

IDENTIFICATION OF RESISTANCE SOURCES TO WITCHES'BROOM DISEASE - CACAO GERmplasm EVALUATION

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This work is part of the first studies that came to guide the recurrent selection processes for the association of resistance genes conducted by the Cacao Research Center, at Ilhéus, Bahia. Aiming to identify sources of witches' broom disease resistance, a set of 573 accessions from the Cacao Germplasm Collection of this Center were evaluated for the variables number of vegetative brooms (VB), floral cushion brooms (CB), and total brooms (TB). In addition, the relationship between the *per se* performance of the accession and their general combining ability was analyzed using data of eight progenies trials, evaluated during several years. The lowest values for VB, CB and TB were observed in Scavina clones and descendants, Amazonian groups, and groups including materials selected for resistance. This study indicated ample possibilities of genetic gain and the occurrence of different resistance genes in the species. Also, indicated evolution of the pathogen, increasing its virulence over resistant materials, and the possibility of predicting the performance of progenies from their parent performances when the differences among them are large.

Key words: *Moniliophthora perniciosa*, plant breeding, *Theobroma cacao*.

Identificação de fontes de resistência à doença de vassoura de bruxa - avaliação de germplasma de cacau. Este trabalho é parte dos primeiros estudos que viriam orientar os processos de seleção recorrente para associação de genes de resistência realizados pelo Centro de Pesquisa do Cacau, em Ilhéus, Bahia. Buscando identificar fontes de resistência à doença da vassoura de bruxa do cacaueiro, avaliou-se um conjunto de 573 acessos da Coleção de Germoplasma de Cacau deste Centro, para as variáveis número de vassouras vegetativas (VV), de vassouras de almofadadas florais (VA) e total vassouras (VT). E a relação entre o desempenho *per se* dos acessos e sua capacidade geral de combinação foi analisada usando dados de oito ensaios de progênies, avaliados durante vários anos. Os menores valores para VV, VA e VT foram observados em clones Scavina e seus descendentes, grupos de clones amazônicos e grupos que incluem materiais selecionados para resistência. Este estudo indicou amplas possibilidades de ganho genético e reforçou a constatação de ocorrência de diferentes genes de resistência na espécie. Além disso, indicou evolução do patógeno, aumentando sua virulência sobre materiais resistentes, e a possibilidade de prever o desempenho de progênies a partir do desempenho dos progenitores quando a diferença entre eles é grande.

Palavras-chave: *Moniliophthora perniciosa*, melhoramento de plantas, *Theobroma cacao*.

Introduction

The introduction of the *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora), the causal agent of the cocoa witches' broom disease (WBD), in the south of Bahia, in association with low international cacao prices, unfavorable currency exchange for the export sector and a period with unfavorable climate conditions, initiated a process which led to a wide reduction of the regional production (Oliveira and Luz, 2012; Santos Filho et al., 2008).

The structure of the breeding program already established and the available knowledge about the resistance of descendants of Scavina-6 clone, the most traditional source of resistance to this disease (Bartley, 1994), allowed, in a short time, the launch of resistant varieties, but, at the beginning, all descendants of the clone Scavina-6 (Yamada et al., 2009).

The central issue for the improvement of cacao in the region, then, became the obvious need to include, in new cultivars, resistance factors different from those used until then, to increase the level of resistance and avoid the evolution of the pathogen with regard to increasing its efficiency to cause damage. This paper aims to reveal one of the early studies for the identification of new sources of resistance for WBD, that would constitute basic elements for the cocoa breeding program conducted by the Cocoa Research Center (CEPEC), at Ilhéus, Bahia, Brazil.

The principal hypothesis of this study was the possibility of identification of new sources of resistance to WBD, or genotypes with chances of carrying different factors of resistance, in the Cacao Germplasm Collection of the Cocoa Research Center (CEPEC), and the search for such genotypes constitutes its scope. A second hypothesis was that the germplasm evaluation is efficient for predicting general combining ability for resistance.

Materials and Methods

A set of 573 cocoa accessions from the Germplasm Collection of, in Ilhéus, Bahia, Brazil, planted between 1978 and 1990, was evaluated. Up to 10 plants per row were considered as representatives of each accession, in a 4 years evaluation (1995 to 1998 - 7

removal periods) for the variables number of vegetative brooms (VB) and number of cushion brooms (CB).

The accessions were established in rows of 10 plants, with few cases of accessions with more than one row repetition, so that the differences between these accessions are inflated by the environmental differences related to their positions and, in the case of the evaluation of the number of brooms, larger plants have a higher number of points likely to be infected. Thereby, the analysis of the differences among the accessions for VB and CB were performed with correction of location effects (block effects), by Mixed Linear Model, through the Best Non-Biased Linear Estimator (BLUE) (PROC MIXED - SAS Institute, 1988, Littell et al., 1996) with subsequent extraction of these effects and analysis of the adjusted values according to a model with the sources of variation: removal period, trunk area (block) and accession, all considered fixed, except the trunk area (PROC GLM - Sas Institute, 1988).

The blocks were defined according to the topography of the area and in order to hold at least 4 rows of repeated clones and from 74 to 185 accessions in total. Only the repeated clones were used to define the block effects and, in removing these effects for the general set of accessions evaluated, the new averages were obtained by means of a rule of three.

The removal period x accession interaction was also tested, through the inclusion of this source of variation in the model (but the previous model was used to analyze the distinctions among clones).

This analysis system allowed greatest increase in repeatability between the rows of the same clone and the greatest correlation between performance *per se* of clones and their overall combining ability, among six different systems tested: 1 - the aforementioned; 2 - the use of the trunk area as a covariate in covariance analyzes based on the variables adjusted for the location effect, as shown; 3 - correction of the location (PROC MIXED) performed from variables already adjusted for the size of the plants, by the values related to the inclination of the regression curves of the variables by the trunk area (subtracting, from each value accounted for each plant and removal period, the product of the coefficient of inclination by the difference between the general average of trunk area and the trunk area of each plant), followed by analysis of the adjusted

variables, according to a model similar to those reported (without the area as a source of variation); 4 - covariance analysis without previous correction for location; 5 - variance analyzes using the values of the trunk areas as blocks, also without previous correction for position; 6 - definition of localization effects based on the consideration of all clones of each block.

