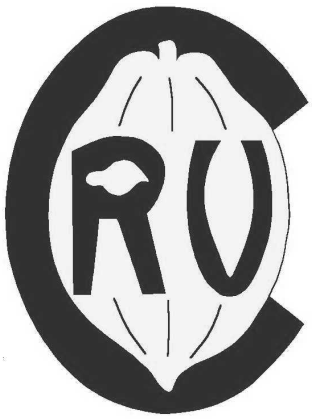
Annual Report 2003



Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
2004

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 only at their first mention.

CRU is a department in the Faculty of Science and Agriculture (FSA) 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 (GORTT) and the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA), UK (now the Biscuit, Cake, Chocolate and Confectionery Association). Sources of additional support for special projects and collaboration from other organisations are listed on the inside front cover of this report.

Projects

The project entitled *Cocoa germplasm conservation and utilisation: a global approach* was due to finish on 31st March 2003, however some outstanding activities took place after that date. The main task was to complete planting of the field trials from the germplasm enhancement programme for resistance to Black Pod disease. For this and maintenance of the field trials, some additional funds were provided to CRU by the United Nations Common Fund for Commodities (CFC). The Supervising Body for the project was the International Cocoa Organisation (ICCO), and it was co-ordinated by the International Plant Genetic Resources Institute (IPGRI). It will be referred to in this report as the "CFC/ICCO/IPRGI Project". By the end of 2003, satisfactory progress had been made in all aspects of the project activities, and we extend our appreciation for the additional funding provided by the CFC. Support from the GORTT and the BCCCA was included in the project as counterpart funding as well as co-financing from the BCCCA, Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) and the World Cocoa Foundation (WCF) (previously the American Cocoa Research Institute (ACRI)). One activity in this project, referred to in previous CRU Annual Reports, is the development of a selection of cacao germplasm (mainly from the ICG,T) with interesting characteristics of resistance to disease, good bean size, good pod index and other valuable traits representing most of the diversity in the genebank. This selection, referred to as the "CFC/ICCO/IPGRI Project Collection", was further refined by Olivier Sounigo in 2003. He has continued to work in CIRAD on achieving this selection, after leaving CRU in August 2001.

The 5-year project to *Evaluate cocoa germplasm for resistance to Witches' Broom disease* was completed on 31st July 2003, by which time over 1,000 accessions had been evaluated for resistance

to Witches' Broom disease (WB). We gratefully acknowledge ACRI's support in providing funding for this project. A proposal to continue the work of screening cacao germplasm for resistance to WB was submitted to the WCF early in 2003. WCF agreed to support this proposal, in the first instance for 12 months from 1st August 2003, with possibility of extending the agreement on a yearly basis.

CRU is participating 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 a tri-partite agreement between CRU, the BCCCA and USDA. During the year, we completed the task of extracting DNA from each accession in the ICG,T, and these samples have been sent to USDA, Beltsville for microsatellite analysis.

The project *To establish the physical, chemical and organoleptic parameters differentiating fine and bulk cocoa* is in its third year. The CFC, with the ICCO as the Supervising Body, supports this project, which involves three other cocoa producing countries (Ecuador, Papua New Guinea and Venezuela). In addition to financial support from the CFC, co-financing is being provided for Trinidad and Tobago from the Ministry of Agriculture, Land and Marine Resources (MALMR), the Guittard Chocolate Company, USA, and Lindt and Sprüngli, Switzerland. The Project Executing Agency is the Instituto Nacional Autonomo de Investigaciones Agropecurias, Ecuador (INIAP), and the project will be referred to as the "CFC/ICCO/INIAP Flavour Project" in this report. The project, which was initiated in 2001, was due to finish in early in 2004. However, it was agreed at a mid-term review in May 2003 to request a one-year extension due to unforeseen delays in some crop harvests and chemical analyses.

Staff news

Herman Ramjewan left in May 2003 when his contract as a Laboratory Assistant expired. He had been working on establishing germplasm enhancement field trials for the CFC/ICCO/IPGRI Project.

Anil Roopchand left in July 2003 when his contract as a Research Technician expired. His work was divided between Plant Pathology - developing an early screening test for Witches' Broom disease, and conservation – updating records, propagation and distribution of clones.

Sharlene Straker left in December 2003 when her contract as a Technical Assistant expired. She had been working on flavour aspects of cocoa liquor in the CFC/ICCO/INIAP Flavour Project.

Sarah Bharath left in December 2003 when her contract as a Technical Assistant expired. She had worked for a number of years in CRU, initially for the CFC/ICCO/IPGRI Project with the germplasm enhancement programme for resistance to Black Pod disease. Later she transferred to the CFC/ICCO/INIAP Flavour Project where she carried out fermentation and drying trials and undertook chemical analyses of bean samples.

Visitors for training

Laura Jacobson came to UWI from January to May 2003 as an exchange student from the University of Wisconsin, USA. During her placement, she undertook a project in CRU on the verification of a selection of clones using Single Sequence Repeat analysis (SSR). She was supervised by Lambert Motilal.

Astrid Breitenstein spent three months in CRU from February to May 2003, when she carried out

[1] Deoxyribonucleic acid

research activities as part of the requirement for a M.Sc. degree in the University of Saarland, Saarbrücken, Germany. She conducted experiments to investigate the reaction of pods and leaves to *Phytophthora palmivora* for a range of cacao genotypes. She was supervised by Dr David Iwaro.

David Secco came to CRU in November 2003 to be trained in aspects of plant pathology for a 7-month period. He is a graduate student from the University of Tours, France and will be working on an early screening test for WB with Jean-Marc Thévenin. During his time in CRU, he also undertook training and participated as a member of a sensory panel to assess cocoa liquor flavour.

Meetings and events

Three representatives from CRU (David Butler, David Iwaro and Jean-Marc Thévenin) participated in a regional planning meeting in Costa Rica in February 2003 to prepare activities for a new project proposal submitted to the CFC on *Cocoa productivity and quality improvement, a participatory approach*. The meeting was hosted by Centro Agronómico Tropical de Investigatión y Enseñanza (CATIE) and was sponsored by potential co-financiers of the new project (ACRI, Masterfoods and USDA). CIRAD supported the attendance of Jean-Marc Thévenin from CRU.

In April 2003, Research Day was held on the St. Augustine Campus to which all departments contributed. One of the purposes of the 3-day event was to bring the Campus into public view and provide a showcase for on-going research in the Science Faculties. All staff in CRU were involved in the event, manning posters, giving an oral presentation (overview) and contributing to publications prepared specifically for Research Day.

