

Assessment of biological and molecular variability between and within field isolates of *Plasmodiophora brassicae*

M. J. Manzanares-Dauleux*†, I. Divaret, F. Baron and G. Thomas

INRA Station d'Amélioration des Plantes, BP 35327, 35653 Le Rheu Cedex, France

Plasmodiophora brassicae is an obligate biotroph that causes clubroot, one of the most damaging diseases of crucifers. Differential cultivars and random amplified polymorphic DNA markers were used to assess the extent of genetic diversity among nine single-gall populations of *P. brassicae* and 37 single-spore isolates (SSI) derived from four of those field samples. Isolates were classified into eight pathotypes, and each isolate was associated with a unique molecular genotype. Virulence and DNA polymorphisms were detected within and between field isolates, and among SSIs from different pathotypes, hosts and geographical origins. The relatively high level of genetic diversity among field isolates was similar to that among SSIs derived from a single-club field isolate. Molecular and pathogenicity-based classifications were not clearly correlated, but isolates belonging to pathotype P1 were clustered. Two RAPD markers were specific to pathotype P1. The finding that genetic differences can occur in *P. brassicae* field isolates will be an important consideration in resistance genetic studies and in choosing breeding strategies to develop durable clubroot resistance.

Keywords: clubroot, crucifers, molecular markers, RAPD, single-spore isolates, virulence

Introduction

Plasmodiophora brassicae is a soilborne, obligate biotroph that causes clubroot, one of the most damaging diseases of crucifers. The life cycle of the parasite consists of two main phases, the first occurring in root hairs, the second in cells of the root cortex and stele leading to gall formation and production of haploid resting spores. The clubs formed inhibit nutrient and water transport, stunt the growth of the plant and increase susceptibility to wilting. *Plasmodiophora brassicae* occurs worldwide and has an extensive host range, which includes the cultivated *Brassica* species and wild crucifers such as *Arabidopsis thaliana* (Koch *et al.*, 1991). Cultural practices and/or chemical treatments have been unsuccessful in protecting crops or have proved too expensive. Therefore the development of resistant cultivars is now considered the most economical and efficient method for the control of clubroot.

Successful breeding and effective deployment of durable plant resistance require an understanding of pathogen diversity and of the way in which virulence evolves in pathogen populations. Very little is known about the extent and mechanisms of variability in *P. brassicae*. Significant differences in pathogenicity

have been recognized among field populations of *P. brassicae* using sets of differential hosts (Williams, 1966; Buczacki *et al.*, 1975; Crute *et al.*, 1980; Voorrips, 1995; Somé *et al.*, 1996; Kuginuki *et al.*, 1999). Variation in virulence was also demonstrated within field isolates (i.e. *P. brassicae* isolated from a single root gall) by using single-spore isolates (Haji Tinggal & Webster, 1981; Jones *et al.*, 1982a; Scott, 1985; Schoeller & Grunewaldt, 1986; Somé *et al.*, 1996). Few alternative molecular methods have been developed to define further the extent of genetic variation within pathogen populations. Polymerase chain reaction (PCR) primers derived from putative repetitive/high-copy-number DNA sequences of *P. brassicae* have been described by Buhariwalla *et al.* (1995) as potential markers of genetic variation. Möller & Harling (1996) used RAPD markers to differentiate three isolates of *P. brassicae*. However, the structure of pathogenic variability has received little attention in *P. brassicae*. To date, no studies have been reported concerning genetic variation within and between field isolates and between different single-spore isolates.

The objectives of the current research were (i) to study the genetic variability within and between *P. brassicae* field isolates in terms of virulence and DNA polymorphism; (ii) to detect genomic polymorphism directly related to pathotype classification; and (iii) to identify molecular markers that can be used for tracking specific isolates of the pathogen.

*To whom correspondence should be addressed.

†E-mail: manzanar@rennes.inra.fr

Accepted 9 October 2000.

Materials and methods

Plasmodiophora brassicae isolates

Forty-six isolates, nine field isolates and 37 single-spore isolates (SSIs) of *P. brassicae* were studied (Table 1). Field isolates were collected from single galls of

naturally infected cauliflower (*Brassica oleracea* conv. *botrytis*), rapeseed (*B. napus* var. *oleifera*) and Chinese cabbage (*B. rapa* spp. *pekinensis*, cv. Granaat) grown in Brittany (north-western France), either in different fields or in the same field but sampled over several years. Single-spore isolates were obtained from four of these field isolates (PbM, K92, SJ92 and Pb137; Table 1) as

