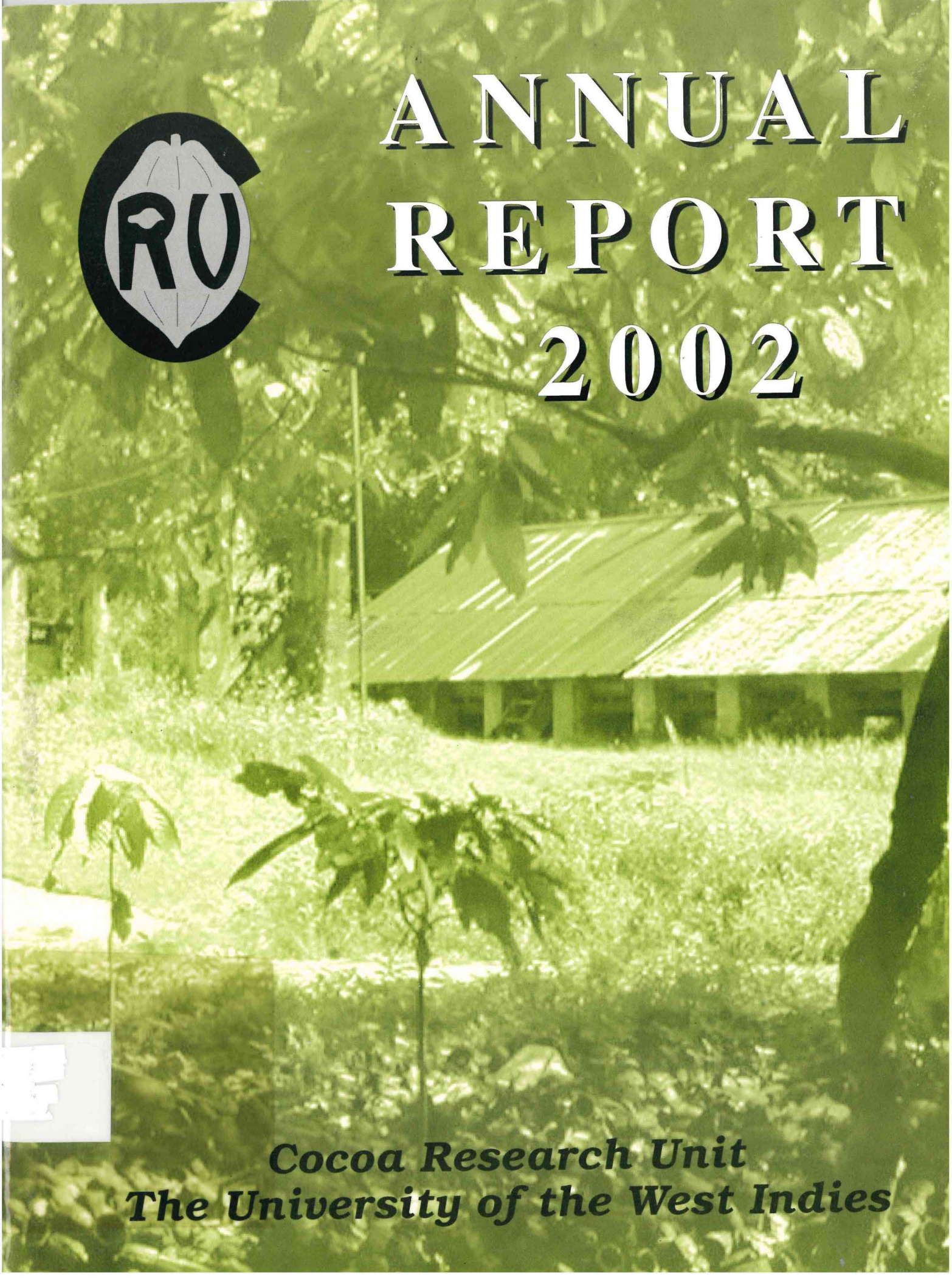
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Cover photograph. "Cocoa houses" for sun drying of beans in a traditional cocoa estate, north Trinidad.

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Field resistance of cocoa to Witches' Broom and Black Pod diseases

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Introduction

As part of the CFC/ICCO/IPGRI Project entitled *Cocoa Germplasm Utilisation and Conservation: a Global Approach*, CRU undertook some field observations in the ICG,T between November 1998 to December 2001 to assess the resistance of cacao clones to Black Pod and Witches' Broom diseases under natural conditions of infection.

Methods

A total of 475 clones from 49 populations were observed (Table 1). The selection of these clones was based on:

- Their known field resistance or susceptibility to Black Pod and Witches' Broom, and their suitability for use as controls,
- Interesting traits other than disease resistance,
- Their resistance to Black Pod disease determined by a detached pod inoculation test.

