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A Sequence-Tagged Linkage Map of *Brassica rapa*

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ABSTRACT

A detailed genetic linkage map of *Brassica rapa* has been constructed containing 545 sequence-tagged loci covering 1287 cM, with an average mapping interval of 2.4 cM. The loci were identified using a combination of 520 RFLP and 25 PCR-based markers. RFLP probes were derived from 359 *B. rapa* EST clones and amplification products of 11 *B. rapa* and 26 Arabidopsis. Including 21 SSR markers provided anchors to previously published linkage maps for *B. rapa* and *B. napus* and is followed as the referenced mapping of R1–R10. The sequence-tagged markers allowed interpretation of the pattern of chromosome duplications within the *B. rapa* genome and comparison with Arabidopsis. A total of 62 EST markers showing a single RFLP band were mapped through 10 linkage groups, indicating that these can be valuable anchoring markers for chromosome-based genome sequencing of *B. rapa*. Other RFLP probes gave rise to 2–5 loci, inferring that *B. rapa* genome duplication is a general phenomenon through 10 chromosomes. The map includes five loci of *FLC* paralogues, which represent the previously reported *BrFLC*-1, -2, -3, and -5 and additionally identified *BrFLC*3 paralogues derived from local segmental duplication on R3.

THE genus Brassica includes oilseed, vegetable, fodder, and condiment crops. *Brassica rapa* (syn. campestris; A genome), *B. napus* (AC genome), *B. juncea* (AB), and *B. carinata* (BC) contribute ∼12% of the global supply of edible vegetable oil (Labana and Gupta 1993). *B. rapa* and *B. oleracea* (C genome) provide many vegetables that contribute to a healthy human diet, being a valuable source of dietary fiber, vitamin C, and other health-enhancing factors such as anticancer compounds (Fahey and Talalay 1995). The Brassica A genome therefore has worldwide importance in agriculture, with the quality and economic value of derived products such as processed oils and kimchi being dependent upon appropriate combinations of alleles. *B. rapa* includes a variety of vegetables crops such as Chinese cabbage, Pakchoi, turnip, and broccoletto as well as oilseed crops such as turnip rape and sarson (Gomez-Campo 1999).

The high degree of neutral DNA polymorphisms of most Brassica species (Figdore *et al.* 1988) has facilitated the development of molecular linkage maps, with at least 15 described to date for *B. oleracea* (Slocum *et al.* 1990; Kianian and Quiros 1992; Lan *et al.* 2000), *B. rapa* (Song *et al.* 1991; Chyi *et al.* 1992; Teutenico and Osborn 1994), *B. nigra* (Lagercrantz and Lydiate 1996), *B. Juncea* (Cheung *et al.* 1997; Pradhan *et al.* 2003), and *B. napus* (Landry *et al.* 1991; Uzunova *et al.* 1995). Where common sets of DNA markers and/or parental genotypes have been used, it has been possible to designate linkage groups according to a common nomenclature (Parkin *et al.* 1995, 2005; Butruille *et al.* 1999; Sebastian *et al.* 2000). Thus for *B. napus* linkage groups N1–N10 representing the A genome correspond to *B. rapa* R1–R10, and linkage groups N11–N19 representing the C genome correspond to *B. oleracea* O1–O9. Bohuon *et al.* (1996) demonstrated that marker order and linkage group structure had been conserved between the diploid (*B. oleracea*) and amphidiploid (*B. napus*) C genomes. In this study, we generated a detailed linkage map using sequenced EST clones derived from tissue-specific libraries of *B. rapa*. To establish the identity of linkage groups corresponding to R1–R10, we used SSR markers from Suwabe *et al.* (2002) and Lowe *et al.* (2004).

The Brassica genomes are closely related to the model plant *Arabidopsis thaliana*, diverging ∼20 MYA (Koch *et al.* 2001), and remain collinear. Comparative mapping of RFLP probes among the three diploid species *B. rapa* ($n = 10$), *B. oleracea* ($n = 9$), and *B. nigra* ($n = 8$) has suggested that genomes of the Brassica species are composed of three rearranged variants of an ancestral genome and descended from a common hexaploid ancestor (Lagercrantz and Lydiate 1996). All comparative studies of Arabidopsis and Brassica to date have revealed extensive duplications, with Arabidopsis segments being conserved an average of three times within

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the diploid Brassica genomes (Truco *et al.* 1996; Lan *et al.* 2000; Lukens *et al.* 2003; Parkin *et al.* 2005). Fiber–FISH mapping has been used to compare a 431-kb Arabidopsis BAC contig with *B. rapa* mitotic chromosomes (Jackson *et al.* 2000). Cytogenetic study using 21 Brassicaceae species revealed that the tribe Brassiceae comprising ~240 species descended from a common hexaploid ancestor that has a similar genome to Arabidopsis (Lysak *et al.* 2005). Comparative genome analysis revealed that genes are reduced by deletion in the triplication blocks in the Brassica genome (O'Neill and Bancroft 2000; Rana *et al.* 2004; Park *et al.* 2005). Recently, we showed the sequence-level indel in four BAC clones that represent a triplicated and segmentally duplicated *FLC* region of *B. rapa* and are homologous with 125 kb of Arabidopsis chromosome 5 (Yang *et al.* 2006).

In this study we demonstrate the conservation of genome segments within and between chromosomes, on the basis of sequence-tagged markers.

MATERIALS AND METHODS

Population development and DNA extraction: $F_{2:3}$ families (40 F_3 seedling) of 134 F_2 lines ("JWF3p") were developed from Chinese cabbage F_1 cultivar Jangwon (*B. rapa* ssp. *pekinensis*). These two biennial inbred parent lines were made available courtesy of the former "Seoul Seed" company (Korea). To induce flowering, all seedling plants were vernalized for 35 days in a cold room at 5° with a 16-hr photoperiod. Tissues were collected from $F_{2:3}$ families after 15 days of growing in greenhouses. Genomic DNA was extracted from the lyophilized tissue following the method of Cho *et al.* (1994), using 1 ml saturated phenol (BM Co.) for every 1-g sample.

Gel electrophoresis and Southern blot analysis: To screen for polymorphisms, an average of 10 μg genomic DNA from the inbred parent lines was digested with seven restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Hin*dIII, *Eco*RV, *Xba*I, and *Sca*I) and fractionated on 0.9% agarose gels. Electrophoresis and Southern blotting were conducted as described by Cho *et al.* (1994). *Bam*HI, *Eco*RI, *Eco*RV, and *Sca*I enzymes were used for digestion of the segregating progeny populations.

