Clubroot resistance derived from the European *Brassica napus* cv. ‘Tosca’ is not effective against virulent *Plasmodiophora brassicae* isolates from Alberta, Canada

Rudolph Fredua-Agyeman¹, Sheau-Fang Hwang¹, Hui Zhang², Igor Falak³, Xiuqiang Huang³ & Stephen E. Strelkov¹*

In this study, clubroot resistance in the resynthesized European winter *Brassica napus* cv. ‘Tosca’ was introgressed into a Canadian spring canola line ‘11SR0099’, which was then crossed with the clubroot susceptible spring line ‘12DH0001’ to produce F₁ seeds. The F₁ plants were used to develop a doubled haploid (DH) mapping population. The parents and the DH lines were screened against ‘old’ pathotypes 2F, 3H, 5I, 6M and 8N of the clubroot pathogen, *Plasmodiophora brassicae*, as well as against the ‘new’ pathotypes 5X, 5L, 2B, 3A, 3D, 5G, 8E, 8J, 5K, 3O and 8P. Genotyping was conducted using a *Brassica* 15K SNP array. The clubroot screening showed that ‘Tosca’, ‘11SR0099’ and the resistant DH lines were resistant to three (2F, 3H and 5I) of the five ‘old’ pathotypes and four (2B, 3O, 8E and 8P) of the 12 ‘new’ pathotypes, while being moderately resistant to the ‘old’ pathotype 8N and the ‘new’ pathotypes 3D and 5G. ‘Tosca’ was susceptible to isolates representing pathotype 3A (the most common among the ‘new’ pathotypes) as well as pathotypes 6M, 5L, 5K and 8J. Linkage analysis and QTL mapping identified a ca. 0.88–0.95 Mb genomic region on the A03 chromosome of ‘Tosca’ as conferring resistance to pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P. The identified QTL genomic region housed the *CRk, Crr3* and *CRd* gene(s). However, the susceptibility of ‘Tosca’ to most of the common virulent pathotypes makes it unattractive as a sole CR donor in the breeding of commercial canola varieties in western Canada.

Rapeseed (*Brassica napus* and *B. rapa*) was the second most important oilseed crop (71.9 MMT) after soybeans (362.0 MMT) worldwide in 2018–2019¹. The leading producers include Canada, the European Union, China, India, Australia, Russia and the United Kingdom (FAOSTAT). In Canada, rapeseed varieties (including *B. juncea*) that contain <2% erucic acid and <30 µmol glucosinolate per gram of air-dried oil-free meal are referred to as canola, derived from “Canadian oil”²,³. According to the Canola Council of Canada, approximately 8.6 million ha of canola were planted in 2019, yielding 19.6 MMT of seed or 28% of the world’s production⁴. The Prairie Provinces, namely Alberta, Saskatchewan and Manitoba, accounted for 28%, 55% and 16%, respectively, of total canola produced in Canada in 2019⁵. The continued profitability of canola has led to monocropping of canola in many regions of the Prairies and, globally, rapeseed production has increased by 12% increase over the past 10 years (FAOSTAT).

Unfortunately, the intensified cultivation of *Brassica* crops worldwide has led to an increased incidence and severity of many diseases and the emergence of virulent isolates of the causal organisms⁶–¹⁰. In Canada, clubroot caused by the obligate parasite *Plasmodiophora brassicae*, has become a major threat to canola production due...
Table 1. Clubroot severity data for ‘11SR0099’ (clubroot-resistant doubled haploid (DH) parent), ‘12DH0001’ (clubroot-susceptible DH parent), DH individual lines and population, B. napus cv. ‘Tosca’, B. rapa subsp. rapifera European Clubroot Differential (ECD) 04, B. rapa var. pekinensis ECD 05, and the B. napus cv. ‘Westar’. Clubroot severity (index of disease, ID %) was significantly (P < 0.05) different in the resistant DH parent (‘11SR0099’) compared to the susceptible DH parent (‘12DH0001’). Clubroot severity was significantly (P < 0.05) different in the Brassica napus cv. ‘Tosca’ compared to the resistant DH parent (‘11SR0099’).

| Pathotype (isolate) | CR DH parent ‘11SR0099’ Mean ± SEM | CS DH parent ‘12DH0001’ Mean ± SEM | Individual DH lines (118 lines) | DH population (10 lines) | B. napus cv. ‘Tosca’ Mean ± SEM | B. rapa ECD 04’ Mean ± SEM | B. rapa ECD 05’ Mean ± SEM | B. napus cv. ‘Westar’ Mean ± SEM |
|---------------------|------------------------------------|-----------------------------------|---------------------------------|--------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                     | Maximum                            | Minimum                           | Mean ± SEM                      | Maximum                  | Mean ± SEM                   | Mean ± SEM                  | Mean ± SEM                  | Mean ± SEM                  |
| 2F                  | 12.63 ± 1.34*                      | 96.07 ± 3.08*                     | 5.25 ± 0.71                     | 100.00 ± 0.00            | 71.31 ± 1.77                | 6.94 ± 2.41                 | 98.96 ± 0.33                | 100.00 ± 0.00                |
| 3H                  | 8.25 ± 2.27*                       | 98.83 ± 1.17*                     | 0.63 ± 0.31                     | 98.37 ± 0.88             | 58.97 ± 2.17                | 6.67 ± 2.13                 | 98.72 ± 0.47                | 98.61 ± 0.43                |
| 5I                  | 22.17 ± 2.69*                      | 99.53 ± 4.79*                     | 9.09 ± 4.77                     | 99.07 ± 0.53             | 75.28 ± 1.64                | 11.76 ± 3.05                | 99.02 ± 0.48                | 100.00 ± 0.00                |
| 6M                  | 91.67 ± 4.41*                      | 93.33 ± 6.67                      | 22.53 ± 8.11                    | 99.01 ± 0.54             | 91.87 ± 0.66                | 50.79 ± 2.78*               | 100.00 ± 0.00                | 100.00 ± 0.00                |
| 8P                  | 10.44 ± 1.38*                      | 97.25 ± 1.66*                     | 0.00 ± 0.00                     | 100.00 ± 0.00            | 65.90 ± 2.33                | 12.50 ± 2.77*               | 98.29 ± 0.31                | 96.30 ± 1.60                |
| 8J                  | 72.22 ± 9.09*                      | 95.64 ± 4.44*                     | 33.33 ± 10.40                   | 100.00 ± 0.00            | 91.34 ± 0.96                | 90.48 ± 1.58                | 98.72 ± 0.47                | 100.00 ± 0.00                |
| 5K                  | 86.31 ± 2.94*                      | 99.25 ± 0.75*                     | 8.33 ± 4.45                     | 99.17 ± 0.83             | 86.67 ± 1.64                | 48.15 ± 3.30                | 97.10 ± 0.87                | 100.00 ± 0.00                |
| 8E                  | 43.61 ± 5.64*                      | 95.02 ± 0.94*                     | 0.00 ± 0.00                     | 100.00 ± 0.00            | 86.67 ± 1.64                | 48.15 ± 3.30                | 97.10 ± 0.87                | 100.00 ± 0.00                |
| 5G                  | 6.89 ± 1.15*                       | 97.25 ± 1.66*                     | 0.00 ± 0.00                     | 100.00 ± 0.00            | 65.90 ± 2.33                | 12.50 ± 2.77*               | 98.29 ± 0.31                | 96.19 ± 0.74                |
Clubroot assessment in DH parents and DH population. There was a significant difference \((p<0.05)\) between the mean ID values of the clubroot resistant DH parent '11SR0099' and the clubroot susceptible DH parent '12DH0001' in 16 of the 18 \(P. brassicae\) isolates, with the exception of pathotypes 6M and 5L (Table 1). The DH parent '11SR0099' was resistant to \(P. brassicae\) pathotypes 2F (12.63% ± 1.34%), 3H (8.25% ± 2.27%), 3I (22.17% ± 2.69%), 2B (2.80% ± 2.80%), 8E (11.94% ± 1.94%), 3O (8.89% ± 1.15%) and 8P (10.44% ± 1.38%). It was moderately resistant to pathotypes 8N (39.97% ± 3.58%), 3D (46.83% ± 2.38%), 5G (43.61% ± 5.64%) and 5C (45.25% ± 2.90%), and susceptible to pathotypes 6M (91.67% ± 4.41%), 5X (L-G1 and L-G2; 73.82% ± 7.15%) and 83.33% ± 3.33%, respectively, 5L (93.33% ± 6.67%), 3A (72.22% ± 9.09%), 5K (86.31% ± 2.94%) and 8J (71.11% ± 4.44%) (Table 1). The difference in the mean ID values between the DH parent '11SR0099' and the \(B. napus\) cv. 'Tosca' was not significant for pathotypes 2F, 3H, 5L, 8N, 5X (L-G1), 5L, 2B, 3A, 3D, 5C, 5G, 8E, 5K and 3O (i.e. except pathotypes 6M, 5X (L-G2), 8J and 8P) (Table 1). Therefore, the disease reactions of the clubroot resistant DH parent '11SR0099' followed a similar pattern as 'Tosca'. In contrast, the DH parent '12DH0001' was susceptible to all 18 isolates (mean ID values in the range of 93.33% ± 6.67% to 100.00% ± 0.00%, Table 1).