In May 2003, David Butler and Darin Sukha attended a mid-term review for the CFC/ICCO/INIAP Flavour Project in Guayaquil, Ecuador. They also took the opportunity to visit our Venezuelan partners in the project at Mérida, Venezuela en route to Ecuador, and the Santa Catalina Experimental Station in Quito, Ecuador. The meetings achieved valuable interactions between all participants from the four cocoa producing countries and excellent progress was made in exchanging experiences and planning the next phase of the project.

Three staff members from CRU (Frances Bekele, David Butler and Darin Sukha) contributed to a Seminar/Exhibition on "Revitalization of the Trinidad and Tobago cocoa industry: targets, problems and options". This one-day event, organised by the Association of Professional Agricultural Scientists of Trinidad and Tobago (APASTT), was addressed by the Minister of Agriculture, Land and Marine Resources and was well attended by all interested parties in the cocoa sector. A great deal of enthusiasm was shown in both the presentations and lively discussions.

CRU made a significant input to the 14th International Cocoa Research Conference held in Accra, Ghana in October 2003. This was an excellent opportunity for those staff who attended (Frances Bekele, David Butler, David Iwaro and Darin Sukha) to interact with colleagues involved in cocoa research world-wide. In addition to the main conference, there were two concurrent satellite meetings; the 4th INGENIC and 4th INCOPED International Seminars which provided a good forum for the debate of current issues in cocoa research. CRU is indebted to the Technical Centre for Agricultural and Rural Cooperation and USDA for providing financial assistance for two people to attend these meetings.

In November 2003, David Butler attended the "CFC round table meeting on commodity development in Latin America and the Caribbean region" in Havana, Cuba. Considerable interest was shown in the Cocoa Working Group, and eight possible projects were identified in the region for further development.

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.

The flavour of cocoa liquor, used in the manufacture of chocolate, is not only determined by the variety, but also by the environment and several post-harvest operations. Different cocoa types require different periods of fermentation and rates of drying to develop the subtle and complex composition of cocoa liquor needed for good quality chocolate. Forastero cocoa generally requires 5-8 days of fermentation to produce rich brown beans with a strong cocoa flavour, whereas Criollo and Trinitario cocoas require less time to ferment. Liquor from Criollo beans has a distinctive nutty flavour, while that from Trinitario is more variable, generally characterised by a fuller cocoa flavour with pleasant ancillaries described as fruity or floral.

Cocoa research began in Trinidad at the Imperial College of Tropical Agriculture (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 the costs of other methods of cryopreservation are prohibitive. The ICG,T is situated on the University Cocoa Research Station (UCRS), a 33 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 (EU), by propagating trees using rooted cuttings from existing collections in Trinidad. These collections had been established at different locations on the island using selected varieties from Trinidad and Tobago, from other national collections and from numerous missions to collect primary germplasm. They include the Imperial College Selections (ICS) which resulted from an exhaustive survey of Trinidad and Tobago carried out by F.J. Pound between1930 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, established by F.J. Pound following his expeditions to the upper Amazon between 1937 and 1942. The trees at Marper are now old and have suffered periods of neglect, however they still serve as an important anchor in confirming the identity of clones in the ICG,T and in replacing material which has proved difficult to establish. In addition, germplasm was available from other expeditions such as the

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

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

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 (10-18 years after establishing the plots) is that plots contain anything from 1 to 16 trees, and some accessions have no survivors. Plots with less than three living trees are considered at risk to genetic erosion. The urgent need to conserve these clones by grafting their budwood onto rootstocks is being addressed, and the grafted plants are being established in clonal gardens. In cases where there is no survivor in UCRS, but the original tree in Marper Farm or elsewhere is still alive, budwood from the original tree is being grafted onto rootstocks. Once established, cuttings can be taken from the grafted plants and rooted to fill gaps in the ICG,T with plants on their own roots. It is important to make a concerted effort to raise plants from rooted cuttings to avoid potential confusion in the future with chupons from rootstocks.

New introductions

The ICG,T is considered to be a dynamic germplasm collection. We are continuously adding accessions from collecting expeditions (when the opportunity arises) or from other national collections. The objective of these inputs is to increase the representation of genetic groups that are currently under-represented in the genebank, thereby creating a balanced collection with maximum genetic diversity. New material is normally introduced through the Barbados Cocoa Quarantine Station (BCQS) however this activity was suspended in 2003 due to financial constraints. A limited amount of material will continue to be introduced Trinidad through the intermediate quarantine station, Reading, UK. 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.

Further acquisitions are proposed when funding permits, from Mexico (Criollo/Trinitario), Costa Rica (CATIE) (Criollo), Guyana (Lower Amazon), French Guiana (Lower Amazon), 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 material 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. Over the last few years we have completed the task of drawing up-to-date maps, and in numbering plots within fields and trees within plots. All this information has been organised in a database, to enable notes about individual trees to be included, and this information is being continuously updated.

Verification

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

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

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

Initially, molecular verification was undertaken using random amplified polymorphic DNA (RAPD) analysis, this being the technique available in CRU when the work started in 1997. Results from the RAPD analysis showed that approximately 70% of the trees tested were true to type. However, more recently results from some RAPD analyses have been shown to be inconsistent, so it is possible that the 30% off-types identified by this technique is an over-estimate. Since 2001, we have adopted microsatellite analysis (otherwise known as Simple Sequence Repeats, SSR) for the verification work. Initially we used SSR on agarose gels, then, in 2002 we acquired the facilities to

use polyacrylamide gel electrophoresis (PAGE) with silver staining, which gives much better resolution of bands. 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) would be 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 54% 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 (IBPGR, now IPGRI) in 1981, but progress was slow and, in recent years, this has been 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 1,050 accessions have now been completed. As they are recorded, the descriptors are entered in a local database and are also sent to the International Cocoa Germplasm Database (ICGD), Reading, UK, for global distribution.

Having reached a point where large numbers of accessions in the ICG,T have been characterised, analyses are possible to examine phenotypic variation among various groups of cacao (such as Upper Amazon Forastero, Refractario, Lower Amazon Forastero, and Trinitario). Furthermore, this large volume of carefully catalogued data could form the basis of new avenues of work in the future. 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 provides 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 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 could 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 plan to obtain 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 will be completed 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 will 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 have been sent to USDA, Beltsville for analysis with an automatic sequencer. In our previous work with RAPD, we analysed 600 accessions in 6 years, and now we expect to analyse 2,300 accessions in 3-4 years. This collaborative effort will therefore accelerate the rate of progress in genetic diversity studies by a factor of six.

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

Evaluation

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

Disease resistance

Two important diseases that affect cacao in Trinidad are Black Pod disease (BP), caused by *Phytophthora* spp., and Witches' Broom disease (WB), caused by *Crinipellis perniciosa* (Stahel) Singer.

