Genetic diversity of cocoa tree *Phytophthora* pathogens

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Before searching for individuals resistant to pod rot disease caused by various species of the genus *Phytophthora*, it is essential to study pathogen diversity. Good knowledge of that diversity provides a clearer understanding of the diversity of attacks, notably of "host x pathogen" interactions that might sometimes complicate the search for resistance. This chapter therefore proposes a review of the genetic diversity of cocoa tree *Phytophthora* pathogens. This genetic diversity is investigated using various recently developed molecular tools. Following an introduction to the different cocoa tree pathogen species, the diversity of the most widespread species will be examined.

The different pathogenic species of the genus *Phytophthora* found on cocoa

Black pod rot is a cocoa disease found worldwide, and was initially thought to be caused by a single species, *P. palmivora* (Butler, 1919). The taxonomic history of *Phytophthora*, which was first described by Butler (1910), has been very eventful.

The first descriptions and identifications of *Phytophthora* isolated from cocoa, coconut or rubber were based on morphological characteristics. These charac-

teristics involved vegetative or reproductive organs (Rosenbaum, 1917; Butler 1925; Ashby, 1922; Turner 1960, 1961), followed by characters linked to the physiology or the pathogenicity of the strains (Tucker, 1931; Gadd, 1924, 1927; Ashby, 1929). Substantial diversity was already found among strains; depending on the criteria used, the strains studied, and the degree of within-species diversity accorded by the author, the limits of the species were often questioned. Some species, such as *P. arecae* and *P. meadii* were included, then withdrawn from the *P. palmivora* group (Tucker, 1931, Waterhouse, 1963). For its part, *P. botryosa* was created from that group (Chee, 1969).

A key stage in the taxonomic history of *P. palmivora* was the cocoa *Phytophthora* workshop held in May 1976 at Rothamsted Experimental Station (UK). The workshop was organized following a presentation by Brasier and Sansome, at the International Cocoa Research Conference (Nigeria, 1975), reporting that at least two chromosomal types of *P. palmivora* existed in West Africa. During those meetings, the *P. palmivora* group was subdivided into four morphological types (MF1, MF2, MF3 et MF4) based on precise morphological criteria and on the size and number of chromosomes; as the other strains of *P. palmivora* did not correspond to any of these morphological types, they were considered to be atypical (Griffin, 1977). A new key for the identification of *Phytophthora* species was then published (Newhook *et al.,* 1978). However, the work by Brasier and Griffin (1979), involving 1,104 strains, 892 of which had been isolated in Nigeria, led those authors to consider morphological type MF3 (5-6 large chromosomes, corresponding to Turner's type 'N', 1960) as a new species: *P. megakarya*. Type MF4 too was a subject of controversy: as similarities were found between MF4 strains and *P. capsici*, Zentmyer *et al.* (1981), Idosu and Zentmyer (1978), Kaosiri (1978) and Kaosiri *et al.* (1978) proposed attaching these strains to *P. capsici* Leonian; Tsao and Alizadeh (1988) then redescribed *P. capsici* in order to include all *P. palmivora* MF4 isolates that are pathogenic on cocoa.

At this stage, it should be noted that in most of the work mentioned, zygote formation was considered to be proof that strains exposed to each other were interfertile. However, Brasier (1972) described the concept of selfing in heteroallelic *Phytophthora* when the oogonia of one strain were in fact fertilized by the antheridia of the same strain, being influenced by the presence of a strain with a complementary mating type, or other factors. It is therefore essential to check that the progenies obtained from a cross between two strains carry the genetic traits of both parents, before concluding on interfertility between those strains, hence that they belong to the same species. Boccas (1981) thus tested crosses between mature species of heteroallelic *Phytophthora* (*P. palmivora, P. megakarya, P. capsici, P. nicotianae* var. *parasitica, P. cinnamomi* and *P. cambivora*). The progenies from those crosses displayed heterogeneous and recombined morphological, phenotypical and physiological traits, thereby suggesting the hypothesis of hybridization between species. However, an analysis of the composition between soluble proteins revealed that those progenies came in fact

from selfing of the parental strains. Hence, with the strains and methods used, there was no hybridization; it therefore seems that there are interspecificity barriers between these different species of *Phytophthora*. However, the formation of selfed oospores increases the chances of a species surviving under adverse environmental conditions.

These analysis techniques have developed and new biochemical and molecular tools are now available for describing intra- and interspecific variability. Techniques such as immunology (Burrel *et al.,* 1966; Savage *et al.,* 1968; Merz *et al.,* 1969), electrophoresis of total proteins (Zentmyer *et al.,* 1977; Erselius and Shaw, 1982; Hansen and Maxwell, 1991), isozyme analysis (Clare and Zentmyer, 1966; Hall *et al.,* 1969; Blaha, 1990; Oudemans and Coffey, 1991*a*, 1991*b*), in situ DNA-DNA hybridization (Goodwin *et al.,* 1989), DNA restriction fragment length polymorphism (RFLP) (Klimczack and Prell, 1984; Carter *et al.,* 1990; Goodwin, 1991; Förster *et al.,* 1987), and random amplification of polymorphic DNA (RAPD) (Nyassé *et al.,* 1999; Sackey *et al.,* 1994), contribute towards more effective discrimination of genotypes and more effective characterization of population structures. In fact, these different methods often give complementary results, enabling more effective characterization of the limits between species. It was in this way that an isozyme determination key was proposed by Ortiz-Garcia (1996), for the *Phytophthora* species involved in cocoa and coconut diseases. This key was drawn up from an isozyme analysis of 220 strains of *Phytophthora,* 150 of which were reference strains from various international collections identified by morphological criteria. Of the 26 isozyme systems studied, 21 proved to be polymorphic, and *Phytophthora* strains pathogenic on cocoa and coconut can be identified by exploiting only 3 loci (Idh-2, Pgi, Mdh-1) (figure 1). This key is based on the extraction of proteins which, unfortunately, cannot be used to take the genome study any further, though new techniques based on DNA analysis do make it possible to fine-tune genome analysis.

The evolution of cocoa pathogenic *Phytophthora* populations over time depending on their geographical origin, type of reproduction, and control methods in the field, is currently being investigated by ITS sequence polymorphism studies (nucleotide sequences of untranscribed intergene regions of ribosomal DNA), (Ducamp *et al.*, 2002; Lee *et al.*, 1993). This ITS-RFLP technique was utilized in comparison to the other techniques used earlier to confirm that the strains described as belonging to species of *P. palmivora* from different host-plants are indeed part of the same species (figure 2). The closest species is *P. megakarya,* which, itself, is also homogeneous, whether it is isolated from cocoa or cola. The species *P. capsici* and *P. citrophthora* are close to the species *P. citricola* and *P. colocasiae*. On cocoa, it is possible to isolate *P. capsici sensu stricto* and a population we shall call *P. capsici* "cocoa", which seems to be particularly adapted to cocoa in terms of aggressiveness. Their ITS sequences differ very little (10 pairs on the 835 studied), but they can cross, giving hybrids (Ortiz-Garcia, 1996). In Brazil, it is possible to isolate *P. citrophthora "cocoa"*, which has a very

similar ITS to that of *P. citrophthora sensu stricto,* which can be isolated from citrus. This technique therefore makes it possible to characterize the different species of *Phytophthora* that are pathogenic on cocoa.

These different methods also provide increasingly precise data that can be used not merely to assess the diversity of *Phytophthora,* but also genetic diversity within the species of *Phytophthora* that are pathogenic on cocoa. These studies may provide a clearer understanding of the differences found between epidemics (Oliveira, 1990). Indeed, the variation in damage from one country to another depends on environmental conditions and the type of material planted, but also on the species of *Phytophthora* involved, or even the strains within species.

