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The "Guiana" genetic group: A new source of resistance to cacao (*Theobroma cacao* L.) black pod rot caused by *Phytophthora capsici*

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ABSTRACT

Black pod rot, caused by Stramenopiles of the genus *Phytophthora*, leads to serious production losses in all cocoa growing zones. In order to reduce the impact of these pests, preference is given to genetic control using resistant varieties, and sources of resistance are actively being sought, particularly in wild cacao trees. Surveys were undertaken in the natural cacao tree populations of south-eastern French Guiana between 1985 and 1995 and an abundant amount of plant material belonging to a particular genetic group, the "Guiana" group, was collected. A great deal of work has shown the merits of this genetic group as a source of resistance to *Phytophthora palmivora* and *megakarya*. We describe here the results of a global study to assess the resistance of the 186 clones in the "Guiana" group "core collection" to a Guianese strain of *Phytophthora capsici* (strain Reg 2-6). This study, which used an efficient methodology (fifteen series of tests on leaf discs and a statistical test adapted to the ordinal nature of the basic data), showed that the "Guiana" genetic group is a major source of resistance to *P. capsici*. Strain Reg 2-6 proves to be particularly virulent, as the Scavina 6 control, an international reference for resistance to *Phytophthora*, is not resistant to it. However, 24 clones of the "Guiana" group are, and 92 have proved to be more resistant than Scavina 6, thereby showing the interest of the group in genetically controlling *P. capsici*.

Thus, of the clones in the Guiana group that are more resistant to *P. capsici* than Scavina 6, some, which are also resistant to *P. palmivora* and/or *Phytophthora megakarya*, and also displaying some other notable qualities, could be incorporated into cocoa genetic improvement programmes in countries where *P. capsici* is rife on cacao trees.

1. Introduction

Black pod rot, caused by several species of Stramenopiles of the genus *Phytophthora*, lead to serious damage in all cocoa growing zones. For sustainable, ecological and economical control, genetic control using resistant varieties is essential; breeders are therefore seeking sources of resistance, particularly in wild cacao trees, in the species' zones of origin. For instance, certain wild cacao trees of south-eastern French Guiana, belonging to the "Guiana" genetic group (Motamayor et al., 2008), collected between 1985 and 1995 (Lachenaud and Sallée, 1993; Lachenaud et al., 1997), have undergone early *Phytophthora palmivora* and *Phytophthora megakarya* resistance tests in various countries. For resistance to *P. palmivora*, after many one-off results (Anonyme, 2004; Paulin et al., 2005; Lachenaud et al., 2007; Paulin et al., 2007, 2010) regarding the merits of GU clones the Camopi and Tanpok river basins, the interest of the group as a whole has been shown (Thevenin et al., 2012). Likewise, for *P. megakarya*, which is only present in Africa, some tests carried out by CIRAD in Montpellier showed the exceptional merits of these cacao trees (Paulin et al., 2008, 2010). In tropical America, another species judged to be predominant (Lawrence et al., 1982; Ducamp et al., 2004) causes black pod rot, *Phytophthora capsici*.

This species has been acknowledged to consist of three close genetic groups, CAP1, 2 and 3 (Ducamp et al., 2004), but some authors have proposed that part of the CAP2 and 3 strains, derived from perennial plants, and especially the cacao tree, is a new species, *Phytophthora tropicalis* (Mchau and Coffey, 1995; Aragaki and Uchida, 2001; Donahoo and Lamour, 2008; Kroon et al. 2012). However, the situation concerning this new species seems still controversial (Bowers et al., 2007).

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As the species *P. capsici* exists in French Guiana, it was possible, and important, to test all the "Guiana" clones in the core collection at Paracou-Combi (Sinnamary, French Guiana) and to select some resistant clones there that can be used directly or as parents in breeding programmes.

2. Material and methods

2.1. Plant material

One hundred and eighty-nine clones were studied: 186 "Guiana" clones and three clones from other groups already used as controls in an earlier study (Thevenin et al. 2012). The "Guiana" clones came from wild mother-trees in the Oyapok, Camopi, Euleupousing, Yaloupi and Tanpok river basins surveyed and collected between 1987 and 1995 (Lachenaud and Sallée, 1993; Lachenaud et al., 1997). The 186 clones represented 17 demes of natural populations, plus one subspontaneous clone (Table 1). All the official authorizations required were obtained for the surveys, as mentioned in earlier work on the same genetic material (Thevenin et al. 2012).

The resistance control was the Scavina 6 clone (= SCA 6), the usual reference in tests on *P. palmivora* (Tahi et al., 2000; Lachenaud et al., 2001; Anonyme, 2001; Pokou et al., 2008; Akaza et al., 2009; Thevenin et al., 2012) and known to be resistant to *P. capsici* in Brazil (Lawrence et al., 1982; Luz et al., 1996). Clone T60/887, which is moderately resistant to *P. palmivora* (Tahi et al., 2006b) was included in the tests. Four clones from French Guiana were used as "susceptibility indicators" to check that the inoculation tests went ahead properly: 3 "Guiana" clones, ELP 40-B, OYA 2-B (highly susceptible to *P. megakarya*; Paulin et al., 2008) and GU 138-A (highly susceptible to *P. palmivora*; Paulin et al., 2010), along with a clone selected from cacao trees formerly grown in French Guiana, GF 24, susceptible to *P. palmivora* (Paulin et al. 2007). All these "susceptibility indicator" clones were confirmed to be susceptible to *P. palmivora* in an earlier study (Thevenin et al. 2012).

