

Resistance of cacao tree to witches' broom disease – A study of inheritance

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ABSTRACT

Pires, J.L.; Luz, E.D.M.N.; Melo, G.S. Resistance of cacao tree to witches' broom disease – A study of inheritance. *Summa Phytopathologica*, v.48, n.3, p.112-120, 2022.

Over a 10-year period, the number of vegetative brooms and floral cushion brooms was evaluated in each plant of progenies from a North Carolina II mating design with the clones: Ca 5, CAB 5003, Chuao 120, ICS 1, Moq 216, Scavina 12, Scavina 6 and SPA 5 as group 1 of progenitors, and CC 10, CCN 34, Cepec 90, CSul 3, Ma 16, RB 36, SGu 26 and SIAL 70 as group 2. Highly significant effects were found for progenitors and interactions among progenitors, indicating that the species *Theobroma cacao* has a high level of genetic diversity for resistance to witches' broom. Results demonstrated the

presence of different genes, alleles or gene combinations among progenitors; the association of various genes, alleles or gene combinations in the progenies, and the presence of additive and dominant effects on the inheritance of these factors. There was also significant loss of inheritance of Scavina 6, the most traditional source of resistance, when it was used alone but not when it was combined with other clones bearing resistance genes or alleles. This demonstrated that gene association increases resistance durability in that source.

Keywords: *Moniliophthora perniciosa*, plant breeding, *Theobroma cacao*

RESUMO

Pires, J.L.; Luz, E.D.M.N.; Melo, G.S. Resistência do cacauzeiro à vassoura de bruxa – um estudo de herança. *Summa Phytopathologica*, v.48, n.3, p.112-120, 2022.

Foram avaliados durante 10 anos o número de vassouras vegetativas e de almofada floral em cada planta das progênes de cruzamentos em delineamento North Carolina II com os clones Ca 5, CAB 5003, Chuao 120, ICS 1, Moq 216, Scavina 12, Scavina 6 e SPA 5, no grupo 1 de progenitores e, CC 10, CCN 34, Cepec 90, CSul 3, Ma 16, RB 36, SGu 26 e SIAL 70 no grupo 2. Efeitos altamente significativos foram obtidos para progenitores e interação entre progenitores indicando que na espécie *Theobroma cacao* existe alta diversidade genética para resistência à vassoura de bruxa. Foi demonstrada

a presença de genes, alelos ou combinações de genes entre progenitores; a associação de vários genes, alelos ou combinações de genes nas progênes; e, a presença de efeitos aditivos e dominantes na herança destes fatores. Constatou-se também perda significativa na herdabilidade da resistência de Scavina 6, a fonte mais tradicional de resistência, quando usada sozinha, mas não nas combinações com outros clones portadores de genes ou alelos para resistência. Isto demonstrou que a associação de genes aumenta a durabilidade da resistência naquela fonte.

Palavras-chave: *Moniliophthora perniciosa*, melhoramento, *Theobroma cacao*

Witches' broom disease (WBD), caused by the basidiomycete *Moniliophthora perniciosa* (Stahel) Aims and Phillips-Mora (former *Crinipellis perniciosa*), is one of the most important diseases affecting the cacao tree, accounting for the majority of yield losses in Brazil in the last three decades (3, 5, 11).

The fungus infects all meristematic tissues: apical buds of leaf flushes – vegetative brooms; flower cushions, which can produce vegetative brooms, abnormal flowers, and parthenocarpic carrot-, strawberry- or custard apple-shaped pods; seedlings and developing pods. Young infected pods suffer hypertrophy and exhibit chlorosis and necrotic lesions (13).

The most important symptoms to evaluate resistance to WBD in adult plants are vegetative brooms (VB), floral cushion brooms (CB), and infected pods. In an evaluation of the germplasm collection of the Cacao Research Center (Cepec)/Executive Committee of the Cacao Crop Plan (Ceplac) (8), VB and CB together showed 0.59 correlation

with the proportion of WBD-infected pods. Genetic gains from the selection of materials resistant to VB and CB will lead to resistance gains for the pods. Less wear and tear on the plants, as well as less damage to flower cushions, will result in less inoculum formation, which can also reduce pod loss.

After the introduction of WBD, the already established breeding program used the available knowledge about the resistance of descendants of Scavina-6 clone, the most traditional source of resistance to WBD, to allow for rapid release of resistant varieties, but at first all descendants of this clone (15). Within a few years, evolution of this fungus caused the breakdown of Scavina resistance in Bahia (4).

The central issue for cacao improvement in that region became the need to include, in new cultivars, resistance factors different from those used until then to increase the resistance level and durability. Thus, new sources of resistance were identified and a recurrent selection program was structured for associating different resistance genes and genes related

to other characters of interest (1, 7-10).

The present study deals with the evaluation of VB and CB in some of the first progenies of Cepec's recurrent selection program, which is currently in progress, aiming at the development of varieties with high productivity, good general characteristics, and vertical and horizontal resistance to the major diseases of cacao. It is based on the hypothesis that there are differences in resistance to WBD among progenitors, as well as differences in additive and non-additive effects on the inheritance, and that it is possible to increase the resistance level and durability by associating alleles or genes favorable to this character.

MATERIAL AND METHODS

This study considers the evaluation of the number of vegetative brooms (VB) and floral cushions brooms (CB), per plant, of progenies from a cross breeding scheme in North Carolina II design with the clones: Ca 5, CAB 5003, Chuao 120, ICS 1, Moq 216, Scavina 12 (Sca 12), Scavina 6 (Sca 6) and SPA 5 as group 1 of progenitors (G1), and CC 10, CCN 34, Cepec 90, Cruzeiro do Sul 3 (CSul 3), Ma 16, RB 36, SGu 26 and SIAL 70 as group 2 (G2).

Natural witches' broom infection in the field was considered in 12 periods over 10 years, and the progenies were represented by two replicates of up to 20 plants each. At each of the 12 periods, the number of VB and CB was counted per progeny plant.

Progeny plants were grafted onto pre-existing adult plants, to accelerate development, and allowed to grow at 3x3m spacing, while some original plants were left to maintain high inoculum pressure. Cultivation was carried out in the traditional way, under the shade of *Erythrina* plants.

