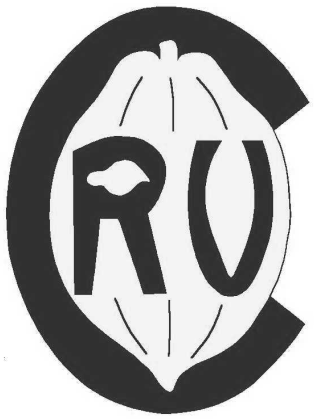
Annual Report 2006



Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
2007

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/BI[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/BI Cocoa Productivity Project". Good progress continued to be made in the third 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 are being evaluated in the field and evaluations of the first and second year progeny for Witches' Broom resistance are at an advanced stage. Peninna Deberdt joined CRU as a visiting scientist from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France (CIRAD) in September 2006 and assumed responsibility for the work on germplasm enhancement for Witches' Broom resistance. Jean-Marc Thévenin was instrumental in planning this work programme before leaving CRU in July 2004 to return to CIRAD, and continued supervising the project up to the time that Peninna assumed her duties. We are grateful to Jean-Marc Thévenin for his continued input during the intervening two years.

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. Since the start of the project, 48 clones from 19 accession groups have been confirmed to be resistant. This provides the opportunity to use 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/Bioversity International

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), through an agreement between USDA and CRU 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 and his team are analysing the DNA with an automatic capillary sequencer using 15 standardised microsatellite primers and analysis of the results for Pound's Upper Amazon and Refractario accessions has proved to be extremely valuable in confirming their genetic groups. We look forward to the completion of this major task of genotyping all our accessions during 2007.

The final workshop of the CFC/ICCO/INIAP[2] Flavour Project to *Establish physical, chemical and organoleptic parameters to differentiate between bulk and fine cocoa* took place in Guayaquil, Ecuador in April 2006. This project involved research teams in Ecuador, Papua New Guinea and Venezuela, and is now completed.

A project entitled *Safeguarding the International Cocoa Genebank, Trinidad: a global resource for the cocoa industry* was approved by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) in May 2005. The project is being jointly funded by the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector and the Cocoa Research Association, UK (CRA), and will be referred to in this report as the "Dutch LNV Project to Safeguard the ICG,T". The main aim is to upgrade the irrigation facilities in the University Cocoa Research Station (UCRS), improve security of the site and re-propagate material at risk of genetic erosion. Good progress was made in these activities in 2006, with the establishment of grafted plants from over 370 accessions, currently not represented in UCRS. In addition, the construction of two large irrigation reservoirs at UCRS is well underway.

A new project *To assess the quality attributes of the Imperial College Selections* was approved by the Dutch LNV in June 2006 for funding by the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector. We welcome the agreement by a number of chocolate manufacturers to collaborate in this project. The first harvest season began in December and is on-going.

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. All cocoa samples for the project were harvested and processed in Trinidad in 2006, and the samples are being analysed in Hamburg.

Staff news

Balram Latchman (Contract Officer I) was appointed in March 2006 to work on the Dutch LNV Project to Safeguard the ICG,T. His main activities were to propagate clones from aging trees in locations outside UCRS and to initiate a programme to raise rooted cuttings with budwood from grafted trees. Balram resigned in August 2006 to take up an alternative post in the petrochemical

[1] Deoxyribonucleic acid
[2] Instituto Nacional Autonomo de Investigaciones Agropecurias (INIAP)

industry.

Naailah Ali was appointed as a Technical Assistant from April to September 2006 to work on the CFC/ICCO/BI Cocoa Productivity Project. She carried out a series of experiments to test the viability of stored pollen.

Peninna Deberdt came to CRU as a visiting scientist from CIRAD on 1 September 2006. She is a specialist in Plant Pathology and is working in the area of germplasm enhancement for resistance to Witches' Broom disease.

Frankie Solomon was appointed as a Technical Assistant from September 2006 to replace Balram Latchman on the Dutch LNV Project to Safeguard the ICG,T. He is working on propagation by rooted cuttings and grafting clones that are vulnerable to genetic erosion.

Wayne Bertrand has been working for CRU as a consultant to advise on the construction of a new irrigation system for the ICG,T. He oversaw the excavation of two irrigation reservoirs in UCRS in April – June 2006 and assisted in the purchase of pumps, pipes and fittings for the new system.

Visitors

Jean-Marc Thévenin visited CRU for 5 days between 7 and 22 February 2006 to review progress on WB research and advise on programmes of work planned at the time that he left CRU in July 2004.

Dr. Bertus Eskes visited CRU from 19 to 26 February 2006 for discussions on progress with the CFC/ICCO/BI Cocoa Productivity Project.

Wim Kokxhoorn and *Loek van Soest* visited CRU from 24 to 28 April 2006. They came on a 'fact finding mission' on behalf of the Dutch LNV in response to several project proposals submitted by CRU for support from the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector.

Carlene Lakhan and *Chad Ramgathe* were hosted as placement students in CRU from 22 May to 28 July 2006. Carlene Lakhan assisted with germplasm enhancement for resistance to Black Pod disease and Chad Ramgathe assisted with work on identity studies by DNA fingerprinting.

Prof. Reinhard Lieberei and *Christina Rohsius* from the Department of Useful Plants and Plant Ecology, University of Hamburg visited CRU from 18 to 21 October 2006. They came to discuss progress in the joint project *Chemical indicators in fermentation of cacao*, and possible future areas of collaboration.

Dr. Chris Turnbull visited CRU from 23 to 26 October for discussions with staff related to the needs for information management in CacaoNet. Chris is responsible for management and development of the International Cocoa Germplasm Database (ICGD) at Reading University, UK.

Sophie Assemat (CIRAD) visited from 28 October to 11 November 2006 to participate in sensory training in CRU for flavour assessment of cocoa liquors. The opportunity was used to train a new sensory panel for CRU that included volunteers from the Ministry of Agriculture, Land and Marine Resources (MALMR) and the private sector.

Meetings and events

Four staff from CRU (David Butler, David Iwaro, Surendra Surejdeo-Maharaj and Michel Boccara) attended the American coordination and resistance testing meeting in Higuerote, Venezuela from 13 to 17 February 2006. This was a regional meeting of the CFC/ICCO/BI Cocoa Productivity Project.

Two staff from CRU (David Butler and Darin Sukha) travelled to Guayaquil, Ecuador in April 2006 for the final workshop of the CFC/ICCO/INIAP Flavour Project (3 – 6 April). However, Darin Sukha was not able to attend the meeting due to visa problems. His presentations on the contribution by Trinidad to the project were delivered by David Butler.

David Butler participated in the Paris Cocoa Meeting, France on 16 - 17 May, the World Cocoa Foundation Partnership Meeting in Brussels, Belgium on 18 May, and the CacaoNet Interim Steering Group Meeting in Brussels on 19 May 2006.

Nine staff from CRU (David Butler, David Iwaro, Frances Bekele, Darin Sukha, Lambert Motilal, Antoinette Sankar, Surendra Surujdeo-Maharaj, Michel Boccara and Pennina Deberdt) participated in the 15th International Cocoa Research Conference, San José, Costa Rica from 9 to 14 October 2006. The majority of these participants also attended satellite meetings of 5th INGENIC[1], 5th INCOPED[2] and/or 1st INAFORESTA[3] workshops on 16 – 17 October. A global genetic resources network for cocoa, "CacaoNet", was launched at the 15th International Cocoa Research Conference. This was an important milestone in encouraging support for conservation and facilitating the utilisation of cocoa genetic resources, which are activities central to CRU's mission.

David Butler participated in the ZDS[4] Chocolate Technology Seminar in Cologne, Germany on 12 – 14 December 2006.

[1] International Group for Genetic Improvement of Cocoa
[2] International Permanent Working Group for Cocoa Pests and Diseases
[3] Grupo internacional dedicado al estudio y mejoramiento de la relación entre cacao, árboles y bosques
[4] Zentralfachschule der Deutschen Süßwarenwirtschaft e. V.

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 in 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[1] (now Bioversity International).

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 if at all possible, 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

[1] International Plant Genetic Resources Institute

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[1]) (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

[1] Centro Agronómico Tropical de Investigatión y Enseñanza

(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

Almost 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 Bioversity International) 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 1,270 accessions have now been completed. As they are recorded, the descriptors are entered in a local database and are also sent to the 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 5-6 years. This collaborative effort will therefore accelerate the rate of progress in genetic diversity studies by a factor of four.

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 *Moniliophthora perniciosa* (Aime and Phillips-Mora) (previously *Crinipellis perniciosa* (Stahel) Singer).

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 conditions 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*). Selections from disease resistant trees in the germplasm enhancement programmes (below) are being 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.

Progeny from crosses in the pre-breeding programme were evaluated for BP resistance with a leaf inoculation method. This permitted early selection of seedlings and comparison of the disease resistance of 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 precocity, vigour, productivity and WB symptoms. Initial results from field observations and detached pod inoculations 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. Other work in CRU aims 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. Some of the selected clones are already available for further distribution to other cocoa-producing countries, and the remainder will be available within two years.

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. Initial selections from BP resistant populations are already being 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, biscuiteries et confiserie de l'UE

Conservation

New cacao introductions into the International Cocoa Genebank, Trinidad

D.R. Butler and J. Joseph

Three clones were transferred to Trinidad from the ICQC,R as budwood during 2006 (Table 1) to replace plants lost following previous transfers.

Table 1. Clones introduced to Trinidad from the International Cocoa Quarantine Centre, Reading in 2006.

Preferred name	Number of plants established
COCA 3308/1 [CHA]	2
EQX 5/J [CHA]	2
PINA 1	1

Twenty eight clones from the greenhouse at UWI were planted in Field 5A at UCRS (Table 2) in September 2006. These had been introduced into Trinidad in recent years, either from the Barbados Cocoa Quarantine Station (Butler and Sukha, 2004) or ICQC,R.

Table 2. Clones planted in fields at UCRS (Field 5A) in 2006.

Preferred Name	Number of trees	Preferred name	Number of trees
BLZ 5	3	LCT EEN 253	4
ELP 16/S3	4	LCT EEN 255	3
ELP 2/S4	4	LCT EEN 312	2
ELP 22/S6	3	LCT EEN 32	4
ELP 35/B	4	LCT EEN 36	2
ELP 40/S9	4	LCT EEN 37/G	4
GS 32	4	LCT EEN 370	11
GS 74	5	LCT EEN 403	3
LCT EEN 122	2	LCT EEN 60	5
LCT EEN 123	6	PA 7 [PER]	3
LCT EEN 133	2	PH 1/5	3
LCT EEN 142	2	PH 2/8	3
LCT EEN 217	3	RB 33/3 [BRA]	4
LCT EEN 220	3	YAL 1/S2	5

References

Butler, D.R. and Sukha, D.A. (2004) Transfer of clones from the Barbados Cocoa Quarantine Station. Page 12 in: *Annual Report 2003*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Re-propagation of cacao germplasm for the ICG,T from original trees in Trinidad

F. Solomon, B. Latchman and D.R. Butler

Introduction

When the ICG,T was initially planted, clones were propagated as rooted cuttings and arranged in plots typically with 16 trees per plot. However, cuttings are most often taken from lateral branches (plagiotropic) and these develop into plants with a shallow root system that can lead to poor establishment. Indeed, a proportion of the plants originally planted in ICG,T did not survive at all and there is now an average of approximately 5 trees per plot.

In 2004, a proposal was sent to the Dutch Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector for a project that included re-propagation of germplasm to reduce the risk of genetic erosion in the ICG,T. The proposal (the Dutch LNV Project to Safeguard the ICG,T) was approved in 2005 and, since then, considerable progress has been made replicating material from original trees in Trinidad. The situation 18 years after the first trees were planted, when the proposal was written in 2004, was that less than 3% of the plots had their full complement of 16 trees. Furthermore, 511 clones for which "original" trees still existed in other locations in Trinidad (Marper Farm, Cheesman Field, and the University Campus) were not represented in UCRS.

Establishment of grafted plants

Cacao germplasm was introduced to Trinidad following expeditions to South America by F.J. Pound in 1937 and 1938. Pods were collected from trees free from symptoms of Witches' Broom disease (caused by *Moniliophthora perniciosa*) and the seed were planted in Barbados for quarantine purposes. Budwood from these seedlings was transferred to Trinidad and budded plants were established in Marper Farm (located in east Trinidad) from 1939 to 1941 in two Blocks; C and D. Originally 1,350 accessions were planted in each block, but a proportion of these were eliminated in the 1940s due to their susceptibility to Witches' Broom disease. Records from 2005 indicate that 247 clones were present in Marper Farm that were not represented in UCRS.

The first priority for propagation by grafting was material in Marper Farm. These trees are now almost 70 years old, and the rate at which trees are dying is increasing with time. Between the time that the project proposal was prepared in 2004, and the grafting was completed in 2006, 33 original trees had already been lost.

It is difficult to find vigorous healthy shoots suitable for grafting on such aging trees as those in Marper Farm. Due to this restriction, the grafting success rate was expected to be generally lower than normal and, for certain clones, it could be difficult to achieve success. Between September 2005 and August 2006, 10 budwood sticks were collected from each designated tree in Marper Farm and brought to the UWI Campus for grafting. After assessing the survival rate for each clone, those with less than 3 surviving plants were repeated. By December 2006, the number of grafted plants surviving from each of 229 clones is shown in Table 1.

Table 1. The number of surviving grafted plants from clones in Marper Farm, currently not represented in UCRS.

Clone name	Source	Number surviving
AM 1/10 [POU]	C360	4
AM 1/13 [POU]	C689	3
AM 1/20 [POU]	C738	5
AM 1/39 [POU]	C156	2
AM 1/42 [POU]	C199	1
AM 1/55 [POU]	C232	7
AM 1/63 [POU]	C112	2
AM 2/7 [POU]	C700	3
AM 2/17 [POU]	C440	8
AM 2/36 [POU]	C7	4
AM 2/39 [POU]	C350	4
AM 2/42 [POU]	C416	3
AM 2/46 [POU]	C427	1
AM 2/68 [POU]	C258	5
AM 2/90 [POU]	C911	4
AM 2/94 [POU]	C924	12
AS 2 [ECU]	C598	6
AS 7 [ECU]	C484	11
B 1/2-8 [POU]	D549	3
B 6/5 [POU]	D196	6
B 7/13 [POU]	D208	1
B 9/10-28 [POU]	D88	5
B 11/3 [POU]	C1070	1
B 18/8 [POU]	D173	6
B 22/5 [POU]	D139	9
CL 9/40	C561	5
CL 9/53	C657	1
CL 91/3	C43	4
CL 91/6	C666	10
CL 10/21	C9	6
CL 13/24	C411	3
CL 13/32	C492	3
CL 19/12	C810	4
CL 19/24	C425	5
CL 19/27	C302	4
CL 19/35	C328	7
CL 19/46	C62	3
CL 19/51	D27	4
CL 27/105	C1073	1
CL 27/116	D362	8
CLM 3	C394	5
CLM 49	C618	4
CLM 61	C458	7
CLM 68	C356	3
CLM 82	C685	4
CLM 85	C61	6
CLM 88	C938	8
CLM 107	D354	5
CLM 111	C933	3
CLM 120	C845	1
CLM 122	C901	4
CRU 63	D83	5
CRU 64	D84	5
CRU 88	D731A	8
CRU 89	D750	6
CRU 116	D644A	6
CRU 134	D809C	5
CRU 135	D809B	11
CRU 152	D620	6
JA 1/28 [POU]	D8	3
JA 2/2 [POU]	C578	6
JA 2/7 [POU]	C515	0
JA 2/8 [POU]	C32	2
JA 2/11 [POU]	C551	2
JA 2/22 [POU]	D6	4
JA 2/26 [POU]	C1141	8
JA 3/3 [POU]	C660	3
JA 3/29 [POU]	C95	7
JA 3/38 [POU]	C1003	8
JA 3/39 [POU]	C1153	4
JA 4/2 [POU]	C558	8
JA 6/12 [POU]	C339	7
JA 6/16 [POU]	D212A	4
JA 8/4 [POU]	C384	3
JA 8/8 [POU]	C195	1
JA 8/18 [POU]	C206	11
JA 9/9 [POU]	C38	4
JA 10/29 [POU]	C374	8
JA 10/31 [POU]	C1095	6
JA 10/40 [POU]	C1082	7
LP 1/23 [POU]	C334	8
LP 1/27 [POU]	C653	3
LP 1/28 [POU]	C766	4
LP 1/31 [POU]	C881	4
LP 1/34 [POU]	C818	5
LP 1/35 [POU]	C838	4
LP 1/36 [POU]	C886	2
LP 1/41 [POU]	C796	4
LP 1/42 [POU]	C816	5
LP 1/47 [POU]	C887	15
LP 1/56 [POU]	C871	8
LP 2/2 [POU]	C615	0
LP 2/3 [POU]	C369	8
LP 2/9 [POU]	D353	2
LP 2/10 [POU]	C889	6
LP 2/16 [POU]	C892	5
LP 3/38 [POU]	C894	7
LP 4/2 [POU]	C580	6
LP 4/41 [POU]	C965	13
LP 5/18 [POU]	C482	3
LP 5/23 [POU]	C888	4
LP 6/7 [POU]	C571	4
LP 6/10 [POU]	C511	3
LP 6/12 [POU]	C373	8
LP 6/16 [POU]	C538	4
LV 15 [POU]	C536	4
LV 36 [POU]	C600	7
LX 15	C216	3
LX 20	C192	2
LX 21	C607	6
LX 49	C1067	8
LZ 9	C226	7
LZ 11	C99	2
LZ 19	C1025	2
MARPER 2	C363A	0
MARPER 5	C782A	2
MARPER 9	C597	3
MARPER 16	D750A	2
MARPER 18	D11	5
MARPER 19	D22	2
MARPER 20	D47	4
MARPER 22	C475	0
MARPER 27	D491A	7
MARPER 28	D647A	6
MARPER 29	D307	6
MARPER 33	D166	6
MARPER 34	D559	5
MARPER 35	D775	4
MARPER 37	D661	3
MARPER 42	D713	5
MARPER 43	D747	3
MARPER 46	D764	10
MARPER 47	C39	7
MARPER 48	D758A	6
MARPER 49	D214A	4
MARPER 50	D790B	2
MARPER 51	D800	2
MARPER 52	D826A	4
MARPER 53	D776A	4
MARPER 54	D777A	6
MO 84	C759	2
MO 94	D594	5
MO 122	D725	2
MO 129	D563	2
MOQ 1/2	C722	0
MOQ 1/5	C490	7
MOQ 1/6	C807	6
MOQ 1/9	C487	10
MOQ 1/16	C541	0
MOQ 1/24	C815	5
MOQ 2/7	C494	1
MOQ 2/8	C124	2
MOQ 2/16	C850	9
MOQ 2/23	D274	4
MOQ 2/33	C781	0
MOQ 2/37	C855	11

Table 1 (cont.)

MOQ 2/38	C865	2	NA 156	D457	9	SCA 7	D655	9
MOQ 3/19	C864	7	NA 214	D418	4	SCA 16	D671	6
MOQ 3/20	C811	2	NA 242	D167	3	SCA 27	D530	8
MOQ 3/22	C785	2	NA 254	D372	4	SJ 1/15 [POU]	C743	2
MOQ 4/5	C249	0	NA 256	D269	4	SJ 1/20 [POU]	C728	10
MOQ 4/15	D315	5	NA 260	D341	5	SJ 1/22 [POU]	C635	7
MOQ 4/17	C984	4	NA 444	D793	5	SJ 1/36 [POU]	C423	4
MOQ 5/18	C814	0	NA 479	D750	1	SJ 1/41 [POU]	D295	2
MOQ 6/11	C151	4	NA 675	C251	2	SJ 2/28 [POU]	D395	8
MOQ 6/29	C765	8	NA 681	C663	6	SLA 23	D539	5
MOQ 6/34	C809	3	NA 694	C64	10	SLA 28	C495	6
MOQ 6/51	C769	4	NA 695	C47	0	SLA 86	C1151	3
MOQ 6/55	C799	4	NA 713	C275	11	SLA 97	C1154	6
MOQ 6/66	C186	8	NA 725	C675	6	SLC 1	C58	2
MOQ 6/70	C748	10	NA 734	D546	2	SLC 11	D504	5
MOQ 6/97	C869	6	NA 747	D360	2	SLC 25	C144	9
MOQ 6/108	C858	2	NA 877	D512	4	SM 2 [POU]	C79	2
NA 38	D122	10	NA 916	D525	0	SM 4 [POU]	C498	0
NA 48	D141	8	NA 929	D499	6	SM 6 [POU]	C496	3
NA 69	D607	6	NA 937	D513	8	SM 7 [POU]	C326	4
NA 79	D612	12	PA 98 [PER]	D295	4	SM 12 [POU]	C1092	2
NA 106	D252	5	PA 167 [PER]	D736	5	SM 13 [POU]	C1068	3
NA 119	D187	4	PA 186 [PER]	D446	6	SM 18 [POU]	D70	5
NA 120	D119	6	PA 203 [PER]	D709	3			
NA 151	D680	2	PA 310 [PER]	D732	2			

Future work

We have now established three or more grafted plants from 175 of the clones that have been propagated from Marper Farm (Table 1). For the remaining 44 clones with less then 3 survivors, budwood will be collected again and the grafting repeated.

There are also 220 accessions on the UWI Campus that are not represented in UCRS and are being multiplied by grafting as part of the Dutch LNV Project to Safeguard the ICG,T. The work is on-going, with a generally better success rate than was possible with the aging trees in Marper Farm.

Acknowledgements

This work has been made possible with funding from the Dutch Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector and CRA, UK.

Evaluation of microsatellites for verification of identities in cacao field genebanks

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

Introduction

The ICG,T is one of the largest cacao germplasm collections containing over 2,000 accessions, each represented by replicated trees in plots in a field genebank. Formally planned in 1982, the genebank was assembled from germplasm, mostly originating from multiple collecting expeditions (1930 onwards) from Amazonian South America, Central America and the West Indies (Kennedy and Mooleedhar, 1993). Mislabelled plants have been identified as a serious problem in germplasm collections (Hurka *et al*., 2004) and have been reported for *Cicer* (Shan *et al*., 2005), French olive (*Olea europaea* L.) (Khadari *et al.*, 2003), persimmon (*Diospyros kaki* Thunb.) (Badenes *et al.*, 2003) and cacao (Figueira, 1998; Risterucci *et al.*, 2001; Motilal and Butler, 2003) among others. Mislabelling within and among accession plots in the ICG,T may have arisen from a combination of factors such as the multiplicity of introductions and plant movements; field mapping and plot demarcation; and the discontinuity in personnel involved in the flow of germplasm material from the original collections and in genebank maintenance.

Mislabelling issues can be resolved by multilocus fingerprinting. A variety of molecular markers are available, but microsatellite markers are well suited being co-dominant (thus allowing the detection of heterozygotes), found throughout the genome, have high allelic variability (Powell *et al*., 1996) and are relatively fast and easy to analyse compared to other DNA markers (Morgante and Olivieri, 1993). Cacao microsatellites developed by Lanaud *et al.* (1999) have been utilised for cacao clone identification (e.g. Figueira, 1998; Risterucci *et al.*, 2001; Saunders *et al.*, 2004; Cryer *et al.*, 2006; Zhang *et al.*, 2006).

