J Plant Growth Regul (2009) 28:265–281
DOI 10.1007/s00344-009-9100-0

Status and Perspectives of Clubroot Resistance Breeding in Crucifer Crops

Elke Diederichsen · Martin Frauen · Enrico G. A. Linders · Katsunori Hatakeyama · Masashi Hirai

Received: 5 March 2009 / Accepted: 10 March 2009 / Published online: 8 May 2009

Abstract Clubroot disease is a major threat to crops belonging to the Brassicaceae. It is controlled most effectively by the use of resistant cultivars. *Plasmodiophora brassicae*, the causal agent, shows a wide variation for pathogenicity, which can be displayed by using differential host sets. Except for *Brassica juncea* and *B. carinata*, resistant accessions can be found in all major crops. Most resistance sources are race-specific, despite some race-independent resistant accessions which can be found in *B. oleracea*. European field isolates from *P. brassicae* display great variation and show a tendency to overcome different resistance sources from either *B. rapa* or *B. oleracea*. At present, resistance genes from stubble turnips (*B. rapa*) are most effective and most widely used in resistance breeding of different Brassica crops. Resistance to *P. brassicae* from turnips was introduced into Chinese cabbage, oilseed rape, and *B. oleracea*. Although most turnips carry more than one resistance gene, the resistant cultivars from other crops received primarily a single, dominant resistance gene having a race-specific effect. Populations of *P. brassicae* that are compatible against most of the used resistance sources have been present in certain European areas for many decades. Such pathogen populations appeared in Japanese Chinese cabbage crops only a few years after the introduction of resistant cultivars. As the spread of virulent *P. brassicae* pathotypes seems to be slow, resistant cultivars are still a very effective method of control in many cropping areas. Mapping studies have revealed the presence of several clubroot-resistance genes in the Brassica A and C genomes; most of these genes are showing race specificity. Only in *B. oleracea* was one broad-spectrum locus detected. Two loci from the A genome confer resistance to more than one pathotype, but not to all isolates. Progress made in the determination of resistance loci should be used to formulate and introduce an improved differential set. Future efforts for breeding *P. brassicae* resistance will focus on durability by broadening the genetic basis of clubroot resistance by using either natural variation or transgenic strategies.

Keywords Clubroot · *Plasmodiophora brassicae* · Resistance · Genetics · Race differentiation · Virulence · Brassica · Raphanus

E. Diederichsen (✉)
Freie Universität Berlin, Institut für Biologie – Angewandte Genetik, Albrecht-Thaer-Weg 6, 14195 Berlin, Germany
e-mail: elked@zedat.fu-berlin.de

M. Frauen
Norddeutsche Pflanzenzucht H.G. Lembke KG, Hohenlieth, 24363 Holtsee, Germany

E. G. A. Linders
Syngenta Seeds B.V., Westeinde 62, 1601 BK Enkhuizen, The Netherlands

K. Hatakeyama
Kyoto Prefectural University, 74 Oji, Kitainayazuma, Seika, Kyoto 619-0244, Japan

M. Hirai
National Institute of Vegetable and Tea Sciences, 360 Kusawa, Ano, Tsu, Mie 514-2392, Japan

Introduction

Clubroot disease of the Brassicaceae has been a major threat to crops belonging to this family for centuries. Brassica oilseeds, cabbage and kale, Chinese cabbage, radishes, and mustards can be infected by *Plasmodiophora brassicae* Wor., the causal agent of clubroot. The disease is

estimated to be present in approximately 10% of all areas where host plants are cultivated (Crête 1981), including Indonesia and Norway, and it is a constant challenge for Brassica breeders. One of the earliest records of clubroot in Europe dates back to the 4th century in Italy (Crisp and others 1989). In Spain the disease was described in the 15th–16th centuries, in England in the 18th century, and by the 19th century clubroot had spread to most European countries, the U.S., and Japan (Colhoun 1958; Yoshikawa and Buczacki 1978). Clubroot is now considered one of the most serious diseases affecting *B. oleracea* crops. Its prevalence ranges from 3.5 to 10% of the land used for these crops worldwide (Crête 1975, 1981), up to 20% in Canada (Landry and others 1992), and up to 70% in Victoria, Australia (Donald and others 2006b), with yield losses conservatively estimated at over 15% in the U.K. (Dixon 1999) (see Dixon, this issue). The disease limits the spread of Brassica crops into those areas where soil and climatic conditions support infection. Although clubroot has been largely a disease of vegetable crops in the last century, it is now of increasing importance for broadacre crops such as oilseed rape (*B. napus* L.) or turnip rape (*B. rapa* L.) as a result of the substantial increase in Brassica oilseed acreage.

Plant resistance is still the most powerful tool for combating clubroot disease. Agricultural control means such as liming are more circumscribed in their effectiveness or applicability. Reducing the frequency of Brassica crops in a rotation will significantly delay the initial incidence of clubroot, although this is not a preferred option of growers faced with an increasing demand for plant oils. In addition, crop rotation alone will not eliminate the disease at infested sites in the short to medium term. Breeding for clubroot resistance (CR) focuses today on Chinese cabbage (*B. rapa* spp. *pekinensis*) in Japan and Korea, oilseed rape in Germany and Sweden, and several *B. oleracea* vegetables for which most CR breeding activities are centered in the Netherlands and in France. Recently released resistant cultivars belong to three Brassica species: *B. napus*, *B. oleracea*, and *B. rapa*. They all possess, however, CR gene(s) that originate from Dutch or Belgian stubble turnips (*B. rapa* spp. *rapifera*). The CR from this source is regarded as race-specific. Therefore, a major aspect in this review is the durability of CR.

Durable resistance was defined by Johnson (1984) as "resistance that remains effective during its prolonged and widespread use in an environment favorable to the disease." To identify a durable resistance source requires that a CR cultivar is in extended use for a long period. Such a cultivar will not exist when considering CR. Therefore resistance geneticists need other ways to estimate the durability of resistance sources, such as significant interactions between host and pathogen genotypes according to a factorial analysis of variance. Vanderplank (1978) suggested determining the ranking orders of host genotypes with as many different pathotypes as possible. Constant ranking orders would form the major criterion for identifying a race-independent resistance type, which could be expected to be durable, with the most resistant genotype being the candidate for a durable resistance source. For clubroot disease, such data have been generated mainly during the 1980s using the European Clubroot Differential (ECD) set (Buczacki and others 1975; Crute and others 1983; Toxopeus and others 1986; see below). The differential screening of *P. brassicae*'s pathogenicity was based, however, on poorly defined resistance genotypes. During the past decades, CR genetics have focused more on the identification and mapping of resistance genes. This knowledge should be used now to analyze the specific effects of defined genes for CR.

Biology of *P. brassicae* and Its Interaction with Hosts

Based on molecular data, taxonomists have reclassified *P. brassicae* into the new taxon Rhizaria, which is a diverse group of amoeboid protists with two different flagellae (Nikolaev and others 2004). *Plasmodiophora brassicae* is neither a fungus nor a slime mold, as previously considered. The general biology of *P. brassicae* was recently reviewed in Dixon (2006). It is widely accepted that the life cycle of *P. brassicae* consists of two infection phases (Karling 1968; Ingram and Tommerup 1972). *Plasmodiophora brassicae*'s lifestyle is strictly biotrophic; only stages that do not grow occur outside the host. After germination of resting spores in the soil, primary zoospores are released; these become attached to the surface of cortical root cells, in particular to the root hairs, and inject their protoplast into the cytoplasm. Inside the host cell cytoplasm the *P. brassicae* amoeba undergoes endomitotic divisions forming a multinucleate primary plasmodium. The primary plasmodium cleaves into sporangiosori (Karling 1968), which give rise to the secondary zoospores. The secondary zoospores are either released into the rhizosphere and then infect the root cortex from outside or they infect neighboring cortical cells directly from inside the root hair (Aist 1977). This second pathway is now considered to have greater relevance. It has been assumed that secondary zoospores fuse and form binucleate zoospores Cook and Schwartz 1930; Tommerup and Ingramm 1971; Ingram and Tommerup 1972). Meiotic divisions in the multinucleate secondary plasmodium, just after karyogamy and before sporangiogenesis, have been described by a number of authors (Tommerup and Ingramm 1971; Ingram and Tommerup 1972; Garber and Aist 1979a, b; Buczacki and Moxham 1980; Braselton 1981). So far these

observations remain the only evidence for sexual stages in *P. brassicae*'s life cycle. Fähling and others (2004) tried to detect recombination between single-spore isolates using RFLP markers but could recover only the parental marker patterns again.

The secondary infection phase, which is initiated by the secondary zoospores, takes place in the cortex. Secondary plasmodia spread inside the root and induce the unorganized growth of the affected tissue, which causes the disintegration of the concentric root structure and of a major part of the vascular system (Mithen and Magrath 1992; Morgner 1995; Kobelt and others 2000). The typical root tumors (clubs) develop and malnutrition and wilting of the plant initiates the final phase. Heavily infected plants will die before flowering. Due to the decay of the root tumors, the resting spores, which are formed after cleavage of the secondary plasmodium, will be released into the soil. The life cycle, the impact of the environment, and the biochemistry of the host–pathogen interaction are considered in other chapters (Kageyama and Asano, this issue; Dixon, this issue; Ludwig-Müller and others, this issue) of this special issue.

Characterization of the Resistance Response

Although the primary phase is rather unspecific and has been observed in resistant (Macfarlane 1955; Voorrips 1992; Morgner 1995) and even in nonhost plants (Bawden 1949; Webb 1949), the secondary infection phase is the period when susceptibility or resistance becomes apparent in all the host species studied so far (Dekhuizen 1979; Kroll and others 1983; Morgner 1995; Kobelt and others 2000; Tanaka and others 2006; Donald and others 2007). Stages belonging to the secondary infection phase are at least quantitatively reduced and delayed in resistant interactions. Kroll and others (1983) compared three radish cultivars that showed quantitative differences in resistance for their degree of colonization by *P. brassicae*. Although secondary plasmodia were present initially in all three cultivars, their development stopped completely only in the wholly resistant cultivar, whereas considerable delay in the development of secondary stages was seen in the partially resistant cultivar. Morgner (1995) studied the histology of *P. brassicae* infections in roots of resistant *B. napus* (ECD-06), *B. rapa* (cv. Chorus), and *B. oleracea* ('Böhmerwaldkohl') hosts and identified secondary stages, particularly the early secondary plasmodia, as present in the cortex 12 days post infection (dpi), but no plasmodia or resting spores were detected at 22 dpi. Apparently, pathogen development was stopped before the completion of the life cycle. The presence of secondary stages in resistant hosts was also described by Kobelt and others (2000) in Arabidopsis and by Donald and others (2007) in a *B. oleracea* breeding line obtained from Syngenta Seeds. Kobelt and others (2000) compared Arabidopsis ecotypes of differing strengths of resistance for their colonization by *P. brassicae* during secondary infection. The most resistant ecotype, Ze-0, restricted the pathogen's development most strongly, leading to a significant delay and reduction of late stages of the life cycle. However, small amounts of resting spores were formed.

