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DNA polymorphism among *Fusarium oxysporum* f.sp. *elaeidis* populations from oil palm, using a repeated and dispersed sequence "Palm"

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Abstract A worldwide collection, of 76 *F. oxysporum* f.sp. *elaeidis* isolates (Foe), and of 21 *F. oxysporum* isolates from the soil of several palm grove was analysed by RFLP. As a probe, we used a random DNA fragment (probe 46) from a genomic library of a Foe isolate. This probe contains two different types of sequence, one being repeated and dispersed in the genome "Palm", the other being a single-copy sequence. All *F. oxysporum* isolates from the palm-grove soils were non-pathogenic to oil palm. They all had a simple restriction pattern with one band homologous to the single-copy sequence of probe 46. All Foe isolates were pathogenic to oil palm and they all had complex patterns due to hybridization with "Palm". This repetitive sequence reveals that Foe isolates are distinct from the other *F. oxysporum* palm-grove soils isolates. The sequence can reliably discriminate pathogenic from non-pathogenic oil palm isolates. Based on DNA fingerprint similarities, Foe populations were divided into ten groups consisting of isolates with the same geographic origin. Isolates from Brazil and Ecuador were an exception to that rule as they had the same restriction pattern as a few isolates from the Ivory Coast, suggesting they may originated from Africa.

Key words *F. oxysporum* f.sp. *elaeidis* · Repetitive sequence · Fingerprint · Oil palm

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Introduction

Fusarium oxysporum is an asexual phytopathogenic fungus. Within this species, host-specific isolates are clustered into *formae speciales* (Booth 1971). This species also includes non-pathogenic isolates able to colonize plant roots and grow in soils as saprophytes (Toussoun 1975; Gordon and Okamoto 1990, 1992). The identification of *F. oxysporum* at the species level is based on morphological characters, but classification into *formae speciales* requires pathogenicity tests which are time consuming. Recently, several *Fusarium* species, including *F. oxysporum* have been analysed using molecular markers such as mitochondrial, ribosomal or repetitive sequences (Manicom et al. 1987, 1990, 1993; Kistler et al. 1991; Kim et al. 1993; Mes et al. 1994), or vegetative compatibility groups (VCGs) (Aloi and Baayen 1993; Katan et al. 1994).

F. oxysporum f.sp. *elaeidis* (Foe) causes the most serious wilt disease of oil palm in many West African countries (Renard et al. 1972). It was first discovered in 1946 at Zaïre (Wardlaw 1946) and is now widespread throughout several African countries (Ivory Coast, Benin, Ghana, Nigeria, Cameroon, Sao-Tome). It recently spread to Brazil (Van de Lande 1983) and Ecuador (1986: Renard, personal communication). On the other hand, this disease has never been reported from south-east Asian countries such as Malaysia which is now the world's leading exporter of oil palm. Identification of this *formae speciales* requires laborious pathogenicity tests for 6–8 months. Therefore, a simplier method is necessary to identify suspected Foe isolates.

Recently, Flood et al. (1992) showed that, based on genomic DNA RFLPs; Foe isolates from Zaïre and Brazil consisted of two distinct populations. Dossa et al. (1991) reported five VCGs corresponding to isolates with geographic origins in West Africa (Benin, Cameroon, Ghana, Ivory Coast, Zaïre).

Such diversity prompted us to survey Foe world populations with molecular markers. We collected a large sample of Foe isolates representative of countries where vas-

cular wilt is endemic (Benin, Brazil, Cameroon, Ecuador, Ghana, Ivory Coast, Nigeria, Sao-Tome, Zaïre). This sample was analysed by fingerprinting with a Foe repeated and dispersed sequence. In order to find a Foe-specific marker, samples of *F. oxysporum* isolated from the soil of distinct palm groves (Cameroun, Ivory Coast, Nigeria, Ecuador, Indonesia and Malaysia) were also investigated with the same probe. These RFLP analyses were combined with pathogenicity tests.

Materials and methods

RFLP analysis

Fungal isolates. The worldwide sample analysed was composed of 76 isolates of *F. oxysporum* collected from diseased oil palm, and of 21 *F. oxysporum* isolates from palm-grove soil, both infected and non-infected (Table 1).

