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Simple Sequence Repeat-Based Comparative Genomics Between *Brassica rapa* and *Arabidopsis thaliana*: The Genetic Origin of Clubroot Resistance

Keita Suwabe,* Hikaru Tsukazaki,* Hiroyuki Iketani,† Katsunori Hatakeyama,* Masatoshi Kondo,* Miyuki Fujimura,* Tsukasa Nunome,* Hiroyuki Fukuoka,* Masashi Hirai‡ and Satoru Matsumoto*,[1]

**Department of Quality Science, National Institute of Vegetable and Tea Science, Ano, Mie, 514-2392, Japan, †Department of Breeding, National Institute of Fruit Science, Tsukuba, Ibaraki, 305-8605, Japan and ‡Faculty of Agriculture, Kyoto Prefectural University, Kitainaazuma, Seika, Kyoto, 619-0244, Japan*

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ABSTRACT

An SSR-based linkage map was constructed in *Brassica rapa*. It includes 113 SSR, 87 RFLP, and 62 RAPD markers. It consists of 10 linkage groups with a total distance of 1005.5 cM and an average distance of 3.7 cM. SSRs are distributed throughout the linkage groups at an average of 8.7 cM. Synteny between *B. rapa* and a model plant, *Arabidopsis thaliana*, was analyzed. A number of small genomic segments of *A. thaliana* were scattered throughout an entire *B. rapa* linkage map. This points out the complex genomic rearrangements during the course of evolution in Cruciferae. A 282.5-cM region in the *B. rapa* map was in synteny with *A. thaliana*. Of the three QTL (*Crr1*, *Crr2*, and *Crr4*) for clubroot resistance identified, synteny analysis revealed that two major QTL regions, *Crr1* and *Crr2*, overlapped in a small region of Arabidopsis chromosome 4. This region belongs to one of the disease-resistance gene clusters (MRCs) in the *A. thaliana* genome. These results suggest that the resistance genes for clubroot originated from a member of the MRCs in a common ancestral genome and subsequently were distributed to the different regions they now inhabit in the process of evolution.

GENUS Brassica is cultivated in most parts of the world. It includes various important agronomical crops such as oilseed rape, cabbage, broccoli, Chinese cabbage, black mustard, and other leafy vegetables. Due to the wide choice of edible forms in the genus, extensive morphological variations such as the head, enlarged root, enlarged stem, color variation, and oil content have been selected for, these parts serving as cultivated foods for human beings. Furthermore, the genetic relation to Brassica has been well studied and is referred to as U's triangle (U 1935). The genomes of three diploid species, *Brassica rapa*, *B. nigra*, and *B. oleracea*, have been designated as A, B, and C, respectively, while those of the amphidiploids, *B. juncea*, *B. napus*, and *B. carinata*, have been designated as AB, AC, and BC, respectively (Iwabuchi *et al.* 1991; Lagercrantz and Lydiate 1996; Snowdon *et al.* 1997). Because such genetic and morphological variation has occurred as the result of a natural hybridization of species, the remarkable diversity of Brassica is an excellent example for understanding how genetic and morphological variation have developed during the evolution of plant genomes.

[1]*Corresponding author:* National Institute of Vegetable and Tea Science, 360 Kusawa, Ano, Mie, 514-2392, Japan. E-mail: ssmats@affrc.go.jp

Recent studies of the model plant *Arabidopsis thaliana* have yielded new insights into plant genetics and genomics. A pattern of chromosomal colinearity has been identified between Arabidopsis and other flowering plants (Grant *et al.* 2000; Rossberg *et al.* 2001). Genomic synteny has been found to be well conserved among a broad array of species, even though genomic size in plant exhibits a wide diversity, *e.g.*, 125 Mb for *A. thaliana* to 125 Gb for *Fritillaria assyriaca* (Bennett and Smith 1976). Physically and functionally conserved gene(s), known as orthologs and paralogs, were also identified (Axelsson *et al.* 2001). Because Brassica and Arabidopsis are in the same family, the Cruciferae, the level of synteny between them provides a good opportunity to study how genetic and morphological variation has developed during the evolution of the genome, including the endurance of certain genetic structures in Arabidopsis and related Brassica species. Such investigation may lead to a better understanding of plant genetics, including molecular biological and physiological aspects that have emerged via evolution.

In past decades, genetic maps of Brassica have been constructed by means of RFLPs, AFLPs, and RAPDs to better understand their genetic makeup (Slocum *et al.* 1990; Landry *et al.* 1991; Kianian and Quiros 1992; Ferreira *et al.* 1994; Uzunova *et al.* 1995). These studies

have contributed to analyses of complicated quantitative traits and to comparisons of the organization of the chromosomes of Brassica. Since the DNA sequences of homologous genes in the related taxa are quite similar, clones have been extensively used as RFLP markers to elucidate the genomic colinearity between species (Osborn and Lukens 2003). The degree of genomic conservation has been elucidated by comparative linkage mapping, and conserved genomic segments have been identified by micro- and macrosynteny studies in various Brassica species (Cavell *et al.* 1998; Lagercrantz 1998; O'Neill and Bancroft 2000; Ryder *et al.* 2001). However, because of the high rate of genomic segment replication, RFLP has been shown at times to detect more than one locus in Brassica genomes (Lagercrantz and Lydiate 1996). Although duplication/triplication of genomic segments permits the evolution of genes, it also leads to considerable complexity in evaluating genomic colinearity within species. For these reasons, a linkage map based on more precise molecular markers that would allow discrimination between homologous and homeologous regions is required for an accurate comparative analysis in Brassica.

Clubroot is one of the most serious diseases that afflict Brassica crops (Crute *et al.* 1980). *Plasmodiophora brassicae*, the causal agent of clubroot, is a soil-borne, obligate pathogen. The pathogen can infect most Brassica species such as oilseed rape, cabbage, Chinese cabbage, and turnip. Several mapping studies have already been reported on *B. oleracea*, *B. rapa*, and *B. napus*, and the major QTL for clubroot resistance have been reported (Figdore *et al.* 1993; Grandclément and Thomas 1996; Kuginuki *et al.* 1997; Voorrips *et al.* 1997; Suwabe *et al.* 2003). Despite these efforts, little information has been made available to allow a comprehensive understanding of the clubroot resistance seen in Brassica. An improved understanding of the genetic mechanisms and the evolution of clubroot resistance would be one of the examples for carrying out genetic analysis of disease resistance in Brassica.

In this article, we describe (i) the construction of a genetic linkage map of *B. rapa* based mainly on SSR markers, (ii) the genomic synteny between *B. rapa* and *A. thaliana* by comparative mapping based on the SSRs in the present map, (iii) QTL analysis for clubroot resistance in two different pathogen isolates, and then we discuss the origin and evolution of clubroot resistance in Brassica by a combination of the QTL and synteny analyses of *A. thaliana*. The result is obtained by comparative genomics, utilizing linkage maps in species possessing complex genomes, and the well established information on model plants.