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. This good rate of progress in mass screening for BP is expected to continue over the next few years, so resistance in a diverse range of cacao will be identified for selection.

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.

An optimised agar-droplet method has been developed as a superior method to assess resistance to WB in both seedlings and in clones (grafted plants). We therefore plan to verify the resistance in promising accessions identified by the spray method, using agar-droplet inoculations. 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 heritability of flavour traits is a new area of investigation in CRU. Work has been initiated to explore the relative contributions of environmental and genetic (maternal and paternal) influences on 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 intermediate quarantine at the University of Reading, UK. 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 Project. In the future, selected populations from germplasm enhancement programmes (below) will be distributed in a similar way.

Germplasm enhancement

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

The progeny from crosses in the pre-breeding programme are evaluated for BP resistance with a leaf inoculation method. This permits early selection of seedlings and comparison of the disease resistance of the parents and progeny at an early stage. The most resistant individuals in the progeny are being planted in field trials for evaluation of performance, not only in terms of BP resistance, but also for precocity, vigour and productivity. 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 is planned for WB, but this depends on the availability of a convenient early screening method for the progeny. Crosses between WB resistant clones will be initiated in 2004, and seedlings will be screened with the agar-droplet inoculation method. Work is also in progress at CRU to develop alternative techniques for early screening of resistance to WB.

Marker assisted selection

Research at CRU in the CAOBISCO[1] project (1995-2000), identified quantitative trait loci (QTL) for resistance to BP based on results of the leaf inoculation method. Selected plants from the same progeny were planted in the field, and we plan to validate the leaf inoculation method with field observations and detached pod inoculations once 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 Monilia (*Moniliophthora roreri*). 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 Monilia).

It is likely that other advanced molecular techniques such as ESTs[2] 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" is being transferred to intermediate quarantine in Reading. We expect to complete the task of sending this elite germplasm to Reading in 2004. This is the end-point of a large body of research in CRU, including morphological and molecular characterisation, evaluation for BP and WB (screening and field observations) and cocoa butterfat determinations. The selected clones will soon be available for further distribution to many cocoa-producing countries.

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

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

[1] Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
[2] Expressed sequence tags

Conservation

Transfer of clones from the Barbados Cocoa Quarantine Station

D.R. Butler and D.A. Sukha

At the Cocoa Research Advisory Committee meeting in December 2002, it was agreed to suspend operations in the BCQS due to the high cost of structural repairs needed in the facility. The most recent consignments of cacao germplasm had been introduced to the BCQS in July 2001, so were due to be released in July 2003.

During 2002, an inventory of all the accessions in the BCQS had been made, and clones were multiplied by grafting with an aim to provide sufficient budwood for transfer to Trinidad. A small number of clones had been in Barbados for many years, but had not previously been successfully transferred to the ICG,T. Others were introduced from Ecuador in May 2001, from Venezuela in July 2001 and from French Guyana in July 2001.

Between May and September 2003, 110 clones (Table 1) were transferred to Trinidad, mainly as budwood but in a few cases where insufficient budwood was available, whole plants were shipped. These clones are now well established in the nursery at UWI and will be multiplied and planted in a clonal garden in 2004.

Table 1. Clones transferred from Barbados to Trinidad in 2003[1].

RIM 23 [MEX]	ELP 30 (T5)	GU 32	LCT EEN 218	LCT EEN 411
RIM 52 [MEX]	ELP 32 (T3)	LCT EEN 30	LCT EEN 219	LCT EEN 413
RIM 9 [MEX]	ELP 32 (T4)	LCT EEN 32	LCT EEN 220	NAPO 3 [CHA]
AMELONADO 2/0-17 [MAY]	ELP 34 (T6)	LCT EEN 33	LCT EEN 221	P 19 [MEX]
BLZ 5 [BLZ]	ELP 34 (T7)	LCT EEN 36	LCT EEN 227	PH 1-1
CC 57	ELP 35 (T4)	LCT EEN 37	LCT EEN 232	PH 1-2
ELP 1 (T3)	ELP 35 (T8)	LCT EEN 49	LCT EEN 234	PH 1-3
ELP 1 (T4)	ELP 40 (T6)	LCT EEN 57	LCT EEN 249	PH 1-4
ELP 10	ELP 40 (T9)	LCT EEN 60	LCT EEN 253	PH 1-5
ELP 10 (T6)	ELP 41 (T5)	LCT EEN 63	LCT EEN 255	PH 2-1
ELP 11 (T1)	ELP 7 (T20	LCT EEN 81	LCT EEN 267	PH 2-2
ELP 11 (T3)	ELP 7 (T7)	LCT EEN 91	LCT EEN 278	PH 2-3
ELP 16 (T3)	ELP 8 (T3)	LCT EEN 107	LCT EEN 312	PH 2-4
ELP 16 (T7)	ELP 8 (T7)	LCT EEN 122	LCT EEN 333	PH 2-5
ELP 18 (T9)	ELP 9 (T2)	LCT EEN 123	LCT EEN 362	PH 2-8
ELP 2 (T4)	ELP 9 (T4)	LCT EEN 133	LCT EEN 364	SIAL 44
ELP 20 (T3)	GDL 2	LCT EEN 142	LCT EEN 368	TAP 10 [CHA]
ELP 20 (T4)	GDL 3	LCT EEN 188	LCT EEN 370	TAP 7 [CHA]
ELP 22 (T10)	GS 32	LCT EEN 189	LCT EEN 376	YAL 1 (T2)
ELP 22 (T6)	GS 74	LCT EEN 193	LCT EEN 378	YAL 5
ELP 28 (T4)	GU 192	LCT EEN 195	LCT EEN 403	YAL 6 (T3)
ELP 28 (T6)	GU 202	LCT EEN 217	LCT EEN 409	YAL 6 (T4)

[1]The tree number, recorded for the majority of clones from French Guyana, is shown in parenthesis after the name.

Tree identification by simple sequence repeats: a synopsis for 2000-2003

L.A. Motilal

Introduction

Work done in the verification section was compiled over a consecutive four-year period (2000 to 2003). The purpose of this synopsis is to facilitate users of the ICG,T and enable CRU staff to have ready access to verification records for them to utilise in making decisions related to their experiments. Grivet and Noyer (2003) stated that the length of the microsatellite (SSR) primers (20 to 25 nucleotides) guarantees specificity since the probability of amplifying another sequence in the genome at random is infinitely low. Furthermore, it appears that the results of fifteen SSR primers are more than sufficient to enable detection of off-types (Risterucci *et al*., 2001). Swanson *et al*. (2003) found that eleven primers were more than sufficient to clearly distinguish each of eight genotypes. The highly polymorphic nature of SSR may be attributed to their high mutation rate and make them excellent candidate DNA markers for fingerprinting studies (see Butcher *et al*., online; Robinson and Harris, 1999; Krutovskii and Neale, 2001; Rossetto, 2001).

