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Understanding the Genetics of Clubroot Resistance for Effectively Controlling this Disease in Brassica Species

Arvind H. Hirani and Genyi Li

Additional information is available at the end of the chapter

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Abstract

Clubroot disease is one of the most serious diseases of Brassica species, which is caused by soil-borne pathogen *Plasmodiophora brassicae* Woronin. Clubroot disease has a long history on vegetable crops belonging to the Brassica species; most recently, this disease is also invading rapeseed/canola crop around the globe. The clubroot disease causes significant yield and quality losses in highly infected fields. Clubroot pathogens invade into the host plant roots and infect root tissues with the formation of abnormal clubs, named as galls, which results in incompetent plant roots to intake water and nutrients and eventually dead plants. As it is a soil-borne disease and accomplishes its disease cycle in two different phases and both phases are highly efficient to damage root system as well as to release more inoculum, there are many challenges to control this disease through chemical and other cultural practices. In general, clubroot disease can be effectively managed by developing resistant cultivars. In this chapter, various resistance sources of clubroot disease in different Brassica species have been discussed with potential applications in canola/rapeseed breeding programs worldwide. Importance of gene mapping and molecular marker development efforts by different research studies for clubroot in *B. rapa*, *B. oleracea*, and *B. napus* has been stressed. Transcriptomic and metabolomic changes occurring during host–pathogen interactions are also covered in this chapter, which would enhance our understanding and utilization of clubroot resistance in Brassica species.

Keywords: brassica species, clubroot resistance, molecular marker development, marker-assisted selection

1. Introduction

The crops in the *Brassicaceae* family are the most economically and nutritionally important for human consumption after cereals [1]. Based on utilities, Brassica species are broadly categorized into oilseed, vegetable, and sources of condiments. In the Brassica crops, canola/rapeseed is the second largest vegetable oil crop after soybean in the world [2]. Globally, rapeseed/canola has been cultivated in about 36.4 M ha with total production approximately 72.5 M tonnes [2]. Among the Brassica species, *B. napus*, *B. rapa*, *B. juncea*, and *B. carinata* provide about 15% of edible vegetable oil supplies around the world [3]. In addition, Brassica species such as *B. oleracea*, *B. rapa*, and *B. napus* supply nutritionally rich green leafy, stem and root vegetables for human daily diets. Brassica species also possess rich genetic diversity with respect to both speciation and the ample morphotypes [4], which designate important species to be investigated for genetic evaluation of plant kingdom. The Brassica crops, however, have significant impact by biotic stresses including diseases and pests, which challenge production and productivity of these crops.

Clubroot is one of the most threatening disease affecting global production and productivity of cruciferous crops including canola/rapeseed and Brassica vegetables. Clubroot disease is caused by the soil-borne obligate biotroph pathogen *P. brassicae* in Brassica crops. Cultivation of different Brassica oilseed and vegetable crops fulfills the host range requirement of the clubroot pathogen, which leads to wide spreading of the pathogen throughout the world. Since the emergence of the clubroot pathogen in vegetable crops of Brassica species, management of the disease has been a big challenge due to the obligate biotroph nature of the pathogen.

2. Impact of clubroot disease in Brassica species

The clubroot disease is not a new one in Brassica crops, it has been historically considered as the most important disease [5]. The origin of the clubroot disease is unknown, but it appears as ancient as its host. Earlier literatures reported the existence of clubroot disease in the 13th century in Spain, and later in 17th century, clubroot disease was also observed in England and subsequently it spread in Scotland, France, Germany, Poland, and other European countries. In Russia, clubroot was first reported in Brassica vegetable crops in 1872 [6]. In Japan, the disease was first recorded in 1890s and now it is one of the major constrains in Chinese cabbage and other Brassica vegetable production [7]. Similarly, this disease was first reported in Australia in the early 1890s [8]. Most of the earlier clubroot disease infections were reported on Brassica vegetable crops.

Mustard/rapeseed crops have similar cultivation history like other Brassica vegetables in different parts of the world; however, there was no evidence of clubroot disease in rapeseed/ mustard crop in earlier time. Before three decades, about 2.5% canola/rapeseed crops were reported with clubroot disease in 18 countries [9, 10]. Since then, canola/rapeseed cultivation expanded significantly due to health benefit properties of its oil. This suggests that clubroot

disease is relatively new in canola/rapeseed compared to other Brassica vegetable crops in which this disease is known from as early as 13th century.

