ARTIGOS

Early selection for resistance to cacao witches’ broom in new parental combinations

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ABSTRACT

Pires, J.L.; Luz, E.D.M.N.; Pimenta Neto, A.A. Early selection for resistance to cacao witches’ broom in new parental combinations. ***Summa Phytopathologica,*** v.47, n.2, p.88-95, 2021.

Progenies from 69 crosses between parents obtained by recurrent selection for resistance and yield were inoculated with *Moniliophthora perniciosa* basidiospores. The symptoms were evaluated and compared with those in the equally inoculated progenies of Catongo, SIC 23 and SCA 6. Forty-three of the new progenies did not differ statistically from SCA 6, while 10 new progenies were statistically better than this control. The female parents that can be highlighted are (P4B x SCA 6), (MA 16 x SCA 6), (CEPEC 86 x SCA 6), and the EET 75 clone. The best male parents were (CAB 214), (CAB 208) and (P4B x OC 67), which did not differ from each other. This study proved the existence of gene combinations between fathers and mothers, the occurrence of additive effects and the dominant inheritance of these factors, which should allow the selection of clones with higher resistance levels and durability.

Keywords: *Theobroma cacao*, plant breeding, *Moniliophthora perniciosa*, genetic control.

RESUMO

Pires, J.L.; Luz, E.D.M.N.; Pimenta Neto, A.A. Seleção precoce para resistência à vassoura-de-bruxa do cacaueiro em novas combinações parentais. ***Summa Phytopathologica,*** v.47, n.2, p.88-95, 2021.

Progênies de 69 cruzamentos entre genitores obtidos por seleção recorrente para resistência e produtividade foram inoculadas com basidiósporos de *Moniliophthora perniciosa*. Os sintomas foram avaliados e comparados com aqueles nas progênies de Catongo, SIC 23 e SCA 6, igualmente inoculadas. Quarenta e três das novas progênies não diferiram estatisticamente de SCA 6, enquanto 10 novas progênies foram estatisticamente melhores do que esse controle. Os genitores femininos que podem ser destacados são (P4B x SCA 6), (MA 16 x SCA 6), (CEPEC 86 x SCA 6) e o clone EET 75. Os melhores genitores do sexo masculino foram (CAB 214), (CAB 208) e (P4B x OC 67), que não diferiram entre si. Este estudo comprovou a existência de combinações gênicas entre pais e mães, a ocorrência de efeitos aditivos e a dominância na herança desses fatores, o que deve permitir a seleção de clones com maiores níveis de resistência e durabilidade.

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

The cocoa tree (*Theobroma cacao* L.) has a high yield potential (1). However, expression of this potential is limited due to several factors, such as unfavorable environmental conditions, unsuitable soil type, genetic material, and especially occurrence of diseases and insects, responsible for annual crop losses that reach approximately one-third of the global production (10).

In Brazil, witches’ broom (WB), caused by the fungus *Moniliophthora perniciosa* (Stahel) Aims & Phillips-Mora, is still the biggest phytosanitary problem for cacao cultivation in the major producing regions of the country, followed by brown rot (*Phytophthora* spp.) and Ceratocystis wilt (*Ceratocystis cacaofunesta* Engelbrecht & Harrington) (16). Economic sustainability of the crop can be obtained through more widespread use of genetically improved varieties or clones that accumulate resistance genes to the main diseases, are productive and have desirable organoleptic characteristics.

According to Bhattacharjee and Kumar (7), only 30% cacao genetic material is genetically improved, mostly consisting of biparental progenies, which would justify the low crop yield, evidencing the urgent demand for new productive and disease-resistant varieties. This is one of the main reasons for the support given in recent years to cacao genetic breeding programs in all cocoa research institutions in the world, as well as for the development of international projects with this objective (9, 10).

In the last two decades, the selection of WB-resistant parents with a high general and specific combining ability, along with the development of populations that contain a pool of genes for resistance, have been the target for cacao breeding in Brazil (2, 5, 11, 14, 15, 19, 23). The cacao populations were formed by progenies obtained in selection cycles for resistance to this disease through crosses between genotypes showing agronomically desirable characteristics and carrying resistance genes, generally from genetically distant sources (12, 19).

To select WB-resistant cacao genotypes, one or two resistant and susceptible genetic materials must be used for comparisons. In Brazil, the most adopted resistant pattern is SCA 6 and the susceptible Catongo (a mutation of ‘Comum’ variety with white seeds) and/or SIC 23 (a ‘Comum’ selection at the Cacao Institute). In previous inoculation studies, all three patterns performed as expected (2, 5, 13,18, 21, 23, 5, 21, 13).

Aiming to increase the genetic base of materials selected for accumulation of WB resistance genes, we performed new crosses including clones resistant to frosty pod rot (*Moniliophthora roreri (*Cif.) H.C. Evans, Stalpers, Samson & Benny 1978), another important cacao disease not yet recorded in the country (quarantine pest A1). Using early selection, this article identifies parents and progenies highlighted for accumulating WB resistance genes, in addition to progenies of clones resistant to frosty pod rot, contributing to the sustainability of cacao crop and preventing the introduction of quarantine pests.

