Theor Appl Genet (2008) 117:759–767
DOI 10.1007/s00122-008-0817-0

ORIGINAL PAPER

Mapping of isolate-specific QTLs for clubroot resistance in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*)

Koji Sakamoto · Atsuo Saito · Nobuaki Hayashida · Goro Taguchi · Etsuo Matsumoto

Received: 21 February 2008 / Accepted: 22 May 2008 / Published online: 9 July 2008

Abstract A number of clubroot resistant (CR) Chinese cabbage cultivars have been developed in Japan using resistant genes from CR European fodder turnips (*B. rapa* ssp. *rapifera*). Clubroot resistance in European fodder turnips are known to be controlled by the combined action of several dominant resistance genes. We have developed three Chinese cabbage clubroot-resistant doubled haploid (DH) lines—T136-8, K10, and C9—which express resistance in different manners against two isolates of *Plasmodiophora brassicae*, M85 and K04. Depending on the isolates, we identified two CR loci, *CRk* and *CRc*. *CRk* was identified by quantitative trait loci (QTL) analysis of an F_2 population derived from a cross between K10 and Q5. This locus showed resistance to both isolates and is located close to *Crr3* in linkage group R3. The other locus, *CRc* was identified by QTL analysis of an F_2 population derived from a cross between C9 and susceptible DH line, 6R. This locus was mapped to linkage group R2 and is independent from any published CR loci. We developed sequence-tagged site markers linked to this locus.

Communicated by C. F. Quiros.

K. Sakamoto (✉) · A. Saito
Takii Plant Breeding and Experiment Station,
Konan, Shiga 520-3231, Japan
e-mail: k-sakamoto@takii.co.jp

N. Hayashida
Division of Gene Research, Shinshu University,
Ueda, Nagano 386-8567, Japan

G. Taguchi
Faculty of Textile Science and Technology,
Shinshu University, Ueda, Nagano 386-8567, Japan

E. Matsumoto
Nagano Vegetable and Ornamental Crops Experiment Station,
Matsushiro, Nagano 381-1211, Japan

Introduction

Clubroot caused by a soil borne fungus *Plasmodiophora brassicae* Woronin, is one of the most serious diseases of cruciferous crops. The heirloom Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) cultivars are highly susceptible to the disease and are destructively damaged in severely infected fields. A number of clubroot resistant (CR) Chinese cabbage cultivars have been developed in Japan using the resistant genes from CR European fodder turnips (*B. rapa* ssp. *rapifera*). Clubroot resistance in European fodder turnips has been investigated and is known to be controlled by few major resistant genes (Toxopeus and Janssen 1975; Wit 1964). Therefore, clubroot resistance in Chinese cabbage cultivars appears to be expressed in the same way as in turnips with certain modifications. Previously published studies had reported five major CR genes (loci) *Crr1*, *Crr2*, *Crr3*, *CRa*, and *CRb*, and their linked markers BRMS088, BRMS096 (Suwabe et al. 2003), OPC11-2S (Hirai et al. 2004), HC352b-SCAR (Hayashida et al. 2008), and TCR05 (Piao et al. 2004), respectively. Comparison of the effects of these CR genes is difficult because of the use of different resistant sources, screening methods, and pathogen isolates. We have developed three CR doubled-haploid (DH) lines of Chinese cabbage—T136-8 (Matsumoto et al. 1998), K10, and C9—which express different kind of resistance against two isolates of *P. brassicae*, M85 and K04. *CRa*, a dominant resistance locus was identified by linkage analysis of an F_2 population derived from a cross between T136-8 and susceptible DH line, Q5 (Matsumoto et al. 1998).

The present study reports two CR loci, which were identified by quantitative trait loci (QTL) analysis of two F_2 populations derived from crosses between resistant DH lines of different sources and susceptible DH lines. We have reported the development of the sequence-tagged site (STS) markers linked to the novel CR loci.

Moreover, we have also discussed the utility of STS markers for marker-assisted selection (MAS) in *Brassica* species and the possibility to use these three clubroot-resistant DH lines for classification of field isolates of *P. brassicae*.

Materials and methods

Plant materials

Two F_2 populations of Chinese cabbage were used to investigate clubroot resistance. One F_2 progeny resulted from a cross between the DH lines K10 and Q5. The CR line K10 derived from the commercial cultivar "CR Kanko" (Nippon Norin Seed Co., Tokyo, Japan) was crossed with a clubroot susceptible line Q5, which has orange–yellow colored flowers and inner leaves of the head. In the other cross combination, two DH Chinese cabbage lines, C9 and 6R were used as parents. The CR line C9 was derived from the European fodder turnip "Debra" crossed with the clubroot susceptible line 6R. A single F_1 hybrid plant was bud self-pollinated to obtain the F_2 progeny. F_2 individuals were bud self-pollinated to obtain the F_3 families that were used to test with *P. brassicae* isolates and to calculate QTL.

Pathogen

Two isolates of *P. brassicae* M85 (Matsumoto et al. 2005) and K04 were used in the resistance tests. Chinese cabbage clubroot-resistant DH lines—T136-8, K10, and C9—expressed resistance in different ways against M85 and K04 (Table 1). The M85 isolate was maintained by infecting of the Chinese cabbage cultivar "Muso" (Takii & Co. Ltd, Kyoto, Japan). K04 which had virulence to commercial CR Chinese cabbage cultivars "Kukai," "Kobo," and "CR Shinki" (Takii & Co. Ltd, Kyoto, Japan) was maintained by infection of the Chinese cabbage cultivar "Kukai."

