Association of genes from different sources of resistance to major cacao diseases

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ABSTRACT

This study aimed to select genotypes resistant to witches’ broom (WB) and black pod (BP), major cacao diseases in Brazil, as well as incorporate resistance genes to moniliasis supplemented by clones EET75 and UF273, forming populations of second-cycle recurrent selection. Moniliophthora perniciosa (2 × 10⁵ basidiospores/mL) was inoculated on 30-day-old seedlings from 72 different progenies, being assessed 60 days later, and a mixture of four isolates of Phytophthora palmivora (3 × 10⁵ zoospores/mL) was inoculated on leaf discs from 58 progenies, observing lesions after seven days. Significant effects of progeny were observed in the tests of resistance to both diseases (p < 0.05). Scavina-6 expressed resistance to both pathogens, 26 crosses did not differ from free-pollinated progenies of Scavina-6 for WB, and ten crosses were higher and 27 similar for BP. Eight crosses were largely resistant to both diseases.

Keywords: Moniliophthora perniciosa; Moniliophthora roreri; Phytophthora palmivora; plant breeding; Theobroma cacao.

INTRODUCTION

Witches’ broom (WB) (Moniliophthora perniciosa (Stahel) Aime & Phillips-Mora) and black pod (BP), caused by three species of Phytophthora (P. palmivora Butler, P. citrophthora (RE Sm. & EH Sm.) Leonian, and P. capsici Leonian), are major cacao diseases in Brazil. Also, another pathogen, Moniliophthora roreri (Cif.) Evans, Stalpers, Samson & Benny, the agent of frosty pod rot (moniliasis disease) of cacao, is an A1 quarantine pest absent with imminent risk of arrival in Brazil (Oliveira & Luz, 2012).

Obtaining genetic material resistant to these diseases with desirable agronomic characteristics, as well as organoleptic qualities that contribute to obtaining adequate chocolate quality, is the main objective of genetic improvement at present (Moreira et al., 2016; Pimenta Neto et al., 2018).

Cacao (Theobroma cacao L.) is a species of Neotropical origin in the Americas that occurs spontaneously from southern Mexico to Bolivia (Monteiro & Ahnert, 2012). This wide geographical range shows distinct edaphoclimatic conditions that allowed the development of vast genetic diversity with a varied population, representing genetic resources with the potential to obtain varieties resistant to diseases. Several cacao populations have been generated at the Cocoa Research Center (Cepec) in Ilhéus, Bahia, to obtain improved genotypes aiming at selecting clones with more durable resistance, which carry genes from different sources of resistance, as well as increasing the genetic basis in order to hinder pathogen evolution (Paim et al., 2006; Yamada et al., 2008; Lopes et al., 2011; Benjamin et al., 2016; Gramacho et al., 2016; Pimenta Neto et al., 2018).

Thus, this study aimed to form populations of second-cycle recurrent selection for resistance to WB using the North Carolina II design, crossing first-cycle selections with genetically distant and productive materials and...
with other desirable genetic characteristics, including clones with resistance to moniliasis. The formed progenies were tested for resistance to WB and BP. From crosses carried out with the combination of genes from different sources of resistance, progenies and parents resistant to major cacao diseases were selected in the formed populations.

**MATERIAL AND METHODS**

1.1 Assessment tests for witches’ broom

Twenty-two genotypes, fifteen mother plants selected in a first-cycle recurrent selection for resistance to WB, and the seven clones CSG70 (6A), BN34 (7A), SJ02 (9), MCB09 (10), RLF1938 (11), EET75 (12), and UF273 (13), being the first five clones selected in farms of the Bahia cacao region and the last two introduced in Brazil, previously selected as resistant to frost pod rot (FPR), were used as genitors. The mother plants were from the following crosses: CSUL3 x CCN10 (1), CAB301 x CCN10 (2), MO20 x CCN34 (3), CAB148 x MO20 (4), CAB157 x MO20 (5), NA33 x RB39 (6), SCA6 x P4B (7), SCA6 x RB36 (8), CEPEC86 x RB36 (1A), CA5 x RB36 (2A), CCN10 x CAB324 (3A), CCN34 x CAB301 (4A), MO20 x AMAIZ15 (5A), TSH1188 x CAB169 (9A), and SCA6 x GU114 (10A). Crosses were carried out to associate genotypes with resistance genes from different sources of Scavina – selections in progenies from CSUL3 x CCN10, CAB301 x CCN10, MO20 x CCN34, CAB148 x MO20, CAB157 x MO20, NA33 x RB39, CEPEC86 x RB36, CA5 x RB36, CCN10 x CAB324, CCN34 x CAB301, and MO20 x AMAIZ15; with resistant genotypes from Scavina – TSH1188 x CAB169, SCA6 x GU114, SCA6 x P4B, SCA6 x RB36, SJ02, MCB09, and RLF1938; and with genotypes selected as resistant to moniliasis – EET75 (12) and UF273 (13). These genotypes, selected for productivity, resistance to WB, resistance to FPR and other characteristics of interest, generated 72 progenies. The origin of the clones is shown in Table 1. Genotypes consisted of three genetic types, assigned with scores 01 and 00, respectively. The type of brooms B, i.e., terminal (TB), axillary (AB), dry (DB), and cotyledonary broom (CB), was also assessed. In addition, AB higher than 1 cm was quantified, and TB length was measured. For the data analysis, the disease index was calculated by the following Luz Index (Rodrigues et al., 2019): DI = TB + (0.1 x TBL) + AB + (0.2 x DB) + CB + (4.3 x DB), where TB is the presence of terminal broom, TBL is the terminal broom length, AB is the presence of axillary broom, NAB = number of axillary brooms higher than 1 cm, CB is the presence of cotyledonary broom, and DB is the presence of dry broom. The coefficient that multiplies DB was defined to allow the plant with dry broom having a DI higher than all the others that did not die. The coefficient for TBL was defined to allow plants with larger terminal broom growing, together with TB, a value close to two, i.e., the double the DI presented by a plant with a very small terminal broom. Similarly, the coefficient for NAB was defined to allow plants with the highest number of large axillary broom having a DI corresponding to twice the DI of plants with only axillary broom lower than 1 cm. The randomized block design was used at each inoculation or test, with 14 plants per replications (56 plants per crossing and inoculation time, repeated once with an equal number of samples).