EST clones used as RFLP probes: Four different tissue-specific libraries were used as a source of RFLP probe. These were prepared from mRNA isolated from immature flowers (BIF), anthers (BAN), roots (BR), and dark-grown seedlings (BDS) of *B. rapa* line Jangwon (Kim *et al.* 1996; Lim *et al.* 2000). Plasmid DNA preparation and nucleotide sequencing were conducted as described by Lim *et al.* (2000). We have used less redundant cDNA clones on the basis of their sequence and BLASTN search in GenBank (National Center for Biotechnology Information). Insert DNA was amplified by PCR using T7 and T3 primers and eluted by QIAGEN (Valencia, CA) gel extraction kits. Probe labeling was conducted by random hexamer labeled with ^{32}P-dCTP (Feinberg and Vogelstein 1983). Hybridization followed the method described by Cho *et al.* (1994). Hybridized filters were washed with three stringency steps (2×, 1×, and 0.5× SSC with 0.5, 0.1, and 0.1% SDS), respectively, and exposed to X-ray film (Fuji, Stamford, CT) for 2–3 days.

Genome sequence tag markers used as RFLP probes: Genome sequence tags (GSTs) representing 24 genes from Arabidopsis chromosomes 4 and 5 were generated by PCR amplication using Arabidopsis ecotype Columbia genomic DNA. The DNA were cloned and sequenced prior to use as RFLP probes. The 10 GSTs derived from Arabidopsis chromosome (chr)4 were At4RPP5 (At4g16860), At4ML1 (At4g21750), At4TR1 (At4g24520), At4CBF2a (At4g25480), At4PRHA (At4g29940), At4CPK5 (At4g35310), At4FAH1 (At4g36220), AtAP2 (At4g36920), At4HLS1 (At4g37580), and At4CESA2 (At4g39350). The other 14 GSTs derived from Arabidopsis chr5, At5HAT2 (At5g4730), At5COR78 (At5g52310), At5PDC2 (At5g54960), At5ILL1 (At5g56650), At5MSI1 (At5g58230), At5NPH3 (At5g64330), AtMYB68 (At5g6579), At5LCY (U50738), and 6 R-EST genes containing a cluster of NBS-LRR resistance recognition motif were used. Two flowering-time genes, *AtFCA* (At4g16280) and *AtLFY* (At5g61850) of Arabidopsis, were developed as probes. As for *B. rapa* genes, 6 flowering-time genes of *B. rapa BrFLC* (AY273164), *BrAGL20* (AY345237), *BrCO* (AY356370), *BrGI* (AY356369), *BrSVP* (AY356366), and *BrFLC*5 gene-specific PCR product (forward primer, 5′-TTACCGC CTCTTTTATCCTTCTC-3′; reverse primer, 5′-CATATAACA ACAAAAACCCCAATC-3′) were used in this genetic map. The 5 function genes of *B. rapa*, *BrGST*, *BrMyrosinase*, *BrSAM*, *BrSLP*, and *BrDFR*I, were surveyed. Characteristics of genetic markers are summarized in Table 1.

PCR-based genetic markers: Twenty-one previously developed SSR marker assays were selected on the basis of their ability to identify known A genome linkage groups in *B. napus* and *B. rapa* (Suwabe *et al.* 2002; Lowe *et al.* 2004). Two SSR markers were developed from BACs containing *BrFLC* sequences and one SSR marker was derived from BACs containing the *BrMAF* gene. One RAPD marker (operon primer S14) was included since it was codominant in this population.

Linkage analysis: Linkage analysis and map construction were carried out using JoinMap 3.0 (van Ooijen and Voorrips 2001). Segregating data were sorted according to locus order for each linkage group using MSExcel. This facilitated detection of errors associated with putative "double-recombinant" events and guided visual checking of original autoradiographs and revision of data points where these had been misscored or typed. All editing operations were recorded and are traceable. Linked loci were grouped on the basis of pairwise LOD values between 5 and 8, and centimorgan distances were estimated with the Kosambi mapping function (Kosambi 1944). Locus order within the LOD grouping was decided through an optimized algorithm using three rounds of linked markers. Multiple segregating loci detected by a probe were indicated by the addition of a suffix (-a, -b, -c, -d) to the locus names. Linkage maps were visualized using MapChart (Voorrips 2002) and PowerPoint.

RESULTS

EST marker characteristics: A total of 551 cDNA clones from four *B. rapa* tissue-specific EST libraries were screened to obtain informative RFLP markers. Of these 440 were polymorphic between the parents. A high degree of polymorphism with such markers has previously been reported within Brassica subspecies (Song *et al.* 1988). To obtain segregating genotypes among the progeny, four restriction enzymes were used, with *Eco*RV found to detect the highest level of polymorphism (Table 2).

The JWF3 *B.rapa* linkage map: This genetic map of *B. rapa* was generated on the basis of 545 markers, 520 RFLPs and 25 PCR-based markers assigned to 10 linkage

TABLE 1

Characteristics of genetic markers in this linkage map

Probe	Marker type	Source	No. of probes	No. of loci	Status	Reference
RFLP	EST_BIF	Immature flower library of *B. rapa* cv. Jangwon	114	157	pBluescript	Kim *et al.* (1996); Lim *et al.* (2000)
	EST_BAN	Anther library of *B. rapa* cv. Jangwon	176	235	pBluescript	
	EST_BDS	Dark-grown seeding library of *B. rapa* cv. Jangwon	45	55	pBluescript	
	EST_BR	Root library of *B. rapa* cv. Jangwon	24	27	pBluescript	
	At4GTS	Chromosome 4-located genes of Arabidopsis cv. Colombia	10	10	pGemT	GenBank
	At5GTS	Chromosome 5-located genes of Arabidopsis cv. Colombia	14	17	pGemT	GenBank
	AtGene	Full length of expressed gene from *A. thaliana*	2	2	pGemT	GenBank
	BrGene	Full length of expressed gene from *B.rapa*	11	17	pGemT	BrFLC_AY273164; BrAGL20_AY345237, etc.
PCR	SSR	R1–R10-located SSR markers	11	11	Primer	Lowe *et al.* (2004)
	SSR	R1–R10-located SSR markers	10	10	Primer	Suwabe *et al.* (2002)
	BrH80A08_FLC1	5′-ttcccaagcttgctggtact-3′ 5′-gagatttccctcgcttgatg-3′	1	1	Primer	KBrH80A08_AC155344
	BrH04D11_FLC2	5′-gcgccaattataaatttgattttc-3′ 5′-tcctcctgaacctggtcttg-3′	1	1	Primer	KBrH04D11_AC155341
	BrH80C09_MAF	5′-cagtgaagttcaaccgcagta-3′ 5′-catgagtgaacataaaacagtgaaa-3′	1	1	Primer	KBrH80C09_A166741
	Random primer	Operon primer S14	1	1	Primer	BM commercial product
Total			421	545		