In Canada, canola/rapeseed is the second most important crop next to the wheat and it is mainly cultivated in the Prairie Provinces. Canola is economically the most important crop for the Canadian agriculture, food, and animal industries. The crop supplies nutritionally healthy edible oil to the food industries [11] along with nutritionally balance seed meal to animal industries. Annually, over 8 M ha canola/rapeseed crop has been grown with greater than 15.6 MT productions in Canada [12]. Canola crop contributes more than $15 billion each year to the Canadian economy [13]. In Canada, clubroot disease has been a problem on Brassica vegetables in producing areas including Ontario, Quebec, British Columbia, and the Atlantic Provinces. Clubroot has been periodically reported in few cases on Brassica vegetables in Alberta and Manitoba over the past 80 years [14]. This situation, however, entirely changed with the discovery of about 12 infected canola fields in Alberta in 2003. Annual survey carried out in Alberta, Saskatchewan, and Manitoba have revealed that clubroot is a much more widespread and serious disease in Canadian canola because canola is one of the major crops in the Prairie Provinces. In 2011, clubroot disease has been confirmed in over 800 fields distributed in most part of the Alberta [15], and from two fields in Saskatchewan [16]. Clubroot disease also reported in the North Dakota state in a few canola fields having patches of >80% plant mortality [17].

Clubroot disease has caused different degree of yield losses in canola/rapeseed fields depending on pressure of the disease and nature of genetic inheritance (susceptible/moderately resistance/ resistance) of canola cultivars planted. Clubroot can cause up to 100% yield loss in heavily infected fields when susceptible canola cultivars are planted [18]. Similarly, about 90% yield loss and 5–6% reduction in oil content was reported in clubroot-infected canola field in Quebec [19]. In a previous publication, Dixon [20] has extensively reviewed clubroot infection in three major Brassica species, *B. oleracea*, *B. rapa*, and *B. napus* based on the survey data [10], and suggested that greater than 10% fields were infected in Australia, Canada, Czechoslovakia, Finland, Germany, Ireland, Netherlands, New Zealand, Norway, Poland, Scotland, United States, and Wales in the early 1980s.

In Asian countries, clubroot disease is widespread in the Brassica species cultivating regions in India, China, Nepal, Bangladesh, Pakistan, Indonesia, and Bhutan. In India, North Eastern part has become widespread due to frequent cultivation of cauliflower and yellow sarson, which are susceptible to clubroot. Similarly, China, Bangladesh, and Nepal are high-risk regions for the clubroot disease, especially for Brassica vegetables, mustard, and rapeseed production.

3. Disease cycle and symptoms

The pathogen *P. brassicae* Wornonin is an obligate biotrophic protist belonging to the class phytomyxea. The pathogen can infect primary and secondary roots at the early stage of plant growth and development that causes significant yield and quality losses. The life cycle of *P.*

