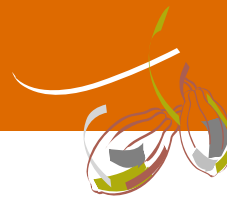


Characterization of *Phytophthora* species from the American continent and the Caribbean that are pathogenic on cocoa



C. Hérail, K. Vezian-Bonnemayre, M. Ducamp
CIRAD-CP, avenue Agropolis, TA 80/02,
34398 Montpellier Cedex 5, France

Contact: claude.herail@cirad.fr

Several species of *Phytophthora* cause cocoa (*Theobroma cacao* L.) black pod rot on the American continent. A study of ITS (Internal Transcribed Spacer) sequences, a region of ribosomal DNA, led to taxonomic separation of the species *P. palmivora*, *P. capsici cacao*, *P. tropicalis* and *P. citrophthora cacao*. In order to fine-tune this species differentiation study, American strains of *Phytophthora* present in the CIRAD collection in Montpellier were characterized for their genetic diversity, mating type and certain physiological characteristics.

Material and methods

After isolation from pods, the strains were purified and added to the CIRAD collection in Montpellier. In all, 384 strains were characterized for their ITS (Internal Transcribed Spacer) sequence, for their isozymes (10 systems studied) and RAPD (Random Amplified Polymorphism DNA) using a protocol developed by Nyassé. For RAPD, 9 primers were used and 45 markers were selected. The mating type of each strain was determined by *in vitro* exposure to compatible species available in the collection. The physiological characteristics of some strains were studied, notably mycelium development and sporulation ability (zoospores and chlamydospores).

Results

- For the species *P. palmivora* (table 1), a single cocoa genotype of the A2 mating type was present on the entire American continent and in the Caribbean—except in Jamaica, where the two strains studied were of the A1 mating type, and in Brazil, where strain 224.1 had different isozyme and RAPD genotypes. The mycelium growth rate was identical between 20°C and 30°C (figure 1) and zoospore production was optimum between 25 and 28°C (figure 2).
- The species *P. capsici cacao* (table 2) was dominant in Mexico; one of its genotypes was present in Trinidad. In Brazil and French Guiana, this species was represented by different genotypes from those in Mexico. The strains were of both mating types and were also compatible with *P. capsici* strains isolated from tomato and pimento. The mycelium also developed well between 20 and 30°C. Zoospore production was maximum at 28°C. It was equivalent to that of the *P. palmivora* strains at 28°C and 30°C, but less at 25°C.
- The species *P. tropicalis* was only characterized on cocoa in Brazil. The strains were of the two mating types, but were not compatible with *P. capsici* and *P. capsici cacao*.

- The species *P. citrophthora cacao* was mostly isolated from cocoa in Brazil, and one strain in Costa Rica. They were sterile with each other and with *P. citrophthora* (pathogen on Citrus) and *P. collocalasiae* (pathogen on taro), which were taxonomically the closest species.

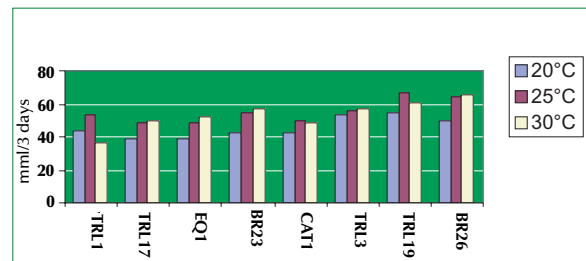


Figure 1. Mycelium growth rate for strains of *P. palmivora* (TRL1, TRL17, EQ1, BR23 and CAT1) and of *P. capsici cacao* (TRL3, TRL19 and BR26) that are pathogenic on cocoa.

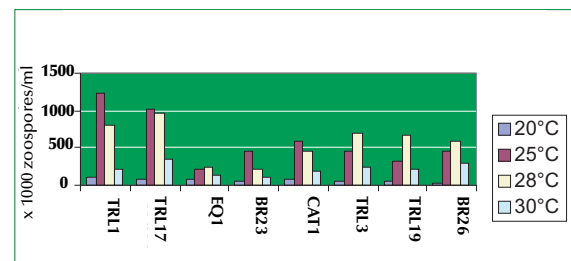


Figure 2. Sporulation ability of strains of *P. palmivora* (TRL1, TRL17, EQ1, BR23 and CAT1) and of *P. capsici cacao* (TRL3, TRL19 and BR26) that are pathogenic on cocoa.