groups covering a total map length of 1287 cM with an average 2.4-cM interval. The 10 linkage groups are most likely to correspond to the 10 chromosomes of *B. rapa* (Figure 1). It was possible to assign each linkage group to a previously determined classification (R1–R10) on the basis of evidence from the location of previously published SSR markers designated to A genome linkage groups within the context of the *B. nap*us (Parkin *et al.* 1995; Lowe *et al.* 2004) and *B. rapa* (Suwabe *et al.* 2002) genetic maps. From a total of 75 mapped A genome SSR markers screened against the parents, 21 displayed polymorphism. Each of the 10 linkage groups had at least one SSR marker that provides an anchor to existing published maps.

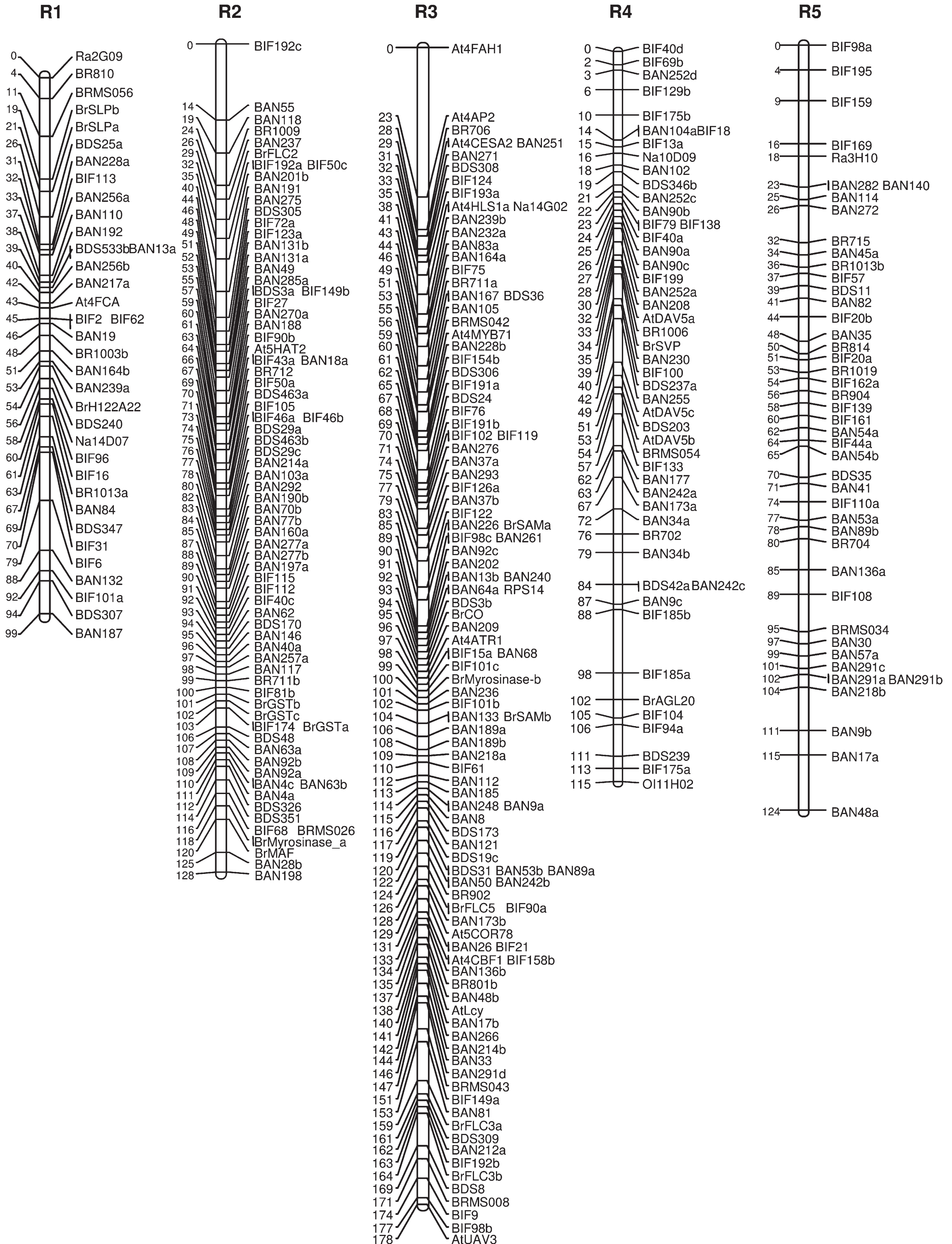
TABLE 2

Summary of the polymorphism detected by EST clones

Source of restriction endonuclease	BIF	BAN	BDS	BR	Total (%)
*Eco*RI	32	45	9	6	92 (25.6)
*Eco*RV	37	65	14	8	124 (34.6)
*Bam*HI	21	29	10	5	65 (18.1)
*Sca*I	24	37	12	5	78 (21.7)
Total	114	176	45	24	359 (100)

The two longest linkage groups R3 (178 cM) and R9 (193 cM) correspond to the two longest groups N3 and N9 of *B. napus* (Lowe *et al.* 2004; Udall *et al.* 2005). Within the Korean Brassica Genome Project (KBGP, http://www.brassica.rapa.org) it had previously been reported that R9 corresponds to cytogenetic chromosome 1 and that R3 corresponds to cytogenetic chromosome 2 (Lim *et al.* 2005). We compared the relative lengths of each linkage group with the length of the corresponding cytogenetic chromosome identified by Lim *et al.* There was good agreement, with a calculated correlation coefficient of 0.87. When the same cytogenetic chromosome lengths were compared with the length of corresponding A genome linkage groups reported for recent genetic maps of *B. napus*, correlation coefficients of 0.66 (Parkin *et al.* 2005) and 0.78 (Udall *et al.* 2005) were obtained.