brassicae consists of two phases; in a primary phase, under favorable conditions, resting spores germinate and produce primary zoospores that penetrate in root hairs and mass production of secondary zoospores occurs in the root hairs. The resting spores are about 3 μm in size and subspherical to spherical in shape and the surface of each resting spore is covered with spines [21]. Mass of primary zoospores is released from each resting spore, spindle-shaped or pyriform, 2.8–5.9 μm long, and biflagellate. When the zoospores come in contact with the surface of a root hair, it penetrates in the cell wall and it is also called root hair infection. Secondary phase of life cycle occurs in the root cortex as a result secondary plasmodia and gall formation occurs as a result restriction in water and nutrient uptake by plants (Figure 1) [21, 22]. The life cycle study of *P. brassicae* in *A. thaliana* reported uninucleate and binucleate myxamoeboid structure production within host cytoplasm that caused cell wall burst and production of secondary plasmodia [23]. During pathogen infection, secondary plasmodia proliferate in roots and plant hormone, especially auxin and cytokinin, biosynthesis altered in the root tissues that causes gall formation (Figure 2) [21]. Infected plants become stunted, yellowish in color, and eventually wilt, which causes severe reduction in yield and quality of crops [24]. Mature secondary plasmodia subsequently develop into resting spores that can survive for 20 years or more [25]. Clubroot disease pressure can significantly increase in those fields where crop rotation frequently includes canola/rapeseed or other Brassica crops. Acidic soil with high soil moisture is the most favorable condition for resting spore germination and subsequent secondary infection.
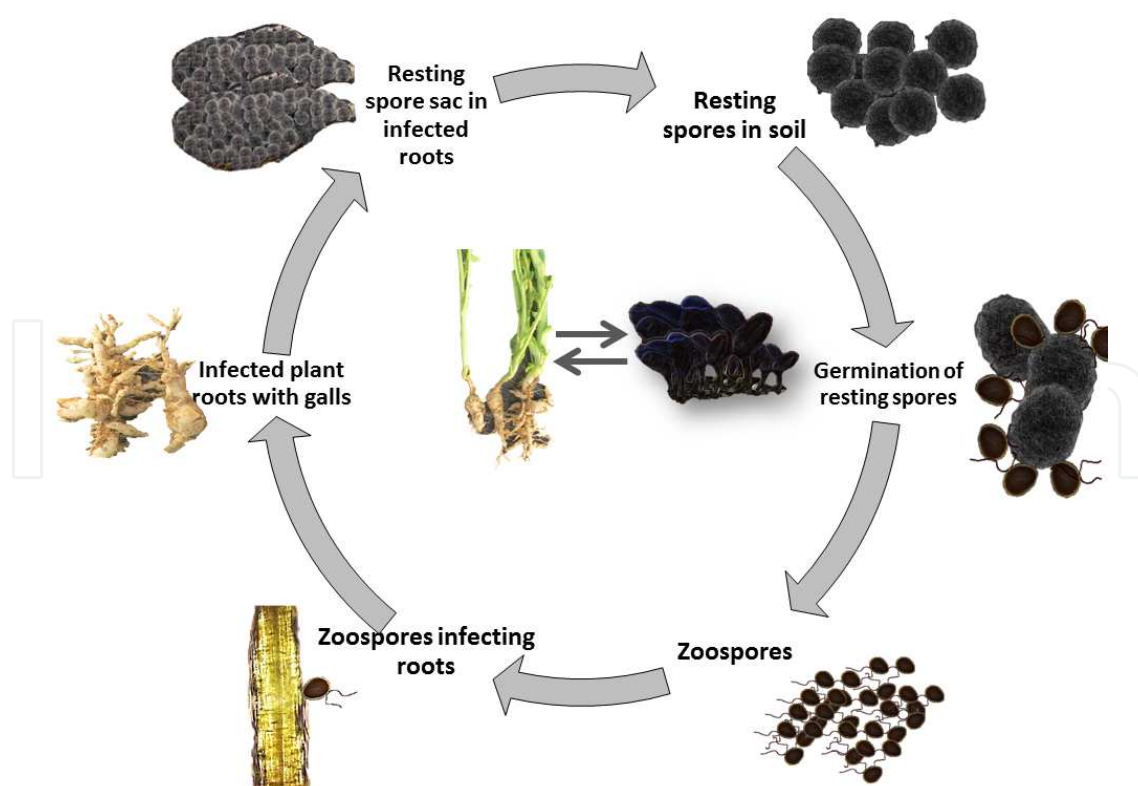
Figure 1. Life cycle of *P. brassicae* and club like gall formation on the roots of Brassica host plant.

Figure 2. Clubroot disease symptoms in highly infected fields. (a) Clubroot-resistant breeding line of *B. rapa* showed no clubroot symptoms on roots, (b) clubroot disease symptoms with large galls on primary and secondary roots in turnip rape line of *B. rapa* under field conditions, (c) clubroot galls on roots of broccoli in *B. oleracea*, and (d) highly infected field of Chinese cabbage in Henan province of China.

4. Biology of *P. brassicae*

The genus *Plasmodiophora* is a monophyletic group with uncertain systematic affinities. The species belonging to this genus possess unique features such as cruciform nuclear division, parasitism, obligate nature, biflagella, heterocont zoospores, and environmentally resistance and long-living resting spores [26]. In this genus, the economically significant member is *P. brassicae*, which hosts Brassica species to cause clubroot disease. The pathogen shows a wide biological range and its populations usually consist of a mixture of different pathotypes [27–30]. Soil environmental factors such as physical, chemical, and biological properties of soil may differentially influence the survival of some physiological races of the pathogen [21, 31]. In European, field isolates of *P. brassicae* display great variation and show a tendency to overcome different resistance sources from either *B. rapa* or *B. oleracea*.

To enhance our understanding of the pathogenicity factors of *P. brassicae* causing clubroot disease on different Brassica hosts, several molecular techniques and tools are employed to determine *P. brassicae* genome size, structure, and number of possible functional genes in the whole genome. Several studies reported use of pulse-field gel electrophoresis (PFGE) to determine the karyotypes for *P. brassicae*. Ito et al. [32] used sheroplasts and differentiated 13 chromosomal bands in the range of 1.9 Mb to 750 kb. Bryan et al. [33] used isolated plasmodia and differentiated six chromosomal bands in the range of 1.7 Mb to 680 kb. Similarly, Graf et al. [34] distinguished 16 chromosomal bands in the range of 2.2 Mb to 680 kb. Based on these studies, it is estimated that the *P. brassicae* total genome size can be 18–20.3 Mb [35].