Analyzes of the distinctions among series, or groups of accessions with the same origin, were carried out in a similar way, with the inclusion of the series or groups as a source of variation - these analyzes are more consistent, as there is some randomization in the physical distribution of the clones of each series or group.

These groups were also analyzed with the measurements at each removal considered as repeated measures, using PROC GLM – MANOVA (SAS Institute, 1988), for the interpretation of the effects of group, accession within group and trunk area, and PROC GLM – REPEATED (SAS Institute, 1988), for the consideration of the effects of period and the interactions period x group and period x accession within group.

Also, for the purpose of distinguishing genes or sets of genes related to resistance, clones listed as having the lowest levels of infection, for the set of accessions evaluated in the 1990s, were reassessed for number of brooms in 2002, together with 145 other genotypes, taken at random to serve as elements of comparison. Analyzes and getting of the adjusted averages were conducted according to the models described. Comparisons were made by the correlations between the averages for each of the two removal period and by ratios between the number of brooms per each clone and the average number of brooms of the 145 controls in each period.

To evaluate the relationship between the *per se* performance of clones and their general combining ability, some of the progeny evaluation tests under conduction at CEPEC were considered: trials 3, 32, 33, 38, 44, 48, 49, and 52 (Monteiro et al., 1995). From these trials the crossings considered were (always with the first group consisting of mothers and the second of fathers):

trial 3 - SIC 19, SIC 813, SIC 823 and TSA 644, in diallel;

trial 32 - BMI 67, Pound 12 and Pa 150 x Ca 4, ICS 1, Ma 15 and UF-613;

trial 33 - IMC 67, Pound 7, Scavina 6 and TSA 644 x CEPEC 11, CEPEC 12 and CEPEC 14;

trial 38 - EET 103, EET 59, ICS 6, Pound 12 and Pound 7 x ICS 8 and Pa 30;

trial 44 - SIAL 169 and SIC 19 x ICS 1, ICS 6, ICS 8 and Pound 7;

trial 48 - SIAL 580, SIAL 70 and SIAL 88 x 41 P, Porcelana, Pqto 87, UF 29, UF 650 and UF 668;

trial 49 - CC 10, OC 77 and RIM 10 x EEG 65, ICS 6 and SIC 328; and trial 52 - Pa 169, Pa 150, SIAL 407 and TSH-1188 x EET 59, EET 61, ICS 89 and ICS 98.

Broom counts and removals were performed, on average, three times a year, and four years of evaluation were considered for trial 52; three for trials 33, 44, and 49; two for trial 3 and 32 and one for trial 38.

The study of these relationships was conducted by analyzing the correlations between the values obtained from the *per se* evaluation of clones in the germplasm collection and indicators of parent performance observed in the progeny evaluation tests: the genetic values of the parents, obtained by the method of the Best Non-Biased Linear Predictor (BLUP) (Littell et al., 1996), from the analysis of all trials together (in this case were used not only the progenies previously listed, but rather all the original progenies of the trials, so that the trials were all connected by common parents - Monteiro et al., 1995), with the trials and blocks within trials as fixed effects, and mother, father, mother x father, mother x trial and father x trial as random effects.

Results and Discussion

1. Distinctions among series or accession groups

The groups or series of genotypes were listed, or formed, according to their origin (International Cocoa Germplasm Database, 1991), with the elements of distinct formation in relation to their series having been separated (Table 1).

The distributions of the considered variables were found to be distinct from the normal distribution by the Kolmogorov-Smirnov test (PROC UNIVARIATE - SAS Institute, 1998) ($D = 0.362, 0.319$ and 0.335 ; for, respectively, CB, VB and TB). Thus, analyzes were conducted with the variables transformed by obtaining

Table 1. List of studied groups

Origin	Group/ Series	Description
State of Acre	AC	Series: CAB, AB, IQ, SE
State of Amazonas	AM	Series: CAB, AM.
State of Bahia	BAH	Selections in varieties from Bahia and E. Santo - series CEPEC
State of Pará	BE	Series BE
State of Amazonas	CA	Series CA
State of Pará Exp. station	CAS	Series CAS
Costa Rica	CC	Series CC
Ecuador	CCN	Series CCN
State of Bahia	CEQ	Series CEPEC - family of unknown origin
State of Bahia - hybrids	CHC	Series CEPEC - Criollo hybrids
State of Amapá	CJ	Series CJ
State of Acre	CSU	Series Cruzeiro do Sul
State of Bahia - hybrids	CTF	Series CEPEC - Trinitario x Forastero Hybrids
State of Espírito Santo	EEG	Series EEG
Ecuador	EET	Series EET
Granada	GS	Series GS
Trinidad	ICS	Series ICS
Peru - Amazon	IMC	Series IMC
State of Amazonas	MA	Series MA
México	MEX	Series P
Ecuador	MOQ	Series MOQ
Peru - Amazon	NA	Series NA
Venezuela	OC	Series OC
Peru - Amazon	PA	Series PA
State of Pará	PAR	Series CAB
Peru - Amazon	PER	Genotypes from Peru - Series: Amazon, MO
Peru - Amazon	POU	Series POUND
Acre	RB	Series RB
México	RIM	Series RIM
State of Roraima	RR	Series CAB
Colombia	SC	Series SC
Peru - Amazon	SCA	Series Scavina
Ecuador and Trinidad	SD1	Scavina 6 descendants - Series: TSA, TSH, EET
State of Bahia	SD2	Scavina 6 descendants - Series CEPEC
Guatemala	SGU	Series SGU
State of Bahia	SIA	Series SIAL
State of Bahia	SIC	Series SIAL
Colombia	SPA	Series SPA
Colombia	SPE	Series SPEC
Costa Rica	UF	Series UF
Venezuela	VE	Series from Venezuela: Chuao, Choroní, PQTO, Playa Alta

their square roots, to approximate their distributions to the normal distribution ($D = 0.228$, 0.167 and 0.171 ; for, respectively, CB, VB and TB) and the groups were distinguished at 0.01% by the F test, with respect to the three variables. For all of them, the effects of removal period, trunk area (block), accession within group, removal period x group and removal period x accession within group were also significant. Therefore, the need for evaluations at different times and the use of the plant size to analyze distinctions regarding resistance to witches' broom has been proven.

