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 in *Brassica*. Different sources of resistance have been found in *B. oleracea*, *B. rapa* and *B. napus*. In this project, oligogenic clubroot resistances introduced from *B. oleracea* var. 'Böhmerwaldkohl' and monogenic resistance from *B. rapa* were genetically mapped in *B. napus*. A doubled haploid (DH) population of rapeseed was developed by crossing a resistant DH-line derived from a resynthesised *B. napus* with the susceptible cultivar Express. The DH population was tested in the greenhouse against seven *P. brassicae* isolates showing low and high aggressiveness towards *B. oleracea*.

An AFLP methodology was employed to identify markers and chromosome regions carrying QTL linked to clubroot resistance in *B. napus*. A genetic map of the whole genome was constructed using 338 AFLPs and 156 SSRs. Nineteen QTL were detected on chromosomes N02, N03, N08, N13, N15, N16 and N19 for 7 different isolates. Two of these were specific for only one *P. brassicae* isolate. The other five isolates showed QTL at least on two different chromosomes. Depending on the isolates, the QTL showed varying phenotypic effects with no clear tendency for one or the other ancestral genome, as it has been postulated before.

Key words: *Brassica napus*, *Plasmodiophora brassicae*, clubroot, QTL, disease resistance

Introduction

Plasmodiophora brassicae causes clubroot disease on cruciferous plant species such as oilseed rape (*Brassica napus* L.), cabbage (*Brassica oleracea* L. *Alba* Group) and common weeds like charlock (*Sinapsis arvensis* L.), thale cress (*Arabidopsis thaliana* (L.) Heyhn.), shepherd's purse (*Capsella bursapastoris* (L.) Medik.), and field pennycress (*Thlapsi arvense* L.). Numerous isolates (field isolates or single spore isolates) with different phenotypic effects and differences in pathogenicity exist (Some et al., 1996, Voorrips, 1996) which make breeding even more difficult. Resistances have been found in *B. oleracea, B. rapa* and *B. napus* which can be classified into two groups: qualitative, dominant resistance (Wit et al. 1964) and quantitative, recessive resistance (Chiang and Crete, 1970, Voorrips, 1996).

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 differential and the resistance race-specific (Gustafson and Fält 1986). A complex type of inheritance, with dominant genes from *B. rapa* and recessive genes from *B. oleracea* can be expected according to Diederichsen et al. (1995). In the publication of Gustafsson and Fält models were proposed based on three, four and five resistance genes, where the most favoured model was based on four genes. Segregation analysis from resynthesised rape seed lines suggests that resistance in *B. napus* is due to 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 on chromosome 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 stability of resistance. The QTL were allocated to the respective *B. napus* chromosomes by using a set of SSR anchor markers.

Material and Methods

A doubled-haploid (DH) population DH914 (N=151) of rapeseed was developed by crossing the resistant DH line 263/11 with the susceptible cultivar Express. The resistant *B. napus* DH line 263/11 was derived via an interspecific cross from 'Böhmerwaldkohl' × ECD-04, hybrid no. 31 = Bwk/04-C31 (Diederichsen and Sacristán, 1996). Six field and one single spore (isolate 1) *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*.

Thirty five AFLP primer combinations were used for genotyping. One hundred fifty six SSR primer pairs were tested for polymorphisms between both parents. Sixtythree were downloaded from the *Brassica* database (http://www.ukcrop.net) and 91 unpublished SSRs were analyzed by the Resistenzlabor Saaten-Union. 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). The PCR products were loaded onto 6-8 % polyacrylamide gels (National Diagnostics Sequagel, Biozym) and separated on a LI-COR Model 4300 DNA sequencer for visualization.

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 was 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 to N19 (Parkin et al., 1995).

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.

