






Resistance to witches' broom in adult plants and progeny of local varieties of cacao in Southern Bahia

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ABSTRACT: Plants of local cacao variety from Southern Bahia were evaluated in order to verify if there are different levels of resistance to witches' broom disease in the study population and if the evaluation of adult plants in the field is a good predictor of these resistance levels. The seedlings were maintained under greenhouse conditions and inoculated with the fungus *Moniliophthora perniciosa*. Evaluations of symptoms occurred 60 days after inoculation. In the field, the same genotypes were evaluated by counting the number of dry brooms per plant, per year, and assessing trunk diameter size. The data were subjected to analysis of variance and averages grouped by the Scott–Knott test, differentiated by Tukey's test and compared by Student's T test. The Scavina-6 and CCN51 clones as well as the Catongo variety were used as resistance, moderate resistance and susceptibility patterns, respectively. Twenty-eight percent of the genotypes were among the most resistant and the genotypes 4119 and 4033 were considered the most susceptible to the disease in both field and greenhouse evaluations. Field evaluation was promising as a predictor of resistance level. The selected materials presented good characteristics for witches' broom resistance, with different levels deserving special attention in the context of reducing the vulnerability of Brazilian cacao cultivation to the effects of the disease, especially in the cacao region of Bahia.

Key words: *Moniliophthora perniciosa*, disease resistance, *Theobroma cacao*, traditional variety.

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INTRODUCTION

Brazil is the seventh largest producer of cacao in the world, having produced around 250,925 tons of cacao beans in the 2019 harvest in a planted area of approximately 578,122 hectares. About 48% of this production takes place in Bahia, the second largest national producer (IBGE 2019).

Among the diseases that affect cacao culture, witches' broom, caused by the fungus *Moniliophthora* (Sin: *Crinipellis*) *perniciosa* (Stahel) is considered, in Brazil, the most devastating (Ploetz 2016). Most of the resistant clones and varieties cultivated in Bahia are descended from the Scavina-6 clone (Pinto and Pires 1998). However, varieties derived from this clone have been reported with symptoms of the disease (Faleiro et al. 2004). Moreover, resistance to witches' broom in local cacao varieties is an object of concern, given the growing niche of fine chocolate market which appreciates the flavor and historical characteristics of beans in these varieties.

Studies have shown that there is variability in the sources of resistance to witches' broom that are distinguished from the descendants of Scavina-6 and that have good agronomic characteristics in genetic materials selected on local farms



(Leal et al. 2008; Lima et al. 2013; Santos et al. 2015). However, few resistant plants have been selected so far and there is a shortage of genetic information about them.

The fine chocolate niche market has grown substantially around the world (Leite 2018), and Brazil has recently been officially recognized by the International Cocoa Organization (ICCO) as a fine cocoa exporting country (Brazil 2019). The origin and quality of cocoa beans along with postharvest factors are critical to achieving excellence chocolate (Afoakwa et al. 2008). Beans from cocoa varieties in Southern Bahia have been internationally recognized for the quality of their sensory characteristics and consequent potential for producing fine or gourmet chocolate.

Studies of identification and selection of new sources of resistance to witches' broom involve steps developed in a greenhouse and/or field (Benjamin et al. 2016; Pimenta Neto et al. 2018). The methodologies are equally important and complementary (not exclusive); however, they may require excessive time and resources when performed together. Field evaluations rely on the natural conditions of infection and disease manifestation and the possibility of expression of the biotic and abiotic interactions inherent to the pathosystem. Thus, the practice can be seen as a good predictor of the resistance level and, being practiced by the producer themselves on their property, results in optimization of resources and time.

Given the above, it is important to increase the genetic diversity of local varieties of cacao plants in Southern Bahia, preventing the eventual evolution of the pathogen and finally contributing to the durability of resistance in these varieties. Therefore, the objectives of this study were to assess whether (i) there are different levels of resistance to witches' broom disease in the study population and (ii) the field evaluation can be seen as a good predictor of resistance levels to this disease.

MATERIAL AND METHODS

Plant material

The plant material consisted of 96 genotypes of traditional local cacao varieties from Bahia cultivated in the "cacaucabruca" agroforestry ecological system in the municipality of Uruçuca, Bahia. Scavina-6 (SCA6) and CCN51 (Colección Castro Naranjal) clones, and the Catongo variety were used as patterns of resistance, moderate resistance and susceptibility to witches' broom, respectively.

