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Sources of resistance to *Phytophthora* pod rot at the International Cocoa Genebank, Trinidad

A.D. Iwaro[1,*], D.R. Butler[1] and A.B. Eskes[2]
[1]*Cocoa Research Unit, The University of the West Indies, St. Augustine, Trinidad and Tobago, West Indies;*
[2]*Centre de Coopération Internationale en Recherche Agronomique pour le Dévelopement (CIRAD), France;*
**Author for correspondence (e-mail: iwaro@hotmail.com, diwaro@fans.uwi.tt; phone/fax: 1-868-662-8788)*

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Abstract

The International Cocoa Genebank, Trinidad (ICG,T) contains about 2000 cacao (*Theobroma cacao* L.) accessions. The purpose of the collection is to provide a source of genes for the genetic improvement of cacao through breeding. In fulfilling this objective, it is imperative that this collection be evaluated to identify major sources of genes for the genetic improvement of important agronomic–economic traits. In this study, 816 cacao accessions were evaluated for resistance to *Phytophthora* pod rot (black pod disease), which causes enormous loss of yield in cocoa production throughout the world.The study identifies promising resistant genotypes that could be exploited in cacao breeding programmes. It further compares the levels of resistance to *Phytophthora* pod rot among two cacao types (wild and cultivated types), three major groups (Forastero, Trinitario and Refractario) and 11 accession groups (B, AM, CL, ICS, IMC, JA, LP, MOQ, NA, PA and TRD) . The distribution of scores for the 816 accessions showed skewness towards the susceptible end of the disease rating scale, indicating that a large proportion (68.9%) of the sample was susceptible (disease rating 6–8) to *Phytophthora* pod rot. However, 12.9% of the sample population was found to be resistant (disease rating 1–3) and 18.2% moderately resistant (disease rating 4–5). Significant differences were observed between the wild and cultivated accessions and among the Forastero, Refractario and Trinitario groups. Marked differences were also observed among 11 accession groups, each of which was represented by at least 20 genotypes. Higher proportions of resistant (17.7%) and moderately resistant (22.6%) genotypes were observed in the wild accessions than in the cultivated varieties (9.4% resistant and 14.4% moderately resistant). The Forastero group, consisting of many wild accessions, was found to contain more resistant (18.0%) and moderately resistant (23.1%) genotypes than either the Trinitario (4.8% resistant and 13.6% moderately resistant) or Refractario (11.3% resistant and 15.4% moderately resistant). Among 11 accession groups, the largest percentage of resistant (24.2%) and moderately resistant (28.8%) genotypes were found in the PA (Forastero). Different accession groups had varying proportions of resistant and moderately resistant genotypes. This is not unexpected in an out-breeding crop such as cacao with a high level of heterogeneity. This finding reinforces the idea of a pre-breeding programme (germplasm enhancement) to accumulate resistance genes over several populations as a strategy for improving the genetic base of resistance in national cacao breeding programmes. One hundred and five promising resistant genotypes were identified among the 816 accessions evaluated. These accessions are potential sources of resistance genes for the development of high-yielding resistant varieties in cacao.

Abbreviations: CFC – Common Fund for Commodities; ICCO – International Cocoa Organisation; IPGRI – International Plant Genetic Resources Institute; ICGD – International Cocoa Germplasm Database; BP – Black pod disease

Introduction

Cacao (*Theobroma cacao* L., family Sterculiaceae) occurs in the wild in the Amazon basin and other tropical areas of South and Central America (Wood 1985). According to Wood (1985), the headwaters of the Amazon should be regarded as the centre of diversity, since great variation in morphological and physiological characters is found in this area. Although considerable genetic diversity exists in cacao, the current cultivated varieties tend to be based on a small portion of the available gene pool (Cope 1976; Wilde et al. 1992). This factor, as well as the possible loss of genetic diversity due to rapid deforestation in the Upper Amazon region of South America prompted the International Plant Genetic Resource Institute (IPGRI) to designate cacao as a priority crop for conservation and characterisation (IBPGR 1981). The International Cocoa Genebank, Trinidad (ICG,T) and the collection at the Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica (CATIE) are regarded as 'Universal Collection Depositories' (IBPGR 1981; Iwaro et al. 2003).