Progenitors were selected from the Cepec's Germplasm Collection for their performance regarding attributes of interest, and the crossing design was defined considering the possibilities of combinations of desired characteristics, genetic distances and possibilities of associating different WBD resistance genes (8, 9). The performance in relation to natural witches' broom infection was a determining factor in the choices of clones from the Upper Amazon: Moq 216, Sca 12, Sca 6, CSul 3, RB 36; Lower Amazon: Ca 5, CAB 5003, Ma 16; Trinitarians: CCN 34, SGu 26; Criollo Chuao 120, and a hybrid of unknown origin: Cepec 90. ICS 1, SPA 5, CC 10 and SIAL 70 showed no resistance in the evaluation (8, 9).

The 12 broom removals and counting periods were considered repeated measures, and data underwent multivariate analysis to determine the effects of progenies, or of G1, G2, and G1 x G2 interaction, and as repeated measures, for the effects of removal period and interactions of progenitors or progenies with removal period using PROC GLM / MANOVA; PROC GLM / REPEATED (14).

RESULTS AND DISCUSSION

The assay used in this study for progeny evaluation is part of the core structure of Cepec's cacao breeding program. Its goal is to link resistance genes to other genes of interest, allowing the selection of new progenitors for new selection cycles and clones to be evaluated in regional trials, identifying new commercial varieties.

There were highly significant effects for the progenitors of group 1 (G1), progenitors of group 2 (G2), and the interaction G1 x G2 considering the number of vegetative brooms (VB) and floral

cushion brooms (CB) per plant (probability of error according to Wilks' Lambda test - $p < 0.0001\%$). Thus, there are additive effects on character inheritance: differences in the general averages or in the general combining ability among progenitors, and non-additive effects: differences in the specific combining abilities or among the averages for progenies of one specific progenitor.

Highly significant differences were also found with both types of brooms for the year or removal period and for the interactions of this removal period with G1, with G2, and with G1 x G2 (Wilks' Lambda - $p < 0.0001$). Therefore, there were differences in the tendencies for the evolution of the number of brooms over time among G1 progenitors, G2 progenitors, and among G1 x G2 combinations.

For both types of broom, highly significant effects were found for progeny and removal period x progeny interaction (Wilks' Lambda - $p < 0.0001$).

There were very clear differences among progenitors in their behavior for VB infection, and of the 28 possible comparisons between two parents (for both groups) only three did not have significant differences, as shown in Tables 1 and 2. These tables exhibit the ratio between the average for each progenitor and the general average for all progenitors at each counting time, as well as the significance of the differences between the sets of averages according to Wilks' Lambda test. Such results indicate the great genetic diversity of the species *Theobroma cacao* for WBD resistance.

Scavina 12 had the best performance among G1 progenitors; it differed from all other progenitors and was followed by Scavina 6, which also distinguished from all other progenitors (Table 1). These clones were collected in Peru and are related to one another (6). Scavina 6 is the most common source of WBD resistance (2), and its inheritance pattern indicates the presence of two dominant alleles with a high effect for this trait (9). Scavina 12 is also an important source of resistance but its superiority to Scavina 6 was unexpected.

Scavina 6 averages ranged from very low in the early years to close to the general averages in the last years (Table 1). For the set of the last six measurements, Sca 6 was no longer significantly different from CAB 5003, Chuao 120 and ICS 9 (Wilks' Lambda - $p > 0.05$, not shown), whereas Scavina 12 was statistically distinct from all other progenitors (Wilks' Lambda - $p < 0.01$, not shown).

The changes found for Scavina 6 progenitor could be attributed to an increment in the inoculum pressure since there was an increase in the average of overall broom (not shown). However, reduced resistance to WBD in descendants of this clone, as a result of the evolution of the pathogen populations, has already been reported (1, 4, 7, 8, 9) and occurred concomitantly with the intensification of planting of the first commercial varieties considered resistant in Bahia State and all descendants of this clone.

The crossings of Scavina 6 with the G2 parents that were expected to have no resistant genes but had the highest general averages (Table 2), CC 10 and SIAL 70, led to sets of averages in the 12 evaluations and in the last six evaluations (Table 3) significantly superior to all other Scavina 6 combinations (Wilks' Lambda - $p < 0.0001$, not shown). This suggests differences between crossings in which only Sca 6 resistant genes were present and crossings in which other important genes were present. Furthermore, the contrast for (Sca 6 x CC 10 and Sca 6 x SIAL 70) x (ICS 1 x CC 10, ICS 1 x SIAL 70, SPA 5 x SIAL 70) (only resistance genes from Sca 6 x no remarkable resistance genes - ICS 1 and SPA 5 were also expected to have no remarkable resistance genes but showed the highest general averages for G1 - Table 1) was significant considering the last six evaluations, and averages were

Table 1 - Ratio between the average of vegetative brooms for each parent in Group 1 and the general average for the eight parents, at each counting period and in general (Mean); significance for the differences between sets of averages according to Wilks' Lambda test.

Progenitor	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CA5	1.01	1.55	1.03	1.26	1.15	1.19	0.88	1.27	1.10	1.08	0.91	1.23	1.14
CAB5003	1.06	0.89	1.31	0.88	0.78	0.78	0.96	0.86	0.93	0.98	0.83	0.78	0.92
CHUAO120	0.91	0.99	0.78	0.95	0.97	0.97	0.83	0.85	0.94	0.87	0.60	1.03	0.89
ICS1	2.45	1.62	1.59	1.49	1.39	1.38	1.08	1.11	1.15	1.16	1.31	1.34	1.42
MOQ216	0.84	0.98	1.51	1.24	1.50	1.49	1.34	1.10	1.13	1.13	0.84	0.59	1.14
SCA12	0.08	0.15	0.11	0.17	0.14	0.14	0.34	0.51	0.58	0.44	0.93	0.54	0.34
SCA6	-0.03	0.19	0.25	0.29	0.28	0.28	0.70	0.76	0.74	1.24	1.06	1.02	0.56
SPA5	1.68	1.62	1.41	1.72	1.79	1.78	1.88	1.53	1.45	1.09	1.52	1.46	1.58

Progenitor	1	2	3	4	5	6	7
CA5	1						
CAB5003	2	**					
CHUAO120	3	ns	ns				
ICS1	4	**	**	**			
MOQ216	5	**	ns	*	**		
SCA12	6	**	**	**	**	**	
SCA6	7	**	**	**	**	**	*
SPA5	8	**	**	**	**	**	**

ns - not significant, * significant at 5%, ** significant at 1% probability of error

Table 2 - Ratio between the average of vegetative brooms for each parent in Group 2 and the general average for the eight parents, at each counting period and in general (Mean); significance for the differences between sets of averages according to Wilks' Lambda test.

