ANNUAL REPORT 2004

Cocoa Research Unit
The University of the West Indies

Annual Report 2004. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies. 71 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

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Cover photograph. Newly established introductions planted at the University of the West Indies, Campus 8 field.

Annual Report 2004

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2005

Progress in resolving identity issues of the cacao resources held in Trinidad

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Introduction

Conservation of genetic resources has become a priority for the world cocoa community. However, the multiplicity of sources of material, of locations of collections and the wide dispersal of end users has resulted in misinterpretation of identities in many instances. Recent molecular techniques for identification of cocoa clones cannot be efficient if collaborative work between research centres is not implemented. The ICG,T is a living reference for verification of material now planted all over the world; nevertheless, to be useful, information collected and published must be precise and correct. Collaborative projects and common strategies could help the efficiency of the work being carried out.

An international collaborative project on DNA fingerprinting of cacao germplasm was started in 2001 with an aim to obtain a fingerprint of each living accession in the Americas. DNA extracted from the most original tree of each accession present in Trinidad has been shipped to the USDA Beltsville Laboratory for analysis by microsatellite markers. Duplicate trees of the UCRS fields have also been tested for mislabelling. Each branch from where sample leaves were taken, was clearly identified (tagged) with a specific blue label, and an FP# (fingerprinting number) was dedicated to each sample. Collections of extra leaf samples have been also undertaken for comparison to existing data.

Priority is given to the analysis of Upper Amazon material such as the "Parinaris" (PA) since these are of particular interest to the international cocoa community.

The PA accession group

It has been reported that the PA accession group originated from 20 pods collected by Pound in 1938 and that 277 PA accessions were planted in Marper Farm from 1939 to 1941 (Pound, 1943). Records available in CRU show that in 1943, 133 PA accessions were recorded in Block D and 11 in Block C, making a total of 144 established clones.

Currently, 92 trees labelled PA are still alive in Block D, including 2 adjacent PA 13 [PER] trees and 2 PA 187 [PER] clones in positions 737 and 482 respectively.

Nine accessions are still alive in Marper Block C and among them PA 293 [PER] which is also present in Marper Block D.

A total of 101 samples were collected including 98 different original trees and 3 replicated trees (PA 13 [PER], PA 187 [PER] and PA 293 [PER]).

Sixteen samples of trees now absent from Marper were collected from the UCRS field as well as 41 replicated samples to check their conformity.

Results

Original trees in Marper fields

The molecular profile from 15 microsatellite primers of the 2 adjacent trees PA 13 [PER] planted in Marper D158 and D159 are identical, showing that they are replicates. The same was found for both examples of PA 293 [PER], one planted in Marper C817 and the other in D762. However, analysis of the data concerning PA 187 [PER] (Marper D737, D482) shows that the trees do not share the same profile (13/15 SSR, D. Zhang, personal communication). Furthermore, leaf samples provided to USDA Miami (J.C. Motamayor, personal communication) shows that the tree in D737 is identical to the tree PA 189 [PER] in position D489. As an interesting footnote, a question mark had been inserted in the notes for this tree dating from 1943.

The DNA profile of PA 205 [PER]suggests that it belongs to the Trinitario group, and it is possible that the leaf sample was obtained from the surviving rootstock.

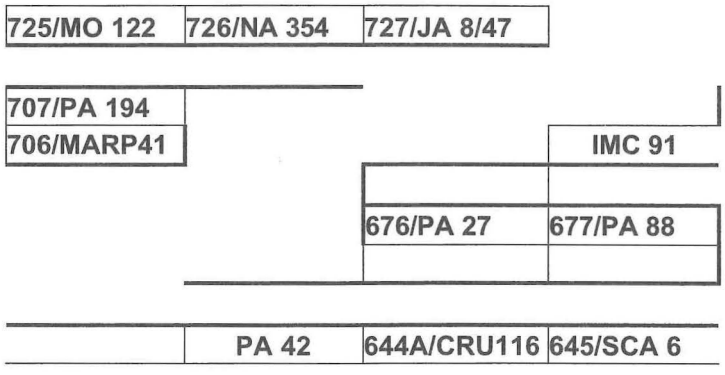
Though no other irregularity has been shown with microsatellite markers among the PA accession group planted in Marper Farm, controversial results could occur if DNA were to be extracted from samples collected at other times to the definitive reference samples sent to Beltsville.

Duplicated trees in UCRS fields

PA 27 [PER], PA 88 [PER] and PA 194[PER]

PA 27 [PER], PA 88 [PER] and PA 194 [PER] were planted contiguously in Marper Block D (Figure 1). PA 194 [PER] has since died. The results of DNA analysis have confirmed that confusion must have occurred during the propagation of these clones when the UCRS fields were established. PA 27 [PER] planted in 5B originates from PA 88 [PER] (Marper D677), while PA 194 [PER] planted in 5B was propagated from PA 27 [PER] (Marper D676). It is interesting to note that the profile of PA 88 [PER] in the ICGD database for RUQ 34 is different from all the above. This clone was transferred to Reading quarantine from the Royal Botanical Gardens, Kew.

Figure 1. Excerpt of the Block D field map in Marper Farm (trees are shown by position number/clone name).