Clubroot severity in the replicated greenhouse experiments was significantly correlated and ranged from \(r=0.60\) to 0.98, \(p<0.0001\) (Table S1). In addition, a significant genotypic effect \((p<0.05)\) and high heritability \((57.68\% \pm 99.94\%)\) were detected in the DH population (Table S1). Thus, the combined data from the individual experiments is presented. Figure 1 shows the frequency distribution of the combined clubroot severity for the 116 DH lines inoculated with 18 \(P. brassicae\) isolates. The Shapiro–Wilk test suggested that none of the data followed a normal distribution, but rather the distributions skewed mostly to the left (Fig. 1). The mean ID ± SEM of the DH lines ranged from 38.97% ± 2.17% to 94.52% ± 0.91% (Table 1). Despite the high mean ID values across all isolates, segregation of clubroot resistance in the DH population was apparent (19.8% to 40.5% R and MR) in the greenhouse experiments carried out with nine (2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P) of the 17 \(P. brassicae\) pathotypes. Segregation for clubroot resistance in the DH population for the remaining eight (6M, 8N, 5X (L-G1 and L-G2), 5L, 3A, 5C, 5K and 8J) pathotypes (represented by nine isolates) was very small \((0.9\%\) to 13.4% R and MR). Chi-square goodness of fit tests showed that segregation for clubroot resistance in the DH population inoculated with all 18 isolates was significantly different \((p<0.05)\) from the expected 1:1 segregation ratio (Table 2). In comparison, Chi-square tests at \(p<0.01\) fit the hypothesized 1:1 segregation ratio for the DH population inoculated with pathotypes 3H, 2B and 8E, and marginally for pathotype 8P (Table 2).

Genetic linkage mapping. The initial filtering steps removed 10,437 (76.1%) of the 13,714 SNP markers. These comprised 445 (3.2%) SNP markers which failed to amplify genomic DNA in the parents, 492 (3.6%) markers that had minor-allele frequency \(≤ 5\%\) and were missing data points. These comprised 445 (3.2%) SNP markers which failed to amplify genomic DNA in the parents, 492 (3.6%) markers that had minor-allele frequency \(≤ 5\%\) and were missing data points. Additive–effect QTL analysis.

QTL analysis conducted by the CIM method with 829 Bin 'Mendelian' markers (Table S4) detected 15 coincident QTL on chromosome A03, which were significantly associated with resistance to \(P. brassicae\) pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P. Based on the \(R^2\) values, five of the QTL were major-effect QTL, nine were moderate-effect QTL and one was a minor-effect QTL. The peak LOD values for the QTL ranged from 6.8 to 48.1 (Table S4). The SNP markers Bn_A03_p14784764 and Bn_A03_p15704830, which were within two-LOD confidence intervals and spanned 20.6 cM (at position 56.6 to 77.2 cM), bordered the genomic region conferring resistance to the nine \(P. brassicae\) pathotypes according to the use of the 'Mendelian' markers (Fig. 3).

QTL analysis conducted with the 1000 'Mendelian' and 'distorted' markers detected 11 coincident QTL, which were associated with resistance to pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P (Table 4). The QTL profiles with the 'Mendelian' and 'distorted' markers (Fig. 2) were similar to those obtained by use of the 'Mendelian' markers. The initial filtering steps removed 10,437 (76.1%) of the 13,714 SNP markers. These comprised 445 (3.2%) SNP markers which failed to amplify genomic DNA in the parents, 492 (3.6%) markers that had minor-allele frequency \(≤ 5\%\) and were missing data points. Additive–effect QTL analysis.

QTL analysis conducted by the CIM method with 829 Bin 'Mendelian' markers (Table S4) detected 15 coincident QTL on chromosome A03, which were significantly associated with resistance to \(P. brassicae\) pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P. Based on the \(R^2\) values, five of the QTL were major-effect QTL, nine were moderate-effect QTL and one was a minor-effect QTL. The peak LOD values for the QTL ranged from 6.8 to 48.1 (Table S4). The SNP markers Bn_A03_p14784764 and Bn_A03_p15704830, which were within two-LOD confidence intervals and spanned 20.6 cM (at position 56.6 to 77.2 cM), bordered the genomic region conferring resistance to the nine \(P. brassicae\) pathotypes according to the use of the 'Mendelian' markers (Fig. 3).

QTL analysis conducted with the 1000 'Mendelian' and 'distorted' markers detected 11 coincident QTL, which were associated with resistance to pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P (Table 4). The QTL profiles with the 'Mendelian' and 'distorted' markers (Fig. 2) were similar to those obtained by use of the 'Mendelian' markers. The initial filtering steps removed 10,437 (76.1%) of the 13,714 SNP markers. These comprised 445 (3.2%) SNP markers which failed to amplify genomic DNA in the parents, 492 (3.6%) markers that had minor-allele frequency \(≤ 5\%\) and were missing data points. Additive–effect QTL analysis.

QTL analysis conducted by the CIM method with 829 Bin 'Mendelian' markers (Table S4) detected 15 coincident QTL on chromosome A03, which were significantly associated with resistance to \(P. brassicae\) pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P. Based on the \(R^2\) values, five of the QTL were major-effect QTL, nine were moderate-effect QTL and one was a minor-effect QTL. The peak LOD values for the QTL ranged from 6.8 to 48.1 (Table S4). The SNP markers Bn_A03_p14784764 and Bn_A03_p15704830, which were within two-LOD confidence intervals and spanned 20.6 cM (at position 56.6 to 77.2 cM), bordered the genomic region conferring resistance to the nine \(P. brassicae\) pathotypes according to the use of the 'Mendelian' markers (Fig. 3).

QTL analysis conducted with the 1000 'Mendelian' and 'distorted' markers detected 11 coincident QTL, which were associated with resistance to pathotypes 2F, 3H, 5L, 2B, 3D, 5G, 8E, 3O and 8P (Table 4). The QTL profiles with the 'Mendelian' and 'distorted' markers (Fig. 2) were similar to those obtained by use of the 'Mendelian' markers.
Figure 1. Frequency distribution of doubled haploid lines derived from the *Brassica napus* cv. 'Tosca' for resistance to *Plasmodiophora brassicae* single-spore isolates (SSIs) representing pathotypes 2F (1), 3H (2), 5I (3), 6M (4) and 8N (5), and field isolates representing pathotypes 5X (L-G1(6) and L-G2(7)), 5L (8), 2B (9), 3A (10), 3D (11), 5C (12), 5G (13), 8E (14), 5K (15), 8J (16), 3O (17) and 8P (18). The SSIs and field isolates were identified prior to and after the introduction of clubroot-resistant canola cultivars in Canada, respectively.
markers (Fig. S1). The SNP markers Bn_A03_p14758285 (57.9 cM) and Bn_A03_p15351982 (73.4 cM) spanned the major QTL identified by the use of the 'Mendelian' and 'distorted' markers (Fig. 3). Based on the $R^2$ values, four of the QTL were major-effect QTL, five were moderate-effect QTL and two were minor-effect QTL. The peak LOD values ranged from 6.7 to 51.2 and the two-LOD confidence interval spanned 15.5 cM (Fig. 3).