Materials and Methods

Details of DNA extraction, PCR[1] protocols and electrophoretic conditions can be found in earlier CRU Annual Reports (Motilal, 2002; Motilal, 2003) and references therein. Trees sampled under the verification project are given in Table 1. SSR bands were deemed equivalent when they resided at the same migratory position in a gel.

Results

Definitive SSR results from 61 accessions are provided in Table 2. Various SSR primers detected differences among accessions. The primers mTcCIR 11, 12, 29, 30, 42, 43, 49, 55, 56, 57, 58, 61 appeared to be most useful in detecting differences. The majority of accessions investigated contain true to type trees. There were four cases (ICS accessions 5, 16, 40 and 73) however, where the reference tree was distinct from all the UCRS material. Two of these are represented by only one true to type tree (ICS 5 & ICS 73); i.e. the reference tree in the San Juan Estate (SJE). Several accessions have results for only a subset of trees because they were assayed in the initial part of the verification programme when only four trees per plot were selected for examination. Also, at this time initial SSR tests were being conducted and these results have been absorbed into the verification programme. Due to this, some accessions have been assessed more than once, sometimes with the same primers, but not always for all trees.

[1] Polymerase chain reaction

Table 1. Sampled trees in the verification programme.

Accession[a]	Number of trees	Location details		
		Field	Section/plot	Tree number or co-ordinates[b]
AM 2/38 [POU]	2	5B	I820	2, 3
AM 2/82 [POU]	4	5B	I806	1-4
AMAZ 12 [CHA]	6	6B	B94	2, 4, 9, 10, 15, 16
B 5/7 [POU]	2 (3)	6A	A27	1, 11
		6B	F458	6
CRU 263	3	6B	F431	3, 12, 15
DE 52/B [TTO]	4	6B	B102	1, 8, 11, 14
DH, DH 1 and DH 2[c]	1each	6B	D231	7
		6B	D232	5
		6B	D246	5
EET 400 [ECU]	15	Campus 9A		x5y2
		Campus 3		x22y9, x22y10
		6B	F456	1-3, 6, 7, 9-11, 13-16
GU 243/H	2	Campus 1A		x1y35
		4A	B196	2
ICS 1	39	Campus 1B		x3y13, x5y5, x5y7, x5y9
		Campus 4		x24y(2-5), x24y(7-11)
		Campus 10		x3y6, x4y(7-9)
		Campus 14		x1y(5-7), x3y3, x5y3, x8/9y(5-6)
		6B	B122	3-16
ICS 4	5	6B	E282	1, 2, 4, 6, 7
ICS 5	5	6B	B124	4, 5, 8, 11, 12
ICS 7	1	6B	B125	1 (this tree subsequently died)
ICS 8	8	Campus 4		x23y7(A,B)[d]
		6B	B111	2, 5, 8, 9, 12, 16
ICS 14	3	6B	E284	1, 4, 10
ICS 15	4	4A	C302	1-4
ICS 16	4	Campus 4		x22y(2-4)
		6B	E345	2
ICS 20	4	4A	C303	1-4
ICS 26	5	Campus 11		x2y10 (A,B)[d]
		6A	A62	4, 5, 6
ICS 28	4	4A	C278	1-4
ICS 30	5	4A	C287	1-3
		6B	E384	8, 13
ICS 36	2	6B	E321	4, 5
ICS 40	8	Campus 4		x20y6
		6B	E287	1, 4, 6, 9-11, 14
ICS 42	4	4A	C309	1-4
ICS 65	3	Campus 12		x6y6(A,B)[d], x6y7
ICS 72	2	6A	A74	9, 12
ICS 73	3	4A	C308	1-3
ICS 82	3	4A	C281	1-3
ICS 87	2	Campus 4		x14y1, x14y2
ICS 92	1	6B	E347	1
ICS 95 subsample	3	Campus 3		x10y1
		Campus 4		x13y (1, 3)
ICS 95 full	38	Campus 3		x10y1
		Campus 4		x13y(1, 3, 7, 9, 11)
		Campus 5		x1y(1, 3, 7, 8, 11), x2y(1, 2, 5, 6), x3y(3-7), x4y(3, 6, 11)
		Campus 9B		x8y(1,4), x9y(4, 5, 7), x10y5
		Campus 14		x6/7y9(A,B)[d]
		6B	B98	1, 3-7, 9, 11, 13, 15

[a]name of accession (preferred name from ICGD); [b]tree numbers in UCRS plots and x,y co-ordinates in Campus fields; [c]DH = DOS HERMANOS; [d](A,B) indicates that two main trunks were sampled.

Table 1 (continued). Sampled trees in the verification programme.

Accession[a]	Number of trees	Location details		
		Field	Section/plot	Tree number or co-ordinates[b]
IMC 57	16	6B	A22	1-15, 18
IMC 57	17	Campus 9A		x15y5
		6B	A22	1-15, 18
IMC 67	44	Campus 1B		x(1-4)y14
		Campus 4		x11y1, x11y2, x11y4
		Campus 5		x5y (2-9), x6y (1-7), x6y(10-12)
		Campus 8		x1y25
		Campus 10		x21y1, x21y2
		6B	A23	1-3, 5-16
JA 5/5 [POU]	3	5B	E379	1-3
JA 5/25 [POU]	7	5B	D232	3, 6, 9, 13-16
JA 6/4 [POU]	8	5B	D320	1-3, 7-10, 14
LX 31	3	5B	C178	1, 8, 9
NA 26	6	5B	E352	1, 4, 5, 7, 10, 12
NA 90	3	5B	F550	1, 3, 8
NA 184	4	5B	G612	1, 3, 4, 8
NA 246	2	5B	E404	2, 7
NA 702	2	5B	G631	2, 3
PA 120 [PER]	13	Campus 9A		x15y(7-9)
		6B	D188	2, 5-7, 9, 10, 13-15
PA 150 [PER]	4	6B	C179	5, 7, 13
PA 165 [PER]	3	5B	F451	1, 3, 7
PA 169 [PER]	12	Campus 11		x15y18
		6B	C180	1, 3, 4, 6, 7, 10-16
PA 175 [PER]	3	5B	F473	4, 6, 8
PA 191 [PER]	3	5B	F536	3, 11, 12
PA 200 [PER]	2	5B	F545	1, 2
PA 218 [PER]	3	6B	C160	1, 2, 3
PA 300 [PER]	2	5B	E407	5, 13
POUND 7/A [POU]	3	6B	A78	4, 6, 9
POUND 18 [POU]	3	6B	F449	1, 10, 12
SCA 6	41	Campus 1B		x(4-7)y22, x(2-4, 6, 7)y21, x(2-7)y19, x(2, 3, 5-7)y18, x(2, 3, 5, 6)y16, x(3-6)y15
		Campus 10		x1y(2, 3, 7)
		6B	A16	1, 4, 5, 7, 8, 10, 12, 14, 15, 16
SCA 11	2	Campus 11		x4y19
		Campus 13		x10y10
SLA 8	3	5B	C145	Tree numbers uncertain
SNK 12	3	Campus 3		x15y2
		6B	B118	14,15
TRD 32	3	4A	A42	1-3
UF 11	3	6B	F434	6, 8, 11
VEN B 47 [ICT]	2	6B	B90	2, 7
WAA	19	Campus 14		x(3-8, 10)y1, x(2, 6, 7, 9, 10)y2, x(6-10)y3, x7y4
		6A	A15	3

