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Analyses of virulence of
European isolates of clubroot
(*Plasmodiophora brassicae* Wor.)
and mapping of resistance genes
in rapeseed (*Brassica napus* L.)



Dissertationen aus dem Julius Kühn-Institut

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Analyses of virulence of European isolates of clubroot (*Plasmodiophora brassicae* Wor.) and mapping of resistance genes in rapeseed (*Brassica napus* L.)

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and Environmental Management

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Abbreviations

AFLP	amplified length polymorphism
BC	backcrossed
cM	centiMorgan
CTAB	cetyltrimethylammonium bromide
cv.	cultivar
DH	doubled haploid
DI	disease index
DNA	deoxyribonucleic acid
ECD	European Clubroot Differential
EU	European Union
LOD	logarithm of odds
MAF	minor allele frequency
MAS	marker-assisted selection
MQM	multiple QTL mapping
OPA	oligo pool assay
OSR	oilseed rape
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
SCAR	sequence characterised amplified region
SNP	single nucleotide polymorphism
SSR	simple sequence repeat
var.	variety

1 Abstract

Clubroot caused by the obligate biotrophic protist *Plasmodiophora brassicae* is a serious soil-borne disease of cruciferous crops including oilseed rape (*Brassica napus* L.). Physiological specialisation of pathogen populations causes differences in pathogenicity, rendering breeding for resistance difficult. Therefore, it is important to get more detailed information on the virulence of *P. brassicae* in Europe. Samples of infected plants were collected all over the main European oilseed rape growing regions and forty-eight isolates were characterised under greenhouse conditions by artificial inoculation on the European Clubroot Differential (ECD) series and the differential set of Somé et al. (1996) followed by optical rating of disease symptoms. In total, 33 different ECD triplet codes were detected of which classifications '16/14/31', '16/31/31' and '17/31/31' were most common. Based on results obtained on the differentials of Somé et al. (1996) P1 is the prevalent pathotype on oilseed rape fields in the maritime region of Northern Europe whereas P3 was most frequently detected in the continental part of Europe. As breeding for resistance is the most powerful tool to control clubroot, broadening of the genetic basis of resistance in oilseed rape is needed. Therefore, clubroot resistances derived from two rutabaga (*Brassica napus* var. *napobrassica*) varieties, i.e., 'Invitation' and 'Wilhelmsburger', were genetically mapped in doubled haploid (DH) populations of crosses to the susceptible oilseed rape (*Brassica napus* L.) cultivar 'Ladoga'. The DH populations were analysed for resistance against two *P. brassicae* isolates showing different virulence patterns in the greenhouse. The segregation ratios indicated the effectiveness of one, two and three resistance genes, respectively, conferring resistance in these DH populations depending on the *P. brassicae* isolate used. Studies on F₁ plants give hint to dominant resistance genes in both donor lines located on chromosomes A03, A05 and A08.

2 General Introduction

2.1 Origin, breeding, cultivation and use of oilseed rape (*Brassica napus* L.)

Oilseed rape (*Brassica napus* L.) is the most widely grown crop species from the crucifer family (FAOSTAT 2014, <http://www.fao.org/faostat/en/#data/QC>). Two subspecies of *B. napus* are grown, i.e., rutabaga (*B. napus* var. *napobrassica*) and *B. napus* var. *napus*, comprising winter and spring oilseed rape as well as forage and vegetable rape forms. The species originated from spontaneous interspecific hybridisation between turnip rape (*B. rapa* L.) and cabbage (*B. oleracea* L.). This resulted in an amphidiploid species including the complete genomes of its two progenitors (Fig. 1).

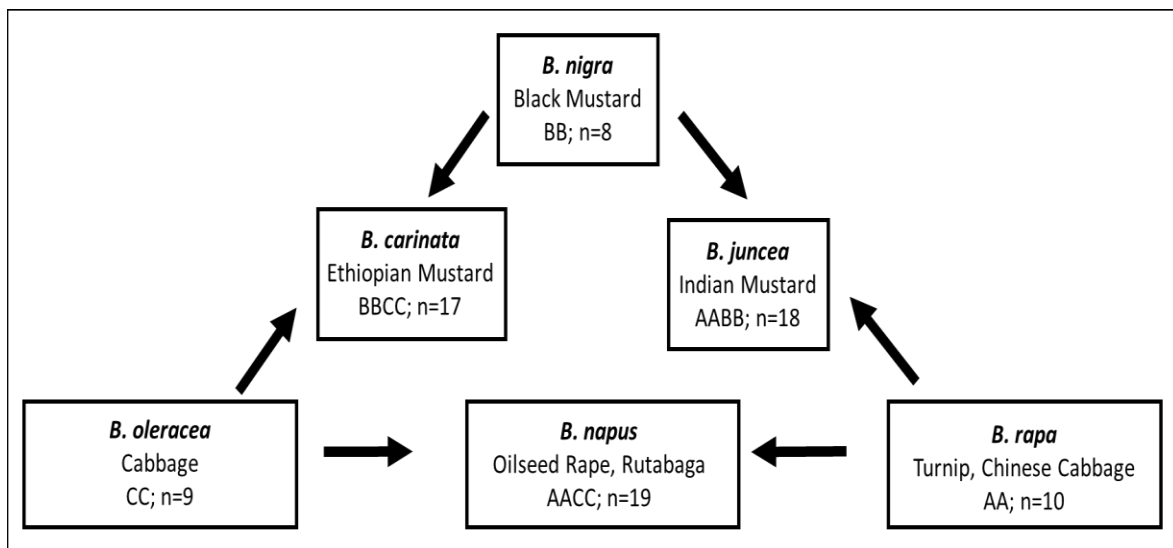


Fig. 1: Relationship between species of the Brassicaceae family and their respective genomes according to the triangle of U (U 1935, modified).

Brassica vegetables and oilseeds have been systematically cultivated for a very long time. Rutabaga beets were already cultivated during the 1st centuries anno Domini and by the 16th century rapeseed was the most important source for lamp oil in Europe (Snowdon et al. 2007). Consequently, during the 18th century rape seed acreage increased and today, oilseed rape is the second widely grown oilseed crop in Europe and worldwide (FAOSTAT 2014, <http://www.fao.org/faostat/en/#data/QC>). Globally, oilseed rape is mainly grown in temperate areas with the largest acreage in Canada, China and Europe. The global production of oil seed rape is about 70 million tons per year of which one third is produced in the European Union (EU; FAOSTAT 2014, <http://www.fao.org/faostat/en/#data/QC>). The

annual rape seed acreage in the EU is slightly above 6 million hectares, whereof France, Germany, Poland and the United Kingdom cover the largest rape seed acreage (Fig. 2).

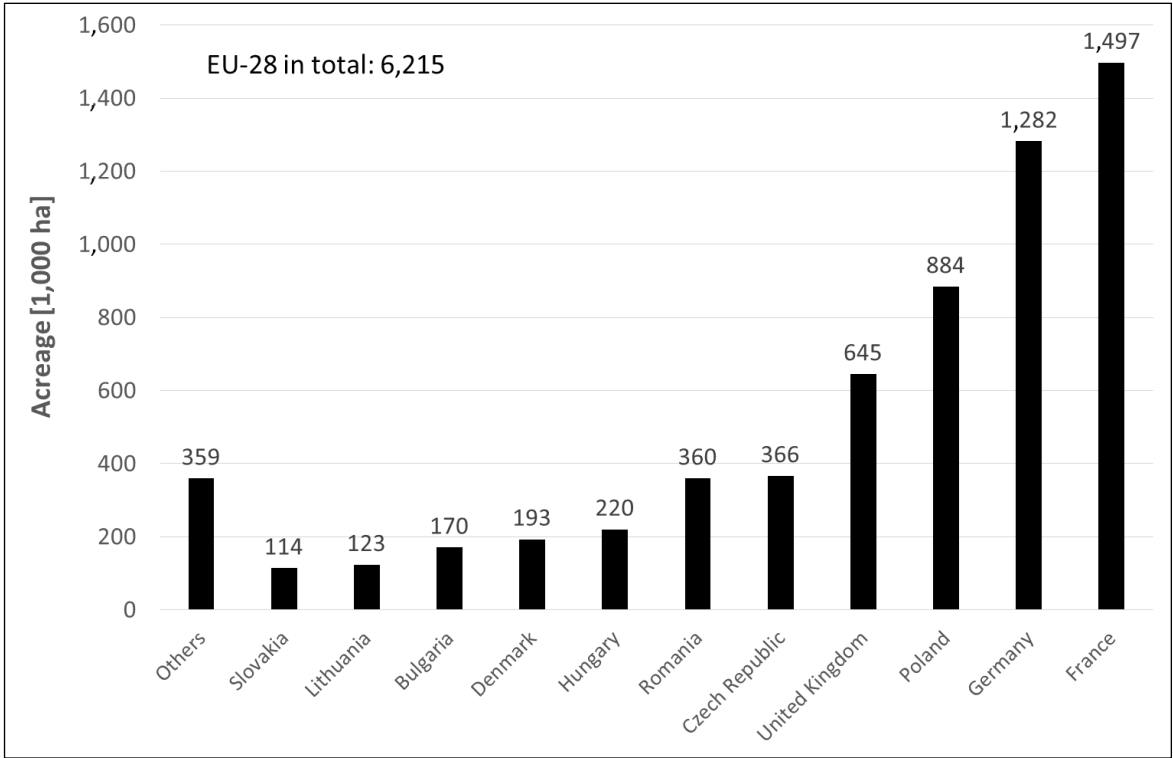


Fig. 2: Acreage of winter oilseed rape in the European Union (EU-28) by country in 2015 (modified after EUROSTAT 2016).

Rapeseed oil today is mainly used for food but also for bioenergy and industrial purposes. Oilseed rape has become a major crop for food during the past decades (Lühs and Friedt 1994; Dusser 2007) due to the reduction of the high erucic acid content, which caused a bitter taste and led to health problems. Furthermore, a strong reduction of glucosinolates facilitated the use of crushing residuals, i.e., the extraction meal or oil cake as a livestock feed. This was achieved in the 1970ies and 1980ies, and respective cultivars were introduced in the Canadian and European market (Brauer and Roebbelen 1989). These achievements in breeding for improved oil and meal quality has been the prerequisite for a global increase in the acreage of oilseed rape (Brauer and Roebbelen 1989). During the 1st decades of “00-Quality” breeding, noteworthy yield increases were obtained by conventional line breeding. But, as considerable heterosis for seed yield in F₁ hybrids of oilseed rape (*B. napus*) has been reported (Paulmann and Roebbelen 1988), two hybridisation systems for fully restored hybrids in winter oilseed rape were developed in Europe. On the one hand the Ogura system

created by INRA France (Bonhomme et al. 1992) and on the other hand the MSL system developed by the plant breeding company NPZ Lembke in Germany (Paulmann and Frauen 1997). Eventually, the first fully restored hybrid cultivar of winter oilseed rape was registered in 1995 in Germany (Frauen and Paulmann 1999; Frauen 2001; Mestries 2001). Since that time, numerous new hybrid cultivars have been developed using one or the other system and meanwhile hybrid cultivars are grown on the majority of oilseed rape (OSR) fields in Europe. As pests and diseases are increasing threats for OSR, several resistances have been introduced into rape seed during recent years, e.g., resistance to *Leptosphaeria maculans*, causal agent of blackleg, and *Plasmodiophora brassicae*, causing clubroot (for details about resistance against clubroot present in oilseed rape cultivars see chapter 2.5).

2.2 *Plasmodiophora brassicae* Woronin

In 1878, the causal agent of clubroot disease was identified and described as *Plasmodiophora brassicae* Woronin by the Russian biologist M. S. Woronin in St. Petersburg (Woronin 1878; Honig 1931; Dixon 2009a). The obligate biotrophic protist *Plasmodiophora brassicae*, a soil-borne pathogen infesting all cruciferous crops including turnips and chinese cabbage (*B. rapa*), oilseed rape and rutabaga (*B. napus*) as well as vegetables like cabbage, sprouts and cauliflower (*B. oleracea*; Diederichsen et al. 2009). Clubroot is one of the economically most important diseases of Brassica crops worldwide (Piao et al. 2009). Biflagellate zoospores of the pathogen infect susceptible host plants through root hairs (Fig. 3).

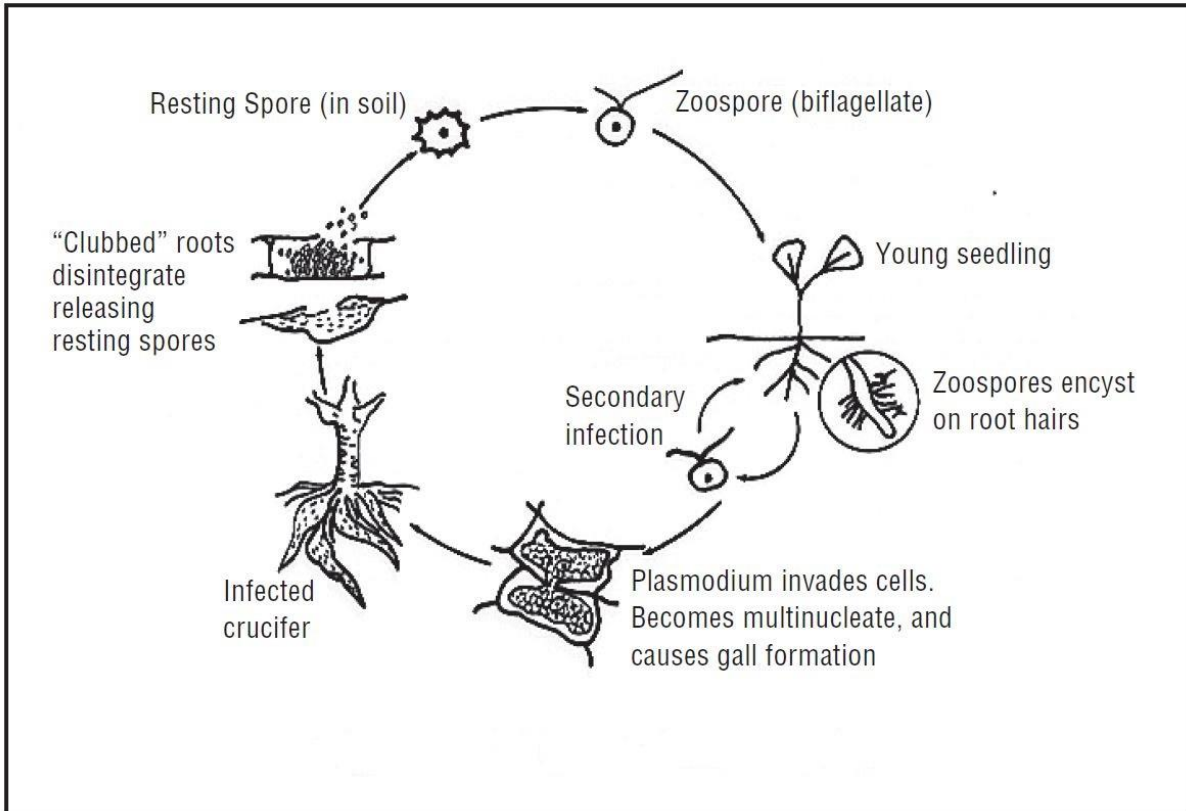


Fig. 3: Life cycle of *Plasmodiophora brassicae* Wor., the causal agent of clubroot disease on oilseed rape (*Brassica napus* L.) and other crucifers according to Miller et al. (1996), modified.

Once in the tissue, zoospores encyst and stimulate abnormal cell enlargement and uncontrolled cell division in infected roots (Gao et al. 2014), resulting in typical disease symptoms as swellings and the formation of root galls (Fig. 4). Consequences are inhibitions on uptake of water and nutrients leading to premature death of the diseased plants (Voorrips 1995) or at least to significant yield losses (Wallenhammar 1998). Once a plant is infected, numerous spores of *P. brassicae* are produced in the clubbed roots. As these tissues decay, resting spores are released into the soil where they remain and infect further host plants (Miller et al. 1996). The longevity of resting spores in the soil is about 20 years (Wallenhammar 1996).



Fig. 4: Symptoms of clubroot disease caused by the obligate biotrophic protist *P. brassicae* in four different developmental stages on roots of oilseed rape (*B. napus* L.) grown under field conditions. Beginning of gall formation on the secondary root system after clubroot infection (A); severe clubroot infestation with relevant galling on the secondary root system (B); strong clubroot infestation with galls on the primary and secondary root system (C); final stage of the infection: the root system has transformed to roundly covering clubroot galls, which start to decay thereby release clubroot resting spores to the environment (D); photos provided by INRA, France (2009).

The spread of the pathogen is initially limited but occurs most frequently via the movement of infested plant material or soil, typically on farm equipment. But, contaminated soil is also moved by wind and water. Additionally, birds or wild animals can serve as a source of infestation of nearby fields causing outbreaks of disease in areas where susceptible crops are planted for the first time (Rennie et al. 2015; Chai et al. 2016).

2.3 Clubroot occurrence and importance for European oilseed rape production

Clubroot is economically relevant in European agriculture and horticulture since the cultivation of host plants started. The pathogen has scattered around the globe by settlers during times of colonisation along with infected transplants (Kuginuki et al. 1999; Dixon 2009a). Meanwhile the disease is present in all areas in which Brassica species are grown, and already by 1950 clubroot was recognised as a major reason for losses in cruciferous vegetables on all continents (Dixon 2009a). During recent years, the importance of clubroot in oilseed rape (*B. napus*) increased along with rising cropping intensity, shorter crop rotations (Dixon 2009a) and a considerable increase of Brassica oilseed acreage (Diederichsen et al. 2009), which was ten times higher in the European Union in 2011 than in Europe 50 years ago (FAOSTAT 2014, <http://www.fao.org/faostat/en/#data/QC>). Clubroot may lead to a total destruction of crops but even in case of fragmentary presence it is affecting yield seriously by a reduction of seed number and oil quality, respectively (Dixon 2009a). Due to the fact, that today one third of the global OSR production derives from the European Union (FAOSTAT 2014, <http://www.fao.org/faostat/en/#data/QC>), the area of cultivation tends to be more and more limited due to the spread of clubroot (Diederichsen et al. 2009). Therefore, clubroot is an important disease in Europe.

Hot spots of clubroot infestation in European OSR growing regions are located in the United Kingdom (Dixon 1999; McGrann et al. 2016), France (Rouxel et al. 1983) and Northern Germany (Fig. 5; Lüders et al. 2011; Diederichsen 2013; Strehlow et al. 2014). But, even in Central and Southern Germany and in other European countries, i.e., Sweden, Poland and the Czech Republic the number of infested fields has increased considerably during the last years (Wallenhammar et al. 2014; Řičařová et al. 2016a; 2016b; Zamani-Noor 2016a). This is a difficult situation for growers as on the one hand a big request for rapeseed oil is present and on the other hand the pathogen limits the spread of OSR to those areas where clubroot is present (Diederichsen et al. 2009). Therefore, resistant cultivars are a prerequisite to ensure rapeseed production (Dixon 2009a).