MATERIALS AND METHODS

Plant materials: A clubroot-resistant (CR) doubled-haploid (DH) line, G004, and a susceptible (CS) DH Chinese cabbage parental line, nou 7 (A9709), were used as the parents for the mapping population (Suwabe *et al.* 2003). An F_2 population consisting of 94 lines was selected randomly and F_3 seeds were obtained from the bud self-pollination of each F_2 line for clubroot-resistance analysis. Young leaves of each F_2 line were freeze dried and stored at −20° until DNA isolation. Three cultivars of Chinese cabbage, "CR Ryutoku" (Watanabe Seed Co., Japan), "Utage 70" (Nozaki Seed Co., Japan) (CR even though they exhibit a breakdown of resistance to "Wakayama-01"), and "Muso" (CS; Takii Seed Co., Kyoto, Japan) were used as controls in the clubroot-resistance analysis. All plants were grown in a greenhouse.

Molecular markers: Complete information on the SSR markers is described in Suwabe *et al.* (2002, 2004). A total of 343 SSR markers were used for the construction of the map. RFLP markers were kindly provided by C. Quiros, University of California (Davis, CA) (Harada *et al.* 1988), and some of the markers were developed originally at the National Institute of Vegetable and Tea Science (NIVTS). RAPD primers were obtained from BEX (Common's primer set, Tokyo), Operon Technologies (Alameda, CA), University of British Columbia (Vancouver, BC, Canada), and Wako Pure Chemical Industries (Osaka, Japan). Some of the primers were originally developed at NIVTS.

To certify the genomic correlations in focused regions within *B. rapa* and *A. thaliana* (see the details in the results section), SNP and/or indel markers were developed using the information in the Arabidopsis database (TAIR: http://www.arabidopsis.org). Single-copy genes in the region of focus in the Arabidopsis genome were selected, and the primers were designed from these genes' nucleotide sequences to amplify the products to ~500 bp. After the confirmation of successful amplification in *A. thaliana*, the PCR products amplified in A9709 and G004 were sequenced and compared with each other in terms of the nucleotide sequences for SNPs and/or indels.

Genetic analysis: For map construction, all molecular markers showing polymorphisms between A9709 and G004 were selected and applied to segregation analysis in the F_2 population.

The SNP and/or indel markers between A9709 and G004 were also applied to this segregation analysis in the F_2 population.

Linkage analysis and map construction: Segregation of each marker in the F_2 population was analyzed by a chi-square test for "goodness-of-fit" to a 1:2:1 (codominant marker) or 3:1 (dominant marker) ratio. Linkage analysis of markers was performed using MAPMAKER/EXP 3.0 (Lander *et al.* 1987). A framework map of codominant markers was constructed by assignment to linkage groups by the "group" and "order" commands with a LOD score of 8.0. Dominant markers were inserted into the intervals in the framework map by the "try" command. The "ripple" command was used to confirm the order of markers in each linkage group. All genetic distances are expressed in centimorgan values as derived by the Kosambi function (Kosambi 1944).

Comparison of SSR loci between *B. rapa* and *A. thaliana*: The nucleotide sequences of each SSR locus in *B. rapa*, 123–1129 bp with an average of 484 bp, were aligned with Arabidopsis genome sequences by BLASTN in TAIR. The methodology for claiming homologous SSR loci was the following: (i) upper and lower nucleotide sequences flanking the SSR were aligned independently, because repeat numbers of SSRs differ greatly between *B. rapa* and *A. thaliana* (Suwabe *et al.* 2004), (ii) the results of each alignment for an SSR locus were compared, and (iii) SSR loci with a consistent homology of flanking upper and lower regions were realigned with the entire nucleotide sequences of SSR loci to calculate a homology

value. We regarded as homologous SSR loci that had flanking upper and lower genomic regions assigned to the same region in the *A. thaliana* genome with a threshold value of $E < 10^{-10}$. When the *E*-value was between 10^{-10} and 10^{-5}, corresponding loci were manually confirmed by the presence of SSR sequences and the homology value of the flanking region. We regarded regions having neighboring SSR loci that were relatively conserved between *B. rapa* and *A. thaliana* as a homologous synteny region.

QTL analysis for clubroot resistance: The *P. brassicae* isolates Wakayama-01 and "Ano-01" were used in this study (Kuginuki *et al.* 1997; Suwabe *et al.* 2003). Wakayama-01 has the highest virulence in our pathogen stock (see the details in Suwabe *et al.* 2003). In contrast, Ano-01 was the lowest, able to infect Muso but not CR Ryutoku or Utage 70. Because of the intermediate reactions of Williams' differential hosts (Williams 1966), the race numbers of the present isolates could not be determined. The inoculum was propagated throughout the infection test and clubs in the infected roots were stored at −20° until required. Resting spores were purified from the clubs according to Williams (1966) and used in the test for clubroot resistance. The tests for clubroot resistance and data evaluation are described in a previous report (Suwabe *et al.* 2003). The root symptoms of each plant were evaluated as follows: grade 0, no symptoms; grade 1, a few small, separate globular clubs on the lateral roots; grade 2, intermediate symptoms; grade 3, severe clubs on the main roots. A disease index (ID) was constructed from the results as the mean grade for 16 F_3 seedlings, and the mean ID for each F_2 line was expressed from two independent tests (a total of 32 seedlings). QTL analysis was performed using MAPMAKER/QTL 1.1 with a LOD threshold of 2.0.

RESULTS

Marker analysis: A total of 200 codominant markers (113 SSRs and 87 RFLPs), exhibiting polymorphisms between the parental lines, were screened for framework markers in the linkage map. Most of the markers segregated with the 1:2:1 Mendelian ratio in the F_2 population; however, 15.5% (31/200) of the markers deviated significantly ($P < 0.05$) from this ratio. In addition, 62 RAPD markers with polymorphisms between the parental lines were screened, and in total 262 markers were ultimately applied to the map.

Construction of the linkage map: The resulting map is composed of 10 linkage groups covering 1005.5 cM (Figure 1). The largest linkage group consists of 36 loci and is a length of 141.6 cM, and the smallest is 11 loci with a length of 45.9 cM (Table 1). Because the *B. rapa* karyotype is composed of 10 chromosomes, these 10 linkage groups seem to correspond to the chromosomes of *B. rapa*. The distances between markers varied from 0 to 24.1 cM, with an average distance of 3.7 cM. SSR markers were distributed throughout the linkage groups with an average distance of 8.7 cM. From the genome size of *B. rapa*, 550 Mb (Arumuganathan and Earle 1991), the average relationship between the physical and genetic distances is ~1 cM = 500 kb.

