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ARTICLE

Cacao parents help their offsprings to fight witches' broom and black pod rot infections

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Abstract: *Searching for new approaches to confer resistance to diseases in plants is one of the main objectives of the Cacao breeding program (CBP). Witches' broom (WB) and black pod rot (BPR) are important cacao diseases in Bahia, Brazil. This study aimed to evaluate the resistance in plants from 40 progenies of genetic crosses between elite clones to natural infection by high levels of Moniliophthora perniciosa and Phytophthora spp. WB and BPR infection assessments were conducted in the field, including evaluation of vegetative brooms (VB), cushion brooms (CB), and total brooms (TB), as well as the percentage of WB-diseased (FRUWB) and BPR-diseased (FRUBPR) fruits. Ten progenies showed resistance to infection in both the canopy and fruits. No significant differences between the progenies were detected for FRUBPR.*

Keywords: *Breeding,* Moniliophthora perniciosa*,* Phytophthora spp.*, resistance source,* Theobroma cacao

INTRODUCTION

Cacao (*Theobroma cacao* L.) is an important agricultural commodity that provides the main raw material for chocolates and other products. Pará and Bahia are Brazil's main cacao bean-producing states, which rank seventh in the global production of cacao beans and are one of the five major chocolate-consuming markets worldwide (Zugaib and Barreto 2015, Leite 2018). In addition, Brazil has been recognized for its superior quality of fine, aromatic cocoa beans that are ideal for producing gourmet, or "premium" chocolates. Therefore, interest in Brazilian beans has increased with the increase in demand for beans of superior quality in the international market (Chiapetti et al. 2021).

Diseases can reduce the quality and productivity of cacao. Since the introduction of cacao farming in the southern region of Bahia, black pod rot (BPR) (*Phytophthora* spp.) has caused damage to cacao trees. Moreover, in 1989, an outbreak and rapid spread of witches' broom (WB) (*Moniliophthora perniciosa* Aime and Phillips-Mora) in plantations caused severe crop destruction (Pereira et al. 1989, Oliveira and Luz 2012). WB and BPR are currently the economically most relevant crop diseases in this region (Oliveira and Luz 2012, Bailey and Meinhardt 2016).

Moniliophthora perniciosa infects the meristematic tissues of cacao, causing a set of conditions in the host, such as hormonal imbalance, hypertrophy, and

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subsequent necrosis of infected tissues (Aime and Phillips-Mora 2005). The climate in the cacao-growing region of Bahia promotes pathogen development and sporulation throughout the year (Luz et al. 2002, Oliveira and Luz 2012). Several methodologies are recommended to control these diseases, including integrated management and the production of adequately resistant genotypes to ensure the extended durability of commercial varieties (Nyassé et al. 2007, Silva et al. 2008). Ideally, new varieties developed for cultivation should carry genes with resistance to major diseases.

Genotypes that accumulate disease resistance genes for WB and subsequently to other diseases have been developed and selected through first and second generation genetic crosses of clones from different origins (Pinto and Pires 1998). These genotypes also possess other agronomically desirable traits that contribute to increased production (Silva et al. 2010, Monteiro and Ahnert 2012, Benjamin et al. 2016). A major challenge in controlling these diseases is the increase in the genetic base of resistant varieties to produce new superior populations that disrupt pathogen evolution and extend the durability of disease resistance (Barreto et al. 2015).

Based on the marker-assisted selection, genome maps have been constructed using various markers to identify several genomic regions associated with disease resistance, such as frosty pod rot disease caused by the clonal pathogen *Moniliophthora roreri* (Cif.) Evans et al. and black pod rot disease caused by *Phytophthora palmivora* (Flament et al. 2001, Brown et al. 2005, Díaz-Valderrama and Aime 2016, Fister et al. 2016, Motilal et al. 2016, Royaert et al. 2016, Navarro et al. 2017, Barreto et al. 2018, McElroy et al. 2018). Conventional breeding is useful for the phenotypic characterization and the development of new populations, which is a fundamental aspect of molecular research. In this study, 40 progenies derived from 21 parental genotypes were evaluated for field resistance over ten years to select plants with the most resistance to natural infection by WB and BPR. Vegetative broom (VB), cushion broom (CB), and total broom (TB) numbers, as well as the percentage of WB-diseased fruits (FRUWB) and BPR-diseased fruits (FRUBPR) were used as screening measures.

