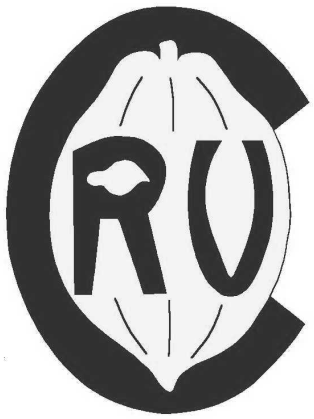
Annual Report 2005



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

CONTENTS

Introduction

Research on cacao at the Cocoa Research Unit (CRU) continues to be centred on the valuable germplasm resources in the International Cocoa Genebank, Trinidad (ICG,T). As in recent years, our activities are summarised in the Overview (next section) and have been grouped under the headings of conservation, characterisation, evaluation and utilisation. However there is considerable overlap and interdependence among these categories so that, for example, characterisation and evaluation depend on conservation, and utilisation depends on effective evaluation. All the current activities in CRU have been mentioned in the Overview, but all our work is not reported in detail every year. Detailed reports are presented from areas where there have been significant findings or progress, so an individual activity may only be reported once every few years.

Details of the Cocoa Research Advisory Committee, staff, publications and visitors and a complete list of acronyms are given at the end of the report. In the text, acronyms will also be defined, normally only at their first mention.

CRU is a department in the Faculty of Science and Agriculture of the University of the West Indies (UWI). Core activities in CRU are made possible by financial support from the Government of the Republic of Trinidad and Tobago and the Biscuit, Cake, Chocolate and Confectionery Association, UK (BCCCA). Sources of additional support for special projects and collaboration from other organisations are listed on the inside front cover of this report.

Projects

The CFC/ICCO/IPGRI[1] project entitled *Cocoa productivity and quality improvement: a participatory approach* started on 1 June 2004 and is referred to in this report as the "CFC/ICCO/IPRGI Cocoa Productivity Project". Good progress continued to be made in the second year of the project. Major components of the activities in CRU are germplasm enhancement for resistance to Black Pod disease (BP) and Witches' Broom disease (WB). Selections for BP resistance from the first round of crosses (1998-2001) are being evaluated in the field and evaluations of the first year progeny for Witches' Broom resistance are at an advanced stage. We are most grateful to Jean-Marc Thévenin, who has continued to supervise the work on germplasm enhancement for Witches' Broom resistance since he returned to the Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France (CIRAD). He was instrumental in planning this work programme before leaving CRU in July 2004, and visited Trinidad in March, June and November in 2005 to guide the work. A replacement from CIRAD for Jean-Marc Thévenin is expected in mid-2006.

The second phase of the project to *Evaluate cocoa germplasm for resistance to Witches' Broom disease* is continuing with support from the World Cocoa Foundation (WCF). In this project, promising clones for resistance to WB are being confirmed and quantified using the agar droplet inoculation method. During 2005, 17 clones were confirmed to be resistant from 13 accession groups. This will assist in providing a wide genetic base for germplasm enhancement and should contribute towards the achievement of durable resistance in countries affected by the disease.

[1] United Nations Common Fund for Commodities/International Cocoa Organisation/International Plant Genetic Resources Institute

CRU is continuing to participate in the project *To develop a DNA[1] fingerprinting database for all major cacao collections in the Americas* with the United States Department of Agriculture (USDA). The project was originally supported jointly by USDA, the BCCCA and CIRAD and in 2005, a direct agreement was drawn up between USDA and CRU to continue the project with inputs from CIRAD. Since the start of the project in 2001, DNA samples from all the accessions held in the ICG,T have been sent to the USDA molecular biology laboratory in Beltsville, USA. Dr. Dapeng Zhang is analysing the DNA with an automatic capillary sequencer using 15 standardised microsatellite primers and has completed the majority of Upper Amazon accessions. In 2005, leaf samples were also collected from the original trees of the Allen collection in the Estacion Experimental Napo (EEN), San Carlos, Ecuador and trees from the Chalmers collection in Estacion Experimental Tropical (EET), Pichilingue. These were brought to Trinidad and DNA is being extracted in CRU in preparation for subsequent analysis by Dr. Zhang using the sequencer in Beltsville.

The CFC/ICCO/INIAP[2] Flavour Project to *Establish physical, chemical and organoleptic parameters to differentiate between bulk and fine cocoa* was further extended in 2005. This project involves research teams in Ecuador, Papua New Guinea and Venezuela, and is now in an advanced stage of writing the final report.

A new project entitled *Safeguarding the International Cocoa Genebank, Trinidad: a global resource for the cocoa industry* was approved by the Begeleidingscommissie Subsidieregeling Duurzame Ontwikkeling Cacao- en Chocoladesector in the Netherlands in May 2005. The project, to be jointly funded by the Dutch Buffer Stock Fund and the Cocoa Research Association, UK (CRA), aims to upgrade the irrigation facilities in the University Cocoa Research Station (UCRS) and re-propagate material at risk of genetic erosion. Up to the end of 2005, progress has been limited by a long delay in the release of funds. However, CRA funds were released in November 2005 and an advance from the Dutch Buffer Stock Fund has been scheduled for early 2006.

A project entitled *Chemical indicators of different fermentation stages of raw cocoa* was approved in October 2005 for support by the Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto-von-Guericke" e.V./Forschungskreis der Ernährungsindustrie e.V. This is a collaborative project between CRU and the Department of Useful Plants and Plant Ecology, University of Hamburg, Germany for completion in 2006.

Staff news

Neerupa Ramnath (Technical Assistant) was appointed for 3 months from January to March 2005 to work on the CFC/ICCO/INIAP Flavour Project carrying out micro-fermentations and preparing cocoa liquor samples for organoleptic assessment. She was re-appointed for another 3-month period in July to assist with pollinations for the CFC/ICCO/IPRGI Cocoa Productivity Project.

Surendra Surujdeo-Maharaj was appointed as a part-time Technical Assistant from April to September 2005 to work on the WCF project on screening for WB.

Lambert Motilal (Contract Officer) registered for a PhD in 2005 under the joint supervision

1 Deoxyribonucleic acid

2 Instituto Nacional Autonomo de Investigaciones Agropecurias (INIAP)

of Drs. Pathmanathan Umaharan (Life Sciences, UWI) and Dapeng Zhang (USDA, Beltsville). Lambert will carry out part of his research in USDA, Beltville, starting in November 2005.

Visitors and trainees

David Cros came to CRU on 15 January 2005 for a five-month training period. He was a student at the "Universitaire de Fouille" in Pointe a Pitre, Guadeloupe and undertook a project to investigate aspects of screening for resistance to Witches' Broom disease as part of the requirement for a Masters degree. The main objective of his research was to assess the impact of plant water stress of the host on infection by *Crinipellis perniciosa* and symptom development.

Three members of Chokladforam, Sweden, came to CRU on 17th January for a two-week training period on general activities in the Unit. During their stay they arranged a tasting demonstration for staff of premium chocolate, wine and rum.

Alisha Omar-Ali and *Frankie Solomon* were hosted as placement students in CRU from 23 May to 22 July 2005. Ms. Omar-Ali assisted with verification studies by DNA fingerprinting and Mr. Solomon assisted with work on pre-breeding for resistance to Witches' Broom disease.

Dr. Jorge de Souza (CEPEC[1]) visited CRU from 9 - 12 August 2005 for discussions on a joint project proposal with David Iwaro. Drs. Iwaro and de Souza are joint recipients of the 2005 John and Ann Niederhauser Endowment (JANE) research award from the American Phytopathological Society. Congratulations and best wishes for success are due to both David Iwaro and Jorge de Souza for the honour of receiving the JANE award.

Xavier Sabau (CIRAD) visited CRU from 18 October to 1 November 2005 to work with Michel Boccara on the CIRAD/CRU project *Study of the genetic and genomic bases of cocoa resistance and quality traits.*

Meetings and events

CRU hosted a planning meeting on 24 - 25 January 2005 for the CFC/ICCO/INIAP Flavour Project with coordinators from INIAP, Ecuador and the Instituto Nacional de Investigaciones Agricolas (INIA), Venezuela.

Two staff from CRU (David Butler and David Iwaro) and one from MALMR[2] (Kamaldeo Maharaj) participated in a *Regional workshop on Moniliasis, Regional Breeding and Resistance Testing Methods (Monilia, Phytophthora pod rot and Witches' Broom),* CATIE[3], Turrialba, Costa Rica. 21-25 February 2005.

Darin Sukha participated in a CFC/ICCO/INIAP Flavour Project workshop *To evaluate and coordinate future actions among researchers involved in chemical analysis,* in Merida, Venezuela from 15 to 18 March 2005.

CRU hosted a CFC/ICCO/INIAP Flavour Project meeting *To discuss and interpret findings of combined country analyses with links to the project objectives* from 24 to 30 June 2005. Participants included collaborators from INIAP, Ecuador and INIA, Venezuela, Plant Research International (PRI), Holland.

[1] Centro de Pesquisa do Cacau, Brazil
[2] Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago
[3] Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica

David Iwaro visited CEPEC, Brazil from 22 to 28 July 2005 for discussions on a joint project proposal with Dr. Jorge de Souza in connection with the JANE research award.

David Butler participated in a *Brainstorming workshop on establishment of a cacao genetic resources network* in Montpellier from 24 to 26 August 2005.

David Butler participated in the BCCCA Cocoa Research Review meeting in Reading, UK on 30th August 2005.

David Butler attended the World Cocoa Foundation partnership meeting, Washington, USA, 11-14 October 2005. The included two satellite meetings: a CacaoNet discussion meeting in Washington and a one-day Science workshop in USDA Beltsville.

Representatives on the Cocoa Research Advisory Committee

At the 42nd meeting of CRAC in January 2005, we were pleased to welcome a Mr. Christophe Montagnon, a new representative for CIRAD and Mr. David Preece, a new representative for BCCCA. This was the last meeting for Mr. Tony Lass, who had represented BCCCA since the committee was established in its present form in 1980.

Mr. Lass had attended every meeting over the last 26 years and his valuable input to both the committee meetings and to the overall activities in CRU will be greatly missed. Largely through his vision, determination and support, CRU has achieved an enviable reputation in cocoa research with the purpose of serving the global cocoa industry. We are greatly indebted to him for his steadfast support and encouragement. We wish him well in his future endeavours.

As a gesture to offer our sincere thanks to Mr. Lass, a reception was arranged in his honour at the Ortinola Great House in Maracas Valley. At this function Mr. Lass presented a historic picture of the property (previously owned by Cadbury Ltd.) to the present owners.

The Cocoa Research Unit – an overview

Cocoa, obtained from cacao (*Theobroma cacao* L.), makes a unique contribution to the flavour and textural properties of chocolate that holds an almost universal appeal to people of all ages. The international cocoa community generally classifies cocoa beans into two broad types. The first is Forastero cocoa, with highly pigmented beans, used in the manufacture of cocoa butter and high volume chocolate lines. These beans, referred to as bulk cocoa, make up over 95% of the world production. The second type is Criollo cocoa, mainly grown in Central and northern South America, whose white or pale violet beans are used to manufacture chocolate of the highest quality. Trinitario is a hybrid of the two types that originated in Trinidad but is now grown in many locations. It provides specific flavour distinctions in fine chocolate. Criollo and Trinitario beans are collectively known as 'fine or flavour' cocoa. There are however exceptions to this generalisation such as Nacional cocoa from Ecuador, which is believed to be a Forastero type classified as fine or flavour. Another group is Refractario, which comprises germplasm selected in Ecuador in the 1920s and 1930s. Selections were made of the few survivors among seedlings that had been infected by Witches' Broom disease.

Cacao was introduced into Trinidad around 1575 and ever since that time has been an integral part of the history of Trinidad and Tobago. Cocoa first became a staple product of Trinidad at the start of the 18th century and from the 1860s to the 1920s it played an essential role in the social and economic development of the society. In 1921 cocoa production in Trinidad and Tobago reached 34,000 metric tonnes per year, making the country amongst the world leaders in cocoa exports. Given the prominent position of Trinidad and Tobago in the international cocoa market at that time and the outbreak of Witches' Broom disease in 1928, a Cocoa Research Scheme was established in Trinidad to provide support for local and international cocoa production.

Cocoa research began in Trinidad at the Imperial College of Tropical Agriculture (ICTA, now UWI) in 1930 and has continued uninterrupted since that time. CRU is responsible for maintenance of the ICG,T around which on-going research activities in the Unit are centred. Cacao germplasm has to be conserved as a living collection, since seeds do not remain viable if they are frozen and other methods of cryopreservation are not yet widely available. The ICG,T is situated at UCRS, a 37 ha site, originally part of the La Reunion Estate at Centeno. Work to establish the ICG,T began in 1982 with support from the European Union, by propagating trees using rooted cuttings from existing collections in Trinidad. These collections had been established at different locations on the island using selected varieties from Trinidad and Tobago, from other national collections and from numerous missions to collect primary germplasm. They include the Imperial College Selections (ICS) which resulted from an exhaustive survey of Trinidad and Tobago carried out by F.J. Pound between 1930 and 1935. About 50,000 high-yielding trees were selected and those bearing small and thick-shelled pods were eliminated. The 100 most productive trees (ICS 1 to 100) were selected from the resulting 1,000 using exact criteria from detailed observations.

A main source of original material for the ICG,T was Marper Farm at Manzanilla, east Trinidad, established by F.J. Pound following his expeditions to the upper Amazon between 1937 and 1942. The trees at Marper are now old and have suffered periods of neglect, however they still serve as an important anchor in confirming the identity of clones in the ICG,T and in replacing material which has proved difficult to establish. In addition, germplasm was available

from other expeditions such as the Anglo-Colombian expedition in 1952-53 and Chalmers' expeditions between 1968 and 1972. By 1994 over 2,000 accessions had been planted in the ICG,T and additional clones are added as they become available. The genebank contains one of the most diverse collections of cacao germplasm in the world and has been designated a Universal Collection by IPGRI.

Since the ICG,T was established, research activities in CRU have been centred on the collection. The ICG,T is considered to be of major importance to the future of world cocoa production, but the potential of the collection cannot be fully exploited unless the accessions are characterised, evaluated, and made available to end users in cocoa-producing countries. Furthermore, information related to the germplasm must be well documented and made readily available in a user-friendly format.

CRU has an interest in all aspects of cacao cultivation, including quality. Our mission is to provide support for the provision of varieties suited to sustainable cocoa production, both locally and globally, by making planting material available with improved traits for high yield potential, disease resistance, high fat content and with good flavour characteristics.

Research efforts at CRU over the last 10 years have been directed towards the task of characterising and evaluating all the accessions in the ICG,T, selecting those with desirable traits and undertaking pre-breeding to produce genetically diverse populations with enhanced characters (such as disease resistance). Below is a summary of achievements and an outline of plans for future research in the medium-term time frame.

Conservation

Maintenance and propagation

If the ICG,T is not well maintained, research progress would become limited, so a balance is necessary between funds directed towards the genebank maintenance and research.
Apart from routine maintenance such as weed control, pruning, shade management, irrigation, security/firewatch, there is a continuous need for re-propagation of clones. When the ICG,T was established, 16 trees of each accession were planted in each plot, however, in the majority of cases, not all the trees grew and some accessions proved very difficult to establish as rooted cuttings. The situation now (12-20 years after establishing the plots) is that plots contain anything from 1 to 16 trees, and some accessions have no survivors. Plots with less than three living trees are considered at risk to genetic erosion. The urgent need to conserve these clones by grafting their budwood onto rootstocks is being addressed, and the grafted plants are being established in clonal gardens. In cases where there is no survivor in UCRS, but the original tree in Marper Farm or elsewhere is still alive, budwood from the original tree is being grafted onto rootstocks. Once established, cuttings can be taken from the grafted plants and rooted to fill gaps in the ICG,T with plants on their own roots. It is important to make a concerted effort to raise plants from rooted cuttings to avoid potential confusion in the future with chupons from rootstocks.

New introductions

The ICG,T is considered to be a dynamic germplasm collection. We are continuously adding accessions from collecting expeditions (when the opportunity arises) or from other national collections. The objective of these inputs is to increase the representation of genetic groups that are currently under-represented in the genebank, thereby creating a balanced collection with

maximum genetic diversity. Towards this end, recent acquisitions (since 1990) are Trinitario populations from other islands in the Caribbean and Central America, Lower Amazon material from French Guiana and Venezuela, wild Criollo material from Belize, and genetically diverse Upper Amazon clones from the John Allen collection, Ecuador. Until 2003, new material was introduced through the Barbados Cocoa Quarantine Station however this activity has been suspended due to financial constraints. Material is now being introduced to Trinidad through the International Cocoa Quarantine Centre, Reading (ICQC,R), UK.

Further acquisitions are proposed when funding permits, from Mexico (Criollo/Trinitario), Costa Rica (CATIE) (Criollo), Guyana (Lower Amazon), French Guiana (Lower Amazon), Columbia, Ecuador and Peru (Upper Amazon) and Brazil (Lower Amazon).

Documentation

New introductions, difficulties of establishment, and filling gaps in the ICG,T mean that field maps and databases need to be continuously updated. Each tree has been assigned a unique number to accurately record the source of samples for research and other purposes. This will avoid confounding issues if trees are identified as off-types subsequent to a research activity, since it will always be possible to return to the same tree within a plot. From 1998 to 2001, we completed the task of drawing up-to-date maps, and in numbering plots within fields and trees within plots. All this information has been organised in a database to enable notes about individual trees to be included, and this information is being continuously updated.

Verification

The task of establishing the ICG,T from ageing trees by use of rooted cuttings was complex and there was ample opportunity for mislabelling to occur. Steps in which errors may have arisen include:

- Collection of budwood for cuttings during the clonal propagation of trees from Marper Farm prior to their planting in the ICG,T or on campus. The budded trees in Marper Farm were already old when the multiplication process started in the 1980s. Many of the trees had multiple trunks, which included rootstock as well as scion material. In addition, some trees have fallen and re-grown in new locations, so these are difficult to identify from the field maps. In other cases, seed may have germinated at the base of the original tree, in which case trunks of seedlings would be difficult to distinguish from the trunk of the original tree.
- Mislabelling of plants in the greenhouse after clonal propagation, e.g. when rooted cuttings were moved from the propagation bin to harden off, or from the hardening-off area to another part of the greenhouse or from the greenhouse to the genebank.

Some off-types have been recognised from the pod morphology, and these trees are being tagged to avoid their mistaken use in research. In recent years, further off-type trees have been identified using DNA sequencing methods, and it is now recognised that all trees being used for research or distribution should be verified by DNA fingerprinting to ensure their correct identity.

Initially, molecular verification was undertaken using random amplified polymorphic DNA (RAPD) analysis, this being the technique available in CRU when the work started in 1997. Results from the RAPD analysis showed that approximately 70% of the trees tested were true to type. However, more recently results from some RAPD analyses have been shown to be

inconsistent, so it is possible that the 30% off-types identified by this technique is an over-estimate. Since 2001, we have adopted microsatellite analysis (otherwise known as Simple Sequence Repeats, SSR) for the verification work. We use two techniques to visualise SSR results; either agarose gels with ethyl bromide staining or polyacrylamide gel electrophoresis with silver staining, which gives much better resolution of bands, but is more costly. SSR analysis for DNA fingerprinting is reported to be reliable, with consistent results between different laboratories.

The task of verifying every tree in the ICG,T (over 11,000 trees) is enormous, so it is necessary to set priorities to arrive at achievable targets in the short- and medium-term. Clones identified as having desirable traits (such as disease resistance, good yield potential, high butterfat content or beans of superior flavour) will be given a high priority for the verification of individual trees within plots.

Characterisation

Morphological characterisation

About half of the accessions in the ICG,T have yet to be fully described. To address this problem, a concerted effort is being made to systematically document each accession using morphological descriptors. Work started in 1990 using a complete list of 65 morphological descriptors developed by the International Board for Plant Genetic Resources (now IPGRI) in 1981, but initial progress was slow and this was superseded by a short list of 22 morphological descriptors developed at CRU. The list includes detailed descriptions of leaves, flowers and fruit for traits that aid identification and/or affect economic yield. It remains a large task even with the short list of descriptors, and the work was further streamlined in 2000 by reducing the sample size of pods from 20 to 10 and that of flowers from 15 to 10. Full descriptions of over 1,180 accessions have now been completed. As they are recorded, the descriptors are entered in a local database and are also sent to the International Cocoa Germplasm Database (ICGD), Reading, UK, for global distribution.

Having reached a point where large numbers of accessions in the ICG,T have been characterised, analyses are possible to examine phenotypic variation among various groups of cacao (such as Upper Amazon Forastero, Refractario, Lower Amazon Forastero, and Trinitario). Furthermore, this large volume of carefully catalogued data should form the basis of new avenues of work. Recently developed techniques allow the possibility of gene association between specific traits (recorded as morphological characters) and well-identified parts of the cacao genome. Such information could lead to rapid advances in selection for desirable traits in plant breeding programmes of the future.

Molecular characterisation

From 1994 to 2001, molecular characterisation was carried out using RAPD analysis, with the completion of over 600 accessions. This technique provided information used to assess the genetic diversity within the germplasm collection. Genetic diversity studies can be used to identify cacao types that are over- or under-represented in the ICG,T, to assess the degree of homogeneity within accession groups, and the genetic distances between them. For cacao, the term population is normally used to refer to accessions sharing the same collection name, but here the term "accession group" will be used. The geographic origin within an accession group can vary from a small estate to a large region. This would naturally affect its genetic diversity.