In the analyzes, 39 groups were considered, and, of these, those formed by selections of the traditional varieties of Bahia (BAH group and SIC, SIAL and EEG series) showed the highest WBD averages (Table 2). SCA and SD1 were not included because their variances within group were lower than 1/7 of the largest variance within group, but their averages were calculated in the same way as the others.

This greater susceptibility for local types could suggest some evolution of the pathogen in the sense of acquiring greater efficiency over the segment of genetic diversity that where more frequent in the region. This, on the other hand, is not confirmed for the variable number of cushion brooms. For CB, such groups show averages below the general average and the positions with the highest levels of susceptibility are occupied by domesticated non-Amazonian groups: RIM, MEX, UF (it is emphasized, however, that the correction for size does not eliminate the possibility that series like these may have a higher proportion of floral cushions).

For VB, CB and TB, the lowest values were observed within Amazonian groups, descendants of Scavina and groups that include materials selected for resistance (the latter: CCN, VE, MOQ, OC - International Cocoa Germplasm Database, 1991).

As a promising aspect with respect to the prospects for genetic improvement, there are, then, the occurrence of several genotype groups from different origins that showed averages with large differences (noting that the values presented result from the square root of the

Table 2. Adjusted means of groups with common origin for the characters: total number of broom (TB), number of cushion brooms (CB) and number of vegetative brooms (VB); number of representatives considered per group (N) and Least Significant Difference (LSD) for Tukey, at 5 %, obtained using the harmonic mean of the number of repetitions for group

Series	N	TB	CB	VB	Series	N	TB	CB	VB	Series	N	TB	CB	VB
AC	5	2.54	1.67	1.30	EET	9	3.37	1.36	2.69	RIM	19	4.96	3.11	1.92
AM	3	2.19	1.27	1.54	GS	4	3.85	2.29	2.57	RR	3	1.65	0.70	1.29
BAH	8	3.91	1.20	3.03	ICS	18	3.45	2.22	2.20	SC	4	3.67	2.71	2.17
BE	11	3.04	1.31	2.01	IMC	9	2.82	1.69	1.76	SCA	2	0.04	0.05	-0.03
CA	6	3.67	1.78	2.62	MA	7	2.57	1.24	1.98	SD1	14	0.25	0.10	0.14
CAS	4	3.00	1.10	2.04	MEX	5	5.03	3.11	2.15	SD2	23	2.64	1.77	1.70
CC	14	3.91	2.04	2.85	MOQ	3	2.54	1.64	1.70	SGU	7	3.54	2.34	2.20
CCN	5	1.38	0.95	0.90	NA	5	2.86	1.32	2.20	SIA	47	3.83	1.20	3.27
CEQ	6	1.23	0.77	0.93	OC	4	2.31	1.37	1.28	SIC	39	4.55	0.84	3.38
CHC	11	3.60	1.42	2.28	PA	28	2.87	1.58	2.05	SPA	7	3.28	1.07	1.95
CJ	7	3.90	2.35	2.74	PAR	8	2.81	0.75	1.87	SPE	4	2.96	1.56	2.24
CSU	9	1.58	0.93	0.89	PER	5	2.71	1.86	1.77	UF	20	4.47	3.03	2.69
CTF	14	2.95	1.68	2.11	POU	11	2.76	1.27	2.06	VE	4	2.16	1.08	1.27
EEG	13	3.93	1.35	3.39	RB	16	3.15	2.01	1.70					
LSD		0.31	0.25	0.25	LSD		0.31	0.25	0.25	LSD		0.31	0.25	0.25

brooms number) in relation to that of other series representing the traditional varieties of the region, or those of series widely used in the formation of hybrid varieties previously distributed by CEPLAC (UF, ICS), which indicates broad possibilities of gain in relation to the material established before the WBD advent. Before the WBD event the breeding program was directed to deploy genotypes with high productivity and not resistance to that disease.

2. Distinctions among accessions

The total set of genotypes for all variables showed no homogeneity of variance among treatments, by the Bartlett test (Martin, 1995), and there were cases of variance equal to zero (accessions that did not show any brooms in the period). Therefore, for the analysis of each variable, the accessions were separated into four groups: group 0, which involved those with zero variance (not analyzed), group 1, those with smaller variances other than zero; group 2, those of intermediate variances and group 3, those of greater variances, so that the differences within each group did not exceed the limit of seven times. Such analyzes were also conducted with the variables transformed by obtaining their square roots, in order to approximate their distributions to the normal distribution.

The general averages of all clones for the transformed variables adjusted for the effect of location

and trunk area were 3.31 (TB), 2.23 (VB) and 1.57 (CB). The averages of the 50 accessions with the lowest values are shown in Table 3, together with the Least Significant Difference (LSD) for each group. The LSD was obtained from the harmonic mean of the number of repetitions, for the model with the sources of variation: removal period, trunk area (block) and clone (means of accessions in group zero were obtained by repeating the analyzes with the junction of this group with group one).

Among the clones listed, there is a clear predominance of accessions recognized as descendants of Scavina (IAC, TSA, TSH, CEPEC - with the exception of those numbered as 89, 90, 94, 504, 515, and 523, all of unknown origin -, and EET, with the exception of EET 45) and inclusion of the two clones of this series that were here evaluated, Scavina 6 and 12. However, the set of outstanding accessions includes materials from different origins, with primacy for wild accessions from the State of Acre (RB, Cruzeiro do Sul and CAB 110, 139), but also occurrence of wild accessions from Peru (Pound, PA), State of Rondônia (CAB 179), Amazonas (MA, CAB 274) and Para (BE, Mocarongo, CAB 23, 28, 37), Ecuador accessions produced (CCN and EQX) and selected in commercial plantations (Moq), Venezuela (Chua), Trinidad (ICS), Central America (Pentagona) and Bahia (SIC and SIAL). The latter with prominence only for cushion brooms.

