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ORIGINAL PAPER

High-density *Brassica oleracea* linkage map: identification of useful new linkages

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Abstract We constructed a 1,257-marker, high-density genetic map of *Brassica oleracea* spanning 703 cM in nine linkage groups, designated LG1–LG9. It was developed in an F2 segregating population of 143 individuals obtained by crossing double haploid plants of broccoli "Early-Big" and cauliflower "An-Nan Early". These markers are randomly distributed throughout the map, which includes a total of 1,062 genomic SRAP markers, 155 cDNA SRAP markers, 26 SSR markers, 3 broccoli BAC end sequences and 11 known Brassica genes: *BoGSL-ALK*, *BoGSL-ELONG*, *BoGSL-PROa*, *BoGSL-PROb*, *BoCS-lyase*, *BoGS-OH*, *BoCYP79F1*, *BoS-GT* (glucosinolate pathway), *BoDM1* (resistance to downy mildew), *BoCALa, BoAP1a* (inflorescence architecture). *BoDM1* and *BoGSL-ELONG* are linked on LG 2 at 0.8 cM, making it possible to use the glucosinolate gene as a marker for the disease resistance gene. By QTL analysis, we found three segments involved in curd formation in cauliflower. The map was aligned to the C genome linkage groups and chromosomes of *B. oleracea* and *B. napus*, and anchored to the physical map of *A. thaliana*. This map adds over 1,000 new markers to *Brassica* molecular tools.

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Introduction

After the construction of the first substantial linkage map of *B. oleracea*, with isozyme loci, pioneered by Arus and Orton (1983), several others have followed using a variety of molecular markers (for review see Quiros 2000 and Quiros and Paterson 2004). Little effort was spent at that time to align these maps across laboratories and to perpetuate the mapping populations used for their construction. However, it was possible to assign some of the linkage groups to their respective chromosomes with alien addition lines (Hu and Quiros 1991; Heneen and Jorgensen 2001). Sebastian et al. (2000) established a consensus map for this species based on perpetuated individuals from two double haploid populations, (cauliflower × Brussels sprouts and broccoli × kale) constructed with 547 RFLP, AFLP and SSR markers . Its nine linkage groups have been now physically assigned to their respective chromosomes by Howell et al. (2002) using FISH. Furthermore, these chromosomes have now been aligned with the linkage groups for both the A and C genomes in *B. napus* by Bohuon et al. (1996), Lowe et al. (2004) and Piquemal et al. (2005). With the availability of EST sequences from Arabidopsis, these were used to construct several maps allowing partial comparison of the *A. thaliana* genome with the *B. oleracea* genome (Kowalski et al. 1994; Lan et al. 2000; Babula et al. 2003). This task was also accomplished by Li et al. (2003) using cDNA

polymorphisms to construct a linkage map in *B. oleracea*, followed by comparative physical mapping to *A. thaliana*. Parkin et al. (2005) has now aligned all linkage groups of *B. napus* to *A. thaliana* with RFLP markers.

We report the construction of a high-density genetic map based on the broccoli × cauliflower F2 population used by Li et al. (2003), adding various types of PCR-based markers and sequences of known genes. Each linkage group has been assigned to their respective chromosomes based on common markers with the Sebastian et al. (2000) and Piquemal et al. (2005) maps and to the chromosomes of *A. thaliana*. Further, the map was used to determine QTLs for curd formation, which segregates in this population. Assignment of the linkage groups of this map to their respective C genome chromosomes adds over 1,000 new markers as mapping tools for *B. oleracea* and *B. napus*. The contribution of a substantial amount of new markers from our map will increase the efficiency of marker-assisted selection and map-based gene cloning in *B. oleracea* and *B. napus*.

Materials and methods

F2 mapping population

To construct this map, we used the same F2 segregating population as that used by Li et al. (2003) to construct a transcriptome map based on cDNA-SRAPs in *B. oleracea*. It was developed by crossing two double haploid lines (broccoli "Early-Big" and cauliflower "An-Nan Early"), then selfing the F1 to make 143 F2 plants, which were used as parents to generate inbreds by single seed descent. In addition to the existing cDNA markers (Li et al. 2003), we added genomic SRAP markers, SSR markers, *B. oleracea* BAC clone sequences (B40L6, B59A4, B59C4) and sequences corresponding to 11 known *B. oleracea* genes.

Genetic markers

A total of 170 primer pairs, including 87 SRAP primers labeled with IRDye 800 or IRDye 700 fluorescent dyes combined with various unlabeled primers (Table 1), were used to amplify genomic DNA in the F2 population following the protocol of Li and Quiros (2001). The sequences of these primers have been published by Sun et al. (2007) (in press). The PCR products were run in 5% polyacrylamide with the Li-Cor Global IR2 4200 sequencing system.

