

Genetic mapping of clubroot resistance genes in oilseed rape

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Abstract Clubroot caused by the obligate biotrophic protist *Plasmodiophora brassicae* is a major disease of *Brassica* species. Clubroot resistances introduced from *B. oleracea* var. ‘Böhmerwaldkohl’ and resistance from *B. rapa* ECD-04 were genetically mapped in oilseed rape (*B. napus* L.). A doubled haploid (DH) population of rape seed was developed by crossing a resistant DH-line derived from a resynthesized *B. napus* with the susceptible cultivar ‘Express’. The DH population was tested in the greenhouse against seven *P. brassicae* isolates showing low and high virulence toward *B. oleracea* or/and *B. rapa*. DH-lines with highest or lowest disease scores were used in a bulked segregant analysis (BSA), and 43 polymorphic AFLPs were identified. A genetic map of the whole genome was constructed using 338 AFLP and 156 anchored SSR

markers. Nineteen QTL were detected on chromosomes N02, N03, N08, N13, N15, N16 and N19 giving resistance to seven different isolates. Race-specific effects were observed for all QTL, none of the QTL conferred resistance to all isolates. The phenotypic variance explained by the respective QTL ranged between 10.3 and 67.5%. All QTL could be assigned to both ancestral genomes of *B. napus*. In contrast to previous reports, a clear differentiation into major QTL from *B. rapa* and minor QTL from *B. oleracea* could not be found. Composite interval mapping confirmed the linkage relationships determined by BSA, thus demonstrating that markers for oligogenic traits can be selected by merely testing the distributional extremes of a segregating population.

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Introduction

The obligate biotrophic protist *Plasmodiophora brassicae* causes clubroot disease on cruciferous plant species such as oilseed rape (*Brassica napus* L.), broccoli (*Brassica oleracea*), cabbage (*Brassica oleracea*) and common weeds like charlock (*Sinapis arvensis* L.), thale cress (*Arabidopsis thaliana* L. Heyhn.), shepherd’s purse (*Capsella bursa-pastoris* L. Medik.), and field pennycress (*Thlaspi arvense* L.) (Karling 1968). Numerous isolates, field isolates or single spore isolates, with different phenotypic effects and differences in pathogenicity exist (Some et al. 1996; Voorrips 1996), making resistance breeding difficult. Manzanares-Dauleux et al. (2001) described the variation for virulence of *P. brassicae* between and within field isolates collected from single galls in Brittany (north-western France). Their populations of *P. brassicae* showed high heterogeneity and eight pathotypes were identified

(P1–P8). Hence, durable resistance can only be achieved by pyramiding different major genes or by using quantitative resistances.

Resistances have been found in *B. oleracea* (C genome), *B. rapa* (A genome), and *B. napus* (AC genome). They can be classified into two groups: qualitative, dominant resistance (Wit and Van de Weg 1964; Crute et al. 1980; James and Williams 1980) and quantitative, recessive resistance (Chiang and Crête 1970; Figdore et al. 1993; Grandclément and Thomas 1996; Voorrips 1996). The *Brassica* A genome carries at least three dominant, race-specific resistance genes. They were named *A*, *B* and *C* by Wit and Van de Weg (1964) and *Pb1*, *Pb2* and *Pb3* by James and Williams (1980); however, correspondence of their gene positions could not be demonstrated. More recent work on *B. rapa* showed that between one and three major genes and one QTL are involved in resistance to clubroot. The major genes were named *CRa* by Matsumoto et al. (1998), *CRb* by Piao et al. (2004), *Crr3* by Hirai et al. (2004), *Crr1* and *Crr2* by Suwabe et al. (2003, 2006). An additional QTL was found by Suwabe et al. (2006) and named *Crr4*.

In *B. oleracea* (C genome), clubroot resistance is often quantitative and race-independent (Voorrips 1996). The cabbage cultivar ‘Böhmerwaldkohl’ expresses a high level of race-independent resistance (Crute 1986). Varying numbers of QTL have been reported for *B. oleracea*. Chiang and Crête (1970) described two resistance QTL named *pb-1* and *pb-2*. Seven RFLP-loci linked to clubroot resistances were reported by Figdore et al. (1993), five of these loci were located on the same linkage group in close distance to each other. Voorrips et al. (1997) found two additively acting loci named *pb-3* and *pb-4*. Recently, data on 18 QTL were published by Rocherieux et al. (2004) after infection with five different isolates. Some of these QTL were common for more than one isolate, but only one QTL (*Pb-Bo1*) was common for all tested isolates.

The studies on clubroot resistance in *B. napus* point to an oligogenic inheritance (Crute et al. 1980). The interaction between *B. napus* and *P. brassicae* is mainly described

as differential, i.e. the degree of resistance varies in a race-specific manner (Gustafsson and Fält 1986). Gustafsson and Fält (1986) proposed models based on three, four and five resistance genes, where the most favoured model was based on four genes. A complex type of inheritance, with dominant genes from *B. rapa* and recessive genes from *B. oleracea* can be expected in resynthesized *B. napus* combining resistance from both ancestral species (Diederichsen et al. 1996). Segregation analysis suggested that resistance in resynthesized *B. napus* was controlled by at least two dominant and unlinked genes (Diederichsen and Sacristán 1996). Manzanares-Dauleux et al. (2000) located one major gene (*Pb-Bn1*) for resistance against two *P. brassicae* isolates on chromosome N03 and for each isolate one additional minor QTL was identified on chromosomes N12 and N19, respectively.

