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ORIGINAL PAPER

A genetic linkage map of *Brassica carinata* constructed with a doubled haploid population

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Abstract *Brassica carinata* is an important oilseed crop with unique favourable traits that are desirable for other *Brassica* crops. However, given the limited research into genetic resources in *B. carinata*, knowledge of the genetic structure of this species is relatively poor. Nine homozygous, genetically distinct accessions of *B. carinata* were obtained via microspore culture, from which two divergent doubled haploid (DH) lines were used to develop a DH mapping population that consisted of 183 lines. The mapping population showed segregation of multiple traits of interest. A genetic map was constructed with PCR-based markers, and a total of 212 loci, which covered 1,703 cM, were assigned to eight linkage groups in the B genome and nine linkage groups in the C genome, which allowed comparison with genetic maps of other important *Brassica* species that contain the B/C genome(s). Loci for two Mendelian-inherited traits related to pigmentation (petal and anther tip colour) and one quantitative trait (seed coat colour) were identified using the linkage map. The significance of the mapping population in the context of genetic improvement of *Brassica* crops is discussed.

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Introduction

Brassica carinata A. Braun (BBCC, $2n = 34$) is an important vegetable and oilseed crop in northeast Africa and is thought to have originated in the Ethiopian plateau (Warwick 2011). Together with two other amphidiploid *Brassica* species, *B. napus* (AACC, $2n = 38$) and *B. juncea* (AABB, $2n = 36$), *B. carinata* is an amphidiploid of recent origin (Quiros et al. 1985). Cytogenetic and molecular evidence suggest that *B. carinata* is derived from spontaneous interspecific hybridization between two diploid species, *B. oleracea* (CC, $2n = 18$) and *B. nigra* (BB, $2n = 16$), followed by subsequent polyploidization (Lukens et al. 2004; Song et al. 1988; UN 1935). The processes of speciation and domestication have resulted in genomic divergence of *B. carinata* from its diploid progenitors.

Brassica carinata has been evaluated as a potential oil crop in many countries outside of East Africa (Warwick et al. 2006). Given its many desirable characteristics, such as resistance to pests and tolerance to diseases and drought, *B. carinata* is suitable for cultivation in harsh environments (Getinet et al. 1996). In addition, transfer of the yellow-seeded trait from lines of *B. carinata* to *B. napus* has been attempted (Meng et al. 1998; Rahman 2001). In fact, as an important source of desirable genes, approximately 100 accessions of *B. carinata* have been used to incorporate components of the C genome of *B. carinata* into traditional *B. napus* to generate new-type *B. napus* (Xiao et al. 2010; Zou et al. 2010).

Extensive collection of *B. carinata* germplasm has been carried out since the 1980s (Engels 1984; Seegeler 1983). However, few studies have assessed the genetic resources within *B. carinata*. Genetic variability was detected in relation to a variety of traits, such as seed quality and interspecific crossability with *B. rapa* (Alemayehu and Becker 2002; Jiang et al. 2007) Although *B. carinata*

accessions show more limited genetic variation than those of *B. juncea* and *B. nigra*, as indicated by amplified fragment length polymorphism (AFLP) markers, *B. carinata* is genetically divergent from the other two species of *Brassica* that contain the B genome (Warwick et al. 2006). Further analysis of the genetic variation in *B. carinata* would be useful to explore the origin and evolution of this species and to enable the utilization of the germplasm in *Brassica* breeding programmes.

Construction of a linkage map with molecular markers is essential for better understanding and utilizing the genetic bases of desirable phenotypic variations. A linkage map of one species could also assist with the resolution of genomic relationships among the related species. Such a linkage map is extremely important in the context of 'U's triangle (UN 1935), which depicts genetic relationships among the six *Brassica* species, whereby three diploid and three tetraploid species are derived from the A, B, and C genomes. A number of linkage maps have been developed for five of the six *Brassica* species of U's triangle, i.e. *B. nigra* (Lagercrantz 1998; Lagercrantz and Lydiate 1995), *B. oleracea* (Iniguez-Luy et al. 2009; Nagaoka et al. 2010), *B. rapa* (Choi et al. 2007; Kim et al. 2009), *B. napus* (Qiu et al. 2006; Wang et al. 2011; Zhao et al. 2011), and *B. juncea* (Panjabi et al. 2008; Ramchiary et al. 2007). Important quantitative trait loci (QTL) that control a variety of key traits have been identified from the linkage maps (Brock et al. 2010; Fan et al. 2010; Jagannath et al. 2011; Lou et al. 2011; Radoev et al. 2008; Shi et al. 2009). However, at present, no linkage map of *B. carinata* is available, although studies of the B genome of *B. carinata* and the genetic improvement of this species have been carried out (Jadhav et al. 2005; Navabi et al. 2011; Struss et al. 1996; Velasco et al. 2003).

The aims of the study reported herein were to develop a doubled haploid (DH) mapping population for *B. carinata* with divergent parental lines, and to construct a linkage map with the DH population and molecular markers to enable linkage groups to be assigned to the B and C genomes. Genetic analysis of three pigment-related traits (petal colour, anther tip colour, and seed coat colour) using this genetic linkage map is also reported in this paper.