Evaluation of symptoms

The resistance evaluation tests occurred in a greenhouse by artificial inoculation and in the field by natural infection. In the greenhouse, open-pollinated seeds were planted in 288 cm³ plastic tubes containing a mixture of sterile soil and plant substrate composed of pine bark, coconut fiber and expanded vermiculite in a 1:1 ratio. The seedlings received the recommended cacao crop treatments, with daily irrigation and pest and disease control. Each seedling was identified in accordance with the field matrix that gave rise to it. Due to heterogeneous fructification and the laboratory limitation on the number of samples to be processed per day, the materials were divided into 12 batches with presence of the three patterns in all of them.

Thirty days after planting, the seedlings were artificially inoculated with suspension of basidiospores of the fungus *M. perniciosa*. The basidiospores were obtained according to the methodology described by Dickstein et al. (1987) and subjected to a viability test proposed by Frias et al. (1995). The inoculation process was performed according to a previously established methodology, in which 30 µL of the inoculum with concentration adjusted to 2×10^5 basidiospores·mL⁻¹ was deposited in 0.2% agar-water medium in the apical bud (Pimenta Neto et al. 2018).

Sixty days after inoculation, each plant was assessed for the number and type of broom. For data analysis, the disease index (DI) proposed by Rodrigues et al. (2019) was calculated following the model (Eq. 1):

$$DI = TB + AB + CB + (4.3 * DB) + (0.1 * LTB) + (0.2 * NAB) \quad (1)$$

Where: TB = presence of terminal broom; AB = presence of axillary broom; CB = presence of cotyledon broom; DB = presence of dry broom; LTB = length of terminal broom; NAB = number of axillary brooms > 1 cm.

In the field, the same genotypes were evaluated for the number of dry brooms (DB) per plant, per year, and the size of trunk diameter, measured at 50 cm from the base of the plant, during the periods of July 2018 and July 2019.

Statistical analysis

A randomized block design (RBD) was used for the data from the field evaluations (DB and trunk diameter). The data were subjected to F-test of analysis of variance and averages grouped by Scott–Knott test ($p < 0.05$).

The disease index (DI) was calculated and a RBD was used to the plants submitted to artificial inoculation in a greenhouse, considering the inoculation dates as blocks. The patterns were present in all dates and the genotypes in only one date. Initially, the data of the patterns were subjected to F-test of analysis of variance and averages differentiated by Tukey's test ($p < 0.05$). As there was no interaction between pattern and date or block, the means of the genotypes DI were corrected by the block effects and compared with the patterns in an analysis with all dates, using the Student's t-test ($p < 0.05$). All analyzes were performed with the aid of R software in version 3.1.0 (R Core Team 2011) and SAS software in version 9.4 (SAS institute 2008).

RESULTS AND DISCUSSION

When evaluating the presence of dry broom in the cacao plants under study, it was verified that there was a significant effect of the analyzed genotypes ($p < 0.05$). In the Scott–Knott test, the genotype distinction formed four resistance groups to witches' broom, with average values of dry broom per plant ranging from none to more than 55 (Table 1), suggesting the existence of different resistance levels. The dry broom count per plant has already been established as an appropriate parameter for assessing resistance to witches' broom and has been used frequently (Silva et al. 2010; Yamada et al. 2013).

The measurements of trunk diameter and dry broom behaved as independent characteristics, since they presented a correlation of -0.01. Theoretically, the correlation between the characteristics had already been reported (Faleiro et al. 2006). However, the edaphoclimatic conditions of the experimental area characterized by the "cacaou-cabruca" planting system (Brazil 2014) is considered to have provided different shading patterns, soil depth, spacing between plants and wind exposure, which may have influenced the result. In particular, the genetic effect of different varieties is believed to be the main component of the variation of expression of the trait analyzed. Moreover, the age of the plants (over 30 years) may have led them to stable stem growth.

Most plants had a number of dry brooms 3 to 6 times lower than the most susceptible group, suggesting that there are different resistance levels in this germplasm (Fig. 1).