Public SSR primer sequences were obtained from http://brassica.bbsrc.ac.uk/cgi-bin/ace/searches/browser/BrassicaDB and some from published papers (Sebastian et al. 2000; Smith and King 2000). A total of 50 SSR primer pairs were screened between two parents. Of these, 24 SSR primer

Table 1 Primer pairs for the SRAP markers used in this study. Primer sequences reported in Sun et al. (2007) (in press)

Marker[a]	Primer
m1–9	me2 + od4
m10–15	dc1 + od4
m16–22	me2 + odd2
m23–27	dc1 + od4
m28–34	em2 + od1
m35–39	em2 + od3
m40–44	em1 + od3
m45–51	em1 + od2
m52–55	dc1 + od2
m56–m59	em1 + od4
m60–64	dc1 + ga30
m65–75	me2 + od11
m76–85	em1 + ga30
m86–94	dc1 + od19
m95–102	em1 + od19
m103–109	em1 + od10
m110–115	dc1 + od15
m116–123	em1 + ga29
m124–125	me2 + od12
m126–129	em2 + od5
m130–134	em2 + od4
m135–138	dc1 + od20
m139–146	em1 + od20
m147–150	dc1 + od21
m151–155	em1 + od21
m156–162	em1 + od22
m163–165	me2 + od8
m166–173	me2 + od5
m174–185	em2 + od26
m186–195	me2 + od26
m196–199	em1 + od30
m200–201	em2 + od32
m202–207	dc1 + od33
m208–210	em1 + od33
m211–215	dc1 + od36
m216–221	dc1 + od35
m222–233	em1 + od35
m234–235	em2 + od20
m236–239	me2 + od15
m240–245	dc1 + od34
m246–256	em2 + od30
m257–264	dc1 + od30
m265–270	me2 + od30
m271–274	em2 + od14
m275–278	me2 + od32
m279–283	me2 + od23
m284–292	em2 + od23

Table 1 continued

Marker[a]	Primer
m293–303	em2 + od15
m304–307	me2 + ga2
m308–315	me2 + ga5
m316–322	me2 + ga6
m323–335	me2 + ga15
m336–341	me2 + ga19
m342–348	me2 + ga22
m349–356	me2 + ga27
m357–363	me2 + ga31
m364–367	me2 + ga32
m368–372	me2 + ga39
m373–381	me2 + ga41
m382–387	me2 + ga42
m388–396	me2 + ga45
m397–398	me2 + sa4
m399–406	me8 + sa7
m407–416	me8 + ga3
m417–425	s12 + pm5
m426–436	ga5 + pm1
m437–446	s12 + pm4
m447–456	od3 + pm5
m457–466	od3 + pm3
m467–481	s12 + pm3
m482–501	s12 + pm1
m502–513	od3 + pm1
m514–519	em1 + od50
m520–523	dc1 + od54
m524–528	dc1 + ga6
m529–532	em1 + od51
m533–540	dc1 + ga22
m541–542	dc1 + od55
m543–544	em1 + od46
m545–551	em1 + od45
m552–555	em1 + od43
m556–570	ga3 + pm1
m571–587	ga3 + pm3
m588–600	ga3 + pm6
m601–611	ga5 + ce12
m612–626	od3 + ce7
m627–635	od3 + ce12
m636–652	o15 + ce12
m653–667	o26 + ce12
m668–681	od26 + pm1
m682–698	od26 + pm5
m699–709	od26 + pm6
m710–729	s12 + pm1
m730–751	s12 + ce7
m752–766	s12 + ce10

Table 1 continued

Marker[a]	Primer
m767–786	s12 + ce12
m787–797	em1 + ga3
m798–820	em1 + ga5
m821–830	em1 + ga6
m831–832	em1 + od24
m833–839	em1 + od38
m840–847	em1 + od39
m848–853	em1 + od40
m854–858	dc1 + ga3
m859–863	dc1 + od54
m864–868	dc1 + od37
m869–876	dc1 + od38
m877–883	dc1 + od39
m884–886	dc1 + od43
m887–894	dc1 + od48
m895–898	dc1 + od49
m899–901	dc1 + ga2
m902–906	dc1 + od40
m907–910	em1 + ga10
m911–918	od8 + pm1
m919–922	ga3 + ce12
m923–927	od8 + ce7
m928–934	od8 + pm4
m935–944	od8 + ce8
m945–949	od8 + ce9
m950–962	o15 + pm1
m963–972	od15 + ce9
m973–979	od26 + sa1
m980–987	od26 + o3
m988–995	s14 + pm3
M996–1005	s14 + c8
M1006–1014	s17 + ga1
M1015–1018	s17 + ga2
M1019–1026	me2 + s9
M1027–1038	me2 + s16
M1039–1045	me8 + s14
M1046–1049	me8 + s15
M1050–1061	em1 + od52
S1–11	dc1 + odd15
S12–20	dc1 + Me2
S21–26	dc1 + Mc4
S27–33	dc1 + odd10
S34–40	dc1 + Mc5
S41–42	Me2 + eM8
S43–53	eM2 + odd15
S54–58	Me8 + odd15
S59–73	dc1 + Mc7
S74–90	eM2 + fc3

Table 1 continued

Marker[a]	Primer
S91–96	eM2 + Mc5
S97–106	dc1 + Me10
S107–110	dc1 + Me1
S111–117	dc1 + fc8
S118–120	dc1 + odd4
S121–127	dc1 + Me8
S128–137	dc1 + Me9
S138–142	dc1 + ga30
S143–154	dc1 + odd30
S155–165	eM1 + ga23
S166–170	eM1 + dc1
S171–172	eM2 + Me9
S173	eM1 + fc3
S174–176	eM2 + Me8
S177–192	dc1 + ga23
S193–202	eM2 + ga23
S203–212	eM2 + ga30
S213–217	eM2 + eM8
S218–221	eM2 + Mc7
S222–228	eM2 + Me2
S229–233	Me8 + ga23
S234–238	Me8 + odd30
S239–246	Me2 + odd30
S250	dc1 + ga29
S251–252	eM2 + odd30
S253–254	Me2 + eM7
S255–260	Me2 + ga23

[a] Marker gel migration and primer sequences provided as supplementary information

pairs showing polymorphism between two parents were run in the F2 population.