[a]name of accession (preferred name from ICGD); [b]tree numbers in UCRS plots and x,y co-ordinates in Campus fields.

Table 2. Matches detected by SSR primers for sixty-one accessions in the ICG,T.

Accession[a]	Reference tree[b]	No. of primers used	No. of primers scored[c]	Match[d]	Primers detecting differences (mTcCIR code)
AM 2/38 [POU]	M C111	15	11	Similar	-
AM 2/82 [POU]	M C904	17	17	Similar	-
AMAZ 12 [CHA]	-	19	14	Similar	-
B 5/7 [POU]	M C1000	17	17	M = 6B ? 6A	44, 45
CRU 263 (MIS)[e]	-	55	44	Similar	-
DE 52/B [TTO]	-	26	21	Similar	-
DH, DH 1 & DH 2[f]	-	2	2	DH = DH2 ? DH1	11, 58
EET 400 [ECU]	-	9	8	Similar	-
GU 243/H	-	14	13	Similar	-
ICS 1	SJE B4, LR (6)	14	14	6B T2 ? T3 All others similar	29, 32, 42, 49, 57, 58, 60, 61
ICS 4	SJE B5	11	10	Similar	-
ICS 5	SJE B5	11	11	SJE ? 6B; all 6B similar	11, 29, 49, 56, 57
ICS 7	SJE B5	13	11	Similar	-
ICS 8	SJE B5	11	10	Similar	-
ICS 14	SJE B2	10	9	Similar	-
ICS 15	SJE B5	5	4	Similar	-
ICS 16	SJE B2	5	3	SJE = U ? 6B	29
ICS 20	SJE B2	2	2	Similar	-
ICS 26	SJE B3	1	1	Similar	-
ICS 28	SJE B5	16	11	Similar	-
ICS 30	-	11	9	Only 6B T13 differ	11, 29, 32, 56
ICS 36	SJE B5	2	2	Similar	-
ICS 40	SJE B2	9	8	SJE = U ? 6B	42, 49, 55, 56, 58, 61
ICS 42	SJE B5	17	12	Similar	-
ICS 65	SJE B3, 4	1	1	B3 = B4 ? U	15
ICS 72	SJE B4	4	4	Similar	-
ICS 73	SJE B4	16	11	SJE ? 4A all 4A trees similar	8, 11, 12, 15, 29, 58
ICS 82	SJE B4	16	13	Similar	-
ICS 87	SJE B1	4	4	Similar	-
ICS 92	SJE B1	4	4	Similar	-
ICS 95 subsample	SJE B1	47	42	Similar	
ICS 95 full	SJE B1	15	15	All similar except 6B T3 & T11	8, 12, 18, 29, 30, 37, 58

[a]Name of accession (preferred name from ICGD); [b]reference tree and location, LR = La Reunion, M = Marper Farm, SJE = San Juan Estate; [c]only consistent unambiguous banding patterns assessed; [d]genotype equivalence, U = UWI Campus; [e]CRU 263 (MIS_TTOICGT_POUND 12/B [POU]); [f]DH = DOS HERMANOS.

Table 2 (continued). Matches detected by SSR primers for sixty-one accessions in the ICG,T.

Accession[a]	Reference tree[b]	No. of primers used	No. of primers scored[c]	Match[d]	Primers detecting differences (mTcCIR code)
IMC 57	-	12	12	T4 differs	15/16[e], 53/54A[e], 100/101[e]; 3, 6, 8, 9, 11, 12, 14, 19, 58
IMC 57	M D588, M D619	2	2	D588 ? D619 ? U = F6B; T4 differs	11, 58
IMC 67	LR (6)	16	16	Similar	-
JA 5/5 [POU]	-	14	14	Similar	-
JA 5/25 [POU]	M C476	13	8	Similar	-
JA 6/4 [POU]	M C699	31	19	Similar	-
LX 31	M D113	12	12	Similar	-
NA 26	M 584	14	14	Only T10 differs	29, 30, 42, 49, 58, 59, 61
NA 90	M 577	14	13	M = T1 ? T3 = T8	42, 43, 56
NA 184	M D823	19	16	Similar	-
NA 246	M D459	15	14	M ? T2 = T7	30, 55, 56, 58
NA 702	M D104	12	12	Similar	-
PA 120 [PER]	M D318	2	2	Only 1 tree differs: Campus 9A x15y9	58
PA 150 [PER]	M D679	27	22	M = T5 = T7 ? T1 ? T13	From 18 SSRs
PA 165 [PER]	M D714	14	14	Similar	-
PA 169 [PER]	M D419	17	17	Similar	-
PA 175 [PER]	-	15	15	T4 = T8 ? T6	43, 61
PA 191 [PER]	-	15	14	Similar	-
PA 200 [PER]	M D710	15	13	Similar	-
PA 218 [PER]	-	15	15	Similar	-
PA 300 [PER]	M D544	14	14	M ? T5 = T13	57
POUND 7/A [POU]	-	14	14	Similar	-
POUND 18 [POU]	-	15	14	T1 ? T10 ? T12	12, 43, 61
SCA 6	M D644 LR (2)	9	9	All similar except for 6B T7	30, 42, 55, 56, 57, 58, 61
SCA 11	M D639	1	1	M ? U	58
SLA 8	-	15	14	T1 = T8 ? T7	42
SNK 12	-	30	12	Similar	-
TRD 32	-	32	19	Similar	-
UF 11	-	15	15	T6 ? T8 ? T11	12, 42
VEN B 47 [ICT]	-	2	2	Similar	-
WAA	-	9	9	Similar	-

[a]Name of accession (preferred name from ICGD); [b]reference tree and location, LR = La Reunion, M = Marper Farm, SJE = San Juan Estate; [c]only consistent unambiguous banding patterns assessed; [d]genotype equivalence, U = UWI Campus; [e]EUROGENTEC code for SSR primer.