Synteny analysis between *B. rapa* and *A. thaliana*: To identify the synteny-possessing region(s) of *B. rapa* and *A. thaliana*, homologous SSR loci in the *A. thaliana* genome were sought using the *B. rapa* nucleotide sequences flanking SSRs. In 113 SSR loci of the linkage map, 74 were identified as having corresponding loci in the *A. thaliana* genome, while the remaining 39 could not be aligned, although a low level of homology ($E > 10^{-5}$) was observed in several genomic regions in *A. thaliana* (Table 2). In homologous SSR loci, 52.7% (39/74) composed a single homologous region in the *A. thaliana* genome, while the other loci represented >2 loci, most of them corresponding to 2 or 3 loci, in the *A. thaliana* genome. The majority of the homologous SSR loci in *A. thaliana* included the same SSR motifs, although most had shorter repeats than those in *B. rapa*, and the flanking sequences likewise exhibited a high rate of homology.

Five chromosomes of *A. thaliana* were separated into short segments and dispersed in various regions in the *B. rapa* map, although no continuous synteny region was observed in linkage group 8 (Figure 1, Table 2). Inversions, tandem duplications, and deletions were identified within some of the synteny regions. The longest synteny region was identified in linkage group 6, between BRMS-245 and BRMS-098 for a length of 47.9 cM, and the shortest regions were found in linkage groups 3 and 5, between BRMS-128 and BRMS-163 and between BRMS-079 and BRMS-051 for a length of 1.6 cM. In total, regions ranging up to 282.5 cM in length in the *B. rapa* map were identified to have synteny to *A. thaliana*. In addition to these synteny regions, certain independent SSR loci were found to be homologous to *A. thaliana*, such as BRMS-215 in linkage group 8 (Table 2), but no correlation was found within the neighboring SSR loci in Arabidopsis (Figure 1). Therefore, the synteny region with *A. thaliana* in the present *B. rapa* map is likely underestimated.

QTL analysis for clubroot resistance: In a previous study, we identified two clubroot resistance loci, *Crr1* and *Crr2*, for a Wakayama-01 isolate, which nearly cosegregated with SSR markers, BRMS-088 and BRMS-096, respectively (Suwabe *et al.* 2003). In the present linkage map, these two markers were located in linkage groups 7 and 6, respectively. Because detailed information about these two loci, such as a precise position and genetic effect, could not be determined in our previous study, we conducted a QTL analysis for a Wakayama-01 isolate using the present linkage map. The ID of F_2 individuals for the Wakayama-01 isolate ranged from "very susceptible" to "resistant," but very susceptible individuals were more frequent than resistant individuals (Figure 2A). From the analysis, three QTL were identified at a LOD threshold of 2.0 (Figure 3A). Among these, two major QTL in linkage group 7, between BRMS-297 and -088, and in linkage group 6, between BRMS-100 and -096, corresponded to *Crr1* and *Crr2* (Suwabe *et al.* 2003), respectively. These accounted for 26.8 and 18.3% of phenotypic variance, respectively (Table 3). In addition, a small QTL found in linkage