The 191 objects (i.e. 189 clones, of which 2 were replicated twice, Scavina 6 and ELP 40-B) all came from the same phenotyping platform, a plot kept under artificial shade (Thevenin et al. 2012) to ensure uniform environmental conditions essential for the tests (Tahi et al., 2007).

Table 1
Distribution by deme of the 186 "Guiana" clones studied (the "Camopi 0" clone is a subspontaneous clone of indeterminate deme but of local origin).

Deme	Nomenclature	Number	% Of total
Borne 7	B7	7	3.8
Camopi 1	GU	27	14.5
Camopi 2	GU	1	0.5
Camopi 3	GU	16	8.6
Camopi 6	GU	1	0.5
Camopi 7	GU	19	10.2
Camopi 8	GU	2	1.1
Camopi 9	GU	40	21.5
Camopi 10	GU	1	0.5
Camopi 12	GU	5	2.7
Camopi 13	GU	10	5.4
Euleupousing	ELP	25	13.4
Kérindioutou	KER	19	10.2
Oyapok	OYA	3	1.6
Pina	PINA	1	0.5
Tanpok	GU	3	1.6
Yaloupi	YAL	5	2.7
Camopi	GU	1	0.5
Total		186	100.0

2.2. Fungal material

The strain of *P. capsici* used, Reg 2-6, was isolated from a pod harvested in an old plot near Régina, in the remnants of some 18th century plantations. It was classed specifically by studying ITS sequences (Vasseur, 2010; Thevenin et al., 2012). The upkeep of the strains and maintenance of their pathogenicity were described by Thevenin et al. (2012).

In order to obtain the inoculum (formation of sporocysts and zoospores), the strains were grown at 24 °C on V8 1/5 + Beta sitosterol medium for 3 days in total darkness, then 7 days in the light. Zoospores were released after thermal shock (cold water + 20 min at 4 °C), then the suspension was calibrated with a Malassez cell. During preliminary tests in the study, the local strains of *P. capsici* proved to be clearly more aggressive than those of *P. palmivora*, involving a concentration of 100,000 zoospores/mL maximum rather than the 300,000 used for *P. palmivora*.

2.3. Experimental protocol

The leaf disc test (Nyassé et al. 1995; Tahi, 2003; Tahi et al. 2000, 2006a,b, 2007) was used, for its good correlation with black pod rot losses in the field. The performance of the clones was estimated by the appearance and area of necrotized patches on the underside of the leaf discs, after depositing 10 μL of a calibrated suspension of zoospores.

The inoculated discs were placed in trays and incubated in the dark at 25 °C. Symptoms were scored after 5 days of incubation, using the scale of Nyassé et al. (1995): Highly Resistant (HR: $0 < \text{score} \leq 1$), Resistant (R: $1 < \text{score} \leq 2$), Moderately Resistant (MR: $2 < \text{score} \leq 2.5$), Susceptible (S: $2.5 < \text{score} \leq 3.5$), Highly Susceptible (HS: $3.5 < \text{score} \leq 5$).

Fifteen series of tests were carried out between February 2011 and November 2013, covering all local seasonal variations, with 10 incubation trays per series, each comprising one leaf disc per. The numbers per series varied from 155 to 191 objects, with an average of 179.4, i.e. 94% of the objects. The series were therefore the equivalent of incomplete blocks.

Details about the sampling of the leaves used in the tests (stage and times) can be found in earlier work (Thevenin et al., 2012).

2.4. Statistical methods

The statistical methods used were the same as those used in similar work on *P. palmivora* (Thevenin et al., 2012). We modelled the link between the scores assigned to each disc by a generalized linear model (GLM, McCullagh and Nelder, 1989) using an ordinal probit link (Agresti, 2002). This model respected the ordinal qualitative nature of the scores. The significance of clone and tray effects was assessed by likelihood ratio tests ($pv = 0$ for each of the effects).

In order to assess clonal differences and construct homogeneity groups, we carried out paired likelihood ratio tests. For each pair of clones, we compared the likelihoods of the probit GLM, assuming successively that the 2 clones had some different and identical effects. Statistical processing was carried out with R software (R Development Core Team, 2011).

3. Results

The general mean after 15 series of tests was 2.69, corresponding to overall "Low Susceptibility". The "susceptibility indicator" clones confirmed their susceptibility to *P. capsici* with average scores of 2.96 for GU 138-A, 3.30 for OYA2-B, 3.33 for GF24 and 3.48 for ELP 40-B (Fig. 1). Scavina 6, with an average score of