The ICG,T contains about 2000 genotypes and is managed by the Cocoa Research Unit (CRU), The University of the West Indies, St. Augustine, Trinidad and Tobago. The majority of accessions in the ICG,T include the Imperial College Selections (ICS) (1930–1934), Pound's 'Ecuador Refractario' collections (1937), Pound's Upper Amazon collections (1938, 1942), the Anglo-Colombian expedition collection (1952–1953), Chalmer's Ecuador Collections (1968–1969), John Allen's London Cocoa Trade Collection (1980–1986) and the CRU's local germplasm collection (1991) (Pound 1935; Chalmers 1972; Allen and Lass 1983; Kennedy and Mooleedhar 1993; Lockwood and End 1993). To increase the representation of Criollo germplasm, CRU has recently acquired relic Criollo populations from Belize (Mooleedhar et al. 1995), making the ICG,T one of the most diverse cacao germplasm collections world wide.

Efficient utilisation of the germplasm collection in ICG,T requires that this material be evaluated for traits of economic interest. One such important trait is resistance to *Phytophthora* pod rot [black pod disease (BP)] which is one of the most prevalent and destructive diseases of cacao (Iwaro et al. 1998). Global losses from BP are enormous and were estimated by Opeke and Gorenz (1974) at about 20–30% of annual cocoa production. However, losses may be as high as 90% at some locations depending on the susceptibility of the cultivated varieties and the prevailing environmental conditions (Adegbola 1981). This illustrates the extent of economic impact of this disease on cocoa production and consequently farmers' income. Although chemical control methods have been developed to reduce yield losses from BP, they are expensive and often beyond the reach of cacao farmers in developing countries (Tan and Tan 1990). The development of high yielding, resistant material is generally agreed to be a more effective and economic control method (Rocha 1974; Soria 1974; Iwaro et al. 2000a), but progress in this direction has been very slow, partly due to the narrow genetic base of most cacao breeding programmes. In order to provide cacao breeders with a wide array of resistance genes, this study was conducted to understand the pattern of variation among cacao genotypes in the ICG,T for resistance to BP. It also seeks to identify promising genotypes or groups of genotypes that could be exploited in breeding as sources of resistance genes to BP for the genetic improvement of cacao.

Materials and methods

Cacao germplasm

Eight hundred and sixteen genotypes, representing 51 accession groups in the ICG,T (Table 1), were evaluated for resistance to *Phytophthora* pod rot. As defined in ICGD, an accession group refers to a group of clones sharing the same group name

Table 1. Cacao accessions assessed in the study.