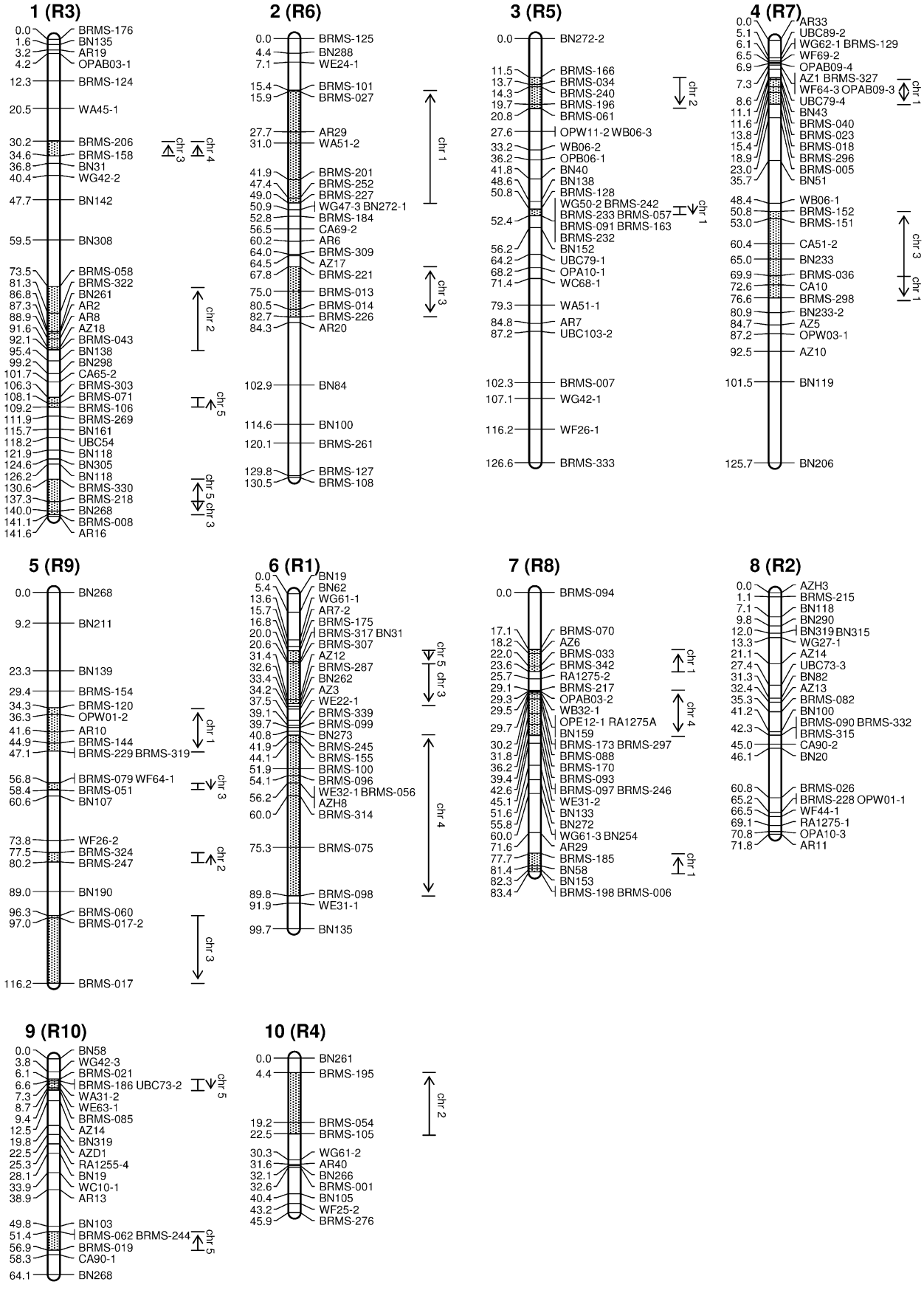
1 (R3)
0.0 BRMS-176
1.6 BN135
3.2 AR19
4.2 OPAB03-1
12.3 BRMS-124
20.5 WA45-1
30.2 BRMS-206
34.6 BRMS-158
36.8 BN31
40.4 WG42-2
47.7 BN142
59.5 BN308
73.5 BRMS-058
81.3 BRMS-322
86.8 BN261
87.3 AR2
88.9 AR8
91.6 AZ18
92.1 BRMS-043
95.4 BN138
99.2 BN298
101.7 CA65-2
106.3 BRMS-303
108.1 BRMS-071
109.2 BRMS-106
111.9 BRMS-269
115.7 BN161
118.2 UBC54
121.9 BN118
124.6 BN305
126.2 BN118
130.6 BRMS-330
137.3 BRMS-218
140.0 BN268
141.1 BRMS-008
141.6 AR16
chr 3
chr 4
chr 2
chr 5
chr 5
chr 3
2 (R6)
0.0 BRMS-125
4.4 BN288
7.1 WE24-1
15.4 BRMS-101
15.9 BRMS-027
27.7 AR29
31.0 WA51-2
41.9 BRMS-201
47.4 BRMS-252
49.0 BRMS-227
50.9 WG47-3 BN272-1
52.8 BRMS-184
56.5 CA69-2
60.2 AR6
64.0 BRMS-309
64.5 AZ17
67.8 BRMS-221
75.0 BRMS-013
80.5 BRMS-014
82.7 BRMS-226
84.3 AR20
102.9 BN84
114.6 BN100
120.1 BRMS-261
129.8 BRMS-127
130.5 BRMS-108
chr 1
chr 3
3 (R5)
0.0 BN272-2
11.5 BRMS-166
13.7 BRMS-034
14.3 BRMS-240
19.7 BRMS-196
20.8 BRMS-061
27.6 OPW11-2 WB06-3
33.2 WB06-2
36.2 OPB06-1
41.8 BN40
48.6 BN138
50.8 BRMS-128
52.4 WG50-2 BRMS-242 BRMS-233 BRMS-057 BRMS-091 BRMS-163 BRMS-232
56.2 BN152
64.2 UBC79-1
68.2 OPA10-1
71.4 WC68-1
79.3 WA51-1
84.8 AR7
87.2 UBC103-2
102.3 BRMS-007
107.1 WG42-1
116.2 WF26-1
126.6 BRMS-333
chr 2
chr 1
4 (R7)
0.0 AR33
5.1 UBC89-2
6.1 WG62-1 BRMS-129
6.5 WF69-2
6.9 OPAB09-4
7.3 AZ1 BRMS-327 WF64-3 OPAB09-3
8.6 UBC79-4
11.1 BN43
11.6 BRMS-040
13.8 BRMS-023
15.4 BRMS-018
18.9 BRMS-296
23.0 BRMS-005
35.7 BN51
48.4 WB06-1
50.8 BRMS-152
53.0 BRMS-151
60.4 CA51-2
65.0 BN233
69.9 BRMS-036
72.6 CA10
76.6 BRMS-298
80.9 BN233-2
84.7 AZ5
87.2 OPW03-1
92.5 AZ10
101.5 BN119
125.7 BN206
chr 1
chr 3
chr 1
5 (R9)
0.0 BN268
9.2 BN211
23.3 BN139
29.4 BRMS-154
34.3 BRMS-120
36.3 OPW01-2
41.6 AR10
44.9 BRMS-144
47.1 BRMS-229 BRMS-319
56.8 BRMS-079 WF64-1
58.4 BRMS-051
60.6 BN107
73.8 WF26-2
77.5 BRMS-324
80.2 BRMS-247
89.0 BN190
96.3 BRMS-060
97.0 BRMS-017-2
116.2 BRMS-017
chr 1
chr 3
chr 2
chr 3
6 (R1)
0.0 BN19
5.4 BN62
13.6 WG61-1
15.7 AR7-2
16.8 BRMS-175
20.0 BRMS-317 BN31
20.6 BRMS-307
31.4 AZ12
32.6 BRMS-287
33.4 BN262
34.2 AZ3
37.5 WE22-1
39.1 BRMS-339
39.7 BRMS-099
40.8 BN273
41.9 BRMS-245
44.1 BRMS-155
51.9 BRMS-100
54.1 BRMS-096
56.2 WE32-1 BRMS-056 AZH8
60.0 BRMS-314
75.3 BRMS-075
89.8 BRMS-098
91.9 WE31-1
99.7 BN135
chr 5 chr 3
chr 4
7 (R8)
0.0 BRMS-094
17.1 BRMS-070
18.2 AZ6
22.0 BRMS-033
23.6 BRMS-342
25.7 RA1275-2
29.1 BRMS-217
29.3 OPAB03-2
29.5 WB32-1
29.7 OPE12-1 RA1275A BN159
30.2 BRMS-173 BRMS-297
31.8 BRMS-088
36.2 BRMS-170
39.4 BRMS-093
42.6 BRMS-097 BRMS-246
45.1 WE31-2
51.6 BN133
55.8 BN272
60.0 WG61-3 BN254
71.6 AR29
77.7 BRMS-185
81.4 BN58
82.3 BN153
83.4 BRMS-198 BRMS-006
chr 1
chr 4
chr 1
8 (R2)
0.0 AZH3
1.1 BRMS-215
7.1 BN118
9.8 BN290
12.0 BN319 BN315
13.3 WG27-1
21.1 AZ14
27.4 UBC73-3
31.3 BN82
32.4 AZ13
35.3 BRMS-082
41.2 BN100
42.3 BRMS-090 BRMS-332 BRMS-315
45.0 CA90-2
46.1 BN20
60.8 BRMS-026
65.2 BRMS-228 OPW01-1
66.5 WF44-1
69.1 RA1275-1
70.8 OPA10-3
71.8 AR11
9 (R10)
0.0 BN58
3.8 WG42-3
6.1 BRMS-021
6.6 BRMS-186 UBC73-2
7.3 WA31-2
8.7 WE63-1
9.4 BRMS-085
12.5 AZ14
19.8 BN319
22.5 AZD1
25.3 RA1255-4
28.1 BN19
33.9 WC10-1
38.9 AR13
49.8 BN103
51.4 BRMS-062 BRMS-244
56.9 BRMS-019
58.3 CA90-1
64.1 BN268
chr 5
chr 5
10 (R4)
0.0 BN261
4.4 BRMS-195
19.2 BRMS-054
22.5 BRMS-105
30.3 WG61-2
31.6 AR40
32.1 BN266
32.6 BRMS-001
40.4 BN105
43.2 WF25-2
45.9 BRMS-276
chr 2

TABLE 1

Characteristic of the *B. rapa* linkage map

Linkage group	Length (cM)	No. of markers			
		SSR	RFLP	RAPD	Total
1	141.6	14	17	5	36
2	130.5	15	8	4	27
3	126.6	14	5	12	31
4	125.7	11	10	11	32
5	116.2	12	6	3	21
6	99.7	14	10	4	28
7	83.4	15	8	7	30
8	71.8	7	11	7	25
9	64.1	6	8	7	21
10	45.9	5	4	2	11
Total	1005.5	113	87	62	262

group 2 accounted for 10.5% of the variance. Because this small QTL was independent of *Crr1*, *Crr2*, and *Crr3* (Hirai *et al.* 2003), it is in fact a novel resistance locus and is designated *Crr4*. The total phenotypic variance of the three QTL was estimated to be 56.4%.

