

ASSESSMENT BY MEANS OF RAPD MARKERS OF VARIABILITY IN *MYCOSPHAERELLA BRASSICICOLA* RESPONSIBLE FOR THE RINGSPOT DISEASE OF CRUCIFERS

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SUMMARY

The genetic variability of the fungus *Mycosphaerella brassicicola* was tested using RAPD markers. We analysed 28 isolates with 17 RAPD primers, generating 176 markers among which we identified 114 polymorphic bands. The cluster analysis revealed the existence of two groups of isolates, one heterogeneous (group II) and the other extremely homogeneous (group I). Most of the isolates in group I exhibited twice as many fragments as the isolates of the heterogeneous group. This difference in fragment number could be due to either diploidy, aneuploidy, heterocaryosis or DNA content. Twenty isolates were in group I thirteen of which had been isolated in Brittany in 1992. Some are probably identical. This suggests that the ringspot epidemic in 1992 was mostly due to group I isolates. Two isolates originating from Brittany behaved as intermediates of both groups. They had the sum of fragments found in both groups. This result suggests that sexual recombinants may occur in the homothallic fungus *M. brassicicola*. No relationships were observed between the genotypes of the isolates and their pathogenicity, host specificity or geographic origin.

RIASSUNTO

VALUTAZIONE DELLA VARIABILITÀ DI *MYCOSPHAERELLA BRASSICICOLA*, AGENTE DELLA MACULATURA ANULARE DELLE CRUCIFERE, MEDIANTE MARKER RAPD. La variabilità genetica del fungo *Mycosphaerella brassicicola* è stata saggiata mediante l'uso di marker RAPD. Sono stati analizzati 28 isolati con 17 primer RAPD che hanno originato 176 marker tra i quali sono state identificate 114 bande polimorfiche. L'analisi cluster ha mostrato l'esistenza di due gruppi di isolati, uno eterogeneo (gruppo II) e l'altro estremamente omogeneo (gruppo I). Molti degli isolati appartenenti al gruppo I

hanno mostrato un numero di frammenti doppio rispetto a quello degli isolati del gruppo eterogeneo. Questa differenza nel numero di frammenti potrebbe essere dovuta a diploidia, ad aneuploidia, ad eterocariosi o al contenuto di DNA. 20 isolati appartenevano al gruppo I e 13 di questi sono stati isolati in Bretagna nel 1992; alcuni di essi sono probabilmente identici. Questo suggerisce che l'epidemia di maculatura anulare verificatasi nel 1992 era per la maggior parte causata da isolati appartenenti al gruppo I. Due isolati provenienti dalla Bretagna hanno evidenziato un comportamento intermedio rispetto ai due gruppi mostrando di possedere la somma dei frammenti trovata in entrambi i gruppi. Questo risultato suggerisce come possano esistere ricombinanti sessuali nel fungo omotallico *M. brassicicola*. Non è stata osservata alcuna correlazione tra il genotipo degli isolati e la loro patogenicità, specificità d'ospite od origine geografica.

Key words: *Mycosphaerella brassicicola*, pathogenicity, genetic variability, RAPD.

INTRODUCTION

Mycosphaerella brassicicola is an ascomycete responsible for the ringspot disease of crucifers. The disease occurs frequently on economically important crops such as oilseed rape, cabbages, brussels sprouts, broccoli and cauliflower. It is especially favoured by mild and humid climates and therefore, severe epidemics were scored during the last three years in Brittany (France), the biggest cauliflower growing region of Europe. The fungus can survive for years on diseased plant debris from which ascospores are released (Dixon 1981). Risks of new epidemics are therefore high, especially with high primary inoculum originating from different host plants continuously grown over the year.

Chemicals such as zineb, captan, maneb, mancozeb, metalaxyl and benomyl provide a good control of the disease, but there is an increased concern about chemical residues in food, particularly in the case of cabbages, broccoli and cauliflower because the consumed

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parts of plants are liable to be sprayed with chemicals. Breeding for resistance is therefore the best alternative for the future years.