Materials and methods

Microspore culture for development of DH parent lines and a mapping population

Thirty accessions of *B. carinata* that show genetic divergence and different degrees of crossability to *B. rapa* were selected from 110 accessions (Jiang et al. 2007) for microspore culture to develop DH plants (Supplementary Table 1). The culture procedure followed that of Möllers et al. (1994) with minor modifications. Isolated microspores were resuspended in 10 ml of 50 mg l^{-1} colchicine solution for 50 h at 30 °C, before the microspore suspensions were incubated in the dark at 25 °C. DH plants of *B. carinata* that showed a high degree of genetic divergence and different degrees of cross-ability to *B. rapa*, and could be distinguished at the morphological level (e.g. by the colour of flowers and seeds or by plant architecture) were crossed with each other. F_1 plants from 16 cross combinations were subjected to a second round of microspore culture to develop DH populations. The cross combination that yielded the highest number of DH plants was selected as the DH mapping population for subsequent study.

Field trial and phenotype evaluation

The DH population, together with the two parents and the F_1 plants, were planted in the experimental field of Huazhong Agricultural University in Wuhan, China, with three random replications in 2009 and two replications in 2010. Each plot contained three rows, 40 cm apart, and there were ten plants in each row with 25 cm between individual plants in the 2-year field trials. The petal colour of each line was recorded after half of the plants had begun to flower, and the anther colour was examined in buds of three plants of each line. Each character (petal colour and anther tip colour) segregated into two distinct types and was recorded as two classes: 1 = yellow, 2 = white (petal), or 1 = red, 2 = purple (anther), respectively. The seed colours of each line formed a continuous range, from bright yellow through pale brown to brown. The colour of self-pollinated seeds from each DH line was classified into seven grades according to the depth of colour, from bright yellow (grade 1) to brown (grade 7).

Detection of molecular marker polymorphism

Amplified fragment length polymorphism markers (Vos et al. 1995) were used to evaluate the genetic distance among the DH lines of *B. carinata* (named as BcDH) derived from microspore culture. Simple sequence repeat (SSR) and intron-based polymorphism (IBP) markers from a variety of resources, together with AFLP markers and sequence-related amplified polymorphism (SRAP) markers (Li and Quiros 2001), were used to construct the genetic linkage map (Supplementary Table 2). Polymorphic fragments from all dominant and co-dominant markers were scored as 1 (present) or 0 (absent) and as A or B, respectively, for further data analysis.

Multivariate analysis

Multivariate analysis was performed as described previously (Chen et al. 2010). Dissimilarity matrices with the

lowest correlation coefficient based on the proportion of shared alleles were calculated using NTSYSpc ver. 2.21o (Rohlf 2008). Hierarchical cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA) as proposed by Sneath and Sokal (1973). The ordination analysis was performed by two-dimensional multidimensional scaling (2D-MDS) using PRIMER 6 software (Clarke and Gorley 2006).

Map construction and QTL analysis

Markers that were polymorphic between the parental lines were amplified and scored in the DH population. A data matrix was generated and imported into JoinMap 4.0 software (Van Ooijen 2006). Linkage analysis and map construction were conducted with a threshold LOD score of 3.0. Markers with a mean Chi-squared value of recombination frequency greater than 3.0 were discarded to guarantee the quality of the map. Genetic distances were calculated as centiMorgans (cM) with the Kosambi mapping function.

Quantitative trait loci for seed coat colour on the genetic map were resolved with the composite interval mapping method using Windows QTL Cartographer 2.5 (Wang et al. 2010), and the presence of QTL was tested with a 10-cM window and five background cofactors, for which the threshold LOD score was determined on the basis of 1,000 permutations at the 5 % significance level. QTL above the threshold LOD score with large genetic effect was regarded as statistically significantly large QTL (SL-QTL), and QTL below the threshold LOD score but repeatedly detected at LOD score higher than 2.0 was regarded as micro-real QTL (MR-QTL) (Long et al. 2007).

BLAST analysis and map alignment with the *Arabidopsis* genome

All available sequences that used to design the SSR and IBP markers were applied to identify homologous genes in the *Arabidopsis* genome. The alignment was performed with the BLASTn program using the default parameter setting (http://blast.ncbi.nlm.nih.gov/) and the *Brassica* BLAST server (http://brassica.bbsrc.ac.uk/BrassicaDB/blast_form.html). The primer sequences were also used in a BLAST search against the *Arabidopsis* genome (http://atidb.org/) to search for homologous gene sequences. The alignment results with the cutoff E value of $1E$-05 were used to determine blocks of synteny or small islands between the *Arabidopsis* chromosomes and *B. carinata* linkage groups. The synteny blocks of the *B. carinata* genome were defined on the basis of at least two closely linked loci within one of conserved blocks that were proposed by Schranz et al. (2006); an island was defined as a region with a gap of greater than 20 cM between adjacent markers (Long et al. 2007; Panjabi et al. 2008).