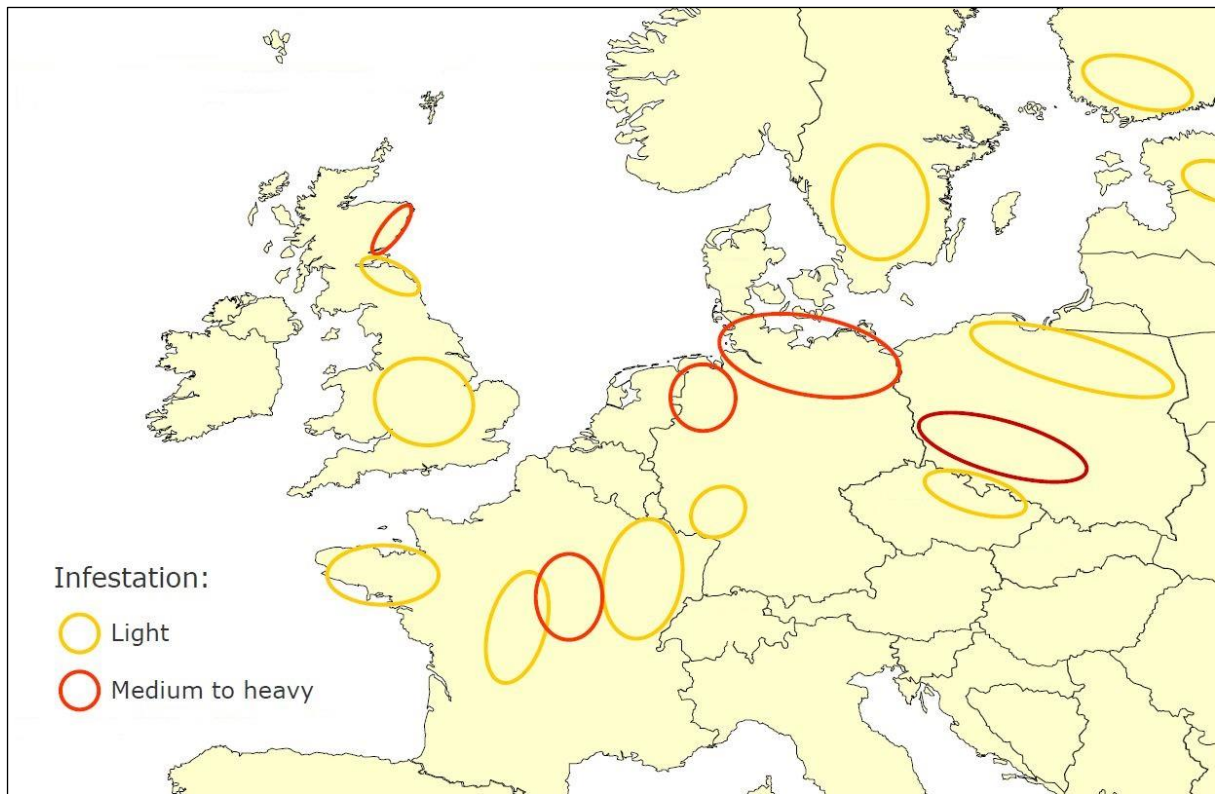


Fig. 5: Estimation of clubroot infestation in European oilseed rape cultivation regions according to Diederichsen (2013), modified. *Yellow rings* indicate areas with light infestations. *Red circles* indicate medium to heavy infestations in the respective area.

2.4 Control of clubroot spread

Agricultural control means like liming with calcium carbonate or the use of calcium cyanamide may reduce the inoculum potential thereby reducing yield losses (Dixon and Wilson 1983; Neuweiler et al. 2009; Zamani-Noor 2016b), but cannot eliminate clubroot from contaminated fields (Tremblay et al. 2005; McGrann et al. 2016). Efficient pesticides are not available or are too expensive for use in large-scale oilseed rape production (Donald and Porter 2009; Strelkov et al. 2011). Reducing the spread by longer crop rotations is only of theoretical relevance because the longevity of resting spores in the soil is about 20 years (Wallenhammar 1996). Therefore resistance is still the most powerful tool for combating clubroot disease and to minimise yield losses (Strelkov et al. 2006; Diederichsen et al. 2009; Yu et al. 2013; Gao et al. 2014).

2.5 Plant resistance against clubroot present in oilseed rape cultivars

The winter-type hybrid cultivar ‘Mendel’ carrying a race-specific clubroot resistance originating from resynthesised *B. napus* (Diederichsen et al. 2009) was the first resistant cultivar released in Germany in 2002 (Anonymous 2013). Since that time, the cultivar ‘Mendel’ itself was used as a source for resistance in many breeding programmes.

Table 1: Hybrid cultivars of winter oilseed rape carrying race-specific clubroot resistance registered in the European Union

No.	Variety	Breeder	Country and Year of Admission ^a
1	Mendel	NPZ	The United Kingdom 2001; Germany 2002; Luxembourg 2007
2	Cracker	NPZ	The United Kingdom 2009
3	Andromeda	Limagrain	Germany 2012
4	Mendelson	NPZ	Denmark 2012; The United Kingdom 2012; Luxembourg 2013
5	SY Alister	Syngenta	Germany 2012; The United Kingdom 2012; Poland 2014
6	Mentor	NPZ	Germany 2014; Denmark 2014; Estonia 2016
7	PT235	Pioneer	Denmark 2014; The United Kingdom 2014
8	PT242	Pioneer	Germany 2014; Denmark 2014
9	Archimedes	Limagrain	Denmark 2015; The United Kingdom 2015; Poland 2016
10	Menhir	NPZ	Germany 2015; The United Kingdom 2015
11	SY Alistorm	Syngenta	Poland 2015
12	Aristoteles	Limagrain	Denmark 2016
13	DK Platinum	Monsanto	Poland 2016
14	SY Alibaba	Syngenta	France 2016
15	Croquet	NPZ	France 2016

^a data from the Plant variety database of the European Commission (http://ec.europa.eu/food/plant/plant_propagation_material/plant_variety_catalogues_databases/search/public/index.cfm?event=SearchVariety&ctl_type=A&species_id=238&variety_name=&listed_in=0&show_current=on&show_deleted=).

Consequently, from 2009 to 2016 approximately a dozen of clubroot resistant winter-type hybrid cultivars (e.g., 'SY Alister', 'Mentor' and 'Archimedes'), which exhibit similar race-specific resistance have been released and widely grown on infested fields in Europe (Diederichsen et al. 2014; Table 1). During the same period several clubroot resistant spring-type canola hybrids (e.g., '73-67RR', 'VR9562GC', '45H29' and '6076CR') based on the same donor have been released in Canada (Rahman et al. 2011; 2014; Hirani et al. 2016).

2.6 Pathotypes overcoming the race-specific clubroot resistance present in oilseed rape

The presence of different physiological races or pathotypes of *P. brassicae* was first reported by Honig (1931) and was supported by Walker (1942). The occurrence of different pathotypes of *P. brassicae* overcoming the resistance of present cultivars is a big challenge in breeding for resistance to clubroot and disease management (Donald et al. 2006; Werner et al. 2008). Virulent clubroot isolates overcoming the resistance of cv. 'Mendel' were already known before this hybrid cultivar was released, and after more than a decade of cropping the frequency of these pathotypes has increased considerably. Mostly, virulent isolates were detected in North-Eastern Germany, but have been identified also in Denmark, Poland, in the UK and, more recently, in Western Canada (Diederichsen et al. 2014; Damsgaard Thorsted and Cordsen Nielsen 2016; Strelkov et al. 2016). The rapid break-down of race-specific resistances is also known for other oilseed rape infecting pathogens, e.g., *Leptosphaeria maculans* (Carpezat et al. 2014).

2.7 Molecular markers and their relevance for plant breeding

Molecular marker techniques have revolutionised plant breeding methods in recent decades (Snowdon and Friedt 2004). One of the main uses of DNA markers has been the construction of linkage maps in order to identify chromosomal regions carrying loci for agronomically important genes and use respective DNA polymorphisms linked to these genes in marker based selection procedures (Collard et al. 2005). Studies on marker-assisted approaches in *B. napus* started in the late 1980s with the development of RFLP (restriction fragment length polymorphism) linkage maps (Landry et al. 1991) and went on with randomly amplified

polymorphic DNA markers (RAPD; Williams et al. 1990), amplified fragment length polymorphisms (AFLP; Vos et al. 1995) followed by simple sequence repeat markers (SSR; Grist et al. 1993). Simple sequence repeat markers, also known as microsatellites, were of special importance because of a high level of polymorphism, high reproducibility and - for the first time – amenability to automation (Mammadov et al. 2012). The next big step towards efficient marker development was the introduction of single nucleotide polymorphism (SNP) markers along with improvements through automated detection, DNA-chip techniques and high-throughput technology (Snowdon and Friedt 2004; Mammadov et al. 2012). As a consequence of technological progress an integrated high density genetic map for oilseed rape (*B. napus*), polymorphic in various genetic backgrounds, has been developed (Delourme et al. 2013) facilitating effective marker-assisted breeding. In breeding of Brassica oilseeds molecular methods have been widely used to map loci for oil content and quality, for abiotic stress tolerance, for male sterility and morphological traits (Snowdon and Friedt 2004; Raman et al. 2013). Respective markers offer the possibility to trace resistance genes in an easier and more efficient way compared to conventional plant breeding approaches (Moulet et al. 2008). Chalhoub et al. (2014) sequenced the genome of *Brassica napus* rendering marker-assisted breeding even more efficient.

2.8 Major goals of the present thesis

Clubroot caused by *P. brassicae* is a serious disease of oilseed rape with increasing importance as the number of infested fields in the European OSR cultivation regions has been constantly increasing during the last years. Breeding for clubroot resistance is the most effective way to control the disease. As different pathotypes that are able to overcome the resistance of present cultivars occur, there is a need to characterise *P. brassicae* pathotypes as well as to search for new resistance genes. Therefore, the objectives of the present thesis were:

1. Characterisation of *P. brassicae* pathotypes found in major European oilseed rape cultivation regions;
2. Characterisation and molecular mapping of new clubroot resistance genes and loci from genetic resources with a focus on the primary gene pool of *B. napus*.

3 A classification survey of *Plasmodiophora brassicae* pathotypes in the main regions of oilseed rape cultivation in Europe

3.1 Introduction

3.1.1 Identification of genetic variation within *P. brassicae* samples by differential sets

As plant genetic resources used for the introgression of clubroot resistance in Brassica species usually carry race-specific resistance genes, determining their efficiency against different pathotypes is of prime importance (Rahman et al. 2011). To achieve this, interactions between host genotypes and prevalent pathogen populations in different cropping regions need to be determined as a prerequisite for resistance breeding (Diederichsen et al. 2009). Such pathotype classifications usually are generated in tests under controlled greenhouse conditions using differential sets of host plants for clubroot disease. The primarily designed set for that reason, published by Williams (1966), defined theoretically 16 pathotypes and was composed of four differential lines, i.e., two lines each of cabbage (*B. oleracea*) and rutabaga (*B. napus* var. *napobrassica*). Since the 1980ies, the European clubroot differential (ECD) series by Buczacki et al. (1975) consisting of 15 genotypes subdivided into five lines each of three different species, i.e., *B. rapa*, *B. napus* and *B. oleracea*, has provided an internationally accepted method for the classification of *P. brassicae* populations (Donald et al. 2006). Therewith, differentiation of theoretically 48 pathotypes is possible by allocation of a 'triplet code' based on the susceptibility of the three host groups to one pathogen population. However, Somé et al. (1996) stated that the existing classification systems were not able to precisely distinguish between *P. brassicae* populations occurring in France, and therefore defined a new differential set of three *B. napus* lines that define eight different pathotypes called P1 to P8. Considerable studies about pathotype classification of *P. brassicae* populations related to different cruciferous host species and different geographical regions of the past decades are listed in Table 2. Ayers (1957) distinguished six pathotypes occurring in Canada and the USA according to their pathogenicity on two rutabaga lines (*B. napus* var. *napobrassica*), one cabbage line (*B. oleracea*) and accessions of *B. rapa*, *B. nigra* and *Sisymbrium altissimum*. Tjallingii (1965) found ten different pathotypes based on experiments with turnip samples (*B. rapa*) from fields in the Netherlands and Belgium.

Table 2: Major items and results of studies on *P. brassicae* pathotype classifications of different Brassica species using varying differential sets of host plants until 2016 in chronological order

No.	Author(s)	Year of Publication	Differential Set Used	No. of Isolates Collected	No. of Pathotype Designations Shown	Origin of Samples	Pathogen Material Extracted from		
							<i>Brassica rapa</i>	<i>Brassica oleracea</i>	<i>Brassica napus</i>
1	Ayers	1957	n/a	n/a	9	Canada, USA	-	X	X
2	Tjallingii	1965	n/a	n/a	9	The Netherlands, Belgium	X	X	X
3	Williams	1966	Williams	124	36	Australia, Asia, Europe, North America, New Zealand	X	X	X
4	Ayers	1972	Williams	160	68	Canada, USA	-	X	X
5	Dobson et al.	1983	ECD	13	13	USA	X	X	-
6	Toxopeus et al.	1986	ECD	n/a	299	Australia, Argentina, Europe, North America, New Zealand	X	X	X
7	Somé et al.	1996	Somé et al.	20	20	France	X	X	X
8	Kuginuki et al.	1999	Kuginuki et al.	36	4	Japan	X	-	-
9	Manzanares-Dauleux et al.	2001	Somé et al.	n/a	9	France	X	X	X
10	Scholze et al.	2002	ECD	42	10	Germany, Switzerland	X	X	X
11	Strelkov et al.	2006	ECD, Williams	9	9	Canada	-	X	X
12	Donald et al.	2006	ECD	41	23	Australia	X	X	-
13	Strelkov et al.	2007	ECD, Williams, Somé et al.	41	10	Canada	-	-	X
14	Osaki et al.	2008	Williams, modification of Kuginuki et al.	28	28	Japan	X	-	-
15	Cao et al.	2009	ECD, Williams, Somé et al.	n/a	17	Canada	-	X	X
16	Strelkov et al.	2016	ECD, Williams, Somé et al.	n/a	4	Canada	-	-	X
17	Řičařová et al.	2016a	ECD, Williams, Somé et al.	30	30	Czech Republic, Poland	-	-	X
18	Řičařová et al.	2016b	ECD, Williams	92	69	Czech Republic, Slovakia	-	X	-
19	Zamani-Noor	2016	ECD, Somé et al.	n/a	49	Germany	-	-	X

n/a: not applicable; X: yes; -: no

Nine pathotypes according to a clearly defined differential set were detected in samples derived from all over the globe (Williams 1966). Subsequently, Ayers (1972) confirmed the presence of six different pathotypes based on 68 samples from Canada and the USA. Dobson et al. (1983), Toxopeus et al. (1986) and Scholze et al. (2002) worked on *P. brassicae* samples from nearly all continents by using the ECD series and they found that the virulence of tested samples was low on *B. rapa* lines, diversifying on *B. napus* and very common on *B. oleracea* hosts. The most common virulence combination mentioned by Toxopeus et al. (1986) was the ECD triplet code '16/31/31', indicating limited virulence towards the *B. rapa* subset, but strong infections on all *B. napus* and *B. oleracea* hosts. Thirty-six and 28 *P. brassicae* samples from *B. rapa* collected in Japan were screened by Kuginuki et al. (1999) and Osaki et al. (2008), respectively. The authors stated that the differential hosts from Williams (1966) and the ECD could not be used to provide clear classifications of these populations and therefore defined a differential set on the basis of Chinese cabbage F₁ cultivars with clear resistance profiles distinguishing four pathotypes. In rapeseed, Somé et al. (1996) analysed 20 samples from diverse hosts in France and found that the most frequent pathotypes were P1 and P4. Manzanares-Dauleux et al. (2001) discovered variability within field samples of *P. brassicae* from France and underlined the importance of using single-spore isolates for virulence surveys and genetic studies. Recent classifications from Europe presented a predominance of Somé's pathotypes P1 and P3 in Germany and Poland whereas in the Czech Republic, depending on the cut-off point used to discriminate between a susceptible and a resistant host reaction, either P2 or P3 were most frequent (Zamani-Noor 2016a; Řičařová et al. 2016a). Canadian studies, mainly based on canola samples from Central Alberta, observed the presence of different pathotypes but, at the same time they pointed out the strong predominance of the single pathotype 3 of the differential set of Williams (Cao et al. 2009; Hwang et al. 2012; Sharma et al. 2013; Strelkov et al. 2006; 2007; 2016). This pathotype corresponds to P2 on the classification by Somé et al. (1996) and '16/15/12' on the ECD series. This observation correlates to a certain extent with the general statement about less diversity in pathogen populations in America and Australia compared to Europe as mentioned by Donald et al. (2006), who identified 23 ECD triplet codes from 41 pathogen samples mainly of *B. oleracea* and *B. rapa* collected in five states of Australia. The most common pathotypes in that study were '16/03/12' and '16/03/31', indicating that virulence

towards *B. rapa* and *B. napus* is limited whereas differential variation was observed in the reaction of *B. oleracea* hosts.

3.1.2 The relevance of pathogen classifications as a prerequisite for resistance breeding

General statements about the geographic distribution of varying pathogenicity of *P. brassicae* are hardly possible for whole Europe as data from international surveys are very limited. But the identification of pathotypes present in the field is essential in order to predict which cultivars can be grown in clubroot infested areas (Donald et al. 2006). Therefore, the primary goal of the present study was to survey the occurrence of pathotypes in the main regions of OSR cultivation in Europe as a prerequisite of breeding for resistance (cf. Kuginuki et al. 1999). The second aim was to evaluate whether clubroot testing under controlled greenhouse conditions is representative of results under field conditions as described by Sharma et al. (2013) or if extensive field testing is necessary to determine functional resistance (Robak and Gabrielson 1988; Dixon 2009b) of plant genetic resources and differential hosts. In this study the terminology related with the pathogen is used as described by Strelkov et al. (2007). In this context the term ‘population’ refers to a collection of *P. brassicae* resting spores prepared from a mixture of clubroot galls of susceptible plants from one individual field and used to inoculate a set of differential hosts.

3.2 Material and Methods

3.2.1 Origin and sampling of the pathogen

During 2009 to 2012 numerous samples of infested root tissue of oilseed rape (OSR) crops were collected as summarised in Table 3. Two populations of *P. brassicae* originated from fields in the UK, two from Denmark, 20 from fields across Germany, one from Poland, four from the Czech Republic, three from Austria and five from France. Five additional populations were obtained from the collections of the Julius Kühn-Institut in Germany and six populations from the Institute of Plant Genetics of the Polish Academy of Sciences in Poznan, Poland. In total, 48 populations of *P. brassicae* were analysed. Actually, 42 samples were extracted from *B. napus* material, five from *B. oleracea* and one sample was taken from mustard roots (*Sinapis arvensis*). Clubroot populations were multiplied in the greenhouse on

artificially inoculated seedlings either of *B. rapa* var. *pekinensis* cv. 'Granaat' or *B. napus* var. *napus* cv. 'Ladoga'. The *P. brassicae* populations examined in the tests are named according to the municipality the field of origin belongs to. In case of having several populations from the identical municipality the place name of the field is added.