This work took a new direction in 2001 when the USDA Fingerprinting Project was initiated. In this project we are generating a DNA fingerprint of each accession in the ICG,T (2,300 accessions), taking a sample from the most original tree of each clone. The analysis is done using 15 SSR primers, selected to cover most of the cacao genome (9 of the 10 chromosomes) and to give good differentiation between clones. The results of these analyses not only provide a means of positively identifying each clone, but also provide data for genetic diversity studies. DNA has been extracted in CRU from each accession, and the samples are being analysed in USDA, Beltsville with an automatic sequencer. In our previous work with RAPD, we analysed 600 accessions in 6 years, and now we expect to analyse 2,300 accessions in 3-4 years. This collaborative effort will therefore accelerate the rate of progress in genetic diversity studies by a factor of six.

Information on genetic diversity within and between accession groups will be vital to the selection of populations for inclusion in germplasm enhancement and breeding programmes of the future.

Evaluation

To assess the value of accessions in the ICG,T, traits that affect the economic yield need to be evaluated. Examples of these traits are disease resistance, bean size, pod index (the number of pods needed to produce 1 kg of dry beans), cocoa butterfat content and flavour potential.

Disease resistance

Two important diseases that affect cacao in Trinidad are Black Pod disease (BP), caused by *Phytophthora* spp., and Witches' Broom disease (WB), caused by *Crinipellis perniciosa* (Stahel) Singer (recently proposed as *Moniliophthora perniciosa* (Aime and Phillips-Mora)).

Mass screening for resistance to BP was started in 1996 using a detached pod inoculation method, which distinguishes pre- and post-penetration types of resistance. Inoculations are carried out with *P. palmivora*, the more aggressive of two species of *Phytophthora* found in Trinidad (*P. palmivora* (Butler) Butler and *P. capsici* Leonian). So far, over 1,400 accessions have been screened at least once and the inoculation has been repeated on 967 accessions. Overall, about 13% of the clones tested are either resistant or moderately resistant to BP, although the proportion of resistant clones is greater in the Forastero group than in the Trinitario group.

In addition to screening by controlled inoculation, the incidence of BP in the field has been observed in the ICG,T. This combination of detached pod inoculations in controlled conditions with field observations over a number of years will provide sound evidence on host resistance to BP.

Mass screening for resistance to WB is being undertaken using a spray inoculation method. This work was started in 1998 using young grafted plants, replicated up to five times to allow inoculations of the same clone to be repeated. The inoculation method had to be adapted for use with grafted plants (as opposed to seedlings) and to the environmental conditions in Trinidad, so early progress in this project was slow. However, about 700 accessions were inoculated in the first phases of this project by July 2003. Results from this work identify clones that are susceptible to WB, but there is a need to verify true resistance to WB where few or no symptoms developed after inoculation. This is because escapes are common with the spray inoculation

method.

Recently, an optimised agar-droplet method was developed that allows resistance to WB to be quantified. We are therefore using agar-droplet inoculations on seedlings or clones (grafted plants) to verify the resistance of promising accessions identified by the spray method. These results will also be combined with field observations in the ICG,T over a number of years.

Quality traits

The percentage butterfat has been determined in over 400 clones from the ICG,T and further determinations are being made in selected clones.

Assessment of flavour is an aspect of evaluation of particular value to cocoa farmers in Trinidad and Tobago who produce 'fine or flavour' cocoa. Sensory assessments are carried out using trained panellists to investigate effects of various post-harvest processes on the flavour attributes of selected accessions. Recent work has demonstrated the consistency of trained panels to give quantitative sensory assessments, and flavour profiles are being documented for a range of accessions. We plan to extend this effort to determine flavour profiles of clones with other desirable traits such as good yield potential and/or disease resistance.

The assessment of flavour traits is an expanding area of investigation in CRU. Work is underway to explore the relative contributions of the growing environment, the environment during post-harvest processing and pollen to flavour.

Utilisation and application

Distribution

Selected cacao accessions from a diverse genetic background with desirable agronomic traits are being distributed to cocoa-producing countries via the ICQC,R. After satisfying the required period in quarantine, these elite accessions will be distributed to a range of cocoa-producing countries, including participants in the CFC/ICCO/IPGRI Germplasm Utilisation Project (*Cocoa germplasm conservation and utilisation: a global approach*). In the future, selected populations from germplasm enhancement programmes (below) will be distributed in a similar way.

Germplasm enhancement

From 1998 to 2002, over 90 accessions were used in a pre-breeding programme to accumulate genes for resistance to BP. Parents were selected by considering their genetic diversity, geographic origin and economically important traits, as well as disease resistance.

The progeny from crosses in the pre-breeding programme were evaluated for BP resistance with a leaf inoculation method. This permits early selection of seedlings and comparison of the disease resistance of the parents and progeny at an early stage. The most resistant individuals in the progeny were planted in field trials and are being evaluated for performance, not only in terms of BP resistance, but also for precocity, vigour, productivity and WB symptoms. Initial results from field observations and the detached pod inoculation method confirm substantially improved resistance in these selections compared to unselected populations. The main objective of the pre-breeding programme is to produce enhanced germplasm that will introduce resistance genes to conventional breeding programmes in various cocoa-producing countries throughout the world.

A similar pre-breeding programme was initiated in 2004 for WB. Progeny from crosses

between WB resistant clones are being screened with the agar-droplet inoculation method. Work is also in progress at CRU to develop alternative techniques for early screening of resistance to WB.

Marker assisted selection

Research at CRU in the CAOBISCO[1] project (1995-2000) identified quantitative trait loci (QTL) for resistance to BP based on results of the leaf inoculation method. Selected plants from the same progeny were planted in the field, and we are now in a position to validate the leaf inoculation method with field observations and detached pod inoculations as the plants come into bearing. Confirmation of the QTL would open the possibility of marker assisted selection in future breeding programmes for BP resistance.

Other work (outside CRU) is underway to search for QTL for resistance to other diseases such as WB and Frosty Pod disease (FP, caused by *Moniliophthora roreri* (Ciferri & Parodi) Evans *et al*.). When this has been completed, it should be possible to use marker assisted selection for germplasm enhancement even for diseases not present in Trinidad (such as FP).

It is likely that other advanced molecular techniques such as expressed sequence tags and microarray analysis will lead to other selection methods in the future. However, the application of such techniques is entirely dependent on reliable datasets for traits of interest. The painstaking ground work at CRU on morphological characterisation, disease resistance screening and evaluation for quality traits has the potential to form a rigorous basis for such future investigations.

Conclusion

Since establishing the ICG,T, substantial progress has been made in research at CRU. A large body of information has been accumulated and documented, some of which has immediate applications, and some of which will form the basis for future investigations. For example, the list of 100 priority clones available in the ICG,T that are part of the "CFC/ICCO/IPGRI Project Collection" has been transferred to the ICQC,R. This is the end-point of a large body of research in CRU, including morphological and molecular characterisation, evaluation for BP and WB (screening and field observations) and cocoa butterfat determinations. The selected clones will soon be available for further distribution to many cocoa-producing countries.

As the work of characterisation and evaluation continues, further selections of priority germplasm will be possible. In addition, practical results from the germplasm enhancement programme will soon be forthcoming after completing some basic field observations. Selections from BP resistant populations will then be sent to intermediate quarantine for further distribution.

The utilisation of the substantial body of information resulting from on-going activities in the development of novel selection methods provides the prospect of an exciting future for cocoa research. The possibility of molecular based selection techniques, together with well-documented information on genetic diversity, could lead to unprecedented progress in cocoa breeding in the foreseeable future.

1 Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE

Conservation

New cacao introductions into the International Cocoa Genebank, Trinidad

D.R. Butler and J. Joseph

Two consignments of budwood were transferred to Trinidad from the ICQC,R during 2005. The success rate of establishing grafted plants was 97% (one clone was lost out of 39); the number of plants established for each clone is given in Table 1.

Table 1. Clones introduced to Trinidad from the International Cocoa Quarantine Centre, Reading in 2005.

Preferred name	No. of plants established	Preferred name	No. of plants established
B 7/A-6 [GUF]	1	KER 3	1
GU 123/V	4	KER 6	4
GU 125/C	3	MAR 9	3
GU 133/C	4	PA 7 [PER]	4
GU136/H	6	PNG 10	2
GU 144/C	4	PNG 110	2
GU 147/H	4	PNG 139	1
GU 154/C	2	PNG 215	2
GU 168/H	4	PNG 224	3
GU 171/C	2	PNG 386	3
GU 207/H	4	PNG 414	4
GU 221/C	2	POUND 4/B [POU]	2
GU 221/H	5	POUND 19/A [POU]	4
GU 239/H	1	RB 47 [BRA]	2
GU 249/H	5	RIM 21 [MEX]	2
GU 259/C	4	RIM 189 [MEX]	4
GU 296/H	1	RIM 39 [MEX]	2
GU 341/H	4	SC 9	5
HONDURAS 1 [GBR]	4	SIAL 93	4

Clones still held in the greenhouse at UWI from germplasm that was transferred to Trinidad from Barbados in 2003 (Butler and Sukha, 2004) are being multiplied. Trees are being planted in the field after establishing at least five plants of each accession in the greenhouse. During 2005, more of these new introductions were planted in Campus 8 at UWI and in Field 5A at UCRS (Table 2).

Table 2. Number of trees planted in fields at UWI (Campus 8) and at UCRS (Field 5A) in 2005.

Preferred Name	No. at UWI	No. at UCRS	Preferred name	No. at UWI	No. at UCRS
CC 57		3	LCT EEN 107	1	2
CRIOLLO 54 [CRI]	2	3	LCT EEN 188		5
ELP 1/S4		6	LCT EEN 193		3
ELP 10/S6	2	2	LCT EEN 218		4
ELP 10/T	1	3	LCT EEN 227		4
ELP 11/S3		5	LCT EEN 267	2	3
ELP 16/S3	2		LCT EEN 278		2
ELP 16/S7		5	LCT EEN 33	2	3
ELP 18/S12		3	LCT EEN 333		3
ELP 20/S3	2	3	LCT EEN 368		3
ELP 22/S10		3	LCT EEN 37		4
ELP 28/S4		6	LCT EEN 413	2	2
ELP 32/S3		5	LCT EEN 49	1	3
ELP 32/S4		5	LCT EEN 57	2	3
ELP 34/S7	2	5	LCT EEN 81	2	4
ELP 35/S4		4	LCT EEN 91		4
ELP 40/B	1	3	MA 12 [BRA]	1	3
ELP 41/S5		5	PH 1/1	2	2
ELP 7/S2		5	PH 1/3	2	3
ELP 8/S3		4	PH 2/4		5
ELP 9/S4		4	RB 49 [BRA]		2
EQX 69 [EQX]		5	RIM 23 [MEX]		9
GDL 3		5	RIM 52 [MEX]	2	3
GF 32	2	3	TAP 10 [CHA]		4
GU 192/A		3	UF 221	2	3
GU 202/A		5	YAL 5/T		4

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Transfer of accessions from the Chalmers and Allen collections, Ecuador to the International Cocoa Quarantine Centre, Reading

D.R. Butler

In 1979, the London Cocoa Terminal Market and the Cocoa Association of London agreed to provide financial support for a project to make a thorough and systematic collection of wild cocoa in the Amazon region of Ecuador. The project, known as the London Cocoa Trade Amazon Project, marked the Golden Jubilee of the London Cocoa Market. It was undertaken by John Allen in two phases; the first from 1980 to 1983 and the second from 1984 to 1985 (Allen and Lass, 1983, Allen, 1987). During the two phases, 23 collecting trips were made that resulted in 281 accessions (represented as either seedlings, clones or both) being established at the Estacion Experimental Napo (EEN) near San Carlos.

There have been numerous attempts over the past twenty years to transfer material from San Carlos, but the overall survival rate is extremely low, especially when transferring budwood for vegetative propagation by the recipients. This difficulty is linked in part to the remote location of San Carlos, which causes excessive delays between the time of collecting budwood and delivering it to any of the world's cocoa quarantine stations.

The concept of propagating clones at San Carlos and transferring whole plants is attractive (Mooleedhar, 2000), but large numbers of mature plants are bulky and expensive to transport.

Micrografting

In 2005, a project was undertaken to apply the micrografting technique (originally developed at CRU, Sreenivasan (1995)) to transfer internationally available cocoa germplasm from Ecuador to ICQC,R as small bared-rooted plants. This included germplasm from the Allen collection in EEN San Carlos and the Chalmers collection at Estacion Experimental Tropical (EET) Pichilingue. If successful, the material would subsequently be brought to the ICG,T after the statutory 2-year quarantine period. In achieving this objective, a number of INIAP staff were trained in the micrografting/hydroponics technique for raising young clonal plants, both at EET Pichilingue and at EEN San Carlos.

In April/May clones that were not represented in the ICG,T were selected for propagation, normally grafting six budsticks of each. In EET Pichilingue, 245 plants were grafted representing 35 clones from the Chalmers collection. In EEN San Carlos, 438 plants were grafted representing 70 clones from the Allen collection and a further 588 plants were grafted from seedlings that represented 369 individual genotypes.

Grafted plants were covered with plastic bags for two weeks to prevent transpiration while the scion/rootstock union was forming. At the end of this period, the bags were removed and the percentage of 'takes' was accessed. The overall success rate in both locations was over 70% but subsequent survival was poor and appeared to be linked to the hydroponic system, probably to nutrients or poor aeration leading to stunted root growth. Grafts were repeated for most of the clones from the Chalmers collection by local staff in EET Pichilingue.

In August, 73 plants (25 clones) from EET Pichilingue and 82 plants (64 clones) from EEN San Carlos were taken to ICQC,R, UK. In Reading some further losses occurred, however by

December 2005, 16 clones were well established in the quarantine centre (Table 1).

Table 1. Surviving clones in the ICQC,R, transferred from Ecuador in 2005 as bare-rooted micrografted plants.

Clone name	Clone name	Clone name	Clone name
NAPO 25	LCT EEN 141	LCT EEN 341/S2	LCT EEN 412
NAPO 30	LCT EEN 166	LCT EEN 366	LCT EEN 415
AGU 31	LCT EEN 237	LCT EEN 38	LCT EEN 429
EBC 135	LCT EEN 254	LCT EEN 401	LCT EEN 74

Acknowledgements

This project would not have been possible without close collaboration from INIAP, and special thanks are due to Dr. Freddy Amores (Head of the Cocoa Programme, INIAP) for arranging facilities to raise seedlings in the hydroponic system both in EET Pichilingue and EEN San Carlos prior to my arrival in Ecuador. He also assigned very capable technical assistants who provided untold assistance with the ambitious propagation programme, and provided transport from resources in INIAP.

In EET Pichilingue, thanks are due to all the staff in the Cocoa Programme who assisted in numerous ways. In particular Juan Agama and Dario Calderon for raising the seedlings, collecting budwood and grafting, and Jonny Espinales, who obtained phytosanitary certificates for the plants to be taken to Reading.

In EEN San Carlos, Nelly Pareres provided facilities and the time of a technical assistant to complete the work. Special thanks are again due to Juan Agama who travelled from Pichilingue and worked long hours over a prolonged period in San Carlos to complete the task. The help from Juan Carlos Cedeño was essential to locate trees in the genebank, and he subsequently cared for the grafted plants between May and August.

A travel grant was provided by UWI and financial support for the materials in Ecuador were kindly provided by the BCCCA.

Many thanks to everyone.

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Identifying marker-trait association in a cacao germplasm collection: a research plan

L.A. Motilal, P. Umaharan and D. Zhang

Introduction

Currently, genetic mapping and genomic research in cacao is largely focussed on pest and disease resistance traits due in part to the economic importance of the cacao pod borer (*Conopomorpha cramerella*, Snellen) , Black Pod disease (caused by *Phytophthora* spp.), Witches' Broom disease (caused by *Crinipellis perniciosa*) and Frosty Pod disease (caused by *Moniliophthora rorei*). Linkage maps containing QTL have been prepared for disease resistance to *P. palmivora* (Risterucci *et al.*, 2003; Flament *et al.*, 2001; Lanaud *et al.*, 2000; Lanaud *et al.*, 2001; Motilal *et al.*, 2002), disease resistance to *C. perniciosa* (Quieroz *et al*., 2003) as well as for the agronomic traits such as early flowering, trunk diameter, jorquette height, vigour, yield components, ovule number and bean traits (Clément *et al*., 2001; Clément *et al.*, 2003a,b; Crouzillat *et al.*, 1996). A single gene for anthocyanin (Crouzillat *et al.*, 1996; Osei *et al.*, 1995) has been mapped as well as a single gene for self-compatibility (Crouzillat *et al*., 1996). Recently, an alternative approach - genetic association mapping has been increasingly applied to plants. This approach explores the statistical relationship between the alleles present in existing collections of germplasm and the traits of interest. Association analysis or linkage disequilibrium (LD) mapping is expected to contribute to finer scale maps with markers applicable to the species of interest rather than to specific crosses (Herr *et al.*, 2000). LD mapping is also recommended for identifying loci for complex traits (e.g. Risch and Merikangas, 1996).

The quality of phenotypic data is one of the most important factors affecting the success of association mapping. Misidentifications are common in cacao genebanks (Figueira, 1998; Motilal and Butler, 2003) including the ICG,T (Sounigo *et al.*, 2001), and tree identity problems must be resolved before data averaged over multiple trees in a field genebank can be utilised. Research towards developing association maps may be conveniently divided into the following main areas:

- Accession selection
- Verification of genetic identities and estimation of population structure
- Microsatellite survey
- Test for marker-trait association

These areas are discussed below in relation to a project formulation and work already conducted.

Accession selection

There are about 2,000 accessions within the ICG,T and CRU has accumulated a wealth of data for many of these accessions. Data collection for the same accession would have been carried out on different trees over years to facilitate the needs of different research interests. In addition, some research interests have focussed on particular accessions determined in part by the availability of plant material. Verification of the germplasm collection therefore requires that each tree be unambiguously identified in the interests of all researchers.

A project investigating the butterfat content of over 390 accessions was carried out at CRU during the period 1993-1998 with funding from American Cocoa Research Institute (Khan, 1997, 1998). The accessions in the butterfat project formed the core group used in the proposed work.

Although the primary objective of the proposed work was to identify markers linked to butterfat content, the opportunity was taken to include other accessions for which information on other traits was available. Accessions were selected to include low and high categories for each selected trait. Disease resistance to *P. palmivora,* for instance, was sorted by scores and accessions ranked at either end of the rating scale were included. The tree numbers from which samples were taken for the butterfat project were uncertain except where only one tree was available. All the trees could therefore have been sampled in cases where an accession was represented by more than one tree. A total of 300 accessions (1,657 trees) belonging to the Forastero and Refractario types were finally selected for this study.

DNA extraction

Mature leaf samples were collected and DNA using the Kobayashi protocol (Kobayashi *et al.*, 1998) with tissue homogenisation achieved in a FastPrep 120V machine (Qbiogene, Inc., USA). DNA was re-suspended in sterile distilled or deionized water. Quantification of DNA was carried out at CRU using a TBS-180 fluorimeter (Turner Biosystems, Inc., USA) with Hoescht dye (Sigma, USA) while DNA quantification at the USDA site was done using Picogreen® dye (Molecular Probes, USA) on a Fluoroskan Ascent (Labsystems, Finland) setup.

The DNA quantification methods were comparable and were highly correlated (r = 0.85; Figure 1) suggesting that the Hoescht dye, while less sensitive than the Picogreen® dye, was similarly reliable for DNA quantification. A survey of the DNA yields found a left-skewed distribution (Figure 2) with a mean of 278 ± 6.6 ng/µL and 173 ng/µL for the modal value.

Verification of genetic identities and estimation of population structure

ICS verification at San Juan Estate

Fifteen (15) primers (mTcCIR 1, 6, 7, 8, 11, 12, 15, 18, 22, 24, 26, 33, 37, 40 and 60) were

Table 1. Identity verification for selected ICS accessions at San Juan Estate, Trinidad.

Accession	Blocks (B) Compared	Output[a]
ICS 10	B3 vs. B5	Differ by at least 11 loci
ICS 11	B1 vs. B2	Similar
ICS 14	B1 vs. B2	Differ by at least 9 loci
ICS 44	B1 vs. B5	Similar
ICS 69	B1 vs. B4	Similar
ICS 75	B2 vs. B4	Similar
ICS 84	B3a vs. B3b vs.B4 vs. B5	B3b differs at 5 loci; all others similar
ICS 86	B2 vs. B4	Similar
ICS 92	B1 vs. B3 vs. B4	B3 differs at 3 loci
ICS 93	B3 vs. B4 vs. B5	Similar
ICS 94	B3 vs. B4	Similar
ICS 97	B2 vs. B3	Differ by at least 12 loci
ICS 100	B2 vs. B5	Differ by at least 9 loci
? = 13	? = 30	? off-types = 6

[a]Differences of 2 or more bp are called to distinguish alleles

Figure 1. Comparison of two DNA quantification methodologies.