Map construction

The map was constructed with the program Joinmap 3.0 (LOD score from 4.0 to 8.0). SRAP markers from genomic DNA were developed for this map (starting with M or S on the actual map). These were combined with 155 cDNA SRAP markers (Li et al. 2003), 26 SSR markers (starting with OL on the map, or named NGA248, LS107, sORA21b, MB4), three BAC end sequences: B40L6 (corresponding to *A. thaliana* At5g23400), B59A4 (At2g03240), and B59C4 (At4g29905) and 11 *B. oleracea* genes as follows: glucosinolate pathway: *BoGSL-ALK, BoGSL-ELONG, BoGSL-PROa , BoGSL-PROb, BoCS-lyase, BoGS-OH, BoCYP79F1, BoS-GT*; resistance to cotyledon stage downy mildew: *BoDM1*; and inflorescence development: *BoCAL* ,and *BoAP1*. Primer sequences used to map these genes are shown in Table 2.

Table 2 Primer sequences for 11 genes included in the map

Gene	Left primer	Sequence	Right primer	Sequence	Resource
BoGSL-ELONG	IPM2	5′-GTG ACG GTG AAC AAT CTC C-3′	IPM9	5′-GTA GTA TTC TCA AAA TCT TGT-3′	Li et al. 2001
BoGSL-ALK	odd48	5′-TTC CAT CAT TTA CTT TCT CAG-3′	ODD12	5′-TTG AAT ATC CAG TGT AAG GTT-3′	Li et al. 2003
BoGSL-PROa	PM87	5′-AGA AGG GTG GTG ATT GTT G-3′	PM132	5′-ACGCATTGTCAGAATGATCT-3′	Gao et al. sequence 2005
BoGSL-PRO-b	PL132	5′-ACGCATTGTCAGAATGATCT-3′	PL581	5′-GTCATAACAATGTGCCGAGT-3′	Gao et al. 2005
BoCS-lyase	LS360F	5′-CGGCAAAAGCAATTCTTAC-3′	LS2230R	5′-CCACTATCCCGACACTATCA-3′	This paper
BoGS-OH	OH621F	5′-GGTACGAACAAGGCTTCTCT-3′	OH1274R	5′-CGGAGTTGAAGAGGAAAACT-3′	This paper
BoCYP79F1	CY713F	5′-TAGGACAAGCGGAGAAAGAT-3′	CY2000r	5′-TTCAGTTTCGACCAGAGAAA-3′	This paper
BoS-GT	GT435F	5′-CACCGTTTGCTCTGTTCTAC-3′	GT1028R	5′-AAAAACCACTGACTTGCTCA-3′	This paper
BoCAL	CALSSRF	5′-GTTAAGTGTGGCGTTAGAGG-3′	CALSSRR	5′-CCTTGGTACATGCCACTGAA-3′	Smith and King 2000
BoAP1	AP1F	5′-AAAGGAGAAAGCCATACAGG-3′	AP1R	5′-AACGTACATTTGCCGAACTA-3′	From cDNA sequence
BoDM1	DM1-F	5′-CTGGTGAATGCCCGCTCT -3′	DM1-R	5′-AAGCGATCAAAGCGGG-3′	This paper
BAC59A	BAC59A4F	5′- TCAACGTGTTAGACCAAAGT TCT-3			This paper
BAC59C4F	BAC59C4F	5′-AATACAAGACGAGACGGCG-3′	BAC59C4R	5′-CCAGAAGCAGGGACAAGT-3′	This paper
B40L6	LA3	5′-TATATGGCTTGGGCAACGA-3′	LA4	5′-GATGCTCTCCTTCTTGTGA-3′	This paper

Alignment of our map to existing maps and assignment of linkage groups to specific chromosomes

A total of 26 SSR markers and 77 SRAP markers were compared with the current *B. napus* and *B. oleracea* maps (Sebastian et al. 2000; Howell et al. 2002; Lowe et al. 2004; Piquemal et al. 2005; Qiu et al. 2006). Following the rationale of Li et al. (2003), 155 cDNA markers, 11 known gene sequences and three BAC-end sequences were use for alignment with *Arabidopsis* chromosomes.

QTL mapping of curd phenotype

We visually scored each of the F2 plants in the greenhouse and F3 families in the field and greenhouse for inflorescence type in three major classes: 1 = broccoli-like, 2 = intermediate and 3 = cauliflower-like based on the scoring system of Labate et al. (2006). QTL determination was carried out by composite interval mapping with the software WinQTLCart 2.0 (Zeng. 1994). The threshold LOD of 2.50 was selected based on a 5% significance level determined by 300 permutations.