Overall, the use of the 'Mendelian' markers and both the 'Mendelian' and 'distorted' markers for the analysis yielded comparable LOD and $R^2$ values, as well as coincident QTL for resistance to nine (2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P) of the 18 isolates used to screen the DH lines. No QTL were detected for resistance to $P$. brassicae pathotypes 6M, 8N, 5X (L-G1 and L-G2), 5L, 3A, 5C, 8J or 5K. Furthermore, the additive effect of the QTL detected in both cases had negative values (Tables 4 and S4). This indicated that the favorable allele for resistance originated from 'Tosca'.

**QTL genomic region.** The 15.5 to 20.6 cM genomic region flanking the QTL region represented an 837.6 Kb (LK031800 at positions 887,529 to 1,725,145b) region on the $B$. napus genome and a 946.6 Kb (A03 chromosome at positions 14,757,826 to 15,704,427 b) region on the of the $B$. rapa genome (Table 5). The SNP markers in this region matched GenBank entries corresponding to proteasome family proteins, Calcium-dependent lipid-binding family proteins, zinc finger containing proteins, multisubstrate pseudouridine synthase, serine/threonine-protein kinase WNK1 and RBK2, HCO3-transporter family proteins, alpha/beta-Hydrolases superfamily proteins, E3 ubiquitin ligase, peptidyl-prolyl isomerase, putative DNA repair protein, electron transport SCO1/SenC family proteins and transcriptional factor B3 family proteins (Table 5). Moreover, the QTL region contained six (LOC103860116, LOC103859010, LOC103859016, LOC103859177, LOC103859225 and LOC103859386) leucine-rich repeat (LRR) kinases, which have been identified as disease resistance-related genes (Table 6).

**Discussion.** In this study, the European winter $B$. napus cv. 'Tosca' exhibited high clubroot resistance to the $P$. brassicae pathotypes 2F, 3H, 5I, 2B and 8E, which was comparable to levels previously reported in 'Mendel'8,15,25. In addition, 'Tosca' exhibited higher levels of resistance to pathotypes 3O and 8P, which were reported to cause increased disease (MR and S, respectively) on 'Mendel'9,15,25. In contrast, 'Tosca' was susceptible to pathotypes 6M, 5X (L-G1 and L-G2), 5L, 3A, 5C and 8J, which caused only minor or moderate disease on 'Mendel'9,15,25. 'Tosca' exhibited moderate resistance to pathotypes 8N, 3D and 5G, as opposed to the complete resistance shown by 'Mendel' to these pathotypes. Both 'Tosca' and 'Mendel' were susceptible to pathotype 5C. Collectively, the results of this
study showed that 'Tosca' was resistant to 7 isolates, moderately resistant to 4, and susceptible to another 7 isolates. In contrast, 'Mendel' was resistant to 10 isolates, moderately resistant to 5, and susceptible to 3 isolates. The differences in the resistance phenotypes of 'Tosca' and 'Mendel' to the same pathotypes in this study and in Fredua-Agyeman et al. respectively, suggest that loci controlling clubroot resistance in the two cultivars might be different. However, different loci can confer resistance to the same *P. brassicae* pathotypes.

QTL mapping is usually carried out with markers that follow expected 'Mendelian' segregation ratios, which in a DH population should be 1:1 for resistance and susceptibility. Xu et al. (2008) reported that QTL mapping could benefit from using all available ('Mendelian' + 'distorted') marker resources. Recently, Coulton et al. reported that markers that showed extreme segregation distortion affected the estimation of recombination between marker pairs and hence should be discarded. In this study, Chi-square goodness of test was used to measure deviation from a 1:1 ratio. By adjusting for *p*-value using the Bonferroni correction, we retained an additional 785 SNP markers for the QTL analysis. The use of the additional 'distorted' markers did not result in much improvement in the QTL profiles compared with the use of only the 'Mendelian' markers at a minimum significance threshold of *p* < 0.05 (Figs. 2 and Fig. S1). In addition, the use of the markers with lower levels of segregation distortion did not affect the order of the genetic map. Based on the 'Mendelian' and 'distorted' markers, however, the genomic region conferring resistance to the nine *P. brassicae* pathotypes mapped to a narrower (15.5 cM) region compared with the use of only the 'Mendelian' markers (20.6 cM). Therefore, as previously reported, it was beneficial for QTL mapping to include both the 'Mendelian' and low 'distorted' markers.

The genomic region identified to confer resistance to *P. brassicae* pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P mapped to the top half of the A03 chromosome in *B. rapa* and *B. napus*. Fredua-Agyeman et al. positioned the *CRk* and *Crr* genes to the top half of the A03 chromosome in *B. rapa* and *B. napus*. The physical position of these genes spans a genomic region of approximately 765 Kb (14,396,950–15,161,430 nt) on the *B. rapa* genome and 1731 Kb (24,338,876–26,070,712 nt) on the *B. napus* genome. The QTL region identified in this study spanned 837.6 to 946.6 Kb, consistent with the values obtained in our previous studies.

A closer inspection of the QTL region indicated that the different-sized fragments of the A03 chromosome were responsible for the resistance to the different *P. brassicae* pathotypes (Fig. 3). By use of the 'Mendelian' and 'distorted' markers, the genomic region of the QTL could be partitioned into at least two CR 'hotspots'. The first CR hotspot comprised the region between the SNP markers Bn_A03_p14758285 (57.9 cM) and Bn_A03_p15004059 (64.8 cM), while the second comprised the region between the SNP markers Bn_A03_p14968153 (67.3 cM) to Bn_A03_p15351982 (73.4 cM). The first hotspot conferred resistance to all nine (2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P) aforementioned pathotypes while the second conferred resistance to pathotypes 2B, 3H, 5I, 2B, 3D, 5G, 8E, 3O, and 8P.

### Table 3

The distribution of 2969 'Mendelian' and 'distorted' SNP markers on 20 linkage groups representing 18 of the 19 chromosomes used to map QTL for clubroot resistance in doubled haploid lines derived from the *Brassica napus* cv. 'Tosca'. Multiple markers that mapped to the same position on the linkage map were put in the same bin.