Accession code	Accession name	Country of origin	Population	Type
ACT [TTO]	Accession Trinidad	Trinidad	Trinitario	Cultivated
AM [POU]	Amalia	Ecuador	Various [R]	Cultivated
AMAZ [CHA]	Amazon	Ecuador	Forastero [U.A.F]	Wild
AX	Crosses in CRU after 1954	Trinidad	Various	Cultivated
B [POU]	Balao	Ecuador	Various [R]	Cultivated
C [TRI]	Crosses in CRU before 1954	Trinidad	Trinitario	Cultivated
CATONGO	Catongo	Brazil	Forastero [L.A.F]	Cultivated
CC	Cacao Centre	Costa Rica	Various	Cultivated
CL	Clementina	Ecuador	Various [R]	Cultivated
CLM	Clementina Mixed	Ecuador	Various [R]	Cultivated
COCA [CHA]	Coca	Ecuador	Forastero [U.A.F]	Wild
CRU [1–155]	Cocoa Research Unit	Peru or Ecuador	Forastero or Various [U.A.F]	Unknown
CRUZ	Cruzeiro do Sul Open Pollinated	Brazil	Forastero [L.A.F]	Cultivated
DE [TTO]	Double Embryo	Trinidad	Various	Cultivated
DOM	Dominica	Dominica	Trinitario	Cultivated
DOPOL	Double Pollination	Trinidad	Various	Cultivated
DR	Djatti Roenggo	Indonesia	Trinitario	Cultivated
E [ECU]	Ecuador	Ecuador	Various	Cultivated
EBC	Expedición Botanica Caqueta	Colombia	Forastero [U.A.F]	Wild
ECNR	Ecuador Cacao National Refractario	Ecuador	Various [R]	Cultivated
EET [ECU]	Estación Experimental Tropical	Ecuador	Various	Cultivated
GS	Grenada selection	Grenada	Trinitario	Cultivated
GU	French Guiana	French Guiana	Forastero [L.A.F]	Wild
ICS	Imperial College Selections	Trinidad	Trinitario	Cultivated
IMC	Iquitos Mixed Calabacillo	Peru	Forastero [U.A.F]	Wild
JA [POU]	Javilla	Ecuador	Various [R]	Cultivated
LCT EEN	London Cocoa Trade Est. Exp. Napo	Ecuador	Forastero [U.A.F]	Wild
LH	Las Hermanas	Trinidad	Trinitario	Cultivated
LP [POU]	La Paz	Ecuador	Various [R]	Cultivated
LV [POU]	Larga Vuelta	Ecuador	Various [R]	Cultivated
LX	Larga Vuelta X	Ecuador	Various [R]	Cultivated
LZ	Larga Vuelta Z	Ecuador	Various [R]	Cultivated
M [ICT]	Miscellaneous/Museum	Trinidad	Various	Cultivated
MAN [BRA]	Manaus	Brazil	Forastero [L.A.F]	Cultivated
MAR	Martinique	Martinique	Trinitario	Cultivated
MATINA	Matina	Costa Rica	Unknown	Cultivated
MO	Morona	Peru	Forastero [U.A.F]	Wild
MOCO	Mocorongo	Brazil	Forastero [L.A.F]	Unknown
MOQ	Moquique	Ecuador	Various [R]	Cultivated
NA	Nanay	Peru	Forastero [U.A.F]	Wild
POUND [POU]	Pound	Peru	Forastero [U.A.F]	Wild
PA [PER]	Parinari	Peru	Forastero [U.A.F]	Wild
PLAYA ALTA [VEN]	Playa Alta	Venezuela	Trinitario	Cultivated
RIM [MEX]	Rosario Izapa Mexico	Mexico	Trinitario	Cultivated
SC [COL]	Selección Colombiana	Colombia	Various	Cultivated
SCA	Scavina	Peru	Forastero [U.A.F]	Wild
SJ [POU]	San Juan	Ecuador	Various [R]	Cultivated
SLA	Santa Lucia A	Ecuador	Various [R]	Cultivated
SLC	Santa Lucia C	Ecuador	Various [R]	Cultivated
SPA [COL]	Selección Palmira	Colombia or Peru	Forastero [U.A.F]	Unknown
SPEC [1–54]	Specimen	Colombia	Forastero [U.A.F]	Wild
SPEC [≥55]	Specimen	Colombia	Unknown	Cultivated
TRD	Trinidad	Trinidad	Trinitario	Cultivated
UF	United Fruit Selections	Costa Rica	Trinitario	Cultivated
VEN B [ICT]	Venezuela B	Venezuela	Trinitario	Cultivated

U.A.F – Upper Amazon Forastero; L.A.F – Lower Amazon Forastero; R – Refractario.
Sources of information: Enriquez and Soria (1967), Bartley and Chalmers (1970), Lockwood and Gyamfi (1979), Kennedy (1985), Mooleedhar et al. (1991), Bekele and Bekele (1996), Wadsworth et al. (1997) and Bartley (2001).

(Wadsworth et al. 1997). The selected accessions are planted at the University Cocoa Research Station, Centeno, Trinidad at an altitude of 15 m above sea level. Shade is provided by trees of *Erythrina* sp. which are planted 6 m apart. The cacao trees are planted 1.8 m apart with up to 16 trees per plot for each accession. The soil type is Cunupia fine sandy clay with restricted internal drainage. Over a 30-year period from 1961, the mean annual rainfall in this region was 2,392 mm, and the average temperature 26 °C. The plants are irrigated as required during the dry season (January–June) each year.

Assessment of pathogen aggressiveness and isolate selection

Five species of *Phytophthora* (*P. palmivora*, *P. megakarya*, *P. capsici*, *P. citrophthora*, and *P. megasperma*) have been identified as causal agents of BP at different locations throughout the world (Brasier and Griffin 1979; Zentmyer 1988; Iwaro et al. 1998). Two of these species (*P. palmivora* and *P. capsici*) are present in Trinidad and Tobago. Isolates of *P. palmivora* and *P. capsici* were evaluated for their aggressiveness on detached pods, and significant differences were found in their reactions, with *P. palmivora* being the most aggressive (Iwaro et al. 1998). Furthermore, there was no interaction between clones and pathogen species and the similarity in the ranking of clones for lesion size allowed resistance to be assessed with either one of the two pathogens. Further experiments showed 5- to 6-fold differences in the aggressiveness of 10 isolates of *P. palmivora* from different locations in Trinidad and Tobago (Surujdeo-Maharaj et al. 2001). However, there was no significant host genotype × isolate interaction, suggesting that resistance found using any one isolate would be equally valid for the other isolates. So far, the ranking of resistance appears to be constant for different isolates in different countries and even species of *Phytophthora*. Van der Vossen (1997) reported that the ranking order for resistance to BP caused by *P. megakarya* in Cameroon and Togo was very similar to that for BP caused by *P. palmivora* in Côte d'Ivoire. These observations suggest that the results of screening for resistance to *Phytophthora* in Trinidad would be relevant to breeding programmes for resistance to BP at other locations. In this study, an aggressive isolate of *P. palmivora*, obtained from a naturally infected cacao pod in Santa Cruz, Trinidad (Surujdeo-Maharaj et al. 2001) was selected for screening of the selected cacao accessions in the ICG,T.