The analysis of variance of the data obtained for the DI considering only the patterns of resistance (SCA6, CCN51 and Catongo) and different dates of inoculation demonstrates that significant effects ($p < 0.01$) were only observed among genotypes. No significant effects were observed for the date of inoculation or for their interaction with genotypes (Table 2; Fig. 2). Therefore, the obtained responses reflected the resistance variability of the cacao genotypes and not that of the inoculum.

As expected, the used patterns have been significantly different from each other by the Tukey's test ($p < 0.05$). The SCA6 clone showed DI of 0.27, while CCN51 showed 0.51 and the Catongo variety showed 0.95 (Table 3). The SCA6 clone was selected in Peru in 1942 and has since been used as a source of resistance to witches' broom in cacao breeding programs (Lopes et al. 2011; Teixeira et al. 2015). In fact, in many progeny tests both in the field and the greenhouse, the clone has shown superior performance for disease resistance, having been used as a pattern of resistance in various studies (Lopes et al. 2011;

Table 1. Mean values of dry brooms in 96 local variety cacao genotypes over two consecutive years.

Genotype	Dry broom (DB)	Genotype	Dry broom (DB)	Genotype	Dry broom (DB)
4015	55.50 ^A	4181	18.50 ^C	4076	7.50 ^D
4132	55.00 ^A	4184	18.50 ^C	4078	7.50 ^D
4119	45.00 ^B	4007	18.00 ^C	4062	7.00 ^D
4033	40.00 ^B	4151	18.00 ^C	4104	7.00 ^D
4163	40.00 ^B	4095	17.00 ^C	4105	6.50 ^D
4186	33.50 ^C	4178	17.00 ^D	4121	6.50 ^D
4091	32.50 ^C	4125	16.00 ^D	4139	6.50 ^D
4187	29.50 ^C	4047	15.00 ^D	4064	6.00 ^D
4162	28.50 ^C	4099	15.00 ^D	4112	6.00 ^D
4009	27.50 ^C	4141	15.00 ^D	4127	5.50 ^D
4063	27.50 ^C	4161	15.00 ^D	4060	4.50 ^D
4185	27.50 ^C	4092	14.50 ^D	4083	4.50 ^D
4042	27.00 ^C	4103	14.00 ^D	4096	4.50 ^D
4056	25.50 ^C	4067	13.50 ^D	4107	4.50 ^D
4140	25.50 ^C	4126	13.50 ^D	4135	4.50 ^D
4043	25.00 ^C	4115	13.00 ^D	4137	4.50 ^D
4098	25.00 ^C	4138	13.00 ^D	4070B	3.50 ^D
4158	25.00 ^C	4179	12.00 ^D	4005	3.00 ^D
4180	24.00 ^C	4109	11.50 ^D	4035	3.00 ^D
4089	23.50 ^C	4086	11.00 ^D	4084	3.00 ^D
4100	23.50 ^C	4110	11.00 ^D	4111	3.00 ^D
4152	22.50 ^C	4177A	11.00 ^D	4057	2.50 ^D
4050	22.00 ^C	4075	10.00 ^D	4077	2.50 ^D
4177B	21.50 ^C	4133	10.00 ^D	4085	2.50 ^D
4031	21.00 ^C	4113	9.50 ^D	4001	2.00 ^D
4045	21.00 ^C	4134	9.50 ^D	4054	1.50 ^D
4044	20.50 ^C	4183	9.50 ^D	4058	1.50 ^D
4156	20.50 ^C	4036	9.00 ^D	4065	1.50 ^D
4176	20.00 ^C	4053	9.00 ^D	4114	1.50 ^D
4090	19.50 ^C	4069	9.00 ^D	4102	1.00 ^D
4153	19.00 ^C	4070A	8.00 ^D	4037	0.00 ^D
4094	18.50 ^C	4059	7.50 ^D	4101	0.00 ^D

Means followed by the same letters are in a same group by Scott-Knott test ($p < 0.05$).

Pires et al. 2012; Sena et al. 2014). In turn, the CCN51 clone selected in Ecuador, is the result of a breeding between ICS-95, IMC-67 and Canelus, and has been considered a carrier of a moderate resistance to witches' broom (Campo and Andía 1997; Boza et al. 2014). Finally, the Catongo variety (*Theobroma leiocarpa* Bernoulli), first found in the mid-1930s in Pirangi, Bahia, is considered an albino mutation of Forastero cacao (Dantas Neto et al. 2005) and is often used as a pattern for susceptibility to witches' broom (Pimenta Neto et al. 2018; Benjamin et al. 2016).

