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Short communication

New sources of resistance to *Phytophthora megakarya* identified in wild cocoa tree populations of French Guiana

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Abstract

Cocoa black pod rot, a disease caused by oomycetes of the genus *Phytophthora*, causes substantial yield losses throughout the world, particularly in Africa with the very aggressive species *Phytophthora megakarya*. In order to reduce the impact of that pathogen, priority is given to genetic control through more resistant cultivars, and breeders are seeking sources of resistance in wild cocoa trees. Wild cocoa trees were surveyed in French Guiana between 1985 and 1995, leading to the collection of abundant plant material from more than 200 mother trees originating from five river basins. We present here the results of tests to assess resistance to the species *P. megakarya* (a species only existing in Africa), conducted at CIRAD in Montpellier, France, on *circa* 40 genotypes collected in the Camopi river basin, along with approximately 20 genotypes from other populations (Kérindioutou, Borne 7, Euleupousing, Pina and Oyapok). The strain used for artificial inoculation was NS269, isolated in Cameroon. Seven cacao clones were classified as "highly resistant" and 29 as "resistant", some of which displayed greater resistance to *P. megakarya* than the reference resistant clone IMC 47. This study suggests that the wild material from French Guiana could play a significant role in controlling *P. megakarya* in Africa and also *Phytophthora palmivora* in all cocoa-producing zones.

Keywords: Cocoa; *Phytophthora megakarya*; French Guiana; Resistance

1. Introduction

Cocoa black pod rot, caused by oomycetes of the genus *Phytophthora*, causes substantial yield losses worldwide, particularly in Africa with the species *Phytophthora megakarya*, which is the most damaging species for the cocoa industry. Although *P. megakarya* only exists in Africa, the species *Phytophthora palmivora* and *Phytophthora capsici* are responsible for the disease in South America. The search for effective control methods was stepped up in 1988, when *P. megakarya* arrived in Ivory Coast, the world's leading cocoa-producing country. Genetic control is a promising solution, but resistant clones are particularly few in number (Nyassé et al., 1995; Thevenin et al., 2004) and breeders are seeking wild cocoa trees naturally displaying disease resistance or tolerance, by collecting from the zones of the species' origin (Amazonia and Guiana shield). The wild cocoa trees of southeastern French Guiana, collected between 1985 and 1995, have undergone various characterization studies: pod and bean morphology, organoleptic quality and agronomic evaluation, including the degree of resistance to diseases in the field or in laboratory or nursery tests (Lachenaud et al., 2007).

The merits of GU clones, which originate from the basins of the Camopi and Tanpok rivers (Lachenaud and Sallee, 1993), in terms of their resistance to *P. palmivora*, were already revealed in earlier work on a sample of the clones (Anon, 2004; Paulin et al, 2005; Lachenaud et al., 2005), whilst one of the clones was classified as "resistant" to *P. megakarya* (Nyassé et al., 1995). In order to confirm those results, a study was carried out at CIRAD in Montpellier to assess the degree of resistance to *P. megakarya* existing in clones originating from several natural populations of French Guiana, using a test

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in which leaves were inoculated with an aggressive strain from Cameroon (NS269). A genetic correlation exists between resistance on leaves and percentage of rotten pods in the field, and it is possible to use the clone value with leaf test to predict the average resistance of seedling progenies, obtained by crossing parental clones (Tahi et al., 2006). The ultimate goal of these studies was to transfer selected clones to Africa for their incorporation in cocoa genetic improvement programmes.

