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Intra- and interspecific variability in *Globodera*, parasites of Solanaceous plants, revealed by Random Amplified Polymorphic DNA (RAPD) and correlation with biological features.

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Summary - Inter- and intraspecific variability among species of *Globodera* parasites of *Solanaceae* plants, were studied by host range and RAPD. Host range was studied among *Nicotiana* species and cultivars within *G. tabacum sensu lato*. RAPD was applied to 26 populations belonging to six putative *Globodera* species. Host range revealed clear differences allowing grouping in accordance with pathogenicity or virulence. 222 RAPD markers were obtained using eight ten bases Operon primers (OPG-1, OPG-2, OPG-3, OPG-4-OPG-5, OPL-3, OPL-4, OPL-6). Different clustering programs and a principal coordinate analysis were performed with RAPD data showing very few differences in the results. These dendrograms clearly revealed the existence of four OTU or species as *G. rostochiensis*, *G. pallida*, *G. tabacum* and *G. "mexicana"*. The clustering of these species was identical with that obtained previously with ribosomal DNA cistron ITS (Internal Transcribed Spacers) restriction analysis. Furthermore, the intraspecific clusterings were in very good accordance with host range grouping and with the hybridisation results published previously.

Résumé - ***Variabilité intra- et interspécifiques chez les* Globodera *parasites des Solanacées, révélée par RAPD (Random Amplified Polymorphic DNA) et comparée aux caractéristiques biologiques*** - La variabilité inter et intraspécifique de *Globodera* parasites des Solanacées est évaluée par leurs gammes d'hôtes et leurs profils RAPD. Divers espèces et cultivars de tabac sont testés vis-à-vis de *Globodera tabacum sensu lato* et de *G. "mexicana"*. Les profils RAPD sont étudiés sur 26 populations appartenant aux six espèces (publiées ou non) de *Globodera*. De fortes différences dans la gamme d'hôtes permettent de classer les populations en groupes de virulence. Huit amorces Operon de dix bases (OPG-1, OPG-2, OPG-3, OPG-4, OPG-5, OPL-3, OPL-4, OPL-6) permettent d'identifier 222 marqueurs RAPD. Divers programmes d'analyses en grappes et en composante principale sont réalisés en utilisant ces marqueurs, avec de très faibles différences de classification entre programmes. Les dendrogrammes obtenus montrent l'existence de quatre unités taxinomiques, à savoir *G. rostochiensis*, *G. pallida*, *G. tabacum* et *G. "mexicana"*. Ils sont semblables à ceux obtenus précédemment avec l'analyse de restriction des ITS. Ils révèlent des apparentements congruents avec ceux déterminés par la gamme d'hôtes et non contradictoires avec les barrières génétiques précédemment mises en évidence par les hybridations *in vitro*.

Keywords : molecular taxonomy, nematode, phylogeny, virulence.

The cyst nematodes belonging to the genus *Globodera*, which parasitise Solanaceous plants, comprise few closely related species : two species from South America are major pathogens of potato, *Globodera rostochiensis* Wollenweber and *G. pallida* Stone; three separate "tobacco" species from north America, *G. tabacum* (Lownsbery & Lownsbery; 1954), *G. virginiae* (Miller & Gray, 1968) and *G. solanacearum* (Miller & Gray, 1972), considered later as a unique species subdivided into three sub-species (Stone, 1983) and some Mexican populations found on wild Solanaceae which were reported as *G. "mexicana"* by Campos-Vela (1967).

Polymorphism research has been done using several criteria : morphology (Stone, 1983; Vlachopoulos & Smith, 1992 ; Mota & Einsenback, 1993*a*, *b*, *c*), pathotypes (Phillips & Trugdgill, 1983), ability to form hybrids (Mugniéry *et al.*, 1992 ; Thiéry *et al.*, 1996), antibodies (Wharton *et al.*, 1983; Schots *et al.*, 1987), isozymes (Phillips *et al.*, 1992 ; Zaheer *et al.*, 1992), 2D-electrophoresis of proteins (Bakker & Bouwman, 1988 ; Bossis & Mugniéry, 1993), DNA analysis like for example Restriction Fragment Length Polymorphism and Dot Blot (Burrows & Perry, 1988; Marshall, 1993), satellite DNA (Stratford *et al.*, 1992; Blok *et al.*, 1995), Internal Transcribed Spacer(ITS) in ribosomic DNA (Ferris *et al*, 1995 ; Thiéry & Mugniéry, 1996) and Random Amplified Polymorphic DNA (Folkertsma *et al.*, 1994).

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The majority of those biochemical and DNA techniques have been successfully applied to identify *G. rostochiensis* and *G. pallida*. Some of them are suitable to discriminate these two species in routine assays (Schots *et al.*, 1987; Bakker *et al.*, 1993).

