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# Participatory cocoa (*Theobroma cacao*) selection in Cameroon: *Phytophthora* pod rot resistant accessions identified in farmers' fields

M.I.B. Efombagn<sup>a,\*</sup>, S. Nyassé<sup>a</sup>, O. Sounigo<sup>b</sup>, M. Kolesnikova-Allen<sup>c</sup>, A.B. Eskes<sup>b</sup>

<sup>a</sup>Institute of Agricultural Research for Development (IRAD), P.O. Box 2067 or 2123, Yaoundé, Cameroon

<sup>b</sup>CIRAD-CP, TA 80/02, 34398 Montpellier Cedex 5, France

<sup>c</sup>Central Biotechnology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria

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#### **Abstract**

As part of a participatory selection programme, promising individual trees were selected in 2004 in cocoa farms of southern and western Cameroon regions for yield and for low incidence of *Phytophthora* pod rot (Ppr) caused by *Phytophthora megakarya*. The aim of this study was to compare the levels of resistance to Ppr between farm accessions (FA), introduced and local genebank accessions (GA). In total, 234 FA were grafted in the nursery together with 22 introduced GA and 73 local GA, and tested for resistance to *P. megakarya* by leaf disc inoculations. The introduced GA, that were reported as resistant to Ppr in other countries, proved to be more resistant than the selected FA and unselected FA and also more resistant than the 3 control clones for Ppr resistance used in the study. However, approximately 10% of the FA were as resistant as the average of the introduced GA, showing the potential of selection for resistance to Ppr in farmers' fields. The average level of resistance of the FA was relatively higher than that of the local GA. The FA selected for yield and low Ppr incidence in the field were more resistant in the leaf disc test than the unselected FA. The use of FA and of farmers' knowledge in the participatory selection process is valuable in obtaining Ppr-resistant cultivars.

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#### 1. Introduction

Cocoa is a perennial crop widely cultivated in Africa, Asia and America. In Cameroon, production, based on smallholder farmers, has been fluctuating around 110 000 and 160 000 tonne over several decades, with a maximum of 180 000 tonne recorded in 2005 (ICCO, 2005). This stagnation is due to several factors, the most important being the pod rot disease caused by *Phytophthora megakarya* (Ppr). In Cameroon, cocoa-breeding activities in the 1960s and 1970s emphasized the development of high yielding hybrid cultivars. These are mainly crosses between introduced Upper Amazon genotypes and accessions selected in the traditional cultivars (seedlings from Trinitario and Lower Amazon types). In spite of the distribution of these cultivars, cocoa plantations in Cameroon continue

to be low yielding, with an average of 200–600 kg of dry cocoa per ha (Efombagn, 2005). One of the main reasons is the high Ppr incidence, with losses of 60–80% of the crop being common in the absence of control measures. The hybrid cultivars are considered by the farmers to be more susceptible to Ppr than the traditional cultivars. Therefore, farmers have continued to be compelled to apply frequent chemical control, with copper-based or systemic fungicides. These are damaging for the environment, increase significantly production cost compared to the limited income from cocoa sales, and are therefore not available to all farmers.

During the 1990s, resistance to Ppr received considerable attention. An early screening test based on leaf disc inoculations was developed (Nyassé et al., 1995) that proved to be correlated with field level of infection (Tahi et al., 2000). From 1998, breeding for resistance is receiving increased attention in Cameroon, including through an international collaborative effort. This included recent

<sup>\*</sup>Corresponding author. Tel.: +33467614467; fax: +33467615581. *E-mail address*: efombagn@yahoo.fr (M.I.B. Efombagn).

introductions of Ppr-resistant accessions through intermediate quarantine at the University of Reading, UK. A participatory cocoa selection programme was initiated to complement conventional breeding activities. Globally, participatory crop improvement emerged recently as an alternative approach for developing countries in response to the recognition that conventional breeding has brought little improvement to small-scale farmers (Lipton and Longhurst, 1989; Kerr and Kolavalli, 1999). Conventional or formal plant breeding programmes conducted in developing countries have been criticized for ignoring indigenous germplasm and failing to breed for adaptation to conditions faced by small-scale farmers (Atlin et al., 2001).

