Fine Mapping a Clubroot Resistance Locus in Chinese Cabbage

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ABSTRACT. There are various clubroot pathogen (*Plasmodiophora brassicae*) resistance genes within *Brassica* species with european turnip (*B. rapa* ssp. *rapifera*) being identified as potentially the best source of resistance for the development of clubroot-resistant cultivars in chinese cabbage (*B. rapa* ssp. *pekinensis*). To use clubroot resistance genes effectively, it is necessary to map these genes so that molecular markers inside or closely linked to these resistance genes can be developed. Using molecular marker-assisted selection, the clubroot resistance genes can be effectively transferred from cultivar to cultivar and from species to species. In this report, one clubroot resistance locus was mapped on linkage group A3 using five segregating populations developed from five chinese cabbage cultivars, suggesting that all the five cultivars shared the same clubroot resistance locus. Furthermore, one of these five chinese cabbage cultivars was used to develop a large segregating population to fine-map this clubroot resistance locus to a 187-kilobp chromosomal region. Molecular markers that are closely linked to the mapped clubroot resistance locus have been developed that can be used for marker-assisted selection in chinese cabbage and canola/rapeseed (*B. rapa* and *B. napus*) breeding programs.

Clubroot, caused by the soilborne pathogen *Plasmodiophora brassicae*, is one of the most devastating diseases for *Brassica* crop production globally. The pathogen causes abnormal cell enlargement and uncontrolled cell division in infected roots, thus leading to clubroot galls on the roots. As a result, nutrient and water uptake in infected roots is inhibited and the growth of the aerial parts of host plants is diminished, affecting crop yield and quality. The pathogen *P. brassicae* was first reported in Russia in 1878 (Karling, 1968). In Asia, this disease was first reported in the 1890s in Japan (Ikegami et al., 1981). The pathogen can survive as resting spores for long periods of time in the soil, making it difficult to control by crop rotation or chemical treatments (Voorrips, 1995). Thus, breeding of resistant cultivars is a very desirable method to control clubroot disease. In Canada, clubroot disease has been recognized as a problem for years in *Brassica* vegetable crops [e.g., broccoli

(*B. oleracea* var. *italica*), cabbage (*B. oleracea* var. *capitata*), and radish (*Raphanus sativus*)] grown in Ontario, Quebec, British Columbia, and Atlantic Canada. This disease was first reported in a canola field near Edmonton, Alberta, Canada, in 2003, and more recently, clubroot disease was confirmed in Saskatchewan and Manitoba.

Clubroot-resistant germplasms have been identified in *B. rapa*, *B. oleracea*, and *B. napus* (Crisp et al., 1989; Crute et al., 1983). The published literature indicates that host clubroot resistance genes in *B. napus* are located in the A genome derived from *B. rapa* (Chiang et al., 1977). In chinese cabbage, most cultivars are susceptible to this disease. To develop resistant cultivars, clubroot resistance genes from clubroot-resistant european turnip have been introduced in chinese cabbage (Yoshikawa, 1981). Earlier studies of clubroot resistance in turnip suggested that there are three independent clubroot resistance loci in *B. rapa* (Buczackiet al., 1975; Toxopeus and Janssen, 1975). Recent molecular marker technology has been used to map independent clubroot resistance loci in *B. rapa*. For example, two loci, *Crr1* and *Crr2*, derived from european turnip ‘Siloga’ were reported by Suwabe et al. (2003). *Crr3* locus derived from european turnip ‘Milan White’ (Saito et al., 2006) and another clubroot resistance locus *Crr4* (Suwabe et al., 2006) were reported. More recently, three clubroot resistance loci,

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CRa, *CRk*, and *CRc*, were mapped by Sakamoto et al. (2008), and *CRb* derived from the clubroot-resistant chinese cabbage cultivar, CR Shinki, was mapped (Piao et al., 2004).

Cloning of clubroot resistance genes allows the comparison of different clubroot resistance loci, which help determine the precise resistance spectra of individual clubroot resistance genes. Currently, two clubroot resistance genes, *CRa* and *Crr1a*, have been confirmed through analysis of mutation in the clubroot resistance genes (Ueno et al., 2012) and introduction of the resistance gene into *Arabidopsis thaliana* (Hatakeyama et al., 2013), respectively. Both genes encode TIR-NBS-LLR proteins, suggesting that these two genes belong to the different members of the same gene family. However, these genes need to be analyzed further through complementary transformation in *Brassica* species. In the current study, we have used molecular markers to localize a clubroot resistance locus to a 187-kilobp (kbp) region on *B. rapa* linkage group A3.

