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Resistance to *Phytophthora palmivora* (Butler) Butler Assessed on Leaf Discs of Cacao (*Theobroma cacao* L.) Hybrid Trees

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Abstract: To select resistant cacao genotypes to *Phytophthora palmivora* and better understand the genetic determinism of resistance to this pathogen, the individual resistance of 133 and 105 two ways cocoa hybrids trees of two related progenies, F1 and F7, respectively, was evaluated by leaf discs test. The F1 and F7 derived from crosses (P7 x ICS100) x C1 (P7 x ICS95) x C1, respectively. P7 is a resistant heterozygous Upper Amazon Forastero clone; ICS100 and ICS95 are less susceptible genetically close Trinitario clones. The common male parent, C1, is a completely homozygous susceptible Lower Amazon Forastero clone Amelonado type. Three series of inoculations were conducted per family and one-hundred and twenty leaf discs taken from each tree were inoculated. The results showed additivity of transmission of resistance to *P. palmivora*, the presence of a transgression for this character and the involvement of several recessive alleles in resistance to *Phytophthora*. Significant differences in levels of resistance were observed between the genotypes of each progeny. The average levels of resistance of F1 and F7 are intermediate between those of both resistant controls SCA6 and PA150 and that of NA79, the susceptible control. In the F1 and F7, respectively, 24 and 05 cocoa trees were more resistant than SCA6 and PA150. These trees may be selected and may serve as potential new genitor clones in *Phytophthora* sp., genetic resistance breeding programs. They may also provide budwoods for replacements in fields.

Key words: Resistance, genetic determinism, *Phytophthora palmivora*, *Theobroma cacao* L., leaf disc test

INTRODUCTION

Phytophthora Black pod is the most worldwide spread and destructive of cocoa diseases (Iwaro *et al*., 1998). It is the first fungal disease affecting cocoa since 1920, especially in West and Central Africa, where some 71.5% of world's cocoa production (ICCO, 2009) are provided by 5-6 millions small holders for whom cocoa is the only one livelihood. Four main species of *Phytophthora*, of the 10 fungi species of the genus *Phytophthora*, are known to cause black pod disease. Among these latter ones, *P. palmivora* is present in all

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cocoa producing countries (Garcia *et al*., 1994). Losses of productions due to black pod are estimated about 30 to 40% (ICCO, 2009) of annual average supply, but may reach 90% depending upon the susceptibility of cultivars and environmental conditions. In Côte d'Ivoire, *P. palmivora* is prevailing more in plantations than *P. megakarya*, the most aggressive species (Cilas *et al*., 2004) reported at the end of 1990s in the Eastern regions, near the Ghanaian border (Koné, 1999). The rot rate varies from 40 and 80% in some cocoa plantations. Copper-based contact or systemic and other fungicides used to control the disease are expensive, polluting for the environment, toxious for farmers and constraining in use. In addition, their optimal efficiency is gained when they are associated with husbandry practices (Akrofi *et al*., 2003) and chemical residues could be accumulated in cocoa beans. Genetic resistance may be a sustainable alternative, effective and economic way to control the disease. Despite much work on resistance to *Phytophthora*, to date no completely resistant cocoa cultivar has been found. Thus, most cultivars are susceptible to black pod (Eskes and Lanaud, 2001) making the selection for resistance to *Phytophthora* inefficient. The percentage of resistant accessions is still low in spite of significant variation observed for genetic resistance in germplasm collections and selection trials (Iwaro *et al*., 2003). Since, resistance is polygenic and so additive (Ndoumbe *et al*., 2001; Nyassé *et al*., 2007), much effort have been undertaken to develop and/or improve its methods of assessment. These methods by carrying out artificial inoculation tests on attached or detached pods, whole leaves (Iwaro *et al*., 1997; Nyassé *et al*., 1995) and leaf discs (Nyassé *et al*., 1995; Tahi *et al*., 2007). Many studies established conditions for performing leaf discs and pod inoculation tests which give significantly correlated results and with field infection levels (Nyassé *et al*., 2002; Tahi *et al*., 2007). The inoculation of leaf discs of many accessions with different *Phytophthora* species, isolates or strains showed high heritability levels (Nyassé *et al*., 2002; Tahi *et al*., 2006a) and suggested that resistances to *P. megakarya* and *P. palmivora* may be correlated (Nyassé *et al*., 1995; Paulin *et al*., 2005). In addition, selection for resistance to *P. palmivora* would be more effective than that to other species. Latter findings are similar to those obtained about resistance QTL detected by Risterucci *et al*. (2003). Tahi *et al*. (2006a) showed that expected genetic gain by leaf discs test is higher than that obtained in natural conditions of infection. Leaf discs test is serving in different research institutes for assessing germplasm and accessions (Risterucci *et al*., 2003; Efombagn *et al*., 2007).