DNA isolation. Approximately 10^6 spores/ml suspensions were inoculated in Roux flasks containing 100 ml of potato dextrose broth supplemented with 0.1% (w/v) yeast extract and 0.1% (w/v) casein hydrolysate. After 48 h growth at 24°C, mycelium was harvested, frozen with liquid nitrogen, lyophilized for 24 h and stored at –20°C. DNA was then extracted according to the CTAB method (Manicom et al. 1987).

Construction of a Foe genomic library. Approximatively 50 µg of genomic DNA from Foe isolate no.16, was digested with *Eco*RI. The resulting fragments were size-fractionated by agarose electrophoresis TAE (40 mM-Tris/acetate, 1 mM-EDTA, pH 8) at 2 V/cm for 18 h. Fragments of 1–3 kb, were electroeluted, ligated into *Eco*RI-digested de-phosphorylated pUC19 (Messing 1983), and used to transform cells of *Escherichia coli* $DH_5\alpha$. Plasmid DNA containing *F. oxysporum* inserts were prepared by the alkaline-lysis protocol of Maniatis et al. (1982).

Southern blots and hybridization. Approximately 6 µg of total genomic *Fusarium* DNA was digested overnight with 40 units of *Eco*RI. The resulting fragments were separated on 0.7% agarose gels at 25 V for 20 h and transferred to nylon membranes (Hybond N, Amersham UK) using a vacuum blotting system (Pharmacia). *F. oxysporum* DNA inserts were amplified by PCR with universal and reverse primers (Bioprobe) and purified with spun columns (Pharmacia). Amplification reactions were performed in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 8.3), with 100 µM each of dATP, dCTP, dGTP and dTTP (Boehringer), 200 nM of primer, 2 units of *Taq* DNA polymerase (Bioprobe) and 30 ng of plasmid DNA, in a final volume of 100 µl. The reaction was performed for 30 cycles in a Braun Thermal Cycler: 1 min at 95°C, 1 min at 55°C and 1 min at 72°C. Amplified DNA fragments were labelled with P^{32} by random priming (Pharmacia kit) and hybridized overnight at 65°C. Three 15-min washes in 2 × SSC, 0.1% SDS and two 15-min washes in 0.2 × SSC, 0.1% SDS at 65°C were performed.

Data analysis. RFLPs were detected by hybridization of probe 46 to *Eco*RI genomic blots. Similarities between DNA fingerprints were calculated using Nei and Li's index (1979): $S_{xy} = 2n_{xy}/(n_x + n_y)$, where n_{xy} is the number of shared fragments, and n_x and n_y are the number of fragments in isolates x and y. Fingerprint groups were defined as group of isolates with patterns having at least 80% similarity.

Pathogenicity test

Plant material. Oil palm seeds, previously warmed for 75 days at 39°C to induce germination, were supplied by I.D.E.F.O.R, Abidjan (Ivory Coast). A total of 945 seeds from cross L431T × L407D were grown in plastic bags containing vermiculite and maintained in a heated greenhouse at 22–30°C, 60–90% relative humidity, until the 2-leaf stage (about 2 months), when they were inoculated with isolates of *F. oxysporum*.

Inoculation. For inoculation, fungal isolates were grown on 100 ml of liquid medium (3 g glucose, 0.2 g Na/NO_3, 0.14 g KH_2/PO_4, 0.075 g Mg/SO_4, 0.001 g Fe $SO_4/7H_2O$ pH 7.5) for 5 days at 25°C. The fungal mats were disrupted, filtered, and adjusted to 8×10^6 spores/ml. The roots of young oil palms were soaked for a few minutes in spore suspension and planted in pots with sand and compost (3/pot). The non-inoculated controls were treated with distilled water. The inoculated plants were maintained for 8 weeks under conditions similar to those described above.

Disease assessment. The inoculated oil palm seedlings were organized in five randomized blocks. In each block, we inoculated nine oil palm seedlings for each isolate. External symptoms, and for some diseased oil palm, internal symptoms, were observed. Disease progress was assessed by counting diseased and dead oil palms once a week for 2 months.

Isolation of F. oxysporum from inoculated oil palm. For each isolate tested, we dissected the bulb of an inoculated oil palm seedling under sterile conditions. Bulb fragments were plated onto PDA gelified medium. Isolates originating from such bulb fragments were characterized for RFLPs using probe 46.