Focus Groups

(a) VEN B 47

This accession is represented by only two trees in Field 6B at UCRS and which reportedly have differently coloured pods. Two primers were used but a difference between these trees was not detected.

(b) ICS 65 and SCA 11

ICS 65 is present in Blocks 3 & 4 of SJE. However, these plants have multiple trunks and like many plants at SJE it is not clear which is the original trunk and/or the USDA/CRU label is not on the same trunk as other pre-existing labels. Furthermore, three plants are shown as ICS 65 on an older map of the Campus fields but as NL (no label) on a newer map. Pods from these trees at both locations were similar in appearance. One SSR was amplified from these (including multiple trunks) and the results clearly separated SJE material from the UWI material.

SCA 11 from Campus (two trees) was compared to the reference tree in Marper (D639) for one primer. The result clearly showed a distinction between the Marper tree and the UWI trees.

(c) DOS HERMANOS

Three plots in Field 6B are contiguous with each other and are labelled as DOS HERMANOS (DH, plot D231), DOS HERMANOS 1 (DH 1, plot D232) and DOS HERMANOS 2 (DH 2, plot D246). Dr B.G.D. Bartley, who had collected these accessions, indicated to CRU that there should be just two groups (personal communication). Two SSR primers (mTcCIR11 and mTcCIR58) were assayed across DNA from one tree of each group. The results confirm Dr Bartley's impression that DH is equivalent to DH 2 but is distinct from DH 1.

(d) PA 150 [PER]

This accession is included in the International Clone Trial of the CFC/ICCO/IPGRI Project. Pod photographs circulated by Dr P. Lachneaud and Dr A.B. Eskes showed morphological differences in terms of multiple characteristics among the samples from various countries. The four trees in Field 6B were compared to that from Marper Farm (D679) with 22 primers. The results clearly separated Field 6B material into three distinct groups. Two trees were similar to the Marper reference (T5 and T7) whilst T1 and T13 were different from each other and from the reference tree. The correct description of PA 150 [PER] pods is rough, cylindrical/oblong with an acute apex and slight or no basal constriction.

(e) JA 5/41 [POU] vs. JA 5/47 [POU]

In Field 5B, plots F454 and D300 are both labelled JA 5/47 [POU]. However, individual trees in plot F454 are labelled JA 5/41. Furthermore, J-M. Thévenin had detected a difference in WB susceptibility between these two plots (personal communication). Two trees of each plot were compared over four SSR primers with reference DNA from trees at Marper Farm (JA 5/41 [POU] from C1118 and JA 5/47 [POU] from C1094). Two groups were found: F454 T1 & T2 were similar to JA 5/41 [POU] and D300 T5 & T8 were distinct from the former group but similar to JA 5/47 [POU] from Marper.

(f) Venezuelan material vs. Trinidadian material

Three accessions ICS 1, ICS 95 and IMC 67 collected by researchers in Venezuela were compared to corresponding material in Trinidad being used in the CFC/ICCO/INIAP Flavour Project.

ICS 1 from Venezuela was compared to two trees (Campus 1B x5y1 and Field 6B B122 T11) from Trinidad. The latter had been previously shown to be similar to ICS 1 from SJE with 14 SSR and one RAPD primer. Thirty-one SSR primers were utilised in the comparison between Venezuelan and Trinidadian material. Only one primer (mTcCIR44) exhibited a different profile for the Venezuelan material.

ICS 95 from Venezuela was compared to an old labelled tree (Campus 9B x8y4). This tree had been shown to be similar to that of ICS 95 in SJE with 15 SSR primers. The Venezuelan material was compared to the Trinidadian material with 14 SSR primers. Three of these exhibited different profiles, with the Trinidadian material being heterozygous and the Venezuelan material being homozygous. The Venezuelan material was heterozygous at six loci that were common to the Trinidadian material.

For IMC 67, one tree at UCRS (Field 6B A23 T1) was used for the comparison. This tree was identical over 16 SSR primers to the IMC 67 at La Reunion Estate. The Venezuelan material was compared to the Trinidadian material with 23 SSR primers and identical banding patterns were obtained.

Discussion

Several key issues have arisen during the course of the verification programme. Perhaps one of the more fundamental but commonly neglected points is that researchers should always note the exact tree(s) from which data/plant material is collected. The trees in the ICG,T are individually identified by the field, section, plot and tree number, and trees on Campus all have designated Cartesian coordinates. At SJE and Marper, each tree can be identified from its map position as well as its individual label. This nomenclature should be carried forward, either directly or coded so that at some future date, data points can be attributed to a specific tree. This may prove useful in detecting off-types from morphological or pathological work. It may also highlight above average performers that could be useful for further experimentation.

The results presented here are composed of SSR results and included only those that are quite definitive. Hence, any differences between alleles which are not readily apparent or which have been problematic in scoring have been ignored. Thus, if there is uncertainty in deciding whether two plants have the same SSR profile for a given primer, then they are deemed equivalent rather than calling a difference. This protocol would also take into account errors introduced by Taq polymerase during amplification (see reviews by Robinson and Harris (1999) and Holton (2001)).

It should be noted that several differences previously detected by RAPD were not distinguished by the SSR primers utilised. Examples of these include WAA, ICS 1, JA 6/4 [POU] and SCA 6. These have already been discussed in a previous report (Motilal, 2002). Thus, the number of SSR primers utilised for verification may need to be reassessed or a method with greater resolving power than agarose electrophoresis may be required. Further work is needed in these areas.

Results for ICS 65 and SCA 11 highlight the imprecision of some maps and even of the labelling of certain trees. In the case of ICS 65 an older field map was available; however the newer map

proved to be correct. Thus, complete reliance should not be placed on older maps. Many events (e.g. the propagation and planting of the wrong tree followed by its subsequent detection) could have occurred which resulted in a map being updated. Keeping complete records, in this case an explanation of why the change from ICS 65 to NL was made, is thus critical to future researchers. In the case of SCA 11, good records (i.e. no conflicts encountered) are available for the two Campus trees present at the time of collection. However, while they were similar to each other, they were different from the tree at Marper Farm. Furthermore, previous work with RAPD had separated SCA 11 material in Field 6B into at least two groups (V1 and V2). The complete results of SCA 11 should prove whether material might have originated from both Campus and Marper material.

The results from the DOS HERMANOS group highlight the importance of obtaining information from scientists involved in the collection of germplasm and serve as a guide to proper collection procedures. One method to overcome this is to ensure that project developers make sufficient allowance for rechecking of collections, prior to their use. Also, other workers may encounter mislabelled plants and their input should also be sourced.