MATERIAL AND METHODS

Plants from 40 different progenies of hybrid cacao trees produced from crosses with the paternals CCN10, CCN34, TSH1188, MO20, or SCA6 were evaluated in the field. The maternal trees crossed with CCN10 (13) were: CAB11, CAB94, CAB148, CAB157, CAB165, CAB169, CAB301, CEPEC90, CSUL3, CSUL8, EET397, MO20, and P4B. With CCN34 (9), the same crossed with CCN10 except for CAB11, CAB94, CAB165, CAB301, and EET397; with this paternal, another maternal was used, CAB324. TSH1188 (10) was crossed with CAB148, CAB157, CAB165, CAB301, CSUL3, CSUL8, EET397, MO20, P4B, and GU257. MO20 (6) was crossed with CAB148, CAB157, CAB169, CAB94, CCN51, and CEPEC90 as mothers, while SCA6 (2) was only crossed with CAB157 and CAB324 as mothers.

These parents were chosen because they are elite clones with desirable agronomic characteristics, including disease resistance, productivity, and self-compatibility (Table 1). CCN10, CCN34, CCN51, CEPEC90, CSUL3, CSUL8, EET397, GU259, TSH1188, MO20, SCA6, and P4B are cacao clones that are well adapted to the main agro-systems of the Bahian cacao growing region. However, CAB11, CAB148, CAB157, CAB165, CAB169, CAB301, CAB324, and CAB94 have descendants from previous crosses with other genetic materials being tested in clonal assays established in some of these agrosystems. The 12 progeny plants were segregated populations that resulted from each of the crosses.

The crosses were designed to generate a new base population. These findings are based on 11 years of evaluations of WB and BPR symptoms in each cacao progeny plant exposed to high levels of infection due to pathogens with no control methods in order to select accessions with high genetic value and disease resistance.

The experimental area was located at the Cacao Research Center (CEPEC/CEPLAC) Arnaldo Medeiros Experimental Station in Ilhéus, BA, Brazil, and all parents were clones of the CEPEC Cacao Germplasm collection. The progenies from the first generation of a recurrent selection cycle were produced by artificial pollination (Pires et al. 1999). The evaluated progenies were planted at 3 × 3 m spacing in the shade of native trees following the cacao-cabruca system. Adult cacao plants infected with WB and BPD were previously grown in this field area, resulting in a high natural inoculum pressure of pathogens. A randomized block design was used with five blocks of 12 plants each, totaling 60 plants per progeny.

The progenies were planted in the fields in 2004, and the evaluation began in 2007 and continued until 2015 by counting the number of VB, CB, WB, and BPR-diseased pods. Plant evaluations were not performed in 2016, but the

Table 1. Origin, genetic group and agronomic value of parental clones of the progenies evaluated for natural infection by *Moniliophthora perniciosa* and *Phytophthora* spp

Clone	Series / Geographic origin	Genetic group	Agronomic value
CCN10	CCN - Castro Naranjal collection, Pichilingue, Ecuador	Trinitarian	Resistance to witches' broom, self-compatible, productivity
CCN34	CCN - Castro Naranjal collection, Pichilingue, Ecuador	Trinitarian	Resistance to witches' broom,
CCN51	CCN - Castro Naranjal collection, Pichilingue, Ecuador	Trinitarian	Resistance to witches' broom, productivity
MO20	MO - Morona, Peru	Amazonian Forastero	Resistance to witches' broom, productivity
SCA6	SCA - Scavina, Ucayali, Peru	Amazonian Forastero	Resistance to witches' broom, self-compatible, productivity
TSH1188	TSH - Trinidad Selection Hibrid, Saint George, Trinidad	Trinitarian	Resistance to witches' broom, black pod rot and ceratocystis wilt, productivity
CAB11	CAB - Cacao from Brazilian Amazon, Rondonia, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB94	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB148	CAB - Cacao from Brazilian Amazon, Acre, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB157	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB165	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB169	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom
CAB301	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom, productivity
CAB324	CAB - Cacao from Brazilian Amazon, Amazon, Brazil	Amazonian Forastero	Resistance to witches' broom
CEPEC90	CEPEC - Cacao Research Center, Bahia, Brazil	Amazonian Forastero	Resistance to witches' broom
CSUL3	CSUL – Cruzeiro do Sul, Acre, Brazil	Amazonian	Resistance to witches' broom and black pod rot, self-compatible productivity
CSUL8	CSUL – Cruzeiro do Sul, Acre, Brazil	Amazonian Forastero	Resistance to witches' broom
EET397	EET - Tropical experimental station, Pichilingue, Ecuador	Trinitarian	Resistance to witches' broom, self-compatible, productivity
P4B	P4B - Pound 4 /B, Loreto, Peru	Amazonian Forastero	Resistance to witches' broom and black pod rot
GU259	GU - Guiana, French	Amazonian Forastero	Resistance to witches' broom