Results

RFLP analysis

In order to find a repetitive sequence, 12 clones, obtained from the genomic library of Foe isolate no. 16, were hybridized separately on Southern blots of *F.oxysporum* f.sp. *elaeidis*. One clone, corresponding to a 2.3-kb *Eco*RI fragment (probe 46), revealed numerous DNA polymorphisms among *F. oxysporum* isolates from diseased oil palms or from palm-grove soils. Hybridization of probe 1 (3′ fragment of probe 46, Fig. 1) to *Eco*RI-digested DNA of Foe isolate no. 16 revealed only one band (Fig. 2, lane 7). As with the complete probe 46, hybridization of probe 2 (5′ fragment of probe 46, Fig. 1) with Foe isolate no. 16 revealed, a multiple banding pattern (Fig. 3, lane 7).

These results show that probe 46 is composed of two different types of sequence, a 5′ repeated and dispersed sequence (named "Palm") and a 3′ unique sequence. Our collection of 76 Foe isolates and 21 *F. oxysporum* isolates from palm-grove soils were examined for RFLPs using probe 46. We detected two distinct patterns:

First, all *F. oxysporum* isolates from palm-grove soils, and one isolate from a diseased oil palm (no. 248), had simple patterns with only one band of different sizes (2–8 kb) corresponding to the 3′ unique sequence of probe 46. Such a pattern shows that these isolates lack sequences homologous to the 5′ repetitive sequence of probe 46 (Fig. 4).

Second, all Foe isolates from diseased oil palms showed a multiple banding pattern (8–29 bands) corresponding to the repetitive sequence of probe 46 (Figs. 3, 5).

To study the genetic diversity of Foe world populations, the patterns of isolates were scored for similarity based on