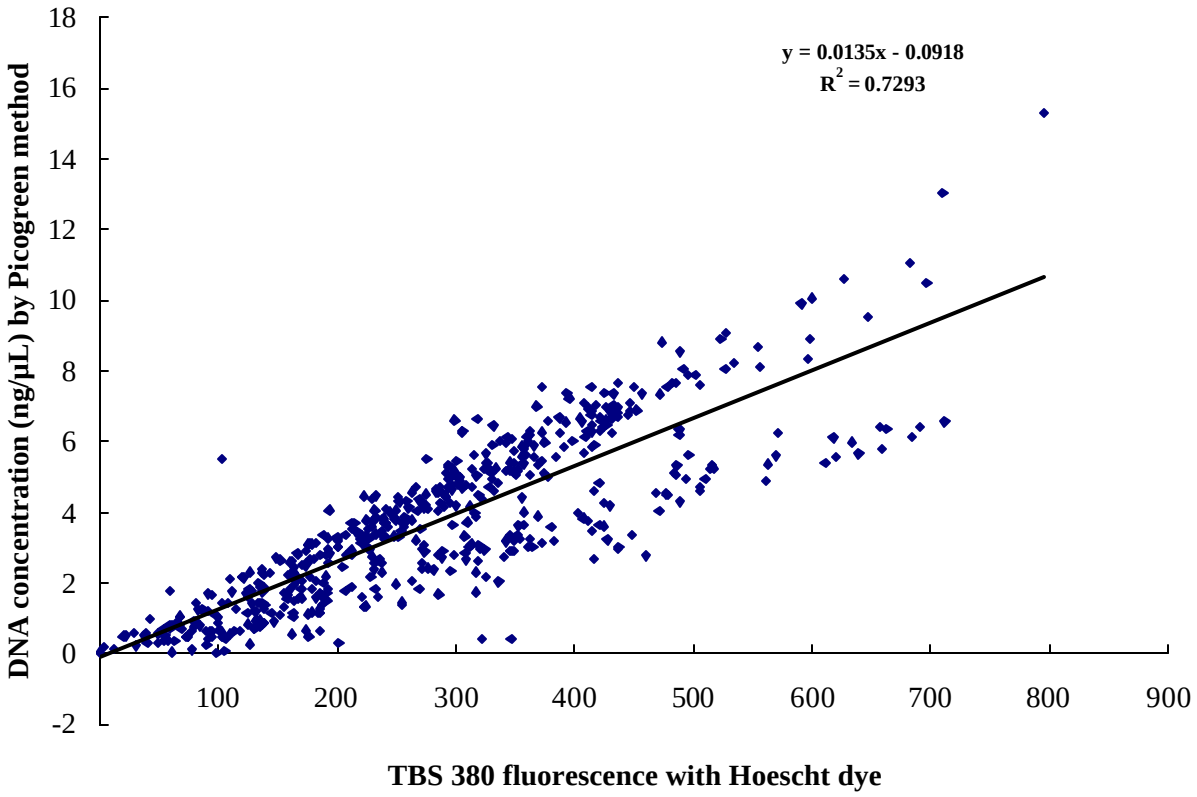
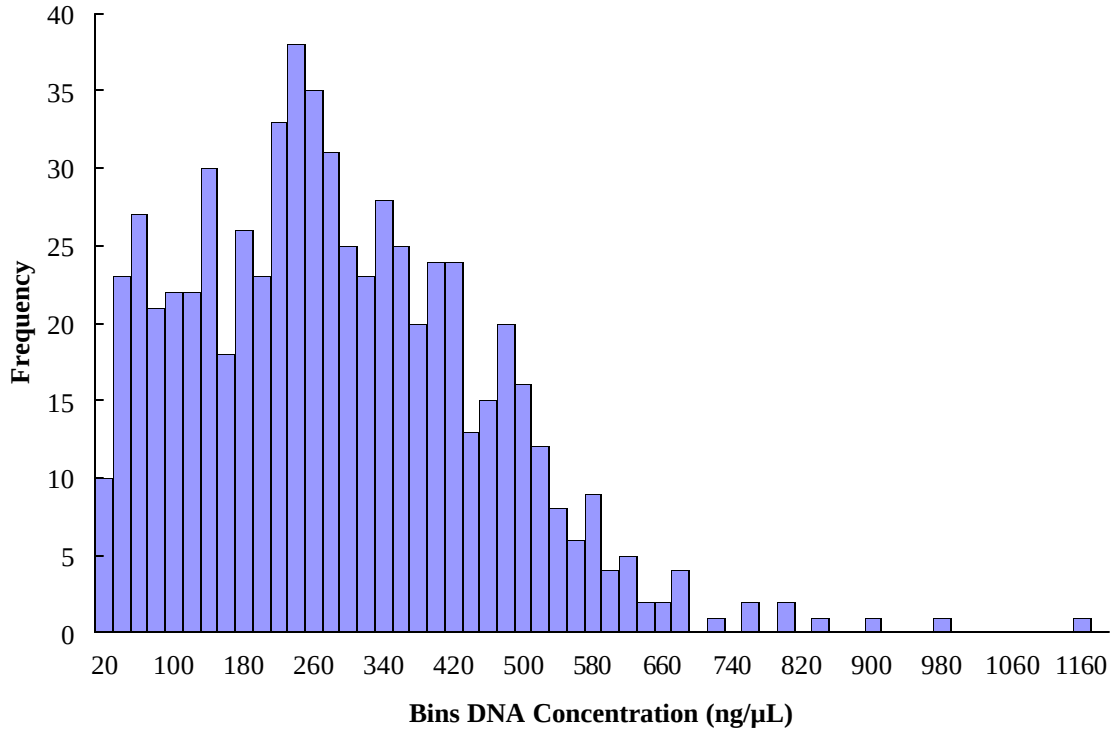


Figure 2. Distribution of DNA yields from 653 cacao leaf extracts.



assessed on the accessions ICS 4, 10, 11, 14, 16, 36, 44, 66, 69, 75, 84, 86, 87, 89, 92, 93, 94, 97 and 100 from San Juan Estate. Samples of ICS 4, ICS 14 (Block 3), ICS 16, ICS 36 (Block 5), ICS 66, ICS 69 (Block 2), ICS 75 (Block 1), ICS 87, and ICS 89 (Block 1) yielded poor results overall (no amplification or poor amplification; background noise too high for automatic peak calling). Amplification patterns from primer mTcCIR11 were poor across many of the ICS samples. The results of the reliable accessions from one round of genotyping are provided in Table 1.

Comparison of these results with those obtained on agarose for the same accessions (Motilal, 2005) revealed that nearly the same decision (92.3% match; 12/13) output in tagging mislabelled accessions was obtained . The only difference was for the B trunk of ICS 84 from Block 3. This sample should be redone since the original picket position appears valid and the trunks appear similar in age. Further, since the trees in the Cheesman Field were propagated by cuttings (Cheesman, 1944) there would be no rootstock problems. Overall, these results suggest that off-type detection on agarose gels is reliable.

Microsatellite assessment in the proposed study

Microsatellite profiles will be compared amongst trees of a particular accession to determine homogeneity and with the reference profile for that particular accession and to determine the validity of the accession nomenclature. Zhang *et al*. (2006) demonstrated that a minimum number of seven microsatellites was required for cacao identification using a capillary electrophoresis system. Twelve of the core 15 microsatellite primers recommended by Saunders *et al*. (2004) were therefore selected based on their potential for use on the agarose system (Motilal, 2003), previously established usefulness in detecting offtypes (Motilal and Boccara, 2004) and based on their information content (Zhang *et al.*, 2006). These primers and their multiplexing for capillary electrophoresis in the current project are listed in Table 2. This formulation is expected to facilitate the speedier detection of heterogeneity within trees sampled from a particular accession as the most informative and discriminant primers have been retained.

Table 2. Selected microsatellite primers for cacao identity verification.

Multiplex Set	**WellRED dye**[a]		
	D2-PA (black)	**D4-PA (blue)**	**D3-PA (green)**
A	CIR7: 150-167[b]	CIR6: 224-253	CIR8: 290-307
B	CIR1: 128-146	CIR33: 265-348	CIR60: 190-218
C	CIR37: 136-187	CIR15: 234-263	CIR26: 285-310
D	CIR12: 165-256	CIR11: 286-321	CIR18: 333-357

[a]Beckman Coulter, Inc., USA [b]Microsatellite code from mTcCIR; primer information details can be obtained online at EMBL-EBI (http://www.ebi.ac.uk); the range of alleles was taken from Saunders *et al*. (2004).

The microsatellite profile data will be used to determine which accessions will be retained for further study and how the data can be used to confirm homogenous groups of trees from which samples were collected for the determination of traits. After the verification of genetic identities, the potential hidden population sub-structure in the targeted population will be assessed using a Bayesian cluster analysis (Pritchard *et al*., 2000).

Microsatellite survey

After verification of identities, selected accessions will be surveyed with numerous microsatellites (> 200 loci) to generate sufficient genotype data for the association study.

Test for marker-trait association

Association mapping takes advantage of an existing population generated as a result of many meioses. Successive recombinations would tend to remove markers not tightly linked with a trait thereby making it easier to detect markers more closely linked to a trait of interest. There are two main approaches to association studies. In the first approach, groups are distinguished with a divergent dichotomy e.g. high vs. low expression and allele frequencies are compared across the groups. In the second, groups are distinguished according to their marker genotypes and phenotypic means are then compared across the groups. There has been some concern raised with regard to false positive results due to population structure, however this can be resolved with appropriate statistical tests that employ independent marker loci (Pritchard *et al*., 2000b and Pritchard, 2001).

Test-case for butterfat data

The distribution of butterfat content deviated from normality ($P < 0.01$) (Figure 3). Overall, butterfat content was 53.5% ± 0.1 (mean ± s.e.m) with a range of 15.4 from a sample of 390 accessions. The median value was 53.7% and the coefficient of variation was 3.9%. The lower and upper quartiles were determined as 52.2 and 54.9 respectively. These quartile values were used to subdivide the butterfat dataset into low and high groups. The resulting dataset showed a significant structuring effect ($?^2 = 79.3$; df = 2; $P < 0.001$) and is illustrated in Figure 4.

Figure 3. Distribution of butterfat content from 390 accessions of the International Cocoa Genebank, Trinidad.

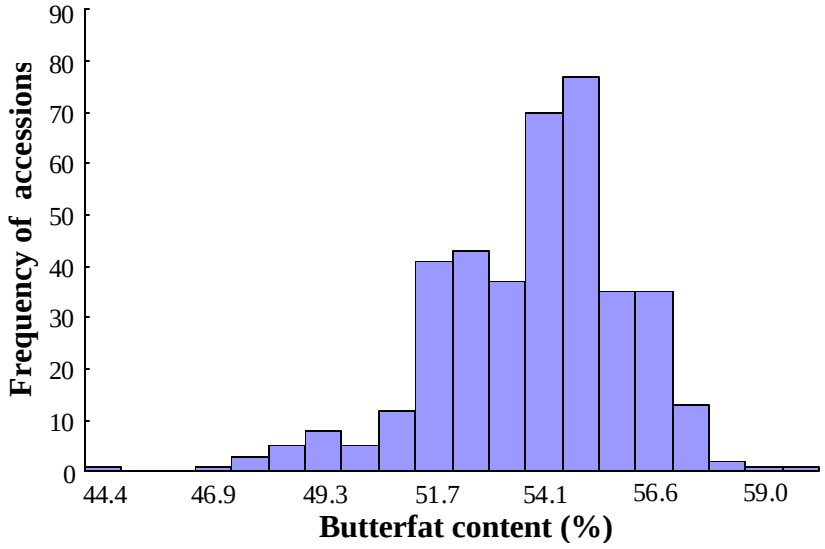
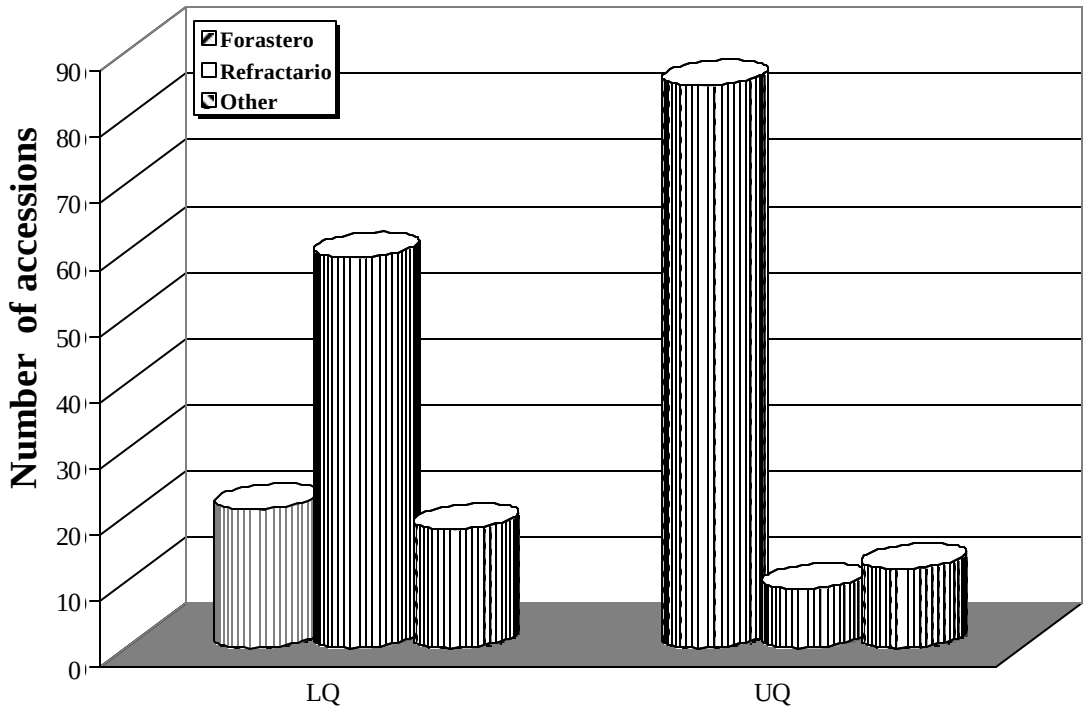


Figure 4. Accession grouping within lower (LQ) and upper (UQ) quartiles for the butterfat data.



Discussion

This project was conceptualised as an extension of the ongoing verification programme at CRU in which every tree of every accession in the ICG,T would be fingerprinted thereby allowing for judicious use of the extant germplasm. By adding many more microsatellite primers it is envisaged that markers could be obtained for traits of interest which would preclude the continued use of mapping populations and pinpoint candidate markers as diagnostic tools which breeders may utilise to select promising progeny from their breeding programmes. The butterfat data appear to have population stratification and would necessitate the application of statistical programs to redress this issue. The generation of marker data would however prove useful in testing for association with a variety of phenotypic traits.

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Progress in resolving identity issues among the Parinari accessions held in Trinidad: the contribution of the collaborative USDA/CRU project

M. Boccara and D. Zhang

Introduction

While searching for trees free of Witches' Broom disease in 1938, Dr. F.J. Pound observed about twenty of them on the left bank of the Marañon River above Parinari in Peru. He collected pods from these trees and seeds were planted in Barbados. After a suitable quarantine period, healthy budwood from the seedlings was forwarded to Trinidad, budded onto rootstock and subsequently planted, mostly in Marper Farm with some on the ICTA campus (now UWI). There is no clear indication about the number of trees selected, pods collected and seeds planted, however records have been kept of the identity of the established 'clones'.

Although 277 Parinari clones are reported to have been introduced to Trinidad (Pound, 1943), records available in CRU show that in 1943 only 147 clones were present in Marper Farm: 136 in Block D and 11 in Block C. Other PA trees were planted on the ICTA campus, among them 2 clones (PA7 [PER] and PA 35 [PER], Bartley pers. comm.) not represented in Marper.

When the ICG,T was established from 1986 to 1994, some PA clones were already missing. Only 113 clones were available, and 16 trees of each of these were planted per plot in UCRS.

Currently, in Marper Farm, 92 trees labelled PA are still alive in Block D, 9 in Block C and in UCRS, 111 clones are replicated in 133 plots (Table 1).

An international collaborative project on DNA fingerprinting of cacao germplasm was started in 2001, and priority was given to the analysis of Upper Amazon material such as the 'Parinaris' since they are of special interest to the international cocoa community.

Achievements

Leaves have been collected from every live tree in Blocks C and D of Marper Farm and from trees in UCRS when absent in Marper. Collection of some extra leaf samples was also undertaken for verification purposes.

A total of 158 samples were collected, including 101 from original trees from Marper fields, 16 from UCRS trees now absent from Marper as well as 41 samples from replicated trees in UCRS to check their conformity.

DNA samples were sent to the USDA-ARS[1] Beltsville laboratory to be analysed with 15 selected SSR primers, following a recommended protocol and guide-lines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS Beltsville laboratory are available for 1,200 clones from the ICG,T, including the PA accessions and have been used for different purposes:

[1] United States Department of Agriculture – Agriculture Research Service

Table 1. List of PA accessions and their locations in 2005.

	Marper Farm		UCRS			Marper Farm		UCRS	
PA 1 [PER]	D 111	+	5B		PA 81 [PER]	D 675	†	4A	+
PA 2 [PER]	D 101	+	5A		PA 82 [PER]	D 666	+	5B	
PA 3 [PER]	D 571	+	--		PA 84 [PER]	D 264	+	5B	+
PA 4 [PER]	C 1108	+	5B		PA 88 [PER]	D 677	+	5B	+
PA 5 [PER]	C 1101	+	--		PA 90 [PER]	D 627	+	5B	
PA 7 [PER]	**	†	--		PA 95 [PER]	D 663	+	5B	+
PA 12 [PER]	D 54	+	5A	+	PA 98 [PER]	D 295	+	--	
PA 13 [PER]	D158/D159	++	5A, 6B		PA 101 [PER]	D 831	†	--	
PA 14 [PER]	D 115	†	--		PA 103 [PER]	D 293	†	--	
PA 15 [PER]	D 596	+	6B		PA 105 [PER]	D 285	+	4A, 5A	+
PA 16 [PER]	D 573	+	6B	+	PA 107 [PER]	D 284	+	5A	
PA 18 [PER]	D 55	+	5A, 6B	+	PA 109 [PER]	D 304	†	6B	
PA 20 [PER]	D 24	+	4A		PA 110 [PER]	D 805	†	--	
PA 24 [PER]	D 401	+	5B	+	PA 111 [PER]	D 783	†	--	
PA 25 [PER]	D 262	†	--		PA 113 [PER]	D 384	+	5A, 5B	+
PA 26 [PER]	D 672	†	--		PA 114 [PER]	D 837	†	5B	+
PA 27 [PER]	D 676	+	5B	+	PA 115 [PER]	D 324	+	5B	
PA 29 [PER]	D 652	+	5B		PA 117 [PER]	D 332	†	--	
PA 30 [PER]	D 266	†	6B	+	PA 118 [PER]	D 288	+	5B	
PA 31 [PER]	D 670	†	--		PA 120 [PER]	D 318	+	6B	+
PA 32 [PER]	D 253	+	5A		PA 121 [PER]	D 393	+	6B	+
PA 33 [PER]	D 257	†	--		PA 123 [PER]	D 462	+	5A	
PA 34 [PER]	D 259	+	5B	+	PA 124 [PER]	D 492	+	4A, 6B	+
PA 35 [PER]	**	†	6B	+	PA 125 [PER]	D 433	+	4A, 5B	
PA 37 [PER]	D 244	+	5A	+	PA 126 [PER]	D 247	+	6B	+
PA 39 [PER]	D 685	†	5A	+	PA 127 [PER]	D 488	†	--	
PA 41 [PER]	D 667	+	6B		PA 128 [PER]	D 443	+	5A	+
PA 42 [PER]	D 643	†	--		PA 132 [PER]	D 357	+	5A	
PA 44 [PER]	D 233	+	6B		PA 134 [PER]	D 442	+	4A, 5A	
PA 45 [PER]	D 261	+	4A, 5A	+	PA 135 [PER]	D 481	+	4A, 5A	
PA 46 [PER]	D 235	+	6B	+	PA 136 [PER]	D 455	+	5A, 5B	
PA 48 [PER]	D 234	†	5A	+	PA 137 [PER]	D 430	+	5A	+
PA 49 [PER]	C 586	+	5A, 5B		PA 138 [PER]	D 842	†	--	
PA 51 [PER]	D 260	+	6B		PA 139 [PER]	D 426	+	4A, 5A	+
PA 52 [PER]	D 654	†	5A	+	PA 140 [PER]	D 439	+	5A	
PA 53 [PER]	D 243	+	5A		PA 141 [PER]	D 463	+	5B	+
PA 56 [PER]	D 238	+	5B		PA 143 [PER]	C 985	†	5A	+
PA 58 [PER]	D 678	†	--		PA 146 [PER]	D 441	†	--	
PA 59 [PER]	D 256	†	--		PA 148 [PER]	D 423	†	--	
PA 61 [PER]	D 121	+	6B		PA 149 [PER]	D 810	+	5B	+
PA 62 [PER]	D 255	+	--		PA 150 [PER]	D 679	+	6B	
PA 63 [PER]	D 348	+	5A	+	PA 151 [PER]	D 790	+	5B	
PA 64 [PER]	D 263	†	--		PA 152 [PER]	D 700	†	--	
PA 65 [PER]	D 507	+	5B	+	PA 156 [PER]	D 447	†	5A	+
PA 66 [PER]	C 880	+	5B		PA 157 [PER]	D 452	†	5B	+
PA 67 [PER]	D 629	+	5A, 5B	+	PA 159 [PER]	D 756	†	--	
PA 68 [PER]	D 638	†	5B	+	PA 165 [PER]	D 714	+	5B	+
PA 70 [PER]	D 634	+	5B	+	PA 167 [PER]	D 736	+	--	
PA 71 [PER]	D 674	†	6B	+	PA 168 [PER]	D 479	+	5A	
PA 72 [PER]	D 633	+	4A, 5B		PA 169 [PER]	D 491	+	6B	
PA 73 [PER]	D 254	+	--		PA 171 [PER]	D 467	+	5A, 6B	

+ Tree(s) alive and DNA sampled -- No record

† Tree dead ** Trees not planted in Marper, but were on the ICTA/UWI campus

Table 1 (continued). List of PA accessions and their locations in 2005.