Unambiguous identification of individuals within germplasm collections is, however, a large-scale project requiring substantial resource and time allocations. Saunders *et al.* (2004) suggested that identity issues in cacao germplasm could be resolved with 15 microsatellite primer pairs (MPPs) which have since been utilised by cacao molecular biologists. However, there are approximately 12,000 trees (2,000 accessions × 6 propagated trees) in the ICG,T to be evaluated over 15 loci, and any effort to resolve identity issues in a timely and cost-effective manner would therefore be welcomed.

One way of making the task more efficient is to reduce the number of SSR loci required for detecting mislabelling in the germplasm collection. Furthermore, many other cacao SSRs have been identified since Saunders *et al.* (2004) made their recommendation and it is quite possible that other MPPs may be more useful than the proposed set. This study was therefore undertaken to determine the composition and minimum number of microsatellite loci required for an accelerated yet reliable protocol for fingerprinting individuals in a cacao germplasm collection.

Materials and methods

Plant material, DNA extraction and quantification

DNA from cacao leaf tissue collected from accessions in the ICG,T (Table 1) was extracted using

the Kobayashi protocol (Kobayashi *et al.*, 1998) using a FastPrep 120V machine (Qbiogene, Inc., California, USA) for maceration. Precipitated DNA was re-suspended in sterile deionised water (SDW) and kept as stock solutions. Dilutions (×100 or as required) of the stock DNA solutions were prepared in SDW and assayed with PicoGreen® (Molecular Probes, Eugene, Oregon, USA) in a Fluroskan Ascent (Labsystems, Finland) system. Final dilutions for experimental manipulations were prepared at 0.2 ng/µL in SDW. Two additional DNA samples (H1 and U1), extracted using a Qiagen DNeasy plant minikit (Qiagen GmbH, 2000), were obtained from a Peruvian collection held by USDA-ARS (Agriculture Research Service), Beltsville.

Table 1. Details of accessions used in this study.

Accession	Position	Group	Country of Origin (Status)
AC 2 [BLZ]	T1	Criollo	Belize (wild)
AC 20 [BLZ]	T1 (fp1032)	Criollo	Belize (wild)
B 9/10-25 [POU]	Marper Farm, C1078	Refractario	Ecuador (cultivated)
BC 3 [BLZ]	T1 (fp1019)	Criollo	Belize (wild)
COCA 3348/44 [CHA]	F6B E374 T2 (fp1047)	Forastero	Ecuador (wild)
CRIOLLO 22 [CRI]	F4A T1	Criollo	Costa Rica (cultivated)
EET 400 [ECU]*	F6B F455 T1	Forastero	Ecuador (cultivated)
ELP 1	T6 (fp950)	Forastero	French Guiana (wild)
GU 241/P	UWI, Campus 1a, x2y33 (fp500)	Forastero	French Guiana (wild)
H 1		Forastero	Peru (cultivated)
HF 8 [BLZ]	T1 (fp987)	Criollo	Belize (wild)
IB 2 [BLZ]*	T1 (fp1020)	Criollo	Belize (wild)
IB 9 [BLZ]	T1 (fp996)	Criollo	Belize (wild)
ICS 75	San Juan Estate, Block 2	Trinitario	Trinidad (cultivated)
ICS 97*	San Juan Estate, Block 1	Trinitario	Trinidad (cultivated)
ICS 100*	San Juan Estate, Block 2	Trinitario	Trinidad (cultivated)
IMC 3*	UWI, Campus 3	Forastero	Peru (wild)
IMC 12*	Marper Farm, C1056	Forastero	Peru (wild)
IMC 16	Marper Farm, D603	Forastero	Peru (wild)
IMC 67	La Reunion Estate	Forastero	Peru (wild)
JA 5/4 [POU]	Marper Farm, C526 (fp2307)	Refractario	Ecuador (cultivated)
JA 5/5 [POU]	Marper Farm, C324 (fp1351)	Refractario	Ecuador (cultivated)
LCT EEN 31	F6A A6 T3 (fp450)	Forastero	Ecuador (wild)
MO 9	Marper Farm, D835 (fp253)	Forastero	Peru (wild)
MO 20*	Marper Farm, D809 (fp254)	Forastero	Peru (wild)
MOQ 6/95*	Marper Farm, C1 (fp582)	Refractario	Ecuador (cultivated)
MXC 67	UWI, Campus 12, x3y6	Criollo	Mexico (cultivated)
NA 702*	Marper Farm, D104 (fp819)	Forastero	Peru (wild)
NAPO 2 [CHA]*	UWI, Campus 7, x8y9 (fp1922)	Forastero	Ecuador (wild)
PA 279 [PER]	Marper Farm, D59 (fp426)	Forastero	Peru (wild)
PA 299 [PER]	Marper Farm, C936 (fp571)	Forastero	Peru (wild)
POR 1 [TTO]*	UWI, Campus 2, x2y12 (fp1897)	Criollo	Venezuela
POUND 7/B [POU]	F6B F407 T3 (fp521)	Forastero	Peru (wild)
SCA 12*	Marper Farm, D205	Forastero	Peru (wild)
SCA 24*	Marper Farm, D569	Forastero	Peru (wild)
SPA 5 [COL]	UWI, Campus 2, x1y15 (fp1817)	Forastero	Colombia or Peru
U 1		Forastero	Peru (cultivated)
UF 613	F4A A93 T2 (fp1237)	Trinitario	Costa Rica (cultivated)
YAL 6		Forastero	French Guiana (wild)

*Accessions with missing allelic data that were not used for microsatellite statistical analyses.

SSR Amplification

The polymerase chain reaction (PCR) mix was composed of 4 µL Eppendorf HotMaster Mix (Brinkmann Instruments Inc., New York, USA) giving 2.5 mM Mg^{2+}, 2 mM total dNTP[1], 0.2 units of Taq polymerase activity at final composition in reaction mix; 0.5 µL of a MPP solution in TE[2] buffer (10 µM each primer; forward primers from Operon Technologies, Inc., Alabama, USA; reverse primers from Proligo Japan KK, Kyoto, Japan); and 5.5 µL of appropriate DNA solution. Each MPP was amplified from separate reaction mixes. Cycling was carried out in GeneAmp PCR System 9700 thermal cyclers (Applied Biosystems, California, USA). A touchdown protocol was utilised. Initial denaturation at 94°C for 5 mins was followed by eight cycles with denaturation at 95°C for 30 secs, annealing at 55°C for 60 secs with reduction by 0.5°C after every cycle and extension at 72°C for one minute. Then 25 cycles with denaturation at 94°C for 30 secs, annealing at 51°C for one minute and extension at 72°C for one minute were performed. A final extension step at 60°C for 15 mins was included to ensure complete adenine addition and the products were held at 4°C until recovery. Each combination of DNA-MPP was amplified at least once.

Capillary Electrophoresis (CE)

Post-PCR bulking was conducted by pooling 1.5 µL of each MPP-PCR product. Sample loading buffer containing 29.8 µL of Hi-Di formamide (Applied Biosystems, Warrington, UK) and 0.2 µL of GenomeLab™ DNA size standard-400 (Beckman Coulter Inc., California, USA) was added to each well. Samples were overlaid with one drop of mineral oil (Beckman Coulter Inc.). Fragments were separated on an 8-capillary CEQ 8000 or 8800 (Beckman Coulter Inc.) according to the manufacturer's recommendations. Products with poor standard profiles (missing bands; improper sizing) were discarded and the appropriate PCR product pools were recomposed and run again to ensure that fragment profiling was suitable for allele sizing. Raw fragment sizes were converted into alleles classes (binning) with the bundled fragment analysis software (Beckman Coulter Inc.).

MPP assessment

Twelve of the 15 recommended MPPs (Saunders *et al.*, 2004) and 22 additional MPPs (Table 2) were assessed on a set of 39 accessions (Table 1) from the Criollo, Forastero and Trinitario groups. PCR and CE were repeated once as described above. Allele binning was as described earlier. Allele data for the 34 MPPs over the 39 accessions were cleaned and sizing discrepancies were manually resolved. Eleven accessions had missing data for at least three loci and were removed from the main analyses; SCA 12 with one missing locus and SCA 24 with two missing loci were also excluded yielding a reduced database of 26 accessions in which two samples lacked allele data at two loci only. Summary statistics were obtained with PowerMarker v3.25 (Liu and Muse, 2005). Probability of identity (PID) values were obtained with GIMLET v.1.3.2 (Valière, 2002). Five groups of MPPs (Saunder's 12, GIMLET's top 12, Most Alleles (6 MPPs), Random (7 MPPs) and Minimal (4 MPPs)) were assessed for their capacity to differentiate by comparison of cumulative probability of identity (PID_{com}) values.

[1] Deoxyribonucleotide triphosphate
[2] Tri-EDTA (ethylenediamine tetraacetic acid)

Table 2. Microsatellite loci information on 26 cacao accessions.

Loci[1]	Number of accessions[2]	Groups separated	Number of alleles	Allele Range (bp[3])	PIC[4]	PID[5]
mTcCIR1	24	6	5	127-157	0.561	0.185
mTcCIR3	26	12	8	212-277	0.775	0.047
mTcCIR6	24	9	6	230-251	0.677	0.095
mTcCIR7	26	9	5	155-163	0.620	0.141
mTcCIR8	22	10	7	290-308	0.675	0.098
mTcCIR9	26	9	8	260-298	0.706	0.085
mTcCIR10	26	11	6	207-218	0.704	0.085
mTcCIR11	26	11	8	286-319	0.711	0.079
mTcCIR12	26	12	8	188-219	0.762	0.054
mTcCIR15	26	13	10	239-264	0.769	0.039
mTcCIR17	26	5	3	274-284	0.472	0.258
mTcCIR18	24	11	8	333-357	0.756	0.056
mTcCIR26	24	10	8	276-310	0.760	0.058
mTcCIR29	26	10	6	160-176	0.726	0.075
mTcCIR30	26	8	4	176-186	0.637	0.128
mTcCIR33	25	15	10	275-347	0.832	0.025
mTcCIR37	26	16	12	136-181	0.840	0.020
mTcCIR42	26	12	8	205-240	0.728	0.062
mTcCIR43	26	11	7	203-215	0.667	0.085
mTcCIR45	26	7	4	288-294	0.531	0.201
mTcCIR55	26	5	3	238-250	0.386	0.348
mTcCIR56	26	9	6	317-368	0.702	0.087
mTcCIR57	26	8	4	248-256	0.585	0.024
mTcCIR58	26	16	12	209-324	0.829	0.066
mTcCIR60	23	12	9	189-215	0.721	0.059
mTcCIR184	26	12	7	118-147	0.748	0.059
mTcCIR210	26	6	4	139-151	0.662	0.116
mTcCIR229	26	10	7	311-327	0.669	0.103
mTcCIR243	26	9	5	128-143	0.695	0.088
mTcCIR244	26	13	9	241-272	0.754	0.055
mTcCIR274	26	11	9	188-277	0.696	0.089
mTcCIR278	26	3	2	99-101	0.361	0.377
S012	26	5	5	264-284	0.578	0.173
S016	26	7	5	202-222	0.581	0.161

[1]Microsatellite code; [2]Number of accessions with allele data; [3]Base pair
[4]Polymorphic information content; [5]Probability of identity
Number of alleles, range and PIC obtained from PowerMarker v3.25 (Liu and Muse, 2005).
PID obtained from GIMLET v1.3.2 (Valière, 2002).

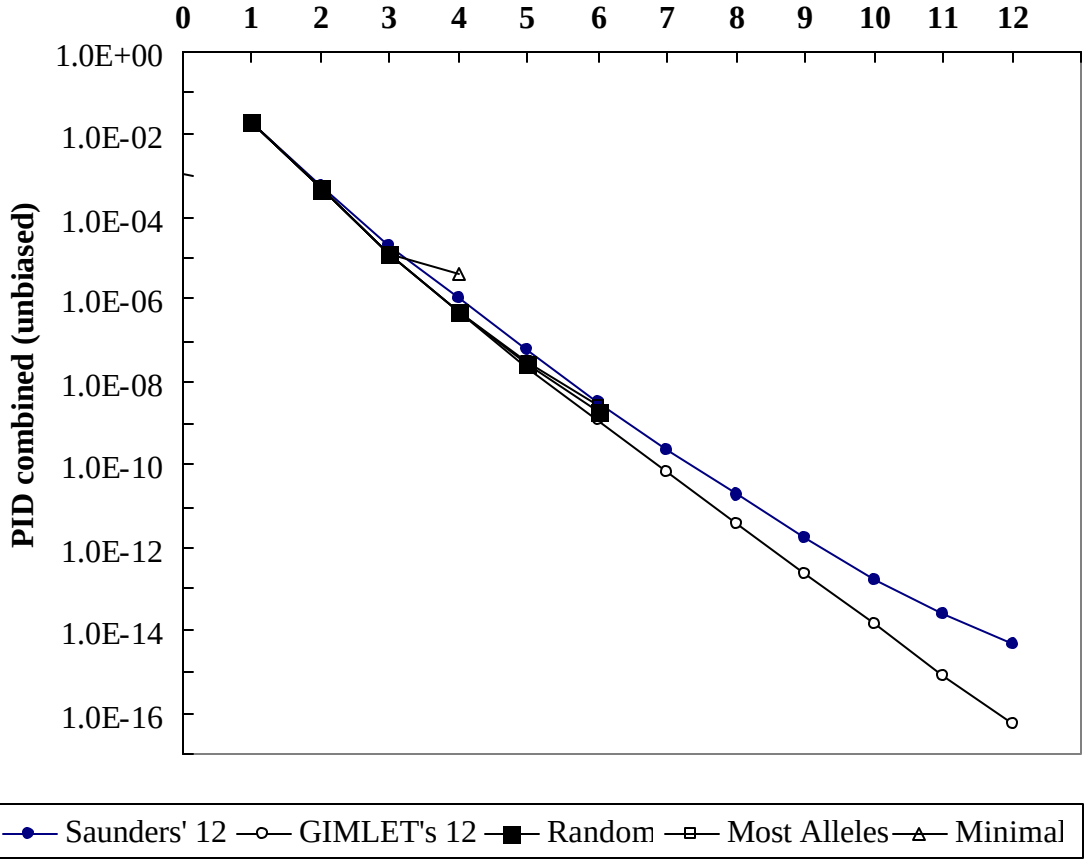
Results

Characteristics of the individual MPPs based on 26 accessions are provided in Table 2. The MPPs, mTcCIR37 and mTcCIR58 generated the most alleles (12) followed by mTcCIR15 and mTcCIR33 (10) and then mTcCIR60, mTcCIR244 and mTcCIR274 with nine alleles. The separation ability of the MPPs was significantly correlated (large, positive; r = 0.93; $P<0.001$) with the number of alleles. The PID values from GIMLET v1.3.2 (Valière, 2002) enabled ranking of the differentiating power of the MPPs. The twelve primers recommended by Saunders *et al*. (2004) were ranked as mTcCIR37, 33, 15, 12, 18, 26, 60, 11, 6, 8, 7 and 1 from most to least

differentiating. The twelve top ranked from the full complement of 34 MPPs were mTcCIR 37, 58, 33, 15, 3, 12, 244, 18, 26, 184, 42 and 60 and included seven of the MPPs recommended by Saunders *et al.* (2004).

As the number of loci increased, the probability of grouping together more than one different accession was decreased (Figure 1), with MPPs from Random, Most Alleles and GIMLET's 12 achieving lower PID_{com} values at a faster rate. The latter gave the most confident result (lowest probability of having two accessions that match each other). Two groups of accessions, each with two samples (BC 3 and HF 8; AC 20 and IB 9) could not be resolved with the primers recommended by Saunders *et al*. (2004) or with the full complement of 34 primers applied to the dataset of 26 accessions (Table 3). The random set of MPPs was unable to resolve the accessions to the same extent as the other MPP groupings even though the number of loci used was greater than that of two other groups. Three MPPs (mTcCIR 33, 37 and 58) were sufficient to assign the accessions to the same identity groupings as the full complement of MPPs used on the same 26 accessions.

Figure 1. Probability of identity (PID) combined over increasing loci number for different sets of microsatellite loci.



Lower values indicate an increased probability that the accessions are different; output from GIMLET v1.3.2 (Valière, 2002). Primer sets were: GIMLET's 12 = mTcCIR 3,12,15,18,26,33,37,42,58,60,184,244; Minimal = mTcCIR 33,37,55,58; Most Alleles = mTcCIR 15,33,37,58,60,184; Random = mTcCIR 6,9,10,45,57,229,243; and Saunder's 12 = mTcCIR 1,6,7,8,11,12,15,18,26,33,37,60;

Table 3. Differentiation ability of select microsatellite loci groupings on 26 accessions.

MPP group[1]	Number separated	% separation	PID_{com}[2]	Unresolved accessions
All 34 loci	24	92.3	1.49×10^{-36}	(AC 20 T1, IB 9 T1); (BC 3 T1; HF 8 T1)
Saunder's 12	24	92.3	4.42×10^{-15}	(AC 20 T1, IB 9 T1); (BC 3 T1; HF 8 T1)
GIMLET's 12	24	92.3	5.31×10^{-17}	(AC 20 T1, IB 9 T1); (BC 3 T1; HF 8 T1)
Random	20	76.9	2.02×10^{-7}	(AC 2 T1, AC 20 T1, BC 3 T1, CRIOLLO 22 F4AT1, HF 8 T1, IB 9 T1); (ELP 1 T6, GU 241/P)
Most Alleles	24	92.3	2.80×10^{-9}	(AC 20 T1, IB 9 T1); (BC 3 T1; HF 8 T1)
Minimal	24	92.3	4.22×10^{-6}	(AC 20 T1, IB 9 T1); (BC 3 T1; HF 8 T1)

[1]Microsatellite primer pairs; Saunder's 12 = mTcCIR 1,6,7,8,11,12,15,18,26,33,37,60; GIMLET's 12 = mTcCIR 3,12,15,18,26,33,37,42,58,60,184,244; Random = mTcCIR 6,9,10,45,57,229,243; Most Alleles = mTcCIR 15,33,37,58,60,184; Minimal = mTcCIR 33,37,55,58.
[2]Combined probability of identity obtained from GIMLET v1.3.2 (Valière, 2002).

When all 39 accessions were utilised a similar result occurred, even though missing values (17 accessions/102 missing alleles) were present in the dataset. The accession IB 2 T1 could not be clearly differentiated in the primer groups Saunder's 12, GIMLET's top 12, Most Alleles, Random and Minimal due to the presence of missing data for the loci mTcCIR 1, 11, 12, 26 and 33. The full complement of primers allocated this accession to a unique group based on the exclusive allele pattern obtained from mTcCIR30. Excluding the identical accessions (HF 8/BC 3; IB 9/AC 20), there were 14 accession pairs that had more than 25 identical loci. Three and eight pairs of Criollo material exhibited 31 and 32 matching loci respectively and the accession pair JA 5/4 vs. JA 5/5 had 27 matching loci. Resolution of the Criollo pairs was achieved with the primers mTcCIR26 (5/11 pairs), mTcCIR33 (10/11 pairs), mTcCIR37 (6/11 pairs) and mTcCIR55 (4/11 pairs). JA 5/4 was resolved from JA 5/5 by seven primers (mTcCIR 7, 8, 29, 45, 58, 229 and 274).

The accession NA 702 was homozygous at 26 of 31 loci (mTcCIR11, 12 and 26 with missing data). In-house data from the USDA/CRU fingerprinting project confirmed the high homozygosity of this accession and provided the missing data to reveal 28 of 34 loci (82.4%) to be homozygous. The MPPs mTcCIR 10, 12, 29, 42, 60 and 274 were heterozygous in this accession.

Discussion

This study examined the possibility of accelerating verification of identities in genebanks by decreasing the number of genotyped loci. Subsumed within this objective was the identification of loci which were most suited for differentiating cacao accessions. Successful and efficient verification of identities in a germplasm collection relies on the judicious use of microsatellite loci. The loci chosen must be able to differentiate among existing and future accession holdings. Furthermore, loci should be used that would maximise differences among accessions. The latter is especially important when highly homozygous material need to be surveyed for mislabelled plants. Results of the primer survey suggested that the primers recommended by Saunders *et al*. (2004) were good discriminatory loci and that seven of these (mTcCIR 12, 15, 18, 26, 33, 37 and 60) were the most useful. Interestingly, three loci (mTcCIR33, 37 and 58) resolved the 26 accessions into the identical groupings generated by a set of 12 of the fifteen primers recommended by Saunders *et al.* (2004).

Furthermore the random set of seven primers underperformed in comparison to other primer

combinations, suggesting that the composition of the MPP set is more important than the number of loci that is used. The latter is important when a low PID is required as increasing the number of loci reduces the match probability. Zhang *et al*. (2006) demonstrated that seven loci could have sufficient differentiation power for cacao accessions. In-house CRU/UWI fingerprinting data revealed that the accessions NA 228 (fp1) and NA 266 (fp25) could not be resolved from each other with the 15 primers recommended by Saunders *et al.* (2004) although the MPPs mTcCIR6 and mTcCIR8 were useful in separating NA accessions from one another. Preliminary work (data not shown) indicated that mTcCIR57, 229 and 243 separated NA 228 from NA 266. This suggests that Type II errors (accessions declared similar when they are really different) may be overlooked in verification work and that this error is due not only to the number of loci used but, more importantly, the types of loci utilised for differentiation purposes.

Table 4. Recommended MPPs for verification work.

Loci	Allele range	Suggested dye	Loci note and advantage
Set L1			
mTcCIR26	276-310	Green	9th ranked locus; resolves Criollo material
mTcCIR33	275-347	Black	3rd ranked locus; resolves Criollo material
mTcCIR37	136-181	Black	1st ranked locus; resolves Criollo material
mTcCIR55	238-250	Blue	33rd ranked locus; resolves Criollo material
Set L2			
mTcCIR8	290-308	Green	22nd ranked locus; differentiates JA 5/4 from JA 5/5; resolves NA material
mTcCIR57	248-256	Blue	27th ranked locus; resolves NA accessions
mTcCIR58	209-324	Black	2nd ranked locus; differentiates JA 5/4 from JA 5/5
mTcCIR229	311-327	Blue	23rd ranked locus; differentiates JA 5/4 from JA 5/5; resolves NA accessions

Loci ranking obtained from GIMLET v1.3.2 (Valière, 2002).

It is suggested that a set of eight MPPs could be used for verification purposes in the ICG,T and that electrophoresis of amplified fragments could be performed by pooling products from four MPPs. In this way, both the number of PCR and CE runs can be substantially reduced. The recommended primers are presented in Table 4. The inclusion of low ranked loci (based on PID values) was justified by their utility in discriminating amongst homozygous material. With this recommended set of primers used on the dataset of 26 accessions, a combined PID of 1.64×10^{-10} would be obtained providing sufficient confidence in match declarations. The adoption of these primers by the cacao community would allow for more rapid and definitive resolution of identities in other cacao germplasm collections. Accessions declared as similar under these circumstances may be considered to be genetically similar (e.g. BC 3/HF 8 and AC 20/ IB 9) and unless morphological or agronomic evidence indicates otherwise may be grouped together as the same accession. However, since the possibility always exists that some other primer (existing or future) may separate these accessions, genebank curators may prefer to keep the accessions separate but flag these as being synonymies.