The results of PA 150 [PER] and JA 5/41 [POU] vs. JA 5/47 [POU] illustrate the importance of networking and the applicability of SSR analysis to reliably resolve problems that are common with widely distributed germplasm. The latter results also illustrate the value of internal networking within an institution, i.e. data collected by one section may be of use to another section. In this case, even if the tree labels had been absent, different WB reactions observed in different plots containing supposedly similar plant material was a good indicator that there may be a mislabelling problem. It is hoped that other sections would be as helpful. While the issue of data sensitivity is appreciated, alerting the verification section to indicators of possible anomalies at an early stage would be of value and could provide pointers to prioritise work of the section.

The results of the Venezuelan comparison strongly suggested that the Venezuelan researchers had a true to type IMC 67 but their ICS 95 was an off-type. ICS 1 in Venezuela appears to be very closely related to its Trinidadian counterpart as it only differed for one out of 31 SSR primers. SSR markers are known to have a high mutagenic rate which are manifested as changes in the number of SSR repeats resulting in detectable polymorphism (Li *et al*., 2002). Thus, the Venezuelan material may be a mutant due to variation at this microsatellite sequence. However the extra band is likely to be an artefact due to stutter from the PCR amplification. If this were so, then by current convention, the ICS 1 tree in Venezuela would be equivalent to the one in Trinidad. Nevertheless, the consistent amplification of this band, present only in the Venezuelan material is interesting since it may point to accessory band being of discriminating value. The stability of this product should be re-checked by extracting DNA from a different leaf from the same tree and repeating the PCR amplification. The validity of scoring consistently reproducible non-SSR products arising from the SSR-PCR may be an area worth examining by the cacao molecular biology community.

References

Butcher, P.A., Glaubitz, J.C., and Moran, G.F. (online) *Applications for microsatellite markers in the domestication and conservation of forest trees.* Available at <www.fao.org/forestry/FOR/FORM/FOGENRES/genresbu/web27-en/micr-e.stm>, retrieved 27 March 2004.

Grivet, L. and Noyer, J-L. (2003) Biochemical and molecular markers. Pages 1-29 in: *Genetic diversity of cultivated tropical plants*, (P. Hamon, M. Seguin, X. Perrier and C. Glaszmann Eds): USA and France: Science Publishers, Inc./CIRAD.

Holton, T.A. (2001) Plant genotyping by analysis of microsatellites. Pages 15-27 in: *Plant Genotyping: the DNA Fingerprinting of Plants*, (R.J. Henry Ed.). [Electronic version]. New York, USA: CAB International. Available online at <http://www.agbiotechnet.com-pdfs>.

Krutovskii, K.V. and Neale, D.B. (2001) *Forest genomics for conserving adaptive genetic diversity*. Forest Genetic Resources Working Papers, Working Paper FGR/3 (E), Forestry Department. Rome, Italy: FAO. Available online at <http://www.fao.org/DOCREP/003/X6884E/x6884e02.htm>. Retrieved 17 May 2004.

Li, Y-C., Korol, A.B., Fahima, T., Beiles, A. and Nevo, E. (2002) Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Molecular Ecology* **11**: 2453-2465.

Motilal, L.A. (2002) Tree identification within the ICG,T. Pages 12-17 in: *Annual Report 2001*. Trinidad and Tobago: Cocoa Research Unit, The University of the West Indies.

Motilal, L.A. (2003) True to type trees in the ICG,T. Pages 12-23 in: *Annual Report 2002*. Trinidad and Tobago: Cocoa Research Unit, The University of the West Indies.

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

Robinson, J.P. and Harris, S.A. (1999) Amplified fragment length polymorphisms and microsatellites: A phylogenetic perspective. Pages 211-224 in: *Which DNA marker for which purpose? Final compendium of the research project Development, Optimisation and Validation of Molecular Tools for Assessment of Biodiversity in Forest Trees*. Europen Union DGXII Biotechnology FW IV Research Programme Molecular Tools for Biodiversity, (E.M. Gillet Ed.), Institut für Forstgenetik und Forstpflanzenzüchtung, Universität Göttingen. Available online at <http://webdoc.sub.gwdg.de/ebook/y/1999/whichmarker/index.htm>. Retrieved 31 March 2004.

Rossetto, M. (2001) Sourcing of SSR markers from related plant species. Pages 211-224 in: *Plant Genotyping: the DNA Fingerprinting of Plants*, (R.J. Henry, Ed.). [Electronic version]. New York, USA: CAB International. Available online at <http://www.agbiotechnet.com-pdfs>.

Swanson, J-D., Lee, A. C. and Guiltinan, M. J. (2003). USDA Cacao DNA fingerprinting ring test: Results from Penn State University. *INGENIC Newsletter* No. 8: 22-24.

Resolving identity issues of cocoa clones using SSR markers

M. Boccara, L.A. Motilal and A. Sankar

Introduction

The ICG,T is an internationally recognised field genebank for cacao and represents an important resource for the world cocoa community. It contains accessions from the early expeditions collected in the 1930s as well as more recent introductions and many local selections. The need for correct identification of every tree in a genebank is imperative (this is highlighted when, for example, information which is gathered from different sources shows discrepancies). Molecular characterisation of cocoa germplasm with the use of SSR markers is a dependable way to confirm identity and to correct identification errors.

Verification has always been an ongoing task in CRU as it is the base of origin for many cultivars grown worldwide.

Material and methods

For the majority of accessions in UCRS, the original tree representing an accession is either in Marper Farm, the Cheesman Field in the San Juan Estate or in the UWI Campus. The creation of the ICG,T at La Reunion Estate was done by planting rooted cuttings or grafted trees into plots containing 4 to 16 trees. Unfortunately, in some cases the identity of the original tree was lost, mislabelling occurred and/or errors were made during the planting process or the drawing of maps.

The USDA/CRU fingerprinting project gave us the opportunity to sample DNA from the most original tree of each accession held in Trinidad. These samples are used as a standard reference for duplicates of each clone.

A modified Kobayashi extraction method (Kobayashi *et al.*, 1998) was used as a standard routine to obtain the DNA. The template quantification was achieved in a Turner Biosystems mini-fluorometer with the Hoechst 33258 dye (Anon 2004).

The accessions were checked by running electrophoresis on 3% agarose gels, stained with ethidium-bromide and photographed under UV light. Eight SSR primers were used to complete the comparisons between samples.

Results and discussion

IMC 47

IMC 47 is a clone present in 20 research centres across the world, but some verification work has shown that clones differed in multiple profiles.