Results

Construction of the map

A 1,257-marker high-density genetic map of *Brassica oleracea* was constructed and spanned 703 cM in nine linkage groups designated LG1–LG9. Most of these markers were randomly distributed throughout the map (Fig. 1). It included a total of 1,062 genomic SRAP dominant markers generated with the 170 primer pairs. On an average, 6.2 SRAP polymorphic markers were produced per primer pair. All 155 cDNA SRAP markers produced by Li et al. (2003) in the same population were integrated into the nine linkage groups of this map. Of the 50 SSR primer pairs screened between two parents, only 24 showed polymorphism. The 24 SSR primer pairs found to be polymorphic between the two parents generated 26 SSR markers. Of these, 26 markers were mapped into the nine linkage groups.

The eight glucosinolate genes, *BoDM1, BoCAL* and *BoAP1* and three BAC clone end sequences were scored as co-dominant markers. *BoGSL-ELONG* and *BoDM1* were mapped on BoLG2, 0.84 cM apart. Giovanelli et al. (2002) reported RAPD marker OMP-750 linked at 3 cM to *BoDM1* in *B. oleracea.* After sequencing of this marker, we found conservation of the chromosome segment containing *BoGSL-ELONG* and OMP-750 in *A. thaliana*. The OMP-750 Arabidopsis homolog is in BAC MOP9 and is 53 Kb apart from At5 g23020, which corresponds to the *BoGSL-ELONG* gene (Gao et al. 2005) (Fig. 2). Downstream from the *BoGSL-ELONG* ortholog, there is a putative disease resistance gene, At5 g23400. Screening of the *B. oleracea* "Early Big" BAC library produced clone B57M17 harboring the gene corresponding to At5 g23400. Sequencing of this gene in *B. oleracea* revealed that it has 1,764 bases, it is intronless and has 86% identity with At5 g23400 (data not shown). A marker developed from sequencing the *Brassica* homolog to this gene on this BAC clone, co-segregated with *BoGSL-ELONG*, revealed further conservation of the chromosomal segment in both species. The mapping progeny did not segregate for downy mildew resistance, therefore it was not possible to confirm whether the At5 g23400 homolog is the true gene for *BoDM1*. Genes *BoCAL* and *BoS-GT* mapped on BoLG3, *BoGSLPROa*, *BoGSL-PROb* (previously named *BoGSL-PRO* and *BoGSL-PROL*, respectively; (Gao et al. 2006) and *BoCYP79F1* mapped on BoLG5. The first two genes were only 0.027 cM apart. They are duplicated gene members of the *MAM* (methylthioalkylmalate synthase) gene family (Gao et al. 2006). *BoAP1-a* and *BoCS-lyase* mapped on BoLG 6. *BoGSL-ALK* and *BoGS-OH* mapped on BoLG9 5.4 cM apart. These two genes are members of the *AOP* (2- oxoacid-dependent dioxygenases) family. The latter corresponds to the *A. thaliana* ortholog (at2 g25450) and has not been previously mapped on Brassica.

Alignments to C genome linkage groups from other *B. oleracea* and *B. napus* maps

Using 22 common SSR markers, we were able to align the linkage groups of our map to the genome specific groups of *B. oleracea* and *B. napus.* The nine linkage groups BoLG1–BoLG9 on our map are equivalent to the *B. oleracea* linkage groups O1–O9 (Sebastian et al. 2000; Howell et al. 2002) and *B. napus* linkage group N11–N19, respectively (Bohuon et al. 1996; Lowe et al. 2004; Piquemal et al. 2005) (Table 3). Of the genomic SRAP markers in our map, 77 had also been included in an ultradense *B. napus* map constructed by Sun et al. (2007) (in press). Thus, it was possible to align both maps, confirming our previous assignment of our linkage groups to the N11 through N19 standardized groups.

Based on the physical assignment of the linkage groups in the map developed by Sebastian et al. (2000) and Howell et al. (2002), our linkage groups BoLG1–BoLG9 are analogous to their linkage groups, which corresponds to the C genome chromosomes 8, 5, 1, 2, 6, 9, 4, 7 and 3, respectively (Fig. 1).

Alignment of *B. oleracea* linkage groups and the *Arabidopsis thaliana* physical map

Our map was compared to the Arabidopsis physical map with 11 gene sequences, three BAC-end sequences, and