Table 1 Characteristics and pathotype designation as determined in this study of 46 field and single-spore isolates (SSI) of *Plasmodiophora brassicae*

Type of isolate	Source field isolate ^a (pathotype)	Host	French district	Year of collection	Isolate	Pathotype ^b		
Field		Rapeseed	Côtes d'Armor	1993	PbM	P1		
		Cauliflower	Finistère	1991	Pb137	P5		
			Ille-et-Vilaine	1992	K92	P2		
				1992	SJ92	P5		
				1993	SJ93	P1		
				1993	K93	P1		
	Chinese cabbage	Finistère	1994	K94	P7			
			1995	K95	P7			
			1996	K96	P7			
		SSI ^c	PbM (P1)	Rapeseed	Côtes d'Armor	1993	Ms6	P1
							M651	P1
							M727	P1
							M746	P1
	M774					P1		
K92 (P2)	Cauliflower		Finistère	1992	K92-42	P1		
					K92-33	P3		
					K92-18	P3		
					K92-16	P4		
					K92-19	P4		
					K92-118	P4		
					K92-8	P6		
					K92-24	P7		
		K92-32			P7			
		Pb137 (P5)			Cauliflower	Finistère	1991	Pb137-436
Pb137-437	P4							
Pb137-514	P4							
Pb137-538	P4							
Pb137-560	P4							
Pb137-524	P5							
Pb137-418	P7							
Pb137-434	P7							
Pb137-439	P7							
Pb137-499	P7							
Pb137-522	P7							
Pb137-532	P7							
Pb137-543	P7							
Pb137-555	P7							
Pb137-566	P7							
Pb137-574	P7							
SJ92 (P5)	Cauliflower	Ille-et-Vilaine	1992	Pb137-576	P7			
				Pb137-483	P8			
				SJ92-225	P1			
				SJ92-336	P1			
				SJ92-256	P4			
	SJ92-233	P5						
	SJ92-370	P7						

^aField isolate from which single-spore isolates (SSIs) were derived.

^bPathotype classification according to the disease reaction of three differential *B. napus* cultivars to the *P. brassicae* isolates (Somé *et al.*, 1996).

^cFor the SSIs, host of origin and year of sampling are those of the original field isolate.

described previously (Manzanares *et al.*, 1994; Somé *et al.*, 1996). All the isolates were propagated on Chinese cabbage cv. Granaat. Clubs were washed and stored at -20°C until required.

Determination of *P. brassicae* pathotypes

Classification of the isolates was performed according to the disease reaction of three differential *B. napus* cultivars as described previously (Somé *et al.*, 1996). Designation of the pathotypes using this set is shown in Table 2. Each host/isolate combination was tested in a randomized complete block design with two replicates and 20 plants per replicate. Eight weeks after inoculation, plants were rated for disease reaction using the Buczacki *et al.* (1975) scale with one supplementary measure (2+): 0, no visible swelling; 1, very slight swelling usually confined to lateral roots; 2, moderate swelling on lateral roots and taproot; 2+, severe clubs on all roots but some roots remain; 3, no root left, only one large gall. A disease index was calculated by summation of the coefficients (0, 25, 50, 75, 100) affecting each plant class frequency (Buczacki *et al.*, 1975). A cut-off point of 25% was used to classify reactions as virulent or avirulent. The tests were repeated at least twice to confirm the consistency of the virulence patterns.

DNA isolation

Genomic DNA of *P. brassicae* was extracted from resting spores. The spores were washed five times in sterile distilled water, dispersed in 100 mM MgCl_2 , 200 mM Tris pH 7.4 buffer and treated with DNase I ($30\ \mu\text{g mL}^{-1}$) for 3 h at 37°C to eliminate host DNA. The solution was centrifuged at 2500 g for 5 min, and the pellet was recovered in 5 mM EDTA, 0.5% SDS, 10 mM Tris pH 7.8 buffer containing $20\ \mu\text{g mL}^{-1}$ proteinase K for 30 min at 37°C . After repeated centrifugations the final pellet was frozen in liquid nitrogen and lyophilized. DNA was extracted from lyophilized resting spores using a modified CTAB protocol (Doyle & Doyle, 1990). Subsequently, DNA was also extracted using the same CTAB protocol from young leaves of plant host Chinese cabbage cv. Granaat. Dried spores or frozen leaves were ground in liquid nitrogen to a fine powder, dispersed in 25 mL hot CTAB extraction buffer, and incubated at 65°C for 30 min. An equal volume of chloroform : octanol (24 : 1, v/v) was added, and after agitation, the mixture was centrifuged at 3000 g for 45 min. The upper aqueous phase was transferred to a further tube containing 16 mL isopropanol, incubated for 1 h at 4°C and centrifuged at 3000 g for 10 min. The pellet was dissolved in 10 mL $1\times$ TE buffer containing $0.5\ \mu\text{g mL}^{-1}$ RNase, and incubated for 1 h at 37°C . Samples were extracted with an equal volume of chloroform : octanol (24 : 1, v/v), and centrifuged at 3500 g for 10 min. The DNA was precipitated with 0.5 vol NaCl 5 M and 3 vol cold