Depending on the availability of plants, one to five trees per clone were selected and tagged.

Table 1. Number of clones assessed within each population.

Population	Number of clones	Population	Number of clones	Population	Number of clones	Population	Number of clones
AGU [CHA]	1	DOM	2	LZ	1	RIM [MEX]	10
AM [POU]	17	DOPOL	1	M	2	SC [COL]	7
AMAZ [CHA]	5	EET [ECU]	6	MAN [BRA]	1	SCA	5
B [POU]	16	G	1	MATINA	1	SJ [POU]	2
CC	6	GS	3	MO	3	SLA	5
CL	13	GU	15	MOQ	14	SLC	2
CLEM	1	ICS	30	NA	83	SM [POU]	1
CLM	4	IMC	25	OC [VEN]	1	SNK	1
COCA [CHA]	1	JA [POU]	29	PA [PER]	53	SPA [COL]	5
CRU	24	LCTEEN	16	PLAYA ALTA [VEN]	1	SPEC	10
CRUZ	2	LP [POU]	14	POUND [POU]	11	TRD	8
DE [TTO]	2	LV [POU]	2	RB [BRA]	1	UF	6

Vegetative infection of Witches' Broom

Three branches of approximately 1.5 m length were selected from each tree to represent the canopy. On each branch, the number of healthy shoots and brooms were recorded. Observations were carried out three times a year.

Pests and diseases on pods

Each tree was observed monthly, ripe pods were harvested and the following variables were recorded:

- Number of healthy pods,
- Number of pods with Black Pod symptoms but no Witches' Broom symptoms, in association with or without other fungi, insects, rodent or bird attack,
- Number of pods with Witches' Broom symptoms but no Black Pod symptoms, in association with or without other fungi, insects, rodent or bird attack,
- Number of pods with both Black Pod and Witches' Broom symptoms on the same pods, in association with or without other fungi, insects, rodent or bird attack.

Analysis of data

A pod was considered as healthy if it did not show any symptoms of Black Pod and Witches' Broom, even if symptoms due to other fungi, insects, rodents or birds were present.

Statistical analysis was performed using Minitab release 12.2 (Minitab Inc.) and NCSS 2001 (NCSS Statistical Software, Utah, USA). Variables studied were: percentage of pods affected by Black Pod, percentage of pods affected by Witches' Broom and percentage of shoots affected by Witches' Broom. Data were subjected to arcsin($\sqrt{}$) transformation before analysis.

For the 61 clones (representing 20 populations) assessed for the entire period of observation (November 1998-December 2001), each tree was characterised by the cumulative data over the period of observations. Each clone was characterised by the average value from the trees representing it.

Correlations between variables were evaluated with the Pearson and Spearman correlation coefficients (for values and ranks, respectively): on a tree per tree basis for the phenotypic correlation and on a clone per clone basis for the genotypic correlation.

The broad sense heritability (H^2_{bs}) was evaluated using the following formulae:

H^2_{bs} = genotypic variance/phenotypic variance

Phenotypic variance = Genotypic variance + Residual variance

Residual variance = MSR

Genotypic variance = (MSC-MSR)/n,

Where: MSC is the mean square for clone

MSR is the mean square for error

and n is the number of trees per clone.

For all clones, including those observed for just 12 or 24 months of the period, analysis of variance (ANOVA) was carried out on the yearly percentage of diseased material, using "year" as co-variate, to take into consideration differences of incidence of the diseases between years. Only trees having produced 10 or more pods per year were included in the analysis on the percentage of pods affected by Black Pod and Witches' Broom diseases (354 clones). Four hundred and seventy two clones were kept for the analysis on shoots.

Results

Field resistance to Witches' Broom and Black Pod diseases

The ANOVA showed a highly significant clonal effect for all three variables: percentage of infected pods by Black Pod, percentage of infected pods by Witches' Broom and percentage of affected shoots by Witches' Broom (Table 2).

Table 2. Analysis of Variance for all clones.

Black Pod

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Probability Level
Year	1	19.77634	19.77634	371.74	<0.001
Clone	353	78.69964	0.2229452	4.19	<0.001
Error	1240	65.96806	5.320005E-02		
Total	1594	166.1637			

Witches' Broom on pods

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Probability Level
Year	1	1.648795	1.648795	57.48	<0.001
Clone	353	32.35797	9.166563E-02	3.20	<0.001
Error	1240	35.56966	2.868521E-02		
Total	1594	69.76682			

Witches' Broom on shoots

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Probability Level
Year	1	5.622244E-03	5.622244E-03	1.95	0.162762
Clone	471	7.988047	1.692383E-02	5.87	<0.001
Error	2193	6.323888	2.88367E-03		
Total	2665	14.3216			

A highly significant effect was also observed for the year of observations for symptoms on pods. This is not surprising since environmental variables such as rainfall vary from one year to another and those factors are known to strongly influence the development and the epidemiology of the diseases. It confirms that several years of observations are necessary to get a true evaluation of the resistance level of the accessions.