The aims of this study were to select resistant cacao genotypes to *P. palmivora* and further understand the genetic determinism of the resistance to the pathogen. Thus, 2 ways related hybrid progenies were assessed regarding their resistance to *P. palmivora* by inoculating foliar discs with suspension of zoospores according to conditions described by Nyassé *et al*. (1995) and Tahi *et al*. (2006a, 2007). Resistance levels of trees were compared within a given progeny and compared with those of resistance control clones assessed at the same time. The aspects of dissimilarity and/or likeness as well as the genetic determinism are discussed.

MATERIALS AND METHODS

Plant Material

Four clones representing two main groups of cacao trees available in CNRA gene banks were used to create the two 2 ways hybrid progenies (families) studied. They are an Upper Amazon Forastero clone (Pound 7 or P7), a Lower Amazon Forastero clone Amelonado type (IFC1 or C1) and 2 Trinitario clones (ICS100 and ICS95) from Trinidad selected by ICTA.

These 2 progenies are part of a trial, completely randomized, in which each genotype is represented by a single tree. The trial comprised overall 5 progenies. Both populations studied have a common male parent, the clone C1. The first family (Family 1 or F1) derived from the cross (P7 x ICS100) x C1. The second one (family 7 or F7) derived from cross (P7 x ICS95) x C1. F1 and F7 comprised, respectively 173 and 183 living plants, but only 133 out of 173 and 105 out of 183 were evaluated. The clone C1, susceptible to black pod and homozygous (Risterucci *et al*., 2003) was selected in Côte d'Ivoire. The clone P7, resistant to *Phytophthora palmivora* (Nyassé *et al*., 2007), was one of the genitors of released hybrid cacao varieties in Côte d'Ivoire, especially in crosses with Lower Amazon Forastero clones (Amelonado). This clone is able to transmit high resistance levels to its offsprings (Tahi *et al*., 2006a). ICS95 and ISC100 are moderately susceptible clones to *Phytophthora* sp. (Nyassé *et al*., 2007).

Three Upper Amazon Forastero clones (SCA6, PA150 and NA79) growing in CNRA/Bingerville research station collections were used as control clones in this study. These same clones served as control in many previous cocoa assessment processes (Tahi *et al*., 2006b; Efombagn *et al*., 2007; Pokou *et al*., 2008). SCA6 and PA150 are resistant whereas NA79 is susceptible to *P. palmivora* (Lachenaud *et al*., 2001).

Experimental Design

The trial, consisted of 2 blocks (block 1 and 2), spreads on 1.25 ha and is set up according a completely randomized design of rows with guards without overhead shade trees. Each block is constituted of 44 rows with 8 rows for each of the 5 families, 2 rows for the control deriving from the cross T60/887 x C1 and 2 guard rows. Each row is constituted of 19 trees with 17 being useful. The spacing was 3×3 m, that gave an overall number of 836 plants block^{-1} with 34 useful for the control, 136 useful for each family and 122 as guards. The trial comprises overall 1672 trees for a density of 1337 trees ha^{-1} with 272 and 68 useful trees, per family and for the control, respectively. Hand weedings were supplemented, if needed, by herbicide treatments. Kalach 360 SL (360 g L^{-1}) at the rate of 10 ml L^{-1} of water was used. Pesticides treatments, by using Thiodan 50 CE at the rates of 12.5 ml L^{-1} of water and 500 mL ha^{-1}, were applied 3 times a year. Regular prunings of chupons as well as parasitic epiphytes like *Loranthus* sp. were conducted. A pruning of basal plagiotropic and orthotropic branches was carried out in Mar 2006.

Fungal Material

A strain of *P. palmivora*, BL7-11-2, was used to prepare the inoculum (zoospore suspension) for artificial inoculations on leaf discs. Its degree of aggressiveness is slightly high with disease scores varying between 3 and 4 for susceptible genotypes (Zéï, 2001). This strain was maintained in the laboratory with regular transfers every 4-6 weeks on 1.5% pea-based agar medium in tubes placed at 26°C in darkness. To maintain its aggressiveness in laboratory, the isolate was periodically re-inoculated onto sterile green mature cocoa pods incubated in 100% humidity and re-isolated on pea-based agar medium in tubes placed at 26°C in darkness. The zoospores formation was performed by incubating these cultures in Roux flasks of 15×25×7 cm for 6 days in complete darkness and alternatively 5 days minimum in dark (12 h) and fluorescent light (12 h) (Tahi *et al*., 2006a, 2007).

