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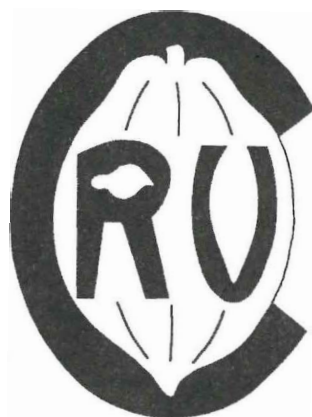
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Cover photograph. Intensively planted cocoa before (back cover) and after (front cover) pruning.

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Progress in germplasm enhancement for Witches' Broom resistance

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Introduction

Germplasm enhancement for resistance to Witches' Broom disease was initiated in July 2004 as an activity in the CFC/ICCO/BI Cocoa Productivity Project. The main objective of this programme is to develop cacao populations with enhanced resistance to Witches' Broom and Black Pod diseases while maintaining a broad genetic base. Crosses were completed over three consecutive years at UCRS, using different sets of parents each year.

Methodology

Design and crosses

Year 1 (May - December 2004) and year 2 (June - December 2005) designs were reported in the CRU Annual Report 2005 (Holder *et al.*, 2006). The year 3 design was similar to that in year 2 with 11 crosses in an incomplete factorial experimental design and seven additional crosses. Furthermore, 15 crosses which either failed in previous years or did not produce sufficient seedlings were repeated.

Screening

Witches' Broom disease

Six to seven-month-old progeny from year 2 crosses aged of 6-7 months together with their parents, represented by seedlings from open-pollinated pods, were screened in four batches (June, July, September and November 2006). The agar droplet technique of inoculation (Surujdeo-Maharaj *et al.*, 2003) was used.

The percentage of plants developing symptoms (incidence) and largest broom-base diameter (BBD) (severity) as well as time to first symptom (TFS) and time to broom initiation (TBI) were observed. In each family, resistant plants (those with low disease incidence and/or low severity) together with a few susceptible ones, were also tested for Black Pod disease resistance.

Black Pod disease

Selected crosses from the first pollination year with the correct interflush-2 stage leaves were screened for resistance to *P. palmivora* using the leaf disc test (Nyassé *et al.*, 1995). The day prior to inoculation, leaves were harvested, 15 mm discs were punched from the leaves and placed in sealed humidified trays. A complete randomised block design was used with three replications (trays) and five discs per tray, *i.e.* three replicates totalling 15 discs per plant. Each experiment was repeated 2 to 3 times (on different dates). A range of control cacao clones was used namely ICS 46, CAS 1, IMC 57 and PA 124 [PER].

Analyses of variance (General Linear Model of SAS, SAS Institute, USA) for effects and interactions between the possible sources of variation were conducted and the degree of susceptibility of controls and plants within each cross was compared with the Newman and Keuls test. Statistically homogeneous groups were identified.

Results and Discussion

Black pod resistance screening of selected year 1 progeny

Table 1a. Leaf disc susceptibility of 4 control clones of cocoa inoculated with *P. palmivora*.

Control clones	Symptom rating ¹	Resistance level ²
ICS 46	4.00 a	S
CAS 1	2.60 b	MR
IMC 57	1.53 c	R
PA 124 [PER]	1.73 c	R

¹Symptom rating was 0 (none) to 5 (full necrosis). Mean symptom ratings followed by the same letter are not significantly different according to the Newman and Keuls test at 5 %.

²Resistance level: S = susceptible; MS = moderately resistant; R = resistant

Table 1b. Number of seedlings from each cross classified according to their level of Black Pod resistance, based on the leaf disc test.