Table 3: Origin of *Plasmodiophora brassicae* populations from main European oilseed rape growing regions collected 2006 to 2012

No.	Country	State/Province/Region/ Voivodeship	<i>Plasmodiophora brassicae</i> Population	Host of Origin	Year of Sampling	Source of Sample
1	Austria	Upper Austria	Antiesenhofen	<i>Brassica napus</i>	2011	Blumenschein, F. (Saatzucht Donau)
2	Austria	Upper Austria	Baumgarten	<i>Brassica napus</i>	2011	Blumenschein, F. (Saatzucht Donau)
3	Austria	Upper Austria	Gurten	<i>Brassica napus</i>	2011	Blumenschein, F. (Saatzucht Donau)
4	Czech Republic	Moravia, Vysocina region	Krizanow	<i>Brassica napus</i>	2012	Marakova, M. (Limagrain)
5	Czech Republic	Moravian-Silesian region	Frychovice	<i>Brassica napus</i>	2011	Adler, M. (Limagrain)
6	Czech Republic	Moravian-Silesian region	Kozmice	<i>Brassica napus</i>	2010	Matus, J. (Limagrain)
7	Czech Republic	Moravian-Silesian region	Sedlnice	<i>Brassica napus</i>	2011	Adler, M. (Limagrain)
8	Denmark	Central Denmark	Flemming	<i>Brassica napus</i>	2010	Kristensen, K.H. (LRO Horsens)
9	Denmark	Southern Denmark	Vojens	<i>Brassica napus</i>	2010	Cordsen Nielsen, G./Olsen, F. (Videncentret)
10	France	Brittany	Paimpol	<i>Brassica oleracea</i>	2006	Henry, M. (Clause)
11	France	Brittany	St. Pol Du Léon	<i>Brassica oleracea</i>	2006	Henry, M. (Clause)
12	France	Centre	Oizon/Le Boulay	<i>Brassica napus</i>	2009	Leis, G. (Limagrain)
13	France	Centre	Oizon/Val Nère	<i>Brassica napus</i>	2011	Leis, G. (Limagrain)
14	France	Pays de la Loire	La Bohalle	<i>Brassica oleracea</i>	2010	Henry, M. (Clause)
15	Germany	Baden-Wuerttemberg	Mudau	<i>Brassica napus</i>	2009	Adam, J. (Limagrain)
16	Germany	Baden-Wuerttemberg	Schutterwald	<i>Brassica napus</i>	2011	Winter, M. (Agrartest)

Table 3 continued

No.	Country	State/Province/Region/ Voivodeship	<i>Plasmodiophora brassicae</i> Population	Host of Origin	Year of Sampling	Source of Sample
17	Germany	Bavaria	Altoetting	<i>Brassica napus</i>	2010	Mitterreiter, M. (AELF Rosenheim)
18	Germany	Bavaria	Eschenbach	<i>Brassica napus</i>	2010	Bremer, A. (Limagrain)
19	Germany	Bavaria	Niederhummel	<i>Brassica napus</i>	2010	Mayer, M. (Euralis)
20	Germany	Hesse	Beselich	<i>Brassica napus</i>	2011	Moeller, W. (LLH Limburg)
21	Germany	Hesse	Graevenwiesbach	<i>Brassica napus</i>	2011	Koehler, J.
22	Germany	Hesse	Ortenberg	<i>Brassica napus</i>	2000	Kraemer, R. and Malorny, M. (JKI Quedlinburg)
23	Germany	Lower Saxony	Balje (Nordkehdingen)	<i>Brassica napus</i>	2010	Dohms, S. (JKI Braunschweig)
24	Germany	Lower Saxony	Hattorf	<i>Brassica napus</i>	2010	Dohms, S. (JKI Braunschweig)
25	Germany	Lower Saxony	Lippoldshausen	<i>Brassica napus</i>	2011	Teuteberg, H.
26	Germany	Lower Saxony	Mielenhausen	<i>Brassica napus</i>	2011	Boettcher, K.
27	Germany	Lower Saxony	Oberode	<i>Brassica napus</i>	2011	Teuteberg, H.
28	Germany	Lower Saxony	Wohld	<i>Brassica napus</i>	2011	Veenker, H. (Hetterich Fieldwork)
29	Germany	Mecklenburg-Vorpommern	Altenkirchen	<i>Brassica oleracea</i>	unknown	Kraemer, R. and Malorny, M. (JKI Quedlinburg)
30	Germany	Mecklenburg-Vorpommern	Gross Schwiesow	<i>Brassica napus</i>	2009	Abel, S. (Limagrain)
31	Germany	Mecklenburg-Vorpommern	Warnitz	<i>Brassica napus</i>	2011	Waldschmidt (LALLF Schwerin)
32	Germany	Rhineland-Palatinate	Gondershausen	<i>Brassica napus</i>	2010	Preiss, U. (DLR Bad Kreuznach)
33	Germany	Rhineland-Palatinate	Niederrotterbach	<i>Brassica napus</i>	2006	Preiss, U. (DLR Bad Kreuznach)
34	Germany	Rhineland-Palatinate	Ravensbeuren	<i>Sinapis arvensis</i>	2006	Preiss, U. (DLR Bad Kreuznach)
35	Germany	Rhineland-Palatinate	Sayn	<i>Brassica napus</i>	2006	Preiss, U. (DLR Bad Kreuznach)
36	Germany	Schleswig-Holstein	Grossensee	<i>Brassica napus</i>	2010	Dabelstein, K. (Hetterich Fieldwork)

Table 3 continued

No.	Country	State/Province/Region/ Voivodeship	<i>Plasmodiophora brassicae</i> Population	Host of Origin	Year of Sampling	Source of Sample
37	Germany	Schleswig-Holstein	Hoisdorf	<i>Brassica napus</i>	2010	Dabelstein, K. (Hetterich Fieldwork)
38	Germany	Schleswig-Holstein	Marne	<i>Brassica oleracea</i>	1992	Kraemer, R. and Malorny, M. (JKI Quedlinburg)
39	Germany	Schleswig-Holstein	Tiebensee	<i>Brassica napus</i>	2009	Kadler, M. (Limagrain)
40	Poland	Greater Poland	Poznan	<i>Brassica napus</i>	2011	Jedryczka, M. (IGR Poznan)
41	Poland	Lower Silesian	Legnica	<i>Brassica napus</i>	2011	Jedryczka, M. (IGR Poznan)
42	Poland	Lower Silesian	Pielgrzymka	<i>Brassica napus</i>	2011	Andrzejewski, B. (Limagrain)
43	Poland	Lubusz	Zielona Góra	<i>Brassica napus</i>	2011	Jedryczka, M. (IGR Poznan)
44	Poland	Opole	Opole	<i>Brassica napus</i>	2010	Jedryczka, M. (IGR Poznan)
45	Poland	Warmian-Masurian	Olsztyn	<i>Brassica napus</i>	2010	Jedryczka, M. (IGR Poznan)
46	Poland	West Pomeranian	Szczecin	<i>Brassica napus</i>	2011	Jedryczka, M. (IGR Poznan)
47	Scotland	Aberdeen	Aberdeen	<i>Brassica napus</i>	2010	Muirhead, J./Booth, E. (SAC Aberdeen)
48	Scotland	Angus	Montrose	<i>Brassica napus</i>	2010	Anon. (Trials Force)

3.2.2 Preparation of pathogen inoculum

P. brassicae clubs were washed and stored at -25°C. Root galls were used freshly or thawed and were homogenised in distilled water in a blender to prepare spore suspensions. The spore suspension was filtered through gauze sieves of 25µm mesh size. Spores were washed three times with distilled water by centrifugation at 3.000 rpm and 4°C for 5 minutes, at 4.000 rpm and 4°C for 7 minutes and finally at 4.000 rpm and 4°C for 10 minutes. Using a Thoma haemocytometer resting spores were quantified and the final suspension was diluted to 0.8 x 10⁸ spores ml⁻¹.

3.2.3 Plant material of differential hosts

Two sets of differential hosts, including the ECD series as described by Buczacki et al. (1975) and the differentials of Somé et al. (1996), were used for pathotype differentiation (Table 4).

Table 4: Differential hosts used to identify *Plasmodiophora brassicae* pathotypes in Europe

Differential Host ^a	Species and Description	Origin of Seed Samples
ECD 01	<i>Brassica rapa</i> var. <i>rapa</i> line 'a'	University of Warwick, UK
ECD 02	<i>Brassica rapa</i> var. <i>rapa</i> line 'b'	University of Warwick, UK
ECD 03	<i>Brassica rapa</i> var. <i>rapa</i> line 'c'	University of Warwick, UK
ECD 04	<i>Brassica rapa</i> var. <i>rapa</i> line 'r'	University of Warwick, UK
ECD 05	<i>Brassica rapa</i> var. <i>pekinensis</i> cv. 'Granaat'	University of Warwick, UK
ECD 06	<i>Brassica napus</i> var. <i>napus</i> cv. 'Nevin'	University of Warwick, UK
ECD 07	<i>Brassica napus</i> var. <i>napus</i> cv. 'Giant Rape'	University of Warwick, UK
ECD 08	<i>Brassica napus</i> var. <i>napus</i> selection ex. 'Giant Rape'	University of Warwick, UK
ECD 09	<i>Brassica napus</i> var. <i>napus</i> New Zealand clubroot resistant rape	University of Warwick, UK
ECD 10	<i>Brassica napus</i> var. <i>napobrassica</i> cv. 'Wilhelmsburger'	University of Warwick, UK
ECD 11	<i>Brassica oleracea</i> var. <i>capitata</i> cv. 'Badger Shipper'	University of Warwick, UK
ECD 12	<i>Brassica oleracea</i> var. <i>capitata</i> cv. 'Bindsachsener'	University of Warwick, UK
ECD 13	<i>Brassica oleracea</i> var. <i>capitata</i> cv. 'Jersey Queen'	University of Warwick, UK
ECD 14	<i>Brassica oleracea</i> var. <i>capitata</i> cv. 'Septa'	University of Warwick, UK
ECD 15	<i>Brassica oleracea</i> var. <i>acephala</i> subvar. <i>laciniata</i> cv. 'Verheul'	University of Warwick, UK
Brutor	<i>Brassica napus</i> var. <i>napus</i> cv. 'Brutor' (spring oilseed rape)	Limagrain, France & IPK, Germany
Mendel	<i>Brassica napus</i> var. <i>napus</i> cv. 'Mendel' (winter oilseed rape)	NPZ, Germany
Clapton	<i>Brassica oleracea</i> var. <i>botrytis</i> cv. 'Clapton'	Syngenta, Germany
Ladoga	<i>Brassica napus</i> var. <i>napus</i> cv. 'Ladoga' (winter oilseed rape)	Limagrain, Germany

^a ECD 01 to ECD 15 denote the differential hosts of the European Clubroot Differential series of Buczacki et al. (1975); hosts ECD 06, ECD 10 and 'Brutor' represent the differential of Somé et al. (1996).

Two of the differentials, ECD 06 and ECD 10, are members of both series. The host *B. napus* var. *napus* cv. 'Brutor' is unique to the differential set of Somé et al. (1996). As suggested by Diederichsen et al. (2009) the differential sets were amended by two genotypes, i.e., *B. napus* var. *napus* cv. 'Mendel' and *B. oleracea* var. *botrytis* cv. 'Clapton'. These cultivars contain monogenic and therefore pathotype-specific clubroot resistances with very similar specificity (Diederichsen et al. 2009). Seeds of all ECD hosts were obtained from the University of Warwick, Genetic Resources Unit (Wellesbourne, Warwick, UK). Seeds of the host *B. napus* var. *napus* cv. 'Brutor' were purchased from Limagrains Europe (Verneuil L'Étang, France) and IPK (Gatersleben, Germany), respectively. The seeds of *B. napus* var. *napus* cv. 'Mendel' were provided by NPZ Lembke (Holtsee, Germany) and seeds of *B. oleracea* var. *botrytis* cv. 'Clapton' were purchased from Syngenta Seeds (Hillscheid, Germany), respectively.

3.2.4 Greenhouse tests for differential virulence

Greenhouse tests, disease assessments and calculation of the respective disease indexes were done according to Glory and Manzaneres-Dauleux (2005). For each combination of genotype x *P. brassicae* population two replicated tests were performed with ten plants per test, each. The various differential hosts were pre-germinated in bulks in potting soil (Einheitserde Classic, Gebr. Patzer, Sinntal, Germany) in a climate chamber at 20°C. After 3 to 4 days in the dark the seedlings were transplanted into 8 x 8 x 8 cm plastic pots at a rate of one seedling per pot (Fig. 6). The pots contained a steam-sterilised mixture of compost (20%-vol.), white peat (27%-vol.) and sand (53%-vol.). The pH of the substrate varied from 5.8 to 6.2. The insecticide granulate 'Exemptor®' (Bayer Crop Science, Germany) containing the active ingredient 'Thiacloprid' was added to the soil mixture at a rate of 400 g m⁻³ in order to protect the seedlings against soil and foliar pests, in particular aphids. The pots were placed in a greenhouse chamber at 18/23°C (night/day temperature), relative humidity of around 60% and a 16h photoperiod. Inoculation was performed 7 to 10 days after transplanting by pipetting 2ml of the spore suspension (0.8 x 10⁸ spores ml⁻¹) in a 10 to 20 mm deep hole in the soil that was pierced with a glass tip near the base of each seedling (Fig. 6).



Fig. 6: Clubroot resistance tests under greenhouse conditions. Young seedlings were planted in plastic pots (A) and, thereafter, inoculated with a suspension containing spores of *P. brassicae* at a concentration of 0.8×10^8 spores ml^{-1} (B); inoculated plants were incubated for seven weeks in the greenhouse chamber (C) and finally, the plants were removed from the pots and scored for clubroot on the roots (D).

After inoculation the pots were kept in jamming water at a height of 4 to 5 cm for 10 to 14 days in order to promote mobility of zoospores. After that, the soil moisture was maintained at field capacity by frequent watering. Seven weeks after inoculation the soil was washed off the roots with tap water. The degree of galling was assessed for each plant on a 5-staged rating scale (Fig. 7). Scoring stages from “0” to “3” are defined as follows: 0=no symptoms, 1=one or a few small galls on the secondary root system, 2= primary root system slightly affected and numerous galls present in the secondary root system, 2+=the primary root system is heavily affected but the extremities of the roots are still of regular shape or a new

root system was developed upstream of the galls, 3=all roots have disappeared and the root system was replaced by a clubroot gall.



Fig. 7: Classification of clubroot symptoms on oilseed rape plants in five stages according to Glory and Manzanares-Dauleux (2005).

Based on all disease severity scores obtained from both replications a disease index (DI) was calculated as follows:

$$DI = \frac{(0*n_0)+(25*n_1)+(50*n_2)+(75*n_{2+})+(100*n_3)}{n_0+n_1+n_2+n_{2+}+n_3}$$

In that formula, “n” is the number of plants in each class. The DI value may range from 0 (no galls) to 100 (all plants tested exhibit class 3 galls). A cut-off point of DI >25 was used to discriminate between virulent and avirulent reactions (Glory and Manzanares-Dauleux 2005). As a measure to eliminate implausible results, all data from trial series in which ‘ECD 05’ and ‘ECD 07’ were both rated with a final DI <50 were not taken into account due to an assumed inadequate level of infection. The characterisation of *P. brassicae* samples on the ECD series was executed as explained by Buczacki et al. (1975). The key information is the description of virulence patterns towards the three subsets of the ECD series, separately. Therefore, the virulence characteristics are expressed by so-called ‘triplet codes’ (e.g., ‘17/21/31’). The first pair of digits represents the virulence on the *B. rapa* subset, the second stands for virulence on the *B. napus* subset and the third digit pair indicates the *B. oleracea* subset, respectively. For that purpose, the differential hosts of each subset are arranged in a

fixed ascending order and to each line a binary and a denary (i.e., 01, 02, 04, 08, and 16) value is assigned (Table 5).

Table 5: European Clubroot Differential series: subsets, differential hosts and their respective binary and denary values according to Buczacki et al. (1975), modified

Subset	Differential host	Binary value	Denary value
<i>B. rapa</i>	ECD 01	2 ⁰	01
	ECD 02	2 ¹	02
	ECD 03	2 ²	04
	ECD 04	2 ³	08
	ECD 05	2 ⁴	16
<i>B. napus</i>	ECD 06	2 ⁰	01
	ECD 07	2 ¹	02
	ECD 08	2 ²	04
	ECD 09	2 ³	08
	ECD 10	2 ⁴	16
<i>B. oleracea</i>	ECD 11	2 ⁰	01
	ECD 12	2 ¹	02
	ECD 13	2 ²	04
	ECD 14	2 ³	08
	ECD 15	2 ⁴	16

If, for example, the five differential hosts of one subset are tested with a *P. brassicae* population and the first, second, third and fifth are susceptible (DI >25; Glory and Manzanares-Dauleux 2005) the value assigned to this host is summed as 1+2+4+16=23 (Buczacki et al. 1975). Therefore, 23 would be the “population value” describing the virulence of a distinct *P. brassicae* population against one of the ECD subsets. The population value is unique for each combination of denary value components. For instance, a *P. brassicae* population with very limited virulence to all subsets would be described as

'16/02/08' (in this case only the susceptible hosts 'ECD 05', 'ECD 07' and 'ECD 14' were diseased) whereas maximum virulence would be described as '31/31/31' (all hosts were strongly diseased).

3.2.5 Field test for clubroot resistance

As some host-pathogen reactions from the greenhouse seemed to be contradictory to observations in infested fields where the respective pathogen population was collected, a survey of field trials (Fig. 8) was carried out in order to compare greenhouse and field observations in an orthogonal scheme using the same set of differential hosts as described for the greenhouse tests.



Fig. 8: Field test for clubroot resistance in Brassica crops. Pre-cultivated host plants were transplanted in a contaminated field during April in 2011 and in 2012 (A); each trial contained 25 genotypes and two replications (B); after an incubation period of 90 days (C) all plants were removed from the soil (D) and the roots were scored for clubroot infection.

3.2.5.1 Locations of field trials

In spring 2011 and 2012 four field trials each were conducted at three locations in Northern Germany and one location in the Centre of France (for details see Table 6).

Table 6: Field trial locations in 2011 and 2012 for the classification of *Plasmodiophora brassicae* pathotypes

Year	Location	Country	Region	Soil Type	Average Annual Rainfall [mm]	Planting Date	Scoring Date	Incubation Period [d]	Irrigated by Hand	No. of Plants per Plot Planted (left) and Rated (right)	
2011	Groß Schwiesow	Germany	MV	loamy sand	542	April 14 & 15	August 14 & 24	126	no	24	18
	Hoisdorf	Germany	SH	sandy loam	620	April 13 & 14	July 11	88	yes	24	17
	Tiebensee	Germany	SH	loamy sand	895	April 14	July 12	89	yes	24	20
	Oizon-Val Nere	France	Centre	clayey loam	734	April 13	July 07	85	no	24	19
2012	Groß Schwiesow	Germany	MV	loamy sand	542	April 27	July 12	76	no	24	24
	Hoisdorf	Germany	SH	sandy loam	620	April 26 & 27	July 12 & 13	76	yes	24	23
	Mielenhausen	Germany	LS	clayey loam	650	April 28	July 25	88	yes	24	22
	Oizon-Le Boulay	France	Centre	clayey loam	734	April 11	July 17 & 18	97	no	24	21

MV: Mecklenburg-Vorpommern; SH: Schleswig-Holstein; LS: Lower Saxony

The soil type varied between locations from loamy sand in Groß Schwiesow (Mecklenburg-Vorpommern) and Tiebensee (Schleswig-Holstein) and sandy loam in Hoisdorf (Schleswig-Holstein) to clayey loam in Mielenhausen (Lower Saxony), Oizon-Val Nère and Oizon-Le Boulay. The average annual precipitation varied between 542 mm in Groß Schwiesow to 620 mm in Hoisdorf, 650 mm in Mielenhausen, 734 mm in Oizon and 895 mm in Tiebensee.

3.2.5.2 Preparation of plant material for field experiments

The seeds of differential hosts were taken from the same seed lots as those used for greenhouse experiments. Plants were pre-germinated in bulks on potting soil (see chapter 3.2.4) in a growth chamber at 20°C. After 3 to 4 days in the dark the seedlings were transplanted into 48 x 48 x 90 mm multi-pot trays (Quickpot, Herkplast, Ering, Germany) in potting soil (see chapter 3.2.4) at a rate of one seedling per pot and 60 pots per tray. Thereafter, plants were raised in the greenhouse at 10°C for 4 weeks and transplanted to the field in the 2 to 4-leaf stage (BBCH 12-14).