Significant effects for DI between 96 cacao genotypes under study were found ($p < 0.01$). The same was not verified for the block ($p > 0.01$) (Table 4). In the Student's t-test, the genotype distinction formed five groups of resistance to witches' broom, based on the DI (Table 5). The genotypes considered highly resistant were those that had a significantly lower DI than CCN51 and Catongo, resistant were those with a significantly lower DI than just the Catongo, and intermediate those that had DI higher than SCA6 or were not significantly different from none. The genotypes considered susceptible were

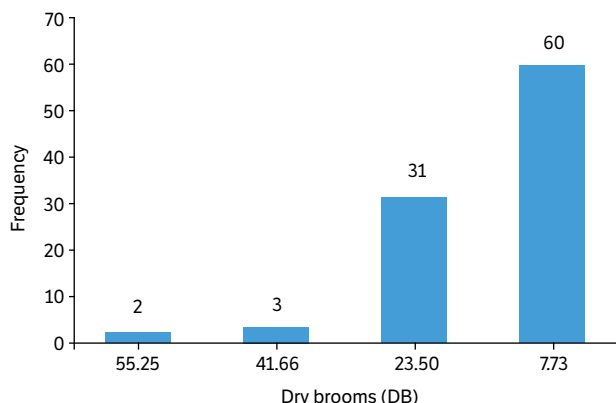


Figure 1. Frequency histogram of 96 local variety cacao genotypes relative to the four witches' broom resistance groups based on the number of dry brooms formed in the Scott-Knott test at 5% probability.

Table 2. Disease index (DI) analysis of variance on resistance patterns, moderate resistance and susceptibility to witches' broom disease, at different inoculation dates with the fungus *Moniliophthora perniciosa*.

Variation sources	DF	Mean SQ	F value	Pr(>F)
Block/Environment	11	0.98	0.87	0.56 ^{ns}
Genotype	2	75.43	68.04	<2e-16 ***
Environment: genotype	22	1.46	1.30	0.16 ^{ns}
Error	1908	1.12		
Correct Total	1943			
DI Mean	0.58			
CV (%)	29.01			

ns: not significant, ***: significant $p < 0.01$ by the F-test.

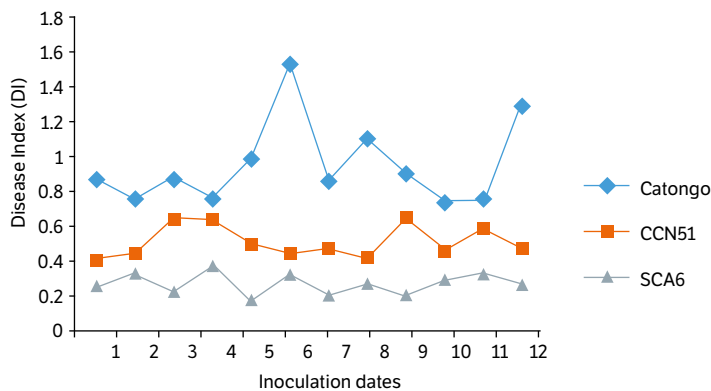


Figure 2. Disease index (DI) of SCA6 and CCN51 clones and Catongo variety in function of different dates of inoculation with the fungus *Moniliophthora perniciosa*.

those with significantly higher DI than SCA6 and CCN51 and, finally, highly susceptible were those with higher DI than all patterns. The variability of resistance to witches' broom in selected genetic materials on local farms has been reported in important studies (Leal et al. 2008; Lima et al. 2013) and has been reinforced in this paper (Table 5). Numerically considering the value of DI, the distribution of genotypes was mostly more resistant than the Catongo variety and less resistant than the SCA6 clone (Fig. 3).

The susceptibility of local cacao varieties of the cacao region of Bahia has already been reported (Lopes et al. 2011). However, promisingly, the present study found 32 genotypes with averages that are inferior to that of Catongo variety, two of which with averages lower than the SCA6 and CCN51 clones. Unfortunately, the Southern Bahia local cacao germplasm

Table 3. Mean values of disease index values (DI) of SCA6 and CCN51 clones and Catongo variety.