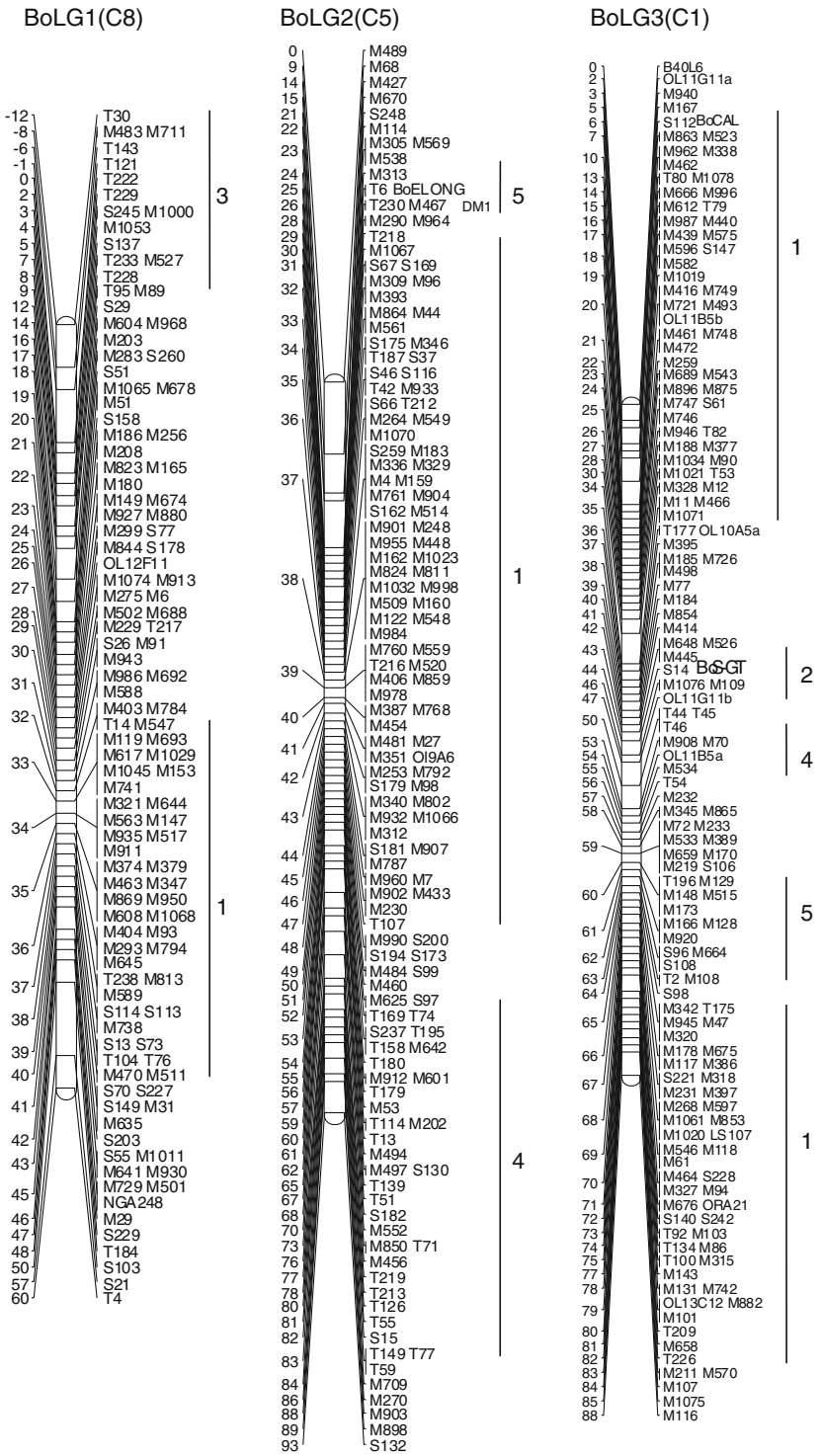


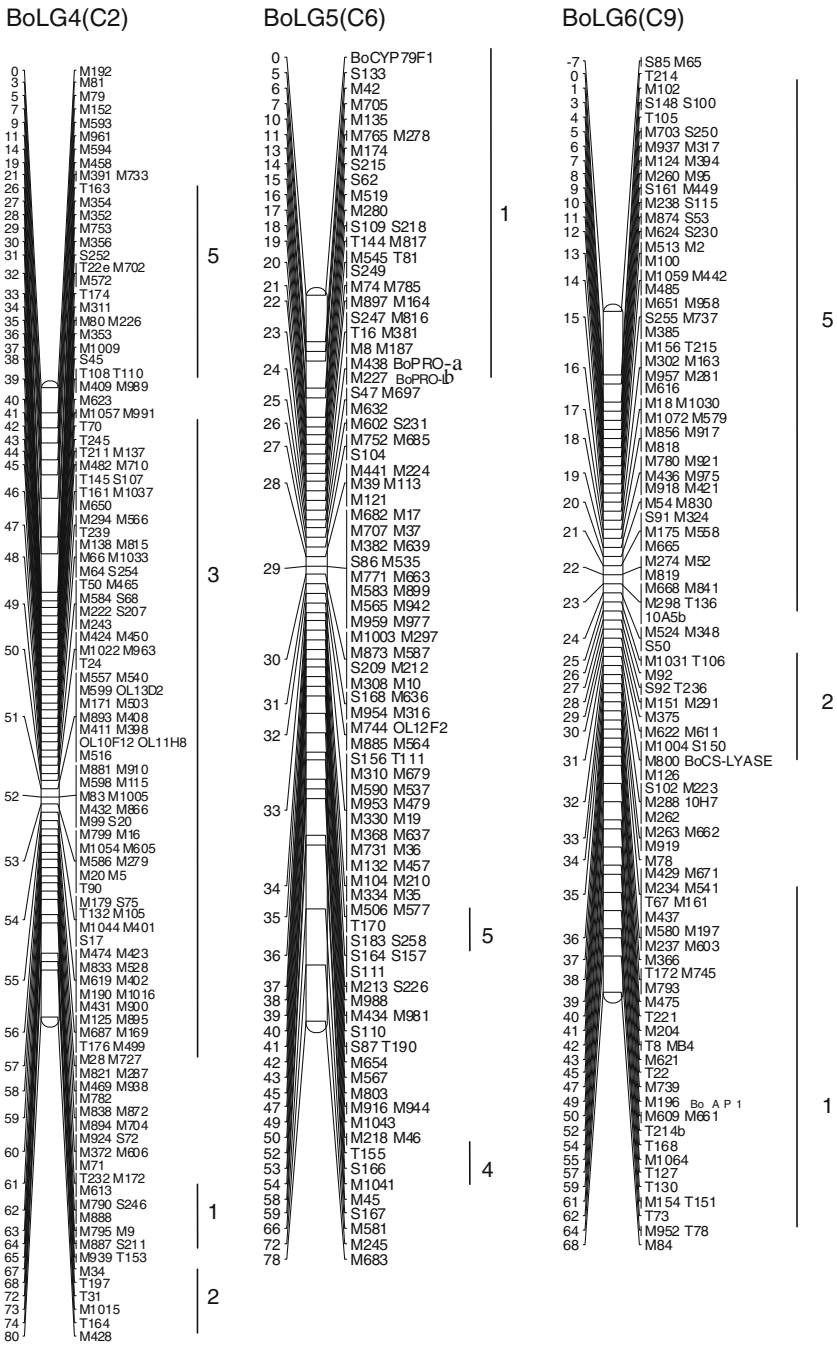
Fig. 1 Linkage groups LG1–LG9 for the *B. oleracea* map and their correspondence to the C genome chromosomes (C1–C9). cM are shown on the *left side*. *Bars* on the *right* indicate homologous segments to chromosomes 1–5 of *A. thaliana*