Progenitor	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CC10	1.88	1.83	1.10	1.78	1.79	1.78	1.47	1.72	1.55	2.05	1.42	1.32	1.64
CCN34	1.58	1.14	0.71	0.98	0.99	0.99	1.12	1.34	0.98	0.78	0.79	0.89	1.02
CEPEC90	0.40	0.45	0.31	0.48	0.35	0.34	0.71	0.64	0.66	0.91	1.16	0.79	0.60
CSUL3	0.43	0.79	0.76	0.55	0.51	0.51	0.55	0.43	0.43	0.30	0.65	0.53	0.54
MA16	1.15	1.01	0.81	0.74	1.01	1.05	0.98	0.79	0.80	0.37	0.52	0.71	0.83
RB36	0.61	0.87	1.22	0.74	0.80	0.79	0.61	0.58	0.78	0.47	0.55	0.43	0.70
SGU26	1.04	0.97	1.64	1.57	1.18	1.17	1.34	1.18	1.46	1.41	1.25	1.74	1.33
SIAL70	0.92	0.93	1.45	1.15	1.38	1.37	1.22	1.33	1.35	1.70	1.68	1.60	1.34

Progenitor	1	2	3	4	5	6	7
CC10	1						
CCN34	2	**					
CEPEC90	3	**	**				
CSUL3	4	**	**	*			
MA16	5	**	ns	**	**		
RB36	6	**	**	**	ns	*	
SGU26	7	**	**	**	**	**	**
SIAL70	8	**	**	**	**	**	**

ns - not significant, * significant at 5%, ** significant at 1% probability of error

higher for the crossings with Sca 6 (Wilks' Lambda - $p < 0.0001$, not shown). This indicates that when Sca 6 resistance genes are isolated, they are no longer effective - breakdown of resistance.

The question then arises: do crossings of Scavina with other sources of resistance continue to perform well solely because of the effect of these other sources, or are there still effects of Scavina inheritance when

in association with other resistance genes or alleles? Accordingly, the contrast (Sca 6 x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, and SGU 26) x (ICS 1 x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, and SGU 26; SPA 5 x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, and SGU 26) was significant (Wilks' Lambda - $p < 0.0001$, not shown) for the first set, which has resistance genes from Scavina 6 and another source, showing

Table 3 - Ratio between the average of vegetative brooms for each progeny and the general average for all progenies, at each counting period.