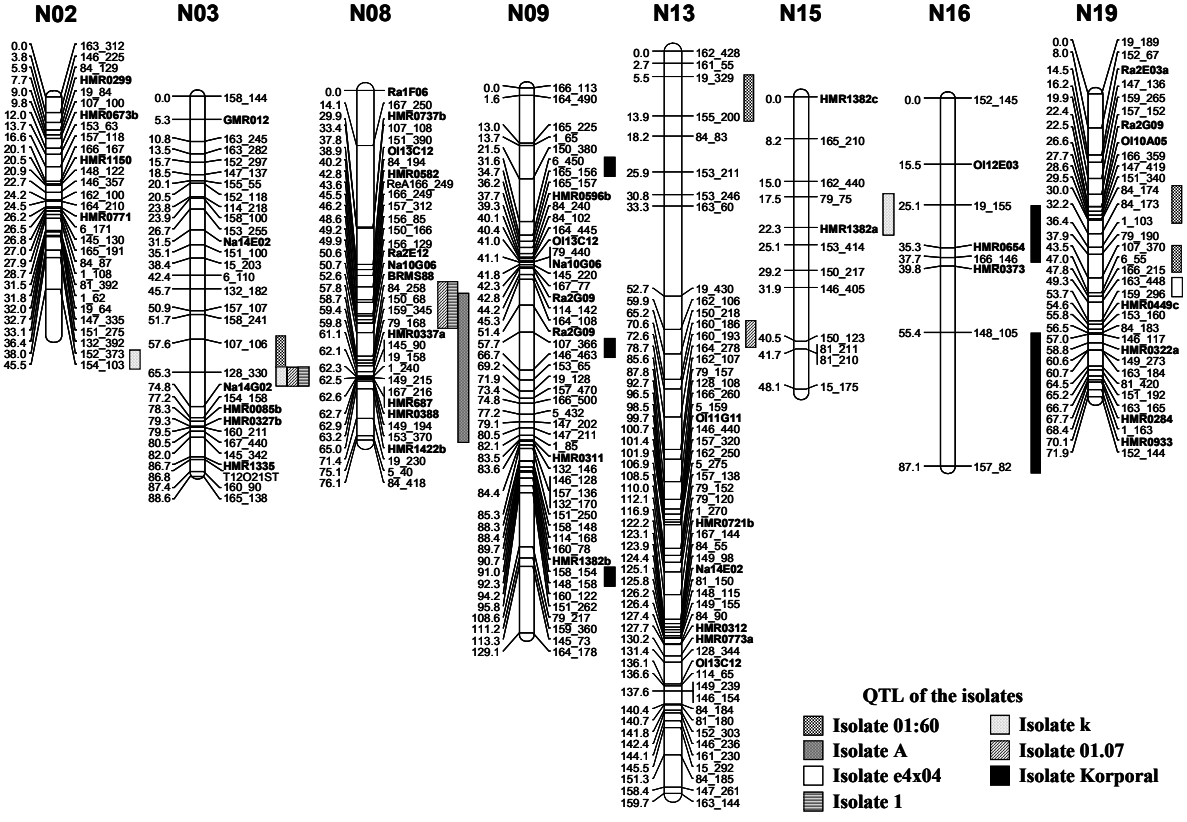

Figure 1. Linkage maps of eight selected *B. napus* chromosomes with QTL for clubroot resistance. The maps comprise AFLPs and SSRs (in bold). The distances in cM are given on the left, the marker numbers on the right. The chromosomes are designated according to the map positions of their SSR anchor markers. Confidence intervals of clubroot QTL are shown on the right with their respective isolate.

Results

For a genome wide detection of clubroot QTL, a comprehensive linkage map of the *B. napus* genome was constructed using the DH914 population. The final linkage map based on the population DH914 consists of 305 AFLPs and 89 SSRs, 27 of these are public markers. The map covers 1570 cM, with an average marker interval of 3.97 cM. The sizes of the linkage groups ranged between 27.2 and 159.7 cM. Due to mapping of SSR anchor markers, 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.

For the seven *P. brassicae* isolates studied, a total of 19 QTL were detected on 8 chromosomes (Table 1). The total phenotypic variance collectively explained by all detected QTL ranged from 20.8 % to 79.6 %. One to five QTL were explained by a single isolate, with LOD scores ranging between 3.53 and 34.39. QTL on N03 and N08 being common for several isolates.