155 cDNA markers. As expected , the alignment between the *B. oleracea* linkage groups and chromosomes of *A. thaliana* (Fig. 1) fully agrees with that reported by Li et al. (2003). However, there was only partial agreement with the alignment reported for the C genome chromosomes of *B. napus* and *A. thaliana* chromosomes by Parkin et al. (2005). Lack of total agreement between the two reports is not unexpected considering that none of the two maps are complete.

QTL mapping of curd phenotype

The frequency distribution for inflorescence type, characterized as broccoli-like versus exhibiting curd formation in

Fig. 1 continued

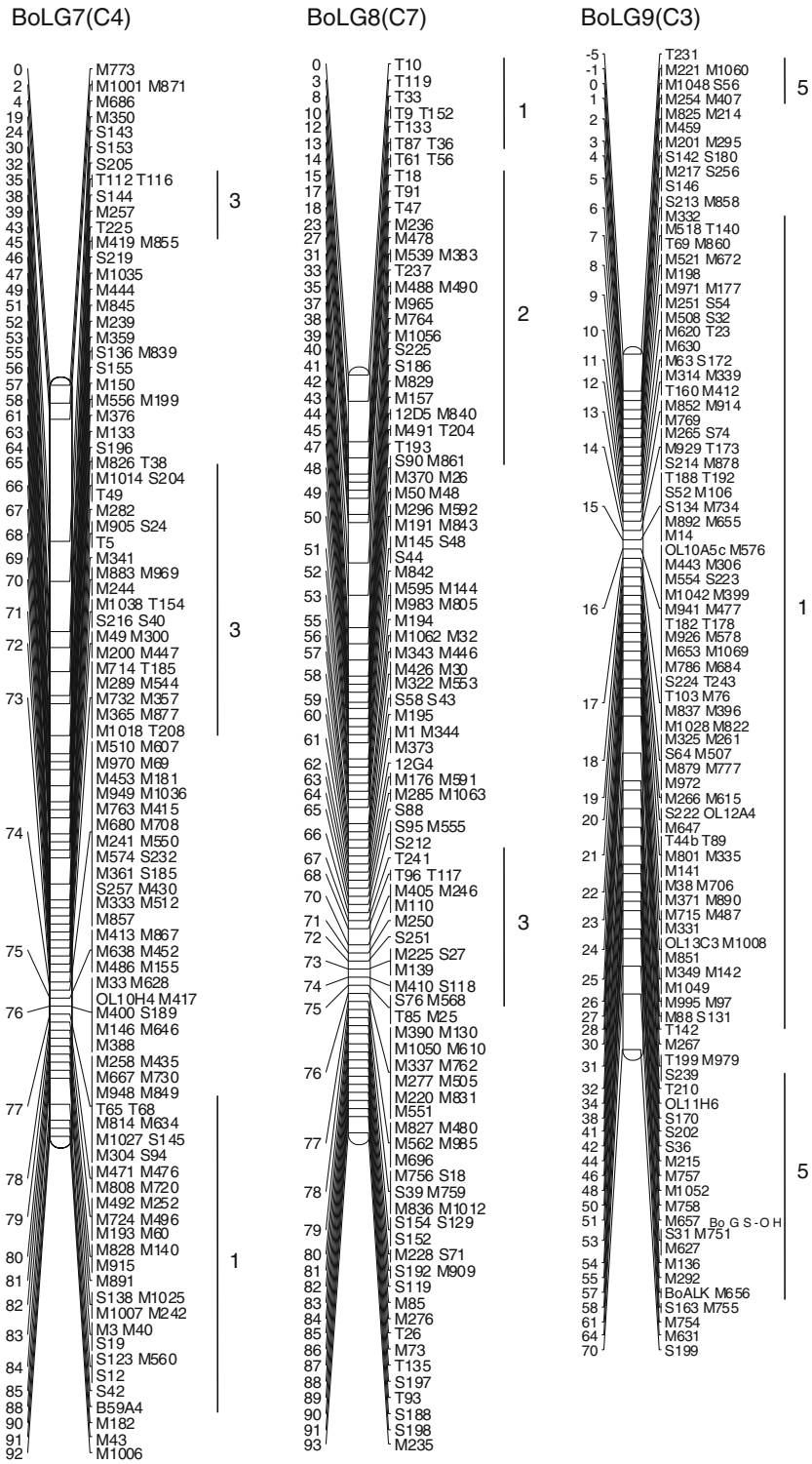


the mapping populations is shown in Fig. 3. Although in general it followed a bell-shaped curve, it was skewed toward the broccoli phenotype. Three chromosomal regions for curd formation were detected in this population by statistically significant QTLs. Two QTLs in BoLG1 explained 21 and 6% of the variation, respectively. These two were 17 cM apart with non-overlapping confidence intervals. The third QTL was on BoLG6 associated with *BoAP1-a* and explained 15% variation. No QTLs were detected in the linkage group regions containing the *BoCAL-a* and *BoGSL-ELONG* genes (Table 4).

Discussion

The number of markers, linkage group coverage and density reported in our map and its alignment to the *B. oleracea* maps of Sebastian et al. (2000) and Howell et al. (2002), and to the *B. napus* maps of Lowe et al. (2004), Piquemal et al. (2005), Parkin et al. (2003, 2005), Qiu et al. (2006) and Sun et al. (2007) (in press) add a significant number of markers to the C genome useful for marker assisted selection and map-based cloning in both species. Basically, we have added 1,257 markers to the *B. oleracea* maps