Often biotrophic pathogens are restricted by a hypersensitive response (HR), which has been defined as "the rapid death of plant cells in association with the restriction of pathogen growth" by Goodman and Novacky (1994). The concept of this reaction is questioned for root pathogens and probably is not fully applicable to CR. Dekhuizen (1979) studied the histology of resistant turnips (*B. rapa*) and observed necrotic areas in infected roots which he interpreted as evidence of a hypersensitive response. Morgner (1995) could not confirm this observation in the CR Chinese cabbage cv. Chorus. Kobelt and others (2000) observed autofluorescence and also necrotic areas in infected roots of resistant Arabidopsis ecotypes. The number of necrotic spots was correlated with the degree of resistance and also to the amount of inoculum; however, the pathogen was not fully restricted by necroses. The time required for necrosis development was much longer than that known for hypersensitive responses to biotrophic leaf pathogens.

The physiologic effects of CR were studied in Arabidopsis by Jubault and others (2008). They found remarkable differences with respect to the regulation of arginine catabolism and polyamine metabolism between a susceptible and a partially resistant ecotype. So far it is not clear whether these different reactions are a part of or a result of the defense reaction. See Ludwig-Müller and others (this issue) for a discussion on metabolism and plant growth regulator events during clubroot disease.

Epidemiology of *P. brassicae*

Successful infection by *P. brassicae* depends on several environmental factors (see Dixon, this issue, for a detailed discussion). Uneven distribution of these environmental conditions in the field can lead to a scattered pattern of infested patches. One major factor that affects disease incidence is soil pH. Infections occur mainly where the pH is below 7. Reports of disease incidence at pH values around 8 are rare but do exist (Colhoun 1953). This may reflect the capacity of *P. brassicae* to adapt, especially when other factors are optimal. Soil humidity is another key factor; the zoospore stages of *P. brassicae*'s life cycle depend on soil water for movement (Dobson and others

1982) and this could explain the prevalence of infections in those patches of a field where drainage is not sufficient. Soil temperature between 20 and 22°C was shown to be optimum for disease severity in the field (Thuma and others 1983; Einhorn and Bochow 1990), although the geographical distribution of the disease leads to the suggestion that *P. brassicae* could adapt itself to different temperature regimes. In summary, infections can be expected when resting spores, young host roots, low pH, and wet and warm weather coincide.

Plasmodiophora brassicae depends on the movement of contaminated material for long-distance dispersal. Opposite to leaf pathogens wind dispersal is only of minor relevance and is expected to depend on the drift of contaminated soil. Medium to long distance spread is assisted by infected transplants of cole crops, farm machinery, animals, and water runoff. Spore dispersal over short distances by active movement in the rhizosphere seems of limited relevance for spreading because both primary and secondary zoospores appear to be incapable of swimming more than a few millimeters (Doyle 1990). Zoospore movements should be sufficient to allow attachment to the nearest root surface. An evolutionary strategy to cope with poor dispersal is longevity and a high reproduction rate, which applies to *P. brassicae*. Resting spores are capable of remaining viable in soil for at least 17 years, with an estimated half-life time of 3.6 years (Wallenhammar 1996). One gram of infected root tissue contains at least 1–2 $\times$ 10^8 spores, and a single host plant can produce root tumors weighing more than 20 g. On the assumption that the plant in this example was initially infected by 10^4 spores, this would mean a 100,000-fold increase in one generation, lasting approximately 6 weeks.

Due to the nonspecificity of the primary infection phase, it has been assumed that some nonhost plants as well as resistant plants reduce the inoculum level in contaminated soils by causing the resting spores to germinate without allowing their multiplication (Yamagishi 1987; Harling and Kennedy 1991; Friberg and others 2006) but the experimental evidence is unclear. The observation of viable *P. brassicae* resting spores coming from infections of what are regarded as nonhost plant species (that is, *Beta vulgaris* or *Tropaeolum majus*) by Ludwig-Müller and others (1999) raises the question: To what extent do other plants as crucifers play a role in the maintenance or increase of the soil inoculum? Detection of soil infestations, a major prerequisite for epidemiologic studies, is achieved mainly by bioassays using highly susceptible plants as indicators, that is, Chinese cabbage or brown mustard (Melville and Hawken 1967; Rouxel and Cadiou 1988). Some PCR-based methods for detecting *P. brassicae* in soil, plant, or water samples have been reported (Ito and others 1997; Faggian and others 1999; Cao and others 2007) and will lead to the development of real-time PCR protocols for the quantitative detection of soil infestation levels (see Faggian and Strelkov, this issue).

Genetic Variation of the Interaction Between *P. brassicae* and Its Hosts

Genetic variation in *P. brassicae* was first demonstrated by Honig (1931) when she compared three cruciferous species for resistance toward *P. brassicae* isolates of different origin. Scheijgrond and Vos (1954) showed variation of *P. brassicae* on hosts from the same species. In the following decades resistant genotypes for the major Brassica crops were identified, except for *B. juncea* and *B. carinata* (Walker and Larson 1948; Gante 1951; Weisaeth 1961; Lammerink 1964; Tjallingii 1965; Ayers and Lelacheur 1972; Crute and others 1980; Brokenshire 1982; Schoeller 1987; Crisp and others 1989; Monteiro and Williams 1989; Williamson and Bradshaw 1989; Bradshaw and Williamson 1991). Resistant accessions were found more frequently in certain crop types, for example, in white cabbage or kale types for *B. oleracea*, or in the storage root-forming types of *B. rapa* and *B. napus*.

The interaction between *P. brassicae* and its hosts has been studied intensively using different tester sets (Table 1). The ECD set (Buczacki and others 1975) has played a major

Table 1 Overview of present clubroot differential sets

Set	No. hosts	Species represented	Known resistance genes	No. pathotypes to be identified
ECD set (Buczacki and others 1975)	15 (12 resistant)	5× *B. rapa* 5× *B. napus* 5× *B. oleracea*	A, B, C in *B. rapa* hosts	48 Theoretical 23 Realistic[a]
Williams (1966)	4	2× *B. napus* 2× *B. oleracea*	Not identified	16
Somé and others (1996)	3	*B. napus*	Not identified	8
Kuginuki and others (1999)	2	*B. rapa*	Not identified	4

[a] Difference between theoretical and realistic numbers is due to complex genotypes and shared R genes between different hosts

role in race designation; it consists of five hosts from each of *B. rapa*, *B. napus*, and *B. oleracea*. Crute and others (1983) analyzed more than 240 internationally collected data sets from ECD tests and concluded that CR in *B. rapa* and *B. napus* was race-specific, whereas the resistance of the *B. oleracea* ECD hosts appeared to be basically nondifferential. *Plasmodiophora brassicae* does not form *formae specialis*; however, isolates might differ in particular with repect to their pathogenicity toward resistant hosts from certain crop species (Crute 1986). Only very few isolates were capable of overcoming the resistance of *B. rapa* hosts, whereas susceptible reactions were very frequent in most *B. napus* and *B. oleracea* hosts. This was still true when Toxopeus and others (1986) published their survey of a worldwide collection of data generated by using the ECD series. So far, pathogenicity for the *B. rapa* ECD hosts appears to be specific for some isolates from Europe and, after introduction of CR *B. rapa* cultivars, from Japan (Williams and Walker 1963; Crute 1986; Donald and others 2006b; Hirai 2006; Strelkov and others 2007). It is remarkable that pathogenicity against *B. oleracea* and some *B. napus* hosts did not occur in isolates with no obvious selective origin, whereas virulence toward *B. rapa* hosts was present in isolates that merely had a selective history in this direction (Toxopeus and others 1986).

Interestingly, pathogenicity toward resistant *B. rapa* hosts seems to be associated with pathogenicity toward resistant *B. napus* hosts, whereas resistant *B. oleracea* hosts are more likely to show heavy infections with *P. brassicae* isolates that were not virulent on most resistant *B. rapa* and *B. napus* hosts (Crute and others 1983). This dissociated pathogenicity might be an expression of different CR mechanisms that must be overcome. On the other hand, this might be a result of CR genes being introduced into *B. napus* from one of its ancestral species, *B. rapa*. Diederichsen and Sacristan (1996) combined CR from *B. rapa* host ECD-04 and *B. oleracea* host ECD-15 in resynthesized *B. napus* lines and found that CR from *B. oleracea* was hardly effective in *B. napus* unless it was combined with CR from *B. rapa*. Thus, the expression of CR from both parental species in *B. napus* differs; CR genes from *B. oleracea* might be present in natural *B. napus* without being noticed. Taken together, CR from *B. rapa* should be regarded as based on strong and efficient but race-specific resistance genes.

Other differential sets consisting of fewer differential hosts have been introduced by Williams (1966), Somé and others (1996), and Kuginuki and others (1999). All sets are sufficient in distinguishing *P. brassicae* isolates, at least in particular geographical regions. Their major characteristics are summarized in Table 1. Many clubroot workers prefer to use fewer hosts for obvious reasons; however, investigations such as those by Crute and others (1983) and Toxopeus and others (1986) did yield valuable information about the interaction of *P. brassicae* with major host crops and this would not have been possible with the use of smaller sets.

All clubroot differential sets lack exact information about the number and identity of resistance genes in the hosts. For the *B. rapa* ECD hosts, combinations of two to three resistance genes (A, B, C) were proposed by Wit (1964) and Toxopeus and Jansen (1975). There are different models of the genetics of the *B. napus* ECD hosts that are based on at least four race-specific genes (Crute and others 1983; Crute 1986; Gustafsson and Fält 1986). It is assumed that most differential hosts represent complex CR genotypes. Combinations of CR genes will lead to an underestimation of the frequency of compatible isolates of *P. brassicae* for individual CR genes and, moreover, of the degree of variation in *P. brassicae*. In Table 2 race-specific reactions of the *B. rapa* ECD hosts are compared with those from *B. napus* lines that represent the *B. rapa* CR genes A, B, or C separately or have a single QTL according to markers mapped by Werner and others (2008). The *B. rapa* hosts showed much fewer compatible interactions than did the monogenic differentials, and even when two genes were overcome individually, their respective combination in a *B. rapa* host still provided at least a quantitative level of CR. At least from a European perspective, the number of *P. brassicae* pathotypes seems to be limited more by the number of differential hosts rather than by a capacity for adaptation. The relatively recent introduction of clubroot into America and Australia in the 19th century, probably via diseased fodder plant material by colonists (Colhoun 1958; Donald and others 2006a), seems to be associated with less diverse pathogen populations in the

Table 2 Race specificity of CR in Brassica hosts displaying CR genes from *B. rapa*

Host	Postulated loci	Disease index with *P. brassicae* isolate			
		China1	Schwaan	H1	Polen1
B. rapa ECD hosts					
ECD-01	B + C	*42*	9	*44*	2
ECD-02	A + C	5	2	29	0
ECD-03	A + B	0	19	*48*	0
ECD-04	A + B + C	0	0	9	0
B. napus hosts					
55–6	A	23	17	*91*	*40*
140–4	B	*82*	*100*	*96*	*99*
102–4	C	*83*	0	*41*	*100*
914–8	(A03 65.3)[a]	*39*	0	*86*	*100*
914–48	(A08 57.8)[a]	10	8	*93*	8

[a] Map position according to Werner and others (2008); susceptible reactions (DI > 33) in italic; testing procedures as in Diederichsen and Sacristan (1996)

New World compared with Europe (Donald and others 2006b).

