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# Detection of genetic resistance to cocoa black pod disease caused by three *Phytophthora* species

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**Abstract** Disease has become a major limiting factor for the production of cacao crops. Black pod disease, which is caused by *Phytophthora* spp., has caused losses of 40 % of the worldwide production of cacao crops. The most efficient way to control black pod disease is to use resistant crop varieties. In this study, a total of 262 genotypes obtained from F1 cacao segregating progeny (TSH 1188 × CCN 51) were evaluated for their genetic resistance to infection by three species of *Phytophthora*. The descriptive estimates of resistance were significant ($p < 0.01$), and a high level of heritability was observed for *Phytophthora* spp. ($h^2 = 0.759$ for *P. citrophthora*, $h^2 = 0.839$ for *P. palmivora*, $h^2 = 0.799$ for *P. capsici*). Statistically distinct homogeneous groups ($p < 0.01$; Scott–Knott) were observed. Ten genotypes that are resistant to *Phytophthora* spp. were identified. The frequency of the individuals within each homogeneous group suggests that resistance to black pod disease is oligogenic. Our results, which suggest that resistance to black pod disease in cocoa trees is most likely oligogenic, have extremely important implications for cocoa breeding programs. Resistance to the various species that cause black pod disease in cocoa is associated with genetic variability. This result is very important for cocoa breeding programs that aim to use molecular markers to increase genetic selection gain per unit time. The genotypes of the cocoa progeny segregating in the F1 generation (TSH 1188 × CCN 51) are very useful in studies aimed at increasing cocoa resistance to black pod disease.

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## Introduction

Cocoa (*Theobroma cocoa* L.) is a perennial tree that is typically grown on small farms in the tropics. The primary cocoa product of interest is the seed, which serves as the raw material for the manufacture of chocolate. Disease has become a major limiting factor for cocoa crops, and three of the main diseases caused by fungi are responsible for an annual 40 % loss of production worldwide: black pod (*Phytophthora spp*.), witches' broom (*Moniliophthora perniciosa*) and frosty pod rot (*Moniliophthora roreri*) (Fulton 1989; Evans 2007). Within the state of Bahia in Brazil (the largest producer of cocoa in the country), black pod disease has had an economic impact since 1993, when production losses of 70–80 % were recorded (Oliveira and Luz 2005). Black pod disease is greatly influenced by unfavorable environmental conditions, particularly during the colder months of the year when the average minimum temperature is less than 20 °C and humidity is greater than 85 % (Oliveira and Luz 2005; Luz and Silva 2001).

Black pod, or *Phytophthora* pod rot, occurs in all cocoa-producing regions and can cause significant production losses depending on the fungal species and area of incidence (Campello and Luz 1981; Bowers et al. 2001). Black pod disease is caused by several species of *Phytophthora*, a genus of the Oomycota belonging to the order Pythiales and the family Pythiaceae. This genus is known as the "plant destroyer," and it consists of more than 60 species that are all considered to be pathogenic and highly destructive (Luz and Silva 2001; Campello and Luz 1981). To date, seven species of *Phytophthora* have been shown to be related to the etiology of black pod disease in the cocoa tree: *P. katsurae* (Ko and Chang), *P. megakarya* (Brasier and Griffin), *P. megasperma* (Dreschler), *P. citrophthora* (R.E. Smith and E.H. Smith), *P. heveae* (Thompson), *P. capsici* (Leonian), and *P. palmivora* (Butler) (Luz and Silva 2001; Luz et al. 2005; Appiah et al. 2004).

The seven species of *Phytophthora* have been shown to be related to black pod disease in the cocoa tree; only four were registered as being the species that causes black pod in Brazil: *P. capsici*, *P. palmivora* and *P. citrophthora* (Campello and Luz 1981). Among the species known to cause black pod disease in Brazil, *P. citrophthora* is the most virulent, followed by *P. palmivora*, and *P. capsici*, which is the least virulent species (Campello et al. 1982; Santos and Mendes 1967; Santos et al. 2009). In the state of Bahia, Brazil, *P. capsici* was the predominant species in 95 % of the attacks on cocoa fruits between 1977 and 1981. However, in the 1980s, variations in the population distribution of *Phytophthora spp*. were observed (Luz and Silva 2001), with a demonstrated tendency for *P. palmivora* and *P. citrophthora* predominance (Luz et al. 2003). *P. heveae* has also been found in Bahia, but its pathogenicity in cocoa trees is considered to be moderate (Luz and Silva 2001).