Based on farmers' knowledge and breeders' inputs, outstanding farm accessions (FA) with respect to low Ppr incidence and high yield were selected through a participatory approach during field surveys conducted in 2004 in the two major cocoa producing areas of Cameroon (Efombagn, 2005). Collection of the selected and unselected FA was carried out, and they were grafted in nurseries on-station for subsequent evaluation. The aim of the study was to compare levels of resistance to Ppr of the selected and unselected FA, evaluated by leaf disc inoculation, with the resistance of introduced and local genebank accessions (GA).

#### 2. Material and methods

# 2.1. Cocoa genotypes

In total, 196 selected FA and 38 unselected FA collected from 120 cocoa farms, 73 local GA and 22 introduced GA (Table 1) were used in this study. Farms composed of hybrids or traditional cultivars were randomly selected in

different localities. The local GA accessions are both cloned F1 progenies (Upper Amazon × Trinitario) and Trinitario clones selected in the field in 1960s and transferred in genebanks at the IRAD Nkoemvone Research Station (southern Cameroon). The introduced GA were part of an international exchange of accessions selected for Ppr resistance, through the intermediate quarantine centre at the University of Reading (RUQ), UK. Among the introduced GA group (Table 1), PNG accessions were created in Papua New Guinea, and the remaining accessions originated from Latin America and provided to RUQ through the International Cocoa Genebank (ICG), Trinidad.

The FA originated from the two main agro-ecological cocoa growing zones of the country designated in this study as "southern" and "western" regions. Selection was made by the farmers in the presence of the breeder. The criteria used by the farmers were the incidence of Ppr or the high yield potential observed in each selected FA over several years of production. Out of the 196 selected FA, 101 trees were classified as 'less infected with Ppr' than the level of infection in the plot and 95 as 'higher yielding' than the level of production in the plot. Furthermore, part of these trees (196 selected FA and 38 unselected FA) were identified by the farmers as belonging to the traditional cocoa cultivars (n = 99) or to hybrid cultivars distributed from seed gardens from the 1970s onward (n = 135). More than 95% of the traditional cultivars are Amelonado like (lower Amazon cocoa type) and the remaining ones are Trinitario like. Hybrid cultivars are trees from F1 or F2 progenies created by mating between Trinitario and Upper Amazon parental clones in seed gardens.

All the accessions were established by grafting in the nursery under uniform shade, which is an important condition to obtain uniform results in the leaf disc test

Table 1 Clones and farm accessions used

Accession groups	Origin	Number of accessions
Farm accessions (FA) <sup>a</sup>	Southern Cameroon	103
	Western Cameroon	131
Local genebank accessions (GA) <sup>b</sup>	Nkoemvone station, Cameroon	73
Introduced genebank accessions (GA) <sup>c</sup>		22
PNG155; PNG197; PNG210; PNG218; PNG299; PNG340	Papua New Guinea (PNG)	6
PA70; PA120; PA150, EET59; EET399; LCTEEN162/5;	International Cocoa Genebank, Trinidad	16
LCTEEN302; IMC20; IMC40; SCA6; SCA9; MA12; B5/7;	(ICG, T)	
POUND7/B; AMAZ15/15; COCA3370-5	· · · · · · · · · · · · · · · · · · ·	
Control clones <sup>d</sup>		3
SCA6 (resistant)	Peru	
SNK413 (moderately resistant)	Nkoemvone, Cameroon	
SNK64 (moderately susceptible)	,	

<sup>&</sup>lt;sup>a</sup>Cocoa trees selected by the farmers in their plantations, in the presence of breeders.

<sup>&</sup>lt;sup>b</sup>Cloned F1 progenies (Upper Amazon X Trinitario) selected on-station and Trinitario clones selected in the field in 1960s and transferred in genebanks at the IRAD Nkoemvone Research Station (Southern Cameroon).

<sup>&</sup>lt;sup>c</sup>Accessions introduced at IRAD (Cameroon) through the intermediate quarantine centre of the University of Reading, UK.

<sup>&</sup>lt;sup>d</sup>Clones selected as controls according to results published by Iwaro et al. (2005) and Nyassé et al. (2003).

