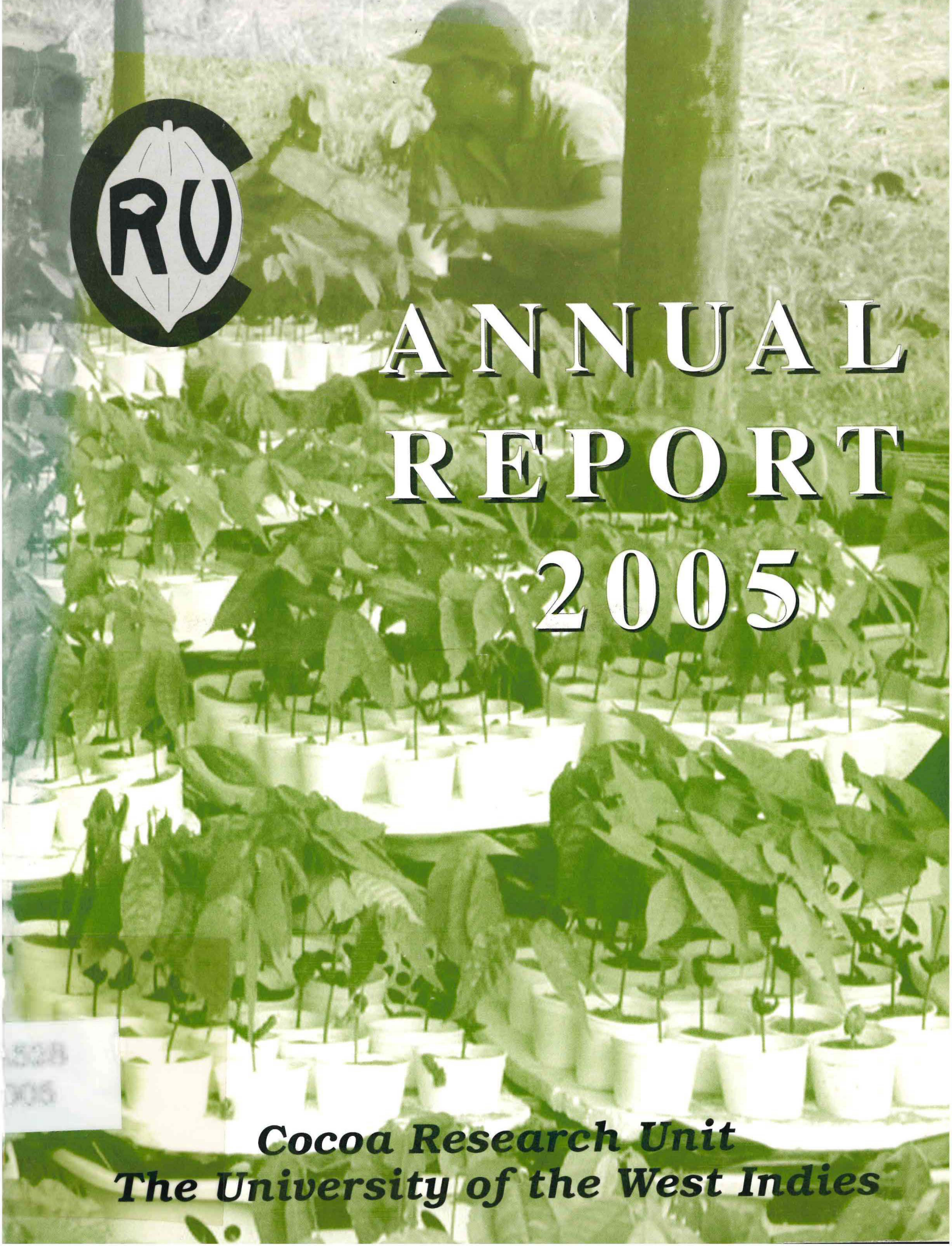

ANNUAL REPORT 2005

Cocoa Research Unit
The University of the West Indies

Annual Report 2005. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies. 83 pp.

The work of CRU is made possible by support from

World Cocoa Foundation, USA

The Biscuit, Cake, Chocolate & Confectionary Association (BCCCA), UK

Cadbury Ltd., UK

United Nations Common Fund for Commodities (CFC)

Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), France

Guittard Chocolate Company, Burlingame, USA

International Cocoa Germplasm Database (ICGD)

Lindt & Sprüngli (International) AG, Switzerland

Ministry of Agriculture, Land and Marine Resources (MALMR), Government of the Republic of Trinidad and Tobago

Masterfoods, UK

United States Department of Agriculture, USA

The University of Hamburg, Germany

The University of Reading, UK

The University of the West Indies (UWI), Trinidad and Tobago and Barbados

Forschungskreis der Ernährungsindustrie e.V.

Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto-von-Guericke" e.V.

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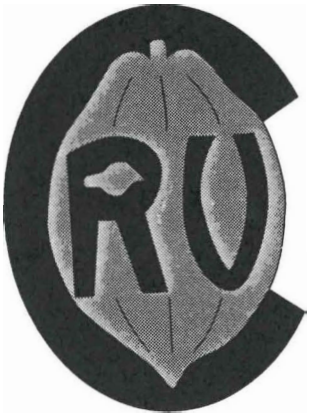
Cover photograph. Cacao seedlings raised in a hydroponic system for micrografting at EEN San Carlos, Ecuador.

Annual Report 2005



Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
2006

Germplasm enhancement for resistance to Witches' Broom disease

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Introduction

Germplasm enhancement for resistance to WB was initiated in July 2004 as an activity in the CFC/ICCO/IPGRI Cocoa Productivity Project. The main objective of this program is to develop cocoa populations with enhanced resistance to WB while maintaining a broad genetic base. Crosses are planned to be completed over three consecutive years at UCRS, using different parents each year.

Methodology

Choice of parents

The choice of parents was based on several criteria. These included level of resistance to WB on shoots estimated from field observations in the ICG,T, results obtained in the nursery when clones were inoculated with a basidiospore suspension and clones with a low percentage of pods affected by WB. A broad genetic base and the chances of favorable alleles being combined was maintained by selecting parents from Forastero, Trinitario and Refractario groups.

Additional traits were considered when selecting parents, including resistance to Black Pod disease, good bean size (dried cotyledon weight ≥1.20g), pod size (>20.0 cm length), bean number (≥ 45) and bean quality (F. Bekele, pers.comm.) when data were available.
Such a choice was partially based on results obtained during the previous CFC/ICCO/IPGRI project *Cocoa germplasm conservation and utilization: a global approach.*

Design and crosses

Year 1 crosses (May – December 2004): twenty-two crosses involving 11 parents (9 resistant and 2 susceptible to WB) were completed. The experimental design was an incomplete diallele Kempthorne and Curnow model (1961) with reciprocal crosses chosen to allow robust statistical analysis to be performed and to show any putative maternal effect. It created a large number of crosses between parents resistant to WB as well as a few control crosses using susceptible parents. In addition, to evaluate and compare the level of resistance of the parental clones, each of them was pollinated with the same highly homozygous clone susceptible to WB (CATONGO). Crosses with few exceptions (GU 114/P female parent located on the UWI campus) were carried out at the ICG,T. Two control clones (IMC 57 and UF 29) were selected to serve as common control clones from one year to another.

Year 2 crosses (June – December 2005): Eleven crosses involving 11 parents resistant to WB were completed using a factorial mating design. In addition, 11 crosses with 21 other parents were carried out without following any specific experimental design.

Screening

Seeds were sown in polystyrene cups filled with a horticultural potting mixture. Seedlings aged 1-2 months were then transferred into garden bags containing top-soil.

The objective was to screen approximately 100 seedlings from each cross. Plants were pruned two weeks before the inoculation to induce bud-break and one shoot per plant was inoculated.

Progeny aged 4-7 months from the year 1 crosses were screened in four batches (March, June and October 2005 and January 2006). We used the agar-droplet technique (Surujdeo- Maharaj *et al.*, 2003), where a drop of inoculum adjusted to 350,000 basidiospores per mL in 0.5% agar was placed on an active axillary bud of each seedling. Plants were incubated at 25-27°C and saturated humidity for 60h, and then moved to the greenhouse area under 70% shade netting.

Variables measured included time to first symptom (TFS), time to broom initiation (TBI), largest broom diameter (BBD), diameter of a healthy shoot (to be used as a co-variable) and percentage of plants infected (incidence). Symptom observation began seven days after inoculation and was done every day for the first month, twice a week for the second month and once a week thereafter. Recording of symptoms was terminated either at the end of the fourth month or when all brooms and swellings had become necrotic.

Results and Discussion

Year 1 crosses (2004/2005)

Although all crosses were attempted, four crosses failed due to various factors including the lack of availability of flowers, bad synchronisation of flowering between female and male parents and losses due to BP.

A total of 2,695 progeny from 2004/2005 crosses were screened for resistance to WB. A proportion of seedlings were lost after inoculation, but 1,930 survived the four-month period of assessment. Most of the plants or the inoculated shoots that died during the experiment belonged to the first inoculation batch (inoculated in March 2005). It is suspected that these plants were not vigorous enough to withstand both pruning and inoculation. A preliminary analysis of variance for TFS, TBI and BBD on surviving plants showed a highly significant family effect for the three variables.