Here, we report on the identification of new QTL for clubroot resistance in *B. napus*, which had been introduced from *B. rapa* and *B. oleracea*. We tested a number of different isolates to determine race specific host–pathogen interactions. The QTL were allocated to the respective *B. napus* chromosomes by using a set of simple sequence repeat (SSR) anchor markers.

Materials and methods

Plant material and pathogen isolates

A doubled-haploid (DH) population DH914 ($N = 151$) of rapeseed was developed by crossing the resistant DH line ‘263/11’ as a female parent with the susceptible cultivar ‘Express’. The resistant DH line ‘263/11’ was derived from a backcross progeny of a resynthesized *B. napus* (*B. oleracea* ‘Böhmerwaldkohl’ × *B. rapa* ECD-04, Diederichsen and Sacristán 1996). Seven different *P. brassicae* isolates were used in this study under controlled greenhouse conditions. The isolates differ in their pathogenicity and thus their ability to infest *B. oleracea*, *B. rapa* and *B. napus*.

Table 1 *Plasmodiophora brassicae* isolates used in this study

Isolate	Number of DH lines tested	Provided by	Interaction with differential hosts (<i>B. rapa</i> , <i>B. oleracea</i>)
01:60	151	I. Happstadius, Svalöv Weibull AB, Sweden	More virulent on <i>B. rapa</i> , less virulent on <i>B. oleracea</i>
01.07	101	I. Happstadius, Svalöv Weibull AB, Sweden	Low virulence
e4x04	150	E. Diederichsen, FU Berlin, Germany	Virulent on <i>B. rapa</i> , less virulent on <i>B. oleracea</i>
k	142	E. Diederichsen, FU Berlin, Germany	Virulent on ECD-01, virulent for <i>B. oleracea</i>
l	148	G. Thomas, INRA Le Rheu, France	Avirulent on ECD-01 to ECD-04, virulent for <i>B. oleracea</i>
a	76	H. Busch, DSV Thüle, Germany	Broad pathogenicity
Korporal	137	G. Korporal, NPZ Hohenlieth, Germany	Virulent on <i>B. rapa</i> , less virulent on <i>B. oleracea</i>

The isolates had been characterized using the ECD-set (Buczacki et al. 1975)

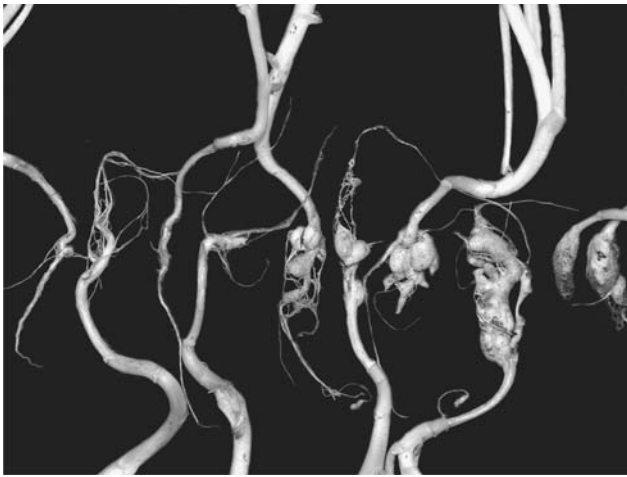


Fig. 1 Oilseed rape roots after infection with *P. brassicae*. The roots are arranged from left to right according to the degree of distorted root growth. The clubroot disease rating scale ranges between 1 (left, resistant) and 9 (right, fully susceptible)

Six field isolates with different virulences were kindly provided by I. Happstadius (Svalöv Weibull, Sweden), H. Busch (DSV, Germany), and G. Korporal (NPZ, Germany) (Table 1). The field isolates were collected from infested rapeseed plants. The isolates were multiplied in Chinese cabbage and infested roots stored at -18°C . Resting spores of *P. brassicae* were isolated from homogenised clubbed roots of Chinese cabbage and diluted to a density between 10^7 and 10^8 spores/ml. In addition, one single spore isolate ‘1’, which was used in this study had been kindly provided by G. Thomas (INRA Le Rheu, France).

The main focus was directed on clubroot reactions with isolate ‘01:60’, as it was showing the least aggressiveness on *B. oleracea* but compatibility toward most *B. rapa* hosts.

Resistance tests

Greenhouse tests were done as described by Diederichsen and Sacristán (1996), except that the disease symptoms were assessed using a scale graded into nine classes (1 = no infection, 9 = heavily infested, Fig. 1). The disease index (DI) was calculated according to the formula

$$\text{DI} = \frac{(2n_2 + 3n_3 + \dots + 9n_9) \times 100}{8 \times N_T}$$

with n_2 through to n_9 being the number of plants in each class showing any degree of infection and N_T being the total number of plants tested. The number of DH lines tested with each isolate is indicated in Table 1. Of each DH line 15 plants per isolate were tested.