Evaluation of resistance to Phytophthora pod rot

A spray inoculation method was used to assess the reaction of fully grown, unripe detached pods (about 4–5 months old) to *P. palmivora* (Iwaro 1997; Iwaro et al. 2000b, 2003). As recommended by Iwaro et al. (2000b), 2–4 pods were tested per genotype in each of two experiments conducted to confirm the reaction of each genotype to *P. palmivora*. Pods were harvested between 07.00 and 10.00 a.m., rinsed in two changes of sterile distilled water and arranged with labels in plastic trays lined with moist tissue in a completely randomised design. Two to four pods from each of two standard genotypes with known reactions to *P. palmivora*, SCA 6 (resistant) and IMC 67 (susceptible) were included in each experiment. These two standard clones and some common accessions have shown consistent results in both the detached pod test (Iwaro et al. 2000a, 2003) and their field reactions to *P. palmivora* infection, measured as percentage pod rot, in Côte d'Ivoire (Tahi et al. 2000) and Trinidad (Thévenin et al. 2003). Zoospore suspensions were prepared from 10-day-old cultures of an aggressive isolate of *P. palmivora* following the method of Lawrence (1978) and the concentration was determined using a haemacytometer and adjusted to 100,000 mL^{-1}. Inoculation was performed by spraying half the surface area of each pod at a distance of 30 cm using a Chromist atomiser (Cat. No. 51901 Spray Unit, Gelman Sciences, Ann Arbor, Michigan), to deliver an average of 1 mL of zoospore suspension on about 150 cm^2 of pod surface area. Control pods were treated with sterile distilled water in place of the zoospore suspension. Trays containing the pods were covered with another tray as a lid and enclosed in a polythene bag to maintain a high relative humidity, and inoculated pods were incubated at 25 °C for 4 days. After incubation, the levels of resistance of the inoculated pods were assessed using the disease rating scale below:

Data analysis

A distribution of scores was plotted to assess the pattern of variation among the 816 genotypes evaluated for resistance to *Phytophthora* pod rot. A χ^2 test was performed to determine the significance of differences between wild and cultivated accessions. Similar tests were also conducted to assess the differences among major groups (Forastero, Refractario and Trinitario) and eleven accession groups (B, AM, CL, JA, LP, ICS, TRD, IMC, MOQ, NA and PA) which were represented by at least 20 genotypes. Subsequently, genotypes were categorised into three classes to determine the percentage of the resistant, moderately resistant and susceptible genotypes among the major groups and accession groups.

For the purpose of this study, accessions selected in the 1920s and 1930s for field resistance to witches' broom under high disease pressure in Ecuador were designated as Refractario. This group does not represent a geographical or genetic population as do the Forastero and the Trinitario. It is an extremely heterogeneous group selected from amongst mixed plantings of apparently resistant and susceptible trees on various commercial plantations (Iwaro et al. 2003). These genotypes were most likely of diverse origins (Bartley 2001). In this study, the Refractarios were treated as a group to determine the impact of the initial selection on black pod resistance. The Forastero group originates throughout the basin of the Amazon and its tributaries, while the Trinitario group comprises of a wide range of hybrids between Criollo and the Forastero (Purseglove 1988; Iwaro et al. 2003).

Results

The distribution of scores for 816 accessions (Figure 1a) shows skewness towards the susceptible end of the disease rating scale. This indicates that a large proportion (68.9%) of the sample