Cross	Code	BP resistance level		
		R	MR	S
ICS 46 × LP 1/45 [POU]	A2	0	3	15
PA 303 [PER] × LP 1/45 [POU]	B1	3	5	3
PA 303 [PER] × SPA 9 [COL]	B2	4	5	4
PA 303 [PER] × CATONGO	B3	5	4	2
JA 3/4 [POU] × SPA 9 [COL]	C1	0	1	0
JA 3/4 [POU] × SLC 4	C2	0	0	4
JA 3/4 [POU] × CATONGO	C3	0	0	1
GU 114/P × SLC 4	D1	1	4	0
GU 114/P × CATONGO	D3	11	3	0
RB 29 [BRA] × CRUZ 7/8	E1	3	2	0
RB 29 [BRA] × PLAYA ALTA 2 [VEN]	E2	1	0	1
RB 29 [BRA] × CATONGO	E3	4	4	2
LP 1/45 [POU] × ICS 46	G1	3	5	3
LP 1/45 [POU] × PA 303 [PER]	G2	6	5	6
LP 1/45 [POU] × CATONGO	G3	0	0	1
SPA 9 [COL] × PA 303 [PER]	H1	2	2	3
SPA 9 [COL] × JA 3/4 [POU]	H2	5	8	4
SPA 9 [COL] × CATONGO	H3	6	5	0
SLC 4 × GU 114/P	I2	0	1	0
CRUZ 7/8 × GU 114/P	J1	6	3	6
CRUZ 7/8 × RB 29 [BRA]	J2	1	0	2
CRUZ 7/8 × CATONGO	J3	4	6	1
PLAYA ALTA 2 [VEN] × RB 29 [BRA]	K1	0	1	0
PLAYA ALTA 2 [VEN] × LCTEEN 90/S-7	K2	1	4	1
PLAYA ALTA 2 [VEN] × CATONGO	K3	1	0	0
IMC 57 × CATONGO	L1	8	2	0

R= resistant; MR = moderately resistant; S = susceptible

Black Pod resistance screening of 20-30% of the progeny most resistant to WB with a few moderately resistant and susceptible plants from year 1 crosses is 95% complete. A total of 208 seedlings from the progenies have been screened and have been categorised into 3 resistance

Table 2a. Percentage of plants showing symptoms, the time of appearance of symptoms and severity of symptoms for year 2 crosses.

Crosses	Code	Total ⁵	Dead Plants (%)	Percentage of plants with ¹			TFS ²		TBI ³		BBD ⁴	
				No symptom	Swelling (no broom)	Broom	N ⁶	Mean (days)	N ⁶	Mean (days)	N ⁶	Mean (mm)
PA 195 [PER] × LP 3/15 [POU]	B1	Failed	-	-	-	-	-	-	-	-	-	-
PA 195 [PER] × (ICS 1 × GU 175/P, tree 28)	B2	29	13.8	0.0	3.5	96.6	29	15.3	28	18.5	28	10.57
CRU 89 × (ICS 1 × GU 175/P, tree 28)	B3	36	19.4	2.8	2.8	94.4	35	14.1	34	19.7	34	9.48
CRU 89 × SJ 1/40 [POU]	B4	81	18.5	3.7	1.2	95.1	78	13.9	77	20.3	77	9.67
AM 2/19 [POU] × SJ 1/40 [POU]	B5	30	23.3	0.0	10.0	90.0	30	14.0	27	19.6	27	8.46
AM 2/19 [POU] × NA 232	B6	119	15.1	5.0	2.5	92.4	113	14.4	110	21.3	110	9.88
MOQ 695 × NA 232	B7	20	10.0	0.0	5.0	95.0	20	14.2	19	18.8	19	11.26
MOQ 695 × (IMC 67 × GU 353/L, tree 64)	B8	38	7.9	0.0	2.6	97.4	38	14.8	37	23.1	37	8.65
B 9/10-25 [POU] × (IMC 67 × GU 353/L, tree 64)	B9	84	7.1	5.6	1.2	92.9	79	13.6	78	18.0	78	8.61
B 9/10-25 [POU] × CL 10/5	B10	98	6.1	4.1	0.0	95.9	94	13.7	94	17.9	94	9.21
LP 3/15 [POU] × CL 10/5	B11	11	9.1	0.0	0.0	100.0	11	13.0	11	17.1	11	11.81
CC 71 × NA 33	A1	35	20.0	0.0	2.9	97.1	35	15.4	34	21.4	34	9.98
PA171 [PER] × TRD 109	A2	147	10.2	6.1	2.0	91.8	138	13.8	135	21.3	135	9.91
PA 126 [PER] × AMAZ 6/3 [CHA]	A3	79	8.9	3.8	1.3	94.9	76	16.0	75	25.0	75	9.68
CRU 80 × MATINA 1/7	A4	60	16.7	0.0	0.0	96.7	58	16.2	58	23.5	58	9.26
MO 9 × PA 150 [PER]	A5	84	4.8	5.9	2.4	91.7	79	13.7	77	18.1	77	9.33
CL 10/15 × (ICS 84 × TSH 1077, tree 49)	A6	92	18.5	1.1	1.1	97.8	91	15.2	90	21.4	90	9.79
IMC 47 × (NA 45 × B 7/21 [POU], tree 83)	A7	106	16.0	1.9	0.9	98.1	105	16.1	104	24.3	104	9.69
NA 399 × (SCA 6 × IMC 67, tree 12)	A8	125	16.8	0.8	4.0	95.2	124	15.2	119	23.1	119	8.91
TRD 32 × NA 471	A9	27	3.7	0.0	0.0	100.0	27	13.1	27	18.6	27	9.46
ICS 35 × SCA 24	A10	Failed	-	-	-	-	-	-	-	-	-	-
TRD 45 × NA 471	A11	68	14.1	10.3	5.9	83.8	61	15.0	57	24.6	57	9.54