3.2.5.3 Planting, incubation and scoring of field trials

Plants were transplanted to the field in April (Table 6) accompanied by their root ball and soil from multi-pot trays at a rate of 24 plants per plot (1 m²) in two randomised replications per location. Plots were irrigated by hand in order to support plant growth and pathogen infection. After an incubation period of 76 to 126 days all plants were removed from the soil in July. The average number of recovered plants per plot varied from 17 to 24. The roots were cleaned on the field with tap water and scored for clubroot symptoms as described by Glory and Manzanares-Dauleux (2005; see chapter 3.2.4). Based on this data the DI was calculated for each differential host as described for the tests under controlled conditions.

3.3 Results

3.3.1 Pathogenicity tests under controlled conditions

During the collection period from 2009 to 2012 nearly 100 clubroot populations were collected, 48 of which were selected for pathotype designation taking into account a well-balanced distribution of geographical origins.

*3.3.1.1 Virulence of *P. brassicae* populations under controlled conditions*

The 48 *P. brassicae* samples analysed caused differential virulence on the 19 host lines (Table 4) of the differential sets. In case of averaging all orthogonally tested hosts ('ECD 01' to 'ECD

15' plus cv. 'Brutor') the level of virulence expressed by the disease index varied from 18 to 64. Furthermore, there were clearly different responses to inoculation among host genotypes. As expected, the lines 'ECD 05' (*B. rapa*), 'ECD 07' (*B. napus*) and 'ECD 14' (*B. oleracea*) were highly susceptible to all *P. brassicae* populations with three exceptions for 'ECD 14'. Additionally, the *B. napus* hosts 'ECD 08', 'ECD 09' and cv. 'Brutor' as well as 'ECD 13' (*B. oleracea*) were also highly susceptible in response to inoculation with the majority of the tested samples. In contrast, the turnip host 'ECD 01' and the resistant OSR cultivars 'Mendel' and 'Clapton' exhibited susceptible responses to only a small number of the tested *P. brassicae* populations. The turnip differentials 'ECD 02', 'ECD 03' and 'ECD 04' were even more resistant to all populations with exception of the sample from Schutterwald in Southern Germany. Finally, the *B. napus* lines 'ECD 06' and 'ECD 10' as well as the *B. oleracea* lines 'ECD 11', 'ECD 12' and 'ECD 15' showed clear differential responses to the set of *P. brassicae* populations and were therefore most informative (supplementary data 1).

3.3.1.2 Pathotype classification on differential sets

The results of the classification of pathotypes in the samples according to Somé et al. (1996) are listed in Table 7. The most frequent pathotypes in Germany were P1 with 13 samples and P3 with seven samples out of a total of 25 samples from Germany. For P2 and P6 two samples each were identified whereas only one population was classified as P5. In the seven samples from Poland P3 was found most frequently (four cases). P1 was identified two times and P4 in only one population. Five samples from France were tested in which P3 was identified in two populations and P2, P4 and P6 once, each. The four samples collected in the Czech Republic were identified as P3 and out of the three populations from Austria two were classified as P3 and one as P2. All four samples from Scotland and Denmark were classified as P1. Altogether, the most frequent pathotypes in Europe are P1 and P3 with 19 populations each out of the 48 samples analysed (summarised data not shown).

The classification in ECD triplet codes is shown also in Table 7. Thirty-three different triplet codes were identified in 48 pathogen samples from seven European countries. A majority of 42 samples exhibited no virulence on *B. rapa* hosts, except the completely susceptible line 'ECD 05'. This is expressed by the triplet code '16/--/--'. In addition to this, five samples were

also virulent on the host 'ECD 01' from the *B. rapa* subset (triplet code '17/--/--'). Finally, just one population from Southern Germany was highly aggressive to *B. rapa* as demonstrated by the figure '31/--/--' (Table 7). Regarding the virulence on *B. napus* hosts, a high level of variation was detected for the tested samples. The lowest figure was expressed by the triplet code '--/02/--'. In that case only the universal susceptible host 'ECD 07' was infected. Highest virulence on the *B. napus* subset was expressed by the triplet code '--/31/--', which indicates severe clubbing on all hosts from the specific group. The ECD triplet code describing the virulence towards *B. napus* hosts is combined with the pathotype designations defined by Somé et al. (1996). The most virulent populations P1 and P5 are represented by the triplet codes '--/31/--' and '--/30/--', respectively, whereas the medium virulent pathotypes P2 and P6 have the ECD designations '--/15/--', '--/03/--' and '--/07/--'. The samples classified as P3 contained different ECD codes for *B. napus* like '--/02/--' describing low virulence as well as '--/06/--' and '--/14/--', which describe medium virulence on the respective group of host genotypes. Finally, the samples classified as pathotype P4 with the lowest virulence received the respective ECD triplet '--/02/--', too. The virulence on *B. oleracea* hosts is represented by the last two digits of the ECD triplet code. Nearly all existing designations from '--/--/01' up to '--/--/31' are found describing a broad variation of gall formation on the respective *B. oleracea* hosts. It was remarkable that some ECD triplet codes were found for several samples whereas other virulence combinations are unique. The most commonly detected classification was '16/14/31' which was found seven times in 48 samples. This pathotype causes clubbing symptoms on 'ECD 05', the *B. napus* hosts 'ECD 07', 'ECD 08' and 'ECD 09' and all *B. oleracea* differentials. The next most frequent triplet code, which was recorded four times in 48, was '16/31/31', which in contrast to the combination mentioned above is infecting all *B. napus* differentials. ECD classification '17/31/31' was found in three samples which additionally showed strong clubroot symptoms on the *B. rapa* host 'ECD 01'.

Table 7: Frequency of occurrence of pathotypes within *Plasmodiophora brassicae* populations collected from oilseed rape fields in Europe

Country	No. of Populations	Pathotype Classification ^a								
		Somé et al. ^b	ECD ^b		16/31/15	16/31/29	16/31/31	17/31/31	31/31/1	
Germany	25	P1 (13)	16/31/02	16/31/07	16/31/12	16/31/15 (2)	16/31/29 (2)	16/31/31 (3)	17/31/31 (2)	31/31/1 3
		P2 (2)	16/15/31 (2)							
		P3 (7)	16/02/30	16/06/01	16/06/12	16/14/12	16/14/31 (3)			
		P5 (1)	16/30/31							
		P6 (2)	16/03/14	16/07/14						
		P1 (2)	16/31/08	17/31/31						
Poland	7	P3 (4)	16/14/15	16/14/30	16/14/31 (2)					
		P4 (1)	16/02/31							
		P2 (1)	16/15/08							
France	5	P3 (2)	16/14/13	16/14/29						
		P4 (1)	16/02/04							
		P6 (1)	16/03/30							
		P3 (4)	16/14/08	16/14/28	16/14/31 (2)					
Austria	3	P2 (1)	16/15/14							
		P3 (2)	16/06/30	16/06/08						
Denmark	2	P1 (2)	16/31/08	17/31/13						
Scotland	2	P1 (2)	16/31/31	17/31/12						

^a pathotype classification as determined on the differential hosts of the European Clubroot Differential set (Buczacki et al. 1975) and Somé et al. (1996). For classification into pathotypes a disease index of DI >25 was used as the cut-off to discriminate between resistant and susceptible host reaction according to Glory and Manzaneres-Dauleux (2005); ^b frequency of appearance in brackets.

The oilseed rape cv. 'Mendel', generally considered as clubroot resistant, was tested in the greenhouse with 47 *P. brassicae* populations, 14 of which (30%) caused clubbing on a level indicating a susceptible reaction (Table 8).

Table 8: Frequency of occurrence of resistant (R) and susceptible (S) host reactions of two resistant cultivars towards different *P. brassicae* populations from European oilseed rape fields under controlled conditions

Pathotype ^a	cv. 'Mendel' ^b			cv. 'Clapton' ^c		
	Σ	R	S	Σ	R	S
P1	18	12	6	10	6	4
P2	4	2	2	4	2	2
P3	19	13	6	13	12	1
P4	2	2	0	1	1	0
P5	1	1	0	1	1	0
P6	3	3	0	n/a	n/a	n/a
Σ	47	33	14	29	22	7

^a as determined by Somé et al. (1996). Neither pathotype P7 nor P8 were detected. For classification into pathotypes a disease index of DI >25 was used as the cut-off to discriminate between resistant and susceptible host reaction according to Glory and Manzanares-Dauleux (2005); ^b oilseed rape (*B. napus*) cultivar with race specific resistance; ^c cauliflower (*B. oleracea*) cultivar with race specific resistance; n/a: not applicable.

These virulent samples were classified as P1, P2, and P3 according to Somé et al. (1996), and ten different ECD triplet codes with varying pathogenicity from '16/06/01' to '31/31/13'. In comparison the resistant cauliflower cv. 'Clapton' showed severe gall formation on seven out of 29 (24%) pathogen populations tested. Among the virulent samples, P1, P2 and P3 were present as well as five different ECD classifications of which the majority exhibited a strong virulence towards *B. oleracea* hosts.

3.3.2 Pathogenicity tests under field conditions

In order to collect information on the effects of environmental conditions on the interaction between different host genotypes and *P. brassicae* populations and to set up cross checkings with greenhouse testing series, four trials on clubroot infested fields were planted in spring 2011 and four trials in 2012, respectively.

3.3.2.1 Pathogenicity of *P. brassicae* populations

The pathogen samples from these origins had already been classified under controlled conditions by that time. In 2012 the level of clubroot infection in Groß Schwiesow and Oizon-Le Boulay was low and the generally susceptible lines 'ECD 05', 'ECD 07' and 'ECD 14' exhibited only very little clubbing. Therefore, the data from these two trials were not taken into account. Between the remaining six trials, a variable degree of infestation was detected (supplementary data 2). The lowest mean disease index of all orthogonally tested hosts (i.e., 'ECD 01' to 'ECD 15' plus cv. 'Brutor') was scored in Tiebensee in 2011 on a level of 17. The highest disease index of 49 was found in Mielenhausen in 2012. Among the differential hosts clearly different levels of susceptibility were detected. The susceptible lines 'ECD 05', 'ECD 07' and 'ECD 14' as well as the *B. napus* hosts 'ECD 06', 'ECD08' and 'ECD09' were highly susceptible in every location whereas the *B. rapa* hosts showed almost no symptoms. The remaining *B. napus* lines 'ECD 10' and 'Brutor' as well as the *B. oleracea* lines 'ECD 11', 'ECD 12', 'ECD 13' and 'ECD 15' differed in susceptibility between the locations. Hybrid cultivars carrying a race-specific resistance, i.e., 'Mendel' and 'Clapton' were not tested in each location but these genotypes showed only minor root symptoms below the threshold disease index of 25.

3.3.2.2 Cross checking of pathotype classification under greenhouse versus field conditions

The comparison of disease response under controlled and field conditions is given in Table 9. The pathotype classification according to Somé et al. (1996) based on greenhouse tests was confirmed in the field for every location, while the categorisation on the ECD was only confirmed in case of *B. rapa* hosts for all locations. Concerning this matter, all samples were virulent only to the generally susceptible host 'ECD05' resulting in '16/--/--'. The ECD code

for *B. napus* generated under controlled conditions was confirmed in three field locations whereas in Groß Schwiesow the pathogenicity in the field was lower compared to the greenhouse, and in Tiebensee the situation was opposite. Regarding the pathogenicity to *B. oleracea* hosts the ECD code obtained in the greenhouse was approved only by the field test in Oizon-Val Nère, while in the four remaining locations the number of diseased hosts in the field was lower than under greenhouse conditions.

Table 9: Pathotype classification of five *P. brassicae* populations under controlled and field conditions and cross checking of disease response on clubroot resistant oilseed rape cultivar 'Mendel'

Location	Pathotype				Disease Index			
	Somé et al.		ECD		cv. 'Mendel'			
	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field		
	n=20	n=48	n=20	n=48	n=20	n=48		
Groß Schwiesow	P1	P1	16/31/31	16/19/08	19	R	7	R
Hoisdorf ^a	P1	P1	16/31/31	16/31/13	60	S	23	R
Tiebensee	P6	P6	16/03/14	16/15/12	4	R	1	R
Mielenhausen	P2	P2	16/15/31	16/15/29	39	S	7	R
Oizon val Nère	P2	P2	16/15/08	16/15/08	14	R	-	-

R: resistant reaction; S: susceptible reaction; ^a field trial: mean value of two years, n=96.

Moreover, the clubroot resistant cv. 'Mendel' was free from disease (DI <25) in greenhouse tests with inoculum from contaminated fields in Groß Schwiesow, Tiebensee and Oizon-Val Nère but susceptible in respective tests with clubroot material from Hoisdorf and Mielenhausen. On the other hand, cv. 'Mendel' remained nearly symptomless under field conditions in all tested locations, except Hoisdorf. But, also in Hoisdorf the disease index was below the threshold. Therefore, cv. 'Mendel' can be classified as resistant in all field trials analysed. It has to be noticed that the disease reaction of the clubroot resistant cv. 'Mendel' was contradictory between greenhouse and field conditions in the case of Hoisdorf, but even more in Mielenhausen.

3.4 Discussion

Nearly 30 years after the report of Toxopeus et al. (1986), the present study is the first international survey on pathogenic variation of clubroot populations in Europe and, actually, one of the first European studies with a focus on oilseed rape. Scholze et al. (2002) focused mainly on selected populations derived from cabbage grown in Germany and Switzerland and the study of Somé et al. (1996) dealt only with French samples. Whereas Řičařová et al. (2016a) and Zamani-Noor (2016a) focused on isolates from Poland, the Czech Republic and Germany, respectively, an overview on the pathogenicity of *P. brassicae* populations originating from OSR growing regions in the European Union as a whole is presented in this thesis.

3.4.1 Distribution of virulence patterns of *P. brassicae* pathotypes found in Europe is different to samples from the Canadian prairies but in accordance with earlier findings

The present research efforts yielded 33 different ECD classifications, of which 65% correspond to triplet codes that were already recorded by Toxopeus et al. (1986). Pathotype P1 (Somé et al. 1996) was detected in a high frequency in Scotland, Denmark, Northern Germany and Poland leading to the conclusion that this is the most prevalent pathotype in OSR fields in Northern Europe whereas P3 was most frequently detected in Poland, the Czech Republic, Austria and Southern Germany and therefore obviously is the most important pathotype in the more continental climates. This pattern of geographical pathotype distribution is in full accordance with the recent findings of Řičařová et al. (2016a) and Zamani-Noor (2016a). The pathotype P1 shows maximum virulence on all *B. napus* hosts from the ECD series whereas P3 turned out to have a lower virulence to that group. The pathotype P6 was mainly found in fields associated with the cultivation of cabbage like La Bohalle (France), where *B. oleracea* breeding is conducted, or Tiebensee, which is located in the cabbage growing region 'Dithmarschen' in Schleswig-Holstein, Northern Germany. In contrast to the recent findings a considerable number of the pathotypes P2 and P4 was found by Somé et al. (1996) in France. This may be caused by the fact that in the latter case the majority of *P. brassicae* samples were taken from *B. oleracea* originating from Brittany in North-Western France, well known as an important growing area for Brassica vegetables. In the present study, two pathogen samples from *B. oleracea* with the same geographic origin

were analysed and one of these was also classified as P4, indicating that pathotype P4 actually plays a relevant role in that region. The pathotypes P2 and P3 are distinguished only by the reaction of the forage rape cv. 'Nevin', which is susceptible to P2 and resistant to P3 populations. It was found in several clubroot areas, e.g., Oizon in France, the 'Innkreis' region in Northwestern Austria and the region around Goettingen, Lower Saxony (Germany) that populations classified as P2 and P3 occur in the same region. Therefore, the pathotype classifications of Somé et al. (1996) and the present study are in accordance. Furthermore, it has to be stated that only two out of five French *P. brassicae* samples analysed in the present study were actually extracted from OSR fields and therefore the relevance of this study for France may be somewhat limited. Moreover, the possibility to compare the physiological specialisation of *P. brassicae* derived from OSR in Europe with the situation in other parts of the world is somewhat restricted, because most of the published studies were done with *P. brassicae* taken from diseased *B. oleracea* or *B. rapa* samples (Dobson et al. 1983; Kuginuki et al. 1999; Scholze et al. 2002; Donald et al. 2006; Osaki et al. 2008). Comparable studies however are from Western Canada where the virulence of *P. brassicae* from canola (double-low spring OSR) was examined. As summarised by Hwang et al. (2012) the predominant pathotype in Alberta was pathotype 3 according to Williams (1966), P2 based on the differential of Somé et al. (1996) or '16/15/12' according to the ECD series (Strelkov et al. 2006; 2007; Xue et al. 2008; Cao et al. 2009). More recently, Strelkov et al. (2016) found four samples of *P. brassicae* with lower virulence, i.e., pathotypes 5 (Williams 1966), P3 according to Somé et al. (1996) and ECD triplet code '16/06/08' in the same area. Nevertheless, as not one single Canadian sample was characterised as P1 based on the set of Somé et al. (1996), the findings indicate only marginal virulence (below the threshold) towards the host genotype 'ECD 10' (*B. napus* var. *napobrassica* cv. 'Wilhelmsburger'), in contradiction to European results. In summary, a broader variation of virulence patterns of European *P. brassicae* samples to *B. napus* is shown in the present study. Evidence for this is stated by the fact that the frequency of occurrence is well balanced between pathotypes P1 and P3; in other words, differences in virulence towards 'ECD 06' and 'ECD 10' between European *P. brassicae* populations are very frequent.

3.4.2 Different thresholds used to discriminate susceptible vs. resistant reactions have no significant effect on the discovery of genetic variation within *P. brassicae* populations

When comparing different studies it has to be taken into account that Canadian authors cited before (mainly from the research group of Stephen Strelkov, University of Edmonton, Alberta, Canada) used a $DI \geq 50$ to discriminate resistant and susceptible host reactions whereas in the present study a value of $DI > 25$ (according to Glory and Manzanares-Dauleux 2005) has been used. In this context, it is necessary to get information whether the higher cut-off-point chosen in the Canadian research may have caused less variation in pathotype classification. In the present study a five-staged scale of disease severity classes and a corresponding formula was used to calculate a disease index. In contrast, Canadian authors used a four-staged scale of disease severity classes according to Kuginuki et al. (1999) and for calculating the disease index a formula as described by Horiuchi and Hori (1980), Dobson et al. (1983) and modified by Strelkov et al. (2006) was applied. It can be assumed that the use of either four or five disease severity classes has no considerable influence on the final determination of a distinct host-pathogen interaction. Based on proper recalculations, it was found that a reduction of the cut-off point from $DI \geq 50$ to ≥ 25 would not cause a relevant change in the actual pathotype classifications by Strelkov et al. (2007) or Cao et al. (2009), respectively. Only one out of ten populations would shift from P2 to P1 in the first study and two populations classified as P3 would shift to P2 in the latter case. On the other hand an increase of the disease index as used in the present study to a corresponding level like chosen in Canada would cause some shifts from P1 to P2 and P3, but would have no effect on the general predominance of pathotypes P1 and P3 in Europe. This evaluation is supported by recent findings of Řičařová et al. (2016a), who compared the effect of different thresholds and reported that only some of the discovered pathotypes were reclassified. Generally, it can be confirmed that choosing a higher disease index value for discrimination between resistant and susceptible host reactions leads to a lower variation in pathotype classification. But, this has no impact on the fact that variation in virulence on *B. napus* hosts is higher within European *P. brassicae* populations than within clubroot samples from Canadian fields.