Materials and Methods

PLANT MATERIALS. A total 40 chinese cultivars and breeding doubled haploid (DH) lines were evaluated for clubroot disease by Canadian field isolates, and five commercial hybrid chinese cabbage cultivars were identified to contain clubroot resistance genes. These five clubroot-resistant hybrid chinese cabbage cultivars, Qulihuang, Sijihuang, Daifeng No. 1, Nongke No. 1, and Huxinbaicai, were obtained from China and were crossed with a susceptible *B. rapa* oilseed accession (BAR) to develop segregating populations. All the segregating populations were developed at the greenhouse facilities of the Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada. All hybrid chinese cabbage cultivars, including Qulihuang, Sijihuang, Daifeng No. 1, Nongke No. 1, and Huxinbaicai, are commercially available in China. To develop segregating populations with these hybrid chinese cabbage cultivars, these F₁ chinese cabbage plants were backcrossed to a susceptible parent to obtain BC₁ segregating populations and selfed to obtain F₂ segregating populations. Three BC₁ and two F₂ segregating populations were used in gene mapping analysis. To fine-map the clubroot resistance locus in chinese cabbage, a large BC₂ mapping population derived from the cross of BAR and 'Qulihuang' was used to select recombinants after the resistance locus was mapped on chromosome A3. All recombinants were selfed to obtain BC₂F₁ progeny and these individual BC₂F₂ seeds were used in lines tested with a clubroot field isolate collected in Canada.

PATHOGEN AND INFECTION. The clubroot pathogen that was collected from infected canola fields near Edmonton, Alberta, Canada, was used to test phenotypes in all parental lines and BC₁, BC₂, BC₃, BC₃F₁, and BC₃F₂ populations. All indoor testing was performed at the University of Guelph, Guelph, Ontario, Canada. Eight to 10 seeds were sown in a pot containing the soil from a known highly infected field, and the disease was scored after plants grew in a greenhouse for 5 weeks. Clubroot disease scoring scale included four levels: 0 for plants without galls on roots, 1 for plants with a few small galls on secondary roots, 2 for plants with a few small galls on both primary and secondary roots, and 3 for plants with many big galls on both primary and secondary roots.

DNA EXTRACTION. A modified CTAB extraction procedure as described by Li and Quiros (2001) was used to extract DNA. To identify recombinants from a large segregating population,

96-deep-well plates were used to extract DNA. Leaves were crushed in liquid nitrogen and 400 µL 2× CTAB was added and incubated at 65 °C for 1.5 h. Then, 400 µL of chloroform was added and shaken vigorously; supernatant was taken after centrifuge at 8000 g_n for 15 min and DNA was precipitated with isopropanol in 96-well plates.

PRELIMINARY GENE MAPPING USING SRAP MARKERS. Sequence-related amplified polymorphism (SRAP) was performed as described by Sun et al. (2007). A four-fluorescent dye color set (6-FAM, VIC, NED, and PET) was used for signal detection using a genetic analyzer (ABI 3130xl; Life Technologies, Carlsbad, CA). The GeneScan™–500 LIZ was used for the size standard (Life Technologies). SRAP primers were selected from the primer set used in the construction of an ultra-dense genetic map in *B. napus* (Sun et al., 2007). Bulk segregant analysis with SRAP markers was performed to locate clubroot resistance loci on the chromosomes. SRAP primer combinations previously used in the construction of *B. napus* genetic map (Sun et al., 2007) were selected to determine resistance loci using eight resistance and eight susceptible lines and 48 resistance and 48 susceptible individual plants. These resistance and susceptible lines were selected from BC₁ segregating populations that were developed from chinese cabbage hybrids. After SRAP markers linked to clubroot resistance were identified, these SRAP markers were compared with those on the ultra-dense genetic map to identify the locations of resistance loci.