The discrimination of the four *Globodera* species or groups cited above done by restriction site analysis in the ITS allows the description of phylogenic relationships inside the *Globodera* genus (Thiéry & Mugniéry, 1996). The main outstanding from this work is to know if such phylogeny, based only on an extremely small part of the genome is reliable. Furthermore the ITS polymorphism was not sufficient to distinguish subspecies, pathotypes and populations in a given species

Numerous examples demonstrated the great potential utility of RAPD polymorphisms in insect population genetics (Chapco *et al.*, 1992), cryptic species identification (Black *et al.*, 1992) and genetic fingerprinting (Black, 1993). RAPD markers have already proved useful for phylogenetic analysis in *Brassica* spp. (Demeke *et al.*, 1992), aphids (Black, 1993) and mosquitoes (Black *et al.*, 1992). RAPD markers should also be useful for analysing population structure and gene flow (Hadrys *et al.*, 1992) or for finding markers linked to resistance genes (Haley *et al.*, 1994).

The purpose of this report was to assess the reliability of RAPD to classify the *Globodera* at the specific and sub-specific levels, and to verify the concordance of the results with those obtained from ITS restriction and those derived from the biological and pathotype studies.

Material and methods

NEMATODES

The origin of five *G. rostochiensis*, six *G. pallida*, six *G. "mexicana"* and nine *G. tabacum sensu lato* populations used in this study is listed in Table 1. Populations of *G. rostochiensis* and *G. pallida* were reared on *Solanum tuberosum* cv. Désirée, and the other populations were reared on *L. esculentum* cv. St Pierre or *S. dulcamara*, in a greenhouse.

PLANTS

Different cultivars and species of *Nicotiana* were tested with all the populations of *Globodera* attacking tobacco and with one population of each potato cyst species. Five replications were made. Greenhouses tests were performed in 1000 cm^3 pots. Inoculum consisted in cysts placed in a nylon bag and corresponding to a density of ten J2/g of soil. *Nicotiana* species (*N. acuminata* and *N. sanderae*), and cvs Hicks and Cocker 254 of *Nicotiana tabacum* were chosen according to the data of Miller and Gray (1972) and Meredith (1978). Furthermore, the cv. PB D6, a French black tobacco cultivar, was used to look at a presumed parasitic adaptation of the two French tobacco populations from Agen and Aiguillon. Four months after planting cysts were extracted from soil by the Kort elutriator followed by sugar centrifugation (Coolen & d'Herde, 1972).

DNA EXTRACTION

Females were extracted by gently washing roots and hand picking. DNA extraction was done as already published (Thiéry & Mugniéry, 1996) according to Folkertsma *et al.* (1994).

RAPD PROCEDURES

PCR reactions were performed in a Perkin Elmer Cetus thermocycler No. 480 in 12.5 µl reactions. The concentrations of dATP, dCTP, dTTP, and dGTP were always 0.1 mM each, buffer was 1X (TrisHCl 10 mM, KCl 50 mM, $MgCl_2$ 1.5 mM, Triton X100, gelatin 0.2 mg/ml), $MgCl_2$ 0.44 mM (approximately 2 mM final), Taq 0.1 U. Template DNA was approximately 15 ng per reaction, primer 200 nM. Randomly chosen primers from the kit G and the kit L of Operon Technologies Inc. (Alameda, CA, USA) were used. Taq polymerase and buffer were purchased from Appligene (Illkirch, France), dNTPs from Boehringer (Mannheim, Germany). The first cycle at 94°C for 5 min was followed by 40 cycles, 1 min at 94°C, 1 min at 35°C and 1 min at 72°C.

Samples of PCR reaction were loaded on a 1.5 % gel (NuSieve-Agarose ; 3-1) in 1X Tris-Boric acid-ethylenediamino-tetra-acetate (TBE), pH 8.0. As a size standard the DNA molecular weight markers λ / HindIII - EcoRI (Boerhinger) and pBR322/HaeIII (Appligene) were used. Electrophoresis was carried in 1XTBE at 110V. Products were visualised by ethidium bromide (0.5 µg/ml) staining and UV transillumination.

Three repeats of each amplification were performed independently, to investigate the reproducibility of reactions. Population markers were chosen only when present without ambiguity in the three repeated amplification reactions. Differences of band brightness were not taken into account. A negative control was included as a test for contamination.

COMPUTING OF RESULTS

For the data analysis of host range and due to the reduced number of differentials, the correspondence analysis procedure was preferred to clustering.

The RAPD data typically contained the information for each population encoded on a separate line with the name of the population followed by a 1 for the presence of a specific band or a 0 for the absence of that band (*e.g.*, Welsh & McClelland, 1990).

The resulting matrix was used as input data to the Phylogeny Inference Package (Phylip, version 3.5c)

Table 1. *Location, code, and host range of the 26 populations of* Globodera.