An important problem for race differentiation is the genetic heterogeneity present on both sides of this host-pathogen interaction. Many host species, such as *B. rapa*, *B. oleracea*, and *R. sativus*, are outbreeders and display heterogenic resistance reactions in crop cultivars as well as in differential hosts. These reactions lead to speculation about the genetic identity of the differential host and to uncertain interpretations of compatibility. Heterogeneity has to be expected even more for *P. brassicae* isolates. In accordance with most clubroot literature, the term "isolate" is used here for a heterogeneous *P. brassicae* field population in the natural state in which it was collected from the field as infected roots and maintained subsequently in susceptible host genotypes. At least in Europe such a field collection has to be regarded as heterogenic. Different pathotypes can be found even inside a root tumor club (Jones and others 1982b). Single resting spore-derived isolates (SSI) have been generated (Buczacki 1977; Jones and others 1982a; Schoeller and Grunewald 1986; Voorrips 1996; Manzanares-Dauleux and others 2000; Fähling and others 2004; Xue and others 2008) and often these differ in pathogenicity in comparison to the original collection. Single-spore isolates (SSIs) are most valuable for genetic analysis. For breeding and selection purposes, specific virulence properties are of greater importance. Clubroot disease reactions in resistance tests frequently give quantitative reactions; this can also apply to the interaction between monogenic CR hosts and SSI. Race-specific CR genes can yield quantitative disease phenotypes, which should not be interpreted as a criterion for race-independent resistance as in other pathosystems.

Crop-Specific Status of CR Breeding

The following sections provide an overview of the current status of CR breeding in major cruciferous crops. It might be helpful to consider the phylogenetic relationship among Brassica species. *B. napus* is a recent, natural amphidiploid hybrid between *B. rapa* and *B. oleracea* (U 1935). According to recent chromosome nomenclature, *B. rapa* has the chromosomes A1-A10 and *B. oleracea* the chromosomes C1-C9 which together form the *B. napus* genome. Both ancestral species of *B. napus* are regarded as still being closely related to each other (Lagercrantz 1998). Interspecific transfer of CR has played an important role in breeding during the last 50 years and also possibly in spontaneous gene flow. Breeding CR benefits strongly from modern breeding methods such as embryo rescue techniques which support interspecific transfer. Major challenges for CR breeding remain the identification and choice of resistance sources and of *P. brassicae* isolates to be used for selection and the management of race-specific resistance. In most Brassica crops hybrid cultivars have a strong position in the seed market, leading to a preference for dominant CR genes.

Breeding for CR in *B. rapa* Crops

In East Asian countries such as China, Korea, and Japan, *B. rapa* displays very wide morphologic variation. Besides the turnip and oil seed types, many kinds of leafy vegetables are grown there. Most relevant is the heading vegetable, Chinese cabbage. As a result of its long cultivation period of up to 150 days, Chinese cabbage is more vulnerable to clubroot compared with short-term crops such as Pak Choi, Mizuna, or Mibuna, which are per se equally susceptible. Flowering vegetables, from which flower buds and stalks are used for cooking, require longer cultivation periods and also suffer badly from clubroot (Hirai and Matsumoto 2007). Efforts in breeding for CR by seed companies have focused mainly on Chinese cabbage. A number of CR Chinese cabbage cultivars were released in Japan, and some CR flowering vegetables and CR turnip cultivars have also been released and are used in commerce. Oilseed turnip rape is the prevalent spring oil crop in areas with short growing seasons like the northern parts of Canada and Scandinavia. Partially resistant lines of this oil crop were developed in Sweden using the Chinese cabbage WR60 as resistance donor (C. Persson, SWSeeds Sweden, personal communication). They have higher yields than susceptible lines on sites infested by *P. brassicae* (Wallenhammar 1996; Wallenhammer and others 2000).

Addressing the clubroot problem in Japan, Dr. H. Yoshikawa of the National Institute for Vegetable, Japan, performed an extensive survey of CR in crucifer crops. He found no CR lines in East Asian germplasm (Yoshikawa 1993). Because *B. rapa* originated in the Mediterranean area, it is reasonable to speculate that the genetic diversity in European germplasm is greater than in East Asian germplasm despite their morphologic diversity. Genetic resources with CR in *B. rapa* were found only in European turnips (Yoshikawa 1993) as was also suggested by Crute and others (1980). European turnips such as cvs. Siloga, Gelria, Milan White, and Debra have been used for breeding different CR cultivars. Some of these turnip cultivars have also been used to select the differential hosts ECD-02 (from cv. Gelria) and ECD-01 (from cv. Debra) of the ECD set (Crute 1986).

A number of CR loci in *B. rapa* have been identified and distinguished with the aid of molecular markers. The first report on this was by Kuginuki and others (1997), who mapped a locus presently known as *Crr1*. A number of studies have been published since on CR in this species. Four more major CR loci have since been reported (Hirai

Table 3 Mapped CR loci in *Brassica rapa* and their linked markers

CR locus[a]	Identified as	Linkage group	Linked markers	Inheritance	References
Cra[b]	Major gene	A03	HC352b-SCAR	Dominant	Matsumoto and others (1998); Hayashida and others (2008)
Crr1	Major gene	A08	BRMS-088	Codominant	Suwabe and others (2003, 2006)
Crr2	Modifier gene	A01	BRMS-096	Codominant	Suwabe and others (2003, 2006)
Crr3	Major gene	A03	OPC11-2S	Codominant	Hirai and others (2004); Saito and others (2006)
CRb	Major gene	A03	TCR05	Codominant	Piao and others (2004)
Crr4	QTL	A06	BRMS-125	Codominant	Suwabe and others (2003, 2006)
CRk[c]	QTL, possibly major gene	A03	OPC11-2S	Codominant	Sakamoto and others (2008)
CRc	QTL, possibly major gene	A02	m6R	Dominant	Sakamoto and others (2008)

[a] Suffixes, a, b, and c in the locus name do not correspond to A, B, and C genes of ECD set, but indicate chronological order of discovery

[b] Very near or identical to *CRb*

[c] Very near or identical to *Crr3*

and others 2004; Piao and others 2004; Saito and others 2006; Suwabe and others 2003, 2006) and have been reviewed recently (Hirai 2006). Table 3 summarizes the available information about eight CR loci or QTL that have been mapped in *B. rapa*. Four of these reside on chromosome A03, probably representing two complex regions that control CR. One region, originally identified by mapping *CRb* and its marker TCR05 (Piao and others 2004), is assumed to be located in the top part of A03 because a QTL derived from cv. Gelria could be allocated around TCR05 and was mapped between 12.5 cM (BRMS124) and 30.2 cM (BRMS206) on the map of Suwabe and others (2006) by M. Hirai (unpublished data). Locus *CRa* was identified as being linked with the SCAR marker HC352b (Matsumoto and others 1998, 2005; Hayashida and others 2008), which has recently been mapped by Sakamoto and others (2008) on A03 in the vicinity of markers that were mapped in this region. Therefore, *CRa* and *CRb* might be allelic or closely linked CR loci. So far, it is not known whether they differ in their race specificity, but they differ in their mode of inheritance (Table 1).

Another region on A03 is highlighted by the *Crr3* locus, which is located between 34.6 cM (BRMS158) and 73.5 cM (BRMS058) on the map of Suwabe and others (2006; see also Saito and others 2006). Recently, Sakamoto and others (2008) reported a large QTL (*CRk*) around OPC11-2S, a marker of *Crr3*. The locus *CRk* was derived from Chinese cabbage cv. CR Kanko, and it is possible that it is identical, allelic, or closely linked to *Crr3*.

Other QTL or major loci that have a strong impact on CR were found on chromosomes A02 [*CRc* (Sakamoto and others 2008) derived from cv. Debra] and A08 [*Crr*1 (Suwabe and others 2003)]. Because only a few resistant turnip cultivars have been used as CR sources, it is likely that additional CR loci will be identified in this crop type. The QTLs for CR residing in the Brassica A genome and originating from turnip (ECD-04) were also mapped in *B. napus* (Werner and others 2008). A QTL on A03 had a strong effect on CR for three *P. brassicae* isolates in this study. This QTL was located at 65.3 cM in the map of Werner and others (2008); it might correspond to *Crr3* or eventually to *Crb*. Their map did not have enough markers in common with the previously mentioned maps, however, so the identity of these loci/QTLs remains to be determined. Furthermore, the correspondence of the QTL on A08 from Werner and others (2008) and *Crr1* (Suwabe and others 2003), which seems to be in close vicinity according to a common marker (BRMS088), requires clarification.

Mapping studies have advanced over the past 10 years but there is little knowledge about the performance or race specificity of each CR gene, and, furthermore, it is not known which CR locus would correspond with those present in CR cultivars. Differential race-specific responses of the *CRk* and *CRc* loci were reported by Sakamoto and others (2008). According to the available information about race-specific effects of CR genes from *B. rapa*, it is most likely that the mapped CR loci confer race-specific resistance, as in Japanese CR cultivars. Kuginuki and others (1999) and Hatakeyama and others (2004) suggested the use of two commercial CR Chinese cabbage cultivars showing opposite interactions with most Japanese field isolates of *P. brassicae* for race designation. The CR genes of these cultivars are unknown, however; therefore, more studies are required to clarify the performance and the broadness of major CR loci identified so far.

Most mapping studies have used a CR cultivar or CR breeding line as a resistant parent. These breeding lines have already been selected and may have only a proportion of those CR genes that might have been present in the original CR source. In this context, a study by Suwabe and

others (2003) is noteworthy. Their study used a doubled-haploid (DH) line, G004, as a resistant parent. This line was selected from DH lines derived from an F_1 between the CR turnip cv. Siloga and a susceptible Chinese cabbage line. Kuginuki and others (1997) selected DH lines with CR similar to that of cv. Siloga. This selection was essential for finding *Crr2* in addition to *Crr1*. The effect of *Crr2* was visible only in the presence of *Crr1*. The *Crr2* locus was lost during the breeding of commercial CR Chinese cabbage, but it was shown to be essential for resistance toward highly virulent pathotypes (Suwabe and others 2003). Finding and mapping CR loci that increase the broadness of CR is the target of future studies. These CR loci will become important in breeding cultivars with more durable resistance.

Breeding CR *B. oleracea* Crops

Vegetable *B. oleracea* has a long history of selection into different crop forms such as cabbage, cauliflower, Brussels sprouts, and broccoli. The earliest descriptions of *B. oleracea* crops date back to the ancient Greeks and Romans who mention stem kales, headed cabbages, and possibly kohlrabi (Thompson 1976; Snogerup 1980). Forage kales (*B. oleracea* var. *acephala*) are one of the most primitive groups used for livestock. The more modern red and white cabbages (*B. oleracea* convar. *capitata* var. *capitata*) trace back to the 12th century in Germany and to the 14th century in England. Savoy (*B. oleracea* convar. *capitata* var. *sabauda*) probably originated from Italy and spread to France and Germany in the 16th–17th centuries (Thompson 1976). In many parts of Europe "ancient" forms, for example, Italian local varieties of broccoli and cauliflower *B. oleracea* var. *botrytis*, are still grown as locally specialized varieties, both as fodder types and for human consumption (Massie and others 1996).