SCAR AND SSR DETECTION. Sequence characterized amplified regions (SCAR) and simple sequence repeat (SSR) markers were designed based on the whole genome sequence of the *B. rapa* database. For SCAR and SSR marker detection, a M13 primer sequence (CACGACGTTGTAAAACGAC) was added to one of two primers of the SCAR markers. The M13 primer was labeled with one of four colored fluorescent dyes (6-FAM, VIC, NED, and PET) for signal detection using a genetic analyzer (ABI 3130xl; Life Technologies). The GeneScan™–500 LIZ was used for the size standard. The polymerase chain reactions (PCRs) for SCAR marker detection were set up in a 10-µL mixture containing three primers, which included two genetic marker primers and one labeled M13 primer. The PCR program was 94 °C 3 min; 94 °C, 1 min; 60 °C with –Δ0.8 °C each cycle 1 min and 72 °C, 1 min for 6 cycles; 94 °C, 1 min; 55 °C, 1 min; and 72 °C, 1 min for 25 cycles. The PCR cocktail included 0.05 µM of marker primers with M13 tail, 0.15 µM of genetic marker primer, and 0.1 µM of labeled M₁₃ primer, 0.5 mM dNTP, 1 µL 10× PCR buffer, 1.5 mM MgCl₂, and 1 U of *Taq* polymerase. The PCR products were separated in the genetic analyzer. The data were collected and analyzed with the ABI GenScan software (Life Technologies) and further transferred into images for scoring using Genographer software (Benham et al., 1999).

SNP MARKER DETECTION. Single nucleotide polymorphism (SNP) detection as described by Rahman et al. (2008) with minor modifications was used. The primers of genetic markers were used to obtain PCR products covering SNP positions. PCR reactions were performed as 94 °C, 1 min; 55 °C, 1 min; and 72 °C, 1 min for 35 cycles. The PCR was set up in a 10-µL volume contained 0.15 µM of primers, 0.5 mM dNTP, 1 µL 10× PCR buffer, 1.5 mM MgCl₂, and 1 U of *Taq* polymerase. The SNaPshot multiplex kit (Life Technologies) was used as the instructions in the kit. Two-microliter SNaPshot products were added in 8 µL formamide containing GeneScan™ 120 LIZ® Size Standard (Life Technologies). The DNA fragments were

analyzed with an ABI 3100xl genetic analyzer. The data were size-analyzed by the ABI GenScan software. Genotypes were scored manually using peak color verification.

Results

GENE MAPPING IN CHINESE CABBAGE. After screening of over 40 chinese cultivars and DH breeding lines, five commercial hybrid chinese cabbage cultivars were identified to contain clubroot resistance genes. These five chinese cabbage cultivars were crossed to a *B. rapa* oilseed accession, which was susceptible to clubroot. Using these five chinese cabbage cultivars, two F₂, and three BC₁ segregating populations were developed to map clubroot resistance genes. After indoor screening, a 3:1 ratio of resistant individuals to susceptible in two F₂ populations and a 1:1 ratio in all three BC₁ populations were statistically analyzed using χ^2 testing. The results suggested that one Mendelian locus controls the clubroot resistance in all five hybrid chinese cabbage cultivars (Table 1). All five segregation ratios were not significantly different from the expected ratios of 3:1 in the F₂ populations and 1:1 in the BC₁ populations for the segregation of a single dominant gene.

MAPPING THE CLUBROOT RESISTANCE LOCUS TO LINKAGE GROUP N3. The ultra-dense SRAP genetic map in *B. napus* constructed by Sun et al. (2007) was used to map the clubroot resistance gene in hybrid chinese cabbage cultivars. Four bulks were pooled from 16 susceptible plants and four bulks from 16 resistant plants in a BC₁ mapping population. All eight pooled DNA samples were analyzed using SRAP molecular markers. To identify clubroot resistance genes, 96 primer pairs were used in the initial screening and two SRAP markers, FAD+PM04-385 and PM88 + BG73-365 were found to cosegregate with the resistance gene in these 32 tested plants. By comparing these two SRAP markers with those SRAP molecular markers on the ultra-dense genetic recombination map, it was found that these two markers corresponded to the molecular markers on linkage group N3 because these two SRAP markers in *B. rapa* shared the same sizes and were amplified with the same primer combinations as in *B. napus*. After testing these two SRAP markers on the two F₂ and three BC₁ segregating populations in *B. rapa*, it was found that these two SRAP markers were linked to the clubroot resistance loci in all five mapping populations, suggesting that the same locus on chromosome A3 conferred clubroot resistance

in all five hybrid chinese cabbage cultivars although they were developed by various breeding companies in China.