Species	Locations / code (country)	Principal host (pathotypes)	Reference number
G. rostochiensis	Scotland (UK)	potato (Ro1)	1
	Noirmoutier (F)	potato (Ro1)	2
	Castellane (F)	potato (Ro1)	3
	Sedan (F)	potato (Ro1)	4
	Mierenbos (NL)	potato (Ro1)	5
G. pallida	Guiclan (F)	potato (Pa2/3)	6
	Saint-Malo	potato (Pa2/3)	7
	Chavornay (CH)	potato (Pa2/3)	8
	Pas-de-Calais (F)	potato (Pa2/3)	9
	HPL1 (NL)	potato (Pa2)	10
	Duddingston (UK)	potato (Pa1)	11
G. "mexicana"	Tlaxcala (ME)	wild solanum	12
	Santa-Ana (ME)	wild solanum	13
	GM5*, Huamantla (ME)	wild solanum	14
	GM6*, Santa-Ana (ME)	wild solanum	15
	GM3* (ME)	wild solanum	16
	GM4* Huamantla (ME)	wild solanum	17
G. tabacum	Aiguillon (F)	tobacco	18
	Agen (F)	tobacco	19
	Connecticut* (USA)	tobacco	20
G. virginiae	GV1* (USA)	tobacco	21
	GV2* (USA)	tobacco	22
	GV3* Crutchlow (USA)	tobacco	26
G. solanacearum	GS1* Watkins (USA)	tobacco	24
	GS2* (USA)	tobacco	25
	GS3* (USA)	tobacco	26

* Populations GM3, GM4, GM5, GM6, GV1, GV2, GV3, GS1, GS2, GS3, GT Connecticut are from L.I. Miller collection (University of Virginia, USA)

program. Genetic distances of Nei and Li (1979) and cluster analysis with Unweighted Pair Group Method with Arithmetic mean (UPGMA) were obtained with the Gendist and Neighbor programs respectively as in Thiéry *et al.* (1996). The KITCH program of Phylip was also used to compute genetic distances of Nei and Li (Gendist).

Correspondence analysis was performed using as variables the genetic distances of Nei and Li (Gendist).

Similarity coefficients were also calculated according to the Nei and Li's (1979) index :

$$F = 2Nab/Na+Nb$$

where *Nab* were the bands shared by populations *a* and *b*, and *Na* and *Nb* were the total number of bands in each population. *D=1-S* values were used to construct a symmetrical distance matrix and this matrix was used to construct a dendrogram using UPGMA with the Neighbor program of Phylip.

Table 2. *Average number of cysts of each population formed on each plant of seven* Nicotiana *species and cultivars, from an inoculum of ten cysts.*

Code of populations	*N. tabacum*					*N. acuminata*	*N. sanderae*
	Hicks	Xanthi	Cocker 254	Kentucky	PB D6		
Ecosse	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Guiclan	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
GM5	31 ± 11	60 ± 36	20 ± 7	70 ± 49	4 ± 3	33 ± 3	0 ± 0
GM6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0	0 ± 0
GM3	288 ± 76	288 ± 56	10 ± 4	274 ± 48	0 ± 0	234 ± 92	2 ± 1
GM4	5 ± 3	5.4 ± 2.5	8 ± 3	6 ± 2	3 ± 2	40 ± 9	5 ± 1
Tlaxcala	0.6 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0 ± 0	0 ± 0	4 ± 3	0 ± 0
Santa Ana	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3 ± 1	0 ± 0
GS1	506 ± 87	557 ± 124	505 ± 89	445 ± 97	11 ± 3	467 ± 91	3 ± 1
GS2	793 ± 110	738 ± 144	602 ± 77	797 ± 53	12 ± 3	852 ± 242	12 ± 2
GS3	397 ± 120	264 ± 38	266 ± 63	286 ± 66	19 ± 4	421 ± 67	3 ± 2
GV1	513 ± 89	506 ± 45	242 ± 47	673 ± 107	29 ± 5	323 ± 69	8 ± 5
GV2	30 ± 10	40 ± 13	22 ± 8	37 ± 7	17 ± 4	635 ± 184	17 ± 4
GV3	174 ± 150	34 ± 6	7 ± 2	23 ± 4	9 ± 4	127 ± 31	25 ± 10
Connecticut	133 ± 31	434 ± 94	61 ± 19	239 ± 45	2 ± 1	245 ± 61	19 ± 12
Aiguillon	242 ± 75	302 ± 35	14 ± 4	239 ± 70	424 ± 95	131 ± 77	53 ± 47
Agen	400 ± 64	684 ± 74	35 ± 12	444 ± 132	746 ± 142	269 ± 42	143 ± 114