(Eskes et al., 2000). The SNK64, SNK413 and SCA6 clones (Table 1) were also grafted to be used as moderately susceptible (MS), moderately resistant (MR) and resistant (R) controls, respectively (Blaha and Lotodé, 1977; Nyassé et al., 1995). Open-pollinated seedlings rootstocks from IMC67 (Upper Amazon clone) were used for grafting. The number of grafted plants per accession varied between three and five.

#### 2.2. Leaf disc inoculation test

The inoculation method and disease rating used in the study was developed by Nyassé et al. (1995). A moderately aggressive isolate of *P. megakarya* available in the Plant Pathology Laboratory at IRAD Nkolbisson Centre (central Cameroon) was used in this study. It was isolated in 2004 from a cocoa plantation situated in the Centre region of the country, and it has been maintained in the laboratory by successive transfers on 1.5% pea-based agar medium. To maintain its aggressiveness, the isolate is periodically inoculated onto cocoa pods.

Three inoculations series were carried out during the wet season and the interval between two inoculation series was 30 d. In each inoculation series (replicate), 2 or 3 leaves approximately 2 months old from non-lignified twigs as described by Nyassé et al. (1995) were collected early in the morning from relatively flexible (green turning to brown) branches of each accession. Only the synchronized flushes of all the plants were used at the same time (replicate). Thirty leaf discs of 15 mm diameter were cut per accession in the afternoon. The 30 discs of each accession were placed upside down in 3 wetted plastic trays, with 10 discs/tray. Due to the limited size of the trays, a total of 100 discs (10 discs/accession) were placed in a tray. The covered trays were incubated in darkness overnight at approximately 25 °C. Inoculations were carried out the next morning with a 10 µl suspension of P. megakarva zoospores calibrated at 300 000 ml<sup>-1</sup>. Symptoms (disease scores—DS) were observed 5 d after inoculation by using a 5-point scale, with 0 = no symptoms; 1 = very small localized penetrationpoints; 2 = small penetration spots, sometimes in a network; 3 = coalescing lesions of intermediate size; 4 = largecoalescing brown patches; and 5 = uniform large dark brown lesions.

# 2.3. Statistical analyses

Three inoculation series (replicates) were carried out, each containing 30 leaf discs of each accession. The statistical unit considered in the analysis was the DS of each leaf disc. Analyses of variance were carried out with S.A.S. (2000) software version 8 to compare accessions or groups of accessions. The Newman and Keuls (Cochran and Cox, 1957) test at 5% probability level was used to compare means of individual accessions and means of groups of accessions. Accessions were grouped according to their type (FA and GA), to their geographic origin

(southern and western FA), to their genetic origin (hybrid and traditional cultivars) and to their agronomic characteristics (high yielding, low Ppr incidence and unselected FA). Spearman's (1904) rank correlation analyses were used to compare the repeatability of results obtained in three different series of inoculations (replicates).

# 3. Results

#### 3.1. Repeatability of test results

The coefficients of rank correlation between the DSs obtained in the 3 series of all 329 accessions were positive and significant,  $\{r1 = 0.83 \ (p = 0.02)\}$ ,  $\{r2 = 0.86, (p = 0.01)\}$  and  $\{r3 = 0.93 \ (p = 0.02)\}$ , between series 1 and 2, 1 and 3, and 2 and 3, respectively. This shows the stability of results obtained in the 3 replicate inoculations.

#### 3.2. Variation in DS within and between accession groups

Reactions to inoculation varied significantly (p=0.001) between all 329 accessions, the 3 control clones, and also within the 4 accession groups (Table 2). The average coefficient of variation (CV) for DSs for all genotypes tested was 33%. CV's varied between 28% and 36% for the accession groups, with the highest CV value being obtained for the FA from the south of Cameroon.

The mean DS also varied significantly between accession groups (Table 3). The introduced GA were much more resistant (average DS = 1.88) than the other accession groups. The local accession groups varied less, but their average DS values were still significantly different: DS = 2.72 for the FA from southern Cameroon, DS = 2.91 for the FA from western Cameroon, and DS = 3.14 for the local GA. The range of variation for DSs was large for all groups (Table 4) and highest for the introduced GA group, from 1.2 for the IMC47 clone to 4.9 for the LCTEEN302 clone.