100% ethanol. After 30 min incubation at 4°C the mixture was centrifuged at 3800 g for 10 min, and the pellet rinsed with 70% ethanol. The DNA was dissolved in 100 μL TE buffer and stored at -20°C until use.

DNA amplification

Approximately 12.5 ng genomic DNA template was used in a 12.5 μL amplification reaction. The reaction mix contained 0.4 units Taq DNA polymerase (Eurobio, Les Ulis, France), $1\times$ polymerase buffer (10 \times), 1.9 mM MgCl_2 , 100 μM dNTPs, and 0.2 μM single decamer primer obtained from Operon Technologies Inc. (Alameda, CA, USA). Amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, USA) programmed for an initial denaturation cycle (94°C for 30 s) followed by 45 cycles of 30 s at 92°C , 1 min at 35°C , and 2 min 30 s at 72°C , with a final extension at 72°C for 5 min. RAPD products were separated on a 1.8% agarose gel. Gels were stained with ethidium bromide and viewed with ultraviolet light. Lambda DNA restricted with *Hind*III and *Eco*RI was included as a molecular weight marker.

Amplifications were also performed with sequence-generated primers (SG) specific to *P. brassicae*, developed by Buhariwalla *et al.* (1995). Sequence-generated primers were synthesized by ISOPRIM (Toulouse, France); primer sequence, reaction mix and amplification conditions were as indicated by Buhariwalla *et al.* (1995).

At least two replicates of the amplification assay were run with different template DNA obtained from different DNA extractions, to ensure the consistency of each DNA band. All amplifications were also performed with DNA from host Chinese cabbage cv. Granaat.

Data analysis

Only highly amplified reproducible bands of *P. brassicae*, clearly distinct from the host bands in

Table 2 Pathotype designation of the *Plasmodiophora brassicae* isolates in the *Brassica napus* differential set^a

Pathotype	Differential host <i>B. napus</i>		
	ECD 6 cv. Nevin	ECD 10 cv. Wilhelmsburger	cv. Brutor
P1	+	+	+
P2	+	-	+
P3	-	-	+
P4	-	-	-
P5	-	+	+
P6	+	-	-
P7	-	+	-
P8	+	+	-

+, Susceptible host reaction; -, resistant host reaction (see Materials and methods).

^aSee Somé *et al.* (1996).

the amplifications patterns, were scored and used for phenetic analysis. Data matrices were generated for the molecular data by scoring the absence of a DNA fragment as 0 and the presence of a band as 1. Genetic distances were calculated with the following formula based on Jaccard's coefficient: $D = 1 - (1,1) / [(1,1) + (0,1) + (1,0)]$, where (1,1) is the number of bands shared by two patterns and (0,1) + (1,0) the number of bands specific to one of the patterns. Mean genetic dissimilarities were calculated among field isolates, among SSIs derived from a single field isolate, and among SSIs according to pathotype, host and geographical origin. Dendrograms were constructed from the distance matrices with the UPGMA option of the program NEIGHBOR of PHYLIP (3.5c) (Felsenstein, 1993). To determine the robustness of the dendrogram branches, 100 bootstrap replications were generated from the data using the program SEQBOOT; the resulting set of trees was synthesized using CONSENSE. Dendrograms were drawn with TREEVIEW (Page, 1996).

Results

Pathogenicity tests

All the isolates were virulent and highly aggressive on the susceptible control Chinese cabbage cv. Granaat (disease index >90%, results not shown). The eight possible pathotypes were identified from the 46 isolates (Table 1). Pathotypes P7 (virulent on ECD10 host) and P1 (virulent on all three hosts) were the most common, comprising, respectively, 36 and 23% of the total sample. Among the nine field isolates, four different pathotypes (P1, P2, P5 and P7) were found. Among the 36 SSIs, seven pathotypes were identified (all except P2). All five SSIs derived from the PbM field isolate showed the same virulence pattern as the original field isolate (P1). In contrast, none of the nine SSIs derived from K92 expressed the same virulence pattern as the original field isolate (P2). Among the 23 SSIs derived from the field isolates SJ92 and Pb137, five different