A model with the sources of variation test or inoculation and progeny was used to analyze differences between progenies in an incomplete block system. Comparisons between the corrected means of progeny for the effects of test or inoculation were performed by the T-test (SAS, 2002). We did not consider to which genetic design or which of the diallels the progeny belonged.

Because progenitor-corrected means are not estimable in the model with the sources of variation mother, father, and test or inoculation (i.e.), the means of...
mother corrected by principle of incomplete blocks are not estimable at the same time for effects of father and test or father, corrected for mother and test because the tests mixed progenies of the three genetic designs. DI correction was applied for each test to analyze differences between progenitors. In this case, the corrected index for each plant is equal to the original index (DI) multiplied by the inverse of the sum of the means of the indices of the three controls in that test and divided by the sum of the overall means of the controls in all tests. Thus, DIs of each plant were corrected for the effect of the test to which they belong by the ratio between the mean DIs of the controls in that test and their overall mean DIs for all tests. The effects of progenitors were analyzed in the model with the sources of variation father and mother for each of the three diallels from the corrected DI. The previous model was used to compare fathers within mothers or mothers within fathers, with uncorrected DI and model with the sources of variation test or inoculation and progeny. After the assessments, diseased plants were incinerated, and healthy plants were selected to further assessment of BP resistance.

1.2 Assessment tests for black pod

Fifty-eight progenies among surviving plants and without the presence of WB symptoms from the previous experiment were selected to be tested for resistance to BP using the leaf disc method (Nyassé et al., 1995). The isolates of \textit{P. palmivora} used were 1744, 1778, 1845, and 1913, obtained from the Arnaldo Medeiros collection at Cepec, originated from cacao pods samples collected in the following counties and years: Uruçuca (2011), Camacan (2011), Mutuípe (2011) and Belmonte (2010), respectively. These isolates were selected based on their high aggressiveness to cacao among 100 \textit{P. palmivora} isolates tested in previous studies (Lessa, 2017). Healthy leaves of surviving plants from crossings were collected and taken to the laboratory of \textit{Phytophthora}, where they were sanitized and 15-mm diameter discs were cut from the leaf blade. These discs were arranged with the abaxial part up in boxes containing foam moistened with sterile water to form a humid chamber and provide favorable conditions for pathogen development.

A 10-µm aliquot of zoospore suspension from the mixture of four isolates, obtained according to the protocol

| Clone     | Abbreviation's mean | Genetic group       | Origin                  |
|-----------|---------------------|---------------------|-------------------------|
| AMAZ15*   | Amazon              | Amazonian           | Iquitos, Peru           |
| BN34      | Boa Nova            | Trinitarian         | Bahia, Brazil           |
| CA5*      | Careiro             | Amazonian           | Amazon, Brazil          |
| CAB148    | Cocoa from Brazilian Amazon | Amazonian | Acre, Brazil           |
| CAB157    | Cocoa from Brazilian Amazon | Amazonian | Acre, Brazil           |
| CAB169    | Cocoa from Brazilian Amazon | Amazonian | Acre, Brazil           |
| CAB301    | Cocoa from Brazilian Amazon | Amazonian | Amazon, Brazil         |
| CAB324*   | Cocoa from Brazilian Amazon | Amazonian | Amazon, Brazil         |
| Catongo*  | Mutation of common cocoa | Amazonian | Bahia, Brazil          |
| CCN10*    | Castro Naranjal collection | Trinitarian | Pichilingue, Ecuador   |
| CCN34*    | Castro Naranjal collection | Trinitarian | Pichilingue, Ecuador   |
| CEPEC86*  | Cocoa Research Center | Amazonian           | Bahia, Brazil           |
| CSG70     | Conjunto Serra Grande | Trinitarian         | Bahia, Brazil           |
| CSUL3*    | Southern cross       | Amazonian           | Acre, Brazil            |
| EET75*    | Tropical experimental station | Trinitarian | Pichilingue, Ecuador   |
| GU114*    | Guiana              | Amazonian           | Haut Camopi, French Guiana |
| MCBC9     | Manoel Carlos Barreto | Trinitario          | Bahia, Brazil           |
| M020*     | Morona              | Amazonian           | Morona, Peru            |
| NA33*     | Nanay               | Amazonian           | Nanay, Peru             |
| P4B*      | Pound 4 / B         | Amazonian           | Loreto, Peru            |
| RB36*     | Rio Branco          | Amazonian           | Acre, Brazil            |
| RB39*     | Rio Branco          | Amazonian           | Acre, Brazil            |
| RLF1938   | Romildo Luiz Fernandes | Trinitarian      | Bahia, Brazil           |
| SCA6*     | Scavina             | Amazonian           | Ucayali, Peru           |
| SIC23*    | Cocoa institute selection | Amazonian | Bahia, Brazil           |
| SJ02      | São José Farm       | Trinitarian         | Bahia, Brazil           |
| TSH1188*  | Selected hybrid in Trinidad | Trinitarian      | Saint George, Trinidad and Tobago |
| UF273*    | United Fruit        | Trinitarian         | Limón, Costa Rica       |