Source: Turnbull and Hadley (2020).

measurements were resumed twice a year in 2017 and 2018, from June to August and November to December, during the peak of broom formation (Luz et al. 2006). The number of vegetative and cushion brooms, as well as infected pods, was counted in each of the 12 progeny plants within a block, and the averages for each block were calculated for each variable.

The following variables were evaluated: mean number of brooms plant^{-1} year^{-1} for each broom type (VB, CB, and TB), FRUWB, and FRUBPR. Sources of variation in the analysis model included year, block, paternal, and maternal, with the t-test used for comparisons of paternal means (corrected for the maternal effect, LSMEAN (SAS 1990) and maternal means (corrected for the paternal effect). The model used for comparison of mean values by Tukey's test at 5% probability had three sources of variations: year, block, and progeny. The model's sources of variation were the year and parent in relation to the effects of maternals among genetic crosses with the same paternal and *vice versa*, and a t-test was used for each parent.

RESULTS AND DISCUSSION

For the structured base population, the effects of year, block, paternal, and maternal as individual parents and the paternal × maternal interaction were found significant for all field variables related to WB and BPR, except for the paternal × maternal interaction for BPR-diseased pods (F-test at 1% probability) (Supplementary file, Table S1-S4). These elements indicate the existence of additive effects (significant paternal and maternal effect) and dominance (significant paternal–maternal interaction) during the inheritance of these characteristics (Pires et al. 2021). All sources were significant for all variables in the model with variation sources of year, block, and progeny (Supplementary file*, Tables S1–S4).

For VB, CB, and TB, the highest number of brooms were formed on progenies of CCN10, CCN34, and TSH1188 as paternals, whereas consistently fewer brooms were counted in the progenies obtained from MO20 and SCA6. The latter two paternals also showed the lowest FRUWB percentage (Table 2). Based on phenotypic and genomic sequence evaluation (Queiroz et al. 2003, Brown et al. 2005) of regions associated with WB resistance, SCA6 contains quantitative trait loci (QTLs) that were inherited by progenies, which could result in long-lasting resistance when crossed with other WB resistant genotypes (Pires et al. 2012, Benjamin et al. 2016, Pimenta Neto et al. 2018). This clone has shown resistance to *Phytophthora* species that cause BPR in Brazil in the studies conducted by Bahia et al. (2015), Barreto et al. (2018), and *Phytophthora megakarya* in a study by Nyadanu et al. (2009). For all WB variables tested, MO20 performed similarly to SCA6, whereas TSH1188, a descendant of SCA6 (Wadsworth et al. 1997), appeared to have lost some of its predecessor's resistance.