Progeny	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CA5XCC10	5.08	3.37	1.48	2.41	3.76	3.74	1.03	2.48	1.39	1.54	1.30	1.19	2.40
CA5XCEPEC90	0.27	0.99	1.01	0.97	0.71	0.71	1.08	1.33	0.65	0.91	1.18	1.25	0.92
CA5XCSUL3	0.33	2.07	0.73	0.75	0.35	0.34	0.48	0.47	0.63	0.75	0.43	0.24	0.63
CA5XMA16	0.00	0.77	0.26	0.60	0.56	0.84	0.28	1.00	0.63	0.43	0.17	1.13	0.56
CA5XRB36	0.35	0.54	0.15	0.37	0.39	0.39	0.18	0.39	0.42	0.22	0.26	0.28	0.33
CA5XSGU26	0.52	2.60	2.61	3.06	1.40	1.39	2.07	1.99	3.89	3.12	1.86	2.66	2.27
CAB5003XCCN34	2.37	1.26	1.00	1.37	0.75	0.74	1.22	1.33	1.23	1.03	1.06	0.69	1.17
CAB5003XCEPEC90	0.31	0.81	0.26	0.36	0.48	0.48	0.81	0.57	0.71	0.71	1.69	0.45	0.64
CAB5003XCSUL3	0.80	0.42	0.56	0.52	0.07	0.07	0.35	0.21	0.35	0.04	0.68	0.34	0.37
CAB5003XMA16	0.00	0.53	0.94	0.13	0.39	0.39	0.35	0.37	0.67	0.95	0.48	0.26	0.45
CAB5003XRB36	0.52	0.76	2.40	0.16	0.88	0.87	0.73	0.50	0.77	0.25	0.24	0.45	0.71
CAB5003XSGU26	0.00	0.33	0.78	1.97	1.21	1.20	2.23	1.64	0.74	3.51	1.05	4.21	1.57
CAB5003XSIAL70	1.07	0.97	1.57	1.27	0.93	0.93	1.12	1.30	1.39	1.95	0.64	0.69	1.15
CHUAO120XCCN34	1.75	1.90	0.49	0.57	0.36	0.36	0.86	0.96	0.88	0.44	0.26	0.65	0.79
CHUAO120XCEPEC90	0.47	0.24	0.00	0.56	0.38	0.38	0.79	0.73	0.80	1.71	0.68	0.89	0.64
CHUAO120XCSUL3	0.42	0.48	0.50	0.48	0.44	0.44	0.51	0.40	0.63	0.11	0.38	0.62	0.45
CHUAO120XMA16	0.00	0.33	0.20	0.16	0.60	0.60	0.68	0.73	1.04	0.00	0.15	1.50	0.50
CHUAO120XSIAL70	0.80	1.55	1.57	1.69	2.21	2.20	0.66	0.94	0.79	0.65	1.37	0.97	1.28
ICS1XCC10	3.26	1.32	0.78	0.98	0.73	0.72	1.99	0.55	1.22	0.62	1.79	1.62	1.30
ICS1XCCN34	5.44	1.43	0.78	1.86	1.69	1.68	0.80	3.29	1.25	2.58	0.70	1.92	1.95
ICS1XCEPEC90	1.40	0.97	0.17	0.56	0.45	0.45	1.05	0.50	1.14	1.12	1.75	0.81	0.86
ICS1XCSUL3	0.40	0.99	0.34	0.23	0.41	0.41	0.26	0.13	0.23	0.27	0.92	0.46	0.42
ICS1XMA16	3.46	2.03	1.45	1.59	2.07	2.06	0.82	0.26	0.42	0.47	0.56	0.49	1.31
ICS1XRB36	2.71	2.64	2.70	2.63	1.45	1.44	0.90	0.50	1.82	0.71	1.28	0.38	1.60
ICS1XSGU26	3.11	1.21	5.22	1.75	2.10	2.09	1.43	1.83	0.54	1.45	1.00	2.05	1.98
ICS1XSIAL70	2.80	1.81	3.91	2.30	2.42	2.41	2.19	3.74	2.26	2.07	1.57	4.70	2.68
MOQ216XCC10	0.47	3.63	3.13	5.25	3.39	3.37	1.20	0.00	1.04	0.83	1.79	0.49	2.05
MOQ216XCCN34	1.24	1.37	0.26	0.00	1.13	1.12	1.38	1.16	0.96	0.31	0.55	0.22	0.81
MOQ216XCEPEC90	0.08	0.33	0.33	0.22	0.20	0.20	0.29	0.33	0.52	1.41	0.07	0.31	0.36
MOQ216XCSUL3	0.00	1.04	3.47	1.31	1.24	1.24	1.23	1.77	0.78	0.56	0.98	0.65	1.19
MOQ216XMA16	0.50	0.53	0.78	0.66	1.60	1.59	2.11	1.04	1.55	0.32	0.57	0.45	0.98
MOQ216XRB36	0.73	1.47	1.74	1.24	1.77	1.76	1.63	1.50	1.80	2.62	1.43	0.88	1.55
MOQ216XSGU26	3.92	1.32	2.03	2.23	0.97	0.96	1.05	1.10	0.84	1.36	0.66	0.52	1.41
MOQ216XSIAL70	0.40	0.24	1.68	2.06	2.49	2.48	1.12	0.94	1.13	0.74	1.11	0.14	1.21
SCA12XCC10	0.00	0.51	0.12	0.05	0.15	0.15	0.20	0.59	0.90	0.54	1.10	0.57	0.41
SCA12XCCN34	0.00	0.08	0.10	0.16	0.12	0.12	0.45	0.57	0.88	0.05	0.88	0.54	0.33
SCA12XCEPEC90	0.00	0.00	0.00	0.00	0.19	0.19	0.28	0.39	0.38	0.94	0.71	0.51	0.30
SCA12XCSUL3	0.52	0.04	0.20	0.16	0.06	0.06	0.12	0.05	0.17	0.14	0.37	0.33	0.19
SCA12XMA16	0.00	0.02	0.06	0.05	0.17	0.17	0.26	0.42	0.50	0.28	0.53	0.35	0.23
SCA12XRB36	0.00	0.21	0.05	0.12	0.14	0.14	0.29	0.47	0.67	0.33	0.58	0.38	0.28
SCA12XSGU26	0.00	0.25	0.39	0.49	0.16	0.16	0.80	1.43	0.99	0.64	1.27	0.92	0.62
SCA12XSIAL70	0.00	0.14	0.00	0.16	0.06	0.06	0.30	0.50	0.53	0.87	1.92	0.39	0.41
SCA6XCC10	0.00	0.92	0.06	1.50	0.69	0.69	2.06	2.45	2.21	4.59	1.64	1.72	1.54
SCA6XCCN34	0.00	0.16	0.00	0.14	0.15	0.15	0.27	0.77	0.46	0.97	0.87	0.33	0.36
SCA6XCEPEC90	0.00	0.00	0.00	0.00	0.03	0.03	0.23	0.17	0.32	0.19	1.13	0.57	0.22
SCA6XCSUL3	0.27	0.00	0.00	0.19	0.55	0.55	0.32	0.16	0.07	0.21	0.21	0.39	0.24
SCA6XMA16	0.00	0.19	0.18	0.00	0.09	0.08	0.39	0.24	0.24	0.13	0.32	0.40	0.19
SCA6XRB36	0.00	0.15	0.16	0.00	0.26	0.26	0.25	0.00	0.27	0.12	0.38	0.19	0.17

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Table 3 - Continuação

Progeny	1	2	3	4	5	6	7	8	9	10	11	12	Mean
SCA6XSGU26	0.00	0.07	0.16	0.13	0.19	0.19	0.16	0.07	0.15	0.21	0.48	0.00	0.15
SCA6XSIAL70	0.00	0.03	0.85	0.11	0.44	0.44	1.93	2.41	2.36	3.99	2.94	3.63	1.59
SPA5XCCN34	2.33	1.90	1.47	2.46	2.72	2.71	3.78	2.74	1.83	1.11	0.86	2.05	2.16
SPA5XCEPEC90	0.57	0.53	0.18	1.11	0.22	0.22	1.15	1.38	1.32	1.30	2.23	1.15	0.95
SPA5XCSUL3	0.00	2.31	1.17	0.98	1.93	1.93	1.35	1.10	0.85	0.00	1.94	2.43	1.33
SPA5XMA16	5.83	4.41	2.54	2.87	2.84	2.83	3.03	2.74	2.20	1.16	1.61	1.13	2.77
SPA5XRB36	0.47	1.17	1.61	1.35	1.00	0.99	0.89	1.35	0.88	0.30	0.64	0.48	0.93
SPA5XSGU26	0.47	0.16	0.65	1.42	2.66	2.65	1.83	0.30	1.77	1.21	1.89	2.56	1.46
SPA5XSIAL70	1.58	1.52	1.80	1.25	2.18	2.17	1.80	0.73	1.93	1.96	1.88	1.41	1.68

the lowest averages in the last six and all 12 evaluations (Table 3). Thus, Sca 6 presented loss of significant effects of the inheritance when acting alone simultaneously with the preservation of significant effects when in association with other resistance genes or alleles (gene pyramiding). These results evidence the importance of recurrent selection processes to improve the level and the durability of resistance to WBD.