Table 3. Adjusted means of 50 accessions selected for the characters: total number of broom (TB), number of cushion brooms (CB) and number of vegetative brooms (VB), group to which the access belongs with respect to variance (G), and Least Significant Difference (LSD) for Tukey, at 5 %, obtained using the harmonic mean of the number of repetitions for access

CLONE	TB	G	CLONE	VB	G	CLONE	CB	G
EET 390	-0.05	0	RB 39	-0.20	3	RB 39	-0.20	3
TSA 654	-0.05	0	TSA 654	-0.12	0	TSH 1188	-0.07	2
SCA 12	-0.03	0	EET 390	-0.11	0	EET 376	-0.02	2
SCA 6	-0.02	0	SCA 6	-0.10	0	TSA 654	-0.02	0
EET 397	-0.02	0	EET 397	-0.10	0	EET 397	-0.02	0
IAC 1	-0.02	0	SCA 12	-0.10	0	EET 390	-0.01	0
EET 377	-0.02	0	EET 377	-0.09	0	SCA 12	-0.01	0
TSA 656	-0.01	0	TSA 656	-0.08	0	TSH 774	-0.01	1
EET 392	0.11	1	IAC 1	-0.04	0	EET 377	0.00	0
TSH 774	0.12	1	EET 392	-0.03	1	SCA 6	0.00	0
CEPEC 42	0.12	1	TSH 774	0.04	1	CEPEC 42	0.00	0
EET 45	0.19	1	TSH 1188	0.09	1	IAC 1	0.01	0
CEPEC 515	0.20	1	CEPEC 42	0.10	1	TSA 656	0.01	0
PENTAGONA	0.22	1	CCN 10	0.14	1	CAB 23	0.03	1
CCN 10	0.23	1	CEPEC 515	0.14	1	EET 45	0.03	1
TSA 641	0.23	1	EET 376	0.15	1	TSA 516	0.04	1
EQX 107	0.23	1	EQX 107	0.16	1	TSA 641	0.05	1
TSH 1188	0.27	1	EET 45	0.17	1	CCN 16	0.06	1
EET 376	0.29	1	PENTAGONA	0.18	1	CEPEC 515	0.06	1
TSA 516	0.30	1	TSA 641	0.19	1	PENTAGONA	0.07	1
CCN 16	0.32	1	CSUL 3	0.21	1	TSA 644	0.07	1
TSA 644	0.37	1	TSA 516	0.22	1	EET 392	0.09	1
CAB 274	0.38	1	CAB 274	0.23	1	TSA 792	0.10	1
TSA 792	0.39	1	TSA 792	0.28	1	CEPEC 1008	0.11	2
CSUL 3	0.39	1	CCN 16	0.29	1	CAB 28	0.11	1
POUND 4B	0.42	1	TSA 644	0.31	1	POUND 4B	0.12	1
CEPEC 90	0.46	2	POUND 4B	0.34	1	CEPEC 25	0.13	1
CEPEC 38	0.49	1	RB 36	0.36	3	CCN 10	0.13	1
CEPEC 1008	0.53	1	CHUAO 120	0.37	3	CAB 274	0.16	1
CEPEC 25	0.53	1	TSH 565	0.37	3	MOCORONGO1 ^{SI}	0.18	3
CAB 179	0.57	1	CEPEC 74	0.38	2	BE 6	0.24	2
CEPEC 94	0.64	2	CEPEC 90	0.38	2	EQX 107	0.25	2
MA 16	0.64	2	CEPEC 38	0.41	1	CEPEC 38	0.26	2
CAB 139	0.71	1	MA 16	0.42	2	CSUL 3	0.26	2
CEPEC 74	0.73	2	CEPEC 25	0.43	1	SIAL 543	0.26	2
RB 39	0.74	3	CEPEC 1008	0.46	1	SIAL CB-2054	0.27	3
TSH 565	0.77	3	CEPEC 94	0.49	2	TSH 565	0.29	2
CAB 23	0.84	1	CAB 179	0.50	1	SIC 628	0.30	3
CEPEC 89	0.88	3	CAB 139	0.51	1	SPA 9	0.30	3
CAB 110	0.89	2	CSUL 7	0.52	3	MA 16	0.31	2
CSUL 8	0.89	2	C 87.56	0.55	2	CEPEC 90	0.31	2
MOQ 216	0.92	2	CAB 110	0.56	2	SIAL CB ^{SI}	0.32	2
CAB 37	0.93	2	CEPEC 504	0.56	3	MA 15S1	0.32	2
C87.56	0.99	2	CSUL 4	0.62	2	SIC 22	0.32	3
PA 169 ^{SI*}	1.02	2	PA 169 ^{SI}	0.64	2	SIC 806	0.33	3
CEPEC 523	1.11	2	CEPEC 89	0.65	3	MOCORONGO2 ^{SI}	0.34	3
ICS 32	1.13	2	MOQ 216	0.68	2	SIC 801	0.35	3
CAB 28	1.17	2	CAB 37	0.72	2	SIC 18	0.35	3
CSUL 4	1.19	2	CEPEC 79	0.73	3	SIC 19	0.35	3
CSUL 7	1.23	3	ICS 32	0.75	2	CSUL 8	0.35	2

Continuation Table 3.

GENERAL AVERAGE ^{1*}	3.31	2.33	1.57
LSD TUKEY 5%			
Group 1	0.49	0.43	0.21
Group 2	0.75	0.66	0.71
Group 3	1.52	1.29	1.19

* Average obtained considering, jointly, all groups of variances and all accesses evaluated.

¹ A seminal access produced by self-fertilization of the access identified by the name before the symbol.

Once again, it is highlighted as an important element for genetic improvement the occurrence of a large number of genotypes of different origins among the resistant ones, and the great difference of their means in relation to the general means of the collection, indicating the perspective of occurrence of different resistance genes and wide possibilities of gain.