Table 1 Origin, haplotype and fingerprint of *F. oxysporum* isolates

Isolates	Location	Source	Origin	Fingerprint/ haplotype[a]	Isolates	Location	Source	Origin	Fingerprint/ haplotype[a]
14	Ecuador: Quininde	a	DO	A2	273	Benin	b	DO	B11
254	Ecuador: Quininde	a	DO	A2	274	Benin	b	DO	B11
255	Ecuador: Quininde	a	DO	A2	275	Benin	b	DO	B11
								DO	
6	Brazil: Denpasa	a	DO	A5	152	Ghana: Pretsea	a	DO	H17
19	Brazil: Denpasa	a	DO	A3	153	Ghana: Kusi	a	DO	H17
175	Brazil: Denpasa	a	DO	A3	154	Ghana: Kusi	a	DO	I18
176	Brazil: Denpasa	a	DO	A3	155	Ghana: Kwae	a	DO	H17
177	Brazil: Denpasa	a	DO	A4	162	Ghana: Kusi	a	DO	H17
178	Brazil: Denpasa	a	DO	A4	163	Ghana	a	DO	J19
179	Brazil: Denpasa	a	DO	A4					
180	Brazil: Denpasa	a	DO	A3	263	Nigeria: Aback	c	DO	F15
181	Brazil: Denpasa	a	DO	A3	264	Nigeria: Aback	c	DO	F15
182	Brazil: Denpasa	a	DO	A3	268	Nigeria: Aback	c	DO	C12
183	Brazil: Denpasa	a	DO	A3	270	Nigeria: Aback	c	DO	F15
					271	Nigeria: Aback	c	DO	F15
16	Ivory Coast: Attingue	a	DO	A1					
71	Ivory Coast: Dabou	a	DO	A6	265	Cameroon: Ndian	c	DO	E14
169	Ivory Coast: Yocoboue	a	DO	A1	266	Cameroon: Ndian	c	DO	E14
170	Ivory Coast: Dabou	a	DO	A3	269	Cameroon: Ndian	c	DO	C12
172	Ivory Coast: Yocoboue	a	DO	A9	272	Unknown origin	c	DO	F15
173	Ivory Coast	a	DO	A7					
174	Ivory Coast: La Mé	a	DO	A3	4	Zaïre: Binga	a	DO	D13
191	Ivory Coast: Yocoboue	a	DO	A8	241	Zaïre	a	DO	D13
192	Ivory Coast: Yocoboue	a	DO	A8					
193	Ivory Coast: La Mé	a	DO	A1	209	Sao-Tome	a	DO	G16
195	Ivory Coast: Dabou	a	DO	A1	210	Sao-Tome	a	DO	G16
196	Ivory Coast: Tieviessou	a	DO	A2	211	Sao-Tome	a	DO	G16
197	Ivory Coast: Ehania	a	DO	A10	212	Sao-Tome	a	DO	G16
198	Ivory Coast: Ehania	a	DO	A10					
199	Ivory Coast: Ehania	a	DO	A10	242	Ivory Coast: Dabou	a	S	
200	Ivory Coast: Ehania	a	DO	A10	246	Ivory Coast: Dabou	a	S	
201	Ivory Coast: Ehania	a	DO	A2	171	Ivory Coast: Dabou	a	S	
202	Ivory Coast: Ehania	a	DO	A2	267	Nigeria	c	S	
203	Ivory Coast: Ehania	a	DO	A2	214	Ivory Coast	a	S	
204	Ivory Coast: Tieviessou	a	DO	A9	215	Ivory Coast	a	S	
205	Ivory Coast: Anguededou	a	DO	A7	216	Ivory Coast	a	S	
206	Ivory Coast: Attingue	a	DO	A1	S317	Ivory Coast	d	S	
213	Ivory Coast: Dabou	a	DO	A1	S310	Ivory Coast	d	S	
243	Ivory Coast: Dabou	a	DO	A9	S519	Ivory Coast	d	S	
244	Ivory Coast: Dabou	a	DO	A9	S514	Ivory Coast	d	S	
245	Ivory Coast: Dabou	a	DO	A9	S201	Ivory Coast	d	S	
247	Ivory Coast: Dabou	a	DO	A3	S610	Ivory Coast	d	S	
249	Ivory Coast: Dabou	a	DO	A7	S135	Malaysia	d	S	
250	Ivory Coast: Dabou	a	DO	A7	S1318	Malaysia	d	S	
251	Ivory Coast: Dabou	a	DO	A1	S133	Malaysia	d	S	
252	Ivory Coast: Dabou	a	DO	A1	S1417	Indonesia	d	S	
253	Ivory Coast: Dabou	a	DO	A1	S1411	Indonesia	d	S	
308	Ivory Coast: Boubo	a	DO	A6	S1718	Ecuador	d	S	
309	Ivory Coast: Boubo	a	DO	A6	S169	Ecuador	d	S	
310	Ivory Coast: Boubo	a	DO	A6	S102	Cameroon	d	S	
311	Ivory Coast: Boubo	a	DO	A6					
312	Ivory Coast: Boubo	a	DO	A6					
248	Ivory Coast: Dabou	a	DO						

[a] The distinct haplotypes are shematized in Fig. 5
DO = Diseased oil palm
S = Soil of palm grove
a CIRAD/CP: Avenue du Val de Montferrand BP 5035,34032 Montpellier Cedex 01
b ORSTOM: Avenue du Val de Montferrand BP 5045, 34032 Montpellier Cedex 01
c Plant Pathology Division, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria
d INRA: 17 rue Sully BV 1540, 21034 Dijon Cedex