# 3.3. Variation for DS among accessions within selected and unselected FA

Based on the DS rating on the 0–5 point scale, 4 resistance classes were defined as follows (Table 4): A = DS of 0–2.0; B = DS of 2.0–3.0, C = DS of 3.0–4.0, and D = DS of 4.0–5.0. Most of the FA were found in the intermediate groups B and C. Out of 234 FA, 21 (9%) were part of group A, 123 (52.6%) of group B, 86 (36.8%) of group C and only 4 (1.7%) of group D. The distribution of DS within the southern and western selected and unselected FA was not similar. More resistant genotypes (15.5% in group A) were found in the accession group of the southern region, compared to only 5.3% of group-A accessions for the western region.

The relative resistance of the FA was compared also to the 3 control clones: SNK64 (MS), SNK413 (MR) and SCA6 (R) (Table 5). The mean DSs for these clones

Table 2
ANOVA for *Phytophthora* pod rot disease scores within farm and genebank accession groups

Accession groups	DF	MS	F	p	CV (%)
Introduced GA	21	62.78	86.55	0.001	35
Local GA	72	38.69	46.86	0.001	28
Selected FA and unselected FA from South Cameroon	102	35.39	35.32	0.001	36
Selected FA and unselected FA from West Cameroon	130	26.99	30.87	0.001	32
All accessions	331	40.50	47.17	0.001	33

Table 3
Mean resistance of farmers and genebank accession groups

Accession group	Inoculation series			Mean
	1	2	3	
Local GA	3.05b	3.02a	3.35a	3.14a <sup>a</sup>
Selected and unselected FA—Western Cameroon	3.21a	2.54b	2.99b	2.91b
Selected and unselected FA—Southern Cameroon	2.82c	2.47b	2.87c	2.72c
Introduced GA	1.90d	1.69c	2.06d	1.88d
Mean	2.75	2.53	2.88	2.72

<sup>&</sup>lt;sup>a</sup>Accession group means with different letters are significantly different according to the Newman and Keuls test at 5% probability.

Table 4
Frequency (%) of farm and genebank accessions for disease score groups in the leaf disc test (0–5 point scale)

Accession group	Disease rating gr	Disease rating groups				
	A	В	С	D		
Genebank material						
Introduced GA	17 (77.2) <sup>a</sup>	3 (13.6)	1 (4.5)	1 (4.5)	1.2-4.9	
Local GA	4 (5.4)	23 (31.5)	40 (54.7)	6 (8.21)	1.3-4.4	
Sub-total	21 (22.1)	26 (27.4)	41 (43.1)	7 (7.4)		
Selected and unselected FA						
Southern Cameroon	16 (15.5)	52 (50.4)	33 (32.0)	2 (1.9)	1.4-4.1	
Unselected FA	14	34	24	1		
Selected FA	2	18	9	1		
Western Cameroon	5 (5.3)	71 (54.2)	53 (40.4)	2 (1.5)	1.2-4.1	
Unselected FA	5	65	51	1		
Selected FA	0	6	2	1		
Sub-total	21 (9.0)	123 (52.6)	86 (36.8)	4 (1.7)		
All accession groups	42 (12.8)	149 (45.3)	127 (38.6)	11 (3.3)	1.2-4.9	

Group A = disease scores of 0-2.0; B = scores of 2.0-3.0; C = scores of 3.0-4.0; and D = scores of 4.0-5.0.

were 3.73, 2.28 and 1.35, respectively. None of the FA was more resistant than SCA6, but 18.8% (45 genotypes) of them were more resistant than the MR control clone (SNK413). Six percent of the FA were more susceptible than the SNK64 clone and they were all unselected FA. The remaining ones were classified between SNK413 and the MS clone (SNK64). Again, more resistant FA were found in the southern region, with 27% of accessions between SCA6 and SNK413, than in the western region, with only 12% of the accessions with this class (Table 5).

# 3.4. Variation for DS between selected and unselected FAS

The average DS value of the unselected FA group was slightly, but significantly, higher than that of the selected FA group (Table 6). None of the selected FA, in the south as well as in the west, was more susceptible than the MS control clone SNK64 (Table 5). The most resistant accession group was made up by the FA selected for low Ppr incidence in the field, with DS of 2.77, in relation to DS of 2.97 for the unselected FA (Table 6). The average resistance of the FA selected for high yield was

<sup>&</sup>lt;sup>a</sup>The values between brackets represent the percentage of genotypes (of each accession group) belonging to the corresponding group.