2. Material and methods

2.1. Plant material

The plant material was derived from wild mother trees collected in the basins of the Camopi, Tanpok and Kérindioutou rivers (Lachenaud and Sallee, 1993), and the Oyapok, Euleupousing and Yaloupi rivers (Lachenaud et al., 1997). The complete set included 59 clones belonging to 13 populations (Table 1): 10 clones from each of the populations Camopi-1, 7 and 9; 7 clones from the Euleupousing population; 6 clones from the Kérindioutou and Camopi 3 populations; 4 from the Borne-7 population; and 1 from each of the Camopi-6, Camopi-12, Oyapok, Pina, Tanpok and Yaloupi populations. Twenty-two GU clones (Camopi and Tanpok) maintained in the greenhouse in Montpellier after 1987 and 20 clones originating from the other rivers provided a random sampling (without selection before tests) of the set of wild Guianan germplasm. Seventeen GU clones (ortets) pre-selected in French Guiana for their excellent reaction to pod rot attacks in the field, after 10 years of observations (Table 1), were also added to those 42 clones, for confirmation purposes. Of the populations with large numbers of individuals in collections, only the Camopi-13 population was not represented in our sample. A clone selected from cultivars formerly cultivated locally, of unknown origin ("primitive" germplasm), GF23, (Lachenaud et al., 1997) was also tested.

The control references used per trial varied from three to five clones, including IMC47 (resistant) and EQX3360, ICS1, OC77, MXC67 and LF1 (susceptible). The degree of resistance in those clones was defined in earlier work (Thevenin et al., 2004).

The 59 Guianan clone grafts grown in a greenhouse in a controlled environment were tested in four successive comparative trials, depending on leaf availability (Table 1):

Trial 1: 23 clones, of which 18 were wild Guianan clones (15 pre-selected in the field) and 5 controls.

Trial 2: 17 clones, of which 12 were wild Guianan clones (2 pre-selected) and 5 controls.

Trial 3: 18 clones, of which 14 were wild Guianan clones (1 pre-selected) and 4 controls.

Trial 4: 30 clones, of which 27 were wild Guianan clones and 3 controls.

Table 1
List of Guianan clones studied in the four trials (in bold, clones pre-selected in the field in French Guiana)

Clone	Population	Trial 1	Trial 2	Trial 3	Trial 4
B7-A2	Borne 7			+	+
B7-B3	Borne 7		+	+	+
B7-B5	Borne 7			+	+
B7-B6	Borne 7		+		
ELP 16-A	Euleupousing		+		
ELP 20-A	Euleupousing		+		
ELP 22-A	Euleupousing		+		
ELP 28-A	Euleupousing			+	
ELP 2-B	Euleupousing			+	
ELP 37-A	Euleupousing			+	
ELP 40-B	Euleupousing			+	
GU 123-V	Tanpok				+
GU 125-C	Camopi 6		+		
GU 129-B	Camopi 7	+			
GU 134-A	Camopi 7	+			
GU 134-B	Camopi 7	+			
GU 134-C	Camopi 7	+			
GU 138-A	Camopi 7	+			
GU 139-A	Camopi 7	+	+		
GU 140-S	Camopi 7				+
GU 143-A	Camopi 7	+			
GU 143-B	Camopi 7	+			
GU 147-P	Camopi 7				+
GU 156-B	Camopi 1	+			
GU 175-V	Camopi 9				+
GU 183-G	Camopi 9				+
GU 185-G	Camopi 9				+
GU 195-V	Camopi 9				+
GU 213-V	Camopi 12				+
GU 226-V	Camopi 3				+
GU 230-B	Camopi 3	+			
GU 230-C	Camopi 3	+			
GU 233-P	Camopi 3				+
GU 237-V	Camopi 3				+
GU 241-V	Camopi 3				+
GU 254-A	Camopi 1	+	+	+	
GU 255-V	Camopi 1	+	+		+
GU 257-E	Camopi 1				+
GU 263-V	Camopi 1				+
GU 265-V	Camopi 1				+
GU 268-A	Camopi 1	+			
GU 269-V	Camopi 1				+
GU 285-A	Camopi 1			+	
GU 285-B	Camopi 1	+			
GU 301-A	Camopi 9	+			
GU 303-B	Camopi 9	+			
GU 307-V	Camopi 9	+	+		+
GU 312-V	Camopi 9				+
GU 329-V	Camopi 9				+
GU 353-V	Camopi 9				+
KER 11-1-A	Kérindioutou				+
KER 1-L	Kérindioutou		+		
KER 3	Kérindioutou				+
KER 6	Kérindioutou			+	
KER 8-R	Kérindioutou			+	
KER 9	Kérindioutou				+
OYA 2-B	Oyapok			+	
PINA	Pina		+	+	
YAL 3	Yaloupi			+	

2.2. Fungal material

Strain NS269 used for inoculation was isolated from a pod harvested in West Province, Cameroon (village of Fako). This strain is of mating type A1. It may have arisen from natural hybridization between two compatible strains, each derived from one of the two populations of the species *P. megakarya*: West Africa and central Africa (Ducamp et al., 2004). Its degree of aggressiveness is among the highest of the strains existing in the CIRAD collection in Montpellier (Thevenin et al., 2004).