Sequence homology: *B. rapa* probe sequences used to establish marker loci were compared against all sequences in GenBank release using BLASTn. The supplemental table (http://www.genetics.org/supplemental/) lists the *B. rapa* loci for which nucleotide sequence homology was determined with a cutoff of 1*E*-12, together with the matching GenBank database accession. The similarity data indicated that 422 (77%) of all loci corresponded to genes of known sequence, of which


R1
0 Ra2G09
4 BR810
11 BRMS056
19 BrSLPb
21 BrSLPa
26 BDS25a
31 BAN228a
32 BIF113
33 BAN256a
37 BAN110
38 BAN192
39 BDS533b BAN13a
40 BAN256b
42 BAN217a
43 At4FCA
45 BIF2 BIF62
46 BAN19
48 BR1003b
51 BAN164b
53 BAN239a
54 BrH122A22
56 BDS240
58 Na14D07
60 BIF96
61 BIF16
63 BR1013a
67 BAN84
69 BDS347
70 BIF31
79 BIF6
88 BAN132
92 BIF101a
94 BDS307
99 BAN187
R2
0 BIF192c
14 BAN55
19 BAN118
24 BR1009
26 BAN237
29 BrFLC2
32 BIF192a BIF50c
35 BAN201b
40 BAN191
44 BAN275
46 BDS305
48 BIF72a
49 BIF123a
51 BAN131b
52 BAN131a
53 BAN49
55 BAN285a
57 BDS3a BIF149b
59 BIF27
60 BAN270a
61 BAN188
63 BIF90b
64 At5HAT2
66 BIF43a BAN18a
67 BR712
69 BIF50a
70 BDS463a
71 BIF105
73 BIF46a BIF46b
74 BDS29a
75 BDS463b
76 BDS29c
77 BAN214a
78 BAN103a
80 BAN292
82 BAN190b
83 BAN70b
84 BAN77b
85 BAN160a
87 BAN277a
88 BAN277b
89 BAN197a
90 BIF115
91 BIF112
92 BIF40c
93 BAN62
94 BDS170
95 BAN146
96 BAN40a
97 BAN257a
98 BAN117
99 BR711b
100 BIF81b
101 BrGSTb
102 BrGSTc
103 BIF174 BrGSTa
106 BDS48
107 BAN63a
108 BAN92b
109 BAN92a
110 BAN4c BAN63b
111 BAN4a
112 BDS326
114 BDS351
116 BIF68 BRMS026
118 BrMyrosinase_a
120 BrMAF
125 BAN28b
128 BAN198
R3
0 At4FAH1
23 At4AP2
28 BR706
29 At4CESA2 BAN251
31 BAN271
32 BDS308
33 BIF124
35 BIF193a
38 At4HLS1a Na14G02
41 BAN239b
43 BAN232a
44 BAN83a
46 BAN164a
49 BIF75
51 BR711a
53 BAN167 BDS36
55 BAN105
56 BRMS042
59 At4MYB71
60 BAN228b
61 BIF154b
62 BDS306
65 BIF191a
67 BDS24
68 BIF76
69 BIF191b
70 BIF102 BIF119
71 BAN276
74 BAN37a
75 BAN293
77 BIF126a
79 BAN37b
83 BIF122
85 BAN226 BrSAMa
89 BIF98c BAN261
90 BAN92c
91 BAN202
92 BAN13b BAN240
93 BAN64a RPS14
94 BDS3b
95 BrCO
96 BAN209
97 At4ATR1
98 BIF15a BAN68
99 BIF101c
100 BrMyrosinase-b
101 BAN236
102 BIF101b
104 BAN133 BrSAMb
106 BAN189a
108 BAN189b
109 BAN218a
110 BIF61
112 BAN112
113 BAN185
114 BAN248 BAN9a
115 BAN8
116 BDS173
117 BAN121
119 BDS19c
120 BDS31 BAN53b BAN89a
122 BAN50 BAN242b
124 BR902
126 BrFLC5 BIF90a
128 BAN173b
129 At5COR78
131 BAN26 BIF21
133 At4CBF1 BIF158b
134 BAN136b
135 BR801b
137 BAN48b
138 AtLcy
140 BAN17b
141 BAN266
142 BAN214b
144 BAN33
146 BAN291d
147 BRMS043
151 BIF149a
153 BAN81
159 BrFLC3a
161 BDS309
162 BAN212a
163 BIF192b
164 BrFLC3b
169 BDS8
171 BRMS008
174 BIF9
177 BIF98b
178 AtUAV3
R4
0 BIF40d
2 BIF69b
3 BAN252d
6 BIF129b
10 BIF175b
14 BAN104aBIF18
15 BIF13a
16 Na10D09
18 BAN102
19 BDS346b
21 BAN252c
22 BAN90b
23 BIF79 BIF138
24 BIF40a
25 BAN90a
26 BAN90c
27 BIF199
28 BAN252a
30 BAN208
32 AtDAV5a
33 BR1006
34 BrSVP
35 BAN230
39 BIF100
40 BDS237a
42 BAN255
49 AtDAV5c
51 BDS203
53 AtDAV5b
54 BRMS054
57 BIF133
62 BAN177
63 BAN242a
67 BAN173a
72 BAN34a
76 BR702
79 BAN34b
84 BDS42a BAN242c
87 BAN9c
88 BIF185b
98 BIF185a
102 BrAGL20
105 BIF104
106 BIF94a
111 BDS239
113 BIF175a
115 Ol11H02
R5
0 BIF98a
4 BIF195
9 BIF159
16 BIF169
18 Ra3H10
23 BAN282 BAN140
25 BAN114
26 BAN272
32 BR715
34 BAN45a
36 BR1013b
37 BIF57
39 BDS11
41 BAN82
44 BIF20b
48 BAN35
50 BR814
51 BIF20a
53 BR1019
54 BIF162a
56 BR904
58 BIF139
60 BIF161
62 BAN54a
64 BIF44a
65 BAN54b
70 BDS35
71 BAN41
74 BIF110a
77 BAN53a
78 BAN89b
80 BR704
85 BAN136a
89 BIF108
95 BRMS034
97 BAN30
99 BAN57a
101 BAN291c
102 BAN291a BAN291b
104 BAN218b
111 BAN9b
115 BAN17a
124 BAN48a