Results

Selection of parental lines and development of a DH mapping population

The efficiency of microspore culture of the 30 selected *B. carinata* accessions was relatively low. Twenty-one of the accessions generated no DH plants at all. This was largely due to the high temperature in the field when the *B. carinata* accessions were flowering in late spring in Wuhan. Plants were regenerated by the formation of embryoids from 9 of the 30 accessions, which corresponded to nine DH families. One healthy plant with fertile pollen and normal seed set was selected from each DH family to produce a BcDH line. A total of 210 AFLP markers polymorphic among nine DH plants were generated from 43 primer pairs and used for genetic distance analysis. Hierarchical cluster analysis showed that the nine BcDH lines clustered in the dendrogram tree in a manner that was consistent with the pattern of their parental accessions (Fig. 1a, b). This indicated that the variation within lines derived from a single accession by microspore culture was generally not significant compared with the variation among accessions. The DH line BcDH76 showed the largest genetic distance from the other BcDH lines and its parental accession, CGN03976 [The Centre for Genetic Resources http://www.cgn.wur.nl/NL/. The Netherlands (CGN)] was also the most distant from the other parental accessions. CGN03976 was collected from farmland on São Tomé Island, an island located off the coast of western Africa (Supplementary Fig. 1), whereas the other accessions were collected originally from Ethiopia in eastern Africa. F_1 plants from the cross between BcDH76 and BcDH64, a BcDH line of eastern African origin, were chosen to develop a DH mapping population (Supplementary Fig. 3).

Consistent with their paternal accessions, BcDH64 and BcDH76 produced flowers with yellow and white petals, respectively (Fig. 2a). The F_1 plants from both reciprocal crosses produced yellow petals, indicating a complete dominance of yellow over white, and petal colour segregated distinctly in the DH mapping population. As a consequence, the DH population was designated the Yellow-BcDH64/White-BcDH76 (YW) population. The two parental lines were also distinguished by two other pigment phenotypes. The yellow-flowered BcDH64 bore a small red spot at the tip of the anther and produced yellow seeds, whereas the white-flowered BcDH76 had a deep purple spot at the anther tip and produced dark brown seeds