MATERIALS AND METHODS

Seeds were obtained from 69 crosses between a group of 11 female parents and a group of 9 male parents. These were plants selected from crosses of previous cycles of recurrent selection for resistance to WB (23), pod characteristics and yield, or known clones also selected for resistance to WB or Frost pod rot, pod characteristics and production. Table 1 lists the main characteristics of each clone used in this study. Number of vegetative and cushion brooms, number of infected and produced pods, pod characteristics and vegetative aspects of plants assessed on a regular basis for at least six years of field cultivation, were used to select plants from the first cycle of recurrent selection (18).

The female parents were six plants selected from a previous cycle of recurrent selection: (CEPEC 86 X SCA 6), (RB 36 X SCA 6), (SGU 26 X SCA 6), (CSUL 3 X SCA 6), (NA 33 X CSUL 7), (MA 13 X SCA 6), and the five clones EET 233, EET 392, EET 92, EET 75 and UF 273. Three selected plants derived from open-pollinated progenies of WB-resistant clones (CAB 270), (CAB 208), (CAB 214); four plants from crosses (NA 33 X RB 39), (CEPEC 90 X CHUAO 120), (P4B X OC 67), (CEPEC 86 X RB 39), and the clones EET 75 and ICS 95 were used as male parents. The seeds were planted in 288cm^3

Table 1. Cacao clones used as F1 parents - name, acronym, origin, major reasons for selection.

CLONES F1	ACRONYM	ORIGIN	REASONS FOR SELECTION
CAB 208	Cacau da Amazônia Brasileira	Brazil, Amazonas	Resistance to witches' broom, productivity
CAB 214	Cacau da Amazônia Brasileira	Brazil, Amazonas	Resistance to witches' broom, productivity
CAB 270	Cacau da Amazônia Brasileira	Brazil, Amazonas	Resistance to witches' broom, productivity
CEPEC 86	Centro de Pesquisas do Cacau	Brazil, Bahia	Resistance to witches' broom, pod characteristics
CEPEC 90	Centro de Pesquisas do Cacau	unknown origin	Resistance to witches' broom
CHUAO 120	Chuao - village	Venezuela	Resistance to witches' broom, productivity, pod characteristics, quality
CSUL 3	Cruzeiro do Sul - municipality	Brazil, Acre	Resistance to witches' broom
CSUL 7	Cruzeiro do Sul - municipality	Brazil, Acre	Resistance to witches' broom
EET 233	Estacion Experimental Tropical	Ecuador, Pichilingue	Resistance to frosty pod rot, productivity, pod characteristics
EET 392	Estacion Experimental Tropical	Ecuador, Pichilingue	Resistance to witches' broom, productivity, pod characteristics
EET 75	Estacion Experimental Tropical	Ecuador, Pichilingue	Resistance to frosty pod rot, productivity, pod characteristics
ICS 95	Imperial College Selections	Trinidad	Resistance to frosty pod rot, productivity, pod characteristics
MA 16	Manaus - municipality	Brazil, Amazonas	Resistance to witches' broom
NA 33	Nanay - river	Peru	Resistance to witches' broom, productivity
OC 67	Ocumare - municipality	Venezuela	Resistance to witches' broom, pod characteristics, quality
P 4B	Pound (collector)	Peru, Iquitos	Resistance to witches' broom
RB 36	Rio Branco - municipality	Brazil, Acre	Resistance to witches' broom
RB 39	Rio Branco - municipality	Brazil, Acre	Resistance to witches' broom, canopy structure
SCA 6	Escavino - family	Peru	Resistance to witches' broom
SGU 26	Selecciones Guatemaltecas	Guatemala, Los brillantes	Resistance to witches' broom, pod characteristics, quality
UF 273	United Fruit selections	Costa Rica	Resistance to frost pod rot, productivity, pod characteristics, quality

tubes containing commercial substrate and soil at the ratio of 3:1. The resulting seedlings were kept in a greenhouse until the second leaf had expanded to 1.5 cm (approximately 30 days). They were then inoculated by depositing in the apical meristem 30 µL of a suspension of $2x10^5$ basidiospores·mL^{-1} *Moniliophthora perniciosa* in 0.3% agar-water. The seedlings were then transferred to a humid chamber at 25°C and 100% relative humidity for 48 h. Subsequently, they were transferred to a greenhouse, where they remained until the end of the evaluations, 60 days after inoculation.

The seeds of the 72 progenies (69 crosses plus three controls) were obtained at different times, requiring several inoculation experiments. Thus, 31 experiments were performed with different number of progenies, which had two to three common controls each (free pollination seeds from the clones Catongo or SIC 23, susceptible, and SCA 6, resistant). In some cases, progenies were repeated.

In the evaluation, value 1 was assigned to the presence and 0 to the absence of brooms, considering: terminal broom (TB), axillary broom (AB), dry broom (DB), and cotyledonary broom (CB). Axillary brooms larger than 1 cm were quantified and the length of terminal broom was measured.