An important aspect regarding the possibilities of gain with indirect selection is the ample coincidence, in the group identified as more resistant, of genotypes with low average numbers of VB and CB, although the correlation between these two variables was not high when considering all the evaluated clones (coefficient equal to 0.33 - statistically significant). Of the 50 accessions listed for VB, 33 were also listed for CB, with SICs and SIALs standing out among the exceptions, so that from the three sets of 50 accessions make up a total of only 68. There are then indicatives of wide possibilities of gain for CB with early selection for VB, since, in the development of young plants, data on VB can be accumulated more quickly.

In turn, regarding the origin of these genotypes, despite the notorious prevalence of Amazonian ancestry, it is noteworthy that seven accessions from Venezuela, Central America and the Caribbean had averages that would distinguish themselves from the

general averages, if considered the LSD (Tukey 5%) for group 3 (the largest LSD), so that such regions cannot be disregarded in the search for sources of resistance (Table 4).

3. Analysis of initial changes in behavior of resistant clones regarding to natural witches' broom infection

Of the 68 clones listed as having the lowest levels of infection for the total set of accessions, 65 were reassessed (the others were disregarded because their representations were reduced to less than three plants, due to deaths from *Ceratocystis cacaofunesta*) for a period of removal in 2002, together with 145 other genotypes, taken at random to serve as link elements in the comparisons.

Analyzing only the 2002 removal for the 210 accessions, these divided into three groups for TB and two for VB and CB, according to the dimensions of the variances within accessions, significant effects of trunk area and accession were observed for all groups and variables (limit of 2% for area and 0.01% for accession). The analyzes with all eight removals (the accessions were divided into three groups for the three variables, as done in the previous analyzes considering the first seven removals) showed significant effects of removal

Table 4. Adjusted means of prominent Trinitarian-Criollo accessions for the characters: total number of brooms (TB), number of cushion brooms (CB) and number of vegetative brooms (VB), and group to which the access belongs with respect to variance (G)

CLONE	TB	G	CLONE	VB	G	CLONE	CB	G
PENTAGONA	0.22	1	PENTAGONA	0.18	1	PENTAGONA	0.07	1
C 87.56	0.99	2	CHUAO 120	0.37	3			
ICS 32	1.13	2	C 87.56	0.55	2			
PLAYA ALTA 4	1.54	3	ICS 32	0.75	2			
ICS 55	1.66	3	OC 67	0.78	2			
			PLAYA ALTA 4	0.93	2			
			ICS 55	0.99	2			

period, accession and interaction: accession x removal period (0.01%), also, for all groups and variables.

The adjusted means of clone by period, when correlated with each other, produced mean coefficients of 0.61 (TB), 0.50 (VB) and 0.55 (CB) for all possible double combinations between each of the first seven periods of removal, and 0.66 (TB), 0.65 (VB) and 0.62 (CB) for the period of 2002 in relation to the previous seven, so that in this superficial approach there are no indications of significant behavioral changes and the differences seem to be related to the effects of the interaction: genotype x environment (accession x removal period).

Similarly, of the 50 accessions selected as those with the lowest number of TB, with the initial seven removals, only the CABs, who may have suffered in this evaluation period the influence of the fact that they are younger (planted in 1989 and 90); the CEPECs 25, 523 and 90; the Cruzeiro do Sul 8; the EET 397; the PA 169^{SI} and the TSH 516 ceased to belong to the group of the best 50 in the 2002 evaluation. They were replaced, primarily by genotypes from groups that had already been noted for their good performance: Cruzeiro do Sul 1 and 2, RB 36, CAB 5003.23, CA 5, BE 6 and OB 52 (from the Brazilian Amazon); CEPEC 91 and 92 (from the same origin as CEPEC 89, 90 and 94); CEPEC 504 and 510 (of unknown origin); CEPEC 79 (descendant of Scavina 6), EET 399 (descendant of Silecial, another classic source of WBD resistance); IMC 76 (from Peru); SGU 26^{SI}, CAS 2^{SI} and SPA 12S. Similar results could be here highlighted for VB.

However, for CB, which in the last removal was the predominant type of broom (averages of the proportions of the number of cushion brooms in relation to the total for the eight removal periods, in the order: 56.7, 29.5, 52.0, 35.8, 36.8, 47.8, 54.8, 58.6), draw the attention the wide changes between the initial seven removals and the eighth one to the proportions between the number of brooms from each selected accession and the average number from the controls, that occurred with the CAB and, most notably, with Scavina's descendants (groups EES - EETs descending from Scavina 6 and TS - TSA and TSH). The CES - CEPEC group, also Scavina descendant of second generation, did not change so strongly, but this group, unlike the preceding ones, included several genotypes previously identified as susceptible (Table 5).

In this last evaluation, although only two of the selected Scavina descendants surpassed the average of the controls for CB and one for VB, while the majority remained with levels of infection well below these averages (Table 6). The change in behavior of several of them suggest that evolution of the pathogen towards the expansion of virulence over resistant materials descendant from Scavina was taking place, which, in this period, were already widely used in commercial plantations. (Note that the proportions between the clone averages and general averages of controls were obtained from variables transformed by obtaining their square roots, so that values less than 100, this value indicating equal means, are overestimated in relation to those that would be found without the transformations and the superiors underestimated, and if these deviations are greater, greatest will be the differences between the means).

The local series also lost much of their distinction in relation to the general average of the controls for the variable CB (Table 5) and all selected accessions in these series lost their prominence (Table 6).

Finally, the different patterns of behavior change over time, with the change in the pathogen populations, indicates the occurrence of different resistance factors, an element that has been confirmed in other studies covering this same period and/or later years (Paim et al., 2006; Albuquerque et al., 2009; Pires et al., 2012 a, b). Similarly, different changes for Scavina's descendants suggest the presence of two alleles of great effect in the same locus, with some descendants inheriting one and others the other, or the preservation of the allele effect when in association with another resistance gene, and both aspects has also been confirmed in later works (Pires et al., 2012 a, b).