	Marper Farm		UCRS			Marper Farm		UCRS	
PA 172 [PER]	D 728	†	--		PA 271 [PER]	C 716	+	5A	
PA 173 [PER]	D 851	†	5B	+	PA 272 [PER]	D 37	+	5A	
PA 175 [PER]	D 738	+	5B	+	PA 275 [PER]	C 764	+	4A	
PA 176 [PER]	D 704	+	4A, 5A		PA 276 [PER]	D 520	†	--	
PA 179 [PER]	D 472	+	5B		PA 279 [PER]	D 59	+	6B	+
PA 181 [PER]	D 460	†	--		PA 281 [PER]	D 28	†	--	
PA 184 [PER]	D 723	+	5B		PA 285 [PER]	D 378	+	--	
PA 185 [PER]	D 735	+	5B		PA 288 [PER]	C 782	†	--	
PA 186 [PER]	D 446	+	--		PA 289 [PER]	C 803	+	4A, 5B	+
PA 187 [PER]	D 482/D737	++	4A, 5B		PA 291 [PER]	D 214	+	6B	+
PA 188 [PER]	D 724	+	5B	+	PA 293 [PER]	C 817	+	--	
PA 189 [PER]	D 489	+	4A, 5B		PA 293 [PER]	D 762	+	4A, 5A	
PA 191 [PER]	D 743	+	5B	+	PA 294 [PER]	D 330	+	5B	+
PA 194 [PER]	D 707	†	4A, 5B	+	PA 295 [PER]	D 371	†	--	
PA 195 [PER]	D 493	†	6B	+	PA 296 [PER]	D 495	+	6B	+
PA 196 [PER]	D 458	+	5B	+	PA 297 [PER]	--	†	6B	+
PA 200 [PER]	D 710	+	4A, 5B	+	PA 299 [PER]	C 936	+	5B	+
PA 202 [PER]	D 453	†	5A	+	PA 300 [PER]	D 544	+	5B	+
PA 203 [PER]	D 709	+	--		PA 301 [PER]	D 733	+	5A	
PA 205 [PER]	D 715	+	5B		PA 303 [PER]	D 500	+	6B	+
PA 206 [PER]	D 745	†	--		PA 310 [PER]	D 732	†	5A	+
PA 207 [PER]	D 731	+	4A		PA 312 [PER]	--	†	6B	
PA 211 [PER]	D 766	+	5B		PA 319 [PER]	D 554	†	--	
PA 218 [PER]	D 708	+	6B		PA 320 [PER]	D 721	†	--	

+ Tree(s) alive and DNA sampled -- No record
† Tree dead ** Trees not planted in Marper, but were on the ICTA/UWI campus

- To assess the population identity of the Parinari group
- To discover potential mislabelling and to find conceivable explanations
- To detect off-type clones in the accession group
- To verify that the duplicate trees are identical
- To place individual trees within appropriate accession groups
- To assess population admixture.

Methods

- Genetic diversity of the 132 PA clones was assessed in relation to the 1,200 clones sampled in the ICG,T, using dissimilarity analysis (DARwin software, 5.0.142) and principal component analysis (PCA)(Genetix software, v.4.03).
- Duplicate trees were assessed by identifying matching multilocus genotypes among PA accessions.
- Mislabelled trees were identified by comparing their multilocus profile to the reference tree or a putative replicate.
- The identities of off-types were sought from matching profiles, and by using all the information available in historical records, publications and maps.

Results

Table 2. Confirmed surviving PA accessions at Marper Farm and UCRS.

Accession	DNA sample number	Field plot location	Accession	DNA sample number	Field plot location
PA 2 [PER]	FP1114	Marper D101	PA 134 [PER]	FP1197	Marper D442
PA 3 [PER]	FP184	Marper D571	PA 135 [PER]	FP392	Marper D481
PA 4 [PER]	FP619	Marper C1108	PA 136 [PER]	FP147	Marper D430
PA 12 [PER]	FP424	Marper D 54	PA 137 [PER]	FP307	Marper D430
PA 13 [PER]	FP821	Marper D158/159	PA 139 [PER]	FP308	Marper D426
PA 15 [PER]	FP177	Marper D596	PA 140 [PER]	FP1201	Marper D439
PA 16 [PER]	FP189	Marp er D573	PA 141 [PER]	FP1249	Marper D463
PA 20 [PER]	FP410	Marper D24	PA 149 [PER]	FP252	Marper D810
PA 24 [PER]	FP384	Marper D401	PA 150 [PER]	FP144	Marper D679
PA 29 [PER]	FP161	Marper D401	PA 151 [PER]	FP114	Marper D790
PA 30 [PER]	FP1634	5B C144 T1	PA 156 [PER]	FP1396	5A D295 T1
PA 32 [PER]	FP219	Marper D253	PA 157 [PER]	FP17	5B F466 T3
PA 34 [PER]	FP333	Marper D259	PA 165 [PER]	FP239	Marper D714
PA 39 [PER]	FP634	5A D264 T1	PA 168 [PER]	FP391	Marper D479
PA 41 [PER]	FP277	Marper D667	PA 169 [PER]	FP118	Marper D491
PA 44 [PER]	FP1141	Marper D233	PA 171 [PER]	FP149	Marper D467
PA 45 [PER]	FP67	Marper D261	PA 173 [PER]	FP13	5B F480 T8
PA 48 [PER]	FP1959	5A D354 T2	PA 175 [PER]	FP266	Marper D738
PA 49 [PER]	FP88	Marper C586	PA 176 [PER]	FP234	Marper D704
PA 51 [PER]	FP212	Marper D260	PA 179 [PER]	FP376	Marper D472
PA 52 [PER]	FP1399	5A D310 T8	PA 184 [PER]	FP229	Marper D723
PA 53 [PER]	FP1169	Marper D243	PA 185 [PER]	FP268	Marper D735
PA 56 [PER]	FP1162	Marper D238	PA 186 [PER]	FP378	Marper D446
PA 61 [PER]	FP208	Marper D121	PA 187 [PER]	FP228	Marper D737
PA 63 [PER]	FP1202	Marper D348	PA 188 [PER]	FP238	Marper D724
PA 70 [PER]	FP278	Marper D634	PA 189 [PER]	FP137	MarperD489
PA 71 [PER]	FP2464	6B D189 T14	PA 191 [PER]	FP265	Marper D743
PA 72 [PER]	FP305	Marper D633	PA 196 [PER]	FP176	Marper D458
PA 73 [PER]	FP325	Marper D254	PA 200 [PER]	FP111	Marper D710
PA 82 [PER]	FP279	Marper D666	PA 202 [PER]	FP1397	5A D309 T1
PA 84 [PER]	FP334	Marper D264	PA 203 [PER]	FP106	Marper D709
PA 88 [PER]	FP294	Marper D677	PA 207 [PER]	FP133	Marper D731
PA 90 [PER]	FP154	Marper D627	PA 211 [PER]	FP248	Marper D766
PA 95 [PER]	FP280	Marper D663	PA 218 [PER]	FP113	Marper D708
PA 98 [PER]	FP205	Marper D295	PA 271 [PER]	FP551	Marper C716
PA 105 [PER]	FP1179	Marper D285	PA 275 [PER]	FP560	Marper C764
PA 107 [PER]	FP1168	Marper D284	PA 279 [PER]	FP426	Marper D59
PA 113 [PER]	FP306	Marper D384	PA 289 [PER]	FP559	Marper C803
PA 115 [PER]	FP1196	Marper D324	PA 291 [PER]	FP50	Marper D214
PA 118 [PER]	FP1180	M arper D288	PA 293 [PER]	FP258	Marper D762
PA 120 [PER]	FP194	Marper D318	PA 294 [PER]	FP1203	Marper D330
PA 121 [PER]	FP1185	Marper D393	PA 296 [PER]	FP162	Marper D495
PA 123 [PER]	FP160	Marper D462	PA 297 [PER]	FP2421	6B D208 T1
PA 124 [PER]	FP1251	Marper D492	PA 299 [PER]	FP571	Marper C936
PA 125 [PER]	FP1200	Marper D433	PA 300 [PER]	FP382	Marper D544
PA 126 [PER]	FP331	Marper D247	PA 301 [PER]	FP270	Marper D733
PA 128 [PER]	FP388	Marper D443	PA 303 [PER]	FP185	Marper D500
PA 132 [PER]	FP389	Marper D357	PA 310 [PER]	FP1958	5A D288 T2

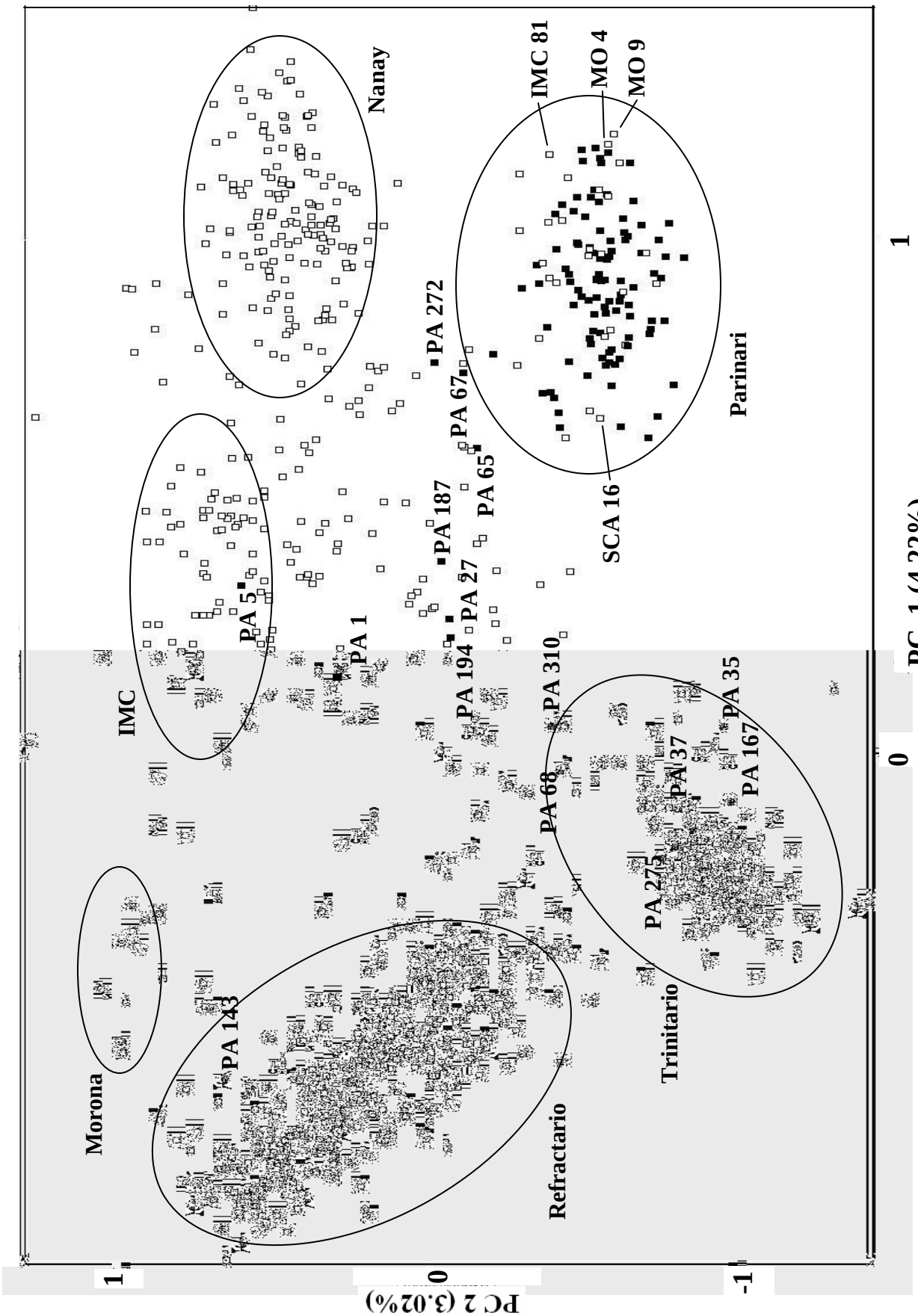


Figure 1. Principal component analysis for 1,200 accessions from the ICG,T. Trees with a PA label are shown as solid points.

Genetic diversity of the PA clones and potential mislabelling

The PCA using the Genetix software (Figure 1) shows clearly that the PA accessions form a group that is distinct from the rest of the clones analysed.

It also shows that:

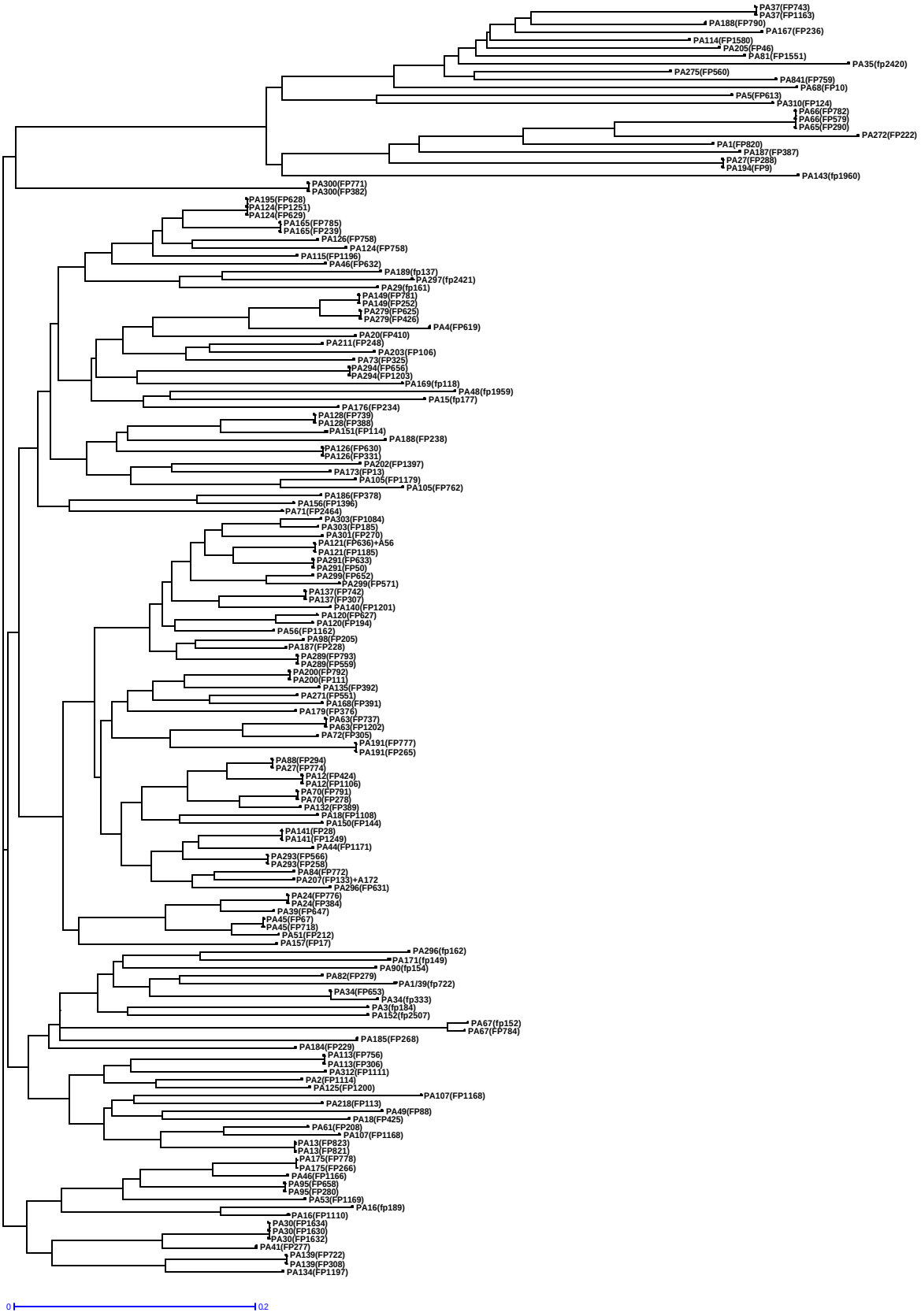
- Some accessions labelled PA belong to other accession groups such as Trinitario and IMC
- Some accessions not labelled as PA fall in the PA group.

Table 3. Confirmed PA clones and identical duplicates at Marper Farm and UCRS.

Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 12 [PER]	FP424	Marper D 54	PA 12 [PER]	FP1106	6B D 200
PA 13 [PER]	FP821	Marper D158	PA 13 [PER]	FP823	Marper D159
PA 16 [PER]	FP189	Marper D573	PA 16 [PER]	FP189	6B D186
PA 24 [PER]	FP384	Marper D401	PA 24 [PER]	FP776	5B F507
PA 30 [PER]	FP1634	5B C144 T1	PA 30 [PER]	FP1632	5B C144 T5
PA 34 [PER]	FP333	Marper D259	PA 34 [PER]	FP653	5B E347
PA 45 [PER]	FP67	Marper D261	PA 45 [PER]	FP718	4A F528
PA 63 [PER]	FP1202	Marper D348	PA 63 [PER]	FP737	5A D265
PA 70 [PER]	FP278	Marper D634	PA 70 [PER]	FP791	5B F489
PA 84 [PER]	FP334	Marper D264	PA 84 [PER]	FP772	5B E388
PA 95 [PER]	FP280	Marper D663	PA 95 [PER]	FP658	5B F460
PA 105 [PER]	FP1179	Marper D285	PA 105 [PER]	FP762	4A F526
PA 113 [PER]	FP306	Marper D384	PA 113 [PER]	FP756	5A D307
PA 120 [PER]	FP194	Marper D318	PA 120 [PER]	FP627	6B D188
PA 121 [PER]	FP1185	Marper D393	PA 121 [PER]	FP636	6B C166
PA 124 [PER]	FP1251	Marper D492	PA 124 [PER]	FP629	6B D192
PA 126 [PER]	FP331	Marper D247	PA 126 [PER]	FP630	6B D198
PA 128 [PER]	FP388	Marper D357	PA 128 [PER]	FP739	5A D272
PA 137 [PER]	FP307	Marper D430	PA 137 [PER]	FP742	5A D274
PA 139 [PER]	FP308	Marper D426	PA 139 [PER]	FP722	4A F529
PA 141 [PER]	FP1249	Marper D463	PA 141 [PER]	FP28	5B F431
PA 149 [PER]	FP252	Marper D810	PA 149 [PER]	FP781	5B F474
PA 165 [PER]	FP239	Marper D714	PA 165 [PER]	FP785	5B F451
PA 175 [PER]	FP266	Marper D738	PA 175 [PER]	FP778	5B F473
PA 191 [PER]	FP265	Marper D743	PA 191 [PER]	FP777	5B F536
PA 196 [PER]	FP176	Marper D458	PA 196 [PER]	FP780	5B E371
PA 200 [PER]	FP111	Marper D710	PA 200 [PER]	FP792	5B F545
PA 279 [PER]	FP426	Marper D59	PA 279 [PER]	FP625	6B D197
PA 289 [PER]	FP559	Marper C803	PA 289 [PER]	FP793	5B F535
PA 291 [PER]	FP50	Marper D214	PA 291 [PER]	FP633	6B C167
PA 293 [PER]	FP258	Marper D762	PA 293 [PER]	FP566	Marper C817
PA 294 [PER]	FP1203	Marper D330	PA 294 [PER]	FP656	5B E389
PA 296 [PER]	FP162	Marper D495	PA 296 [PER]	FP631	6B D207
PA 299 [PER]	FP571	Marper C936	PA 299 [PER]	FP652	5B E398
PA 300 [PER]	FP382	Marper D544	PA 300 [PER]	FP771	5B E407
PA 303 [PER]	FP185	Marper D500	PA 303 [PER]	FP1084	6B D211

The Cluster analysis of the 158 DNA samples of PA labelled accessions using the DARwin software (Figure 2) provided additional information:

Figure 2. Dendrogram of dissimilarity analysis run on 158 PA accessions samples.