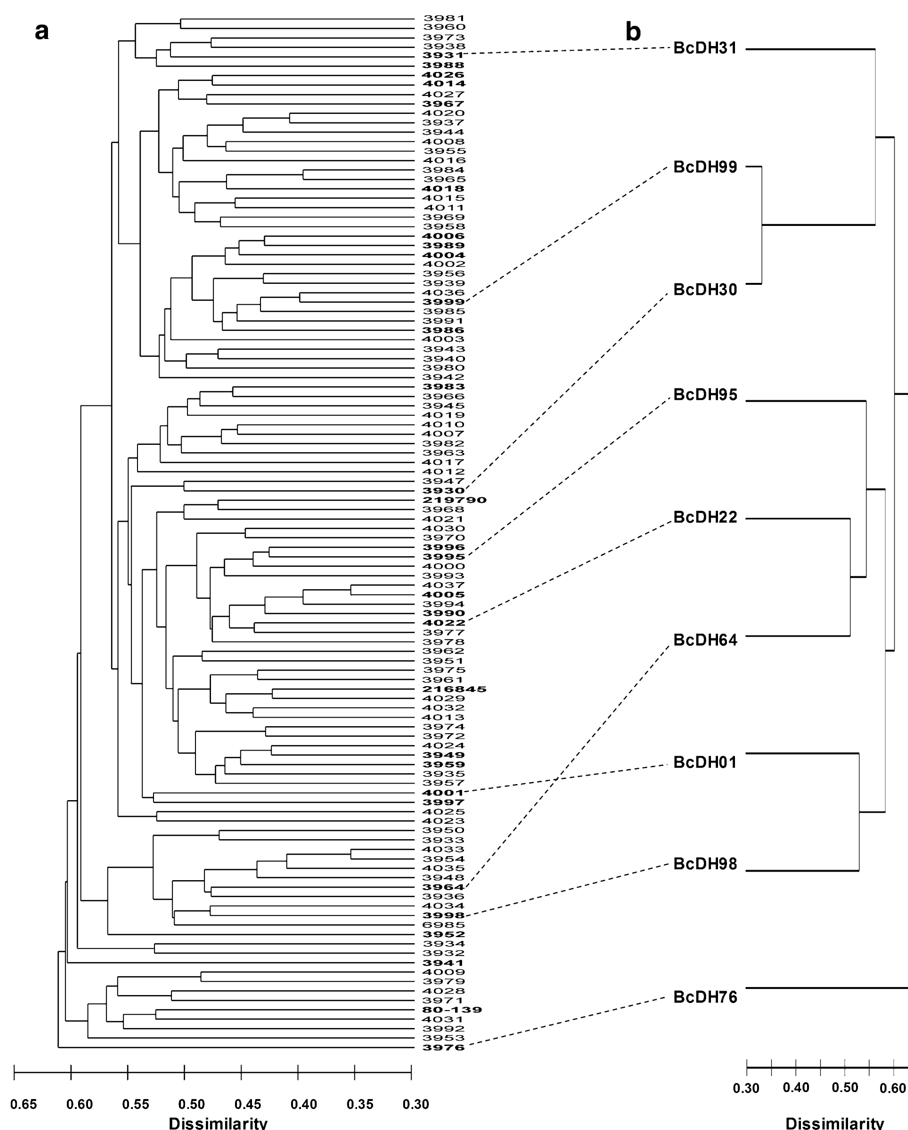

Fig. 1 Genetic similarity of *B. carinata* accessions and their derived DH lines as evaluated with AFLP markers. **a** Similarity of the 30 accessions (*highlighted* in *boldface*) that were used to generate the BcDH lines among the 110 accessions of *B. carinata* that were studied by Jiang et al. (2007). The *first four letters* (CGN0) of the original serial number for each of the 110 accession is omitted. The dendrogram is reconstructed with the software Primer 6 using the data from Jiang et al. (2007). **b** Genetic similarity of the nine BcDH lines derived from the nine microspore-cultured accessions *highlighted* in (**a**). The *dashed lines* between the dendrograms in **a** and **b** indicate the pedigree relationships between the accessions and corresponding derived BcDH lines

(Fig. 2b, c). The purple anther tip was dominant in the F_1 plants, and seed colour was semi-dominant in F_1 plants.

Construction of the YW linkage map

We surveyed a total of 1,685 SSR primer pairs that had been identified in different *Brassica* crops, namely, *B. napus*, *B. nigra*, *B. oleracea*, *B. rapa*, and *B. juncea*, and found that only 10.6 % showed polymorphism between the two parents of the YW population. Consequently, the 183 DH lines of the YW population were genotyped by using 151 SSR markers plus 61 other molecular markers (i.e. 44 AFLP, 12 SRAP, and 5 IBP). The total 212 markers were distributed in 17 linkage groups with an LOD score value greater than 3.0 (Fig. 3; Table 1). The total genetic length of the map was 1,703 cM with an average distance of 8.0 cM between adjacent loci.

The deviation of marker segregation from the expected ratio was analysed with a Chi-squared test. Most of the 212 mapped markers segregated in the expected 1:1 ratio in the YW population, but 23 % (48/212) of the markers deviated significantly ($P < 0.05$) from the expected ratio, and most of these markers (42/48) were skewed towards the maternal line, BcDH64, and formed clusters on linkage group B5, B8, C1, C2, C3, and C5. However, four markers (from e6m9-80 to e6m2-250) formed a cluster on linkage group B6 (Fig. 3) and were skewed towards the paternal line, BcDH76.

A set of SSR markers that were anchored on linkage groups of the B genome of *B. juncea* (Ramchiary et al. 2007) and C genomes of *B. napus* (Cheng et al. 2009; Qiu et al. 2006; Wang et al. 2011) and *B. oleracea* (Iniguez-Luy et al. 2009) were used to assign the linkage groups of *B. carinata* to the equivalent linkage groups of the B/C genomes in the above species. As a result, four linkage groups of *B. carinata* were assigned to their equivalent

Fig. 2 Flower and seed colour phenotypes in two DH lines of *B. carinata* and their F_1 hybrid progeny. The *upper* and *central images* show the differences in pigmentation in the petals and anther tip, respectively. The traits of *yellow* petals in BcDH76 and the *purple* spot on the anther in BcDH64 were dominant in the F_1 generation. The *lower images* show the differences in seed coat *colour* between the two DH lines and the intermediate phenotype of the F_1 population (colour figure online)

linkage groups in the B genome (B2, B4, B6, and B7), and nine linkage groups were assigned to their equivalent linkage groups in the C genome. The other four linkage groups of the B genome were identified on the basis of conserved building blocks (Table 1). Consequently, eight linkage groups in the map of *B. carinata* were assigned to the equivalent ancestral haploid chromosome of *B. nigra* (BB, $n = 8$) on the basis of reference maps for *B. juncea* (Panjabi et al. 2008), and nine linkage groups were assigned to the C genome on the basis of reference maps for *B. oleracea* and *B. napus* (Table 1). The SSR markers that originated from *B. rapa*, *B. oleracea*, and *B. napus* were more likely to be mapped to the C genome of *B. carinata*, whereas the markers that were developed from *B. nigra* and *B. juncea* tended to be located in the B genome. However, some markers that were derived from *B. oleracea*, *B. rapa*, and *B. napus* were mapped to the B genome, which indicated that the B genome of *B. carinata* contained some regions that were homologous with the A and C genomes.