Results

As expected, the two potato cyst species did not multiply on *Nicotiana* species (Table 2). The two native Mexican populations, *i.e.*, Santa Ana and Tlaxcala did not multiply on *N. tabacum* as did GM6. The general multiplication of GM3 looks as the north America populations. A small development was observed with GM5 and GM4. To be sure that a small multiplication or no multiplication at all was a reality and not an artefact due to a bad viability of the inoculum, additional experiment was conducted using tomato as control. Results confirm the absence of multiplication of the two native Mexican populations and of GM6, the small multiplication of GM5 and GM4 and the high multiplication of GM3 on *N. tabacum*. General development of the north American populations and of the two French ones was fairly good. The French black tobacco cultivar cv. PB D6 clearly distinguished Aiguillon and Agen populations from all others which were unable to multiply on it. Cultivars Hicks and Cocker 254, found by Meredith (1978) to allow the development of *G. rostochiensis* were unable to multiply the Scotish population. Surprisingly, cv. Cocker 254 allowed only a very small multiplication of the two French populations and of the Connecticut population. It differentiated clearly *G. virginiae* GV1 and all the *G. solanacearum* populations which developed fairly well. Multiplication of GV2 and GV3 was variable: small for GV2 except on *N. acuminata*, small but consistent for GV3 (Table 2).

Populations which multiplied poorly or not at all (GM4, Ecosse, Guiclan, Santa Ana and Tlaxcala) were not used for the factorial analysis procedure. This analysis indicated two principal axis (Fig. 1). The bidimensional representation using the two first axis showed very close relationships between the two French populations, and between GM3, Connecticut, the three *G. solanacearum* and *G. virginiae* GV1 populations. Distance between GV2 and GV3 from all the other populations and between themselves was great. The impact of the different tobacco species and cultivars on the nematode populations is shown with a two first axis representation of the data (Fig. 1). Cultivars Hicks, Xanthi and Kentucky expressed few differences. *N. sanderae* and mainly cv. PB D6 are important for the discrimination of the French populations. *N. acuminata* is important to discriminate GV3 and mainly GV2.

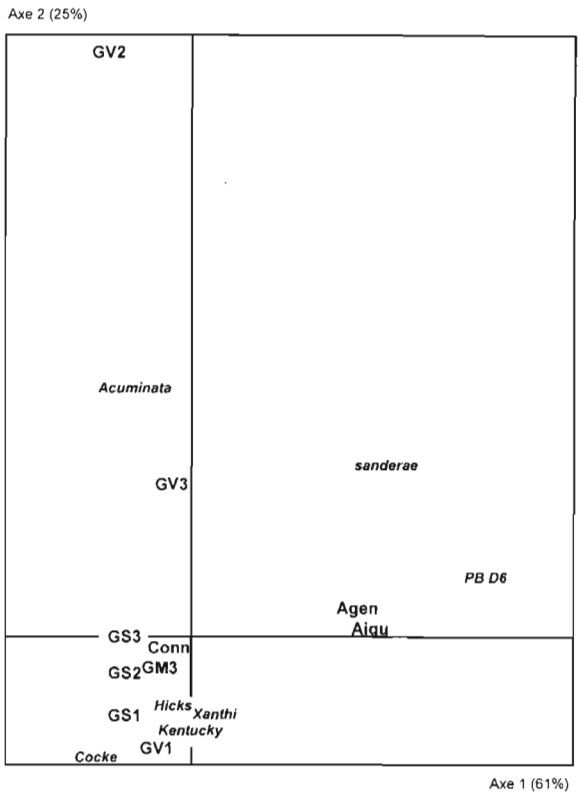

Fig. 1. *Factorial analysis on the number of cyst formed on seven tobacco plants for ten populations of* G. tabacum *complex. Tobacco plants :* N. tabacum *(cultivars : Hicks, Xanthi, Cocker 254, Kentucky and PBD6),* N. acuminata *(Acumina) and* N. sanderae. *Nematode populations : Connecticut (Conn), Aiguillon (Aigu) , Agen, GV1, GV2, GV3, GS1, GS2, GS3 and GM3.*

The amplified DNA fragments were in the range of 180 to 3500 bp, with an average of six to seven consistent amplification products per population and per primer. The five G kit primers used (G1 to G5) gave 126 markers and the three L kit primers (L3-L4 and L6) gave 96 markers with the 26 nematode populations, giving 222 as the total number of markers. Two gels photos containing the 26 populations patterns obtained with primers G6 and G8 are shown in Fig. 2A and B, respectively.

Few markers were monomorphic between the 26 populations of the six species, but the majority showed interspecific polymorphism. Intraspecific polymorphism was revealed in a smaller proportion by some primers.