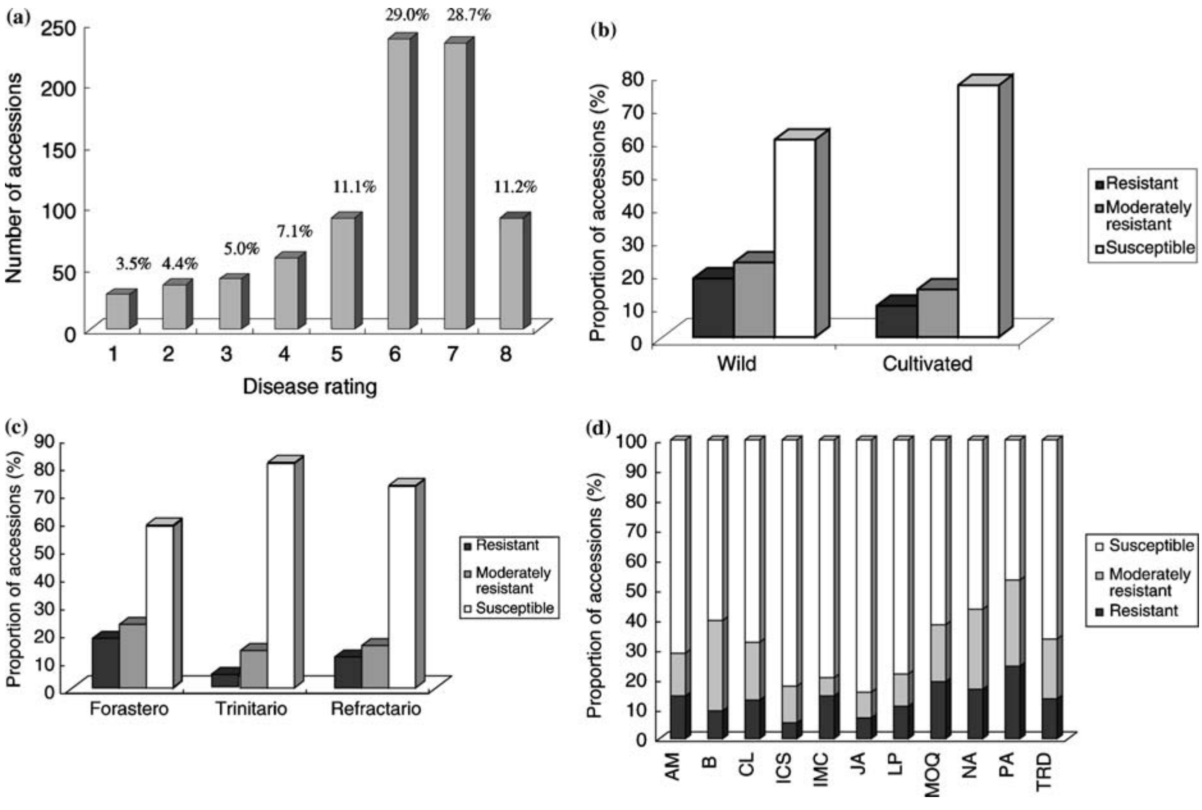

Figure 1. Distribution of scores for resistance to *Phytophthora* pod rot for (a) 816 cacao accessions in ICG,T; (b) two cacoa types (wild and cultivated types); (c) three major groups (Forastero, Trinitario and Refractario); (d) 11 cacao accession groups.

population was susceptible (disease rating 6–8) to the isolate of *P. palmivora* used for screening. However, 12.9% of the sample population was found resistant (disease rating 1–3) and 18.2% moderately resistant (disease rating 4–5).

The wild accessions (288 genotypes) were significantly different ($p < 0.001$) from the cultivated accessions (466 genotypes) in their reactions to inoculation with *P. palmivora* (Figure 1b). Accessions of spontaneous origin (wild) had higher proportions of resistant (17.7%, disease rating 1–3) and moderately resistant (22.6%, disease rating 4–5) genotypes than the cultivated ones (9.4% resistant and 14.4% moderately resistant). Cultivated accessions contained a greater proportion of susceptible genotypes (76.2%, disease rating 6–8) than the uncultivated group (59.7%, disease rating 6–8) (Figure 1b).

Figure 1c shows the levels of resistance to *Phytophthora* among 294 Forastero, 147 Trinitario and 266 Refractario genotypes. The Amazon Forastero group was significantly different ($p < 0.001$) from the Refractario and Trinitario groups (Table 2). Higher proportions of resistant and moderately resistant accessions were recorded in the Forastero (18.0% resistant and 23.1% moderately resistant) than in the Trinitario (4.8% resistant and 13.6% moderately resistant) and Refractario (11.3% resistant and 15.4% moderately resistant) groups. While the difference between Forastero and Refractario was significant at 1% level, a similar test between Forastero and Trinitario was significant at 0.1% level (Table 2) showing a greater difference between the latter groups. The Trinitarios and Refractarios were however not significantly different to each other in their responses to inoculation with *P. palmivora* (Table 2).

Table 2. Chi-square values for comparison among cacao major groups, types and accession groups for resistance to *Phytophthora* pod rot.

