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Genetics of Clubroot Resistance in *Brassica* Species

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Abstract Clubroot disease, caused by the obligate plant pathogen *Plasmodiophora brassicae* Wor., is one of the most economically important diseases affecting *Brassica* crops in the world. The genetic basis of clubroot resistance (CR) has been well studied in three economically important *Brassica* species: *B. rapa*, *B. oleracea*, and *B. napus*. In *B. rapa*, mainly in Chinese cabbage, one minor and seven major CR genes introduced from European fodder turnips have been identified. Mapping of these CR genes localized *Crr1* on R8, *Crr2* on R1, *CRc* on R2, and *Crr4* on R6 linkage groups of Chinese cabbage. Genes *Crr3*, *CRa*, *CRb*, and *CRk* mapped to R3, but at two separate loci, *CRa* and *CRb* are independent of *Crr3* and *CRk*, which are closely linked. Further analysis suggested that *Crr1*, *Crr2*, and *CRb* have similar origins in the ancestral genome as in chromosome 4 of *Arabidopsis thaliana*. Genetic analysis of clubroot resistance genes in *B. oleracea* suggests that they are quantitative traits. Twenty-two quantitative trait loci (QTLs) were mapped in different linkage groups of *B. oleracea*. In *B. napus*, genetic analysis of clubroot resistance was found to be governed by one or two dominant genes, whereas resistance conferred by two recessive genes is reported. The quantitative analysis approach, however, proved that they are polygenic. In total, at least 16 QTLs have been detected on eight chromosomes of *B. napus*, N02, N03, N08, N09, N13, N15, N16, and N19. The chromosomal location of the other six QTLs is not clear. Cloning of any of these QTLs or resistance loci was not, however, possible until recently. Progress in genomics, particularly the techniques of comparative mapping and genome sequencing, supplements cloning and allows improved characterization of CR genes. Further development of DNA markers linked to CR genes will in turn hasten the breeding of clubroot-resistant *Brassica* cultivars.

Keywords *Brassica* crops · *Plasmodiophora brassicae* · Clubroot · Resistance · Genetic analysis · DNA marker · Genetic mapping · QTL · Genomics

Introduction

Clubroot disease, caused by the soilborne, obligate plant pathogen *Plasmodiophora brassicae* Wor. infects all cruciferous vegetable and oil crops, including *Brassica rapa*, *B. oleracea*, *B. napus,* and other *Brassica* species. This disease is one of the most economically important diseases of *Brassica* crops worldwide. The pathogen causes abnormal cell enlargement and uncontrolled cell division of infected roots, thus deforming them with characteristic clubs. As a result, nutrient and water uptake by infected roots is inhibited; the growth of the aerial parts of host plants becomes stunted, the aerial parts become yellowish in color and wilt in direct sunlight; and crop yield and quality are reduced (see both Dixon articles, this issue). *Plasmodiophora brassicae* was first reported in Russia in 1878 by Woronin (see Karling 1968). The disease was widely recorded in 18th century in England and illustrations are found in European herbals. In Korea, the first report of clubroot disease was in 1920, whereas in Japan it was noted as early as the 1890s (Ikegami and others 1981). Now this

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disease is one of the most serious problems in Chinese cabbage production in Korea and elsewhere.

The ability of this pathogen to survive in soil as resting spores for long periods makes it difficult to control by cultural practices or chemical treatments (Voorrips 1995). Thus, breeding of resistant cultivars is a desirable means of minimizing crop losses, especially when they are incorporated into systems of integrated control (see Diederichsen and others, this issue; Faggian and Strelkov, this issue; Donald and Porter, this issue). Sources of resistance have been identified and the genetic basis for resistance were studied in detail in *B. rapa*, *B. oleracea*, *B. napus*, and the model plant *Arabidopsis thaliana.* Clubroot resistance (CR) genes from European fodder turnips (*B. rapa*) have been introduced into Chinese cabbage (Yoshikawa 1981) and a number of resistant F_1 hybrid cultivars were subsequently released. In turnip, clubroot resistance has been found to be controlled by three independent dominant genes, each conferring resistance to different *P. brassicae* pathotypes (Wit 1964; Tjallingii 1965; Toxopeus and Janssen 1975; James and others 1978; Crute and others 1980). Genetic analysis of clubroot resistance has been done for *B. oleracea* and *B. napus* (Chiang and Crête 1970, 1976; Chiang and others 1977; Crute and others 1980, 1983; Gustafsson and Fält 1986; Diederichsen and others 2006). Separate studies reported clubroot resistance as either qualitative (Yoshikawa 1981; Kuginuki and others 1997; Piao and others 2002) or quantitative (Suwabe and others 2003, 2006), depending on the genotypes of Chinese cabbage studied. Resistance has been reported as quantitative involving recessive (Crute and others 1983; Voorrips and Visser 1993) or dominant alleles (Laurens and Thomas 1993; Grandclément and others 1996) in several *B. oleracea* hosts. Breeding programs aimed at producing CR cultivars attempted to introduce CR genes from clubroot-resistant turnip into Chinese cabbage, from clubroot-resistant turnip with or without resistant sources from *B. oleracea* into oil seed rape, and from clubroot-resistant kale and other crop types into subspecies of *B. oleracea*. Several clubroot-resistant cultivars have been released for each of the *Brassica* species with varying degrees of success. They are challenged by the expansion of physiological races of *P. brassicae* following classical "boom and bust" epidemiology. To increase the durability of clubroot-resistant cultivars, the combination of the different CR genes into a single line will be an indispensable means for breeding cultivars with resistance to a broader spectrum of physiological races.