*Phytophthora palmivora* and *P. capsici* have a pantropical distribution; thus, they are key cocoa tree pathogens, and they are the species most associated with the loss of production due to black pod in the world (Nyassé et al. 1995). *P. palmivora* can infect almost all the parts of the cocoa plant, making it one of the most important plant pathogens in warmer climates worldwide (Iwaro et al. 1997). *P. capsici* is the only species that has not yet been isolated from the roots of cocoa or its surrounding soil (Luz et al. 1992). Its life cycle is assumed to occur entirely in the shoots of the cocoa tree, and it survives on infected branches and leaves. *P. citrophthora* requires minimal time for zoospore germination and for the penetration of unwounded detached pods (Campello et al. 1982).

Cocoa pods are susceptible to black pod disease at all developmental stages. The infection begins with small chlorotic spots that become dark brown and then stretch rapidly to form dark-brown lesions. The spots can fully overtake the fruit's surface in 10–14 days, depending on the cocoa clone and *Phytophthora spp*. involved (Luz and Silva 2001). The pod eventually becomes black and mummified and develops a characteristic fishy smell. In the advanced stages of infection, the fungus invades the pod's internal tissue and causes discoloration and deformation of the cocoa beans (Bowers et al. 2001; Santos and Mendes 1967), which makes the beans unsuitable for industrial use. Gregory and Maddison (1981) claimed that rain is an essential factor for sporangia dispersion and infection by *Phytophthora spp*.; according to Medeiros (Medeiros 1967), the presence of sacking coverage is

correlated with an increased disease incidence. Infected pods remaining on the plant are another source of inocula; a single infected pod can produce eighty thousand new zoosporangia of *P. palmivora*/cm$^2$ after 5 days of infection under appropriate conditions. In Papua New Guinea, it has been demonstrated that flying beetles are an important component of the disease cycles of *P. palmivora* cocoa diseases (Konam 2004). Beetles are involved in the dissemination of the pathogen, which results in the development of pod rot lesions. It has been shown that beetles can rapidly increase the pool of secondary inocula during an epidemic (Konam 2004).

Measures that have been used to control black pod disease include phytosanitary pruning and the application of fungicide. The phytosanitary pruning, while effective, adds to production costs; fungicide applications increase the risk of environmental pollution. The use of resistant clones and suitable crop management, including pruning and sanitation, is necessary for sustainable cocoa yields and is a more effective alternative for the control of this disease (Nyassé et al. 2003; Pokou et al. 2008). However, successful resistance to black pod disease appears to be unachievable, and many existing commercial cocoa genotypes remain susceptible due to the prevalence of different *Phytophthora spp*. in cocoa crop regions, the frequent and aggressive differentiation of *Phytophthora spp*. and the lack of genetic variability in breeding programs for black pod tolerance (Bartley 1986). In Papua New Guinea, resistance to *P. palmivora* appears to be under polygenic control, predominantly by additive genetic effects, with four genes estimated to control resistance in the leaves (Dias 2001). In Brazil, cocoa resistance to *Phytophthora spp*. is likely oligogenic (Luz et al. 1996) and Iwaro et al. (1997) demonstrated that two levels of resistance to *P. palmivora* occur: at the penetration level, which controls the lesion number, and at the post-penetration level, which determines the lesion size.

Various methods of assessing planting materials have been tested to determine their genetic resistance to black pod disease. In addition to field observations, different types of artificial inoculation tests have been used (Nyassé et al. 1995; Iwaro et al. 1998), and considerable effort has been expended for the development and improvement of resistance evaluation methods, including the inoculation of pods, leaves and leaf discs (Nyassé et al. 1995; Cilas and Despreaux 2004; Iwaro et al. 2006; Nyassé et al. 2002; Tahi et al. 2000, 2006). The leaf disc test has recently become a routine method for screening for resistance to black pod disease. When conducted under standardized conditions, this test is significantly correlated with field infection levels (Nyassé et al. 2003; Tahi et al. 2000, 2006). Thus, the selection of resistant planting materials can be considerably accelerated using leaf or leaf disc inoculation.

