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

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## **The collaborative CRU/USDA cocoa DNA fingerprinting project: progress report**

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### **Introduction**

CRU is a key partner in the USDA cocoa fingerprinting project to confirm the identity of each *Theobroma cacao* L. accession in the Americas. Approximately 2,300 of the estimated 8,000-10,000 accessions held in the diverse collections on the continent are present in the ICG,T. CRU has agreed to provide a DNA sample of the most original tree of each accession in Trinidad to the USDA Beltsville laboratory to be analysed by 15 SSR primers.

The original trees of many accessions in the ICG,T were planted in Marper Farm in the 1940s as budded plants. Since then, a large number of trees have died and for many others chupons have grown from the rootstock into mature trunks. In some cases, re-growths from fallen trees have resulted in upright stems in different locations to the original tree. Some labels have been lost and some may have been replaced on stems without certainty.

The ICS population was planted in the Cheesman Field at San Juan Estate in the 1950s. Five replicates of each of the 100 selected accessions were established in blocks. Since then, missing trees have been replaced by supply trees without any identification (since the field is part of a commercial estate). However, about 60% of the accessions are still present in at least one block.

Other accessions in the ICG,T at UCRS, were propagated from trees established in the UWI campus fields. Although the original trees were individually labelled, there is a continuous need for updating the maps and verifying the identity of every tree.

In addition, there is a continuous enrichment of the ICG,T when accessions are introduced from other countries through the BCQS.

### **Tasks and actions**

Accuracy of the work is key to the success of the project, and every step of the process has been followed with the greatest care.

#### Identification of the trees to be sampled

Field maps for all locations (Marper Farm, Cheesman Field, UCRS and UWI Campus) have been updated since 1998, and an individual tree label has been attached to each tree, with a unique identification/position number.

Cross-checking of pod and leaf morphology with that of trees from the oldest original lists has been very useful to eliminate wrong interpretation of identity.

#### Collection of samples

Once the identity of the accession had been ascertained by morphological observations, one or two healthy mature leaves were collected and the DNA was extracted. A distinctive blue embossed label, indicating the accession name, the position and the tree number where

appropriate, was secured on the branch from where the leaf sample had been procured; when available, immature and mature pods were collected from the same stem, for digital photographing at CRU.

#### DNA extraction

The D<sup>2</sup> Biotechnologies DNA extraction method, recommended by the USDA Beltsville laboratory, or the Kobayashi DNA extraction method (Kobayashi *et al.*, 1998) was used as standard routine.

### **Accomplishments**

#### Field maps and tree identity

Approximately 2,300 cacao accessions are present in the various fields of the ICG,T. Normally their clone name has been used which relates to their origin or the name of their discoverer; however, when the genebank was established at UCRS in the 1980s, 155 of the accessions in Marper had lost their identity, and CRU codes from CRU 1 to CRU 155 were assigned to them. In the most recent revision of the Marper maps, 55 other accessions could not be positively identified. These accessions were designated with a MARPER name, from MARPER 1 to MARPER 55. The most recent checks have shown that 1,193 different accessions are still alive in Marper Farm (out of 2,700 originally planted).

The latest verifications using pod or flower characterisations in the Cheesman Field in San Juan Estate and in UCRS have shown that verifying the maps and checking the conformity of the trees individually are two crucial elements in the maintenance of up-to-date records.

#### Collection of leaf samples

Young or mature healthy leaves were collected and packaged in individually labelled brown paper bags. As recommended by USDA, marking of the branch from where the sample has been taken, ensures the accuracy of sampling.

#### DNA extraction

Extraction was carried out as soon as possible to prevent leaf damage and fungal infections. The quality of the DNA extracts was checked on a regular basis; agarose gels were run and 1:125 dilutions were amplified very successfully with SSR primers.

Aliquots of 50 µL were prepared for sending to the USDA Beltsville laboratory, and duplicates are being stored at CRU for domestic studies.

#### Pod photographs

When available, pods were collected for photographing. Two pods, one fully-grown but immature and one ripe, were taken from the same stem from which the leaf sample had been collected. They were carefully wrapped in plastic bubble bags to prevent bruising and were brought to CRU for digital imaging.



**Table 1. Number of leaf samples, DNA samples and pod photographs obtained from various locations in Trinidad.**

Location	Field	Leaf samples	DNA samples	Pod photographs
Marper farm	Block C	714	503	251
Marper farm	Block D	533	476	257
San Juan Estate	Cheesman Field	61	61	58
UCRS	4A	293	291	39
UCRS	5A	115	108	29
UCRS	5B	238	238	12
UCRS	6A	20	20	12
UCRS	6B	261	261	93
UWI	Campus fields	315	206	39
UWI	Greenhouses	36	36	0
UWI	BCQS	133	133	0
<b>Total</b>		<b>2,658</b>	<b>2,333</b>	<b>790</b>

## Conclusion

Leaf samples from practically every different identified accession have been taken and DNA extraction has been completed from 88% of these. A total of 2,135 samples, in two shipments have been sent to the USDA Beltsville laboratory, and these are currently being processed for molecular analysis. The fingerprinting project is proving to be of a great value to CRU. Already, inventories have been revised and the collections will be characterised. When further results become available, it will be possible to prepare/compile a comprehensive database of the cacao plant material held in different locations around the world.

## Reference

Kobayashi, N., Horikoshi, T., Katsuyama, H., Handa, T., and Takayanagi, K. (1998) A simple and efficient DNA extraction method for plants, especially woody plants. *Plant Tissue Culture and Biotechnology* 4(2): 76-80.