The crossings of Scavina 6 with CCN 34, Cepec 90, CSul 3, Ma 16, RB 39 and SGu 26 did not differ significantly (Wilks' Lambda - $p > 0.05$, not shown).

Considering the 12 evaluations, Sca 6 x CC 10 and Sca 12 x CC 10 (Table 3) were significantly different (Wilks' Lambda - $p < 0.0001$, not shown), as well as SCA 6 x SIAL 70 and SCA 12 x SIAL 70, while the crossings with Sca 12 showed the best results. Therefore, Scavina 12 resistance genes acting alone outperformed Sca 6 resistance genes under the same conditions. However, in the last six counts, these crossings with Sca 12 also had a certain increase in the infection ratio (Table 3) and, although remaining numerically better, they no longer differed significantly from those with Sca 6 (Wilks' Lambda - $p > 0.05$, not shown).

In numerical values, the best Sca 12 crossing was that with the Upper Amazon CSul 3, whereas the worst one was that with the Trinitarian SGu 26 (Table 3), but the differences among all Sca 12 crossings were not significant (Wilks' Lambda - $p > 0.05$, not shown).

Chua 120 and CAB 5003 were two other G1 progenitors that outperformed the average (Table 1). Chua 120, a Criollo, had only one combination showing general average higher than that of the experiment: with SIAL 70, and the contrast of this crossing with the set of the other Chua 120 crossings was significant (Wilks' Lambda - $p < 0.05$, not shown). The general averages of CAB 5003 were lower than the general average of the experiment in four combinations: with Cepec 90, CSul 3, Ma 16, and RB 36, and higher in three combinations: with CCN 34, Sgu 26, and SIAL 70 (Table 3); the contrast between these two sets was significant (Wilks' Lambda - $p < 0.0001$, not shown).

For G2, the best results were obtained with CSul 3, which showed significant differences, except from RB 36; Cepec 90, which stood out from the rest; RB 36, which had significant differences, except from CSul 3; and Ma 16, which showed significant differences, except from CCN 34 (Table 2).

Only two of CSul 3 crossings, with SPA 5 and Moq 216, had general averages higher than the overall average of the experiment. The other six crossings including CSul 3 had total averages that were significantly lower than the overall average (Table 3); the difference between these two sets was significant (Wilks' Lambda - $p < 0.01$, not shown).

Cepec 90 did not produce any crossing with an overall average higher than that of the experiment (Table 3), but there was a significant

difference in the contrast among the three crossings showing lower averages (with Sca 6, Sca 12, and Moq 216) and the three crossings presenting higher averages (with Ca 5, ICS 1, and SPA 5) (Wilks' Lambda - $p < 0.001$, not shown).

The averages of progenies generated from RB 36 were low with Sca 6, Sca 12 and Ca 5, close to the general average with CAB 5003 and SPA 5, and high with ICS 1 and Moq 216 (Table 3); the three possible contrasts between groups were highly significant (Wilks' Lambda - $p < 0.0001$, not shown).

Ma 16 had very low averages in five combinations, with Sca 6, Sca 12, CAB 5003, Chua 120, and Ca 5; intermediate general average in one combination, with Moq 216, and high averages in two combinations, with ICS 1 and SPA 5 (Table 3). The differences between groups for the three possible contrasts were highly significant (Wilks' Lambda - $p < 0.0001$, not shown).

There was also a wide diversity of additive effects for CB, but not as much as that for VB. This could be due to the lower incidence of CB, compared to VB, in the experiment; the general average of VB per plant was 1.51 times greater than that of CB (not shown). Furthermore, six of the 28 possible comparisons between parents of each group were not significant for G1 and eight were not significant for G2 (Tables 4 and 5).

For G1, the best performance considering the 12 evaluations, in numerical values, was that of Sca 12, which did not differ from Sca 6 (Table 4). In the last six evaluations, Sca 12 was superior to Sca 6, which was not different from Moq 216 and was inferior to Chua 120, which in turn had the lowest averages of CB (Wilks' Lambda - $p < 0.01$, not shown). The proportions of CB for Sca 6 and Sca 12, similarly to those of VB, increased in the last evaluations but decreased for Chua 120 and Moq 216.

Considering the last six evaluations, Sca 6 crossings with CC 10 and SIAL 70 had significantly higher sets of averages (Table 6) than all other Sca 6 combinations (for all possible comparisons between two averages - Wilks' Lambda - $p < 0.001$, not shown). For CB, as observed for VB, there were differences between crossings in which only Sca 6 resistance genes should be present and crossings in which resistance genes from other sources should be present. Furthermore, in the last six evaluations, the contrast (Sca 6 x CC 10 and SCA 6 x SIAL 70) x (ICS 1 x CC 10, ICS 1 x SIAL 70, SPA 5 x SIAL 70) (resistance genes only from Scavina x no important resistance genes) was significant (Wilks' Lambda - $p < 0.0001$, not shown), and averages were higher for the pair of crossings with Sca 6 (Table 6). This reinforces that Scavina resistance genes alone no longer have a significant effect: breakdown of resistance.

The contrast (Sca 6 x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, SGu 26) x (ICS 1 x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, SGu

Table 4 - Ratio between the average of floral cushion brooms for each parent in Group 1 and the general average for the eight parents, at each counting period and in general (Mean); significance for the differences between sets of averages according to Wilks' Lambda test.