By analysis of the Ano-01 isolate, the ID of the F_2 individuals ranged from very susceptible to resistant (Figure 2B), and two QTL in linkage groups 7 and 2 were identified at a LOD threshold of 2.0 (Figure 3B). The regions of these two QTL were in accordance with *Crr1* and *Crr4* and accounted for 71.7 and 15.9% of the variance, respectively (Table 3). The total phenotypic variance of these two QTL was estimated to be 74.6%.

With the synteny map data from *B. rapa* and *A. thaliana*, two major QTL regions, *Crr1* and *Crr2*, were found to be aligned to the same genomic region of chromosome 4 in *A. thaliana* (Figure 4). The region that includes *Crr2* corresponds to the Arabidopsis BAC clones T5K18 (BRMS-096) and F17L22 (BRMS-100). In the *Crr1* region, BRMS-173 and BRMS-297 correspond to Arabidopsis BAC T6K21, but BRMS-088 displays only a low homology to Arabidopsis BACs. The region corresponding to *Crr2* in the Arabidopsis genome ranges to 970 kb and includes 273 open reading frames (ORFs). Of these, 59 ORFs exist as a single-copy gene in the Arabidopsis genome. All primer pairs designed for the 59 ORFs were successful in *A. thaliana*, and the 30 pairs also successfully amplified the corresponding fragments in the *B. rapa* mapping parents, A9709 and G004. A total of 12 markers were designed from these fragment sequences and named *B*rassica *S*NPs/indels from *A*rabidopsis (BSA) (Table 4). They included SNPs and/or indels between A9709 and G004. Three markers, BSA 3, BSA4, and BSA5, were mapped to the region of *Crr2* in linkage group 6 of *B. rapa*. Three other markers, BSA1, BSA2, and BSA7, were mapped to the *Crr1* region in linkage group 7. Additional BSA markers (BSA6, BSA8, and BSA9) developed from the synteny region of BRMS-100 and BRMS-155 in Arabidopsis were also mapped to the corresponding region in the *B. rapa* map.

DISCUSSION

The SSR-based linkage map of *B. rapa*: The present linkage map consists mainly of SSR markers and 10 linkage groups that correspond to the chromosome number of *B. rapa*, with an average distance between markers of 3.7 cM (Figure 1). One of the most conspicuous characteristics of the present map is the mapping of the 113 SSR markers. As indicated in the human linkage map (Dib *et al.* 1996), SSR markers have certain outstanding characteristics: (i) a codominant nature of high information value on genetic analysis; (ii) most can detect a single locus, while RFLP probes have a tendency to detect more than one locus in Brassica; (iii) SSR loci are well conserved among various *B. rapa* accessions, and most can be applied to related species such as *B. oleracea*, *B. napus*, and *A. thalinana* with high accuracy (Suwabe *et al.* 2002, 2004); and (iv) their genotype can be detected by a simple PCR technique. That is, in Brassica, SSR markers make it possible to carry out accurate genetic analysis with a simple technique, and moreover, the correspondence of the linkage groups and/or the linkage segments in the different maps can be compared with great clarity. In fact, SSR markers in the present linkage map could be used to assign linkage groups to the internationally agreed chromosomal nomenclature of *B. rapa*, R1–R10 (Figure 1, G. Teakle and G. King, personal communication). Comparative genomics elucidates not only the chromosomal organization within and between species, but also the evolutional processes of genomes. Several reports have described the conservation and colinearity of small chromosomal segments between various Cruciferous species, such as *B. napus* and *A. thaliana*, and have predicted extensive rearrangements of chromosomal segments before/after the differentiation of these species (Conner *et al.* 1998; Sadowski and Quiros 1998; Quiros *et al.* 2001;

Figure 1.—Linkage map of *B. rapa* and comparative map with *A. thaliana*. The 10 linkage groups are represented by vertical bars. Recombination distances are given in Kosambi centimorgans on the left side of the linkage groups, and the locus names are given on the right side. The number in parentheses at the top of each linkage group, R1–R10, indicates the internationally agreed chromosomal nomenclature of *B. rapa*. The synteny regions with *A. thaliana* (stippled areas) are shown to the right of the *B. rapa* linkage groups as vertical lines with chromosome numbers (chr 1 to chr 5). The arrowheads indicate the order of the corresponding region in the *A. thaliana* chromosomes. Double-headed arrows indicate the recombinant nature in the corresponding regions of *B. rapa* and *A. thaliana*. Marker types are indicated by first one to four letters: BRMS, SSR; A and BN, RFLP; and CA, OP, RA, UBC, and W, RAPD marker.