Table 3 Primers used for the molecular characterization of *Plasmodiophora brassicae*, number of polymorphic bands analysed and bands specific to one isolate or to isolates belonging to one pathotype

Primer			Number of bands analysed	Specific bands	
				Marker	Isolate
Operon (OP)	A04	AATCGGGCTG	1		
	A07	GAAACGGTG	6		
	A10	GTGATCGCAG	1		
	A13	CAGCACCCAC	5	A13-400	All isolates belonging to pathotype P1
	J20	AAGCGGCCTC	9		
	K10	GTGCAACGTG	5		
	K20	GTGTCGCGAG	5		
	L02	TGGGCGTCAA	4		
	L05	ACGCAGGCAC	2		
	L10	TGGGAGATGG	4		
	L12	GGGCGTACT	6		
	L14	GTGACAGGCT	4	L14-1250 L14-1200	K95 All isolates belonging to pathotype P1
	L18	ACCACCCACC	4		
	M05	GGGAACGTGT	3		
	M14	AGGGTCGTTC	8		
N06	GAGACGCACA	2			
R02	CACAGCTGCC	2			
R12	ACAGGTGCGT	2			
R15	GGACAACGAG	6			
SG ^a	HKB 17/9	CGTGGTTCCAATG	4		
	HKB 17/33	GCATCGTCTG	1		
	HKB 23/52	CGTGCCTACTTAGG	6		
	RFM3	AAAGTGCCGGTTACCAGCAA	2		
	RFM4	GATATCGAATTCGGTGCCCT	2		
	RFM7	AAAGTGCCGGTTACCAGCAACTGA	5	RFM7-907	SJ92-225
	RFM8	GGAACAAGCTCGAAGCCATG	1		
	RFM9	AGGTGAGGAGAAATGTCTC	2		
	RFM10	CACGTACCGAACCTAACGAG	1		

^aSG, specific *P. brassicae* primers developed by Buhariwalla et al. (1995).

pathotypes were found (P1, P4, P5, P7 and P8), with only two isolates expressing the same virulence pattern as the original field isolates (P5).

Different virulence patterns were found for some of

the field isolates sampled from the same field and on the same host (K93, K94, K95 and K96 sampled at the same site (Kerdevez, Finistère) from Chinese cabbage, and SJ92 and SJ93 sampled at the same site (Saint