Variable	Source of variance	df	Chi-square value
Phytophthora pod rot	Major groups:		
	Forastero, Trinitario, Refractario	4	29.51***
	Forastero, Trinitario	2	24.79***
	Forastero, Refractario	2	13.01**
	Trinitario, Refractario	2	5.56 n.s.
	Types:		
	Wild, Cultivated	2	23.37***
	Accession groups:		
	AM, B, CL, ICS, IMC, JA, LP, MOQ, NA, PA, TRD	10	38.13***

, * Significant at 1 and 0.1% levels, respectively; n.s. not significant.
The frequencies of genotypes for disease ratings were combined into three categories (1–3, 4–5, 6–8) for comparison among the main groups and the two types of cacao assessed. For comparison among accession groups, the frequencies of genotypes for disease ratings were combined into two categories (1–5, 6–8).

Figure 1d shows the distribution of scores for resistance among 11 accession groups, each with at least 20 genotypes tested. Significant differences ($p < 0.001$) were observed among the 11 accession groups (Table 2). Higher percentages of resistant (24.2%) and moderately resistant (28.8%) accessions were observed in the PA group than in the other accession groups (Figure 1d). The greatest proportions of susceptible accessions were found in the ICS (82.5%) and the JA (84.5%) accession groups (Figure 1d).

Table 3 shows the χ^2 values and the levels of significance for paired comparisons among 11 accession groups. Despite the fact that the Refractario and the Trinitario groups were not significantly different in the previous test, the paired comparisons among accession groups showed a significant difference between the accession groups B (Refractario) and ICS (Trinitario) and between JA (Refractario) and TRD (Trinitario) (Table 3). This is illustrated in Figure 1d where there are greater proportions of resistant (9.3%) and moderately resistant (30.2%) accessions in the B than in the ICS (5.3% resistant and 12.3% moderately resistant) accession group. Conversely, the TRD had higher proportions of resistant (13.3%) and moderately resistant (20.0%) accessions than the JA (6.9% resistant and 8.6% moderately resistant).

Although a significant difference was observed between the Refractarios and the Forasteros (Table 2), the paired comparisons between accession groups from the Refractario and the Forastero main groups were not always significant. The IMC group (Forastero) was not significantly different from five Refractario accession groups (AM, MOQ, CL, JA, LP), while the B group (Refractario) was not significantly different from the NA and PA groups (Forastero). Also, the NA group was not significantly different from the AM, B, CL and MOQ groups (Refractario) (Table 3). However, the NA and PA groups (Forastero) were

Table 3. Similarity matrix among 11 cacao accessions groups based on chi-square test.

	AM	B	CL	ICS	IMC	JA	LP	MOQ	NA	PA
B	0.89									
CL	0.09	0.41								
ICS	1.37	6.01**	2.48							
IMC	0.66	4.04*	1.42	0.14						
JA	2.03	7.45**	3.37	0.09	0.44					
LP	0.42	2.97	0.98	0.24	0.02	0.57				
MOQ	0.50	0.01	0.19	3.65	2.41	4.65*	1.82			
NA	1.96	0.17	1.19	10.65***	7.44**	12.69***	5.38*	0.19		
PA	4.74*	1.90	3.66	16.60***	12.57***	18.98***	9.62**	1.42	1.49	
TRD	0.15	0.29	0.01	2.77	1.64	3.71*	1.16	0.12	0.94	3.21

*, **, *** Significant at 5, 1 and 0.1%, respectively.
Other χ^2 values are not significant.

significantly different from the JA and LP groups (Refractario) (Table 3). In addition, the PA group was significantly different from the AM group. In the latter cases, the NA and PA groups consistently showed greater proportions of resistant and moderately resistant accessions than the AM, CL, JA and LP groups. However, there was no significant difference between the MOQ group (Refractario) and the PA and NA groups (Forastero).

Although a significant difference ($p < 0.01$) was observed between the Trinitario and Forastero groups (Table 2), paired comparisons between accession groups from the Trinitario and Forastero were not always significantly different (Table 3). The TRD group (Trinitario) was not significantly different from any of the three Forastero accession groups tested (IMC, NA and PA). In addition, the IMC group was not significantly different from either of the Trinitario accession groups (ICS and TRD) (Table 3). However, the ICS group was significantly different from the NA and PA groups. NA and PA had higher percentages of resistant and moderately resistant accessions than ICS (Figure 1d).