The variation in BPR among the five paternals was less significant, as CCN34 was the only paternal that differed significantly from the others. For VB, the maternals with the highest mean values were EET397 and GU259 (6.82 and 6.61, respectively). The lowest mean was observed for CAB157 (1.57), which did not differ significantly from that of CAB94 and CSUL3 (Supplementary file, Table S5). For VB, there were many differences between the maternals. The

Table 2. Individual comparisons among corrected mean values for paternal-variables: vegetative brooms, cushion brooms and total brooms; percentages of witches' broom-diseased fruits and black pod rot-diseased fruits

Vegetative Brooms

Paternal	Mean	(1) CCN10	(2) CCN34	(3) MO20	(4) SCA6	(5) TSH1188
(1) CCN10	3.74			*	*	*
(2) CCN34	3.55			*	*	
(3) MO20	2.42					*
(4) SCA6	2.68					*
(5) TSH1188	3.47					

Cushion Brooms

Paternal	Mean	(1) CCN10	(2) CCN34	(3) MO20	(4) SCA6	(5) TSH1188
(1) CCN10	2.91		*	*	*	
(2) CCN34	3.24			*	*	
(3) MO20	1.72					*
(4) SCA6	2.14					*
(5) TSH1188	3.13					

Total Brooms

Paternal	Mean	(1) CCN10	(2) CCN34	(3) MO20	(4) SCA6	(5) TSH1188
(1) CCN10	6.65			*	*	
(2) CCN34	6.79			*	*	
(3) MO20	4.14					*
(4) SCA6	4.82					*
(5) TSH1188	6.60					

Percentage of witches' broom-diseased fruits

Paternal	Mean	(1) CCN10	(2) CCN34	(3) MO20	(4) SCA6	(5) TSH1188
(1) CCN10	17.98		*	*	*	*
(2) CCN34	16.07			*	*	
(3) MO20	14.07					
(4) SCA6	12.72					*
(5) TSH1188	15.60					

Percentage of black pod rot-diseased fruits

Paternal	Mean	(1) CCN10	(2) CCN34	(3) MO20	(4) SCA6	(5) TSH1188
(1) CCN10	10.29		*			
(2) CCN34	11.90			*	*	*
(3) MO20	9.48					
(4) SCA6	10.15					
(5) TSH1188	10.16					

* The mean for the parents shown in the vertical column is significantly lower than that for the parents in the horizontal line. * The mean of the parents in the vertical column is significantly higher than that of the parent in the horizontal line. Evaluated by the procedure LSMEANS (SAS 1990) by the t-test at 5% error probability in the affirmation of the differences among means; LSMEANS_SAS: compare the average of means that need to be corrected using the incomplete block principle; 1-5: is the numeration of the paternal represented in the vertical column repeated by their respective numeration in the horizontal line.

average infection in EET397, a SCA6 descendant, and a WB-resistant clone was greater than that of 14 out of the 16 maternals tested (Wadsworth et al. 1997). This demonstrates that the maternals selected for generating an improved population were strong. Similarly, CCN51, another resistant clone, showed significantly higher infection rates than 11 of the maternals.

For CB, EET397 had the highest mean (7.10) out of all the other maternals. The lowest mean value with no statistical differences was observed for CSUL3, MO20, P4B, and CAB157 (1.10, 1.18, 1.25, and 1.59, respectively). The first three maternals differed from all others (Supplementary file, Table S5). EET397 showed the highest mean (13.92) for TB (vegetative + cushion brooms), followed by CAB11 (11.4), and GU259 (9.55). Each of the three maternals were distinct from the others. CSUL3 showed the smallest mean (2.85), even though it did not differ significantly from those of CAB157 (3.16) or CAB94 (3.37) (Table 3). For this variable, MO20 and P4B responded well among the maternals. CSUL3 is a BPR-resistant clone (Luz et al. 1996) collected from Cruzeiro do Sul (Acre State), and its progenies have shown resistance to WB in field evaluations (Benjamin et al. 2016). Pereira et al. (2021) observed the high performance of CSUL3 as a parent, which was resistant to vegetative and floral cushion brooms, demonstrating its ability to reduce the infection potential of the pathogen and its evolution.

The percentage of diseased cacao fruits was determined by counting the total number of fruits per plant and their progeny and separating them into healthy, WB-diseased, and BPR-diseased fruits. The maternal with the highest percentage of FRUWB was GU259 (21.53%), which was statistically equal to those of CSUL3, CAB11, and EET397. CAB148, CSUL8, CAB169, CAB165, CAB94, CEPEC90, P4B, and CAB301, which were found statistically equal to FRUWB, showed the lowest percentage of fruits affected by *M. perniciosa* (12.08, 12.51, 12.62, 13.09, 13.28, 13.76, 14.04, and 14.38, respectively) (Table 3). CSUL3 responded well to VB, CB, and TB; however, in terms of diseased pods, it was comparable to GU259, CAB11, and EET397, which showed the poorest results in TB. This indicates that separate resistance mechanisms might function during the production of broom and diseased pods. Regarding BPR, CAB169, CSUL8, CAB165, CAB324, CEPEC90, CAB148, CAB157, CAB301, CAB11, P4B, and MO20 showed the lowest mean values (Table 3).