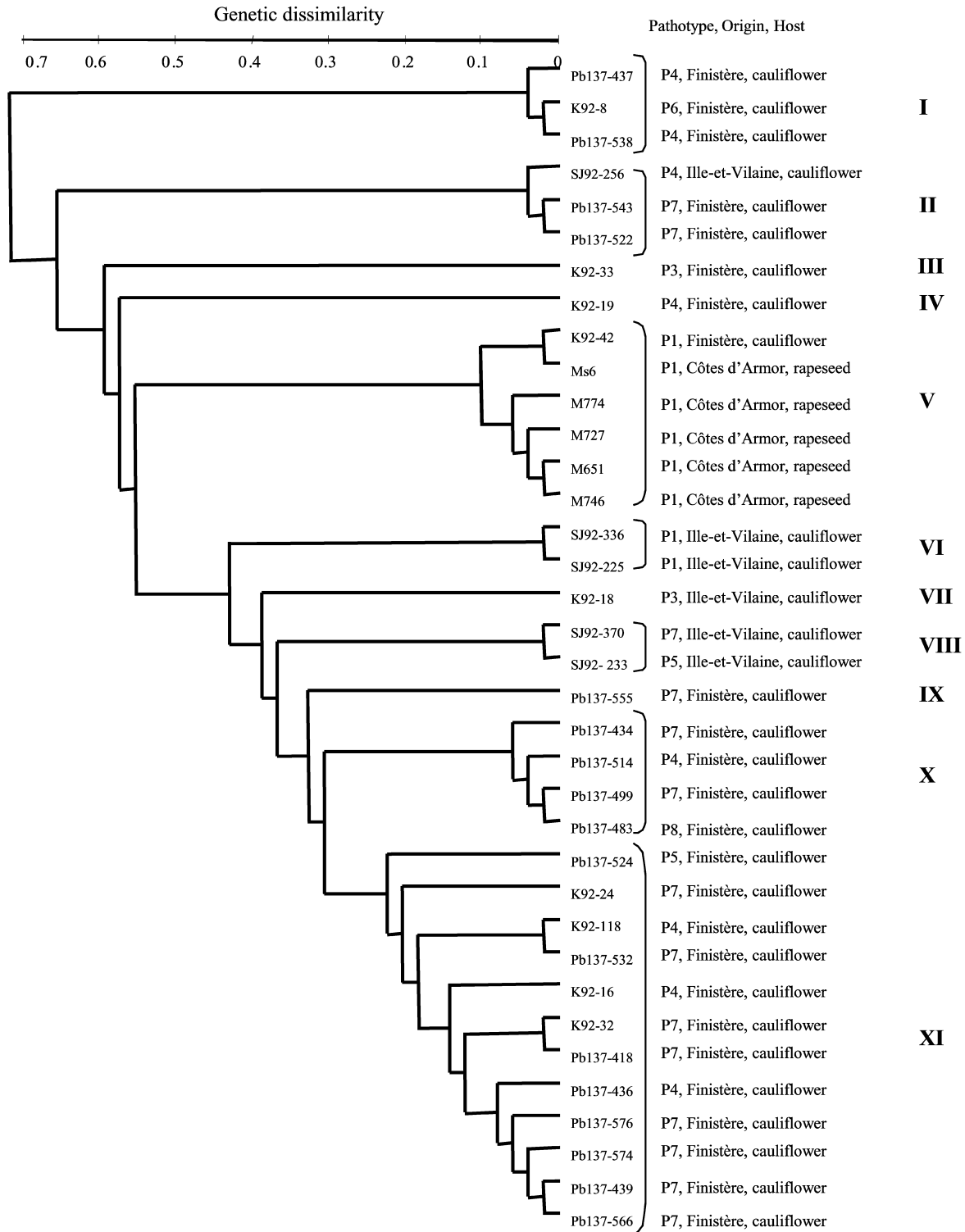


Fig. 1 Dendrogram of 37 single-spore isolates of *Plasmodiophora brassicae* based on 103 random amplified polymorphic DNA (RAPD and SG) loci, using the unweighted pair group arithmetic mean (UPGMA) program of PHYLIP (3.5c).

Table 4 Genetic distances among groups of single-spore isolates of *Plasmodiophora brassicae* using RAPD and SG data according to the field isolate from which SSIs were derived, pathotype, host and geographical origin

Level of comparison	Sublevel	Number of isolates	Genetic dissimilarity ^a
Field isolate	PbM	5	0.273
	K92	9	0.368
	SJ92	5	0.384
	Pb137	17	0.341
Pathotype	P1	8	0.326
	P3	2	0.474
	P4	8	0.434
	P5	2	0.323
	P6	1	–
	P7	14	0.290
	P8	1	–
	Host	Cauliflower	31
Rapeseed		5	0.273
Geographic origin	Côtes d'Armor	5	0.273
	Finistère	26	0.352
	Ille-et-Vilaine	5	0.384

^aAverage Jaccard coefficient of dissimilarity calculated among isolates of each sublevel.

Jouan, Ille-et-Vilaine) from cauliflower but collected in different years. Of the four field isolates obtained from infected cauliflower, three pathotypes were identified. There was no obvious pattern linking virulence with host genotype or geographical origin.

Molecular characterization

Thirty-seven random primers and 10 SG primers were initially tested on a set of 16 isolates. All the amplifications were also performed on DNA from Chinese cabbage cv. Granaat. From this initial screening, primers were selected on the basis of (i) reproducible and polymorphic patterns yielded, and (ii) clear distinction between host and pathogen amplification products. Nineteen Operon primers and nine SG primers that consistently generated distinct reproducible polymorphic amplicons were retained for the molecular analysis (Table 3). A total of 103 polymorphic intense reproducible bands were amplified using the DNA from 45 isolates as templates. The size of the DNA fragments ranged from 0.2 to 2.1 kb. All RAPD profiles were fully reproducible in at least two independent assays using two independent DNA extractions.

Each SSI and field isolate had a unique multilocus molecular genotype. When the fingerprints of the 45 isolates were compared, only four bands were identified as pathotype- or isolate-specific (Table 3). Two primers (OPL14 and OPA13) gave profiles that matched the pathotype P1 classification based on the differential host set used. Isolate-specific markers were found for two isolates (K95 and SJ92-225).

The mean genetic dissimilarity obtained for the 45 isolates was 0.375 (CV = 15.53%). The mean genetic

distance among field isolates (0.346) was similar to the mean genetic distance within field isolates (0.341), except for isolate PbM from rapeseed in Côtes d'Armor, for which the variability within was lower than among field isolates (Table 4). Genetic distances were also calculated among SSIs grouped according to pathotype, host and geographical origin (Table 4). Whatever the grouping, a high level of genetic diversity was present among SSIs.