These data were used to calculate the disease index (DI) of each plant, using the following formula (20):

DI = TB + (0.01 * TBL) + AB + (0.1 * NAB) + CB + (4.0 * DB),

TB = value for the presence or absence of terminal broom (0 or 1);

TBL = terminal broom length;

0.01 = fraction that, multiplied by the length of the largest broom in the experiment, results in 1;

AB = value for axillary broom;

NAB = number of axillary brooms larger than 1 cm;

0.1 = fraction that, multiplied by the highest number of axillary brooms larger than 1 cm observed in a plant in the experiment, results in 1;

CB = value for cotyledonary broom;

DB = value for dry broom;

4.0 = index that makes the value for dry broom (4.0 * DB) higher than TB + (0.01 * TBL) + AB + (0.1 * NAB) + CB of any plant in the experiment.

Progeny effects were analyzed according to an incomplete block design with the sources of variation: trial or experiment (block) and progeny. All progenies were compared with the controls according to T test for corrected means (22).

The following effects were also analyzed: mother, father, and mother x father interaction, adopting a model with these effects and trial or experiment as sources of variation.

Corrected parent means are not estimable in this model, i.e., mother means cannot be corrected for the effects of father and trial at the same time, or father means cannot be corrected for mother and trial, simultaneously. Thus, for comparisons between parents, the DI was corrected for each trial, as follows: the corrected index for each plant is equal to the original index multiplied by the inverse of the sum of the means of the controls in that trial, divided by the sum of the generals means of controls in all tests. Thus, the DIs of each plant were corrected for the effects of the trial to which they belong by the ratio between the mean DIs of the controls in that trial and the mean DIs of the controls for all trials. With corrected DIs, parent effects were analyzed in a model with the sources of variation: father and mother; means (22) were compared according to T Test.

For comparisons between the means of fathers within mother, or mothers within father, and the evaluation of their effects, each mother and each father were analyzed separately, using the model with the source of variation father for analyses within each mother, and the source of variation mother for analyses within each father, with the corrected DI.

However, to simplify and reduce the number of tables, the means of fathers within mother, or mothers within father, presented here are only the ones calculated from the previous model, with DI corrected by LSmeans, with the sources of variation: trial or experiment and progeny (Table 2), each one contrasted only with the controls.

RESULTS AND DISCUSSION

Significant effects were found for experiment and progeny, with F probability lower than 0.0001, or significant F at 0.01% (not shown).

The three controls had averages, corrected for the experiment effects, increasing from SCA 6 to SIC 23 and from SIC 23 to Catongo, all different from each other, according to T test (Table 2).

The crosses between the 11 mothers and nine fathers, generating a variable number of progenies for each of them, resulted in 69 progenies, which were contrasted through the disease index (DI); corrected for the effects of the experiment, as well as for the effects of progenies from open pollination of the clones Catongo and SIC 23 (susceptibility patterns) and SCA 6 (resistance pattern). Only one progeny was as susceptible as that of Catongo [free UF 273 x (NA 33 x RB 39)], and 9 did not differ statistically from the progeny of SIC 23 (Table 2). Three of them included the father (CEPEC 90 x CHUAO 120), three included the father (NA 33 x RB 39), and two the father ICS 95.

ICS 95 also produced a progeny with high DI, which significantly differed from all controls (progeny UF 273 X ICS 95). The same occurred for the progenies [(CEPEC 86 X SCA 6) X CAB 270], [(SGU 26 x SCA 6) X CAB 270], (EET 392 X EET 75), and [(NA 33 x CSUL 7) X (CEPEC 90 x CHUAO 120)]. The father (CAB 270) (plant selected from a free pollination progeny of the clone CAB 270) also generated one of the 10 progenies that did not differ from that of SIC 23. Thus, among progenies presenting the worst behavior, there is clear predominance of some progenitors, indicating parent effect for resistance, which will be discussed later.

The free pollination progeny of SCA 6 maintained the characteristic of resistance to *M. perniciosa* and did not differ statistically from 43 of the newly attained progenies. Of these, 20 had DIs numerically smaller than that of the Scavina progeny.

Ten progenies had statistically lower means than that of Scavina 6 progeny: [(CEPEC 86 X SCA 6) X EET 75], [(RB 36 x SCA 6) X (CEPEC 90 x CHUAO 120)], [(RB 36 x SCA 6) X EET 75], [(NA 33 x CSUL 7) X (P4B x OC 67)], [(MA 16 x SCA 6) X CAB 214], [(MA 16 x SCA 6) X (CEPEC 86 x RB 39)], [(P4B x SCA 6) X CAB 208], [(P4B x SCA 6) X ICS 95], [EET 233 X CAB 214] and [UF 233 X (CEPEC 90 x CHUAO 120)].

Seven of them descend from clones collected in the Brazilian Amazon and selected as resistant to witches' brooms: CAB 208 and CAB 214 (2, 16) – both are selections from progenies of open pollination of these clones, as well as RB 36, RB 39 and CSUL 7 – Acre State collections. Such results confirm that the Brazilian Amazon is a consistent source of resistance to this disease (2, 15, 16, 21). Four of the ten best progenies descend from clones selected for resistance to frosty pod rot, EET 75, EET 233 and UF 273 (3, 14, 17), which indicates possible gains with indirect selection – gains for resistance to one disease with the selection for another disease.