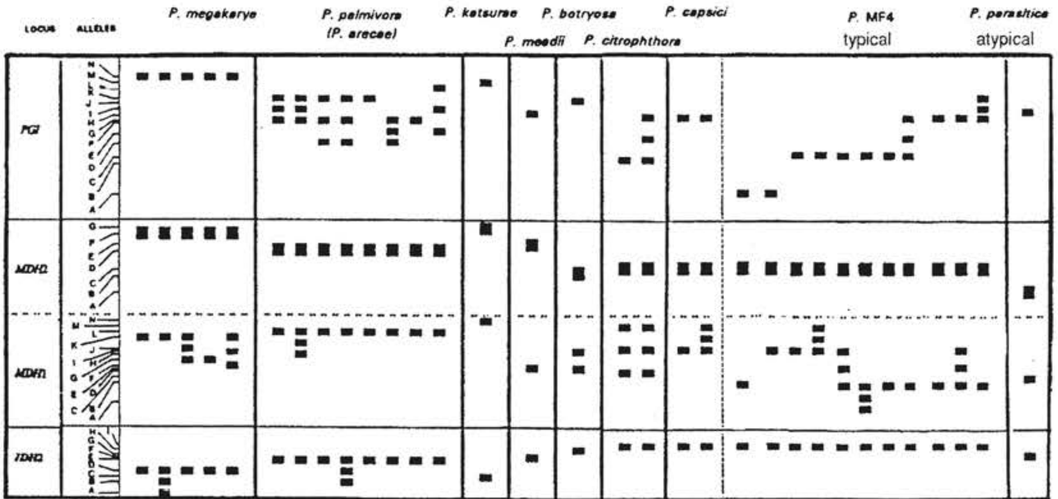

Figure 1. Biochemical key for the determination of Phytophthora *species parasitizing cocoa and coconut.*

Idh-2	if allele C	*P. katsurae*	Pgi	if allele A	*P. MF4 typical*
	if allele D	*P. megakarya*		if allele B	*P. citrophthora* Smith & Smith Leonian
	if allele E	*P. palmivora/P. arecae*		if allele C	*P. MF4 typical*
	if allele F	*P. meadii*		if allele G	Mdh-1
	if allele G	*P. parasitica*		if allele B	*P. MF4 atypical*
	if allele H	*P. botryosa*		if others	*P. capsici*
	if allele I				

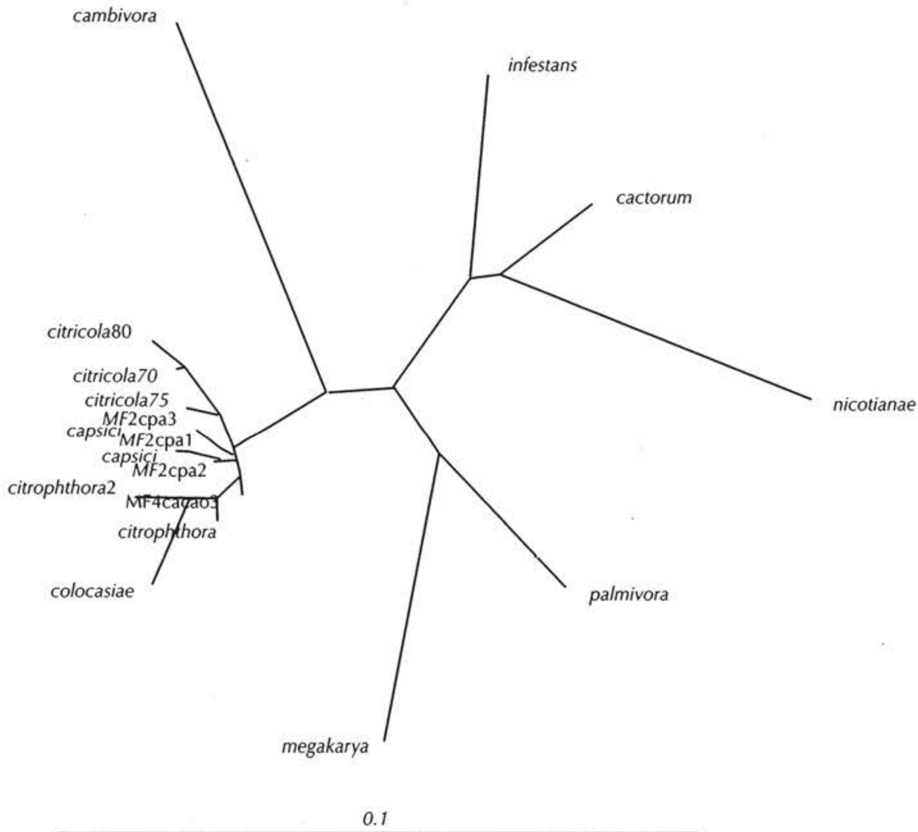

Figure 2. Classification of the different Phytophthora *species from ITS sequences.*

Genetic diversity of *P. palmivora*

The species *P. palmivora* exists virtually throughout the world's cocoa-growing zone, even though it is not always the majority species (*P. capsici* being the most common in Latin America and *P. megakarya* in Central Africa). Neither is it rare to see different attack levels within the same species, depending on the strains used (Surujdeo-Maharaj *et al.*, 2001; Appiah *et al.*, 2002).

Genetic studies were recently carried out on *P. palmivora* along with taxonomic studies; they date from the 1990s and focused on isozyme criteria (Oudemans and Coffey, 1991; Ortiz-Garcia, 1996).

Oudemans and Coffey (1991*b,c*), working on 393 strains of 12 species of *Phytophthora,* studied interspecific and intraspecific diversity by isozyme analysis. An initial conclusion indicated that no distinction could be made between the

species *P. palmivora* and *P. arecae*. The 100 *P. palmivora* strains studied, which came from such varied hosts as *Theobroma cacao, Cocos nucifera, Carica papaya, Durio zibethinus* etc., and from different regions of the world (Asia, Africa, Latin America), proved to be relatively uniform: only 2 loci (PGI and IDH-1) out of 18 were polymorphic. UPGMA analysis (Sokal and Sneath, 1963) using Rogers' genetic distance (1972) modified by Wright (1978), gave a maximum distance of 0.3 between strains of the *P. palmivora* group and the genetic diversity measured with Nei's index (1978) was evaluated at 0.08 within this group.

The work by Mchau and Coffey (1994*a,b*) on 93 strains of *P. palmivora* and 6 strains of *P. arecae*, also originating from highly varied hosts and countries, gave 18 ETs, established from 6 polymorphic loci (PGI, HEX-2, IDH-1, MDH-1, PEP and SOD), the most polymorphic loci being once again PGI and IDH-1. As previously, the *P. arecae* strains shared the ETs most represented in *P. palmivora*. Maximum genetic diversity was found in strains isolated from coconut and durian in Indonesia, Malaysia and Thailand. As these plants came from Southeast Asia, the authors propose this region as the centre of origin of *P. palmivora*.

Ortiz-Garcia (1996) worked on 631 strains of *P. palmivora* and *P. arecae* from different parts of the world (West Africa, Latin America, Caribbean, Southeast Asia and the Pacific), isolated from *Theobroma cacao, Cocos nucifera*, and from soil in coconut plantings. Forty-six different ETs were obtained using 7 loci. Once again, the strains morphologically identified with *P. arecae* shared common ETs with *P. palmivora*. A cross carried out between a *P. palmivora* strain and a *P. arecae* strain revealed genetic recombination, thereby indicating interfertility between the two "species". Nei's genetic diversity index was then evaluated at 0.229.

An examination of the UPGMA dendrogram established from the matrix of Rogers distances showed that there was no preferential distribution of strains according to their geographical origin or those from which they came (figure 3). However, examination of a sub-set comprising 179 coconut strains and 69 cocoa strains, all from Southeast Asia, showed that these two populations are genetically differentiated (they are separated by a distance of 0.202) and suggested the existence of parasitic specialization within this species. This parasitic specialization was confirmed by crossed artificial inoculations.

Indeed, tests carried out at Balai Penelitian Kelapa, Manado, Indonesia, showed that strains isolated from pods more easily attacked pods (between 55 and 94% success rate) than coconuts (between 0 and 22%), whilst strains isolated from coconut attacked pods and nuts indifferently (between 11 and 94% for pods and between 28 and 55% for coconuts). However, in the latter case, lesions developed much more quickly on nuts (lesion diameters of between 22 and 62 mm, 8 days after inoculation) than on pods (between 1 and 15 mm) (unpublished results). These results tally with those obtained by Steer and Coates-Beckford (1990) and by Warokka and Maskar (1991).