Leaves Collection and Discs Confection

Four healthy green leaves about 2 months of age were taken between 6:30 am and 9:00 am (Tahi *et al*., 2007) per hybrid plant and control clone. They are attached to peduncles

and semi-lignified plagiotropic twigs growing in medium-shaded zones in the canopies (Nyassé *et al*., 1995; Tahi *et al*., 2006b). Leaves of each tree were placed in a numbered plastic bag in which a few drops of distilled water were sprayed before hand. Leaves were then placed in an ice box containing humidified plastic foam for maintaining at 100% relative humidity. In laboratory, leaves being placed in bags were kept in the dark till next morning. This was done to minimize any effect of leaf sampling time that may occur with large time lapses between harvesting of leaves (Tahi, 2003). The following morning, 10 discs of 15 mm in diameter were cut in the lamina of each leaf. The 40 discs from the same tree were mixed and aligned in randomized rows of 10 discs, abaxial face up (Nyassé *et al*., 1995), on wetted plastic foam in each of the four trays of 70×60×10 cm (length×width×height) used. The plastic foam of one cm thick was imbibed with 2.5 L of distilled water (Tahi *et al*., 2006a).

Preparation of Inoculum and Inoculation of Leaf Discs

The inoculation was carried out immediately, after leaf discs confection. Forty milliliter of sterile distilled water, maintained at 4°C, were added to a culture of the isolate in Roux flask incubated on V8 medium at 26°C, alternately, for 5 days minimum in the dark and under white light. The liberation of zoospores by sporocystes was induced by keeping the culture in the fridge for 15 min then under incandescent light of 60 Watts (220-240 volts) for 40 min minimum at lab room temperature. The suspension was calibrated with a heamacytometer or MALASSEZ cell (SOVIREL, Paris, France). The final concentration of zoospores in the inoculum was adjusted to 3.10^5 zoospores per ml (Nyassé *et al*., 1995) with distilled water conserved at 4°C. The abaxial leaf surface, which is more susceptible than the adaxial one and through which natural infections more frequently occur (Iwaro, 1995) was inoculated by depositing in its middle, using a micropipette, 10 μL of spore suspension (corresponding to a drop). The discs of each plant, placed in rows, were inoculated transversally to the rows, so as to inoculate a disc per plant successively (Nyassé *et al*., 1995) and to randomize any effect of the spore batch over the different genotypes (Tahi *et al*., 2006a). The trays containing the inoculated leaf discs were sealed hermetically with dark plastic film to maintain darkness inside and 100% relative humidity. The covered batches were placed in laboratory for inoculated discs incubation for 7 days, avoiding direct sun light, at a controlled temperature of about 26-28°C with an air conditioner (Nyassé *et al*., 2002; Tahi *et al*., 2007). After incubation, symptoms due to zoospores attacks that occurred on leaf discs were scored according to the necrosis size (Nyassé *et al*., 1995; Nyassé, 1997) using the following scale:

0 = No symptoms
1 = Very small localized penetration points
2 = Small penetration spots, sometimes in a network
3 = Coalescing lesions of intermediate size or web like patch
4 = Large coalescing brown patches or mottled patch
5 = Uniform large dark brown lesions, expanding outside the area covered by the inoculation droplet or true patch

Three inoculation series were conducted for each family, F1 and F7, from October 2006 to January 2008, in the phytopathology laboratory of CNRA research station in Bingerville/Côte d'Ivoire. The first serie was realized between October 2006 and January 2007, the second between July and August 2007 and the third between November 2007 and January 2008. Due to the limited capacity of the batches, trees of each family were split into 3 sets (set 1, 2 and 3). So, a given serie comprised 3 inoculations trials corresponding to the

3 sets per family, which gave 9 inoculations trials per family for the 3 series and in total 18 inoculations trials for both families. During a given series, the 3 inoculations trials within a family were carried out either continuously or alternately with the 3 series of the other family. Four replicates, represented by the 4 batches used, were carried out for each set (per inoculations trial) with the same number of individuals from a serie to another. When performing each of the 18 inoculations trials, the 3 control clones were subjected to the same treatment like the hybrids. One-hundred and twenty leaf discs were inoculated per genotype for all the 3 series.