| Chromosome | Linkage group | Number of SNP markers used for QTL mapping | Total map length/(cM) | Marker density/cM |
|------------|---------------|------------------------------------------|-----------------------|-------------------|
| A01        | 1             | 245                                      | 134.4                 | 1.8               |
| A02        | 2             | 71                                       | 62.1                  | 1.1               |
| A03        | 3             | 231                                      | 182.4                 | 1.3               |
| A04        | 4             | 185                                      | 163.9                 | 1.1               |
| A05        | 5             | 20                                       | 14.2                  | 1.4               |
| A06        | 6             | 289                                      | 188.2                 | 1.5               |
| A07        | 7             | 204                                      | 77.9                  | 2.6               |
| A08        | 8             | 248                                      | 127.4                 | 1.9               |
| A09a       | 9             | 56                                       | 77.1                  | 0.7               |
| A09b       | 10            | 70                                       | 66.8                  | 1.0               |
| A10        | 11            | 183                                      | 100.9                 | 1.8               |
| C01        | 12            | 152                                      | 57.7                  | 2.6               |
| C02        | 13            | 153                                      | 169.5                 | 0.9               |
| C03        | 14            | 232                                      | 156.6                 | 1.5               |
| C04        | 15            | 31                                       | 20.6                  | 1.5               |
| C05        | 16            | 143                                      | 150.4                 | 1.0               |
| C07        | 17            | 232                                      | 184.4                 | 1.3               |
| C08a       | 18            | 122                                      | 72.7                  | 1.7               |
| C08b       | 19            | 31                                       | 24.3                  | 1.3               |
| C09        | 20            | 71                                       | 83.3                  | 0.9               |
| Total or average | | 2969                                           | 2114.8                | 1.4               |
| Identified QTL | Pathotype | Expt | Peak | Conf Interval | Left SNP Marker | Right SNP marker | LOD | Additive | R² (%) |
|---------------|-----------|------|------|---------------|----------------|-----------------|-----|----------|-------|
| Bn.A3P2F.Crr3/CRk/CRd 1.1 | 2F | 1 | 61.5 | 60.4–63.6 | Bn_A03_p14583041 | Bn_A03_p15149454 | 12.2 | 30.8 | 17.1 |
|                  |           | 2 | 62.5 | 61.1–63.6 | Bn_A03_p14870270 | Bn_A03_p15149454 | 15.9 | 26.3 | 18.6 |
|                  |           | 3 | 61.5 | 60.4–63.3 | Bn_A03_p14583041 | Bn_A03_p15149454 | 20.1 | 31.3 | 34.5 |
| Pooled          |           |  | 62.5 | 61.5–63.5 | Bn_A03_p14870270 | Bn_A03_p15149454 | 19.2 | 29.5 | 37.2 |
| Bn.A3P3H.Crr3/CRk/CRd 1.1 | 3H | 1 | 62.5 | 61.6–63.6 | Bn_A03_p14870270 | Bn_A03_p15149454 | 29.0 | 33.8 | 49.9 |
|                  |           | 2 | 62.5 | 61.5–62.8 | Bn_A03_p14870270 | Bn_A03_p15149454 | 36.7 | 38.7 | 93.3 |
|                  |           | 3 | 62.5 | 61.7–63.1 | Bn_A03_p14870270 | Bn_A03_p15149454 | 36.8 | 39.4 | 90.6 |
| Pooled          |           |  | 62.5 | 62.2–63.1 | Bn_A03_p14885241 | Bn_A03_p15149454 | 41.6 | 37.2 | 92.5 |
| Bn.A3P5L.Crr3/CRk/CRd 1.1 | 5L | 2 | 61.5 | 61.4–64.6 | Bn_A03_p14870270 | Bn_A03_p15004059 | 10.7 | 27.8 | 27.7 |
|                  |           | 3 | 61.5 | 59.8–63.7 | Bn_A03_p14583041 | Bn_A03_p15004059 | 10.0 | 28.2 | 19.3 |
| Pooled          |           |  | 61.5 | 60.2–63.3 | Bn_A03_p14885241 | Bn_A03_p15149454 | 10.4 | 25.9 | 18.3 |
| Bn.A3P2B.Crr3/CRk/CRd 1.1 | 2B | 1 | 57.4 | 55.8–58.4 | Bn_A03_p14583041 | Bn_A03_p14968153 | 16.2 | 30.3 | 23.2 |
|                  |           | 2 | 64.7 | 62.2–67.8 | Bn_A03_p14885241 | Bn_A03_p15237693 | 6.7 | 40.4 | 19.4 |
|                  |           | 3 | 64.7 | 63.7–65.7 | Bn_A03_p14888403 | Bn_A03_p14968153 | 9.3 | 42.5 | 15.6 |
| Pooled          |           |  | 62.5 | 61.5–64.6 | Bn_A03_p14870270 | Bn_A03_p15004059 | 8.2 | 41.5 | 15.5 |
| Bn.A3P2B.Crr3/CRk/CRd 1.2 | 2B | 1 | 62.5 | 60.5–65.7 | Bn_A03_p14583041 | Bn_A03_p14968153 | 16.2 | 30.3 | 23.2 |
|                  |           | 2 | 64.7 | 63.7–66.3 | Bn_A03_p14888403 | Bn_A03_p14968153 | 7.5 | 20.4 | 11.5 |
|                  |           | 3 | 62.5 | 60.5–65.2 | Bn_A03_p14583041 | Bn_A03_p14968153 | 10.4 | 24.8 | 16.0 |
| Pooled          |           |  | 61.5 | 60.3–63.4 | Bn_A03_p14583041 | Bn_A03_p15149454 | 13.0 | 25.6 | 15.8 |
| Bn.A3P3D.Crr3/CRk/CRd 1.1 | 3D | 1 | 77.2 | 75.9–80.6 | Bn_A03_p15704830 | Bn_A03_p16126013 | 15.8 | 31.4 | 36.4 |
| Bn.A3P3D.Crr3/CRk/CRd 1.2 | 3D | 1 | 61.7 | 58.7–65.7 | Bn_A03_p14583041 | Bn_A03_p14968153 | 8.3 | 20.6 | 34.2 |
|                  |           | 2 | 61.7 | 58.7–65.7 | Bn_A03_p14583041 | Bn_A03_p14968153 | 9.5 | 20.7 | 37.8 |
|                  |           | 3 | 61.7 | 58.7–66.3 | Bn_A03_p14583041 | Bn_A03_p14968153 | 8.0 | 21.6 | 32.4 |
| Pooled          |           |  | 61.7 | 58.7–66.3 | Bn_A03_p14583041 | Bn_A03_p14968153 | 9.0 | 20.1 | 36.7 |
| Bn.A3P5G.Crr3/CRk/CRd 1.1 | 5G | 1 | 61.5 | 61.5–72.0 | Bn_A03_p14583041 | Bn_A03_p14968153 | 8.3 | 20.6 | 34.2 |
|                  |           | 2 | 61.5 | 64.4–69.1 | Bn_A03_p14927037 | Bn_A03_p15237693 | 25.2 | 40.5 | 67.6 |
|                  |           | 3 | 64.7 | 64.5–69.7 | Bn_A03_p14927037 | Bn_A03_p15265791 | 24.3 | 42.2 | 84.5 |
| Pooled          |           |  | 64.7 | 64.4–69.2 | Bn_A03_p14927037 | Bn_A03_p15237693 | 32.8 | 41.3 | 90.5 |

Continued
3D, 5G, 8E and 8P. In the case of the use of only the ‘Mendelian’ markers, three CR hotspots could be delimited. The first hotspot, between the SNP markers Bn_A03_p14780270 (56.6 cM) to Bn_A03_p14927037 (63.7 cM), conferred resistance to pathotypes 2F, 2B, 3D, 5G and 3O. The second CR hotspot, between SNP markers Bn_A03_p14870270 (63.7 cM) to Bn_A03_p14968153 (67.0 cM), conferred resistance to all nine pathotypes. The third CR hotspot, between SNP markers Bn_A03_p15237693 (69.7 cM) and Bn_A03_p15704830 (77.2 cM), conferred resistance to 8 of the 9 pathotypes, with the exception of pathotype 2B.