4. Relationships between the per se performance of clones and their general combining ability

The genetic values of the parents, obtained by the method of the Best Non-Biased Linear Predictor (BLUP) (Littell et al., 1996), from the analysis of all trials together (in this case were used not only the progenies previously listed, but rather all the original progenies of the trials, so that the trials were all connected by common parents (Monteiro et al., 1995), with the trials and blocks within trials as fixed effects,

Table 5. Relations between the number of total, vegetative, and cushion brooms of groups of accesses and averages of the 145 controls (RELTB, RELVB and RELCB: 1 for the eighth removal, in 2002, and 0 for the previous seven, until 1999), and number of clones in the group (N)

GRUPO	N	RELTB0	RELTB1	RELVB0	RELVB1	RELCB0	RELCB1
AC	3	127.3	120.0	114.8	91.8	125.0	123.6
AM	2	114.6	116.2	158.4	132.1	88.7	114.8
BE	5	84.2	44.7	113.7	60.6	40.0	62.1
CA	6	140.9	88.8	202.2	93.0	86.5	105.0
CAB	7	28.6	111.0	45.3	95.8	14.4	108.4
CCN	4	55.3	83.1	72.1	68.9	53.9	86.5
CEP	29	102.2	101.7	147.1	101.8	73.5	101.9
CEQ	6	44.3	41.6	67.7	26.3	37.9	45.7
CES	11	46.5	52.8	66.1	46.1	32.0	62.5
CJ	2	103.5	66.6	158.2	98.2	67.0	79.9
CSU	11	63.4	48.7	68.3	22.8	54.4	72.1
CTF	8	94.3	113.3	136.2	148.2	66.5	107.6
EEG	3	133.3	93.4	226.3	135.5	64.3	98.7
EES	5	2.2	27.6	-2.7	2.9	0.5	56.4
EET	3	75.0	49.8	84.7	65.2	39.0	68.2
ICS	7	80.5	110.1	110.9	115.8	64.2	96.4
IMC	3	101.6	159.3	131.7	61.4	93.6	131.6
MA	4	68.1	68.7	105.1	62.5	40.6	84.3
MOQ	2	89.9	97.1	122.1	87.4	81.0	83.2
MT	7	84.5	94.9	146.7	115.2	42.7	104.4
PA	11	85.0	112.2	128.8	98.0	58.3	114.7
PAR	6	151.0	53.0	196.7	91.4	66.6	79.0
PER	4	98.8	125.2	135.3	116.5	89.8	123.7
POU	4	88.8	69.0	118.4	79.7	66.5	76.7
RB	10	113.3	120.8	118.6	61.0	95.5	115.7
RIM	6	192.4	149.0	150.5	120.7	182.8	148.0
RR	3	61.4	48.6	96.5	56.0	33.7	72.3
SCA	2	-1.1	-4.7	-7.4	-13.8	-0.2	14.8
SGU	2	60.8	33.9	73.2	21.6	38.6	47.7
SIA	8	96.0	69.7	148.1	90.4	35.6	82.8
SIC	15	157.9	75.8	234.1	163.4	39.3	69.2
SPA	5	111.6	114.5	150.7	66.7	68.2	113.5
TS	9	9.7	30.7	10.6	8.3	2.7	56.9

Encoding of series or groups as shown in Table 1 - with the additions: TS - Scavina descendants of the TSA and TSH series; EES - Scavina descendants of the EET series; CES - Scavina descendants of the CEPEC series.

and mother, father, mother x father, mother x trial and father x trial as random effects.

In the analyzes for determining the genetic values of the parents on the progeny trials (by the method of the Best Non-Biased Linear Predictor; Littell et al., 1996), the treatments considered presented, for the variables VB and CB, variances whose differences between the smallest and the largest do not exceed the value of seven times, with the exception of Trial

33, for the variable CB, which reached the limit of eight times (VB and CB were transformed by obtaining their square roots, in order to approximate their distribution curves of normal curves). Considering, however, that this case involved a progeny of the clone Scavina 6, which show a resistance pattern, and that the most important element here is obtaining the means of parents for comparison with the means obtained from clones, and not the comparison among parents, such progeny was not excluded from the analyzes.

Taking the level of probability of 0.05 (significance at 5%) as the reference standard, the effects of year, parents (SIC 19, SIC 813, SIC 823 and TSA 644) and interaction among parents were found to be significant for VB, and only year, for CB, in trial 3; year and mother (IMC 67, Pound 12 and PA 150) for VB and CB, in trial 32; year, mother (IMC 67, Pound 7, Scavina 6 and TSA 644), mother x father and year x mother, for VB and CB in trial 33; mother (EET 103, EET 59, ICS 6, Pound 12 and Pound 7) and father (ICS 8 and Pa 30), for VB and CB in trial 38; year, mother (SIAL 169 and SIC 19), father (ICS 1, ICS 6, ICS 8 and Pound 7), year x mother, for CB, and year, mother, father, father x mother and year x

father, for VB, in trial 44; year, mother (SIAL 580, SIAL 70 and SIAL 88), father (41 P, Porcelana, Pqto 87, UF 29, UF 650 and UF 668) and mother x father, for VB and CB in trial 48; year, father (EEG 65, ICS 6 and SIC 328), mother x father and year x father, for VB, and year, father, and year x father, for CB, in trial 49; and year, mother (Pa 169, Pa 150, SIAL 407 and TSH-1188), father (EET 59, EET 61, ICS 89 and ICS 98), mother x father and year x mother, for VB and CB in trial 52.

Table 6. Reason for the selection of the clone in the first seven removals - total brooms, vegetative brooms, or cushion brooms (SEL), and relations between the number of total, vegetative, and cushion brooms of the clone and averages of the 145 controls (RELTB, RELVB and RELCB: 1 for the eighth removal, in 2002, and 0 for the previous seven, until 1999)