Genotype	DI
Catongo	0.95±1.29 ^A
CCN51	0.51±1.05 ^B
SCA6	0.27±0.79 ^C

Means followed by the same letters are in a same group by Tukey's test ($p < 0.05$).

Table 4. Disease index (DI) analysis of variance to witches' broom of 96 genotypes of local cacao varieties and 3 resistance patterns at different dates.

Variation sources	DF	Mean SQ	F value	Pr(>F)
Block/Environment	11	0.98	0.71	0.73 ^{ns}
Genotypes	98	8.50	6.13	3.18e-06 ***
Error	7018	1.38		
Correct Total	7127			
DI Mean	0.71			
CV (%)	23.26			

ns: not significant, ***: significant $p < 0.01$ by the F-test.

suffered losses of up to 90% in the 1990s, when the disease showed the highest damage to cacao cultivation in the region (Oliveira and Luz 2005). Interestingly, even after 30 years since the first reports of the disease at the field (Pereira et al. 1989), some of these varieties are still present in crops. There are lines of studies that argue that this is due to the random crossing of these plants with resistant clones distributed on farms by the Executive Commission of the Cacao Plant (Centro de Pesquisa da Lavoura Cacaueira – CEPLAC), since the arrival of the disease in the region (Lopes et al. 2011). However, the possibility that there are different sources of Scavina-6 resistance in the genetic background in question is not ruled out. This is because the gene combination phenomenon from random crossing in cacao cultivation has already been verified (Benjamin et al. 2016). Additionally, it is possible that there is greater genetic diversity of cacao accesses in commercial plantations in Bahia compared to the genotypes corresponding to the alleged parents used to produce hybrid cacao varieties (Leal et al. 2008).

The genotypes 4031 and 4037 showed, with significant difference, DI means lower than the CCN51 and Catongo variety, suggestive to strong resistance to witches' broom. Approximately 34% of the genotypes showed DI means significantly lower than the Catongo variety and with no statistical difference with the CCN51 and SCA6 clones, also worthy of prominence in the selection for resistance. Approximately 9% of the genotypes showed DI means that did not differ significantly from any patterns used in the experiment. It is also noteworthy that 25% of the 96 evaluated genotypes had higher DI means than the SCA6 and CCN51 clones, but without statistical difference with the Catongo variety, being therefore considered susceptible to witches' broom. Finally, approximately 7% of the genotypes had higher DI means than all the patterns, suggesting a high susceptibility to the mentioned disease (Table 5).

There was a significant ($p < 0.05$) moderate correlation (0.31) between the variables DI and DB. Although the interpretation of the magnitude of the correlation coefficient depends on specific application context, the found value suggests that the dry broom count per plant is a good predictor for evaluation of resistance level to witches' broom. However, the same per se does not exclude the importance of progeny studies in greenhouses which are conducted in order to prevent escape.

Promisingly, the genotypes 4037, 4057, 4070B, 4078, 4069, 4083, 4059, 4113, 4076, 4101, 4139, 4111, 4134, 4065, 4141, 4138, 4103, 4058, 4084, 4121, 4127, 4102, 4005, 4137, 4054, 4060, 4047, 4099 showed greater resistance in both greenhouse and field evaluations, while the genotypes 4119 and 4033 were among the most susceptible in both assessments.

According to the morphoagronomic characterization of this population, the genotypes with resistant behavior, 4057, 4070B, 4078, 4069, 4083, 4059, 4076, 4065, 4058, 4084, 4054, 4060, 4099, are classified as *cacao comum*, while 4113, 4101, 4139, 4111,

Table 5. Individual comparison among adjusted means values of witches' broom disease index (DI) from 96 genotypes of local cacao varieties versus the SCA6 (resistance pattern) and CCN51 (moderate resistance pattern) clones and Catongo variety (susceptibility pattern) at inoculated with the *Moniliophthora perniciosa* fungus, and error probability for the difference observed between the means, by the t-test.