Fig. 1 continued



(Table 3). The level of polymorphism of genomic SRAP markers between broccoli and cauliflower is high, so similar levels are expected between other more divergent *Brassica* crops, such as broccoli and kale (Li and Quiros 2001). Although most of these markers are dominant, they could be quite efficient for marker-assisted selection when associated in repulsion phase to genes targeted for selection (Haley et al. 1994). Regarding the usefulness of a high-marker density map for map-based-cloning, it can be estimated based on genome size and map length, that 1 cM corresponds to approximately 800 Kb in *B. oleracea*. However, this value must be taken conservatively considering the variation in density along the chromosomes. An example of this is the positional cloning of gene *BoGSL-ALK*

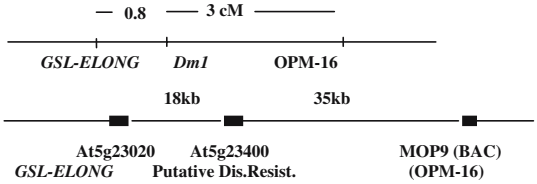


Fig. 2 Maps for *BoGSL-ELONG* and *BoDM1*. *Top*, genetic map; *bottom*, physical map.OPM-750 is RAPD marker reported by Giovannelli et al. (2002)

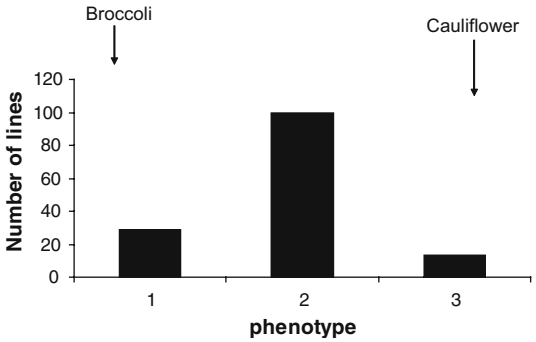


Fig. 3 Histogram showing phenotype distribution for inflorescence type in F2 mapping population. (phenotype 1 = broccoli; phenotype 2 = intermediate; phenotype 3 = cauliflower)

tagged with a marker at 1.4 cM, but at a physical distance of less than 100 Kb (Li and Quiros 2003).

Further, anchoring this map to the *A. thaliana* physical map is an important asset because the latter could serve as a useful source of additional markers to saturate specific segments carrying a gene(s) of interest in *B. oleracea*. Discrepancies of alignment between the C genome chromosomes of *B. oleracea* and *B. napus* might reflect chromosomal structural changes during alloploidization and stabilization of *B. napus* (Song et al. 1995).