Recent advances in molecular biology especially the increasing genomic information such as development of molecular markers, comparative mapping between related species, and transcriptomic analysis, offer promises for identifying, localizing, cloning, and functionally characterizing genes of interest. This review discusses details of the genetics of clubroot resistance in *Brassica* species, mapping and tagging of CR genes using a number of molecular markers, comparative mapping and the current status of the application of recently available genomics tools for identifying genes involved in CR.

Genetic Basis of Clubroot Resistance in *Brassica* Species

In the three major cultivated *Brassica* species, *B. rapa*, *B. napus*, and *B. oleracea*, clubroot disease has become a cause of serious concern because of rising crop failures (see Dixon, this issue). To breed clubroot-resistant cultivars of these species, a number of *Brassica* germplasms were evaluated. This allowed sources of resistance to be identified in *B. oleracea, B. napus*, and *B. rapa*. Furthermore, aspects of the *Brassica-P. brassicae* relationship related to host resistance were studied. Inheritance of resistance to clubroot varied between these species.

Brassica rapa

The breeding of clubroot-resistant cultivars of Chinese cabbage (*B. rapa* ssp. *pekenensis*) has been impeded because most cultivars are highly susceptible to clubroot disease (Yoshikawa 1981; Cho and others 2002). The identification of resistant sources in European fodder turnips (*B. rapa* ssp. *rapifera*) (Karling 1968; Buczacki and others 1975; Crute and others 1983; Crisp and others 1989) allowed the transfer of CR genes from European fodder turnip to Chinese cabbage (Yoshikawa 1981). The introgression of CR genes from European fodder turnip cultivars, including Gelria R, Siloga, Debra, and Milan White, has provided and broadened the genetic diversity of Chinese cabbage. The European Clubroot Differential (ECD) hosts 01–04 represent the spectrum of resistance to physiologic pathotypes of *P. brassicae* (Toxopeus and Janssen 1975; Toxopeus and others 1986; Buczacki and others 1975). These have been used as resistant sources in breeding clubroot-resistant cultivars either in Chinese cabbage or in *B. napus*.

In these studies, at least three independent dominant genes, which conferred differential (race-specific or vertical) resistance to particular pathotypes of *P. brassicae*, were suggested as present in turnip genotypes (Wit and Van de Weg 1964; Tjallingii 1965; Toxopeus and Janssen 1975; James and others 1978; Crute and others 1980). Yoshikawa (1993) demonstrated that clubroot resistance of European fodder turnips, including cv Siloga, was controlled mainly by a major gene and a few minor genes. James and others (1978) identified three independent

dominant genes that conferred resistance in three *B. rapa* genotypes to race 6 of *P. brassicae.* Crute and others (1980) also demonstrated that three genes controlled resistance in turnip. This suggests that clubroot resistance in *B. rapa* is controlled by several genes independently. Because clubroot-resistant cultivars of Chinese cabbage were released by introducing CR genes from clubroot-resistant European fodder turnip, it is believed that clubroot-resistant cultivars of Chinese cabbage have the potential for introducing one to several genes from turnip. Recently published data obtained by marker trait analysis confirmed that at least eight CR loci are present in *B. rapa* (Suwabe and others 2003, 2006; Hirai and others 2004; Piao and others 2004; Sakamoto and others 2008).

Brassica oleracea

Clubroot disease causes severe losses to both quality and quantity of *B. oleracea* crops, including cauliflower, broccoli, kale, and others. Breeding of resistant cultivars could be the most effective method for controlling clubroot disease; consequently, different research groups identified various sources of clubroot resistance through the screening of germplasm (Crute and others 1980; Dixon and Robinson 1986; Dixon and others 1986; Dixon 1988; Crisp and others 1989; Dias and others 1993; Voorrips and Kanne 1997a; Manzanares-Dauleux and others 2000b; Carlsson and others 2004). In contrast to *B. rapa,* completely resistant accessions have only rarely been identified in *B. oleracea*, although a large number of accessions were screened. Crisp and others (1989) evaluated about 1000 *B. oleracea* accessions. Resistant sources were confirmed in some kales, including the curly marrow stem and 1000-head types, and in cabbage, including Böhmerwaldkohl, Bindsachsener cabbage, Badger Shipper, and Ladoszkaya cabbage. Some open pollinated Brussels sprouts, including cvs Cambridge, Continuity, Rubine, and Catskill, and forms of southern European cabbage, cauliflower, and broccoli exhibited lower levels of susceptibility. Among the kale, cabbage, and winter cauliflower accessions evaluated, only some kale accessions, mainly the leafy and short leafy kale groups, exhibited high levels of resistance to clubroot, whereas all cabbage and cauliflower accessions were susceptible (Manzanares-Dauleux and others 2000b). Some of these resistant sources are widely used in breeding programs for *B. oleracea*.