The alignment of *B. carinata* with *Arabidopsis thaliana* (*At*) genome was conducted with the sequences flanking SSR motifs, or by direct BLASTn using the primer sequences. Of the mapped 151 SSR and 5 IBP marker loci, 76 % (119/156) had at least one *At* homologous and the homologous loci were converted into the 24 hypothetical genomic blocks for At and *Brassica* lineages. The collinear blocks were defined by at least two *At* loci from the same block region. Six of the nine C linkage groups and five of the eight B linkage groups have collinear blocks with one *At* chromosome (Fig. 3). Collinear blocks were represented corresponding to the homologous linkage groups in B genome in *B. nigra* and *B. juncea* and in C genome in *B. olercea* and *B. napus*. The R block and two small islands, O and Q, at C9 were in correspondence to the C9 in *B. napus*. However, the collinear blocks were altered in the

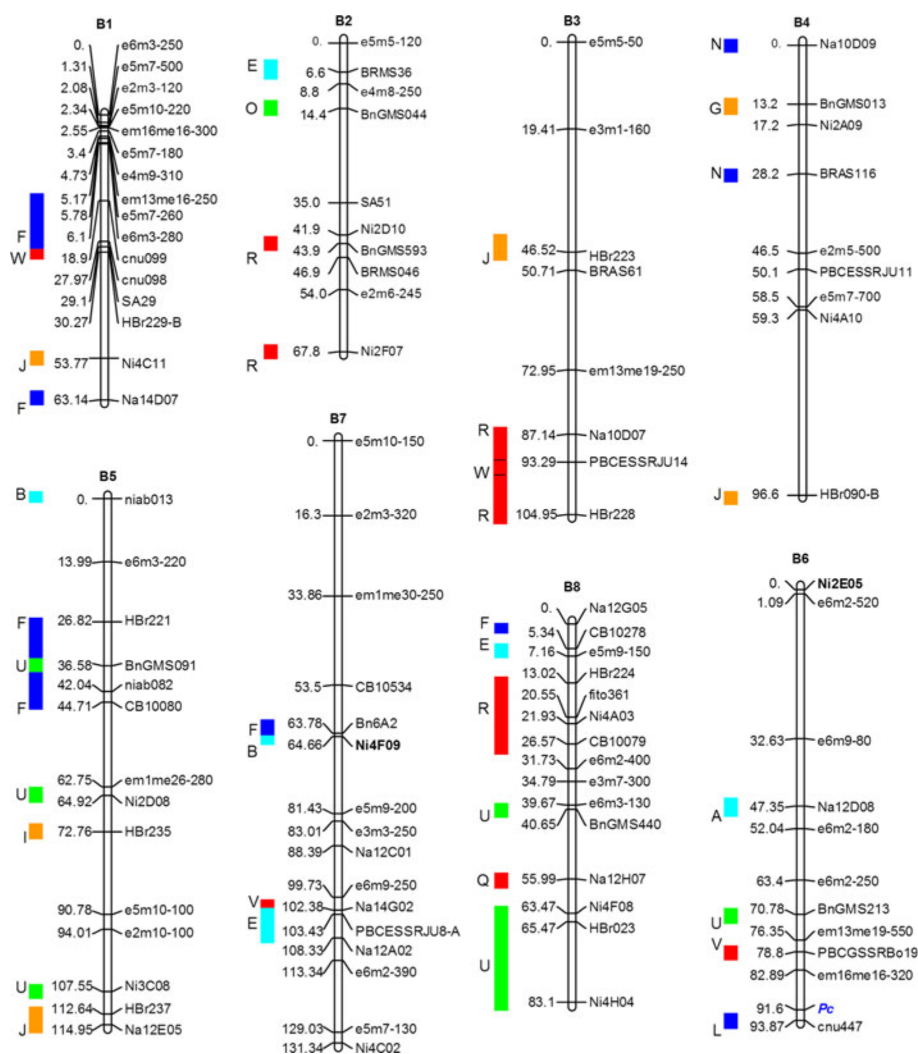

Fig. 3 Linkage map of *B. carinata* constructed with the YW population. The nomenclature for the linkage groups corresponds to that of the B genome of *B. juncea* and the C genome of *B. napus*, respectively. Loci that are *highlighted in boldface* are anchored markers that indicate the linkage groups reported previously (Cheng et al. 2009; Iniguez-Luy et al. 2009; Qiu et al. 2006; Ramchiary et al. 2007; Wang et al. 2011). The segments that were homologous to blocks and islands in the *Arabidopsis* genome are represented by *coloured bars* that correspond to the five *At* chromosomes and are shown between the linkage groups and *capital letters*, which represent the Brassicaceae building blocks. QTL for seed coat colour identified in linkage groups of C3, C9 and C4 are presented above each linkage group, respectively. QTL score of the first year and the second year are shown in *red* and *green lines*, the threshold LOD value (3.0) for 2 years trail is shown as *dashed line* (colour figure online)

C3 linkage group, in which the up and bottom regions changed into J block. In total, there were seven conserved building blocks identified in five B linkage groups and six building blocks in six linkage groups of the corresponding reference maps mentioned above, although due to limited available markers, small syntenic islands were identified on the six remaining linkage groups (Table 1).

Mapping of markers and QTL for three pigment-related traits

The traits of petal colour and anther spots displayed Mendelian segregation in the YW population. The ratio of plants with yellow petals to those with white petals was approximately 1:1 ($\chi^2 = 0.92$, $P = 0.05$). The ratio of plants with a red spot at the anther tip to those with a deep purple spot was also approximately 1:1 ($\chi^2 = 1.58$, $P = 0.05$) over the course of the 2year experiment. The loci that controlled petal colour (*Pc*) and anther colour (*Ac*) were mapped on linkage groups B6 and C9, respectively (Fig. 3).