Test for clubroot resistance

Inoculation tests were carried out in glasshouses as previously described (Matsumoto et al. 1998). Fifteen seeds per F_3 family were sown in a plastic tray with inoculated soil (two replications). Inoculum was adjusted to 3.0×10^5 spores/g of soil. Parental lines were used as resistant and susceptible controls. Thirty days after sowing the plants were evaluated for clubroot infection. Disease symptoms were scored as follows: 0, no symptoms; 1, a few small clubs on lateral roots; 2, larger clubs on lateral roots and/or slight swelling of main roots; 3, severe galling of tissues of both lateral and main roots. The disease index for each F_2 individual was calculated from the mean grades of two F_3 progeny tests.

Table 1 Resistance responses of DH and F_1 cultivars of Chinese cabbage against *P. brassicae* isolates, M85 and K09

Variety	Isolate	
	M85	K04
F_1		
Muso	S	S
Kukai	R	S
DH		
Q5	S	S
6R	S	S
T136-8	R	S
K10	R	(R)
C9	S	R

R resistant, no symptoms, disease index was scored 0, *(R)* partial resistant, a few small clubs on lateral roots, disease index was scored 1, *S* susceptible, larger clubs on lateral roots and/or slight swelling of main roots and severe galling of tissues of both lateral roots and main roots, disease indexes were scored as 2 and 3

DNA extraction and marker analysis

DNA was extracted from the leaves of 98 and 60 F_2 plants derived from K10 × Q5 and C9 × 6R according to the cetyltrimethylammonium bromide method (Murray and Thompson 1980). Restriction fragment length polymorphism (RFLP) analysis was performed as previously described (Matsumoto et al. 1998). Random amplification of polymorphic DNA (RAPD) analysis was performed using 12-mer random primers (Wako Pure Chemicals, Osaka, Japan). Amplified fragment length polymorphism (AFLP) analysis was performed using the AFLP Analysis System I kit (Invitrogen Corp., Carlsbad, CA, USA). Simple sequence repeat (SSR) markers of *B. rapa* were amplified as previously reported (Suwabe et al. 2002, 2006). The STS markers for *CRa* (Hayashida et al. 2008), *CRb* (Piao et al. 2004), *Crr3* (Hirai et al. 2004), and self-incompatibility (Sakamoto and Nishio 2001) were also analyzed. Another STS marker, m6R (forward: 5′-CCTCTTGGAAAACCCATGAA-3′, reverse: 5′-GCAATTATTGGCCTGTTCGT-3′) was designed from CR susceptible line, 6R, specific fragment (Accession number AB435409, fragment size: 890 bp) which was derived from a bulk segregant analysis (Michelmore et al. 1991) of F_2 population derived from C9 × 6R.

Construction of linkage map

Linkage analysis for the molecular markers and the clubroot resistance genes was performed using JoinMap 3.0 (Biometris, Wageningen, The Netherlands). Segregation ratios of markers were tested for deviations from the expected Mendelian ratios using the chi-square test implemented by Joinmap 3.0 at a significance level of $\alpha = 0.05$. Loci that were completely linked were identified and removed from the data set before the groups was determined. Linkage groups (LGs) were established with a minimum logarithm of odds (LOD) threshold of 4.0. Genetic distances between markers were estimated by Kosambi function (Kosambi 1944).

QTL analysis for clubroot resistance

The QTL detection was performed with the QTL Cartographer software version 2.5 (Basten et al. 2005) using composite interval mapping (CIM). CIMs were performed at LOD threshold values which were estimated by means of a permutation test with 1,000 permutations.

Results

Construction of linkage maps

The linkage map of the F_2 progeny derived from K10 × Q5 has been constructed using the AFLP, RAPD, RFLP, STS, and SSR markers. In the AFLP analysis, 96 polymorphic markers were detected in the products generated from 21 primer pairs. In the RAPD analysis, 88 polymorphic markers were detected from 24 primers. In the RFLP analysis, 112 polymorphic loci were detected using 86 probes. 22 polymorphic markers were detected in the SSR and STS analysis. When a total of 318 polymorphic markers were analyzed, 236 markers were distributed into 12 LGs, covering 640.3 cM and the average interval between markers was 2.7 cM (Table 2; Fig. 1a). The presence of SSR markers BRMS144, BRMS018, BRMS026, BRMS088, BRMS173, BRMS001, BRMS158, BRMS232, BRMS096, BRMS013 (Suwabe et al. 2006), and NiF02 (Lowe et al. 2004) indicated that LG 1, 2, 3, 4, 5, 6, 7, 8, 9, and 11 correspond to R9, 7, 2, 8, 4, 3, 5, 1, 10 and 6 of the internationally agreed *Brassica* reference LG.