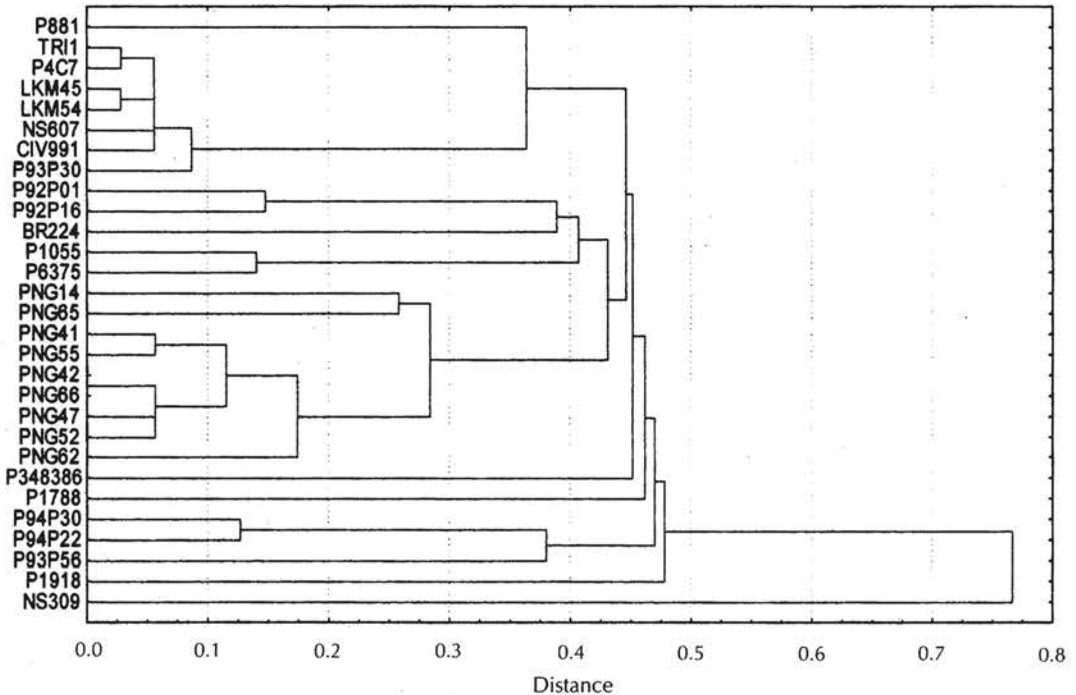

Figure 3. Genetic diversity of P. palmivora.

P. palmivora: Cocoa tree strains: P881: Jamaica (A1), TRI 1: Trinidad (A2), P4C7: Cuba (A2), LKM 45: Malaysia (A2), LKM 54: Malaysia (A2), CIV 991: Ivory Coast (A2), NS 607: Cameroon (A2), P93P30: Indonesia-South Sumatra (A2), P92P01: Indonesia-North Sulawesi (A2), P92P16: Indonesia-North Sulawesi (A2), BR 224: Brazil (A2), PNG 14, PNG 65, PNG 41, PNG 55, PNG 62: Papua New Guinea.

Coconut strains: P94P30, P94P22, P93P56: Indonesia.

Others: P1055: rubber, Thailand (A1), P6325: durian, Malaysia (A1), P34386: bamboo, USA (A1), P1788: papaw, Hawaii (A1), P1819: vanilla, Polynesia.

P. megakarya : NS309: cocoa, Cameroon (A1).

The cocoa population study (198 strains) revealed genetic proximity between the strains from West Africa and those from Latin America (distance of 0.016). Maximum genetic diversity was found in the Southeast Asian population (0.229, minimum: 0.104 in the Pacific), and more particularly in the North Sulawesi region of Indonesia (0.258, minimum: 0.163 in North Sumatra). Similar results were obtained with the coconut population, for which maximum genetic diversity was found in the regions of North and Central Sulawesi, in Indonesia.

To conclude, all this different research argues in favour of merging *P. palmivora* and *P. arecae* in a single species, *P. palmivora,* and of a centre of origin for the species in Southeast Asia. However, it disagrees with the work by Zentmyer (1988), who based his conclusions on diversity linked to the morphological traits of *P. palmivora* and who, on observing that most of the host plants were of American origin, proposed Central America as the centre of origin of *P. palmivora*.

Latin America and West Africa could therefore be two zones into which *P. palmivora* was introduced from Southeast Asia, right from the first exchanges of planting material between those regions (Harries, 1978; Wood, 1991). The Pacific islands would seem to be another, older, centre of introduction, from Southeast Asia, linked to maritime trading by Polynesians (Harries, 1978). Wood (1991) states that "Criollo" cocoa trees from Venezuela were first of all introduced into Sulawesi and the "Criollos" from Mexico were introduced into the Philippines, and that it is from one of those regions that the cocoa tree was then introduced into Java. Ortiz-Garcia (1996) believes that the structuring found in Southeast Asia comes from an adaptation of naturally present *P. palmivora* strains. Adaptation, then differentiation, would seem to depend on the material planted; the genetic proximity of Javan strains with those from Sulawesi, and of those from the Philippines with those from the other regions of Indonesia would seem to be related to the two types of Criollo initially introduced.

Recent DNA study techniques should make it possible to confirm all these results. In fact, enzyme electrophoresis can be used to distinguish charge differences between proteins, but cannot detect amino acid substitutions if the protein charge is not modified. Moreover, these enzymes do not always form a representative sample of the genome (Hartl, 1987). However, studying DNA, which is a source of genetic variability, makes it possible to establish a veritable identity card for each individual, thereby going right to the heart of the genome.

Of the work currently under way on cocoa tree *Phytophthora,* we would mention the RAPD studies undertaken by Sackey *et al.* (1999), who suggest the existence of genetic variations between and within *P. megakarya* and *P. palmivora*. Recent genetic diversity studies in the same laboratory, using RAPD according to the protocol used by Nyassé (1997) on 28 strains of *P. palmivora* isolated from different plants (figure 3), showed that the strains from bamboo, papaw, coconut and vanilla, were clearly separate from strains isolated from cocoa. The strains isolated from rubber and durian were closer to the strains from cocoa; being of opposite mating types, their crossing when these crops are grown together might lie at the origin of further diversification of the species *P. palmivora* (Ducamp, 2002).

Genetic diversity of *Phytophthora megakarya*

The species *P. megakarya* is clearly distinct from the other species of *Phytophthora* through the size of its chromosomes. It seems endemic to Africa, since it has never been detected on other continents (Ortiz-Garcia *et al.,* 1994). It is found in Cameroon, Gabon, São Tomé, Nigeria, Togo and Ghana, and appeared in Ivory Coast in 2000. This species often exists alongside *P. palmivora* (Brasier and Griffin, 1979; Zentmyer, 1988). In Cameroon, the characterization of more than 2,000 *Phytophthora* isolates seems to indicate that *P. megakarya* is virtually alone responsible for pod rot (Nyassé, 1992), though *P. palmivora* was mentioned in that country at the

end of the 1970s (Bakala, 1981) and mixes with *P. palmivora* exist in some plots. In West Africa, *P. megakarya* is spreading westwards and it has been reported in Togo (Djiekpor *et al.*, 1982), followed by Ghana (Dakwa, 1988; Luterbacher and Akrofi, 1994), and consequently more recently in Ivory Coast (Kébé, pers. comm.).

Phytophthora megakarya is the pod rot pathogen that causes most damage on the species *Theobroma cacao* L. Losses have reached 80% in Cameroon (Despréaux *et al.*, 1988; Berry and Cilas, 1994) and Gabon (Anon., 1990), and 100% losses were reported in Ghana (Dakwa, 1988). In Togo, 80% of cocoa plantations have been infected by this species (Djiekpor *et al.*, 1982). In comparison, the incidence of pod rot due to *P. palmivora* is more limited, with losses of around 20-30%. For optimum control of *P. megakarya*, it is necessary to acquire a clearer understanding of the pathogen's epidemiology, reproduction and diversity. The last aspect takes on particular importance for characterizing host-pathogen interactions, and therefore for developing efficient breeding strategies for resistance to this disease.

It proved important to study isolates of the species *P. megakarya* from the African countries in which it has been reported, using several types of markers (biochemical and molecular). From the results of earlier work, it is in fact possible to choose the tools required to study *P. megakarya* diversity. Isozymes appear to be a powerful tool for describing the intraspecific and interspecific diversity of cocoa *Phytophthora* (Blaha, 1990; Nyassé, 1992; Blaha, 1994). An isozyme analysis of 15 *P. megakarya* isolates from Nigeria and Cameroon with 16 enzyme systems enabled a clear separation of the isolates from the two countries (Oudemans and Coffey, 1991*a*). The intraspecific diversity of *P. megakarya* was characterized from mitochondrial DNA on a sample of 12 isolates (Förster *et al.*, 1990); this study enabled effective differentiation between the isolates from Cameroon and those from Nigeria. The genetic diversity of 161 isolates from various African countries (Cameroon, Gabon, São Tomé, Nigeria, Togo and Ghana) was studied using 13 isozyme systems and 9 RAPD primers, after determining the compatibility of their mating types. The degree of correlation between the two types of markers was determined by the Mantel test. Although RAPD are not the most appropriate markers for genetic studies of populations on diploid individuals, these molecular markers do provide a rapid overall picture of population structure. We therefore used them as a quick way of screening a large number of genotypes on numerous loci.