GROUP	CLONE	SEL	RELTB0	RELTB1	RELVB0	RELVB1	RELCB0	RELCB1
C	C 87.56	TB	35.9	28.7	40.0	-3.5	37.5	66.9
CAB	CAB 110	TB	32.3	192.3	41.1	90.4	22.4	153.3
CAB	CAB 139	TB	25.9	89.6	37.2	75.7	22.2	105.2
CAB	CAB 179	TB	20.7	73.7	36.3	22.3	22.0	93.0
CAB	CAB 23	TB	30.7	111.9	60.9	209.8	1.5	94.3
CAB	CAB 274	TB	14.0	112.5	16.9	64.3	8.2	81.6
CAB	CAB 28	TB	42.6	67.9	72.1	110.3	5.6	93.5
CAB	CAB 37	TB	33.7	129.0	52.8	97.8	18.7	137.9
CCN	CCN 10	TB	8.4	3.0	10.3	-2.2	6.8	6.8
CES	CEPEC 1008	TB	19.2	27.3	33.4	8.4	5.5	39.5
CES	CEPEC 25	TB	19.4	53.5	31.5	80.8	6.6	48.6
CES	CEPEC 38	TB	17.7	13.7	30.0	6.9	13.2	30.2
CES	CEPEC 42	TB	4.5	5.9	7.3	-6.0	0.1	8.7
CEP	CEPEC 515	TB	7.2	0.3	10.5	-4.6	3.4	3.4
CEP	CEPEC 523	TB	40.3	53.4	64.9	31.5	28.0	81.7
CES	CEPEC 74	TB	26.5	10.5	27.5	-3.6	26.5	27.5
CEQ	CEPEC 89	TB	32.2	13.7	47.4	-0.8	28.4	34.4
CEQ	CEPEC 90	TB	16.8	75.3	27.6	6.8	16.2	74.9
CEQ	CEPEC 94	TB	23.2	3.9	35.6	4.6	24.1	7.3
CSU	CSUL 3	TB	14.4	18.1	15.7	-7.5	13.5	40.2
CSU	CSUL 4	TB	43.4	33.2	45.5	-9.9	18.7	68.6
CSU	CSUL 7	TB	44.8	32.7	37.8	-4.3	41.2	58.0
CSU	CSUL 8	TB	32.6	76.3	56.4	52.5	18.3	101.5
EES	EET 376	TB	10.5	37.9	10.7	12.1	-1.2	72.5
EES	EET 377	TB	-0.7	8.0	-6.3	1.3	-0.1	47.1
EES	EET 390	TB	-1.8	-2.1	-8.4	-6.7	-0.5	27.5
EES	EET 392	TB	4.0	-6.7	-2.0	-14.8	4.9	4.2
EES	EET 397	TB	-0.9	100.7	-7.4	22.8	-0.9	130.6
EET	EET 45	TB	6.9	10.9	12.8	2.5	1.8	15.2
EQX	EQX 107	TB	8.4	0.8	11.9	1.8	13.2	3.4
IAC	IAC 1	TB	-0.7	23.8	-3.2	2.6	0.3	35.8
ICS	ICS 32	TB	41.3	38.3	55.1	4.0	40.7	52.4
MA	MA 16	TB	23.5	26.2	31.1	7.4	15.9	43.3
MOQ	MOQ 216	TB	33.5	38.4	49.7	68.6	27.6	35.1
PA	PA 169 ^{S1}	TB	37.1	146.5	46.5	.	21.6	129.5
POU	POUND 4B	TB	15.5	5.4	25.0	6.1	6.4	9.3
RB	RB 39	TB	26.9	-23.2	-14.4	-15.3	-10.1	13.1
SCA	SCA 12	TB	-1.2	-3.8	-7.3	-13.8	-0.4	24.0
SCA	SCA 6	TB	-0.9	-5.6	-7.5	-13.8	0.1	5.7
TS	TSA 516	TB	11.0	86.6	16.2	57.6	2.1	112.8
TS	TSA 641	TB	8.4	0.8	13.9	-4.2	2.7	4.0
TS	TSA 644	TB	13.4	29.7	22.5	17.0	3.8	34.0
TS	TSA 654	TB	-1.7	30.0	-8.8	-14.8	-1.2	63.0
TS	TSA 656	TB	-0.4	31.5	-5.8	23.9	0.5	63.6
TS	TSA 792	TB	14.3	12.9	20.4	-12.6	4.9	45.1
TS	TSH 1188	TB	9.9	39.7	6.9	-4.3	-3.5	83.2
TS	TSH 565	TB	27.9	49.4	27.1	16.2	15.0	73.7
TS	TSH 774	TB	4.3	-4.3	3.2	-4.5	-0.3	32.4
BE	BE 6	CB	70.9	2.4	69.1	-1.3	12.3	13.9
MA	MA 15S1	CB	60.3	52.3	108.9	56.9	16.6	64.2
PAR	MOCORONGO1 ^{S1}	CB	136.6	68.2	166.7	154.4	9.3	93.4
PAR	MOCORONGO2 ^{S1}	CB	159.1	67.5	209.5	188.4	17.8	117.3

Continuation Table 6.

SIA	SIAL 543	CB	48.4	46.2	93.5	85.0	13.5	46.3
SIA	SIAL CB-2054	CB	110.5	23.4	108.1	35.9	13.8	70.1
SIA	SIAL CBS1	CB	108.1	45.2	111.4	56.4	16.4	91.6
SIC	SIC 18	CB	185.7	51.3	266.6	.	18.2	76.9
SIC	SIC 19	CB	164.6	65.7	219.0	154.3	18.3	82.4
SIC	SIC 22	CB	162.1	55.6	216.9	140.5	16.7	72.7
SIC	SIC 628	CB	181.3	84.3	256.0	198.4	15.5	88.3
SIC	SIC 801	CB	187.9	79.4	266.8	184.5	18.0	93.5
SIC	SIC 806	CB	165.9	68.4	225.8	152.7	17.0	92.7
SPA	SPA 9	CB	117.0	63.9	123.8	123.9	15.5	97.8
CEP	CEPEC 504	VB	69.2	9.6	41.1	61.4	35.1	69.2
CES	CEPEC 79	VB	52.1	8.7	53.4	-4.4	50.7	23.0
RB	RB 36	VB	59.6	34.2	26.3	-7.4	27.3	71.2

Encoding of series or groups as shown in the previous Table.

TB - selected by the total number of brooms; VB - selected by the number of vegetative brooms and not included in the top 50 for TB; CB - selected by the number of cushion brooms and not included in the top 50 for TB and the top 50 for VB.