On the other hand, several molecular marker techniques were employed to investigate virulent pattern of the *P. brassicae* population derived from single-spore isolate or field isolates [36–38]; however, the number of distinguishing patterns were very low and that could not correlate with virulence patterns. In continuous efforts, two RAPD markers [39] and one SCAR marker [40] were identified, which correlate to isolates of pathotype 1. Yet there are no sets of molecular markers that can distinguish other pathotypes from field isolates which make clubroot-resistance breeding intriguing.

5. Host–pathogen interactions

a.   During resting spore germination

In soil environment, host–pathogen interactions begin at the early seedling stage when host plant root exudates are present, which induces germination of resting spores [41] and releasing of primary zoospores. The role of root exudates as stimulants for resting spore germination was examined and confirmed in different research studies [42–44]. In contrast, substantial studies by Kowalski and Bochow [45] reported that the stimulant effect for germination is not confined to the specific host of *P. brassicae*. This finding was supported by the evidence of root exudates from Brassica host (broccoli) and non-Brassica host (ryegrass), both stimulated spore germination [46]. Studies also reported that some specific stimulants such as caffeic acid, coumalic acid, corilagin, and others could stimulate resting spore germination in Chinese cabbage [47, 48]. All these studies suggest that the Brassica species have unique root characteristics which permit pathogen invasion and subsequent infection for the disease development. Resting spore germination is observed stimulated by root exudates in other species, but zoospores could not establish primary infection.

b.   During disease development and gall formation

Earlier studies reported that regulation of phytohormones plays an importance role in the formation of massive galls on roots. Rapid increase in both cytokinin and auxin biosynthesis was observed during secondary infection and gall formation in the infected roots of *B. rapa* [49–51]. Brassica species contain high aliphatic, indole, and aromatic glucosinolates may play a vital role in disease development and gall formation because conversion of indole-3-methyl glucosinolate to indole-3-acetonitrile is thought to be the main pathway of auxin synthesis in

infected root tissues [52, 53]. Studies suggested that induction in nitrilase activity which cleavages indole-3-acetonitrole to indole-3-acetic acid occurred in infected roots [54]. Elevated cytokinin biosynthesis was also observed in secondary plasmodia during gall formation [51, 55]. Pedras et al. [56] reported production of 45 different metabolites in *B. napus* infected by *P. brassicae*, which suggested that canola roots under biotic stress produce a complex blend of phytoalexins and other antimicrobial metabolites as defensive mechanisms. However, limited information about metabolomic interaction between host and pathogen is available during gall formation in both susceptible and complete clubroot resistance disease reactions.

6. Identification of clubroot resistance in Brassica species and their relatives

Brassica species are the major sources that are used to identify clubroot resistance. In the *Brassica* genus, three diploid species are the natural progenitors of three amphidiploid species, which is the famous triangle of U, explaining the evolutionary relationship of Brassica species. The close evolutionary relationship of Brassica species suggests that it is relatively easy to transfer clubroot resistance from species to species through interspecific hybridization and gene introgression. Extensive searching for the clubroot resistance has been performed in Brassica species, especially *B. rapa*, *B. oleracea*, and *B. napus*, and the European turnips in *B. rapa* are found to contain dominant resistance and those clubroot resistance sources have been widely used *in B. rapa* and *B. napus* breeding.

In *B. rapa*, there are various types of vegetables such as Chinese cabbage, Shanghai Pak-choy, and turnip. Clubroot disease causes heavy yield losses in Chinese cabbage production in Eastern Asian countries, especially in Japan, South Korea, and China. Fortunately, European turnip contains dominant clubroot resistance which is commonly used in Chinese cabbage hybrid cultivar development through crosses of Chinese cabbage and resistant European turnips. The clubroot resistance in European turnips has been extensively tested and genetically analyzed under field conditions or using artificial inoculation under controlled environmental conditions [57]. Before 1960, breeders in the Netherlands developed various clubroot-resistant turnip cultivars which were used to control the most serious disease in fodder turnip production, and also those clubroot-resistant turnip cultivars were used to differentiate pathogen and study clubroot infection under different field conditions [57–59]. For example, the European Clubroot Differential (ECD) set has been selected [59] and are currently used by other researchers.