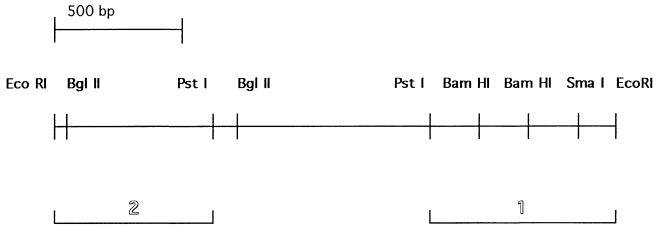

Fig. 1 Partial restriction map and position of probes 1 and 2 from clone 46

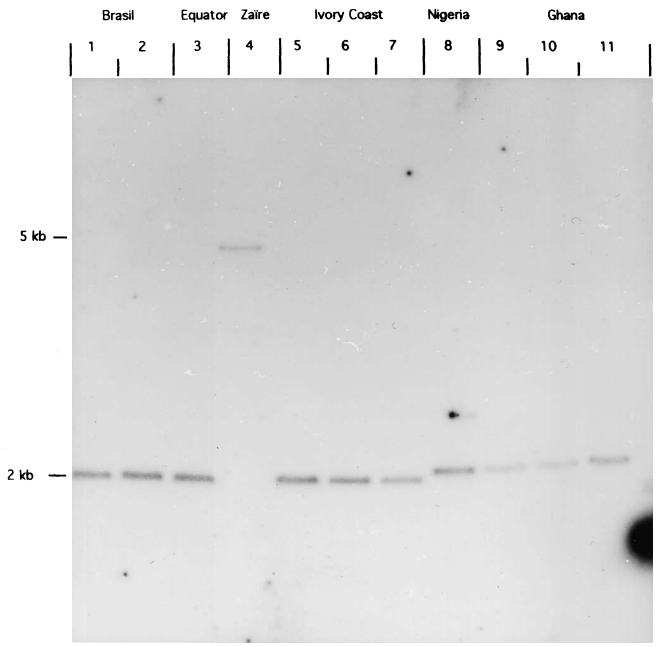

Fig. 2 Southern blot of *F. oxysporum* f.sp. *elaeidis* DNA digested with *Eco*RI and probed with probe 1 (3′ fragment of probe 46). The size of DNA fragments is indicated on the left in kilobases (kb). * Countries of origin: isolates 6 (*lane 1*), 19 (*lane 2*), 14 (*lane 3*), 4 (*lane 4*), 71 (*lane 5*), 170 (*lane 6*), 16 (*lane 7*), 268 (*lane 8*), 152 (*lane 9*), 155 (*lane 10*), and 162 (*lane 11*)

the presence or absence of DNA fragments ranging in size from 0.5 to 7 kb. Similarities between DNA fingerprints for all pairwise comparisons are shown in Table 2. Isolates with more than 80% of shared fragments were classified into the same fingerprint group, whereas isolates with less than 60% of shared fragments were considered as separate groups. All isolates could be classified into ten fingerprint groups (A through J). Most of these groups corresponded to isolates from the same country. For example, isolates from Benin, from Zaïre, from Cameroun, from Nigeria, and from Sao-Tome belong respectively to groups B, D, E, F and G. In each of these groups, the isolates have the same haplotype (see Table 1).

The isolates from Ivory Coast and from Ghana reveal much more polymorphism: the isolates from Ivory Coast all belong to group A but eight distinct haplotypes were observed (with 80–97% of similarity); the isolates from Ghana belong to three distinct groups, H, I, and J. For some

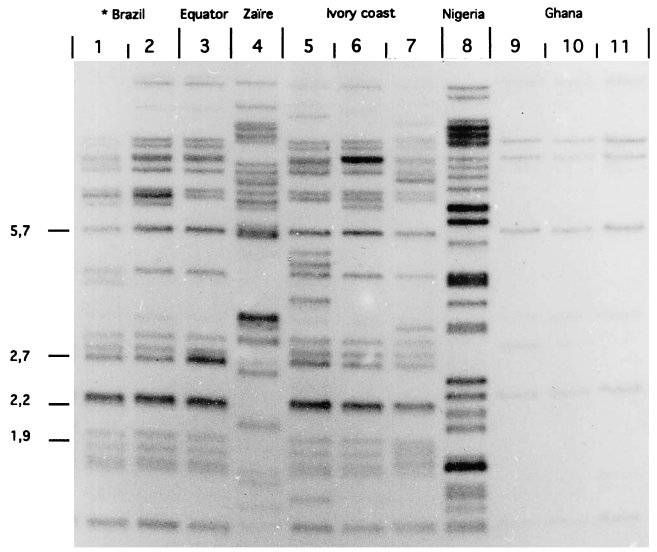

Fig. 3 Southern blot of *F. oxysporum* f.sp. *elaeidis* DNA digested with *Eco*RI and probed with clone 46. The size of DNA fragments is indicated on the left in kilobases (kb). * Countries of origin: isolates 6 (*lane 1*), 19 (*lane 2*), 14 (*lane 3*), 4 (*lane 4*), 71 (*lane 5*). 170 (*lane 6*), 16 (*lane 7*), 268 (*lane 8*), 152 (*lane 9*), 155 (*lane 10*), and 162 (*lane 11*)