IDENTIFICATION OF CANDIDATE GENES. Because the clubroot resistance locus was mapped on linkage group N3 corresponding to chromosome A3 in chinese cabbage, the bacterial artificial chromosome (BAC) clone sequences on chromosome A3 (Mun et al., 2008) were selected to develop additional molecular markers for the clubroot resistance genes. BAC clones were selected and primers were designed in accordance with the BAC sequences to develop SNP, SSR, and SCAR markers (Table 2). The physical locations of these molecular markers were identified by blasting the whole *B. rapa* genome sequence (Table 2) (*Brassica rapa* Genome Sequencing Project Consortium, 2011).

To fine-map the resistance locus, two flanking markers, M1 and M16, were used to identify 134 recombinants from 8400 individual plants in a large BC₂ population developed from parents BAR and 'Quli Huang'. Using 16 SSR, SCAR, and SNP molecular markers, these recombinants were analyzed to identify 119 recombination events. Phenotypes of 54 recombination BC₂F₁ lines were collected and compared with the genotypes of 16 molecular markers to fine-map the resistance locus (Fig. 1). There was no recombinant between molecular marker M9 and the resistance locus, whereas nine and six recombination events were detected between the resistance locus and molecular markers M8 and M10, respectively. The physical distance between molecular markers M8 and M10 on chromosome A3 is 187 kbp.

Discussion

In this study, a clubroot resistance locus in *B. rapa* was fine-mapped. This clubroot resistance locus showed a typical Mendelian segregation of 3:1 in two F₂ populations and 1:1 in three BC₁ populations. Therefore, it is highly possible that all hybrid chinese cabbage cultivars used in this study share the same resistance locus, although further work is necessary to confirm this assumption through sequencing and plant transformation. The fine-mapped clubroot resistance locus was located to the same region where a clubroot resistance gene has been recently cloned (Ueno et al., 2012). It is possible that the fine-mapped clubroot resistance locus in this study might be the same as the cloned clubroot resistance gene, which was

Table 1. Backcross and selfed mapping populations developed with five hybrid chinese cabbage cultivars and a susceptible rapeseed.

Mapping populations ^z	Clubroot symptom scoring (0 to 3 scale) ^y				Expected ratio ^x	P value ^w
	0	1	2	3		
BAR × Quli Huang	29	1	7	30	1:1	0.39 NS
BAR × Sijihuang	28	0	9	23	1:1	0.61 NS
BAR × Daifeng No. 1	41	0	7	39	1:1	0.59 NS
Nongke No. 1	38	1	2	11	3:1	1 NS
Huxinbaicai	44		2	16	3:1	0.46 NS
BAR	0	0	0	44		

^zBAR is the susceptible parent used to produce first three backcross populations. All five chinese cabbage cultivars (Quli Huang, Sijihuang, Daifeng No. 1, Nongke No. 1, and Huxinbaicai) were F₁ hybrids and the resistance loci in these five cultivars were heterozygous according to phenotypic segregation.

^y0 = no galls on roots, 1 = a few small galls on secondary roots, 2 = a few small galls on both primary and secondary roots, 3 = many big galls on both primary and secondary roots.

^xUsing scorings 0 and 1 as the resistant group and scorings 2 and 3 as the susceptible group, χ^2 testing of a 1:1 ratio of resistant and susceptible groups in backcross populations, and 3:1, in selfing populations was performed.

^wNS = Non-significant according to the probability values in χ^2 testing of resistant and susceptible groups described previously.

Table 2. Fine mapping primers designed with sequences of bacterial artificial chromosome (BAC) clones and their physical positions on chromosome A3 of *Brassica rapa*.