Statistical Analysis

The average disease scores obtained for each hybrid genotype in a set for all 4 batches and in a given serie were corrected by multiplying with an adjustment coefficient. This allows us to analyze all scores together and obtain a better estimation of the genetic value of plants in the same progeny. The coefficient of adjustment is the ratio of the mean score for the 3 control clones obtained over all inoculations of the serie over the mean value of the same 3 control clones obtained for the set. The corrected disease mean score of the 10 discs for each hybrid or each control clone per batch was used in statistical analyses. Normality of average scores distributions and homogeneity of variances were verified using Shapiro-Wilk test following the univariate procedure of SAS. The leaf discs test results repeatability was checked by calculating Pearson's and Spearman's coefficients of correlation between the 3 inoculations series conducted for each of the 3 sets of plants within a given family using the procedure CORR of SAS. Pearson's coefficient of correlation was used to compare mean scores of a plant whereas Spearman's rank correlation coefficient was used to compare ranks of that plant. The effects of genotypes, families, series as well as their interactions were tested using the SAS v6.12 (1993) package with procedure GLM. All corrected scores were analyzed together in a type III of a one-way Analysis of Variance (ANOVA) with regard to the differences in sizes of the sets of plants tested. The multiple comparisons of individual corrected mean scores was performed using the Newman and Keuls test at 5% probability level. Trees were grouped in resistance or susceptibility classes according to the 5 levels applied by Paulin *et al.* (2008) and Thevenin and Motilal (1998). The genetic gain was determined by selecting trees more resistant than SCA6. Successful Infection rate (% SI) was estimated per family. That is the percentage of number of plants with disease mean scores superior to 2.5 over the total number of plant tested per family. The Index of Resistance to Black Pod (IRBP) was also estimated.

RESULTS

Distribution of Disease Scores

The statistical analysis showed normal distributions ($p>0.05$) of corrected average disease scores in both families, F1 and F7. The plot of the corrected average scores of plants of each family, F1 and F7, in responses to infection by *P. palmivora* against the numbers of these plants is shown in Fig. 1. On this plot, frequencies distributions were continuous following normal distributions patterns.

Repeatability of Results

Pearson's Correlation Coefficient

The Pearson's coefficient of correlation obtained for the three inoculations series concerning F1 were positive and highly significant ($r_1 = 0.2056$, $p = 0.0001$), ($r_2 = 0.2920$,

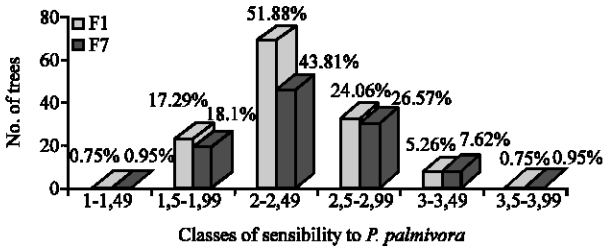

Fig. 1: Frequencies distributions of corrected mean scores of resistance to *P. palmivora* obtained on foliar discs for the 133 and 105 individuals of both families F1 and F7

Table 1: Coefficients of correlation and associated probabilities for mean scores obtained between the 3 series of inoculations for the 133 and 105 trees of both families F1 and F7

	Pearson's correlation coefficient		Spearman's correlation coefficient	
Family	Serie 1	Serie 2	Serie 1	Serie 2
F1				
Serie 2	0.2056*** (0.0001)		0.2033*** (0.0001)	
Serie 3	0.2920*** (0.0001)	0.2836*** (0.0001)	0.2871*** (0.0001)	0.2588*** (0.0001)
F7				
Serie 2	0.2232*** (0.0001)		0.2026*** (0.0001)	
Serie 3	0.3269*** (0.0001)	0.3290*** (0.0001)	0.3322*** (0.0001)	0.2693*** (0.0001)

***Significant at $p<0.0001$. Values between parentheses are probabilities associated to the different coefficients of correlation

$p = 0.0001$) and ($r_3 = 0.2836$, $p = 0.0001$) between series 1 and 2, 1 and 3, 2 and 3, respectively. The Pearson's coefficient of correlation obtained for the 3 inoculations series concerning F7 were positive and highly significant ($r_1 = 0.2232$, $p = 0.0001$), ($r_2 = 0.3269$, $p = 0.0001$) and ($r_3 = 0.3290$, $p = 0.0001$) between series 1 and 2, 1 and 3, 2 and 3, respectively. Albeit fairly low, all coefficient of correlation values were significant, in both families. The coefficients of correlation between series 1 and 3, on one hand and between series 2 and 3 on the other hand, were higher than that obtained between series 1 and 2 (Table 1).