Only a few studies were carried out on the ringspot disease during the last decade. Zornbach (1990) analysed pathogenicity of 29 isolates from Germany, France and The Netherlands on different host accessions. He did not find any physiological specialization and concluded that the isolates differed only for aggressivity. Van Den Ende (1993b) however showed the existence of physiological races in The Netherlands. He also found variations in host responses depending on plant age. Some line-isolate combinations exhibited a more susceptible reaction at an older (leaf 6) than at a younger growth stage (leaf 4). He also described susceptible plants which became resistant when they were at an older growth stage. It can be suggested that growth-dependent specific compounds involved in resistance expression may explain the observed variations.

Since *M. brassicicola* has no conidial stage and produces few ascospores *in vitro*, mycelial fragments are often used as inoculum in pathogenicity tests (Zornbach, 1990; Van Den Ende, 1992, 1993; Van Den Ende and Frinking, 1993). Van Den Ende and Frinking (1993) showed that the use of such inocula led to longer incubation periods and fewer lesions than with ascospores.

We have screened local accessions of cauliflower with 52 isolates originating from France and other countries and noticed differences in pathogenicity. We estimated the genetic diversity of these isolates using Random Amplified Polymorphic DNA (RAPD) markers. Such markers are widely used to assess variability in plant pathogenic microorganisms (Guthrie *et al.*, 1992; Johanson *et al.*, 1994; Tham *et al.*, 1994; Zimand *et al.*, 1994; Crowhurst *et al.*, 1995; Kolmer *et al.*, 1995; Lilja *et al.*, 1996; Yang and Kharbanda, 1996). This is the first report on the use of RAPD markers in the assessment of the variability of *M. brassicicola* isolates. Results obtained are discussed in relation to pathogenicity of the isolates.

MATERIAL AND METHODS

Fungal isolates and their culture. Twenty-eight isolates were used in this study. They all derived from single ascospore cultures and were stored on sterile dried filter paper pieces as already described for *Mycosphaerella fijiensis*, *M. musicola* (Carlier *et al.*, 1994) and *Magnaporthe grisea* (Silué *et al.*, 1992). This type of storage avoids variations due to repeated subculturing. Most of them were isolated in 1992 from cauliflower in Brittany. The characteristics of each isolate are given in Table 1. Because *M. brassicicola* produces only a few ascospores

in vitro but no conidia, all DNA extractions were therefore made from fresh mycelia obtained from liquid cultures. For this purpose, 250 ml flasks were used and filled with 150 ml V8 sterile medium: 200 ml centrifuged V8 juice, 2.5 g saccharose, 2.5 g yeast extract, 2 g agar and completed up to 1 litre. Each flask was agitated at 140 rpm at 20°C and 16 h light after inoculation. After 11-15 days of culture, the entire solution of each flask was either centrifuged (4000 rpm) or filtered. In both cases, the pellet of mycelium was then freeze dried.

Cauliflower accessions and their inoculation. Seventeen cauliflower accessions were tested. Plants were grown in the greenhouse where the temperature remained between 15°C (nights) and 25°C (days). For each cultivar, five seeds were sown in a pot (2 pots per cultivar) containing a potting mixture (Pinceland, Trio BV, Holland). At the second true leaf stage, they were watered with a 15% fertilizer solution (Solufeed ICI Agrochemicals, UK). They were inoculated 30 days after sowing. Inoculations were repeated at least twice.

For inoculum preparation, V8 plates were first inoculated with 3-4 implants. All inocula were prepared using young mycelium (maximum one month old cultures) fragments obtained using either a waring blender or a pestle and mortar. Inocula contained 2% carboxymethyl-cellulose and their concentrations were of 10⁵ fragments per ml. Each fragment could be considered as an infection unit.

Inoculations were carried out by spraying approximately 3 ml of inoculum per plant using compressed air (0.5 bar). Van Den Ende (1992) sprayed the same quantities. Inoculated plants were then moved for three days into a humid room where relative humidity was close to saturation, and temperature maintained at 15°C nights and under 25°C days. After this treatment, plants were kept in the greenhouse under the same temperature conditions and a 12 h light treatment was applied till to disease rating.