Acknowledgements

Stephen Pinney, Sue Mischke and Eric Tillson are thanked for their assistance in the laboratory and for suggestions during this work.

References

Badenes, M., Garcés, A., Romero, C., Romero, M., Clavé, J., Rovira, M. and Llácer, G. (2003) Genetic diversity of introduced and local Spanish persimmon cultivars revealed by RAPD markers. *Genet. Resour. Crop Evol*. **50**: 579-585.

Cryer, N.C., Fenn, M.G.E., Turnbull, C.J. and Wilkinson, M.J. (2006) Allelic size standards and reference genotypes to unify international cocoa (*Theobroma cacao* L.) microsatellite data. *Genet. Resour. Crop Evol*. **53**: 1643-1652.

Figueira, A. (1998) Homononymous genotypes and misidentification in germplasm collections of Brazil and Malaysia. *INGENIC Newsletter* **4**: 4-8.

Hurka, H., Neuffer, B. and Friesen, N. (2004) Plant genetic resources in botanical gardens. Pages 35-44 in: *Proc. 21st International Symposium on Breeding Ornamentals, Part II* (G. Forkmann, S. Michaelis Eds) *Acta. Hort*. **651.**

Kennedy, A.J. and Mooleedhar, V. (1993) Conservation of cocoa in field genebanks– the International Cocoa Genebank, Trinidad. In: *Proc. Intl. Workshop on Conservation,Characterisation and Utilisation of Cocoa Genetic Resources in the 21st Century*, Port of Spain, Trinidad, September 13-17, 1992, The Cocoa Research Unit, Port of Spain, Trinidad, pp. 21-23.

Khadari, B., Breton, C., Moutier, N., Roger, J.P., Besnard, G., Bervillé, A. and Dosba, F. (2003) The use of molecular markers for germplasm management in a French olive collection *Theor. Appl. Genet*. **106**: 521-529.

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

Lanaud, C., Risterucci, A.M., Pieretti, I., Falque, M., Bouet, A. and Lagoda, P.J.L. (1999) Isolation and characterization of microsatellites in *Theobroma cacao* L. *Mol. Ecol.* **8**: 2141-2152.

Liu, K. and Muse, S.V. (2005) PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**: 2128-2129.

Morgante, M. and Olivieri, A.M. (1993) PCR-amplified microsatellites as markers in plant genetics. *Plant J*. **3**: 175-182.

Motilal, L. and Butler, D. (2003) Verification of identities in global cacao germplasm collections. *Genet. Resour. Crop. Evol.* **50**: 799-807.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996) The comparison of RFPL, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*. **2**: 225-238.

Qiagen GmbH. (2000) DNeasy Plant Mini Kit and DNeasy MaxiKit Handbook 08/2000. Germany: Qiagen GmbH.

Risterucci, A.M., Eskes, B., Fargeas, D., Motamayor, J.C. and Lanaud, C. (2001) Use of microsatellite markers for germplasm identity analysis in cocoa. Pages 25-33 in: *Proceedings of the 3rd International Group for Genetic Improvement of Cocoa (INGENIC) International Workshop on the New Technologies and Cocoa Breeding*. 16th-17th October 2000, Kota Kinabalu, Malaysia: INGENIC, UK.

Saunders, J.A., Hemeida, A.A. and Mischke, S. (2004) Selection of international molecular standard for DNA fingerprinting. *Theor. Appl. Genet*. **110**: 41-47.

Shan, F., Clarke, H.C., Plummer, J.A., Yan, G. and Siddique, K.H.M. (2005) Geographical patterns of genetic variation in the world collections of wild annual *Cicer* characterized by amplified fragment length polymorphisms. *Theor. Appl. Genet*. **110**: 381-391.

Valière, N. (2002) GIMLET: A computer program for analyzing genetic individual identification data. *Mol. Ecol. Notes* **2**: 377-379.

Zhang, D., Mischke, S., Goenaga, R., Hemeida, A.A. and Saunders, J.A. (2006). Accuracy and reliability of high-throughput microsatellite genotyping for cacao clone identification. *Crop Sci*. **46**: 2084-2092.

The contribution of the collaborative USDA/CRU project to resolve identity issues for trees in Marper Farm with missing labels

M. Boccara and D. Zhang

Introduction

Two fields in Marper Farm (Blocks C and D) were established by F.J. Pound following his expeditions to the upper Amazon between 1937 and 1942. After establishment, a survey was conducted in 1943 to check surviving trees and the infection rate of Witches' Broom disease. As a general rule, 2 replicate trees were planted in contiguous rows, one was discarded after assessment and a location number was given to the remaining one.

However, according to the 1943 records, some tree labels were already missing, and others were subsequently lost. When leaf samples were collected for DNA extraction in the USDA/CRU project, trees with no labels were given "MARPER" names.

Currently, in Marper Farm, 20 trees labelled MARPER are still alive in Block C, and 31 in block D; despite the lack of information about their identity, these trees are being replicated in the LNV Project to Safeguard the ICG,T (Table 1).

A main goal of the international collaborative project on DNA fingerprinting of Cocoa germplasm, which was started in 2001, was to confirm the identity of all trees in the ICG,T, and this led to renewed interest in the "MARPER" clones.

Achievements

Since 2001, leaves have been collected from every live tree in Blocks C and D of Marper Farm, in addition to other original accessions.

DNA samples were sent to the USDA-ARS Beltsville laboratory to be analysed with 15 recommended SSR primers, following the recommended protocol and guidelines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS Beltsville laboratory are available for 1,400 clones from CRU, including 49 "MARPER" clones and have been used for different purposes:

? To identify the individual trees
? To place trees within appropriate accession groups.

Table 1. List of "MARPER" accessions and their locations.

Accession	Field location	DNA sample number	Comments
MARPER 1	C372	fp2549	Same location number as SJ 1/42 [POU] dead
MARPER 2	C363A	fp2527	Extra tree next to LP 3/2 [POU] location C363
MARPER 3	C1011	fp2602	Same location number as B 6/11 [POU] dead
MARPER 4	C782	fp2546	Tree PA 288 [PER] missing after landslide in 1943
MARPER 5	C782A	fp2542	Extra tree next to MARPER 4
MARPER 6	C783	fp2544	Tree LP 1/51 [POU] missing after landslide in 1943
MARPER 7	C784	fp2545	Tree MOQ 2/18 missing after landslide in 1943
MARPER 8	C216	fp2525	Same location number as LX 15 dead
MARPER 9	C597	fp2566	Same location number as CL 9/12 dead
MARPER 10	C895	fp2539	Same location number as AM 2/88 [POU] dead
MARPER 11	C492	fp2529	Same location number as CL 13/32 dead
MARPER 12	C942	fp2540	Same location number as AM 2/84 [POU] dead
MARPER 13	C748	fp2575	Same location number as MOQ 6/70 dead
MARPER 14	C622	fp2552	Same location number as LP 1/6 [POU] dead
MARPER 15	C449	fp2028	Same location number as CL 9/47 dead
MARPER 16	D750A	fp667	Extra tree next to NA 540 location D 751 dead
MARPER 17	C660	fp2368	Same location number as JA 3/3 [POU] dead
MARPER 18	D11	fp394	Label missing in 1943
MARPER 19	D22	fp414	Label missing in 1943
MARPER 20	D47	fp419	Label missing in 1943
MARPER 21	D31	fp80	Label missing in 1943
MARPER 22	C475	fp2345	Same location number as AM 2/49 [POU] dead
MARPER 24	D119	fp77	Same location number as NA 120 dead
MARPER 25	D122	fp82	Same location number as NA 38 dead
MARPER 27	D491A	fp84	Extra tree between NA 251 and PA 169
MARPER 28	D647A	fp76	Extra tree between IMC67,IMC45 and NA 157
MARPER 29	D307	fp69	Label missing in 1943
MARPER 30	D212A	fp86	Same location number as JA 6/16 [POU] dead
MARPER 31	D208	fp75	Same location number as B 7/13 [POU] dead
MARPER 33	D166	fp78	Same location number as B 18/9 [POU] dead
MARPER 34	D559	fp150	Label missing in 1943
MARPER 35	D755	fp690	Label missing in 1943
MARPER 37	D661	fp281	Label missing in 1943
MARPER 38	D167	fp318	Same location number as NA 242 dead
MARPER 39	D168	fp321	Same location number as B 22/15 [POU] dead
MARPER 40	D680	fp85	Same location number as NA 151 dead
MARPER 41	D706	fp227	Label missing in 1943
MARPER 42	D713	fp320	Extra tree next to MO 4 location D 684 dead
MARPER 43	D747	fp263	Label missing in 1943
MARPER 44	D251	fp74	Same location number as B 14/14 [POU]
MARPER 45	D251A	fp83	Extra tree between NA 98 and SLC 24
MARPER 46	D764	fp251	Label missing in 1943
MARPER 47	C39	fp1270	Same location number as AM 1/38 [POU] dead
MARPER 48	D758A	fp244	Extra tree between PA 159 [PER] and B 14/17 [POU] dead
MARPER 50	D790B	fp674	Extra tree next to PA 151 [PER] location D 750 alive
MARPER 51	D800	fp668	Same location number as NA 345 dead
MARPER 52	D826A	fp711	Extra tree between NA 232 and NA 300 dead
MARPER 53	D776A	fp670	Same location number as NA 406 dead
MARPER 54	D777A	fp675	Same location number as NA 537 dead
MARPER 55	C249	fp1365	Same location number as MOQ 4/5 dead

Methods

Genetic grouping of the "MARPER" clones was assessed in relation to the 1,400 clones sampled in the ICG, T, using dissimilarity analysis (DARwin software, 5.0.142) and Principal Component Analysis (Genetix software, v.4.03).

The similarity of DNA profiles was examined and used in combination with all information available in historical records, publications and maps.

Results

Genetic diversity of the MARPER clones

The Principal component analysis (PCA) using the Genetix software (Figure 1) shows that:

- ? Some accessions labelled MARPER fall in the PA group
- ? Some accessions fall in the NA group
- ? Some accessions fall in the Refractario group
- ? Some belong to other genetic groups such as Trinitario
- ? Many accessions cannot be assigned to a distinctive group.

The Cluster analysis of the 49 "MARPER" DNA samples using the DARwin software and the detailed comparison of their multilocus profile provided additional information (Table 2).

Table 2. Assignments to groups of "MARPER" accessions.

Accessions clustered with PA accessions						
MARPER 4	fp2546	C782		MARPER 5	fp2542	C782A
MARPER 27	fp84	D491A		MARPER 42	fp320	D713

Accessions clustered with Refractarios accessions						
MARPER 11	fp2529	C492		MARPER 12	fp2540	C942
MARPER 19	fp414	D22		MARPER 20	fp419	D47
MARPER 21	fp80	D31		MARPER 43	fp263	D747

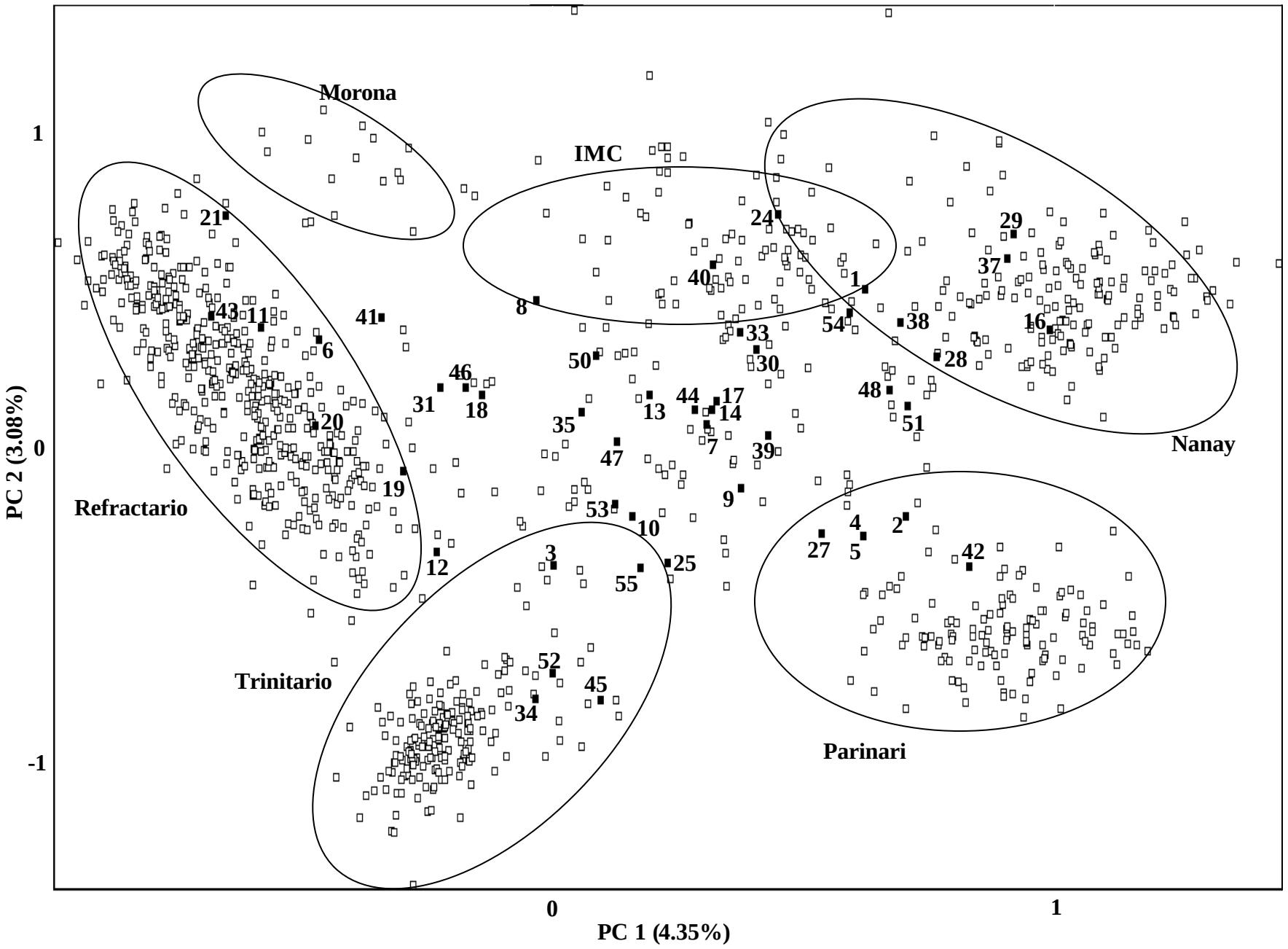
Accessions clustered with NA accessions						
MARPER 16	fp613	D750A		MARPER 28	fp76	D647A
MARPER 29	fp69	D307		MARPER 37	fp281	D661
MARPER 38	fp318	D167				

Accessions clustered with Trinitarios accessions						
MARPER 3	fp2602	C1011		MARPER 25	fp82	D122
MARPER 34	fp150	D559		MARPER 35	fp690	D755
MARPER 45	fp83	D251A		MARPER 47	fp1270	C39
MARPER 52	fp711	D826A		MARPER 55	fp1365	C249

Trees showing a PA profile

MARPER 4 location C782 and MARPER 5 location C782A share the same profile and belong to the PA group; the original tree PA 288 [PER] planted in that position went missing after a

Figure 1. Principal component analysis for 1,400 accessions from the ICG,T. Trees with a MARPER label are shown as solid points.



landslide: these two trees could have re-grown from it.

MARPER 27 location D491A is an extra tree growing next to two PA accessions which are still alive (PA 189 [PER], PA 169 [PER]) and three PA accessions now dead (PA 127 [PER], PA 157 [PER] and PA 202 [PER]), the latter two accessions having been duplicated in UCRS. Profile comparisons show that MARPER 27 is not a duplicate of any of its neighbours, but it belongs to the PA group. It could be a seedling of one of them or even the missing clone PA 127 [PER].

MARPER 42 location D713 shows a PA profile and matches for 14/15 markers the profiles of MO 4 and IMC 41. We suggested in the last annual report (Boccara *et al.*, 2005) that these identical trees could have been propagated from a PA accession seedling instead of from their mother-trees now dead: MARPER 42 could be that seedling.

Trees showing a NA profile

The accession labelled MARPER 16 location D750A is growing where NA 540 (now dead) was planted; molecular analysis concerning the duplicate NA 540 tree in field 5B shows a Trinitario profile, indicative of propagation mistakenly done from the rootstock. MARPER 16 is probably the real NA 540 or could be a seedling from that tree.

MARPER 28 location D647A, and two of its neighbours IMC 67 location D647 and IMC 45 location D648 show NA profiles. All these trees, originally planted on a very steep terrain and now lying on the ground, could be regrowths or seedlings originating from NA 157 location D649. The replicate trees of NA 157 in UCRS should be checked.

The labels of trees planted in D307 and D661 were missing in 1943, and the names MARPER 29 and MARPER 37 have been assigned to them. DNA analysis shows that these trees belong to the NA group.

MARPER 38 is a tree growing at the position D167 and was tentatively renamed NA 242 from the 1943 records. This result confirms that it is in the NA group and it is likely to be NA 242.

Trees showing a Refractario profile

MARPER 11, fp2529 location C492 is growing where, according to the 1943 records, CL13/32 was originally planted. Since it shows a Refractario profile, it is probably CL13/32. This is also the case of MARPER 12 location D942; it was a neighbour of AM 2/84 [POU] now missing.

The names MARPER 19, MARPER 20, MARPER 43 were given to trees without labels planted in D22, D47 and D747 respectively: the DNA profile analysis shows that all 3 trees belong to the Refractario group.

The profile of the tree MARPER 21 growing in D31 matches perfectly its neighbour, B 7/3 [POU] in D30: they are duplicate trees. Their similar morphology had previously been noted.

Trees showing a Trinitario profile

The dissimilarity analysis shows that eight MARPER accessions are closely related and all show a Trinitario profile, implying that they are rootstock. (Figure 2).

MARPER 3, MARPER 25, MARPER 47, MARPER 55 occupy locations where the original accession has disappeared, whilst MARPER 45 and MARPER 52 are extra trees. MARPER 34 and 35 had lost their identification labels in 1943.

Trees not falling in any group

Even if the results of DNA analysis did not place with certainty the remaining MARPER clones in

Figure 2. Trees showing a Trinitario profile; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.

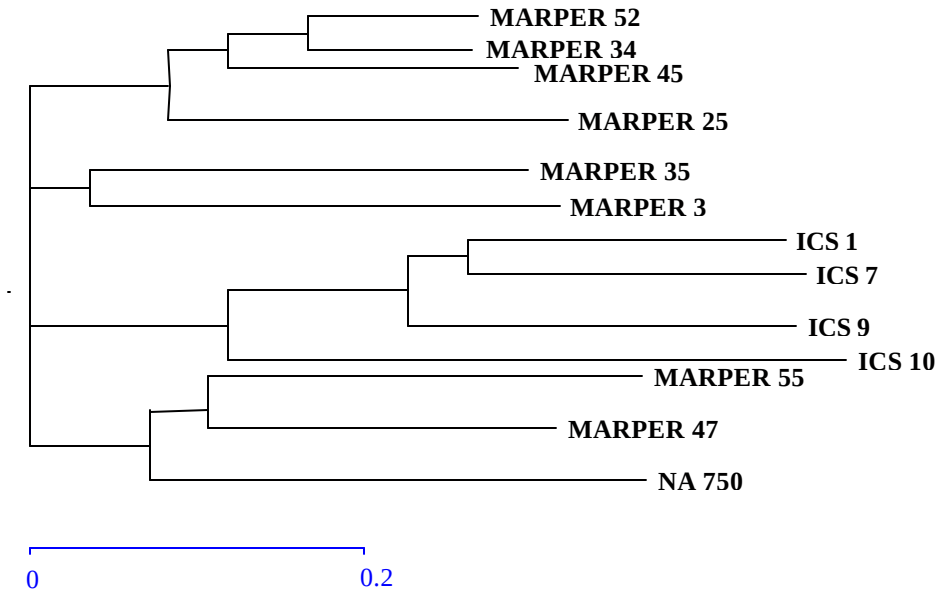
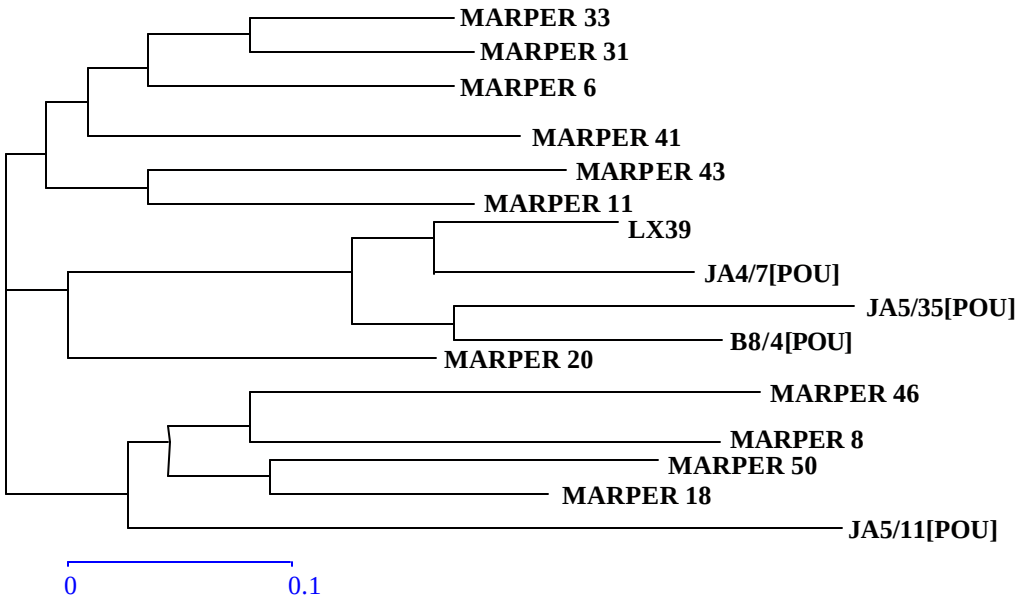


Figure 3. Trees showing a Refractario-like profile; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.



a predefined group, the dendrogram constructed with the DARwin software (Figure 3) shows eight accessions in the same cluster as Refractarios (Table 3). Phenotypic diversity analysis could be very valuable for further assessment.

According to the PCA the remaining **"**MARPER" trees (Table 4) do not cluster clearly in any predefined group; however, the dendrogram constructed with the DARwin software shows that they are closely related (Figure 4).

Table 3. "MARPER" accessions possibly belonging to the Refractario group.

Accession	Field location	DNA sample number
MARPER 6	C783	fp2544
MARPER 8	C216	fp2525
MARPER 11	C492	fp2529
MARPER 18	D11	fp394
MARPER 31	D208	fp75
MARPER 33	D166	fp78
MARPER 41	D706	fp227
MARPER 46	D764	fp251
MARPER 50	D790B	fp674

Figure 4. Trees not falling into any group; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.