Disease resistance is a complex trait and may involve the interaction of subunits of the same gene or different genes. In this study, the QTL region contained several genes including LRR kinases. Mutation studies in Arabidopsis showed that the interaction between the LRR and the kinase domains of the ERECTA (ER) gene were required for resistance to rot caused by Plectosphaerella cucumerina. In addition, the QTL region identified in this study contained transcriptional factor family proteins. In rice, the interaction of transcription activator-like effector (TALE) proteins and transcription factor IIA small subunit was reported to determine resistance or susceptibility to bacterial leaf blight and bacterial leaf streak. Epistatic interaction between the CR genes

| Identified QTL | Pathotype | Expt | QTL positions (cM) | Left SNP Marker | Right SNP marker | LOD | Additive | R² (%) |
|---------------|-----------|------|--------------------|----------------|------------------|-----|----------|--------|
| Bn.A3P3O.Crr3/CRk/CRd 1.1 | 3O | 1 | 62.5 | 61.2–63.5 | Bn_A03_p14780270 | Bn_A03_p15149454 | 13.3 | −40.5 | 19.8 |
| | | 2 | 62.5 | 61.5–63.5 | Bn_A03_p14780270 | Bn_A03_p15149454 | 21.0 | −37.1 | 35.1 |
| | | 3 | 62.5 | 61.4–64.2 | Bn_A03_p14780270 | Bn_A03_p15006559 | 15.5 | −31.6 | 23.2 |
| | | Pooled | 62.5 | 61.5–63.6 | Bn_A03_p14780270 | Bn_A03_p15149454 | 22.2 | −37.3 | 31.7 |
| Bn.A3P8P.Crr3/CRk/CRd 1.1 | 8P | 1 | 62.5 | 61.5–63.5 | Bn_A03_p14780270 | Bn_A03_p15149454 | 51.2 | −43.2 | 95.0 |
| | | 2 | 63.7 | 62.2–65.7 | Bn_A03_p14885241 | Bn_A03_p14968153 | 38.0 | −39.3 | 46.1 |
| | | 3 | 62.5 | 61.5–63.5 | Bn_A03_p14780270 | Bn_A03_p15149454 | 43.1 | −40.8 | 90.2 |
| | | Pooled | 62.5 | 61.5–63.5 | Bn_A03_p14780270 | Bn_A03_p15149454 | 46.4 | −41.5 | 92.7 |

Table 4. Summary of QTL on chromosome A03 associated with clubroot resistance in doubled haploid lines derived from the Brassica napus cv. ‘Tosca’ inoculated with different Plasmodiophora brassicae pathotypes using ‘Mendelian’ and ‘distorted’ markers. aQTL positions based on two-LOD support intervals for 99% confidence interval (CI), (Lander and Botstein).

Figure 2. QTL likelihood profile of the A03 chromosome of Brassica napus obtained by use of both ‘Mendelian’ and low ‘distorted’ markers. The peak regions indicate genomic regions conferring resistance to nine Plasmodiophora brassicae pathotypes, 2F (a), 3H (b), 5I (c), 2B (d), 3D (e), 5G (f), 8E (g), 3O (h) and 8P (i). Clubroot resistance in the DH lines was derived from the Brassica napus cv. ‘Tosca’. The LOD scores are indicated on the y-axis and the QTL names positioned at the peak of each profile.

In the case of the use of only the ‘Mendelian’ markers, three CR hotspots could be delimited. The first hotspot, between the SNP markers Bn_A03_p14784764 (56.6 cM) to Bn_A03_p14927037 (63.7 cM), conferred resistance to pathotypes 2F, 2B, 3D, 5G and 3O. The second CR hotspot, between SNP markers Bn_A03_p14870270 (63.7 cM) to Bn_A03_p14968153 (67.0 cM), conferred resistance to all nine pathotypes. The third CR hotspot, between SNP markers Bn_A03_p15237693 (69.7 cM) and Bn_A03_p15704830 (77.2 cM), conferred resistance to 8 of the 9 pathotypes, with the exception of pathotype 2B.

Disease resistance is a complex trait and may involve the interaction of subunits of the same gene or different genes. In this study, the QTL region contained several genes including LRR kinases. Mutation studies in Arabidopsis showed that the interaction between the LRR and the kinase domains of the ERECTA (ER) gene were required for resistance to rot caused by Plectosphaerella cucumerina. In addition, the QTL region identified in this study contained transcriptional factor family proteins. In rice, the interaction of transcription activator-like effector (TALE) proteins and transcription factor IIA small subunit was reported to determine resistance or susceptibility to bacterial leaf blight and bacterial leaf streak. Epistatic interaction between the CR genes
CRa/CRbKato on the A03 chromosome and the Crr1 genes on the A08 chromosome of B. rapa and B. napus was reported to confer clubroot resistance in Brassica35. The fact that the QTL region contained several genes suggests the possibility of various interactions within subunits of the same genes and also amongst different genes. This is further complicated by the identification of the CR genes CRk29, Crr330,31 and CRd32 in the QTL region introgressed from 'Tosca'. Therefore, mutation studies are needed to confirm whether the CR gene(s) introgressed from 'Tosca' are three different genes or alleles of the same gene.

The genome region conferring clubroot resistance derived from 'Mendel' was reported by Fredua-Agyeman and Rahman18 to be located on the A03 chromosome at positions 24,376,817 to 24,684,311b in B. rapa and 40,936,414 to 41,929,968 b in B. napus. The CR loci from 'Mendel' conferred resistance against the old P. brassicae pathotypes 2F, 3H, 5I, 6M and 8N from Alberta, Canada. In contrast, the CR loci derived from 'Tosca' in this study was located upstream (14,396,950–15,161,430 nt in B. rapa and 24,338,876–26,070,712 nt in B. napus) of the genomic region conferring clubroot resistance in 'Mendel'. Thus, the genomic hotspot regions reported in 'Tosca' (this study) and that reported in 'Mendel'18 are different.

In conclusion, the Swedish B. napus cv. 'Tosca' is resistant to multiple P. brassicae pathotypes, including isolates representing the 'old' pathotypes 2F, 3H and 5I as well as the 'new' pathotypes 2B, 3O, 5G, 8E and 8P. This host also exhibited moderate resistance to isolates representing the 'old' pathotype 8N and the 'new' pathotypes 3A, 5K and 8J. Unfortunately, 'Tosca' was susceptible to isolates representing the 'old' pathotype 6M and the 'new' pathotypes 5X, 5L, 3A, 5K and 8J. This is the first report on the genomic loci controlling clubroot resistance in the Brassica napus cv. 'Tosca'. The resistance was shown to be different from the clubroot resistance derived from 'Mendel'. The increased clubroot severity on 'Tosca', especially in response to pathotypes 3A and 3D, which constitute the bulk of the virulent pathotypes (note: pathotype 3H is still most prevalent of all pathotypes) in Alberta, makes the cultivar unattractive as the sole CR donor in the breeding of commercial canola varieties in Canada. However, the CRk, Crr3 and CRd gene(s) present in 'Tosca' could be stacked with other CR genes present in additional resistance resources such as 'Mendel', ECD 02 and ECD 04.

Materials and methods

**Plant materials.** One hundred sixteen doubled haploid (DH) lines obtained from F1 plants of the cross '11SR0099' (clubroot resistant) × '12DH0001' (clubroot susceptible) were used as the mapping population. The CR parent '11SR0099' is a spring-type canola line derived from a spring canola × winter canola cv. 'Tosca' cross, while the CS parent '12DH0001' is a spring-type canola line with good agronomy and quality characteristics. Seeds of the DH parents and lines were provided by Corteva AgriScience (Caledon, ON, Canada), while seeds of a 'Tosca' , used as a resistant (negative) control in the inoculation experiments, were obtained from Prof. Ann-Charlotte Wallenhammar (Swedish University of Agricultural Sciences, Skara, Sweden). The European Clubroot Differential (ECD) 04 (B. rapa subsp. napobrassica), which exhibits broad-spectrum resistance to many Canadian isolates of P. brassicae25, was included as resistant (negative) control in the inoculation experiments, while ECD 05 (B. rapa var. pekinensis 'Granat')36 and B. napus cv. 'Westar'30 were included as susceptible (positive) controls.