- 96 accessions labelled PA are grouped together in a cluster (Figure 2)
- Among the 41 pairs of original trees and their duplicates, 37 are matching (Table 3)
- Some PA labelled accessions and their homonymous duplicates, are not identical even though they belong to the PA group (Table 4a)
- Some PA labelled accessions and their duplicate trees were both found to be off-type (Table 4b)
- Some PA accessions share the same profile but bear different names (Table 4c)
- Some PA accessions have a duplicate which is an off-type (Table 4d)
- There is a cluster of "off-type" PA clones (Table 5).

Table 4a. PA confirmed accessions and PA non-identical duplicates.

Confirmed PA homonymous mislabelled clones					
Accession I	**DNA sample number**	**Field plot location**	**Accession II**	**DNA sample number**	**Field plot location**
PA 18 [PER]	FP425	Marper D55	PA 18 [PER]	FP1108	6B C145
PA 46 [PER]	FP1166	Marper D235	PA 46 [PER]	FP632	6B C159 T9

Table 4b. Confirmed off-type PA accessions and identical duplicates.

Confirmed off-type PA identical clones sampled from different plot and field					
Accession I	**DNA sample number**	**Field plot location**	**Accession II**	**DNA sample number**	**Field plot location**
PA 37 [PER]	FP1163	Marper D244	PA 37 [PER]	FP743	5A D253 T5
PA 65 [PER]	FP290	Marper D507	PA 66 [PER]	FP579	Marper C880
PA 66 [PER]	FP579	Marper C880	PA 66 [PER]	FP782	5B E356 T2
PA 27 [PER]	FP288	Marper D676	PA 194 [PER]	FP9	5B F513 T1

Table 4c. Confirmed PA identical accessions.

Confirmed PA synonymous mislabelled clones					
Accession I	**DNA sample number**	**Field plot location**	**Accession II**	**DNA sample number**	**Field plot location**
PA 124 [PER]	FP1251	Marper D492	PA 195 [PER]	FP628	6B C165 T1
PA 88 [PER]	FP294	Marper D677	PA 27 [PER]	FP774	5B E423 T4

Table 4d. Confirmed PA accessions and off-type duplicate.

Homonymous mislabelling					
Accession I	**DNA sample number**	**Field plot location**	**Accession II**	**DNA sample number**	**Field plot location**
PA 187 [PER]	FP387	Marper D482	PA 187 [PER]	FP228	Marper D737
PA 188 [PER]	FP238	Marper D724	PA 188 [PER]	FP790	5B F494 T9

Table 5. Distribution of the PA off-type accessions.

Accessions clustered with Trinitario accessions					
PA 37 [PER]	FP1163	Marper D225	PA 205 [PER]	FP46	Marper D715
PA 188 [PER]	FP790	5B F494 T11	PA 35 [PER]	FP2420	6B D225 T3
PA 167 [PER]	FP236	Marper D736	PA 275 [PER]	FP560	Marper C764
PA 114 [PER]	FP1580	5B F514 T3	PA 68 [PER]	FP10	5B E369 T14
PA 81 [PER]	FP1551	4A F527 T2	PA 841 [PER]	FP759	4A F517 T4

Accession clustered with IMC accessions			
PA 5 [PER]	FP613	Marper C1101	

Other PA off-type accessions					
PA 1 [PER]	FP820	Marper	PA 65 [PER]	FP290	Marper D507
PA 66 [PER]	FP579	Marper C880	PA 187 [PER]	FP387	Marper D482
PA 27 [PER]	FP288	Marper D676	PA 194 [PER]	FP9	5B F513 T1
PA 143 [PER]	FP1960	5A D342	PA 310 [PER]	FP124	Marper D732

Mislabelling analysis

PA labelled accessions

Trees with PA labels that we found to be off-types are examined below to search for feasible explanations of the mislabelling.

Trees showing a PA profile

PA 195 [PER] planted in UCRS Field 6B, plot C165 is a duplicate of PA 124 [PER].The original PA 195 [PER] now missing, was in position Marper D493, adjacent to the original PA 124 [PER] clone in position D492. It is almost certain than budwood was mistakenly taken from PA 124 [PER] rather than PA 195 [PER].

PA 27 [PER] planted in UCRS field, plot E423 was not propagated from the clone PA 27 [PER] in Marper D676, but instead from the contiguous tree PA 88 in D677.

Trees showing a Trinitario profile

PA 37 [PER] from Marper D244 and the duplicate tree sampled from the UCRS both showed a Trinitario profile, implying that propagation was done from rootstock.

Whereas the PA 275 [PER] tree, Marper C764 shows a Trinitario profile, the neighbouring tree in C765, MOQ 6/29, shows a PA profile; the tree labelled PA 275 [PER] is rootstock and the tree labelled MOQ 6/29 is probably PA 275 [PER]. MOQ 6/29 was not propagated when the ICG,T was established at UCRS.

PA 205 [PER], Marper D715 and the neighbouring tree PA 167 [PER], Marper D736 have both been detected earlier by morphological observation to be rootstock. While PA 167 [PER], Marper D736 was not duplicated when the ICG,T was established, PA 205 [PER], Marper D715 and PA 275 [PER], Marper C764 were; morphological observation and DNA analysis should be performed to assess the identity of the replicate trees.

Accessions PA 35 [PER], UCRS Field 6B, plot D225 T3, PA 68 [PER], UCRS Field 5B, plot E369 T14, PA 81 [PER], UCRS Field 4A, plot F527 T2, PA 114 [PER], UCRS Field 5B, plot F514 T3 must have been all propagated from rootstock; the mother trees are now missing.

Tree 11 of PA 188 [PER] in UCRS Field 5B, plot F494 shows a Trinitario profile, although the fingerprint of the mother tree in Marper D724 shows the PA profile: this demonstrates that budwood for this tree was taken from the rootstock instead from the grafted tree. There is a need to authenticate the 14 other trees of the UCRS plot.

PA 841 [PER] had been already detected as being an off-type by morphological criteria and has been renamed CRU 4A/1.

Trees showing an IMC accession profile

The results of DNA analysis shows that the tree PA 5 [PER], Marper C1101 belongs to the IMC group. However, analysis of the DNA from the tree immediately next to it, CL19/49 infers that it belongs to the PA group and could be PA 5. The fact that the profile of the tree IMC 22 position C1102 matches NA 8, position C1058, makes plausible the explanation of the following mislabelling:

The tree in C1100 is PA 5 [PER] and the tree in C1101 is IMC 22, the tree in C1102 being a duplicate of NA 8, C1058 (Figure 3).

The duplicate trees in UCRS need to be checked.

Figure 3. Excerpt of the Block C field map in Marper Farm (trees are shown by position number/clone name).

1099/IMC 9	**1100/CL19/49**	**1101/PA 5**	**1102/IMC 22**

1057/B 8/9			
	1058/NA 8	**SM 11**	**1059/B 16/1**

Other PA off-type accessions

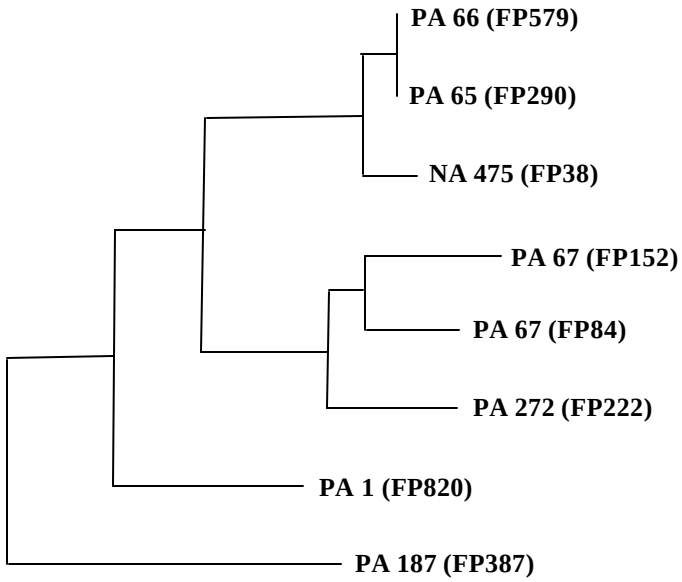
PA 3/10 [PER] (FP124) in Marper D732 is confirmed as an off-type; morphological observations had already led to the renaming of the tree as CRU 88. However the sample from PA 310 [PER] in UCRS Field 5A, plot D288, matches the PA group.

PA 143 [PER] (FP1960) collected from UCRS Field 5A, plot D342 T2 shows a profile that falls in the Refractario group. It is worth noting that the mother tree in Marper C985, now dead, was the first tree in the row, as was the case for PA 3/10 [PER]; there could have been some imprecision near the limits of the fields. A similar explanation could be given for the tree labelled PA 272 [PER], also an off-type, position D37 planted in the first row of the field.

In the dendrogram constructed with the DARwin software including all the 1,200 samples, 6 other off-type accessions were grouped in the same cluster as PA 272 [PER] (Figure 4). These clones are clearly separated from the PA group and there is some ambiguity about their sources for propagation in the ICG,T:

- PA 65 [PER] Marper D507 and PA 66 [PER] Marper C880 are duplicate trees.
- PA 194 [PER] planted in UCRS Field 5B was propagated from PA 27 [PER], Marper D676, which is an off-type. The accession labelled PA 27 [PER] in UCRS was collected from the neighbouring Marper tree PA 88 [PER]. PA 27 [PER], PA 88 [PER] and PA 194 [PER] were planted contiguously in Marper, but PA 194 [PER] has since died.

Figure 4. Excerpt of the dendrogram of dissimilarity analysis run on 1,200 DNA samples from cacao accessions in the ICG,T.



- Analysis of the data shows that PA 187 [PER], Marper D737 differs from PA 187 [PER], Marper D762. A question mark had been inserted in the notes for the tree in D737, dating from 1943.
- PA 272 [PER] and PA 1 [PER] are also off-types and have been propagated in UCRS: an assessment of all the duplicate trees is required.

Accessions not labelled as PA

The dissimilarity analysis (Figure 4) and the principal component analysis (Figure 1) of the 1,200 DNA samples shows that some accessions not labelled as PA, are genetically related to the PA clones (Table 6). These are shown as open points within the Parinari group in Figure 1.

Table 1. Original or duplicated accessions with a PA profile at Marper Farm and UCRS.

Clones with PA-like profile					
NA 423	FP262	Marper D757	MO 81	FP764	Marper D192
NA 759	FP32	5B H711 T15	IMC 41	FP1069	6B F418 T1
NA 851	FP21	5B F475 T2	IMC 81	FP1635	6B F421 T2
NA 534	FP11	5B G630 T1	B9/10-33 [POU]	FP299	Marper D632
NA 387	FP745	5A D251 T2	B21/6[POU]	FP1204	Marper D395
NA 312	FP795	5B G614 T2	CL 19/49	FP1603	Marper C1000
NA 372	FP216	Marper D417	CL 19/51	FP66	Marper D27
NA 176	FP1662	4A D389 T4	MOQ 6/29	FP2103	Marper C765
NA 686	FP750	6A B105 T5	SCA16	FP284	Marper D671
MO 4	FP36	5B B111 T3	SLA 16	FP2707	5B D242 T8
MO 9	FP253	Marper D835			

Original trees potentially mislabelled

The MO 9 (FP253) DNA sample collected from the tree in Marper D835 shows a profile close to the adjacent accession PA 149 [PER] located in Marper D810. MO 9 could be a seedling of PA 149 [PER] as well as the 4 other unidentified surrounding trees, renamed CRU 134, CRU 135, CRU 136 and CRU 137.

The B 9/10-33 [POU] clone in Marper D632 shows a PA profile, and could be a seedling of the contiguous tree, PA 72 [PER] in Marper D633 that has a similar profile. This hypothesis of mislabelling is reinforced by the fact the tree in the next row (Marper D601), IMC 16 is also an off-type and shows a Nanay profile. The tree in Marper D602, now dead, was NA 105.

The analysis of B 21/6 [POU] (Marper D395) shows that this tree is a duplicate of the PA 140 [PER] tree planted in D439, just opposite and in the next row.

NA 423 (Marper D757) was planted next to PA 159 [PER], now dead, which could be its true identity. Similarly CL 19/51 in Marper D27 was the neighbour of a dead PA tree, as is NA 372 in D417.

The tree in Marper D671 bearing both labels SCA 16 and SLA 16, and its replicate tree in UCRS labelled SLA 16, share a PA profile; PA 31 [PER] and PA 26 [PER], now dead, were formerly planted in the vicinity.

The trees labelled MO 81 still present on the Campus fields should be sampled to compare with the MO 81 accession planted in Marper showing a PA profile.

Replicated mislabelled accessions

The dissimilarity analysis of DNA fingerprints shows that the MO 4 and IMC 41 accessions planted in UCRS are identical duplicates of a PA accession. Mother-trees of these accessions were planted contiguously in Marper D683 and D684, but are now dead. These trees could have been propagated from a seedling issued from one of the PA accessions nearby, PA 200 [PER] or PA 207 [PER]. Two trees CRU 86 and Marper 42, whose identities need to be clarified, are still alive in the vicinity.

The accession IMC 81 planted in UCRS field 6B, shares the same PA profile as the above, even though there is no evidence that such an accession was ever planted in Marper Farm; its identity was probably mistaken for IMC 41.

Tree 5 of the UCRS Field 6A, plot B105 shows a PA profile, although the fingerprint of the mother tree in Marper C383 shows the correct profile of NA 686. There is a need to authenticate the other NA 686 trees planted in fields 5A and 6A at UCRS.

The accessions NA 534 (FP11) and NA 387 (FP745) established in UCRS, share the same PA profile. The mother-trees, now dead, of these accessions were planted contiguously in Marper D781 and D782; the only living tree in the vicinity is an unidentified Marper 35 accession.

As NA 475 growing in Marper D469 was identified as being rootstock when collection of samples was undertaken, only the DNA from the NA 475 accession growing in UCRS 5B plot F534 was analysed. The result shows that budwood for propagation was taken from PA 65 [PER] located in the next row in Marper.

NA 176 growing in UCRS Field 4A plot D389 has the same profile as the original PA 176 [PER] accession planted in Marper D704. Mislabelling must have occurred at the time of the replication.

NA 312 growing in UCRS Field 5B plot G614 has the same profile as the PA 312 [PER] accession planted in UCRS Field 6B plot D209, although there is no record of an original tree in

Marper with this clone name.

Discussion and conclusion

From the genetic diversity revealed by the analysis of SSR profiles, Parinari accessions can be clearly identified as a distinct group of accessions.

Phenotypic diversity observed and analysed in CRU (Bekele *et al.*, 2005), has been reinforced by this work: for example, IMC 41 described as phenotypically closely linked to PA 171 [PER] and PA 303 [PER], has now been recognized as a member of the PA group by molecular analysis. "Low branching habit and dense canopy with long, dark leaves", is shared by the PA 13 [PER] and PA 107 [PER] accessions (Bartley, 2005); the dissimilarity analysis of the DNA shows that these 2 clones are very closely linked.

The use of 15 markers has been efficient in completing the unambiguous identification of accessions amongst the group, and to detect population admixture. The analysis confirmed 96 PA clones as being correct, whilst only 6 cannot be included in the group.

For mislabelled accessions, feasible explanations can be found in most cases.

More verification of duplicated trees will be needed to reduce the risk of erroneous distribution from UCRS. It is valuable to know that the clones MO 4 and MO 9, selected to be propagated and later distributed to 13 producing countries as part of the CFC Project Collection, happen to be seedlings of PA clones.

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Characterisation

Morphological characterisation of accessions from the International Cocoa Genebank, Trinidad – recent highlights

F.L. Bekele, G.G. Bidaisee and J. Bhola

Introduction

The objective of this study was to examine variation in the expression of 25 morphological traits recorded for 1,180 accessions from the International Cocoa Genebank, Trinidad (ICG,T). Particular attention was paid to the characters of economic interest such as bean size, number, weight and pod index (a component of yield potential[1]). These results will facilitate selection of superior accessions of potential value for genetic enhancement of cacao as well as the management of the ICG,T (Bekele, 1999; Bekele and Bekele, 1996; Bekele *et al*., 1994; 2006; Iwaro *et al*., 2003; 2005).

Materials and methods

At the end of December 2005, 1,180 accessions representing 71 accession groups from the ICG,T had been fully characterised in terms of 25 morphological traits (Table 1), as described in earlier Annual Reports, and by Bekele and Butler (2000) and Bekele *et al*. (2006). The numbers of accessions characterised in terms of the various categories of descriptors were as follows:

Leaf	1,750
Flower	1,764
Fruit	1,198

Data for the fully characterised accessions were subjected to statistical analyses to examine the variation within traits and among the accessions studied. Descriptive statistics for each descriptor studied (Table 2) were generated using MINITAB (Minitab Inc., 1997).

One hundred and twenty-five accessions, representing 29 accession groups (Table 3), were observed to have pod index values less than or equal to 20.5, and are considered to have good yield potential. These were examined using principal component analysis (PCA) and cluster analysis (CA) (NTSYSpc Ver. 2.10b, (NTSYS, 2000)). The data were first standardised since varied scales of measurement were associated with the descriptors used (Table 1). In order to display the relationships among the accessions in three dimensions, 3-D PCA was performed on a correlation matrix of the data. Similarity matrices were generated to perform CA. The group average method (UPGMA - unweighted pair-group method using arithmetic means) was used to perform CA as recommended for this type of dataset (mixed continuous and categorical) (Sneath and Sokal, 1973). A dendrogram was used to depict, in two dimensions, the inter-relationships among the favourable accessions studied.

[1] Yield potential is defined as the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and with pests, diseases, weeds and other stresses effectively controlled.

Table 1. Descriptors for characterisation - their states and sample sizes.

Descriptor	State [sample size, n]
Flower, anthocyanin intensity in column of pedicel	1=green, 2=reddish, 3=red [n=10].
Flower, sepal length (mm) [n=10]	
Flower, anthocyanin intensity on ligule	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Flower, ligule width (mm) [n=10]	
Flower, anthocyanin intensity in filament	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Flower, style length (mm) [n=10]	
Flower, ovule number [n=10]	
Fruit, (pod) shape	1= oblong, 2= elliptic, 3=obovate, 4= orbicular [n=10], 5= other.
Fruit, basal constriction	0=absent, 1=slight, 2=intermediate, 3=strong, 4=wide shoulder [n=10]
Fruit, apex form	1=attenuate, 2=acute, 3=obtuse, 4=rounded, 5=mammillate, 6=indented [n=10]
Fruit, surface texture (rugosity or degree of wartiness)	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, anthocyanin intensity in mature ridges	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, ridge disposition	1=equidistant, 2=paired [n=10]
Fruit, primary ridge separation	1=slight, 2=intermediate, 3=wide [n=10]
Fruit, pod wall hardness [n=10]	3= = 1.6 MPa, 5 = > 1.6 MPa = 2.0 MPa, 7= > 2.0 MPa
Fruit, length (cm) [n=10]	
Fruit, width (cm) [n=10]	
Seed (bean), number [n=10]	
Seed, shape	1=oblong 2=elliptic 3=ovate
Seed, cotyledon colour	1=white, 2=grey, 3=light purple, 4=medium purple, 5=dark purple, 6=mottled [n=40]
Wet bean weight (total) (g) [n=10]	
Cotyledon length (cm) [n=20].	
Cotyledon width (cm) [n=20].	
Cotyledon weight (g) [n=20]	
Pod index (the number of pods required to produce 1 kg of dried cocoa) [n=10]	

Results

Considerable variation was observed for all the characters studied (Table 2), as was done previously at CRU (Bekele and Bekele, 1996; Bekele *et al*., 2006; Iwaro *et al*., 2003) and reported in earlier Annual Reports. The coefficients of variation for the quantitative traits ranged from 9.1% to 25.6% (Table 2).

All of the quantitative characters except bean and sepal length, including the characters of economic interest, *viz*., bean number, cotyledon weight and pod index, were normally distributed ($P < 0.005$). These ranged from 17.2 to 59.3; 0.44 g to 1.84 g and 13.9 to 91, respectively. UF 11 had the lowest pod index value (13.9) (Table 4). This was outstanding when compared to the overall mean of 28, which was recorded for the 1,180 accessions studied (Table 2). However, UF 11 is highly susceptible to Witches' Broom disease and this may limit its usefulness for breeding. The groups with the largest proportions of accessions with low pod index values were JA (18.4%), CRU (17.6%), ICS (12.8%) and IMC (8.8%). Accessions with favourable pod index values were found among the groups well represented in the ICG,T, *viz*., Forasteros, Trinitarios and Refractarios (Tables 3 and 4).

Table 2. Descriptive statistics for the descriptors used to characterise 1,180 accessions from the ICG,T.