Molecular marker analysis

AFLPs were analyzed essentially as described by Vos et al. (1995), except that a non-radioactive method for fragment detection was used (LI-COR Model 4300). For BSA (Michelmore et al. 1991), DNA from both parents, pooled DNA of eight DH lines with $\text{DI} < 10$ and pooled DNA of eight DH lines with $\text{DI} = 100$ (isolate ‘01:60’) were used. One hundred and sixty-five *Pst*I and *Mse*I AFLP primer combinations (PC) were screened for polymorphisms between the two parents and the bulks. Primer combinations that were polymorphic between resistant and susceptible bulks were used for an initial identification of resistance loci. For complete genome genotyping all PC that were polymorphic between parents were applied.

One hundred and fifty-six SSR primer pairs were tested for polymorphisms between both parents. Sixty-three SSR primer pairs, indicated by the prefixes Na, Ni, Ol, and Ra were downloaded from the *Brassica* database (<http://brassica.bbsrc.ac.uk>) and 91 unpublished SSRs (prefixes GMR, HMR) were analyzed by the Saaten-Union Resistenzlabor GmbH, Hovedissen which made the genotyping data available for this study. In addition the SSR markers BRMS88 and BRMS96 were used because they had been reported to be linked to two clubroot resistance loci in *B. rapa* (Suwabe et al. 2003). PCR for the SSRs from the *Brassica* database was performed according to Lowe et al. (2004) except that one primer had an M13 tail at its 5' end, and PCR was performed with 0.5 pmol fluorescent dye (IRD-700 or IRD-800, MWG Biotech, Ebersberg, Germany) labelled M13. For genotyping the BRMS markers the protocol of Suwabe et al. (2003) was used. The PCR fragments were labelled with IRD-800, loaded onto 6–8% polyacrylamide gels (National Diagnostics Sequagel, Biozym) and separated on a LI-COR Model 4300 DNA sequencer (Lincoln, NE, USA) for visualization.

Statistical analysis, linkage and QTL analysis

For each marker locus, both homozygous classes were compared with each other. Multiple comparisons of least-square means (LSMEANS) were carried out with SAS, version 9.0 (SAS Institute 1999, Cary, USA) using phenotypic and genotypic data. LSMEANS of the homozygous classes for the DI were calculated for each isolate separately. Significant differences between LSMEANS of ‘263/11’ and cv. ‘Express’ were calculated with the procedure GLM using a Tukey–Kramer adjustment for multiple comparisons.

Linkage map construction was performed using the program Joinmap 3.0 (Van Ooijen and Voorrips 2001). A

LOD threshold of 3.5 and a rec value of 0.4 were used to group loci. Recombination frequencies were transformed to centiMorgans (cM) using the Kosambi mapping function (Kosambi 1944). Linkage groups with two or more SSR markers of known map positions were assigned to their respective rapeseed chromosomes following the standard nomenclature N01–N19 (Parkin et al. 1995).

The program package PLABQTL v.1.2 (Utz and Melchinger 1996) was used to analyse QTL. In order to avoid false positives, we set a stringent LOD score of 3.5 as the threshold value for the detection of QTL. The COV SELECT statement was used to identify important markers as cofactors. These cofactors are associated with putative QTL on a chromosome. Statistical interactions among detected QTL were estimated with this program.

Results

Clubroot infection experiments

Between 76 and 151 DHs from the population DH914 including the parents were inoculated with seven clubroot isolates (Table 1). Among the DH populations, the DI ranged between 0 and 100 with the exception of isolate ‘Korporal’, where the lowest DI value was 42. Disease reactions with different *P. brassicae* isolates are illustrated as frequency distributions in Fig. 2. Parental reactions were as expected with isolates ‘01.07’, ‘1’, ‘k’, and ‘a’, with DH ‘263/11’ being resistant and cv. ‘Express’ being susceptible. Opposite to expectations, cv. ‘Express’ reacted moderately resistant with isolate ‘e4x04’, and DH ‘263/11’ was susceptible against the recently isolated *P. brassicae* population ‘Korporal’.

Population DH914 showed isolate-specific segregation patterns. Most isolates (‘e4x04’, ‘1’, ‘k’, ‘a’, ‘01.07’) led to bimodal segregations with a cut off point around DI = 50, indicating the presence of at least one gene having a major effect. Isolates ‘k’, ‘01.07’ and ‘1’ led to a segregation ratio of approximately 3:1 and showed correlated disease reactions (r between 0.89 and 0.94, data not shown), indicating two genes having major effects toward these isolates. Reactions with isolate ‘e4x04’ correlated significantly only with those from isolate ‘01:60’ ($r = 0.46$, data not shown), although both isolates differed by their segregation patterns. Segregation with isolate ‘e4x04’ indicated the presence of a single gene having a qualitative effect against this isolate. Isolate ‘01:60’ gave a segregation with ca. 50% of completely susceptible DH-lines and 50% of lines showing different levels of susceptibility/resistance, indicating the presence of genes conferring minor and quantitative effects.