Disease assessment. Disease rating was made 28-30 days after inoculation. For this purpose, a five point scale was used. In this scale, ratings were made according to percentage of diseased leaf surface previously assessed by means of a camera followed by a software analysis. The following ratings correspond to: 1= 0%, 2= up to 5%, 3= 6-20%, 4= 21-50% and 5= more than 50%. Rating 1 corresponds to nonpathogenic isolates and the others correspond to different levels of aggressivity. Similar plant genotypes rated 1-2 are resistant and those rated 3 are moderately resistant (MR). Moderate susceptibility (MS) was assigned to genotypes rated 4 and full susceptibility (very susceptible, VS) to rating 5.

Table 1. Origins and characteristics of the tested isolates.

Isolate designation	Date of isolation or reception	Origin of diseased leaves	Cultivar of origin (crops)	Source
MB2, MB3	13.01.92	St-Pol-de-Léon (France)	INRA 3 (CF)	This study
MB5, MB6, MB7	"	"	INRA 5 (CF)	"
MB8, MB9	"	"	Vidoke (CF)	"
MB10	"	"	Castlegrant (CF)	"
MB11, MB12	"	"	Dova (CF)	"
MB16	"	"	AY 1040 (CF)	"
MB18	17.01.92	"	INRA7 (CF)	"
MB20, MB24, MB25, MB28	"	"	" isolated on a unique lesion	"
MB34	"	"	Arbon (CF)	"
MB35	"	"	20N (CF)	"
MB37	4.03.92	Germany	? (CB)	provided by Dr. Van Den Ende ²
MB38	"	Denmark	? (CB)	"
MB39	"	The Netherlands	? (CB)	"
MB40	"	United Kingdom	? (CB)	"
MB46	25.03.92	St-Pol-de-Léon (France)	Jakez (CF)	This study
MB47	"	"	"	"
MB50, MB51, MB52	5.05.92	USA	? (CB)	diseased leaves provided by Dr. Mike Deries ³
MB54	22.10.93	Plougonvelin (France)	? (CB)	This study

¹ CF: cauliflower, CB: cabbages.² Agricultural University, Dept. of Phytopathology, Binnenhaven 9, Wageningen, The Netherlands³ Washington State University, Research & Extension Center, Puyallup, WA 98371-4998, USA

DNA extraction. DNA was prepared according to Carlier *et al.* (1994). Briefly, 300 mg of mycelia are wrapped in aluminium foils, frozen in liquid nitrogen and then crushed in a mortar under liquid nitrogen. The resulting fine powder is then transferred into a 50 ml test tube containing 7 ml of extraction buffer (0.7 M NaCl, 10 mM EDTA, 50 mM Tris- HCl, pH 8, 1% CTAB) and 70 μ l of β -mercaptoethanol. The mixture is incubated at 65°C for 1 hour. Five ml of chloroform/isoamyl alcohol are then added and the emulsified mixture is centrifuged for 30 min at 2000 *g* at 20°C. The lower (organic) phase is discarded and one volume of isopropanol is added to the upper (aqueous) phase in order to precipitate the DNA. After 10 min at room temperature, the mixture is centrifuged (30 min, 2000 *g*, 20°C). The DNA pellet is washed with 70% ethanol and then dried and resuspended in 200 μ l of Tris-EDTA. The DNA solution is treated with 10 μ l of RNase A (10 mg ml⁻¹) for 1 hour at 37°C. DNA concentration is measured using a fluorometer TKO 100.