Marker name	Marker type ^z	Primer sequence ^y	BAC name ^x	Primer position on A3 ^w
M1	Del/Ins	M ₁₃ GTTGGTAGTGGTAAATGAGT CGAATCTACTAGATTTCCACT	KBrH097C05	23985172
M2	Del/Ins	M ₁₃ GATAGACAAAGGTGTACAGT TGGTACTTCTATGGAAATGT	KBrH091P11	24001784
M3	Del/Ins	*M ₁₃ CTTCATAGAGCTAAGCATGT ATGTACTGAACCAAGATCCT	KBrB039B11	24059462
M4	Del/Ins	M ₁₃ ATAGATTGACAGTCTGACCT AAATATCCACCACGTACAGT	KBrB039B11	24059505
M5	Del/Ins	M ₁₃ GGACCTTTTGTTCATTGTCT CAATACGTTGATTTAGTGGTGT	KBrB009M08	24129546
M6	Del/Ins	M ₁₃ GCAAATGGATATGCAACTGT ATGTACTGAACCAAGATCCT	KBrH069E01	24059579
M7	SNP	CTTTCTCCGTATGAATCAGGT CTTCTTAGTTGCTGTACGTGT T ₍₁₃₎ ATATGGAACGTCGTGGGTTCT	KBrB039B11	24192903
M8	Del/Ins	M ₁₃ AGAGACAGAAATTGCAACTC GCGCTTAATGAATACTCTCT	KBrH059N21	24262671
M9	Del/Ins	M ₁₃ GTCAACGAACCACTAGACT TAAAGCTGGCTTGTCGATCT	KBrH059N21	24311757
M10	Del/Ins	M ₁₃ AATAGAAGTTATAACGTACGAT TTAATAAGGTTTACTTCAACCT	KBrB045E11	24450356
M11	Del/Ins	M ₁₃ CACAAATTCAATGCAACGCT AACAAGTCAATAGCTAGTAGT	KBrH102F05	24459499
M12	SNP	CTTTGGGATAAAACCACACT TAAGGAAGCCAGCAAATGGT T ₍₁₀₎ CAAGCACAGACTGGTTGAG	KBrH071D21	24482525
M13	Del/Ins	GAATTGTCACAAAAATGCATCT M ₁₃ CTAGTTTGGAAAGGTATTGT	KBrB045E11	24492907
M14	SNP	CTTCTCGAAAAGCTCCTTGT TTGTGGCTGTTGATGAGGAT T ₍₁₀₎ CCAAAATCTACTTCGGCTAAT	KBrB045E11	24540957
M15	SNP	ATTTAGGAGGAAGAATGAACC ATCCAGTCTACTTTAGCGTTT T ₍₁₀₎ GTTTTTAATCAAACGACTAGTTTAGC	KBrH129J18	24615366
M16	Del/Ins	M ₁₃ ATTCCAAGATTTGGACCTCT GATGTGAGTATTCCAATGGT	KBrH129J18	24684421

^zDel/Ins = deletion/insertion; SNP = single nucleotide polymorphism.

^yM₁₃ = 5' CACGACGTTGTAAAACGAC 3', T(n) = SNP detection primers and “n” means the number of nucleotides “T” added to the detection primers to get more nucleotides that are easily separated using a genetic analyzer (ABI 3130xl; Life Technologies, Carlsbad, CA).

^xSequences of the BAC clones on chromosome A3 described by Mun et al. (2008) were used to design primers in previous columns.

^wNumbers represent the physical locations of the first nucleotide of the primers in previous columns in the genome sequence of chromosome A3.

confirmed through gene mapping and mutation analysis (Ueno et al., 2012). Because there are three candidates in this clubroot resistance locus, it is necessary to further sequence the whole region and test each individual member of the gene family through plant transformation so any differences among these three gene members can be confirmed.

Earlier studies of clubroot resistance in turnip indicated that three independent clubroot resistance genes were present in *B. rapa* (Buczacki et al., 1975; Toxopeus and Janssen, 1975), whereas various resistance loci were reported later by different research groups. Five clubroot resistance loci have been reported in *B. rapa* using molecular markers (Piao et al., 2004; Suwabe et al., 2006) and eight loci were further reported by Sakamoto et al. (2008). The clubroot resistance loci, *Crr1* and *Crr2*, of *B. rapa* share colinearity with chromosome 4 in *A. thaliana*, which corresponds to two linkage groups A1 and

A8 of *B. rapa*, respectively (Suwabe et al., 2006). The locus *Crr3* was fine-mapped by Saito et al. (2006) and is located on the same chromosome A3 in *B. rapa*, and analysis of *Crr3* shows that *Crr3* is syntenic with chromosome 3 of *A. thaliana*. Piao et al. (2004) mapped *CRb* in a region that is homologous to chromosome 4 of *A. thaliana*. Moreover, three more clubroot resistance genes, *CRA*, *CRk*, and *CRc*, were reported by Sakamoto et al. (2008).