The detailed genetics of clubroot resistance were studied in *B. oleracea* using either diallel crossing methods or segregating population. Most of these studies concluded that inheritance of this trait in *B. oleracea* is polygenic (Table 1). Yoshikawa (1993), who worked with progenies of crosses between cv Böhmerwaldkohl and a susceptible cabbage, demonstrated that at least one to four genes controlled this trait. Voorrips and Visser (1993) found that the inheritance of resistance to clubroot was recessive based on the genetic analysis of the 11 F_1 populations derived from crosses between resistant sources, including three accessions of Böhmerwaldkohl, cv Badger Shipper, two lines derived from 'Larson 8353 T', cv Resistant Detroitand, and four accessions of curly kale and susceptible cabbage, respectively. The inheritance of resistance to clubroot in kale was studied by Laurens and Thomas (1993). They concluded that the inheritance of resistance is controlled by many dominant alleles with a predominance of additive genetic effects with incomplete dominance. Based on qualitative and quantitative analyses of the F_1, F_2, and backcross progenies of four crosses derived from four sources of resistance and one common susceptible doubled haploid line, Voorrips and Kanne (1997b) suggested the different interpretation of inheritance of clubroot resistance. Of the four resistances studied, one was controlled by two complementary genes. Chiang and Crête (1970) agreed that resistance was controlled by two loci.

Brassica napus

Early work on clubroot resistance in swedes (*B. napus*) suggested that genotypes, including cvs Wilhelmsburger

Table 1 Summary of classical genetic analysis of clubroot resistance in *Brassica oleracea*

Resistant source	Population	Test (pathotype)	Genes involved	Reference
Böhmerwaldkohl	F_2, BC	Glasshouse (field isolate)	Four genes	Yoshikawa 1993
Böhmerwaldkohl	F_2, BC	Glasshouse (field isolate)	Additive and recessive	Crute and Pink 1989
Kale accessions	Diallel	Glasshouse (field isolate)	Many dominant genes	Laurens and Thomas 1993
Böhmerwaldkohl, Badger Shipper, Larson 8353 T, Resistant Detroitand, Curly kale	F_1	Glasshouse (field isolate)	Recessive	Voorrips and Visser 1993
Cabbage	F_1, F_2, BC_1	Glasshouse (field isolate, single spore isolate)	Two complementary genes	Voorrips and Kanne 1997a, b
Resistant Detroit			Two genes	
Bindsachsener			Two genes	
Curly kale			More than two genes	

and Studsgaard Bangholm, exhibited resistance under certain environmental conditions (Karling 1968; Dixon and others 1972). These sources are used to breed clubroot-resistant cultivars of *B. napus*.

Genetic studies of resistance to clubroot in swedes have used different sources of resistance, including Wilhelmsburger, clubroot-resistant rape, Wye swede group, York swede, and Ditmar S2 swede (Lammerink 1967; Johnston 1970; Ayers 1972; Ayers and Lelacheur 1972). These authors agreed that the inheritance of resistance in *B. napus* is controlled by one or two single independent dominant genes. At least one of these genes is shared by several sources. Two recessive genes with additive effect were also identified in Danish Giant swede. Gustafsson and Fält (1986) suggested that four resistance genes are present in the *B. napus* ECD hosts. Based on genetic analysis using partial or genome-wide marker surveys in several *B. napus*, over 20 quantitative trait loci for CR were identified (Manzanares-Dauleux and others 2000a; Werner and others 2008). Because *B. napus* is a natural amphidiploid between *B. oleracea* and *B. rapa*, a question arises as to where these resistance genes are located and their origins. It is suggested that genes for resistance in *B. napus* are located in the A genome from *B. rapa* (Chiang and others 1977).

Genetic Mapping of Clubroot Resistance Genes in *Brassica* Species

So far, a number of DNA markers linked to CR loci in the three cultivated species *B. rapa*, *B. oleracea*, and *B. napus* have been developed by several research groups. The details of CR QTLs and their number and location in specific chromosomes in each species are discussed here.

Brassica rapa

Genetic analysis and genetic mapping of CR genes are well studied in *B. rapa*. All eight possible CR genes present in *B. rapa* have been identified through QTL mapping by research groups using a range of resistant sources and marker systems (Table 2). Kuginuki and others (1997) identified three random amplified polymorphic DNA (RAPD) markers linked to a CR locus using turnip cv Siloga as a source of resistance and 36 double-haploid (DH) lines derived from five F_1 plants. These markers were converted into sequence tagged site (STS) markers (Kikuchi and others 1999). In subsequent mapping studies, this locus was designated as *Crr1* (Suwabe and others 2003). Matsumoto and others (1998) mapped the CR gene *CRa* to an interval of about 34 cM between two restriction fragment length polymorphism (RFLP) markers on linkage group 3 using ECD02 as a resistance source. Suwabe and others (2003) identified *Crr1* and *Crr2*, two loci that originated from cv Siloga, using F_2 populations and simple sequence repeat (SSR) marker systems. They concluded that these two loci are complementary for clubroot resistance. Inoculation of test plants with mild and virulent isolates of *P. brassicae* showed that plants with a homozygous resistance locus had greater resistance to clubroot than those having a heterozygous resistance locus. In addition to *Crr1* and *Crr2*, *Crr4,* a weak QTL, was detected using a similar F_2 population (Suwabe and others 2006). Hirai and others (2004) identified and mapped a novel