Discussion

The identification of potentially race-nonspecific resistance in the AC genome of *B. napus* was possibly the most interesting finding of this study, since Crute et al. (1983) and Gustafson and Fält (1986) hypothesised a qualitative resistance in *B. napus*. Previous studies for clubroot resistance in *B. napus* suggests that the resistance is due to at least two dominant and unlinked genes (Diederichsen et al. 1996) or one major dominant gene, designated *Pb-Bn1*, located on top of chromosome N03 and one minor-effect QTL (Manzanares-Dauleux et al. 2000). It is also worthwhile to determine by further comparison analysis whether the genes (QTL) mapped here are identical to any of the clubroot resistance genes previously reported for *Brassica*.

The present results suggest that three clubroot resistance genes (*Crr1* on N08, Suwabe et al. 2006, *Crr3* on N03, Hirai et

al. 2004 and *CRb* on N03, Piao et al. 2004) and one QTL (*Pb-Bn1* on N03, Manzanares-Dauleux et al. 2000) identified from the C-genome correspond to two major QTL identified in *B. napus*. The SSR marker BRMS88 which is linked with *Crr1* was located on the same chromosome, however, not within the confidence interval of the QTL. The positions of *Crr3* or *CRb* maybe identical to the region of clustered QTL on N03 in the population DH914. But also for these genes further analysis is needed to confirm this hypothesis.

The resistance gene *Crr2* linked with the SSR marker BRMS96 located on chromosome N01 of *B. rapa* as well, as the QTL *Crr4* (Suwabe et al., 2006) located on chromosome N06 of *B. rapa* and *B. napus* were not linked to any QTL in our DH population DH914 tested with seven different isolates. In the future, DH lines associated with QTL against specific *P. brassicae* isolates will be selected and used as donor lines for breeding rapeseed with durable resistance.

Table 1. Results from QTL mapping. QTL positions were calculated using the marker map from DH population DH914 with 396 molecular markers. Phenotypic data came from tests with seven *P. brassicae* isolates.

No.	Isolate	QTL symbol	Chr.	Marker[a]	Position[b]	LOD	R^{2c}	R^2t^{d}	Additive Effecte[e]	Parent[f]
1	Korporal	Korp_1	N09	6_450	31,6	3,95	12,4	21,6	-7.868	-
2		Korp_2	N09	107_366	57,7	4,03	12,7	21,6	6.683	-
3		Korp_3	N09	158_154	91	6,0	18,3	21,6	4.942	263/11
4		Korp_4	N16	19_155	25,1	6,21	18,8	21,6	6.214	263/11
5		Korp_5	N16	148_105	55,4	3,71	11,7	21,6	-4.508	Express
6	k	k_1	N02	152_373	38	5,91	17,6	56,9	8.498	263/11
7		k_2	N03	128_330	65,3	34,39	67,5	56,9	35.318	263/11
8		k_3	N15	79_75	17,5	4,87	14,7	56,9	-6.823	Express
9	01.07	01.07_1	N03	128_330	65,3	12,92	44,5	79,6	32.847	263/11
10		01.07_2	N08	84_258	57,8	5,5	22,2	79,6	12.843	263/11
11		01.07_3	N13	160_186	70,6	7,38	28,6	79,6	16.523	263/11
12	1	1_1	N03	128_330	65,3	22,93	51,3	74,0	28.696	263/11
13		1_2	N08	84_258	57,8	6,6	18,7	74,0	17.745	263/11
14	01:60	01:60_1	N03	107_106	57,6	5,61	15,8	20,8	14.031	263/11
15		01:60_2	N13	19_329	5,5	4,05	11,7	20,8	6.279	263/11
16		01:60_3	N19	84_174	30,0	3,53	10,3	20,8	-8.917	Express
17		01:60_4	N19	107_370	43,5	6,61	18,4	20,8	-19.051	Express
18	e4x04	e4x04_1	N19	163_448	49,3	17,81	42,3	60,4	-38.429	Express
19	a	a_1	N08	79_168	59,8	6,26	31,9	43,8	12.400	263/11

[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.

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