Spearman's Correlation Coefficient

The Spearman's rank coefficient of correlation obtained for the 3 inoculations series concerning F1 were positive and highly significant ($r_1 = 0.2033$, $p = 0.0001$), ($r_2 = 0.2871$, $p = 0.0001$) and ($r_3 = 0.2588$, $p = 0.0001$) between series 1 and 2, 1 and 3, 2 and 3, respectively. The Spearman's rank coefficient of correlation obtained for the 3 inoculations series concerning F7 were positive and highly significant ($r_1 = 0.2026$, $p = 0.0001$), ($r_2 = 0.3322$, $p = 0.0001$) and ($r_3 = 0.2693$, $p = 0.0001$) between series 1 and 2, 1 and 3, 2 and 3, respectively. Albeit fairly low, all coefficient of correlation values were significant, in both families. The coefficients of correlation between series 1 and 3 and between series 2 and 3, were higher than that obtained between series 1 and 2. Moreover, the coefficient of correlation between series 1 and 3 was higher than that obtained between series 2 and 3 (Table 1).

In F1, series 1 and 3 were ranged in the same resistance group, a, with respective index, 2.4975 and 2.4581, superior to that of serie 2 that is 2.0757 (Table 2). In F7, series 1 and 3 were ranged in two different resistance groups, b and a, respectively. In this latter family, index of series 1 and 2, which are 2.2638 and 2.2834, respectively, are inferior to that of serie 3 being 2.4868.

Table 2: Variations of mean scores of both families (F1 and F7) and of the 3 control clones (SCA6, PA150 and NA79) for all inoculations series

Family and clones	Inoculations series			
	1	2	3	Global mean
F1	2.4975	2.0757	2.4581	2.3438
F7	2.2638	2.2834	2.4868	2.3446
SCA6	1.7608 (with F1)	1.5450 (with F1)	1.8108 (with F1)	1.7028 (with F1)
	1.6500 (with F7)	1.4692 (with F7)	2.1308 (with F7)	1.7400 (with F7)
PA150	2.1792 (with F1)	1.7392 (with F1)	2.0400 (with F1)	1.9845 (with F1)
	1.7758 (with F7)	1.5091 (with F7)	1.9392 (with F7)	1.7362 (with F7)
NA79	2.9358 (with F1)	2.5558 (with F1)	2.6958 (with F1)	2.7300 (with F1)
	2.5717 (with F7)	2.5550 (with F7)	2.8858 (with F7)	2.6652 (with F7)

Table 3: Global susceptibilities to *P. palmivora* measured on leaf discs in both progenies F1 and F7 compared with those of control clones SCA6, PA150 and NA79

F1, F7 and control clones	N	CV (%)	Mean	Class
F1 and the 3 controls				
SCA6	36	4.6290	1.7056	a
PA150	36	10.6126	1.9861	b
F1	1596	14.0777	2.3438	c
NA79	36	6.2012	2.7292	d
F7 and the 3 controls				
PA150	36	2.4852	1.7414	a
SCA6	36	6.4455	1.7500	a
F7	2596	15.1957	2.3446	c
NA79	36	2.3275	2.6708	

N: No. of scores; CV: Coefficient of variation; Mean: Mean score. Different letters indicate significant differences according to Newman and Keuls test, at 5 % probability applied to correct average scores

Comparisons of Resistance Levels of Both Families with Those of Control Clones

The overall average reaction of hybrids plants of both families F1, F7 and of the three control clones SCA6, PA150 and NA79, comparatively to the three inoculations series is shown in Table 2. At the probability of 5%, Newman and Keuls test showed that resistance levels of the two families F1 and F7 were superior to that of the susceptible control NA79 and inferior to those of control clones SCA6 and PA150, known to exhibit high resistance levels to *Phytophthora* sp. The clone SCA6 was the most resistant followed by PA150, when used to assess the plants of family F1. The clones SCA6 and PA150 exhibited the same resistance level when used to assess plants of family F7. The clone NA79 remained susceptible.