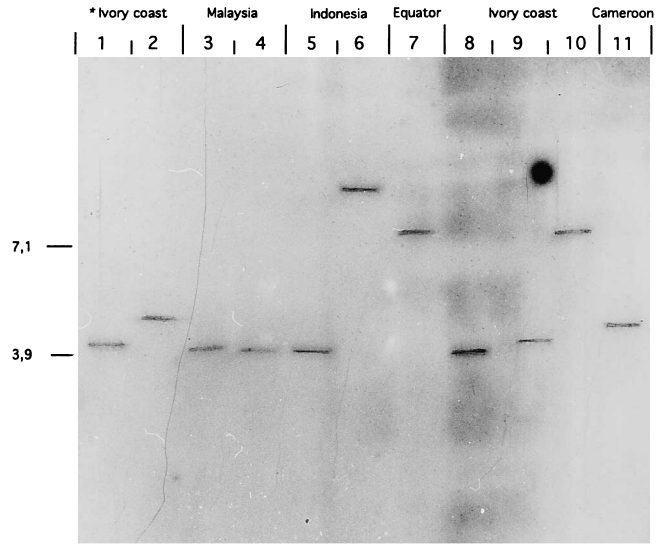

Fig. 4 Southern blot of *F. oxysporum* from soil; DNA digested with *Eco*RI and probed with clone 46. The size of DNA fragments is indicated on the left in kilobases (kb). * Countries of origin: isolates: S317 (*lane 1*), S310 (*lane 2*), S1318 (*lane 3*), S135 (*lane 4*), S1417 (*lane 5*), S1411 (*lane 6*), S1718 (*lane 7*), S519 (*lane 8*), S514 (*lane 9*), S201 (*lane 10*), and S102 (*lane 11*)

isolates, however, the groups do not correspond exactly to geographic origin. For example, isolate 269 (Cameroon) has exactly the same restriction pattern as isolate 268 (Nigeria) which formed group C. All the isolates from South America (Brazil and Ecuador) belong to the same group as the isolates from Ivory Coast (group A): four distinct haplotypes (A2, A3, A4, A5) are observed, some of these hap-

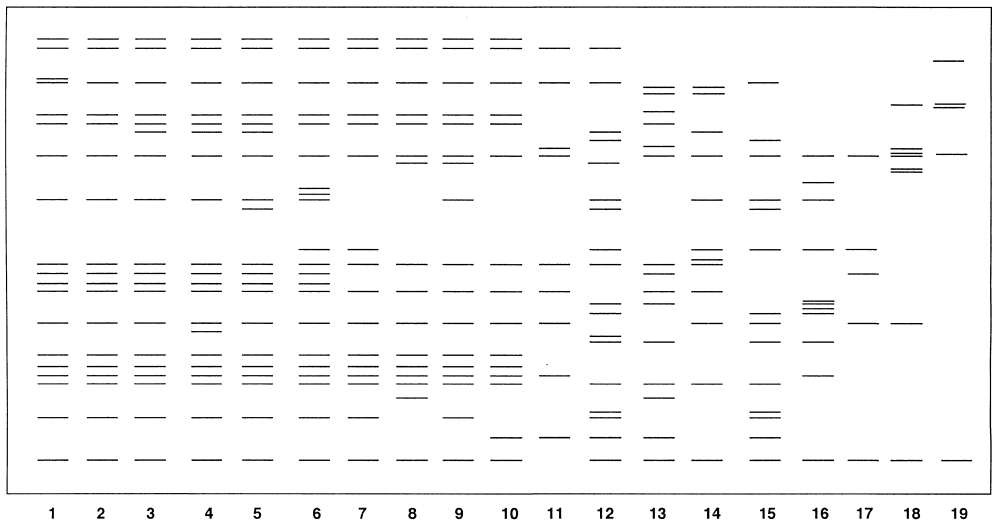

Fig. 5 The distinct haplotypes are schematised in this diagram: haplotypes *1 to 10* contain isolates from Ivory Coast, Brazil and Ecuador; haplotype *11*, isolates from Benin; haplotype *12*, isolates 268 and 269 respectively from Nigeria and Cameroon; haplotype *13*, isolates from Zaire; haplotype *14*, isolates from Cameroon; haplotype *15*, isolates from Nigeria; haplotype *16*, isolates from Sao-Tome; haplotypes *17 to 19*, isolates from Ghana