Among the Refractarios assessed, significant differences were observed between the B and JA groups, and MOQ and JA groups (Table 3). However, the MOQ and B accession groups were not significantly different from each other. Both accession groups had higher proportions of resistant and moderately resistant accessions than JA (Figure 1d). The two Trinitario accession groups tested (TRD and ICS) were significantly different from each other, TRD showing a greater proportion of resistant and moderately resistant accessions than the ICS group (Figure 1d).

Within the Forastero group, the NA and PA accession groups were not significantly different to each other, but each differed from IMC (Table 3). Both NA and PA had greater proportions of resistant and moderately resistant accessions than the IMC group (Figure 1d).

Discussion

The range of variability observed among the genotypes tested for resistance to *Phytophthora* pod rot showed that there are abundant genetic resources in the ICG,T that could be exploited in breeding for black pod resistant cultivars. The evidence in this study of more resistant genotypes among the wild types is in agreement with earlier suggestions on the sources of resistance to BP of cacao (Soria 1974). Soria (1974) indicated that there were no reports of fruits infected by *P. palmivora* from any of the expeditions to collect wild cacao in the Amazon basin, but noted that this does not rule out the presence of this fungus in the wild Amazonian populations of cacao since absence of infection could be a reflection of the low level of inoculum within the field. The results of this study show that although the Amazon wild cacao types are good sources of resistance to *P. palmivora*, 58.8% of those tested were found susceptible. It appears that lack of disease could not be interpreted as immunity or total resistance to *P. palmivora*. Desrosiers and Diaz (1957) suggested that the lack of *P. palmivora* infection in cacao-growing areas near the Equator, like the West Coast of Ecuador (and the Amazon basin) may be due to a combination of environmental conditions, which are possibly unfavourable to *Phytophthora* epiphytotics. They noted that the rainy season in these areas is during the hottest part of the year while the cooler period coincides with dry months. Nevertheless, the results of this study show that wild cacao of spontaneous origin possesses valuable sources of resistance genes that could be exploited to broaden the genetic base of black pod resistance in cacao breeding programmes. The high level of susceptibility among the cultivated accessions suggests that they were probably produced from susceptible parents, so that resistance is less concentrated in most of them and hence the need for further improvement through breeding.

In agreement with the above findings, the Forasteros, most of which are wild types of spontaneous origin in this study, had a higher percentage of resistant and moderately resistant genotypes than the Trinitario and Refractario groups. These comprised of selected, cultivated material. The Trinitarios are hybrids between Criollo and Forastero and are highly heterogeneous and were selected by and large for their yield potential. The Refractarios, on the other hand, were selected for resistance to witches' broom disease. The different selection criteria imposed on these groups would probably have a significant influence on their genepool. The genepool for Refractario should have an increased frequency of witches' broom resistance genes, while Trinitario should be richer in genes for good yield potential. These selection criteria may have limited favourable alleles for black pod resistance, and could account for the lower proportion of resistant genotypes observed among the Refractarios and Trinitarios, compared to the Forastero group.

Analysis conducted on 11 accession groups, each represented by at least 20 clones showed that resistance genes are widely distributed among cacao accession groups in all the major groups tested. Different accession groups had varying proportions of resistant and moderately resistant accessions. This is not unexpected in an out-breeding crop such as cacao with a high level of heterogeneity. This finding reinforces the idea of a pre-breeding programme (germplasm enhancement) to accumulate resistance genes over several populations as a strategy to improve the genetic base of resistance in national cacao breeding programmes.

Although no significant difference was observed between the Trinitario and Refractario groups, some accession groups in these two major groups were found to be very different. This shows that the two major groups, although similar on average, may possess different levels of resistance and probably different resistance genes within accession groups.

This study also revealed that the grouping of accessions into three main categories does not mean that the accession groups in the same major group should necessarily have similar proportions of resistant genotypes. The greatest proportions of moderately resistant and resistant genotypes were recorded in the PA and NA accession groups in the Forasteros. TRD was most promising group in the Trinitarios, while MOQ had the greatest proportion of resistant and moderately resistant accessions in the Refractarios. Earlier investigations suggested that the PA and NA groups from the Parinari and Nanay regions are important sources of resistance to BP (Soria 1974; Iwaro et al. 2003). In addition to these two accession groups from Peru, which are both Forasteros, this study showed that the TRD (Trinitario) and MOQ (Refractario) from Trinidad and Ecuador, respectively, are also good sources of resistance to BP of cacao. Since the TRD and MOQ groups probably originate from quite different source to PA and NA, they may possess different genes for resistance to BP. It would therefore seem prudent to try to combine these resistance genes in a pre-breeding programme.