Variation of Foliar Resistance Within Progenies and between Control Clones

Reactions to *P. palmivora* by inoculating leaf discs with suspension of zoospores varied significantly ($p = 0.0001$) between the 133 plants of F1, between the 105 plants of F7 and the 3 control clones (Table 4). Significant effects «serie» ($p = 0.0001$), on one hand and highly significant interactions serie x genotype ($p = 0.0001$), on the other hand, were observed in F1 and F7. The corrected average disease scores varied from 1.4875 to 3.7125 and from 1.4908 to 3.5800 in F1 and F7, respectively. The average Coefficients of Variation (CV) of disease scores were 14.0777 and 15.1957% in F1 and F7, respectively (Table 3). The highest average CV for SCA6, PA150 and NA79 were 6.4455, 10.6126 and 6.2012% (Table 3), respectively.

The grouping of the plants based on their resistance or susceptibility levels is shown as follows (Table 5). In both families, no plant was classified Highly Resistant (HR). On the contrary, in F1 25 trees were classified resistant (R), 69 Moderately Resistant (MR), 38 susceptible (S) and 1 highly susceptible (HS). Likewise, in F7 22 trees were classified resistant (R), 44 moderately resistant (MR), 38 susceptible (S) and 1 highly susceptible (HS).

Table 4: Analysis of variance of corrected average scores obtained for the 133 and 105 trees of both progenies F1 and F7

	F1				F7			
Source of variation	df	MS	F	Pr > F	df	MS	F	Pr > F
Serie	2	28.8743	265.23	0.0001	2	6.4128	50.52	0.0001
Genotype	132	1.8656	17.14	0.0001	104	2.1551	16.98	0.0001
Genotype x serie	264	0.7336	6.74	0.0001	208	0.7873	6.20	0.0001
Error	1197	0.1089	-	-	945	0.1269	-	-
Total	1595	-	-	-	1259	-	-	-

df: Degree of freedom; MS: Mean square; F: Calculated value of Fischer's test

Table 5: Distributions of trees of both progenies (F1 and F7) according to the resistance levels established by Paulin *et al.* (2008)

	Resistance levels					
Family	HR	R	MR	S	HS	Disease interval index
F1	0 (0%)	25 (18.80%)	69 (51.88%)	38 (28.57%)	01 (0.75%)	1.4875 à 3.7125
F7	0 (0%)	22 (16.54%)	44 (33.08%)	38 (28.57%)	01 (0.75%)	1.4908 à 3.5800

Highly resistant (HR): 0≤Index≤1; Resistant (R): 1<Index≤2; Moderately Resistant (MR): 2<Index≤2.5; Susceptible (S): 2.5<Index≤3.5; Highly Susceptible (HS): 3.5<Index≤5. The index corresponds to average scores obtained for a given plant or a family. Values between parentheses represent the respective percentages of the plants per resistance level

Table 6: Distributions of individuals of both progenies F1 and F7 according to their reactions to *P. palmivora* measured on leaf discs and compared with those of the 3 controls (SCA6 and PA150 resistant and NA79 susceptible)

	No. of individuals with resistance level	
Family	Superior or equal to that of resistant clone having the highest disease note	Inferior or equal to that of NA79
F1	24 (18.06%)	21 (15.79%)
F7	05 (04.76%)	21 (20%)

Values between parentheses represent the percentage of the individuals

The resistance levels of plants of each progeny were compared with those of the three control clones. In that respect, 24 and 5 trees, of F1 and F7, respectively, were more resistant than the control clone having exhibited the highest disease score. This resistant clone was either SCA6 or PA150. Twenty-one plants of each progeny were more or equally susceptible as the susceptible control (NA79) (Table 6). In F1, 67 plants, representing 50.34%, were more resistant than the entire family. Likewise in F7, 58 plants, representing 55.24%, were more resistant than the entire family.

The Newman and Keuls test ranged, at the threshold of 5%, the plants of F1 in 65 classes and those of F7 in 44 classes. Each class comprised at most 3 plants in F1 as well as in F7. The average sensibility score of plants varied from a class to another.

Variation of Foliar Resistance Between Progenies

The analysis of variance performed with the global average disease scores, minima and maxima scores and the coefficients of variation in F1 as well as in F7 showed no significant difference ($p>0.05$). So, the same structure was observed in both progenies regarding the different resistance or susceptibility classes of the plants.

Index of Resistance to Black Pod (IRBP)

The index of resistance to black pod (IRBP) was expressed as the percentage of resistant plants to black pod. The so-called trees i.e., the highly resistant (HR), resistant (R) and Moderately Resistant (MR) were used for calculating this index (Table 5). In F1 and F7, respectively, 94 and 66 plants were considered. Thereby, IRPB was 70.68% in F1 while it was 62.86% in F7.

