













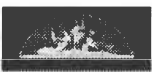





# ANNUAL REPORT 2005

***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.

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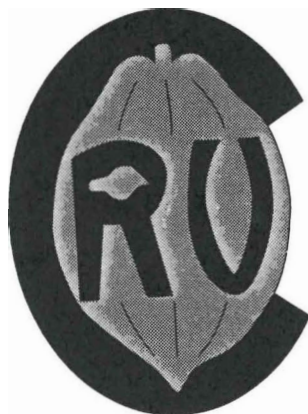
	World Cocoa Foundation, USA		Ministry of Agriculture, Land and Marine Resources (MALMR), Government of the Republic of Trinidad and Tobago
	The Biscuit, Cake, Chocolate & Confectionary Association (BCCCA), UK		Masterfoods, UK
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**Cover photograph.** 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**

## Development of a cacao clones manual

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### Introduction

The aim of the cacao clones manual project is to produce a guide to all of the accessions in the International Cocoa Genebank, Trinidad (ICG,T) with photographs of fruit (pods) and descriptor data. Supplementary information such as DNA marker profiles and historical information will be included based on availability.

Data from ongoing morphological and molecular studies will be combined to produce a cohesive, coherent source of information on clones that can be used to quickly identify any particular accession. Characteristics that can be used to distinguish individual cacao clones are morphological descriptors such as pod colour, pod size, pod yield or number of beans per pod, and molecular profiles such as SSR markers.

Users of cacao material may not know what pods of a particular clone should look like. Pod appearance is diverse and varies significantly among clones. It is difficult to identify a clone just by looking at a picture of its fruit, and descriptor information may not be readily available. There are few comprehensive hardcopy/softcopy resources on cacao clones and one may have to search numerous sources to find the information required to identify a clone or to describe it from just an accession name.

Resources with information on cacao clones include databases such as the ICGD produced by the University of Reading (Wadsworth and Harwood, 2000) and Pound's notes (1934, 1935 and 1936). The ICGD contains information on all known clones throughout the world and comes in two versions. The ICGD CD-ROM is the most complete version and has many photographs but not for all clones. The online version is still being updated. Pound's notes are helpful but they do not describe all the accessions or provide pictures.

There is no manual with comprehensive information on accessions that covers the entire collection of the ICG,T although there is a new guide for the ICS recently published by Johnson *et al.* (2004)

The project described here aims to produce a manual of all the cacao accessions of the ICG,T. The focal point will be pod photographs (of fully grown ripe and unripe pods) for each accession. The photographs will provide a quick and easy reference for persons wishing to identify a specific clone, and a selection of basic morphological descriptors will aid identification. If available, Simple Sequence Repeat (SSR) fingerprint with SSR marker details will enhance the utility and broaden the target audience of the manual.

### The manual

#### Project Progress

The manual was first envisaged as a single publication, however progress to date suggests this will result in a long delay in production. The first CD-ROM edition of the Cacao Clones Manual will therefore be launched with approximately 400 accessions in order that the vital information already collected and compiled can be made available without further delay. The first version is

in the final stages of editing for publication.

#### *Identity verification and other delays*

Possible mislabelling/identity issues in the ICG,T, pose a serious challenge to the completion of the manual. True identity is crucial to the manual's purpose as an authentic reference and the project team is dedicated in their resolve to safeguard the credibility of the manual. This has resulted in longer delays than anticipated in completing basic data for all the accessions of the ICG,T. Pod availability has caused delays in identity verification for some accessions and this in turn slows both image processing and the completion of descriptor data records. In addition, a number of major changes in the processing of images and data were decided on since the inception of the project, which has impacted on the project completion time.

The format used to present descriptor data was changed to reflect more standardised botanical nomenclature. The decision was made to change the image format from Joint Photographic Experts Group (JPEG/JPG) to tagged image file format (TIFF) because it provides the most economical file size without sacrificing the quality of the final printed image. However TIFF images sometimes require an extra step to process because of frequent parsing errors (which appear to be hardware-related) and are therefore more time-consuming to produce than JPG images. The use of TIFF images became the cause of another change in the manual because some web browsers will not display these images, while others may selectively display them. Therefore, an add-in (active-x control or a plug-in) will be required on some computers. The add-in chosen to solve this issue is AlternaTIFF because it is freely available (user registration required) for use and distribution. The manual had to be reformatted, however, to include coding to allow the AlternaTIFF software (Medical Informatics Engineering, 2005) to be used with any AlternaTIFF-enabled web browser.