(*Turnbull & Hadley, 2019).
of the Luz et al. (2008) was adjusted to a concentration of 3 × 10⁵ zoospores/mL and placed on the center of each leaf disc. The boxes were closed and incubated at 25 °C in the dark for seven days, when the assessment was performed using a scoring scale developed by Nyassé et al. (2002) with values varying from 0 to 5. The disease severity index (DI) was determined for each genotype from the scores using the equation of McKinney (1923): Infection index (%) = [Σ (scale degree × frequency) × 100]/[(total number of units × maximum scale degree)].

Two experiments were set up with all the 58 progenies in a randomized block design with four replications containing ten discs per clone, totaling 40 discs inoculated per clone and experiment. The analysis of differences between means of progenies was conducted under the model with the sources of variation experiment and treatment, without considering the genetic designs. The model experiment, mother, and father was used to analyze differences between progenitors for each of the three diallels. The previous model was used to analyze mother within father or father within mother.

RESULTS AND DISCUSSION

1.1 Assessment for witches’ broom

The proposal presented here for defining the disease index for the early assessment of cacao seedlings took into account a very important factor: the dry broom. Therefore, plant death due to the disease was considered in this study. In addition, the methodology gives greater or lesser weight to the types of brooms formed according to the number of axillary broom and size of terminal broom. This formula also included the presence of cotyledonary broom because of the relatively high frequency of this type of symptom in plants from some genotypes.

Significant effects for inoculation test (p = 0.0415) and progeny (P < 0.0001) were observed by the F-test, with ten inoculations tests with different progenies in each test and three controls in all tests. As a primary and lesser weight to the types of brooms formed according to the number of axillary broom and size of terminal broom. This model also included the presence of cotyledonary broom because of the relatively high frequency of this type of symptom in plants from some genotypes.

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Table 2: Mean disease index of witches’ broom in cacao seedlings from crossings (DI) and the probability of error (P) for rejecting the hypothesis of equality between means of each progeny and controls by the T-test

| Crossings                                           | Disease index (DI) | CAT 1.878526 | SIC23 2.134295 | SCA6 0.275166 |
|-----------------------------------------------------|--------------------|--------------|----------------|--------------|
| MCB09 (10) x EET75 (12)                             | 0.26236770         | <.0001       | <.0001         | 0.9676       |
| MCB09 (10) x UF273 (13)                             | 0.98332349         | <.0001       | <.0001         | <.0001       |
| RLF1938 (11) x EET75 (12)                           | 1.21532022         | <.0001       | <.0001         | <.0001       |
| RLF1938 (11) x UF273 (13)                           | 1.16995865         | <.0001       | <.0001         | <.0001       |
| CEPEC86 x RB36 (1A) x SCA6 x GU114 (10A)            | 0.26231648         | <.0001       | <.0001         | 0.9299       |
| CEPEC86 x RB36 (1A) x RLF1938 (11)                  | 0.30737087         | <.0001       | <.0001         | 0.8015       |
| CEPEC86 x RB36 (1A) x EET75 (12)                    | 0.77984161         | <.0001       | <.0001         | 0.0009       |
| CEPEC86 x RB36 (1A) x UF273 (13)                    | 1.27083093         | <.0001       | <.0001         | <.0001       |
| CEPEC86 x RB36 (1A) x CSG70 (6A)                    | 0.17017603         | <.0001       | <.0001         | 0.4494       |
| MCB09 (10) x EET75 (12)                             | 0.26231648         | <.0001       | <.0001         | 0.9299       |
| MCB09 (10) x UF273 (13)                             | 0.98332349         | <.0001       | <.0001         | <.0001       |
| RLF1938 (11) x EET75 (12)                           | 1.21532022         | <.0001       | <.0001         | <.0001       |
| RLF1938 (11) x UF273 (13)                           | 1.16995865         | <.0001       | <.0001         | <.0001       |

To be continued...
Among the 26 crossings that were similar to Scavina-6, CEPEC86 x RB36 (1A) should also be highlighted, as it also appears four times in the list when combined with RLF1938 (11), CSG70 (6A), SCA6 x GU114 (10A), and TSH1188 x CAB169 (9A).

The two clones resistant to moniliasis used in the experiments (EET75 and UF273) formed five progenies as resistant as the standard: [MCB09 (10) x EET75 (12)], [CSUL3 x CCN10 (1) x UF273 (13)], [ICA5 x RB36 (2A) x UF273 (13)], [CAB157 x MO20 (5) x UF273 (13)], and [NA33 x RB39 (6) x EET75 (12)], which indicates their great potential also for WB. Pimenta Neto et al. (2018) also found that five of the offspring of the clones EET75 (12) and UF273 (13) crossed with other genetic materials generated progenies with very low WB index, three of them crossed with EET75 (12) and two with UF273 (13).