Table 2 Genetic mapping of clubroot resistance loci in *Brassica rapa*

Resistant source	Population	Isolate[a]	Locus	Types of DNA marker	Flanking markers	Chromosome with interval (cM)	Reference
ECD02	F_2	Race 2	*CRa*	RFLP STS	HC352b, HC181 HC352b-SCAR	R3 (15 cM)	Matsumoto and others (1998) Sakamoto and others (2008)
Gelria R	F_2	Race 4	*CRb*	SCAR	TCR05, TCR09	R3 (3. 0 cM)	Piao and others (2004)
Siloga	F_2	Ano-o1 (race 2), Wakayama-01	*Crr1* *Crr2* *Crr4*	SSR SSR RFLP	BRMS-297, BRMS-088 BRMS-100, BRMS-096 BN288D, WE24-1	R8 (1.6 cM) R1 (2.2 cM) R6 (2.7 cM)	Suwabe and others (2006)
Milan White	F_3	Ano-01	*Crr3*	STS	OPC11-1S, OPC11-2S	R3 (10 cM)	Hirai and others (2004)
Debra	F_2 F_2	M85 (race 2), K04	*CRk* *CRc*	STS AFLP	HC688, OPC11-2S E14M3-02, E15M4-006	R3 (9.1 cM) R2 (5.1 cM)	Sakamoto and others (2008)

[a] Isolate characterization based on the Williams' classification (Williams 1966)

locus named *Crr3*, which originated from the clubroot-resistant turnip cv Milan White. Another CR locus, *CRb*, derived from turnip cv Gelria R, was mapped in an interval of 3 cM using sequence characterized amplified region (SCAR) markers converted from amplified fragment length polymorphism (AFLP) markers (Piao and others 2004). Homology analysis of SCAR markers with that of *Arabidopsis* sequence database identified that the region containing the *CRb* locus is homologous to the central part of the *A. thaliana* chromosome 4 (Fig. 1). These are also homologs with the region of the *B. rapa* chromosome containing *Crr1* and *Crr2* CR QTL. *Brassica rapa* BAC clones containing these SCAR markers have been identified and located on a specific chromosome in clubroot resistance cv CR Shinki through fluorescent in *situ* hybridization (FISH) (Fig. 2). Colocalization of these BAC clones was found in *B. rapa* chromosome 1. Cloning of the *CRb* gene is in progress. This locus is independent of *Crr1*, *Crr2*, *Crr3*, and *Crr4* (Saito and others 2006). Recently, Sakamoto and others (2008) identified *CRk* and *CRc* loci derived from turnip cv Debra using QTL analysis of two F_2 populations and two isolates of *P. brassicae*. In total, eight CR loci were mapped and allocated to five different chromosomes of *B. rapa*. The genes *Crr1*, *Crr2*, *Crr3*, *Crr4*, and *CRc* are mapped to R8, R1, R3, R6, and R2,

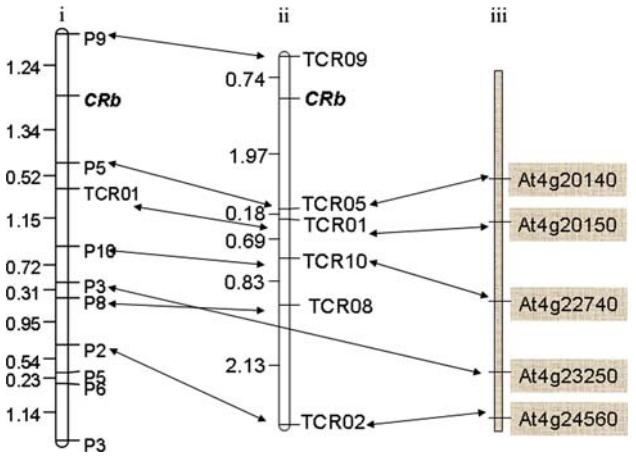

Fig. 1 Identification of the homologous region of *B. rapa* linkage group containing *CRb* locus with that of *Arabidopsis thaliana* chromosome 4 (Piao and others 2004). (i) AFLP linkage group containing *CRb* locus, (ii) Linkage group converted to SCARs and CAPS markers, (iii) Homologous *Arabidopsis thaliana* chromsome 4