The computing analysis of the 222 markers on the 26 populations with the Nei distances of Gendist and UPGMA gave a four clusters dendrogram (Fig. 3A). The four groups were constituted by *G. tabacum sensu lato*, *G. rostochiensis*, *G. pallida* and some Mexican populations. *G. pallida* and *G. "mexicana"* groups cluster together just as do *G. tabacum sensu lato* and *G. rostochiensis*. In the *G. pallida* group the Duddingston population, a Pa1 pathotype, was far from the other *G. pallida* populations belonging to Pa2/3 pathotypes. Mierenbos, a Dutch Ro1 pathotype, appeared to be more distant from the Ro1 French pathotypes. In the *G. tabacum sensu lato* group two sub-clusters were identified ; one with the *G. t. solanacearum* and *G. t. virginiae* and the Mexican GM4 ; the other sub-cluster with *G. t. tabacum*, the two French populations, and the Mexican GM3. However *G. virginiae* group appeared to be more polymorphic showing GV1 population in the *G. solanacearum* group while GV2 and GV3 populations formed the *G. virginiae* group. Two Mexican populations GM3 and GM4 seemed to be very different from the other four Mexican populations. GM3 was grouped with *G. tabacum sensu stricto*, while GM4 was joined to *G. virginiae* (GV2 - GV3) group. The two French populations found on tobacco belong to the *G. tabacum sensu stricto* group.

The principal coordinate analysis confirmed the existence of four very separate groups, corresponding to the different species (Fig. 4). Both GM3 and GM4 clearly belonged to the tobacco group. The Duddingston population appeared far from the other *G. pallida* populations. In the two projections, GV2, GV3 and GM4 were very close or confused. In order to measure the confidence of the dendogram, a boot-strapping was made by 500 fold repeatedly resampling the data. The consensus dendogram obtained was exactly the same as in Fig. 3A and the robustness may be shown by the high value of the data expressed in percent at each node.

Discussion

The RAPD technique showed a great number of advantages: it required no prior knowledge of the molecular biology of the organisms to be investigated (Welsh & McClelland, 1990), its assay was relatively simple, rapid and independent of gene expression (Goodwin & Anis, 1991) and a small amount of material was needed to perform the reaction (Pinochet *et al.*, 1994). RAPD originate mainly from non codant DNA and so completely verify the neutral theory of Kimura reported by Margalé *et al.* (1994). However reliability is questionnable. The annealing temperature and the $MgCl_2$ concentration were choosen to minimise the variability (Caswell-Chen *et al.*, 1992; Rothuizen & Wolferen, 1994). Since the reproducibility of quantitative differences might not be as consistent, only band-presence *vs* band-absence was analysed in this study. Furthermore co-migration of nonhomologous products, if widespread, could be