The *P. megakarya* isolates studied

One hundred and sixty-one isolates were collected from naturally infected pods in Cameroon (72), Gabon (11), Ghana (10), Nigeria (50), São Tomé (14) and Togo (4) between 1982 and 1995 (table 1). The distribution of the sampling zones is indicated in figure 4. In Cameroon, samples were taken from all the production zones. In Nigeria, samples were taken on six different agricultural stations, with the help of O.A. Olunoyo, K. Badaru and E.B. Esan from the Cocoa Research Institute of Nigeria. The isolates from Ghana and Togo were collected from

Table 1. Regions sampled for *Phytophthora megakarya* in West and Central Africa, number of isolates collected, year of collect, mating types detected and isozymes and RAPD genotypes identified.

Location	Nb[1]	Year [2]	Mating type[3]	Isozyme genotype[3]	RAPD genotype[3]
Cameroon (72, 19, 28)[4]					
Fako (FK)	7	1995	A1	I33(3), I35(2), I136(2)	R7, R8(3), R28(2), R29
Haut Nyong (HN)	5	1995	A1	I31(5)	R17(4), R18
Haute Sanaga (HS)	6	1995	A1	I31(6)	R17(6)
Manyu (MA)	5	1995	A1	I31(4), I34	R1, R31, R33, R34(2)
Mbam (MB)	15	1994 & 1995	A1	I1, I4, I9, I10, I14 I31(5), I32(4), I33	R11, R12, R13, R14(3), R15, R17(4) R18, R20, R27, R39
Mefou (MEF)	5	1990 & 1994	A1	I1(5)	R17(5)
Meme (MM)	9	1994 & 1995	A1	13, 17, I31(3), I37(4)	R28(4), R30, R31, R32, R33(2)
Mfoundi (MF)	4	1989 & 1994	A1(3), A2	I1(3), I2	R17(2), R21, R26
Ndé (NDE)	1	1994	A1	I7	R22
Ndian (ND)	3	1995	A1	I37(3)	R28(3)
Ntem (NT)	1	1994	A1	I8	R24
Nyong et Kellé (NK)	1	1994	A1	I1	R19
Nyong et Mfoumou (NM)	6	1995	A1	I31(6)	R17(5), R18
Nyong et So'o (NS)	1	1994	A1	I1	R17
Océan (OC)	2	1990 & 1994	A1	15, I11	R25, R44
Unknown (isolate 184)	1	-	A2	I15	R16
Gabon (11, 5, 6)[4]					
Koulamoutou (KO)	2	1982	A1	I12, I20	R40, R41
Makokou-Est (MA)	3	1982	A1	I12(3)	R41, R42, R43
Oyem-CM (OYC)	1	1982	A1	I17	R43
Oyem-Est (OYE)	5	1982	A1	I12(2), I18, I19(2)	R12, R24, R41(3)

(Contd.)

Location	Nb[1]	Year[2]	Mating type[3]	Isozyme genotype[3]	RAPD genotype[3]
São Tomé (14, 4, 4)[4]					
Clara Dias (CL)	1	1994	A1	I21	R35
Pedroma (PE)	3	1994 & 1995	A1	I21(2), I30	R36(3)
Poto (PO)	7	1994 & 1995	A1	I7, I9, I21(2), I30(3)	R35(2), R36(4), R38
Queluz (QU)	3	1995	A1	I30(3)	R35, R37(2)
Nigeria (50, 9, 8)[4]					
Ibeku (IBE)	9	1995	A1	I22(9)	R1(9)
Ibule (IBU)	6	1995	A1, A2(5)	I24, I25, I26, I27(3)	R4(5), R5
Idi-Ayunre (ID)	15	1995	A1	I22(13), I28(2)	R1(10), R2, R3(2), R10(2)
Ikom (IK)	6	1995	A1	I22(6)	R1(6)
Owena (OW)	3	1995	A1	I22, I28, I29	R1, R6, R9
Uhonmora (UH)	10	1995	A1	I22(9), I23	R1(10)
Unknown (isolate P1663)	1	-	A1	I13	R1
Ghana (10, 2, 2)[4]					
Ashanti (AS)	1	1994	A1	I13	R4
Brong Ahafo (BR)	5	1993	A1	I13(5)	R1(5)
Volta (VO)	2	1994	A1	I16(2)	R1(2)
Western (WE)	2	1994	A1	I13(2)	R1(2)
Togo (4, 1, 2)[4]					
Kloto (KL)	2	1988 & 1991	A1	I13(2)	R1(2)
Litimé (LI)	2	1991	A1	I13(2)	R1, R2

1. Number of isolates collected.
2. Isolate collection year(s).
3. A number in brackets corresponds to the number of isolates for a given genotype when there is more than one. For mating type, this is valid only when type A2 is present.
4. (x, y, z); x = Total number of isolates from the country; y = Number of isozyme genotypes; z = Number of RAPD genotypes.

several production zones in those countries; they were supplied by S.T. Sackey (Cocoa Research Institute of Ghana) and E.K. Djiekpor (Institut Togolais de la Recherche Agronomique) respectively. From Cameroon, two reference strains with known mating types were studied: strain 309, of mating type A1, collected from the Mbam zone and strain 184, of mating type A2 (Blaha, 1995), along with two strains, NS130 and NS131, taken from naturally infected cola fruits (*Cola nitida*). The mating types were determined by exposing each strain to strains 309 and 184, characterized as being type A1 and A2 respectively, on carrot-based culture medium (Ribeiro, 1978). The existence of oospores was noted between the 15th and 30th days after inoculation.

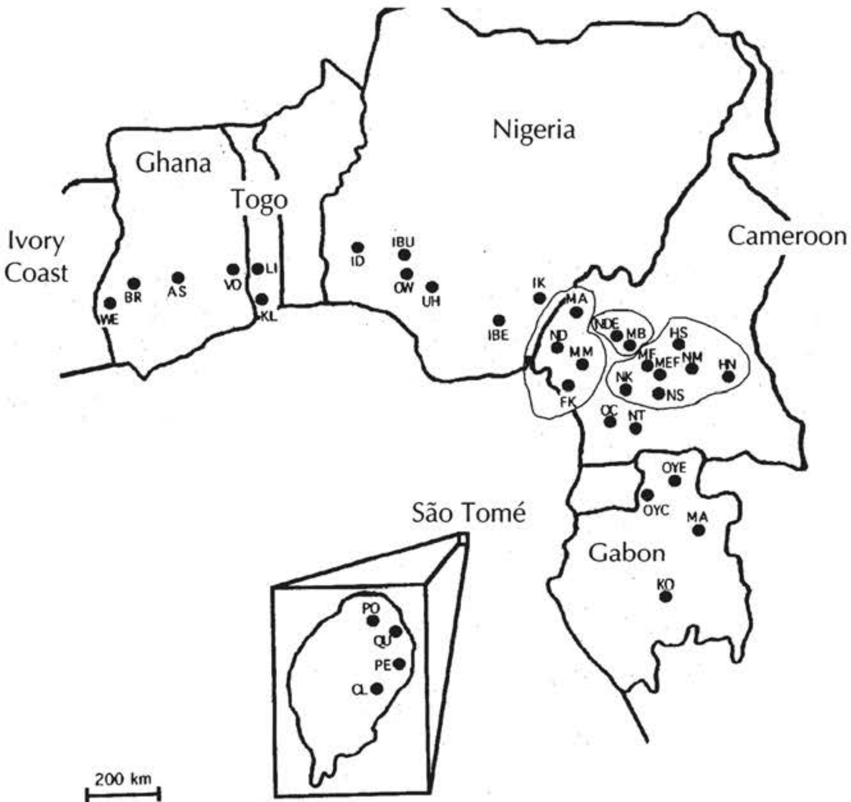

Figure 4. Distribution of sampled P. megakarya *strains.*

The analyses: isozymes and RAPD, material and methods

The different methods used to produce mycelium, and the isozyme and RAPD analysis techniques, have been described (Nyassé *et al.*, 1999). For each allozyme, the configurations observed were considered as different modalities of the same genetic descriptor, since it was impossible to identify the loci and alleles

for several of them. For RAPD, we considered that each band represented a locus. Each polymorphic band was given a score of 1 for presence and 0 for absence. Similarity indexes were calculated between all the possible pairs of isolates by simple matching. The calculations were carried out with Numerical Taxonomy System (NTSYS) software (Rohlf, 1993). The correspondence between the similarity matrix of the isozymes and that for RAPD was examined by the Mantel test (1967). Significance levels were determined from 500 permutations. The distances between genotypes were summarized using two statistical techniques. Firstly, factorial correspondence analyses (FCA) were carried out with Addad software (Escofier and Pagès, 1988). Secondly, classifications were performed with NTSYS, using the simple matching index and the unweighted pair-group method using arithmetic averages (UPGMA). The reliability limits of the nodes produced by the dendrograms were evaluated using the robust bootstrap technique with Winboot software (Yap and Nelson, 1996). A measurement of genetic diversity in each region was given by:

$G=1 / \sum p_i^2$

where p_i is the frequency of genotype i in the region considered (Stoddart and Taylor, 1988). For a given region, G is equal to 1 when all the isolates have the same genotype and is equal to 1/N when all the isolates are represented by a single genotype. The maximum possible diversity percentage (G/N) is used to compare regions with different sample sizes (McDonald *et al.*, 1994; Drenth *et al.*, 1996).

Genetic diversity from isozyme analysis

Thirteen enzyme systems were studied on 161 strains. Ten of them gave between two and four different configurations (ICD: 2, MDH1: 3, MDH2: 4, G6PDH: 3, MPI: 2, HK: 3, FUM: 3, ADA: 2, PEP(L-T): 3, PEP(G-L):3) and three were monomorphic (figure 5). These analyses therefore gave a total of 28 polymorphic descriptors, enabling the isolates to be classed into 36 different phenotypes. The number of isolates with the same phenotype varied from 1 to 38. Three phenotypes, I22, I31 and I13, were very frequent, since they alone accounted for around 50% of the isolates. Only 18 isolates had unique phenotypes. Nineteen different phenotypes were detected in Cameroon, five in Gabon, four in São Tomé, nine in Nigeria, two in Ghana and one in Togo. There were two phenotypes common to São Tomé and Cameroon and one to Nigeria, Ghana and Togo. The factorial correspondence analysis identified groups (figure 6).

– Two main groups stood out along the first axis, explaining 29% of total variability. This first group comprised phenotypes corresponding to the West African isolates (Togo, Ghana, Nigeria) and the second group comprised the isolates from Central Africa (São Tomé, Gabon, Cameroon). One isolate, I34, had an intermediate phenotype between the two previous groups. This isolate had an enzyme configuration typical of Central Africa for three isozymes, PEP(L-T)-1, PEP(G-L)-1 and MDH2-2, but it was the only Central African isolate displaying

the two enzyme configurations ADA-2 and HK-3, which are very frequent in West Africa. This isolate came from the Manyu region of Cameroon near the Nigerian border.

– Axis 2 explained 12% of the variability and separated the West African isolates into two groups, with two intermediate isolates. One group comprised isolates collected at Ibule in Nigeria, the other comprised genotypes scattered throughout West Africa.

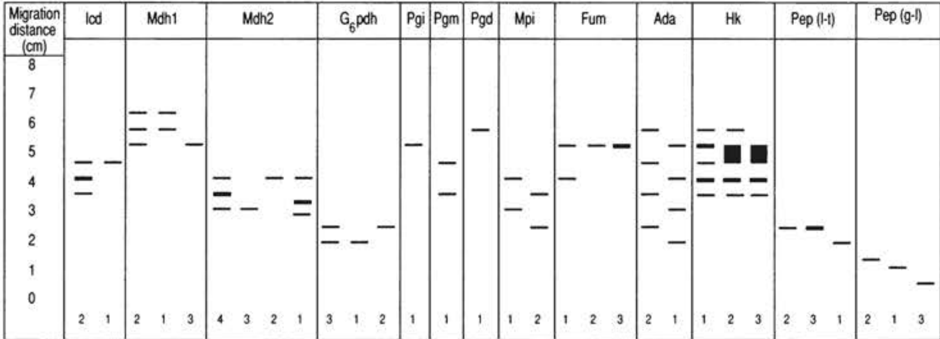

Figure 5. Description of the observed patterns for the 13 isozymes in P. megakarya.

The analysis of the UPGMA classification provided a complementary point of view. It also distinguished between the same two geographical groups indicated by the first FCA axis (figure 7). Intermediate isolate I34 is attached to the West African group. The bootstrap values associated with the nodes corresponding to the two groups were 43% and 46% for the West African and Central African groups respectively. These values became 83% and 76% when intermediate genotype I34 was removed from the analysis. No structure was detected within each group.

Genetic diversity from RAPD analysis

Nine RAPD primers revealed 33 reliable polymorphic bands among the 161 isolates. The size of the amplified products ranged from 0.5 to 3.0 KB. The isolates were divided into 44 RAPD genotypes. The number of isolates displaying the same RAPD genotype varied from 1 to 50. Two genotypes were very frequent: R1 corresponding to 49 isolates from West Africa and one isolate from western Cameroon, and R17 corresponding to 27 isolates from Cameroon. For the remaining genotypes, the number of isolates per genotype was less than nine. Twenty-eight different RAPD genotypes were identified in Cameroon, six in São Tomé, four in Gabon, eight in Nigeria, two in Ghana and two in Togo. One genotype was common to Nigeria, Togo and Ghana, another was common to Nigeria and Togo, and one was common to Nigeria and Ghana.

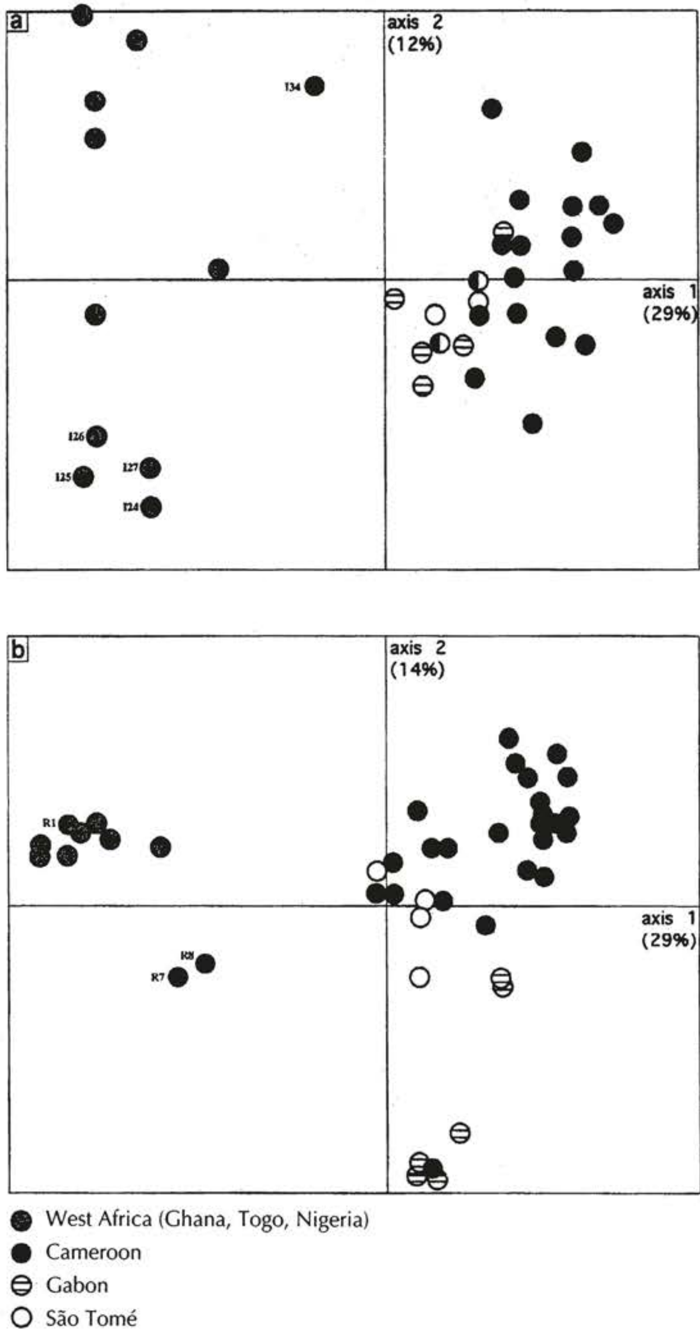

Figure 6. Plots of the first two axes generated by the Factorial Correspondence Analysis conducted on the isozymes (a) and RAPD (b) genotype data.