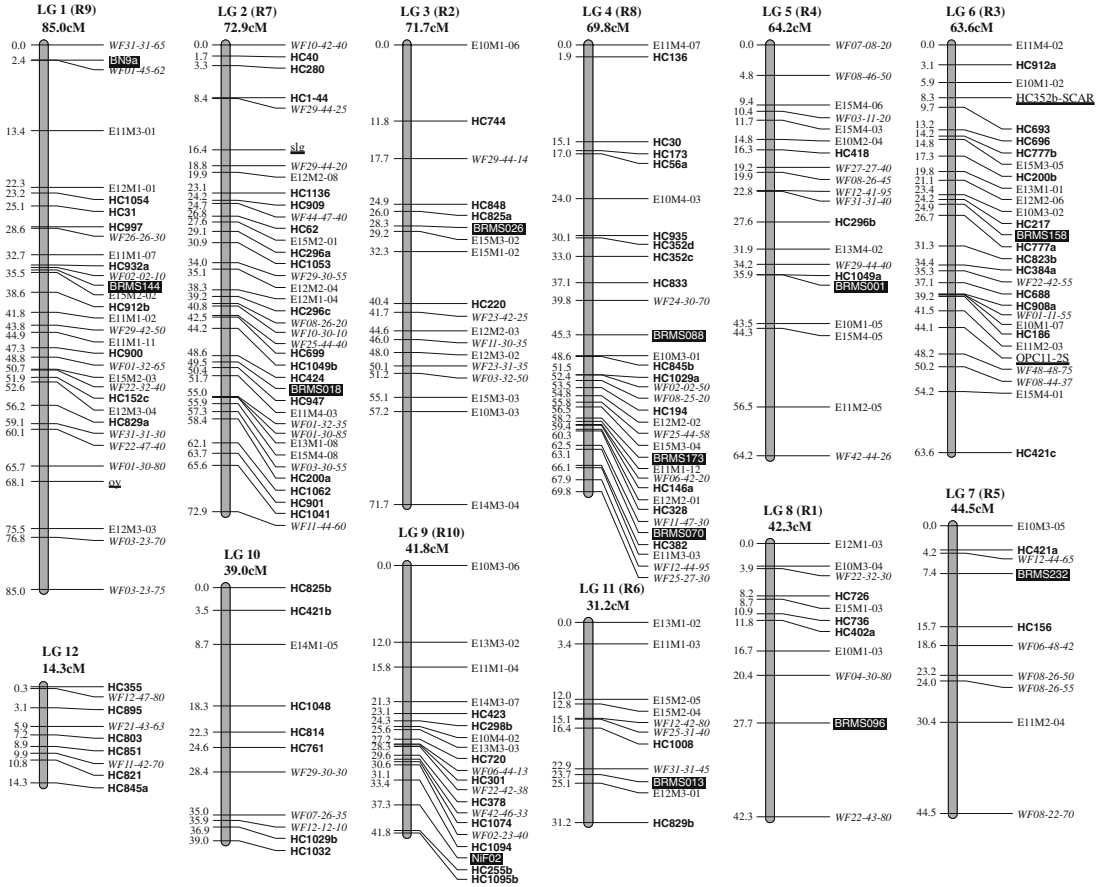
The linkage map of the F_2 progeny derived from C9 × 6R have been constructed using 160 AFLP, 3 RAPD, 7 RFLP, 5 STS, and 12 SSR markers which were screened from the polymorphic markers of 266 AFLP, 3 RAPD, 15 RFLP, 5 STS, and 21 SSR. These markers were distributed into 10 LGs, covering 485.9 cM, and the average interval between the markers was 2.6 cM (Table 2; Fig. 1b). The presence of anchor SSR markers BRMS129, BRMS056, BRMS013, BRMS054, BRMS007, BRMS057, BRMS242, BRMS026, BRMS082, BRMS088, BRMS173 and BRMS106 (Suwabe et al. 2006) indicated that LG 1, 2, 3, 4, 6, 7, 8 and 9 corresponds to R7, 1, 6, 4, 5, 2, 8 and 3 of the internationally agreed *Brassica* reference LGs.