Descriptor	Mean	Standard Error	Coefficient of variation (%)	Minimum value	Maximum value
LIGULE COLOUR				0	7
FILAMENT COLOUR				0	7
PEDICEL COLOUR				1	3
SEPAL LENGTH (mm)	7.6	0.025	11.4	5.1	10.5
LIGULE WIDTH (mm)	2.44	0.009	12.5	1.49	3.83
OVULE NUMBER	43.7	0.16	12.3	33.0	62.0
STYLE LENGTH	2.27	0.009	14.8	1.21	3.56
MATURE POD RIDGE COLOUR				0	7
POD SHAPE				1	5
POD BASAL CONSTRICTION				0	4
POD APEX FORM				1	6
POD SURFACE TEXTURE				0	7
POD RIDGE DISPOSITION				1	2
POD RIDGE PAIR SEPARATION				1	3
BEAN COLOUR				1	5
BEAN SHAPE				1	3
POD LENGTH (cm)	15.9	0.05	11.6	10.6	22.6
POD WIDTH (cm)	8.1	0.02	9.3	6.0	11.1
WET BEAN WEIGHT (g)	56.4	0.39	23.6	20.2	102.0
BEAN NUMBER	39.1	0.17	14.9	17.2 (CL 27/96)	59.3 (IMC 39)
COTYLEDON WEIGHT (g)	0.97	0.006	20.7	0.44 (B 9/10-28)	1.84 (UF 11)
COTYLEDON LENGTH (cm)	2.15	0.006	9.1	1.37	2.72
COTYLEDON WIDTH (cm)	1.21	0.004	10	0.63	1.56
POD INDEX	28	0.21	25.6	13.9 (UF 11)	91.1 (B 9/10-35)

Table 3. Accession groups with accessions displaying favourable pod index (PI) values.

Accession Group	Number of accessions with PI ? 20.5 (total observed)	Proportion of the 125 accessions with PI = 20.5	Accession Group	Number of accessions with PI ? 20.5 (total observed)	Proportion of the 125 accessions with PI = 20.5
AM [POU]	6 (50)	4.8	MATINA	1 (3)	0.8
B [POU]	4 (66)	3.2	MOQ	2 (44)	1.6
CC	1 (3)	0.8	NA	5 (151)	4.0
CL [POU]	2 (49)	1.6	PA [PER]	3 (107)	2.4
CLM [POU]	1 (14)	0.8	POUND [POU]	2 (24)	1.6
CRU	22 (68)	17.6	SC	1 (3)	0.8
EET [ECU]	3 (11)	2.4	SCA	1 (11)	0.8
FSC	1 (1)	0.8	SD	1 (1)	0.8
GS	2 (17)	1.6	SILECIA	1 (1)	0.8
ICS	16 (58)	12.8	SJ [POU]	2 (24)	1.6
IMC	11 (57)	8.8	SLA	2 (18)	1.6
JA [POU]	23 (97)	18.4	SNK	1 (1)	0.8
LCT EEN	1 (13)	0.8	TRD	2 (26)	1.6
LP [POU]	4 (53)	3.2	UF	3 (13)	2.4
M	1 (3)	0.8			

Table 4. The twenty most promising accessions in terms of pod index values from among the 1,180 observed.

Accession group	Accession number	Suffix	Pod index	Cotyledon weight	Bean number
UF	11		13.9	1.84	39
UF	12		14.9	1.77	38
CRU[1]	153		15.2	1.60	41
CRU	147		15.3	1.43	46
JA [POU]	5	/36	15.5	1.40	46
CRU	122		15.5	1.47	44
CRU	34		15.6	1.37	47
CRU	138		15.6	1.35	47
ICS	60		15.6	1.64	39
JA [POU]	5	/7	15.7	1.41	45
ICS	68		16.0	1.26	50
IMC	97		16.0	1.25	50
CRU	51		16.1	1.41	44
NA	81		16.1	1.49	42
ICS	43		16.1	1.64	38
CRU	38		16.3	1.26	49
CRU	35		16.4	1.43	43
CRU	73		16.4	1.23	50
SILECIA	8		16.4	1.40	44
PA [PER]	205		16.5	1.40	43

[1]It is interesting to note that CRU accessions (origin unknown) comprise almost 50% (9 out of 20) of the best accessions in terms of pod index.

Figure 1a. Principal component plots of the 125 accessions with favourable pod index values.

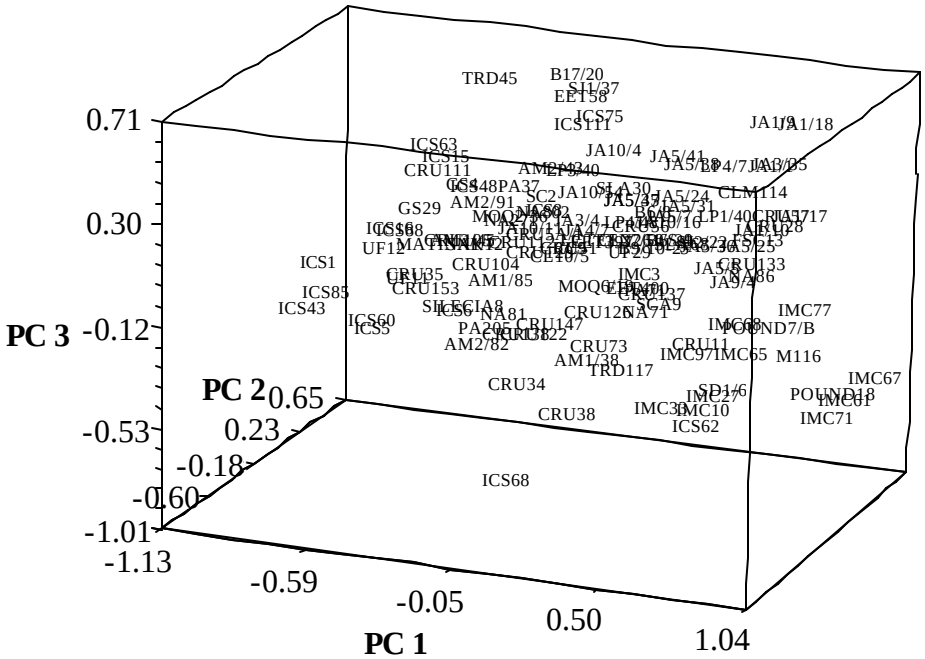
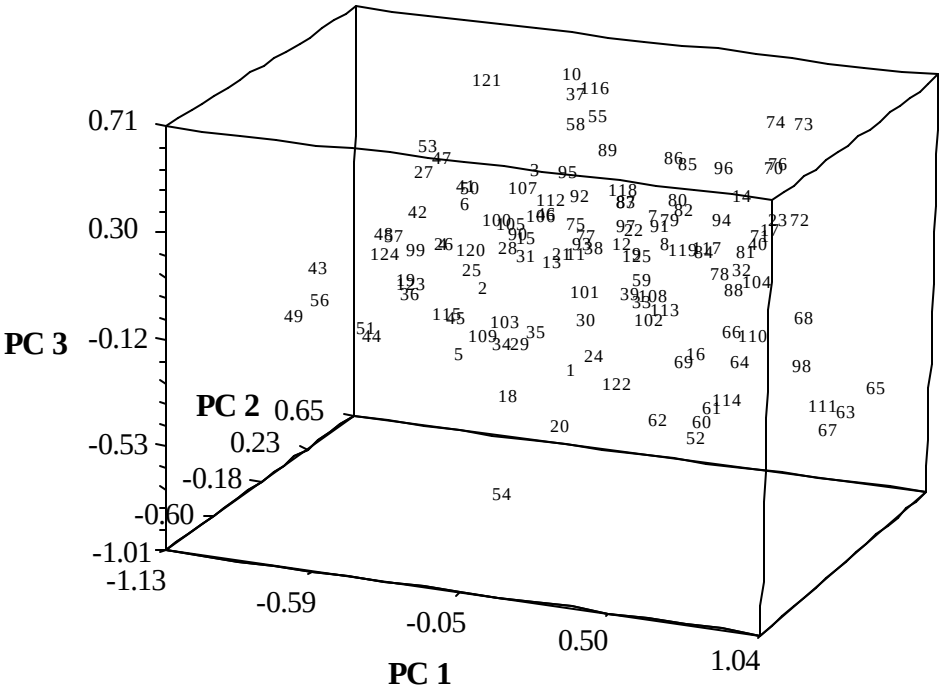


Figure 1b. Principal component plots of the 125 accessions with favourable pod index values.

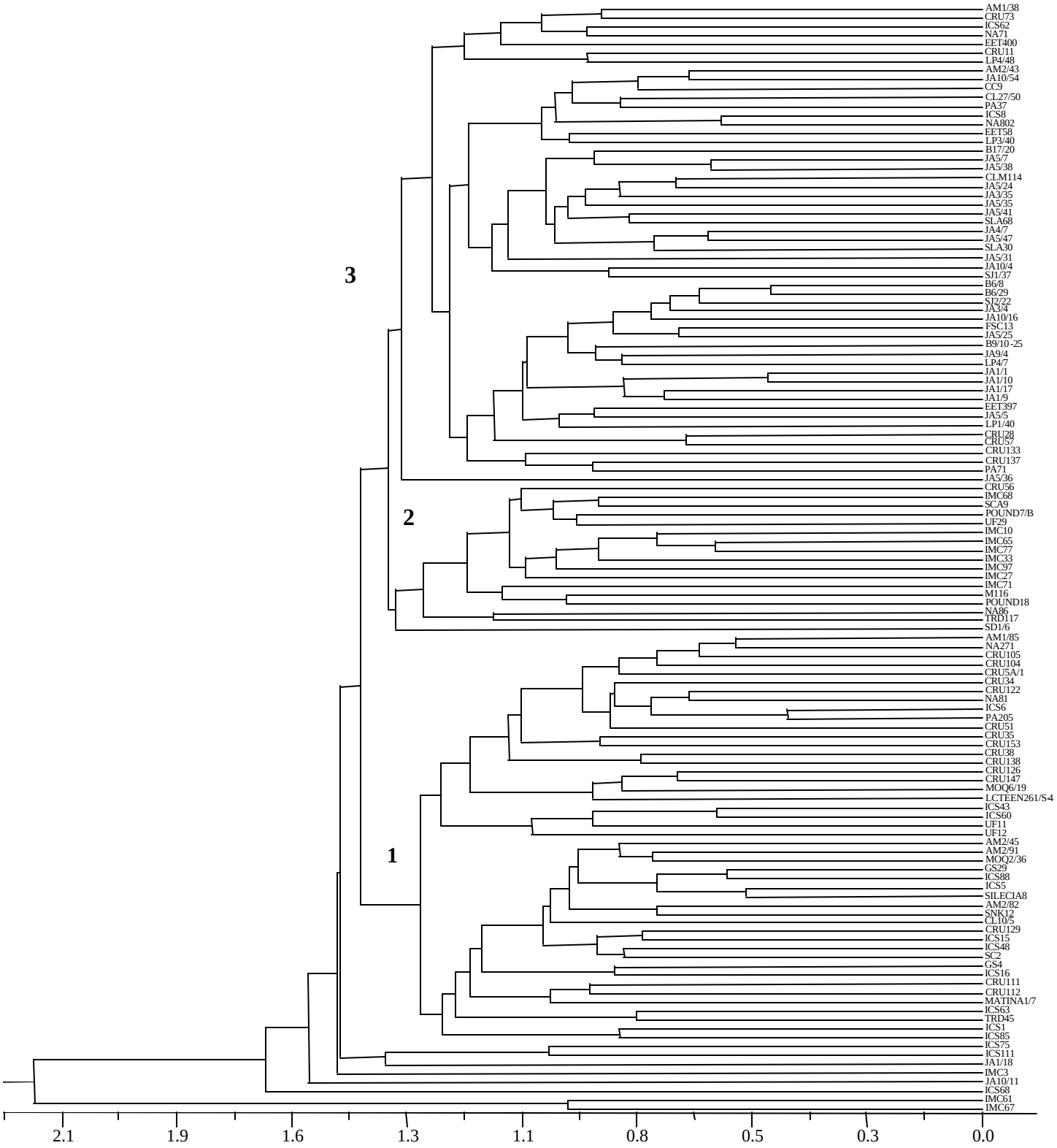


Legend for Figure 1b.

AM 1/38 [POU]	1	CRU 56[1]	22	ICS 1	43	IMC 65	64	JA 5/38 [POU]	85	NA 802	106
AM 1/85 [POU]	2	CRU 57	23	ICS 5	44	IMC 67	65	JA 5/41 [POU]	86	PA 37	107
AM 2/43 [POU]	3	CRU 73	24	ICS 6	45	IMC 68	66	JA 5/47 [POU]	87	PA 71	108
AM 2/45 [POU]	4	CRU 104	25	ICS 8	46	IMC 71	67	JA 9/4 [POU]	88	PA 205	109
AM 2/82 [POU]	5	CRU 105	26	ICS 15	47	IMC 77	68	JA 10/4 [POU]	89	POUND 7/B[POU]	110
AM 2/91 [POU]	6	CRU 111	27	ICS 16	48	IMC 97[1]	69	JA 10/11 [POU]	90	POUND 18 [POU]	111
B 6/8 [POU]	7	CRU 112	28	ICS 43	49	JA 1/1 [POU]	70	JA 10/16 [POU]	91	SC 2	112
B 6/29 [POU]	8	CRU 122	29	ICS 48	50	JA 1/10 [POU]	71	JA 10/54 [POU]	92	SCA 9	113
B 9/10-25 [POU]	9	CRU 126	30	ICS 60	51	JA 1/17 [POU]	72	LCTEEN 261/S-4	93	SD 1/6	114
B 17/20 [POU]	10	CRU 129	31	ICS 62[1]	52	JA 1/18 [POU]	73	LP 1/40 [POU]	94	SILECIA 8	115
CC 9	11	CRU 133	32	ICS 63	53	JA 1/9 [POU]	74	LP 3/40 [POU]	95	SJ 1/37	116
CL 27/50	12	CRU 137	33	ICS 68	54	JA 3/4 [POU][1]	75	LP 4/7 [POU]	96	SJ 2/22	117
CL 10/5	13	CRU 138	34	ICS 75	55	JA 3/35 [POU]	76	LP 4/48 [POU]	97	SLA 30	118
CLM 114	14	CRU 147	35	ICS 85	56	JA 4/7 [POU]	77	M 116	98	SLA 68	119
CRU 5A/1	15	CRU 153	36	ICS 88[1]	57	JA 5/5 [POU][1]	78	MATINA 1/7	99	SNK 12	120
CRU 11	16	EET 58 [ECU]	37	ICS 111	58	JA 5/7 [POU]	79	MOQ 2/36	100	TRD 45	121
CRU 28	17	EET 397 [ECU]	38	IMC 3	59	JA 5/24 [POU]	80	MOQ 6/19	101	TRD 117	122
CRU 34	18	EET 400 [ECU]	39	IMC 10	60	JA 5/25 [POU]	81	NA 71[1]	102	UF 11	123
CRU 35	19	FSC 13	40	IMC 27	61	JA 5/31 [POU]	82	NA 81[1]	103	UF 12	124
CRU 38	20	GS 4	41	IMC 33	62	JA 5/35 [POU]	83	NA 86	104	UF 29	125
CRU 51	21	GS 29	42	IMC 61[1]	63	JA 5/36 [POU]	84	NA 271	105		

[1]Plot contains possibly mis-labelled trees

Figure 2. Dendrogram depicting the relationships among 125 accessions with favourable pod index values. Three distinct clusters are denoted by numbers in bold.



The phenotypic relationships among the accessions with favourable pod index values are depicted in Figures 1a, 1b and 2. In Figure 2, ICS 6 (Trinitario) and PA 205 [PER] are phenotypically very similar; the dissimilarity coefficient (DC) discriminating between them is 0.45. This is noteworthy because Boccara (2005) reported that PA 205 [PER] belongs to the Trinitario group based on its DNA profile. Similarly, B 6/8 [POU] and B 6/29 [POU] are closely related, as are JA 1/1 [POU] and JA 1/10 [POU] (DC of approximately 0.48). ICS 5 and SILECIA 8 are ranked next in terms of similarity (DC = 0.5). As expected, the Nicaraguan Criollos, ICS 43 and 60, are also closely related (DC = 0.58). In addition, IMC 65 and IMC 75; ICS 8 and NA 802; GS 29 and ICS 88; AM 1/85 [POU] and NA 271; and the Refractarios, JA 5/7 [POU] and JA 5/38 [POU], and JA 4/7 [POU] and JA 5/47 [POU] were similarly closely related (DC = 0.58). When a DC of 0.65 was applied, AM 1/85 [POU], NA 271 and CRU 105 were clustered together.

There were three main clusters, along with 2 paired and 4 unlinked accessions when a DC of 1.28 was applied. The cluster furthest from the x-axis, labelled 3, almost exclusively contains Refractarios. The cluster labelled 2 contained almost exclusively Forasteros (IMC 68, 10, 65, 77, 33, 97 27, 71, NA 86, POUND 18 [POU] and 7/B [POU] and SCA 9) and that labelled 1 contained many Trinitarios (ICS, UF and GS), accessions of unknown origin (CRU) along with some Refractarios and three Forasteros including PA 205 [PER]. The Refractarios are well represented (46 accessions) among the 125 accessions with favourable pod index values. The general pattern of association suggests a clear separation between the recognised genetic groups studied (Trinitario and Forastero), and among the Trinitarios, Forasteros and Refractarios. Similar findings were obtained by Bekele *et al*. (2006) and Sounigo *et al*. (2005b).

Divergent accessions may be screened for potentially heterotic combinations. It is possible to detect such accessions in Figure 1 (a and b). ICS 68, ICS 1, ICS 85, ICS 43, TRD 45, B 17/20 [POU], SJ 1/37 [POU], EET 58 [ECU], ICS 75, ICS 111, JA 1/9 [POU], JA 1/18 [POU], IMC 77, M 116, POUND 18 [POU], IMC 67, IMC 61 and IMC 71 were among these. IMC 3 and PA 37 [PER][1] are other favourable accessions (Figure 1a and b), which were found to be unique among 111 Upper Amazon clones studied by Bekele *et al*. (2005). In Figure 2, IMC 61, IMC 67, ICS 68, JA 10/11 [POU], IMC 3, JA 1/16 [POU], JA 5/36 [POU], SD 1/6 and JA 1/18 [POU] were unlinked at DC = 1.28. The phenotypic uniqueness of IMC 61, IMC 67, ICS 68, and JA 1/18 [POU] was apparent in both multivariate analyses.

It is interesting to note that Motilal (2005) reported that JA 1/9 [POU], JA 1/18 [POU] and JA 1/17 [POU] were found to be equivalent in the USDA/CRU Fingerprinting Project. In this study, JA 1/9 [POU] and JA 1/18 [POU] were close to each other and fairly close to JA 1/17 [POU] in Figure 1a and b, and JA 1/17 [POU] and JA 1/9 [POU] were fairly close (DC= 0.7), but far removed from JA 1/18 [POU] in Figure 2.

Discussion and Conclusion

Detailed consideration of the phenotypic relatedness among the clones studied along with information on their allelic richness or gene frequencies will be useful for selecting core and working collections (Sounigo *et al*., 2005a), where the objective is to preserve as much diversity

[1] PA 37 [PER] was grouped with Refractarios in cluster 3, Figure 2 and was profiled as a Trinitario by Boccara and Zhang (2006) (page 34 in this Report).

as possible relative to the larger collection (Bekele *et al.*, 2004). Information on diversity patterns is also important when formulating strategies for future collections in the wild aimed at increasing the genetic diversity of cacao conserved *ex situ.* A tentative link has already been established between geographic origin and genetic grouping in cacao (Bekele *et al*., 2005; 2006). This suggests that future collection expeditions in the wild should be conducted in yet unexplored or under-represented areas. The apparent uniqueness of Guianese clones (Lachenaud *et al*., 2001) attests to the usefulness of this strategy.

The identification of potentially heterotic groups that may produce transgressive segregants[1] will be useful when selecting candidate parents for further germplasm enhancement. Furthermore, accessions combining low pod index (Table 4) with a high level of resistance to BP (Iwaro *et al.* 2003; 2005) and/or WB and other desirable traits are potential candidates for inclusion in international clone trials to assess their adaptability in different environments.

Research at CRU continues to demonstrate that the ICG,T is an invaluable resource for cacao genetic improvement. It is envisaged that studies to identify high-yielding, disease-resistant genotypes within it will ultimately lead, through successful breeding, to a reduction in the cost of cocoa production. It is also prudent to develop ideotypes (ideal plant types)[2] to safeguard against yet unknown pests, diseases and challenges to successful cocoa cultivation.

Acknowledgements

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[1] Transgressive segregation may be more likely to occur when parents in a cross are less similar, allowing different favourable alleles to be combined in the off-spring.

[2] An idealised multi-trait characterisation; for example, a plant with low pod index value, high butterfat content, and resistance to BP and WBD.

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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[1] product information and SSR primer details. AlternaTIFF software (with instructions for installation) will be included on the CD for computers that require it.