Rates of Successful Infections (% SI)

The rates of Successful Infections (% SI) were arcsine square-root transformed (arcsine v√SI) before analysis, since these proportions were not established from the same number of individuals. In each family, 39 plants had disease mean scores superior to 2.5 (Table 5). The rates of successful infections were, 29.32 and 37.14%, in F1 and F7, respectively.

Genetic Gain

The clone SCA6 is known for exhibiting high resistance level to *Phytophthora* sp. In this study, its reaction remained stable with both progenies. The individuals with resistance levels higher than that of SCA6 were chosen for genetic gain estimating. Thus, in F1 the genetic gain was 69.40% for 7 plants more resistant than the control SCA6. Likewise in F7, genetic gain was 69.30% for 5 plants more resistant than the control SCA6.

DISCUSSION

This study is the first that was carried out using leaf discs test in standardized conditions described by Nyassé *et al.* (1995) and Tahi (2003) to assess resistance to *Phytophthora palmivora* of two related cacao progenies of adult hybrid plants with the aim of selecting individual trees growing in natural environment.

The continuous variation observed in scores distributions for both progenies indicated that foliar resistance to *Phytophthora palmivora* is polygenically determined, hence additive. Oligogenic inheritance of resistance to *Phytophthora* was also established by Ndoumbé *et al.* (2001), Tahi *et al.* (2006a) and Nyassé *et al.* (2007).

In each progeny studied, differences in the reactions of plants to *P. palmivora* measured by the leaf discs test were highly significant through the three inoculations series. The important numbers of classes of plants established, 66 in F1 and 44 in F7, are an index. Such differences were reported in previous studies with plants maintained in nurseries conditions (Tahi *et al.*, 2007; Efombagn *et al.*, 2007) and in field (Tahi *et al.*, 2006b). All inoculations series were highly and positively correlated. However, the Pearson's coefficients of correlation varied from 0.2056 to 0.2920 and from 0.2232 to 0.3290 in F1 and F7, respectively. The Spearman's coefficients of correlation varied from 0.2033 to 0.2871 and from 0.2926 to 0.3322, in F1 and F7, respectively. Furthermore, significant serie effects and significant interaction genotype x serie effect were observed. These effects were preferentially due to the weak values of coefficients of correlations obtained. Tahi *et al.* (2006a) shown that leaves of clonal trees planted in rows in field were less influenced by environmental variations than leaves of hybrid cacao trees planted in totally randomized single-tree mating design. The weak coefficient of correlation values could be explained by the fact that these correlations were estimated between relatively high numbers of individual plants (133 and 105). Similar values of correlation were observed by Iwaro *et al.* (1997) in 32 families and Flament *et al.* (2001) in two related families. In our study, the evaluation was performed on individual adult plants maintained in natural selection conditions. In these conditions, since cacao trees do not always flush at the same time, it was not always easy to find on all trees, at the same time, much leaves, in suitable development stage. In addition, the inoculations series were carried out at different periods of the year. The different environmental conditions influenced slightly the absolute stability of the reactions of the plants. Efombagn *et al.* (2007) and Pokou *et al.* (2008) did not observe similar effects evaluating entire families maintained in nurseries. In entire families where each genotype is represented by many trees, choosing plants carrying numerous leaves suited for performing leaf discs test is possible. This choice

is easier when progenies are set up in strictly controlled environments like greenhouses or nurseries. In latter conditions, correlation values were higher (Tahi *et al*., 2006b; Efombagn *et al*., 2007; Pokou *et al*., 2008).