Table 2. Disease index of symptoms caused by *Moniliophthora perniciosa* in cacao seedlings from crosses among progenitors selected for resistant to *M. perniciosa* and *M. roreri*; desirable agronomic characteristics, and significance according to T test for contrasts with controls.

CROSSES PROGENIES	DI LSmeans[1]	No.	CONTROLS		
			CATONGO	SIC 23	SCA 6
(CEPEC 86 x SCA 6) X (CAB 270)	0.604	1	**	**	**
(CEPEC 86 x SCA 6) X (CAB 208)	0.282	2	**	**	ns
(CEPEC 86 x SCA 6) X (CAB 214)	0.283	3	**	**	ns
(CEPEC 86 x SCA 6) X (CEPEC 90 x CHUAO 120)	0.221	4	**	**	ns
(CEPEC 86 x SCA 6) X (CEPEC 86 x RB 39)	0.305	5	**	**	ns
(CEPEC 86 X SCA 6) X EET 75	0.084	6	**	**	*
(RB 36 x SCA 6) X (CAB 270)	0.748	7	**	ns	**
(RB 36 x SCA 6) X (CAB 208)	0.450	8	**	**	ns
(RB 36 x SCA 6) X (CAB 214)	0.388	9	**	**	ns
(RB 36 x SCA 6) X (CEPEC 90 x CHUAO 120)	0.030	10	**	**	**
(RB 36 x SCA 6) X (CEPEC 86 x RB 39)	0.492	11	**	**	ns
(RB 36 x SCA 6) X EET 75	-0.083	12	**	**	**
(SGU 26 x SCA 6) X (CAB 270)	0.570	13	**	**	**
(SGU 26 x SCA 6) X (CAB 208)	0.306	14	**	**	ns
(SGU 26 x SCA 6) X (CAB 214)	0.353	15	**	**	ns
(SGU 26 x SCA 6) X (CEPEC 90 x CHUAO 120)	0.647	16	**	ns	*
(SGU 26 x SCA 6) X (P4B x OC 67)	0.312	17	**	**	ns
(SGU 26 x SCA 6) X (CEPEC 86 x RB 39)	0.508	18	**	**	ns
(CSUL 3 x SCA 6) X (CAB 270)	0.323	19	**	**	ns
(CSUL 3 x SCA 6) X (CAB 208)	0.392	20	**	**	ns
(CSUL 3 x SCA 6) X (CAB 214)	0.302	21	**	**	ns
(CSUL 3 x SCA 6) X (NA 33 x RB 39)	0.166	22	**	**	ns
(CSUL 3 x SCA 6) X (P4B x OC 67)	0.432	23	**	**	ns
(CSUL 3 x SCA 6) X (CEPEC 86 x RB 39)	0.766	24	**	ns	**
(CSUL 3 x SCA 6) X ICS 95	0.826	25	**	ns	**
(NA 33 x CSUL 7) X (CAB 270)	0.446	26	**	**	ns
(NA 33 x CSUL 7) X (CAB 208)	0.312	27	**	**	ns
(NA 33 x CSUL 7) X (CAB 214)	0.218	28	**	**	ns
(NA 33 x CSUL 7) X (CEPEC 90 x CHUAO 120)	0.558	29	**	**	**
(NA 33 x CSUL 7) X (P4B x OC 67)	-0.026	30	**	**	**
(NA 33 x CSUL 7) X (CEPEC 86 x RB 39)	0.122	31	**	**	ns
(NA 33 x CSUL 7) X EET 75	0.203	32	**	**	ns
(MA 16 x SCA 6) X (CAB 270)	0.125	33	**	**	ns
(MA 16 x SCA 6) X (CAB 208)	0.388	34	**	**	ns
(MA 16 x SCA 6) X (CAB 214)	-0.199	35	**	**	*
(MA 16 x SCA 6) X (NA 33 x RB 39)	0.706	36	**	ns	*
(MA 16 x SCA 6) X (CEPEC 90 x CHUAO 120)	0.189	37	**	**	ns
(MA 16 x SCA 6) X (CEPEC 86 x RB 39)	-0.027	38	**	**	**
(MA 16 x SCA 6) X ICS 95	0.367	39	**	**	ns
(P4B x SCA 6) X (CAB 270)	0.229	40	**	**	ns
(P4B x SCA 6) X (CAB 208)	0.085	41	**	**	**
(P4B x SCA 6) X (CAB 214)	0.174	42	**	**	ns
(P4B x SCA 6) X (NA 33 x RB 39)	0.232	43	**	**	ns
(P4B x SCA 6) X (CEPEC 86 x RB 39)	0.367	44	**	**	ns
(P4B x SCA 6) X EET 233	0.247	45	**	**	ns