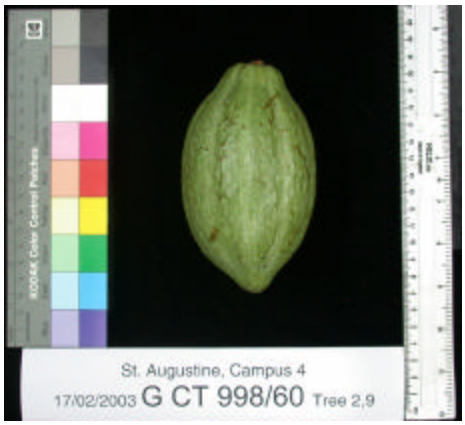
Preparation of content

Photographs

Photographs for the manual were obtained either from digital images of freshly picked pods (using the CRU Nikon 2.1MP[2] 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

[1] PCR - polymerase chain reaction
[2] 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[1] 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

[1] 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

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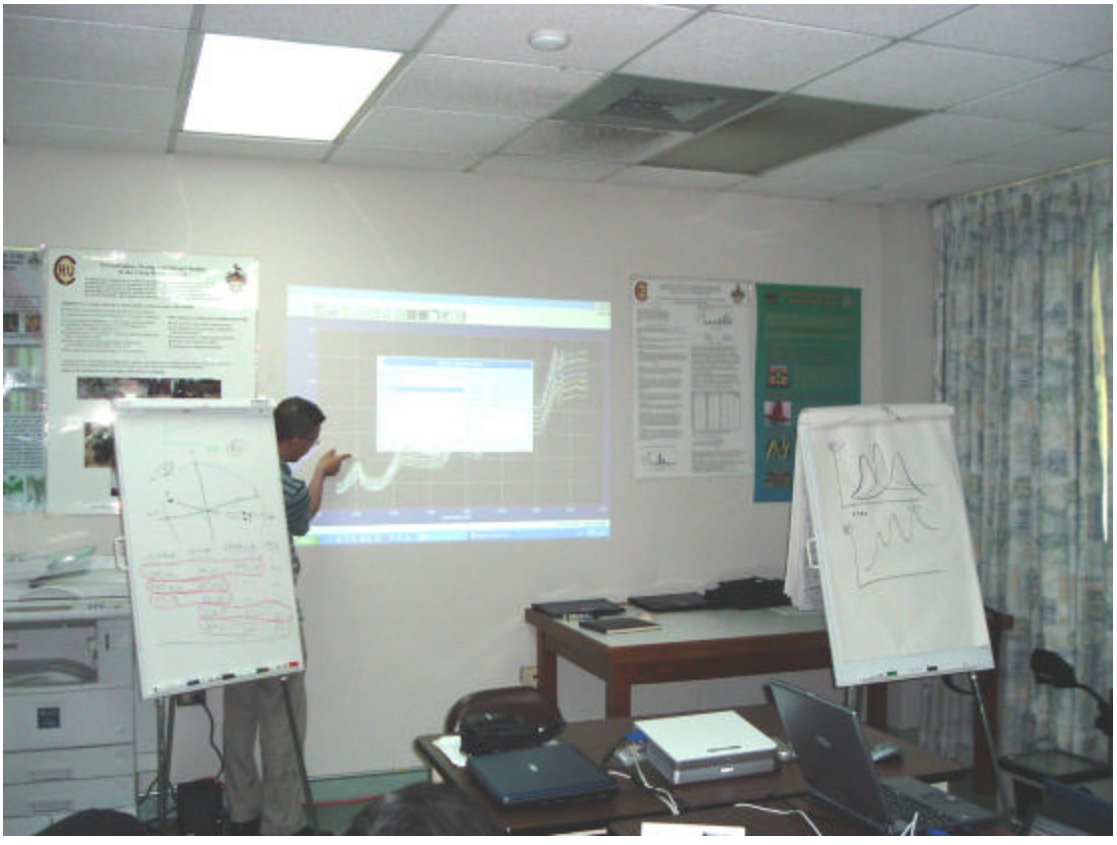
Evaluation



Trends in flavour profiles of the common clones for the CFC/ICCO/INIAP Flavour Project

D.A. Sukha and D.R. Butler

Introduction

The CFC/ICCO/INIAP Flavour Project "To establish physical, chemical and organoleptic parameters to differentiate between fine and bulk cocoa" was initiated in 2001 with participants from four fine or flavour producing countries *viz*. Ecuador, Papua New Guinea (PNG), Trinidad and Tobago and Venezuela. The research conducted in this project was completed in 2005 and this article presents some interesting trends in flavour profiles of common clones used in the project. Cocoa liquors of common clones grown in Ecuador, Papua New Guinea (PNG) and Venezuela, each processed in their respective country of origin, were sent to Trinidad for evaluation by the taste panel at the CRU. This allowed direct comparison by the same sensory panel of the same clones grown and processed in different countries. It was not possible to have the same common clones present in all project member countries. However, the wide range of genotypes grown in the ICG,T made it possible for every common clone to be compared with the same clone in Trinidad. After morphological and molecular verification of the different accessions, the following clones, shown in Table 1, were selected as the 'bulk' and 'fine or flavour' common clones present in at least two countries for the project.

Table 1. Country × common clone distribution for the CFC/ICCO/INIAP Flavour Project.

Country	'Bulk' common clones		'Fine or flavour' common clones		
	IMC 67	SCA 6	ICS 1	ICS 95	EET 400 [ECU]
Ecuador	Present	Absent	Absent	Present	Present
Papua New Guinea	Absent	Present	Present	Absent	Absent
Trinidad	Present	Present	Present	Present	Present
Venezuela	Present	Absent	Present	Absent	Absent

Materials and methods

Sample verification

Verification of the common clones between Trinidad and Venezuela was done using pod photographs and molecular analysis of leaf samples using Simple Sequence Repeats (SSR) carried out at CRU on gels. Other molecular verification was done at PRI, The Netherlands and USDA, USA using capillary sequencers.

Sample preparation

Detailed accounts of the protocol followed for sample preparation in Trinidad *viz*. fermentation and drying (primary processing) and roasting and milling (secondary processing) as well as the organoleptic assessment procedure can be found in the proceedings of the workshop to establish working procedures for the CFC/ICCO/INIAP Flavour Project (Sukha, 2001a, 2001b) and in

previous reports (Sukha *et al*., 2003, 2004).

Subtle variations in the protocol occurred in Ecuador, PNG and Venezuela where the common clones were roasted at 145°C × 30 minutes versus 140°C × 30 minutes for samples from Trinidad, however, experiments in the project to compare oven calibrations showed that this temperature difference would have a minor effect on flavour, if any. A mortar and pestle mill was used to prepare the cocoa liquor in Trinidad and PNG whilst liquor was produced in Ecuador and Venezuela by blending coarsely ground nibs for 3 hours in a liquidiser. During blending in the liquidiser the motor part of the unit (bottom half) was changed periodically to prevent it from overheating.

Liquors were assessed by a trained panel of at least six persons in a sensory design that incorporated hidden reference liquors to check panellist consistency between repetitions. Randomly selected three-digit codes were assigned to cocoa liquors and the order of tasting liquors was randomised over three repetitions to minimise carry-over effects. No two panellists received liquors in the same order in any given evaluation session. Sensory profiles were recorded for eight cocoa flavour attributes using 10-cm line scales with a possible range of scores from 0 to 10, the higher numbers denoted stronger flavour intensities.

Data analysis

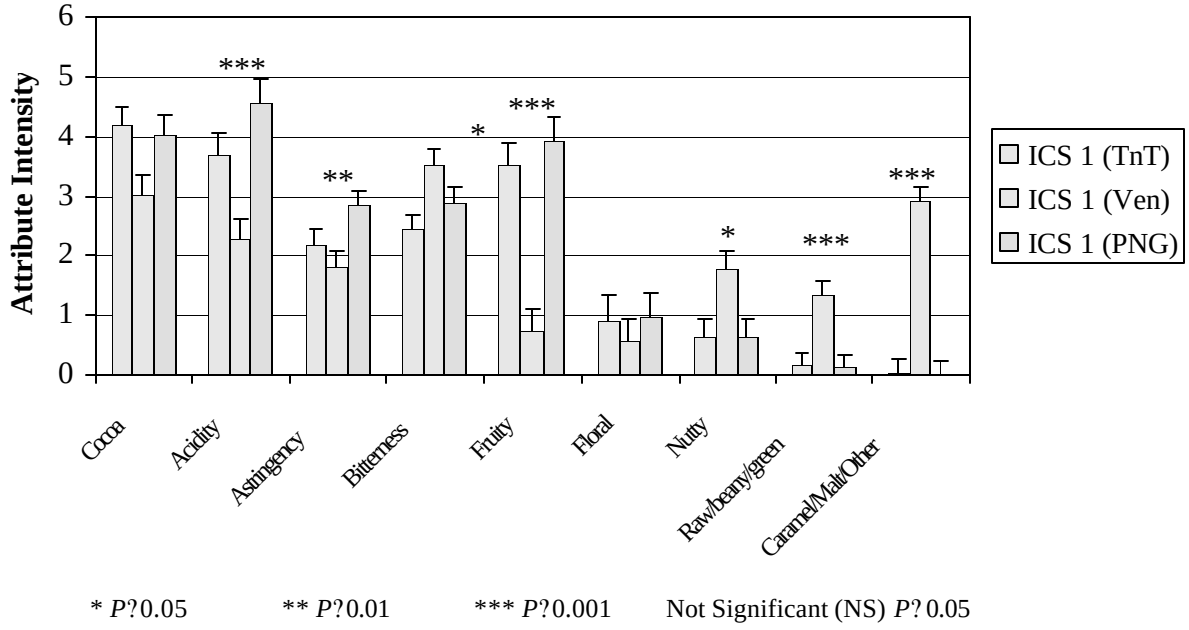
Individual flavour attribute scores from the profiling forms were entered into a data template in Microsoft® Excel. Mean flavour profiles and the standard errors of the mean were calculated. Variance components were investigated using restricted maximum likelihood (REML) variance estimation with Genstat 4.24 DE (VSN International) to determine the significance of treatment effects and interactions. Principal component analysis (PCA) and cluster analysis using the group average method (UPGMA – unweighted pair-group method using arithmetic means) were performed on the pooled data using either GenStat 7.0 (VSN International) or palaeontological statistics software (PAST) Version 1.34 (Hammer *et al*., 2001) and graphical representation was carried out in Microsoft Excel or PAST.

Results

Flavour profiles of each common clone grown in different countries are compared in Figures 1-4. Averages for Trinidad were generated for common clone samples assessed over three crop years (2002 - 2004) whilst averages from Ecuador and PNG were from liquors received for the 2003 and 2004 crop years. No liquor samples were received for the 2002 crop year. Common clone data from the 2004 crop year alone are presented for Venezuela because it was only in this year that organoleptic evaluations incorporated the "caramel/malt" flavour attribute into the sensory vocabulary. Liquors received from Venezuela in the previous crop years were exhausted so re-tasting using the updated vocabulary was not possible.

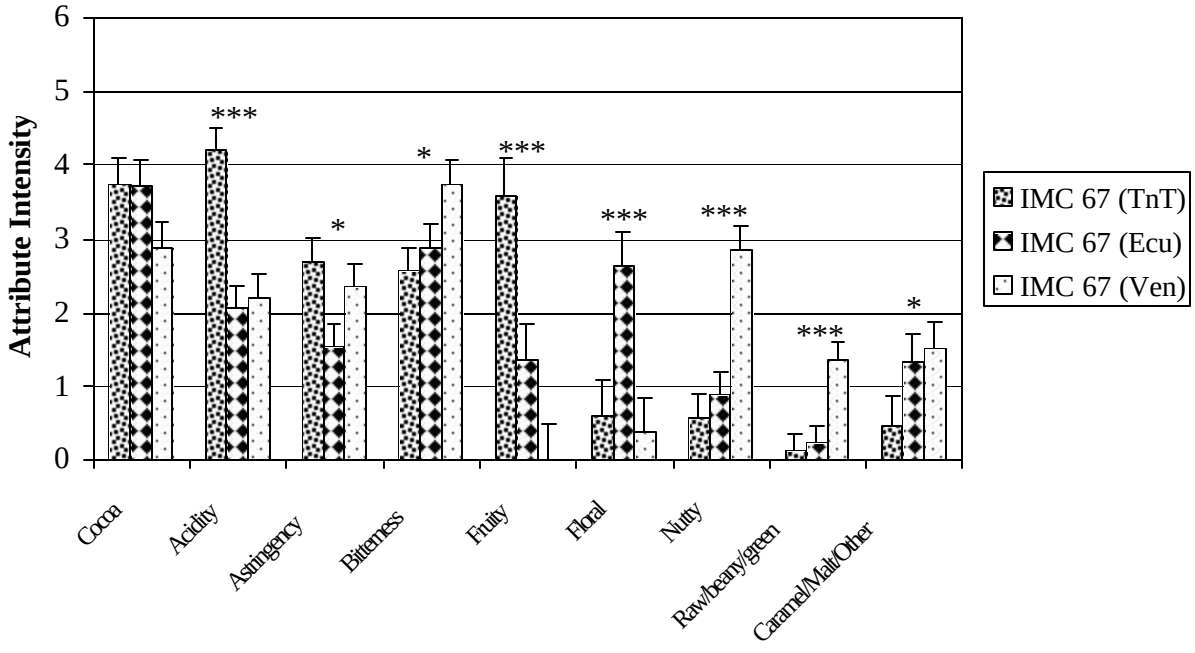
Figure 1 shows the average flavour profiles for ICS 1 grown in Trinidad, PNG and Venezuela. REML analysis showed that all flavour attributes except cocoa and floral flavours varied significantly (P?0.001 – P?0.05) between countries (see significance values on the

Figure 1. Average flavour profiles of ICS 1 grown in Trinidad, PNG and Venezuela.



* *P*?0.05 ** *P*?0.01 *** *P*?0.001 Not Significant (NS) *P*? 0.05

Figure 2. Average flavour profiles of IMC 67 grown in Trinidad, Ecuador and Venezuela.



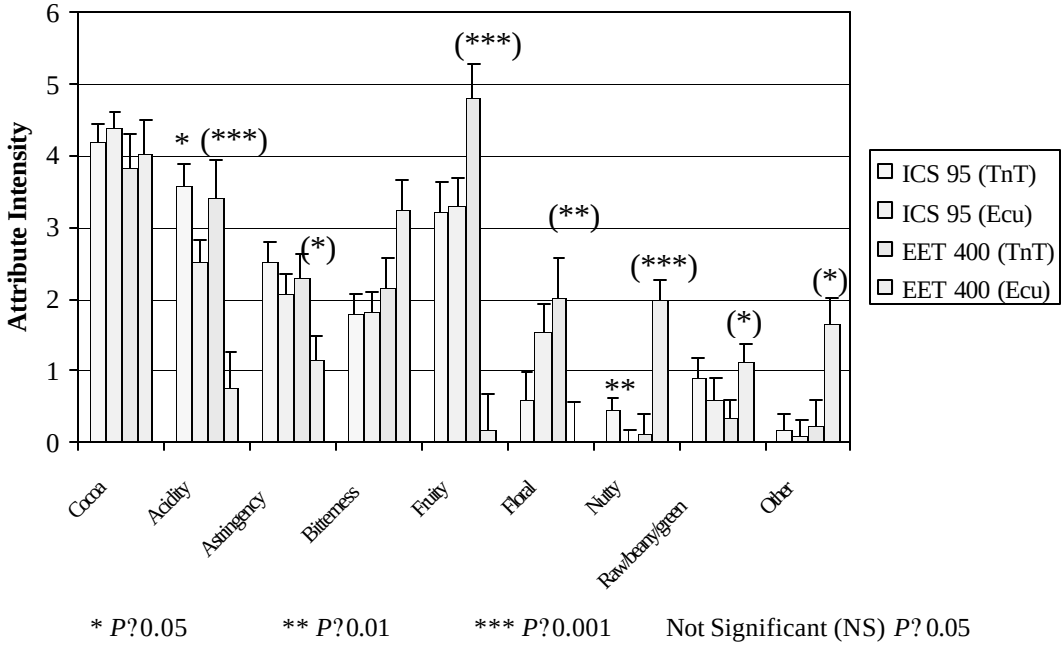
* *P*?0.05 *** *P*?0.001 Not Significant (NS) *P*? 0.05

graph). ICS 1 grown in Venezuela was very different to ICS 1 grown in both Trinidad and PNG with lower cocoa, acid and fruity flavours, and higher bitter, nutty and raw/beany/green flavours.

In addition there was a marked caramel/malt flavour unique to the sample grown in Venezuela. The average flavour profiles for IMC 67 grown in Trinidad, Ecuador and Venezuela are presented in Figure 2 and reveal that all flavours except cocoa varied significantly (*P*?0.001 – *P*?0.05) between countries. IMC 67 grown in Trinidad had significantly (*P*?0.001) higher acid and fruity flavour attributes whilst IMC 67 grown in Ecuador had the highest floral flavour. Again, for the sample grown in Venezuela the caramel/malt flavour was detected as well as higher bitter, nutty and raw/beany/green flavours than the other two countries.

The common clones ICS 95 and EET 400 [ECU] were grown in both Ecuador and Trinidad, and their average flavour profiles are presented jointly in Figure 3. Significance values from REML analysis for EET 400 [ECU] are presented in brackets whilst those for ICS 95 are not. Acid and nutty flavours varied significantly (*P*?0.01 and *P*?0.05) for ICS 95 with acidity being higher in the Trinidad sample.

Figure 3. Average flavour profiles of ICS 95 and EET 400 [ECU] grown in Trinidad and Ecuador. Significance values for EET 400 [ECU] are presented in brackets whilst those for ICS 95 are not.



* *P*?0.05 ** *P*?0.01 *** *P*?0.001 Not Significant (NS) *P*?0.05

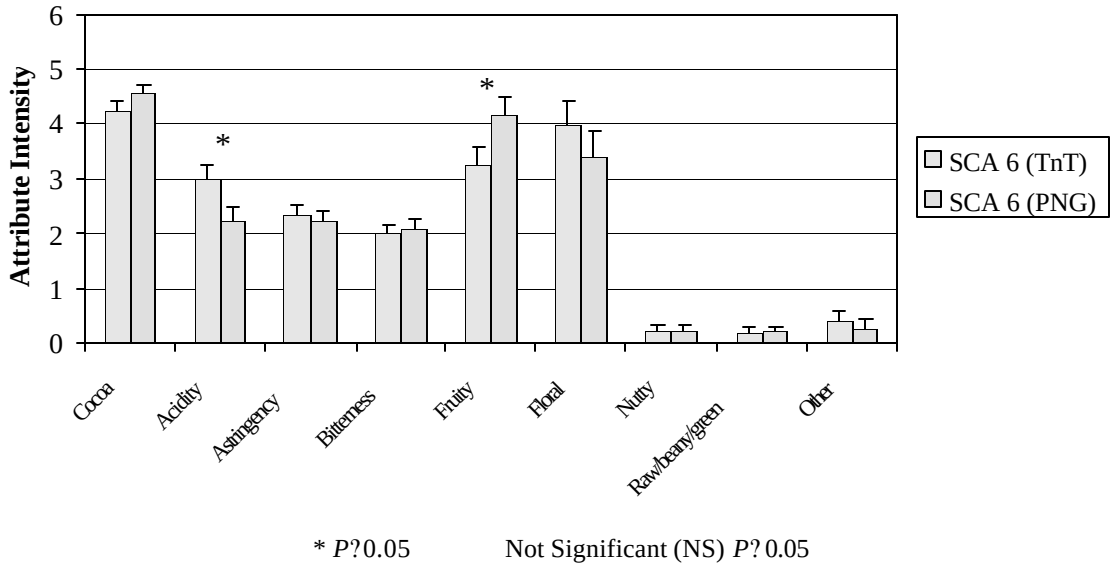
All flavour attributes except cocoa flavour and bitterness varied significantly (*P*?0.001- *P*?0.05) between EET 400 [ECU] grown in Trinidad and Ecuador. Acidity (*P*?0.001), astringency (*P*?0.05), fruity (*P*?0.001), and floral (*P*?0.001) flavours were significantly higher for EET 400 [ECU] grown in Trinidad, whilst nutty (*P*?0.001), raw/beany/green (*P*?0.05) and 'other' (*P*?0.05) flavours were higher in EET 400 [ECU] grown in Ecuador.

SCA 6 was common to both Trinidad and PNG and the average flavour profiles presented in Figure 4 reveal that acid and fruity flavours varied significantly (*P*?0.05) between the two different origins with acidity being marginally higher in Trinidad and fruity higher in PNG. The floral attribute was strong and the overall flavour profile was very similar in the samples from

both countries.