The most promising families for TFS include PA 303 [PER] × SPA 9 [COL], RB 29 [BRA] × CRUZ 7/8 and JA 3/4 [POU] × SPA 9 [COL], whereas the most promising families for BBD include PA 303 [PER] × LP 1/45 [POU], LP 1/45 [POU] × ICS 46 and PLAYA ALTA 2 [VEN] × RB 29 [BRA] (Table 1).

Full analyses of the diallele are underway to estimate genetic parameters and to assess any maternal effect. A selection of plants based either on the absence of WB symptoms, a long incubation time or small BBD will be evaluated for their level of resistance to *Phytophthora* using the leaf disc test (Nyassé *et al.*, 1995) and planted in the field.

Table 1. Percentage of plants showing symptoms, time for the appearance of symptoms and severity of symptoms for year 1 crosses.

Cross	Code	Total [5]	Dead plants (%)	Percentage of plants with [1]			TFS [2]		TBI [3]		BBD [4]	
				no symptoms	swelling (no broom)	broom	n [6]	Mean (days)	n	Mean (days)	n	Mean (mm)
ICS 46 × LCT EEN 90/S-7	A1	Failed	-	-	-	-	-	-	-	-	-	-
ICS 46 × LP 1/45 [POU]	A2	154	19.5	4.8	1.6	93.5	118	13.9	116	19.7	116	9.8
ICS 46 × CATONGO	A3	18	33.3	8.3	0.0	91.7	11	14.4	11	18.7	11	11.4
PA 303 [PER] × LP 1/45 [POU]	B1	53	43.4	6.7	10.0	83.3	28	12.5	25	18.6	25	7.0
PA 303 [PER] × SPA 9 [COL]	B2	89	38.2	10.9	5.5	83.6	49	18.3	46	25.9	46	9.8
PA 303 [PER] × CATONGO	B3	106	17.0	3.4	3.4	93.2	85	16.1	82	22.5	82	9.9
JA 3/4 [POU] × SPA 9 [COL]	C1	20	40.0	16.7	0.0	83.3	10	21.9	10	30.3	10	12.2
JA 3/4 [POU] × SLC 4	C2	76	67.1	8.0	16.0	76.0	23	16.3	19	20.0	19	9.3
JA 3/4 [POU] × CATONGO	C3	108	42.6	6.5	0.0	93.5	58	14.3	58	17.4	58	10.1
GU 114/P × SLC 4	D1	76	21.1	1.7	1.7	96.7	59	14.3	58	18.6	58	11.1
GU 114/P × CRUZ 7/8	D2	40	0.0	17.5	2.5	80.0	33	13.7	32	18.1	32	11.2
GU 114/P × CATONGO	D3	96	4.2	2.2	0.0	97.8	90	14.7	90	18.2	90	9.7
RB 29 [BRA] × CRUZ 7/8	E1	94	31.9	7.8	3.1	89.1	59	18.8	57	27.4	57	10.4
RB 29 [BRA] × PLAYAALTA 2 [VEN]	E2	67	37.3	14.3	0.0	85.7	36	15.1	36	18.6	36	11.3
RB 29 [BRA] × CATONGO	E3	211	18.5	4.1	1.2	94.8	165	15.3	163	20.0	163	10.4
LCTEEN 90/S-7 × ICS 46	F1	24	50.0	0.0	8.3	91.7	12	12.3	11	16.4	11	12.4
LCTEEN 90/S-7 × PLAYAALTA 2 [VEN]	F2	17	0.0	11.8	0.0	88.2	15	13.3	15	16.1	15	11.3
LCTEEN 90/S-7 × CATONGO	F3	Failed	-	-	-	-	-	-	-	-	-	-
LP 1/45 [POU] × ICS 46	G1	153	12.4	5.2	4.5	90.3	127	12.9	121	18.0	121	7.7
LP 1/45 [POU] × PA 303 [PER]	G2	140	17.9	10.4	0.9	88.7	103	13.9	102	20.5	102	8.4
LP 1/45 [POU] × CATONGO	G3	106	38.7	6.1	1.5	92.3	61	14.8	60	20.8	60	9.1
SPA 9 [COL] × PA 303 [PER]	H1	121	29.7	1.2	3.5	95.3	84	14.6	81	18.1	81	8.5
SPA 9 [COL] × JA 3/4 [POU]	H2	263	40.3	5.7	1.3	93.0	148	15.7	146	20.9	146	9.7
SPA 9 [COL] × CATONGO	H3	117	39.3	4.2	1.4	94.4	68	17.3	67	23.1	67	10.1
SLC 4 × JA 3/4 [POU]	I1	61	27.9	2.3	2.3	95.5	43	14.9	42	19.3	42	12.9
SLC 4 × GU 114/P	I2	37	37.8	13.0	0.0	87.0	20	15.6	20	20.3	20	11.2
SLC 4 × CATONGO	I3	Failed	-	-	-	-	-	-	-	-	-	-
CRUZ 7/8 × GU 114/P	J1	167	18.0	7.3	2.2	90.5	127	15.4	124	21.0	124	8.3
CRUZ 7/8 × RB 29 [BRA]	J2	29	51.7	7.1	7.1	85.7	13	16.8	12	22.5	12	8.3
CRUZ 7/8 × CATONGO	J3	112	33.0	2.7	2.7	94.7	73	15.7	71	20.6	71	9.9
PLAYAALTA 2 [VEN] × RB 29 [BRA]	K1	14	7.1	7.7	7.7	84.6	12	15.2	11	19.8	11	8.0
PLAYA ALTA 2 [VEN] × LCT EEN 90/S-7	K2	Failed	-	-	-	-	-	-	-	-	-	-
PLAYA ALTA 2 [VEN] × CATONGO	K3	53	22.6	19.5	0.0	80.5	33	13.4	33	17.3	33	10.4
IMC 57 × CATONGO	L1	73	32.9	12.2	4.1	83.7	43	14.9	41	19.5	41	9.7