Isolate ‘Korporal’ was the most virulent isolate and both parents were heavily attacked. However, their progeny

showed disease indices between 50 and 100, indicating transgressive segregation.

Bulked segregant analysis

In a first step to identify markers linked to clubroot disease resistance genes, AFLPs were determined between distributional extremes of the DH population with low (<10) and high DI (=100) analyzed with isolate ‘01:60’. This strategy avoids construction of large marker maps focusing on the target QTL only. The two parents were used as controls; 142 out of 165 primer combinations were analyzed giving rise to 1,207 fragments. Of these, 35 primer combinations revealed polymorphisms between the parents and between the DH bulks. In total, 43 polymorphic AFLPs were found, 14 linked to the ‘263/11’ allele and 29 linked to the ‘Express’ allele as determined from their presence in the respective bulks. The multiple comparisons of least-square means calculated for each AFLP revealed significant differences for 21 markers ($\alpha \leq 0.05$), giving a first indication on their linkage to clubroot resistance genes. In a next step, genetic linkage between these markers was calculated and a map was constructed. As expected, most of the markers detected by BSA were not randomly distributed among the genome. Thirty-six markers were clustered on five linkage groups. These results demonstrate that markers linked to major resistance QTL could be identified by bulked segregant analysis (Fig. 3).

Construction of a genetic map

For anchoring clubroot resistance QTL on the *B. napus* chromosomes, a comprehensive linkage map was constructed using the DH914 population. Among 35 AFLP primer combinations, 338 segregating AFLPs were found. On average, ten bands per primer combination were polymorphic, indicating that AFLP analysis after *PstI* digest has a potential to generate a high number of polymorphisms.

To assign linkage groups to rapeseed chromosomes, previously mapped SSRs were used as anchor markers. In total, 156 SSR primer combinations were tested for polymorphisms between the parents of the DH914 population. Among 63 publicly available SSRs, 32 showed at least one polymorphism. The assignment of SSRs to fragments of the expected size as described in previous publications turned out to be difficult, due to various levels of polymorphism ranging from 1 to 8 amplification products with an average of 3.5 alleles per locus. A total of 16 SSRs were suitable for genotyping the DH plants, providing 25 polymorphic markers. To verify their chromosomal assignment, additional SSRs from Saaten-Union Resistenzlabor were