Table 2 Characteristics of *B. rapa* likage map

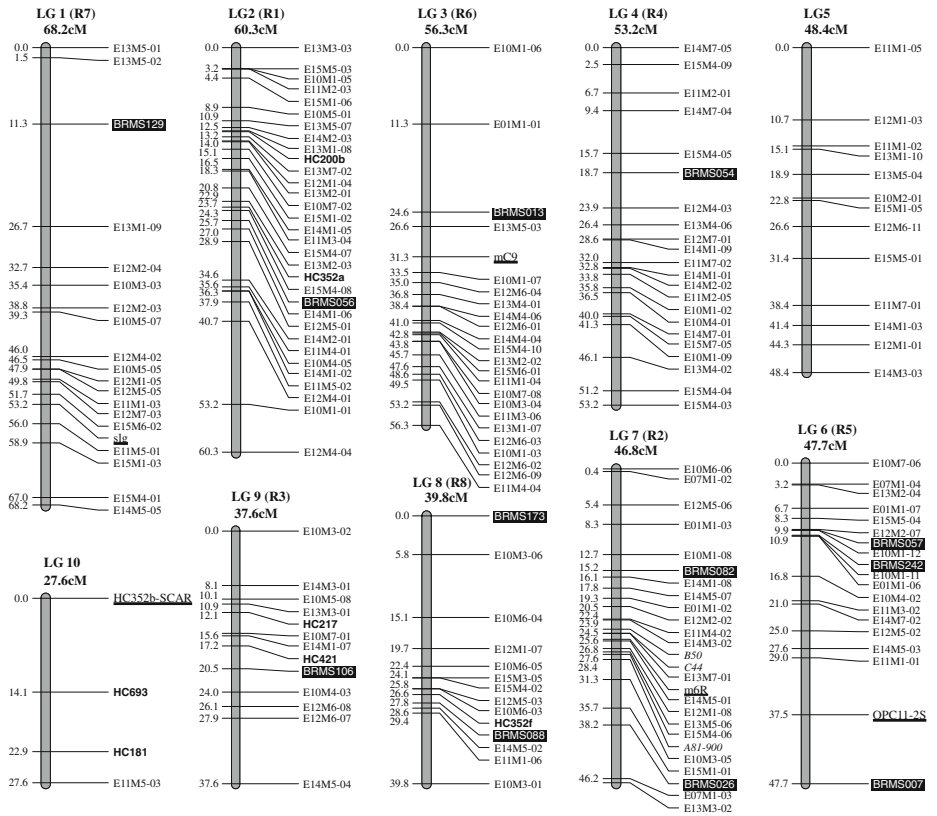
Linkage Group	Length (cM)	No. of markers					
		AFLP	RAPD	RFLP	STS	SSR	Total
(K10 × Q5)							
1	85.0	8	12	8	1	2	31
2	72.9	7	12	17	1	1	38
3	71.7	8	5	4	0	1	18
4	69.8	7	8	14	0	1	30
5	64.2	7	9	3	0	1	20
6	63.6	9	4	11	2	1	27
7	44.5	2	5	2	0	1	10
8	42.3	4	3	3	0	1	11
9	41.8	6	4	9	0	1	20
10	39.0	1	3	7	0	0	11
11	31.2	5	3	2	0	1	11
12	14.3	0	3	6	0	0	9
Total	640.3	64	71	86	4	11	236
(C9 × 6R)							
1	68.2	18	0	0	1	1	20
2	60.3	29	0	2	0	1	32
3	56.3	22	0	0	1	1	24
4	53.2	21	0	0	0	1	22
5	48.4	13	0	0	0	0	13
6	47.7	15	0	0	1	3	19
7	46.8	20	3	0	1	2	26
8	39.8	11	0	1	0	2	14
9	37.6	10	0	2	0	1	13
10	27.6	1	0	2	1	0	4
Total	485.9	160	3	7	5	12	187

QTL analysis for clubroot resistance

With the interval mapping analysis for the F_2 progeny derived from K10 × Q5, one putative QTL for clubroot resistance to the two isolates, M85 and K04 was detected and localized on LG 6 (R3). The largest QTL effect (LOD 20.3) for clubroot resistance to M85 was significantly detected in the interval between 27.7 and 50.2 cM on LG 6 (R3), based on the LOD threshold of 8.1 in the permutation test. The largest QTL effect (LOD 14.5) for clubroot resistance to K04 was also detected in the same region, based on the LOD threshold of 6.7 in the permutation test. The effects of the QTLs detected are listed in Table 3. Published


LG 1 (R9)
85.0cM
LG 2 (R7)
72.9cM
LG 3 (R2)
71.7cM
LG 4 (R8)
69.8cM
LG 5 (R4)
64.2cM
LG 6 (R3)
63.6cM
LG 12
14.3cM
LG 10
39.0cM
LG 9 (R10)
41.8cM
LG 11 (R6)
31.2cM
LG 8 (R1)
42.3cM
LG 7 (R5)
44.5cM

a


LG 1 (R7)
68.2cM
LG2 (R1)
60.3cM
LG 3 (R6)
56.3cM
LG 4 (R4)
53.2cM
LG5
48.4cM
LG 10
27.6cM
LG 9 (R3)
37.6cM
LG 8 (R8)
39.8cM
LG 7 (R2)
46.8cM
LG 6 (R5)
47.7cM

b

◀ **Fig. 1** Linkage map of *B. rapa* obtained from F_2 plants derived from K10 × Q5 cross (**a**) and C9 × 6R cross (**b**). RFLP, AFLP, RAPD, SSR and STS are indicated by different characters, *ariel bold*, *ariel*, *italic*, *white in black box* and *underlined*. Locus names are indicated on the *right side* of LGs and map distances in centi-Morgans are on the *left*. The number in parentheses at the top of each linkage group, *R1–R10*, indicates the internationally agreed *Brassica* reference LG

CR gene linked markers BRMS088, BRMS096, HC352b-SCAR and TCR05 were not detected in this region (Fig. 2a). This result indicates that these QTLs are independent of the CR genes *Crr1*, *Crr2*, *CRa* and *CRb*. This CR locus was named *CRk*. *Crr3* linked marker OPC11-2S was located in this region. *Crr3* is possibly involved within the location of QTLs obtained here.

With the interval mapping analysis for the F_2 progeny derived from C9 × 6R, one putative QTL localized on LG 7 (R2), involved clubroot resistance to the K04 isolate (Table 4). This QTL (LOD 24.0) was located between E01M1-03 and E12M1-08 (map distance 27.4 cM), based on the LOD threshold of 5.3 in the permutation test. None of the published CR loci were included in R2 and CR linked markers, BRMS088, 096, OPC11-2S, HC352b-SCAR, and TCR05 were not detected in this region (Fig. 2b). This result indicates that this QTL is independent of the CR genes *Crr1*, *Crr2*, *Crr3*, *CRa*, *CRb*, and *CRk*. This new CR locus was named *CRc*.