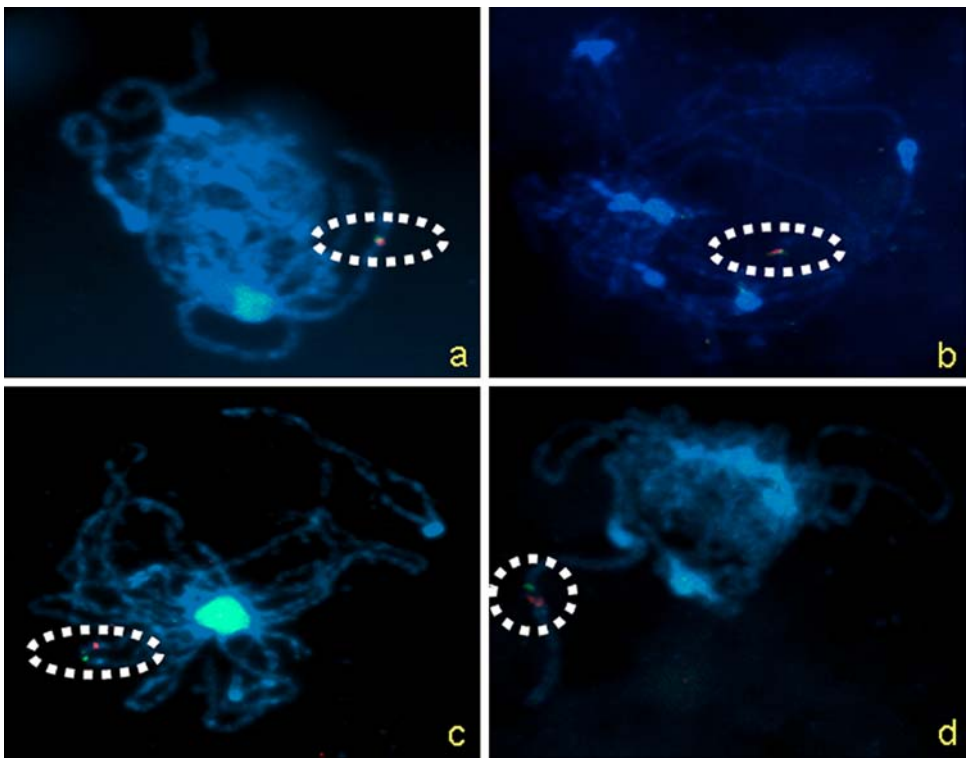

Fig. 2 FISH localization of BAC clones containing SCAR markers (TCR05 and TCR09) linked to clubroot resistance locus in *B. rapa* CR Shinki genome. **a** BAC clones KBrH060E03 (*green*) and KBrH097J16 (*red*) from same contig harboring TCR05 marker showing localization in the same position in *B. rapa* CR Shinki chromsome 1. **b** BAC clones KBrH083J12 (*green*) and KBrH103M15 (*red*) from same contig harboring TCR09 marker showing localization in same region. **c** BAC clones KBrH97J16 (*green*, harboring TCR05) and KBrH88B11 (*red*, harboring TCR09) from different contig linked to TCR05 and TCR09 markers showing localization in different regions of the same chromosome. **d** BAC clones KBrH115F22 (*green*, harboring TCR05) and KBrH144K19 (*red*, harboring TCR09) from different contig linked to TCR05 and TCR09 markers showing localization in different regions of the same chromosome

respectively, of the internationally agreed upon *B. rapa* reference genetic map. It is noteworthy that *CRa*, *CRb*, and *CRk* with *Crr3* are mapped on the same linkage group of R3, but they are not located in the same chromosome region, except for *CRk* and *Crr3*.

Brassica oleracea

So far, a number of DNA markers linked to CR loci in *B. oleracea* have been developed by several research groups. In independent mapping experiments two to nine QTLs have been identified (Table 3) (Landry and others 1992; Figdore and others 1993; Grandclément and Thomas 1996; Voorrips and others 1997; Moriguchi and others 1999; Rocherieus and others 2004; Nomura and others 2005). Two QTLs, *CR2a* and *CR2b*, showing resistance to race 2 of *P. brassicae* and contributing 58 and 15%, respectively, of the phenotypic variation, were identified using swede cv Wilhelmsburger as a resistance source (Landry and others 1992). Three QTLs showing resistance to race 7 were identified using broccoli (Figdore and others 1993). Voorrips and others (1997) identified two QTLs, *pb-3* and *pb-4*, and a minor QTL contained in cv Bindsachsener using a multiple QTL mapping approach which analyzed the fresh weight of galls. The additive effects of two major loci were responsible for 68% of the difference between the parents and for 60% of the genetic variance among the means of DH lines. One QTL for resistance to clubroot disease was identified on linkage group 3 using resistant kale (K269) under conditions of natural infection (Moriguchi and others 1999). Based on the quantitative analysis of an F_3 family using controlled environments and four single-spore isolates and one field isolate from four *P. brassicae* isolates, Rocherieus and others (2004) found two to five QTLs depending on the pathotype used. Of the nine QTLs fully identified, *Pb-Bo1* is common to all isolates and accounts for 20.7–80.7% of the phenotypic variation, whereas the rest were specific to one, two, or three isolates. Nomura and others (2005) identified three QTLs, QTL1, QTL3, and QTL9, using a kale line (K-269) as the resistant parent, which was similar to that used by Moriguchi and others (1999). Therefore, one of these QTLs is probably similar to the one QTL that is located at the end of LG3. The SCAR markers converted from RAPD and RFLP markers linked to these QTLs were evaluated for F_2 and F_3 plants. It was observed that F_2 individuals with three QTLs expressed very high clubroot resistance, similar to that of the kale parent, whereas the F_2 and F_3 plants carrying a single QTL expressed only intermediate resistance (Nomura and others 2005).

In summary, at least 22 QTLs have been found in *B. oleracea* so far. The discovery of several CR QTLs indicates that the clubroot resistance in *B. oleracea* is controlled at several QTLs, further confirming the complex genetic basis of clubroot resistance in *B. oleracea*. Because these mapping studies used different CR sources and isolates, although the primer and marker sequence are disclosed, the comparison of these QTLs is impossible. To understand the genetics and genomics of CR loci in *B. oleracea* in detail, development of common PCR-based markers is required. In addition, comparative studies suggested that the genomes of *Brassica* species have evolved from a common ancestor. It is worthwhile investigating whether CR genes in *Brassica* species might have common origins and how their mechanisms of evolution are maintained. Comparative studies of CR genes or their linked markers should provide new insights into these processes.