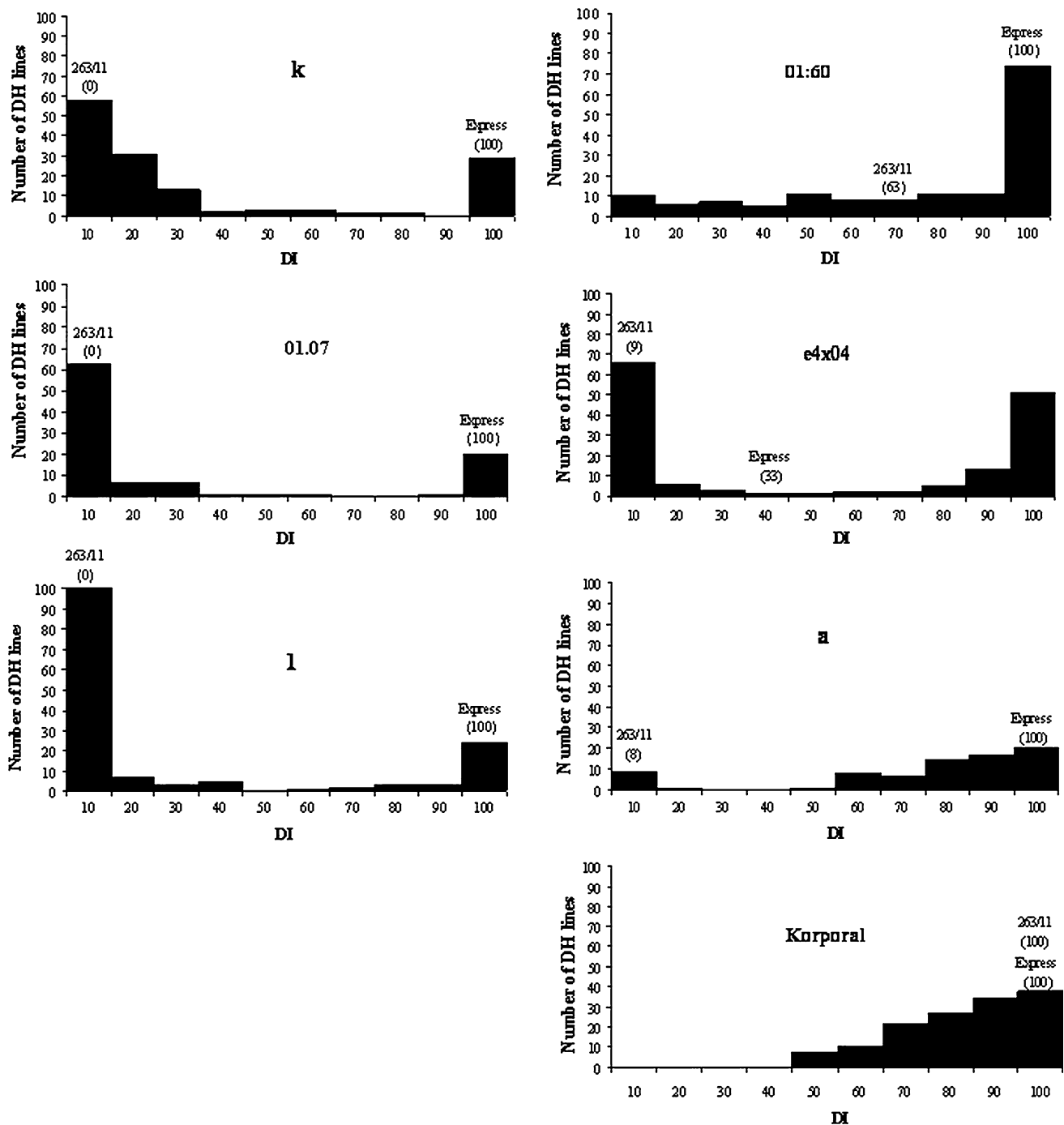


Fig. 2 Frequency distribution of disease indices (DI) after testing between 76 and 151 DH lines of the population DH914 with seven different *P. brassicae* isolates ('k', '01.07', 'l', '01:60', 'e4x04', 'a'

and 'Korporal'). Disease indices ranged between 0 (no infection) and 100 (severely infected)

also incorporated into the map. The final linkage map based on the population DH914 consists of 305 AFLPs and 89 SSRs. The map covers 1,570 cM, with an average marker interval of 3.97 cM. The sizes of the linkage groups ranged between 27.2 and 159.7 cM. A substantial number of the linked loci (20.9%) showed segregation ratios, which deviated significantly ($P < 0.05$) from the expected 1:1

segregation ratio. Among the 83 loci with distorted segregation ratios, 63.9% had an abundance of '263/11' alleles, whereas 36.1% were skewed toward the 'Express' allele. Most of the skewed markers were mapped on chromosomes N03, N09 and N13. N03 and N13 showed an excess of '263/11' alleles while N09 skewed toward 'Express' alleles. Due to mapping of SSR anchor markers,