In the dendrogram constructed, 11 groups (I–XI) not consistently clustered (low bootstrap values) were observed (Fig. 1). These clusters could not be related to pathotype, host or geographical origin.

Discussion

The results of this study indicate that the populations of *P. brassicae* in Brittany are highly heterogeneous for both virulence and DNA pattern. Among the field isolates analysed, four different pathotypes were found. Among the 37 SSIs, seven pathotypes were found. The eight possible pathotypes that can be identified with the differential set used were detected in the restricted sample. Among these pathotypes, one (P8) had not been reported previously. The identification of the new pathotype was achieved with SSIs. These results confirm the high degree of variation for virulence found previously amongst French *P. brassicae* collections (Somé *et al.*, 1996), and found by others around the world (Crute *et al.*, 1980; Linnasalmi & Toivainen, 1991; Voorrips, 1995; Kuginuki *et al.*, 1999). As pathotype identification depends on host genotype, isolates identified as a determined pathotype can be separated into more than one pathotype when more differential cultivars are used. Therefore natural

P. brassicae variation for virulence is probably much greater than the variation reported here. The molecular markers distinguished many more genotypes than did differential hosts, each isolate having a unique molecular genotype. The RAPD and SG primers were selected to obtain as many polymorphic amplicons specific to one isolate or group of isolates as possible. It is obvious that selection of polymorphic bands leads, in contrast to the virulence study, to an overestimation of genetic dissimilarities between *P. brassicae* isolates; such amplicons would be of interest for the development of molecular epidemiological tools. Amplification of *P. brassicae* with arbitrary primers has proved to be successful for characterizing genetic diversity, as already established by Möller & Harling (1996). Accurate choice of Operon primers and standardized conditions of *P. brassicae* DNA extraction produced simple amplification patterns that could be clearly interpreted, and with selected bands different from the host plant patterns, as with the SG primers (Buhariwalla *et al.*, 1995).

Virulence and DNA polymorphisms were detected within and between field isolates, and among SSIs from different pathotypes, hosts and geographic origins. General mean genetic dissimilarity among 46 isolates of *P. brassicae* was high compared with genetic dissimilarity found in other obligate biotrophic fungi (Délye *et al.*, 1997; Jennings *et al.*, 1997), and in pathogens for which no sexual stage has been observed under field conditions (González *et al.*, 1998; Hsiang & Mahuku, 1999); comparable levels of genetic dissimilarity were observed among populations of the facultative stem canker pathogen *Leptosphaeria maculans* (Mahuku *et al.*, 1997). The relatively high level of genetic diversity was similar among and within field isolates, i.e. genetic distances among SSIs derived from a single-club field isolate were similar to genetic distances among field isolates from different host and geographical origins. The life history of *P. brassicae* is not entirely understood, and there are uncertainties about some aspects of its population biology. There is still doubt concerning the relative roles of asexual propagation (secondary zoospores reinfesting root hairs) (Buczacki, 1983; Naiki *et al.*, 1984; Mithen & Magrath, 1992) and sexual propagation in the *P. brassicae* cycle, and where and how meiotic recombination takes place (fusion of secondary zoospores, Ingram & Tommerup, 1972; myxamoebae fusion, Mithen & Magrath, 1992). The degree of global variability (virulence and DNA patterns) assessed in the populations of *P. brassicae* studied, and the high level of genetic diversity observed at a very small scale (among SSIs from a single-club field isolate), support the assumption that recombination plays an important role in the cycle of the pathogen.

If variability for virulence is distributed at a very fine scale (a club), as indicated by the results presented here, the field isolates in this study were very complex composite populations (a mix of genotypes), supporting

earlier reports (Jones *et al.*, 1982b; Toxopeus *et al.*, 1986). Moreover, among the SSIs derived from a single-club field isolate, genotypes were detected that possessed more and less virulence than the original field isolate, and some genotypes (pathotype P2) could not be obtained as an SSI. These findings suggest that some kind of inhibition during pathotyping of the field isolates and selection for specific pathotypes during the SSI extraction process must be taking place. Similar results were obtained by Jones *et al.* (1982a) and Voorrips (1996), who explained this either by mechanisms of competition between pathogenic and nonpathogenic genotypes for infection sites, or by systems of resistance induced by nonpathogenic genotypes. These results underline the importance of using single-spore isolates instead of field isolates for both genetic studies of resistance and virulence surveys of the pathogen. For example, if virulent genotypes are masked (inhibited) by avirulent ones in a club, a plant could be selected as resistant to only the major component of the heterogeneous pathogen isolate (Somé *et al.*, 1996; Voorrips, 1996). Bulking isolates would therefore mask much of the variability present and result in an artificially homogeneous population. However, single-spore isolation is not feasible in the routine identification of the virulence patterns of a large number of *P. brassicae* collections because of the time required and the variable success rate (Haji Tinggal & Webster, 1981; Jones *et al.*, 1982a; Scott, 1985; Manzanares *et al.*, 1994; Narisawa *et al.*, 1996; Voorrips, 1996).