TABLE 2

Homologous SSR loci in *B. rapa* and *A. thaliana*

SSR marker	*A. thaliana*		
	Chromosome	BAC clone	*E*-value
BRMS-001	5	F3F24. F21E10	0.81
BRMS-005	5	MUD21, MSN2	4.00*E*-07
BRMS-006	1	T25K16	4.00*E*-39
	4	F1104	1.00*E*-08
	3	F21F14	4.00*E*-05
BRMS-007	3	K2019	0.008
BRMS-008	3	K1G2	0.11
BRMS-013	2	T16F16	0.063
BRMS-014	3	F1I16	0.043
	2	T2G17	0.043
BRMS-017	3	K20I9	4.00*E*-08
BRMS-018	2	F24L7	1.5
BRMS-019	5	F21E1, F8F6	4.00*E*-65
BRMS-021	5	MBG8	6.00*E*-07
BRMS-023	1	T2P11	3.00*E*-13
BRMS-026	1	F3N23	0.014
BRMS-027	1	F10F5	6.00*E*-13
BRMS-033	1	F5D21, F19C24	8.00*E*-34
BRMS-034	2	F18O19	7.00*E*-43
BRMS-036	1	F12A21	6.00*E*-55
	1	F21J9	2.00*E*-23
	3	F20C19	2.00*E*-08
	1	F3F19	5.00*E*-06
BRMS-040	1	F2J7	2.00*E*-19
	1	F24J5	7.00*E*-10
BRMS-043	2	F4P9	0.035
BRMS-051	3	T5N23	2.00*E*-34
BRMS-054	2	F4P9	2.00*E*-05
	2	T9J23	6.00*E*-04
	2	F17A22	6.00*E*-04
BRMS-056	5	K18I23, MOP10	0.009
BRMS-057	5	MUF9	0.31
BRMS-058	2	T6D20	5.00*E*-42
	4	F22L13, F20D10	3.00*E*-28
BRMS-060	3	K20I9	4.00*E*-09
BRMS-061	2	F4L23	5.00*E*-12
BRMS-062	2	T27E13	2.00*E*-33
	5	MHF15	2.00*E*-15
	5	T2I1	1.00*E*-06
BRMS-070	1	F20D21	7.00*E*-41
BRMS-071	5	T6G21	0.49
BRMS-075	2	T28M21, T5I7	4.00*E*-04
BRMS-079	3	MOD1	6.00*E*-52
	3	T17F15	1.00*E*-50
	3	T26G12	5.00*E*-34
	4	AP21, AP22	3.00*E*-04
BRMS-082	2	F17K2	0.001
BRMS-085	5	MRI1, MTI20	6.00*E*-19
BRMS-088	4	C6L9	0.009
	4	T1J24	0.15
BRMS-090	2	T32G6, F4P9	0.88
	5	K9B18, MUF9	0.88
BRMS-091	4	F1C12	0.27
	5	T1G16	0.27
BRMS-093	5	T1M15	0.024
BRMS-094	5	MXC20	5.00*E*-28
BRMS-096	4	T5K18	3.00*E*-07
BRMS-097	4	T30C3	0.028
BRMS-098	4	T19K4	*E*-104
BRMS-099	1	F13A11, F2H10	5.00*E*-04
BRMS-100	4	F17L22	5.00*E*-13
BRMS-101	1	F10F5	9.00*E*-34
BRMS-105	2	F4P9	3.00*E*-07
BRMS-106	5	MAC 12	2.00*E*-07
BRMS-108	3	T4C21	0.89
	2	T103	0.89
	4	F4C21	0.89
	3	F1M23	0.89
BRMS-120	1	F8K4	9.00*E*-15
BRMS-124	2	T32G6	0.18
	5	F7K24	0.18
	5	T24G5	0.18
	1	F5M6	0.18
BRMS-125	4	F18A5	0.19
	5	F22D1	0.19
	4	T1J24	0.19
BRMS-127	3	T4C21	0.58
	4	F4C21	0.58
BRMS-128	2	T32F6	2.00*E*-23
	1	YUP8H12	4.00*E*-12
BRMS-129	4	T7B11	1.00*E*-17
	5	MKD15	3.00*E*-15
	1	T14P4	1.00*E*-14
	3	T17J13	8.00*E*-13
	5	K11I1, F10E10	7.00*E*-04
BRMS-144	1	F1N18	0.006
BRMS-151	3	MAA21	8.00*E*-25
BRMS-152	3	NAA21	3.00*E*-33
BRMS-154	2	T30D6	4.00*E*-16
BRMS-155	1	T23E23	0.05
BRMS-158	3	MJG19(T7)	2.00*E*-20
	4	T27D20(T3)	1.00*E*-06
BRMS-163	1	T12O21	5.00*E*-26
BRMS-166	2	F7D19, F23E6	2.00*E*-40
BRMS-170	4	L23H3	3.00*E*-17
BRMS-173	4	T6K21	2.00*E*-22
BRMS-175	5	T32M21, T19N18	7.00*E*-15
BRMS-176	5	T32B03	8.00*E*-36
	3	T28G19, F21A14	5.00*E*-31
BRMS-184	3	MPE11	2.00*E*-05
BRMS-185	1	T21E18	5.00*E*-22
	2	T28P16	2.00*E*-06
BRMS-186	5	F5O24	0.44
BRMS-195	3	F18O21, F27K19	4.00*E*-19
	2	T3G21, T7M7	4.00*E*-16
BRMS-196	2	T14P1	4.00*E*-19
BRMS-198	5	MJE4	4.00*E*-05
	1	T14P4 ('3 only)	1.00*E*-16
BRMS-201	1	F7H2	4.00*E*-75
BRMS-206	3	MUH15	5.00*E*-33
	4	FCAALL	3.00*E*-13
BRMS-214	4	F17A13	8.00*E*-67

(*continued*)

TABLE 2

(Continued)

SSR marker	*A. thaliana*		
	Chromosome	BAC clone	*E*-value

(*continued*)

TABLE 2

(Continued)

SSR marker	*A. thaliana*		
	Chromosome	BAC clone	*E*-value
BRMS-215	5	T2L20	1.00*E*-27
	1	F8D11, F13A11	3.00*E*-07
	1	F2G19, F8G22	2.00*E*-04
BRMS-217	4	F6I18, MAL21, F16B22	0.15
BRMS-218	5	NED24, F8F6	4.00*E*-44
	3	T22K18	3.00*E*-20
BRMS-221	3	MVA11	0.16
BRMS-226	3	K17E12	2.00*E*-67
BRMS-227	1	T26J12	0.017
BRMS-228	5	MSL3	0.003
BRMS-229	1	T4K22	4.00*E*-35
BRMS-232	4	T13J8	0.49
BRMS-233	5	T25O11	0.027
BRMS-240	2	F18O19	6.00*E*-43
BRMS-242	3	F4B12	0.26
BRMS-244	5	T28J14	2.00*E*-35
	5	MRI1	2.00*E*-04
BRMS-245	4	FCAALL	2.00*E*-54
	5	MQL5	1.00*E*-12
BRMS-246	4	T19F6	4.00*E*-21
BRMS-247	2	F27L4	4.00*E*-25
	4	AP21, AP22	2.00*E*-05
BRMS-252	1	F6A14	3.00*E*-18
BRMS-261	5	MLN1	7.00*E*-16
BRMS-269	2	T1B3	0.024
	1	F9I5	0.024
BRMS-276	5	K15O15	1.00*E*-40
BRMS-287	3	MCB17	8.00*E*-50
BRMS-296	1	F5M15	2.00*E*-20
BRMS-297	4	T6K21	3.00*E*-23
BRMS-298	1	T17F3	1.00*E*-64
	1	F20P5	1.00*E*-12
BRMS-303	5	MNC17	1.00*E*-33
BRMS-307	3	T21B14, T23B7	5.00*E*-32
	5	MPH15	3.00*E*-15
BRMS-309	5	K7J8	0.015
BRMS-314	4	F17A13	7.00*E*-67
BRMS-315	5	MRO11 (T3)	0.065
	5	K5J14 (T7)	0.019
BRMS-317	3	T23B7, T21B14	2.00*E*-31
	5	MPH15	9.00*E*-15
BRMS-319	1	F1N18	3.00*E*-35
BRMS-322	2	F6E13	1.00*E*-12
BRMS-324	2	F17H15, F3N11	3.00*E*-10
BRMS-327	3	F24K9	0.13
BRMS-330	5	MYH9	0.26
BRMS-332	2	T22F11, F23J3, T24P13	1.2
BRMS-333	3	T4P13	1.00*E*-31
	1	F10C21, T16O9	5.00*E*-06
BRMS-339	3	F9K21, T14D3	0.002
BRMS-342	1	T28K15	4.00*E*-26
	1	F5O11	1.00*E*-19