Genotype	DI Mean	SCA6	CCN51	Catongo	Class
		X=0.27	X=0.51	X=0.95	
4031	-0.01	0.1391	0.0064	<0.0001	HR
4037	0.12	0.4119	0.0384	<0.0001	HR
4057	0.14	0.4858	0.0515	<0.0001	R
4070B	0.14	0.4959	0.0535	<0.0001	R
4078	0.15	0.5176	0.0578	<0.0001	R
4069	0.16	0.5479	0.0641	<0.0001	R
4083	0.25	0.9228	0.1779	0.0002	R
4059	0.27	0.9851	0.2044	0.0003	R
4113	0.28	0.9725	0.2240	0.0004	R
4076	0.28	0.9584	0.2309	0.0004	R
4101	0.29	0.9231	0.2485	0.0005	R
4139	0.31	0.8623	0.2815	0.0007	R
4111	0.31	0.8533	0.2867	0.0007	R
4134	0.31	0.8477	0.2899	0.0007	R
4065	0.35	0.6729	0.4075	0.0016	R
4141	0.37	0.6095	0.4595	0.0021	R
4138	0.37	0.5926	0.4744	0.0023	R
4103	0.39	0.5222	0.5416	0.0033	R
4058	0.42	0.4259	0.6497	0.0053	R
4140	0.43	0.3973	0.6863	0.0062	R
4084	0.44	0.3840	0.7041	0.0067	R
4121	0.44	0.3701	0.7232	0.0072	R
4127	0.46	0.3326	0.7784	0.0090	R
4102	0.49	0.2603	0.9007	0.0140	R
4007	0.51	0.2062	0.9890	0.0204	R
4056	0.53	0.1734	0.9116	0.0263	R
4005	0.54	0.1637	0.8868	0.0285	R
4137	0.54	0.1573	0.8698	0.0301	R
4054	0.55	0.1389	0.8185	0.0354	R
4060	0.56	0.1232	0.7710	0.0412	R
4047	0.57	0.1171	0.7515	0.0438	R
4099	0.58	0.1079	0.7209	0.0482	R
4042	0.58	0.1024	0.7018	0.0512	I
4001	0.58	0.0999	0.6929	0.0526	I
4156	0.59	0.0895	0.6544	0.0594	I
4132	0.59	0.0894	0.6540	0.0595	I
4089	0.59	0.0889	0.6519	0.0599	I
4158	0.60	0.0850	0.6368	0.0628	I
4133	0.61	0.0766	0.6028	0.0699	I
4092	0.62	0.0642	0.5481	0.0832	I

Continue...

Table 5. Continuation.

Genotype	DI Mean	SCA6	CCN51	Catongo	Class
		X=0.27	X=0.51	X=0.95	
4162	0.63	0.0587	0.5219	0.0906	I
4009	0.64	0.0488	0.4717	0.1067	I
4179	0.66	0.0418	0.4322	0.1217	I
4115	0.67	0.0334	0.3803	0.1455	I
4110	0.68	0.0310	0.3645	0.1539	I
4105	0.69	0.0277	0.3416	0.1670	I
4104	0.71	0.0220	0.2978	0.1964	I
4125	0.73	0.0162	0.2479	0.2390	I
4036	0.73	0.0147	0.2341	0.2530	I
4094	0.74	0.0136	0.2232	0.2649	I
4015	0.74	0.0125	0.2118	0.2783	I
4135	0.75	0.0108	0.1941	0.3009	I
4186	0.78	0.0075	0.1546	0.3631	I
4177B	0.78	0.0072	0.1502	0.3713	I
4075	0.78	0.0071	0.1486	0.3743	I
4161	0.79	0.0066	0.1430	0.3854	I
4085	0.79	0.0063	0.1377	0.3962	I
4178	0.80	0.0055	0.1273	0.4190	I
4187	0.80	0.0054	0.1256	0.4230	I
4095	0.81	0.0040	0.1029	0.4828	I
4163	0.84	0.0029	0.0842	0.5445	I
4096	0.84	0.0027	0.0809	0.5571	I
4126	0.84	0.0025	0.0757	0.5778	I
4045	0.86	0.0018	0.0620	0.6409	I
4114	0.88	0.0014	0.0512	0.7015	I
4176	0.90	0.0009	0.0372	0.8031	S
4077	0.92	0.0006	0.0298	0.8726	S
4053	0.93	0.0005	0.0273	0.8999	S
4033	0.94	0.0004	0.0233	0.9482	S
4062	0.96	0.0003	0.0181	0.9750	S
4098	0.96	0.0003	0.0159	0.9367	S
4086	0.98	0.0002	0.0131	0.8814	S
4152	1.00	0.0001	0.0090	0.7797	S
4100	1.00	0.0001	0.0087	0.7704	S
4184	1.02	<0.0001	0.0073	0.7274	S
4070A	1.03	<0.0001	0.0056	0.6615	S
4035	1.04	<0.0001	0.0049	0.6293	S
4151	1.04	<0.0001	0.0046	0.6169	S
4181	1.08	<0.0001	0.0024	0.4820	S
4064	1.09	<0.0001	0.0023	0.4702	S
4063	1.10	<0.0001	0.0019	0.4369	S
4153	1.15	<0.0001	0.0007	0.2982	S