A limited number of studies have been performed to compare the susceptibility of cocoa trees to various *Phytophthora spp.* (Luz et al. 1996). One important strategy for determining durable resistance involves the development or selection of varieties with a large number of genes associated with resistance to black pod disease. Thus, the aim of this study was to evaluate the genetic resistance of the progeny from a cross of Trinidad selected hybrids and Coleccion Castro Naranjal clones (TSH 1188 × CCN 51) in response to an infection by *Phytophthora spp*.

## Materials and methods

The biological materials for this experiment were obtained from a cocoa population and consisted of leaves from 262 genotypes obtained from the segregation of F1 progeny derived from a cross between the clones TSH1188 and CCN51. This population is maintained at the MARS Center of Cocoa Science (MCCS) located in Barro Preto, Bahia at 14°42′45.021171″S and 39°22′13.008369″W. The TSH1188 and CCN51 clones were selected because they have contrasting agronomic traits for productivity, sexual incompatibility and disease resistance. Moreover, their genotypes are included in an international research program that involves institutions in Brazil, Costa Rica and the United States. The clone TSH1188 (Trinidad selected hybrids) produces rough red fruits (CEPEC 1987) and has genetic self-incompatibility. TSH1188 originated from a cross involving the IMC67, ICS1, SCA6 and P18 clones. The clone CCN51 (Coleccion Castro Naranjal) produces purplish-red immature fruits that become yellow-orange when ripe with slightly wrinkled rinds and clear purple interior seeds. CCN51 is derived from an F1 plant obtained from a cross between IMC67 and ICS95 crossed with the native Ecuadorian clone “Canelos.” CCN51 is related to the Iquitos, Criollo and

Amelonado genetic types and has been an ideal parent in several breeding and selection programs worldwide (Pugh 2005; Boza et al. 2014). Furthermore, CCN51 is resistant to witches' broom. We obtained individuals by controlled pollination, and the female flowers were protected for 24 h before pollination to avoid pollen contamination. Each seedling was identified and planted in several 3 × 3 m plots in rows of 25 plants each.

The artificial inoculation method and disease ratings used for the *Phytophthora spp*. in this study were developed by (Nyassé et al. 1995). Two inoculation series were conducted during the wet season with an interval of 30 days. In each inoculation series (replicate), leaves that were approximately 2 months old were collected from non-lignified twigs early in the morning. Twenty 15 mm diameter leaf discs were then cut with a semi-automatic cutter. The 20 discs of each accession were placed upside down in 8 plastic 70 × 60 × 10 cm trays on 1 cm wetted foam imbibed with 0.5 L of distilled water per tray. Each box contained a maximum of 45 of the cocoa genotypes of the F1 population and the clones TSH1188, CCN51, Sca6 and Catongo in all of the trials.

The characterization of the genetic resistance of cacao trees in response to infection by *Phytophthora spp.* was performed with the *P. palmivora*, *P. capsici* and *P. citrophthora* species. These three species were used to represent the species of interest in the South of Bahia. The experimental isolates were obtained from the *Phytophthora* culture collection in the *Phytophthora* laboratory (Phytolab) of CEPLAC-CEPEC. We used the isolates 692, 1043 and 2196 for the species *P. capsici*, *P. citrophthora* and *P. palmivora*, respectively. The zoospore suspensions for each species were obtained from *Phytophthora spp*. cultures grown on Petri dishes with carrot-water medium (*P. citrophthora*) or carrot-agar medium (*P. palmivora* and *P. capsici*) for at least 7 days. The zoospore suspensions s were obtained by adding sterile water to the plates, maintaining them at 4 °C for 25 min and exposing them to room temperature for 20 min. Each leaf disc was inoculated with 10 µL of a *Phytophthora spp*. zoospore suspension at a concentration of $3 \times 10^5$ mL-1 zoospores, which was calculated using a Neubauer chamber. The boxes were closed to maintain a relative humidity of approximately 100 %, and the temperature was kept between 24 and 26 °C. In addition, the leaf discs were not exposed to light sources. We observed the symptoms 7 days after inoculation (Santos et al. 2009) using the 5-point scale proposed by Nyassé et al. (1995) where 0 = no symptoms, 1 = very small localized penetration points, 2 = small penetration spots, occasionally in a network, 3 = coalescing lesions of intermediate size, 4 = large coalescing brown patches, and 5 = uniform large dark brown lesions (Fig. 1). Because it permits a more detailed classification of the resistance gradient in cocoa trees, the resistance gradient of the *Phytophthora spp*. was measured using a disease index (DI) (Santos et al. 2009). The DI proposed by (Mckinney 1923) varies from 0 to 1 and was calculated as follows:

$$DI = \frac{\Sigma(IL * LD)}{TNLD * HIL}$$

where IL = the infection level estimated for each leaf disc; LD = the number of leaf discs per IL; TNLD = the total number of leaf discs; and HIL = the highest infection level on the scale (Santos et al. 2009).

Statistical analyses were performed using the BioEstat 5.0 (Ayres et al. 2005) and SisVar 5.3 (Furtado 2011) programs with a randomized block design model:

$$y_i = \mu + t_i + b_j + \varepsilon_{ij}$$

where $y_i$ corresponds to the level of black pod infection, µ is the intercept, $t_i$ is the effect of treatment $i$, $b_j$ is the fixed effect of box j associated with treatment $i$, and $\varepsilon_{ij}$ is a random error term.

## Results

An analysis of variance of the DI (disease index) (Mckinney 1923) obtained from the leaf disc test indicated that the resistance levels were significantly ($p < 0.05$) influenced by the cocoa genotype and *Phytophthora spp*. isolate (Table 1) used. This result makes the selection of genotypes that are less susceptible to *Phytophthora spp*. more efficient. The genotype × *Phytophthora spp*. interaction effects were significant ($p < 0.05$). Considering the experimental conditions in this study, the observed variations are most likely associated with genetic origins. The estimates for the three evaluated *Phytophthora* species were significant, confirming that diverse cocoa genotypes react differently to specific *Phytophthora spp*. In


**Fig. 1** Leaf discs showing the progression of black pod symptoms observed by a 5-point scale (Nyassé et al. 1995): *0* no symptoms, *1* very small localized penetration points, *2* small penetration spots, sometimes in a network, *3* coalescing lesions of intermediate size, *4* large coalescing brown patches, and *5* uniform large dark brown lesions

**Table 1** Variance analysis of the disease index for black pod resistance in a cocoa population obtained from the F1 progeny segregation (CCN-51 × TSH-1188)

| Variation source | GL | Mean square | | |
|---|---|---|---|---|
| | | *P. citrophthora* | *P. palmivora* | *P. capsici* |
| Genotypes | 256 | 0.632999* | 0.514387* | 0.96913* |
| Boxes | 7 | 0.026635 | 0.023987 | 0.134886 |
| Error | 1792 | 0.016364 | 0.015005 | 0.025257 |
| Mean | | 0.597257 | 0.519167 | 0.410817 |
| CV (%) | | 21.42 | 23.59 | 38.68 |

* Significant ($p < 0.05$)

short, the means for *P. citrophthora* were higher than those of *P. palmivora*, which were higher than the means for *P. capsici*.

The CV (coefficient of variation) showed average values with little variation among the assessed species, indicating the consistency of the methodology used to evaluate the three *Phytophthora spp*., although the experiment assessing *P. capsici* was less consistent than the experiments assessing the other two species (Table 1).