3.4.3 Pathotype classifications of *P. brassicae* populations give hint for differential host genotypes to be used as donors for clubroot resistance

Finally, the pathotypes give hint, if any of the differential hosts can potentially be used as a donor of resistance for breeding. The present classifications related to *B. rapa* hosts correspond to published studies (Dobson et al. 1983; Toxopeus et al. 1986; Scholze et al. 2002; Strelkov et al. 2006; Donald et al. 2006; Cao et al. 2009) and indicate that turnip lines 'ECD 01' to 'ECD 04' are of great interest for breeding novel resistant Brassica crops in Europe. On the contrary, the *B. napus* lines 'ECD 07' to 'ECD 09' as well as the cv. 'Brutor' cannot be recommended for further efforts, but 'ECD 06' and 'ECD 10' are suggested to be appropriate resistance donors for regions in Europe, where the pathotype P3 (Somé et al. 1996) prevails. For Western Canada only 'ECD 10' from the *B. napus* subset can be recommended as a suitable donor of resistance. Regarding the *B. oleracea* subset of the ECD series, genotype 'ECD 15' (curly cale cv. 'Verheuil') is most promising. But, taking into account that clubroot resistance from *B. oleracea* is genetically more complex the use for resistance breeding in OSR will be more difficult and may therefore be avoided (Diederichsen et al. 2009). However, on the other hand complex resistance may be more durable.

3.4.4 Differences in environmental conditions affect the clubroot disease reaction depending on the host genotype

In greenhouse trials like in the present study, it was detected that 30% of the *P. brassicae* samples caused susceptible reactions on the clubroot resistant OSR cv. 'Mendel' (Table 8). In this respect, Diederichsen and Frauen (2012) have already mentioned that *P. brassicae* populations virulent to the race-specific resistance of cv. 'Mendel' are present in Germany and particularly in the North-Eastern region of Mecklenburg-Vorpommern. Recently, Zamani-Noor (2016a) tested 49 *P. brassicae* populations for virulence on cv. 'Mendel' in the greenhouse and found 15 isolates from all over Germany to be moderately or highly virulent. This is in accordance with the percentage of virulent pathotypes detected in the present study. Also Strelkov et al. (2016) observed variation in the response of cv. 'Mendel' to clubroot populations under controlled conditions. The latter authors tested four populations from Alberta, Canada, and cv. 'Mendel' turned out to be susceptible to three of these. But, in contrast to that findings from the greenhouse no considerable clubbing indicating a

susceptible host-reaction on cv. 'Mendel' was noticed during previous field experiments by Limagrain GmbH (Edemissen) at several locations in Northern Germany for years (Abel 2010, pers. communication). However, *P. brassicae* derived from these locations caused severe clubbing on cv. 'Mendel' in the greenhouse. Therefore, it was questioned whether pathotype classifications of *P. brassicae* populations conducted under controlled and field conditions would be comparable at all. While the low virulence towards fodder turnip (*B. rapa*) hosts from ECD series and pathotype classifications according to Somé et al. (1996) were fully confirmed in field tests, the classification of the *B. napus* subset from ECD series fitted only in three out of five trials and the *B. oleracea* subset exhibited no corresponding classifications (Table 9). Therefore, the assumption that testing under controlled conditions will yield the same results as under field conditions (Sharma et al. 2013) is questionable based on our results as for some *B. napus* and *B. oleracea* hosts from the ECD series clear differences were observed. Particularly, the clubroot resistant cv. 'Mendel' exhibited different reactions. Resistant reactions were detected in field experiments in Hoisdorf and Mielenhausen, while clear susceptibility in the respective greenhouse tests was observed (Table 9). This observation is by trend in accordance with results of Sharma et al. (2013), who examined the performance of the spring canola cultivar '45H29' with race-specific clubroot resistance under field and controlled conditions with a comparable pathotype in Canada. The authors detected an increased disease severity level under controlled conditions, which, in contrast to the present study, remained below the threshold value. The susceptible host reaction in the greenhouse might be caused by the higher temperature compared to field conditions as described by Robak and Gabrielson (1988), who observed that the clubroot resistance of cauliflower lines was temperature sensitive and was overcome at 20°C. This might be an explanation for the observations with the pathogen population from Mielenhausen, where the reaction of cv. 'Mendel' was clearly susceptible in the greenhouse but the roots remained nearly symptomless under field conditions. At Hoisdorf the discrimination between the different environments was rather slight: While the greenhouse trials revealed clear susceptibility, the average disease index of all field experiments with cv. 'Mendel' showed resistance, although, close to the cut-off between a resistant and a susceptible host reaction (DI=23). Presumably, in this case the lower temperature in the field compared to the greenhouse may have resulted in a slower development of clubroot symptoms as observed by Sharma et al. (2011), who found that temperature affected every

aspect of the development of *P. brassicae* in root tissues. The optimum temperature for the pathogen development was assessed at 25°C, i.e., slightly above the greenhouse temperature in the present study. Gossen et al. (2012) found that in addition to a moderate soil temperature an adequate level of soil moisture must be present for the development of severe clubroot symptoms. This is guaranteed under controlled conditions by frequent watering, but is not always ensured in the field. During greenhouse experiments by Narisawa et al. (2005) the soil moisture content largely affected the disease level of Chinese cabbage plants depending on the pathogen spore density, but showed no differences across three moisture treatments in the field. Hamilton and Crête (1978) have shown that clubroot infections and disease development occurred above soil moisture levels of 25 to 40% of the water-holding capacity of the respective soil type used under controlled conditions. These findings were recently confirmed by Dohms et al. (2013), who observed a very small variation between moisture treatments from 40 to 100% of the water-holding capacity 21 days after inoculation, but detected serious effects of different temperature levels. The latter authors concluded that just a minimum moisture level in the rhizosphere is required by *P. brassicae* to infect host plants. As soil moisture levels above a threshold of 30 to 40% of the water-holding capacity are ubiquitous in most arable soils of the temperate zone, it might be assumed that the influence of soil moisture on clubroot infection and disease development is of minor relevance compared to the temperature. Earlier, Nieuwhof and Wiering (1963), based on their work with *B. oleracea* genotypes came to the conclusion that a direct effect of temperature and soil moisture on clubroot resistance is not present. But, they reported that differences in infection conditions, spore loads and period of exposure to inoculum have a reliable effect on disease severity of clubroot. The recent findings show that the race-specific clubroot resistance of cv. 'Mendel' may be overcome by some populations of *P. brassicae* depending on temperature. It is suggested that interactions between host resistance, clubroot pathotype and temperature may be present since the disease index on cv. 'Mendel' in Mielenhausen was much below the value recorded in Hoisdorf although the conditions were quite suitable for the pathogen in both locations. Therefore, future studies on resistance of cv. 'Mendel' to different populations of *P. brassicae* should take the temperature regime as a varying parameter into account.

3.4.5 Further efforts for an improvement of differential sets to define *P. brassicae* pathotypes

As the race-specific clubroot resistance firstly utilised in the hybrid OSR cultivar ‘Mendel’ is the only one used in European oilseed breeding up to now, it is suggested to include this genotype in a new differential set to monitor the occurrence of virulent populations. On the other hand, the inclusion of cv. ‘Clapton’ suggested by Diederichsen et al. (2009) is not considered as essential because the resistance pattern in the present study was very similar to cv. ‘Mendel’. But perhaps, not too much effort should be invested into a further improvement of differential tester sets as the rather imprecise *P. brassicae* pathotyping systems of the future will presumably be replaced by better molecular diagnostic tools based on the *P. brassicae* genome sequence (Schwelm et al. 2015; 2016).

3.4.6 Status and perspective of the present clubroot resistance derived from cv. ‘Mendel’

Diederichsen and Frauen (2012) reported that the pathogen populations from Schleswig-Holstein (SH) are still avirulent to cv. ‘Mendel’, but in the present study it turned out that at Hoisdorf (SH) a tendency to overcome this resistance is present. This confirms the assumption of Sauermann (2015) that virulent clubroot pathotypes are already established in that region, but have not yet been detected. Nevertheless, during the present survey 70% of the classified populations were avirulent to cv. ‘Mendel’ in the greenhouse and there was no indication for virulence on respective fields except for Hoisdorf. Thus, it seems that this resistance is still (more or less) effective. However, attention should be paid to cultural strategies to manage clubroot, e.g., sanitation of farm machinery, sound crop rotations, later sowing in case of winter OSR and perhaps the application of calcium cyanamide fertilizer (Strelkov et al. 2011; Diederichsen 2013; Zamani-Noor 2016b), because if this resistance will be overcome, no further oilseed rape cultivation may be possible unless broader resistances will be developed and implemented in new adapted OSR cultivars (Diederichsen et al. 2014).

3.5 Summary of the *Plasmodiophora brassicae* classification survey

Clubroot caused by the obligate biotrophic protist *P. brassicae* is a serious soil-borne disease of cruciferous crops including OSR. It causes galls on roots leading to premature death of the

host plants. The organism remains infective in the soil as resting spores can survive 20 years or even longer. The traditional hot spots of clubroot infestation on oilseed rape in Europe are mainly located in the United Kingdom (UK), France and Northern Germany. Due to the raise of acreage within the last decades, contaminated fields are currently detected nearly all over the main areas of OSR cultivation in Europe. Physiological specialisation of pathogen populations causes differences in pathogenicity so that breeding for resistance is difficult. To collect more detailed information on the occurrence and the virulence of *P. brassicae* as a prerequisite for efficient breeding for resistance, samples of infected plant material were taken from locations all over the main OSR growing regions in Europe. The collection contains samples from the UK, France, Denmark, Poland, the Czech Republic, and Austria and from numerous locations across Germany. Forty-eight out of nearly 100 plant samples were analysed under greenhouse conditions by using artificial inoculation and performing optical ratings of disease symptoms. The European Clubroot Differential (ECD) series and the differential set from Somé et al. (1996) were used for the classification of pathotypes, respectively. Differences in pathogenicity were clearly demonstrated. The virulence of these pathotypes to the *B. rapa* genotypes of the ECD is rather low. For the *B. napus* and *B. oleracea* genotypes the situation is different as considerable variation with respect to virulence to the different hosts within each group was detected. In total, 33 different ECD triplet codes were detected, whereof most commonly encountered classifications were '16/14/31', '16/31/31' and '17/31/31'. Based on the differentials of Somé et al. (1996) it can be suggested, that P1 is the prevalent pathotype in OSR fields in the maritime region of Northern Europe whereas P3 was most frequently detected in more continental areas. This distribution pattern was recently confirmed by several independent publications cited above. Furthermore, the present classifications related to *B. rapa* hosts correlate well with already published results. Those findings indicate that turnip lines 'ECD 01' to 'ECD 04', but particularly the latter, are of great interest in breeding of all Brassica crops. In contradiction, the *B. napus* lines 'ECD 07' to 'ECD 09' as well as the cv. 'Brutor' cannot be recommended for further efforts but 'ECD 06' and 'ECD 10' are suggested to be appropriate resistance donors for the region where pathotype P3 (Somé et al. 1996) is predominant. Regarding the *B. oleracea* subset of the ECD series, genotype 'ECD 15' (curly kale cv. 'Verheuil') was most promising, but due to partial resistance difficult to use in breeding. Under controlled conditions 30% of *P. brassicae* populations regardless of the respective pathotype were

virulent on cv. 'Mendel', probably carrying a monogenic resistance. Based on a subsequent comparison of infections under greenhouse and field conditions, evidence for a considerable impact of environmental conditions on host-pathogen interactions, particularly for cv. 'Mendel', was shown. Therefore, greenhouse tests may lead in some cases to an overestimation of virulence. Therefore, growing of OSR cultivars with race-specific clubroot resistance in the majority of contaminated fields in Europe must currently not be disclaimed. But, farmers have to pay attention to useful cultural strategies to manage clubroot, e.g., the permanent evaluation of durability of resistance.

4 Phenotypic characterisation and genetic mapping of clubroot resistance in rapeseed (*Brassica napus* L.)

4.1 Breeding for clubroot resistance and genetic resources

The combination (pyramiding) of different clubroot resistance genes has been considered the most efficient way to increase the durability of resistance to a broader spectrum of physiological races of *P. brassicae* (Piao et al. 2009; Chen et al. 2013). Many Brassica germplasms have been screened to possibly identify effective resistance genes as a prerequisite for clubroot resistance breeding. Genes and QTL involved in clubroot resistance were identified in *B. napus* itself but more often in its ancestral species *B. oleracea* and *B. rapa* (Fig. 9; adapted from Piao et al. 2009).

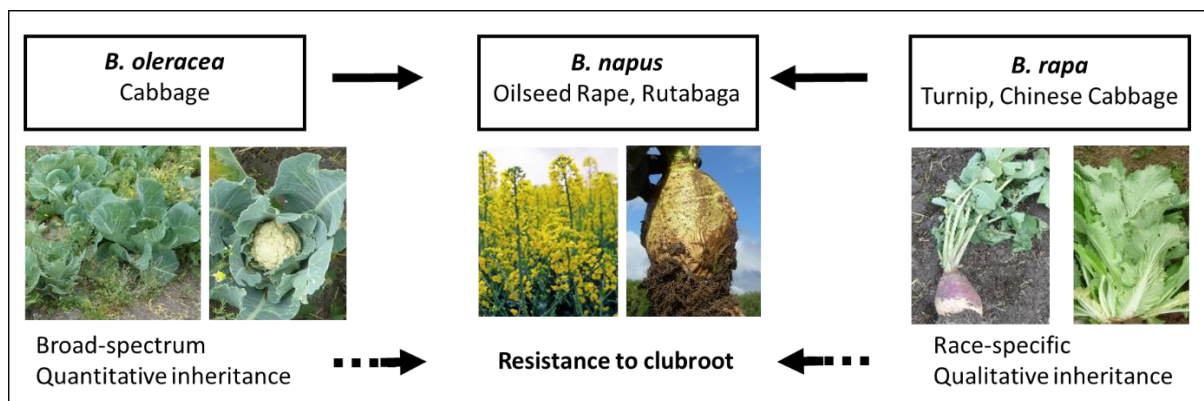


Fig. 9: Types of *B. oleracea* and *B. rapa* as genetic resources for clubroot resistance of *B. napus*.

Regarding *B. rapa* eight resistance gene loci, initially introduced from European fodder turnip (*B. rapa* ssp. *rapifera*) genotypes ‘ECD02’, ‘Gelria R’, ‘Siloga’, ‘Milan White’ and ‘Debra’, were localised on chromosomes A01, A02, A06 and A08, while on linkage group A03 four loci were mapped. These genetic loci were named *Crr1*, *Crr2*, *Crr3*, *Crr4* (Suwabe et al. 2003; 2006; Hirai et al. 2004) and *CRa*, *CRb*, *CRc*, *CRk*, respectively (Matsumoto et al. 1998; Piao et al. 2004; Sakamoto et al. 2008). In contrast to *B. rapa*, completely resistant genotypes have only rarely been identified in *B. oleracea*. But, quantitative trait loci (QTL) for clubroot resistance were found on all C-genome chromosomes except C07 (Piao et al. 2009). Resistance sources from the *B. oleracea* gene pool used in breeding programs are the landraces ‘Bindsachsener’ (Voorrips et al. 1997) and ‘Böhmerwaldkohl’ (Nieuwhof and Wiering 1962; Manzanares–

Dauleux et al. 2000a; Werner et al. 2008), the cabbage cultivars ‘Richelain’ (Chiang and Crete 1989) and ‘Badger Shipper’ (Buczacki et al. 1975) and the inbred line ‘C10’ (Grandclément and Thomas 1996; Rocherieux et al. 2004). Diederichsen et al. (2009) stated that clubroot resistance in *B. oleracea* is genetically more complex and therefore difficult to be used in breeding. Alternatively, the clubroot resistant cauliflower (*B. oleracea* var. *botrytis* L.) cultivar ‘Clapton’ was bred by using an introgression from Chinese cabbage (*B. rapa*) cv. ‘Parkin’ into *B. oleracea*. However, this transfer requires laborious embryo rescue procedures, and the resulting resynthetic lines often exhibit agronomic disadvantages and – particularly important for oilseed rape – unfavourable fatty acid composition of the seed oil (erucic acid content) and high glucosinolate content in the seed and meal. Therefore, there is an urgent need to identify clubroot resistance genes in the primary gene pool of *B. napus*. Early studies reported that rutabaga (*B. napus* var. *napobrassica*) varieties like ‘Wilhelmsburger’, ‘York’ and ‘Ditmar S2’ under certain environmental conditions exhibit clubroot resistance, which is controlled by one or two independent dominant gene loci (Lammerink 1967; Ayers and Lelacheur 1972). During the period from 1975 to 1996 the resistance from the European clubroot differential host ‘ECD 04’ (*B. rapa*) was transferred via embryo rescue to rutabaga (*B. napus* var. *napobrassica*). In case of cv. ‘Invitation’ evidence was provided for the presence of, at least, one of the three postulated dominant genes derived from ‘ECD 04’ (Bradshaw et al. 1997). During internal clubroot resistance tests with numerous potential donor lines and a large set of *P. brassicae* samples the resistance of the rutabaga varieties ‘Wilhelmsburger’ and, particularly, ‘Invitation’ was confirmed. Therefore, this study aimed at mapping loci involved in clubroot resistance present in rutabaga cultivars.

4.2 Material and Methods

4.2.1 Plant material and pathogen isolates

Two clubroot resistant rutabaga cultivars (*B. napus* var. *napobrassica*) were selected as resistance donors for crosses to susceptible rapeseed cultivars. Subsequently, two doubled haploid (DH) populations were developed by microspore culture in the Limagrain Europe laboratories in Rilland, The Netherlands, as described by Lichter (1982) with minor

modifications. The first DH population ‘R103’ was created by crossing the clubroot resistant rutabaga cv. ‘Invitation’ as a female parent with the susceptible oilseed rape cultivar ‘Ladoga’. The second DH population ‘R106’ derived from a cross of the same susceptible OSR cultivar as a female parent with the clubroot resistant rutabaga cv. ‘Wilhelmsburger’. Residual F₁ plants were used for resistance tests (see below). The seeds of cv. ‘Ladoga’ and cv. ‘Invitation’ were supplied by Limagrain GmbH (Germany) and Limagrain UK, respectively, whereas cv. ‘Wilhelmsburger’ seeds were derived from the gene bank of the University of Warwick, UK.

Out of population ‘R103’ a total of 103 DH lines were analysed genotypically in order to build the genetic map whereas phenotype data from the resistance test with the *P. brassicae* isolate ‘Tiebensee’ were only present for 102 individuals and for 73 individuals in case of the test against *P. brassicae* isolate ‘Vojens’, respectively. Out of DH population ‘R106’ a total of 395 DH lines were phenotyped and used for mapping.

Two different *P. brassicae* isolates collected from infested oilseed rape fields were used for resistance tests and phenotyping in this study (Table 10).

Table 10: Origin and pathotype classification of *Plasmodiophora brassicae* isolates used for resistance tests in the present study

<i>P. brassicae</i> Isolate	Pathotype on ECD Series	Pathotype		Country of Origin	State/Region	Host of Origin	Year of Sampling	Source
		on Differential Set of Somé et al. (1996)						
‘Tiebensee’	16/03/14	P6		Germany	Schleswig-Holstein	<i>Brassica napus</i>	2009	Kadler, M. (Limagrain)
‘Vojens’	16/31/08	P1		Denmark	Southern Denmark	<i>Brassica napus</i>	2010	Cordsen Nielsen, G./ Olsen, F. (Videncentret)

The clubroot isolates differ in their pathogenicity patterns on the European Clubroot Differential series (Buczacki et al. 1975) and the differential set of Somé et al. (1996) and thus, their ability to infest *B. napus* and *B. oleracea* hosts. The isolate ‘Tiebensee’ exhibits moderate virulence on the *B. napus* and *B. oleracea* hosts of the ECD series and is classified

as pathotype P6 in the set of Somé et al. (1996). The narrow virulence spectrum regarding *B. napus* hosts of that isolate makes it possible to detect a maximum number of resistance genes in this group of genotypes (Gustafsson and Fält 1986). In contrast, the second clubroot isolate 'Vojens' shows high virulence on the *B. napus* hosts and is therefore classified as P1 (Somé et al. 1996) whereas the virulence on the *B. oleracea* hosts from the ECD series is rather low.