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Table 2. *continuation...*

CROSSES PROGENIES	DI LSmeans[1]	No.	CONTROLS		
			CATONGO	SIC 23	SCA 6
(P4B x SCA 6) X EET 75	0.351	46	**	**	ns
(P4B x SCA 6) X ICS 95	-0.059	47	**	**	**
(P4B x SCA 6) X UF 273	0.222	48	**	**	ns
EET 233 X (CAB 214)	-0.202	49	**	**	**
EET 233 X (CEPEC 90 x CHUAO 120)	0.958	50	**	ns	**
EET 233 X ICS 95	0.539	51	**	*	ns
EET 392 X CAB 270	0.733	52	**	**	**
EET 392 X (CEPEC 90 x CHUAO 120)	0.693	56	**	ns	*
EET 392 X (CEPEC 86 x RB 39)	0.494	57	**	**	ns
EET 392 X EET 75	0.555	58	**	**	**
EET 392 X ICS 95	0.980	59	*	ns	**
EET 75 X CAB 270	0.279	60	**	**	ns
EET 75 X CAB 214	0.221	61	**	**	ns
EET 75 X (NA 33 x RB 39)	0.166	62	**	**	ns
EET 75 X (CEPEC 90 x CHUAO 120)	0.324	63	**	**	ns
ICS 95 X (CEPEC 90 x CHUAO 120)	0.522	64	**	**	ns
UF 273 X CAB 208	0.812	68	**	ns	**
UF 273 X CAB 214	0.138	69	**	**	ns
UF 273 X (NA 33 x RB 39)	1.413	70	ns	**	**
UF 273 X (CEPEC 90 x CHUAO 120)	0.123	71	**	**	*
UF 273 X ICS 95	0.691	72	**	*	**
CATONGO	1.385	65	.	**	**
SIC 23	0.935	67	**	.	**
SCA 6	0.301	66	**	**	.

[1] DI LSmeans - disease index obtained through the quantification and measurement of symptoms: TB - Presence of terminal broom; LTB - Length of the terminal broom; AB - Presence of axillary broom; NAB - Number of axillary brooms greater than 1cm; CB - Presence of cotyledonary broom, and DB - Presence of dry broom, were used in the formula: DI = TB + (0.01 * TBL) + AB + (0.1 * NAB) + CB + (4.0 * DB) (Rodrigues et al. 2019), correcting the DIs of each plant for the effect of trial by LSmeans - statistical software SAS. ** Significant effects at 0.01%, according to T test. * Significant effects at 0.05%, according to T test.

(CAB 208), UF 273 and other parents of the 10 best progenies are also among the parents of some of the progenies presenting the worst behavior, indicating significant effects for the specific combining ability or the father-mother interaction. In addition, significant effects were found, at 0.01% F probability, for this interaction in a model that contemplated it, as well as for father, mother and trial (not shown).

The present results are analogous to those reported by Pimenta Neto et al. (18), who analyzed part of the progenies presented here. They are not identical due to changes in the methodology for DI calculation. Here, the differences among parents were also evaluated (below), which did not occur in the cited article.

Correcting the DIs of each plant for the effect of the trial to which they belong, by the ratio among the mean DIs of the controls in that trial and their mean DIs for all trials, and using a model with the sources of variation: mother and father, which allows obtaining the means of mothers corrected for the effects of father (Table 3), and vice versa, significant effects of fathers and mothers were again found, at 0.01% probability.

Analyzing individually each of the mothers and each of the fathers, significant effects were observed for father within mother for all mothers, showing F probability of 5% or less, except for [SGU 26 x SCA 6], which had 0.058, or 5.8%, error probability of F, as well as for mother within father for all fathers (not shown).

The most noticeable mothers were (P4B x SCA 6), (MA 16 x SCA 6), (CEPEC 86 x SCA 6), and EET 75, which did not differ from each other, except (CEPEC 86 x SCA 6), which differed statistically from (P4B x SCA 6); the latter was the female parent that had the lowest mean DI (Table 3).

(P4B x SCA 6) generated nine progenies, seven of which did not perform differently from the resistance pattern, and two, [(P4B x SCA 6) x CAB 208] and [(P4B x SCA 6) x ICS 95], had a mean DI lower than that of SCA 6 progeny (Table 2). Of the seven progenies generated by (MA 16 x SCA 6), two were more resistant than the progeny of SCA 6, [(MA 16 x SCA 6) x CAB 214] and [(MA 16 x SCA 6) x (CEPEC 86 x RB 39)]; four had the same reaction to the pathogen as this standard, and one [(MA 16 x SCA 6) x (NA 33 x RB 39)] behaved similarly to the SIC 23 pattern. EET 75 generated four progenies, all of which were not different from SCA 6 progeny. Six progenies originated from the crosses that had (CEPEC 86 x SCA 6) as mother. Of these, the progeny resulting from the cross with the male parent ETT 75 was superior; four

Table 3. Disease index of symptoms caused by *Moniliophthora perniciosa* in cacao seedlings – average of mothers, and significance according to T test for contrasts between mothers.