In this research, a dominant clubroot resistance gene was fine-mapped on A3 of *B. rapa*. A series of markers were developed for this clubroot resistance locus. A large BC₂F₁ population including over 8000 individuals was screened to identify recombinants. The dominant clubroot resistance gene was further fine-mapped to a small chromosome region. Two markers, M8 and M10, were used to narrow the clubroot resistance genes into a 187-kbp physical region where three

Fig. 1. Map position of a clubroot resistance locus on the physical map constructed using the positions of molecular markers M1 to M16 on chromosome A3. Each distance [kilobps (kbp)] represents the physical distance of two markers (e.g., the distance of M1 and M2 is 16.6 kbp). The numbers in the last panel represent distribution of 119 recombination events, which were identified in 134 recombinants. These recombinants were identified using molecular markers M1 and M16 in a BC₂ population developed from a cross between cultivars BAR and 'Qulihuang' (*Brassica rapa*) (population size = 8400 individuals). According to phenotypic data, the clubroot resistance locus (R gene on the physical map) on chromosome A3 cosegregated with molecular marker M9 because no recombination event between M9 and the clubroot resistance locus was identified in the current mapping population.

putative R genes encoding TIR-NBS-LLR proteins can be identified using the 187-kbp sequence to blast against *A. thaliana* genes.

To introgress the clubroot resistance genes from *B. rapa* to *B. napus* canola, three markers were developed to screen interspecific progenies derived from a cross of chinese cabbage carrying the clubroot resistance gene with *B. napus* canola, which is susceptible to clubroot disease. After three generations of crossing and backcrossing, the clubroot resistance gene was successfully introduced into canola through marker-assisted selection (F. Gao, A.H. Hirani, J. Liu, Z. Liu, and G. Li, unpublished data).

Breeding of clubroot-resistant hybrid chinese cabbage cultivars is based on the assumption that clubroot resistance is controlled by a single dominant locus (Yoshikawa, 1993). In this context, a simple backcross is an effective way to introduce

one dominant clubroot resistance gene to chinese cabbage cultivars. However, infection of some clubroot-resistant cultivars has been reported in some production areas in Japan (Kuginuki et al., 1999), indicating that the breakdown of the introduced clubroot resistance trait happened. Development of new cultivars with multiple clubroot resistance genes is therefore desirable. Most present clubroot-resistant chinese cabbage cultivars in Japan and China are F₁ hybrids between clubroot-resistant and susceptible parents (including five hybrid cultivars used in this study). Therefore, they would be heterozygous at the clubroot resistance locus. Some clubroot resistance loci show partial dominance and clubroot resistance homozygotes are more resistant than heterozygotes (Suwabe et al., 2006). In addition, the variation in the clubroot pathogen leads to more virulent populations of *P. brassicae* (Hatakeyama et al., 2004), so a single clubroot resistance gene may not be enough to protect *Brassica* crops from clubroot infection. Breeding of clubroot-resistant cultivars may require the accumulation of more than two clubroot resistance genes in a single cultivar, and pyramiding clubroot resistance genes using marker-assisted selection may be an ideal strategy for this complex breeding procedure. The molecular markers developed in the present study are codominant PCR-based markers. Their polymorphisms are easily detected with various methods. Thus, these molecular markers can be used effectively in marker-assisted pyramiding of clubroot resistance genes in chinese cabbage and other *Brassica* crops.

In the mapping analysis of a simply inherited gene, an exact determination of genotypes of the trait is needed. However, the evaluation of host plant resistance to a fungal disease is generally affected by environmental factors such as temperature, humidity, and soil pH. In the clubroot disease evaluation, both the humidity level in the soil and the temperature can influence the pathogen establishment and further disease development from initial inoculation (Hamilton and Crete, 1978). We observed that one of 40 F₂ plants that were only used to construct the two bulks showed resistance when inoculation was performed on F₂ progeny. However, all progeny derived from this plant showed a susceptible phenotype, indicating that the genotype of an individual BC₂ plant cannot be precisely scored. In general, this problem could be resolved by the use of line testing of BC₂F₁ families to determine the genotypes of BC₂ individuals for producing corresponding BC₂F₁ families.

In conclusion, single dominant clubroot resistance locus was mapped and fine-mapped in five hybrid chinese cabbage cultivars and molecular markers were developed that can be deployed for marker-assisted selection of this locus for canola breeding programs. Furthermore, this locus was fine-mapped to a 187-kbp physical region of chromosome A3 and revealed three R genes. Functional characterization of these three genes through canola transformation needs to be confirmed further.

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