Progress in identifying and monitoring variability in *P. brassicae* is possible by use of molecular markers that are isolate-specific or pathotype-specific. Results reported here, however, indicated that there was no clear relationship between the molecular analysis and the pathogenicity tests. Overall, the heterogeneous group of isolates could not be classified according to pathotype, host or geographical origin. The lack of correlation between virulence and/or DNA patterns and host origin (*B. oleracea*, *B. rapa* or *B. napus*) confirmed earlier studies showing no evidence for a pathogenic specialization of *P. brassicae* to a particular host species (Crute *et al.*, 1983). The low correlation between virulence and RAPD patterns may be due to the high degree of DNA polymorphism among isolates within pathotypes. The evolution of the pathotype seems to be independent of the evolution of neutral DNA markers, as shown for other pathogens (Chen *et al.*, 1993). Isolates classified here as pathotype P1 (virulent on all hosts) were clustered, although at low levels of similarity. Furthermore, two primers (OPL14 and OPA13) gave polymorphic patterns that matched the biological classification. A SCAR-PCR marker from the OPL14-1200 RAPD fragment differentiating the RAPD pattern of the isolates belonging to pathotype P1 has recently been developed (Manzanares-Dauleux *et al.*, 2000), and further work is in progress to identify specific markers of other pathotypes.

In conclusion, the finding that extensive genetic differences occur in *P. brassicae* populations should be an important consideration in choosing breeding tactics to develop durable clubroot resistance. Molecular markers that are genotype-specific can be useful for studying how hosts, cultural practices and environment affect the genetic structure of *P. brassicae* populations.

Acknowledgements

The authors are grateful to Drs D. Andrivon, H. Brun, A.M. Chèvre and E. Jenczewski for helpful discussions and critical review of the manuscript. The authors thank P. Glory and I. Brunot for technical assistance.