Table 2 Percentage similarity[a] of 46 DNA fingerprint and group assignments among isolates of Foe

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1		97	95	92	92	90	86	80	89	82	50	38	39	44	41	27	35	10	15
2			97	95	95	92	88	82	91	85	52	39	40	45	42	28	36	10	16
3				92	92	90	86	80	89	82	50	43	39	44	41	27	35	10	15
4					95	88	83	80	86	80	48	37	38	42	40	26	33	10	15
5						88	83	80	86	80	48	37	38	48	40	26	33	10	15
6							81	80	84	80	47	41	37	47	44	31	32	10	15
7								88	90	90	56	41	36	55	45	30	40	10	17
8									91	90	56	41	36	48	32	22	40	10	17
9										88	54	40	47	50	44	21	38	10	17
10											58	36	48	43	40	23	42	10	18
11												22	31	36	33	20	31	10	12
12													34	45	73	41	9	15	8
13														53	31	29	26	10	8
14															50	33	35	10	20
15																46	32	10	15
16																	27	10	11
17																		40	36
18																			15
46 fingerprint group[b]	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B	C	D	E	F	G	H	I	J

[a] Percentage similarity of 46 DNA fingerprints was calculated using Nei and Li's formula (1979). Bold numbers indicate >80% similarity. Isolates with <60% shared fragment were designated as distinct groups; isolates within a group with minor differences, <20%, were considered as haplotypes

[b] Letter indicates fingerprint group and numbers indicate haplotype within a group

lotypes (A2, A3) are present in some isolates from the Ivory Coast.

Pathogenicity test

Within each fingerprint group, we choose at least one representative isolate to be tested for pathogenicity. Except for Brazil and Ecuador, isolates from all geographic origins have been analysed.

Fifteen Foe isolates from diseased oil palm (206, 205, 248, 274, 152, 153, 154, 155, 162, 163, 264, 265, 269, 4, 209) and four *F. oxysporum* from palm-grove soils (246, 267, S133, S169) were assayed for pathogenicity to oil palm (Table 3).

The four *F. oxysporum* isolated from soil and one isolate (no. 248) from the Ivory Coast which was obtained from diseased oil palm did not cause any symptoms of disease. These isolates are considered to be non-pathogenic. The 14 other isolates from diseased oil palm produce symptoms in 20–60% of inoculated oil palms. In order to compare the restriction patterns of each isolate tested, before and after inoculation, we decided to re-isolate each isolate tested from the bulb of the inoculated plant (healthy or diseased). All pathogenic isolates were re-isolated from diseased palms. The re-isolated isolates proved to be identi-

Table 3 Pathogenicity test: the number and % of diseased oil palm per isolate is indicated. An isolate was considered a non-pathogen when the % of diseased oil palm was zero

Isolates	Origin	Healthy	Diseased	Dead	% Of diseased oil palm	Total
F. oxysporum isolated from diseased oil palm						
205	Ivory Coast	30	8	3	20	41
206	Ivory Coast	0	11	11	50	22
248	Ivory Coast	36	0	2	0	38
274	Benin	0	13	11	54	24
152	Ghana	1	22	15	58	38
153	Ghana	19	16	3	50	38
154	Ghana	8	21	10	54	39
155	Ghana	3	17	16	47	36
162	Ghana	4	9	11	38	24
163	Ghana	5	17	10	53	32
265	Cameroon	29	10	1	25	40
269	Cameroon	7	21	8	58	36
264	Nigeria	1	13	15	45	29
4	Zaire	1	17	14	53	32
209	Sao-Tome	9	18	12	46	39
Control		41	0	2	0	43
F. oxysporum isolated from soil						
soil 16 no. 9	Ecuador	34	0	2	0	36
soil 13 no. 3	Malaysia	32	0	3	0	35
246	Ivory Coast	36	0	4	0	40
267	Nigeria	32	0	4	0	36

cal to the originally inoculated isolates by a comparison of their probe-46 fingerprints. On the other hand, non-pathogenic isolates could be not re-isolated from inoculated plants which, except for isolate 267, showed no symptoms of disease.