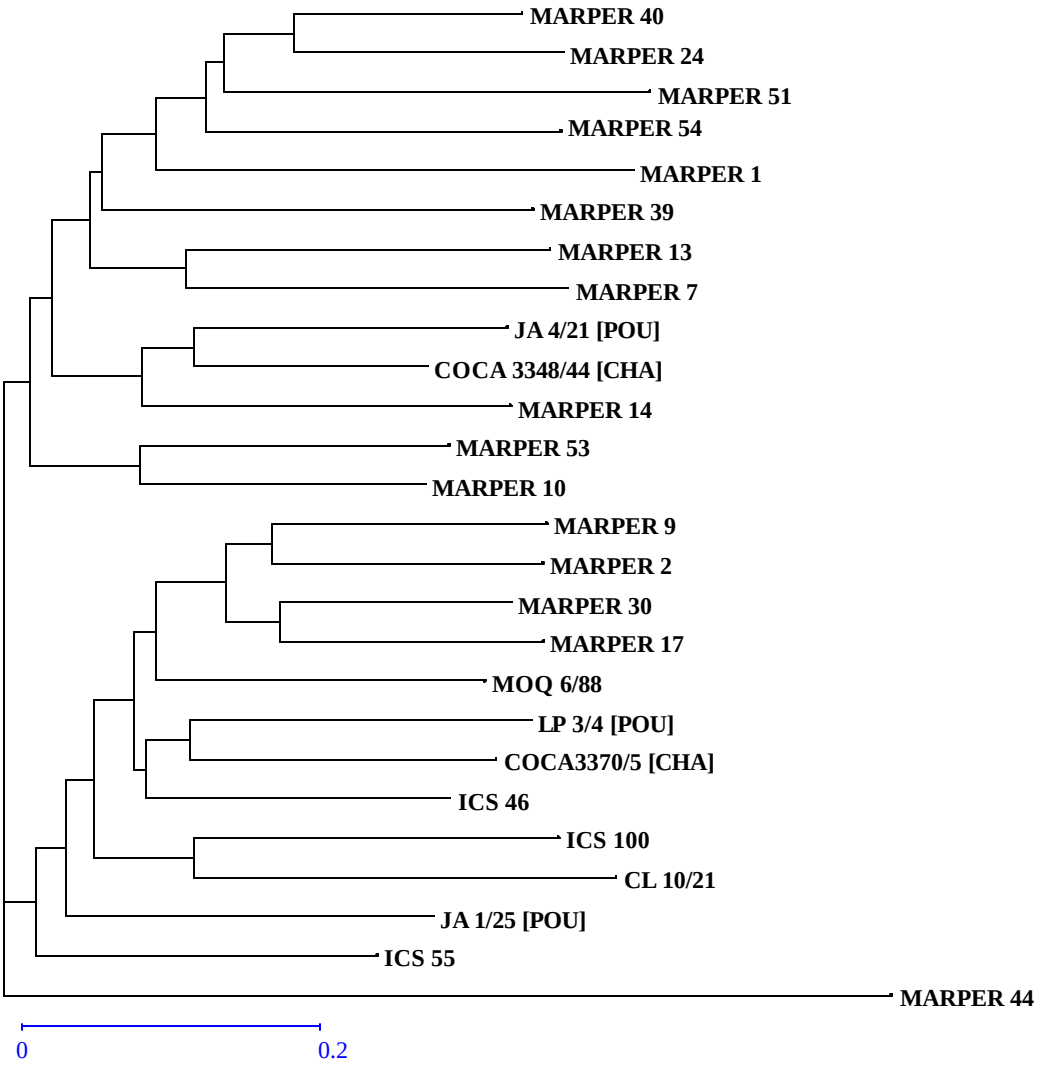


Table 4. "MARPER" accessions not assigned to a predefined group.

Accession	Field location	DNA sample number
MARPER 1	C372	fp2549
MARPER 2	C363A	fp2527
MARPER 7	C784	fp2545
MARPER 9	C597	fp2566
MARPER 10	C895	fp2539
MARPER 13	C748	fp2575
MARPER 14	C622	fp2552
MARPER 17	C660	fp2368

Accession	Field location	DNA sample number
MARPER 24	D119	fp77
MARPER 30	D212A	fp86
MARPER 39	D168	fp321
MARPER 40	D680	fp85
MARPER 44	D251	fp74
MARPER 51	D800	fp668
MARPER 53	D776A	fp670
MARPER 54	D777A	fp675

It is noteworthy that each of these trees, without exception, is occupying a location where the original accession is reported dead. One explanation could be that they are non-Trinitario surviving rootstock, or spontaneous hybrids from upper Amazon or Refractario accessions.

We found out that the clone MARPER 44, erroneously renamed B14/14 [POU] to match the old records, does not belong to the Refractario group: its MARPER name must be reinstated.

Discussion and conclusion

The use of 15 markers has been efficient in completing the unambiguous identification of accessions unlabelled 60 years ago. While more than one half of the "MARPER" clones can be assigned to an accession group, others were shown to be Trinitario rootstock, and the remaining clones may be hybrids or non-Trinitario rootstocks.

After assessment, clones of interest should be duplicated and transferred to UCRS for safe conservation.

Acknowledgements

We thank Antoinette Sankar for DNA sample preparation, Frances Bekele for sharing her knowledge on morphological traits, and the USDA-ARS Beltsville team for the efforts made to process the samples and generate molecular profile data.

References

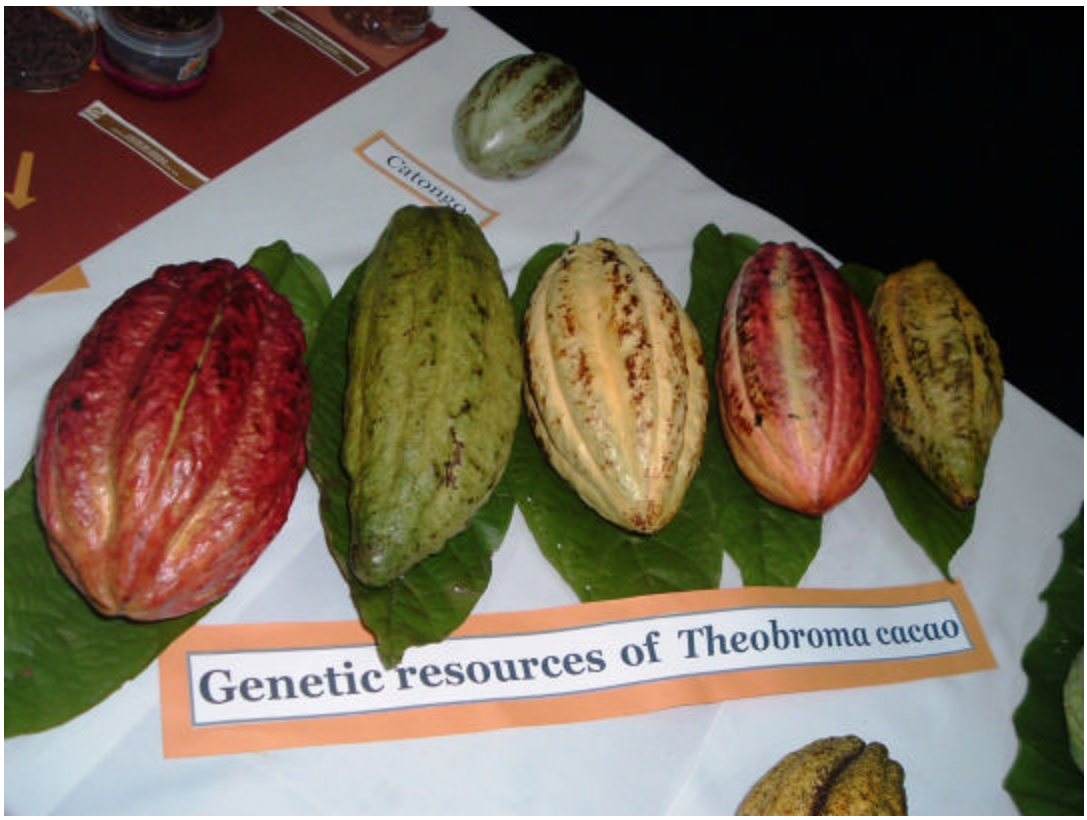
Belkhir K. et al. (1996-2004) GENETIX V.4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

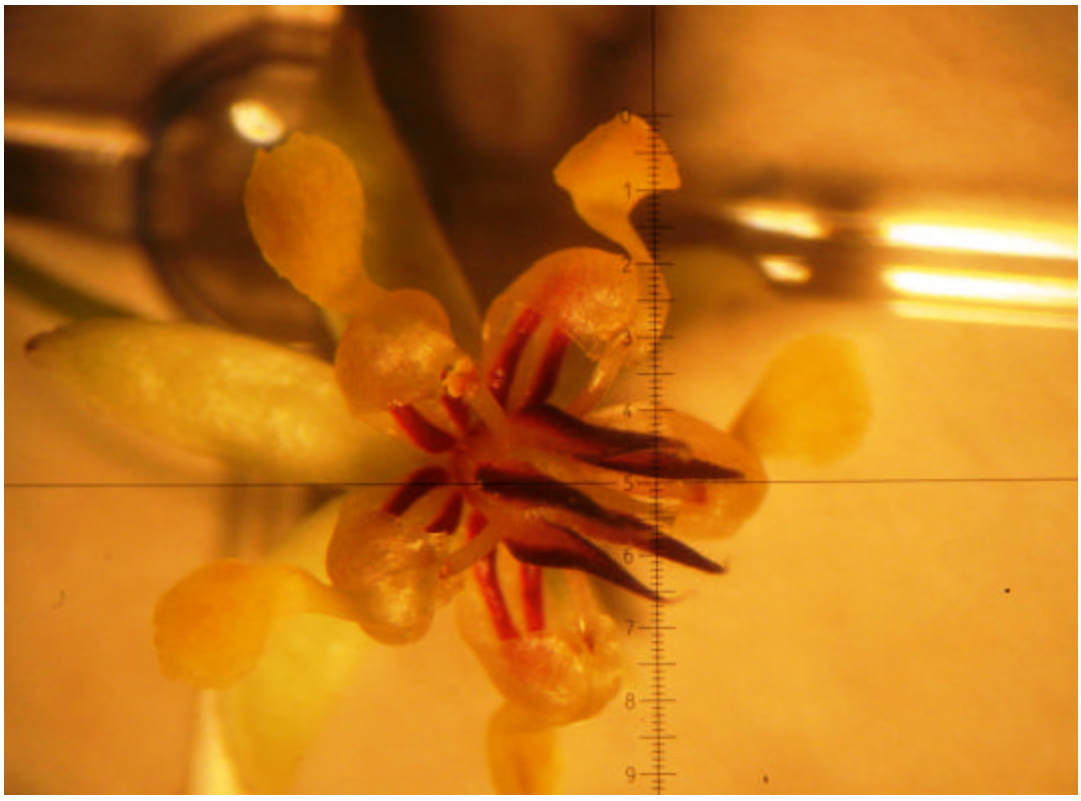
Boccara, M., Zhang, D. (2006) Progress in resolving identity issues among the Parinari accessions held in Trinidad: the contribution of the collaborative USDA/CRU project. Pages 25-37 in: *Annual Report 2005.* St Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Perrier, X., Flori, A., Bonnot, F. (2003) Data analysis methods. Pages 43-76 in: *Genetic diversity of cultivated tropical plants* (P. Hamon, M. Seguin, X. Perrier and J.C. Glaszmann Eds). Montpellier, France: Enfield Science.

Saunders, J.A. (2000) USDA DNA Fingerprinting programme for identification of *Theobroma cacao* accessions. Pages 108-114 in: *Proceedings of the International Workshop on New Technologies and Cocoa Breeding.* 16th-17th October 2000, Malaysia: INGENIC, UK.

Characterisation





A comparative morphological study of two Trinitario groups from the International Cocoa Genebank, Trinidad

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

Introduction

The indigenous *type* of cacao in Trinidad and Tobago is Trinitario, a hybrid of Criollo and Forastero (Cheesman, 1944), which were introduced into the country in 1525 and 1757, respectively (Bekele, 2004). Currently, the cacao trees cultivated in Trinidad and Tobago are mainly Trinitario and Trinidad Selected Hybrids (TSH). The latter were produced by MALMR in a very successful, recurrent breeding programme that spanned a period of over 30 years (Kennedy *et al*., 1987). Genes for resistance to Witches Broom (WB) and *Ceratocystis* wilt were incorporated through the use of SCA 6 and IMC 67 as parents, respectively. ICS 1 and 95 were selected as parents for heavy bearing, large bean weight and flavour (Freeman, 1969). Several TSH clones have been identified with increased resistance to the aforementioned diseases and favourable agronomic traits. TSH 1188 is widely recognised for its tolerance to WB, and twelve TSH selections and their progenies have been made available to farmers. In more recent times, old plantation trees (including ICS clones) have been replaced by these newer commercial varieties on some farms in Trinidad and Tobago (Abdul-Karimu *et al*., 2003; Maharaj *et al*., 2005). However, local farmers have expressed concern over their unknown flavour, which was not assessed until recently (Sukha *et al*., 2005), and several have preferred to continue to plant the traditional Trinitario cacao known to produce beans with the distinctive flavour important in the marketing of Trinidad and Tobago cocoa. The farming community may be unaware that the aforementioned ICS clones were used as parents in the TSH breeding programme. MALMR can ensure preservation of the distinctive "Trinidad" flavour in the beans of commercial planting material by incorporating more traditional (Trinitario) varieties in future breeding cycles.

Criollo and Trinitario beans are collectively designated as “fine or flavour” cocoa, which has a high demand among manufacturers of fine chocolates throughout the world, and commands premium prices on the world market. The niche market for fine or flavour cocoa comprises roughly 5% of the global cocoa market, and Trinitario (flavour) cocoa accounts for about 15% of the global trade. Trinidad and Tobago is an exclusive producer of fine or flavour cocoa, which commands a premium of 80 USD to 1,000 USD per tonne over the London markets (ICCO website, http://www.icco.org/economics/, accessed on June 19, 1993; CCIB[1] pers. comm.). This demonstrates that Trinidad and Tobago has a comparative advantage in the marketing of its cocoa. This cocoa is characterised by a full cocoa flavour with pleasant ancillary flavours such as molasses, liquorice, caramel and raisin, and is simply described as fruity (Mooleedhar, 1995). Some chocolate manufacturers consider this cocoa as superior and of the highest quality. It is mainly used in the manufacture of specialty products. There is a high demand for all the cocoa Trinidad and Tobago can produce, and an expansion of production and increased investment in the industry would undoubtedly be beneficial to the economy of the country.

Over the last three decades, cocoa production, exports, acreage under cultivation and farmer participation in Trinidad and Tobago have been declining steadily (Bekele, 2004). Currently,

[1] Cocoa and Coffee Industry Board, Trinidad and Tobago

production seems to have stabilised at 1.0 - 2.3 million kgs per annum. The value of cocoa exports for 2005-2006 was TTD 19,369,000 (US$3,100,000)(Cocoa and Coffee Industry Board of Trinidad and Tobago (CCIB), pers. comm.). There is an official policy currently being implemented to rehabilitate the local cocoa industry, and several initiatives have been undertaken by MALMR and the CCIB. In an effort to support these initiatives, CRU has undertaken studies to provide information that would benefit the local cocoa industry. This study was conducted to provide botanical and agronomic information on traditional cocoa varieties planted in Trinidad and Tobago, *viz*., the Imperial College Selections (ICS) and the TRD collection. Data on the characters of economic interest such as cotyledon weight and yield potential[1] (as measured by pod index - the number of pods required to produce 1 kg of dried cocoa) (Table 1) are of particular value to the local industry. They are crucial for selecting suitable candidates for future germplasm enhancement activities that will facilitate the planned rehabilitation and expansion of the local cocoa industry (Huntings Technical Services, 1999).

The Imperial College Selections (ICS)

The Imperial College Selections were selected by Dr. F.J Pound from among 50,000 cacao trees in Trinidad as a representative sample with outstanding characteristics. These selections represent over 500 years of cultivation and farmer selection. The selection criteria were high yield and good quality in terms of bean size (Pound, 1936). A subset of promising trees was observed over a 2-year period in farmers' fields. One hundred trees were finally selected based on pod production per tree, pod index, annual yield and average bean weight (1.0-1.2 g). Thirty-six trees were selected in 1933-34, and 64 in 1935 (Pound, 1934-1936). Among the more promising ICS clones identified in earlier work at CRU were ICS 1, 6, 8, 39, 45, 60, 89 and 98 (Posnette, 1986).

Johnson *et al*. (2004) found that ICS 10, 35, 46, 57, 77, 80 and 100 were genetically distinct from the remaining 57 ICS clones at San Juan Estate, Gran Couva, Central Trinidad, and recommended them for inclusion in future germplasm enhancement research.

The Trinidad Selections (TRD)

The TRD accessions were selected and collected by Dr. John Warren and Thakurani Cassie on 10 abandoned cocoa estates in Trinidad between 1991 and 1992 (Cassie, pers. comm.). The aim was to obtain relic/old Trinitario/Criollo material. Ten trees per estate were sampled. Collection sites included Lopinot, Sangre Grande, Cumaca, Blanchisseuse, Maracas (St. Joseph), Mt. St. Benedict, Tabaquite, Rio Claro and two other estates in North-East Trinidad. A total of 119 clones were collected of which 68 have survived to date:

TRD 1-24 were collected in the abandoned cacao fields near Mt. St. Benedict;
TRD 25-36 were collected along Waterfall Road in Maracas Valley;
TRD 37 – 48 – site unknown
TRD 49 – 60 and 61-72 were collected in April 1992 (site unknown);

[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.

TRD 73 – 84 were collected in May 1992 (site unknown);
TRD 85-107 – site unknown
TRD 108-119 were collected in Lopinot in July 1992.

Materials and Methods

In this study, morphological characterisation data for 61 ICS and 29 TRD accessions were collated according to the convention of Bekele *et al.* (2006). The 25 descriptors used (Table 1) were selected from the International Board for Plant Genetic Resources (now Bioversity International) descriptor list for cocoa (Anon., 1981) based on analyses described by Bekele *et al*. (1994) and Bekele and Butler (2000). They have been used for routine characterisation of cacao accessions at the ICG,T (Bekele and Bekele, 1996; Iwaro *et al.,* 2003; Bekele *et al.,* 2006).

Table 1. Descriptors used for morphological characterisation.

Descriptor	State and 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, 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, 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]	

The ICS and TRD accessions studied are listed in Table 2.

Table 2. Trinitario accessions included in the comparative study.

Group name	Accessions
ICS	1(used widely as a pollen donor), 4, 5, 6, 7, 8, 10, 12, 14, 15, 16, 22, 24, CRU 269 (MIS_TTOICGT_ICS 25), 26, 30, 35, 39, CRU 267 (MIS_TTOICGT_ICS 40), 41, 42, 43, 46, 48, 53, 57, 58, 59, 60, 61, 62, 63. 65, 66, 67, 68, 69, 70, 73, 75, 76, 77, 80, 83*, 84, 85, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 97, 98, 99, 100, 111(ICS 1 × ICS 6)
TRD	1, 2, 3, 5, 7, 8, 24, 2930, **32**, 35, 41, 44, **45,** 49, 60, **85**, 86, 88, 94, 99, **109**, 111, 112, 113, 115, 116, 117, 118

Legend: * Tree 3 at the ICG,T was recently found to be putatively authentic (trees 2 and 4 were characterised using morphological descriptors and the data used when selecting the CFC/ICCO/IPGRI Project Collection (Sounigo *et al*., 2005));
ICS 35 was included in the CFC/ICCO/IPGRI Project Collection;
Underlined accessions have trees which are putatively mislabelled within plots at the ICG,T (19 clones);
The clones highlighted in bold were included in the CFC/ICCO/IPGRI Project Collection (Sounigo *et al*., 2005).
TRD 32 has been found to be genetically identical to POUND 7 [POU].

Descriptive statistics for the descriptors/traits studied were generated using MINITAB (Minitab Inc., 1997). The Mood median test (Minitab Inc., 1997) was performed to compare the medians for each trait obtained for the two accession groups studied. In order to display the phenotypic relationships among the accessions in three dimensions, 3-D[1] PCA was performed on a correlation matrix of the data recorded for the descriptors (Table 1) using NTSYS-pc (NTSYS, 2000). The data were first standardised to eliminate the effects of different scales of measurement.

Results

The TRD and ICS clones studied were found to be very similar morphologically (Tables 3a and b), apart from weakly significant differences ($P < 0.05$) in pod length and cotyledon width. This similarity was also observed in the PCA plot of the accessions in which the majority of TRD clones were interspersed with ICS clones (Figure 1). TRD 8, 94, 109, 111, 112 and 113 were less closely associated with the ICS clones, and TRD 1, 7 and 35 were rather distinct from them. Roughly 26 of the ICS clones were quite distinct from the TRD clones. These include ICS 1, 22, 35, 68, 43, 60 and 86 (Figure 1).

The results of the Mood median test (Minitab Inc., 1997) applied to the pod index values for ICS and TRD (not significantly different) are shown below as an example:

[1] Three dimensional

Chi-Square = 2.49 DF = 1 *P* = 0.114

Individual 95.0% Confidence Intervals

Accession	N<Median	N>Median	Median	-----+---------+---------+---------+-
ICS	34	27	23.41	(----------*---------)
TRD	11	18	25.04	(----------*----------)
				-----+---------+---------+---------+-
				22.4 24.0 25.6 27.2

Overall median = 23.77
(N = number of accessions)

For pod length and cotyledon width, the Mood median test revealed significant differences between the two groups (*P* = 0.019).

The TRD and ICS accessions with the most promising cotyledon weights (? 1.0 g) and pod index values (? 21) are presented in Table 4.

Table 3a. Descriptive statistics for the qualitative descriptors used to characterise 61 ICS and 29 TRD accessions from the ICG,T.

