linked markers at that time], C4, and C9 in previous studies. It seems that the QTL accounting for seed colour that was identified in this study will be unique. Previous research has shown that seed colour is correlated with brighter crude oil, higher protein, and lower fibre content in seed meal of *Brassica* oilcrops (Getinet et al. 1996; Stringam et al. 1974). Therefore, this QTL may have major implications in improving oil and meal quality attributes in *B. carinata* and related species. Further research is required to fine map the major QTL to develop 'diagnostic' markers for marker-assisted breeding.

Flowering time is another important trait that we focused on in this study. *B. carinata* has been grown as a spring crop which flowers under a long-photoperiod condition in Africa for 1,000 years and is also well adapted to areas in Ethiopia at elevations between 2,200 and 2800 m (Malik 1900; Warwick et al. 2006; Warwick 2011). However, the cultivation of *B. carinata* in regions with low elevations, mild winters, and short day-length conditions is limited due to the late flowering time. In the present study, six flowering time QTL, including the major QTL (*qFT.C6-2*) and the consistent QTL (*qFT.B4-2*), were identified in winter-cropped environments. This information would be extremely useful for researchers who wish to breed new cultivars of *B. carinata* that can adapt to a semi-winter-cropped environment where low

level of vernalisation is required and plants often flower in early spring such as in India, central parts of China, Middle Europe, and Australia. The QTL information revealed from this dense genetic map could also be compared with the QTL identified from other related species of *Brassica* via the conserved block analysis. For example, a major QTL controlling the flowering time in winter-cropped environments was located at the E block onto linkage group C6. This QTL corresponded to a major flowering time QTL for the winter-crop environment in *B. napus* (Long et al. 2007). The *BnFT*, a gene homologue to *FT* (*Flowering time locus T*) which accounts for florigen in *A. thaliana* (Corbesier et al. 2007), was also located in this QTL region and corresponds to the *BnFT* locus in *B. napus* (Wang et al. 2009). This result suggests that the *FT* homologue may also play an important role in flowering time in *B. carinata.* These findings can be further exploited for its genetic improvement. We also detected several QTL for flowering time onto linkage groups C2, C3, C4, C7, and C8, similar to those detected in other *Brassica* species carrying the C genome, such as *B. napus* and *B. oleracea* (Axelsson et al. 2001; Lan and Paterson 2000; Long et al. 2007; Raman et al. 2013a, 2014).

For the first time, we identified eight major QTL for flowering time on the linkage groups of B1, B2, B4, and B5 of *B. carinata*. To our knowledge, only a few QTL accounting for the flowering time have been reported in the *Brassica* species carrying the B genome (Axelsson et al. 2001; Lagercrantz et al. 1996). These results may enable researchers to identify important flowering time-related genes underlying QTL and to further compare the gene network related to the flowering time among different *Brassica* crops.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that the study comply with the current laws of the countries in which experiments were performed.

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