Optimised amplification procedure. In order to perform reliable experiments and because we had no previous molecular work experience on this fungus, we first optimized the different parameters involved in the RAPD protocol. We tested two different DNA extraction protocols (Carlier *et al.*, 1994 ; Brygoo, personal communication) which all give similar results. We therefore chose the easier and the more rapid one. We then used three to five different concentrations of all the components of the reaction, *i.e.* from 5 to 100 ng of DNA, from 0.25 mM to 0.75 mM of primers, from 1.25 mM to 3.75 mM of MgCl₂, from 0.05 mM to 0.15 mM of dNTPs and from 0.1 to 2 units of *Taq* polymerase. The optimised conditions resulting from these experiments are described below. They are rather conventional. The utilisation of two instead of one unit of *Taq* polymerase normally used in our laboratory seemed to give us more robust RAPD patterns.

RAPD profiles were obtained as follows : 30 ng of template DNA were mixed with 0.5 μ M oligonucleotides (Operon Technologies Inc., Alameda, USA), 2.5 mM MgCl₂, 2 Units of *Taq* polymerase (Promega, USA), 0.1 mM dNTPs (Pharmacia) and 1x buffer provided as 10x by the manufacturer. The amplification reactions were performed using a M.J. Research thermocycler (PTC 100) under the following conditions: a first denaturation step at 94°C for 30 s, then 45 cycles at 94°C for 30 s, 35°C for 1 min and 72°C for 2 min. The PCR products are then analysed by electrophoresis in 2% agarose gels. The gels are stained with ethidium bromide.

Although our amplification profiles appeared to be reliable and unambiguous, we systematically confirmed our data. Therefore all primer/template DNA combinations have been performed at least twice.

RFLP-PCR of the Internal Transcribed Spacer (ITS) rDNA sequences. We amplified the ITS sequences using the protocol described by White *et al.* (1990) in combination with the universal primers NS7 and ITS4. The total volume of each reaction was 100 μ l and 20 μ l served for the restriction experiments. These experiments were performed on four isolates of each group. We used 18 restriction enzymes (both 4 or 6 bp cutters). The amplification/digestion products were analysed on agarose gels as described above.

Data analysis. RAPD profiles were recorded on Polaroid films. Polymorphic bands were scored as present or absent and fed into a Macintosh computer. The matrix analysis was performed using the 'PAUP' software (Phylogenetic Analysis Using Parsimony, Swofford 1993) according to the author's recommendations. Due to the number of taxa in this study, we used a heuristic (approximate) algorithm including a stepwise addition of taxa followed by the rearrangement of the trees via 'branch swapping' techniques. A bootstrap procedure was used to assess the solidity of each tree connection and hence the validity of the tree obtained.

RESULTS

RAPD. We used 17 randomly chosen oligonucleotides to prime the amplification of 28 different isolates of *M. brassicicola*. We also included two control lanes: one for a blank experiment (no DNA added in the amplification mixture) and one for a RAPD experiment with cauliflower as the source of template DNA. The 17 primers generated patterns containing 5 to 22 reproducible bands with an average of 10 bands per primer. Depending on the primer, we observed from 3 to 18 polymorphic bands with an average of 6. Overall, we identified 114 polymorphic markers. In all RAPD experiments, we observed two distinct types of patterns, when comparing the number of fragments. For the more complex group (group I), we obtained (Fig.1) twice as many fragments (10 to 22) as in the other group (5 to 10). Isolates in group II have fragments found in isolates of group I and new ones. They behave as if they were in another taxonomic group (species or subspecies).