The cabbage cv. Badger Shipper was one of the first CR cabbage cultivars introduced in North America in the 1960s (Crute and others 1980). The resistance was thought to be based on a single gene group with an unknown number of genes (Chiang and Crête 1976). This resistance, however, was readily broken after introduction in the field. However, in the absence of the resistant cultivar for 3 years, the pathogen population seemed to lose pathogenicity to cv. Badger Shipper, suggesting that there are adverse fitness costs associated with pathogenicity (Crute and others 1980). Other attempts to introduce resistant *B. oleracea* cultivars include broccoli and cabbage lines developed in North America (Baggett 1983; Baggett and Kean 1985) that are resistant to various pathotypes present in North America, resistant cabbage cultivars released in Norway based upon resistance from the primitive cabbage cultivar Böhmerwaldkohl (Weisaeth 1977), and a cabbage landrace from the Shetland Islands [Dixon 1988; for a more complete overview see Crute and others (1980) or Voorrips (1995)].

In Canada, the *B. napus* swede cv. Wilhelmsburger was chosen as a means to introduce resistance into *B. oleracea* (Chiang and others 1977; Chiang and Crête 1983), as the prevalent races from the U.S. and Canada seem to lack virulence to most of the *B. napus* differential hosts (Chiang and Crête 1989). A single resistant BC_1 plant with $n = 26$ was selected for introgression of CR into the cabbage cultivar Badger Shipper and the cabbage line 8-41 (Chiang and others 1977, 1980; Landry and others 1992). They both possess resistance against race 6, similar to cv. Wilhelmsburger (Chiang and Crête 1976; Williams 1966). This work resulted in the release of two CR cultivars, Acadie and Richelain (Chiang and Crête 1985, 1989). The first disease resistance QTLs that were mapped in Brassica were reported in 1992 by Landry and others, who found two QTLs for CR in this material.

The CR introduced by Chiang and Crête seems of only limited value for European conditions because of the high frequency of compatible isolates (see above). Toxopeus and others (1986) concluded that the turnip *B. rapa* ECD hosts had the strongest and broadest resistance. Within *B. oleracea* many resistance sources have been described (for example, Crute and others 1980; Crisp and others 1989; Hansen 1989; Voorrips and Visser 1993; Manzanarez-Dauleux and others 2000). Only a few lines or cultivars have reached the commercial state (Crute and others 1980; Voorrips 1995). Linkage drag between CR and undesired traits such as reduced shelf-life has been reported for some *B. oleracea* CR sources (Crute and others 1980). Although CR from *B. oleracea* is regarded as mainly nondifferential, it is less used in breeding than race-specific CR from *B. napus* or *B. rapa*. The most recent CR *B. oleracea* cultivars carry race-specific CR that originates from *B. rapa* (see below). There are reasons for this. CR from *B. oleracea* is genetically more complex, mainly recessive, and therefore difficult to use in breeding, particularly in hybrid breeding. In addition, *P. brassicae* isolates that can completely overcome the resistance of all *B. oleracea* ECD hosts are very common (Crute and others 1983; Toxopeus and others 1986).

In different QTL analyses of CR in *B. oleracea*, major and minor QTLs were found (Voorrips and others 1997; Moriguchi and others 1999; Rocherieux and others 2004; Werner and others 2008). It is unknown whether some of these QTLs are allelic or identical because most of them have not been mapped onto a reference map. Rocherieux and others (2004) identified several isolate-specific QTLs and one major QTL that conferred resistance to all five *P. brassicae* isolates used in their study. Werner and others (2008) mapped QTLs from *B. rapa* and *B. oleracea* after introduction into *B. napus* and identified eight QTLs in the *B. oleracea* C genome, all showing race specificity. Despite

its nondifferential appearance, it has to be assumed that CR from *B. oleracea* is likely to be based on a mixture of race-specific and race-independent CR loci.

For hybrid cultivars based on cytoplasmic male sterility (CMS), the use of recessive CR would require that all three lines involved in breeding (CMS female, maintainer, and male line) possess identical CR alleles for several loci. To fix such a complex CR in breeding lines would inevitably require the application of molecular markers for all involved loci and, furthermore, dihaploidization techniques, making this a very long-term, expensive, and difficult breeding process. There would still be a risk of creating only partial and race-dependent resistance. For these reasons the CR sources in *B. oleracea* were considered to be too complex by breeders from Syngenta Seeds B·V., who decided to use the *B. rapa* CR source. The use of this source in *B. oleracea* made it necessary to overcome the species barrier for stable introgression of CR from the A into the C genome. The first interspecific crosses were made in 1987 between a broccoli inbred line and Chinese cabbage cv. Parkin, which possesses a single dominant resistance gene (Fig. 1). Embryo rescue (Harbert 1969) was used to obtain F_1 and BC_1 plants. Nine resistant BC_2 plants were derived from a single BC_1 plant. The expected 1:1 segregations were found only in the BC_4, suggesting that the CR gene had been introgressed in the C genome. A backcross program in cauliflower, cabbage, and Brussels sprouts was then initiated; it included several years of backcrosses, selections, line development, and test crosses. Field tests evaluating the resistance in *B. oleracea* background under field conditions were carried out for several years at naturally infested locations in Europe. In all tests the CR entries remained resistant, whereas the susceptible controls were heavily infected (E. Linders, data not shown). Trials performed in India, Australia (Donald and others 2006a, b), and other countries yielded similar results. These results indicated that the resistance gene was as effective in the

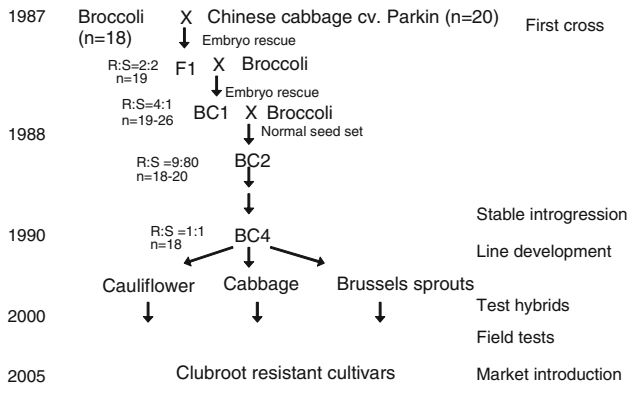

Fig. 1 Introgression of clubroot resistance from *B. rapa* cv. Parkin into *B. oleracea* crops at Syngenta Seeds B·V.; chromosome numbers and segregation ratios for resistance are given for initial generations

B. oleracea background as in the original *B. rapa* cultivar and encouraged introduction of cultivars into the market.

The CR cultivars were introduced to the market in 2005 (for example, cauliflower cvs. Clapton and Clarify and cabbage cvs. Kilaton and Tekila) and well received by growers. The resistance from *B. rapa* does not cover all isolates of *P. brassicae* (Toxopeus and others 1986), however. A small number of isolated infections were observed. Medium to severe attacks occurred at some locations in Germany, Poland, and Brittany, France. This situation had already been encountered with *B. rapa* cv. Parkin before introduction of the new cultivars. Often infections of the CR cultivars are quantitative with small clubs as reported for CR Chinese cabbage cultivars (Osaki and others 2008). Compatible isolates sometimes have a reduced fitness on susceptible *B. oleracea* hosts as compared with a standard isolate not capable of overcoming *B. rapa* genes. An isolate that was capable of infecting CR cv. Parkin from Indonesia did show a strongly reduced reproduction rate on three susceptible cabbage cultivars (Table 4). Donald and others (2007) studied the histology of the Clapton-like resistance and showed that the pathogen was inhibited in the cortex; for some isolates this interaction may be more quantitative than qualitative. Fitness costs associated with certain pathogenicity factors, as has been observed with cv. Badger Shipper (see above, Crute and others 1980), or interaction between pathotypes may be present and certainly more research is needed to elucidate this.

When advising growers, they must be told that resistance will not work against all pathotypes. Including cv. Clapton in a new differential set would help to monitor the occurrence of compatible races. In the current situation it is important that growers use this resistance in combination with existing local husbandry measures such as liming,

Table 4 Reproduction rate[a] of a standard isolate compared to an Indonesian isolate capable of infecting CR Chinese cabbage cv. Parkin on three cabbage entries with different levels of susceptibility

Entry	Spores/g infected root		Spores/plant		Mean score (0–5)	
	Stand.	Ind.	Stand.[b]	Ind.[c]	Stand.	Ind.
Marathon	595	199b*	1809	347b	5.0	4.3b
Cab. 1	563	248b	802	158a	4.9	3.4ab
Cab. 2	468	68a	792	64a	4.6	2.5a
Mean	542b	172a	1134b	189a	4.8b	3.4a

[a] Reproduction rate = No. of spores × 10^6 per gram of infected root or per plant

[b] Stand. = race 4, Nord-Ditmar Germany

[c] Ind. = Indonesian

* Different letters indicate significant differences between means according to two-way ANOVA and Tukey test after square root transformation; $n = 14$, $p < 0.05$. Scoring scale: 0 = no symptoms, 5 = fully diseased

calcium fertilizers (for example, calcium cyanamide and calcium nitrate), drainage, and fungicides (if allowed) (Donald and others 2006a) in an integrated disease control strategy to prevent further erosion (see Donald and Porter, this issue). Breeding combinations with resistance sources from *B. oleracea* is an additional future option, but the technical and scientific difficulties mentioned earlier make the solely genetic solution a real challenge.

CR Breeding in *B. napus*

Brassica napus is a recent amphidiploid Brassica species with very little intraspecific variation and no wild accessions. Both ancestral species, *B. rapa* and *B. oleracea*, are still important genetic resources for increasing the genetic variation in *B. napus*. Although traditional high-erucic-acid and high-glucosinolate *B. napus* oilseed crops had only limited use (for example, for lamp oils in the past centuries), modern double low cultivars of oilseed rape and its Canadian variant canola have experienced a dramatic boom in production since 1978. Due to its improved oil and meal quality, oilseed rape has become the major oil crop in Europe, Canada, Australia, and China. Its acreage has reached more than 6×10^6 ha in 2007 within the European Union alone. In Europe and Canada, oilseed rape or canola, respectively, is now often grown every third year in a rotation. The use of rapeseed oils for biofuel has significantly contributed to the value of this crop and its growing acreage.

Clubroot disease was originally most important to the storage-root-forming *B. napus* type spp. *napobrassica* (swede or rutabaga) and to fodder rape (*B. napus* spp. *oleifera* var. *annuum*) compared with winter oilseed rape because their growing period better matches the pathogen's temperature requirements. As a result of the spread of oilseed rape into areas where soil conditions are more conducive, clubroot incidences are more frequent in this crop. In a Swedish survey, 72% of 190 soil samples from oilseed rape fields tested positive for the presence of *P. brassicae* (Wallenhammar 1996). In naturally infested fields, Wallenhammar and others (1999) found up to 50% yield reduction in spring oilseed rape. In Canada, the first incidences of clubroot in *B. napus* were recorded in 2003 in Alberta and the disease is still spreading in this region (Strelkov and others 2005; Xue and others 2008).