Mislabelling of other accessions

Many different cases of mislabelling can occur, especially when records are not available, labels are misplaced and maps are wrongly interpreted.

Suspected mislabelling in Marper Farm

The preliminary results of the USDA/BCCCA/CRU Fingerprinting Project showing accessions with identical profiles suggested that mislabelling of some trees probably occurred at the time of planting in Marper Farm. That may be the case for a few neighbouring clones, however results have to be carefully interpreted as sampling leaves for DNA extraction could also be a reason for errors (Table 1).

Table 1. Neighbouring trees in Marper Farm sharing the same DNA fingerprinting profile. For each row of the table, accession I was found to be identical to accession II.

Accession I			Accession II		
Clone name	**Fingerprinting number**	**Marper location**	**Clone name**	**Fingerprinting number**	**Marper location**
AM 1/19 [POU]	FP#2145	C923	AM 2/92 [POU]	FP#1606	C924
AM 2/17 [POU]	FP#2022	C440	CLM 65	FP#2302	C438
AM 2/3 [POU]	FP#1439	C105	MOQ 6/46	FP#1296	C106
AM 2/68 [POU]	FP#1275	C258	CL 78/9	FP#2007	C297
AM 2/70 [POU]	FP#1338	C236	LX 20	FP#695	C192
B 8/8 [POU]	FP#1602	C1068	LX 41	FP#2089	C1088
LP 1/56 [POU]	FP#2156	C916	SJ 1/33 [POU]	FP#2582	C917

Suspected mislabelling in UCRS fields

As all the material planted in the UCRS fields is a multiple replication of an original tree, other mislabelling could have occurred during propagation and establishment of these clones.

The accession names used for some plots in the UCRS fields are shown to be invalid when the DNA fingerprinting profile of the tree (accession I) matches that of another accession (accession II), and the original mother tree of "accession I" had either never been recorded in Trinidad, or had died long before the establishment of the UCRS plots (Table 2). In these cases, the mistake must have been made in writing the wrong accession name.

Identity verification of individual trees within plots of UCRS is being confirmed by comparing the SSR marker profiles with the reference tree where possible, or by comparing individual trees within the same plot.

Table 2. Mislabelled plots in the University Cocoa Research Station, Centeno.

Accession I				Accession II			Original tree		
Clone name	Finger-printing number	Location in UCRS		Clone name	Finger-printing number	Position in Marper	Clone name	Position in Marper	Status
		Field	Plot						
AM 1/29 [POU]	FP#1716	5B	I804	AM 1/10 [POU]	FP#1969	C360	AM 1/29 [POU]	C577	Dead
B 22/7 [POU]	FP#1257	5B	A32	B 22/17 [POU]	FP#52	D127	B 22/7 [POU]	D162	Dead
JA 3/39 [POU]	FP#1702	5B	F512	JA 3/37 [POU]	FP#2081	C1120	JA 3/39 [POU]	C1123	Dead
JA 5/19 [POU]	FP#1712	5B	F427	JA 5/18 [POU]	FP#2032	C420	JA 5/19 [POU]	C804	Dead
LP 4/45 [POU]	FP#1673	4A	E473	LP 4/41 [POU]	FP#2172	C965	LP 4/45 [POU]	C879	Dead
LX 1[a]	FP#1682	4A	D335	MOQ 6/5	FP#1294	C92	LX 1	C91	Dead
MOQ 2/17	FP#1570	5B	C197	MOQ 4/17	FP#1804	C984	MOQ 2/17	C865 and C866	Dead
AM 2/60 [POU]	FP#1559	5B	I760	AM 1/60 [POU]	FP#1313	C179	AM 2/60 [POU]	Was never in Marper	
B 5/5 [POU]	FP#1942	5A	B99	B 5/3 [POU]	FP#1599	C1023	B 5/5 [POU]	Was never in Marper	
B 2/34 [POU]	FP#1569	5B	A45	B 23/4 [POU]	FP#1489	D383	B 2/34 [POU]	Was never in Marper	
IMC 81	FP#1635	6B	F421	MO 81[b]	FP#1188	D192	IMC 81	Shouldn't exist	
NA 33[c]	FP#1669	4A	D371	NA 833	FP# 297	D640	NA 33	Was never in Marper	

[a]LX 1 scions were taken from the neighbouring tree in Marper C92.
[b]The accession MO 87 present in 4A, recently added to the CRU database, is also a replicate of MO 81.
[c]The accession NA 33 planted in field 4A, selected as a priority clone in the CFC/ICCO/IPGRI Germplasm Utilisation Project is identical to NA 833. However the SSR profile does not match the ICGD NA 33 (RUQ 822), provided by CIRAD. There is no record of NA 33 ever having been planted at Marper Farm.

Conclusion

Analysis of SSR profiles of the PA accession group showed that there are very few mistakes with the identity of original trees. More results regarding upper Amazon material will add to our knowledge of the genetic diversity of this group. International exchange of data has greatly improved the effectiveness of resolving identity ambiguities.

References

Pound, F.J. (1943) First Report on the selection of cacao trees for resistance to Witches' Broom disease. Unpublished report, Ministry of Agriculture, Trinidad.