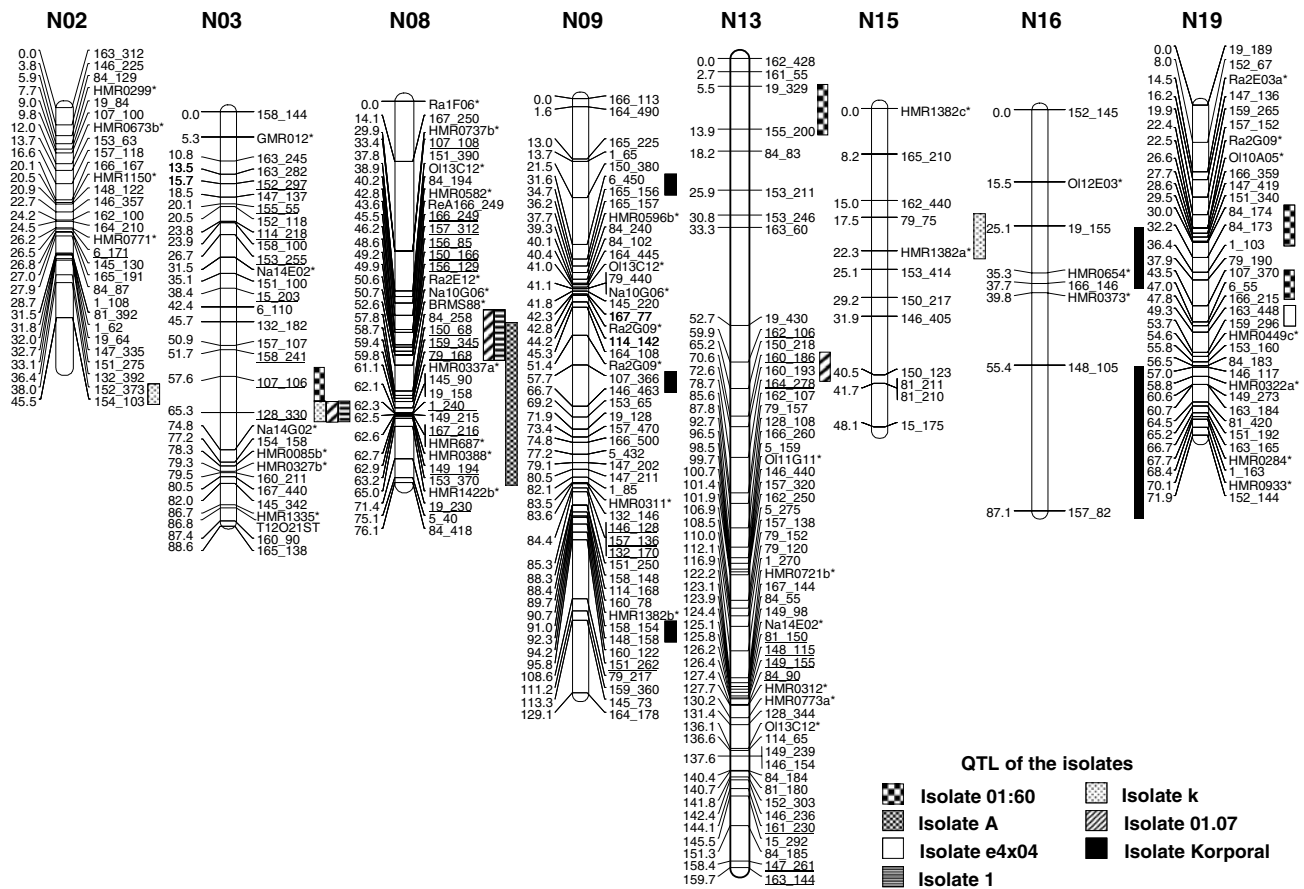


Fig. 3 Linkage maps of eight selected *B. napus* chromosomes with QTL for clubroot resistance. The maps comprise AFLPs and SSRs (with asterisk). Prefixes of SSR names indicating the source are described under [Materials and methods](#). AFLPs detected after BSA are underlined. The distances in centiMorgan are given on the left, the

marker numbers on the right. The chromosomes are designated according to the map positions of their SSR anchor markers. Confidence intervals of clubroot QTL are shown on the right with their respective isolate

19 linkage groups could be assigned to their respective rapeseed chromosomes. The two SSR markers BRMS88 and BRMS96 which had been reported to be linked to clubroot resistance in *B. rapa* (Suwabe et al. 2006) could be integrated on chromosomes N08 and N01.

QTL mapping

For the seven *P. brassicae* isolates studied, a total of 19 QTL were detected on eight chromosomes (Table 2; Fig. 3). The total phenotypic variance collectively explained by all detected QTL ranged from 20.8 to 79.6%, depending on the respective isolate. Between one and five QTL were identified by a single isolate, with LOD scores ranging between 3.53 and 34.39 (Table 2). Three QTL were identified with isolates ‘k’, *PbBn-k-1* on chromosome N02, *PbBn-k-2* on N03 and *PbBn-k-3* on N15. Altogether these QTL explained 56.9% of the genetic variance. The QTL *PbBn-k-2* explained the highest proportion of the

phenotypic variance (67.5%) among all QTL detected here. *PbBn-k-1* and *PbBn-k-2* had positive additive effects indicating that the resistance level was increased by the parental allele from ‘263/11’. Negative additive effects for *PbBn-k-3* implicated that the desirable allele came from the parent cv. ‘Express’.

Resistance to isolate ‘Korporal’ is due to five QTL, located on chromosomes N09 and N16, jointly explaining 21.6% of the variation. Three QTL associated with resistance to isolate ‘01.07’ are located on chromosomes N03, N08, and N13. Collectively, these QTL explained 79.6% of the total phenotypic variance, the highest value found among all QTL detected.

Two QTL were detected for isolate ‘1’ on N03 and N08 and accounted for 74.0% of the phenotypic variance. For isolate ‘01:60’, which was used for bulked segregant analysis, four QTL were identified on chromosomes N03, N13 and N19, collectively explaining the lowest proportion of the total phenotypic variance with 20.8%. Only one QTL was detected for isolates ‘a’ and ‘e4x04’ each. The QTL

Table 2 Results from QTL mapping

Number	Isolate	QTL symbol	Chromosome	Marker ^a	Position ^b	LOD	R^2 ^c	R^2t ^d	Additive effect ^e	Parent ^f
1	Korporal	<i>PbBn-Korp-1</i>	N09	6_450	31.6	3.95	12.4	21.6	−7.868	–
2		<i>PbBn-Korp-2</i>	N09	107_366	57.7	4.03	12.7		6.683	–
3		<i>PbBn-Korp-3</i>	N09	158_154	91.0	6.00	18.3		4.942	263/11
4		<i>PbBn-Korp-4</i>	N16	19_155	25.1	6.21	18.8		6.214	263/11
5		<i>PbBn-Korp-5</i>	N16	148_105	55.4	3.71	11.7		−4.508	Express
6	k	<i>PbBn-k-1</i>	N02	152_373	38.0	5.91	17.6	56.9	8.498	263/11
7		<i>PbBn-k-2</i>	N03	128_330	65.3	34.39	67.5		35.318	263/11
8		<i>PbBn-k-3</i>	N15	79_75	17.5	4.87	14.7		−6.823	Express
9	01.07	<i>PbBn-01.07-1</i>	N03	128_330	65.3	12.92	44.5	79.6	32.847	263/11
10		<i>PbBn-01.07-2</i>	N08	84_258	57.8	5.50	22.2		12.843	263/11
11		<i>PbBn-01.07-3</i>	N13	160_186	70.6	7.38	28.6		16.523	263/11
12	1	<i>PbBn-1-1</i>	N03	128_330	65.3	22.93	51.3	74.0	28.696	263/11
13		<i>PbBn-1-2</i>	N08	84_258	57.8	6.60	18.7		17.745	263/11
14	01:60	<i>PbBn-01:60-1</i>	N03	107_106	57.6	5.61	15.8	20.8	14.031	263/11
15		<i>PbBn-01:60-2</i>	N13	19_329	5.5	4.05	11.7		6.279	263/11
16		<i>PbBn-01:60-3</i>	N19	84_174	30.0	3.53	10.3		−8.917	Express
17		<i>PbBn-01:60-4</i>	N19	107_370	43.5	6.61	18.4		−19.051	Express
18	e4x04	<i>PbBn-e4x04-1</i>	N19	163_448	49.3	17.81	42.3	60.4	−38.429	Express
19	a	<i>PbBn-a-1</i>	N08	79_168	59.8	6.26	31.9	43.8	12.400	263/11