MOTHER	DI	No.	1	2	3	4	5	6	7	8	9	10	11
(CEPEC 86 x SCA 6)	0.277	1	.	**	**	ns	ns	ns	**	**	ns	ns	**
(RB 36 x SCA 6)	0.471	2	**	.	ns	ns	*	**	**	**	**	**	**
(SGU 26 x SCA 6)	0.430	3	**	ns	.	ns	ns	**	**	**	**	**	**
(CSUL 3 x SCA 6)	0.378	4	ns	ns	ns	.	ns	**	**	**	ns	**	**
(NA 33 x CSUL 7)	0.340	5	ns	*	ns	ns	.	*	**	**	ns	*	**
(MA 13 x SCA 6)	0.221	6	ns	**	**	**	*	.	ns	**	ns	ns	**
(P4B x SCA 6)	0.145	7	**	**	**	**	**	ns	.	**	**	ns	**
EET 233	0.755	8	**	**	**	**	**	**	**	.	**	**	ns
EET 392	0.285	9	ns	**	**	ns	ns	ns	**	**	.	ns	**
EET 75	0.215	10	ns	**	**	**	*	ns	ns	**	ns	.	**
UF 273	0.705	11	**	**	**	**	**	**	**	ns	**	**	.

[1] DI LSmeans - disease index obtained through the quantification and measurement of symptoms: TB - Presence of terminal broom; LTB - Length of the terminal broom; AB - Presence of axillary broom; NAB - Number of axillary brooms greater than 1cm; CB - Presence of cotyledonary broom, and DB - Presence of dry broom, were used in the formula: DI = TB + (0.01 * TBL) + AB + (0.1 * NAB) + CB + (4.0 * DB) (Rodrigues et al. 2019), correcting the DIs of each plant for the effect of the trial to which they belong, based on the ratio between the mean DIs of the controls in that trial and their mean DIs in all trials, and for the effect of the father based on LSmeans - statistical software SAS. ** Significant effects at 0.01%, according to T test. * Significant effects at 0.05%, according to T test.

progenies were not different from SCA 6 progeny, and one, with high mean DI, differed from this pattern but also from Catongo and SIC 23. Rodrigues et al (21) classified both the reverse cross of (P4B x SCA 6) and the same selected plant of (CEPEC 86 x SCA) among the best female parents when testing selections from progenies of other crosses from a first cycle of recurrent selection as parents.

Regarding the ancestry of these matrices, SCA 6 and P4B were collected by Pound; the first one was collected in the Ucayali River region, and the second one in Iquitos, Peru (8). The clone Scavina 6, per se, as well as the offspring, was practically immune to witches' broom in Trinidad, where it was first evaluated, becoming the most important and used source of resistance to this disease from then on (4). P4B was also an excellent parent for this characteristic (6). The clone MA 16 was collected in the state of Amazonas (8) and can be considered a resistance source of the type Low Amazon; CEPEC 86 was selected in a cacao plantation with traditional varieties in the Jequitinhonha River region, Bahia State, and the clone EET 75 was developed by INIAP - Pichilingue, Ecuador (8).

Of the nine male parents tested, the lowest mean DIs were found for (CAB 214), (CAB 208) and (P4B x OC 67), which did not differ from each other (Table 4). Another parent, (CEPEC 86 x RB 39), did not differ statistically from (P4B x OC 67).

(CAB 214), a plant selected from a free pollination progeny of the CAB 214 clone, generated 11 progenies. Of these, two progenies – from the crosses with (MA 16 x SCA 6) and EET 233 – were more resistant, and seven were not different from the progeny of SCA 6. Of the eight progenies of (CAB 208), six behaved according to the resistance pattern, one – from the cross with (P4B x SCA 6) – was more resistant, and one – from the cross with UF 273 – did not differ from the progeny of SIC 23. Only three progenies that had P4B x OC 67 as father were tested,

Table 4. Disease index of symptoms caused by *Moniliophthora perniciosa* in cacao seedlings – average of fathers, and significance according to T test for contrasts between fathers.

FATHER	DI	No.	1	2	3	4	5	6	7	8	9
(CAB 270)	0.425	1	.	**	**	**	ns	**	ns	ns	ns
(CAB 208)	0.231	2	**	.	ns	**	**	ns	**	**	**
(CAB 214)	0.155	3	**	ns	.	**	**	ns	**	**	**
(NA 33 x RB 39)	0.605	4	**	**	**	.	**	**	**	*	ns
(CEPEC 90 x CHUAO 120)	0.433	5	ns	**	**	**	.	**	ns	ns	ns
(P4B x OC 67)	0.233	6	**	ns	ns	**	**	.	ns	**	**
(CEPEC 86 x RB 39)	0.358	7	ns	**	**	**	ns	ns	.	*	*
EET 75	0.474	8	ns	**	**	*	ns	**	*	.	ns
ICS 95	0.537	9	ns	**	**	ns	ns	**	*	ns	.

[1] DI LSmeans - disease index obtained through the quantification and measurement of symptoms: TB - Presence of terminal broom; LTB - Length of the terminal broom, AB - Presence of axillary broom; NAB - Number of axillary brooms greater than 1cm; CB - Presence of cotyledonary broom, and DB - Presence of dry broom, were used in the formula: DI = TB + (0.01 * TBL) + AB + (0.1 * NAB) + CB + (4.0 * DB) (Rodrigues et al. 2019), correcting the DIs of each plant for the effect of the trial to which they belong, based on the ratio between the mean DIs of the controls in that trial and their mean DIs in all trials, and for the effect of the mother based on LSmeans - statistical software SAS. ** Significant effects at 0.01%, according to T test. * Significant effects at 0.05%, according to T test.

of which two did not differ from SCA 6, and one – which had (NA 33 x CSUL 7) as mother – was more resistant than the resistant pattern after inoculation with the pathogen. Eight progenies had the matrix (CEPEC 86 x RB 39) as male parent, of which one did not differ from SIC 23, six did not differ from SCA 6, and one – from the crosses with (MA 16 x SCA 6) – differed from the last pattern, showing greater resistance.