The content of the manual has been updated and will now include a glossary of terms mainly relating to the descriptor data. This was considered a necessity for users who may be unfamiliar with the terminology.

#### Target Audience

The manual is intended to be a helpful reference with basic information on each accession of the ICG,T for researchers, collectors, breeders and any other users of these clones. The pod photographs will allow immediate visual verification of users' samples and will be a valuable aid to resolve problems with unidentified or mislabelled trees. The descriptor data provided will serve to guide users' expectations on the features of individual clones and the SSR data will permit positive identification in the case of ambiguity.

#### Contents

The basic content of an entry for each accession will consist of the photographs of fully grown ripe and unripe pods and qualitative descriptor data. Supplementary information to be included if available will be quantitative descriptor data, historical information relating to origin of collection and genetic molecular data. The possibilities for inclusion of molecular data include



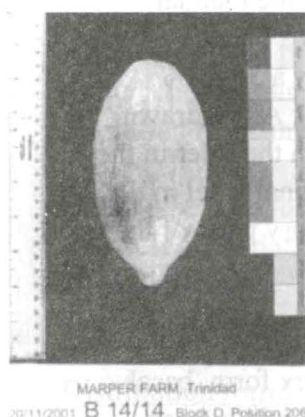
SSR marker information such as SSR-PCR<sup>1</sup> product information and SSR primer details. AlternATIFF software (with instructions for installation) will be included on the CD for computers that require it.

### Preparation of content

#### *Photographs*

Photographs for the manual were obtained either from digital images of freshly picked pods (using the CRU Nikon 2.1MP<sup>2</sup> camera or Elizabeth Johnson's personal digital camera), or from scans of pod photographs provided by F.L. Bekele taken with a conventional camera.

**Figure 1. Scan of photograph  
courtesy F.L. Bekele.**



**Figure 2. Digital photograph of a freshly  
picked pod by L.A. Motilal.**



Pods were collected when available from trees in the field that:

- a) Were in the appropriate position where the field map matched the field conditions
- b) Had a blue label (used to indicate the branch for DNA sampling, usually on the correct trunk, but not always)
- c) Had no blue label if they appeared to be the original trunks
- d) Were not on the map, but present in the field
- e) Presented multiple trunks (sometimes but not always the original trunk could be easily distinguished)

Collection was made to cover all living trees of an accession in the field. If trees had multiple trunks, then pods were collected from several if not all possible trunks, and the descriptor section would make the decision on which was the correct pod form and hence the correct trunk of that accession. This is particularly important in cases where the rootstock overtakes the scion (especially since some rootstocks in Marper Farm can bear green pods instead of only red pods as was previously thought (M. Boccara, pers comm.).

There was no linearity in deciding how to collect but rather that collection be made from fruit-bearing trees from as many accessions as possible in the time available, with multiple

<sup>1</sup> PCR - polymerase chain reaction

<sup>2</sup> Mega pixel

collections being from accessions with more than one trunk.

Repeated collections were sometimes necessary (same accession collected on different trips) either because of concerns about the state of fruit (not photogenic; distorted or diseased) or because of identity issues which arose after transfer of the pods from the field to the laboratory. On a few occasions, duplicate collections were made in error. An accession then could have more than one photo but this gave us the opportunity to select the best possible photo, and the descriptor section the chance to make a more general description instead of focusing on one pod. This also allowed us to decide which pod was more typical of the accession. Since it is not possible to do this in the field, photographing several pods allowed us to choose the best examples.

Paper labels were printed to be included with each pod when the photographs were taken. Pods were photographed on the same day as harvesting or after no more than two days. The digital pictures were then downloaded from the camera card and processed for the manual.

To prepare the photograph for the manual, the pod image was inserted into a blank Microsoft® PowerPoint slide and reformatted to fit the size of the slide usually a 36-40% reduction (necessary because the photographs were much larger than the slide). A pre-drawn scale (cm) was then pasted into the same slide and stretched or shrunk to match the ruler in the pod photograph. A text box was added to correspond with and replace the original label in the image. Finally, the file was cropped to remove the old rule and other unnecessary parts of the original image and then saved as a TIFF image.