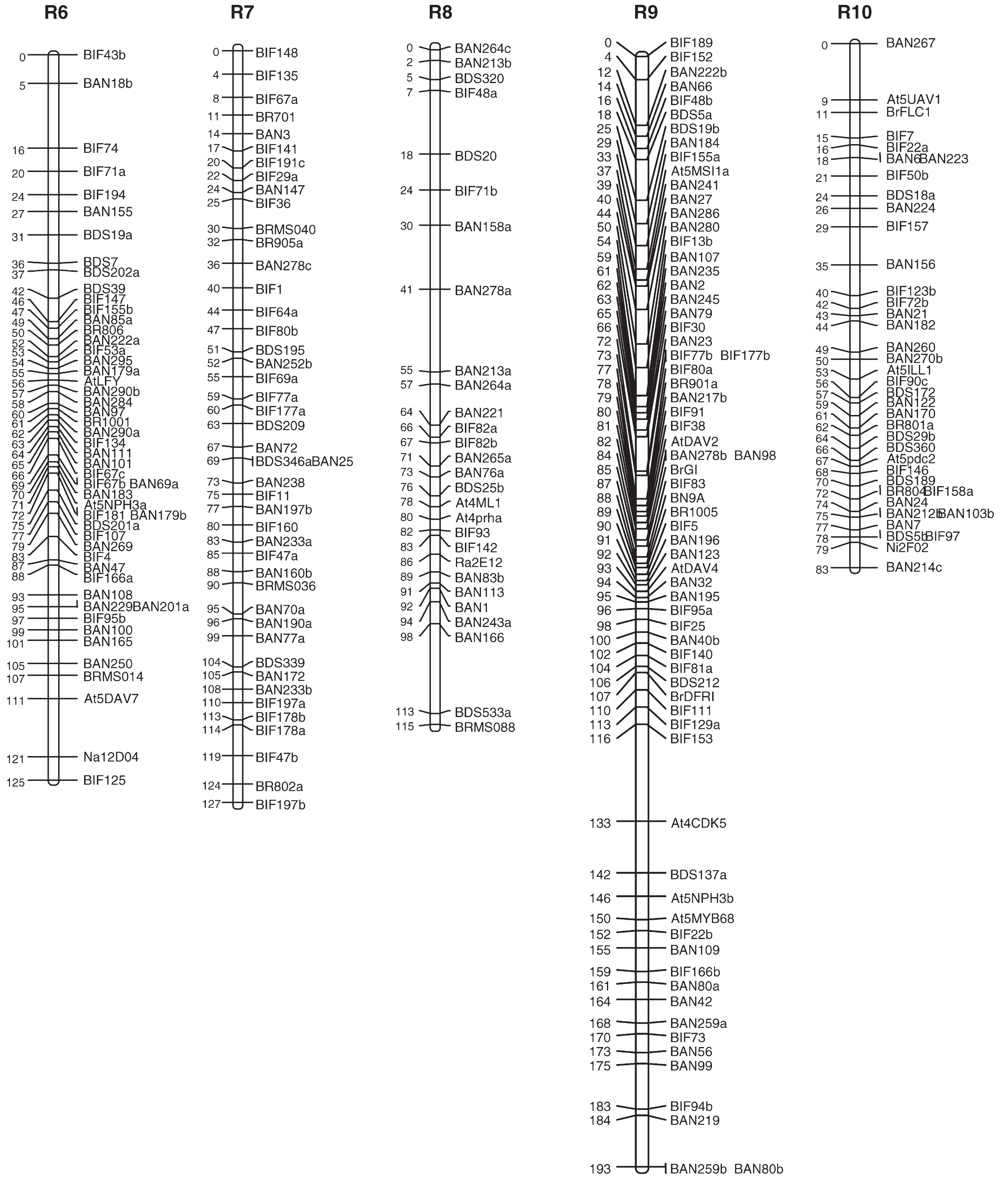


Figure 1.—Composite detailed genetic linkage map of *Brassica rapa*.

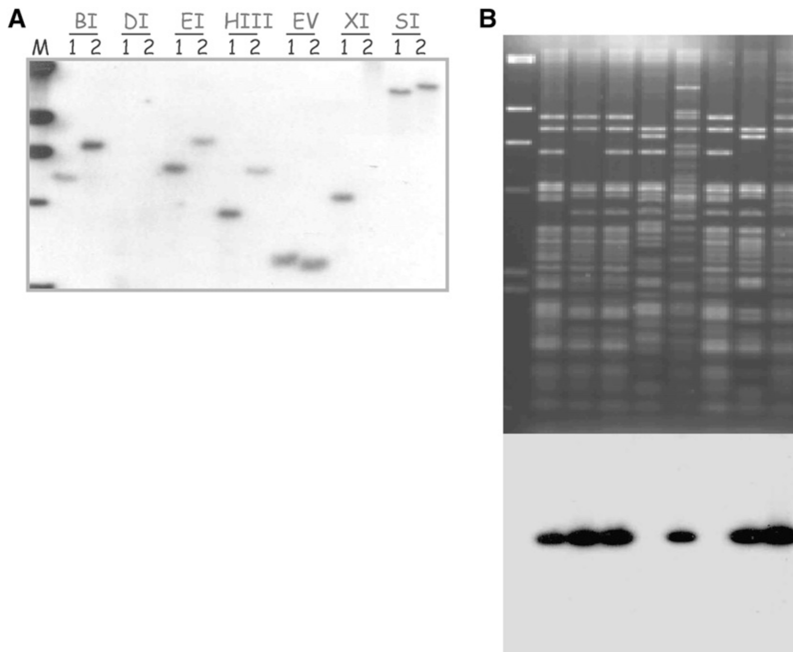


Figure 2.—Examples of single-locus genes of a parent survey, colony hybridization, and confirmation by Southern blotting to positive BAC fingerprints using BAN235 that it is located on R9 (61 cM). (A) RFLP autoradiography of parent genomic DNA digested with seven restriction enzymes using a BAN 235 single-locus probe. (B) Autoradiography of *Hin*dIII fingerprint of positive BAC clones using a BAN235 probe.

89 aligned with Arabidopsis expressed, putative, or hypothetical protein-coding sequences. A study of the hit sequence based on their organisms showed that 317 of 422 (75%) had highest sequence homology to the Arabidopsis genome and 97 of 422 (23%) were matched on four species of Brassica, which were *B. rapa* (39/97), *B. napus* (32/97), *B. oleracea* (19/97), and *B. juncea* (7/97). Only 2% (8 of 422) showed the sequence homology on other organisms, including on rice. The probe sequences used to generate the marker loci appeared to represent a wide range of gene classes, including regulatory factors and structural genes involved in membrane transport, signal transduction, cell cycle regulation, carbon metabolism, stress response, DNA synthesis, and fatty acid metabolism.

Single-locus genes: From the screening of RFLP probes against parental lines using seven restriction enzymes, 12% of clones gave single hybridization fragments, and 62 of these were incorporated into the linkage map. Probe BAN235 (Figure 2A) mapped to a single locus on R9, and this was confirmed by screening the probe to an 11× genome coverage *Hin*dIII BAC library (Park *et al.* 2005). Positive hybridization signals were detected for 14 BAC clones, and the isolated DNA was digested with *Hin*dIII enzyme. The resultant fingerprints were consistent with the BACs forming a single contig by Southern analysis using the same probe, BAN235 (Figure 2B).