Association between the markers and clubroot resistance

As for the relationship between CR and linked marker genotypes in the F_2 population between K10 and Q5, the K10 homozygote exhibited a high level of resistance to M85 and insufficient resistance to K04 on each marker (Fig. 3a). The Q5 homozygote was susceptible to the two isolates.

From genetic and phenotypic analyses of the F_2 progeny of C9 × 6R, we found a STS marker, m6R closely linked to *CRc* (Fig. 4). m6R was designed using the sequence of 890 bp fragment which was amplified in susceptible bulk. A bulk segregant analysis was conducted in the F_2 progeny of C9 × 6R using a 12-mer random primer (5′-TGGC-CTCTTGGA-3′). Susceptible and resistant bulks were including ten individuals of susceptible and resistant, respectively. Thus, m6R is a dominant marker for susceptible. About 761 bp fragments were found in 6R homozygote and heterozygote but not in C9 homozygote. C9 homozygote exhibited complete resistance to K04 and susceptibility to M85 and 6R homozygote exhibited susceptible to the two isolates on each marker (Figs. 3b, 4).

Discussion

We identified three clubroot resistant loci *CRa*, *CRk*, and *CRc* for two different *P. brassicae* isolates, M85 and K04. These loci nearly co-segregated with three markers HC352b-SCAR, HC688, and m6R, respectively. *CRa* was derived from fodder turnip line ECD-02 (Matsumoto et al. 2005), a member of the European Clubroot Differential set (Buczacki et al. 1975). *CRk* and *CRc* were derived from fodder turnip "Debra." CR Chinese cabbages have been bred using European turnips "Gelria R," "Siloga," "Debra," and "Milan White" (Hirai 2006). *Crr1*, *Crr2*, and *Crr4* were derived from "Siloga" (Kuginuki et al. 1997, Suwabe et al. 2003, 2006). *Crr3* was derived from "Milan White" (Hirai et al. 2004; Saito et al. 2006). *CRb* was derived from ECD-01 (Piao et al. 2004). The precise relationship of the CR loci should be described using polymorphic anchor markers, such as microsatellite and STS markers. The distribution of microsatellite markers indicated that *CRa* and *CRk* were located in R3 of the internationally agreed *Brassica* reference LGs (Kim et al. 2006) but independent of each other. *Crr3* was also located in R3 and its linked marker OPC11-2S (Hirai et al. 2004) was mapped in the QTL region of *CRk* in our analysis. This means that *CRk* and *Crr3* may be near each other but not be the same locus because these two loci were derived from different CR origins and inoculated with different isolates and conditions. Fine mapping should be needed to identify the relationship between these loci. The linked markers for *CRb* were

Table 3 Summary of QTLs detected for clubroot resistance against two different isolates of *P. brassicae* using CIM

Isolate	Linkage group	Closest marker	Position (cM)	LOD	R^2 (%)	Additive effect	Dominance effect
M85	LG6 (R3)	HC688	37.1	18.6	61.4	1.16	0.64
	LG6 (R3)	HC186	39.3	15.1	55.8	1.08	0.56
	LG6 (R3)	E11M2-03	42.6	18.1	65.0	1.21	0.36
	LG6 (R3)	OPC11-2S	46.2	20.3	71.1	1.24	0.42
K04	LG6 (R3)	HC688	37.1	14.1	56.5	0.54	0.38
	LG6 (R3)	HC186	39.3	11.3	50.2	0.51	0.34
	LG6 (R3)	E11M2-03	42.6	13.7	57.1	0.57	0.31
	LG6 (R3)	OPC11-2S	46.2	14.2	58.6	0.57	0.31