In the segregated population, seed colour showed a continuous distribution of phenotypes, from dark brown to bright yellow, and thus was treated as a quantitative trait. The seed colour observed in the 2 years showed a very high correlation ($r = 0.95$), and the heritability (h^2) of seed colour estimated from the variance component was also high (97.38 %). Four QTL for seed colour were identified from three linkage groups, and all of them belonged to the C genome. The largest QTL on linkage group C3 was repeatedly detected for each of the 2009 and 2010 trials explaining 30 % of the phenotypic variation. There were two QTL located adjacently on C9, one with smaller effect but repeatedly detected and the other only detected in 2009 with higher LOD value (5.4). One QTL at LOD value 2.5–2.6, slightly lower the threshold of 3.0, repeatedly detected on C4, was regarded as a micro-real QTL (MR-QTL) (Fig. 3; Table 2).

Fig. 3 continued

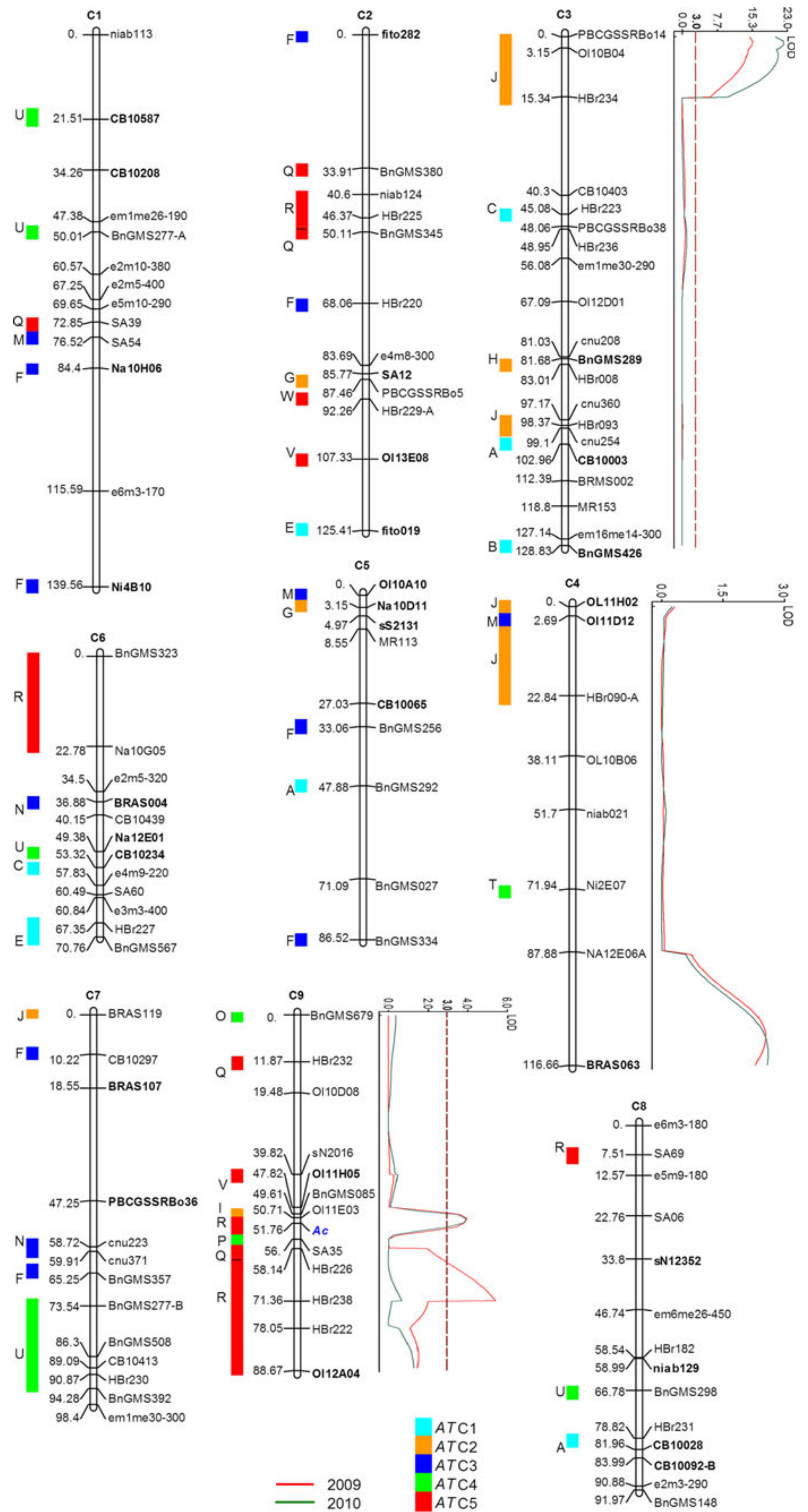

Table 1 Characterization of the linkage groups of *B. carinata* constructed with the YW population