References

- Buczacki ST, 1983. *Plasmiodiophora*: an inter-relationship between biological and practical problems. In: Buczacki ST, ed. *Zoosporic Plant Pathogens – A Modern Perspective*. London, UK: Academic Press, 161–91.
- Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA, 1975. Study of physiological specialization in *Plasmiodiophora brassicae*: proposals for attempted rationalization through an international approach. *Transactions of the British Mycological Society* 65, 295–303.
- Buhariwalla H, Greaves S, Magrath R, Mithen R, 1995. Development of specific primers for the amplification of polymorphic DNA from the obligate root pathogen *Plasmiodiophora brassicae*. *Physiological and Molecular Plant Pathology* 47, 83–94.
- Chen X, Line RF, Leung H, 1993. Relations between virulence variation and DNA polymorphism in *Puccinia striiformis*. *Phytopathology* 83, 1489–97.
- Crute IR, Gray AR, Crisp P, Buczacki ST, 1980. Variation in *Plasmiodiophora brassicae* and resistance to clubroot disease in brassicas and allied crops – a critical review. *Plant Breeding Abstracts* 50, 91–104.
- Crute IR, Phelps K, Barnes A, Buczacki ST, Crisp P, 1983. The relationship between genotypes of three *Brassica* species and collections of *Plasmiodiophora brassicae*. *Plant Pathology* 32, 405–20.
- Délye C, Laigret F, Corio-Costet MF, 1997. RAPD analysis provides insight into the biology and epidemiology of *Ucinula necator*. *Phytopathology* 87, 670–7.
- Doyle JJ, Doyle JL, 1990. Isolation of plant DNA from fresh tissue. *Focus* 12, 13–5.
- Felsenstein J, 1993. PHYLIP (PHYLogenetic Inference Package), Version 3.5c (distributed by the author). Seattle, Washington, USA: Department of Genetics, University of Washington.
- González M, Rodríguez R, Zavala ME, Jacobo JL, Hernández F, Acosta J, Martínez O, Simpsom J, 1998. Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. *Phytopathology* 88, 292–9.
- Haji Tinggal SH, Webster J, 1981. Technique for single spore infection by *Plasmiodiophora brassicae*. *Transactions of the British Mycological Society* 76, 187–90.
- Hsiang T, Mahuku GS, 1999. Genetic variation within and between southern Ontario populations of *Sclerotinia homoeocarpa*. *Plant Pathology* 48, 83–94.
- Ingram DS, Tommerup IC, 1972. The life history of *Plasmiodiophora brassicae* Woron. *Proceedings of the Royal Society, London B* 180, 103–12.
- Jennings JM, Newton AC, Buck KW, 1997. Detection of polymorphism in *Puccinia hordei* using RFLP and RAPD markers, differential cultivars, and analysis of the intergenic spacer region of rDNA. *Journal of Phytopathology* 145, 511–9.
- Jones DR, Ingram DS, Dixon GR, 1982a. Characterization of isolates derived from single resting spores of *Plasmiodiophora brassicae* and studies of their interaction. *Plant Pathology* 31, 239–46.
- Jones DR, Ingram DS, Dixon GR, 1982b. Factors affecting tests for differential pathogenicity in populations of *Plasmiodiophora brassicae*. *Plant Pathology* 31, 229–38.
- Koch E, Cox R, Williams PH, 1991. Infection of *Arabidopsis thaliana* by *Plasmiodiophora brassicae*. *Journal of Phytopathology* 132, 99–104.
- Kuginuki Y, Yoshikawa H, Hirai M, 1999. Variation in virulence of *Plasmiodiophora brassicae* in Japan tested with clubroot-resistant cultivars of Chinese cabbage (*Brassica rapa* L. spp. *pekinensis*). *European Journal of Plant Pathology* 105, 327–32.
- Linnasalmi A, Toiviainen A, 1991. Occurrence of clubroot and *Plasmiodiophora brassicae* Wor. races in Finland. *Journal of Agricultural Science in Finland* 63, 415–34.
- Mahuku GS, Goodwin PH, Hall R, Hsiang T, 1997. Variability in the highly virulent type of *Leptosphaeria maculans* within and between oilseed rape fields. *Canadian Journal of Botany* 75, 1485–92.
- Manzanares MJ, Laurens F, Baron F, Thomas G, 1994. Production of single spore isolates of *Plasmiodiophora brassicae*. *Cruciferae Newsletter* 16, 131–2.
- Manzanares-Dauleux MJ, Barret P, Thomas G, 2000. Development of a pathotype specific SCAR marker in *Plasmiodiophora brassicae*. *European Journal of Plant Pathology* 106, 781–7.
- Mithen R, Magrath R, 1992. A contribution to the life history of *Plasmiodiophora brassicae*: secondary plasmodia development in root galls of *Arabidopsis thaliana*. *Mycological Research* 96, 877–85.
- Möller M, Harling R, 1996. Randomly amplified polymorphic DNA (RAPD) profiling of *Plasmiodiophora brassicae*. *Letters in Applied Microbiology* 22, 70–5.
- Naiki T, Kawaguchi C, Ikegami H, 1984. Root hair reinfection in Chinese cabbage seedlings by the secondary zoospores of *Plasmiodiophora brassicae* Woronin. *Annals of the Phytopathological Society of Japan* 50, 216–20.
- Narisawa K, Kageyama K, Hashiba T, 1996. Efficient root infection with single resting spores of *Plasmiodiophora brassicae*. *Mycological Research* 100, 855–8.
- Page RDM, 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12, 357–8.
- Schoeller M, Grunewaldt J, 1986. Production and characterization

- of single spore derived lines of *P. brassicae* Woron. *Cruciferae Newsletter* 11, 110–1.
- Scott ES, 1985. Production and characterization of single-spore isolates of *Plasmodiophora brassicae*. *Plant Pathology* 34, 287–92.
- Somé A, Manzanares MJ, Laurens F, Baron F, Thomas G, Rouxel F, 1996. Variation for virulence on *Brassica napus* L. among *Plasmodiophora brassicae* collections from France and derived single-spore isolates. *Plant Pathology* 45, 432–9.
- Toxopeus H, Dixon GR, Mattusch P, 1986. Physiological specialization in *Plasmodiophora brassicae*: an analysis by international experimentation. *Transactions of the British Mycological Society* 87, 279–87.
- Voorrips RE, 1995. *Plasmodiophora brassicae*: aspects of pathogenesis and resistance in *Brassica oleracea*. *Euphytica* 83, 139–46.
- Voorrips RE, 1996. Production, characterization and interaction of single-spore isolates of *Plasmodiophora brassicae*. *European Journal of Plant Pathology* 102, 377–83.
- Williams PH, 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. *Phytopathology* 56, 624–6.