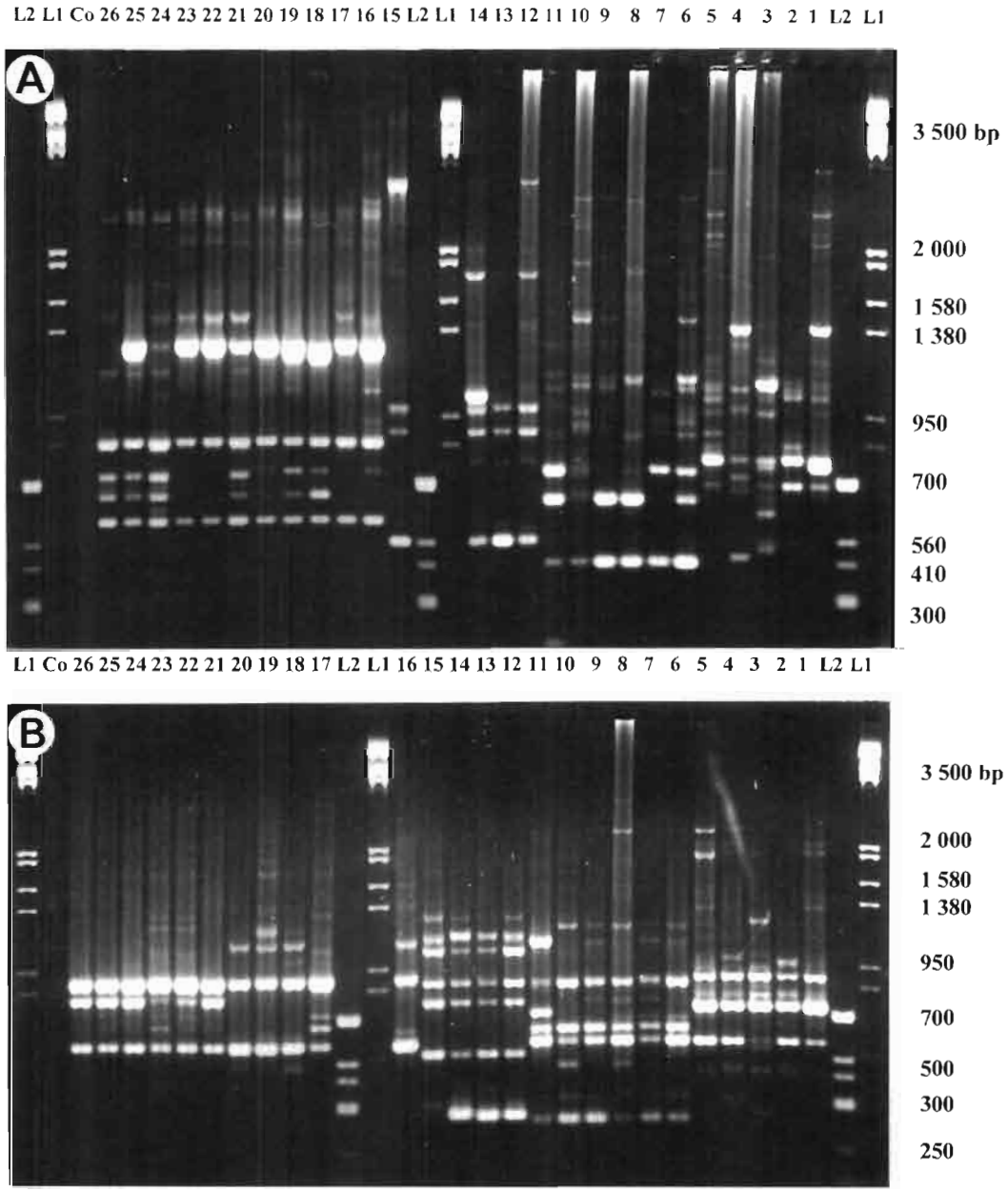

Fig. 2. *RAPD patterns of 26 populations with primer OPG-6 (A) and OPG-8 (B) - From 1 to 26 : Ecosse, Noirmoutier, Castellane, Sedan, Mierenbos, Guiclan, Saint-Malo, Chavornay, Pas-de-Calais, HPL1, Duddingston, Tlaxcala, Santa-Ana, GM5, GM6, GM3, GM4, Aiguillon, Agen, Connecticut, GV1, GV2, GV3, GS1, GS2, GS3. Co : control sample without template DNA. L1 : (DNA digested by HindIII and EcoRI. L2 : plasmid pBR322 digested by HaeIII. Molecular weights are indicated in basepairs.*

regarded as a "noise" factor in the calculation of S % (Chapco *et al.*, 1992). Codominant alleles were occasionally detected, presumably arising through small deletions or insertions within the amplified region (Black, 1993). However, the size polymorphisms due to insertion or deletion cannot be not taken into account as in Williams *et al.* (1990) because the size differences were usually too difficult to be differentiated from the variation typically seen from lane to lane within the agarose gels. Considering the number of

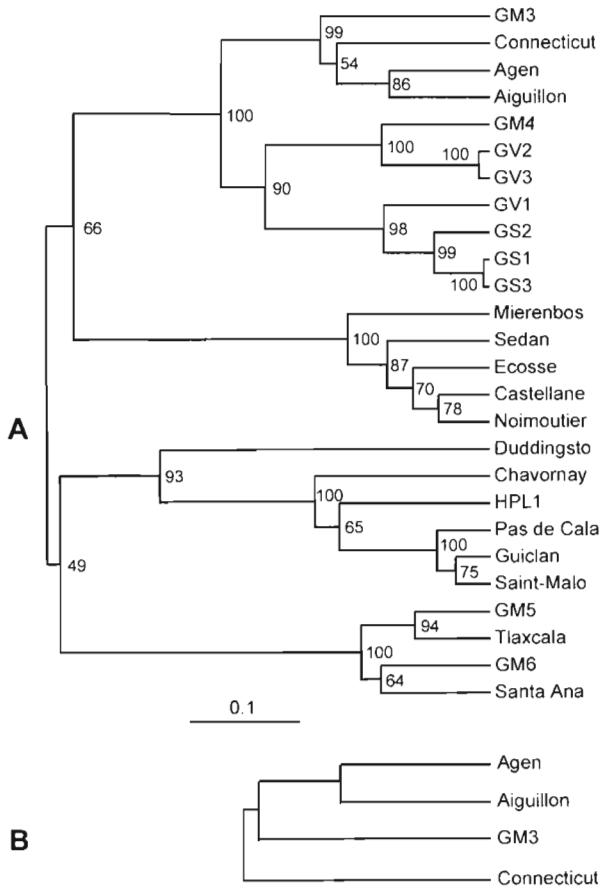

Fig. 3. *Dendrogram of the 26* Globodera *populations based on 222 RAPD markers. A: Construction with the Nei & Li distances and UPGMA (Gendist and Neighbor, PHYLIP3.5c); B: Construction with the similarity coefficients (S) and the UPGMA. The unique part differing from Nei and Li (Gendist) distances construction is showed.*

markers used, it may be supposed that all these factors have a very small impact on the final cluster.