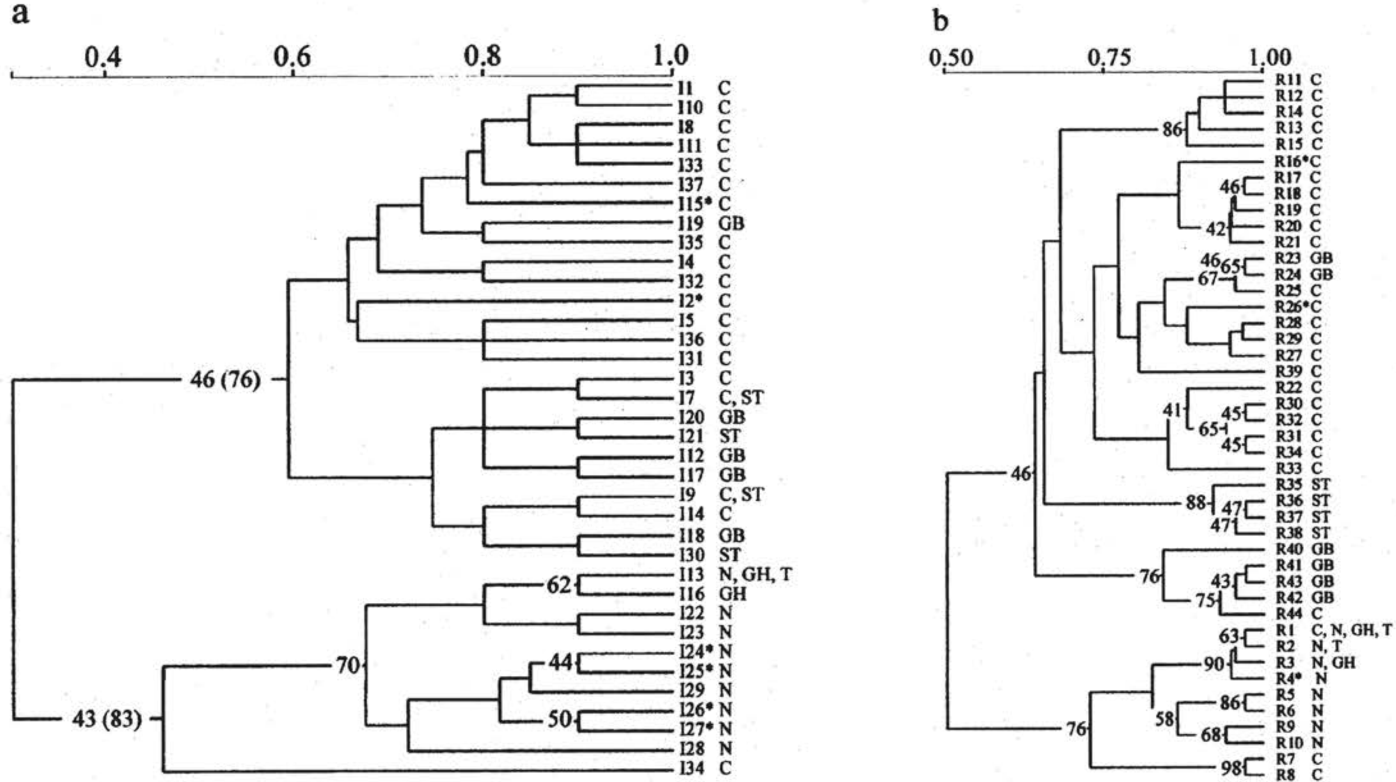

Figure 7. UPGMA dendrograms performed with the isozymes (a) and RAPD (b) genotype data. Bootstrap node values are given when they exceed 40%. The countries where genotypes have been detected are indicated as follows: C: Cameroon, GB: Gabon, GH: Ghana, N: Nigeria, ST: São Tomé, T: Togo. Genotypes including isolates of the A2 mating type are identified with an asterisk. On the isozyme dendrogram, values in brackets are obtained when genotype I34 is removed from the analysis.

Axis 1 of the factorial correspondence analysis, which explained 29% of total variability, separated the set of isolates into two groups. The first group was heterogeneous and contained 34 genotypes corresponding to the isolates collected in Central Africa (São Tomé, Gabon, Cameroon). The second group comprised eight genotypes corresponding to all the isolates collected in West Africa (Togo, Ghana, Nigeria), containing an isolate of genotype R1, sampled in the Manyu region of western Cameroon. This group also contained two genotypes, R7 and R8, corresponding to the isolates collected at Fako in Cameroon, near the Nigerian border. These two genotypes were closely linked to the West African genotypes, but had some specificities. In particular, they displayed four specific bands of the Central African genotypes, which were absent from the eight West African genotypes. A few genotypes of the first group were divided into sub-groups along axes 2, 3 and 4.

Axis 2 explained 14% of the variability. It made it possible to distinguish an initial sub-group containing four genotypes from Gabon (R40, R41, R42, R43) and one from Ocean in southern Cameroon (R44).

Axis 3 explained 12% of the variability and made it possible to distinguish a second sub-group containing four genotypes from São Tomé (R35, R36, R37, R38).

Axis 4 explained 11% of the variability and brought out a third sub-group containing four genotypes from Mbam (R11, R12, R13, R14), a sampling zone located in Central Cameroon.

The UPGMA analysis indicated two main groups corresponding to those distinguished by axis 1 of the FCA (figure 7). The bootstrap values associated with the nodes of the West African and Central African isolates were 76% and 46% respectively. The Central African group was therefore more heterogeneous, and sub-groups revealed by axes 2, 3 and 4 corresponded to the genotypes from Gabon and southern Cameroon, and also to those from São Tomé and Mbam.

Correspondence between the isozyme and RAPD analyses

The correspondence between the similarity matrices obtained for isozymes and RAPD on all the isolates was estimated by the Mantel test. The normal Mantel statistic Z was $r = 0.82$ with $p = 0.004$ (calculated from more than 500 permutations), indicating good general agreement between the two datasets. The correspondence between the isozyme and RAPD analyses is shown in diagram 5. The total number of genotypes determined simultaneously on the isozymes and RAPD amounted to 63, of which 47 corresponded to the central African isolates and 16 to the West African isolates. Nevertheless, four genotypes did not respect this geographical classification. It involved five isolates collected at Manyu and Fako in western Cameroon, near the

Nigerian border. In fact, they had intermediate configurations between the two previously mentioned groups. These isolates (NS259, NS269, NS270, NS267 and NS268) had intermediate characteristics for the isozymes and the RAPD.

For the isolates from São Tomé, the structuring found with RAPD analysis was different from that found with isozyme analysis; this situation revealed a problem with isozyme analysis power, as they were less numerous. In fact, with isozymes, the isolates from São Tomé were identical or closely linked to those from Cameroon, while with RAPD these isolates were clearly differentiated from all the others. In addition, the isolates from Gabon were separated into two groups based on both types of markers (figure 7). The first group contained two isolates from Oyem in eastern Gabon, while the second contained all the other isolates collected in Gabon. However, these two groups remained closely linked to the isolates from southern Cameroon: Ocean and Ntem.

Geographical distribution of genotypic diversity

Calculation of the genetic diversity indexes G and G/N, based simultaneously on isozymes and RAPD, showed that genetic variability was lower in West Africa than in Central Africa (table 2). In West Africa, variability distribution was heterogeneous; it was very low in Ghana, Togo, at Idi-Ayunre (western Nigeria) and at Ikom, Ibeku and Uhonmora (eastern Nigeria). Two isozymes associated with genotype RAPD R1 were strongly dominant in these two regions (I13, R1) and (I22, R1). At Ibule and Owena, in central eastern Nigeria, genetic variability appeared to be much greater, though few isolates were sampled. In Central Africa, diversity was high in the three countries studied: Cameroon, São Tomé and Gabon (table 2). In Cameroon, where the largest number of isolates was collected, variability depended on the region considered. Three geographical groups were formed: region 1 corresponding to the western mountainous region, region 2 to the east of the Bamiléké plateau and region 3 to the east of the River Sanaga. Genetic diversity was much greater in regions 1 and 2 than that estimated in region 3. In region 3, two genotypes were very frequent: (I31, R17) and (I1, R17). Greater variability could be detected by studying the 23 places in which several isolates were collected from the same plot. In four of those places, two different genotypes were detected with isozymes (two cases in West Africa and two cases in Cameroon) and in 11 places, from two to three different genotypes were detected with RAPD (two cases in West Africa and nine cases in Cameroon).