Regarding the performance of fathers and mothers used in the three tests, MO20 x CCN34 (3) was the best mother in diallel 01, with no difference only from NA33 x RB39 (6) (Table 3). Benjamin et al. (2016) used this last crossing in field assessments and concluded that RB39 is a highly promising source of resistance to WB, promoting the durability of this character when combined with other sources. This latter progenitor did not differ from the progenitors CSUL3 x CCN10 (1), CAB301 x CCN10 (2) and MO20 x CCN34 (3) which also showed low DI means.

The best father for crossings with MO20 x CCN34 (3) was SCA6 x P4B (7), but this crossing only differed significantly (p < 0.05) from the crossing with UF273 (13), which was, regarding resistance, selected only for moniliasis. The crossing [(MO20 x CCN34 (3)) X UF273 (13)] showed no ancestry of Scavina. The parents SCA6 x RB36 (8), RLF1938(11), and SJ02 (9), all with ancestry of Scavina, generated progenies with means close to that generated by the progenitor SCA6 x P4B (7) (means shown in Table 2 and probability of error for rejecting the hypothesis of equality between means not shown). The best parents for crossings with NA33 x RB39 (6) were SCA6 x RB36 (8) and EET75 (12), both crossings significantly different from the two worst: SJ02(9) and RLF1938(11), which are very contrasting results when compared to those of the crossings with MO20 x CCN34 (3).

| Crossings                                              | Disease index (DI) | Controls         |                  |                  |                  |
|---------------------------------------------------------|--------------------|------------------|------------------|------------------|------------------|
| MO20 x AMAZ15 (5A) x SCA6 x GU114 (10A)                | 0.43917588         | <.0001           | <.0001           | 0.2079           |
| MO20 x AMAZ15 (5A) x EET75 (12)                        | 1.24824115         | <.0001           | <.0001           | <.0001           |
| MO20 x AMAZ15 (5A) x UF273 (13)                        | 1.82716434         | 0.7939           | 0.1176           | <.0001           |
| MO20 x AMAZ15 (5A) x CSG70 (6A)                        | 0.69640765         | <.0001           | <.0001           | 0.0494           |
| MO20 x AMAZ15 (5A) x BN34 (7A)                         | 1.26681025         | 0.0001           | <.0001           | <.0001           |
| MO20 x AMAZ15 (5A) x TSH1188 x CAB169 (9A)             | 0.74904369         | <.0001           | <.0001           | 0.0086           |
| CAB157 x MO20 (5) x MCB9 (10)                          | 0.93062792         | <.0001           | <.0001           | 0.0015           |
| CAB157 x MO20 (5) x RLF1938 (11)                       | 0.36615577         | <.0001           | <.0001           | 0.5336           |
| CAB157 x MO20 (5) x EET75 (12)                         | 1.8094936          | 0.5907           | 0.0109           | <.0001           |
| CAB157 x MO20 (5) x UF273 (13)                         | 0.64574296         | <.0001           | <.0001           | 0.0569           |
| CAB157 x MO20 (5) x SCA6 x P4B (7)                     | 1.03421936         | <.0001           | <.0001           | <.0001           |
| CAB157 x MO20 (5) x SCA6 x RB36 (8)                    | 0.5973135          | <.0001           | <.0001           | 0.1240           |
| CAB157 x MO20 (5) x SJ02 (9)                           | 0.61125419         | <.0001           | <.0001           | 0.0107           |
| NA33 x RB39 (6) x MCB9 (10)                            | 0.78204961         | <.0001           | <.0001           | 0.0002           |
| NA33 x RB39 (6) x RLF1938 (11)                         | 1.13390423         | 0.0002           | <.0001           | <.0001           |
| NA33 x RB39 (6) x EET75 (12)                           | 0.52477423         | <.0001           | <.0001           | 0.0660           |
| NA33 x RB39 (6) x UF273 (13)                           | 0.62723668         | <.0001           | <.0001           | 0.0079           |
| NA33 x RB39 (6) x SCA6 x P4B (7)                       | 0.86889884         | <.0001           | <.0001           | <.0001           |
| NA33 x RB39 (6) x SCA6 x RB36 (8)                      | 0.49357779         | <.0001           | <.0001           | 0.1651           |
| NA33 x RB39 (6) x SJ02 (9)                             | 1.33420130         | 0.0001           | <.0001           | <.0001           |
| SJ02 (9) x EET75 (12)                                  | 1.00411401         | <.0001           | <.0001           | <.0001           |
| SJ02 (9) x UF273 (13)                                  | 0.94675223         | <.0001           | <.0001           | <.0001           |
| CATONGO(1)                                             | 1.87852665         | 0.0008           | <.0001           | <.0001           |
| SIC23(2)                                               | 2.13429554         | 0.0008           | <.0001           |                  |
| SCA6(2)                                                | 0.27516669         | <.0001           | <.0001           |                  |

(1) Susceptibility control; (2) Resistance control.
Regarding the overall means of parents of diallel 01, the best parents were the mother SCA6 x RB36 (8) and clones RLF1938 (11) and UF273 (13), not distinct from each other and significantly different from all others.