---

**Figure 3.** Single-nucleotide polymorphism (SNP) markers and different-sized genomic fragments on the 24.3–26.1 Mb region of the Brassica napus genome (ca. 14.7–15.7 Mb on the Brassica rapa genome), conferring resistance to Plasmodiophora brassicae pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P.
### Table 5. Description of the single-nucleotide polymorphism (SNP) markers flanking the major QTL associated with resistance to *Plasmodiophora brassicae* pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P.

| SNP marker | Overlapping gene(s) | Species | Genomic location | SNP marker position | Expect (E)-value | Description of gene functions |
|------------|---------------------|---------|------------------|--------------------|-----------------|-------------------------------|
| Bn_A03_p11473296 | BnaA03g27790D | B. napus | LK031800 | 2009415 | 2009519 | 3.2e−50 | Proteasome family protein |
| Bn_A03_p14355646 | BnaA03g27480D | B. napus | LK031800 | 2118648 | 2118712 | 7.6e−26 | Uncharacterized protein Mb2253c-like |
| Bn_A03_p14758285 | BnaA03g28540D | B. rapa | A03 | 14773527 | 14773591 | 2e−21 | Calcium-dependent lipid-binding family protein |
| Bn_A03_p14583041 | BnaA03g28150D | B. napus | LK031800 | 1898103 | 1898167 | 4.6e−29 | Zinc finger CCCH domain-containing protein |
| Bn_A03_p14870270 | BnaA03g28800D | B. napus | LK031800 | 1628327 | 1628431 | 7.3e−52 | Multisubstrate pseudouridine synthase |

### Table 6. List of leucine-rich-repeat (LRR) receptor-like kinases in the QTL genomic region associated with resistance to *Plasmodiophora brassicae* pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O and 8P.

| Gene ID | Symbol | Chromosome | Position | Description of gene functions |
|---------|--------|------------|----------|-------------------------------|
| 103860116 | LOC103860116 | A03 | 14449648 | 14453765 | LRR receptor-like serine/threonine-protein kinase RPK2 |
| 103859010 | LOC103859010 | A03 | 14678662 | 14682582 | Putative DNA repair protein |
| 103859018 | LOC103859018 | A03 | 14707594 | 14710622 | Electron transport SCO1/SenC family protein |
| 103859177 | LOC103859177 | A03 | 15327289 | 15373533 | Zinc finger family protein |
| 103859225 | LOC103859225 | A03 | 15349521 | 15349674 | E3 ubiquitin ligase |

The collection of plant materials and all conducted experiments complied with relevant guidelines/regulations of the University of Alberta, Canada and International Treaty for Plant Genetic Resources guidelines and legislation.

**Pathogen isolates and inoculum preparation.** Eighteen *P. brassicae* isolates were used in inoculation experiments under controlled conditions in the greenhouse. These consisted of five single-spore isolates (SSIs) (SACAN-ss3, SACAN-ss4, ORCA-ss4, AbtJE-ss1 and ORCA-ss2), classified as pathotypes 2F, 3H, 5I, 2B, 3D, 5G, 8E, 3O, 3P, 8P, respectively. One field isolate, L-G1 = L-G2, represented the same pathotype (5X). The 18 isolates (representing isolates of...
17 pathotypes) were maintained as frozen (−20 °C) root galls of the universally susceptible host ECD 05 until needed. Clubroot inoculum was prepared following Fredua-Agyeman et al.\textsuperscript{25} by macerating root galls using a variable-speed blender, filtering the spore suspension through three layers of cheesecloth, and adjusting the final resting spore concentration to 1 × 10^6 spores/ml with sterile distilled water. Each batch of inoculum was stored at 4 °C and used within 24 h after preparation.

**Evaluation of DH lines for clubroot resistance.** Inoculation experiments were performed in two greenhouses at the at the Crop Diversification Centre North (CDCN), Alberta Agriculture and Forestry (AAF), Edmonton, Canada. Briefly, 15–20 seeds of the DH parents (‘11SR0099’ and ‘12DH0001’), each DH line, ‘Tosca’, ECD 04, ECD 05 and ‘Westar’, were placed on moistened Whatman No. 1 filter paper in Petri dishes and kept at room temperature under natural light\textsuperscript{29}. After 7 days, 8–12 seedlings of each genotype were inoculated by dipping their entire roots in a resting spore suspension for about 10–20 s\textsuperscript{27} and then transplanted into 13 × 13 × 15 cm (L × B × D) pots filled with Sunshine Mix #4 Aggregate Plus soilless Mix (Sungro Horticulture Canada Ltd)\textsuperscript{25,28}. To minimize disease escape, 1 ml of additional inoculum was dispensed into the potting mixture surrounding each seedling with a micropipette\textsuperscript{19}. The plants were kept in the greenhouse maintained under a 16 h photo-period and day and night temperatures of 20–25 °C and 15–18 °C, respectively. The pots were arranged in a randomized complete block design (RCBD) in 225 × 104 × 20 cm (L × B × D) trays\textsuperscript{38}. The potting mixture were kept saturated with slightly acidic water (pH ≈ 5.5–6.9) for the first 2 weeks, and then watered daily and fertilized every 2 weeks with 200 ppm Plant Prod 20–20–20 (N–P–K) with micronutrients (Plant Products, Leamington, ON, Canada).

Eight weeks after inoculation, the plants were uprooted, washed and assessed for disease severity on a 0–3 scale\textsuperscript{40}, where: 0 = no galling, 1 = a few small galls on the lateral roots, 2 = moderate galling on the lateral roots but not on the main root, and 3 = severe galling on both the lateral and main root. Disease severity symptoms, measured as an index of disease (ID, 0–100%), were calculated following Strelkov et al.\textsuperscript{20} as shown below:

\[
ID(\%) = \frac{\sum (n \times 0 + n \times 1 + n \times 2 + n \times 3)}{N \times 3} \times 100
\]

where \(n\) = number of plants in each symptom severity class and \(N\) is the total number of plants. The inoculation experiments were repeated two times. Based on the mean ID values of the combined data ± the standard error (SEM), the DH lines were classified as resistant (R) (Mean ID ± SEM ≤ 30%), moderately resistant (MR) (30% < Mean ID ± SEM ≤ 50%) or susceptible (S) (Mean ID ± SEM > 50%) as per the recommendations of the Western Canada Canola/Rapeseed Recommending Committee (WCC/RCC)\textsuperscript{19}.

**Statistical analyses of phenotypic data.** Statistical analyses of the disease severity data for the individual experiments and the combined data were conducted with SAS v. 9.4 (SAS Institute, United States) as described by Fredua-Agyeman et al.\textsuperscript{25,28}. In brief, the PROC CORR function was used to determine the correlation among the mean ID values for each DH line, parents and checks for each pathotype in the three experiments. Broad sense heritability (\(H^2\)), which is the ratio of total genetic variance to phenotypic variance, was estimated from variance components from Analysis of Variance (ANOVA)\textsuperscript{41,42}. The PROC MEANS function was used to calculate the mean ID, standard error of the mean (SEM), minimum and maximum ID for each genotype and isolate investigated. The PROC FREQL function was used to count the number of accessions that were resistant (Mean ID ± SEM ≤ 30%), moderately resistant (30% < Mean ID ± SEM ≤ 50%) or susceptible (Mean ID ± SEM > 50%) to each P. brassicae isolate based on the combined data, while SigmaPlot (SYSTAT Software Inc., San Jose, California, USA) was used to create histograms. The Shapiro–Wilk test was used to test for normality in the phenotypic data\textsuperscript{43}. The \(\chi^2\) test for goodness-of-fit for the 1:1 ratio expected for a DH population was determined through Chi-Square goodness of fit tests (\(\chi^2\)) at \(p \leq 0.05\) for each of the 18 P. brassicae isolates. Differences in the mean ID values of all DH lines to pairs of the 18 P. brassicae isolates were compared with Tukey’s test at \(p \leq 0.05\).

**Genotyping with SNP markers.** The parental lines and the DH population were genotyped using the Brassica 15 K array, which contained 13,714 SNPs, at SGS TraitGenetics GmbH, Gatersleben, Germany, according to the manufacturer’s protocols\textsuperscript{44}. The software TASSEL v5.2.2.5\textsuperscript{45} was used to perform SNP filtering by deleting failed SNP reactions, setting minor allele frequency (MAF) to ≤ 0.05 and removing markers missing data for > 5% of the accessions. Segregation distortion was determined through a \(\chi^2\) test for goodness-of-fit for the 1:1 ratio expected for a DH population. A minimum significance threshold of \(p < 0.05\) was used for markers that followed expected ‘Mendelian’ ratio, while adjusted Bonferroni correction \(p\)-values (\(\alpha/n\), where \(\alpha = \text{level of significance}\), \(n = \text{number of markers}\)) were used to select markers with ‘minor’ segregation distortion\textsuperscript{46}. Markers that showed extreme segregation distortion were discarded.