As mentioned above, significant differences among the progeny of 40 crosses were observed for VB, CB, TB, FRUWB, and FRUBPR. However, these differences were not detectable for the last variable while using the mean significant difference (MSD) in Tukey's test at a 5% probability for mean separation. However, they were observable in the individual comparisons by the t-test (Supplementary file, Table S6–S10 because of the size of the matrices in these comparisons). The MSD for each variable was used to divide the mean of the crosses into groups. Regarding WB resistance, 14 crosses (MO20 × CAB157, SCA6 × CAB157, MO20 × CAB169, TSH1188 × CAB94, MO20 × CAB94, SCA6 × CAB324, CCN10 × CAB157, CCN10 × CSUL3, MO20 × CEPEC90, CCN34 × CSUL3, TSH1188 × CAB157, CCN10 × CAB94, CCN34 × CAB157, and MO20 × CAB148) showed the lowest means for all WB-related variables that were analyzed. The progeny of cross between MO20 × CAB157 showed the lowest numerical mean for VB, CB, and TB but was not statistically different from 13 other progenies mentioned above (Table 4). There was a strong correlation between the lowest VB and CB values presented by the progenies, indicating the possibility of increased CB resistance with early VB selection in young plants. For the progenies, five of the 14 progenies had MO20, three had CCN10, and two pairs had SCA6, CCN34, or TSH1188 as the paternal. Meanwhile, six, three, and two plants out of the 14 superior progenies had CAB157, CAB94, CSUL3 as the maternal, respectively. Maternals CAB169, CAB324, and CEPEC90 had one progeny in the top 14.

The highest mean values of the three broom types occurred for the progenies of TSH1188 × EET397, which were statistically equal to those of CCN10 × EET397 with respect to the mean of VB and TB, and those of CCN10 × CAB11, along with TSH1188 × GU259 in terms of mean of VB. Ten of the progenies with lower VB and CB mean values also showed a lower number of WB-diseased pods, consisting of MO20 × CAB157, SCA6 × CAB157, MO20 × CAB169, TSH1188 × CAB94, MO20 × CAB94, SCA6 × CAB324, MO20 × CEPEC90, CCN10 × CAB94, CCN34 × CAB157, and MO20 × CAB148 (Table 4). Therefore, indirect selection for pod infection by *M. perniciosa* may be possible, but further research is required to confirm this hypothesis. A total of 12 progenies produced the highest percentage of diseased fruits, CCN34 × CSUL3, CCN10 × CSUL3, and CCN10 × CAB157 developed fewer CBs.

Plants of the progeny TSH1188 × EET397 were also susceptible to fruit infection, whereas CCN10 × EET397, CCN10 × CAB11, and TSH1188 × GU259 were susceptible to broom formation (Table 4). For FRUBPR, no significant difference between the progenies was detected; however, the progenies showed a higher percentage of FRUWB than FRUBPR.

Table 3. Individual comparison among corrected mean values for maternal-variables: total brooms (vegetative brooms + cushion brooms), percentages of witches' broom-diseased fruits and black pod rot-diseased fruits

Total Brooms (VB + CB)