4.2.2 Preparation and supply of inoculum of *P. brassicae*

Clubroot galls were multiplied in the glasshouse on artificially inoculated seedlings either of *B. rapa* var. *pekinensis* cv. 'Granaat' or *B. napus* var. *napus* cv. 'Ladoga'. Preparation of inocula was conducted as described in chapter 3.2.2.

4.2.3 Resistance phenotyping of DH populations and parental lines

For each combination of DH line x *P. brassicae* isolate five plants were phenotyped in two replications, each. Greenhouse tests, disease assessments and calculation of the respective disease indexes were done according to Glory and Manzanares-Dauleux (2005). Phenotyping was conducted as described in detail in chapter 3.2.4.

4.2.4 SNP marker origin and selection

A set of 1,109 SNP markers were used to genotype the two DH populations. This set of markers was selected from the global set of SNPs available at Limagrain by two criteria: a) marker quality, i.e., call rate and minor allele frequencies (MAF); b) genetic mapping data in order to get a set of SNPs evenly distributed over the whole *B. napus* genome.

4.2.5 DNA extraction and SNP genotyping

Leaf discs were sampled in the greenhouse with a puncher (4 mm diameter) and dried for 48 hours at room temperature in 96-deepwell plates with covers containing dehydrated silica gel. The DNA was extracted from leaf tissue at the Limagrain Europe laboratories (Research

Chappes Centre, France) according to Rogers and Bendich (1994) using a modified CTAB method. The quantification of DNA was done on an Applied Biosystems 7900 system (lifetechnology) with the Quant-iT™ PicoGreen® Assay (Invitrogen, Carlsbad, USA) and DNA concentrations were adjusted to 50 ng μL^{-1} for each sample. Fifty μL of genomic DNA per sample were used for genotyping. Genotyping was conducted with a custom designed oligo pool assay (OPA) of SNP markers on the Illumina BeadXpress Reader using the 'GoldenGate Genotyping Assay for VeraCode Manual Protocol' (Illumina Inc., San Diego, USA) according to Fan et al. (2003). The Genome Studio software (Illumina Inc., San Diego, USA) was used for automatic allele calling for each locus. The clusters were manually edited if necessary. Technical replicates and signal intensities were checked and the most reliable calls were included in further analyses. In addition to the GoldenGate Genotyping Assay, specific markers were used to enrich the marker density of chromosomes using KBiosciences Competitive Allele-Specific polymerase chain reaction (KASPar) SNP genotyping assays in the Limagrain Europe laboratories using the protocol and the consumables recommended by KBiosciences (LGC Genomics, formerly KBiosciences, Teddington, UK).

4.2.6 Genetic map construction

Genotypic data were revised by removing all loci and individuals containing more than 30% of missing data, and the data matrix was converted to an a-b-u matrix (a= allele of the female parent, b= allele of the male parent, u=missing data). Segregation of each marker was tested by Chi² test for goodness of fit (1:1; P=0.05). Linkage map construction was performed using the program Joinmap 4.0 (van Ooijen 2006). A LOD threshold of 3.5 and a rec value smaller than 0.4 were used to group loci. Identification of linkage groups is based on SNP markers published on an integrated *B. napus* map by Delourme et al. (2013). The numbering of linkage groups follows the denomination A01 to A10 and C01 to C09 as used for the integrated SNP-based genetic map provided by Delourme et al. (2013). This is synonymous to the standard nomenclature N01 to N19 (Parkin et al. 1995). Recombination frequencies were transformed to map distances between markers [cM] by using the Kosambi mapping function (Kosambi 1944).

4.2.7 QTL mapping

The program MapQTL 5 (van Ooijen 2004) was applied for mapping clubroot resistance loci in those DH populations in which resistance does not conform to a monogenic mode of inheritance. The Multiple QTL Mapping (MQM) approach (Jansen 1993, 1994, 2007; Jansen and Stam 1994) was used. For each QTL the few markers offering the peak LOD score were set as cofactors. To avoid false positive signals, a LOD score of 3.0 was set as the minimum value for the detection of significant QTL.

4.2.8 Statistical analyses

Chi² tests were applied to assess the observed proportions of resistant and susceptible progenies with the respective expected segregation ratio of each combination of DH population x *P. brassicae* isolate. The probability of error was set to alpha=5%.

4.3 Results

4.3.1 Phenotypic variation of disease reaction

The resistance reaction of the donor lines was the crucial factor for selection of the *P. brassicae* isolates to be used for phenotyping. The non-resistant parent cv. 'Ladoga' was, in fact, completely susceptible to both isolates (DI=3 and DI=49) whereas both presumed resistant parents turned out to be fully resistant to the isolate 'Tiebensee' (DI=1/0). 'Invitation' (DI=0) was resistant to the isolate 'Vojens' whereas cv. 'Wilhelmsburger' (DI=51) was susceptible to the isolate 'Vojens' (Table 11).

Table 11: Disease indexes of parental lines of DH populations and differential hosts from the set of Somé et al. (1996) in clubroot resistance tests with two different *P. brassicae*

isolate Genotype	Disease Index ^a			
	Differential Host	Parental Line	<i>P. brassicae</i> Isolate 'Tiebensee'	<i>P. brassicae</i> Isolate 'Vojens'
Nevin/ECD06	X		37 (s)	45 (s)
Brutor	X		18 (r)	57 (s)
Wilhelmsburger/ECD10	X	X	0 (r)	51 (s)
Invitation		X	1 (r)	0 (r)
Ladoga		X	63 (s)	49 (s)

^a a disease index of DI>25 was set as the cut-off between resistance (r) and susceptibility (s) according to Glory and Manzanares-Dauleux (2005).

Disease reactions of the two DH populations after inoculation with respective *P. brassicae* isolates are presented in Fig. 10. Within the DH population 'R103' the disease index ranged between DI=0 and DI=75 for the isolate 'Tiebensee' (Fig. 10A) whereas a maximum range between DI=0 and DI=100 was observed for the isolate 'Vojens' (Fig. 10B). Regarding the DH population 'R106' the disease index ranged between DI=0 and DI=91 after inoculation with the *P. brassicae* isolate 'Tiebensee' (Fig. 10C). Parental lines reacted as expected.

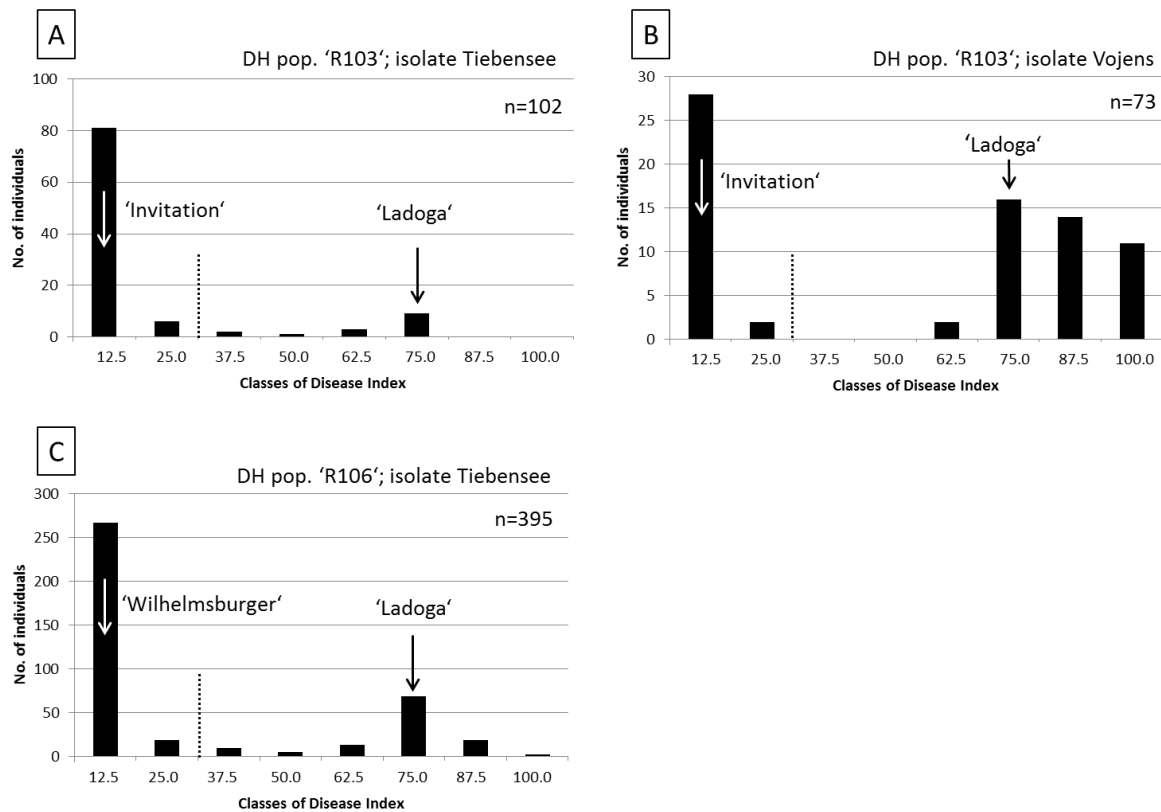


Fig. 10: Frequency distributions of the disease index for clubroot infection of two segregating DH populations of *B. napus* tested with two different *P. brassicae* isolates under controlled greenhouse conditions. Disease indexes of the tested individuals ranged from DI=0 (no infection) to DI=100 (severely infected). The DH population 'R103' was phenotyped with the *P. brassicae* P6-isolate 'Tiebensee' (A) and the P1-isolate 'Vojens' (B), respectively. The DH population 'R106' was tested with the P6-isolate 'Tiebensee' (C). The clubroot reactions of parental lines of the DH populations are indicated by *arrows*. The resistant parents were free from infection (DI=0) in all the trials whereas the disease index of the susceptible parent ranged from DI=65 to DI=68. The inscription of the axis of abscissae indicates the maximum value of each disease index class. The *dotted lines* show the cut-off points between resistant and susceptible reactions (DI>25) according to Glory and Manzaneres-Dauleux (2005). "n" indicates the total number of DH lines tested per DH population and *P. brassicae* isolate.

Segregation analyses were conducted and a disease index of DI>25 was used as the cut-off between resistant and susceptible reaction as proposed by Glory and Manzaneres-Dauleux (2005) (Table 12). The respective segregation ratios indicated the presence of three and two resistance genes against the *P. brassicae* isolate 'Tiebensee' in the DH populations 'R103' and 'R106', respectively.

Table 12: Segregation ratios and χ^2 values of DH populations ‘R103’ and ‘R106’ tested with *P. brassicae* isolates ‘Tiebensee’ and ‘Vojens’, respectively. Applying a probability of error of $\alpha=0.05$ the critical χ^2 value is $\chi^2_{(1; 0.95)}=3.84$

DH Population	<i>P. brassicae</i> Isolate	No. of Individuals		Expected Segregation Ratio	No. of Genes	χ^2
		Resistant ^a	Susceptible ^a			
‘R103’	‘Tiebensee’	87	15	7:1	3	0.45
‘R103’	‘Vojens’	30	43	1:1	1	2.32
‘R106’	‘Tiebensee’	278	117	3:1	2	4.50 ^b

^a a disease index of DI>25 according to Glory and Manzaneres-Dauleux (2005) was used as the cut-off between resistant and susceptible host reaction; ^b by using a disease index of DI>50 according to Strelkov et al. (2007) as the cut-off between resistant and susceptible host reaction the χ^2 value would change to $\chi^2=0.24$.

In contrast, the segregation observed for DH population ‘R103’ in resistance tests with isolate ‘Vojens’ indicated the presence of one major gene. In order to obtain additional information on the mode of inheritance of resistance against the isolate ‘Tiebensee’ 29 F₁ plants of the cross ‘Invitation’ x ‘Ladoga’ and 60 F₁ plants of the cross ‘Ladoga’ x ‘Wilhelmsburger’ were tested. All plants turned out to be resistant indicating at least one dominant resistance gene.

4.3.2 Discovery of genetic loci for clubroot resistance

4.3.2.1 Genetic linkage maps

For mapping clubroot resistance, linkage maps for the two DH populations were constructed. The resulting genetic map of the DH population ‘R103’ comprised 427 SNP markers on 20 linkage groups covering 1,552.3 cM with an average marker distance of 3.6 cM and 34 gaps >10 cM (Table 13). The linkage group C02 was split in two parts due to the low number of mapped markers in the critical region. Regarding DH population ‘R106’ the map contained 409 SNPs on 19 linkage groups, covering 1,654.5 cM, with a mean distance between two markers of 4.0 cM and a number of 41 gaps >10 cM.

Table 13: Description of the genetic linkage maps constructed for the *B. napus* DH populations 'R103' and 'R106'

DH Population 'R103'					DH Population 'R106'				
Linkage Group	Length [cM]	No. of SNPs	Mean Marker Distance [cM]	No. of Gaps >10cM	Linkage Group	Length [cM]	No. of SNPs	Mean Marker Distance [cM]	No. of gaps >10cM
A01	65.4	25	2.6	1	A01	90.7	28	3.2	1
A02	22.5	10	2.3	1	A02	78.6	16	4.9	3
A03	119.0	36	3.3	1	A03	139.8	33	4.2	4
A04	51.0	21	2.4	1	A04	48.7	24	2.0	0
A05	92.1	23	4.0	2	A05	84.3	25	3.4	2
A06	63.6	33	1.9	0	A06	110.9	29	3.8	2
A07	100.2	26	3.9	2	A07	87.3	19	4.6	3
A08	45.8	24	1.9	1	A08	53.5	22	2.4	1
A09	75.1	17	4.4	0	A09	72.7	19	3.8	2
A10	69.0	24	2.9	1	A10	66.5	24	2.8	2
C01	124.1	27	4.6	5	C01	78.1	21	3.7	4
C02_1	13.6	5	2.7	0	C02	65.6	9	7.3	1
C02_2	17.4	4	4.3	0	-	-	-	-	-
C03	183.1	36	5.1	6	C03	138.5	30	4.6	4
C04	127.5	26	4.9	4	C04	95.8	22	4.4	3
C05	96.0	20	4.8	3	C05	74.4	16	4.7	2
C06	77.7	15	5.2	1	C06	94.0	16	5.9	2
C07	77.3	19	4.1	2	C07	92.1	21	4.4	1
C08	59.4	14	4.2	2	C08	128.2	18	7.1	3
C09	72.4	22	3.3	1	C09	54.8	17	3.2	1
Total	1,552.3	427	3.6	34	Total	1,654.5	409	4.0	41

The size of the linkage groups varied between 13.6 and 183.1 cM in case of DH population 'R103' and between 54.8 and 139.8 cM for 'R106', respectively. The number of markers per group ranged from four to 36 in case of 'R103' and from nine to 33 for 'R106', respectively.

On both maps the linkage groups A03 and C03 showed the highest number of markers while A02 and C02 contained the lowest number of markers.

4.3.2.2 Mapping of genetic loci for clubroot resistance

Generally, genetic loci for disease resistance can be mapped in two different ways: Either as a Mendelian trait, if the resistance is caused by a single (or few) major gene(s) or as a quantitative trait with the aid of QTL mapping, if several or many loci are contributing to the overall level of resistance resulting in a quantitative phenotypic variation. Consequently, the distribution of phenotypic disease scores is a crucial criterion for the decision on the appropriate approach. Due to the segregation ratios observed in the present study, which give hint to the presence of two or three major genes, respectively, QTL mapping has been applied to identify these loci. The segregation ratio observed for the DH population 'R103' to *P. brassicae* isolate 'Vojens' gives hint to a single major gene (Table 12). This resistance gene was mapped on linkage group A03 at position 15.9 cM. The resistance locus mapped 9.0 cM proximal of the SNP marker R-0073926 and 2.3 cM distal of the marker R-0090205 (Fig. 11a). As this resistance gene derived from the rutabaga cv. 'Invitation' and determines clubroot resistance towards the *P. brassicae* isolate 'Vojens', the locus was designated as 'R-PbBn-IN-VO'.

During the multiple QTL mapping (MQM) mapping analysis of the segregating DH population 'R103', three genomic regions were identified as linked with resistance to *P. brassicae* isolate 'Tiebensee'. One locus mapped in the telomeric region of chromosome A03 close to 'R-PbBn-IN-VO'. The second locus was detected at position 44.8 cM of linkage group A05 with the closest markers R-0088922 and R-0090227. The third resistance locus effective against *P. brassicae* isolate 'Tiebensee' was located on chromosome A08 at 29.5 cM (Fig. 11A).

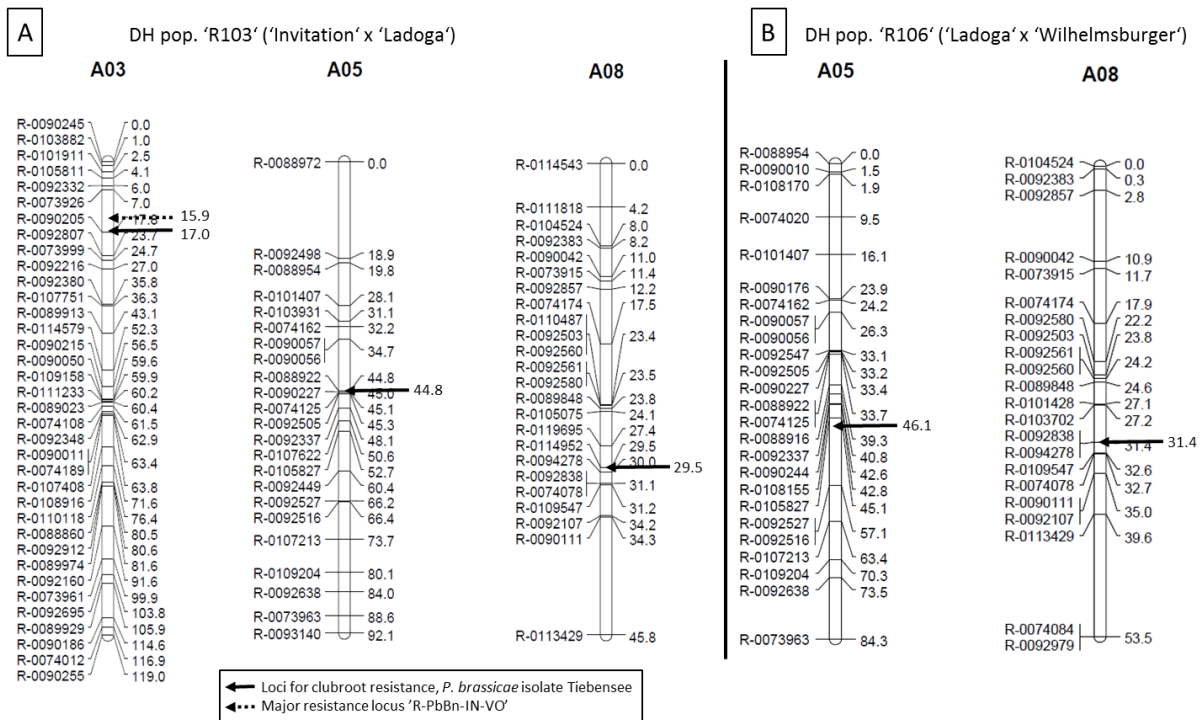


Fig. 11: Chromosomal locations of loci conferring resistance to clubroot isolate 'Tiebensee' identified in the DH populations 'R103' ('Invitation' x 'Ladoga'; A) and 'R106' ('Ladoga' x 'Wilhelmsburger'; B). Respective mapping positions [cM] are indicated by *black arrows*. The *interrupted arrow* indicates the mapping position [cM] of the major resistance locus 'R-PbBn-IN-VO'.