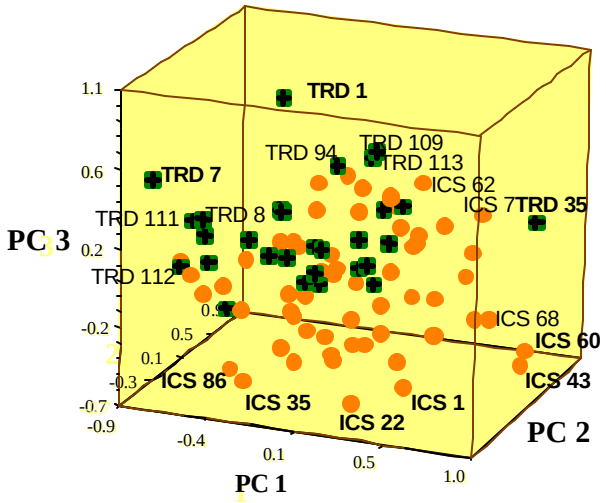
Descriptor	Accession group	Mean	Min. value	Max. value
Flower ligule colour	ICS TRD	3 3	0 0	7 7
Pedicel column colour	ICS TRD	3 3	1 2	3 3
Filament colour	ICS TRD	2 2	0 0	7 7
Mature Pod ridge colour	ICS TRD	2 1	0 0	7 5
Pod apex form	ICS TRD	2 4	1 2	6 6
Pod basal constriction	ICS TRD	2 2	0 0	3 3
Pod Shape	ICS TRD	2 2	1 2	5 4

Descriptor	Accession group	Mean	Min. value	Max. value
Pod surface texture	ICS TRD	5 4	0 0	7 7
Pod furrow disposition	ICS TRD	2 2	1 2	2 2
Pod furrow separation	ICS TRD	3 2	1 1	3 3
Cotyledon Colour	ICS TRD	4 4	3 3	5 5
Cotyledon shape	ICS TRD	2 2	1 1	3 3
Pod wall hardness	ICS TRD	6 6	3 5	7 7

Table 3b. Descriptive statistics for the quantitative descriptors used to characterise 61 ICS and 29 TRD accessions from the ICG,T.

Descriptor	ICS accessions			TRD accessions		
	Mean	Standard error	Coefficient of variation (%)	Mean	Standard error	Coefficient of variation (%)
Sepal length (mm)	7.8	0.11	10.5	7.9	0.13	8.8
Ligule width (mm)	2.5	0.05	14.5	2.4	0.06	12.2
Ovule number	41.8	0.77	14.4	43.1	1.25	15.6
Style length (mm)	2.1	0.03	11.5	2.2	0.34	8.4
Pod length (cm)	16.9	0.24	10.9	15.6	0.37	12.7
Pod width (cm)	8.2	0.08	7.3	8.1	0.09	6.0
Total wet bean weight (g)	65.7	1.68	20.0	62.3	2.40	21
Bean number	38.9	0.77	15.5	38.8	0.97	13.4
Cotyledon weight (g)	1.1	0.03	20.0	1.0	0.04	19
Cotyledon length (cm)	2.3	0.02	7.8	2.24	0.03	6.9
Cotyledon width (cm)	1.3	0.02	10.3	1.2	0.02	10.3
Pod index	23.8	0.66	21.6	26.5	1.10	22.8

Figure 1. PCA plot depicting phenotypic relationships among the ICS and TRD clones studied.



Legend

TRD clones **ICS clones** **PC – Principal component**

PC 1 accounted for 25% of the total phenotypic variation expressed, while PC 1 and 2 accounted for 41.3 % and the first three components for 51.1%.

Table 4. List of ICS and TRD accessions with favourable Pod Index and cotyledon weights.

Accession	Cotyledon weight (g)	Pod Index	Accession	Cotyledon weight (g)	Pod Index
TRD 35	1.59	15.3	ICS 63	1.31	19.6
ICS 60	1.64	15.6	TRD 117	1.04	19.6
ICS 68	1.26	15.9	ICS 85*	1.24	19.7
ICS 43	1.64	16.1	TRD 45	1.41	19.7
ICS 5*	1.37	16.9	ICS 8	1.26	19.9
ICS 62*	1.09	16.9	ICS 1	1.29	19.9
ICS 7*	1.43	17.1	ICS 48	1.38	20.1
ICS 16	1.38	17.3	ICS 15	1.44	20.5
ICS 6	1.33	17.5	ICS 83*	1.19	20.5
ICS 88*	1.52	18.3	ICS 92	1.18	20.7
ICS 75	1.41	18.7	TRD 44	1.12	20.8
ICS 111	1.46	19.0	TRD 2	1.14	20.9
ICS 93	1.32	19.4	TRD 109	1.14	20.9

* Potential mislabelling problems appear to exist within the accession plot at the ICG,T

Discussion and Conclusion

A close phenotypic relationship (morphological similarity) was observed between the two Trinitario groups studied as was indicated by the significant overlap of ICS and TRD accessions in Figure 1. This suggests that during the TRD collection exercise germplasm morphologically similar to (or possibly derived from) ICS were collected on the old abandoned estates.

TRD 1, 7 and 35 and ICS 1, 22, 35, 43, 60 and 86 were found to be most distinct morphologically (Figure 1). Johnson *et al.* (2004) found ICS 35 to be genetically distinct from the other 56 ICS accessions they studied. It would be interesting to examine the genetic distances between the ICS and TRD groups, and the genetic relationships among the TRD accessions once morphological characterisation of the 39 uncharacterised TRD accessions has been completed.

Future Direction

The use of promising ICS and TRD clones to incorporate with select TSH clones in future local germplasm enhancement programmes is recommended. Outstanding TSH clones can be crossed with Trinitarios (ICS, TRD or other) that combine good bean size, yield potential and other favourable traits with the distinctive Trinidad and Tobago cocoa flavour since MALMR also considers bean quality (flavour) as an important criterion in its breeding programme. A study to identify (using SSR markers) Trinitario clones for potentially heterotic[1] combinations in local trials should be undertaken. ICS 10, 35, 46, 57, 77, 80 and 100 were suggested as likely candidates (Johnson *et al*. 2004) and may be tested along with other genetically distant clones, as well as promising clones such as ICS 63, 76, 83 and 95, and TRD 45, 85 and 109 (Sounigo *et al*.,

[1] The average of the progeny is better than the average of the parents.

2005; Table 4). The incorporation of all of these favourable genes in planting material distributed to farmers should augur well for the future of the local cocoa industry.

Acknowledgements

We wish to acknowledge the Government of Trinidad and Tobago and BCCCA for financial support; Drs. Isaac Bekele, David R. Butler and Anthony J. Kennedy, Prof. John A. Spence, Drs. Thayil N. Sreenivasan, Adeniyi D. Iwaro, Elizabeth S. Johnson and Albertus B. Eskes for their support, advice and encouragement; and William Mollineau, Vindar Badall, Arlene Richardson-Drakes, Sandra Samnarine, Charmela Jagroop and the many other technical assistants, who have assisted with data collection at various times over the years.

References

Abdul-Karimu, A., Butler, D.R., Iwaro, A.D., Sukha, D.A., Bekele, F., Mooleedhar, V. and Shripat, C. (2003) Farmers' perceptions of cocoa planting material and factors affecting the cocoa industry in Trinidad and Tobago. *Tropical Agriculture (Trinidad)* Vol. **80:** 261-266.

Anon. (1981) *Genetic resources of cocoa*. (AGP: IBPGR/80/56) IBPGR Working Group on genetic resources of cocoa. Rome: IBPGR Secretariat, 25pp.

Bekele, F.L. (2004) The history of cocoa production in Trinidad and Tobago. Pages 4-12 in: *Proceedings of the APASTT Seminar – Exhibition entitled Re-vitalisation of the Trinidad & Tobago Cocoa Industry*. 20 September 2003, St. Augustine, Trinidad: APASTT, The University of the West Indies.

Bekele, F.L. and Bekele, I. (1996) A sampling of the phenetic diversity in the International Cocoa Genebank of Trinidad. *Crop Science* **36** (1), 57-64.

Bekele, F. and Butler, D.R. (2000) Proposed list of cocoa descriptors for characterisation. Pages 41-48 in: *Working procedures for cocoa germplasm evaluation and selection*. *Proceedings of the CFC/ICCO/IPGRI Project Workshop*, (A.B. Eskes, J.M.M. Engels and R.A. Lass Eds). 1-6 February 1998, Montpellier, France: IPGRI.

Bekele, F.L., Kennedy, A.J., Mc David, C., Lauckner, B. and Bekele, I. (1994) Numerical taxonomic studies on cacao (*Theobroma cacao* L. in Trinidad. *Euphytica* **75**: 231-240.

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 and Crop Evolution* **53** (5): 933-948.

Cheesman, E.E. (1944) Notes on the nomenclature, classification and possible relationships of cacao populations. *Tropical Agriculture (Trinidad)* **21:** 144-159.

Freeman, W.E. (1969) Some aspects of the cocoa breeding programme. Pages 1-15 in: *Proceedings of the Agricultural Society of Trinidad and Tobago*, December 1968.

Huntings Technical Services (1999) Rehabilitation of the cocoa industry investment preparation study. *Final Report:* Government of the Republic of Trinidad and Tobago.

Iwaro, A. D., Bekele, F.L. and Butler, D.R. (2003) Evaluation and utilisation of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. *Euphytica* **130**: 207-221.

Johnson, E., Bekele, F. and Schnell, R. (2004) *Field Guide to the ICS Clones of Trinidad*. Serie Técnica Manual técnico No. 54. Turrialba, Costa Rica: Tropical Agricultural Research and Higher Education Center. 32pp. (ISBN 9977-57-398-0).

Kennedy, A. J., Lockwood, G. Mossu, Simmonds, N.W. and Tan, G.Y. (1987) Cocoa breeding: past, present and future. *Cocoa Growers Bulletin* **38**: 5-22.

Maharaj, K. Indalsingh, T., Ramnath, D. and Cumberbatch, A. (2005) High density planting of cacao: the Trinidad and Tobago experience. Pages 171-182 in: *Proceedings of the International Workshop on cocoa breeding for improved production systems*, (F. Bekele, M.J. End and A.B. Eskes Eds).19-21 October 2003, Accra Ghana: INGENIC and the Ghana Cocoa Board.

Minitab Inc. (1997) MINITAB User's Guide 2: *Data analysis and quality tools*. Release 12 for Windows. USA: Minitab Inc.

Mooleedhar, V. (1995) "Fine" flavour cocoa – a Trinidadian and Tobagonian tradition. *Cocoa Research Unit Newsletter* **2**: 6.

NTSYS (2000) *NTSYSpc ver 2.10b users guide*. USA: Applied Biostatistics Inc.

Posnette, A.F. (1986) Fifty years of cocoa research. In Trinidad and Tobago. St. Augustine: The Cocoa Research Unit, The University of the West Indies. (ISBN 976-620-001-7).

Pound, F.J. (1934) The Progress of Selection. Pages 25-28 in: *Third Annual Report on Cacao Research*, 1933. Trinidad and Tobago: Government Printery.

Pound, F.J. (1935) The Progress of Selection. Pages 7-11in: *Fourth Annual Report on Cacao Research*, 1934. Trinidad and Tobago: Government Printery.

Pound, F.J. (1936) The Completion of Selection. Pages 7-16 in: *Fifth Annual Report on Cacao Research*, 1935. Trinidad and Tobago: Government Printery.

Sounigo, O.S., Bekele, F., Iwaro, D., Thévenin, J-M., Bidaisee, G., Umaharan, R., Sankar, A., Sukha, D., Boccara, M., Butler, D.R., Eskes, A.B. (2005) Description of the CFC/ICCO/IPGRI project collection. Pages 21-32 in: *Proceedings of the 14th International Cocoa Research Conference*. 13-17 October 2003, Accra, Ghana: COPAL.

Sukha, D.A., Ramnath, N. and Butler. D.R. (2005) Trends in flavour profiles and pyrazine compounds of cocoa liquor samples evaluated over three growing seasons for the CFC/ICCO/INIAP Flavour Project. Pages 53-61in: *Annual Report for 2004*. St. Augustine, Trinidad: Cocoa Research Unit, the University of the West Indies.

Cacao clones manual: Introducing version 1.1

A.A. Sankar, G.G. Bidaisee, L.A. Motilal and E.S. Johnson

Introduction

The first output of the ongoing cacao clones manual (CCM) project is entitled "Cacao Clones Manual - Version 1.1" to reflect that it is the first in a series of publications from the cacao clones manual project. CCM Version 1.1 features data for 290 accessions from the ICG,T. The project team decided to publish the manual in multiple versions/editions because complete data sets for a sufficient number of accessions have been prepared for the first release and this information is valuable to users of the ICG,T therefore it is important to make it available in a timely manner. Version 1.1 will be distributed as one volume on a CD-ROM[1]. Version 1.1 will be the first step in providing a comprehensive reference to the ICG,T for the cacao community and in particular for users of germplasm from the ICG,T.

As with Version 1.1, each version of the manual will consist of photographs of fully grown pods (ripe and unripe) with descriptor data and, where available, other information such as molecular and/or historical information. If addition information to that included in Version 1.1 becomes available, this will be added in subsequent versions. Furthermore, additional CD-ROMs will be released when a sufficient number of photographs of new clones and descriptions are completed.

Materials and Methods

Preparation of content

Photographs

Photographs for Version 1.1 were either digital images of freshly harvested pods (captured using a Nikon® 3.3 Megapixel camera or other similar digital camera), or previously created scans of pod photographs taken with a conventional camera obtained from the 'Descriptor' album compiled by the morphological characterisation team.

For each accession, pods were collected when available from trees in the field that could be verified as accurate through field maps and trunk labels. If pods were not available from verified trees, multiple pods were collected and later evaluated to select an accurate representative pod.

Paper labels were printed to be photographed with each pod. Pods were photographed on the same day or no more than two days after collection to minimise degradation of pod appearance. The digital pictures were then edited for the manual as described below.

To prepare the photograph for the manual, the pod image was inserted into a blank Microsoft® PowerPoint slide with a custom canvas size of 968 × 726 mm. A pre-drawn scale was 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. The file was edited further and cropped to remove the old rule and other unnecessary parts of the original image and saved as a TIFF[2] image. This exported image was then cropped in Adobe Photoshop 5.0 LE to trim the excess space around it and the text label replaced to avoid the aliasing effect.

[1] Compact disc – read only memory
[2] Tagged image file format

Table 1. Accessions in CCM Version 1.1 with both qualitative and quantitative descriptor data.

Group name	Accession
AM	1/37, 1/68, 1/95, 2/13, 2/18, 2/21, 2/53, 2/70, 2/83, 2/88, 2/92
B	5/7, 6/3, 9/10-18, 9/10-25, 9/10-32, 12/1, 13/5, 17/1
CL	9/7, 9/19, 10/7, 10/10, 10/11, 13/13, 19/10, 19/39, 19/49, 27/27, 27/58, 27/76, 27/109, 78/2
CLEM	/S 62-1
CLM	90, 97, 114
COCA	3348/44
EET	58, 59, 272
FSC	13
GU	114/P, 175/P, 195/P, 241/P, 243/H, 271/P, 277/G, 300/P, 305/P, 307/F, 310/P, 335/P, 351/P, 353/L
ICS	1, 8, 16, 25, 30, 35, 4, 40, 43, 44, 46, 48, 57, 60, 63, 65, 66, 69, 75, 80, 84, 86, 87, 90, 92, 94, 95, 97, 98, 100, 111
IMC	16, 20, 23, 27, 30, 41, 44, 49, 50, 53, 54, 65, 71, 96, 107
JA	1/2, 1/12, 1/29, 2/8, 3/4, 3/11, 4/7, 4/17, 5/2, 5/5, 5/9, 5/10, 5/26, 5/34, 5/35, 5/41, 6/7, 9/92, 10/12
LCT EEN	31, 68/S2, 162/S-1010, 326
LP	1/4, 1/40, 1/45, 2/13, 3/29, 4/5, 4/8, 4/10, 4/24, 4/38, 4/39, 4/52
LX	16, 41, 44, 47
MATINA	1/7
MO	14, 20, 81, 118
MOQ	1/25, 5/28, 5/34, 5/35, 6/10, 6/30, 6/41, 6/44, 6/67, 6/87, 6/103
NA	3, 8, 12, 26, 70, 110, 111, 142, 155, 218, 232, 312, 326, 372, 387, 399, 423, 669, 672, 694, 712, 715, 717, 739, 758, 802, 804, 867
PA	13, 15, 20, 24, 32, 34, 39, 41, 46, 51, 61, 63, 67, 71, 73, 84, 107, 113, 118, 120, 124, 125, 126, 132, 136, 141, 175, 185, 196, 271, 279, 289, 291, 294, 296, 299, 300, 303
POUND	9/B, 12/A, 15/A, 26/C, 32/A
SCA	9, 12, 19, 23, 24
SJ	1/18, 1/40, 2/7
SLA	30
SPEC	194/15, 194/48
UF	29, 654

Descriptor data

Descriptors compiled for inclusion in this version were qualitative (for all accessions): pod colour, shape, and apex; basal constriction; surface texture; ridge pair separation, and quantitative (for some accessions only): pod length and width; bean number; cotyledon length, width and weight and pod index. Data were averaged from 10 pods for the quantitative descriptors. Descriptor data were recorded on the day of collection or one day after.

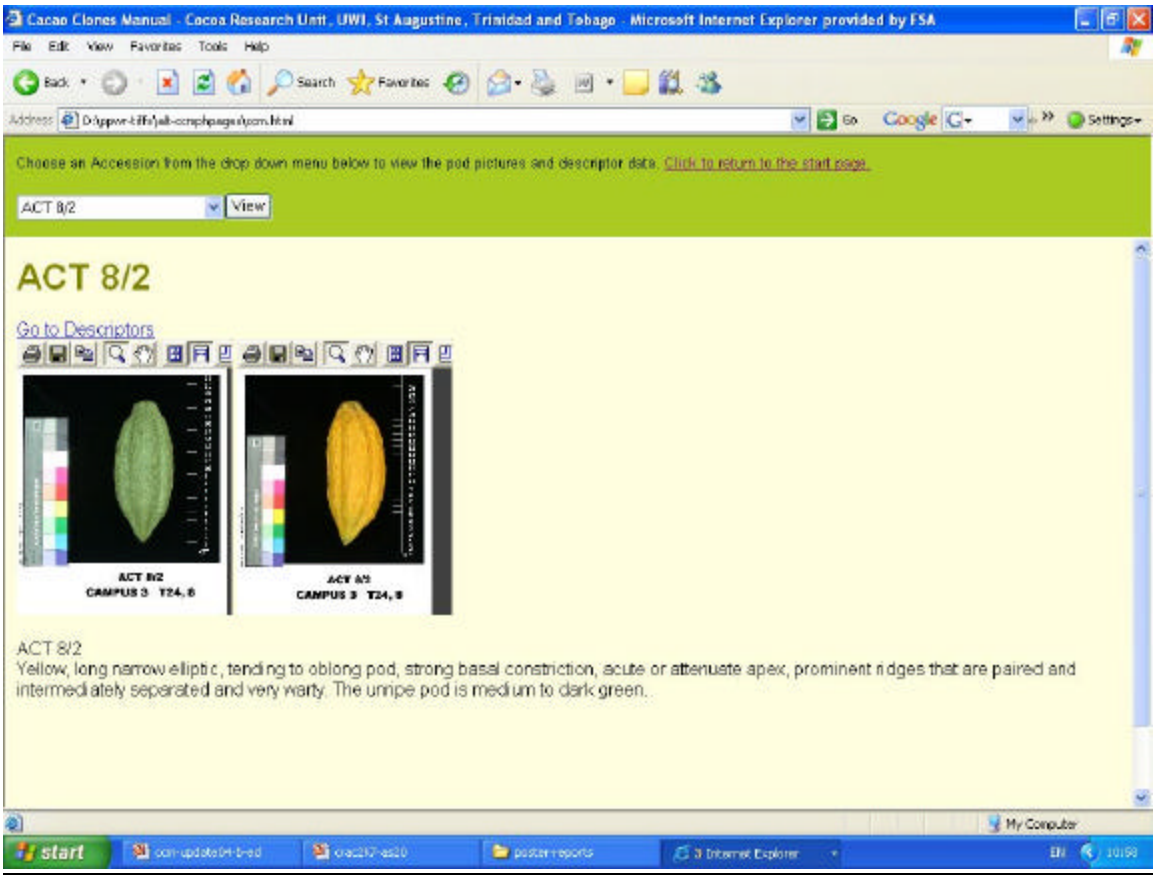
Content

Version 1.1 is comprised of a single volume on CD-ROM containing information for 290 accessions, including two photographs for each accession (580 photographs). An entry for each accession in Version 1.1 therefore consists of the photographs of a fully grown ripe and unripe pod and qualitative descriptor data. All accessions have qualitative descriptor data and for many accessions there is both qualitative and quantitative data. However quantitative data sets were

Table 2. Accessions in CCM Version 1.1 with only qualitative descriptor data.

Group name	Accession
AM	1/33, 1/55, 2/41
AMAZ	5/2
B	23/9
CL	10/23, 13/41, 19/11, 27/71
CLM	116
ICS	7, 9, 10, 28, 93
IMC	13
JA	2/26, 3/16, 3/37, 6/9, 9/13, 10/40
LP	1/29, 3/38, 4/11
MAN	15/2
MOQ	1/5, 1/21, 2/8, 2/33, 4/21, 6/29
NA	39, 61, 254, 339, 695, 766
PA	98, 186
POUND	21/B
SJ	1/4, 1/20, 1/34, 2/28
SLA	13
SLC	1

Figure 1. Screen showing HTML dual frame format of CCM Version 1.1.



incomplete for some accessions and therefore excluded. Supplementary information was not included in this version. AlternaTIFF software (with instructions for installation, Medical Informatics Engineering, 2005) will be included on the CD-ROM for computers that require it.

Format

The simple structure chosen for the manual is a style-sheet formatted 2-frame frameset HTML (hypertext markup language) web page consisting of two horizontal subspaces or frames (Figure 1). The top frame contains a 'drop-down' menu from which an accession can be selected by clicking on the accession name 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 and the selection confirmed by clicking the *"View pod photo"* button.

Image format
TIFF was selected because it is the most economical format for file size that guarantees the highest possible quality when the image is printed on a suitable printer.

Descriptor data format
The descriptor data are presented as text in a simple paragraph and table style with tabs to align quantitative data. HTML 'PRE' tags were used with style sheet formatting to format the descriptor data text.

CD-ROM credits for Version 1.1

Version 1.1 is a compilation of data from several sources therefore the CD-ROM credits for this version are as follows:

- CRU
- USDA (Elizabeth Johnson)

The following citation is proposed for bibliographic purposes:
Sankar, A., Bidaisee G.G., Motilal, L.A., Johnson, E.S., Bekele, F.L., Boccara, M. and Butler, D.R. Cacao Clones Manual CD-ROM Version 1.1 St. Augustine: Cocoa Research Unit, The University of the West Indies, 2007

How to use the CCM CD-ROM

Notes for first use

The computer system requirements for CCM Version 1.1:

- CD-ROM drive
- Internet browser such as Internet Explorer, Netscape & others
- Operating system: Windows 2000, XP

The manual has not been tested on operating systems older than Windows 2000 and XP therefore these are the only two operating systems that can be recommended for viewing the manual. To browse Version 1.1, insert the CD-ROM into a compatible drive. The CCM Version 1.1 CD-ROM is configured to autorun and will therefore launch the default internet browser to display the welcome page. Internet Explorer 7 may have a "full page view" problem on some

computers but it is solvable by resetting the browser. If not previously installed on the computer being used, it may be necessary to install the activeX plugin for Internet Explorer, AlternaTIFF, to view the manual. Two forms of installation are available: automatic or manual.

Automatic installation requires internet access (*i.e.* computer must be online) and after installation is complete, registration is then required before it can be used. Registration also requires internet access either on the computer where AlternaTIFF is installed or at another computer. An alternative to this form of registration is the purchase of site licenses, which are available at $10 USD per copy so that no registration will be required. All of this information and more can be found in the "frequently asked questions" on the website for AlternaTIFF at www.alternatiff.com.