Discussion

We used a random clone (probe 46) from a Foe genomic library and have shown that the 5′ and 3′ fragments of probe 46 correspond to a repeated and to a unique sequence respectively. Two repetitive sequences isolated from *F. oxysporum* have already been identified as transposable elements (Daboussi et al. 1992; Julien et al. 1992). We are currently studying the nature of the repetitive sequence of probe 46. We used probe 46 as a molecular marker to examine the genetic structure of Foe world populations. We analysed a large sample of isolates (76 *F. oxysporum* isolates obtained from oil palm and 21 *F. oxysporum* isolates from palm-grove soils), representative of countries where this vascular wilt is endemic.

Two kinds of restriction patterns were observed: a multiple restriction pattern in all Foe isolates corresponding to the repeated 5′ "Palm" sequence of probe 46 and a simple restriction pattern in all non-pathogenic isolates from palm-grove soils corresponding to the unique sequence of probe 46. These results showed that this repetitive dispersed sequence ("Palm") is specific for Foe. Therefore it is a very good marker to identify Foe isolates. The only exception to this rule, isolate 248 (which was obtained from diseased oil palm, and shows a restriction pattern similar to the non-pathogenic isolates), was shown to be non-pathogenic to oil palm. This result seems to indicate the presence of a non-pathogenic isolate in an oil palm which does not show any symptom of disease.

The repetitive sequence "Palm" was also used to fingerprint Foe isolates. Isolates were grouped according to the similarity of their DNA fingerprints (at least 80% similarity). Isolates within a fingerprint group were further divided into haplotypes based on minor differences in the DNA fingerprint (80–97% similarity). These groups generally correspond precisely to their geographic origin. This is the case for countries such as Sao-Tome, Benin, Zaïre, Ivory Coast (one group per country) and Ghana (three groups). It does not completely hold true for Nigeria and Cameroon, mainly composed of isolates from groups F and E respectively. We detected two isolates, one from each country, (Nigerian isolate 268 and Cameroon isolate 269), which have the same restriction pattern and belong to group C.

The polymorphism observed in Ivory Coast and in Ghana could be due to the method of sampling. In the case of the isolates from Ghana, two of them, 153 and 154, come from the same tree whereas they belong to two distinct groups H and I. This result seems to show that two distinct isolates can infect the same tree.

With regard to the diversity of African isolates, it was very surprising to find that South American isolates (Brazil and Ecuador) were very similar to some isolates from the Ivory Coast. This result indicates that these isolates have a common ancestor. Vascular wilt appeared recently in Brazil (Van de Lande 1983) and Ecuador (1986: Renard, personal communication). Ivory Coast is a producer and exporter of seeds and of pueraria (a leguminous plant used in palm groves to avoid erosion and as a supplier of nitrogenous substances). The similarity of restriction patterns observed could be explained by a recent introduction of an Ivory Coast isolate to Brazil or Ecuador. Several factors could be responsible for the transmission of *F. oxysporum* f.sp. *elaeidis* over such long distances, including humans, cover plants, or contamination by seeds and pollen (Flood et al. 1990). Fingerprinting is a very good tool to detect such introductions.

The observations of distinct groups in isolates of *F. oxysporum* f.sp. *elaeidis* complement previous studies. The study of DNA polymorphisms and VCGs (Flood et al. 1992) in Brazilian and Zaïre isolates revealed two distinct groups. Dossa et al. (1991) identified five VCGs among African Foe: one specific VCG for Ivory coast, Ghana, and Zaïre respectively. On the other hand, Benin isolates were in the same VCG as Ivory Coast isolates, as were Brazilian and Ecuadorian isolates (Dossa, personal communication). Our results are in agreement with these data and complement previous experiments obtained using different markers and techniques: the sample that we analysed were more representative than that of Flood et al. and, compared to VCGs it is evident that probe 46 can distinguish between Ivory Coast and Benin isolates. The Foe African isolates show the highest diversification implying that Africa is a diversity area for this fungus. This hypothesis would be in agreement with an African origin of the oil palm.

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