2.3. Inoculation and symptom scoring

The degree of resistance of the clones was assessed by inoculating leaf discs with a suspension of *P. megakarya* zoospores (Nyassé et al., 1995). Four comparative trials were carried out with several replicates repeated in time, in accordance with the protocol described by Tahi et al. (2000): Five replicates for the first three trials and three replicates for the fourth. For each replicate, five leaves per clone were used. A total of 10 discs were inoculated per leaf, i.e. a total of 50 inoculated discs to assess the resistance of a clone per replicate, and 150 or 250 per trial.

Inoculation was carried out in the laboratory by depositing a 10 µl drop of suspension, with a zoospore concentration of 200,000 ml^{-1}, on the underside of each leaf disc. The inoculated discs, placed in trays, were incubated in the dark at 25 °C. Symptoms were scored 7 d after inoculation using the lesion scoring scale developed by Nyassé et al. (1995). Resistance levels were defined as follows: highly resistant (HR) ($0 < \text{score} \leqslant 1$), resistant (R) ($1 < \text{score} \leqslant 2$), moderately resistant (MR) ($2 < \text{score} \leqslant 2.5$), susceptible (S) ($2.5 < \text{score} \leqslant 3.5$), highly susceptible (HS) ($3.5 < \text{score} < 5$).

In order to compare the trials with each other, scores were adjusted by calculating a coefficient which was the ratio of the mean of control clones in one trial to the general mean of the control clones. An initial adjustment was calculated for trials 1, 2 and 3, using control clones IMC47, EQX3360 and GU 254-A. For adjustment to trial 4, a second coefficient was used, calculated from the means of IMC47.

2.4. Statistical analyses

Statistical analyses were carried out with XLStat software. Comparisons of the means per trial were made with the Bonferroni correction, at 5% probability.

3. Results

3.1. Statistical analysis of clone effects

As the four trials involved a choice of different clones, an analysis of variance and clone classification were performed individually for each trial.

Table 2
Analysis of variance parameters for clone effects

	Trial 1	Trial 2	Trial 3	Trial 4
Number of replicates	5	5	5	3
Mean	1.94	1.82	2.75	2.34
Score of resistant control (IMC47)	1.22	1.34	1.36	1.43
Number of GU clones R or TR	16	11	4	16
Total variance	2.96	5.59	3.83	3.05
df clone	22	16	17	29
F clone	15**	51**	31**	19**

**Significant at $P < 0.01$.

The analyses of variance revealed highly significant clone effects in each trial, and quite high levels of resistance, reflected in a fairly low average score per trial (Table 2).

After adjustment of the scores among the four trials, the clone classification shown in Table 3 reveals that many of the tested clones were classed as "resistant" (49%) or "highly resistant" (12%). Thirteen clones were classified as more resistant than IMC 47, the reference resistant clone, seven of which were classified as "highly resistant" (score < 1). It should be noted that each of those seven clones came from a different population (Tanpok, Kérindioutou, Euleupousing, Borne-7, Camopi-3, 6 and 9).

4. Discussion and conclusion

This study made it possible to determine the degree of resistance to *P. megakarya* existing in 59 Guianan clones belonging to 13 natural populations. The results show that 61% of the clones (36 clones) in this sample were classified as "resistant" or "highly resistant". The resistant nature of the control clone IMC 47 was confirmed, with a value of 1.27, and 13 Guianan clones had a lower value. Five clones were "moderately resistant", 15 "susceptible" (25%) and 3 "highly susceptible". Among the six populations represented by at least six clones, the four Camopi populations (Cam-1, 3, 7 and 9), with "resistant" or "highly resistant" percentages varying from 70% to 83%, seemed to be more resistant, overall, than the Euleupousing population (42%) and especially the Kérindioutou population (17%). For the Camopi-1 population, a single clone was judged to be susceptible (GU265-V), whereas all the others were resistant. For the Kérindioutou population, the reverse situation was found: a single clone was judged to be resistant (KER1-L), all the others were susceptible. Yet, microsatellite markers showed those populations, especially the Kérindioutou population, to be highly homogeneous (unpublished data). For their part, the "highly resistant" clones seemed to be well distributed because, in our sample, each of them belonged to a different population.