Maternal	Mean			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	VB	CB	TB																
(1) CAB11	5.84	5.56	11.40		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
(2) CAB148	2.64	3.56	6.20			*	*			*	*	*	*	*		*	*		
(3) CAB157	1.57	1.59	3.16				*	*	*	*		*	*		*	*	*	*	*
(4) CAB165	2.18	2.00	4.18								*	*	*			*	*	*	
(5) CAB169	2.41	2.41	4.82								*	*	*	*		*	*		
(6) CAB301	2.60	1.89	4.49								*	*	*	*		*	*		
(7) CAB324	2.22	2.21	4.43								*	*	*	*		*	*	*	
(8) CAB94	1.67	1.70	3.37									*	*		*	*	*	*	*
(9) CCN51	3.36	2.33	5.69											*	*	*	*	*	*
(10) CEPEC90	3.53	2.77	6.30											*	*	*	*	*	*
(11) CSUL3	1.75	1.10	2.85												*	*	*	*	*
(12) CSUL8	2.30	2.44	4.74													*	*	*	
(13) EET397	6.82	7.10	13.92															*	*
(14) GU259	6.61	2.94	9.55															*	*
(15) MO20	2.75	1.18	3.93																
(16) P4B	2.51	1.25	3.76																

Percentage of witches' broom-diseased fruits

Maternal	Mean	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
(1) CAB11	18.87		*	*	*	*	*		*		*		*			*	*
(2) CAB148	12.08			*				*		*		*		*	*	*	
(3) CAB157	14.24											*		*	*		
(4) CAB165	13.09											*		*	*		
(5) CAB169	12.62											*		*	*		
(6) CAB301	14.39											*		*	*		
(7) CAB324	15.19											*		*	*		
(8) CAB94	13.28											*		*	*		
(9) CCN51	15.94														*		
(10) CEPEC90	13.76											*		*	*		
(11) CSUL3	19.73												*			*	*
(12) CSUL8	12.51													*	*		
(13) EET397	18.55															*	*
(14) GU259	21.53															*	*
(15) MO20	14.72																
(16) P4B	14.09																

Percentage of black pod rot-diseased fruits

Maternal	Mean	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
(1) CAB11	10.20									*							
(2) CAB148	9.30								*	*		*		*	*		
(3) CAB157	9.78								*	*				*	*		
(4) CAB165	8.57								*	*		*		*	*		
(5) CAB169	8.35								*	*		*		*	*		
(6) CAB301	10.07									*							
(7) CAB324	8.73								*	*		*		*	*		
(8) CAB94	11.72										*		*				
(9) CCN51	14.63										*		*			*	*
(10) CEPEC90	8.83											*		*	*		
(11) CSUL3	11.65												*				
(12) CSUL8	8.37													*	*		
(13) EET397	12.32																
(14) GU259	13.22																
(15) MO20	10.35																
(16) P4B	10.25																

* The mean for the parents shown in the vertical column is significantly lower than that for the parents in the horizontal line. * The mean of the parents in the vertical column is significantly higher than that of the parent in the horizontal line. Evaluated by the procedure LSMEANS (SAS 1990) by the t-test at 5% error probability in the affirmation of the differences among means; 1-16: is the numeration of the maternals represented in the vertical column repeated by their respective numeration in the horizontal line.

Genetic material can influence the level of aggression of *Phytophthora* species, considering that the most aggressive isolates likely prevailed in the field owing to the variations incurred over the years (Lessa et al. 2020).

Rodrigues et al. (2020) selected plants from progenies of five genetic crosses (CSUL3 × CCN10, CAB301 × CCN10, MO20 × CCN34, CAB148 × MO20, and CAB157 × MO20) as maternals for the second cycle of recurrent selection, which

Table 4. Mean values corrected by LSMEANS_SAS (1990). Mean significant difference (MSD) for progenies in the evaluation of the number of field brooms with natural infection by *Moniliophthora perniciosa* and percentage of fruits diseased with witches' broom and black pod rot, and grouping of categories formed by relationships between limits of the highest and lowest mean values and respective MDS