The reactions of the parental clones inoculated with *Phytophthora spp*. were compared with the reactions of the clones that were used as controls, namely, Sca6 (resistant) and Catongo (susceptible). The efficiencies of clone Sca6 as a resistance standard and clone Catongo as a susceptible standard were observed. Furthermore, clone CCN51 showed a moderate resistance reaction to *Phytophthora spp*., whereas clone TSH1188 showed full susceptibility, and the estimates for clone TSH1188 did not differ significantly from that of the standard susceptible clone Catongo (Table 2). The phenotypic estimates obtained by analysis of variance (ANOVA) for the parental clones (TSH1188 and CCN51) and the segregating progeny are shown in Table 2. When the averages of the parental clones were compared to the average of the segregating progeny, we observed that the average of the progeny was different from the averages of the parental clones, suggesting the existence of dominant epistasis in black pod resistance. High heritability ($h^2$)

**Table 2** Statistical comparison of the mean disease indices for black pod resistance in the parental clones (TSH-1188 and CCN-51), the resistance (Sca-6) and susceptibility (Catongo) standards and the cocoa genotypes obtained from the F1 progeny segregation (CCN-51 × TSH-1188)

| | SCA-6 | CCN-51 | TSH-1188 | Catongo | F1 | $h^2$ |
|---|---|---|---|---|---|---|
| *P. citrophthora* | 0.000 a | 0.375 b | 0.908 c | 1.000 c | 0.597 | 0.759 |
| *P. palmivora* | 0.000 a | 0.295 b | 0.886 c | 0.933 c | 0.519 | 0.839 |
| *P. capsici* | 0.025 a | 0.121 a | 0.815 c | 0.854 c | 0.407 | 0.799 |

Means followed by identical letters do not differ significantly ($p < 0.05$)

was observed in the leaf disc test for a full-sibs family: $h^2 = 0.759$ in *P. citrophthora*, $h^2 = 0.839$ in *P. palmivora* and $h^2 = 0.799$ in *P. capsici* (Table 2), indicating a low level of environmental influences on the plant-pathogen relationship.

An analysis based on a Scott-Knott mean comparison test allowed for the identification of significantly different homogeneous groups ($p < 0.01$) (Table 3; Fig. 2). These groups reflect the variability of the analyzed genotypes with respect to their degree of resistance to black pod disease. The cocoa genotypes of the homogeneous groups A, B and C for *P. citrophthora* and *P. palmivora* and, A and B for *P. capsici* are potential sources of resistance for the development of elite cocoa genotypes. In general, these groups have mean levels of severity ranging from 0 to 0.3 (Table 4). In this study, we were able to identify potential sources of resistance from the F1 progeny (TSH1188 × CCN 51). Thus, of the 262 genotypes tested for resistance to black pod in cocoa trees, 10 genotypes showed resistance to the 3 evaluated *Phytophthora spp*. (Table 4). Studies have shown that selecting for resistance to a single species (*P. palmivora*, for example, which is common in all cocoa crop regions) provides genetic gains that improve the resistance to black pod disease caused by other *Phytophthora spp.* (Risterucci et al. 2003). In this study, the selection of the individuals most resistant to *P. palmivora* led to a genetic gain of 15 %; similarly, genetic gains of 37 % for *P. citrophthora* and 26 % for *P. capsici* were also achieved (Table 4).

**Table 3** Univariate clustering of the cocoa genotypes that were assessed for their resistance to black pod disease

| Group* | *P. citrophthora* | | *P. palmivora* | | *P. capsici* | |
|---|---|---|---|---|---|---|
| | Clone | Mean | Clone | Mean | Clone | Mean |
| A | 85 | 0.021 | 76 | 0.035 | 102 | 0.032 |
| B | 55 | 0.150 | 41 | 0.234 | 74 | 0.300 |
| C | 28 | 0.298 | 33 | 0.364 | 21 | 0.467 |
| D | 26 | 0.409 | 35 | 0.493 | 20 | 0.636 |
| E | 18 | 0.555 | 19 | 0.599 | 25 | 0.789 |
| F | 28 | 0.680 | 28 | 0.701 | 15 | 0.965 |
| G | 13 | 0.810 | 16 | 0.798 | | |
| H | 3 | 0.943 | 8 | 0.892 | | |

* Mean significant (Scott-Knott test, $p < 0.05$)