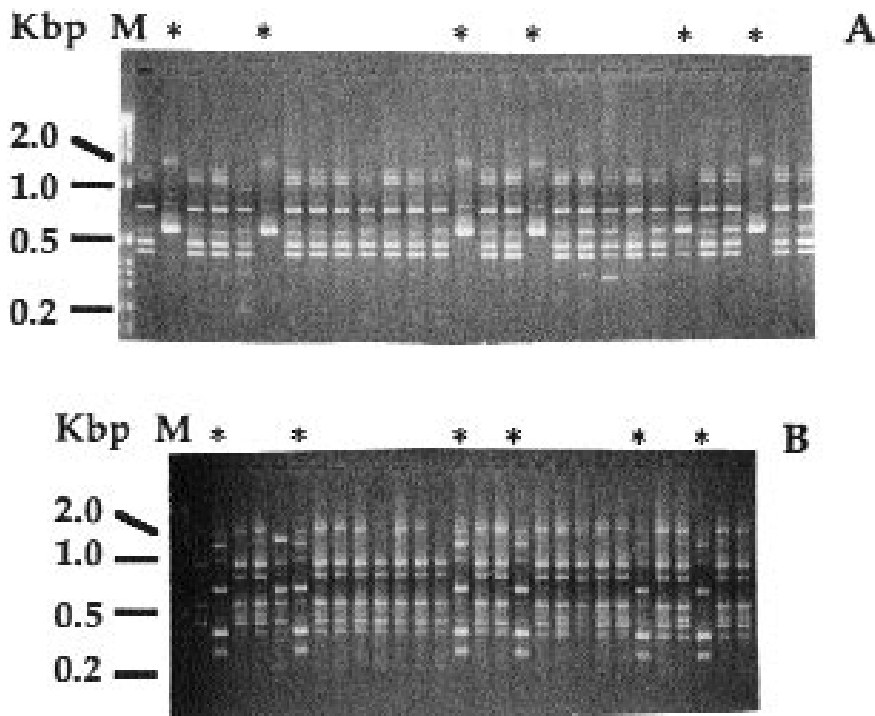


Fig.1. RAPD profiles of the 28 isolates of *M. brassicicola* using random primers E12 (A) and E3 (B). Each lane represents an isolate and they were ranged according to the increasing number of their designation. The existence of two very distinct groups of isolates showing a different amount of amplification products (group I exhibits an average of a twice as many RAPD bands as compared to group II) is clearly illustrated. The * indicate isolates belonging to group II. From lane 2 to 29: MB2, MB3, MB5, MB6, MB7, MB8, MB9, MB10, MB11, MB12, MB16, MB18, MB20, MB24, MB25, MB28, MB34, MB35, MB37, MB38, MB39, MB40, MB46, MB47, MB50, MB51, MB52, MB54. The DNA bands were separated on a 2% agarose gel and stained with ethidium bromide.

Amplification of ITS (rDNA) sequences. It has been established that the ITS region of the rRNA genes are highly polymorphic between species and monomorphic between individuals of the same species (Bruns *et al.*, 1991; Henson and French, 1993). The RAPD data analysis showed the existence of two distinctive groups amongst *M. brassicicola* isolates. In order to verify that we were dealing with isolates of the same species, we amplified the ITS sequences using primers NS7 and ITS4 (White *et al.*, 1990) and digested the amplification products with restriction enzymes. We were unable to detect any polymorphism between isolates from these two groups (data not shown) and concluded they belong to the same species.

Cluster analysis. Using the PAUP software, we were able to analyse the matrix data obtained with 114 markers obtained when comparing 28 isolates of *M. brassicicola*. We obtained a consensus tree after having performed 100 bootstraps to assess the reliability of the different branches. As stated above, two types of populations emerged from this analysis. One includes 8 isolates which are genetically divergent. The other one is composed of 20 very similar isolates (Figs 1 and 2). In this group, some isolates differ by only one fragment. Examples of similar isolates are: MB5, MB52; MB6, MB9; MB10, MB16, MB25, MB28, MB35, MB37, MB54. Most of these isolates originated from Brittany and some genotypes may have been isolated several

times. On the other hand MB37 and MB54 which are respectively from Germany and Brittany have comparable pathogenicity patterns and belong to the same RAPD group (group I). The climate in Brittany favours the disease and this region might be a diversification area for the pathogen. In fact, previous studies (data not published) showed important variations in pathogenicity among the isolates. It is therefore possible that some pathotypes have migrated from Brittany to other growing regions.

Isolates MB7 and MB12 behave as intermediates between isolates of group I and group II. They have bands of both groups (Fig. 2).