The genetic map location of the 62 single-locus markers and their corresponding BLAST comparison data are shown with an asterisk (*) next to their locus name in the supplemental table (http://www.genetics.org/supplemental/). Of these, 26% (17/62) had no sequence similarity to any sequence in GenBank, indicating that these may be Brassica unique genes, and 25% (16/62) displayed highest similarity to sequences corresponding to uncharacterized gene models within the Arabidopsis genome (expressed protein, unknown protein, or full-length cDNA). Single-locus-specific probes are distributed across all *B. rapa* chromosomes with no apparent clustering.

Duplicated marker and homologous linkage groups: The remainder of the RFLP probes detected more than one segregating locus, with an average of 1.31 loci per probe (520/396). A total of 102 of the 396 mapped probes gave rise to multiple loci (229), with an average of 2.25 loci per probe. Eighty-one detected 2 loci, 18 detected 3, 2 detected 4, and a single probe detected 5 loci. Of these, 72 probes revealed locus duplication (164 loci) of two or three copies on different linkage groups. The pattern of duplications within the *B. rapa* genome was revealed by comparing ordered clusters of loci derived from common gene probes. At the top of the largest linkage group R3 with five loci duplicated R1 and the middle region of R3 corresponds to sections of R4 and R5, whereas the lower region corresponds to sections represented by 10 loci on R2 and 7 loci on R10. Four marker loci, spanning 19 cM at the top of R4, are also duplicated within R7 and R9 where the four duplicated loci span 17 and 11 cM, respectively. Most chromosomal parts show two or three duplication blocks (Figure 3). These relationships between homeologous chromosome segments provide good evidence for a series of historical segmental duplication events in this genome. However, since all genetic mapping experiments are based on polymorphism of genetic markers, the fine detailed pattern of duplications or triplication is incomplete due to the presence of monomorphic or dimorphic markers.

Comparison of flowering-time-related genes: Six genes involved in regulating flowering time isolated from

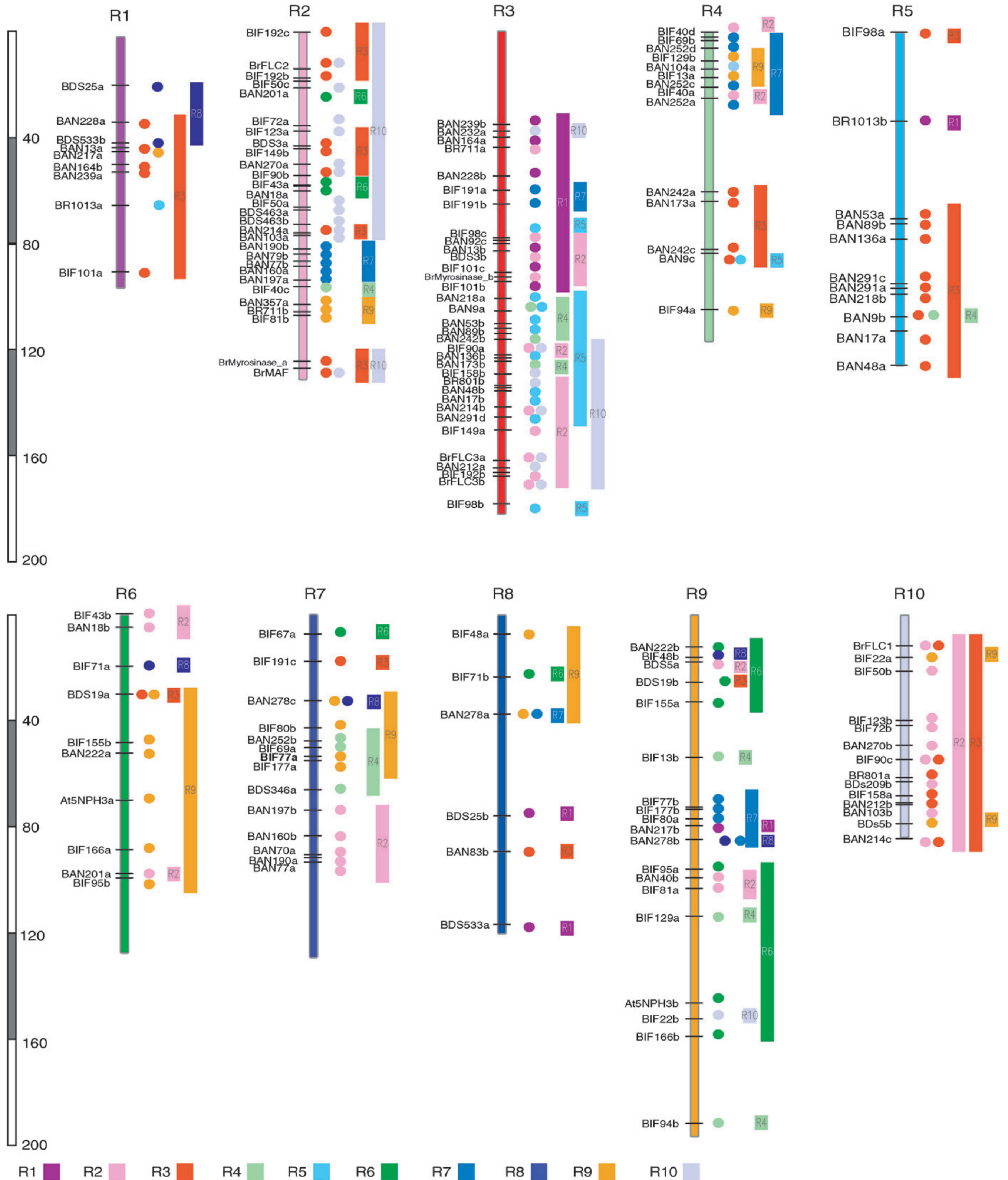


FIGURE 3.—Interchromosomal duplication of *Brassica rapa*. Circles show duplicated loci of the same markers. Each linkage group is assigned 10 different colors.