The father SCA6 x RB36 (8) was four times among the most resistant crossings when combined with mothers CSUL3 x CCN10 (2), MO20 x CCN34 (3), CAB157 x MO20 (5), and NA33 x RB39 (6). The father RLF1938 (11) also four times appeared, being the mothers CEPEC86 x RB36 (1A), MO20 x CCN34 (3), CCN34 x MO20 (4), and CAB157 x MO20 (5). Clone UF273 (13) appeared in this ranking with three satisfactory combinations with the mothers CEPEC86 x RB36 (1), CA5 x RB36 (2A), and CAB157 x MO20 (5).

The best mother in diallel 02 was CEPEC86 x RB36 (1A), which did not differ only from CCN10 x CAB324 (3A). The worst was the mother MO20 x AMAZ15 (5A), but the five did not present large differences in absolute values (Table 3).

The best fathers for mothers CEPEC86 x RB36 (1A) and CCN10 x CAB324 (3A) and overall means of diallel 2 were SCA6 x SGU114 (10A) and TSH1188 x CAB169 (9A). Clone RLF1938 (11), which is a selection carried out in a farm of the region, with probable ancestry of Scavina and also crossed with the first mother, as the two previous fathers, generated progenies with performance similar to that of the resistance pattern. In fact, the four crossings that had SCA6 x SGU114 (10A)

| P | D | C | CM | C (1) | C (2) | C (3) | C (4) | C (5) | C (6) |
|---|---|---|-----|------|------|------|------|------|------|
| M | CSUL3 x CCN10 (1) | 0.814 | 0.9227 | 0.0348 | <0.001 | 0.0522 | 0.6551 |
| M | CAB301 x CCN10 (2) | 0.807 | 0.9227 | 0.0440 | <0.001 | 0.0481 | 0.7343 |
| M | MO20 x CCN34 (3) | 0.627 | 0.0348 | 0.0440 | <0.001 | 0.0004 | 0.0821 |
| M | CAB148 x MO20 (4) | 1.242 | <0.001 | <0.001 | <0.001 | 0.0010 | <0.001 |
| M | CAB157 x MO20 (5) | 0.948 | 0.0522 | 0.0481 | 0.0004 | 0.0010 | 0.0193 |
| M | NA33 x RB39 (6) | 0.784 | 0.6551 | 0.7343 | 0.0821 | <0.001 | 0.0193 |
| M | MCBC9 (10) | 1.019 | <0.001 | 0.1705 | 0.0031 | 0.5228 | <0.001 |
| M | RLF1938 (11) | 0.666 | <0.001 | <0.001 | 0.3453 | <0.001 | 0.4226 |
| M | EET75 (12) | 1.151 | 0.1705 | <0.001 | <0.001 | 0.0262 | 0.0004 |
| M | UF273 (13) | 0.743 | 0.0031 | 0.3453 | <0.001 | 0.0053 | 0.0962 |
| M | SC6A x P4B (7) | 0.965 | 0.5228 | <0.001 | 0.0262 | 0.0053 | <0.001 |
| M | SC6A x RB36 (8) | 0.601 | <0.001 | 0.4226 | <0.001 | 0.0962 | <0.001 |
| M | SJ02 (9) | 0.945 | 0.4087 | 0.0004 | 0.0171 | 0.0156 | 0.7901 |
| M | CEPEC86 x RB36 (1A) | 0.653 | 0.0469 | 0.1747 | 0.0439 | <0.001 |
| M | CA5 x RB36 (2A) | 0.832 | 0.0469 | 0.4019 | 0.5620 | 0.2351 |
| M | CCN10 x CAB324 (3A) | 0.744 | 0.1747 | 0.4019 | 0.6955 | 0.0171 |
| M | CCN34 x CAB301 (4A) | 0.775 | 0.0439 | 0.5620 | 0.6955 | 0.0203 |
| M | MO20 x AMAZ15 (5A) | 0.959 | <0.001 | 0.2351 | 0.0171 | 0.0203 |
| M | SCA6 x GU114 (10A) | 0.342 | 0.0003 | <0.001 | <0.001 | 0.0054 | <0.001 |
| M | RLF1938 (11) | 0.649 | 0.0003 | 0.0002 | <0.001 | 0.7424 | <0.001 |
| M | EET75 (12) | 0.977 | <0.001 | 0.0002 | 0.0099 | 0.0005 | 0.0641 |
| M | UF273 (13) | 1.219 | <0.001 | <0.001 | 0.0099 | 0.9858 | <0.001 |
| M | TSH1188 x CAB169 (9A) | 0.498 | 0.0692 | 0.0871 | <0.001 | 0.2508 | <0.001 |
| M | MCB90 (10) | 1.069 | 0.3605 | 0.8606 |
| M | RLF1938 (11) | 1.232 | 0.3605 | 0.8606 |
| M | SJ02 (9) | 1.035 | 0.8606 | 0.2424 |
| F | C | (10A) | C (11) | C (12) | C (13) | C (6A) | C (7A) | C (9A) |
| F | SCA6 x GU114 (10A) | 0.342 | 0.0003 | <0.001 | <0.001 | 0.0054 | <0.001 |
| F | RLF1938 (11) | 0.649 | 0.0003 | 0.0002 | <0.001 | 0.7424 | <0.001 |
| F | EET75 (12) | 0.977 | <0.001 | 0.0002 | 0.0099 | 0.0005 | 0.0641 |
| F | UF273 (13) | 1.219 | <0.001 | <0.001 | 0.0099 | 0.9858 | <0.001 |
| F | CSG70 (7A) | 0.616 | 0.0054 | 0.7424 | 0.0005 | <0.001 | 0.2508 |
| F | BN34 (7A) | 1.222 | <0.001 | <0.001 | 0.0641 | 0.9858 | <0.001 |
| F | TSH1188 x CAB169 (9A) | 0.498 | 0.0692 | 0.0871 | <0.001 | 0.2508 | <0.001 |
| F | MCB90 (10) | 1.069 | 0.3605 | 0.8606 |
| F | RLF1938 (11) | 1.232 | 0.3605 | 0.8606 |
| F | SJ02 (9) | 1.035 | 0.8606 | 0.2424 |