Assessment of pathogenicity. Results obtained are listed in Table 2. In general, isolates exhibited different pathogenicity. However, it can be noted that 13 isolates *e.g.* MB5, MB8, MB9, MB20, MB37 are nonpathogenic to all seven accessions tested. Most of these isolates belong to RAPD group I but some of them such as MB8 are in group II (Table 2). This low pathogenicity is not origin dependent since this group includes isolates from different countries and were isolated either from cauliflower or cabbages. Isolates MB7, MB10 and MB38 are the most pathogenic ones. Again their pathogenicity is origin independent. MB38 is from Denmark and was isolated from an unknown cabbage accession (Table 1). The remaining two isolates are from cauliflower accessions grown in Brittany.

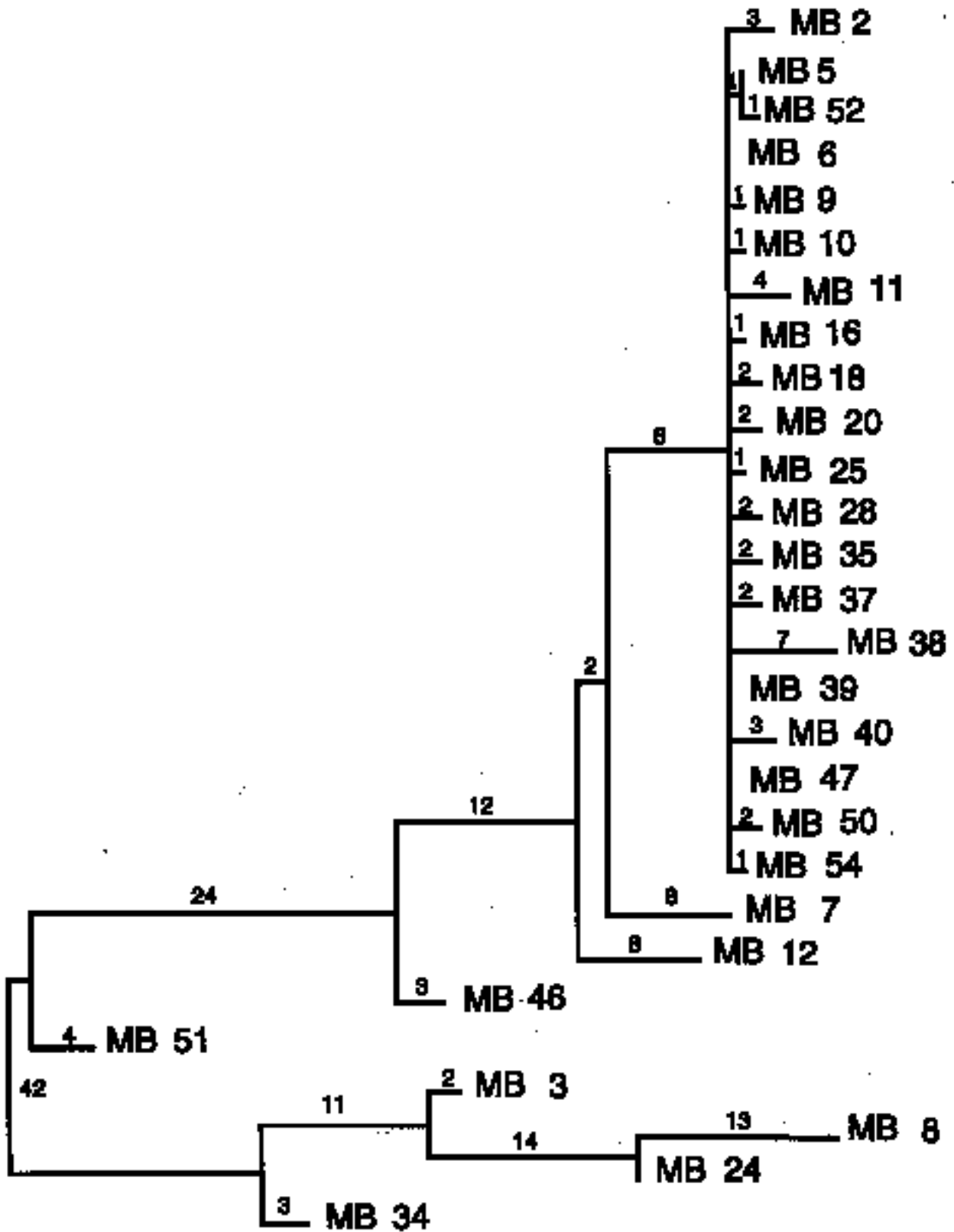


Fig. 2. Cluster obtained using the PAUP software. This cluster was established after having scored 114 polymorphic RAPD bands on 28 isolates of *M. brassicicola*. Two distinct groups of isolates are clearly shown with the number of bands.

Table 2. RAPD and disease indexes (DI) obtained with 23 *M. brassicicola* monoascospore isolates assessed on seven cauliflower accessions.

Cultivars	Isolates of <i>M. brassicicola</i> tested ¹																						
	2	5	6	7	8	9	10	16	18	20	24	28	34	35	37	38	39	40	46	47	50	52	54
48.9.5	1.3	1.1	2.3	3.6	1.0	1.0	2.5	2.0	2.0	1.0	1.4	2.0	1.1	1.0	1.0	3.5	1.3	1.0	1.1	1.1	1.0	1.0	1.0
48.9.4	1.0	1.0	2.3	3.3	1.0	1.0	3.7	1.3	2.1	1.0	1.1	2.5	1.3	1.1	1.0	2.8	1.1	1.0	1.1	1.0	1.0	1.0	1.0
48.9.16	1.0	1.0	3.6	2.2	1.0	1.0	2.7	1.5	1.4	1.0	1.5	1.8	1.1	1.1	1.0	3.4	1.7	1.0	1.1	1.0	1.0	1.0	1.0
Ami 41	1.1	1.0	2.7	2.1	1.0	1.0	1.4	1.7	2.3	1.0	1.0	1.0	1.0	1.2	1.0	1.7	1.4	1.0	1.1	1.0	1.0	1.0	1.0