Table 5
Relative resistance of genebank (introduced GA and local GA) and farm (selected FA and unselected FA) accessions in comparison to the resistant (R), moderately resistant (MR) and moderately susceptible (MS) control clones

Accession groups	Number and percentage of accessions				
	More resistant than SCA6 (R)	Intermediate between SCA6 (R) and SNK413 (MR)	Intermediate between SNK413 (MR) and SNK64 (MS)	More susceptible than SNK64	
Genebank accessions (GA)					
Introduced GA	3 (13.6) <sup>a</sup>	17 (77.3)	1 (4.5)	1 (4.5)	22
Local GA	0	7 (9.5)	53 (72.6)	13(17.8)	73
Subtotal	3 (3.1)	24 (24.2)	54 (56.8)	14 (14.7)	95
Selected and unselected FA					
Southern Cameroon	0	28 (27.1)	70 (67.9)	5 (4.8)	103
'Less Ppr infected' FA	0	13	32	0	45
'High-yielding' FA	0	8	21	0	29
Unselected FA	0	7	17	5	29
Western Cameroon	2 (1.5)	16 (12.2)	104 (79.3)	9 (6.8)	131
'Less Ppr infected' FA	1	9	46	0	56
'High-yielding' FA	1	7	58	0	66
Unselected FA	0	0	0	9	9
Subtotal	2 (0.8)	44 (18.8)	174 (74.3)	14 (5.9)	234
All accession groups	5 (1.5)	68 (20.6)	228 (69.3)	28(8.5)	329

<sup>&</sup>lt;sup>a</sup>The values between brackets represent the percentages of genotypes belonging to the corresponding class within each accession group.

Table 6
Mean resistance of farm accessions according to their characteristics (high yielding, low Ppr incidence and unselected FA) and to the genetic origin (traditional and hybrids trees)

Group of accessions	Origin of farm accessions		Mean	Disease score range	
	Southern Cameroon	Western Cameroon		runge	
Type of selection					
Unselected FA	2.81a	3.13a	2.97a	1.87-4.19	
'High-yielding'	2.69b	2.93b	2.86b	1.20-4.17	
FA					
'Less Ppr	2.67b	2.85c	2.77c	1.34-3.92	
infected' FA					
Cultivar					
Traditional	2.86a	2.98b	2.89a	_	
Hybrids	2.70b	3.09a	2.76b		

intermediate between the unselected FA and the FAs selected for low Ppr incidence. However, the difference between FA selected for high yield and FA selected for low Ppr was not significant in the southern region (Table 6).

# 3.5. Comparison between resistance of FA and GA

As expected, most of the introduced GA were resistant to *P. megakarya*, with 77.2% showing mean DS value under the score 2 of the disease rating scale of the leaf disc test (Table 4). This compares to 9% of the selected and unselected FA having their DS values below 2% and 5.4% of the local GA.

In Table 5, 13.6% of the introduced GA were more resistant than the R control clone SCA6 and 77.3% were grouped between the R and the MR control clones (SNK413). These percentages were respectively 0.8% and 18.8% for the FA, and respectively 0% and 9.5% for the local GA.

# 3.6. Variation for DS between traditional and hybrid cultivars within selected and unselected FA

The mean DS values of the FA belonging to the two major types of cultivars (traditional or hybrid cultivars) found in cocoa farms in southern and western Cameroon are given in Table 6. The mean DS value for all FA was slightly higher for the traditional than for the hybrid cultivars. However, the traditional FA were slightly more resistant than the hybrid FA in the western region, and slightly more susceptible in the southern region.

# 4. Discussion

The high repeatability observed between the three series of leaf disc inoculations suggested that the test has been carried out under uniform conditions. Similarly conducted leaf disc experiments in Cameroon and in Côte d'Ivoire have shown significant correlations with field Ppr incidence (Nyassé et al., 2002; Tahi et al., 2006). Therefore, the differences in resistance observed in our study are expected to have implications for field Ppr incidence. Furthermore, the level of resistance of the control clones Sca6 (R), SNK64 and SNK413 (MR) in our study agrees with earlier results (Iwaro et al., 2005; Nyassé et al., 2003), so the

comparisons of the GA and FA with these clones appear to be valid.