B. oleracea vegetables such as cabbage, broccoli, and cauliflower are tested to identify clubroot resistance. As the clubroot resistance in *B. oleracea* was analyzed, the results in genetic analyses showed that susceptibility was dominant over resistance, and recessive genes were inferred to explain the inheritance of clubroot resistance in diallel analysis [60]. In another diallel analysis of F_1 kale populations, it was also found that additive effects are inferred based on the assessment of broad sense inheritability [61]. In addition, there are several other investigations on the clubroot resistance in *B. oleracea*; and in most cases, recessive inheritance of clubroot

resistance was identified. For example, 71 accessions of cabbage, broccoli, and curly kale were tested and most of them showed some levels of resistance to clubroot, while all the F_1 populations of these resistant and susceptible *B. oleracea* accessions were susceptible [62, 63]. Further analysis indicated that multiple loci are involved in the clubroot resistance in *B. oleracea*, but it was not easy to determine how many loci control clubroot resistance in the analysis of F_1, F_2, and backcross populations of *B. oleracea* [64]. Moreover, 44 landraces of Portuguese coles (*B. oleracea*) were tested to identify clubroot resistance and three accessions showed high levels of clubroot resistance [65].

In *B. napus*, rutabaga cultivars are identified to contain dominant clubroot resistance. In one report, the clubroot resistance in rutabaga was suggested to be controlled by one dominant resistance gene [66]. To investigate the diversity of clubroot pathogen (*P. brassicae*), the Williams differential set was suggested; [67] and in this set, there are two rutabaga accessions that show clubroot resistance in several reports [66, 68, 69]. Vigier et al. [70] tested 31 cultivars and breeding lines of spring canola under controlled environmental conditions and found that several Swedish accessions showed clubroot resistance, but the resistance was not recovered in the subsequent progenies. In another report, the clubroot resistance from rutabaga was transferred into cabbage through interspecific hybridization and results indicated that all the F_1 hybrids were resistant to clubroot disease [71].

Radish (*Raphanus sativus*) is a Brassica relative and there are several reports that focus on the identification and transfer of clubroot resistance to Brassica species. Rowe [72] tested 68 radish cultivars and breeding lines collected from several countries and found that all Japanese and most Dutch radish cultivars were completely resistant to clubroot. Akaba et al. [73] used *B. napus*–radish chromosome additional lines to analyze clubroot resistance and found that one chromosome additional line, the c-type, showed a high level of clubroot resistance. More recently, quantitative resistance loci (QTL) mapping for clubroot resistance in radish has been performed and one major gene on one linkage group was found to control the high level of clubroot resistance in radish [74].

As discussed earlier, European turnips contain dominant clubroot resistance genes which makes gene mapping easier than in *B. oleracea* varieties. To control clubroot disease, Chinese cabbage hybrid cultivars were developed by introducing clubroot resistance from European turnips into Chinese cabbage in Japan [75]; and currently, clubroot-resistant Chinese cabbage cultivars containing turnip clubroot resistance genes are being used in Japan, South Korea, and China.

7. Genetic mapping of clubroot resistance

All the first generation of molecular markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) are used to map clubroot resistance in various Brassica species [76–83]. Landry et al. [80] used RFLP markers detected two QTLs in *B. oleracea*. Figdore et al. [79] used RFLP markers and associated several linkage groups to clubroot resistance in *B. oleracea*. Grandclément and Thomas [82] used RAPD markers and

analyzed QTL for clubroot resistance in broccoli and cauliflower, and identified some RAPD markers significantly linked to clubroot resistance. Moreover, Voorrips et al. [83] used RFLP and AFLP markers, detected two genes for clubroot resistance in *B. oleracea* doubled haploid lines. Rocherieux et al. [84] performed QTL analysis in *B. oleracea* and detected isolate-specific and broad spectrum QTLs, suggesting that the clubroot resistance in *B. oleracea* is genetically complicated and molecular marker-assisted selection might be not so effective. More recently, Nagaoka et al. [24] performed QTL mapping using a DH line population between resistant cabbage and susceptible broccoli and detected two major QTL for clubroot resistance on chromosomes O2 and O5 and minor QTLs on chromosome O2, O3, and O7. Similar to the conclusion drowned from conventional genetic analysis, clubroot resistance in *B. oleracea* is most likely due to the effects of multiple minor genes and molecular marker-assisted selection might be not as effective as that in Chinese cabbage which contains dominant resistance genes introduced from European turnips.