In case of DH population 'R106', two genomic regions linked with resistance to *P. brassicae* isolate 'Tiebensee' were identified. The peak position of the first locus was detected at 46.1 cM on chromosome A05, which is 1.00 cM distal of marker R-0105827 and 11.02 cM proximal of marker R-0092527. The second locus was detected at position 31.4 cM of linkage group A08 with the closest marker being R-0092838 (Fig. 11B).

4.4 Discussion

4.4.1 Genes and QTL known from mapping studies on clubroot resistance in *B. napus*

The first relevant study on mapping of clubroot resistance in *B. napus* was published by Manzanares-Dauleux et al. (2000b). A major resistance gene together with a QTL was mapped on linkage group DY4 which is synonymous to A03 (Werner 2007). Additional minor

QTL were identified on linkage groups DY2 (C02) and DY15 (C09). However, compared to more recent studies the *P. brassicae* isolates used were of very low virulence. Therefore, clubroot resistance loci from the donor 'Darmor-bzh' can be hardly used for improving resistance of oilseed rape. Nevertheless, it is important to underline that the main locus for clubroot resistance mapped by Manzanares-Dauleux et al. (2000b) is located on linkage group A03 in the genomic region representing the highest density of loci involved in clubroot resistance in both *B. napus* and *B. rapa* according to Piao et al. (2009) and the present study. As the review of Piao et al. (2009) contains all loci linked to clubroot resistance in *B. napus* up to that time, an update on major genes and QTL being effective to more than one *P. brassicae* isolate is given in Table 14.

Table 14: Comparison of mapped major genes and QTL (with effects against several *P. brassicae* isolates) for clubroot resistance in DH populations of *B. napus* reported in the literature with the mapping results of the present study

Reference	Resistance Donor (<i>B. napus</i>)	Ancestral Donor (<i>B. rapa</i>)	<i>P. brassicae</i> Isolate	Resistance Loci	DNA Marker Type	Linkage Group
Manzanares-Dauleux et al. (2000b)	'Darmor-bzh'	Unknown	K92-16 (P4 [#]); Pb137-522 (P7 [#])	Pb-Bn1	RFLP, RAPD	A03 (DY4)
Diederichsen et al. (2006)	DH47/19	ECD04	1 (SSI) + field isolates	Gene A from <i>B. rapa</i>	AFLP, SSR	A08 (MS06)
Werner et al. (2008)	263/11	ECD04	1 (SSI); 01.07; k; 01:60	PbBn-1-1; PbBn-01.07-1; PbBn-k-2; PbBn-01:60-1	AFLP, SSR	A03
Werner et al. (2008)	263/11	ECD04	1 (SSI); 01.07; a	PbBn-1-2; PbBn-01.07-2; PbBn-a-1	AFLP, SSR	A08
Zhang et al. (2016)	12-3	ECD04	Pathotype 3 ^{##}	Linked to CRa	SCAR	A03
Hasan and Rahman (2016)	Rutabaga cv. 'Brookfield'	Unknown	Pathotype 3 ^{##}	Close to Crr1a	SSR	A08
Present study	Rutabaga cv. 'Invitation'	ECD04	Vojens (P1 [#]); Tiebensee (P6 [#])	R-PbBn-IN-VO	SNP	A03

[#] Pathotype based on the differential of Somé et al. (1996); ^{##} Pathotype based on differential of Williams (1966).

4.4.2 Results on clubroot resistance of cv. 'Mendel'

Diederichsen et al. (2006) reported about the genetics of clubroot resistance of the OSR hybrid cultivar 'Mendel'. In this case the resistance donor 'ECD 04' (*B. rapa* ssp. *rapifera*) was used to develop resynthesised *B. napus*, which then was backcrossed to different elite *B. napus* varieties. According to Buczacki et al. (1975) the donor 'ECD 04' contains three dominant resistance genes. The presence of three dominant, race-specific clubroot resistance genes in a BC generation derived from a resynthesised *B. napus* with the donor 'ECD 04' was also reported by Diederichsen et al. (1996). Later, Diederichsen et al. (2009)

concluded that two of the three clubroot resistance genes derived from 'ECD 04' have been lost during the breeding process leading to cv. 'Mendel'. Therefore, these authors stated that the resistance of 'Mendel' is due to one major gene, only. Anyhow, additional recessive genes would probably have no phenotypic effect on resistance in a hybrid cultivar. The monogenic clubroot resistance of 'Mendel' was finally located on linkage group MS06. The locus was reported to be closely linked to SSR markers HMR307, HMR337 and HMR388 (Diederichsen et al. 2006). Werner et al. (2008) also worked with progenies of 'ECD 04' and used SSR markers HMR0337a, HMR0388 and others to map clubroot resistance. These markers mapped very close to a major QTL on linkage group A08 related to resistance to three different clubroot isolates. Thereupon, it is speculated that linkage group MS06 (Diederichsen et al. 2006) is synonymous to A08, based on the localisation of respective markers. Consequently, it may be possible that the resistance gene in cv. 'Mendel' (Diederichsen et al. 2006) and the major QTLs described by Werner et al. (2008) on linkage group A08 are identical. However, this hypothesis would have to be proven by allelism tests.

4.4.3 Clubroot resistance in DH population 'R103' with the donor 'Invitation'

In a study of Diederichsen et al. (1996) cuttings of a backcross population derived from a resynthesised *B. napus* with the resistant parent 'ECD04' were phenotyped in resistance tests with four different *P. brassicae* isolates. The segregation ratio of resistant versus susceptible lines differed depending on the isolate. The first and the second isolate revealed a 1r:1s ratio, indicating one effective resistance gene, each. Segregation ratios observed with a third fit to a 3r:1s segregation, suggesting two effective resistance genes. Finally, after inoculation with the fourth isolate the population segregated in a 7r:1s ratio, indicating three dominant resistance genes. Similar observations were made in the present study during resistance tests with two different clubroot isolates and the DH population 'R103' with the donor 'Invitation', whose resistance was derived from 'ECD 04', too (Bradshaw et al. 1997). In detail, phenotyping after inoculation with the very distinct isolate 'Vojens' (pathotype P1) gave hint for only one resistance gene, while for the moderate virulent isolate 'Tiebensee' (pathotype P6) three loci for clubroot resistance were found. These comparable findings demonstrate that the number of clubroot resistance loci detectable in in the same rapeseed genotype strongly depends on the virulence pattern of the *P. brassicae* isolate used for

testing. This observation should be kept in mind whenever the count of clubroot resistance genes is subject of debate. Anyway, the oilseed rape 'Mendel' and rutabaga 'Invitation', the resistance donor of the DH mapping population 'R103,' share the same ancestor for clubroot resistance, i.e., 'ECD 04' (Bradshaw et al. 1997; Diederichsen et al. 2006). Therefore, it is useful to compare the facts about 'Mendel' in the literature (see above) with the findings from the present study. The mapping of the DH population 'R103' revealed three gene loci for clubroot resistance in total: One locus each on linkage groups A03, A05 and A08. Therefore, the question arises, whether the locus detected in population 'R103' on chromosome A08 is identical to the resistance locus of 'Mendel'. For the present study, SNP markers have been used whereas the mapping studies of Diederichsen et al. (2006) and Werner et al. (2008) were conducted with AFLP and SSR markers. Therefore, the chromosomal locations cannot be identified and compared exactly. Consequently, further studies and particularly allelism tests would be needed to prove this hypothesis.

4.4.4 Clubroot resistance in DH population 'R106' and the donor 'Wilhelmsburger'

Since decades, the old German rutabaga (*B. napus* var. *napobrassica*) cultivar 'Wilhelmsburger' is in the focus of clubroot resistance research. As this genotype is known for differential resistance reactions it was included into the European Clubroot Differential (ECD) set and named 'ECD 10' (Buczacki et al. 1975). 'Wilhelmsburger' was used as resistance donor to set up the segregating DH population 'R106' used in the present work as a possible resource for improving clubroot resistance in oilseed rape. Lammerink (1967) phenotyped the *B. napus* cultivars 'Clubroot Resistant Rape', 'Wilhelmsburger' and progenies derived from crosses of both lines for clubroot resistance with a *P. brassicae* isolate named "Race B". It turned out that 'Clubroot Resistant Rape' was susceptible whereas 'Wilhelmsburger' was resistant. Based on the 3r:1s segregation ratio of the respective F₂ population, the presence of one resistance gene in 'Wilhelmsburger' was postulated (Lammerink 1967). However, the full name of this accession was 'New Zealand Wilhelmsburger swede' and it was mentioned that the genotype had been selected in New Zealand. Therefore, it is unknown whether the author really tested the original German rutabaga variety 'Wilhelmsburger', and it is therefore questionable, if the findings of Lammerink (1967) are directly comparable to the results obtained with cv. 'Wilhelmsburger' in the present study.

Ayers and Lelacheur (1972) crossed cv. 'Wilhelmsburger' as a female with the susceptible variety 'Laurentian' and phenotyped the offspring with two clubroot isolates characterised by Williams (1966) as races 2 and 3. In both cases, all F_1 plants were resistant, i.e., resistance is inherited in a dominant manner. Further, segregation ratios from resistance tests of the F_2 population showed that after inoculation with race 2 two genes segregated for clubroot resistance, whereas only one gene segregated for resistance to race 3. Crute et al. (1983) worked with more than 200 data sets on resistance of *B. napus* genotypes out of the ECD collection tested against different clubroot isolates from around the world. Based on their data, the authors developed different hypotheses ranging from a minimum of three up to a total of five clubroot resistance genes that can be found in the ECD *B. napus* lines. Thereof, the model based on four resistance genes explained the highest counts of observed disease reactions. Regarding the variety 'Wilhelmsburger' the authors concluded that it contained three clubroot resistance genes. Gustaffson and Fält (1986) tested segregating F_2 populations derived from crosses between ten clubroot resistant *B. napus* lines with one susceptible genotype, each, against four Scandinavian clubroot races to investigate the genetics of host resistance. In order to detect all of the resistance genes, they used a clubroot isolate with limited virulence potential named "Pb3". The segregation ratio of an F_2 progeny derived from a cross of resistant cv. 'Wilhelmsburger' and the susceptible cv. 'Doon Spartan' gave hint for the presence of two dominant resistance genes. In another test the authors used the highly virulent clubroot isolate "Pb7" and identified only one dominant resistance gene in the same F_2 progeny. Based on these observations, the authors concluded that the number of detectable resistance genes strictly depends on the virulence pattern of the clubroot isolate used for inoculation. In detail, they confirmed that infection with an isolate of limited virulence pattern led to a higher number of detected clubroot resistance genes compared to an isolate with stronger virulence. Additionally, the authors confirmed the hypothesis of Crute et al. (1983) of a maximum of four genes in total that cause clubroot resistance in all *B. napus* genotypes of the ECD collection. However, considering the genotype 'ECD 10', it is obvious that the two conclusions are contradictory. Crute et al. (1983) expected three genes in that genotype, while Gustafsson and Fält (1986) confirmed only two effective resistance loci. The incongruity between these two assumptions has already been commented by Diederichsen et al. (2009), who stated that even the Scandinavian isolate with a limited virulence pattern had the capability to overcome the third resistance gene.

In our studies, the P6 clubroot isolate 'Tiebensee' (ECD code '16/03/14') was chosen for resistance tests because of its limited virulence pattern and the expectation to detect the maximum number of resistance genes here. But, according to the pathotype classification or ECD code (Table 2) it is evident that the isolate 'Tiebensee' can overcome the resistance of the differential genotype 'ECD 06' (cv. 'Nevin') resulting in the fact that the common gene of cultivars 'Nevin' and 'Wilhelmsburger' (Crute et al. 1983) could not be detected. Finally, both clubroot resistance genes of cv. 'Wilhelmsburger' located on chromosomes A05 and A08 agree with the findings of empiric models set up and published more than 30 years ago (Ayers and Lelacheur 1972; Crute et al. 1983; Gustafsson and Fält 1986). Thereupon, it is assumed that cv. 'Wilhelmsburger' contains at least one additional resistance gene, which however could not be discovered in the present study. Finding this particular resistance locus would have required additional phenotyping and mapping based on a further *P. brassicae* isolate characterised as pathotype P3 or P4 on the set of Somé et al. (1996) assuming that they are not virulent against cultivar 'Nevin'.

4.4.5 Comparison of the resistance loci of 'Invitation', 'Wilhelmsburger' and 'Mendel'

The pedigree of 'Invitation' as resistance donor of the DH population 'R103' is well known (Bradshaw et al. 1997) and can be traced back to 'ECD 04'. This is not the case for 'Wilhelmsburger', where only is known that the resistance was derived from Dutch or Belgian stubble turnips (*B. rapa ssp. rapifera*, Diederichsen et al. 2009). The locus most interesting in breeding for clubroot resistance detected in the present work may be the major gene 'R-PbBN-IN-VO' on chromosome A03 of 'Invitation'. In contrast, no resistance locus has been found on chromosome A03 in 'Wilhelmsburger', but in both genotypes one resistance locus was mapped on chromosome A05. However, the respective loci on linkage group A05 may differ due to different flanking markers (Fig. 11). On chromosome A08 the situation is somewhat different since the loci detected in both donors share one of the flanking markers, i.e., R-0094278. Besides this, the marker R-0092838, which maps exactly at the position of the locus detected in 'R106', mapped only 1.6 cM distally of that detected in 'R103'. Therefore, these genetic loci may be identical. To prove this, allelism tests would have to be conducted. But, if the assumption of a common locus in that region of 'Invitation' and 'Mendel' will be correct, it must also be true for cultivars 'Wilhelmsburger' and

'Mendel'. On the other hand, numerous clubroot isolates were identified in the pathotype survey, which caused different resistance reactions in the differential hosts 'Wilhelmsburger' ('ECD 10') and 'Mendel'. If 'Wilhelmsburger' had the same resistance gene as 'Mendel' on chromosome A08 and an additional locus on A05, this variety should be resistant to the same isolates as 'Mendel'. But, the results of phenotyping tests during the pathotype characterisation issue were actually different. In all tests conducted, pathotype P1 was identified 18 times (see chapter 3.3.1.2; Table 8). Consequently, in all these cases 'Wilhelmsburger' was susceptible because pathotype P1 is defined to overcome the resistance of all differential hosts, including 'Wilhelmsburger' (Somé et al. 1996). But, remarkably, 'Mendel' turned out to be susceptible only against six out of the 18 isolates described as P1. To survey the identity of resistance genes, F₂ progenies derived from crosses of 'Wilhelmsburger' and a Mendel-derived clubroot resistant inbred line were tested for clubroot resistance (data not shown). The segregation of resistant and susceptible plants in this F₂ population was in accordance with the expected ratio of 15r:1s. Consequently, the clubroot resistance locus of 'Wilhelmsburger' and 'Mendel' on linkage group A08 should be different. But the information content of these data is limited since results are obtained on single plants. To confirm these results, F₃ progeny tests have to be conducted.

4.4.6 Genetic core regions for clubroot resistance in *B. napus*

By genetic mapping of clubroot resistance in oilseed rape, Werner et al. (2008) identified 19 QTL in total, spread over seven chromosomes of the A and C genomes of *B. napus*. But QTL that contributed resistance to more than one isolate were identified on linkage groups A03 and A08 only (Table 14). Diederichsen et al. (2014) summarised the state of scientific knowledge on clubroot resistance loci in *B. napus* and pointed out that most of the effective loci involved are mapped on linkage groups A03 and A08. All additional QTL exhibited resistance effects to only one isolate and are of low relevance in breeding, therefore. In summary, this confirms the earlier recommendation of Chiang et al. (1977) to search for clubroot resistance genes in the A genome of the ancestral donor, *B. rapa*. Meanwhile, it has been shown that clubroot resistance genes in resynthesised *B. napus* transferred from donors of the *B. rapa* gene pool cause resistance even in the modified genetic background (Bradshaw et al. 1997, Diederichsen et al. 2006). Correspondingly, Werner (2007) stated that

major genes from *B. rapa* were re-detected on the respective chromosomes A03 and A08 in resynthesised *B. napus* progenies. And recently, Hirani et al. (2016) proved that molecular markers can be effectively deployed for MAS of clubroot resistance in diverse germplasm of *B. rapa* and *B. napus*, respectively. Therefore, it is relevant for resistance breeding in *B. napus* to mention that six out of a total of eleven discovered clubroot resistance genes were mapped on chromosome A03 of *B. rapa* and three additional genes were detected on chromosome A08 of *B. rapa* (Piao et al. 2009; Cho et al. 2012; Kato et al. 2012; Hatakeyama et al. 2013). Because of different marker technologies used, it cannot be concluded that the clubroot resistance genes identified in the A genome of *B. napus* in the present study are the same as those detected in *B. rapa*. However, the genomic regions, where clubroot resistance genes are detected in *B. napus* and *B. rapa*, respectively, give strong hints to similar, if not the same genes.

4.4.7 Outlook on breeding for clubroot resistance in *B. napus* rapeseed

The findings presented in this study fully support the state of knowledge published in literature. For an application of the results in breeding for clubroot resistance in oilseed rape, it is particularly recommended to exploit the major gene locus of cv. 'Mendel' in future resistance breeding programs, since that locus still provides an adequate level of resistance in many OSR growing areas across Europe, where clubroot is a major threat. But, at the same time it will be of outstanding importance to diversify the oilseed rape germplasm and elite material with regard to clubroot resistance. In order to stabilise the clubroot resistance in parental lines and future hybrid cultivars, it is recommended to focus on the major resistance gene 'R-PbBn-IN-VO' and additional resistance loci from 'Invitation' and 'Wilhelmsburger'. These gene loci may be combined with the major resistance gene of 'Mendel' by pyramiding in various combinations. A further useful source of resistance will be the genotype 'ECD 04'. For future research, a detailed comparison of newly discovered clubroot resistance genes to already known ones should be conducted, in order to improve the efficiency of marker-based breeding. In this respect, detailed tests for allelism have to be conducted and new resistance loci need to be fine mapped as a prerequisite for gene cloning (Gao et al. 2014). To achieve this, besides high resolution mapping populations, a large number of molecular markers for the target regions is needed. Respective markers can be

developed from the full sequence of the *B. napus* genome (Chalhoub et al. 2014), or primers from candidate genes for chromosomes A03 and A08 may be used (Gao et al. 2014; Hasan and Rahman 2016; Zhang et al. 2016). On top of that, the recent knowledge about the full sequence of the pathogen genome will allow a detailed analysis on the interactions within the Brassicaceae - *P. brassicae* pathosystem, which may support future resistance breeding (Schwelm et al. 2015; 2016).

4.5 Summary for characterisation and genetic mapping of clubroot resistance in rapeseed

Clubroot caused by the obligate biotrophic protist *Plasmodiophora brassicae* is a serious soil-borne disease of cruciferous crops. Based on resting spores the longevity of the pathogen in the soil is estimated at about 20 years. Agricultural control means like liming with calcium carbonate or the use of calcium cyanamide may reduce the inoculum potential and save yield, but cannot eliminate clubroot from a contaminated field. Therefore, particularly for a large-scale crop as OSR there are no economically reasonable control measures once a field has been infested. Consequently, breeding for clubroot resistance is the most powerful tool to control the disease. Up to now, only one race specific resistance has been incorporated in adapted cultivars. Therefore, broadening the genetic base of resistance in OSR is needed. Potential donors for clubroot resistance are mainly known from *B. oleracea* and *B. rapa*, ancestral parents of *B. napus*. But, also within the primary gene pool of *B. napus* itself resistance is known. Therefore, clubroot resistances from two rutabaga (*Brassica napus* var. *napobrassica*) varieties, i.e., cv. 'Invitation' and 'Wilhelmsburger' were genetically mapped in DH lines of crosses to the oilseed rape (*Brassica napus* L.) cv. 'Ladoga' using a set of 1,109 SNP markers. The DH populations were phenotypically analysed for resistance against two *P. brassicae* isolates showing different virulence patterns in the greenhouse. The segregation ratios indicated the presence of one, two and three genes, respectively, conferring resistance depending on the *P. brassicae* isolate and the DH population. Studies with F₁ progenies showed the presence of a dominantly inherited resistance gene in both donor lines. A genetic map of each DH population of the whole genome was constructed using 427 and 409 polymorphic SNP markers, respectively. Loci conferring resistance were mapped on chromosomes A03, A05 and A08. The high level of resistance expressed by cv. 'Invitation' to the highly virulent P1 isolate 'Vojens' is due to a major gene located on linkage group A03,

which was named 'R-PbBn-IN-VO'. Within the same DH population three loci conferring resistance to the moderately virulent P6 isolate 'Tiebensee' were mapped on chromosomes A03, A05 and A08. Towards the latter isolate, resistance derived from cv. 'Wilhelmsburger' was mapped on chromosome A05 and A08. Respective loci can be used for pyramiding with the monogenic resistance derived from cv. 'Mendel'. Furthermore, the loci derived from cv. 'Wilhelmsburger' are especially suited for regions in Europe, where the pathotype P3 prevails and for the Canadian prairies.