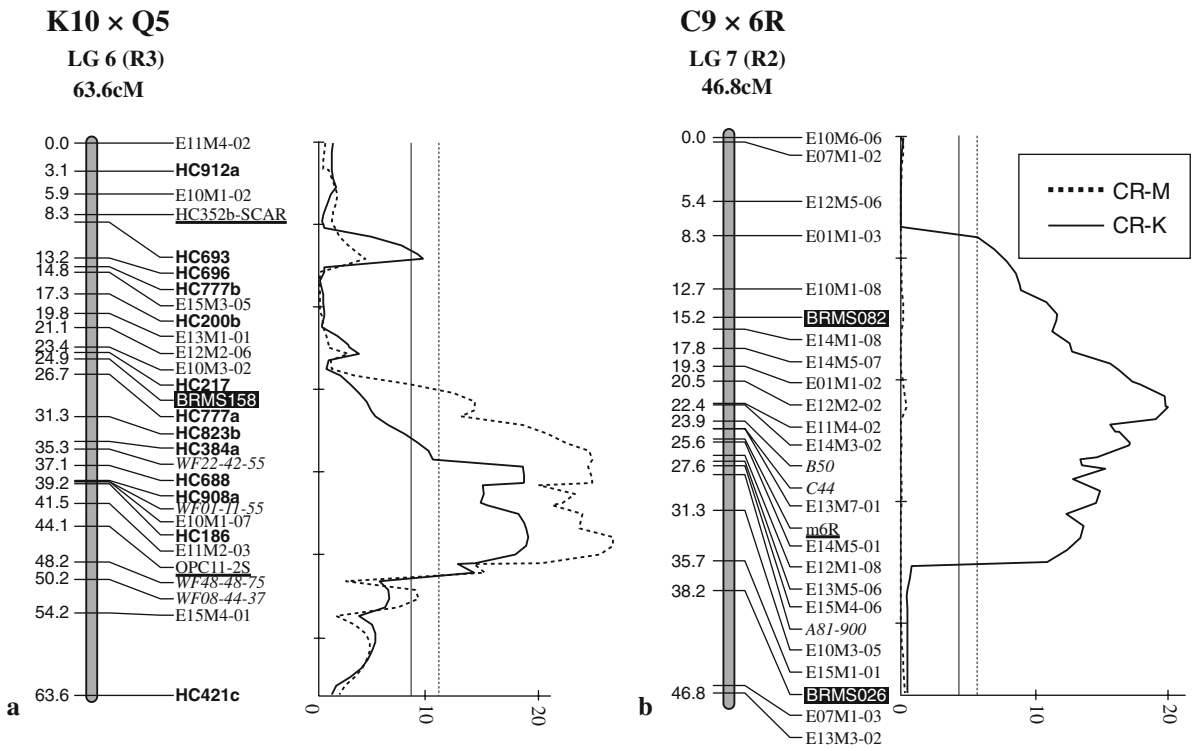


Fig. 2 **a** A partial linkage map of the region containing *CRk* and QTL analysis for clubroot resistance. *Left* a linkage map containing the *CRk* locus. Locus names are indicated on the *right side* of LGs and map distances in centi-Morgans are on the *left*. RFLP, AFLP, RAPD, SSR and STS are indicated by different characters, *ariel bold*, *ariel*, *italic*, *white in black box* and *underlined*. *Right* the LOD profile of QTL for the CR trait. LOD score and map distance are indicated on the *x* and *y* axes, respectively. *Thick broken line* and *thick solid line* indicate LOD plots of resistance to M85 and K04, respectively. *Thin broken line* and *thin solid line* indicate LOD thresholds of 8.1 (resistance to M85) and 6.7 (resistance to K04), respectively. **b** A partial linkage map of the region containing *CRc* and QTL analysis for clubroot resistance. *Left* a linkage map containing the *CRc* locus. Locus names are indicated on the *right side* of LGs and map distances in centi-Morgans are on the *left*. RFLP, AFLP, RAPD, SSR and STS are indicated by different characters, *ariel bold*, *ariel*, *italic*, *white in black box* and *underlined*. *Right* the LOD profile of QTL for the CR trait. LOD score and map distance are indicated on the *x* and *y* axes, respectively. *Thick broken line* and *thick solid line* indicate LOD plots of resistance to M85 and K04, respectively. *Thin broken line* and *thin solid line* indicate LOD thresholds of 7.0 (resistance to M85) and 5.3 (resistance to K04), respectively

Table 4 Summary of QTLs detected for clubroot resistance against K04 isolate of *P. brassicae* using CIM

Linkage group	Closest marker	Position (cM)	LOD	R^2 (%)	Additive effect	Dominance effect
LG7 (R2)	E14M3-02	22.5	24.0	72.1	1.22	0.06
LG7 (R2)	m6R	25.5	20.3	68.5	1.17	0.10
LG7 (R2)	E15M4-06	27.6	17.8	66.0	1.15	0.14

mapped to R3 (Saito et al. 2006), but the location of that locus was not clarified because *Crr3* linked marker, TCR05 was not polymorphic in our population. The linked markers for *Crr1*, *Crr2*, and *Crr4* were mapped to the LG R8, R1, and R6, respectively (Suwabe et al. 2003, 2006). In this study, we mapped the novel CR loci *CRc* in R2, based on the distribution of common markers (Suwabe et al. 2006). *CRc* shows resistance to clubroot K04, but susceptibility to M85 which was identified as race 2 according to William's classification (Williams 1966).

The effect of resistant loci could be identified as qualitative or quantitative traits corresponding to the available pathogen isolates. The resistance responses to the pathogens are not simple because the composition of field isolates is not homogeneous. *P. brassicae* isolated from a single root gall, is composed of various mixed genotypes (Crute et al. 1980; Tjallingii 1965; Wit and Van de Weg 1964). The use of homogeneous isolates, e.g. proliferated single-spore isolates, can simplify the complex nature of interaction between the clubroot resistance genes and *P. brassicae* races. In *B. oleracea*, two to five QTLs were identified using four single-spore isolates and a field isolate under controlled conditions (Rocherieux et al. 2004). In *B. rapa*, the genetics of resistance was analyzed using field isolates consisting of