In Chinese cabbage, major clubroot resistance loci that are introduced from European turnips have been mapped (Table 1). These clubroot resistance loci are named as *Crr1*, *Crr2*, *Crr3*, and *Crr4* and also *CRa*, *CRb*, *CRc*, and *CRk* in a dozen of investigations, suggesting that there might be eight independent loci [81, 85–87]. Two clubroot resistance loci, *Crr1* and *Crr2* were mapped using SSR markers [75, 88] and a third locus, *Crr3* was identified using RAPD markers, which suggested that there are three independent clubroot resistance loci in Chinese cabbage [85, 87]. Using RFLP markers, a genetic map was constructed and a clubroot resistance locus, *CRa*, was mapped on linkage group 3 [77], and SCAR and CAPS markers were used to map another locus, *CRb*, on chromosome R3 [81]. Moreover, three clubroot resistance loci *CRa*, *CRc*, and *CRk* have been added in the list of clubroot resistance through molecular marker-assisted selection [89].

Due to the complex genome structure of *B. napus*, QTL mapping for clubroot resistance is necessary in *B. napus* (Table 1). It is quite common to find the clubroot resistance in *B. napus* that does not segregate as a typical Mendelian trait as in *B. rapa*. Although one major locus was mapped on chromosome N3, two minor QTL on chromosomes N12 and N19 were identified for clubroot resistance in *B. napus* [90]. In another report, [91] a DH line population derived from a cross of clubroot-resistant synthetic *B. napus* and susceptible canola was used to perform QTL mapping for clubroot resistance. The synthetic *B. napus* contains dominant clubroot resistance from European turnip ECD4 and may be medium resistance from *B. oleracea*. They identified a total of nineteen QTLs on chromosomes N02, N03, N08, N13, N15, N16, and N19 for clubroot resistance, and surprisingly, there were four QTLs with LOD values of over 11, of which three were located on chromosome N3 and one on N19 and the proportion of the phenotypic variance explained by each QTL was over 40%. Their data suggested that the major QTLs might come from the C genome of *B. oleracea*, which is contradictory to previous reports where major clubroot resistance genes in *B. napus* come from the A genome of *B. rapa*.

8. Fine mapping and cloning of clubroot resistance genes

As the whole genome sequencing and molecular marker development in Brassica species advances [92], those previously identified clubroot resistance loci in Chinese cabbage have

been fine mapped and some clubroot resistance genes have been eventually cloned (Table 1). The *Crr3* locating on chromosome R3 was first mapped to a small genetic region between 0.35 cM genetic distance using 888 F_2 individual plants [85]. In another report, the clubroot resistance locus *CRa* has been further analyzed to identify the candidate gene [93]. Over 1,600 F_2 individual plants were used to select 80 recombinants using two closely linked molecular markers. Further analysis of those recombinants allowed identifying one open reading frame located on chromosome R3, which belongs to a typical resistance gene family and encodes a TIR-NBS-LRR protein [93]. More recently, there are two other independent reports that focused on fine mapping of clubroot resistance loci on chromosome R3. The *CRb* clubroot resistance locus which was described to be effective to *P. brassicae* isolates No. 14, a very aggressive isolate in Japan, has been fine mapped [94]. Using over 2,000 F_2 individual plants and F_3 progeny testing, 92 F_2 recombinants between two closely linked molecular markers were identified. The analysis of these 92 F_2 recombinants suggested that the *CRb* clubroot resistance locus might be the same as the *CRa* locus and the *CRa* and *CRb* clubroot resistance loci are different from the clubroot resistance locus *Crr3* [94]. Similarly, gene mapping of five Chinese cabbage cultivars was performed and all these hybrid cultivars were found to contain the same clubroot resistance locus on chromosome R3 [95]. They further fine mapped the clubroot resistance locus in Chinese cabbage to a 187 kilo-base pair (kb) chromosomal region using a large segregating population with over 8,000 individual plants. Molecular markers which are closely linked to the mapped clubroot resistance locus have been developed and those molecular markers can be used in marker-assisted selection to breed Chinese cabbage with clubroot resistance.

Characterization of clubroot resistance genes offers opportunities for further understanding clubroot resistance and interactions of resistance genes and pathogens. Hatakeyama et al. [86] cloned one clubroot resistance gene *Crr1a* on chromosome R8 and confirmed the resistance through plant transformation. Some transgenic *B. rapa* plants are resistant while others are susceptible, suggesting that the *Crr1a* gene might not explain the whole clubroot resistance in the original locus. They also found that *Crr1a* and *Crr1b* were tandem repeats in the same locus and both genes encode typical resistance gene proteins with TIR-NBS-LRR structures.