Only one clone, EET 75, was used as a father and as a mother parent. Although EET 75 did not have the same significance as a father as it did as a mother, two of its five progenies were superior to SCA 6 – from the crosses with (CEPEC 86 x SCA 6) and with (RB 36 x SCA 6) – and two were not different from the progeny of SCA 6. One progeny, from the crosses with EET 392, behaved worse than SCA 6 but better than the susceptible patterns. EET 75 and UF 273 stood out among the parents generating resistant crosses to black pod rot, implying that these parents may also have resistance genes to this other important cacao disease in Brazil (21).

Regarding parental ancestry, CAB 208, CAB 214 and RB 39 were collected in the Brazilian Amazon, the first two in Purus River, Amazonas State, and the third one in the Acre River, Acre State (8). All of them had already shown to be good parents for resistance to witches' broom (2, 5, 16, 24). OC 67 was collected in Ocumare de la Costa, Venezuela (8), and was the only Criollo type among the mentioned ascendants.

The significant differences for the means of progenies of different mothers and different fathers, the significant effect for the mother x father interaction, and the significant differences among progenies of the same mothers or the same fathers prove the existence of different genes or gene combinations among fathers and mothers, the association of different genes or gene combinations in the progenies, and the occurrence of additive and dominant effects in the inheritance of these factors. The association of different resistance genes to increase the level and durability of this character in commercial varieties is the central objective of the breeding program conducted at the Cocoa Research Center of CEPLAC – and the increase in resistance durability due to the association of different genes was confirmed by Pires et al. (19).

Moreover, the great variety of ascendants in the progenies that were not different or superior to the progeny of the resistance pattern indicates wide possibilities for selection of clones for commercial planting with different genetic bases of resistance. The cultivation of varieties with different resistance bases is another element that hinders the evolution of the pathogen and, consequently, increases the durability of resistance.

This study proved the existence of different genes or gene combinations among clones for resistance to witches' broom, as well as the association of different genes or gene combinations in the progenies and the occurrence of additive and dominant effects in the inheritance of these factors.

In addition, ten progenies carrying WB resistance genes were selected from various sources that were more resistant than SCA 6, four of which were from parents carrying frost pod rot resistance genes; at least seven parents outperformed in producing progenies with high levels of resistance. More progenies behaving similarly to SCA 6, with different genetic bases, are also useful in selecting clones for commercial planting.