Continue...

Table 5. Continuation.

Genotype	DI Mean	SCA6	CCN51	Catongo	Class
		X=0.27	X=0.51	X=0.95	
4067	1.16	<0.0001	0.0006	0.2707	S
4090	1.21	<0.0001	0.0002	0.1610	S
4112	1.24	<0.0001	0.0001	0.1215	S
4043	1.25	<0.0001	<0.0001	0.1067	S
4183	1.30	<0.0001	<0.0001	0.0652	S
4050	1.31	<0.0001	<0.0001	0.0540	S
4177A	1.31	<0.0001	<0.0001	0.0534	S
4109	1.36	<0.0001	<0.0001	0.0283	HS
4119	1.37	<0.0001	<0.0001	0.0258	HS
4107	1.57	<0.0001	<0.0001	0.0011	HS
4091	1.58	<0.0001	<0.0001	0.0008	HS
4185	1.65	<0.0001	<0.0001	0.0002	HS
4044	1.81	<0.0001	<0.0001	<0.0001	HS
4180	1.91	<0.0001	<0.0001	<0.0001	HS
SCA6	0.27	.	0.0003	<0.0001	R
CCN51	0.51	0.0003	.	<0.0001	MR
Catongo	0.95	<0.0001	<0.0001	.	S

HR: highly resistant; R: resistant; I: intermediate; S: Susceptible; HS: highly susceptible; MR: moderate resistant.

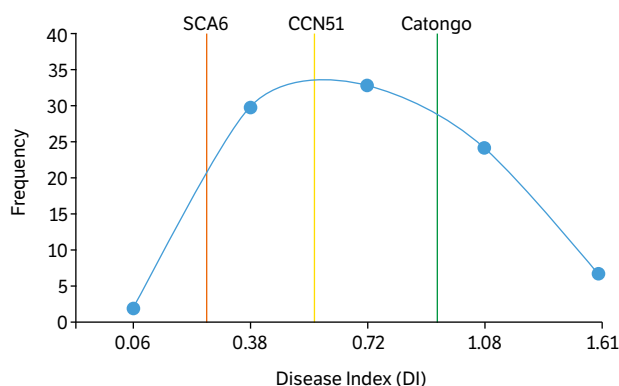


Figure 3. Frequency distribution of 96 local variety cacao genotypes and 3 resistance patterns, relative to the 5 witches' broom resistance groups formed in the disease index (DI) by the t-test.

4134, 4141, 4138, 4103, 4121, 4127, 4102, 4137 are classified as *Maranhão* and 4005, 4047 and 4037 as *Parazinho*. On the other hand, among the genotypes with susceptible behavior, 4119 is classified as *Maranhão* while 4033 as *Parazinho*. It is interesting to note that approximately 46% of the genotypes among the most resistant are classified as *cacao comum* and approximately 43% as *Maranhão*. This draws attention to the varieties of *cacao comum* and *Maranhão* in the context of resistance to witches' broom.

CONCLUSION

There are different levels of resistance to witches' broom in the germplasm under study, compatible with the existence in this genetic background of sources of resistance to the disease in question that are possibly different from those traditionally

used in cacao genetic breeding programs. Therefore, it is essential to continue selection work in local farms and conduct subsequent studies of gene identification with effects on resistance in this population, especially for the 28 genotypes that showed high or moderate resistance.

The report of the occurrence of significant correlation between the two types of tests (field evaluation by counting dry brooms and artificial inoculation in a greenhouse), indicate the effectiveness of both. Thus, the methodology of field evaluation is a good predictor of resistance levels to this disease.

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