However, there was no significant effect of year for observations on shoots, suggesting that observations for a short period could be sufficient to assess resistance, providing the disease is already well developed in the field and trees are well established.

Correlations between diseases

An ANOVA was performed on the 61 clones that were observed during the whole period of the experiment (3 years). It showed a highly significant clonal effect for all variables (Table 3).

Table 3. Analysis of variance for the 61 clones observed for 3 years.

Percentage of Black Pod disease

Source	DF	Seq SS	Adj SS	Adj MS	F-ratio	*P*
Clone	60	11.48689	11.46943	0.19116	8.98	<0.001
Tree	4	0.00244	0.00244	0.00061	0.03	0.998
Error	209	4.44950	4.44950	0.02129		
Total	273	15.93883				

Percentage of Witches' Broom disease on pods

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Clone	60	4.57004	4.57190	0.07620	5.41	<0.001
Tree	4	0.02483	0.02483	0.00621	0.44	0.779
Error	209	2.94389	2.94389	0.01409		
Total	273	7.53876				

Percentage of Witches' Broom disease on shoots

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Clone	60	1.066380	1.065729	0.017762	10.58	<0.001
Tree	4	0.002395	0.002395	0.000599	0.36	0.839
Error	209	0.350943	0.350943	0.001679		
Total	273	1.419718				

Table 4. Incidence of Black Pod and Witches' Broom diseases on 61 clones observed during 3 years.

Clone	BP (%)	WB Pod (%)	WB Shoot (%)
AM 1/73 [POU]	12.3 abcde	5.2 abcde	0.13 ab
AM 2/65 [POU]	20.2 abcdefg	6.8 abcdefg	0.13 ab
AMAZ 12 [CHA]	37.9 defghij	0.9 ab	0.00 a
B 12/1 [POU]	8.0 ab	2.2 abcd	0.19 abc
B 13/5 [POU]	19.2 abcdef	3.2 abcde	0.37 abcd
B 5/3 [POU]	15.3 abcde	9.9 bcdefg	0.70 abcd
CL 10/10	21.5 abcdefg	8.1 abcdefg	0.43 abcd
CL 19/10	17.2 abcdef	3.2 abcde	0.15 abc
CLEM /S-62-1	20.7 abcdefg	6.6 abcdef	0.00 a
CRU 101	7.9 ab	2.8 abcde	0.00 a
CRU 119	62.8 ijk	10.8 bcdefg	4.51 efgh
CRU 124	9.8 abcd	14.8 efg	2.80 efg
CRU 19	23.8 abcdefg	2.1 abcd	0.05 a
CRU 96	77.3 k	13.2 efg	2.04 cdefg

Clones followed by the same letter are not significantly different according to the Tukey-Kramer multiple-comparison test at a 95% confidence level.

Table 4 (cont.). Incidence of Black Pod and Witches' Broom diseases on 61 clones observed during 3 years.