According to Chapco *et al.* (1992) and Folkertsma *et al.* (1994), the statistic $S = 2Nab / Na+Nb$ is at present the most unambigous formula to express inter- or intraspecific similarity until a theoretical framework is developed for the variation generated by the RAPD assay. However, there was no significant differences between *i)* the dendrogram obtained with Nei and Li (1979) Gendist formula based on frequencies and *ii*) the dendrogram issued from similarity coefficients. A comparison of the results obtained with a Fitch and Margoliash analysis using the KITCH program of Phylip revealed no consistent differences with the UPGMA analysis. The three dendrograms issued from Nei and Li (1979) (Gendist)-UPGMA, similarity coefficients-UPGMA and Nei and Li (1979) (Gendist)-Fitsch and Margoliash analyses did not reveal any significant difference. The Gendist Nei and Li (1979) formula based on allele frequencies is probably less appropriate to compute RAPD data than similarity coefficients. Although, the dendrogram obtained with the Nei and Li formula of Gendist is similar to the dendrogram issued from the D values analysis. The unique and small difference concerned GM3 and Connecticut clustering (Fig. 3B).

Clustering the RAPD markers result in the Fig 3A and 3B. The four main species identified by ITS (Thiéry & Mugniéry, 1996) are clearly separated. At the species level RAPD results were partly in accord with the 2-DGE (Bossis & Mugniéry, 1992) in that it showed *G. rostochiensis* far from *G. pallida* and pointed out the closeness of *G. pallida* and *G. "mexicana"*. The RAPD technique revealed that the *G. rostochiensis* group was nearer to the *G. tabacum* group than to the *G. pallida* and *G. "mexicana"* cluster, while this was not the case in the 2-DGE dendrogram were *G. tabacum* appeared to be the more distant group. These results were also in accordance with the hybridisation results. This is not in accord with Ferris *et al.* (1995), probably because they clustered with sequences of the ITS, obtained with an automatic sequencer. Interesting is the fact the same relationships obtained by the both ITS and RAPD is in apparent contradiction with the origin of the *Globodera* species. *G. rostochiensis* is the closest relative of *G. tabacum* despite their origins are so distant. Also morphologically *G. tabacum* is closest to *G. pallida*, which is not the case with proteins, ITS and RAPD data.

We clearly distinguished the four main *Globodera* species. A good resolution of the technique at the species level has also been reported with other nematode genera in previous studies (Caswell *et al.*, 1992; Cenis, 1993). But the data produced here allowed the distinction of populations within a same species. However, in general the patterns were quite similar within species, resulting in a majority of specific *vs* intraspecific markers. This was also found by other authors (Welsh & McClelland, 1990). The result was that interspecific was more reliable than intraspecific clustering.

At the species level RAPD results were partly in accord with the 2-DGE (Bossis & Mugniéry, 1992) in that it showed *G. rostochiensis* far from *G. pallida* and pointed out the closeness of *G. pallida* and *G. "mexicana"*. The RAPD technique revealed that the *G. rostochiensis* group was nearer to the *G. tabacum* group than to the *G. pallida* and *G. "mexicana"* cluster, while this was not the case in the 2-DGE dendrogram were *G. tabacum* appeared to be the more distant group.