Brassica napus

Currently, at least 22 QTLs involving clubroot resistance have been proposed in *B. napus* (Table 4). Manzanares-Dauleux and others (2000a) mapped the major gene *Pb-Bn1*, which confers resistance to two single-spore isolates (SSI) of *P. brassicae*, onto linkage group DY4. Based on the quantitative resistance expressed against each SSI, they also found at least two additive QTLs on chromosomes DY4 and DY15, respectively. In addition, epistatic interactions between nine regions with or without additive effects have been located. The total phenotypic variation accounted for by additive and epistatic QTLs ranged from 62% to 81.4% depending on the *P. brassicae* isolate used. Analyses of double-haploid (DH) populations, in which CR genes are derived from ECD04, identified one major gene and two recessive genes (Diederichsen and others 2006). A resynthesized *B. napus* was developed by crossing cv Böhmerwaldkohl (*B. oleracea*) and ECD-04 (*B. rapa*). From this, the CR DH line "263/11" was obtained (Diederichsen and others 1996). Werner and others (2008) analyzed the DH population derived from a cross of 263/11 and the susceptible cv Express using seven *P. brassicae* isolates. Nineteen QTLs expressing resistance to seven isolates were detected on eight chromosomes: N02, N03, N08, N09, N13, N15, N16, and N19. All QTLs were found to be race-specific. The total phenotypic variation accounted for ranged from 20.8% to 79.6% depending on the pathogen isolate used. Among the 19 QTLs detected, four were closely linked to each other on chromosome N03, three were linked also to chromosome N08. In *B. rapa*, genes *CRa*, *CRb*, *CRk*, and *Crr3* are located on chromosome R3, which corresponds with N03 in *B. napus*. Genes *CRk* and *Crr3* are located in the similar region of *PbBn-k-2*, *PbBn-1-1*, *PbBn-01:60-1* on N03. However, genes *CRa* and *CRb* are independent from them. *PbBn-01.07-2*, *PbBn-l-2*, and *PbBn-a-1* are linked to BRMS088 on chromosome N08 in *B. napus*, which is also linked with

Table 3 Genetic mapping of clubroot resistance loci in *Brassica oleracea*

Resistant source	Population	Isolate	Loci	Types of DNA marker	Flanking markers	LG with interval (cM)	Reference
Wilhelmsburger	F_2	Race[b] 2	*CR2a*	RFLP	2NF11, 2ND3	LG6 (22)	Landry and others (1992)
			CR2b		3NE4a, 3ND3	LG1 (12)	
Broccoli (OSU[a] CR-7)	F_2	Race 7	3	RFLP	14a	LG1	Figdore and others (1993)
					48	LG4	
					177b	LG9	
Kale (C10)	F_2	16/31//31[c]	At least 2	RAPD	OPL6-780, OPB11-740, OPA18-14900, OPA4-700, OPE20-1250, OPA1-1880, OPA16-510	–	Grandclément and others (1996)
Bindsachsener	DH	Field isolate	*pb-3*	RFLP, AFLP	4NE11a	LG3	Voorrips and others (1997)
			pb-4		2NA8c	LG1	
Kale (K269)	F_2	Race 1 and 3	1	RAPD, RFLP	WG6A1, WG1G5	LG3 (2.6)	Moriguchi and others (1999)
Kale (K269)	F_2	Three field isolates	QTL1	SCAR	SCA02a 2	LG1	Nomura and others (2005)
			QTL3		SCB50b,SCB74c	LG3	
			QTL9		SOPT15a, SCA25	LG9	
Kale (C10)	F_2	P1 (Ms6, eH), P2 (K92), P4 (K92-16), P7 (Pb137-522)[d]	*Pb-Bo1*	RAPD, RFLP, ACGM	Ae05.8800, T2	LG1 (18.4)	Rocherieus and others (2004)
			Pb-Bo2		PBB38a, r10.1200	LG2 (19.3)	
			Pb-Bo3		ae15.100, RGA8.450	LG3 (13.9)	
			Pb-Bo4		ELI3.983, aa9.983,	LG4 (3.1)	
			Pb-Bo5a		PBB7b, ae05.135	LG5 (32.5)	
			Pb-Bo5b		ELI3.115, a18.1400	LG5 (12.0)	
			Pb-Bo8		c01.980, t16.500	LG8 (10.2)	
			Pb-Bo9a		aj16.570, W22B.400	LG9 (24.1)	
			Pb-Bo9b		a04.1900, ae03.136	LG9 (1.4)	

[a] OSU = Oregon State University

[b] Isolate characterization based on Williams' classification (Williams 1966)

[c] Isolate characterization based on ECD set (Buczacki and others 1975)

[d] Isolate characterization based on Somé and others (1996)

Table 4 Genetic mapping of clubroot resistance loci in *Brassica napus*

Resistant source	Population	Isolate	Loci	Types of DNA marker	Flanking markers	LG with interval (cM)	Reference
Winter dwarf line	DH	P4 (K92-16)[a]	*Pb-Bn1*	RAPD	OPG03.960, OPV09.2100	DY4 (8.3)	Manzanares-Dauleux and others (2000a)
		P7 (Pb137-522)	two QTL		OPC18.1250, OPD20.760	DY2 (11.8)	
					OPQ01.930, OPG13.950	DY15 (3. 7)	
ECD04	DH	1	One dominant and at least two recessive	SSR	HMR337, HMR388, HMR307	MS06 (4.0)	Diederichsen and others (2006)
Böhmer waldkohl, ECD-04	DH	01:60[b]	*PbBn-01:60-1*	AFLP	158_241, 128_330	N03 (13.6)	Werner and others (2008)
		01:07	*PbBn-01:60-2*		19_329, 155_200	N13 (8.4)	
		e4x04a	*PbBn-01:60-3*		84_174, 1_103	N19 (6.4)	
			PbBn-01:60-4		107_370, 166_215	N19 (4.3)	
			PbBn-01:07-1		107_106, 128_330	N03 (7.7)	
			PbBn-01:07-2		84_258, 79_168	N08 (2.0)	
			PbBn-01:07-3		160_186, 160_193	N13 (2.0)	
			PbBn-e4x04-1		163_448, 159_296	N19 (4.4)	
			PbBn-a-1		84_258, 153_370	N08 (5.4)	
Böhmer waldkohl, ECD-04	DH	l	*PbBn-l-1*	AFLP	107_106, 128_330	N03 (7.7)	Werner and others (2008)
		k	*PbBn-l-2*		84_258, 79_168	N08 (2.0)	
		Korporal	*PbBn-k-1*		154_103, 152_373	N02 (7.5)	
			PbBn-k-2		107_106, 128_330	N03 (7.7)	
			PbBn-k-3		79_75, HMR1382a	N15 (4.8)	
			PbBn-Korp-1		6_450, 165_156	N09 (3.1)	
			PbBn-Korp-2		107_366, 146_363	N09 (9.0)	
			PbBn-Korp-3		158_154, 148_158	N09 (1.3)	
			PbBn-Korp-4		19_155, 166_146	N16 (12.6)	
			PbBn-Korp-5			N16 (31.7)	