Progenitor	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CA5	1.40	1.68	1.52	1.17	1.21	1.29	0.73	0.94	1.06	1.07	0.85	0.97	1.16
CAB5003	0.31	0.76	0.82	0.72	1.05	1.04	1.25	1.26	0.91	1.00	0.66	0.76	0.88
CHUAO120	1.71	0.92	0.46	0.44	0.61	0.61	0.48	0.36	0.84	0.40	0.50	0.59	0.66
ICS1	0.06	2.11	1.46	0.69	1.24	1.22	0.97	1.67	1.17	1.27	1.27	1.42	1.21
MOQ216	0.22	0.52	1.75	1.83	1.27	1.25	1.23	0.95	0.78	0.81	0.71	0.73	1.00
SCA12	0.03	0.07	0.34	0.16	0.22	0.22	0.56	0.62	0.55	0.74	1.09	0.47	0.42
SCA6	0.35	0.24	0.33	0.43	0.29	0.28	0.76	0.73	0.86	1.32	1.09	1.07	0.65
SPA5	3.93	1.68	1.31	2.56	2.12	2.09	2.01	1.47	1.83	1.39	1.83	2.00	2.02

Progenitor	1	2	3	4	5	6	7
CA5	1						
CAB5003	2	*					
CHUAO120	3	Ns	ns				
ICS1	4	Ns	**	**			
MOQ216	5	**	ns	ns	**		
SCA12	6	**	**	*	**	**	
SCA6	7	**	**	*	**	**	ns
SPA5	8	**	**	**	**	**	**

ns - not significant, *significant at 5%,** significant at 1% probability of error

Table 5 - Ratio between the average of floral cushion brooms for each parent in Group 2 and the general average for the eight parents, at each counting period and in general (Mean); significance for the differences between sets of averages according to Wilks' Lambda test.

Progenitor	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CC10	3.56	1.82	0.69	1.32	0.96	0.94	1.88	2.14	1.54	1.99	1.53	1.58	1.66
CCN34	0.23	0.82	0.72	1.04	1.35	1.35	0.73	1.07	0.89	0.91	0.95	0.85	0.91
CEPEC90	0.61	0.11	0.45	0.40	0.59	0.58	0.75	0.92	0.79	0.92	1.12	1.15	0.70
CSUL3	2.05	1.37	2.54	1.81	0.93	0.91	1.16	0.74	0.75	0.44	0.90	1.14	1.23
MA16	1.04	0.91	0.35	0.77	0.79	0.87	0.47	0.32	0.57	0.21	0.34	0.47	0.59
RB36	0.36	0.94	0.73	0.80	1.01	1.00	0.65	0.87	0.75	0.68	0.64	0.61	0.75
SGU26	0.04	1.17	1.71	1.10	1.26	1.24	1.44	0.88	1.42	1.57	1.10	1.01	1.16
SIAL70	0.12	0.86	0.82	0.76	1.11	1.11	0.94	1.08	1.29	1.29	1.42	1.20	1.00

Progenitor	1	2	3	4	5	6	7
CC10	1						
CCN34	2	**					
CEPEC90	3	**	*				
CSUL3	4	**	**	**			
MA16	5	**	ns	**	**		
RB36	6	**	ns	**	**	ns	
SGU26	7	**	ns	**	**	**	ns
SIAL70	8	**	ns	ns	**	**	*

ns - not significant, * significant at 5%,** significant at 1% probability of error

26; SPA x CCN 34, Cepec 90, CSul 3, Ma 16, RB 39, SGU 26) (set with resistance from Sca 6 and another clone x set with resistance from another clone) was significant in the last six evaluations (Wilks' Lambda - $p < 0.0001$, not shown), while the first set had the lowest averages (Table 6). This demonstrates once again that Sca 6 has the loss of its significant effect of inheritance when acting individually and the preservation of this significant effect when in association with other resistance genes or alleles.

In the last six evaluations, the crossings with Sca 6 and Sca 12 x CC 10 (Table 6) were significantly different, as well as those with SCA 6 x SIAL 70 and SCA 12 x SIAL 70 (Wilks' Lambda - $p < 0.001$, not shown), and Sca 12 had the best results. This demonstrated once again differences in Sca 6 and Sca 12 inheritances while acting alone.

Only one Sca 12 combination, considering the 12 evaluations, had an overall average greater than the experiment average: SGU 26 (Table 6), which also had the highest numerical value for VB (Table

Table 6 - Ratio between the average of floral cushion brooms for each progeny and the general average for all progenies, at each counting period.