Linkage group[a]	Length (cM)	Number of loci		Conserved building blocks[b]
		Total	Anchored markers	
B1	63.1	16	0	**F, f** (F, Q, D, F)[c]
B2	83.1	15	1	e, r (E, R, U)
B3	105	8	0	j, **w, R** (O, J, W, R)
B4	96.6	9	1	n, j (N, J)
B5	67.8	10	0	**F**, **J** (F, J)
B6	115	14	1	a (D, A, B, C)
B7	131.3	16	1	E (A, B, U, E)
B8	93.9	12	0	**R, U** (G, H, I, R, U)
C1	139.6	13	4	u, f (U, F)
C2	125.4	12	4	R, w, q (R, W, Q, X)
C3	128.8	20	3	J (R, W, J, F, U)
C4	116.7	8	3	J (J, I, J)
C5	86.5	9	4	a, f (A, F)
C6	70.8	12	3	c, E (C, E)
C7	98.4	13	2	U (H, K, Q, U)
C8	92	14	4	u, a (C, U, A, N, A)
C9	88.7	13	2	o, q, v, p, R (O, Q, X, D, V, P, W, R)
Total	1,702.5	214	28	13 (34)[d]

[a] The nomenclature for linkage groups corresponds with the designation of each linkage group in the B genome of *B. juncea* (Panjabi et al. 2008; Ramchiary et al. 2007) and the C genome of *B. napus* (Cheng et al. 2009; Qiu et al. 2006; Wang et al. 2011), respectively

[b] The conserved blocks highlighted in boldface were used to assign the linkage groups to equivalent linkage groups in the B genome of *B. juncea* and the C genome of *B. napus*

[c] Letters in brackets are conserved building blocks identified in equivalent linkage groups in the B genome of *B. juncea* and the C genome of *B. napus* reported previously (Panjabi et al. 2008)

[d] The number in brackets is the sum of the numbers of conserved blocks and islands

Table 2 QTL for seed coat colour in the YW doubled haploid mapping population

QTL code	Linkage group	Year of trial	Confidence interval (cM)	Additive effect	Variation (R^2)	LOD score
qSC1	C3	2009	0–3.2	1.06	0.27	15.48
		2010	0–3.0	1.29	0.37	22.95
qSC2	C4	2009	96.7–114.9	0.48	0.05	2.55
		2010	98.4–114.9	0.42	0.04	2.61
qSC3	C9	2009	49.3–53.7	0.54	0.06	3.95
		2010	49.4–52.8	0.52	0.05	3.90
qSC4	C9	2009	66.3–73.2	0.61	0.09	5.4

Discussion

A DH mapping population of *B. carinata*, designated the YW population, was developed in the present study. Two DH lines with distinct morphological phenotypes were used to generate the mapping population, namely BcDH64 (with yellow petals, a red spot on the anther tip, and yellow seeds) and BcDH76 (with white petals, a purple spot on the anther tip, and brown seeds). The distinct niches and geographical isolation of the two accessions were reflected in their genetic dissimilarity, as indicated in the dendrogram for the 110 accessions of *B. carinata* (Fig. 1 and Supplementary Fig. 2). A complete linkage map of *B. carinata* was constructed with the YW population, and loci that controlled three pigment-related traits (two qualitative and one quantitative) were identified in the map. Each linkage group in the map was assigned to its homologous chromosome of the B and C genomes of other *Brassica* species. The map could be used as a guide for the transfer of genetic and genomic information from

well-studied *Brassica* species (e.g. *B. napus* and *B. oleracea*) to *B. carinata*, which would help to obtain a better understanding of the genetic structure of *B. carinata*. Moreover, the paternal parents of the two DH lines (CGN03964 and CGN03976) showed higher and lower interspecific crossability (Jiang et al. 2007), respectively. BcDH64 and BcDH76 also showed a similarly divergent compatibility when crossed with *B. rapa* (data not shown). The identification of interspecific compatibility in DH lines derived from the YW population in the future would be very useful for the transfer of genes between the A, B, and C genomes for the purpose of increasing the genetic diversity of *Brassica* crop species.

Despite the strong selection of divergent parents in developing the mapping population, the intrinsically limited genetic variation within *B. carinata* as compared with other *Brassica* species (Warwick et al. 2006), as observed in the present study (Fig. 1a), still made it difficult to construct a high-density linkage map. We surveyed a total of 1,685 SSR primer pairs, of which only 10.6 % showed polymorphism between the two parents of the YW population, and only 153 markers (8.16 %) were mapped on the YW linkage map. With the rapid development of next-generation sequencing (Niedringhaus et al. 2011), a higher-density genetic map could be obtained in the near future by using high-throughput marker technology, e.g. diversity arrays technology (DArT) (Raman et al. 2012; Rodríguez-Suárez et al. 2011), Infinium (Peiffer et al. 2006) or genotyping by sequencing (Varshney et al. 2009). Therefore, the YW mapping population, together with the two DH parents, would provide a valuable resource for further genetic studies on *B. carinata* and for genome sequencing of that species.