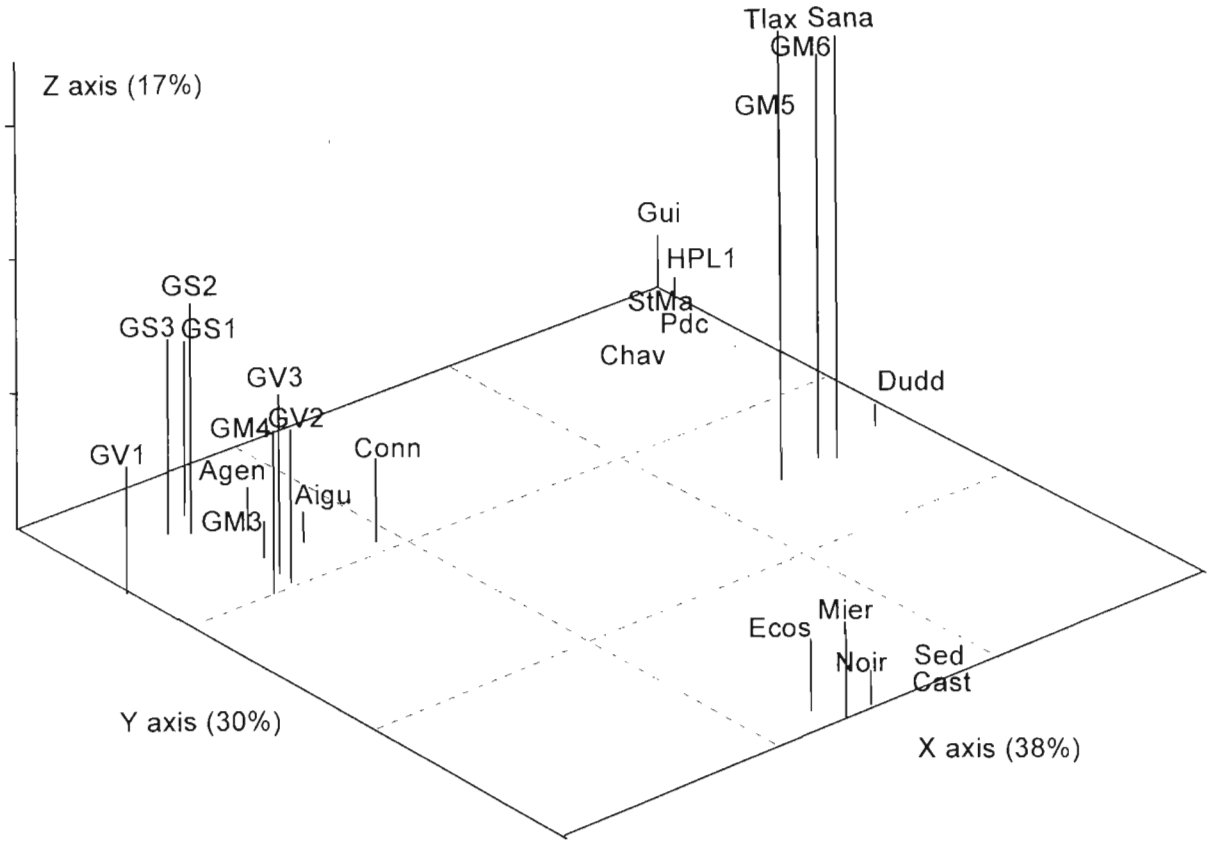

Fig. 4. *Principal coordinate analysis (ACP) on the RAPD results, using the same 222 markers used for the dendrogram construction. Tlaxcala (Tlax), Santa-Ana (Sana), GM3, GM4, GM5, GM6, Guiclan (Gui), HPL1, Saint-Malo (StMa), Pas-de-Calais (Pdc), Chavornay (Chav), Duddingston (Dudd), GS1, GS2, GS3, GV1, GV2, GV3, Connecticut, (Conn), Aiguillon (Aigu), Agen, Mierenbos (Mier), Scotland (Ecos), Noirmoutier (Noir), Castellane (Cast) and Sedan (Sed).*

This was also in accordance with the hybridisation results.

The seven tobacco cultivars and species used in this study differentiated the *G. tabacum* sub-groups. The subspecies grouping in the *G. tabacum* species with the host range analyses presented a great number of similarities with the RAPD classification. The main difference seemed to concern *G. solanacearum* and GV1 populations grouped with GM3 and Connecticut population in the host range analysis, while not appearing with *G. tabacum sensu stricto* in the RAPD dendrogram. Identification of GV1 as *G. virginiae* made by L.I. Miller was based on so faint characters that a wrong determination may be possible (Stone, 1993; Mota & Eisenback, 1993*a, b, c*). Folkertsma *et al.* (1994) showed that the RAPD data of *G. rostochiensis* populations were in concordance with the pathotype classifications, while the RAPD clustering of *G. pallida* populations showed only limited resemblance to their pathotype classification. The same authors argued that the limited number of differential potato clones used to classify pathotypes of *G. pallida* was too small to properly reflect the genetic variation of *G. pallida* populations in Europe. This is probably the case with the set of *Nicotiana* used in this study. A similar situation has also been noted in *Meloidogyne* spp. in which host preference was not revealed by RAPD classification (Cenis, 1993), and with the grouping of *Pratylenchus vulnus* populations based on similarity of bands which did not correlate with the similarity based on host preference (Pinochet *et al.*, 1994). Bakker *et al.* (1993) argued that the genetics of virulence may be restricted to few genes, so virulence polymorphisms should be treated as any other character and not be given too much weight in classifying populations. For this reason we did not use the host

data for clustering, considering to the risk of reaching a wrong conclusion due to the small number of differentials.

The number of European populations used belonging to *G. rostochiensis* and *G. pallida* species is not sufficient to look for a correlation between geographical origin and RAPD clustering. Other authors looking at the intraspecific variability did not find a good correlation between RAPD data grouping and geographical origin of populations (Caswell-Chen *et al.*, 1992; Pinochet *et al.*, 1994; Folkertsma *et al.*, 1994).

The existence of four *Globodera* species and a trend for subspecies in *G. tabacum* is a reality. The great polymorphism observed among the *G. tabacum* species could be explained by the fact that the majority of the populations used in this study came from USA where the species originate. At the opposite the *G. pallida* and *G. rostochiensis* populations used came from some initial introductions into Europe and this could explain the small variability observed among those two species.

The RAPD technique presents a great number of advantages : it is neutral, the dendrogram generated is in accord with the ITS dendrogram, there is no contradiction with the genetic barriers (hybridisation) and, furthermore, a good correlation appears with the virulence results.

At present in order to work on a probably larger polymorphism we are including in the RAPD dendrogram several *G. pallida* populations from Peru from which this species originate. Furthermore, other Mexican populations, a *G. rostochiensis* pathotype Ro2 and a pure *G. pallida* Pa2 pathotype are included.

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