QTL positions were calculated using the marker map from DH population DH914 with 396 molecular markers. Phenotypic data are from tests with seven *P. brassicae* isolates

^a Left-flanking marker

^b Estimated position of the QTL using CIM method along the linkage group

^c R^2 is the proportion of the phenotypic variance explained by each QTL after accounting for co-factors

^d R^2t is the total phenotypic variance explained by all detected QTL

^e Additive effects indicate an additive main effect of the parent contributing the higher value allele: positive values indicate that higher value alleles are from parent 263/11 and the negative values indicate that higher value alleles are from parent “Express”

^f Parental allele which increased the resistance level

were detected on chromosomes N08 and N19 and explained between 43.8 and 60.4% of the phenotypic variances, respectively. The QTL *PbBn-a-1* had positive additive effects, whereas *PbBn-e4x04-1* had negative effects. Statistical interactions between QTL were tested for each isolate and appeared to be significant only for resistance to isolate ‘k’, ‘01.07’ and ‘1’ between the QTL *PbBn-k-2* to *PbBn-k-3* (N03–N15), *PbBn-01.07-1* to *PbBn-01.07-2* (N03 to N08) and *PbBn-1-1* to *PbBn-1-2* (N03–N08).

Discussion

We have identified 19 QTL for clubroot resistance distributed across the rapeseed genome. Surprisingly, positive alleles, which decreased the infection rates, were derived not only from the resistant donor but also from the susceptible parent ‘Express’. QTL with strong effects (LOD > 10) were localised on chromosomes N03 and

N19, respectively. Both QTL seem to confer broad-spectrum resistance because they were effective against more than one isolate. Anchoring of our linkage groups with SSRs enables comparative studies with previously found clubroot resistance QTL.

Identification of resistance-linked markers was performed in two steps. First only distributional extremes of the segregating DH population were used to identify informative AFLPs. This strategy, known as bulked segregant analysis (Michelmore et al. 1991), offers a quick approach for the identification of markers linked to genes of interest. Its potential even for polygenic characters could be demonstrated. Most of the markers identified by BSA targeted the chromosomes with major QTL or could be used as cofactors in composite interval mapping (CIM). However, no BSA marker could be identified for chromosomes N15, N16 and N19, where minor QTL were mapped by CIM, thus demonstrating the limitations of the BSA.

Despite resistance being inherited in a polygenic manner, clubroot resistance in our material has to be regarded

as isolate-specific. Two QTL which are located on chromosomes belonging to the A genome (N03 and N08), had a broader effect, although compatible races of *P. brassicae* to these QTL were present amongst those we used. No QTL could be detected having a similar broad spectrum of resistance as it is known for one of the parents of the resynthesized *B. napus* line, the cabbage landrace ‘Böhmerwaldkohl’ (Nieuwhof and Wiering 1962). In resynthesized *B. napus* clubroot resistance from the *B. oleracea* parent was only detectable in combination with resistance from certain *B. rapa* parents (Diederichsen and Sacristán 1996), but not in resynthesized lines originating from crosses between ‘Böhmerwaldkohl’ and susceptible *B. rapa*. This indicated epistatic effects between loci from the different genomes. In conclusion, the genetic analysis of clubroot resistance from the *B. oleracea* cultivar ‘Böhmerwaldkohl’ introduced into *B. napus* seems only feasible in the parental species because in the amphidiploid species the activity of the parental resistance genes is masked due to epistatic interactions.

Clubroot resistance loci in the A genome have been reported previously. In most cases they originate from European turnips like in our case, such as the host ECD-02 or cv. ‘Milan White’, which were used to introduce clubroot resistance into Japanese Chinese cabbage cultivars. Therefore it is feasible to assume co-localization or identity of at least some loci. Suwabe et al. (2003, 2006) identified resistance loci on chromosomes R08 (*Crr1*), R01 (*Crr2*) and chromosome R06 (*Crr4*, QTL). We also identified three QTL on rapeseed chromosome N08 (*PbBn-01.07-2*, *PbBn-1-2* and *PbBn-a-1*); however, they did not coincide with *Crr1*, as judged from the location of marker BRMS88, which is closely linked to *Crr1*. This marker was mapped outside the confidence interval of the respective QTL. There was also no correspondence between our QTL positions and QTL previously mapped in *B. rapa*. Suwabe et al. (2003, 2006) had located *Crr2* on chromosome R01 and *Crr4* on chromosome R06. In our study, no QTL could be found on the corresponding *B. napus* chromosomes N01 and N06. Sakamoto et al. (2006) had mapped three clubroot resistance loci (*CRa*, *CRc* and *CRk*) in Chinese cabbage. Although *CRc* was mapped in the middle of chromosome R02, it is unlikely that this QTL coincides with our QTL *PbBn-k-1* because this QTL is located at the bottom of N02.