Table 3: Mean performance of *Moniliophthora perniciosa* infection of fathers and mothers for the three studied genetic designs and the probability of error (P) for rejecting the hypothesis of equality between means by the T-test.

P: parental; D: diallel; C: crossings; CM: corrected mean; M: mother; F: father.
as father and four of the five with TSH1188 x CAB169 (9A) were as resistant as SCA6. The exception was observed for the crossing with the mother MO20 x AMAZ15 (5A), whose only resistant crossing was with 10A (Table 2).

Diallel 03 showed no significant difference between the means of the mothers SJ02 (9), MCB09 (10), and RLF1938 (11), all clones selected in farms of the cacao region of Bahia due to their productivity and resistance to WB, as well as probable ancestry of Scavina. No significant differences were also observed between the two fathers of diallel 03, EET75(12) and UF273(13).

1.2 Assessment for black pod

The tests with leaf discs suggested by Nyassé et al. (1995) has been widely used for the assessment of resistance to cacao diseases (Santos et al., 2011; Bahia et al., 2015; Barreto et al., 2015), showing a high reliability regarding BP behavior in fruits (Pires et al., 1997; Santos et al., 2009). The species *P. palmivora* was used in resistance tests because it is a common cosmopolitan species in all cocoa producing regions (Luz et al., 2001). In addition, Risterucci et al. (2003) demonstrated that the selection to a single predominant species, such as *P. palmivora* in Bahia (Luz et al., 2018), provides significant genetic gains of resistance to the disease.

The means of infection caused by *P. palmivora* showed that differences regarding susceptible controls were not as clear as those found for *M. perniciosa*. This result was expected because the selection in the previous cycle for BP was primarily indirect by the priority selection for resistance to WB or moniliasis. In addition, many of the progenies did not differ or even surpassed, in average, the means of the mothers SJ02 (9), MCB09 (10), and RLF1938 (11), [MO20 x AMAZ15 (5A) X EET75 (12)], [CAB301 x CCN10 (2) X EET75 (12)], [CAB301 x CCN10 (2) X SJ02 (9)] and [CAB301 x CCN10 (2) X SJ02 (9)]] and [CSUL3 x CCN10 (1) X EET75 (12)] (Tables 1 and 2). Two of these progenies, i.e., [CSUL3 x CCN10 (1) x UF273 (13)] and [(NA33 x RB39 (6)) x EET75 (12)], can carry resistance genes to moniliasis and are, therefore, essential for preventive breeding to this disease in Brazil. Fifteen crossings with sources of resistance to moniliasis stood out as resistant to BP, being eight with UF273 (13) and seven with EET75 (12).

Regarding the general combining ability in progenitors, the best mothers in diallel 01 were CAB148 x MO20 (4) and CSUL3 x CCN10 (1), not differing from each other and with significantly lower means than all other

Table 4: Mean disease index of black pod in cacao disc leaves (DI) and the probability of error (P) for rejecting the hypothesis of equality between means of each progeny and controls by the T-test

| Crossings                  | Disease index (DI) | CAT 51.71   | SIC23 52.54 | SCA6 37.29 |
|----------------------------|--------------------|-------------|-------------|------------|
| MBCBC (10) x               | 64.00              | 0.0070      | 0.0119      | <0.001     |
| RLF1938 (11) x             | 27.00              | <0.001      | 0.0001      | 0.0238     |
| RLF1938 (11) x             | 40.00              | 0.0102      | 0.0059      | 0.5511     |
| CEPEC86 x RB36 (1A) x      | 55.50              | 0.4040      | 0.5150      | <0.001     |
| CEPEC86 x RB36 (1A) x      | 69.25              | 0.0001      | 0.0003      | <0.001     |
| CEPEC86 x RB36 (1A) x x TSH1188 x CAB169 (9A) | 53.50              | 0.6933      | 0.8329      | 0.0004     |
| CSUL3 x CCN10 (1) x RLF1938 (11) | 31.00          | <0.001      | <0.001      | 0.1664     |
| CSUL3 x CCN10 (1) x EET75 (12) | 50.25              | 0.7482      | 0.6140      | 0.0045     |
| CSUL3 x CCN10 (1) x UF273 (13) | 28.75              | <0.001      | <0.001      | 0.0605     |

To be continued...