Breeding for CR in *B. napus* focused initially on swedes, where resistant types had been identified by Colhoun (1958) and Ayers and Lelacheur (1972). Swedes have been used to introduce CR into *B. oleracea* (Chiang and others 1977, see above) and received CR genes from *B. rapa* by interspecific hybridization (Lammerink 1969; Williamson and Bradshaw 1989; Bradshaw and others 1997). Fodder rape cultivars with CR were bred after selection of resistant individuals (Lammerink 1964; Johnston 1970); some of these are included in the ECD set. The genetics of CR in the ECD *B. napus* differentials (ECD-06 to ECD-10) has been studied by Gustafsson and Fält (1986), who postulated that four race-specific genes are present in these hosts (Table 5). Based on differential pathogenicity patterns with many isolates, Crute and others (1983) postulated a slightly different allocation of CR loci. Genes with the same numbers in both models may not be identical. The difference between both models is most probably due to the choice of *P. brassicae* isolates that were used in the Swedish study. By using an isolate with the capability to overcome the gene "R3" *sensu* Crute and others (1983), the effect of this gene cannot be observed; this would explain the identification of only one gene in ECD-06 and two in ECD-10 by Gustafsson and Fält (1986).

Several attempts were made to introduce CR from both ancestral species into *B. napus*. Diederichsen and Sacristan (1994) combined different sources of CR from *B. rapa* and *B. oleracea*. The expression of CR in resynthesized *B. napus* forms was compared (Diederichsen and Sacristan 1996). Both CR sources were less efficient in the *B. napus* background compared with the original parent; in particular, CR from *B. oleracea* appeared to be strongly diluted, indicating the presence of epistatic factors in the *B. rapa* genome. Combining the most resistant ancestral parents led to resynthesized *B. napus* forms that were resistant against all isolates of *P. brassicae* that were available in their study, representing a level and broadness of CR that had not been present previously in *B. napus*. Bradshaw and others (1997) crossed an autotetraploid of the *B. rapa* host ECD-04 with a tetraploid kale and selected a CR swede after backcrossing; in 1996 the CR cultivar cv. Invitation was released to the UK seed market.

Two CR oilseed rape cultivars, cv. Mendel and cv. Tosca, were introduced to the European seed market in 2000. Both cultivars possess race-specific CR (Diederichsen and others 2003) originating from a resynthesized *B. napus* form (Diederichsen and others 2003; Happstadius and others 2003). The hybrid cultivar Mendel has yields comparable with other cultivars from the same breeding period. In Germany, its market share has been increasing from 0.7% in 2001 to 2% in 2006. Its breeding process is

Table 5 CR genotypes postulated for *B. napus* ECD hosts

B. napus ECD host	Gustafsson and Fält (1986)	Crute and others (1983)
ECD-06	CR2	R2 + R3
ECD-08	CR1 + CR3	R1
ECD-09	CR1 + CR2	R1 + R2
ECD-10	CR1 + CR4	R1 + R3 + R4

illustrated in Fig. 2. Resistance was initially combined in a resynthesized *B. napus* by crossing two ECD hosts, *B. rapa* ECD-04 and *B. oleracea* ECD-15, with the aid of ovule culture (Diederichsen and Sacristan 1994). After backcrossing, dihaploidization, and selection with a highly virulent isolate of *P. brassicae*, a resistant DH line was identified and used for breeding and genetic analysis. The latter confirmed the presence of three dominant, race-specific CR genes from ECD-04 in a backcross population (Diederichsen and others 1996), as expected. Further backcrosses and selections with undefined *P. brassicae* isolates originating from infested crops led to the resistant parent of the hybrid cultivar Mendel. Subsequent mapping of CR in cv. Mendel revealed one major locus plus two recessive genes (Diederichsen and others 2006), indicating that two dominant CR genes were lost during breeding. The resistance of cv. Mendel is efficient in most cropping areas. Compatible populations of *P. brassicae* were known to exist before its release and occasionally infected crops of cv. Mendel have been observed. In many cases the infected individuals were identified to be volunteers of previous crops. However, because cv. Mendel's resistance is based on only one major gene (recessive genes are unlikely to contribute in a hybrid cultivar), it is marketed carefully by the breeder. It is recommended only in those cases where clubroot infections have already been encountered and should not be grown as insurance against potential disease incidence. Control strategies involving the integrated use of pyramids of control methods (see Donald and Porter, this issue) will help to prolong the usefulness of this cultivar.

Identification of race-independent CR genes from *B. oleracea* in *B. napus* was attempted by using as the CR source a resynthesized *B. napus* form that originated from a

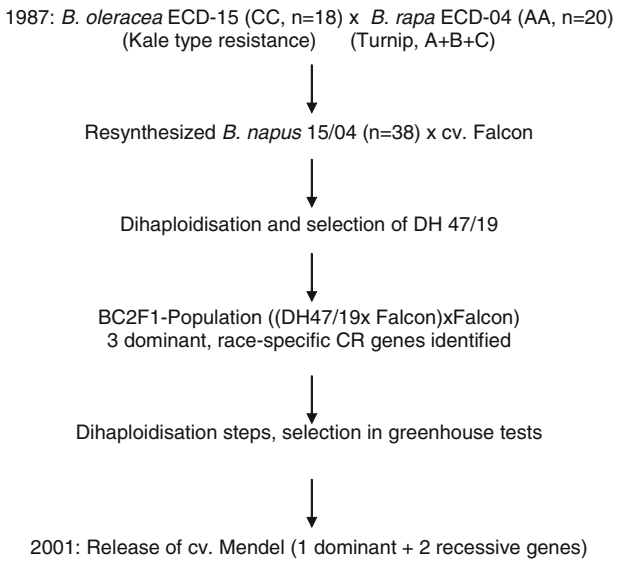

Fig. 2 Breeding of *B. napus* cv. Mendel by Norddeutsche Pflanzenzucht H.G. Lembke KG

cross between ECD-04 and the cabbage landrace Böhmerwaldkohl. Nineteen QTLs were detected in a DH population (Werner and others 2008), all showing race specificity. There was no indication of race-independent QTLs, which were assumed to be present in the *B. oleracea* C genome. The only strong QTL in the C genome that could be detected in this material originated from the susceptible parent cv. Express and conferred CR to only one isolate of *P. brassicae*. Some QTLs had overlapping confidence intervals on chromosomes A03 and A08 and conferred CR against more than one isolate. Based on this study, the QTLs with the broadest effects against *P. brassicae* pathotypes in *B. napus* could be expected to be located in the *B. rapa* A genome.

Manzanares-Dauleux and others (2000) identified resistance against a SSI in the *B. napus* dwarf line Darmor-*bzh*. They mapped one major gene and two QTLs in a DH population. As the linkage groups of their map had not been allocated to either the A or the C genome, it cannot be concluded whether CR from Darmor-*bzh* originated from *B. rapa* or *B. oleracea*. As CR from Darmor-*bzh* has not been observed in the field so far, it seems likely that the major gene has a race-specific effect that has already been overcome in the field. The authors identified some interesting epistatic interactions between the CR loci and other genomic regions, highlighting the value of mapping studies for improving our understanding of resistance genetics.

CR in Radish (*Raphanus sativus* L.)

Radish, a cruciferous crop closely related to Brassica, originated in the Mediterranean region (Kitamura 1958; Tsunoda 1980), but today is more important in East Asia where a wide variety of genotypes are found (Kaneko and others 2007). Yoshikawa (1993) surveyed CR in radishes and found that most Japanese cultivars are highly resistant, except for some cultivars with bolting resistance, such as cvs. Tokinashi or Ninengo. Some northern Chinese cultivars were highly susceptible. European radish cultivars such as Comet and White Icicle showed mild symptoms or were resistant (Yoshikawa 1993). Rat-tail radish, which is grown in tropical Asia for the consumption of its pods, is resistant to the pathogen (M. Hirai, unpublished data). Resistance to clubroot seems to be widespread in this species, but there are reports of clubroot outbreaks in radish in the U.S. (Thuma and others 1983) and more recently in Japan (Horikoshi and Tairako 2002). In Japan, radish was believed for a long time to be totally immune to clubroot. Therefore, radish is often used as a cleaning crop for fields contaminated with *P. brassicae* (Murakami and others 2000). The susceptible cultivars were newly bred, so it is possible that some CR genes were lost during breeding.

Because of its relevance, it is desirable to unravel the genetic basis of CR in radish. Kamei and others (2007) recently reported on a QTL analysis of radish CR using a Japanese radish as the resistant parent and a Chinese cultivar as the susceptible parent. They found that the trait is controlled by one locus or closely linked loci in a single linkage group. Using nine types of monosomic addition lines of radish, Akaba and others (2008) showed that only one addition type had clear resistance, suggesting that CR is controlled predominantly by a single radish chromosome. Therefore, it seems likely that only a single locus controls a large part of radish CR. Considering the broadness of CR from radish, simple inheritance was unexpected. Resistant radishes can be bred by a simple backcross method starting with a Japanese radish as a donor plant. Mass selection is risky, however, when the trait is dominant.

If CR from radish could be transferred into Brassica species, it might be valuable for breeding highly resistant Brassica cultivars. Some efforts to transfer CR to Brassica have been reported using sexual or somatic hybrids between radish and Brassica species (Hagimori and others 1992; Hagimori 1995; Xing and others 1989). Recombination between Brassica and Raphanus chromosomes would be a precondition for a successful introgression. Attempts to introgress other traits such as restorer genes for cytoplasmic male sterility into Brassica did show, however, that this is a rare event (Sakai and others 1996; Delourme and others 1998).

Transgenic Strategies to Generate CR

This review has not yet discussed the subject of transgenic opportunities for the creation of CR. For a biotrophic pathosystem the use of pathogen-induced expression of a resistance gene that confers a HR reaction could be an interesting control strategy. Fuchs and Sacristan (1996) identified a gene that confers CR in the *Arabidopsis thaliana* ecotype Tsu (*RPb1*) which leads to a HR-like defense reaction (Kobelt and others 2000). The cloning of *RPb1* is about to be completed (Rehn and others 2008; J. Siemens, TU Dresden, personal communication), which might lead to novel strategies of using natural resistance genes, for example, in less related species such as *B. oleracea*. Cloning of CR genes might allow identification of the molecular basis of race differentiation in *P. brassicae*. Although the resistance conferred by transgenic strategies based on the use of resistance genes from heterologous interactions is often not sufficient for commercialization (Hennin and others 2001), such an approach might be more promising when targeting the original pathogen in a related host species. The current regulatory and political environment is likely to make a traditional breeding approach more realistic, particularly when considering the size of the market for clubroot-resistant cultivars.

Relevant progress has been made in the characterization of the interaction by *P. brassicae* with its hosts on the molecular level, for example, by studying effects of *P. brassicae* infection on transcripts (Devos and others 2005; Siemens and others 2006). The latter study demonstrated the dependence of clubroot infections on increased levels of cytokinin (see Ludwig-Müller, this issue). Consequently, transgenic Arabidopsis lines overexpressing cytokinin-degrading proteins showed a quantitative level of clubroot resistance against the four isolates used in their study (Table 6). The intimate relationship between *P. brassicae* and its hosts suggests a high degree of dependency of the pathogen on its host. Future studies will show whether this opens opportunities to design transgenic strategies and to what extent these might provide more durable resistance.