[a] Isolate characterization based on Somé and others (1996)

[b] Isolate characterization based on ECD set (Buczacki and others 1975)

Crr1 on R8 in *B. rapa*. Further studies of these loci, using common markers, might explain whether they are identical. The QTLs located on N03 and N19 contribute strong effects and confer broad-spectrum resistance.

Genomic Approaches for the Identification of Clubroot Resistance Genes

Although many studies have identified CR genes through genetic analysis and QTL mapping, none of these loci have yet been cloned in any of the *Brassica* species. Because *P. brassicae* infects all *Brassica* species and the model plant *Arabidopsis thaliana*, it is possible to apply currently available genomics tools and techniques for the cloning and characterization of CR genes though the following approaches.

Comparative Mapping and Identification of Candidate Genes

The whole-genome sequence information available for *Arabidopsis* has been used for comparative genome analysis of *Brassica* species. Comparative mapping between *Brassica* and *Arabidopsis* has revealed a conservation of gene order in small chromosomal blocks despite inversions and large-scale deletions (Cavell and others 1998; O'Neill and Bancroft 2000). This information has been used to align linkage groups containing CR genes in *B. rapa* with those of *Arabidopsis* chromosomes. Genes *Crr1*, *Crr2*, and *CRb* are in synteny with the central region of chromosome 4 of *A. thaliana* (Suwabe and others 2006 and Fig. 1). Recent studies by Jubault and others (2008) of *A. thaliana* have identified one QTL for clubroot resistance in this region, suggesting the presence of functionally active candidate gene(s). This region of an *Arabidopsis* chromosome has clusters of the CR genes such as leucine-rich repeats (LRRs) and nucleotide-binding sites (NBSs). Examples are *RPP* for resistance to *Peronospora parasitica* (downy mildew), *RPS* for a resistance to *Pseudomonas syringae* (bacterial blight), and *ACD*, which accelerates cell death in response to pathogen infection (Suwabe and others 2006). It has been suggested that CR genes may be members of these clusters of resistance genes. The genes *Crr1*, *Crr2*, and *CRb* however, are distributed on three different chromosomes in *B. rapa*, R8, R1, and R3, respectively (Saito and others 2006; Suwabe and others 2006). Based on this observation, it has been suggested that the evolution of CR genes occurs by one of two routes: First, clubroot resistance was originally controlled by a single major gene in the ancestral genome, which later differentiated and diverged as functionally duplicate genes during the course of evolution in the *Brassica* genome (Suwabe and others 2006). A second route might be that the resistance genes for clubroot were originally clustered in that region in the ancestral genome which was later distributed into different genomic regions following chromosomal rearrangement in *Brassica*. Current *Brassica* species, which diverged 17–18 million years ago from *Arabidopsis*, are evolutionarily believed to be derived from whole-genome triplication and rearrangement of one ancestral genome (Lagercrantz 1998; O'Neill and Bancroft 2000; Yang and others 2006). This hypothesis explains why these CR genes are dispersed and located on different chromosomes in *B. rapa*. Saito and others (2006) suggested that the genomic region around *Crr3* exhibits homology to the top of the long arm of *Arabidopsis* chromosome 3, and possibly also to *CRk* (Sakamoto and others 2008). They concluded that *Crr3* has a different origin from that of *Crr1*, *Crr2*, and *CRb*. Gene *CRk* is independent of the CR genes *Crr1*, *Crr2*, *CRa*, and *CRb* but has a similar QTL region with *Crr3* (Sakamoto and others 2008). Another novel CR locus, *CRc*, which is independent of all other CR loci, is located on chromosome R2 (Sakamoto and others 2008). Fuchs and Sacristán (1996) mapped a CR locus (*RPB1*) in *Arabidopsis* chromosome 1. Their mapping for partial clubroot resistance identified two QTLs in chromosome 5 in F_2 and four QTLs in the RIL population, one each in chromosome 1 and 4 and two in the chromosome 5 of *A. thaliana* (Jubault and others 2008). The CR QTL region in chromosome 5 colocalized with that containing several resistance gene clusters. These resistance genes could be candidates for clubroot disease resistance. Fine mapping and detailed analysis of the levels of expression possessed by these genes would help to identify specific ones capable of conferring clubroot resistance.