Progeny	1	2	3	4	5	6	7	8	9	10	11	12	Mean
CA5XCC10	5.45	5.42	1.02	0.66	0.88	0.87	0.56	1.21	0.89	1.32	1.68	1.08	1.75
CA5XCEPEC90	0.00	0.00	0.68	0.52	1.04	1.03	0.86	1.56	0.85	1.06	0.84	1.06	0.79
CA5XCSUL3	0.00	3.70	4.82	2.83	1.26	1.25	0.91	0.39	1.14	1.32	1.54	1.04	1.68
CA5XMA16	0.00	0.51	0.00	0.08	1.32	1.88	0.31	0.07	0.43	0.33	0.02	0.40	0.45
CA5XRB36	1.23	0.10	0.05	0.06	0.64	0.63	0.28	0.39	0.38	0.66	0.05	0.91	0.45
CA5XSGU26	0.00	3.22	3.66	2.31	0.98	0.97	1.84	2.86	3.38	2.06	0.91	1.18	1.95
CAB5003XCCN34	0.00	0.42	0.28	0.45	1.48	1.46	0.49	1.56	0.65	0.58	0.40	0.68	0.70
CAB5003XCEPEC90	0.00	1.02	0.00	0.11	0.57	0.56	2.27	0.61	0.98	0.59	1.97	0.25	0.74
CAB5003XCSUL3	0.00	0.44	1.26	0.07	1.03	1.02	0.67	1.62	0.52	0.23	0.33	0.79	0.66
CAB5003XMA16	0.00	0.00	0.00	0.30	0.56	0.55	0.03	0.78	0.30	0.48	0.17	0.26	0.29
CAB5003XRB36	0.00	1.05	2.30	1.64	1.63	1.61	1.04	1.32	1.26	1.92	0.84	0.91	1.29
CAB5003XSGU26	0.00	0.00	0.00	0.00	1.86	1.84	0.44	0.00	0.75	2.38	0.73	2.21	0.85
CAB5003XSIAL70	0.00	0.98	0.44	0.64	0.03	0.03	2.06	1.62	1.06	0.85	0.19	0.66	0.71
CHUAO120XCCN34	0.00	1.52	0.86	0.68	1.28	1.27	0.28	0.39	0.94	0.23	0.18	0.77	0.70
CHUAO120XCEPEC90	2.80	0.00	0.44	0.39	0.20	0.20	0.70	0.28	0.98	0.42	0.71	0.85	0.66
CHUAO120XCSUL3	0.00	1.39	0.70	0.05	0.08	0.08	0.16	0.21	0.51	0.05	0.82	0.52	0.38
CHUAO120XMA16	0.00	0.00	0.00	0.00	0.12	0.12	0.26	0.39	0.62	0.07	0.36	0.44	0.20
CHUAO120XSIAL70	0.00	0.65	0.44	0.57	1.00	0.99	0.10	0.11	0.51	0.15	0.25	0.38	0.43
ICS1XCC10	0.00	0.00	0.77	0.00	0.93	0.92	0.52	0.00	0.49	0.53	0.29	1.10	0.46
ICS1XCCN34	0.00	0.25	0.13	0.33	0.62	0.61	0.67	2.86	1.04	0.71	0.10	1.03	0.70
ICS1XCEPEC90	0.00	0.44	1.75	0.39	0.83	0.82	1.01	2.84	1.27	2.49	2.73	2.30	1.41
ICS1XCSUL3	0.00	2.72	2.63	0.42	0.20	0.20	0.76	0.50	0.71	0.25	0.77	1.73	0.91
ICS1XMA16	0.00	1.74	0.77	0.50	1.00	0.99	0.45	0.45	0.51	0.23	0.58	0.00	0.60
ICS1XRB36	0.00	3.60	0.91	0.86	1.48	1.46	1.25	2.91	1.59	0.62	0.93	0.48	1.34
ICS1XSGU26	0.00	5.59	2.81	2.81	3.41	3.37	0.23	0.26	0.65	3.88	1.46	0.88	2.11
ICS1XSIAL70	0.00	4.19	1.92	0.37	2.91	2.88	1.70	1.76	2.18	1.92	1.53	2.98	2.03
MOQ216XCC10	0.00	0.00	0.00	0.74	1.39	1.38	0.35	0.00	1.02	0.26	0.44	0.00	0.47
MOQ216XCCN34	0.00	1.02	0.64	0.00	0.77	0.77	0.35	0.13	0.29	0.40	0.53	0.59	0.46
MOQ216XCEPEC90	0.00	0.00	0.06	0.17	0.27	0.27	0.19	0.26	0.40	0.31	0.10	0.07	0.17
MOQ216XCSUL3	0.00	0.65	10.51	5.87	2.32	2.30	4.59	4.13	1.06	0.53	1.71	1.77	2.95
MOQ216XMA16	0.00	0.00	0.00	1.18	0.54	0.53	0.51	0.24	0.75	0.41	0.20	0.48	0.40
MOQ216XRB36	0.00	1.02	0.68	1.87	1.03	1.02	0.78	1.47	0.73	2.59	1.36	1.32	1.16
MOQ216XSGU26	0.00	0.30	1.07	0.40	1.86	1.84	1.78	0.62	0.58	0.26	0.00	0.71	0.79
MOQ216XSIAL70	0.00	0.44	1.64	1.77	2.19	2.17	1.30	0.33	1.17	0.26	0.83	0.06	1.01
SCA12XCC10	0.00	0.23	0.06	0.15	0.11	0.11	1.01	1.74	1.07	1.32	1.32	0.41	0.63
SCA12XCCN34	0.00	0.00	0.00	0.22	0.38	0.37	0.35	0.83	0.67	0.99	1.88	0.69	0.53
SCA12XCEPEC90	0.00	0.00	0.00	0.00	0.00	0.00	0.21	1.08	0.22	0.26	0.13	0.03	0.16
SCA12XCSUL3	0.00	0.19	0.24	0.06	0.15	0.14	0.79	0.05	0.36	0.31	0.40	0.52	0.27
SCA12XMA16	0.00	0.00	0.05	0.00	0.03	0.03	0.34	0.06	0.37	0.15	0.29	0.32	0.14
SCA12XRB36	0.00	0.00	0.09	0.15	0.11	0.11	0.26	0.64	0.40	0.20	0.46	0.39	0.23
SCA12XSGU26	0.00	0.13	2.17	0.66	0.77	0.77	1.79	0.72	0.93	2.38	2.36	0.96	1.14
SCA12XSIAL70	0.00	0.10	0.53	0.00	0.17	0.17	0.05	0.68	0.68	1.04	2.20	0.39	0.50
SCA6XCC10	1.40	0.76	0.38	1.59	0.40	0.39	3.42	3.96	2.38	4.32	2.21	2.90	2.01
SCA6XCCN34	0.00	0.00	0.00	0.13	0.20	0.19	0.14	0.12	0.15	2.07	0.87	0.16	0.34
SCA6XCEPEC90	0.00	0.00	0.00	0.00	0.11	0.11	0.20	0.18	0.44	0.26	0.77	1.04	0.26
SCA6XCSUL3	0.00	0.00	0.11	0.00	0.00	0.00	0.20	0.00	0.23	0.08	0.08	0.32	0.08
SCA6XMA16	0.00	0.36	0.05	0.00	0.03	0.03	0.36	0.00	0.48	0.14	0.58	0.70	0.23
SCA6XRB36	0.00	0.20	0.15	0.07	0.06	0.06	0.06	0.00	0.53	0.11	0.43	0.12	0.15

continua...