However, these figures need to be confirmed by a larger study, which is currently being launched in French Guiana with local strains of *P. palmivora* on 184 wild clones, the most resistant of which will then be tested in Montpellier

Table 3
General classification of the Guianan clones, with adjusted mean values (in bold, reference clones): $0<HR\leqslant 1< R\leqslant 2< MR\leqslant 2.5< S\leqslant 3.5< HS<5$

Clone	Adjusted mean	Degree of resistance
GU 123-V	0.16	HR
GU 226-V	0.59	HR
KER 1-L	0.60	HR
GU 195-V	0.67	HR
GU 125-C	0.71	HR
B7-B6	0.84	HR
ELP 20-A	0.89	HR
GU 269-V	1.03	R
ELP 16-A	1.06	R
GU 255-V	1.11	R
GU 241-V	1.13	R
ELP 22-A	1.18	R
B7-B5	1.19	R
IMC47	1.27	R
GU 301-A	1.34	R
GU 134-B	1.34	R
GU 263-V	1.35	R
GU 254-A	1.37	R
GU 285-B	1.43	R
B7-B3	1.43	R
GU 143-B	1.47	R
GU-353-V	1.50	R
GU 183-G	1.53	R
GU 134-C	1.55	R
GU 143-A	1.58	R
GU 156-B	1.60	R
GU 139-A	1.65	R
GU 285-A	1.76	R
GU 175-V	1.79	R
GU 268-A	1.80	R
GU 303-B	1.80	R
GU 230-B	1.88	R
GU 307-V	1.91	R
GU 147-P	1.94	R
GU 230-C	1.96	R
GU 140-S	1.99	R
GU 237-V	2.00	R
GU 257-E	2.08	MR
B7-A2	2.18	MR
GU 213-V	2.21	MR
GU 233-P	2.28	MR
GU 185-G	2.37	MR
KER 11.1-A	2.54	S
KER 6	2.57	S
PINA	2.62	S
GU 134-A	2.63	S
YAL 3	2.64	S
GU 129-B	2.66	S
GU 329-V	2.67	S
ELP 28-A	2.71	S
ELP 2-B	2.81	S
ELP 37-A	3.04	S
KER 3	3.08	S
OC77	3.10	S
KER 8-R	3.16	S
MXC67	3.16	S
GU 312-V	3.17	S
GU 138-A	3.26	S
GF23	3.28	S
ICS1	3.36	S
GU 265-V	3.40	S
EQX3360	3.56	HS
ELP 40-B	3.61	HS
KER 9	3.66	HS
OYA 2-B	3.73	HS

with *P. megakarya*. Out of the 17 ortets pre-selected in French Guiana, 14 (82%) proved to be "resistant" after leaf tests, whilst 3 were "susceptible". The local "primitive" clone, GF 23, of unknown origin, was classified as susceptible.

The wild Guianan clones of French Guiana are therefore a new and important source of resistance to *P. megakarya*, a species for which known and available resistant clones are particularly few in number (IMC 47, SNK 413, UPA 134; Nyassé et al., 1995; Thevenin et al., 2004). It is reasonable to think that they would make it possible to achieve significant genetic progress when used as parents in cocoa tree-breeding schemes for resistance to *P. megakarya* in Africa.

Moreover, a comparison of the results obtained with isolates NS269 (*P. megakarya*) and NS607 (*P. palmivora*) in the same leaf test trial demonstrated that a good correlation of the level of resistance to the two species exists (Paulin et al., 2005). The clones resistant to *P. megakarya* identified in our study should also be resistant to *P. palmivora*.

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