Perspectives of Clubroot Resistance Genetics and Breeding

Several examples demonstrate the rapid adaptation of *P. brassicae* to a widespread CR source, as occurred for the

Table 6 Interaction of lines overexpressing cytokinin-degrading enzymes (cytokinin oxidases/dehydrogenases *AtCKX1*, *AtCKX2*, *AtCKX3*) and wild-type Col-0 with different isolates of *Plasmodiophora brassicae* (Siemens and others 2006)

Line	Isolate	Disease index	Ri/Rni
Col-0	eH	99.2	4.78 ± 0.86
Col-0	1CK	100.0	4.19 ± 0.83
Col-0	k1	96.9	2.58 ± 0.60
Col-0	e2	91.4	7.70 ± 0.94
35S::AtCKX1	eH	31.03 (s)	1.15 ± 0.28
35S::AtCKX1	1CK	50.6 (s)	0.66 ± 0.31
35S::AtCKX1	k1	40.8 (s)	0.99 ± 0.23
35S::AtCKX1	e2	62.5 (s)	1.29 ± 0.43
35S::AtCKX2	eH	90.07 (ns)	2.61 ± 0.80
35S::AtCKX2	1CK	100.0 (ns)	1.97 ± 0.53
35S::AtCKX2	k1	77.9 (s)	1.36 ± 0.45
35S:AtCKX2	e2	83.3 (ns)	3.39 ± 0.82
35S::AtCKX3	eH	61.6 (s)	1.23 ± 0.10
35S::AtCKX3	1CK	71.0 (s)	1.81 ± 0.65
35S::AtCKX3	k1	79.0 (s)	1.77 ± 0.51
35S::AtCKX3	e2	68.8 (s)	1.83 ± 0.49

The disease indices (DI), mean values, and standard deviations of the root index Ri/Rni (fresh weight of infected root/fresh weight of noninfected root) are given; (s) or (ns) = significant or not significantly different from Col-0 ($\alpha = 0.05$) according to Kruskal–Wallis test and mean rank comparison

cabbage cultivar 'Badger Shipper,' CR Chinese cabbage, and CR radish in Japan (see above). Most of these CR cultivars possessed monogenic resistance; this applies also to the more recent CR cultivars such as cv. Clapton (*B. oleracea*) and cv. Mendel (*B. napus*). A comparison of cv. Mendel and cv. Clapton with a number of different *P. brassicae* isolates indicated that their resistance showed very similar race specificity (Diederichsen, unpublished data). It is possible that both cultivars coincidentally have the same CR locus. From the perspective of managing resistance, it should be stressed that oilseed rape and cabbage have hardly any overlapping cropping areas. The prospects for the durability of the present resistant cultivars are unsettling and the question must be asked: Is CR breeding going to enter a phase of boom-and-bust cycles for different race-specific CR genes, thereby spending valuable genetic resources? The spread of clubroot in Canadian canola fields and the presence of compatible isolates toward current CR cultivars create the demand for a new generation of resistant cultivars.

For a realistic estimation of the likelihood of a widespread occurrence of compatible isolates of *P. brassicae*, many factors have to be considered. For most of these factors we have only very rough estimations. Studies of compatible isolates of *P. brassicae* are needed to understand whether the pathogen's loss of fitness is a frequent or isolated phenomenon. For some genetical properties within *P. brassicae*, such as the likelihood of mutations and recombination in the pathogen, there is no knowledge at all. The frequency of virulent isolates for cvs. Mendel and Clapton is still low, although compatible isolates were already present at the time of cultivar release. The surveys of pathogenicity in *P. brassicae* made by Crute and others (1983) and Toxopeus and others (1986) were based on hosts with more than one CR gene. Therefore, a higher frequency of compatible isolates could be expected for cv. Mendel or cv. Clapton than for the *B. rapa* ECD hosts, which was below 10% of all tested *P. brassicae* isolates. On the other hand, the new CR cultivars are not yet used widely and they are less often repeatedly grown at the same site than Asian CR Chinese cabbage cultivars. Furthermore, *P. brassicae* spreads itself not very efficient. These factors should provide breeders with some time to apply strategies to increase the durability of CR.

Future CR cultivars will need a broader genetic basis for resistance to *P. brassicae*, and it is the task of breeders and scientists to cooperate in the identification and characterization of sources that can be expected to contribute significant durable resistance. An important resource to characterize resistance is a differential set that combines relevant resistance sources and that could be used by most clubroot workers. Major progress has been made in map-based identification of CR genes or QTLs. This knowledge should be used to clarify, for example, the identity of certain loci and the broadness of their effect. An ideal differential set should contain hosts of known monogenic resistance genotypes that represent relevant resistance loci for different areas. Furthermore, it should be possible to easily maintain pure hosts, which is difficult with outbreeding crops. Double haploid (DH) lines extracted from a mapping population would be ideal, as these combine homozygosity with the possibility of testing for the presence of a respective locus with markers. Efforts need to be made to balance public and private interests to find an agreement on the composition of such a differential set.

Another aspect of CR cultivars is their use as potential catch crops with the aim of reducing soil inoculum levels (Yamagishi and others 1986). A resistant cultivar would encourage the germination of *P. brassicae* resting spores without allowing them to multiply. This way the number of resting spores is thought to decline during the growth of the CR crops. However, as long as there is no reliable race-independent CR cultivar, this is a risky strategy that would significantly contribute to reductions in the durability of current resistance sources by selecting compatible pathotypes. Resistant cultivars should be grown only for the commercial crop itself. Clubroot control will always require the whole spectrum of integrated control components, such as crop rotation, liming, boron, calcium cyanamide or calcium nitrate applications, hygiene, and drainage. The resistant cultivar has a central role in this strategy.

Acknowledgments The authors thank Prof. G. R. Dixon and Dr. N. Kubo of Kyoto Prefectural University for their valuable comments on the manuscript.

References

Aist JR (1977) *Plasmodiophora brassicae*—the organism and life cycle: what we think we know, what we know, and what we need to find out. In: Woronin +100, International Conference on Clubroot, University of Wisconsin, Madison, pp 11–15

Akaba M, Kaneko Y, Hatakeyama K, Ishida M, Bang SW, Matsuzawa Y (2008) Evaluation of clubroot resistance in *Raphanus sativus* monosomic addition lines. In: Proceedings 113th meeting of Japanese Society of Breeding. Breed Res, vol 10, p 205

Ayers GW, Lelacheur KE (1972) Genetics of resistance in rutabaga to races of *Plasmodiophora brassicae*. Can J Plant Sci 52:897–900

Baggett JR (1983) Clubroot-resistant cabbage breeding lines Oregon 100, 123, 140, and 142. Hortic Sci 18:112–114

Baggett JR, Kean D (1985) Clubroot-resistant broccoli breeding lines OSU CR-2 to OSU CR-8. Hortic Sci 20:784–785

Bawden FC (1949) Department of Plant Pathology, Report from Rothamstead experimental station, 1948, pp 55–56

Bradshaw JE, Willliamson CJ (1991) Selection for resistance to clubroot (*Plasmodiophora brassicae*) in marrowstem kale (*Brassica oleracea* var. *acephala* L.). Ann Appl Biol 119:501–511

Bradshaw JE, Gemmell DJ, Wilson RN (1997) Transfer of resistance to clubroot (*Plasmodiophora brassicae*) to swedes (*Brassica*

napus L. var. *napobrassica* Peterm) from *B. rapa*. Ann Appl Biol 130:337–348

Braselton JP (1981) Karyotypic analysis of *Plasmodiophora brassicae* based on serial thin sections of pachytene nuclei. Can J Bot 60:403–408

Brokenshire T (1982) The reaction of forage Brassicas to nine populations of *Plasmodiophora brassicae* Woron. from S.E. Scotland. Ann Appl Biol 100:92–93

Buczacki ST (1977) Root infections from single resting spores of *Plasmodiophora brassicae*. Trans Br Mycol Soc 69:328–329

Buczacki ST, Moxham SE (1980) Karyogamy in *Plasmodiophora brassicae*. Trans Br Mycol Soc 75:439–444

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth GR (1975) Study of physiological specialization in *Plasmodiophora brassicae*: proposals for attempted rationalisation through an international approach. Trans Br Mycol Soc 65:295–303

Cao T, Tewari J, Strelkov SE (2007) Molecular detection of *Plasmodiophora brassicae*, causal agent of clubroot of crucifers in plant and soil. Plant Dis 91:80–87

Chiang MS, Crête R (1976) Diallel analysis of the inheritance of resistance to 6 of *Plasmodiophora brassicae* in cabbage. Can J Plant Sci 56:865–868

Chiang MS, Crête R (1983) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* spp. *capitata*). V. The inheritance of resistance. Euphytica 32:479–483

Chiang MS, Crête R (1985) Acadie: a clubroot resistant cabbage cultivar. Can J Plant Sci 65:223–235

Chiang MS, Crête R (1989) Richelain: a clubroot resistant cabbage cultivar. Can J Plant Sci 69:337–340

Chiang MS, Chiang BY, Grant WF (1977) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* var. *capitata*). I. Interspecific hybridization between *B. napus* and *B. oleracea* var. *capitata*. Euphytica 26:319–336

Chiang BY, Chiang MS, Grant WF, Crête R (1980) Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*B. oleracea* spp. *capitata*). IV. A resistant 18-chromosome B1 plant and its B2 progenies. Euphytica 29:47–55

Colhoun J (1953) Observations on the incidence of clubroot disease of Brassica in limed soils in relation to temperature. Ann Appl Biol 40:639–644

Colhoun J (1958) Clubroot disease of crucifers caused by *Plasmodiophora brassicae* Woron. A monograph. Phytopathol Paper 3, pp 1–109

Cook WRI, Schwartz EJ (1930) The life-history, cytology and method of infection of *Plasmodiophora brassicae* Wor., the cause of the finger-and-toe disease of cabbages and other crucifers. Philos Trans R Soc Lond 218:283–314

Crête R (1975) Distribution and importance of cruciferae growing areas and clubroot in 1974. Clubroot Newsl l3:3

Crête R (1981) Worldwide importance of clubroot. Clubroot Newsl 11:6–7

Crisp P, Crute IR, Sutherland RA, Angell SM, Bloor K, Burgess H, Gordon PL (1989) The exploitation of genetic resources of *Brassica oleracea* in breeding for resistance to clubroot (*Plasmodiophora brassicae*). Euphytica 42:215–226

Crute IR (1986) The relationship between *Plasmodiophora brassicae* and its hosts: the application of concepts relating to variation in inter-organismal associations. Adv Plant Pathol 5:1–52

Crute IR, Gray AR, Crisp P, Buczacki ST (1980) Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in Brassicas and allied crops—a critical review. Plant Breed Abstr 50:91–104

Crute IR, Phelps K, Barnes A, Buczacki ST, Crisp P (1983) The relationship between genotypes of three Brassica species and collections of *Plasmodiophora brassicae*. Plant Pathol 32:405–420