Cross			**Number of brooms**						**%**		**%**	
			VB		**CB**		**TB**		**FRUWB**		**FRUBPR**	
TSH1188	×	EET397	7.13	a	8.83	a	15.96	a	20.41	a	13.36	a
CCN10	×	EET397	7.37	a	6.33	c	13.70	a	20.15	a	11.31	a
CCN10	×	CAB11	6.40	a	5.84	c	12.24	c	21.56	a	10.12	a
TSH1188	×	GU259	6.91	a	3.44	c	10.35	c	21.84	a	12.99	a
CCN34	×	CAB148	3.54	c	4.63	c	8.17	c	18.02	a	13.51	a
CCN10	×	CEPEC90	4.97	c	3.15	c	8.12	c	18.98	a	9.83	a
TSH1188	×	CAB148	3.02	c	4.43	c	7.45	c	9.58	b	8.03	a
CCN34	×	CAB169	3.23	c	4.21	c	7.44	c	14.99	b	9.74	a
CCN34	×	CEPEC90	3.81	c	3.53	c	7.34	c	15.88	b	9.97	a
CCN10	×	CSUL8	3.06	c	3.44	c	6.50	c	15.73	b	8.31	a
CCN10	×	MO20	4.03	c	2.10	c	6.13	c	21.28	a	11.93	a
TSH1188	×	CAB324	2.77	c	3.26	c	6.03	c	12.16	b	8.12	a
CCN10	×	CAB148	2.46	c	3.46	c	5.92	c	11.76	b	8.59	a
CCN10	×	CAB165	2.83	c	2.95	c	5.78	c	12.10	b	8.58	a
CCN10	×	CAB301	3.08	c	2.36	b	5.44	c	17.09	a	11.14	a
TSH1188	×	CAB301	2.97	c	2.22	b	5.19	c	14.72	b	8.83	a
CCN10	×	CAB169	2.88	c	2.17	b	5.05	c	14.66	b	7.78	a
CCN34	×	P4B	3.18	c	1.62	b	4.80	c	14.27	b	12.54	a
CCN34	×	CAB324	2.31	b	2.43	b	4.74	c	14.83	b	9.62	a
CCN34	×	CSUL8	2.45	c	2.24	b	4.69	c	12.56	b	9.83	a
CCN10	×	P4B	2.90	c	1.71	b	4.61	c	14.29	b	8.82	a
TSH1188	×	P4B	2.73	c	1.77	b	4.50	c	17.26	a	10.64	a
TSH1188	×	CAB165	2.40	c	1.99	b	4.39	c	16.02	b	8.28	a
TSH1188	×	MO20	2.98	c	1.41	b	4.39	c	11.96	b	9.25	a
MO20	×	CAB148	2.08	b	2.25	b	4.33	b	12.78	b	8.36	a
CCN34	×	CAB157	1.89	b	2.32	b	4.21	b	14.71	b	12.16	a
CCN10	×	CAB94	2.04	b	2.16	b	4.20	b	13.37	b	8.86	a
TSH1188	×	CAB157	1.99	b	2.08	b	4.07	b	16.88	c	10.06	a
MO20	×	CCN51	2.61	c	1.42	b	4.03	b	14.75	b	13.71	a
CCN34	×	MO20	2.54	c	1.50	b	4.04	b	15.40	b	11.29	a
CCN34	×	CSUL3	2.32	b	1.72	b	4.04	b	17.63	a	11.57	a
MO20	×	CEPEC90	2.08	b	1.66	b	3.74	b	9.42	b	7.36	a
CCN10	×	CSUL3	2.16	b	1.36	b	3.52	b	24.80	a	12.89	a
CCN10	×	CAB157	1.89	b	1.35	b	3.24	b	18.90	a	10.23	a
SCA6	×	CAB324	1.70	b	1.44	b	3.14	b	16.28	b	9.23	a
MO20	×	CAB94	1.52	b	1.54	b	3.06	b	14.61	b	12.42	a
TSH1188	×	CAB94	1.67	b	1.37	b	3.04	b	14.51	b	13.17	a
MO20	×	CAB169	1.43	b	1.20	b	2.63	b	10.96	b	8.28	a
SCA6	×	CAB157	1.11	b	1.35	b	2.46	b	8.28	b	8.84	a
MO20	×	CAB157	0.99	b	0.87	b	1.86	b	12.81	b	7.56	a
MSD			1.35		1.68		2.52		8.49		7.26	

MSD: Mean significant difference by Tukey's test at 5% probability, calculated from the harmonic mean of the number of repetitions; means followed by the same letter in the columns are not statistically different by Tukey's test at the 5% probability level.

were among the 40 plants evaluated in this study. The authors sought to select genotypes resistant to WB and BPR using genitors with previous associations of genes providing resistance to WB from different sources. Progenies were evaluated by conducting artificial inoculation in a greenhouse (WB) and on leaf discs (BPR). The authors selected MO20 × CCN34 as the maternal for WB, and CAB148 × MO20 (4) and CSUL3 × CCN10 (1) for BPR.