According to the classification criteria used by Paulin et al. (2008), cocoa genotypes can be considered to be highly resistant ($0 < \text{score} \leq 1$), resistant ($1 < \text{score} \leq 2$), moderately resistant ($2 < \text{score} \leq 2.5$), susceptible ($2.5 < \text{score} \leq 3.5$) or highly susceptible ($3.5 < \text{score} < 5$). In this study, modifications were made to the DI (Mckinney 1923) obtained from the leaf disc test. Our results show that there are differences in the aggressiveness of *Phytophthora spp*. against different cocoa genotypes, and among the assessed progeny, 125 were genotypes resistant to *P. citrophthora*, 140 genotypes were resistant to *P. palmivora,* and 176 genotypes were resistant to *P. capsici*. These cocoa genotypes are useful for future studies that aim to increase resistance to black pod disease in cocoa trees. The clone used as the standard of resistance (SCA6) was assigned ($DI = 0.0$) to the resistance group, verifying the use of this clone as a resistance standard (Fig. 2). Clusters C and D for *P. capsici* (38 genotypes) and clusters C, D and E for *P. citrophthora* (48 genotypes) and *P. palmivora* (56 genotypes) have genotypes with intermediate resistance (severity level $0.3 < ID \leq 0.6$). A susceptibility to black pod disease (severity level $0.6 < ID \leq 1.0$) was observed in 76 genotypes for *P. citrophthora*, 61 genotypes for *P. palmivora* and 43 genotypes for *P. capsici* in clusters E, F, G and H. The clone used as the susceptibility standard (Catongo; $DI = 1$) was assigned to the susceptible group (Fig. 2). These results reflect the differences found for the three species in the ANOVA. With respect to the parental clones, CCN51 was assigned to the group with black pod resistance, and TSH1188 was assigned to the group with black pod susceptibility. In addition, it was observed that the distributions of the cocoa genotypes in each severity level did not follow normal distributions, suggesting that resistance to black pod disease may be due to the presence of oligogenic resistance genes in cocoa trees.

## Discussion

The leaf disc test applied in this study has been widely used to assess resistance to black pod disease in cocoa trees (Pokou et al. 2008; Iwaro et al. 2003; Tahi et al. 2000; Paulin et al. 2008; Surujdeo-Maharaj et al. 2001; Tahi et al. 2007; Efombagna et al. 2007; Santos et al. 2011; Silva et al. 2008). A positive correlation

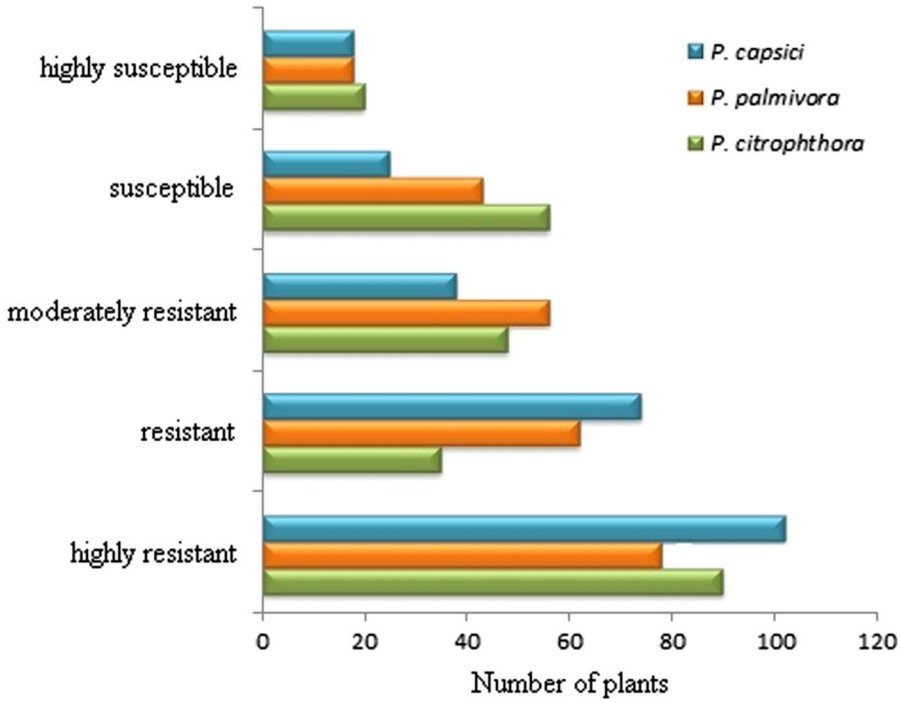


**Fig. 2** Distribution of the number of trees assessed as resistant/susceptible to black pod disease according to the classification criteria used by Paulin et al. (2008)