CRa was mapped on top of R03, as well as *CRb* (Piao et al. 2004) and the QTL *Pb-Bn1* at the corresponding *B. napus* chromosome N03 (Manzanares-Dauleux et al. 2000). In this region no QTL was identified with our population DH914.

There was an indication for corresponding map positions between *B. rapa* QTL and QTL found in our study. The resistance locus *CRk* (Sakamoto et al. 2006) was identified next to *Crr3* (Hirai et al. 2004) at the bottom of

chromosome R03 which is matching to the position of a cluster of broad spectrum QTL on N03 found in this study. Whether these QTL are identical remains to be demonstrated by using common markers and spore isolates.

QTL, which had a broader effect against more than one isolate (e.g., on N03 and N08) seemed to be more complex in respect to their system of resistance, as it is indicated by neighbouring confidence intervals of the QTL when analyzed with another isolate. Complex structures of resistance loci can be regarded as a result of resistance gene evolution processes (Michelmore and Meyers 1998), leading to closely linked genes that vary, for instance, in their race specificity. *P. brassicae* is a pathogen which has been shown to vary extremely in respect to race-differentiation (Crute et al. 1983). This creates a demand for corresponding variation on the host side, which coincides with the number of resistance loci that have been identified in *Brassica* species.

Clubroot resistance in population DH914 against isolate ‘e4x04’ was explained by only one major QTL originating from cv. ‘Express’, although clubroot resistance has never been observed before in this cultivar. Furthermore, the resistant reaction of the parent DH ‘263/11’ against isolate ‘e4x04’ cannot be explained by any QTL originating from this parent, such as QTL on N03 or N08. Based on the specific virulence of this isolate toward major genes from *B. rapa* and its low virulence toward *B. oleracea* hosts, it should be assumed that resistance in DH ‘263/11’ to this isolate is due to resistance from *B. oleracea*. Taken together, it seems possible that resistance from ‘Böhmerwaldkohl’ might still be present in DH914, but that it is based on multiple genes having only minor effects, which are masked by stronger QTL of different origin.

The model plant *Arabidopsis thaliana* is a good host of *P. brassicae* and a number of studies have been made to unravel the genetics of resistance in this species. A major gene for clubroot resistance *RPB1* had been located on chromosomes 1 (Fuchs and Sacristán 1996). The gene is presently being cloned from its position on chromosome 1. Comparative studies between clubroot resistances in *A. thaliana* and *Brassica* spp. may help to understand the evolutionary origin of clubroot resistance loci. Comparison of the QTL positions from this study with the genome region *RPB1* in *Arabidopsis* showed no collinearity to this region of the chromosome (data not shown).

The recent paper by Saito et al. (2006) showed some microsynteny of a region linked to clubroot resistance loci *Crr3* to sequences located on *A. thaliana* chromosome 3. Suwabe et al. (2006) reported synteny between genomic regions from *B. rapa* housing the resistance loci *Crr1* and *Crr2* and a single region on chromosome 4 of *A. thaliana* which carries a cluster of disease resistance genes (major recognition complexes, MRCs) in the *A. thaliana* genome

(Holub 1997; Speelman et al. 1998). Due to these findings, the authors suggested two hypotheses about the evolution of clubroot resistance during evolution of the *Brassicaceae*: (1) in the progenitor genome, resistance was controlled by a single major gene, which was later duplicated to give rise to two functionally different genes. (2) Alternatively, an unknown number of clubroot resistance genes clustered within the ancestral genome were later separated into two different genomic regions due to major chromosomal rearrangements.

The main problem in breeding resistant cultivars is the large variation for pathogenicity in *P. brassicae*. Two resistant oilseed rape cultivars, ‘Mendel’ and ‘Tosca’, have been released; however, since their resistances are working in a race-specific manner the cultivars may not possess durable resistance. The identification of new QTL for clubroot resistance in our study will initiate the development of diagnostic markers for the rapid identification of resistant material. This information will facilitate gene pyramiding and the marker-assisted selection of durable resistance to clubroot in *Brassica*. AFLP markers are of limited use for large-scale selection programs. Therefore, we will convert clubroot-linked AFLPs into locus-specific PCR based markers. It remains to be tested in the future whether any of the QTL presented here is identical to those clubroot resistance genes previously reported for *B. rapa*, *B. oleracea* and *B. napus*. A number of DH lines with resistance to most of the tested isolates have been selected from population DH914 due to the presence of favorable marker alleles. They will be used as parents for breeding rapeseed cultivars with more durable clubroot resistance.

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