Distribution of mating types

Of the 159 isolates for which the mating type was determined, 153 were type A1 and 6 were type A2: one in Mfoundi in Cameroon and five at Ibule in Nigeria (table 1). This confirms the results previously obtained by Maddison and Griffin (1981), indicating the predominance of mating type A1 in

Table 2. Genotypic diversity G and percentage of maximum possible diversity G/N of geographical samples of *P. megakarya* observed at different scales. Computation of G is based on the multilocus isozyme and RAPD genotypes.

Location	No. of isolates	*G*	(G/N) %
Central Africa	97	15.0	16
Cameroon	72	10.0	14
Region 1*	24	7.4	31
Region 2*	16	9.2	57
Region 3*	28	2.6	9
Gabon	11	5.3	48
São Tomé	14	6.1	44
West Africa	64	3.0	5
Nigeria	50	2.0	4
Ibeku	9	1.0	11
Ibule	6	3.0	50
Idi-Ayunre	15	1.3	9
Ikom	6	1.0	17
Owena	3	3.0	100
Uhonmora	10	1.2	12
Togo	4	1.6	40
Ghana	10	1.9	18

* see text.

P. megakarya. This observation argues in favour of a primarily asexual type of reproduction in this species. In Central Africa, mating type A2 of the Mfoundi isolate corresponded to genotype (I2, R26), which was not linked to any genotype of mating type 1, be it with isozymes or RAPD (figures 6 and 7). This was also true for the reference strain (184), which was used to identify the mating types and which was of genotype (I15, R16). In West Africa, Ibule was the only place where mating type A2 was detected. At that sampling site, five out of six isolates had that type, which was therefore dominant. The five A2 isolates had the same RAPD genotype (R4), but different isozymes (I24, I25, I26 and I27). Of them, only two isozymes were polymorphic, ICD and MPI, with two band configurations for each of them. The four genotypes therefore corresponded to the two possible pairs of combinations. The isolate of mating type A1 was of genotype (I27, R5), it differed from genotype R4 through the existence/absence of two bands. These indexes indicated that sexual reproduction seems possible in the Ibule region.

Discussion

The intraspecific genetic variation of *P. megakarya* was studied with isozymes and RAPD, using 161 isolates collected from six African countries. This study led to the identification of two strongly differentiated genetic groups. These two groups were divided either side of a line roughly corresponding to the

border between Cameroon and Nigeria, with one group corresponding to isolates from West Africa and the other to isolates from Central Africa. This geographical structuring tallied with the results obtained by Oudemans and by Coffey (1991*a*) with isozymes, and those obtained by Förster *et al.* (1990) with mitochondrial DNA collected from isolate samples from Cameroon and Nigeria.

Five isolates collected near the contact zone displayed bands specific to each of the two groups for different isozyme of RAPD markers. This indicated possible exchanges between these two groups, through sexual reproduction or through heterokaryosis. This study also confirmed the overall dominance of mating type A1, already observed by Maddison and Griffin (1981), in the two genetic groups. The substantial disparity in frequency between the two mating types argued in favour of predominantly asexual reproduction. This was corroborated by the high frequency of a few genotypes in extensive geographical zones in both West Africa and Cameroon. Nevertheless, the other modes of reproduction cannot be ruled out locally:

– the existence of the two mating types at Ibule in Nigeria, and the enzymatic polymorphism revealed by isozymes indicate that sexual reproduction is possible in that zone,

– the intermediate genotypes, originating from the border between Cameroon and Nigeria, might be the result of hybridizations or of heterokaryosis,

– in the Volta region of eastern Ghana, selfing could have occurred. The two isolates collected in that region had the genotype I16. It only differs from I13, the only other genotype found in Togo and Ghana, through a single allozyme, ICD, which has simple genetic determinism with one locus and two alleles. I16 is homozygous (configuration ICD-1) and might result from selfing of I13, which is heterozygous (configuration ICD-2).

The overall genetic diversity found in West Africa (Nigeria, Togo, Ghana) is low. Few genotypes have been detected in Ghana and Togo, either with isozymes or RAPD. Moreover, all the genotypes from those two countries also existed in Nigeria, apart from one (I16). *P. megakarya* seems to be spreading westwards on the continent. The species was reported for the first time in 1982 in Togo (Djiekpor *et al.*, 1982) then in 1985 in Ghana (Dakwa, 1988; Luterbacher and Akrofi, 1994). It is likely that this extension is primarily due to vegetative propagation of a small number of individuals. *P. megakarya* was recently detected in Ivory Coast, a serious threat for that country, the world's leading cocoa producer.

In Central Africa, overall genetic diversity is greater than in West Africa. No clear structuring was revealed with isozymes, and the genotypes from São Tomé and Gabon appeared to be very similar to those from Cameroon. With RAPD, geographical structuring of the isolates clearly appeared. The difference in structuring found between isozymes and RAPD, might be due to a difference in the number

of loci explored (around 10 with isozymes and 33 with RAPD) or to a difference in the evolution rates of the sequences targeted by these two methods.

The division of *P. megakarya* into two highly differentiated groups, corresponding to two geographical zones, can be explained by the so-called refuge zone theory. Although its most important host from an economic viewpoint, *Theobroma cacao*, comes from Latin America, *P. megakarya* is probably endemic in Africa. In fact, *T. cacao* is a Sterculiaceae and, notably in this study, it was shown that *P. megakarya* can also be isolated from fruits of other Sterculiaceae, such as *Cola nitida,* which comes from West Africa. Consequently, as there are numerous wild Sterculiaceae in African tropical forests, the transfer of *P. megakarya* from an indigenous species to cocoa seems to be a reasonable hypothesis.

In addition, during the Quaternary Period, glacial cycles induced an arid climate in the tropical zones, reducing the dense African forest to a series of refuges, in places where rainfall had remained high (Maley, 1996). This led to fragmentation in the distribution of indigenous species and their pathogens, involving isolation and differentiation of populations. Once milder conditions returned, the forest species spread out from their refuge zones. In the zone covered by this study, two main biogeographical domains resulting from these climate events can be distinguished based on the distribution of plant species (White, 1979; Berthaud, 1984) and animal species (Moreau, 1966). The boundary between these two domains is in eastern Nigeria, near the Cameroon border, and corresponds approximately to the geographical separation between the two *P. megakarya* groups detected in this study. The convergence seen in the structuring of diversity between different plant, animal and fungus taxons in Africa is a fresh argument in favour of ancient development of *P. megakarya* in this region.

Genetic diversity of the pathogen and host-pathogen interactions

Work on the genetic diversity of *Phytophthora* indicates that the species *P. megakarya* is currently the predominant species in Cameroon and Nigeria, since very few *P. palmivora* isolates have been detected in those countries. This suggests that the species *P. megakarya* could also become the predominant species in the other African countries, where it currently exists alongside *P. palmivora* (Togo, Ghana, Gabon, São Tomé and Ivory Coast). With the dissemination of *P. megakarya*, it can be feared that there will be an increase in rot damage in those countries, as currently seen in Ghana. It is also to be feared that this species will invade Ivory Coast, the world's leading cocoa producer, from the east of the country where the first *P. megakarya* have been reported.

Two distinct geographical groups have been identified, between which there has apparently been very little mixing, indicating limited isolate mobility between the two geographical regions. This is undoubtedly due to the geographical barrier of eastern Nigeria, to which can be added the natural division at the river Sanaga in

Cameroon. The latter barrier might explain the difference between the isolates from Bafia and those from sites located south of the river Sanaga.

Although *P. megakarya* multiplication seems to be primarily asexual, there are indications that other mechanisms involving recombination (sexual reproduction or mitotic recombination) are possible locally at Ibule in Nigeria and in western Cameroon. Genetic recombinations are potentially a threat, since they are a source of variability, notably for strain pathogenicity. This has been shown in *P. infestans* (Drenth *et al.*, 1994). This could also be the case for *P. megakarya*, as strain NS269, the most aggressive in the study, is genetically an intermediate strain between the two populations that might be derived from a recombination.