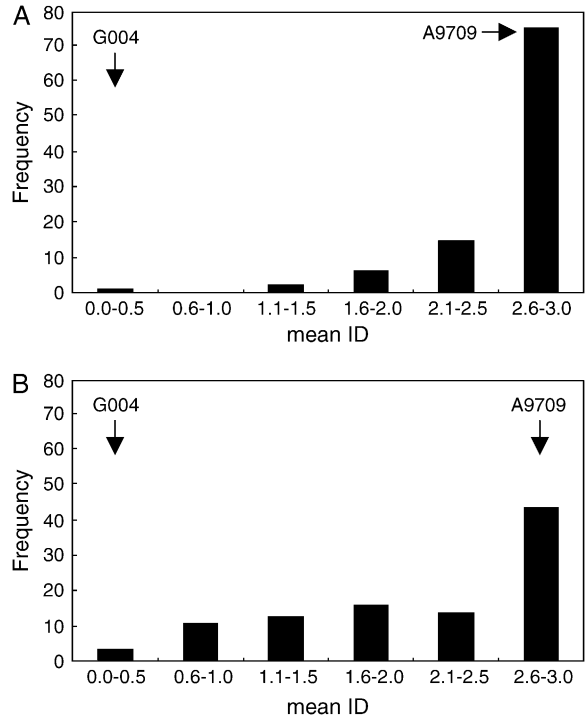

FIGURE 2.—Frequency distribution of disease index (ID) for clubroot resistance in the F_2 population. (A) "Wakayama-01" isolate; (B) "Ano-01" isolate.

BABULA *et al.* 2003; LUKENS *et al.* 2003). In Brassica, linkage maps have been constructed by RFLP, RAPD, and AFLP markers and contributed to various genetic analyses, although to date alignment of the different maps has been conducted exclusively on the basis of RFLPs.

The comparative map of *B. rapa* and *A. thaliana*: *B. rapa* and *A. thaliana* belong to the same family, the Cruciferae. They originated from a common ancestor and differentiated into related genera ~14.5–20.4 million years ago (YANG *et al.* 1999). As indicated in previous studies (refer to OSBORN and LUKENS 2003), a region should exist in common in the chromosomes in these species. Arabidopsis has a relatively small genome (125 Mb), resulting from an exceptionally low rate of repetitive sequences and high gene density (MEYEROWITZ 1992). In contrast, the genome of *B. rapa* is far larger (550 Mb) than that of *A. thaliana* and has a high rate of rearrangements (KOWALSKI *et al.* 1994). It is not surprising that the complex rearrangements and overlaps of the Arabidopsis genome exist in the genome of Brassica. The present results reveal that small genomic segments of Arabidopsis are dispersed throughout the entire *B. rapa* genome (Figure 1). Furthermore, we found no synteny in linkage group 8, although BRMS-215 exhibited a high homology ($E < 10^{-10}$) to certain parts of chromosomes 1 and 5 in *A. thaliana*. These results illustrate the complex nature of the chromosomal rearrangements that occurred before/after differentiation of Arabidopsis and Brassica. They also support a previous report that *A. thaliana* and Brassica originated from a common ancestor and differentiated with certain chromosomal segments conserved

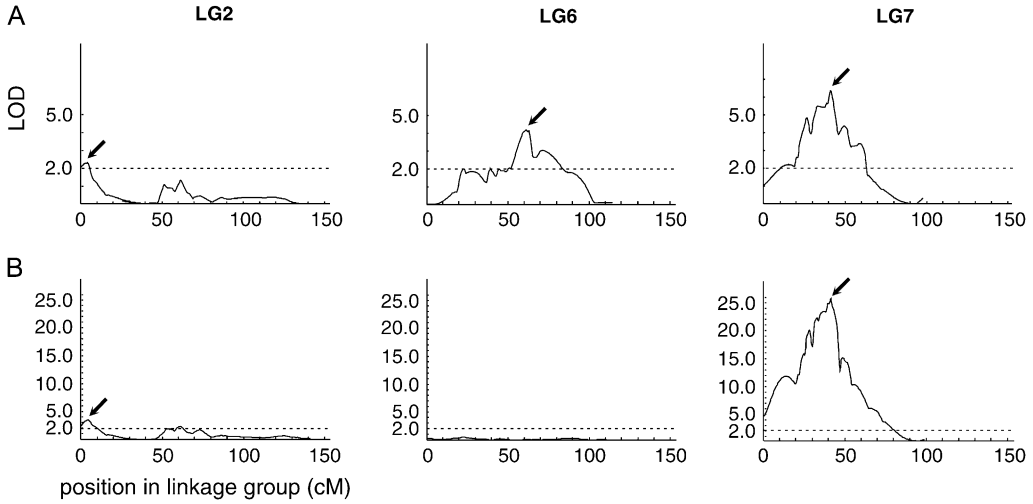

FIGURE 3.—QTL LOD plots for clubroot resistance in *B. rapa*. The LOD scores for QTL affecting clubroot resistance are presented along with the linkage groups 2, 6, and 7. The LOD threshold of 2.0 is indicated. (A) "Wakayama-01" isolate; (B) "Ano-01" isolate.

(YANG *et al.* 1999). Together with earlier reports on *B. oleracea* (BABULA *et al.* 2003), the situation is more complex than has been previously expected in Brassica. These results also support the complexity of the Brassica genome.

Clubroot resistance in *B. rapa* and its genetic origin: *Crr1* in linkage group 7 and *Crr4* in linkage group 2 were effective in the two different pathogen isolates used in this study (Figure 3, Table 3). These results suggest that these two QTL are commonly effective for resistance to clubroot in *B. rapa* and play key roles in resistance. There are two components to the mechanism of disease resistance. The first is a common pathway that is involved in responses against pathogen attack (MEYERS *et al.* 1999). Many genes would participate in such a cascade, such as genes in the signaling pathways and in the production of resistance factor(s). Although the pathological response mechanisms of *Crr1* and *Crr4* are not clear at present, key genes for resistance do reside in these two QTL regions. The second component is a determinant for specificity in recognition during plant–pathogen interactions and/or an enhancing factor for the expression of a more potent resistance. In this study, *Crr2* was specifically effective against the Wakayama-01 isolate, the most virulent. The expression of resistance to the Ano-01 isolate was effective without *Crr2*, however (Figure 3, Table 3). One possible explanation for this result is that *Crr2* is an enhancer for the resistance expressed by *Crr1* and/or *Crr4*, rather than a determinant for recognition specificity. That is, clubroot resistance in *B. rapa* is controlled by two components, a common pathway that has a central role in resistance and an additional component that is an enhancer for more powerful resistance.