Table 6 - continuação

Progeny	1	2	3	4	5	6	7	8	9	10	11	12	Mean
SCA6XSGU26	0.00	0.00	0.00	0.00	0.19	0.18	0.03	0.00	0.04	0.32	0.00	0.00	0.06
SCA6XSIAL70	0.00	0.00	0.32	0.54	1.05	1.04	1.12	2.34	2.53	3.42	3.25	2.83	1.54
SPA5XCCN34	0.00	2.10	2.40	4.33	4.01	3.97	3.86	3.71	3.57	0.79	2.33	2.76	2.82
SPA5XCEPEC90	0.00	0.00	0.47	0.99	1.11	1.10	1.16	1.20	1.80	2.93	2.49	3.46	1.39
SPA5XCSUL3	41.21	3.05	3.07	13.37	4.18	4.14	5.50	1.17	3.07	1.06	3.93	4.41	7.35
SPA5XMA16	4.91	5.15	2.40	3.78	2.15	2.13	1.29	1.37	1.12	0.46	0.84	0.66	2.19
SPA5XRB36	0.00	1.91	1.05	1.05	1.98	1.96	1.75	1.51	1.22	0.63	1.55	0.80	1.28
SPA5XSGU26	0.00	0.00	0.00	0.25	0.77	0.77	2.27	0.39	2.50	1.45	1.60	1.54	0.96
SPA5XSIAL70	0.00	0.46	0.23	0.64	1.35	1.33	1.12	1.09	1.65	1.98	1.46	1.68	1.08

3). The SGU 26 combination differed significantly from the other Sca 12 combinations (Wilks' Lambda - $p < 0.05$, not shown). Furthermore, the contrast between a set of combinations with very low values, Ma 16, Cepec 90, Rb 36, and CSul 3, differed significantly from a set showing moderate averages, CC 10, CCN 34 and SIAL 70 (Table 6) (Wilks' Lambda - $p < 0.001$, not shown). The differences in resistance genes of other progenitors may be discernible, even for clones with a high additive effect.

Chuafo 120, the third best parent in G1, did not differ in numerical values from Ca 5, CAB 5003, and Moq 216 (Table 4), but all of their combinations had averages that were significantly lower than the general average (Wilks' Lambda - $p > 0.05$, not shown). CAB 5003 had only one combination with higher average than the experiment average (Table 6), and there were no significant differences among its combinations (Wilks' Lambda - $p > 0.05$, not shown). Moq 216 had low average combinations with CC 10, CCN 34, Cepec 90, Ma 16, and SGU 26 (Table 6), which differed significantly from the high averages from combinations with CSul 3, RB 36, and SIAL 70 (Wilks' Lambda - $p < 0.0001$, not shown).

As regards G2 parents, only Cepec 90 had a significant increase in the proportion of CB in the last six evaluations, which also occurred for VB (Tables 6 and 3) – this material is suspected to be a Scavina descendant. The resistance factors of the other G2 parents, considered sources of resistance, differed from those of Scavina.

Ma 16 had the lowest average of CB and did not differ significantly from CCN 34 and RB 36 in the 12 evaluations (Table 5), or from RB 36 in the last six evaluations (Wilks' Lambda- $p > 0.05$, not shown). Except in the combination with SPA5, it performed well or very well in all combinations (Table 6), and the contrast between these two sets was significant (Wilks' Lambda- $p < 0.0001$, not shown).

The parent with the second best average performance was Cepec 90, which differed from all other parents considering the 12 evaluations, except for SIAL 70. Such an increase in the proportional incidence of CB over time has previously been observed for clones of local varieties in Bahia, as is the case for SIAL 70, simultaneously with the increase in Scavina descendants (8).

In the most recent evaluations, Cepec 90, similarly to Sca 6, showed a clear increase in the proportion of CB, when combined with parents lacking important resistance genes, ICS 1 and SPA 5. However, it maintained good performance with Sca 6 and Sca 12 (Table 6), and there were significant differences between these groups (Wilks' Lambda - $p < 0.0001$, not shown), as observed for VB. These findings make sense if we consider Cepec 90 to be a descendant of Sca 6, and Sca 6 to have two resistance alleles (9), showing resistance loss when alone but resistance

preservation when in combination. Cepec 90 also kept its resistance in the crossing with Moq 216; the set of three combinations showing higher averages, compared to these first three, with Ca 5, CAB 5003, and Chuafo 120, also differed significantly from the set with ICS 1 and SPA 5 (Wilks' Lambda - $p < 0.0001$, not shown).

The progenitor with the third best averages was RB 36, which did not differ either from CCN 34, Ma 16, and SIAL 70 in all evaluations (Table 5), or from CCN 34 and Ma 16 in the last six evaluations (Wilks' Lambda - $p > 0.05$, not shown). RB 36 performance was good with Sca 6, Sca 12 and Ca 5 but poor with CAB 5003, ICS 1, Moq 216, and SPA 5 (Table 6); the contrast between the two sets was significant (Wilks' Lambda - $p < 0.0001$, not shown).

CCN 34 had the fourth best performance, differing only from CC10, Cepec 90 and CSul 3 in the 12 evaluations (Table 5) and from CC 10, CSul 3 and Ma 16 in the last six evaluations (Wilks' Lambda - $p > 0.05$, not shown). CCN 34 had one combination with SPA 5, which yielded very high averages, and six combinations showing averages lower than the general averages (Table 6); the contrast between the first combination and the set of the other six combinations was significant (Wilks' Lambda - $p < 0.0001$, not shown).

For the general averages of progenies, VB and CB had a 0.49 correlation (significant at $p < 0.0001$).

The present experiment produced new progenitors, several of them confirmed to have good resistance performance (10, 12), for a second generation of recurrent selection and subsequently a third one. In regional trials, the clones selected from the first and second generations of recurrent selection have been evaluated to define new varieties for commercial planting.

The significant differences in the means of progenies of different progenitors, the significant effects on progenitor interaction, and the significant differences among the progenies of each parent show the presence of different genes, alleles or gene combinations among progenitors, and the association of different genes, alleles or gene combinations among the progenies, as well as the additive and dominant effects in the inheritance of these factors. Differences in progenitors and progenies were found for trends in the evolution of the number of brooms over the evaluation period. Furthermore, according to the present findings, *Theobroma cacao* has a high genetic diversity for resistance to witches' broom disease.

Moreover, the loss of a significant effect from the inheritance of the most traditional source of resistance, Scavina 6, when acting alone, and the simultaneous preservation of this significant effect when Scavina 6 is associated with other resistance genes or alleles, proved the action of gene association in increasing the durability of its effects. These findings

emphasize the importance of recurrent selection processes to increase the level and persistence of resistance to witches' broom disease.

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