Notes for use at all times

Active content must be "allowed" once each time the CD-ROM is launched to make the pod pictures display properly. Also, you may observe a pop-up message that you need to press the spacebar to re-activate the control if the menu or photos become un-clickable (i.e. if mouse does not respond to clicks).

Protocol changes

Image editing adjustments

During development of the CCM, it was determined that the default size of a PowerPoint® slide could be increased to improve the (final) resolution of the output TIFF image and this change was therefore implemented in the photo-editing stage of manual production.

Aliasing was a problem that could only be solved by modifying the text label in PhotoShop LE® instead of in PowerPoint®. One other factor that could affect aliasing is the choice of computers, however all the computers available in CRU for use gave the same problem of aliasing.

Suggested changes

Photography
For future versions of the CCM, we plan to modify the way we capture photographs digitally to help maximise efficiency by reducing the number of editing steps required and to also reduce the size of the output image file. To minimise the size of the image file without sacrificing resolution, the area between pod and both the ruler and colour scale can be decreased, that is, a smaller area can be maintained each time pod photography is set up, or a standard can be established of around 1.5 or 2 cm space on each side of the pod. This will not only minimise file size of the original raw file but will also lessen the amount of editing required to reduce the overall file size.

Editing
For future versions, we plan to adopt a standard size canvas for each photo to give immediate visual size approximation of the pod (suggested by C. Turnbull, pers. comm.). Increases in canvas size will inflate file size for some accessions however and this will contribute to storage

capacity limitations, so future versions may have fewer clones on each CD-ROM.

Compatibility issues

Future versions of Windows may create a problem for this version and future versions of the manual if the use of TIFF images and AlternaTIFF is continued (Medical Informatics Engineering, 2005). The following online quotes were found on the subject:

> "AlternaTIFF is compatible with the current versions of most of the popular web browsers for Windows operating systems. While MIE intends to make a reasonable effort to make sure that this remains the case, we cannot guarantee that future web browsers or operating systems will be compatible with AlternaTIFF."
>
> "Due to a questionable court ruling, Microsoft is planning to make changes to Internet Explorer that will affect the user experience, and/or require changes to web pages, when ActiveX controls are used. Refer to <http://support.microsoft.com/kb/912945> and <http://msdn.microsoft.com/ieupdate>."

A solution to this issue may be to revert to the JPG/JPEG (Joint Photographic Experts Group) image format, because the screen resolution of JPG files is adequate; and also supply a print-quality TIFF image (linked from the "jpeg" file) for printing purposes.

Conclusion and future prospects

To continue the development of the manual, pod collection and digital photography remain a priority to create new datasets for additional accessions which are not yet represented. Pod image editing and descriptor data compilation will be prioritised to complement existing data and maximise the number of completed accessions. A simple change in the protocol for digital capture should increase the efficiency. However, the rate of progress will be limited by pod collection and the availability of suitable pods. Scanning photographs from the Descriptor album will be useful to supplement the existing dataset and increase the number of completed accessions. A priority list for scanning was created from a list of those accessions missing pod pictures in order to achieve this. It is necessary to produce supplementary data for inclusion in the next version (version 1.2) and to address issues of compatibility with Macintosh (and future versions of Windows) Operating systems regarding the autorun feature and TIFF display.

Acknowledgements

The project was suggested by Mr. R.A. Lass, and we are grateful to financial support from the Biscuit, Cake, Chocolate and Confectionary Association and Cadbury Schweppes plc. Special thanks are due to E.S. Johnson for additional photographs provided with USDA funding.

References

Johnson, E., Bekele, F. and Schnell, R. (2004) *Field guide to the ICS clones of Trinidad.* Serie Manual Tecnico No. 54. Tropical Agricultural and Higher Education Center. Turrialba, Costa Rica: CATIE.

Medical Informatics Engineering. (2005) *AlternaTIFF 1.7.4 : A TIFF image viewer for Windows Web Browsers*. www.alternatiff.com

Pound, F.J. (1934) The progress of selection. Pages 25-28 in: *Third Annual Report on Cacao Research*, 1933. Trinidad and Tobago: Government Printery.

Pound, F.J. (1935) The progress of selection. Pages 7-11 in: *Fourth Annual Report on Cacao Research*, 1934. Trinidad and Tobago: Government Printery.

Pound, F.J. (1936) The completion of selection. Pages 7-16 in: *Fifth Annual Report on Cacao Research*, 1935. Trinidad and Tobago: Government Printery.

The Software Patch (2006) www.softwarepatch.com/tips/autorun.html.

Wadsworth, R.M. and Harwood, T. (2000) *International Cocoa Germplasm Database ICGD version 4.1*. London, UK: The London International Financial Futures and Options Exchange and The University of Reading.

Evaluation

Linking near infrared reflectance spectroscopy and ultraviolet excitation/emission spectra to flavour in cocoa – current status and future prospects

D.A. Sukha, E.N. van Loo[1], H. Jalink[1], D.R. Butler, F. Amores[2] and G. Ramos[3]

Introduction

One of the many components of the CFC/ICCO/INIAP Flavour Project "To establish physical, chemical and organoleptic parameters to differentiate between fine and bulk cocoa" was to analyse the spectral properties of cocoa using Near Infrared Reflectance Spectrometry (NIRS) and Light Amplification by the Stimulated Emission of Radiation technology. The premise behind this component was to determine if NIRS or Ultraviolet Excitation/Emission Spectra (UV-fluorescence) can be used as fast and non-destructive techniques to distinguish between "fine or flavour" and "bulk" cocoa or can be linked to specific quality attributes in cocoa samples.

Plant Research International (PRI), Wageningen was contracted to do these analyses for the CFC/ICCO/INIAP Flavour Project and they conducted NIRS analysis on hundreds of bean samples and UV-fluorescence spectral analysis on a limited set of bean samples. The relationships between spectral data and taste and fermentation traits were then analysed and this allowed us to assess the potential of NIRS and UV-fluorescence to discriminate between different classes of cocoa. The article presented here summarises the main findings of comparisons between the Trinidad taste panel data and spectral analysis as well as the ability of NIRS to distinguish origins and genetic groups of "fine or flavour" and "bulk" cocoa. To conclude, some future prospects for the application of spectrometric techniques to flavour in cocoa are briefly discussed.

Materials and methods

NIRS analysis

NIRS measures the absorption of near infrared light by a sample by comparing the reflectance of a standard white surface with the reflectance of the sample. Light is emitted by a lamp that has a high and constant yield in the infrared range. This light from a tungsten lamp is filtered using a monochromator that only transmits light of a specified wavelength. The wavelength of light that is transmitted can be increased in 2 nm intervals over the wavelength range from 1100 nm to 2500 nm. The components and design of NIRS instrumentation are similar to UV-visible absorption spectrometers.

For this project, PRI used a Bran & Luebbe InfrAlyzer 500 with a mononchromator grating. Measuring one NIRS spectrum in the range of 1100-2500 nm with a wavelength interval of 2 nm took about 90 seconds, which allowed time to empty and fill the next sample cuvette. Different

[1]Plant Research International (PRI), The Netherlands
[2]Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP), Ecuador
[3]Instituto Nacional de Investigaciones Agrícolas (INIA), Venezuela

absorption spectra were obtained for different compounds and the total spectrum is the 'sum' of all spectra for individual compounds in a sample. Statistical analysis is needed to reveal correlations with the sample traits of interest. A number of sample traits were correlated but only organoleptic data are being considered in this report.

UV-fluorescence

The potential of UV-fluorescence spectrometry to characterise ground cocoa bean powders was investigated in a preliminary study using a small sample set. There are hundreds of chemical compounds present in cocoa beans and determining which chemical compounds are related to specific flavours of interest involves extensive "wet chemistry" and chromatographic techniques. Many plant compounds (such as chlorophyll) show fluorescence. Fluorescence is the process where photons excite electrons in molecules, these electrons then achieve an unstable higher energy status. When the electrons return to their original more stable energy level, they emit a photon. The excitation and emission of photons occur at more or less specific wavelengths and emitted photons always have less energy (longer wavelengths) than the excitation photons. This is very wavelength specific for different compounds and can be used to characterise compounds/traits in samples based on unique excitation and emission spectra. The overall fluorescence spectrum 'sums up' all individual fluorescence spectra of individual compounds. With NIRS the measurement is a function of one wavelength (a 2-dimensional picture) whilst in UV-fluorescence, the measurement is a function of both the excitation and emission wavelength (a 3-dimensional picture).

Considerations

In the process of handling many samples – as in this project – from different sources and by different research groups, there is a serious threat of mixing up of samples. Unique sample coding used by all project partners was used to reduce the risk of such mix ups. However, incidental mix ups are still possible, and in this project it proved that a few samples were mixed up. Interestingly, the problem was identified in one of the results from the NIRS investigation. In the PCA to find out whether grouping for different genotypes could be found on the basis of the NIRS spectra, some samples of genotypes were grouped with totally different genotypes. On the basis of that finding, it was tested whether the identification of these samples was correct and it proved that indeed these samples were misidentified (an order change between an original list of genotypes and the list in the NIRS database). After correcting this, the samples were grouped uniformly according to genotype, showing the power of NIRS in characterising samples. Apart from sample identification, standardised sample presentation was of utmost importance since NIRS and UV-fluorescence are very sensitive to particle size distribution of the powder, and to the temperature and humidity of the sample.

Sample preparation

Sample preparation was standardised where approximately 20 beans were taken at random from a sample. These beans were peeled manually usually without difficulty except for under-fermented beans. After the shells were removed, beans were ground in a lab/coffee grinder for

10 seconds using a fixed protocol. The ground sample was stored for 24 hours in an air conditioned laboratory to stabilise before the NIRS spectra was taken.

Exactly two weeks after the ground powder was produced for NIRS analysis, the same powder was used for UV-fluorescence spectra. The ground sample was placed in a quartz cup and a UV light from a xenon lamp, passed through a monochromator set to the excitation wavelength, was aimed at a 45° angle towards the sample. Fluorescent light coming from the sample was passed through a monochromator set to the emission wavelength to a light sensor. From this we were able to determine excitation and emission spectra at specific wavelengths for a sample. The fluorescence levels at different excitations were related to the organoleptic results from the same samples.

Data analysis (NIRS and UV-fluorescence data)

The NIR spectrophotometer measures the amount of light *not* absorbed by the sample, i.e. the reflectance (R). The absorbance (= log (1/R) is linearly correlated with the concentration of a compound (at least at low concentrations). From chemometrical theory, this concept is known as "Beer's Law" and is the reason that the NIRS raw data is transformed to give absorbance.

Quantitative calibrations are aimed at predicting the concentration or the quantitative level of a quality trait in unknown samples. Calibrations are a standard data analysis technique with NIRS to remove vertical shifts in spectra which are non-informative. Improvements in calibration/prediction can be obtained by using running averages over several wavelengths and by using the slope (the first derivative) of the spectral line rather than the absolute value.

Multiple linear regression (MLR) models based on a learning set of samples with NIRS spectra and known values of the target trait were used as a calibration dataset. A calibration model was generated from this calibration dataset which was then tested (validated) using test samples for which NIRS spectra and values of the target trait were also known. To achieve this, a step-up system was applied on the datasets used, new wavelengths were added to or dropped from the regression model to increase the accuracy of the calibration model (minimise the residual mean standard error). In this study, most calibration models were based on MLR models derived from the best subset of four wavelengths. The standard error of prediction for validation (SEPval), based on the differences between predicted and actual values for the validation samples, is usually higher than the standard error of prediction for calibration (SEPcal). The closer SEPval is to SEPcal, the more robust the prediction model.

For the UV-fluorescence data, linear regression or bilinear regression models were found that related emissions levels to the various flavour attributes in the samples.

In order to discriminate between different genotypes and origins with NIRS, PCA was used. This correlated factors or variables to NIRS absorption at all wavelengths, using linear transformation of the NIRS data. This was done on normalised data for all wavelengths to avoid difficulties when the average and standard deviation for different wavelengths were different.

Results

NIRS analysis

Table 1 presents a summary of the quantitative NIRS calibration and validation models for all

the flavour attributes in the samples from Trinidad. The quality of calibrations has been evaluated by placing them in one of three classes *viz.* "poor", "qualitatively useful" and "quantitatively useful", where 0 = poor distinction between groups, 1 implies that qualitative distinction of the groups is possible, whilst 2 = quantitative prediction of the groups is possible. More details about limits defining each group are described in footnote "a" to Table 1.

Table 1. NIRS calibration and validation results for samples from Trinidad

Flavour attribute	Minimum score	Maximum score	SEPcal	SEPval	SEPcal as % of range	Multiple correlation coefficient r	Quality of calibration[a]
Cocoa	2.89	6.49	0.57	0.78	16%	0.72	2
Acidity	1.13	6.50	0.72	0.52	13%	0.82	2
Astringency	0.82	3.29	0.36	0.40	15%	0.51	0
Bitterness	1.29	3.94	0.36	0.41	14%	0.82	2
Fruity	0.56	5.25	0.67	1.10	14%	0.83	1
Floral	0.17	7.04	1.10	2.40	16%	0.66	1
Nutty	0.00	2.33	0.30	0.41	13%	0.84	2
Raw/beany/green	0.00	1.50	0.28	0.31	19%	0.57	1

[a]Quality of calibration is assessed in three classes: 0 = poor; 1 = qualitative distinction of groups possible; 2 = quantitative prediction possible. Poor (0) is indicated when maximally one of the following criteria is met: $r > 0.75$, SEPcal/range = 18% and SEPval < 1.5 × SEPcal. Qualitative good (1) is indicated when two of these three criteria are met. Quantitative good (2) is indicated when all three criteria are met. Poor (0) is also given when SEPval > 2.5 × SEPcal or $r < 0.55$.

Good quantitative calibrations were observed for cocoa, acid, bitter and nutty flavours whilst only qualitative distinction of groups was possible with fruity, floral and raw/beany/green flavours. Astringency was the only flavour attribute that was poorly calibrated in this dataset.

Figure 1 shows how well the calibration model predicts a set of independent samples for cocoa flavour and the spread of samples along a fairly wide range of scores for this attribute. The closer SEPval is to SEPcal implies that the prediction model is more robust and in this instance the residual mean standard error of calibration = 0.57 whilst that of validation = 0.78. The solid circles identify the calibration samples whilst the open circles identify the validation samples.

Numerous other calibrations with different settings were tried with the same dataset, but none of them gave such a good prediction model. The dataset for this model (Figure 1) is not large (38 samples) since it is based on data from Trinidad and Tobago only.

From Figure 1 we see that the "bulk" Ghana reference samples have the highest cocoa flavour and are well separated. The "bulk" common clone IMC 67 is not separated from the rest as it has an intermediate to low cocoa flavour score. In fact, this sample was notably fruity and acid.

Figure 1. Calibration/validation plot for cocoa flavour.

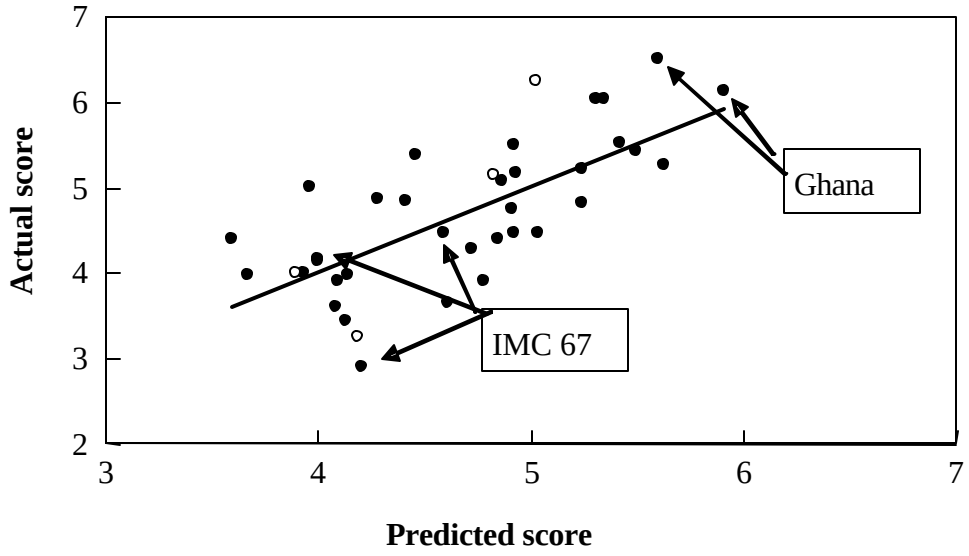
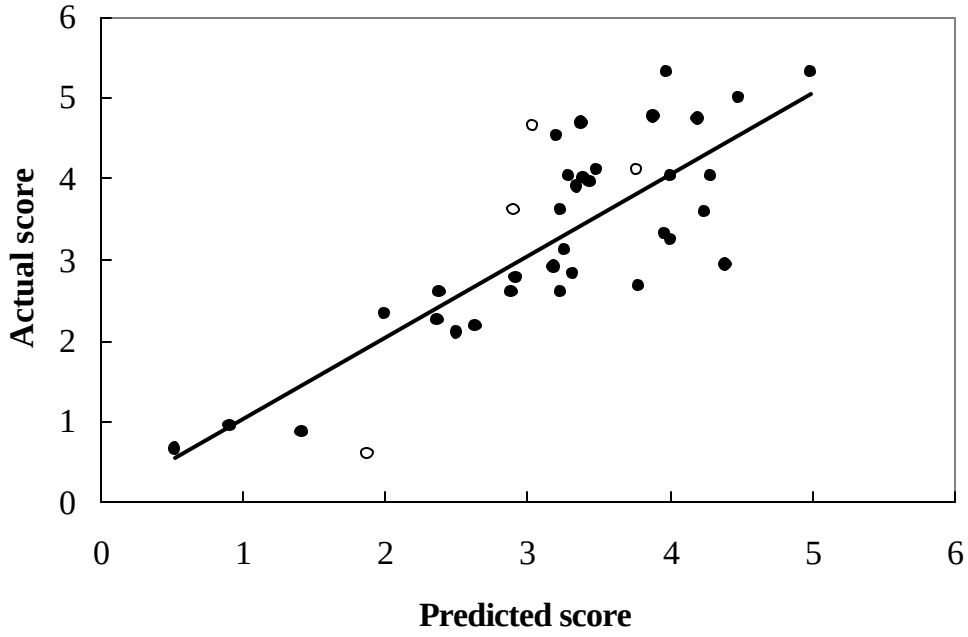


Figure 2. Calibration/validation plot for fruity flavour.



For fruity flavour (Figure 2) the difference between the SEPval to SEPcal values was close to double that for cocoa flavour so the predictive model is weaker. On the basis of this calibration

only qualitative classification is possible. Again, the solid circles identify the calibration samples whilst the open circles identify the validation samples. The validation samples show a larger error, indicating that either 'fruity' cannot be predicted by NIRS, or that a much larger sample dataset with more samples having lower scores for fruity flavour is necessary to capture this trait well with NIRS.

UV-fluorescence analysis

The fluorescence levels at different excitations were related to the Trinidad taste panel results and Figures 3 and 4 show linear relationships for cocoa and fruity flavours respectively.

Figure 3. UV-excitation/emission: cocoa flavour.

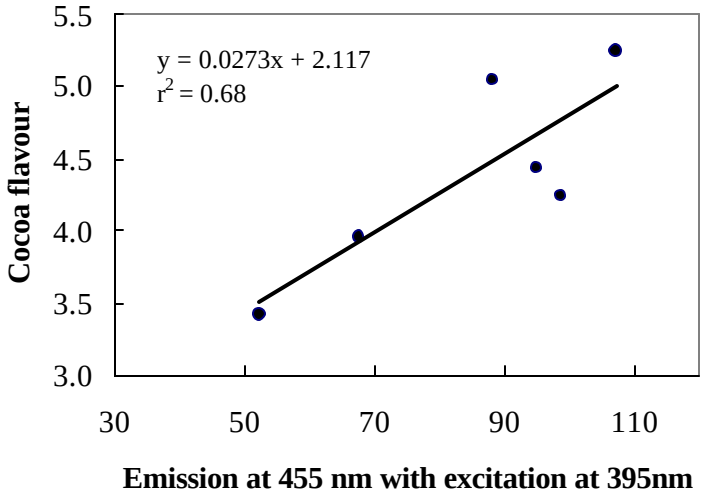
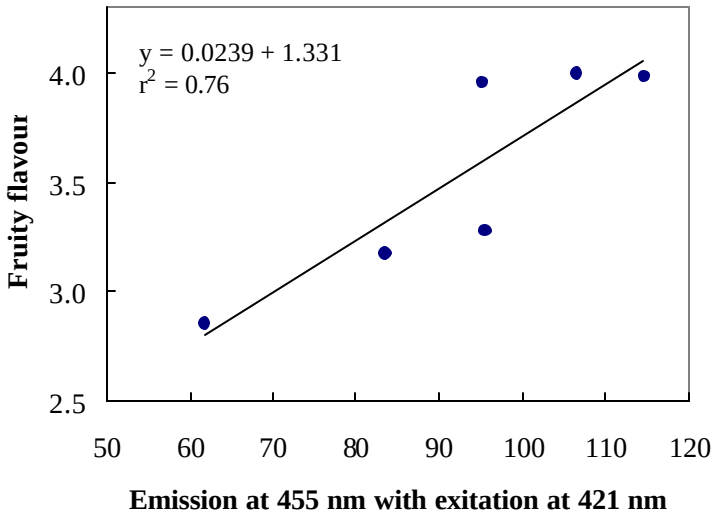


Figure 4. UV-excitation/emission: fruity flavour.