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### Crossings

| Crossings | Disease index (DI) | CAT | SIC23 | SCA6 |
|-----------|-------------------|-----|-------|------|
| CSUL3 x CCN10 (1) x SCA6 x P4B (7) | 30.00 | <.0001 | <.0001 | 0.1089 |
| CSUL3 x CCN10 (1) x SCA6 x RB36 (8) | 39.50 | 0.0074 | 0.0042 | 0.6269 |
| CSUL3 x CCN10 (1) x SJ02 (9) | 22.75 | <.0001 | <.0001 | 0.0014 |
| CA5 x RB36 (2A) x RLF1938 (11) | 36.50 | 0.0009 | 0.0004 | 0.8617 |
| CA5 x RB36 (2A) x EET75 (12) | 22.00 | <.0001 | <.0001 | 0.0008 |
| CA5 x RB36 (2A) x UF273 (13) | 47.50 | 0.3544 | 0.2673 | 0.0249 |
| CA5 x RB36 (2A) x TSH1188 x CAB169 (9A) | 71.50 | <.0001 | <.0001 | <.0001 |
| CAB301 x CCN10 (2) x MBC9 (10) | 35.25 | 0.0003 | 0.0002 | 0.6531 |
| CAB301 x CCN10 (2) x EET75 (12) | 28.25 | <.0001 | <.0001 | 0.0469 |
| CAB301 x CCN10 (2) x UF273 (13) | 41.75 | 0.0287 | 0.0178 | 0.3266 |
| CAB301 x CCN10 (2) x SCA6 x P4B (7) | 61.75 | 0.0247 | 0.0430 | <.0001 |
| CAB301 x CCN10 (2) x SCA6 x RB36 (8) | 55.00 | 0.4688 | 0.5884 | 0.0001 |
| CAB301 x CCN10 (2) x SJ02 (9) | 19.75 | <.0001 | <.0001 | 0.0001 |
| CCN10 x CAB324 (3A) x SCA6 x GU114 (10A) | 66.75 | 0.0010 | 0.0018 | <.0001 |
| CCN10 x CAB324 (3A) x RLF1938 (11) | 42.25 | 0.0377 | 0.0238 | 0.2753 |
| CCN10 x CAB324 (3A) x EET75 (12) | 31.5 | <.0001 | <.0001 | 0.2027 |
| CCN10 x CAB324 (3A) x UF273 (13) | 29.50 | <.0001 | <.0001 | 0.0867 |
| CCN10 x CAB324 (3A) x TSH1188 x CAB169 (9A) | 76.00 | 0.0008 | 0.0015 | <.0001 |
| MO20 x CCN34 (3) x RLF1938 (11) | 40.50 | 0.0139 | 0.0082 | 0.4801 |
| MO20 x CCN34 (3) x SCA6 x P4B (7) | 44.00 | 0.0901 | 0.0605 | 0.1401 |
| CCN34 x CAB301 (4A) x SCA6 x GU114 (10A) | 37.75 | 0.0022 | 0.0012 | 0.9196 |
| CCN34 x CAB301 (4A) x RLF1938 (11) | 50.00 | 0.7069 | 0.5759 | 0.0053 |
| CCN34 x CAB301 (4A) x EET75 (12) | 26.50 | <.0001 | <.0001 | 0.0178 |
| CCN34 x CAB301 (4A) x UF273 (13) | 37.50 | 0.0018 | 0.0010 | 0.9634 |
| CCN34 x CAB301 (4A) x SCA6 x P4B (7) | 54.75 | 0.5032 | 0.6269 | 0.0001 |
| CCN34 x CAB301 (4A) x SCA6 x UF273 (13) | 57.50 | 0.2027 | 0.2753 | <.0001 |
| CCN34 x CAB301 (4A) x SCA6 x GU114 (10A) | 34.25 | 0.0001 | <.0001 | 0.5032 |
| CAB148 x MO20 (4) x MCBC9 (10) | 20.00 | <.0001 | <.0001 | 0.0002 |
| CAB148 x MO20 (4) x RLF1938 (11) | 20.00 | <.0001 | <.0001 | 0.0002 |
| CAB148 x MO20 (4) x SCA6 x P4B (7) | 35.00 | 0.0003 | 0.0001 | 0.6140 |
| MO20 x AMA215 (5A) x SCA6 x P4B (7) | 27.00 | <.0001 | <.0001 | 0.0238 |
| MO20 x AMA215 (5A) x EET75 (12) | 56.75 | 0.2673 | 0.3544 | <.0001 |
| MO20 x AMA215 (5A) x UF273 (13) | 46.00 | 0.2092 | 0.1502 | 0.0556 |
| CAB157 x MO20 (5) x MCBC9 (10) | 37.25 | <.0001 | <.0001 | 0.9903 |
| CAB157 x MO20 (5) x EET75 (12) | 63.25 | 0.0113 | 0.0187 | <.0001 |
| CAB157 x MO20 (5) x UF273 (13) | 45.00 | 0.1401 | 0.0973 | 0.0901 |
| CAB157 x MO20 (5) x SCA6 x P4B (7) | 45.00 | 0.0003 | 0.0006 | <.0001 |
| CAB157 x MO20 (5) x SCA6 x RB36 (8) | 68.25 | <.0001 | <.0001 | <.0001 |
| CAB157 x MO20 (5) x SCA6 x RB36 (8) | 82.00 | <.0001 | <.0001 | 0.0469 |
| NA33 x RB39 (6) x MCBC9 (10) | 41.50 | 0.0249 | 0.0153 | 0.3544 |
| NA33 x RB39 (6) x RLF1938 (11) | 45.00 | 0.1401 | 0.0973 | 0.0901 |
| NA33 x RB39 (6) x EET75 (12) | 37.50 | 0.0018 | 0.0010 | 0.9634 |
| NA33 x RB39 (6) x UF273 (13) | 71.75 | <.0001 | <.0001 | <.0001 |
| NA33 x RB39 (6) x SCA6 x P4B (7) | 36.75 | 0.0010 | 0.0005 | 0.9051 |
| NA33 x RB39 (6) x SCA6 x RB36 (8) | 49.00 | 0.5511 | 0.4357 | 0.0102 |
| NA33 x RB39 (6) x SJ02 (9) | 43.50 | 0.0712 | 0.0469 | 0.1721 |
| SJ02 (9) x EET75 (12) | 31.25 | <.0001 | <.0001 | 0.1839 |
| SJ02 (9) x UF273 (13) | 44.00 | 0.0901 | 0.0605 | 0.1401 |
| CATONGO(1) | 51.71 | 0.7315 | 0.7315 | <.0001 |
| SIC23(2) | 52.54 | 0.7315 | <.0001 | <.0001 |
| SCA6(2) | 37.29 | <.0001 | <.0001 | <.0001 |