**Construction of genetic linkage maps.** Two ‘draft’ linkage maps were constructed using the minimum spanning tree map (MSTMap) program with the following parameters: logarithm of odds (LOD) value of 10.0, a maximum distance between markers of 15 cM and Kosambi mapping function\textsuperscript{47,48}. The first linkage map was constructed only with markers that fit the expected 1:1 Mendelian ratio, while the second linkage map was constructed using markers that fit the 1:1 Mendelian ratio expected for a DH population and markers that showed ‘minor’ segregation distortion. In both cases, markers that mapped to the same position were placed in the same bin, with only one of the markers retained for linkage analysis. MAPMAKER/EXP v. 3.0b\textsuperscript{49} was run at a logarithm of odds (LOD) score ≥ 3.0 and recombination fraction (\(\Theta\)) value ≤ 0.40 to ‘refine’ the marker order obtained by the MSTMap software. Recombination fractions were converted to centiMorgans (cM) using the
Kosambi mapping function. Linkage groups were assigned to chromosomes based on the SNP sequence information provided by SGS TraitGenetics GmbH.

**Additive effect QTL mapping.** Quantitative trait loci (QTL) mapping was conducted separately with only the ‘Mendelian’ markers and with ‘Mendelian’ and ‘distorted’ markers together. The QTL analyses were carried out by composite interval mapping (CIM)\(^{38}\) using WinQTL Cartographer v. 2.5\(^{31}\). The program was run at a walking speed of 1 cM and with the following settings: forward–backward regression method, a window size of 2.0 cM, five background markers as cofactors, 1000 permutations and \(p < 0.05\). The significance level required to declare a QTL was set at LOD \(\geq 5.0\). Locations of putative QTL were estimated based on two-LOD support intervals for a 99% confidence interval (CI)\(^{32}\).

The QTL designations were of the order genus (1 letter), species (1 letter), chromosome number (1 letter), pathotype name (3 letters), closest published gene(s) (3–6 letters) and QTL number (2 letters)\(^{35,33}\). The percentage of phenotypic variation \(R^2\) explained by each QTL was calculated. QTL were arbitrary assigned as major-, moderate- or minor-effect QTL when the \(R^2\) explained > 50%, 25–50% or < 25% of the phenotypic variation, respectively. The additive effects of each QTL were calculated by deducting the phenotypic average of all individuals with the susceptible DH parent allele from all individuals with the resistant DH parent allele.

**Identification of candidate genes.** The physical positions of the SNP markers in the QTL CI region were mapped to the *B. napus*, *B. rapa* and *B. oleracea* reference genomes deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov). Candidate genes present in the QTL two-LOD confidence interval were identified by BlastN searches (E-value ≤ E\(^{-20}\), minimum identity of sequence ≥ 95%) of the three *Brassica* genome sequences.

**Ethical approval.** On behalf of all authors, the corresponding author states that the collected seeds and all conducted experiments in this study complied with relevant guidelines/regulations of the University of Alberta, the Canada Food Inspection Agency and International Treaty for Plant Genetic Resources guidelines and legislation.

**Data availability**

The datasets generated during the current study are available in the manuscript or the supplementary materials. The SNP used for genotyping is available can be downloaded from https://static-content.springer.com/esm/art%3Ai.1007%2Fps80122-016-2746-7/MediaObjects/122_2016_2746_MOESM3_ESM.pdf or from Clark et al.\(^{44}\)