Both progenies were overall more susceptible to *P. palmivora* than the resistant control clones SCA6 and PA150, but less susceptible than the susceptible clone NA79. However, each progeny included both more resistant trees than the two resistant controls and more susceptible trees than NA79. So, effective selection of plants using leaf discs test is possible with important genetic gains estimated about 70% compare to SCA6, despite the significant serie effects and interaction genotype x serie effect observed. In both progenies, IRPB was markedly superior to IR, especially for F1 in which the ratio was 2.41. More than the half of the number of the plants of each progeny was more resistant than the entire family. We reported 50.34 and 55.24% of plants in F1 and F7, respectively. Further, some plants were more resistant than the grand-parent P7 whereas some others were more susceptible than the male parent C1, taking into account data obtained in previous studies carried out according to the same assessment process (Kébé, 1997). Transgressive variation for foliar resistance to *P. palmivora* was noted. This phenomenon was also signalized by Iwaro *et al*. (1997). In that respect, the transgressive segregation observed is attested by the additive nature of resistance inheritance. Selections of resistant plant material to *P. palmivora* may be envisioned using leaf discs test in all hybrid progenies. Thevenin and Motilal (1998) and Paulin *et al*. (2008) established, in two similar studies, two resistance classification scales. The proportions of plants absolutely resistant obtained by adopting a given scale were identical to those obtained with the other scale. These proportions were 18.06 in F1 and 16.54% in F7. They are close to that (18.75%) of a cross between two double heterozygous genotypes involving two unlinked loci. The same proportion was obtained (19.75%) when plants of both progenies are pooled. These resistant plants might represent a part of individuals expressing the recessive allele of one gene and the dominant allele of the other gene. This leads to infer that several recessive alleles would be responsible of the resistance to *Phytophthora*. The different combinations of these alleles, based on their number and nature, might support the different categories of resistant plants i.e., the Highly Resistant (HR), the Resistant (H) and the Moderately Resistant (MR). The higher the number of these recessive alleles in a genotype the higher the resistance level of that genotype. Warren and Pettitt (1994) indicated that foliar resistance in cacao to *P. palmivora* was influenced by a minimum of 5 unlinked heterozygous loci. These alleles would be from nuclear origin as indicated by the vertical transmission of the resistance and the absence of cytoplasmic inheritance found out in Côte d'Ivoire by Tahi *et al*. (2006a) and Costa Rica by Rodriguez *et al*. (1985). Many genotypes HR, R and MR exist and are scattered in all germplasms worldwide owing to the likely much greater number of allele's combinations. This assertion is confirmed by the discovery of other trees more resistant than SCA6 by Efombagn *et al*. (2007), Pokou *et al*. (2008) in cocoa collections and farmers' accessions in Cameroun and in Côte d'Ivoire and by Paulin *et al*. (2008) in wild cocoa trees populations of French Guiana.

Similarity of reactions of plants of both populations and the high segregation observed in plants reactions were predictable. Indeed, the progenies are relatives since they derived from the same cross pattern and further, the resistant clone P7 and the susceptible clone C1 were used as female grand-parent and male parent, respectively. The difference results from the male grand-parent crossed with P7. In case of F1, the clone ICS100 was used whereas in the case of F7, the clone ICS95 was used. They are two moderately resistant clones belonging to the same cocoa group (Trinitario) and selected in the same conditions in

Trinidad. Cocoa trees of these two progenies are then half-sib. Although, the plants of both progenies were not evaluated together i.e., in the same batches, the comparison of their different scores is applicable since these plants grew in the same trial and were assessed following the same process. The different assessment series were performed at the same period with the same controls.

The ranking of the three control clones was identical to those established by Tahi *et al.* (2006b), Efombagn *et al.* (2007) and Pokou *et al.* (2008). SCA6 was the most resistant followed by PA150 when controls were treated jointly with cacao trees of F1. SCA6 and PA150 exhibited the same resistance level like in Nyassé *et al.* (2007) when they were treated jointly with cacoa trees of F7. The susceptible clone NA79 remained susceptible. However, this latter clone was classified highly susceptible according to the resistance levels scale established by Paulin *et al.* (2008). This same clone was classified moderately susceptible according to the resistance levels scale adopted by Thevenin and Motilal (1998). The weak values of the average coefficients of variation observed for the control clones shows the good stability of their reactions vis-à-vis to *P. palmivora*.

CONCLUSIONS

The results revealed important variations in the reactions assessed on leaf discs of adult cacoa plants of each progeny growing in natural environment to *P. palmivora*. Some trees exhibiting higher resistance levels than resistant controls, SCA6 and PA150, were identified. These trees may be selected, owing to the performance mentioned above, or subjected to further evaluations for confirmation and to serve as potential new genitor clones in *Phytophthora* sp. genetic resistance breeding programs. They may also provide budwoods for replacements in fields.

Present results also confirmed the polygenic nature of resistance to *Phytophthora*. Moreover, they suggest the only implication of numerous recessive alleles whom the different combinations would determine the different resistance levels of genotypes. Mapping these alleles, by investigating the existing cocoa populations, will speed up and enhance the improvement of this trait in cocoa. Molecular markers such as Simple Sequence Repeat (SSR or microsatellites), highly polymorphic, abundant and widely distributed in genome could be most convenient for this type of study.

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