B. rapa (*FLC*, *GI*, *CO*, *SVP*, *AGL20*, and *VRN1*) and two from Arabidopsis (*LFY* and *FCA*) were used as RFLP probes. Most probes hybridized to two or three major bands, and some of them were polymorphic between the parents. It was not possible to map any paralogous loci of *VRN1* due to the presence of three major monomorphic bands when tested with seven restriction enzymes. The *BrFLC* (AY273164) isolated from *B. rapa* cv. Maeryuk (F_1) detected two polymorphic segregating bands and an additional three monomorphic bands between the two parents. Two distinct polymorphic loci *BrFLC*-a and *BrFLC*-b were mapped 5 cM apart on the long-arm telomere region of R3. *BrFLC-a* was found to correspond to *BrFLC3* sequences within the BAC clone KBrH117M18 (AC146875) and *BrFLC-b* to KBrH52O08 (AC155342). This was determined by Southern hybridization to BAC *Hin*dIII fingerprints. Two SSR markers, BrH80A08_*FLC1* (KBrH080A08, AC155344) and BrH04D11_*FLC2* (KBrH004D11, AC155341) were derived from two individual BAC sequences. They were classified on the basis of results of colony hybridization, *Hin*dIII fingerprints, and hybridization pattern using the *BrFLC* gene as probe. BrH80A08_*FLC1* was located on the short arm of R10 and BrH04D11 was assigned to the short arm of R2, substantiating the positions determined by Schranz *et al.* (2002). A synthetic GST probe, designated from the second exon to the fifth exon of *BrFLC*5, showed a single polymorphic band and was mapped to a position 33 cM away from *BrFLC3*a. All of the duplicated *BrFLC* genes are located near telomeres (of R2, R3, and R10), with linked markers in the distal regions usually showing skewed segregation, mostly toward the maternal genotype. Data are consistent with the map location reported in a previous study (Schranz *et al.* 2002), although we report here one more *BrFLC*3 paralogue derived from recent segmental duplication and the reverse map orientation. An SSR marker derived from BrH80C09_*MAF* (KBrH80C09, AC166741) was mapped to the long arm of R2 and appears to correspond to the VFR1 locus on R2 (Schranz *et al.* 2002).

DISCUSSION

Genetic linkage map: A detailed genetic linkage map of the Brassica A genome has been constructed, on the basis of 134 *B. rapa* $F_{2:3}$ families. It contains 545 loci and most of them were detected by RFLP analysis using sequenced EST probes from four different tissue-specific libraries (474 loci/359 probes). Additional gene-specific probes were derived from Arabidopsis chromosome 4 (10 loci/10 probes) and chromosome 5 (17 loci/14 probes). Several flowering-time genes from Arabidopsis (2 loci/2 probes), *B. rapa* (7 loci/6 probes), and *BrMyrosinase*, *BrDFRI*, *BrGST*, *BrSAM*, and *BrSLP* (10 loci/5 probes) functional genes were mapped on this linkage map. The detection of 1.31 loci per RFLP probe (520/396) closely matches that reported (1.27 and 1.34 from 220 and 269 probes, respectively) for previous *B. rapa* maps (Song *et al.* 1991; Chyi *et al.* 1992). In contrast, Udall *et al.* (2005) observed a higher level of polymorphism with codominant and dominant segregation in the amphidiploid *B. napus*, where there are twice as many potential loci.

The accumulated set of sequence-tagged genetic markers provides a valuable source of information for study and navigation of the Brassica A genome, not only in *B. rapa* but also in the context of *B. napus* and *B. juncea*. Since the model dicotyledonous plant, *A. thaliana*, is closely related to Brassica the genus and share, on average, 87% sequence identity (Cavell *et al.* 1998), there is an expectation that understanding the genetic control of basic biological processes in Arabidopsis can be transferable to other species (Lagercrantz 1998). However, Brassica EST markers that do not correspond to genes derived from other species are of additional value, as they provide insight into the identity and location of novel gene functions, which may be related to the well-characterized adaptability and plasticity of this crop genus.

We have obtained an average marker density of 2.4 cM. *B. rapa* has the smallest diploid Brassica genome, estimated at 529 Mb (Johnston *et al.* 2005). Thus we calculate that the current map provides a genetic marker on average at least every 1 Mb. This information may be exploited in at least two ways. First, within the ongoing *B. rapa* genome-sequencing project (Yang *et al.* 2005) 62 single-locus gene markers are now available that will assist in the isolation and confirmation of "seed" BACs, as well as provide anchored markers to span between adjacent BAC contigs to integrate the physical map. Second, there is the prospect of benefiting from the rich source of biological information and genetic resources from Arabidopsis functional genomics research to benefit Brassica crop plants.

Multiple duplicated *FLC* genes of *B.rapa*: The MADS-box flowering-time regulator FLC, located at the top of chromosome 5 of Arabidopsis, has a repressive function role on flowering time (Michaels and Amasino 1999). There are some differences on the number of orthologous or paralogous *BrFLC* loci with that of Schranz *et al.* (2002). Using the *BrFLC* gene as an RFLP probe, we obtained two polymorphic and three monomorphic bands. These two loci were located at the telomere of the long arm of R3, whereas Schranz *et al.* were able to assign only one locus to R3. This difference appears to result from the use of a backcross population by Schranz *et al.*, where heterozygote genotypes are not detected. In contrast, the JWF3 is an F_3 pooled population (40 seedlings per line) that is able to represent F_2 segregation. We were able to detect eight recombinant genotypes in the population between two alleles of *BrFLC*3-a and -b, where one was homozygous for one parent line (genotype A), and the other represented the heterozygote