The first sequenced plant species *A. thaliana* shares a common ancestor with the family Brassicaceae and provides a good opportunity to understanding of the genome structure and evolution of this family (Arabidopsis Genome Iniative 2000). Previous comparative mapping studies showed that the *Brassica* crops including the three diploid species *B. rapa*, *B. nigra* and *B. oleracea* had undergone triplication since their divergence from *A. thaliana* about 20 million years ago (Lysak et al. 2005; Yang et al. 1999). The triplicate genome variations and rearrangements might contribute the morphological plasticity of *Brassica* species (The *Brassica rapa* Genome Sequencing Project Consortium 2011). The more recent comparative analysis across the three *Brassica* genomes suggested a high level of chromosomal colinearity. In this study, the segmental organization of *B. carinata* was analysed by 24 conserved At genomic blocks. The order of the blocks in *B. carinata* genome was compared and proved to be consistent with each corresponding linkage group of the available maps of *B. nigra*, *B. juncea*, *B. olercea* and *B. napus* (Panjabi et al. 2008; Parkin 2011). Our mapping study revealed that the R block showed obvious triplication in both B genome (B2, B3 and B8) and C genome (C2, C6 and C9), which provide further evidence of genome triplication from *B. carinata*. The genome structure and organization could be further revealed among different *Brassica* species in U's triangle when more informative linkage maps are available.

Brown seeds are common in *Brassica* oilseed crops. However, in *B. rapa*, yellow-seeded cultivars show a higher oil content than brown-seeded cultivars (Jönsson 1978). Genetic and anatomical studies in *B. rapa*, *B. juncea*, and *B. carinata* showed that, compared with brown-seeded genotypes, yellow-seeded genotypes usually contain a lower content of pigment in the seed coat, a thinner seed shell, and lower fibre content. Consequently, yellow-seeded rapeseed yields brighter crude oil and seed meal with a higher protein content and lower fibre content (Getinet et al. 1996; Stringam et al. 1974). In *B. napus*, an oil crop that is cultivated worldwide, the seeds are black or brown. No naturally occurring yellow-seeded germplasm exists in *B. napus*, although it is the most important *Brassica* oilseed crop. However, yellow-seeded *B. napus* was much more unstable and more likely affected by environmental factors such as temperature and had no advantage in seed retention (Deynze et al. 1993; Tang et al. 1997). Currently, there is no oilseed rapeseed with yellow seeds being widely used, although yellow-seeded cultivars have been developed through introgression with related yellow-seeded *Brassica* crops and show increased oil content (Piotrowska et al. 2003).

The colour of the seed coat is a quantitative trait in yellow-seeded *Brassica*. Three QTL that control the yellow-seed character in *B. juncea* were identified in the A6, A10, and B4 linkage groups (Mahmood et al. 2005). Padmaja et al. (2005) identified three SSR markers that showed a significant association with yellow-seed colour in *B. juncea*, of which one marker, Ni4F11, was anchored on the B3 linkage group. A major QTL for yellow seeds was mapped on the N18 (C8) linkage group in a population of *B. napus* in which the yellow-seed trait was introgressed from *B. rapa* (Badani et al. 2006). Jiana Li's research group developed three mapping populations with *B. napus* as a common parent, into which the yellow-seed trait was introduced from *B. juncea* (Fu et al. 2007; Yan et al. 2009). QTL for yellow-seed colour were identified on three linkage groups of the A genome (A5, A6, and A9) in one of the populations and on the N4 (A4), N8 (A8), N12 (C2), and N16 (C6) linkage groups in another population (Fu et al. 2007), and on the N4 (A4), N8 (A8), and N16 (C6) in the third population (Yan et al. 2009). None of these QTL that were reported previously was identical to linkage groups C3, C4, and C9, in which three SL-QTL and one MR-QTL for the yellow-seed trait were detected in the YW

population. This finding suggests that the genes that regulate the yellow-seed trait in *B. carinata* are unique and divergent from those that regulate the trait in other *Brassica* species. However, some QTL controlling this trait were identified on linkage group B3, A4, B4, and A9. Then the QTL observed on C3, C4, and C9 in this study could be syntenic with the one of the other species. The precise comparison of the *Brassica* sequences will help to know whether the same genes are present in all these regions.

Considering that there are more than 40,000 protein coding genes in the A genome of *B. rapa* (The *Brassica rapa* Genome Sequencing Project Consortium 2011), many more genes should be found in the genomes of tetraploid of *B. carinata*. The genes that control the yellow-seed trait in *B. carinata* are only a tiny proportion of the B and C genomes, and it should be possible to identify many more valuable genes in the *B. carinata* genome that are associated with a wide range of agronomical traits.

In comparison with other *Brassica* species in U's triangle, *B. carinata* has received relatively little attention, but it does contain many potentially useful traits. The doubled haploid population in *B. carinata* and the genetic linkage map would be a very valuable addition to *Brassica* resources under the context of *B. carinata* as an important crop in some parts of the world and the source of elite alleles in *Brassica*.

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