### *Descriptor Data*

Descriptors to be included in the manual are qualitative: pod colour, shape, apex form, basal constriction, surface texture, ridge disposition and quantitative: pod length and width, bean number, cotyledon length, width and weight and pod index. Data were averaged from 10 pods for these descriptors. Descriptors were recorded on the day of collection or the day after. The established protocol of averaging quantitative descriptor data over 10 pods imposed a long processing time for quantitative data due to the low availability of pods in many cases.

### Format

Several different options were considered before selecting the document format for the manual (Table 1). One possibility was a database, which has the advantage of allowing one to store pod photos in a separate directory instead of in the main file thus reducing the potential size of that file and reducing the time for the file to load on slow computers. This advantage would be lost if a Microsoft® Word or PowerPoint document was used.

**Table 1. Comparison of possible document formats for cacao clones manual**

HTML (Hypertext Markup Language)	Database	Word, Powerpoint
Simpler to produce	Microsoft® Access is complicated	Layout options too simplistic
Almost universally portable	Not universally portable	Not universally portable
File size small	File size large (even with separate image files)	File size large
User-friendly	Less user-friendly	User-friendly

The hypertext markup language (HTML) option offers reduced file size compared to databases and other formats, although the changing dynamics of HTML and the limitations of different browsers to display this format can impose restrictions. Ultimately the decision was made to use HTML despite these potential problems since it is the most universally acceptable and easily accessible format, making the manual a readily portable document.

The manual is made up of a style-sheet formatted HTML 2-frame frameset web page consisting of two horizontal subspaces or frames. The top frame contains a 'drop-down' menu from which accessions will be selected and the second frame contains the area in which information (pod photographs, descriptor data and other information) will load when an accession is selected for viewing by clicking on the accession name and the "Submit/View" button.

#### *Image format*

After comparing print-outs of JPG and TIFF images created from the same PowerPoint slides the format chosen was TIFF because it is the most economical format for file size that has a high resolution to ensure that printed images are the highest possible quality.

#### *Descriptor data format*

The descriptor data will be presented in a simple paragraph and table style with tabs to align quantitative data. 'PRE' tags<sup>1</sup> will be used with a style sheet formatting to format the descriptor data content for consistency in the fonts used throughout the manual.

#### Distribution format

The manual will be published on CD-ROMs (for Windows®) that will auto-run upon insertion into any CD-ROM drive (The Software Patch, 2006). The advantages of this distribution format include the following:

- 650-680MB of data on one disc
- Easily portable
- Low production cost

#### **Project Update**

Since the initiation of the project in 2002, more than 1,679 pod images have been processed, selected from over 2,000 original/raw photographs. More than 990 accessions have been described. There are 411 completed pages each containing two images per accession (one ripe pod and one unripe pod), qualitative descriptor information and some quantitative descriptors.

There are approximately 500 accessions for which data are partially complete; missing either one pod image or descriptor data or for which the identity of the representative pod must be verified. Final edits/proofing in progress are mainly related to descriptor text display, headings and editing code for web browser support. The name selected for the first edition will infer that one should anticipate future versions e.g. Cacao Clones Manual Version 1.1 or a variant of this.

The first version will consist of data for 411 accessions of the ICG,T:

- 822 TIFF images

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<sup>1</sup> Pre-formatted tags



- Qualitative descriptors for all 411 accessions
- Additional: quantitative descriptors for some accessions
- 'AlternaTIFF' software
- Glossary of terms

The title text for the front page of the first version will read: "CCM Version 1.1: A CD-ROM publication of photographs and descriptor data for selected accessions of the International Cocoa Genebank, Trinidad (ICG,T)".

## Conclusion

The cacao clones manual project has proven to be a useful and worthwhile exercise even while it is still in progress. It is the only venture of this nature to combine photographs and descriptor data for the entire germplasm collection of the ICG,T and will therefore be a very important original resource for vital information on these accessions. The need to identify an accurate representative pod for each particular accession has revealed some identity issues in CRU's fields, which may not have been discovered for some time even with ongoing verification work because priority trees for this work are those being used in other research projects.

Progress with the manual is mainly limited by pod availability. However, the decision to publish the manual in a series of versions will ensure that the valuable resource of information already assembled for various accessions of the ICG,T can be made available without unnecessary delay. In the future, the manual will provide both phenotypic and molecular characterisation data within easy reach and will be an important tool not only to alleviate mislabelling problems, but to serve as a handy desktop reference to the accessions of the ICG,T.

## Acknowledgements

The project was suggested by Mr. Tony Lass and is funded jointly by the BCCCA and Cadbury Sweppes plc.

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