Transcriptomic/Expression Analysis

To date, the complete genome sequence of only one species of Brassicaceae family, *Arabidopsis thaliana*, is available. This is why progress is slow in detailed studies of genomics of clubroot resistance in *Brassica* even though considerable progress has been made in genetic analysis and QTL mapping of CR genes. This problem would be overcome, however, with the availability of the *Brassica rapa* genome sequence in the near future. Furthermore, it has been reported that gene sequence identity of *Brassica* and *Arabidopsis* varies from 75% to 90% (Quiros and others 2001). Therefore, sequence information from the model species will greatly enhance cloning and characterization of CR genes in other *Brassica* species because the partial sequences of several accessions of *A. thaliana*, showing degrees of response to the pathogen *P. brassicae* collected worldwide, are available now (Fuchs and Sacristán 1996; Siemens and others 2002; Nordborg and others

2005; Alix and others 2007). Only a few studies in transcriptomic/expression levels have been completed, however, with respect to disease development. These include investigation of involvement of several metabolic pathways in disease pathogenesis, such as hormonal regulation by auxins (Grsic and others 1999, Neuhaus and others 2000), cytokinins (Devos and Prinsen 2006; Siemens and others 2006), and trehalose synthesis (Brodman and others 2002). Grsic and others (1999) observed that de novo indole-3-acetic acid (IAA) biosynthesis plays a role in symptom development during later disease stages; they suggested that jasmonic acid, which increased during club development, may be involved in the upregulation of enzymes involved in IAA synthesis. Neuhaus and others (2000) further supported this hypothesis by showing a delay in clubroot development after transforming *A. thaliana* with an antisense construct of *Nitrilase* 1 and 2, enzymes required for auxin biosynthesis. Siemens and others (2006) investigated host gene expression during clubroot development in *A. thaliana* using an ATH1 microarray at two points in time: an early initial stage of infection and a later stage at which 60 % of the host root cells were colonized. More than 1000 genes were observed as being associated with the growth and cell cycle and sugar phosphate metabolism, and defense genes that were differentially expressed between infected versus control plants were observed (see Ludwig-Müller and others, this issue; Siemens and others, this issue). Upregulation of auxin biosynthesis genes such as nitrilases and members of the *GH3* family and downregulation of cytokinin homeostasis were observed. It was further observed that lines overexpressing cytokinin oxidase/hydrolases were resistant to clubroot thereby strongly suggesting cytokinin is a key factor in clubroot development.

Of the 312 genes identified as defense and disease-resistant-related genes, only 5 and 7% were upregulated at the first and the second points in time. This functional analysis of gene expression in clubroot-resistant and susceptible lines provided initial preliminary information. To strengthen further the understanding of genes and genetic networks involved in the mechanisms of clubroot disease and host interaction, more detail analyses at the transcriptomic level are needed.

Conclusions and Perspectives

Plant protection delivered via the seed using CR sources provides one of the most straighforward and environmentally sustainable solutions to clubroot control. The available data, taken together, suggest that the inheritance of clubroot resistance is either qualitative or quantitative in *Brassica* species. In total, over 55 CR loci have been detected based on mapping studies. These CR loci are expressed as both major and minor effects and show race-specific resistance. Among them, 16 are distributed on chromosomes 1, 2, 3, 6, 8, and 9 of the A genome and 8 on chromosomes 3, 5, 6, and 9 of the C genome. The remaining 31 CR loci are located on the C genome but their precise position is unclear. Some of these CR genes might be identical or resident as clusters in one region of the genome. More studies are needed to clarify the exact positions on the chromosomes and to understand the relationships between and among these loci by using common markers. Combined use of recently available molecular tools and techniques such as fine mapping, comparative genomics, and detailed analyses of transcriptomes from candidate genes in the CR QTL region or at the whole-genome level would be an appropriate approach from which to clone and characterize more CR genes more rapidly. This would help in development of pathogen race-specific functional candidate gene markers which could be used at CR loci and would permit pyramiding CR genes into one inbred line of *Brassica* species by marker-assisted selection (MAS). Cloning of these genes would give insights that could unveil the origins of CR genes and elucidate the mechanisms of host–pathogen interactions. Moreover, the availability of single-spore isolates of *P. brassicae* would allow differentiation of the CR genes.

So far, the nomenclature of CR genes has not been standardized in *Brassica* species. In *B. rapa*, there are *Crr* series (*Crr1* to *Crr4*) and *CR* series (*CRa*, *CRb*, *CRc*, and *CRk*). In *B. oleracea*, there are *CR2a* and *CR2b*, *Pb* series, *Pb-Bo* series, and others. In *B. napus*, there are *Pb-Bn* and *PbBn* series. An international standardization of CR loci nomenclature is needed to avoid confusion between and within the different *Brassica* species and to differentiate the CR loci. We recommend the use of *PbBr*, *PbBo*, and *PbBn* for clubroot resistance loci found in *B. rapa*, *B. oleracea*, and *B. napus*, respectively (*Pb* is derived from *P. brassicae*, *Br* from *B. rapa*, *Bo* from *B. oleracea*, and *Bn* from *B. napus*, respectively).

At least three disease-resistance loci of the ancestral genome, that is, *Crr1*, *Crr2*, and *CRb* (together considered as one locus), *Crr3* and *CRkI* (together another locus), and *RPB1* (third locus), and three unclear loci, *CRa*, *CRc*, and *Crr4*, might be involved in the evolution of CR genes in cruciferous plants. According to the hypothesis of hexa-polyploidization applied to *Brassica* species (Lagercrantz and Lydiate 1996; Lagercrantz 1998), there is the possibility that they originated from one common gene. The cloning and comparison of these genes at the nucleotide level would help to unravel stages in the evolution of CR genes. The cloning of the *CRb* and *Crr3* genes is underway (Piao and others 2006; Saito and others 2006).

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