Dekuijzen HM (1979) Electron microscopic studies on the root hairs and cortex of a susceptible and a resistant variety of *Brassica campestris* infected with *Plasmodiophora brassicae*. Neth J Plant Pathol 85:1–17

Delourme R, Foisset N, Horvais R, Barret P, Champagne G, Cheung WY, Landry BS, Renard M (1998) Characterisation of the radish introgression carrying the *Rfo* restorer gene for the *Ogu*-INRA cytoplasmic male sterility in rapeseed (*Brassica napus* L.). Theor Appl Genet 97:129–134

Devos S, Vissenberg K, Verbelen J-P, Prinsen E (2005) Infection of Chinese cabbage by *Plasmodiophora brassicae* leads to a stimulation of plant growth: impacts on cell wall metabolism and hormonal balance. New Phytol 166:241–250

Diederichsen E, Sacristan MD (1994) The use of ovule culture in reciprocal hybridization between *B. campestris* L. and *B. oleracea* L. Plant Breed 113:79–82

Diederichsen E, Sacristan MD (1996) Disease responses of resynthesized *Brassica napus* L. lines carrying different combinations of resistance to *Plasmodiophora brassicae* Wor. Plant Breed 115:5–10

Diederichsen E, Wagenblatt B, Schallehn V, Deppe U, Sacristan MD (1996) Transfer of clubroot resistance from resynthesised *Brassica napus* into oilseed rape—identification of race-specific interactions with *Plasmodiophora brassicae*. In: Proceedings of the International Symposium on Brassicas. Acta Hortic, vol 407, pp 423–429

Diederichsen E, Deppe U, Sacristàn MD (2003) Characterization of clubroot resistance in recent winter oilseed rape material. In: Proceedings of the 11th International Rapeseed Congress, vol 1. Copenhagen, Denmark, pp 68–70

Diederichsen E, Beckmann J, Jörg Schondelmeier J, Dreyer F (2006) Genetics of clubroot resistance in *Brassica napus* 'Mendel'. Acta Hortic 706:307–311

Dixon GR (1988) Shetland cabbage as a source of resistance to *Plasmodiophora brassicae*. In: Proceedings of the 5th International Congress of Plant Pathology, 20–27 August 1988. Int Soc Plant Pathol, Kyoto, Japan, p 178

Dixon GR (1999) Keeping clubroot on the run. Grower 29:28–29

Dixon GR (2006) The biology of *Plasmodiphora brassicae* Wor.: a review of recent advances. Acta Hortic 706:271–282

Dobson RL, Gabrielson RL, Baker AS (1982) Soil water matrix potential requirements for root-hair and cortical infection of Chinese cabbage by *Plasmodiophora brassicae*. Phytopathology 7:1598–1600

Donald EC, Porter IJ, Faggian R, Lancaster RA (2006a) An integrated approach to the control of clubroot in vegetable brassica crops. Acta Hortic 706:283–300

Donald EC, Cross SJ, Lawrence JM, Porter IJ (2006b) Pathotypes of *Plasmodiophora brassicae*, the cause of clubroot, in Australia. Ann Appl Biol 148:239–244

Donald EC, Jaudzems G, Porter IJ (2007) Pathology of cortical invasion by *Plasmodiophora brassicae* in clubroot resistant and susceptible *Brassica oleracea* hosts. Plant Pathol 57:201–209

Doyle P (1990) Sequences in the primary stages of *Plasmodiophora brassicae* (video). Audio Visual Centre, University College Dublin, Dublin

Einhorn G, Bochow H (1990) Influence of temperature and soil pH value on the pathogenesis of clubroot (*Plasmodiophora brassicae* Wor.). Arch Phytopathol Pflanzenschutz 26:131–138

Faggian R, Bulman SR, Lawrie AC, Porter IJ (1999) Specific polymerase chain reaction primers for the detection of *Plasmodiophora brassicae* in soil and water. Phytopathology 89:392–397

Fähling M, Graf H, Siemens J (2004) Characterization of a single-spore isolate population of *Plasmodiophora brassicae* resulting from a single club. J Phytopathol 152:438–444

Friberg H, Lagerlöf J, Rämert B (2006) Usefulness of nonhost plants in managing *Plasmodiophorarassicae*. Plant Pathol 55:690–695

Fuchs H, Sacristan MD (1996) Identification of a gene in *Arabidopsis thaliana* controlling resistance to clubroot (*Plasmodiophora brassicae*) and characterization of the resistance response. Mol Plant Microbe Interact 9:91–97

Gante T (1951) Hernieresistenz bei Weißkohl. Z Pflanzenzüchtung 30:188–197

Garber RC, Aist JR (1979a) The ultrastructure of mitosis in *Plasmodiophora brassicae* (Plasmodiophorales). J Cell Sci 40:89–110

Garber RC, Aist JR (1979b) The ultrastructure of meiosis in *Plasmodiophora brassicae* (Plasmodiophorales). Can J Bot 57:2509–2518

Goodman RN, Novacky AJ (1994) The hypersensitive reaction in plants to pathogens. APS Press, St. Paul, MN, USA

Gustafsson M, Fält AS (1986) Genetic studies on resistance to clubroot in *Brassica napus*. Ann Appl Biol 108:409–415

Hagimori M (1995) Clubroot disease resistance and other characteristics of the progeny of somatic hybrid between Japanese radish and cauliflower. Acta Hortic 392:77–80

Hagimori M, Nagaoka M, Kato N, Yoshikawa H (1992) Production and characterization of somatic hybrids between Japanese radish and cauliflower. Theor Appl Genet 84:819–824

Hansen M (1989) Genetic variation and inheritance of tolerance to clubroot (*Plasmodiophora brassicae* Wor.) and other quantitative characters in cabbage (*Brassica oleracea* L.). Hereditas 110:13–22

Happstadius I, Ljungberg A, Kristiansson B, Dixelius C (2003) Identification of *Brassica oleracea* germplasm with improved resistance to *Verticillium* wilt. Plant Breed 122:30–34

Harbert DJ (1969) A simple effective embryo culture technique for *Brassica*. Euphytica 18:425–429

Harling R, Kennedy SH (1991) Biological control of *Plasmodiophora brassicae* using a bait crop. Mededelingen – Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 56:159–170

Hatakeyama K, Fujimura M, Ishida M, Suzuki T (2004) New classification methods for *Plasmodiophora brassicae* field isolates in Japan based on resistance of F1 cultivars of Chinese cabbage (*Brassica rapa* L.) to clubroot. Breed Sci 54:197–201

Hayashida N, Takabatake Y, Nakazawa N, Aruga D, Nakanishi H, Taguchi G, Sakamoto K, Matsumoto E (2008) Construction of a practical SCAR marker linked to clubroot resistance in Chinese cabbage, with intensive analysis of HC352b genes. J Jpn Soc Hortic Sci 77:150–154

Hennin C, Höfte M, Diederichsen E (2001) Functional expression of *Cf9* and *Avr9* genes in *Brassica napus* induces enhanced resistance to *Leptosphaeria maculans*. Mol Plant Microbe Interact 14:1075–1085

Hirai M (2006) Genetic analysis of clubroot resistance in *Brassica rapa*. Breed Sci 56:223–229

Hirai M, Matsumoto S (2007) *Brassica rapa*. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 5, Vegetables. Springer-Verlag, Berlin, pp 129–134

Hirai M, Harada T, Kubo N, Tsukada M, Suwabe K, Matsumoto S (2004) A novel locus for clubroot resistance in *Brassica rapa* and its linkage markers. Theor Appl Genet 108:639–643

Honig F (1931) Der Kohlkropferreger (*Plasmodiophora brassicae* Wor.). Gartenbauwissenschaft 5:116–225

Horikoshi N, Tairako K (2002) Occurrence of clubroot in Japanese radish caused by *Plasmodiophora brassicae* Woronin in Fukushima. Annu Rep Soc Plant Prot North Jpn 53:58–60

Ingram DS, Tommerup IC (1972) The life history of *Plasmodiophora brassicae* Wor. Proc R Soc London 180:103–112

Ito S, Maehara T, Maruno E, Tanaka S, Kameya-Iwaki M, Kishi F (1997) Development of a PCR-based assay for the detection of *Plasmodiophora brassicae* in soil. J Phytopathol 147:83–88

Johnson R (1984) A critical analysis of durable resistance. Annu Rev Phytopathol 22:309–330

Johnston TD (1970) A new factor for resistance to club root in *Brassica napus* L. Plant Pathol 12:156–158

Jones DR, Igram DS, Dixon GR (1982a) Characterization of isolates derived from single resting spores of *Plasmodiophora brassicae* and studies of their interaction. Plant Pathol 31:239–246

Jones DR, Igram DS, Dixon GR (1982b) Factors affecting tests for differential pathogenicity in populations of *Plasmodiophora brassicae*. Plant Pathol 31:229–238

Jubault M, Hamon C, Gravot A, Lariagon C, Delourme R, Bouchereau A, Manzanares-Dauleux MJ (2008) Differential regulation of root arginine catabolism and polyamine metabolism in clubroot-susceptible and partially resistant *Arabidopsis* genotypes. Plant Physiol 146:2008–2019

Kamei A, Tsuro M, Kubo N, Hirai M (2007) QTL analysis of clubroot resistance in radish (*Raphanus sativus* L.). In: Proceedings of the 111th meeting of the Japanese Society of Breeding. Breed Res, vol 9, p 83

Kaneko Y, Kimizuka-Takagi C, Bang SW, Matsuzawa Y (2007) Radish. In: Kole C (ed) Genome mapping and molecular breeding in plants, vol 5, Vegetables. Springer-Verlag, Berlin, pp 141–160

Karling JS (1968) The plasmodiophorales. Hafner Publishing, New York

Kitamura S (1958) Cultivars of radish and their change. In: Nishiyama I (ed) Japanese radish. Japan Society for the Promotion of Science, Tokyo, pp 1–19

Kobelt P, Siemens J, Sacristan MD (2000) Histological characterisation of the incompatible interaction between *Arabidopsis thaliana* and the obligate biotrophic pathogen *Plasmodiophora brassicae*. Mycol Res 104:220–225

Kroll TK, Lacy GH, Moore LD (1983) A quantitative description of the colonization of susceptible and resistant radish plants by *Plasmodiophora brassicae*. Phytopathol Z 108:97–105

Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica 98:149–154

Kuginuki Y, Yoshikawa H, Hirai M (1999) Variation in virulence of *Plasmodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Eur J Plant Pathol 105:327–332

Lagercrantz U (1998) Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. Genetics 150:1217–1228

Lammerink J (1964) Pathologic specialisation of *Plasmodiophora brassicae* Wor. NZ J Agric Res 7:37–41

Lammerink J (1969) Inter-specific transfer of clubroot resistance from *Brassica campestris* L. to *B. napus* L. NZ J Agric Res 13:105–110

Landry BS, Hubert N, Crête R, Chiang MS, Lincoln SE, Etoh T (1992) A genetic map for *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin). Genome 35:409–420

Ludwig-Müller J, Bennett RN, Kiddle G, Ihmig S, Rüppel M, Hilgenberg W (1999) The host range of *Plasmodiophora brassicae* and its relationship to endogenous glucosinolate content. New Phytol 141:443–458