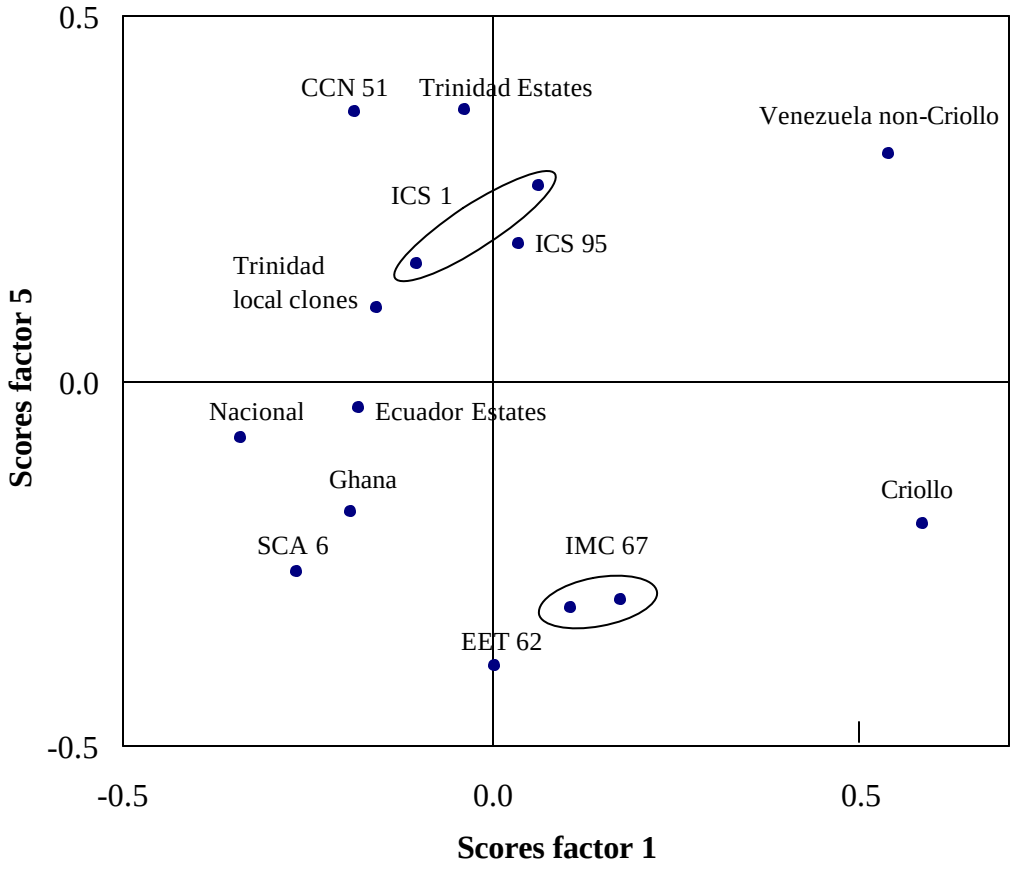


A high correlation was found between cocoa flavour (Figure 3) and the emission at 455 nm using data from a single excitation wavelength (395 nm) only. From the linear regression model, the r value was 0.81 with an r^2 value of 0.68. It was also found that UV-emission at 455 nm correlated highly ($r = 0.87$ and $r^2 = 0.76$) with fruity flavour (Figure 4) when the excitation wavelength was 421 nm.

Distinguishing origins and genotypes

Figure 5 shows the results of a PCA where groups of samples were averaged for each country. This provides a summary of the possibilities for distinguishing origins and genetic groups.

Figure 5. PCA plot summarising of the possibilities for clustering, distinguishing origins and genetic groups.



Best discrimination was found by plotting PCA factors 1 and 5 (Figure 5). The analysis shows that the samples from Venezuela (Venezuela non-Criollo and Criollo) had very different NIRS spectra and were well separated from the rest of the samples. There was also great difference between the Criollo and non-Criollo samples as they occupy different quadrants of the PCA plot. SCA 6, IMC 67, EET 62 and the Ghana reference sample are all grouped together in the PCA

plot and Nacional and Ecuador estate samples fell between this group and the Trinitario samples. The Trinidad local clones, Trinidad estate samples and the ICS genotypes are all in the second quadrant of the PCA plot. CCN 51 from Ecuador is a Trinitario/Forastero hybrid and quite distinct from the Nacional and Ecuador estates. Although Figure 5 shows that the PCA analysis could clearly distinguish average values for the different genotype/origin classes it was not possible to use the PCA model for a fully reliable prediction of the class to which a single sample belonged. This was due to some variation within groups which caused overlap.

Discussion and Conclusion

The results from this study suggest that NIRS can be used to predict some flavour attributes better than others. The exploratory research on a more limited dataset showed very high correlations with single UV excitation/emission wavelengths for certain flavour attributes of Trinidad samples however no attempt was made to validate these relationships. This is nevertheless a highly promising technique that warrants further testing on an expanded dataset. The same can be said for NIRS where too few data points from either a limited sample dataset or range in scores for the flavour attribute contributed to weak calibration/prediction models. Therefore a more extensive database is required to confirm the application of these spectrometric techniques to flavour characterisation.

From a global project view, differentiating between different origins and genetic groups on the basis of NIRS was possible within this sample dataset. We found good discrimination between groups of local clones from different regions, as well as CCN 51 versus the Nacional clones from Ecuador. On the basis of cocoa flavour we were able to distinguish between the "bulk" Ghana reference and regional varieties known to be "fine or flavour". We also had very good discrimination between the Criollo and non-Criollo types in Venezuela as well as between Trinitario in Trinidad and Ecuador Nacional genotypes. These promising findings suggest that more tests with NIRS using larger datasets would be worthwhile for separation between genotypes.

What is the future of applying NIRS and UV-fluorescence technology to flavour and quality in cocoa? If we want to utilise spectrometric techniques to address issues in flavour and quality we need to start routine collection of a large number of commercial samples and local clones with corresponding organoleptic data. Many flavour attributes had fairly good NIRS calibrations, but the accuracy of predictions allows only a classification into broad classes at this stage. This was a function of the size of the dataset, but it also depends on the quality and consistency of organoleptic data.

To implement a NIRS or UV-fluorescence based system for quality assessments on a country or region-wide basis, we need to firstly analyse the cost and benefit of such a venture. A prudent approach might be to test its commercial application on a pilot scale or demonstration basis as part of a broader quality control system. This will allow both cocoa producers to build up an adequate database and chocolate manufacturers to acquire confidence in the system. The system would be continually evolving as the database expands and test samples with robust reference evaluations are used to improve the calibration/prediction models.

Acknowledgements

The financial support, assistance and collaboration of the United Nations Common Fund for Commodities, Anecacao, Ecuador, ACIAR[1], Australia, CONICIT[2], Venezuela, Guittard Chocolate Co., California, USA, Lindt & Sprüngli, Switzerland, Masterfoods UK, MALMR, Trinidad and Tobago, the American Cocoa Research Institute, USA, Nestlé Product Testing Centre, York, UK, Paul Manickchand Estates Ltd. (Trinidad), Produce Marketing Associates Ltd. (Trinidad) and all sensory panellists are gratefully acknowledged participation in this project. The collaboration of CCI[3], Papua New Guinea, in providing bean samples and country clones as well as commercial cocoa liquors for sensory assessment is also gratefully acknowledged.

[1] Australian Centre for International Agricultural Research
[2] Consejo Nacional de Investigaciones Científicas y Tecnológicas
[3] Cocoa Coconut Institute, Papua New Guinea

Evaluation of cocoa germplasm for resistance to Witches' Broom disease

R. Umaharan and S. Surujdeo-Maharaj

Overview

In order to acquire information on Witches' Broom resistance, screening is being carried out with the aim of achieving the following objectives:

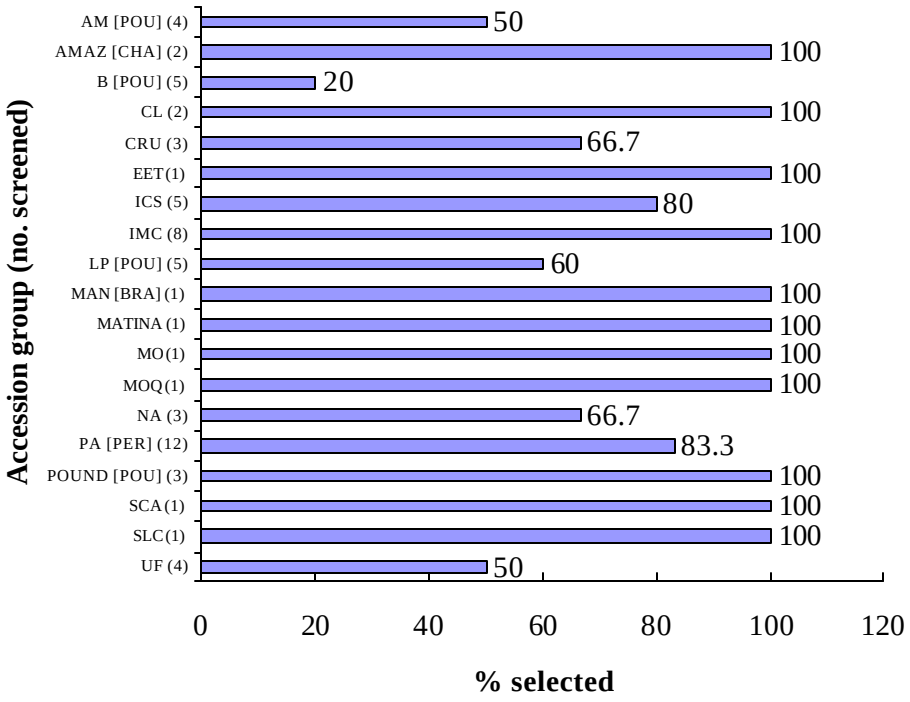
- To identify promising clones in the ICG,T by mass screening for WB resistance.
- To confirm and quantify the level of resistance of promising clones by agar droplet inoculation.

The protocol for screening for resistance to WB disease is a two-tiered system. Cacao clones are collected from the ICG,T as budwood and top-grafted in the greenhouse. The plants are then screened manually, using a spray inoculation technique. They are sprayed with a suspension of WB basidiospores, using a Preval Sprayer (Precision Valve Corp., NY, U.S.A), which delivers a fine spray to the developing shoots of the plant (Umaharan *et al.,* 2005). The plants are then observed over a four-month period and data are collected on the proportion of plants showing symptoms, how long the symptoms take to develop (incubation period) and symptom severity. Clones selected from mass screening with promise of resistance are further confirmed using the agar droplet technique (Surujdeo-Maharaj *et al*., 2003) where the inoculum (spore suspension) is delivered with a pipette to each developing shoot using an agar based medium.

Results

From January to December 2006, 43 clones were screened for resistance and 29 were selected for further confirmation screening. Overall results obtained from screening have shown that there is considerable variation for resistance to WB (ranging from highly resistant to highly susceptible) within the collection of cacao held at the ICG,T. From 1998 to 2006, 1,040 individual clones have been grafted from the ICG,T in preparation for screening. Out of this, a total of 727 clones have been screened for resistance to Witches' Broom disease using spray inoculation techniques. These experiments represent 24 series of inoculations, where approximately 30 clones plus two controls were inoculated per series. Of those screened, assessment of resistance has been completed for 600 clones and 294 were selected for further confirmation. Seventy one clones have been screened for confirmation of resistance and 48 clones belonging to 19 accession groups (Figure 1) were selected and confirmed as showing resistance to WB disease under the experimental conditions at CRU. Figure 1 shows the proportion of clones selected from the 19 accession groups as a percentage of the number of clones screened in each.

Figure 1. Frequency of clones with confirmed resistance to Witches' Broom disease.



Most of the resistance has been observed in populations belonging to Upper Amazon Forastero and Refractario cacao types. Many of these populations were collected from trees selected for lack of symptoms to Witches' Broom, so the proportion of resistant clones is greater than that expected in an unselected population. The current project has also been able to identify those accessions which are definitely susceptible to the disease.

The impact of these results is that promising clones which have been identified can now be recommended for use in other research programmes, such as germplasm enhancement in the CFC/ICCO/BI Cocoa Productivity Project. The majority of the resistant clones from phase one of the project have already been transferred to Bahia, Brazil. Those from more recent findings are being transferred to the ICQC,R for further distribution to end-users.

Future

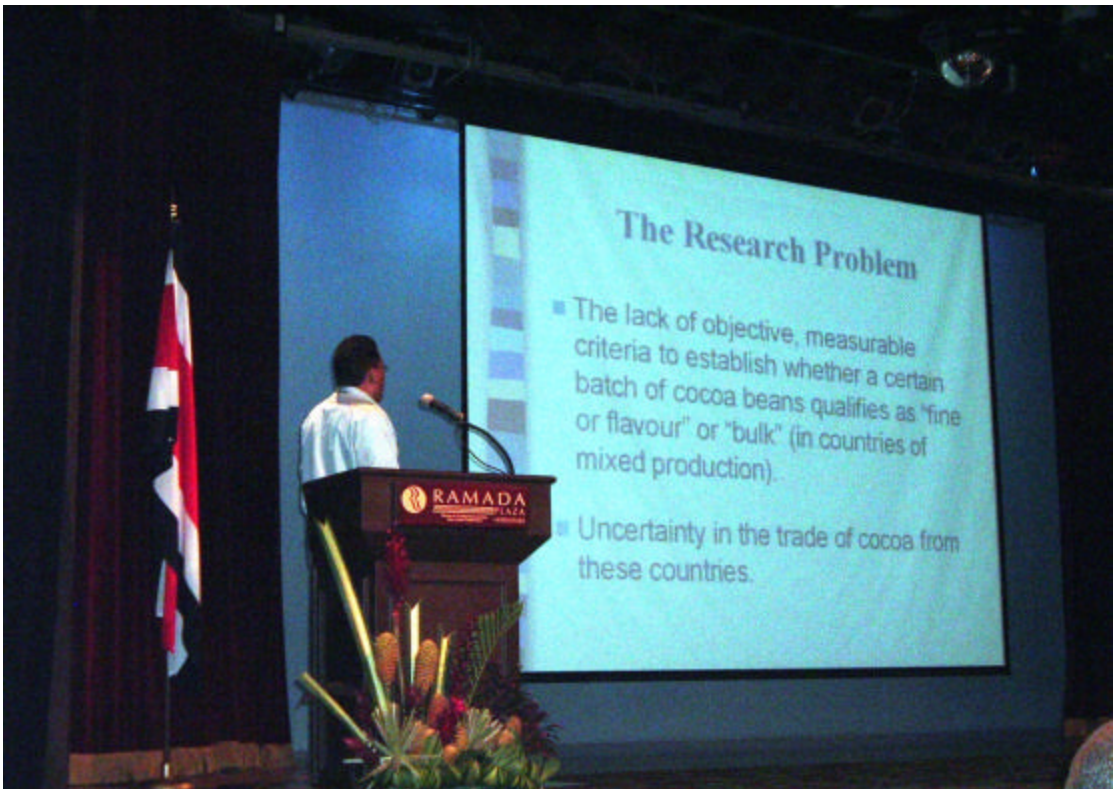
During the next year, the focus will be on confirmation screening of the clones selected by mass screening.

References

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

Umaharan, R., Thévenin, J-M., Holder., A., and Bhola., J. (2005) Evaluation of cocoa germplasm for resistance to Witches' Broom disease. Pages 44-47 in: *Annual Report 2004*. St Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Utilisation



Progress in germplasm enhancement for Witches' Broom resistance

P. Deberdt, A. Holder, V. Jadoo and J-M. Thévenin

Introduction

Germplasm enhancement for resistance to Witches' Broom disease was initiated in July 2004 as an activity in the CFC/ICCO/BI Cocoa Productivity Project. The main objective of this programme is to develop cacao populations with enhanced resistance to Witches' Broom and Black Pod diseases while maintaining a broad genetic base. Crosses were completed over three consecutive years at UCRS, using different sets of parents each year.

Methodology

Design and crosses

Year 1 (May - December 2004) and year 2 (June - December 2005) designs were reported in the CRU Annual Report 2005 (Holder *et al*., 2006). The year 3 design was similar to that in year 2 with 11 crosses in an incomplete factorial experimental design and seven additional crosses. Furthermore, 15 crosses which either failed in previous years or did not produce sufficient seedlings were repeated.

Screening

Witches' Broom disease

Six to seven-month-old progeny from year 2 crosses aged of 6-7 months together with their parents, represented by seedlings from open-pollinated pods, were screened in four batches (June, July, September and November 2006). The agar droplet technique of inoculation (Surujdeo-Maharaj *et al*., 2003) was used.

The percentage of plants developing symptoms (incidence) and largest broom-base diameter (BBD) (severity) as well as time to first symptom (TFS) and time to broom initiation (TBI) were observed. In each family, resistant plants (those with low disease incidence and/or low severity) together with a few susceptible ones, were also tested for Black Pod disease resistance.

Black Pod disease

Selected crosses from the first pollination year with the correct interflush-2 stage leaves were screened for resistance to *P. palmivora* using the leaf disc test (Nyassé *et al.*, 1995). The day prior to inoculation, leaves were harvested, 15 mm discs were punched from the leaves and placed in sealed humidified trays. A complete randomised block design was used with three replications (trays) and five discs per tray, *i.e.* three replicates totalling 15 discs per plant. Each experiment was repeated 2 to 3 times (on different dates). A range of control cacao clones was used namely ICS 46, CAS 1, IMC 57 and PA 124 [PER].

Analyses of variance (General Linear Model of SAS, SAS Institute, USA) for effects and interactions between the possible sources of variation were conducted and the degree of susceptibility of controls and plants within each cross was compared with the Newman and Keuls test. Statistically homogeneous groups were identified.

Results and Discussion

Black pod resistance screening of selected year 1 progeny

Table 1a. Leaf disc susceptibility of 4 control clones of cocoa inoculated with *P. palmivora.*

Control clones	Symptom rating[1]	Resistance level[2]
ICS 46	4.00 a	S
CAS 1	2.60 b	MR
IMC 57	1.53 c	R
PA 124 [PER]	1.73 c	R

[1]Symptom rating was 0 (none) to 5 (full necrosis). Mean symptom ratings followed by the same letter are not significantly different according to the Newman and Keuls test at 5 %.
[2]Resistance level: S = susceptible; MS = moderately resistant; R = resistant

Table 1b. Number of seedlings from each cross classified according to their level of Black Pod resistance, based on the leaf disc test.

Cross	Code	BP resistance level		
		R	MR	S
ICS 46 × LP 1/45 [POU]	A2	0	3	15
PA 303 [PER] × LP 1/45 [POU]	B1	3	5	3
PA 303 [PER] × SPA 9 [COL]	B2	4	5	4
PA 303 [PER] × CATONGO	B3	5	4	2
JA 3/4 [POU] × SPA 9 [COL]	C1	0	1	0
JA 3/4 [POU] × SLC 4	C2	0	0	4
JA 3/4 [POU] × CATONGO	C3	0	0	1
GU 114/P × SLC 4	D1	1	4	0
GU 114/P × CATONGO	D3	11	3	0
RB 29 [BRA] × CRUZ 7/8	E1	3	2	0
RB 29 [BRA] × PLAYA ALTA 2 [VEN]	E2	1	0	1
RB 29 [BRA] × CATONGO	E3	4	4	2
LP 1/45 [POU] × ICS 46	G1	3	5	3
LP 1/45 [POU] × PA 303 [PER]	G2	6	5	6
LP 1/45 [POU] × CATONGO	G3	0	0	1
SPA 9 [COL] × PA 303 [PER]	H1	2	2	3
SPA 9 [COL] × JA 3/4 [POU]	H2	5	8	4
SPA 9 [COL] × CATONGO	H3	6	5	0
SLC 4 × GU 114/P	I2	0	1	0
CRUZ 7/8 × GU 114/P	J1	6	3	6
CRUZ 7/8 × RB 29 [BRA]	J2	1	0	2
CRUZ 7/8 × CATONGO	J3	4	6	1
PLAYA ALTA 2 [VEN] × RB 29 [BRA]	K1	0	1	0
PLAYA ALTA 2 [VEN] × LCTEEN 90/S-7	K2	1	4	1
PLAYA ALTA 2 [VEN] × CATONGO	K3	1	0	0
IMC 57 × CATONGO	L1	8	2	0

R= resistant; MR = moderately resistant; S = susceptible

Black Pod resistance screening of 20-30% of the progeny most resistant to WB with a few moderately resistant and susceptible plants from year 1 crosses is 95% complete. A total of 208 seedlings from the progenies have been screened and have been categorised into 3 resistance

Table 2a. Percentage of plants showing symptoms, the time of appearance of symptoms and severity of symptoms for year 2 crosses.

Crosses	Code	Total[5]	Dead Plants (%)	Percentage of plants with[1]			TFS[2]		TBI[3]		BBD[4]	
				No symptom	Swelling (no broom)	Broom	N[6]	Mean (days)	N[6]	Mean (days)	N[6]	Mean (mm)
PA 195 [PER] × LP 3/15 [POU]	B1	Failed	-	-	-	-	-	-	-	-	-	-
PA 195 [PER] × (ICS 1 × GU 175/P, tree 28)	B2	29	13.8	0.0	3.5	96.6	29	15.3	28	18.5	28	10.57
CRU 89 × (ICS 1 × GU 175/P, tree 28)	B3	36	19.4	2.8	2.8	94.4	35	14.1	34	19.7	34	9.48
CRU 89 × SJ 1/40 [POU]	B4	81	18.5	3.7	1.2	95.1	78	13.9	77	20.3	77	9.67
AM 2/19 [POU] × SJ 1/40 [POU]	B5	30	23.3	0.0	10.0	90.0	30	14.0	27	19.6	27	8.46
AM 2/19 [POU] × NA 232	B6	119	15.1	5.0	2.5	92.4	113	14.4	110	21.3	110	9.88
MOQ 695 × NA 232	B7	20	10.0	0.0	5.0	95.0	20	14.2	19	18.8	19	11.26
MOQ 695 × (IMC 67 × GU 353/L, tree 64)	B8	38	7.9	0.0	2.6	97.4	38	14.8	37	23.1	37	8.65
B 9/10-25 [POU] × (IMC 67 × GU 353/L, tree 64)	B9	84	7.1	5.6	1.2	92.9	79	13.6	78	18.0	78	8.61
B 9/10-25 [POU] × CL 10/5	B10	98	6.1	4.1	0.0	95.9	94	13.7	94	17.9	94	9.21
LP 3/15 [POU] × CL 10/5	B11	11	9.1	0.0	0.0	100.0	11	13.0	11	17.1	11	11.81
CC 71 × NA 33	A1	35	20.0	0.0	2.9	97.1	35	15.4	34	21.4	34	9.98
PA171 [PER] × TRD 109	A2	147	10.2	6.1	2.0	91.8	138	13.8	135	21.3	135	9.91
PA 126 [PER] × AMAZ 6/3 [CHA]	A3	79	8.9	3.8	1.3	94.9	76	16.0	75	25.0	75	9.68
CRU 80 × MATINA 1/7	A4	60	16.7	0.0	0.0	96.7	58	16.2	58	23.5	58	9.26
MO 9 × PA 150 [PER]	A5	84	4.8	5.9	2.4	91.7	79	13.7	77	18.1	77	9.33
CL 10/15 × (ICS 84 × TSH 1077, tree 49)	A6	92	18.5	1.1	1.1	97.8	91	15.2	90	21.4	90	9.79
IMC 47 × (NA 45 × B 7/21 [POU], tree 83)	A7	106	16.0	1.9	0.9	98.1	105	16.1	104	24.3	104	9.69
NA 399 × (SCA 6 × IMC 67, tree 12)	A8	125	16.8	0.8	4.0	95.2	124	15.2	119	23.1	119	8.91
TRD 32 × NA 471	A9	27	3.7	0.0	0.0	100.0	27	13.1	27	18.6	27	9.46
ICS 35 × SCA 24	A10	Failed	-	-	-	-	-	-	-	-	-	-
TRD 45 × NA 471	A11	68	14.1	10.3	5.9	83.8	61	15.0	57	24.6	57	9.54

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

Table 2b. Percentage of plants showing symptoms, the time of appearance of symptoms and severity of symptoms for parents (year 2).