This study has identified different sources of resistance to BP in the ICG,T. This information will allow breeders to exploit complementary resistance genes from genotypes of different origins and exploit inter-population heterosis. This information has been used within the past five years to select resistant parental material for an on-going germplasm enhancement project at the CRU, Trinidad. The results of this study also provide invaluable information for the selection of genotypes for the CFC/ICCO/IPGRI project collection (Sounigo et al. 2000). The additional information presented here on sources of resistance to BP will benefit cacao breeders in the development of high yielding, resistant varieties (Table 4).

Table 4. List of resistant genotypes to *Phytophthora* pod rot.

Accession	Rating[a]	SE	Accession	Rating[a]	SE
CL 10/5	1	0.20	PA 125 [PER]	2	0.73
CRU 100	1	0.00	PA 136 [PER]	2	0.75
CRU 12	1	0.14	PA 150 [PER]	2	0.29
CRU 19	1	0.00	PA 218 [PER]	2	0.58
CRU 87	1	0.00	PA 30 [PER]	2	0.48
CRUZ 7/8	1	0.16	PA 95 [PER]	2	0.48
EBC 148/S-301	1	0.00	POUND 7/A [POU]	2	0.42
EET 272 [POU]	1	0.00	SCA 3	2	0.42
EET 48 [ECU]	1	0.00	SJ 1/40 [POU]	2	0.34
GU 114/F	1	0.00	TRD 32	2	0.44
ICS 41	1	0.00	TRD 85	2	0.39
ICS 69	1	0.17	AM 1/53 [POU]	3	0.75
ICS 70	1	0.00	AM 2/6 [POU]	3	0.50
IMC 20	1	0.00	AMAZ 12 [CHA]	3	0.43
IMC 36	1	0.00	B 13/6 [POU]	3	0.42
IMC 41	1	0.00	B 23/6 [POU]	3	0.29
IMC 47	1	0.00	B 6/3 [POU]	3	0.56
JA 3/4 [POU]	1	0.13	B 6/8 [POU]	3	0.45
MOQ 6/82	1	0.23	CL 19/10	3	0.32
MOQ 6/95	1	0.00	CL 19/31	3	0.29
NA 168	1	0.18	CL 19/49	3	0.63
NA 227	1	0.21	COCA 3370/5 [CHA]	3	0.20
NA 399	1	0.00	CRU 78	3	0.53
NA 719	1	0.00	CRU 85	3	0.34
PA 46 [PER]	1	0.17	DE 52/B [TTO]	3	0.42
POUND 4/A	1	0.20	JA 5/11 [POU]	3	0.23
SCA 6	1	0.00	JA 6/4 [POU]	3	0.50
SLC 4	1	0.00	LV 20 [POU]	3	0.40
AM 2/82 [POU]	2	0.49	LX 32	3	0.56
AM 2/92 [POU]	2	0.40	MO 4	3	0.40
CLM 96	2	0.40	MO 83	3	0.33
CRU 124	2	0.29	MOQ 6/19	3	0.42
CRU 48	2	0.32	NA 235	3	0.60
CRU 80	2	0.42	NA 326	3	0.33
CRU 89	2	0.26	NA 534	3	0.28
CRUZ 7/14	2	0.37	NA 699	3	0.49
EET 59	2	0.50	NA 7/10	3	0.18
IMC 50	2	0.24	NA 702	3	0.87
IMC 76	2	0.46	NA 715	3	0.63
IMC 94	2	0.25	PA 120 [PER]	3	0.30
JA 5/47 [POU]	2	0.44	PA 157 [PER]	3	0.40
LP 3/4 [POU]	2	0.37	PA 27 [PER]	3	0.30
LP 3/5 [POU]	2	0.63	PA 279 [PER]	3	0.31
LP 4/43 [POU]	2	0.37	PA 289 [PER]	3	0.58
LP 5/14 [POU]	2	0.37	PA 293 [PER]	3	0.37
LX 31	2	0.43	PA 70 [PER]	3	0.48
MOQ 2/18	2	0.53	PA 90 [PER]	3	0.48
NA 104	2	0.29	POUND 2/A [POU]	3	0.49
NA 184	2	0.31	SPEC 18/6	3	0.40
NA 217	2	0.60	SPEC 194/75	3	0.43
NA 3	2	0.42	TRD 13	3	0.42
NA 312	2	0.29	TRD 88	3	0.43
PA 124 [PER]	2	0.37			

[a]Based on the mean of scores for clonal reaction to *P. palmivora*.

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