(1) Susceptibility control; (2) Resistance control.
Table 5: Mean performance of *Phytophthora palmivora* infection of fathers and mothers for the three studies genetic designs and probability of error (P) for rejecting the hypothesis of equality between means by the T-test.

| P D C | CM | C (1) | C (2) | C (3) | C (4) | C (5) | C (6) |
|-------|----|-------|-------|-------|-------|-------|-------|
| M     |    |       |       |       |       |       |       |
| C     | 32.157 | 0.0057 | <.0001 | 0.2118 | <.0001 | <.0001 | 1 |
| M     | 39.615 | 0.0057 | 0.0545 | 0.0008 | <.0001 | 0.0081 | |
| M     | 46.279 | <.0001 | 0.0545 | <.0001 | 0.0888 | 0.9639 | |
| M     | 27.871 | <.0001 | 0.0008 | <.0001 | <.0001 | <.0001 | |
| M     | 51.936 | <.0001 | <.0001 | 0.0888 | <.0001 | 0.0222 | |
| M     | 46.428 | <.0001 | 0.0081 | 0.9639 | <.0001 | 0.0222 | |
| F     |    |       |       |       |       |       |       |
| C     | 31.407 | 0.1100 | 0.0003 | <.0001 | <.0001 | <.0001 | 0.1395 |
| F     | 36.655 | 0.0664 | 0.1779 | 0.3982 | 0.0004 | <.0001 | |
| F     | 42.992 | <.0001 | 0.0047 | 0.0010 | 0.0193 |       | |
| F     | 45.468 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| F     | 54.555 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| F     | 26.742 | 0.1395 | 0.0043 | <.0001 | <.0001 | <.0001 | <.0001 |

For the fathers, the best performances were observed for clones SJ02 (9) and MCB09 (10), with means not statistically distinct and lower than those of the other progenitors. The third of the selections carried out in a farm, the clone RLF1938 (11), also presented a low mean infection, not differing from MCB09 (10). From the four crossings with SJ02 (9), two were among the best treatments, with the mothers CAB301 x CCN10 (2) and NA33 x RB39 (6).

In diallel 02, CCN34 x CAB301 (4A) was the best mother and EET75 (12) the best father, differing from the other progenitors. This mother, when combined with TSH1188 x CAB169 (9A), had a lower mean DI when compared to Scavina-6, with no statistical difference from each other.

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EET75 (12) appears as the father in two of the most resistant treatments.

For diallel 03, RLF1938 (11) and SJ02 (9) were comparatively better mothers than MCB09 (10), which presented a corrected mean higher than the others did. The fathers EET75 (12) and UF273 (13) did not differ from each other.

Clone SJ02 (9) contributed to the formation of three of the most resistant progenies to BP when crossed with mothers CSUL3 x CCN10 (1), CAB301 x CCN10 (2), and CAB157 x MO20 (5). The ancestry CSUL3 has been standing out as a progenitor in other tests for resistance to WB (Marita et al., 2001; Silva et al., 2010; Benjamin et al., 2016) and also for BP in field tests (Pires et al., 1997), as well as in the artificial inoculation on fruits with *P. palmivora* (Luz et al., 1996).

**CONCLUSION**

These results allow the early selection in the establishment of recurrent selection tests, future plant selection, which will be tested as clones and assessed regarding the possibility of becoming commercial varieties, and selection of progenitors for the next recurrent selection cycle. They also provide information on the potential of germplasm that can be used in other breeding programs. In addition to contributing to cacao farming in Bahia, they may also be useful for cacao farming in other regions.

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The authors declare that there is no conflict of interests regarding the possibility of becoming commercial varieties, and selection of progenitors for the next recurrent selection cycle. They also provide information on the potential of germplasm that can be used in other breeding programs. In addition to contributing to cacao farming in Bahia, they may also be useful for cacao farming in other regions.

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