Clone	BP (%)	WB Pod (%)	WB Shoot (%)
EET 58 [ECU]	21.8 abcdefg	12.3 cdefg	1.60 bcdef
EET 59 [ECU]	22.9 abcdefg	14.9 efg	3.45 efgh
ICS 1	37.4 defghij	10.6 bcdefg	1.36 abcde
ICS 40	39.1 efghij	2.5 abcde	0.29 abcd
ICS 70	68.5 jk	7.8 abcdefg	0.11 a
ICS 80	43.1 efghijk	5.1 abcde	0.17 abc
ICS 84	21.5 abcdefg	6.1 abcdef	2.13 defg
ICS 95	24.8 bcdefgh	12.3 cdefg	0.44 abcd
IMC 103	50.2 fghijk	7.6 abcdefg	0.13 ab
IMC 16	25.2 bcdefgh	3.3 abcde	0.10 a
IMC 47	8.1 ab	5.0 abcde	0.15 abc
IMC 57	25.0 bcdefgh	4.6 abcde	0.00 a
IMC 6	32.1 cdefghi	4.2 abcde	0.00 a
JA 1/21 [POU]	4.1 a	7.8 abcdefg	0.62 abcd
JA 5/25 [POU]	19.6 abcdefg	10.2 bcdefg	0.34 abcd
LP 1/45 [POU]	21.5 abcdefg	6.2 abcdef	0.00 a
LP 4/24 [POU]	17.2 abcdef	4.1 abcde	1.14 abcde
LX 31	3.4 a	11.4 bcdefg	0.60 abcd
MAN 15/60 [BRA]	5.4 a	1.6 abc	0.00 a
MOQ 5/5	29.3 bcdefgh	7.6 abcdefg	0.00 a
NA 142	10.0 abcd	8.0 abcdefg	0.71 abcd
NA 149	40.8 efghij	13.0 efg	0.12 ab
NA 178	30.7 cdefghi	7.5 abcdefg	0.00 a
NA 226	60.5 ghijk	5.2 abcde	0.00 a
NA 342	16.8 abcde	2.7 abcde	0.51 abcd
NA 387	23.3 abcdefg	1.5 abc	0.33 abcd
NA 45	54.0 ghijk	26.1 g	6.15 gh
NA 672	27.7 bcdefgh	24.2 fg	0.92 abcde
NA 680	8.5 ab	2.8 abcde	0.21 abcd
NA 756	33.9 cdefghij	15.6 efg	0.00 a
NA 8	13.8 abcde	4.7 abcde	0.08 a
PA 120 [PER]	9.1 abc	0.0 a	0.13 ab
PA 151 [PER]	46.3 fghijk	0.7 ab	0.00 a
PA 156 [PER]	21.3 abcdefg	1.5 abc	0.00 a
PA 169 [PER]	19.0 abcdef	1.7 abc	0.00 a
PA 195 [PER]	18.3 abcdef	0.7 ab	0.00 a
PA 202 [PER]	17.9 abcdef	1.0 ab	0.91 abcde
PA 218 [PER]	10.5 abcd	12.0 bcdefg	0.00 a
PA 296 [PER]	5.3 a	0.0 a	0.00 a
PA 303 [PER]	10.4 abcd	1.6 abc	0.00 a
PA 34 [PER]	8.3 ab	0.9 ab	0.64 abcd
PA 67 [PER]	10.6 abcd	3.2 abcde	0.00 a
PA 70 [PER]	24.3 bcdefgh	2.8 abcde	1.84 cdef
POUND32/A [POU]	9.3 abc	7.6 abcdefg	0.09 a
SCA 6	16.4 abcde	0.6 ab	0.05 a
SPEC 185/4	6.3 ab	4.5 abcde	0.46 abcd
UF 11	33.5 cdefghij	25.9 fg	7.70 h

Clones followed by the same letter are not significantly different according to the Tukey-Kramer multiple-comparison test at a 95% confidence level.

The Tukey-Kramer multiple-comparison test compares the means of clones and shows groups of clones significantly different from each other, using a confidence level of 95% (Table 4).

Coefficients of Spearman and Pearson for both genotypic and phenotypic correlations are not very high and are significant at a 95% level of probability, except for the genotypic correlations between Black Pod and Witches' Broom on shoots, which are not significant at this level (Table 5). The highest correlation is obtained with the percentage of pods affected by Witches' Broom and the percentage of shoots affected by Witches' Broom, where 34.8 % of the variation in one form of the disease can be explained by variation of the other form at the clone level. At the tree level, this correlation is lower (r^2= 13.8 %).

Table 5. Correlations between variables.

Pearson coefficients	Phenotypic correlation		Genotypic correlation	
	r	*P*	r	*P*
BP/WB Pod	0.34	<0.001	0.34	0.008
WB Pod/WB Shoot	0.37	<0.001	0.59	< 0.001
BP/WB Shoot	0.21	<0.001	0.23	0.073

Spearman coefficients	Phenotypic correlation		Genotypic correlation	
	r	*P*	r	*P*
BP/WB Pod	0.31	<0.001	0.32	0.012
WB Pod/WB Shoot	0.29	<0.001	0.45	<0.001
BP/WB Shoot	0.12	0.045	0.05	0.679

Correlations between Black Pod and Witches' Broom diseases on pods are not very high suggesting that resistance to these diseases may be governed by different genes.
Broad sense heritability has been estimated at 0.50 and 0.68 for Witches' Broom on pods and on shoots, respectively and at 0.64 for Black Pod; at least half of the variation observed between clones can therefore be attributed to a genetic effect.

Among clones showing a percentage of pods infected by Black Pod and Witches' Broom diseases lower than 10%, one accession (MAN 15/60 [BRA]) has a very good level of resistance to the vegetative form of Witches' Broom, but has unfortunately a high Pod Index (36.9) and a low dry cotyledon weight (0.63 ±0.12 g). From the clones in this study, IMC 47 is the only clone presenting good characteristics both in terms of disease resistance and yield potential.