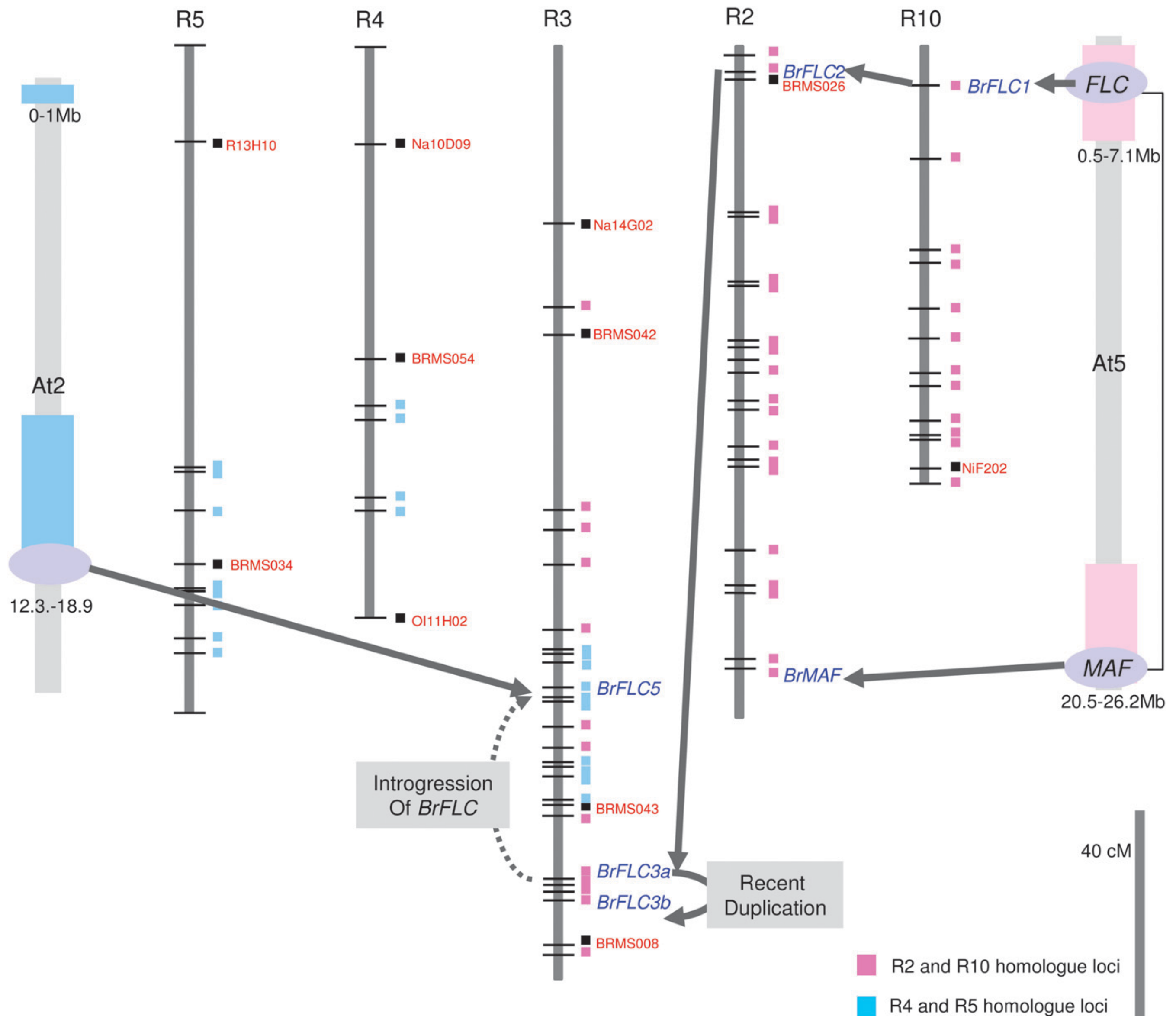


Figure 4.—Comparative mapping of *B. rapa* linkage groups containing replicated *FLC* genes to the Arabidopsis genome. Vertical lines represent homeologous linkage groups in *B. rapa*, two loci of Br*FLC*3a and *FLC*3b are the polymorphic segregating RFLP genotype data using the *BrFLC* gene (AY273164), and the other three, Br*FLC1*, Br*FLC2*, and Br*MAF*, are the segregated genotype data using developed SSR markers from BACs containing *FLC* and *MAF* genes. R2, R3, and R10 show the sequenced homology to Arabidopsis chr5 (0.5–7 Mb and 20.5–26.2 Mb, respectively). Originated *FLC* of Arabidopsis is triplicated at the top of R10 and R2 and at the bottom of R3, and a local duplication event has happened at the bottom of R3. The BrFLC5 gene mapped region shows R4 and -5 homeologues and corresponds to Arabidopsis chr3 (0–1 Mb and 12.3–18.9 Mb, respectively).

(H), resulting in a map interval of 5 cM on R3. Other monomorphic *BrFLC* fragments were detected using SSR markers derived from *B. rapa* BACs that contain *BrFLC* genes. Sequences of four BACs, KBrH080A08, KBrH004D11, KBrH117M18, and KBrH52O08 containing *BrFLC*1, -2, -3a, and -3b, respectively, are collinear to the FLC region at 3.0–3.35 Mb of Arabidopsis chromosome 5 with indels (Yang *et al.* 2006). This genetic map surrounding *BrFLC*1, -2, -3a, and -3b shows synteny between each linkage group and with the 3-Mb region of Arabidopsis chromosome 5 (Figure 4). Meanwhile *BrFLC*5 is identified in a BAC clone KBrH038M21 (not submitted yet) that is collinear with 12.7–12.91 Mb of Arabidopsis chromosome 2 and the genetic position was determined 33 cM away from *BrFLC*3a on the long arm of R3. From this we infer that this genomic segment was replicated by an insertion within the homeologous region of R3 and this region and mostly shows the homeologous blocks to R4 and R5. Another BAC clone KrH80C09 corresponds to the MAF gene locus (At565050) within the 25.8- to 26.2-Mb region of Arabidopsis chromosome 5 (Yang *et al.* 2006).

Comparison of flowering-time genes of *B. rapa*, *B. napus*, and *A. thaliana* and QTL of *B. rapa* have been reported (Osborn *et al.* 1997; Kole *et al.* 2001). A major QTL, "VFR2" has been shown to correspond to *BrFLC1*, while the QTL "FR1" corresponded to *BrFLC2* (Kole *et al.* 2001). An additional QTL "FR2" corresponds to *BrFLC5*, although another vernalization response QTL1 "VFR1" was not accounted for by a corresponding flowering-time-related gene (Schranz *et al.* 2002). A BAC clone KBrH80C09, containing a tandem array of three *MAF* genes (Yang *et al.* 2006) and mapped on the long arm of R2, suggests that this gene may play a role for QTL of VFR1.

The genomes of Brassica species have triplicated counterparts to corresponding homeologous segments of Arabidopsis (O'neill and Bancroft 2000; Rana *et al.* 2004; Lysak *et al.* 2005). Almost 88% of triplicated genes near the FLC regions returned to a single-copy or a two-copy state by deletion (Yang *et al.* 2006). Because of this reason, hybridization data using a single EST probe might have limitations for inferring genome duplication. But, overall distributions of duplicate or triplicate regions are detected from the hybridization data of multiloci EST markers (Figure 3), suggesting that the genome-level triplication might have happened in the ancestor of Brassica.

The KBGP is currently underway and is aiming to generate the first complete Brassica chromosome sequence of R9 (cytogenetic chromosome 1) (www.brassic.rapa.org). We have selected nine seed BACs through BAC library screening using single-locus EST markers. FISH and sequence information generally coincided with our expectations. The complete set of 62 locus-specific single-copy EST markers will be valuable markers for the primary anchoring of "seed" BACs for each linkage group.

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