5 Final Conclusions

Clubroot resistance of current European OSR cultivars is solely based on the monogenic resistance derived from the cultivar 'Mendel'. Therefore, the objective of this work was the genetic mapping of new clubroot resistance genes from the primary gene pool of *B. napus* as a prerequisite to breed OSR varieties with increased resistance durability suitable for the European areas of cultivation. Since resistance donors from the primary gene pool of *B. napus* were described as having race specific effects it was necessary to conduct a monitoring of *P. brassicae* pathotypes present in the European OSR growing areas prior to genetic mapping of resistance genes. The monitoring should identify the most common pathotypes defining the required resistance profile of new OSR varieties. It turned out that pathotypes P1 and P3 are most prevalent in Europe. Isolate-specific effects of the discovered loci for clubroot resistance were detected. Based on the knowledge about pathotypes occurring in different areas of the world, it is possible to select the best suited donors of resistance for breeding clubroot resistant OSR cultivars. The resistance donor 'Invitation' proved to be resistant to the majority of tested clubroot isolates and a new major resistance gene on chromosome A03 was discovered. As the monogenic resistance of 'Mendel' is located on chromosome A08, the major resistance gene located on A03 from 'Invitation' can be used for broadening the genetic basis of clubroot resistance in European OSR varieties. In contrast, several populations of *P. brassicae* tested were virulent (including all pathotype P1 isolates) to the resistance donor 'Wilhelmsburger'. It is known from the literature that in Canada, in contrast to Europe, *P. brassicae* pathotype P1 does not play a major role. Therefore, the new resistance loci detected in cv. 'Wilhelmsburger' are mainly recommended to be used in clubroot resistance breeding programs focussing on Canadian areas of canola cultivation. Finally, closely linked SNP markers for all identified resistance loci were detected in this study, which can be used in marker-assisted breeding programs pyramiding resistance loci.

In this study pathotyping of *P. brassicae* populations as well as the resistance testing of various donors and resistant OSR cultivars to different clubroot populations were conducted under different environmental conditions, i.e., in the greenhouse and in the field. The main findings hereof were: i) pathotype classifications from the greenhouse were mostly confirmed in field tests; ii) the disease reactions of the majority of resistance donors were

the same in both environments; but iii) the disease index describing disease incidence and severity was higher in the greenhouse compared to the field, and iv) few genotypes, thereof the clubroot resistant OSR cultivar 'Mendel', were differing in their disease reactions depending on the environment. Under controlled conditions, 'Mendel' was susceptible to 30% of the *P. brassicae* isolates, while a susceptible reaction was rarely found in the field tests. This shows, that greenhouse tests may lead in some cases to an overestimation of virulence patterns of *P. brassicae* isolates, e.g., in case of cv. 'Mendel', but that most likely genotypes being resistant in greenhouse tests will also be resistant in the field.

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8 Supplementary Data

Supplementary data 1: Disease index induced by *Plasmodiophora brassicae* populations from Europe on the European Clubroot Differential set (ECD), the hosts from Somé et al. (1996) and two resistant cultivars under controlled conditions and respective pathotype classifications

No.	Country	State/Province/Region/ Voivodeship	Location	Host of origin	Disease index ^a on differential host														Pathotype ^b						
					ECD 01	ECD 02	ECD 03	ECD 04	ECD 05	ECD 06	ECD 07	ECD 08	ECD 09	ECD 10	ECD 11	ECD 12	ECD 13	ECD 14	ECD 15	Brutor	Mendel	Clapton	Mean of genotypes ^c	ECD	Somé et al. (1996)
1	Germany	Baden-Wuerttemberg	MUDAU	<i>Brassica napus</i>	0	0	0	0	60	36	75	10	4	4	20	50	75	75	24	15	5	n/ ^a	28	16/07/14	P6
2	Germany	Baden-Wuerttemberg	SCHUTTERWALD	<i>Brassica napus</i>	44	74	49	74	70	40	78	71	72	31	38	21	51	47	11	68	61	41	52	31/31/13	P1
3	Germany	Bavaria	ALTOETTING	<i>Brassica napus</i>	5	0	5	0	52	41	68	63	73	68	31	41	65	35 ^d	58	5	0	0	38	16/31/29	P1
4	Germany	Bavaria	ESCHENBACH ^d	<i>Brassica napus</i>	10	0	8	0	60	48	68	65	63	35	0	18	33	0	38	8	3	3	28	16/31/02	P1
5	Germany	Bavaria	NIEDERHUMMEL	<i>Brassica napus</i>	15	1	1	0	66	15	61	69	6	9	8	13	33	7	69	6	n/ ^a	25	16/06/12	P3	
6	Germany	Hesse	BESELICH	<i>Brassica napus</i>	59	21	8	6	83	89	88	85	79	63	74	50	84	46	91	88	56	64	17/31/31	P1	
7	Germany	Hesse	GRAEVENWIESBACH	<i>Brassica napus</i>	8	4	11	0	74	3	84	50	51	15	41	33	69	30	65	44	31	38	16/14/31	P3	
8	Germany	Hesse	ORTENBERG ^d	<i>Brassica napus</i>	23	6	5	0	75	3	78	80	78	13	92	50	80	67	73	65	n/ ^a	51	16/14/31	P3	
9	Germany	Lower Saxony	BALJE (NORDKEHDINGEN)	<i>Brassica napus</i>	17	9	8	1	76	34	75	83	81	36	51	18	62	26	74	8	3	45	16/31/29	P1	
10	Germany	Lower Saxony	HATTORF	<i>Brassica napus</i>	8	14	0	0	71	76	72	73	69	64	34	28	55	21	59	55	44	44	16/31/15	P1	

Supplementary data 1 continued

No.	Country	State/Province/Region/ Voivodeship	Location	Host of origin	Disease index ^a on differential host															Pathotype ^b					
					ECD 01	ECD 02	ECD 03	ECD 04	ECD 05	ECD 06	ECD 07	ECD 08	ECD 09	ECD 10	ECD 11	ECD 12	ECD 13	ECD 14	ECD 15	Brutor	Mendel	Clapton	Mean of genotypes ^c	ECD	Some et al. (1996)
11	Germany	Lower Saxony	LIPPOLDSHAUSEN	<i>Brassica napus</i>	0	0	0	1	75	43 ^d	76	74	70	8	55	44	68	70	30	67	39	36	42	16/15/3 1	P2
12	Germany	Lower Saxony	MIELENHAUSEN	<i>Brassica napus</i>	10	0	4	0	75	29	80	76	73	18	57	42	67	54	53	70	39	38	44	16/15/3 1	P2
13	Germany	Lower Saxony	OBERODE	<i>Brassica napus</i>	23	0	0	0	75	14	75	65	68	14	55	28	61	64	30	56	28	23	39	16/14/3 1	P3
14	Germany	Lower Saxony	WOHLD	<i>Brassica napus</i>	5	5	14	3	70	20	74	74	75	33	54	51	84	61	66	66	9	4	47	16/30/3 1	P5
15	Germany	Mecklenburg- Vorpommern	ALTENKIRCHEN ^e	<i>Brassica oleracea</i>	55	0	0	0	100	100	100	100	10	0	97	90	0	0	10	0	0	n/ a	78	17/31/3 1	P1
16	Germany	Mecklenburg- Vorpommern	GROß SCHWIESOW	<i>Brassica napus</i>	22	0	0	0	73	63	70	74	65	59	33	50 ^d	55	65	30	49	19	n/ a	44	16/31/3 1	P1
17	Germany	Mecklenburg- Vorpommern	WARNITZ ^d	<i>Brassica napus</i>	0	0	0	0	78	80	73	75	68	83	3	13	73	80	25	75	0	0	45	16/31/1 2	P1
18	Germany	Rhineland-Palatinate	GONDERSHAUSEN	<i>Brassica napus</i>	12	1	0	0	68	31	69	64	66	43 ^d	26	27	58	46	13	66	25	n/ a	36	16/31/1 5	P1
19	Germany	Rhineland-Palatinate	NIEDERROTTERBACH	<i>Brassica napus</i>	6	0	3	0	74	9	73	76	37	17	9	14	29	41	9	68	6	n/ a	29	16/14/1 2	P3
20	Germany	Rhineland-Palatinate	RAVENSBEUREN	<i>Sinapis arvensis</i>	19	0	0	0	38	1	63	33	13	0	31	4	13	15	8	50	26	n/ a	18	16/06/0 1	P3
21	Germany	Rhineland-Palatinate	SAYN	<i>Brassica napus</i>	4	1	6	0	63	33	68	73	71	36 ^d	29	36	70	72	55	76	6	n/ a	43	16/31/3 1	P1
22	Germany	Schleswig-Holstein	GROßENSEE ^d	<i>Brassica napus</i>	0	0	0	0	75	68	95	75	75	56	75	69	89	8	0	75	0	0	48	16/31/0 7	P1
23	Germany	Schleswig-Holstein	HOISDORF	<i>Brassica napus</i>	13	4	6	1	73	74	74	74	72	68	46	41	71	74	46	45	60	n/ a	49	16/31/3 1	P1
24	Germany	Schleswig-Holstein	MARNE	<i>Brassica oleracea</i>	0	0	5	0	75	24	76	3	4	4	9	54	75	66	49	26	8	1	29	16/02/3 0	P3

Supplementary data 1 continued

No.	Country	State/Province/Region/ Voivodeship	Location	Host of origin	Disease index ^a on differential host														Pathotype ^b							
					ECD 01	ECD 02	ECD 03	ECD 04	ECD 05	ECD 06	ECD 07	ECD 08	ECD 09	ECD 10	ECD 11	ECD 12	ECD 13	ECD 14	ECD 15	Brutor	Mendel	Clapton	Mean of genotypes ^c	ECD		
25	Germany	Schleswig-Holstein	TIEBENSEE	<i>Brassica napus</i>	0	0	0	0	67	37	64	1	0	0	0	21	53	60	67	24	18	4	n/ a	25	16/03/1 4	P6
26	Poland	Greater Poland	POZNAN	<i>Brassica napus</i>	0	0	0	0	46	70	68	53	76	56	13	15	18	49	18	48 ^d	5	0	32	16/31/0 8	P1	
27	Poland	Lower Silesian	LEGNICA	<i>Brassica napus</i>	0	0	3	0	75	3	80	69	79	4	63	60	70	85	21	79	14	0	43	16/14/1 5	P3	
28	Poland	Lower Silesian	PIELGRZYMIKA	<i>Brassica napus</i>	0	0	4	0	68	10	70	20	0	4	43	49	66	75	29	13	4	1	28	16/02/3 1	P4	
29	Poland	Lubusz	ZIELONA GÓRA	<i>Brassica napus</i>	0	0	6	0	70	20	80	74	75	10	45	51	64	75	68	74	16	0	45	16/14/3 1	P3	
30	Poland	Opole	OPOLE	<i>Brassica napus</i>	0	0	3	0	75	9	80	76	74	4	29	40	69	76	34	74	11	0	40	16/14/3 1	P3	
31	Poland	Warmian-Masurian	OLSZTYN	<i>Brassica napus</i>	0	0	0	0	64	13	61	51	54	9	24	48 ^d	53	55	48 ^d	45	3	1	32	16/14/3 0	P3	
32	Poland	West Pomeranian	SZCZECIN	<i>Brassica napus</i>	34	8	15	0	71	75	75	79	59	75	59	58	68	76	55	74	74	64	55	17/31/3 1	P1	
33	France	Brittany	PAIMPOL ^d	<i>Brassica oleracea</i>	0	0	0	0	38	5	55	5	0	0	0	0	30	15	5	0	8	n/ a	10	16/02/0 4	P4	
34	France	Brittany	ST. POL DU LÉON ^d	<i>Brassica oleracea</i>	0	0	0	0	68	10	70	60	45	3	30	23	31	63	3	93	43	19	31	16/14/1 3	P3	
35	France	Centre	OIZON/LE BOULAY	<i>Brassica napus</i>	3	0	0	0	50	20	66	59	60	11	29	22	56	66	28	51	10	n/ a	33	16/14/2 9	P3	
36	France	Centre	OIZON/VAL NERE	<i>Brassica napus</i>	3	0	0	3	65	71	80	40	36	10	14	3	25	50	10	71	14	0	30	16/15/0 8	P2	
37	France	Pays de la Loire	LA BOHALLE	<i>Brassica oleracea</i>	0	0	0	0	50	41	69	0	0	0	20	49	58	53	33	13	0	n/ a	24	16/03/3 0	P6	
38	Czech Republic	Moravia, Vysocina Region	KRIZANOW ^d	<i>Brassica napus</i>	5	0	3	0	73	18	100	70	75	20	13	25	70	80	53	75	23	0	42	16/14/2 8	P3	

Supplementary data 1 continued

No.	Country	State/Province/Region/ Voivodeship	Location	Host of origin	Disease index ^a on differential host															Pathotype ^b					
					ECD 01	ECD 02	ECD 03	ECD 04	ECD 05	ECD 06	ECD 07	ECD 08	ECD 09	ECD 10	ECD 11	ECD 12	ECD 13	ECD 14	ECD 15	Brutor	Mendel	Clapton	Mean of genotypes ^c	ECD	Some et al. (1996)
39	Czech Republic	Moravian-Silesian region	FRYCHOVICE	<i>Brassica napus</i>	3	1	1	0	75	23	79	80	72	70 ^d	79	50	70	71	29	76	4	8	45	16/14/3 ₁	P3
40	Czech Republic	Moravian-Silesian region	KOZMICE	<i>Brassica napus</i>	0	5	5	0	70	1	59	66	61	14	40	28	48	34	26	64	9	n/ _a	32	16/14/3 ₁	P3
41	Czech Republic	Moravian-Silesian region	SEDLNICE ^d	<i>Brassica napus</i>	0	0	0	0	50	23	68	45	65	3	25	0	25	30	0	63	8	0	25	16/14/0 ₈	P3
42	Austria	Upper Austria	ANTIESENHOFEN	<i>Brassica napus</i>	8	1	0	0	70	45 ^d	74	76	43	10	17	26	44	58	4	75	16	1	34	16/15/1 ₄	P2
43	Austria	Upper Austria	BAUMGARTEN	<i>Brassica napus</i>	4	0	0	0	75	4	62	65	9	4	0	10	10	46	3	74	4	0	23	16/06/0 ₈	P3
44	Austria	Upper Austria	GURTEN	<i>Brassica napus</i>	3	0	0	0	74	12	78	48	3	5	6	29	50 ^d	46	30	38	36	0	25	16/06/3 ₀	P3
45	Denmark	Central Denmark	FLEMMING	<i>Brassica napus</i>	45	4	6	0	69	75	73	68	40	61	30	14	49	55	13	64	74	n/ _a	41	17/31/1 ₃	P1
46	Denmark	Southern Denmark	VOJENS	<i>Brassica napus</i>	9	0	3	3	49	45	56	56	53	51	3	5	20	54	11	57	8	n/ _a	30	16/31/0 ₈	P1
47	Scotland	Aberdeen	ABERDEEN	<i>Brassica napus</i>	41	13	0	0	70	68	60	56	61	61	3	13	26	54	25	68	11	n/ _a	39	17/31/1 ₂	P1
48	Scotland	Angus	MONTROSE	<i>Brassica napus</i>	0	1	0	0	63	69	69	56	58	30	44	41	58	53	44	77	5	n/ _a	41	16/31/3 ₁	P1

^a mean of two replicates (n=20; 2x10)

^b as determined on the differential hosts of the European Clubroot Differential set (Buczacki et al., 1975) and Somé et al. (1996). For classification into pathotypes, an index of disease of D>25 was used as the cut-off between a resistant and a susceptible reaction.

^c average of all orthogonally tested hosts ECD 01 to ECD 15 plus cv. 'Brutor'

^d only one replicate available (n=10)

^e phenotypic rating by Henry, M. (Clause)

n/a no data

Supplementary data 2: Disease index of *Plasmodiophora brassicae* on the European Clubroot Differential Set (ECD), the hosts from Somé et al. (1996) and two resistant cultivars under field conditions and respective pathotype classification

Year	Location	Disease index ^a on differential host															Pathotype ^b					
		ECD 01	ECD 02	ECD 03	ECD 04	ECD 05	ECD 06	ECD 07	ECD 08	ECD 09	ECD 10	ECD 11	ECD 12	ECD 13	ECD 14	ECD 15	Brutor	Mendel	Clapton	Mean ^c	ECD	Somé et al. (1996)
2011	GROß SCHWIESOW	2	0	0	0	48 ^d	63	72	24	16	38	4	2	11	34	0	43 ^d	7	n/a	22	16/19/08	P1
	HOISDORF	7	1	0	0	88	61	82	86	75	70	42	31	55	81	11	74	24	n/a	46	16/31/15	P1
	OIZON-VAL NÈRE	0	0	0	0	38	40	33	55	42	11	0	3	1	30	2	34	n/a	n/a	20	16/15/08	P2
	TIEBENSEE	3	0	0	0	42	30 ^d	50	32 ^d	35 ^d	10	5	7	41	43	3	1	1	n/a	17	16/15/12	P6
2012	GROß SCHWIESOW ^e	2	0	0	0	51	15	27	16	3	22	0	0	12	14	0	11	1	5	11	-	-
	HOISDORF	1	3	0	0	93	60	74	63	59	46	28	11	55	44	3	71	22	4	38	16/31/13	P1
	MIELENHAUSEN	4	1	0	0	97	36	98	99	99	12	33	22	60	91	27	72	7	13	49	16/15/29	P2
	OIZON-LE BOULAY ^e	0	0	0	0	9	4	27	32	38	1	0	0	10	6	0	11	n/a	0	9	-	-

^a mean of two replicates (n=48; 2x24)

^b as determined on the differential hosts of the European Clubroot Differential set (Buczacki et al., 1975) and Somé et al. (1996). For classification into pathotypes, an index of disease of DI>25 was used as the cut-off between a resistant and a susceptible reaction.

^c average of all orthogonally tested hosts ECD 01 to ECD 15 plus cv. 'Brutor'

^d only one replicate available (n=24)

^e in 2012 the level of clubroot infection in Groß Schwiesow and Oizon-Le Boulay was that low that the general suspects ECD 05, ECD 07 and ECD 14 exhibited clubbing on a level far below the threshold to call it a susceptible response. Therefore the data from those locations were no longer put into account and no pathotype was classified.

n/a no data

Erklärung

gemäß der Promotionsordnung des Fachbereichs 09 vom 07. Juli 2004 § 17 (2)

„Ich erkläre: Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe.

Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht.

Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“



Braunschweig, im Dezember 2016