F45.10.4	1.0	1.0	1.0	1.3	1.0	1.0	1.0	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
7.339.3.7	1.7	1.0	2.9	2.8	1.0	1.0	3.6	1.7	3.3	1.0	1.4	2.4	1.3	1.1	1.0	4.3	1.0	1.1	1.0	1.0	1.0	1.0	1.0
Belot	1.3	1.0	2.6	4.0	1.0	1.0	3.5	2.8	1.8	1.0	1.1	2.6	1.1	1.1	1.0	3.2	1.4	1.1	1.3	1.0	1.0	1.5	1.0
Mean	1.2	1.0	2.5	2.8	1.0	1.0	2.6	1.7	2.0	1.0	1.2	1.9	1.1	1.1	1.0	2.9	1.3	1.0	1.1	1.0	1.0	1.1	1.0
RAPD group	I	I	I	II	II	I	I	I	I	I	II	I	II	I	I	I	I	I	II	I	I	I	I

¹ Names assigned to all isolates begin with MB for *M. brassicicola*. Isolates MB3, MB11, MB12, MB25 and MB51 were not tested.

In general, pathogenicity of the isolates in our study is low (Table 2). The use of mycelial fragments instead of ascospores as inocula was shown by Van Den Ende and Frinking (1993) to lead to fewer lesions being formed. We may have underestimated pathogenicity of the isolates used. *M. brassicicola* produces few ascospores *in vitro* as well as in plants (Zornbach, 1990; Van Den Ende and Frinking, 1993). Further studies are needed to improve *in vitro* ascospore production. Another reason for the observed low pathogenicity may be resistance of the cultivars tested. One of them, F45.10.4, is resistant to all isolates (Table 2).

DISCUSSION

The RAPD technology seems to be well adapted for the molecular studies of fungi such as *M. brassicicola*. In order to estimate the genetic variability of the different *M. brassicicola* isolates available in our laboratory, we used 17 primers generating 114 polymorphic markers amongst a total of 176 bands under our experimental conditions. We stopped the experiments after the use of 17 primers because we noticed that the structuration of our sample of isolates did not evolve further. Indeed the cluster obtained after 15 RAPD experiments was almost identical as the one we present here.