The two QTL regions containing CR loci, *Crr1* and *Crr2*, were aligned to the same region of chromosome 4 in *A. thaliana* and overlapped with each other (Figure 4). These results suggest that a progenitor resistance gene(s) existed in a common ancestral genome and resided in the same region of the chromosome, although it is not known whether they are functional against clubroot in either the ancestral species or Arabidopsis. These results suggest two hypotheses about the evolution of the clubroot resistance. One is that clubroot resistance was originally controlled by a single major gene, which differentiated and diverged into two functionally different, duplicate genes, during evolution of the genome in Brassica. If so, it might gain a diversification of the resistance mechanism in response to differences in pathogenicity. This could also lead to an increased complexity of the resistance mechanism. In maize, the *rp1* complex for common rust resistance has evolved with a duplication and recombination of genes in the evolution of a family of resistance genes, leading to a diversification of resistance (COLLINS *et al.* 1999). Another possibility is that the resistance genes

TABLE 3

Position and effect of QTL for clubroot resistance in *B. rapa*

Isolate	Linkage group	Marker interval	LOD	% variation
Wakayama-01	7	BRMS-297–BRMS-088	6.4	26.8
	6	BRMS-100–BRMS-096	4.1	18.3
	2	BN288D–WE24-1	2.3	10.5
Ano-01	7	BRMS-297–BRMS-088	25.7	71.7
	2	BN288D–WE24-1	3.5	15.9

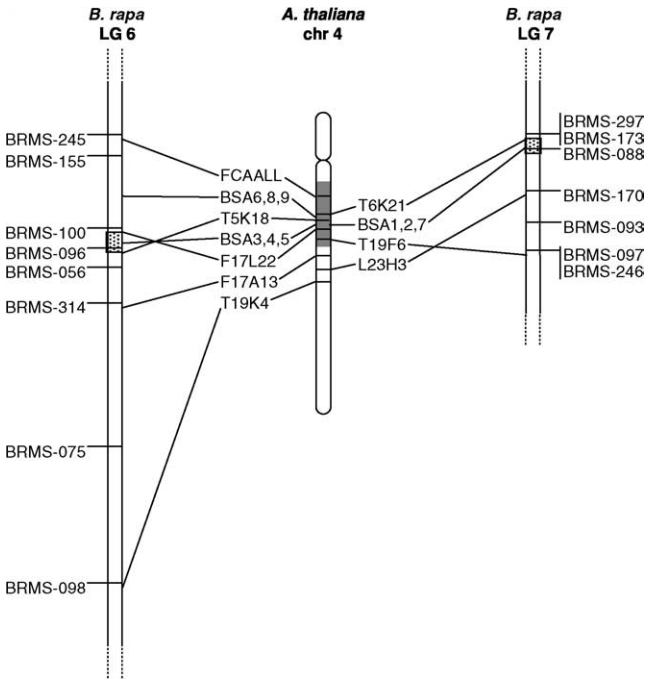

FIGURE 4.—Synteny map of the *Crr1* and *Crr2* regions (stippled area) with *B. rapa* and *A. thaliana*. The linkage groups (LG) of *B. rapa* and the chromosome (chr) of *A. thaliana* are presented as vertical bars. The Arabidopsis BAC clones that correspond to the SSR loci of *B. rapa* are connected by lines. The shaded region indicates the cluster of disease-resistance genes (MRC-H) in the Arabidopsis chromosome.

for clubroot were originally clustered in that genomic region of the ancestral genome and distributed into two different genomic regions following chromosomal rearrangements in Brassica. A clustering of disease-resistance genes has been observed in certain genomic regions in *A. thaliana* (HOLUB 1997) and also in other plant genomes (HULBERT *et al.* 2001). A region in chromosome 4 of *A. thaliana*, a focus of the present study, was previously identified as one of the clusters of disease-resistance genes, termed the major recognition complexes (MRCs) (HOLUB 1997; SPEULMAN *et al.* 1998). In this region (MRC-H), disease-resistance genes with characteristic motifs, such as leucine-rich repeats (LRRs) and nucleotide-binding sites (NBSs), are clustered, for example, *RPP* for a resistance to *Peronospora parasitica* and *RPS* for a resistance to *Pseudomonas syringae*, as well as *ACD*, acceleration of cell death in response pathogen infection. These genes confer resistance to viral, bacterial, and fungal pathogens. The clustering of resistance genes confers an advantage, because it helps maintain resistance while allowing novel specificities to evolve. The resistance genes for clubroot may be members of just such a resistance gene cluster, and species-specific evolution of the resistance mechanism may have occurred in Brassica. At present, although as yet inconclusive, both models would eventuate in a diversity of resistance mechanisms to clubroot.

In another, diploid Brassica species, *B. oleracea*, two QTL, CR2a and CR2b have been identified as being involved in clubroot resistance (LANDRY *et al.* 1992). These account for 58 and 15% of phenotypic variability. VOORRIPS *et al.* (1997) also reported similar results with *pb-3* and *pb-4*. These characteristics in *B. oleracea* seem to be consistent with the characteristics in *B. rapa*. Because the genomes of *B. rapa* and *B. oleracea* are thought to have evolved from a common ancestor, it is interesting to inquire whether clubroot resistance in *B. rapa* and *B. oleracea* might likewise have a common origin and be maintained by similar mechanisms. Further comparative studies of *B. rapa* and *B. oleracea* should provide new insights into this hypothesis.

TABLE 4

SNP/indel markers constructed at the homologous *Crr2* region in *A. thaliana*

Marker	Primer	Product size (bp)	Acc. no.	Predicted homologous protein
BSA1	CAGTCTAAAGGTGATCTTGCAGAGG ACCTGATGAGCAGCAATAATAACCA	402	AT4G19550	Expressed protein
BSA2	TGTTAGACATCAAAGAGGGCTTGAG TCTCAAAGACGATAATGAACCCAAA	489	AT4G19900-2	Glycosyl transferase related
BSA3	CATTTGAGATTAAGATCAGGCGATG TCGAGTTTGGTCTCTGTGAAAAATC	491	AT4G20350	Expressed protein
BSA4	TAACGCTGTTGGTCCAATTCTAGTG CCACAGGATCTTTCCTCCTACCTAA	475	AT4G20760	Short-chain dehydrogenase Reductase family protein
BSA5	ATTCTTTGATATTGAGCCATGTGGT TCCAGAGAGATACACAGTTCTCATCA	589	AT4G21520	Transduction family protein WD-40 repeat family
BSA6	ACTCCTCTGGAAATGCTCTTGAACT ATTACTGATTGAGGGCCTTCTTGAC	435	AT4G21820	Calmodulin-binding family protein
BSA7	AAGCAGAAGGGTTCTTGTCTGAACT TCACTCTGAGACATGAGGACACTTG	410	AT4G21865	Expressed protein
BSA8	GTGATCTTCTTCATGCTGTTGTTCC ACACCATCTTGAGAGTTGGTTGTTG	590	AT4G22930	Dihydroorotase
BSA9	CATTGCCGGTGAAGCTGAAG CACAAGTCCACAAAGTTAACACACG	683	AT4G27430	COP1-interacting protein 7

Our results provide evidence for a genetic effect of QTL in clubroot resistance and genomic correlation with Arabidopsis. They could lead to new insights into the complicated mechanisms of clubroot resistance in Brassica. Further genetic analysis using genomic information from Arabidopsis will move us toward a comprehensive understanding of clubroot resistance in Brassica and should yield valuable information for the deliberate breeding of clubroot-resistance crops in Brassica. More generally, they will contribute to the understanding of the emergence of resistance gene clusters in the course of evolution.

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