Analyzing all the trials together (in this case, not only the listed progenies were used, but all the original progenies of the trials, so that they were all connected by common parents; Monteiro et al., 1995), and estimating the genetic values of the parents by the Best Non-Biased Linear Predictor method (BLUP) (Littell et al., 1996), the correlations between these genetic values and their respective *per se* values, for clones whose progenies showed significant differences in the previous analyzes, were 0.65, 0.63, 0.64 (significant at 1%) and 0.44 (probability of 0.89) for the variable VB, the general group of mothers and, respectively, the chosen methodology, determination of clone averages without correction, correction only by the trunk area and correction also by location using all accessions in each block. For fathers correlations were not significant.

Also for CB there was no significance for the correlations with the group of fathers (at 5%) and, for this variable, the correlation coefficients for the group of mothers were, respectively, regarding the four methodologies, in the same sequence: 0.70, 0.66, 0.65 (all significant at 1%) and 0.54 (probability of 0.04 or 4%).

The group of mothers, unlike that of fathers, includes the clone Scavina 6 and descendants, who are markedly more WBD resistant than the others, and the difficulty of reaching high correlation coefficients when these are not included (for example: group of fathers) indicates limitations regarding the possibility of predicting the performance of progenies from the

performance of clones, when the differences among these clones are less pronounced. This aspect, however, is expected, and even very resistant clones can produce progenies with an inadequate response if the resistance allele is predominantly recessive. On the other hand, the evaluated set did not involve any of the Scavina non-descending clones identified as among the most resistant ones and, even so, some correlation was observed: correlation coefficient of 0.69 (probability of 0.02) for CB with the group of mothers; eliminating Scavina 6 and its descendants, with the other methods, in the same order, the values observed were: 0.56; 0.49 and 0.47, all not significant at 5%. For VB, the correlation coefficients for the group of mothers, with the elimination of Scavina 6 and its descendants, were reduced to: 0.36; 0.33; 0.26 and 0.12 (for the four methods, in the same order), all not significant.

Best performing clones in this study were selected to form the basis of the recurrent selection program initiated in this period, and many proved to be excellent materials for resistance, *per se* or as parents (Pires et al., 2012 a, b; Silva et al., 2010; Yamada et al., 2014; Pires, Luz e Pimenta Neto, 2021).

Conclusions

It was possible to identify the groups of genotypes most resistant to witches' broom disease; verify that the evaluation for witches' broom disease cannot be performed in a single or in a few periods; understand that there are wide possibilities of genetic gain in

relation to the material established before the disease advent; perceive that it was taking place an evolution of the pathogen in the sense of increasing virulence over resistant materials descendants from the most important source of resistance until then; notice the occurrence of different resistance factors (different genes and alleles); realize that there are clear association between the performance of progenies and the performance of the original clones, when the differences among the clones are large, and prove that corrections for local and plants size produced increments in the correlation coefficients between *per se* performance and general combining ability in the evaluation of germplasm established without randomization. All these finding are very important for the cacao genetic improvement program.

Literature Cited

- ALBUQUERQUE, P. S. B. et al. 2009. Novel sources of witches' broom resistance (causal agent *Moniliophthora perniciosa*) from natural populations of *Theobroma cacao* L. from the Brazilian Amazon. *Euphytica* 172:125-138.
- BARTLEY, B. G. 1994. A review of cacao improvement. Fundamental methods and results. In: International Workshop on Cocoa Breeding Strategies, Kuala Lumpur, Malaysia, 1994. Proceedings. Malaysia, Kuala Lumpur, INGENIC. pp.16
- INTERNATIONAL COCOA GERMPLASM DATABASE. 1991. Reading: University of Reading. Available at: <http://www.icgd.rdg.ac.uk>. Accessed on 05 Dez 2021.
- LITTELL, R. C. et al. 1996. SAS System for Mixed Models. Cary, NC: Statistical Analysis System Institute. 633p.
- MARTIN, F. G. 1995. Statistical design and analysis. Gainesville, Florida, University Copy Center. UF:2-4.
- MONTEIRO, W. R. et al. 1995. Desenvolvimento e avaliação de cultivares híbridas de cacau. Informe de Pesquisas, 1987/90. CEPLAC, Ilhéus, Bahia. pp.19-23.
- OLIVEIRA, M. L.; LUZ, E. D. M. N. 2012. Principais doenças do cacau e seu manejo. In: Valle, R. R. (Ed.) Ciência, tecnologia e manejo do cacau. Ceplac, Brasília, DF. pp.187-275.
- PAIM, V. R. L. M. et al. 2006. Sources of resistance to *Crinipellis perniciosa* in progenies of cacao accessions collected in the Brazilian Amazon. *Scientia Agricola* 63:572-578.
- PIRES, J. L. et al. 2012a. Association among sources of resistance to witches' broom disease for the increment of the level and durability of the character. *Agrotrópica (Brasil)* 24:27-30.
- PIRES J. L. et al. 2012b. New genes of resistance to witches' broom identified by the behavior of different clones over time. In: International Cocoa Research Conference. Lagos, Nigeria 2012. Proceeding. Lagos, Nigeria. COPAL.
- PIRES J. L.; LUZ E. D. M. N.; PIMENTA NETO, A. A. 2021. Early selection for resistance to cacao witches' broom in new parental combinations. *Summa Phytopathologica* 47:88-95.
- SANTOS FILHO, L. P. et al. 2008. Produção de cacau e a vassoura-de-bruxa na Bahia. *Agrotrópica (Brasil)* 20:73-82.
- SAS INSTITUTE INC. SAS/STAT. 1988. User's Guide Release 6.03. Cary, NC: Statistical Analysis System Institute. 1028p.
- SILVA, S. D. V. M. et al. 2010. Parent selection for cacao resistance to witches' broom. *Pesquisa Agropecuária Brasileira* 45:680-685.
- YAMADA, M. M. et al. 2009. Parent pair analysis of cacao trees selected in farms for resistance to *Moniliophthora perniciosa* using microsatellites. *Agrotrópica (Brasil)* 21:123-126.
- YAMADA, M. M. et al. 2014. Ocorrência de vassoura-de-bruxa em progênies de cacau selecionadas pelo programa de melhoramento na Estação Experimental Joaquim Bahiana. *Agrotrópica (Brasil)* 26:197-202.