Use of the leaf test to measure the intraspecific aggressiveness of the pathogen should be considered, as results on leaves can be positively correlated with the level of attacks seen in the field. Pathogenicity tests carried out on leaves with a sample of 11 *P. megakarya* strains revealed a large variation in the degree of aggressiveness, but there did not seem to be any link between genetic diversity and the aggressiveness of the strains. This tallied with attack levels, which can also be high in all the countries affected by the disease.

Host-parasite interaction studies suggest that the relations between *P. megakarya* strains and cocoa clones are only slightly specific or not at all. This might be explained by the fact that contact between the parasite and its host is recent. The cocoa tree was introduced into Africa in 1857 and it was not until 1979 that *P. megakarya* was identified with certainty on cocoa (Brasier and Griffin, 1979). Another reason might be the fact that *P. megakarya* is a parasite that attacks several host plants. Often with polyphagous parasites, pathosystem specificity is less marked. Further work is required to acquire a clearer understanding of possible interactions between cocoa trees and strains of *Phytophthora* spp.

Conclusion and prospects

Studies on the genetic diversity of *P. megakarya* are continuing. Some of this work is being conducted in the CIRAD plant pathology laboratory in Montpellier, notably with a view to monitoring the evolution of pathogenic populations. The most recent results confirm those presented in this book. New strains have been studied and the most recent classification is proposed in figure 5.

The different results obtained by Nyassé (1997) have been confirmed and, in particular, the methodology has been fine-tuned:

– As regards the West African population, genotypes R1, R2 and R3 obtained by Nyassé can be grouped into a single genotype R1. Strain NS259 from the Cameroon-Nigeria border is therefore identical to those existing in Nigeria. Strain NS328 representing the five strains collected in 1999 in that border zone, is included in the West African strains. We shall call it R2. In that zone, it seems that the only genotypes that can be characterized are of the West African

type for RAPD. It would seem paramount to monitor this possible extension throughout western Cameroon.

– The other new strains characterized, which came from the other zones of Cameroon, are all of the Central African type for RAPD. Genotypes R13, R14 and R15 can be grouped into a single genotype R13. The same applies for genotypes R17, R18 and R20, which should be grouped in genotype R17. Genotypes R22, R31, R32 and R34 will be grouped in genotype R22. Genotype R31 will be attributed to genotype 2.

– The strains from Gabon are well grouped in a single group, with one strain from Cameroon, but isolated on the Gabon border.

– The strains from São Tomé are also placed in a single group.

Phytophthora capsici

Other *Phytophthora* species affect cocoa trees, causing fruit rot. In Brazil and India, there have been attacks by *P. citrophthora*, and *P. capsici* is seen as the dominant pathogen in Latin America and the Caribbean.

Although UPGMA classification of the isozyme patterns on five loci showed a genetic relation between *P. capsici* and *P. citrophthora* (common allele is C for Mdh-2, I for Idh-2 and F for Sod-2) (Oudemans and Coffey, 1991*a*; Oudemans *et al.*, 1994; Mchau and Coffey, 1995; Ortiz-Garcia, 1996), the analysis involving eight loci (Pgi, Mdh-1, Mdh-2, Idh-2, Sod-2, Me, Pgm and G_6pdh) separated the two species *P. capsici* and *P. citrophthora* into four groups, three of which were more closely linked (Ortiz-Garcia, 1996):

- *P. citrophthora* at a distance of 0.46 (strains from *Citrus* and rubber),
- *P. capsici* at a distance of 0.36, but which subdivided into three subgroups:
  - strains of *P. capsici* from market garden plants and strains of "typical group A" *P. capsici* strains from pepper and cocoa,
  - "typical group B" *P. capsici* from cocoa (allele Mdh-1 different from "typical group A" strains),
  - "atypical" *P. capsici,* with strains from pepper and cocoa.

Following this study, two initial certainties prove founded: firstly the parallel between typical gr. B *P. capsici* and atypical *P. capsici* in agreement with the work by Goodwin *et al.* (1990) and by Mchau and Coffey (1994*b*) showing that these morpho-taxonomic groups are linked more to each other than to *P. citrophthora* Smith & Smith (strains from Citrus*)*; secondly the division of *P. capsici* strains (from cocoa and pepper) into three closely linked genetic units belonging to the same species, *P. capsici*.

Other information will be acquired by using genetic parameters of the populations (Ortiz-Garcia, 1996).

The structuring of the isozyme group *P. capsici* typical group A was studied on seven geographical populations: *P. capsici* from market garden plants, 26 strains (Mediterranean countries and North America, including the southern USA and northern Mexico), and from pepper, 5 strains (Southeast Asia), *P. capsici* typical group A from cocoa, 42 strains (northern Amazonia, eastern Brazil, southern Mexico).

The allele frequencies showed that the typical group A *P. capsici* population from eastern Brazil could be distinguished from all the other populations through the predominance of alleles A for Pgi and H for Me. However, the *P. capsici* populations from the Mediterranean countries, North America and Southeast Asia had in common a homozygous GG pattern, and allele G was not found with the typical group A *P. capsici* populations. The allele differentiation test and the Rogers distances consequently led to a division into three genetic groups defined by:

– a link between *P. capsici* strains from the Mediterranean countries and North America, though stronger than with *P. capsici* strains from Southeast Asia,

– individualization of typical group A *P. capsici* strains from cocoa in eastern Brazil,

– grouping of typical group A *P. capsici* strains from cocoa in northern Amazonia and southern Mexico.

The within-population genetic parameters applied to three *P. capsici* populations from eastern Brazil (Bahia), typical group A (10 strains), typical group B (14 strains) and atypical (16 strains), showed that the typical group B *P. capsici* population had the highest percentage of polymorphic loci and greater genetic and genotypic diversity. These last two parameters differed little from those of the atypical *P. capsici* population. The allele differentiation test between populations, which was highly significant for five out of six loci in each pair of populations (allele A for Pgi only being present with the typical group A *P. capsici* population), the Rogers distances and the coefficient of genetic divergence (both high), confirmed that the three populations are distinct.

The structuring studied on 173 typical group A *P. capsici* strains from southern Mexico, originating from four geographical sites, coastal Tabasco (17 strains), central Tabasco (93 strains), western Tabasco (23 strains) and northern Chiapas/southern Tabasco (40 strains), revealed very low genetic diversities (under 10% on 8 loci within each of the populations).

The third certainty, given by the study of between and within-population structuring, on both a world and Latin American level, is the diversification of *P. capsici* strains into three large groups:

– *P. capsici* from temperate regions (strains from market garden crops) or tropical zones, Southeast Asia (strains from pepper), Trinidad, Costa Rica (strains from cocoa) and typical group A *P. capsici* from northern Amazonia and southern Mexico (strains from cocoa),

– typical group A *P. capsici* from eastern Brazil (strains from cocoa),

– typical group B *P. capsici* and atypical *P. capsici* (strains from cocoa).

To conclude, all these genetically linked groups and subgroups would seem to have a common ancestor from Amazonia and, for the majority of them, the greater between-population diversity and existence of unusual alleles such as A for Pgi are found in eastern Brazil. However, outside Latin America, these taxonomic groups are dispersed: *P. capsici* in Southeast Asia on pepper, in Central America and the Caribbean on cocoa, and in temperate regions on plants such as tomato and capsicum, though the origin is Latin America; typical *P. capsici* and atypical PMF4 on pepper and on cocoa in West Africa and Southeast Asia.

The first numerous trading exchanges between continents, which led to major mixing of plants, and also of their parasites, might explain this worldwide dispersion. New constraints, be they edapho-climatic or plant-related, might well have favoured the adaptation and evolution of individuals capable of finding a "return trip" to their zones of origin (Ortiz-Garcia, 1996). A detailed analysis of allozymes can rapidly provide an evaluation of the genetic diversity of a species (Forster and Coffey, 1991), since over and above the species level, isolates displaying common electrophoretypes segregate into sub-groups assimilable in distinct populations, which often cannot be differentiated through their morphological traits alone. Like *P. palmivora* / *P. arecae* (Oudemans and Coffey, 1991*b*, 1991*c*; Blaha *et al.*, 1994; Mchau and Coffey, 1994*a*) and *P. megakarya* (Nyassé, 1999), *P. capsici* would seem to comprise populations derived from the same phylum but having acquired a specific evolution, through isolation due to particular events. The risks of introduction, which are sources of notorious cocoa crop losses in some countries, such as Brazil, call for constant surveillance and strict quarantine for other producing countries yet to be affected by *Phytophthora capsici sensu* Tsao.

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