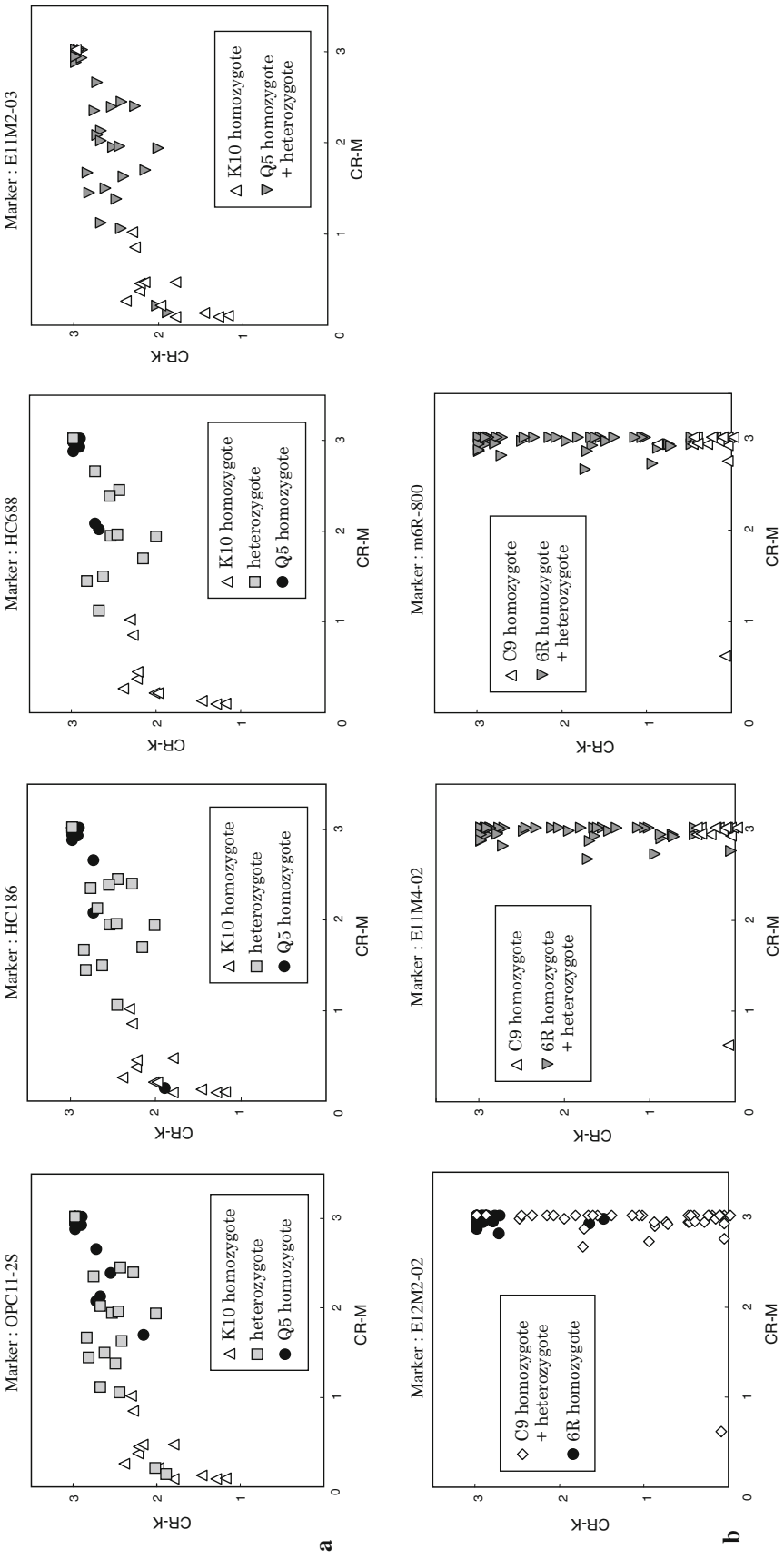


Fig. 3 **a** Relationships between clubroot resistance against different two isolates of *P. brassicae* and genotypes of K10 × Q5 cross on each marker. *Numerals* indicate the disease index (*0* resistant, *3* susceptible). **b** Relationships between clubroot resistance against different two isolates of *P. brassicae* and genotypes of C9 × 6R cross on each marker. *Numerals* indicate the disease index (*0* resistant, *3* susceptible)

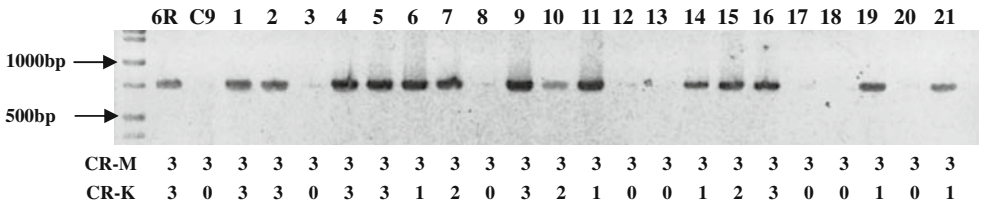


Fig. 4 Agarose gel electrophoresis profiles for the STS marker m6R and means of disease index (*0* resistant, *3* susceptible) of F_3 plants

various mixed pathotypes. It is important to clarify the genetic backgrounds of resistance to each pathotype; however, exhaustive analyses for single-spore isolates are too laborious. We have developed three clubroot-resistant DH lines T136-8, K10, and C9 which are homozygous for the CR loci *CRa*, *CRk*, and *CRc*, respectively. *CRa* corresponds to the M85 isolate, while *CRc* to K04. *CRk* exhibits pleiotropic effects to each pathogen. These DH lines may be useful in classification of the field isolates of *P. brassicae*, because they have discriminative resistance to different pathotypes and permanently clone themselves.