**Table 4** Cocoa genotypes selected for their resistance to *Phytophthora spp.* according to differential selection (the relationship between the means of the selected individuals and the progeny)

| | *P. citrophthora* | *P. palmivora* | *P. capsici* |
|---|---|---|---|
| MCCS MP01 198 | 0.000 | 0.000 | 0.000 |
| MCCS MP01 245 | 0.138 | 0.025 | 0.100 |
| MCCS MP01 250 | 0.000 | 0.025 | 0.063 |
| MCCS MP01 255 | 0.000 | 0.000 | 0.125 |
| MCCS MP01 289 | 0.000 | 0.000 | 0.000 |
| MCCS MP01 295 | 0.000 | 0.000 | 0.025 |
| MCCS MP01 304 | 0.125 | 0.038 | 0.050 |
| MCCS MP01 342 | 0.000 | 0.000 | 0.000 |
| MCCS MP01 626 | 0.244 | 0.271 | 0.000 |
| MCCS MP01 69 | 0.050 | 0.000 | 0.000 |
| MCCS MP01 786 | 0.100 | 0.013 | 0.000 |
| MCCS MP01 83 | 0.188 | 0.088 | 0.000 |
| MCCS MP01 90 | 0.300 | 0.238 | 0.200 |
| Mean | 0.088 | 0.054 | 0.043 |
| Selection differential | 15 | 10 | 11 |

Mean not significant (Scott-Knott test, $p < 0.05$)

between the data obtained by this method, and the data obtained by field analyses has been observed. In addition, anatomical similarities between the abaxial leaf side and the cocoa pod epidermis were also observed (Nyassé et al. 1995; Santos et al. 2009; Nyassé et al. 2002; Tahi et al. 2000, 2006; Iwaro et al. 1997). Therefore, this analysis provides a rapid and early assessment of resistance levels.

The coefficient of variation (CV) uses average values, but according to Costa et al. (2000), it is necessary to consider the nature of the study variables and experimental design when estimating the CV for

an experiment because the variables related to disease resistance generate wide variation when estimating the CV (Garcia 1989). The CV values ranged from 21 to 38 % in this study (Table 1), and in other cocoa tree studies the observed CV values ranged from 27 to 164 % (Efombagna et al. 2007; Ndoumbe-Nkeng et al. 2004; Bahia 2007). The experimental conditions in this study involved the selection of leaves at a developmental stage similar to that of plants grown under the same environmental conditions through the maintenance of humidity (100 %), temperature (25 °C) and the elimination of light sources, thus, these conditions helped to standardize any environmental influences.

The diverse genetic resistance of the cocoa tree genotypes in this study in response to infection by *Phytophthora spp.* has been observed by other authors (Campello et al. 1982; Luz and Yamada 1985). Campello et al. (1982) studied the response to *Phytophthora spp.* infection in 'comum' cocoa pods and found that *P. citrophthora* isolates were the most virulent, *P. palmivora* isolates showed intermediate virulence, and *P. capsici* isolates were less virulent than the *P. citrophthora* and *P. palmivora* isolates. (Luz and Yamada 1985) observed that when cocoa clones were inoculated with different *Phytophthora spp.*, those that were susceptible to black pod disease exhibited variations in the relative diameters of the lesions caused by the disease (x for *P. capsici*, 2x for *P. palmivora* and 3x for *P. citrophthora*); thus, they concluded that the lesion diameter size caused by *Phytophthora spp.* in cocoa pods is the result of pathogen/host/environment interactions, and variations may occur in each environment.

The moderate resistance to *Phytophthora spp.* shown by CCN51 can be explained by the presence of genes from the clone IMC67, which is included in the genealogy of the CCN51 clone. These genes could contribute to the expression of resistance in CCN51 (Santos et al. 2007). When the averages of the parental clones are compared with the average of the segregating progeny, the mean of the population is closer to the mean of the more resistant parent, the CCN51 clone. This supports the hypothesis of a dominant genetic action for resistance.