Although the parents chosen to form the population were based on disease resistance, a variability in WB resistance was observed in the progenies. Similar findings have been reported previously. Benjamin et al. (2014) observed that some progenies derived from resistant parents did not inherit WB resistance genes, most likely due to inefficient parental selection. Silva et al. (2010) found that clone CCN51, which is considered to be WB-resistant, did not have a strong ability to combine with other parents. The two evaluated progenies with SCA6 as a parent, retained the main characteristics of this clone and demonstrated high resistance to WB. However, the behavior of the ten progenies with TSH1188 as a parent, which is an SCA descendant, varied depending on the resistance of the maternal. While testing the progenies from crosses of other sources with SCA6 for resistance to WB, Pereira et al. (2021) confirmed the additive effect of SCA6 resistance when crossed with WB-resistant maternals.

The following results were found for the effect of maternals crossed with different paternals and *vice versa* (t-test and F-test, Supplementary files S11–S28 and S29–S71). Crosses with parents CCN10, CCN34, MO20, and TSH1188 showed significant effects on maternals (differences between progenies of the same paternal and different maternals) with respect to VB, CB, and TB. The maternals crossed with SCA6 as the paternal differed only in VB, and the effect of maternal was significant for the variable FRUWB with the crosses of parents CCN10, SCA6, and TSH1188, but there were no significant differences between the crosses with parents CCN34 and MO20. For the FRUBPR variable, there were significant differences between maternals crossed with the paternal TSH1188.

For the VB, CB, and TB variables, differences between progenies of the same maternal and different paternals were verified in crosses with CAB148, CEPEC90, CAB169, MO20, CAB324, and CAB157 as the maternal. There were no differences between crosses with P4B for the variables VB, CB, TB, FRUWB, and FRUBPR and between crosses with CAB169, CAB324, and CAB94 as maternal for the variable FRUWB. There were no significant differences in the FRUBPR variable between the tested maternals CAB148, CEPEC90, CAB169, MO20, CAB324, P4B, CAB157, and CAB94. Other maternals were not considered because they had fewer than three crossings.

Genetic variability in the cacao population is an important feature of breeding programs for the selection of superior genetic materials. Variations seeking high levels of heritability produced significant results in the selection of disease-resistant progenies in cacao (Nyadanu et al. 2017). The resistance traits to one or both of the diseases studied were most likely to get inherited from the parents, who were initially chosen to form the base population. This study selected 10 progenies among the 40 tested under high field levels of WB over 11 years based on resistance to VB, CB, TB, and pod infection, and WB resistance genes from different sources. These progenies, along with four others that were resistant to the development of VB and CB, could be used to select clones for commercial planting. Two paternals (MO20 and SCA6) and three maternals (CAB157, CAB94, and CSUL3) were prominent in producing disease resistant descendants.

The genetic materials chosen in the study were suitable for inducing precocity and resistance in susceptible cacao clones in commercial plantations, either as grafts or rootstocks. Previous research on cacao and other fruit trees has found heritability and the contribution of additive genetic effects (Reyes-Herrera et al. 2020, Fernández-Paz et al. 2021, Canas-Gutiérrez et al. 2022). The selection allows new early tests for resistance to witches' broom and black pod rot, both in seedlings and nurseries, as a strategy to accelerate the reproductive age of plants. With the use of genomic selection, it is possible to potentiate selection in forest tree species to improve the yield rate of plants in terms of reproduction, selection, and genetic gain (Grattapaglia et al. 2018, Volpato et al. 2021).

To reduce the risk of differential responses of the selected resistant plants to environmental variations, these genetic materials will be tested in at least five farms located in the main agrosystems of the cacao growing region of Bahia. This is a standard component of cacao breeding and is used for other perennial crops. This will aid in determining the adaptability of the chosen materials to the environment, excluding the effects of the genotype and environment interaction (Cruz et al. 2014, Oliveira et al. 2020), to ensure a long-term resistance in the face of potential pathogen population variants.

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