Eight CR loci have been reported in *B. rapa*, including this study. These loci apparently show varied phenotypic responses to the pathogen, though the exact mutual effects have not been evaluated. The epistatic interactions in the expression of clubroot resistance in this species should be important. In *B. napus*, significant epistatic interactions were found between CR loci with and without main effects, and between alleles either from the resistant parent or from each parent (Manzanares-Dauleux et al. 2000). The DH lines which are homozygous for CR loci and their linked markers may be useful in detection of the interaction between each CR gene.

Many CR Chinese cabbage cultivars were bred using a major gene for clubroot resistance, but the obligate biotoroph agent *P. brassicae* is composed of various mixed pathotypes in infected soils. Infection of CR cultivars has been reported in some production areas in Japan (Kuginuki et al. 1999). It means that a single CR gene is not enough to protect *Brassica* crops from some field isolates of *P. brassicae*. We have to accumulate a number of CR genes for breeding widely usable cultivars with high resistance. Many STS (or SCAR) markers linked to each CR genes have already been developed. Markers assisted selection (MAS) may be an effective strategy for accumulating CR genes in the Chinese cabbage and other *Brassica* crops.

Acknowledgments The authors thank Dr. S. Matsumoto of National Institute of Vegetable and Tea Science (NIVTS) for offering information on the SSR markers of *B. rapa* (BRMS).

References

Basten CJ, Weir BS, Zeng ZB (2005) QTL cartographer: a reference manual and tutorial for QTL mapping. North Carolina State University, Department of Statistics, Raleigh

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA (1975) Study of physiologic specialization in *Plasmodiophora brassicae*: proposals for attempted rationalization through an international approach. Trans Br Mycol Soc 65:295–303

Crute IR, Gray AR, Crisp P, Buczacki ST (1980) Variation in *Plasmodiophora brassicea* and resistance to clubroot disease in *Brassica* and allied crops. A critical review. Plant Breed Abstr 50:91–104

Hayashida N, Takabatake Y, Nakazawa N, Aruga D, Nakanishi H, Taguchi G, Sakamoto K, Matsumoto E (2008) Construction of a practical SCAR marker linked to clubroot resistance in Chinese cabbage, with intensive analysis of HC352b genes. J Jpn Soc Hortic Sci 77:150–154

Hirai M (2006) Genetics analysis of clubroot resistance in *Brassica* crops. Breed Sci 56:223–229

Hirai M, Harada T, Kubo N, Tsukada M, Suwabe K, Matsumoto S (2004) A nobel locus for clubroot resistance in *Brassica rapa* and its linkage markers. Theor Appl Genet 108:639–643

Kim JS, Chung TY, King GJ, Jin M, Yang TJ, Jin YM, Kim HI, Park BS (2006) A sequence-tagged linkage map of *Brassica rapa*. Genetics 174:29–39

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica 98:149–154

Kuginuki Y, Yoshikawa H, Hirai M (1999) Variation in virulence of *Plasmodiophora brassicea* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Eur J Plant Pathol 105:327–332

Lowe AJ, Moule C, Trick M, Edwards KJ (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108:1103–1112

Manzanares-Dauleux MJ, Delourme R, Baron F, Thomas G (2000) Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theor Appl Genet 101:885–891

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Euphytica 104:79–86

Matsumoto E, Hayashida N, Sakamoto K, Ohi M (2005) Behavior of DNA markers linked to a clubroot resistance gene in segregating populations of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). J Jpn Soc Hortic Sci 74(5):367–373

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Piao ZY, Deng YQ, Choi SR, Park YJ, Lim YP (2004) SCAR and CAPS mapping of *CRb*, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Theor Appl Genet 108:1458–1465

Rocherieux J, Glory P, Giboulot A, Boury S, Barbeyron G, Thomas G, Manzanares-Dauleux MJ (2004) Isolation-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theor Appl Genet 108:1555–1563

Saito M, Kubo N, Matsumoto S, Suwabe K, Tsukada M, Hirai M (2006) Fine mapping of the clubroot resistance gene, *Crr3*, in *Brassica rapa*. Theor Appl Genet 114:81–91

Sakamoto K, Nishio T (2001) Distribution of *S* haplotypes in commercial cultivars of *Brassica rapa*. Plant Breed 120:155–161

Suwabe K, Ikehashi H, Nunome T, Kage T, Hirai M (2002) Isolation and characterization of microsatellites in *Brassica rapa* L. Theor Appl Genet 104:1092–1098

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Fujimura M, Nunome T, Fukuoka H, Matsumoto S, Hirai M (2003) Identification of two loci for resistance to clubroot (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. Theor Appl Genet 107:997–1002

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M, Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319

Tjallingii F (1965) Testing clubroot-resistance of turnips in the Netherlands and the physiologic specialization of *Plasmodiophora brassicae*. Euphytica 14:1–22

Toxopeus H, Janssen AMP (1975) Clubroot resistance in turnip II. The slurry screening method and clubroot races in the Netherlands. Euphytica 24:751–755

Williams PH (1966) A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56:624–626

Wit F (1964) Inheritance of reaction to clubroot in turnips. Hortic Res 5:47–49

Wit F, Van De Weg M (1964) Clubroot-resistance in turnips (*Brassica campestris* L.). I. Physiologic races of the parasite and their identification in mixtures. Euphytica 13:9–18