Macfarlane I (1955) Variation in *Plasmodiophora brassicae* Woronin. Ann Appl Biol 43:297–306

Manzanares-Dauleux MJ, Divaret I, Baron F, Thomas G (2000) Evaluation of French *Brassica oleracea* landraces for resistance to *Plasmodiophora brassicae*. Euphytica 113:211–218

Massie IH, Astley D, King GJ (1996) Patterns of genetic diversity and relationships between regional groups and populations of Italian landrace cauliflower and broccoli (*Brassica oleracea* L. var. *botrytis* L. and var. *italica* Plenck). Acta Hortic 407:45–54

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage *(Brassica rapa* ssp. *pekinensis*). Euphytica 104:79–86

Matsumoto E, Hayashida N, Sakamoto K, Ohi M (2005) Behavior of DNA markers linked to a clubroot resistance gene in segregating populations of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). J Jpn Soc Hortic Sci 74:367–373

Melville SC, Hawken RH (1967) Soil testing for club root in Devon and Cornwall. Plant Pathol 16:145–147

Mithen R, Magrath R (1992) A contribution to the life history of *Plasmodiophora brassicae*: secondary plasmodia development in root galls of *Arabidopsis thaliana*. Mycol Res 96:877–885

Monteiro AA, Williams PH (1989) The exploration of genetic resources of Portuguese cabbage and kale for resistance to several *Brassica* diseases. Euphytica 41:215–225

Morgner M (1995) Mikroskopische Untersuchungen der Reaktionen empfindlicher und resistenter Genotypen verschiedener *Brassica*-Arten gegenüber *Plasmodiophora brassicae* (Wor.), dissertation, Institut für Angewandte Genetik, Freie Universität Berlin

Moriguchi K, Kimizuka-Takagi C, Ishii K, Nomura K (1999) A genetic map based on RAPD, RFLP, isozyme, morphological markers and QTL analysis for clubroot resistance in *Brassica oleracea*. Breed Sci 49:257–265

Murakami H, Tsushima S, Akimoto T, Murakami K, Goto I, Shishido Y (2000) Effects of growing leafy daikon (*Raphanus sativus*) on populations of *Plasmodiophora brassicae* (clubroot). Plant Pathol 49:584–589

Nikolaev SI, Berney C, Fahrni JF, Bolivar I, Polet S, Mylnikov AP, Aleshin VV, Petrov NB, Pawlowski J (2004) The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. Proc Natl Acad Sci USA 101:8066–8071

Osaki K, Tanaka S, Ito S (2008) Pathogenicity of *Plasmodiophora brassicae* populations from small, spheroid, resistant-type clubroot galls on roots of clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*). J Gen Plant Pathol 74:242–245

Piao ZY, Deng YQ, Choi SR, Park YJ, Lim YP (2004) SCAR and CAPS mapping of CRb, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Theor Appl Genet 108:1458–1465

Rehn F, Arbeiter A, Galfe N, Reinhardt S, Siemens J (2008) RPB1-mediated clubroot resistance in *Arabidopsis thaliana*. Plant Protect Sci 45:32 (Abstract)

Rocherieux J, Glory P, Giboulot A, Boury S, Barbeyron G, Thomas G, Manzanares-Dauleux MJ (2004) Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theor Appl Genet 108:1555–1563

Rouxel F, Cadiou M (1988) Estimation des populations de *Plasmodiophora brassicae* Wor. dans les sols. Agronomie 8:653–656

Saito M, Kubo N, Matsumoto S, Suwabe K, Tsukada M, Hirai M (2006) Fine mapping of the clubroot resistance gene *Crr3* in *Brassica rapa*. Theor Appl Genet 114:81–91

Sakai T, Liu HJ, Iwabuchi M, Kohno-Murase J (1996) Introduction of a gene from fertility restored radish (*Raphanus sativus*) into *Brassica napus* by fusion of X-irradiated protoplasts from a radish restorer line and iodacetoamide-treated protoplasts from a cytoplasmic male-sterile cybrid of *B. napus*. Theor Appl Genet 93:373–379

Sakamoto K, Saito A, Hayashida N, Taguchi G, Matsumoto E (2008) Mapping of isolate-specific QTLs for clubroot resistance in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Theor Appl Genet 117:759–767

Scheijgrond W, Vos H (1954) Investigations on the susceptibility to club root. Euphytica 3:125–139

Schoeller M (1987) Untersuchungen zur Resistenz von Kulturcruciferen gegen den Erreger der Kohlhernie, *Plasmodiophora brassicae* Wor., dissertation, Universität Hannover, Germany

Schoeller M, Grunewald J (1986) Production and characterization of single spore derived lines of *Plasmodiophora brassicae* Woron. Cruciferae Newsl 11:110–111

Siemens J, Keller I, Sarx J, Kunz S, Schuller A, Nagel W, Schmülling T, Parniske M, Ludwig-Müller J (2006) Transcriptome analysis of *Arabidopsis* clubroots indicate a key role for cytokinins in disease development. Mol Plant Microbe Interact 19:480–494

Snogerup S (1980) The wild forms of *Brassica oleracea* group ($2n = 18$) and their possible relations to the cultivated ones. In: Tsunoda S, Hinata K, Gomez Campo C (eds) Brassica crops and wild allies: biology and breeding. Japan Scientific Societies Press, Tokyo, pp 121–132

Somé A, Manzanares MJ, Laurens F, Baron F, Thomas G, Rouxel F (1996) Variation for virulence on *Brassica napus* L. amongst *Plasmodiophora brassicae* collections from France and derived single-spore isolates. Plant Pathol 45:432–439

Strelkov SE, Tewari JP, Hartmann M, Orchard D (2005) Clubroot on canola in Alberta in 2003 and 2004. Can Plant Dis Surv 85:72–73

Strelkov SE, Manolii VP, Cao T, Xue S, Hwang SF (2007) Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta. Can J Phytopathol 155:706–712

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Fujimura M, Nunome T, Fukuoka H, Matsumoto S, Hirai M (2003) Identification of two loci for resistance to clubroot (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. Theor Appl Genet 107:997–1002

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M, Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319

Tanaka S, Hiroaki Mido H, Ito S (2006) Colonization by two isolates of *Plasmodiophora brassicae* with differing pathogenicity on a clubroot-resistant cultivar of Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*). J Gen Plant Pathol 72:205–209

Thompson KF (1976) Cabbages, Kales, etc. In: Simmonds NW (ed) Evolution of crop plants. Longman Group Limited, London, pp 49–52

Thuma BA, Rowe RC, Madden LV (1983) Relationships of soil temperature and moisture to clubroot (*Plasmodiophora brassicae*) severity on radish in organic soil. Plant Dis 67:758–762

Tjallingii F (1965) Testing clubroot-resistance of turnips in the Netherlands and the physiologic specialization of *Plasmodiophora brassicae*. Euphytica 14:1–22

Tommerup IC, Ingramm DS (1971) The life-cycle of *Plasmodiophora brassicae* Wor. in Brassica tissue cultures and in intact roots. New Phytol 70:327–332

Toxopeus H, Jansen AMP (1975) Clubroot resistance in turnip II. The 'slurry' screening method and clubroot races in the Netherlands. Euphytica 24:751–755

Toxopeus H, Dixon GR, Mattusch P (1986) Physiological specialization in *Plasmodiophora brassicae*: an analysis by international experimentation. Trans Br Mycol Soc 87:279–287

Tsunoda S (1980) Eco-physiology of wild and cultivated forms in *Brassica* and allied genera. In: Tsunoda S, Hinata K, Gomez-Campo C (eds) Brassica crops and wild allies. Japan Scientific Societies Press, Tokyo, pp 109–132

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilisation. Jpn J Bot 7:389–452

Vanderplank JE (1978) Genetic and molecular basis of plant pathogenesis. Springer-Verlag, Berlin

Voorrips RE (1992) Root hair infection by *Plasmodiophora brassicae* in clubroot resistant and susceptible genotypes of *Brassica oleracea*, *B. rapa* and *B. napus*. Neth J Plant Pathol 98:361–368

Voorrips RE (1995) *Plasmodiophora brassicae*: aspects of pathogenesis and resistance in *Brassica oleracea*. Euphytica 83:139–146

Voorrips RE (1996) Production, characterization and interaction of single-spore isolates of *Plasmodiophora brassicae*. Eur J Plant Pathol 102:377–383

Voorrips RE, Visser DL (1993) Examination of resistance to clubroot in accessions of *Brassica oleracea* using a glasshouse seedling test. Neth J Plant Pathol 99:269–276

Voorrips RE, Jongerius MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor Appl Genet 94:75–82

Walker JC, Larson RH (1948) Development of clubroot resistance in cabbage. Phytopathology 43:28

Wallenhammar AC (1996) Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol 45:710–719

Wallenhammar AC, Johnsson L, Gerhardson B (2000) Agronomic performance of partly clubroot-resistant spring oilseed turnip rape lines. J Phytopathol 148:495–499

Webb PCR (1949) Zoosporangia, believed to be those of *Plasmodiophora brassicae*, in the root hairs of non-cruciferous plants. Nature 163:60

Weisaeth G (1961) Zur Züchtung hernieresistenten Weißkohls II. Prüfung auf Resistenz und Leistung von Inzuchtlinien aus mitteleuropäischen resistenten Herkünften. Z Pflanzenzüchtung 46:20–45

Weisaeth G (1977) Hodelkålsortene 'Respla' og 'Resista' foredlinger med klumprotresistens. Gartneryrket 67:937–939

Werner S, Diederichsen E, Frauen M, Schondelmaier J, Jung C (2008) Genetic mapping of clubroot resistance genes in oilseed rape. Theor Appl Genet 116:363–372

Williams PH (1966) A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56:624–626

Williams PH, Walker JC (1963) Races of clubroot in North America. Plant Dis Rep 47:608–611

Williamson CJ, Bradshaw JE (1989) Sources of resistance to *Plasmodiophora brassicae* used in breeding programmes at SCRI. XIIth Eucarpia Congress. Vort Pflanzenz 15:17/12

Wit F (1964) Inheritance of reaction to clubroot in turnips. Hortic Res 5:47–49

Xing GM, Long MH, Tanaka S, Fujieda K (1989) Clubroot resistance in Brassicoraphanus (hybrid of cabbage × radish). Plant Dis 33:189–194

Xue S, Cao T, Howard RJ, Hwang SF, Strelkov SE (2008) Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. Plant Dis 92:456–462

Yamagishi H (1987) Cultivation of clubroot-resistant strains to suppress population density of resting spores of *Plasmodiophora brassicae* in the soil. Jpn Agric Res Q 21:36–41

Yamagishi H, Yoshikawa H, Ashizawa M, Hida K, Yui S (1986) Effects of resistant plants as a catch crop on the reduction of resting spores of clubroot (*Plasmodiophora brassicae* Worn.) in soil. J Jpn Soc Hortic Sci 54:460–466

Yoshikawa H (1993) Studies on breeding of clubroot resistance in cole crops. Bull Natl Res Inst Veg Ornam Plants Tea Jpn Ser A 7:1–165

Yoshikawa H, Buczacki ST (1978) Clubroot in Japan: research and problems. Rev Plant Pathol 57:253–257