Several linkages described in this paper are of particular interest. For the downy mildew resistance gene *BoDM1*, we populated the BoLG 2 region with three markers including the gene *BoGSL-ELONG*. Some of these markers should prove more useful than others previously described (Giovannelli et al. 2002) for marker-assisted selection to develop cotyledon stage downy mildew resistance. Another interesting linkage on BoLG9 was for the *AOP* gene family members *BoGSL-ALK* and *BoGSL-OH*. These genes act sequentially in the side chain modification of aliphatic glucosinolates, the first directing desaturation to produce alkenyl glucosinolates and the second one their subsequent hydroxylation (Li and Quiros 2003). In *A. thaliana*, there are three *AOP* genes in triplicate, *GS-OH (AOP3), GS-ALK (AOP2)* and *AOP1* (unknown function) (Gao et al. 2004). Similar to *A. thaliana*, in *B. oleracea*, *BoGSL-ALK* and *BoAOP1* are next to each other, but both are duplicated in tandem and the sequence corresponding to the gene *GS-OH* is absent (Gao et al. 2004). Evidently in *B. oleracea*, although genes *BoGSL-OH* and *BoGSL-ALK* lay on the same chromosome they are not contiguous as in *A. thaliana*. Another conserved linkage is between the *MAM* gene family members *BoGSL-PROa* and its duplicate *BoGSL-PROb* involved in the synthesis of 3 carbon side chain glucosinolates (Gao et al. 2006). These are homologs of the same *Arabidopsis* gene (At1 g18500) located at the top of chromosome 1.

Among the glucosinolate pathway genes that we mapped, *BoGSL-ALK*, *BoGSL-ELONG* and *BoGSL-PRO*

Table 3 Marker statistics for *B. oleracea* map and number of aligned markers with other genetic maps

General information						Aligned markers				
Linkage group	SRAP(M,S)	CDNA marker(T)	SSR	Markers for genes and BACs	Total	An integrated map[a]	Total	Aligned SSR[b]	Aligned SRAP[c]	Total
BoLG1	101	15	2	0	118	46	164	2	4	6
BoLG2	114	26	1	2	143	74	217	1	5	6
BoLG3	127	17	7	3	154	101	255	5	16	21
BoLG4	130	21	4	0	155	56	211	4	12	16
BoLG5	119	7	1	3	130	64	194	1	20	21
BoLG6	111	18	3	2	134	42	176	2	1	3
BoLG7	129	11	2	2	144	55	199	2	5	7
BoLG8	106	23	2	0	131	47	178	2	4	6
BoLG9	125	17	4	2	148	64	212	3	10	13
Total	1062	155	26	14	1257	549	1806	22	77	99

[a] Markers in *B. oleracea* map http://www.brassica.bbsrc.ac.uk/, based on Sebastian et al. (2000)

[b] To *B. napus* maps of Lowe et al. (2004); Piquemal et al. (2005) and Qiu et al. (2006)

[c] To *B. napus* map of Sun et al. (2007) (in press)

Table 4 Chromosomal location of segments involved in curd development detected by QTL analysis

	Linkage group	Distance	Confidence interval	Linked marker	LOD	Variation (%)
QTL-1	LG1	21.36 cM	15–26 cM	M688	10.98	21
QTL-2	LG1	38.61 cM	35– 43 cM	S203	4.32	6
QTL-3	LG6	57.19 cM	54–62 cM	*BoAP1*	5.30	15

have been previously cloned and their function assessed (Gao et al. 2003, 2004, 2006; Li and Quiros 2002, 2003). For the rest of the genes, we only mapped their sequences. Although there were enough differences in their sequences in both parental plants for each of these genes to follow their segregation in the progeny, we could not follow segregation for their glucosinolate phenotype. This was due to the fact that the phenotypes of these genes are based more on glucosinolate amount and not quality, and the broccoli and cauliflower parents of our population have similar amounts for most of the glucosinolates controlled by these genes. The only exception was for gene *BoGSL-OH*, whose phenotype is presence/absence of the glucosinolate progoitrin. As expected, a major QTL for the presence of this glucosinolate was found in the map location for gene *BoGSL-OH* on LG 9 (data not shown). In any case, the sequences and flanking markers of the genes that could not be associated with specific glucosinolate segregation in our mapping population could be used in other populations, segregating for glucosinolate amount and composition. Application of marker-assisted selection in these populations will be helpful for the development of plants with specific glucosinolate profiles and content.

Although the intention of this paper was not to perform an exhaustive QTL analysis for curd formation, as was reported by Lan and Paterson (2000), we took advantage of the fact that we used two double haploid plants as the parents of the mapping population to do a general analysis to compare with the results from previous studies. Our phenotypic analysis involved only visual scoring for inflorescence type and did not include detailed measurements as done by Lan and Paterson (2000). The three chromosome segments detected by QTL analysis in our population explain 42% of the total phenotypic variation for inflorescence type. Two of these segments were on LG1, approximately 17 cM apart, but their confidence intervals did not overlap, thus indicating their independence. The other segment was on LG6. Only the latter fell on a major gene predicted to be involved in curd formation in cauliflower, *BoAP-a1* (Smith and King 2000; Purugganan et al. 2000). The peak of this QTL is located at 57.19 cM and the *BoAP1* sequence is located at 56.8 cM on LG6, which is in agreement with previous reports indicating that this gene plays a role in inflorescence architecture. We did not find any association with the *BoCAL-a* sequence, which is another predicted gene of similar function (Smith and King 2000; Purugganan et al. 2000). Thus, our results agree with those of Labate et al. (2006), who found that the *BoCAL-a* gene actually provides very little contribution to the cauliflower phenotype. Additionally, Lan and Paterson (2000) detected at least 67 loci, distinguishing broccoli from cauliflower in a much more exhaustive analysis, including not only of curd morphology, but also of size, shape and other related traits. Thus, it is clear from Lan and Paterson (2000), Labate et al. (2006) and from our study that additional genes must be involved in curd development.

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