The highly significant variation for disease resistance among FA and GA agrees with work on resistance to Ppr done by Blaha and Lotodé (1977), Nyassé et al. (1995, 2003), Nyassé (1997) and Iwaro et al. (2003). The great variation observed would allow for effective selection of less susceptible genotypes to the disease in any of the local GA, selected FA and unselected FA.

Approximately 10% of the selected FA were as resistant as the average of the introduced GA (Table 4), showing the potential of selection for resistance to Ppr in FA. Further use of the most resistant FA should be made in cocoa breeding in Cameroon, by testing these genotypes as potential new clonal cultivars or as parents of new hybrid cultivars.

The validity of the knowledge of farmers in identifying FA with low Ppr incidence was corroborated by the significantly lower average DS value observed for these trees in relation to the unselected FA group in the leaf disc test (Table 6). Although significant, the difference in resistance (0.2 DS points relative to the average of 3 for the entire FA group) was not large. This suggests that possibly only a part of the differences in disease incidence perceived by farmers in the field is due to differences in intrinsic resistance to Ppr (resistance to infection). Other factors such as escape from infection or variation in the field environment may have played a role. It is concluded that the leaf disc test is a necessary tool to detect more effectively the accessions with highest resistance to Ppr infection among the FA.

Interestingly, there was also a small but significant difference in the leaf disc test between the unselected FA group and the FA group selected by the farmers for yield (0.11 DS point). This might be explained by the fact that FA with lower disease incidence are expected to produce more healthy pods when disease pressure is high. In other words, field resistance to Ppr is an important component of yield.

The results showed little differences in average DS values between the traditional and hybrid cultivars grown in Cameroon. This result does not tally with farmers' assertions about the higher field susceptibility to Ppr of hybrids compared to traditional cultivars. This difference might be explained by mechanisms linked to disease escape. Firstly, the hybrid trees were often planted for infilling of open spaces in adult traditional cocoa plantations. The younger hybrid trees produce pods nearer to the soil than the older traditional cultivars, and may therefore be more exposed to the primary inoculum coming from the soil, as reported by Gregory and Maddison (1981). Secondly, the hybrids are recognized, even by the farmers, as producing more pods than the traditional cultivars, which would favour the transmission of the inoculum from pod to pod. The pod yield has been shown to be environmentally correlated to the level of Ppr infection (Berry and Cilas, 1994). Thirdly, the traditional cultivars resemble the

Amelonado type, that in Vanuatu has shown disease escape in hybrid cocoa cultivars, related to a different and more reduced period of pod production in the year (Jagoret et al., 1995). The same might be the case in Africa. Fourthly, the duration of pod maturation may be an important factor as this relates to the duration of exposure to the pathogen. In Cameroon, most of the released hybrids cultivars were created using Trinitario parental clones that were shown to have relative long periods of pod maturation (Berry and Cilas, 1994; Efombagn et al., 2004).

The mean DS value of the introduced GA group was much lower than that of the local GA and FA groups. The high average susceptibility of the local GA group may be related to the fact that it was selected for high yield potential rather than for resistance to Ppr. The introduced GA group was mainly selected in the ICG in Trinidad with the detached pod inoculation test, using an isolate of *P. palmivora* (Iwaro et al., 2003). This suggests a good correlation of the leaf disc and detached pod test results and also little interaction between cocoa genotypes and the *Phytophthora* species involved (*P. palmivora and P. megakarya*). Therefore, further exchange of Ppr resistant genotypes between cocoa collections in different countries should be beneficial for cocoa breeding and should be encouraged.

#### 5. Conclusion

Potential sources of resistance to *P. megakarya* were detected in accessions selected in farmers' field in Cameroon, based on farmers' information and on the leaf disc test. The most resistant FA were much less susceptible than local GA included in this study. Therefore, they could significantly contribute to obtaining Ppr resistant cultivars, along with the resistant introduced GA. The results show that farmers' knowledge is a useful input in cocoa breeding programmes.

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