¹percentage based on the number of live plants only; ²Time to first symptom; ³Time to broom initiation; ⁴broom-base diameter (mm);

⁵Total number of seedlings inoculated; ⁶Number of seedlings evaluated

Table 2b. Percentage of plants showing symptoms, the time of appearance of symptoms and severity of symptoms for parents (year 2).

Parents	Total ⁵	Dead Plants (%)	Percentage of plants with ¹			TFS ²		TBI ³		BBD ⁴	
			No symptom	Swelling (no broom)	Broom	N ⁶	Mean (days)	N ⁶	Mean (days)	N ⁶	Mean (mm)
AM 2/19 [POU]	8	37.5	12.5	0.0	87.5	7	12.3	7	15.1	7	9.36
AMAZ 6/3 [CHA]	31	12.9	6.5	9.7	83.9	29	14.3	26	22.7	26	7.56
AMEL 2/0-17 [MAY]	5	0.0	0.0	0.0	100.0	5	14.4	5	27.4	5	12.14
B 9/10-25	20	5.0	0.0	0.0	100.0	20	13.5	20	19.8	20	10.46
CC 71	32	21.9	3.1	3.1	93.8	31	14.0	30	18.6	30	10.06
CL 10/5	35	2.9	11.4	0.0	88.6	31	13.8	31	18.6	31	8.76
CRU 80	26	15.4	11.5	3.9	84.6	23	14.3	22	28.5	22	10.74
CRU 89	34	8.8	0.0	0.0	100.0	34	12.4	34	16.3	34	10.28
IMC 47	14	28.6	7.1	0.0	92.9	13	13.5	13	21.6	13	9.75
IMC 57	49	12.2	4.1	6.1	89.8	47	13.7	44	20.8	44	7.46
IMC 6	2	0.0	50.0	0.0	50.0	1	14.0	1	23.0	1	8.60
LP 3/15 [POU]	11	27.3	18.2	0.0	81.8	9	15.6	9	19.7	9	9.16
MATINA 1/7	17	17.7	17.7	0.0	82.4	14	14.4	14	22.6	14	9.59
MO 9	38	13.1	10.5	2.6	86.8	34	14.5	33	20.6	33	7.45
MOQ 6/95	22	13.6	4.6	0.0	95.5	21	13.9	21	19.8	21	10.00
NA 232	31	9.7	3.2	0.0	96.8	30	12.9	30	15.7	30	13.52
NA 399	15	13.3	0.0	0.0	100.0	15	13.5	15	18.7	15	9.73
PA 126 [PER]	31	6.5	3.2	12.9	83.9	30	14.0	26	19.9	26	9.06
PA 150 [PER]	21	9.5	4.8	4.8	90.5	20	16.0	19	24.6	19	8.41
PA 171 [PER]	36	11.1	5.6	8.3	86.1	34	14.4	31	22.9	31	9.56
PA 195 [PER]	10	10.0	10.0	10.0	80.0	9	15.8	8	18.3	8	7.94
SJ 1/40 [POU]	38	7.9	7.9	5.3	86.8	35	12.8	33	19.6	33	8.95
TRD 45	27	25.9	18.5	7.4	74.1	22	19.1	20	25.7	20	8.60
UF 29	56	28.6	3.6	0.0	96.4	54	13.4	54	18.5	54	9.96

¹percentage based on the number of live plant only; ²Time to first symptom; ³Time to broom initiation; ⁴broom-base diameter (mm);

⁵Total number of seedlings inoculated; ⁶Number of seedlings evaluated

level groups (resistant [R], moderately resistant [MR], and susceptible [S]). The data analysis showed a highly significant interaction between symptom ratings and dates ($P < 0.0001$). For each date, a symptom rating average was calculated for each plant using the symptom rating scale developed by Nyassé (Nyassé *et al.*, 1995). To take account of the interaction date \times symptom rating, a Newman and Keuls test was done for each date and homogeneous groups were identified for individual plants including control clones (see controls classification for a given date in Table 1a). The controls grouped into different resistance level classes specifically [S] for ICS 46, [MR] for CAS 1, and [R] for IMC 57 and PA 124 [PER] clones. As the ranking of controls was stable among the dates, it was possible to classify each plant according to its resistance level (Table 1b). A total of 76 seedlings from 19 families were classified as resistant to BP.