Species*	Isozyme genotypes	RAPD genotypes	Mating type
<i>P. palmivora</i>			
Brazil (4)			
BR1 (3)	26	5	A2
224.1 (1)	17	1	A1
Jamaica (2)			
P881	26	6	A1
P80	20	6	A1
Colombia (6)			
Col. 1 (6)	26	5	A2
Costa Rica (19)			
CAT1 (19)	26	5	A2
Cuba (8)			
2C-65 (8)	26	2	A2
French Guiana (4)			
GLY 9432	26	5	A2
Guatemala (5)			
Guat.1	26	5	A2
Honduras (6)			
H1	26	5	A2
Trinidad (12)			
TRL1	26	5	A2
Dominican Rep. (24)			
Rd21	26	5	A2
Haiti (5)			
Hai 1	26	5	A2
Ecuador (2)			
EQ1	26	5	A2
Venezuela (1)			
VEN1	26	5	A2

* confirmation of the species by an ITS sequences. study (x) number of strains characterized.

Species*	Isozyme genotypes	RAPD genotypes	Mating type
<i>P. capsici cacao</i>			
Brazil (11)			
BR147 (9)	23	8	A1
BR124 (1)	26	9	A2
BR157 (1)	24	6	A1
Mexico (225)			
Mex 1 (125)	28	10	A1
MPA 21.1 (12)	28	10	A2
Mex 3.4 (65)	30	11	A1
Mco 9.2 (4)	30	11	A2
Mco 15.3 (14)	29	12	A1
M93115 (1)	29	12	A2
M93109 (1)	25	13	A2
M93100 (5)	27	14	A2
Trinidad (7)			
TRI 3 (7)	28	10	A1
French Guiana			
Guy 335 (1)	36	15	A1
<i>P. tropicalis</i>			
Brazil (10)			
BR23 (4)	13	5	A2
BR29 (1)	14	6	A1
BR132 (2)	11	5	A2
BR134 (1)	15	7	A1
<i>P. citrophthora cacao</i>			
Brazil (12)			
B7 (6)	a5	2	ST
B3 (2)	a8	1	ST
BR104 (3)	a6	3	ST
BR162 (1)	a7	1	ST
Costa Rica (1)			
Sca6.1 (1)	a9	4	ST

* confirmation of the species by a study of ITS sequences. (x) number of strains characterized.

Table 1. Isozyme, RAPD and mating type characterization of American strains of *Phytophthora palmivora* pathogenic on cocoa

Table 2. Isozyme, RAPD and mating type characterization of American strains of *Phytophthora capsici cacao*, *P. tropicalis* and *P. citrophthora cacao* pathogenic on cocoa.

Discussion and conclusion

- This work enabled the characterization of American strains of *Phytophthora* existing in the CIRAD collection in Montpellier that are pathogenic on cocoa.
- Only one *P. palmivora* genotype was characterized on cocoa for virtually all the zones studied, suggesting a single introduction of that pathogen from its zone of origin, Southeast Asia. Another introduction occurred in Brazil. The strains reproduced clonally (the A2 mating type only), except in Jamaica, where A1 strains were isolated, which may have come from coconut, for which the majority of strains are of the A1 mating type. Jamaica would therefore seem to be a priority for monitoring.
- P. capsici cacao* strains had a mostly clonal mating type, with strong sexual reproduction possibilities in Mexico. Production with oospore formation may enable this pathogen to survive for long periods under adverse conditions.
- This information on reproduction methods is important for more effectively establishing control methods against each species in the field.
- In Brazil, it will be important to clearly define the geographical distribution of the 4 species present, for which the epidemiological cycles are different, in order to improve control methods against each species.



Centre de coopération internationale en recherche agronomique pour le développement