Based on the previous reports and whole genome sequencing data, clubroot resistance loci on chromosome 3 in *B. rapa* also contain multiple genes that encode TIR-NBS-LRR proteins. The complexity of those clubroot resistance loci needs to be investigated further. When a clubroot resistance locus contains multiple genes encoding the similar proteins, it becomes challenging to know how each individual gene plays a role in the clubroot resistance and how they contribute to the differences of alleles from various resistant sources. It is necessary to further dissect those complex clubroot resistance loci and investigate each individual gene to understand the functional properties of those loci. Therefore, gene functional analysis for clubroot resistance is still an important research focus in Brassica species.

9. Understanding the mechanism of clubroot disease resistance

The formation of galls on primary and secondary roots is typically characteristic of clubroot disease. The modification of root structure and decaying of root galls eventually damages plant

root systems so the plants may completely die or dramatically reduce productivity. Arabidopsis is a model plant and relative to the Brassica species, thus it has been successfully used in clubroot research. Malinowski et al. [96] investigated the relationship of cell division, gall formation, and clubroot disease development in Arabidopsis. Using those genes involved in cell division as molecular markers, their data suggested that reducing gall formation by inhibiting cell division would not prevent pathogen from finishing the life cycle while large galls may help pathogens produce more resting spores.

The expression of genes involved in the progression of clubroot disease may change so transcriptome analysis can be used to pinpoint the dynamic changing of gene expression in metabolic pathways for clubroot disease development. Schuller et al. [97] used laser micro-dissection and microarray analysis to check the changes of gene expression and found that the genes involved in the metabolism of plant hormones, especially auxin, cytokinin, and brassinosteriod, and plant defense-related hormones such as jasmonate and ethylene were differentially regulated. In another microarray analysis in Arabidopsis, Jubault et al. [98] observed that the major differences of gene expression in partial resistance interaction and susceptible interaction of the same Arabidopsis accession inoculated with two different clubroot isolates. The results showed that reduced or delayed metabolomic changes by pathogen and early induced classical defense responses were the major scenarios leading to partial clubroot phenotype instead of full susceptibility. More recently, Chu et al. [99] used RNA sequencing technology to identify over 2,000 genes that were expressed differentially in clubroot-resistant and susceptible plants. They found that those genes involved in defense responses such as jasmonic acid, ethylene, callose deposition, and indole glucosinolates were upregulated, and the expression of some genes in the pathway of salicylic acid did not show changes while the genes in the auxin biosynthesis and cell growth and development showed reduced expression in clubroot-resistant plants. By inducing clubroot resistance with an endophytic fungus, *Heteroconium chaetospira*, Lahlali et al. [100] detected the upregulation of genes involved in plant defense interaction such as PR-2 and genes in phenylpropanoid biosynthesis, and in the metabolism of plant hormones such as jasmonic acid, auxin, and ethylene using qPCR. Moreover, Verma et al. [101] performed miRNA analysis using miRNA-based microarray to detect differentially expressed miRNA during clubroot development. They further predicted the targets of those differentially expressed miRNA which belong to transcription factors, plant hormone-related and stress-related genes. In general, the data collected in those reports are quite preliminary and more research are required to know how each individual dominant clubroot resistance gene interacts with some avirulence genes in pathogen and eventually the interaction changes the expression of downstream genes which leads to clubroot resistance.

10. Transferring clubroot resistance through molecular marker-assisted selection in canola

Canola is one of the most important oilseed crops, and clubroot disease becomes a major limiting factor in canola production worldwide [102]. To develop resistant canola cultivars, several resistant sources such as European turnips, Chinese cabbage, and rutabaga cultivars

are available and the resistance in these sources are dominant, which makes it easier to transfer clubroot resistance through interspecific and intraspecific hybridization. Rutabaga cultivars have been identified as clubroot-resistant sources [66, 67, 69]. However, the genetics of clubroot resistance in rutabaga is complicated so it is difficult to develop molecular markers that are closely linked to the dominant clubroot resistance genes.

In Chinese cabbage, the dominant clubroot resistance from European turnips has been successfully used to develop clubroot-resistant Chinese cabbage. Since the gene mapping has been performed extensively in Chinese cabbage, molecular markers closely linked to clubroot resistance loci that are used in gene mapping can be easily selected to transfer clubroot resistance genes in the development of Chinese cabbage cultivars through molecular marker-assisted selection. Since canola, the amphidiploid *B. napus*, has a very complex genome, most of the molecular markers developed in *B. rapa* may not be polymorphic and cannot be directly used in canola. Additional efforts are required to develop molecular markers in canola when the mapped clubroot resistance loci in Chinese cabbage are transferred into canola. Currently, most clubroot resistance genes in European turnips have not been intensively investigated and mapping and cloning of these clubroot resistance genes in European turnips will allow using these genes effectively and efficiently in canola breeding.