The high estimate heritability ($h^2$) observed in this study shows that resistance to the various species that cause black pod disease in cocoa trees was minimally influenced by the environment (Raizer and Vencovsky 1999), which indicates that the observed phenotypic variance was associated with genetic variability. In addition, the high $h^2$ values validate the use of these genotypes in future studies designed to increase the resistance of cocoa trees to black pod disease.

An analysis based on a Scott-Knott mean comparison test allowed for the identification of significantly different homogeneous genotype groups ($p < 0.01$) (Table 3; Fig. 2). These groups reflect the variability of the analyzed genotypes with respect to their degrees of resistance to black pod disease. This observed variability among cocoa genotypes with respect to their degrees of resistance to black pod disease indicates that this population is important for genetic mapping studies designed to identify the quantitative trait loci (QTLs) associated with resistance to black pod disease. This result also corroborates the observation that cocoa resistance to *Phytophthora spp*. has distinct levels.

This study also served to identify potential sources of resistance obtained from the F1 progeny (TSH1188 × CCN51) that can be evaluated for durable resistance in resistance experiments by artificial inoculation in the greenhouse or in the field. Other authors have contributed to the discovery of genotypes resistant to *Phytophthora spp.*; for example, Luz et al. (1996) tested 82 cocoa genotypes from CEPEC's Germplasm Collection in Itabuna, Bahia State for resistance to *Phytophthora spp*., and only 2 genotypes showed promising levels of resistance to the 3 species (*P. capsici*, *P. palmivora* and *P. citrophthora*). Successful black pod disease resistance seems unattainable. This problem is most likely a consequence of the existence of several *Phytophthora spp.* that cause black pod disease in cocoa growing regions, the diverse aggressiveness of *Phytophthora spp.*, and the lack of genetic variability in breeding programs.

Cacao resistance to *Phytophthora spp*. has been described as a quantitative resistance (Micheli et al. 2010); the results of this study suggest that black pod resistance in cocoa trees may be an oligogenic trait, most likely determined by a limited number of genes, corroborating with studies performed by Luz et al. (1996); Bahia (2007) and Peixoto et al. (2014) that indicate the possibility of oligogenic resistance to black pod disease in cocoa trees. For example, in Papua New Guinea, resistance to *P. palmivora* seems to be under oligogenic control, predominantly by the additive effects of four genes in its leaves (Bartley

1986). In this study, we observed that the average resistance of the F1 progeny differed from that of the parental clones, suggesting the existence of dominant epistasis in black pod resistance and observed a non-normal distribution of the cocoa genotypes at each severity level shown in Fig. 2. The characteristics that indicate oligogenic inheritance are very important for plant breeding, particularly for finding molecular markers aimed at increasing genetic selection gain per unit time (Peixoto et al. 2014).

Our results on the genetic resistance of cocoa to black pod disease caused by *Phytophthora spp* suggest that strategies for cocoa breeding programs can be considered in the following manner: first, select cocoa genotypes for the most aggressive *Phytophthora* species in the cocoa region where the cocoa genotypes are being selected; second, the previously selected genotypes should be evaluated with infections from the most predominant *Phytophthora* species in the same region; finally, the selected genotype should be evaluated by infection with other *Phytophthora* species in the region. This strategy could accelerate the process of selecting cocoa genotypes resistant to black pod disease caused by multiple *Phytophthora spp*. in breeding programs for specific cocoa regions. It should be noted that there might be differences in the resistance of different plant genotypes related to each of the three *Phytophthora* species reported in this study.

In summary, we have identified ten genotypes that are resistant to *Phytophthora spp.* that are important for the selection of black pod resistance genes. This population is of great value for genetic mapping studies for the identification of resistance-related QTLs (quantitative trait loci). Furthermore, our experimental results have suggested that the resistance to black pod disease found in cocoa may be an oligogenic trait, most likely determined by a limited number of genes. This finding is extremely important for cocoa breeding programs that aim to use molecular markers to increase genetic selection gain per unit time. In addition, the leaf disc test used in this study allowed us to ascertain the extent of cocoa clone CCN51's resistance and cocoa clone TSH1188's susceptibility to *Phytophthora spp.*

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