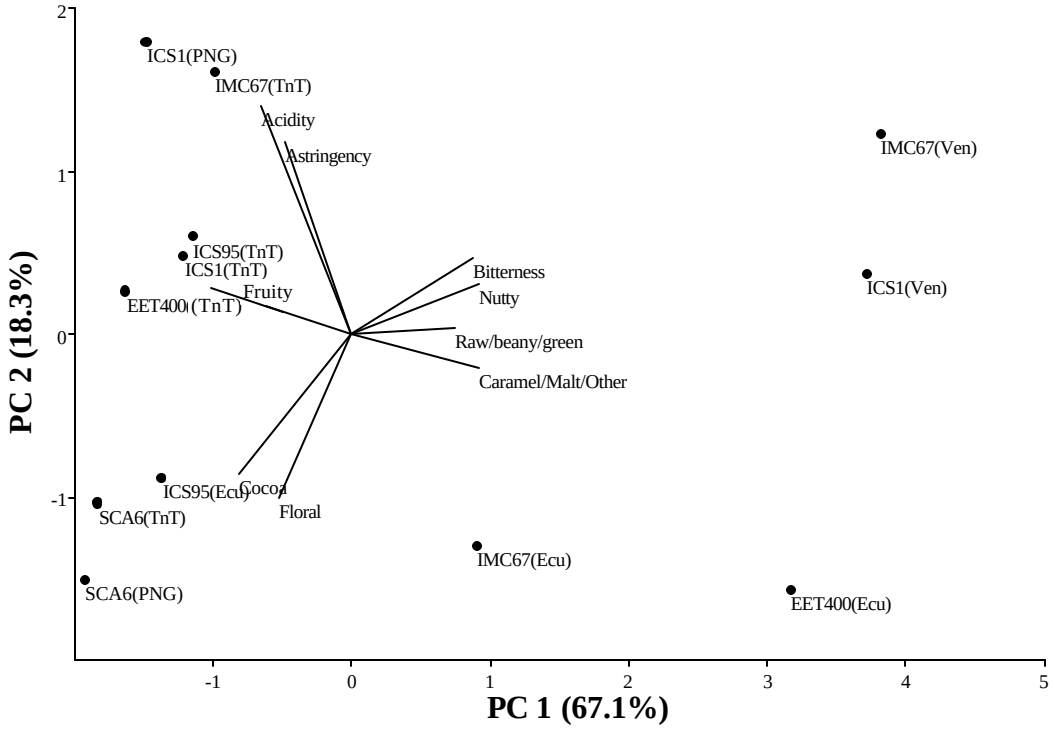
The average flavour attributes of all the common clone samples from the four countries were combined for PCA analysis to see if there were any groupings of the different samples. The PCA plot presented in Figure 5 reveals that two principal components accounted for 85.5% of

Figure 4. Average flavour profiles of SCA 6 grown in Trinidad and PNG.



* *P*?0.05 Not Significant (NS) *P*? 0.05

Figure 5. PCA plot of average flavour scores for common clone samples grown in Trinidad, Ecuador, PNG and Venezuela.



the total variation with principal components 1 and 2 representing 67.1% and 18.3% of the variation respectively.

There were clear groupings according to country of origin regardless of the common clone and no separation according to 'bulk' or 'fine or flavour' common clones. From Ecuador, IMC 67 and ICS 95 tended to be strong in floral and EET 400 [ECU] strong in nutty flavours. Common clone samples from Venezuela were also grouped separately and were associated with caramel/malt, bitter and raw/beany/green flavours. The samples from Trinidad and PNG were closely grouped since, as noted earlier, they were generally similar to each other and associated with acid and fruity flavours.

Discussion and Conclusion

The results from the common clones reveal the interesting pattern of a strong impact on flavour of the same clones grown and processed in different countries. There appears to be a trend for each country to have a characteristic set of flavour attributes that are superimposed on the genotype in question. ICS 1 samples from PNG, Trinidad and Venezuela had flavour attributes that were typical for each country *viz.*, strong fruity and acid flavours for PNG and Trinidad, and caramel/malt flavours for Venezuela. In the same way, comparing Ecuador and Trinidad, EET 400 [ECU] was more fruity and floral when grown in Trinidad and more nutty when grown in Ecuador. IMC 67 was more acid in Trinidad and more floral in Ecuador. There were definite organoleptic differences between the common clones but no clear differences between the bulk and fine or flavour common clones.

The results from this common clone comparison provide strong evidence of marked environmental effects on flavour in addition to genotypic effects. Aspects of the environment that could affect flavour include climatic and edaphic influences of the growing environment, xenia effects as well as conditions during fermentation and drying. This finding is potentially very important from a marketing perspective since it establishes the applicability of the concept of "*terroir*" to cocoa. This quintessentially French term has no precise English translation and it defines the French *Appellation Contrôlée* system for wines. Essentially "*terroir*" refers to the total natural environment (in the case of wines) of any viticultural site. According to Robinson (1994), it is generally agreed that the following factors determine "*terroir*":

- Climate, as measured by temperature and rainfall
- Sunlight energy or insolation, received per unit of land surface area
- Geology and pedology, determining the soil's basic physical and chemical characteristics
- Hydrology or soil water relations.

The holistic combination of all these factors gives each site its own unique "*terroir*" regardless of methods of growing or processing. There may be, however, a need to consider the possibility that that fermentation mass may have an influence on the flavour of the micro-fermented samples, and this needs further investigation.

Marketing of origin specific cocoa is a phenomenon which cannot be ignored and the results from this study provides evidence to support the contribution of environment to flavour and quality attributes of different cocoa genotypes. This leads us to consider applying the concept "*terroir*", that has been well established for wines, to cocoa.

Acknowledgements

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Utilisation

Progress report on germplasm enhancement for resistance to Black Pod disease

A.D. Iwaro and V. Singh

Background

A germplasm enhancement programme was initiated in 1998 as part of the CFC/ICCO/IPGRI Germplasm Utilisation Project. The main objective of the programme is to accumulate genes for resistance to Black Pod disease (BP) in small populations that could be exploited by cacao breeders for the improvement of BP resistance in new cacao varieties. Between 1998 and 2001, 136 resistant/moderately resistant genotypes were selected and used in 96 bi-parental crosses (36 Forastero, 17 Refractario, 20 Trinitario and 23 mixed). Progenies (3,486 seedlings) were raised in the greenhouse and screened for resistance to *P. palmivora* using a leaf disc test (Nyassé *et al*., 1995). Between 2000 and 2003, 1,026 plants including resistant, moderately resistant and susceptible genotypes (control) were established under old cacao trees in Field 14 at the La Reunion Estate, Centeno. In addition, 70 parental genotypes involved in the first two batches of crosses established in 1998 and 1999 were planted in Field 14. A replicate of the same population was planted in a newly established field (Field 7) at UCRS as a backup and to increase pod production for the evaluation of pod resistance and bean traits (bean number and bean weight). In 2005, genotypes bearing pods in Fields 14 and 7 were assessed for resistance to BP under field conditions and in the laboratory using the detached pod test (Iwaro *et al*., 2003). Genotypes with sufficient pods were also evaluated for bean number and bean weight. In addition, all the genotypes established in the two fields were assessed for resistance to Witches' Broom disease (WB) under field conditions. The methods adopted and the results obtained are discussed below.

Methodology

Field observations for resistance to Black Pod and Witches' Broom diseases

In 2005, 821 genotypes (progeny population) in Fields 14 and the replicates (778 plants) in Field 7 were assessed for the following characteristics in 5 rounds of field observations.

- Number of healthy pods per tree/genotype
- Number of diseased pods due to *Phytophthora* infection per tree/genotype
- Number of trees/genotypes free from WB
- Number of trees/genotypes with WB
- Number of Witches' Brooms per tree/genotype

The percentages of genotypes infected by BP and WB and the levels of infection per genotype were determined from the data collected.

Assessment for resistance to *P. palmivora* using the detached pod test

Fully grown unripe pods (2-4 pods per genotype) from 231 genotypes (progeny population) were harvested and evaluated for resistance to *P. palmivora* using the detached pod test described by Iwaro *et al*., 2003.

Evaluation of bean number and bean weight

A maximum of ten healthy, well-developed, ripe pods were harvested from 303 genotypes for the determination of bean number and bean weight. Average bean number per genotype was determined by counting the number of seeds (excluding flat beans) in the available pods and dividing the total number of seeds by the number of pods. Average wet bean weight per genotype was determined by dividing the wet bean weight of the available pods by the total number of beans. Average dry bean weight per genotype was estimated using a 40% conversion ratio (Freeman, 1969).

Results and Discussion

Field observations for resistance to Black Pod and Witches' Broom diseases

Black Pod symptoms were recorded in 52 (17%) of the 303 genotypes bearing pods in Field 14 (Table 1). However, only 5 (2%) of the 207 genotypes bearing pods in Field 7 had BP symptoms (Table 2). The high level of infection in Field 14 (old cacao field) as compared to Field 7 (newly

Table 1. Field observations conducted in Field 14 (Centeno).

Batch	Date of establishment	No. of genotypes established	No. of genotypes alive	No. of genotypes with pods	No. of genotypes with BP	No. of genotypes with WB
1	2000	316	261	167	32 (19%)	164 (63%)
2	2001	339	279	135	20 (15%)	156 (56%)
3	2003	182	175	0	0 (0%)	24 (14%)
4	2003	189	106	1	0 (0%)	13 (12%)
	Total:	1,026	821	303	52 (17%)	357 (43%)

BP - Black Pod disease WB - Witches' Broom disease

Table 2. Field observations conducted in Field 7 (UCRS).

Batch	Date of establishment	No. of genotypes established	No. of genotypes alive	No. of Genotypes With pods	No. of genotypes with BP	No. of genotypes with WB
1	2001	204	169	112	4 (4%)	4 (2%)
2	2002	171	123	36	1(3%)	7 (6%)
3	2002	310	263	23	0 (0%)	2 (1%)
4	2002	275	224	36	0 (0%)	0 (0%)
	Total:	960	778	207	5 (2%)	13 (2%)

BP - Black Pod disease WB - Witches' Broom disease

established field) is likely to be due to the high inoculum pressure in Field 14 and to the greater number of pod bearing trees. The result of Field 14 suggests that 83% of the progeny population had good potential to resist *Phytophthora* infection in the field. This result is subject to confirmation by the detached pod test to ascertain the level of inherent resistance in the genotypes that were free from infection (83%) and those with low levels of infection among the 17% showing BP symptoms in the field.

Witches' Broom symptoms were observed in 357 (43%) of the 821 genotypes in Field 14. Only 13 (2%) of the 778 plants in Field 7 had WB symptoms probably due to the lower level of inoculum in this newly established field. The results from field observations in Field 14 provide an opportunity for a negative selection against WB. However, it is imperative that the field results be confirmed using the agar-droplet inoculation method. This test will be carried out at a later stage in the programme.

Assessment of resistance to *P. palmivora* using the detached pod test

Among the 231 genotypes (progeny population) evaluated for resistance to BP using the detached pod test, 73 genotypes (32%) were found to be resistant (disease rating 1 - 3) (Table 3).

Table 3. Distribution of scores for resistance to *P. palmivora* in the progeny population (231 genotypes) and ICG,T sub-population (500 genotypes).

Population	Disease rating															
	1		2		3		4		5		6		7		8	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Progeny	21	9.1	20	8.7	32	13.9	49	21.2	36	15.6	39	16.9	20	8.7	14	6.1
ICG,T	20	4.0	34	6.8	24	4.8	47	9.4	61	12.2	109	21.8	142	28.4	63	12.6

No visible lesions (disease rating 1) were observed on 21 genotypes, while 20 genotypes had 1 - 5 localised lesions (disease rating 2). Thirty-two genotypes had 6 - 15 localised lesions (disease rating 3), 49 genotypes had more than 15 localised lesions (disease rating 4), and 36 had 1 - 5 expanding lesions (disease rating 5). The combined disease ratings 4 and 5 gave 85 moderately resistant genotypes (37%). Seventy-three genotypes (32%) were classified as susceptible. In this category, 39 genotypes had 6 - 15 expanding lesions (disease rating 6), 20 had more than 15 expanding lesions (disease rating 7), and 14 had fast-expanding coalesced lesions (disease rating 8).

Table 3 shows that the subset of the progeny population (231 genotypes) possessed 32% resistant (disease rating 1 - 3) and 37% moderately resistant genotypes (disease rating 4 - 5), while a subset of the ICG,T population (500 genotypes) had 16% resistant and 21% moderately resistant genotypes (Iwaro *et al*., 2003). The subset of the progeny population shows a considerably higher frequency of resistant genotypes and consequently a higher level of resistance alleles than the base population (Table 3). The subset of the progeny population shows a significant reduction in the frequency of susceptible genotypes (32%) compared to the subset from ICG,T which had 63% susceptible genotypes. This shows a significant improvement in BP resistance in the composition of the new population. It further shows the effectiveness of the selection criteria imposed on the base population for the selection of the parental genotypes and confirms that resistance is heritable.

As may be expected, the result of the detached pod test indicated that the frequency of susceptible genotypes was higher than the 17% observed in the field survey conducted in Field 14 (Table 1). As a confirmatory test for BP resistance, the detached pod test indicated that 32% of the progeny population (Table 3) was susceptible to BP as compared to 17% showing BP

symptoms in field observations (Table 1). This shows that absolute reliance cannot be placed on field observations alone in which a susceptible genotype may escape infection due to a number of factors. Based on the detached pod test, 68% of the progeny population (Table 3) was found to be resistant and moderately resistant. This shows significant improvement over the base population due to the first cycle of the germplasm enhancement programme.

Evaluation of progeny population for bean number, bean weight and pod index

Three hundred and three genotypes were assessed for bean number and bean weight. Those genotypes with a minimum of five pods (149 genotypes) were classified into three groups (large, intermediate and small) for bean number, bean weight and pod index.

Five (3%) of the 149 genotypes had large (>45) bean number, while 99 (67%) possessed intermediate (36 - 45) bean number. Forty-five genotypes (30%) had small (<36) bean number. Small bean number suggests low yield potential and is undesirable in breeding. Table 4 shows that the percentage of genotypes with small bean number in the progeny population (30%) is not significantly different from that of 581 genotypes (31%) in ICG,T. About 70% of the progeny population (149 genotypes) had large to intermediate bean number. This suggests that selection for large to intermediate bean number will be effective in the progeny population.

For bean weight, 24 (16%) of the 149 genotypes had large (>1.20g) bean weight, while 98 (66%) possessed intermediate (0.81 - 1.20g) bean weight. About 18% (27 genotypes) had small (<0.81g) bean weight. A relatively lower percentage of genotypes with low bean weight (18%)

Table 4. Percentage of genotypes in three categories of bean number, bean weight and pod index in the progeny population (149 genotypes) and ICG,T sub-population (581 genotypes).

Character	Category	Rating	Progeny population (149 genotypes)		*ICG,T sample population (581 genotypes)	
			Number	Percentage	Number	Percentage
Bean number	Large	> 45	5	3.4	99	17.0
	Intermediate	36 - 45	99	66.4	302	52.0
	Small	< 36	45	30.2	180	31.0
Bean weight	Large	> 1.20 (g)	24	16.1	64	11.0
	Intermediate	0.81 - 1.20 (g)	98	65.8	395	68.0
	Small	< 0.81 (g)	27	18.1	122	21.0
Pod Index	High	> 35.0	23	15.4	80	13.8
	Intermediate	20.1 - 35.0	108	72.5	445	76.6
	Low	< 20.1	18	12.1	56	9.6

*Iwaro *et al*., 2003

was observed in the progeny population as compared to 21% in the ICG,T sample population (Table 4). The percentage of genotypes with large to intermediate bean weight was higher (82%) in the progeny population than in the ICG,T sample population (79%). This result suggests that selection would be effective for large to intermediate bean weight in the progeny population. It also indicates that resistance to BP could be combined with large to intermediate bean weight.

Table 4 did not show any major difference in the proportions of low to high pod indices between the progeny population and the ICG,T sub-population. This shows that selection for resistance to BP had no significant impact on the genes controlling yield potential in the enhanced population. Selection would therefore be effective for good yield potential within the enhanced population as the frequency of genes affecting yield potential remains similar in both the ICG,T sub-population and the enhanced population.

Conclusion

So far, the germplasm enhancement programme has progressed satisfactorily within the last eight years. An increase in the frequency of resistant individuals confirms the effectiveness of the selection criteria and the overall strategy being adopted in the programme. As the evaluation exercise progresses, more promising genotypes are likely to be identified which combine good yield potential with resistance to BP. These promising genotypes will be used as base parents for the second cycle of the germplasm enhancement programme. In addition, some of the promising genotypes from the first cycle will be transferred to the ICQC,R for distribution as potential sources of genes for resistance to BP.

Based on the results obtained in the first cycle, one can be optimistic that further improvement could be achieved in the second cycle of the programme. The end product of the programme would allow cacao breeders to combine good yield potential with an acceptable level of resistance to BP in new cacao varieties.

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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 a*l., 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 a*l., 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] × PLAYA ALTA 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
LCT EEN 90/S-7 × ICS 46	F1	24	50.0	0.0	8.3	91.7	12	12.3	11	16.4	11	12.4
LCT EEN 90/S-7 × PLAYA ALTA 2 [VEN]	F2	17	0.0	11.8	0.0	88.2	15	13.3	15	16.1	15	11.3
LCT EEN 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
PLAYA ALTA 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.

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BUILDING

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Publications and presentations

Refereed Journals

Bekele, F.L., Bekele, I., Butler, D.R. and Bidaisee, G.G. (2006) Patterns of morphological variation in a sample of cacao (*Theobroma cacao* L.) germplasm from the International Cocoa Genebank, Trinidad. *Genetic Resources & Crop Evolution* **53 (5)**: 933-948.

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Sukha, D.A. Fermentation index as a parameter of cocoa bean quality. Presented at: Evaluation and coordination meeting related to the chemical aspects of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Mérida, Venezuela. 15th – 18th March 2005.

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Sukha, D.A. Anthocyanins and Flavanols in cocoa beans. Presented at: Evaluation and coordination meeting related to the chemical aspects of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Mérida, Venezuela. 15th – 18th March 2005.

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Sukha, D.A. Results from individual and combined analysis of pH, Temperature and Physical bean attributes (including cut test) in samples from Trinidad and Tobago. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24th – 30th June 2005.

Sukha, D.A. Links in results from individual and combined statistical analysis of Fermentation Index. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24th – 30th June 2005.

Sukha, D.A. Links in results from individual and combined statistical analysis of pH, titratable and volatile acidity, volatile and non volatile organic acids and butter fat. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24th – 30th June 2005.

Sukha, D.A. Links in results from individual and combined statistical analysis of polyphenols, anthocyanins, procyanidins and tannins. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24th – 30th June 2005.

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Sukha, D.A. Links in results from individual and combined statistical analysis of alkyl pyrazines and other volatile compounds. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24th – 30th June 2005.

Sukha, D.A. Links in results from individual and combined statistical analysis of organoleptic results from Ecuador, Papua New Guinea, Trinidad and Venezuela. Presented at: Meeting to discuss and interpret findings of combined country analyses with links to objectives of the CFC/ICCO/INIAP Flavour Project: To establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. St. Augustine, Trinidad. 24^{th} – 30^{th} June 2005.

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Davrieux, F., Assemat, S., Sukha, D.A., Portillo, E. Boulanger, R., Bastianelli, D. and Cros, E. (2005) Genotype characterisation of cocoa into genetic groups through caffeine and theobromine content predicted by NIRS. 12^{th} International Conference on Near Infrared Spectroscopy. Sky City Auckland, New Zealand. 10-15 April 2005.

Visitors to CRU in 2005

David Preece	Cadbury International Ltd. UK
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Maja Berthas	Chokladforam, Stockholm, Sweden
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Nelly Vingerhoets	International Cocoa Organization, UK
Martin Gilmour	Masterfoods UK, Slough, UK
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Beryl Hunter	High Bentham, North Yorkshire, England
Janet Momsen	University of California, Davis, USA

Acronyms and abbreviations

ACRI	American Cocoa Research Institute, USA
BBD	Broom-base diameter
BCCCA	Biscuit, Cake, Chocolate and Confectionery Association, London, UK
bp	base pair
BP	Black Pod disease
CA	Cluster analysis
CAOBISCO	Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
CATIE	Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica
CCM	Cacao clones manual
CD-ROM	Compasct disc – read only memory
CEPEC	Centro de Pesquisas do Cacau, Brazil
CFC	United Nations Common Fund for Commodities
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France
CIRAD-CP	Centre de Coopération Internationale en Recherche Agronomique pour le Développement -Culture Pérennes, France
CRA	Cocoa Research Association, UK
CRU	Cocoa Research Unit, Trinidad and Tobago
DC	Dissimilarity coefficient
DNA	Deoxyribonucleic acid
EEN	Estacion Experimental Napo
EET	Estacion Experimental Tropical
FP	Frosty pod disease
HTML	Hypertext markup language
ICCO	International Cocoa Organisation, London, UK
ICGD	International Cocoa Genebank Database
ICG,T	International Cocoa Genebank, Trinidad
ICQC,R	International Cocoa Quarantine Centre, Reading, UK
ICTA	Imperial College of Tropical Agriculture
INIA	Instituto Nacional de Investigaciones Agricolas, Venezuela
INIAP	Instituto Nacional Autonomo de Investigaciones Agropecurias, Ecuador
INGENIC	International Group for Genetic Improvement of Cocoa
IPGRI	International Plant Genetic Resources Institute, Rome, Italy
JANE	John and Ann Niederhauser Endowment research award
JPEG/JPG	Joint Photographic Experts Group
LD	linkage disequilibrium
MP	Mega pixel
MALMR	Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago
P	Probability
PAST	Palaeontological statistics software
PCA	Principle component analysis
PCR	Polymerase chain reaction
PI	Pod Index
PRI	Plant Research International, Holland
QTL	Quantitative trait loci
R	Correlation coefficient
RAPD	Random amplified polymorphic DNA
REML	Restricted maximum likelihood variance estimation
SSR	Simple sequence repeats
TIFF	Tagged image file format
TFS	Time to first symptom
TBI	Time to broom initiation

UCRS	University Cocoa Research Station
UE	Union Européenne
UPGMA	Unweighted pair-group method using arithmetic means
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture – Agriculture Research Service
UWI	The University of the West Indies
WB	Witches' Broom disease
WCF	World Cocoa Foundation, USA