[1] percentage based on the number of alive plants only
[2] Time to first symptom
[3] Time to broom initiation
[4] broom-base diameter
[5] Total number of seedlings inoculated
[6] Number of seedlings

Year 2 crosses (2005/2006)

In year 2, 20 successful crosses out of 22 were completed (Tables 2 and 3) and beans from 90 pods have been planted. The bean number has been unexpectedly low (small pods), and it is hypothesised that the high concentration and frequent application of Kocide could have led to this phenomenon. Open-pollinated pods from the parents and the controls IMC 57 and UF 29 have been collected as well. Seedlings will be screened using agar-droplet inoculation starting in mid-2006.

Table 2. Number of beans planted from year 2 crosses – factorial mating design.

		Male					
		LP 3/15 [POU]	ICS 1 × GU 175/P (T28)	SJ 1/40 [POU]	NA 232	IMC 67 × GU 353/L (T64)	CL 10/5
Female	PA 195 [PER]	0	36				
	CRU 89		42	94			
	AM 2/19 [POU]			32	142		
	MOQ 6/95				21	63	
	B 9/10-25 [POU]					111	101
	LP 3/15 [POU]						11

Table 3. Number of beans planted from year 2 crosses – additional crosses.

Female	Male	No. of progeny
CC 71	NA 33	69
PA 171 [PER]	TRD 109	169
PA 126 [PER]	AMAZ 6/3 [CHA]	125
CRU 80	MATINA 1/7	96
MO 9	PA 150 [PER]	89
CL 10/15	ICS 84 × TSH 1077 (T49)	135
IMC 47	NA 45 × B 7/21 [POU] (T83)	134
NA 399	SCA 6 × IMC 67 (T12)	232
TRD 45	NA 471	76
ICS 35	SCA 24	0
TRD 32	NA 471	48

Conclusion

In conclusion, the majority of crosses were successful and those that were unsuccessful and those with less than 30 seedlings will be repeated in year 3 if flowers are available. In addition, a new set of crosses for year 3 will be completed.

The experimental design (incomplete diallele Kempthorne and Curnow, 1961) used in year 1 allows statistical analysis which aims to provide information on heritability and maternal effects, while the factorial mating design with additional bi-parental crosses in year 2 will produce a larger number of progeny from more parents. The use of these different experimental designs therefore will provide us not only with populations with enhanced resistance to WB and BP, but will also provide us with a greater understanding of the genetics of resistance to WB.

References

Kempthorne, O. and Curnow, R. N. (1961) The partial diallele cross. *Biometrics* **17**: 229 -250.

Nyassé, S., Herail, C. and Blaha, G. (1995) Leaf inoculation as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytopthora* black pod disease. *Crop Protection* **14 (8)**: 657-663.

Surujdeo- Maharaj, S., Umaharan, P., Butler, D. R. and Sreenivasan, T. N. (2003) An optimized screening method for identifying levels of resistance to *Crinipellis perniciosa* in cocoa (*Theobroma cacao*). *Plant Pathology* **52**: 464-475.