From year 1 cross, a total of 57 individuals from 16 families were selected as being resistant to both WB and BP.

Year 2 crosses (2005/2006)

A total of 1,370 individuals in progenies from year 2 crosses and 609 seedlings representing the parental clones were screened for resistance to WB (Tables 2a and 2b). The most promising families for TFS include PA 126 [PER] \times AMAZ 6/3 [CHA], CRU 80 \times MATINA 1/7 and IMC 47 \times (NA 45 \times B 7/21 [POU], tree 83), whereas the most promising families for BBD include AM 2/19 [POU] \times SJ 1/40 [POU], MOQ 695 \times (IMC 67 \times GU 353/L, tree 64) and B 9/10-25 [POU] \times (IMC 67 \times GU 353/L, tree 64) (Table 2a).

In each family, the plants are being classified in 3 resistance level groups (R, MR and S), according to the different parameters recorded. Resistant plants are those that either develop no WB symptoms, have a long TFS or a small BBD.

Year 3 crosses (2006/2007)

In year 3, 15 crosses from the years 1 and 2 that were not successful or had too few individuals in progenies, were repeated successfully in year 3 (Table 3a). An additional 11 crosses from year 3 (factorial mating design with additional crosses) were also successful (Tables 3b and 3c). Five other crosses in year 3 failed due to the lack of synchrony of flowers on male and female trees later in the flowering season.

Seedlings will be screened using the agar droplet inoculation method starting in mid-2007.

Conclusion

From year 1 crosses, 98% of WB resistant seedlings have been screened for BP with the leaf disc test. A total of 76 individuals from 19 crosses were classified as resistant to BP and 57 individuals from 16 crosses were classified as resistant to both WB and BP.

From year 2 crosses, 1,370 individuals and 609 seedlings representing the parental clones have been screened for WB and these will be classified according to their level of resistance. The most WB resistant plants with a few moderate and susceptible plants from year 2 will be evaluated for their level of resistance to *P. palmivora* using the leaf disc test.

Table 3a. Number of beans planted from repeats of crosses from years 1 and 2.

Female	Male	No. of seedlings
CRU 89	(ICS 1 × GU 175/P) tree 28	127
CRUZ 7/8	RB 29 [BRA]	55
GU 114/P	CRUZ 7/8	91
GU 114/P	SLC 4	78
ICS 35	SCA 24	20
IMC 57	CATONGO	156
LCTEEN 90/S-7	CATONGO	26
LCTEEN 90/S-7	PLAYA ALTA 2[VEN]	24
LP 3/15 [POU]	CL 10/5	31
MOQ 6/95	NA 232	89
PA 195 [PER]	(ICS 1 × GU 175/P) tree 28	70
PA 195 [PER]	LP/315 [POU]	82
SLC 4	CATONGO	47
SLC 4	GU 114/P	20
UF 29	CATONGO	57

Table 3b. Number of beans planted or pods harvested from year 3 crosses in an incomplete factorial mating design.

Female/Male	MO 20	NA 702	LP 3/4 [POU]	CRU 90	GU 261/P	IMC 31
EET 399 [ECU]	Failed	Failed				
LV 20		1 pod	2 pods			
CRU 104			Failed	Failed		
B 12/1 [POU]				1 pod	1 pod	
MAN 15/60 [BRA]					2 pods	149 seedlings
MO 20						Failed

Table 3c. Number of beans planted from additional crosses in year 3.

Female	Male	No. of seedlings/pods
MO 9	LCT EEN 46	177 seedlings
JA 5/5 [POU]	CC 41	3 pods
CL 10/3	ICS 35	1 pod
COCA 3370/5 [CHA]	PA 39 [PER]	2 pods
ICS 35	CL 10/3	3 pods

Good success was obtained (early in the flowering season) for repeat crosses from years 1 and 2. Poor synchrony of flowers later in the season was a limitation, so a number of the year 3 crosses need to be repeated.

Selections from the year 1 and year 2 progenies with resistance to both WB and BP will be planted in the field in 2007.

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