REFERENCES

1. Adeyemi, A.A. Evaluation of the formulated mixture of glyphosate and terbuthylazine in the control of weeds in mature cocoa plantation. **Nigeria Journal of Tree Crop Research,** Abuja, v.4, p.62-68, 2000.
2. Albuquerque, P.S.B.; Silva, S.D.V.M.; Luz, E.D.M.N.; Pires, J.L.; Vieira, A.M.C.; Demétrio, C.G.B.; Pascholatti, S.F.; Figueira, A. Novel sources of witches' broom resistance (causal agent *Moniliophthora perniciosa*) from natural populations of *Theobroma cacao* from the Brazilian Amazon. **Euphytica,** Wageningen, v.172, p.125-138, 2010.
3. Arciniegas, A.; Phillips-Mora, W. Caracterización de Genotipos Superiores de Cacao Seleccionados por el Programa de Mejoramiento Genético del CATIE por Su Rendimiento Y/O Resistencia a Moniliasis. In: 15th Internac-tional Cocoa Research Conference, 2006, San Jose, Costa Rica. Proceedings. Lagos, Nigeria: Cocoa Producers' Alliance, 2006.
4. Bartley, B.G. A review of cacao improvement. Fundamental methods and results. In: Internation Workshop on Cocoa Breending Strategies, 1994 Kualla Lumpur, Malaysia. Proceedings. Paris, France: INGENIC 1994. P.3-17
5. Benjamim, C.S.; Luz, E.D.M.N.; Monteiro, W.R.; Santos Filho, L.P.; Santos, W.O. Avaliação de progênies de clones de cacaueiros (série CP) quanto à resistência a *Moniliophthora perniciosa*. **Agrotrópica**, Ilhéus. v. 26, p. 17-26, 2014.
6. Benjamim, C.S.; Luz, E.D.M.N.; Santos, W.O.; Pires, J.L. Cacao families and parents selected as resistant to natural infection of *Moniliophthora perniciosa*. **Crop Breeding and Applied Biotechnology,** Viçosa, v.16, n.2, p.141-146, 2016.
7. Bhattacharjee, R.; Kumar, P.L. Cacao. In: Kole, C. **Genome mapping and molecular breeding in plants**. Berlin: Springer, 2007. p.127-142.
8. End, M.J.; Wadsworth, R.M.; Hadley, P. **International Cocoa Germplasm Database**. United Kingdom: Reading University, 1994. 366p.
9. Eskes, B.; Efron, Y. Global approaches to cocoa germplasm utilization and conservation. Final report of the CFC/ICCO/IPGRI project on "Cocoa Germplasm Utilization and Conservation: A Global Approach" (1998-2004). Amsterdam, The Netherlands/London, UK/Rome, Italy: CFC/ICCO/IPGRI, 2006.
10. Gutiérrez, O.A.; Campbell, A.S.; Phillips-Mora, W. Breeding for Disease Resistance in Cacao. In: Bailey, B.A.; Meinhardt, L.W. (eds). **Cacao Diseases**, **A History of Old Enemies and New Encounters**. New York: Springer, 2016, p.567-609.
11. Lima, E.M.; Pereira, N.E.; Pires, J.L.; Barbosa, A.M.M.; Corrêa, R.X. Genetic molecular diversity, production and resistance to witches' broom in cacao clones. **Crop Breeding and Applied Biotechnology,** Viçosa, v.13, n.2, p.127-135, 2013.
12. Lopes, U.V.; Monteiro, W.R.; Pires, J.L.; Clement, D.; Yamada, M.M.; Gramacho, K.P. Cacao breeding in Bahia, Brazil: Strategies and results. **Crop Breeding and Applied Biotechnology,** Viçosa, v.11, p.73-81, 2011.
13. Marssaro, A.L.; Montejo-Díaz, A.; Montaño-Orellana, V.M.; Luz, E.D.M.N.; Corrêa, R.X. Resistance to witches' broom in adult plants and progeny of local varieties of cacao in Southern Bahia. **Bragantia,** Campinas. v. 79, p. 421-432, 2020.
14. Morera, J.; Paredes, A.; Mora, A. Germoplasma de cacao en el CATIE entre 1947 y 1991, **Programa II: Generacion y transferencia de tecnologia**. IICA, San Jose, Costa Rica, 1991, 49p.
15. Paim, V.R.L.M.; Luz, E.D.M.N.; Pires, J.L.; Silva, S.D.V.M.; Souza, J.T.; Albuquerque, P.S.B.; Santos Filho, L.P. Sources of resistance to *Crinipellis perniciosa* in progenies of cacao accessions collected in the Brazilian Amazon. **Scientia Agricola,** Piracicaba, v.63, n.6, p.572-578, 2006.
16. Oliveira, M.L.; Luz, E.D.M.N. Principais doenças do cacaueiro e seu manejo. In R. R. Valle (ed) **Ciência, tecnologia e manejo do cacaueiro**. Brasília: Ceplac, 2012. p. 187-275.
17. Phillips-Mora, W.; Castillo, J.; Arciniegas, A.; Mata, A.; Sánchez, A.; Le-andro, M.; Astorga, C.; Motamayor, J.; Guyton, B.; Seguine, E.; Schnell, R. Overcoming the main limiting factors of cacao production in Central America through the use of improved clones developed at CATIE. In: 16th International Cocoa Research Conference, 2009, Bali, Indonesia. Proceedings. Lagos, Nigeria: Cocoa Producers' Alliance, 2009.
18. Pimenta Neto, A.A.; Laranjeira, D.; Pires, J.L.; Luz, E.D.M.N. Selection of cocoa progenies for resistance to witches' broom. **Tropical Plant Pathology,** Brasília, n.43, p.381-388, 2018.

19. Pires, J.L.; Melo, G.R.P.; Yamada, M.M.; Gramacho, K.P. Association among sources of resistance to witches' broom disease for the increment of the level and durability of the character. In: 6th International Cocoa Research Conference, 2009, Bali, Indonesia. Proceedings. Lagos, Nigeria: Cocoa Producers' Alliance, 2009. p.431-435.
20. Rodrigues, G.S.; Pires, J.L.; Luz, E.D.M.N. Índice de severidade da vassoura de bruxa para avaliar genótipos de cacaueiro inoculados artificialmente. **Agrotrópica,** Itabuna, v.31, p.255-258, 2019.
21. Rodrigues, G.S.; Pires, J.L.; Luz, E.D.M.N. Association of genes from different sources of resistance to major cacao diseases. **Revista Ceres**, Viçosa, v. 67, p. 383-394, 2020.
22. SAS INSTITUTE INC. **SAS/STAT User's Guide**. Release 6.03. Cary, NC: SAS Institute Inc, 1028p., 1988.
23. Silva, S.D.V.M.; Luz, E.D.M.N.; Pires, J.L.; Yamada, M.M.; Santos Filho, L.P. Parent selection for cocoa resistance to witches'broom. **Pesquisa Agropecuária Brasileira,** Brasília, v.45, p.680-685, 2010.
24. Yamada, M.M.; Pires, J.L.; Faleiro, F.G.; Santos, R.F. Ocorrência de vassoura-de-bruxa em progênies de cacaueiro selecionadas pelo programa de melhoramento na Estação Experimental Joaquim Bahiana. **Agrotrópica,** Itabuna, v.26, p.197-202, 2014.