Parents	Total[5]	Dead Plants (%)	Percentage of plants with[1]			TFS[2]		TBI[3]		BBD[4]	
			No symptom	Swelling (no broom)	Broom	N[6]	Mean (days)	N[6]	Mean (days)	N[6]	Mean (mm)
AM 2/19 [POU]	8	37.5	12.5	0.0	87.5	7	12.3	7	15.1	7	9.36
AMAZ 6/3 [CHA]	31	12.9	6.5	9.7	83.9	29	14.3	26	22.7	26	7.56
AMEL 2/0-17 [MAY]	5	0.0	0.0	0.0	100.0	5	14.4	5	27.4	5	12.14
B 9/10-25	20	5.0	0.0	0.0	100.0	20	13.5	20	19.8	20	10.46
CC 71	32	21.9	3.1	3.1	93.8	31	14.0	30	18.6	30	10.06
CL 10/5	35	2.9	11.4	0.0	88.6	31	13.8	31	18.6	31	8.76
CRU 80	26	15.4	11.5	3.9	84.6	23	14.3	22	28.5	22	10.74
CRU 89	34	8.8	0.0	0.0	100.0	34	12.4	34	16.3	34	10.28
IMC 47	14	28.6	7.1	0.0	92.9	13	13.5	13	21.6	13	9.75
IMC 57	49	12.2	4.1	6.1	89.8	47	13.7	44	20.8	44	7.46
IMC 6	2	0.0	50.0	0.0	50.0	1	14.0	1	23.0	1	8.60
LP 3/15 [POU]	11	27.3	18.2	0.0	81.8	9	15.6	9	19.7	9	9.16
MATINA 1/7	17	17.7	17.7	0.0	82.4	14	14.4	14	22.6	14	9.59
MO 9	38	13.1	10.5	2.6	86.8	34	14.5	33	20.6	33	7.45
MOQ 6/95	22	13.6	4.6	0.0	95.5	21	13.9	21	19.8	21	10.00
NA 232	31	9.7	3.2	0.0	96.8	30	12.9	30	15.7	30	13.52
NA 399	15	13.3	0.0	0.0	100.0	15	13.5	15	18.7	15	9.73
PA 126 [PER]	31	6.5	3.2	12.9	83.9	30	14.0	26	19.9	26	9.06
PA 150 [PER]	21	9.5	4.8	4.8	90.5	20	16.0	19	24.6	19	8.41
PA 171 [PER]	36	11.1	5.6	8.3	86.1	34	14.4	31	22.9	31	9.56
PA 195 [PER]	10	10.0	10.0	10.0	80.0	9	15.8	8	18.3	8	7.94
SJ 1/40 [POU]	38	7.9	7.9	5.3	86.8	35	12.8	33	19.6	33	8.95
TRD 45	27	25.9	18.5	7.4	74.1	22	19.1	20	25.7	20	8.60
UF 29	56	28.6	3.6	0.0	96.4	54	13.4	54	18.5	54	9.96

[1]percentage based on the number of live plant only; [2]Time to first symptom; [3]Time to broom initiation; [4]broom-base diameter (mm);
[5]Total number of seedlings inoculated; [6]Number of seedlings evaluated

level groups (resistant [R], moderately resistant [MR], and susceptible [S]). The data analysis showed a highly significant interaction between symptom ratings and dates ($P < 0.0001$). For each date, a symptom rating average was calculated for each plant using the symptom rating scale developed by Nyassé (Nyassé *et al*., 1995). To take account of the interaction date × symptom rating, a Newman and Keuls test was done for each date and homogeneous groups were identified for individual plants including control clones (see controls classification for a given date in Table 1a). The controls grouped into different resistance level classes specifically [S] for ICS 46, [MR] for CAS 1, and [R] for IMC 57 and PA 124 [PER] clones. As the ranking of controls was stable among the dates, it was possible to classify each plant according to its resistance level (Table 1b). A total of 76 seedlings from 19 families were classified as resistant to BP.

From year 1 cross, a total of 57 individuals from 16 families were selected as being resistant to both WB and BP.

Year 2 crosses (2005/2006)

A total of 1,370 individuals in progenies from year 2 crosses and 609 seedlings representing the parental clones were screened for resistance to WB (Tables 2a and 2b). The most promising families for TFS include PA 126 [PER] × AMAZ 6/3 [CHA], CRU 80 × MATINA 1/7 and IMC 47 × (NA 45 × B 7/21 [POU], tree 83), whereas the most promising families for BBD include AM 2/19 [POU] × SJ 1/40 [POU], MOQ 695 × (IMC 67 × GU 353/L, tree 64) and B 9/10-25 [POU] × (IMC 67 × GU 353/L, tree 64) (Table 2a).

In each family, the plants are being classified in 3 resistance level groups (R, MR and S), according to the different parameters recorded. Resistant plants are those that either develop no WB symptoms, have a long TFS or a small BBD.

Year 3 crosses (2006/2007)

In year 3, 15 crosses from the years 1 and 2 that were not successful or had too few individuals in progenies, were repeated successfully in year 3 (Table 3a). An additional 11 crosses from year 3 (factorial mating design with additional crosses) were also successful (Tables 3b and 3c). Five other crosses in year 3 failed due to the lack of synchrony of flowers on male and female trees later in the flowering season.

Seedlings will be screened using the agar droplet inoculation method starting in mid-2007.

Conclusion

From year 1 crosses, 98% of WB resistant seedlings have been screened for BP with the leaf disc test. A total of 76 individuals from 19 crosses were classified as resistant to BP and 57 individuals from 16 crosses were classified as resistant to both WB and BP.

From year 2 crosses, 1,370 individuals and 609 seedlings representing the parental clones have been screened for WB and these will be classified according to their level of resistance. The most WB resistant plants with a few moderate and susceptible plants from year 2 will be evaluated for their level of resistance to *P. palmivora* using the leaf disc test.

Table 3a. Number of beans planted from repeats of crosses from years 1 and 2.

Female	Male	No. of seedlings
CRU 89	(ICS 1 × GU 175/P) tree 28	127
CRUZ 7/8	RB 29 [BRA]	55
GU 114/P	CRUZ 7/8	91
GU 114/P	SLC 4	78
ICS 35	SCA 24	20
IMC 57	CATONGO	156
LCTEEN 90/S-7	CATONGO	26
LCTEEN 90/S-7	PLAYA ALTA 2[VEN]	24
LP 3/15 [POU]	CL 10/5	31
MOQ 6/95	NA 232	89
PA 195 [PER]	(ICS 1 × GU 175/P) tree 28	70
PA 195 [PER]	LP/315 [POU]	82
SLC 4	CATONGO	47
SLC 4	GU 114/P	20
UF 29	CATONGO	57

Table 3b. Number of beans planted or pods harvested from year 3 crosses in an incomplete factorial mating design.

Female/Male	MO 20	NA 702	LP 3/4 [POU]	CRU 90	GU 261/P	IMC 31
EET 399 [ECU]	Failed	Failed				
LV 20		1 pod	2 pods			
CRU 104			Failed	Failed		
B 12/1 [POU]				1 pod	1 pod	
MAN 15/60 [BRA]					2 pods	149 seedlings
MO 20						Failed

Table 3c. Number of beans planted from additional crosses in year 3.

Female	Male	No. of seedlings/pods
MO 9	LCT EEN 46	177 seedlings
JA 5/5 [POU]	CC 41	3 pods
CL 10/3	ICS 35	1 pod
COCA 3370/5 [CHA]	PA 39 [PER]	2 pods
ICS 35	CL 10/3	3 pods

Good success was obtained (early in the flowering season) for repeat crosses from years 1 and 2. Poor synchrony of flowers later in the season was a limitation, so a number of the year 3 crosses need to be repeated.

Selections from the year 1 and year 2 progenies with resistance to both WB and BP will be planted in the field in 2007.

References

Holder, A., Thévenin, J-M., Boccara, M., Jadoo, V. and Solomon, F. (2006) Germplasm enhancement for resistance to Witches' Broom disease. Pages 68-72 in: *Annual Report 2005.* St Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

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

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

Cocoa Research Advisory Committee

Prof. Lawrence Wilson, *Chairman*
Dept. of Food Production,
The University of the West Indies
St. Augustine, Trinidad and Tobago

Dr. David Butler, *Ex-officio*
Director, Cocoa Research Unit,
The University of the West Indies,
St. Augustine, Trinidad and Tobago

Dr. Bhoendradatt Tewarie, *Representative*
Principal, The University of the West Indies,
St. Augustine, Trinidad and Tobago

Mrs. Lylla Bada
Bursar, The University of the West Indies,
St. Augustine, Trinidad and Tobago

Prof. Dyer Narinesingh
Dean, Faculty of Science and Agriculture,
The University of the West Indies,
St. Augustine, Trinidad and Tobago

Ms. Philippa Forde, *Representative*
Permanent Secretary,
Ministry of Agriculture, Land and Marine Resources (MALMR)
Port of Spain, Trinidad and Tobago

Mr. David Preece, *Representative*
Biscuit, Cake, Chocolate and Confectionery Association (BCCCA),
London, UK

Mr. Christophe Montagnon, *Representative*
Centre de coopération internationale en recherche agronomique
pour le développement, Departement cultures pérennes (CIRAD-CP),
Montpellier Cedex 1, France

Cocoa Research Unit staff 2006

Research staff

David Butler *PhD* Director

Frances Bekele *MPhil* Research Fellow

Gillian Bidaisee *MSc* Contract Officer I

David Iwaro *PhD* Research Fellow
Plant Pathologist/Breeder

Lambert Motilal[1] *MPhil* Contract Officer I

Antoinette Sankar *MSc* Contract Officer I

Darin Sukha[1] *MSc* Junior Research Fellow

Romina Umaharan *MPhil* Contract Officer I (part-time)

Balram Latchman *MSc* Contract Officer I (March – August)

Support staff

Junior Bhola Laboratory Assistant

John Joseph Laboratory Assistant

Annelle Holder *MPhil* Technical Assistant

Gangadeen Ramdhanie Senior Laboratory Assistant

Vindra Singh *BSc* Technical Assistant

Eusebius Solozano Laboratory Assistant

Naailah Ali *BSc* Technical Assistant (April – September)

Frankie Solomon[1] *BSc* Technical Assistant (From September)

Sarah Bharath *BSc* Technical Assistant (part-time from October)

Surendra Surujdeo-Maharaj[1] *BSc* Technical Assistant (from September)

Visiting scientists

Michel Boccara *PhD* Molecular Biologist
CIRAD-CP, France

Peninna Deberdt *PhD* Phytopathologist
CIRAD-CP France

Administrative staff`

Claudia Lyons Secretary

Phulmatee Hetai Messenger/cleaner

[1]Registered as a post-graduate student with the University of the West Indies

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.

Iwaro, A.D., Butler, D.R. and Eskes, A.B. (2006) Sources of resistance to Phytophthora pod rot at the International Cocoa Genebank, Trinidad. *Genetic Resources & Crop Evolution* **53 (1)**: 99-109.

Sukha, D.A., Butler, D.R., Umaharan, P. and Boult, E. (2007) The use of an optimised organoleptic assessment protocol to describe and quantify different flavour attributes of cocoa liquors made from Ghana and Trinitario beans. *European Food Research and Technology*. Published online at http://dx.doi.org/10.1007/s00217-006-0551-2

Zhang, D., Boccara, M., Mische, S., Motilal, L., Leamy, E. and Butler, D.R. (2007) Assessing genetic identity, sibship structure, and population admixture in an Upper Amazonian cacao germplasm group using microsatellite markers. *Tree Genetics and Genomes* (in press).

Conferences and Workshops

Papers presented

Bekele, F.L. and Bidaisee, G.G. Morphological Trait Assessment of 1180 accessions in the International Cocoa Genebank, Trinidad. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Iwaro, A.D., Bharath, S., Bekele, F.L., Butler, D.R. and Eskes, A.B. Assessment of genetic gain in a germplasm enhancement programme for resistance to Black Pod disease. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Lanaud, C., Fouet, O., Gramacho, K., Argout, X., Legrave, T., Sabau, X., Vincker, P., da Silva, C., Loor, G., Lopes, U., Cascardo, J., Courtois, B., Kuhn, D., Schnell, R., Babin, R., Sounigo, O., Ducamp, M., Deberdt, P., Verica, J., Gultinan, M., Alemanno, L., Machado, R., Phillips, W., Micheli, F., Gestera, S., Maximova, S., Boccara, M., Butler, D., Rosenquist, E., Gilmour, M., Glaszmann, J-C. Producing and sequencing a large collection of cocoa expressed sequence tags (ESTs) enriched in full length cDNA. Presented at Plant and Animal Genome XV, San Diego, USA, 13 – 17 January 2007.

Lanaud, C., Fouet, O., Gramacho, K., Argout, X., Legrave, T., Sabau, X., Risterucci, A.M., Vincker, P., Da Silva, C., Loor, G., Lopes, U., Cascardo, J., Courtois, B., Kuhn, D., Schnell, R., Bailey, B., Babin, R., Sounigo, O., Ducamp, M., Paulin, D., Deberdt, P., Verica, J., Gultinan, M., Alemanno, L., Machado, R., Phillips, W., Micheli, F., da Silva Gestera, A., Clement, D., Maximova, S., Butler, D.R., Rosenquist, E., Gilmour, M., Glaszmann, J-C. A large EST resource for *Theobroma cacao* including cDNAs isolated from various organs and under various biotic and abiotic stresses. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Rohsius, C., Andersson, M., Niemenak, N., Sukha, D.A., Lieberei, R. Fermentation quality and its dependence on the testa structure and transport processes. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Sukha, D.A. Organoleptic parameter results from Trinidad and Tobago. Presented at: Final workshop of the CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Guayaquil, Ecuador, 3 – 6 April 2006.

Sukha, D.A. Results from combined analyses of Organoleptic parameters in relation to project objectives. Presented at: Final workshop of the CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Guayaquil, Ecuador, 3 – 6 April 2006.

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: Final workshop of the CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Guayaquil, Ecuador, 3 – 6 April 2006.

Sukha, D.A. Results from individual and combined analysis of volatile and non-volatile chemical compounds in samples from Trinidad and Tobago. Presented at: Final workshop of the CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Guayaquil, Ecuador, 3 – 6 April 2006.

Sukha, D.A. Trends in pyrazine and other volatile compound results from all project member countries. Presented at: Final workshop of the CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. Guayaquil, Ecuador, 3 – 6 April 2006.

Sukha, D.A., Butler, D.R., Amores, F., Jiménez, J.C., Ramos, G., Gomez, A., Zambrano, A., Hollywood, N. and Ravushiro, J. The CFC/ICCO/INIAP cocoa project "To establish the physical, chemical and organoleptic parameters to differentiate between fine and bulk cocoa" – some highlights from the organoleptic component. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Surujdeo-Maharaj, S. and Umaharan, P. Genetics of resistance to witches' broom disease caused by Crinipellis perniciosa in cacao. Presented at the APS.CPS.MSA Joint meeting on Biological Interactions and Biological Crossroads, Quebec City, Canada, 29 July – 2 August 2006.

Surujdeo-Maharaj, S. Assessment of the Agar-droplet inoculation method for screening resistance to witches' broom disease of cacao. Presented at the American coordination and resistance testing meeting, Higuerote, Venezuela, 13 – 17 February 2006.

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D.R. Evidence of polygenic inheritance of resistance to witches' broom disease caused by *Moniliophthora perniciosa* in *T. cacao* L. Presented at the 5th INCOPED International Seminar on Developing Effective Sustainable Crop Protection Systems for Cocoa Production, San Jose, Costa Rica, 15 – 17 October 2006.

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D.R. Studies on the resistance to witches' broom disease caused by *Moniliophthora perniciosa* in *Theobroma cacao* L. Presented at the 5th INGENIC Workshop on Cocoa Breeding for Farmers' Needs, San Jose, Costa Rica, 15 – 17 October 2006.

Zhang, D., Boccara, M., Mischke, S., Motilal, L.A., Kolesnikova-Allen, M., Cryer, N., Turnbull, C., Lanaud, C., Philips-Mora, W. and Butler D.R. Detection of mislabeled germplasm in cocoa collections using DNA fingerprinting with SSR markers. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Posters presented

Boccara, M., Zhang, D., Mischke, S., Motilal, L.A., Sankar, A. and Butler D.R. Verification of clone identity in the International Cocoa Genebank, Trinidad using molecular markers. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Iwaro, A.D., Thévenin, J-M., Butler, D.R. and Eskes, A.B. Validation studies on the detached pod test and leaf disc inoculation method for the assessment of cacao resistance to *Phytophthora* infection. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Johnson, E.S., Philips, W., Bekele, F.L. Zhang, D. and Schnell, R.J. Field guide to the UF clones of Costa Rica. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Motilal, L.A., Khan, N., Sukha, D.A., Bekele, F.L., Iwaro, A.D., Bidaisee, G.G., Umaharan, P., Grierson, L.H. and Zhang, D. Assessment of cocoa (*Theobroma cacao* L.) bean traits for the identification of superior accessions for germplasm enhancement. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Motilal, L.A., Zhang, D., Umaharan, P. and Mischke, S. Association mapping for butterfat content in the tree crop *Theobroma cacao* L. Presented at "From Functional Genomics of Model organisms to Crop Plants for Global Health", Washington, USA, 3 – 5 April 2006.

Sabau, X., Loor, G. Boccara, M., Fouet, O., Argout, X., Leganre, T., Risterucci, A.M., Vincker, P., da Silva, C., Jimenez, J.C., Cros, E., Boulanger, R. and Lanaud, C. Expression of genes involved in linalool synthesis during seed development and fermentation of Nacional and Trinitario varieties. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Sankar, A.A., Bidaisee, G.G., Motilal, L.A., Johnson, E.S. and Butler, D.R. Cacao Clones Manual. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Stoll, L., Rohsius, C., Niemenak, N., Sukha, D.A. and Lieberei, R. Formation of the bitter testing ?-aminobutyric acid (GABA) in the course of fermentation and germination processes. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Sukha, D.A., Butler, D.R. and Davrieux, F. Some environmental effect of the flavour attributes of selected cocoa (*Theobroma cacao* L.) varieties. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D.R. Inheritance of resistance to Witches' Broom disease caused by *Crinipellis perniciosa* in *Theobroma cacao* L. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Thévenin, J-M., Holder, A., Butler, D.R., Cilas, C. and Eskes, A.B. Application of an early screening test for Witches' Broom resistance in cocoa progenies. Presented at the 15th ICRC, San José, Costa Rica, 9 – 14 October 2006.

Visitors to CRU in 2006

David Preece	BCCCA/Cadbury Schweppes plc. UK
Christophe Montagnon	CIRAD Montpellier, France
Cynthra Persad	Ministry of Agriculture, Research Division, Centeno
Robert Steinberg	Scharffen Berger Chocolate, Berkeley, California, USA
Bradley Kintzer	Scharffen Berger Chocolate, Berkeley, California, USA
Justin Gurley	Port of Spain, Trinidad
Dieter Weisskopf	Lindt & Sprüngli (International) AG
René Dardel	Lindt & Sprüngli (Switzerland) Ltd
Wilhelm Kessler	Walter Matter SA, Switzerland
Jürgen Rausch	Rausch Schokoladen GmbH, Berlin, Germany
Stefan Vervliet	Puratos - Belcolade, Belgium
Petra Rausch	Fassbender & Rausch Schokoladen, Berlin, Germany
Robert Rausch	Fassbender & Rausch Schokoladen, Berlin, Germany
Biki Khurana	Basic Service Group, Mainz, Germany
Francis D'Hoore	Puratos - Belcolade, Germany
Alexander Morozoff	CocoaAroma Magazine, San Francisco, USA
Loek van Soest	Genebank Advisor, Holland
Wim Kokxhoorn	Masterfoods and Ministry of Agriculture, Holland
Christopher Fulton	CARICAP Coral Gables, Florida, USA
Yoshihiro Funayama Morinaga	Tokyo, Japan
Monica Felipe	Mitsubishi, Caracas, Venezuela
Fernando Puliti	Mitsubishi International, New York, USA
Amy Roda	USDA APHIS PPQ CPHST SHRS, Miami, Florida, USA
Antonio Francis	FANU, Tallahassee, Florida, USA
Henric Sjostrom	Stockholm, Sweden
Veronica Eklund	Stockholm, Sweden
Duane Dove	Tobago/Sweden
Reinhard Lieberei	University of Hamburg, Germany
Christina Rohsius	University of Hamburg, Germany
Claude Villaume	CIRAD, Guadeloupe
Chris Turnbull	Reading University, UK
David Severson	University of Notre Dame, USA
Stefan Reichl	Rausch Schokolade Berlin, Germany
Sophie Assemat	CIRAD-CP Montpellier, France
Jean-Francois Julia	CIRAD-CP, Mexico
Luc Baudouin	CIRAD-CP Montpellier, France
Pierre Costet	Valrhona, Tain L'Hermitage, France
Larry Placide	Barataria, Trinidad
Inteaz Alli	McGill University, Montreal, Canada
Ulrike Krauss	CABI, Trinidad and Tobago

Acronyms and abbreviations

ACIAR	Australian Centre for International Agricultural Research
BBD	Broom-base diameter
BCCCA	Biscuit, Cake, Chocolate and Confectionery Association, London, UK
BI	Bioversity International
bp	base pair
BP	Black Pod disease
CAOBISCO	Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
CATIE	Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica
CCI	Cocoa Coconut Institute, Papua New Guinea
CCIB	Cocoa and Coffee Industry Board, Trinidad and Tobago
CCM	Cacao clones manual
CD-ROM	Compact disc – read only memory
CE	Capillary Electrophoresis
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
CONICIT	Consejo Nacional de Investigaciones Científicas y Tecnológicas, Venezuela
CRA	Cocoa Research Association, UK
CRU	Cocoa Research Unit, Trinidad and Tobago
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
fp	DNA sample number (fingerprint number)
FP	Frosty pod disease
HTML	Hypertext markup language
INAFORESTA	Grupo internacional dedicado al estudio y mejoramiento de la relación entre cacao, árboles y bosques
ICCO	International Cocoa Organisation, London, UK
ICGD	International Cocoa Germplasm Database
ICG,T	International Cocoa Genebank, Trinidad
ICQC,R	International Cocoa Quarantine Centre, Reading, UK
ICTA	Imperial College of Tropical Agriculture
INCOPED	International Permanent Working Group for Cocoa Pests and Diseases
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
JPEG/JPG	Joint Photographic Experts Group
LNV	Ministry of Agriculture, Nature and Food Quality, Holland
MALMR	Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago
MLR	Multiple linear regression
MPP	microsatellite primer pair
NIRS	Near Infrared Reflectance Spectrometry
P	Probability
PCA	Principle component analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PID	Probability of identity
PRI	Plant Research International, Holland
QTL	Quantitative trait loci
r	Correlation coefficient

R	Reflectance
RAPD	Random amplified polymorphic DNA
SEP	Standard error of prediction
SSR	Simple sequence repeats
SDW	Sterile distilled water
TIFF	Tagged image file format
TE	Tri-EDTA (ethylenediamine tetraacetic acid)
TFS	Time to first symptom
TBI	Time to broom initiation
UCRS	University Cocoa Research Station
UE	Union Européenne
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture – Agriculture Research Service
UV	Ultraviolet
UWI	The University of the West Indies
WB	Witches' Broom disease
WCF	World Cocoa Foundation, USA
ZDS	Zentralfachschule der Deutschen Süßwarenwirtschaft e. V., Germany