Received: 21 March 2021; Accepted: 16 June 2021

**References**

1. Canola Digest. Canola Market Snapshot 2019. https://canoladigest.ca/november-2019/canola-market-snapshot-2019/. Accessed 5 Dec 2020.
2. Busch, L., Gunter, V., Mentele, T., Tachikawa, M. & Tanaka, K. Socializing nature: Technoscience and the transformation of rapeseed into canola. *Crop Sci.* 34, 607–614 (1994).
3. Rakow, G. Dedication: Richard K. Downey-designer of Canola. *Plant Breed. Rev.* 18, 1–12 (2000).
4. Canola Council of Canada. Markets and statistics. Canola industry at a glance. https://www.canolacouncil.org/marks--stats/.
5. Li, H., Sivasithamparam, K. & Barbetti, M. J. Breakdown of *Plasmodiophora brassicae* in *Brassica napus* rapeseed by *Leptosphaeria maculans* field isolates in Australia. *Plant Dis.* 87(6), 752 (2003).
6. Sprague, S. J. et al. Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. *Eur. J. Plant Pathol.* 114, 33–40 (2006).
7. Mei, J. et al. Identification of resistant sources against *Sclerotinia sclerotiorum* in Brassica species with emphasis on *B. oleracea*. *Euphytica* 177, 393–399 (2011).
8. Strelkov, S. E., Hwang, S.-F., Manoli, V. P., Cao, T. & Feindel, D. Emergence of new virulence phenotypes of *Plasmodiophora brassicae* on canola (*Brassica napus*) in Alberta, Canada. *Eur. J. Plant Pathol.* 145(3), 517–529 (2016).
9. Neik, T. X., Barbetti, M. J. & Bailey, J. Current status and challenges in identifying disease resistance genes in *Brassica napus*. *Front Plant Sci.* 8, 1788 (2017).
10. Hwang, S. F. et al. Influence of resistant cultivars and crop intervals on clubroot of canola. *Can. J. Plant Sci.* 99, 862–872 (2019).
11. Strelkov, S. E. et al. Occurrence and spread of clubroot on *Brassica napus* in Canada. *Can. Plant Dis. Surv.* 100, 117–120 (2020).
12. Ziesman, B. et al. Clubroot survey and known occurrence of clubroot in Saskatchewan. *Can. Plant Dis. Surv.* 41(S1), 159–161 (2019).
13. Frosé, R. D., Derksen, H., Guo, X. & McLaren, D. L. Monitoring and occurrence of clubroot in Manitoba in 2018. *Can. Plant Dis. Surv.* 99, 179 (2019).
14. Peng, G. et al. Sources of resistance to *Plasmodiophora brassicae* (clubroot) pathotypes virulent on *canola*. *Can. J. Plant Pathol.* 36, 89–99 (2014).
15. Strelkov, S. E. et al. Virulence and pathotype classification of *Plasmodiophora brassicae* populations collected from clubroot resistant *Brassica napus* (canola) in Canada. *Can. J. Plant Pathol.* 40(2), 284–298 (2018).
16. Hollman, K. B., Hwang, S. F., Manoli, V. P. & Strelkov, S. E. Pathotypes of *Plasmodiophora brassicae* collected from clubroot resistant *Brassica napus* (canola) cultivars in western Canada in 2017–2018. *Can. J. Plant Pathol.* https://doi.org/10.1080/07060661.2020.1851893 (2021).
17. Diederichsen, E., Frauen, M., Linders, E. G. A., Hatakeyama, K. & Hirai, M. Status and perspectives of clubroot resistance breeding in crucifer crops. *J. Plant Growth Regul.* 28, 265–281 (2009).
18. Fredua-Agyeman, R. & Rahman, H. Mapping of the clubroot disease resistance in spring *Brassica napus* canola introgressed from European winter canola cv. *Mendel*. *Euphytica* 211(2), 201–213. https://doi.org/10.1007/s10681-016-1730-2 (2016).
19. Frauen, M. *A new clubroot resistant variety in winter oilseed rape.* in *Proceedings 10th International Rapeseed Congress*, Canberra (1999).
20. Strelkov, S. E., Tewari, J. P. & Smith-Degenhardt, E. Characterization of Plasmodiophora brassicae populations form Alberta, Canada. Can. J. Plant Pathol. 28, 467–474 (2006).
21. Xue, S., Cao, T., Howard, R. J., Hwang, S.-F. & Strelkov, S.E. Isolation and variation in virulence of single-spore isolates of Plasmodiophora brassicae from Canada. Plant Dis 92(3), 456–462 (2008).
22. Strelkov, S. E. et al. The spread of clubroot on canola in Alberta in 2014. Can. Plant Dis Sur. 95, 155–158 (2015).
23. Strelkov, S. E. et al. The occurrence and spread of clubroot on canola in Alberta in 2015. Can. Plant Dis. Sur. 96, 163–165 (2016b).
24. Strelkov, S. E. et al. Occurrence and spread of clubroot on canola in Alberta in 2016. Can. Plant Dis. Sur. 97, 164–165 (2017).
25. Fredua-Agyeman, R., Hwang, S.-F., Strelkov, S. E., Zhou, Q. & Feindel, D. Potential loss of clubroot resistance genes from donor parent Brassica rapa subsp. rapifera (ECD 04) during doubled haploid production. Plant Pathol. 67(4), 892–901 (2018).
26. Xu, S. Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180(4), 2201–2208 (2008).
27. Coulton, A. et al. Segregation distortion: Utilizing simulated genotyping data to evaluate statistical methods. PLoS ONE 15(2), e0228953 (2020).
28. Fredua-Agyeman, R., Yu, Z., Hwang, S. F. & Strelkov, S. Genome-wide mapping of loci associated with resistance to clubroot in Brassica napus ssp. napobrassica (rutabaga) accessions from Nordic countries. Front. Plant Sci. 11, 742. https://doi.org/10.3389/fpls.2020.00742 (2020).
29. Matsumoto, E., Ueno, H. & Aruga, D. Accumulation of three clubroot resistance genes through marker-assisted selection in Chinese cabbage (Brassica rapa ssp. pekinensis). J. Jpn. Soc. Hortic. Sci. 81, 184–190 (2012).
30. Hirai, M. et al. A novel locus for clubroot resistance in Brassica rapa and its linkage markers. Theor. Appl. Genet. 108, 639–643 (2004).
31. Saito, M. et al. Fine mapping of the clubroot resistance gene, Crr3, Brassica rapa. Theor. Appl. Genet. 114, 81–91 (2006).
32. Pang, W. et al. Identification and mapping of the Clubroot resistance gene Cre1 in Chinese cabbage (Brassica rapa ssp. pekinensis). Front. Plant Sci. 9, 653 (2018).
33. Llorente, F., Alonso-Blanco, C., Sanchez-Rodriguez, C., Jorda, L. & Molina, A. ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. Plant J. 2005(43), 165–180 (2005).
34. Hui, S. et al. TALE-carrying bacterial pathogens trap host nuclear import receptors for facilitation of infection of rice. Mol. Plant Pathol. 20(4), 519–532 (2019).
35. Fredua-Agyeman, R., Jiang, J., Hwang, S. F. & Strelkov, S. QTL mapping and inheritance of clubroot resistance genes derived from Brassica rapa subsp. rapifera (ECD 02) reveals resistance loci and distorted segregation ratios in two F2 populations of different crosses. Front. Plant Sci 11, 899. https://doi.org/10.3389/fpls.2020.00899 (2020).
36. Buczacki, S. T. et al. Study of physiological specialization in Plasmodiophora brassicae: Proposals for attempted rationalization through an international approach. Trans. Br. Mycol. Soc. 65, 295–303 (1975).
37. Nieuwhof, M. & Wiering, D. Testing cabbage plants for clubroot resistance. Euphytica 10(2), 191–200 (1961).
38. Fredua-Agyeman, R. et al. Identification of Brassica accessions resistant to ‘old’ and ‘new’ pathotypes of Plasmodiophora brassicae from Canada. Plant Pathol. 68(4), 708–718 (2019).
39. Voorrips, R. E. & Visser, D. L. Examination of resistance to clubroot in accessions of Brassica oleracea using a glasshouse seedling test. New J. Plant Pathol. 99, 269–276 (1993).
40. Suginuki, Y., Yoshikawa, H. & Hirai, M. Variation in virulence of Plasmodiophora brassicae in Japan tested with clubroot resistant cultivars of Chinese cabbage (Brassica rapa L. ssp. pekinensis). Eur. J. Plant Pathol. 105, 327–332 (1999).
41. Dudley, J. W. & Moll, R. H. Interpretation and use of estimates of heritability and genetic variances in plant breeding. Crop Sci 9, 257–262 (1969).
42. Nyquist, W. E. & Baker, R. J. Estimation of heritability and prediction of selection response in plant populations. Crop. Rev. Crop Sci. 10(3), 235–322. https://doi.org/10.1080/0735268910932313 (1991).
43. Shapiro, S. S. & Wilk, M. B. An analysis of variance test for normality (complete samples). Biometrika 52(3–4), 591–615 (1965).
44. Clarke, W. E. et al. A high-density SNP genotyping array for Brassica napus and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. Theor. Appl. Genet. 129, 1–13 (2016).
45. Bradbury, P. J. et al. TASSEL: Software for association mapping of complex traits in diverse samples. Bioinformatics 23(19), 2633–2635 (2007).
46. Bonferroni, C. E. Teoria statistica delle classi e calcio delle probabilit. Publ. Istit. Sup. Sci. Econ. Commerc. Firenza 8, 3–62 (1936).
47. Kosambi, D. D. The estimation of map distances from recombination values. Ann. Eugen 12, 172–175 (1944).
48. Wu, Y., Bhat, P. R., Close, T. J. & Lonardi, S. Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS Genet. 4, e1000212. https://doi.org/10.1371/journal.pgen.1000212 (2008).
49. Lande, R. & Estes, B. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181 (1987).
50. Churchill, G. A. & Doerge, R. W. Empirical threshold values for quantitative trait mapping. Genetics 138, 963–971 (1994).
51. Wang, S., Basten, C. J., & Zeng, Z. B. Windows QTL Cartographer 2.5. (Department of Statistics, North Carolina State University, 2011).
52. Lander, E. S. & Botstein, D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121, 185–199 (1989).
53. Østergaard, L. & King, G. J. Standardized gene nomenclature for the Brassica genus. Plant Method 4(10), 1–4. https://doi.org/10.1186/1746-4811-4-10 (2008).

Acknowledgements
The authors thank the Alberta Crop Industry Development Fund, and the Canadian Agricultural Partnership Agriculture (CAP) Program (Agriculture and Agri-Food Canada and the Canola Council of Canada) for financial support of this research. They are also grateful to George Turnbull, Jo-Ann Berry and Cameron Stevenson of Alberta Agriculture and Forestry (AAF) for their logistical support. The Crop Diversification Centre North (AADF) provided in-kind support in the form of access to greenhouse facilities.

Author contributions
R.F.A. Clubroot tests against Plasmodiophora brassicae pathotypes 6M, 8N, 5X, 5L, 2B, 3A, 3D, 5G, 8E, 5C, 8J, 5K, 3O and 8P. Phenotypic and molecular data analysis, linkage map construction, QTL mapping and writing of the manuscript. S.F.H. Principal Investigator, grant application, provision of clubroot galls of the different pathotypes, supervision and provision of technical support for greenhouse experiments and revision of the manuscript. H.Z. Clubroot tests with Plasmodiophora brassicae pathotypes 2F, 3H and 5I. I.F. Crossing of parents to produce F1 hybrids and development of doubled haploid (DH) mapping population. H.X. Crossing of parents to produce F1 hybrids and development of DH mapping population. S.E.S. Principal Investigator, grant application, provision
of clubroot galls of the different pathotypes, supervision and provision of technical support for greenhouse experiments and revision of the manuscript.

**Funding**

Institutions supporting this project were listed in acknowledgement section.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-93327-0.

**Correspondence** and requests for materials should be addressed to S.S.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

[Open Access] This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021