Polymorphic markers represent about 65% of the amplified products. Our RAPD studies showed the existence of two distinct groups of isolates (Fig. 2). Apart from 2 isolates (MB7 and MB12), the two groups do not have many bands in common (Fig. 2). Isolates MB7

and MB12 are intermediate between both groups. They have the sum of the fragments found in both RAPD groups. It can be speculated that they are sexual recombinants obtained through outcrossing occurring in the homothallic fungus *M. brassicicola*. The existence of such a discrepancy in the amount of amplification products is rather unusual. As a matter of fact, being in the presence of haploid mycelia, one would expect to observe an equilibrium in the number of loci amplified from different isolates of the same species. The possibility that the number of RAPD primers used in this assay is too small does not seem to be relevant. In fact, the same observation was made with all primers used. Possible reasons of the existence of two groups in our sample of isolates are diploidy, aneuploidy or stable heterocaryons, the last being less probable.

Our data also showed that isolates of both RAPD groups co-existed in the same cauliflower growing area, on the same host and during the same season. For example, isolates MB5 and MB7 were isolated from cauliflower in January 1992 at Saint Pol-de-Leon. They belong respectively to group I and II and MB5 is not pathogenic to all cultivars tested while MB7 attacked three of them. Furthermore, isolates MB20 and MB24, belonging to different RAPD groups (Fig. 1) were found on the same leaves (Table 1) and develop the same epidemic. These results support the hypothesis that sexual recombination might occur in nature.

In all countries concerned in this study (Denmark, France, Germany, The Netherlands, the United Kingdom and the United States of America), we could find

isolates of group I. One can speculate that isolates shown to be homogeneous in this study may have the same origin. This could be possible since infected heads of cauliflower or cabbages may be marketed in countries where they were not produced. For instance, isolate MB40 originating from the United Kingdom may have migrated to Brittany or vice versa.

Isolates of group II originated only from France and the United States of America. Since group I and II and intermediate isolates are found in Brittany, it can be speculated that isolates of group II have moved from France to other countries. To test, this hypothesis it is necessary to analyze collections of isolates from countries other than France (*i.e.* USA).

The 28 isolates have different geographic origins (Table 1), different levels of pathogenicity (Table 2) and were isolated from either cauliflower or cabbages (Table 1). There is, however, no relation between host, geographic origin or pathogenicity and the genotypes. Especially, isolates MB6, MB28 from Brittany and MB38 from The Netherlands grouped in the same RAPD profile category (group I) are amongst the most aggressive ones. Isolate MB7 originating from Brittany and belonging to group II is also highly pathogenic. Although most nonpathogenic isolates were isolated in France, our data tend to suggest that pathogenicity is origin independent. The RAPD technology seems to be useless for characterizing either pathogenicity or origins of our isolates. Crowhurst *et al.* (1995) showed that the 39 isolates of *Fusarium oxysporum* from Angsana (*Pterocarpus indicus*) isolated at 19 different locations in Singapore could be shared in two groups according to their RAPD patterns. Furthermore, they also distinguished two groups in five isolates of *F. oxysporum* f.sp. *redolens*. No relation to pathogenicity was however discovered.

In the pathosystem *Oryza sativa*-*Magnaporthe grisea*, DNA fingerprinting (Levy *et al.*, 1991, 1993; Zeigler *et al.*, 1995) has shown that molecular groups are linked with pathotypes.

Based on our RAPD results, we can now perform our screening for resistance in cauliflower accessions. For example, in our forthcoming screening tests, we can choose only a few isolates of group I since they are identical. This strategy can speed up the screening for resistance and help identify accessions resistant to all isolates in this group. On the other hand all eight isolates of group II should be used in our tests. Pathogenicity tests combined with our molecular analysis of others samples of *M. brassicicola* may provide more information on its populations structure. This information is necessary for the development of strategies to control the epidemics caused by this fungus.

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