Brassica species (R sources)	Populations	LG	QTL/genes	Reference
B. rapa (Chinese cabbage)	BC$_1$	A03	*Rcr1* fine mapped	[103]
B. rapa (Chinese cabbage)	F$_2$	A03	*CRb* fine mapped	[104]
B. rapa (G004 line)	F$_2$	A08	*Crr1a* fine mapped	[86]
B. rapa (Chinese cabbage)		A03	*CRa* fine mapped	[93]
B. oleracea (Anju)	DH	O2, O5,	pb-Bo(Anju)1, pb-Bo(GC)1	[24]
B. napus (synthetic line)	DH	N02, N03, N08, N13, N15, N16 and N19	Nineteen QTL identified on different LGs	[91]
B. oleracea (kale)	F$_{2:3}$	LG1, 2, 5	Nine QTL (Pb-Bo1 to Pb-Bo9) with phenotypic variance 20-88%	[84]
B. rapa (Shinki)	F$_2$	A03	*CRb*	[81]
Brassica oleracea (Bindsachsener)	DH	-	Two QTL (pb-3 and pb-4)	[83]
B. rapa (Chinese cabbage)	F$_2$, BC$_1$	A03	CR gene fined mapped	[95]
B. rapa (turnip line)	F$_2$	A03, A08	Two major QTL (Pb-Br3, Pb-Br8 and	[105]
B. rapa (European turnip)	F$_{2:3}$	A03	*Crr3*	[85, 87]
B. rapa (G004)	F$_2$	A06	*Crr4*	[88, 106]
B. rapa (Chinese cabbage)	F$_2$	A03 and A02	*CRk and CRc*	[107]

Table 1. Clubroot resistance QTL/gene mapped/fine mapped in different Brassica species by different research studies

11. Management of clubroot resistance for effective utilization

The Brassica genomes (A, B, and C genome) are crucially important to provide novel genetic inheritance for economically important traits that can be used for the overall improvement of crop production and quality. For example, single genome of diploid *B. rapa* (A-genome) holds more than 230 R-gene sequences in 16 gene families [108], among which over 8 genetic loci have been identified in different research studies, which have functional properties for clubroot disease resistance. There are possibilities of the existence of more R-genes specific to the clubroot disease resistance and their allelic variations in different genetic pools or wild relative species. Effective utilization of resistance loci and their allelic variations may enhance the durability of resistance against clubroot disease in different Brassica species.

As a long history of clubroot disease revealed relatively high evolutionary patterns of the pathogen, *P. brassicae*. In various cultivating geographical regions of Brassica crops, persistence of *P. brassicae* pathotypes with high levels of pathogenicity poses challenges to breed durable clubroot-resistant cultivars. The breakdown of clubroot-resistant cultivars has become a serious problem in Chinese cabbage and leafy cabbage in China, Korea, and Japan [109, 110]. Effective management of resistance genetic resources in breeding novel cultivars could enhance the performance of resistance loci in different Brassica species for sustainable, more durable, and cost-effective control of the clubroot disease.

12. Summary and prospects of clubroot disease control

Crop plants are always challenged by various biotic and abiotic stresses. In agriculture cropping system, plant protection is being delivered using different approaches such as chemical control, various agronomic practices, biological control, integrated pest management (IPM), and cultivation of resistance cultivars. Among these approaches, resistance cultivars are the most economical, environmentally sustainable solution to control different diseases including clubroot in Brassica species. Previous studies suggested that the inheritance of clubroot resistance is either qualitative or quantitative in Brassica species. Recently mapped clubroot resistance genetic loci and closely linked molecular marker to these loci can be used for marker-assisted selection in clubroot resistance breeding of Brassica species. Extensive use of recently available resistance sources can be combined with molecular tools and new technologies such as gene/QTL mapping, fine mapping, gene cloning, comparative genomics and analysis of transcriptomic profiles through next-generation sequencing, which could enhance our understanding of clubroot resistance mechanism. Novel information can help controlling clubroot disease in an effective way, so yield losses would be reduced and the quality of Brassica crop product would be improved.

Author details

Arvind H. Hirani and Genyi Li[*]

*Address all correspondence to: genyi.li@umanitoba.ca

Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

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