In Trinidad, IMC 47 trees are present in different locations:

- One tree in Marper Farm, position D 242
- One tree in UWI Campus 11, coordinates x5y12
- Eleven trees in Field 6B, plot F401 at UCRS

- Two trees in Field 6B (T10 and T14) had been verified and considered as true to type by CIRAD (Risterucci, 2001).

Our verification has included the tree from Marper Farm, the tree from UWI Campus and several trees from Field 6B at UCRS.

Results

Although the tree in Marper Farm is different from the one on Campus, trees 10, 13 and 14 in Field 6B have a similar profile to the Campus tree; trees 1, 3, 4, 11, 12 share the same profile, but are different to both the Marper and the Campus tree profiles. Preliminary results from Reading University indicate that RUQ 849 which is the IMC 47 held in the Reading quarantine facility has an identical profile to the Campus tree.

ICS 83 and ICS 95

These clones are included in the CFC/ICCO/IPGRI Project Collection. DNA samples from trees in the Cheesman Field (ICS 95 Block 2 and ICS 83 Block 5) were sent to USDA Beltsville for the fingerprinting project and have been analysed.

Results

The preliminary results provided by USDA have shown that ICS 83 and ICS 95 could be duplicates, as they are sharing the same profile. DNA analysis from a tree of the ICS 83 plot in UCRS has a different profile to both the San Juan Estate trees. This shows an example of mislabelling and/or planting error, as ICS 83 and ICS 95 are neighbours in block 5.
The pods of ICS 83 in UCRS are partially pigmented whereas the pods of ICS 95 are very dark red, matching Pound's descriptions (Pound, 1936). It follows that budwood for the propagation of ICS 83 should be taken from the UCRS plot.

ICS 45 and ICS 46

The only ICS 45 tree remaining in Cheesman Field, although standing at the correct position according to the map, bears an old label, which reads ICS 46. There is a reference ICS 46 tree in Block 2.

Results

The experiment has shown by comparison of profiles that this tree is not identical to the ICS 46 tree present in Block 2, and is likely to have been mislabelled.

MOQ 1/12 and CRU 10

Two trees in Marper Farm Block C (positions C205 and C259) are labelled as MOQ 1/12. According to hand written records from 1943, the tree in position C260 is also a MOQ 1/12; however, the most recent map and listing refer to this tree as CRU 10.

Results
SSR analysis has shown that the trees in C259 and C260 share the same profile; on the other hand, the C205 tree shows a different profile. According to this evidence, the C260 tree should be re-assigned the name MOQ 1/12, whereas the tree in C205 should be re-named.

MOQ 2/18 and MARPER 7

The tree originally planted in Marper C784 was MOQ 2/18, but the tree in this position was later named MARPER 7, since the original identity had been lost. There are two trees in UCRS Field 5B, Plot C171, labelled as MOQ 2/18, and one in Campus 3, coordinates x10y10.

Results
Preliminary results have shown that tree 10, from 5B, MOQ 2/18 from Campus, and MARPER 7, all show different profiles.

AM 2/88 [POU] and MARPER 10

According to the 1943 records, the clone AM 2/88 [POU] was established in two locations in Marper, C895 and D404; since then the tree in C895 has been renamed MARPER 10.

Results
Differences were found in SSR profiles (differ in 5 out of 8): the distinction between these two trees has been confirmed.

CL 9/11 and CL 91/1

The tree planted in Marper Farm, position C520, was recorded as CL 9.11, while the one in position C678 has been listed as CL 911. The trees propagated in UCRS 4A, named CL 9/11, could have originated from one location or the other.

Results
Although the tree in C678 is now missing, the fact that the profile of the trees in 4A match that from MARPER C520 for 8/8 SSR primers, suggests that the source tree was Marper C520.

SLA 16, SCA 16 and SLA 23

The tree planted in position D671 in Marper Farm, listed as SCA 16 in the 1943 records, bears a SLA 16 label (probably due to confusing handwriting in the early records). Plot D242 in Field 5B is labelled SLA 16, although an old map of 5B shows this plot as SLA 23.

Results
SSR analysis showed that DNA from the original tree in Marper D671 matches the tree sampled in Plot D242 Field 5B. The DNA of the SLA 23 reference tree from Marper is dissimilar, thus confirming that the tree in 5B is probably SCA 16.

CL 9/17 and CL 19/17

The 1943 records from Marper Farm list two trees in position C56, one is CL 9.17 and the other is CL 19.17. Although the updated map names the tree in this position as CL 9/17, the trunk bears a CL 19/17 label.

DNA samples from the Marper C56 tree, from a CL 9/17 tree in Field 5B plot A24, and from a CL 19/17 tree in Field 5B plot I731 were checked.

Results

SSR profiles from trees in the two plots in Field 5B (A24 and I731) are the same but both differ from the Marper C56 tree. It is possible that the trees in 5B were propagated from the adjacent CL 19/17 tree, now missing.

Conclusion

Analyses of SSR profiles have allowed us to resolve issues of identity ambiguity for a selection of trees and clones in the ICG,T fields. Improper naming or labelling can be corrected and updated.

This new information is of particular value for accessions that are already widely distributed, or for those which have been selected for current research activities, and may be distributed in the future.

References

Anon (2004) The TBS-380 Mini-Fluorometer Method for DNA Quantification Using Hoechst 33258 Dye. <*http://www.turnerbiosystems.com/doc/appnotes*>. Retrieved 12 May 2004.

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

Risterucci, A-M., Eskes, A.B., Fargeas, D., Motamayor, J.C., and C. Lanaud (2001) Use of Microsatellite Markers for Germplasm Identity Analysis in Cocoa. Pages 25-33 in: *Proceedings of the International Workshop on New Technologies and Cocoa Breeding*. 16-17 October 2000, Kota Kinabalu, Sabah, Malaysia: INGENIC.

Pound, F.J. (1936) Cocoa Research: The completion of selection, 1935. Pages 7-16 in: *Fifth Annual Report on Cocoa Research,1935*. Trinidad: ICTA.

Characterisation

A comparison of the phenotypic diversity of two samples from the International Cocoa Genebank, Trinidad

F. L. Bekele, G. Bidaisee and N. Persad

Introduction

The existence of diversity in a germplasm collection is a prerequisite for genetic improvement (Bekele and Bekele, 1996), and facilitates the identification of potentially heterotic groups. A comparison of the phenotypic diversity of the provisional CFC/ICCO/IPGRI Project Collection with that of a larger sample of characterised accessions from the ICG,T is presented in this report along with the results of an examination of the inter-relationships among the clones from the Project Collection. The objective of this Collection was to select approximately 100 "superior" clones, representing broad genetic diversity, for distribution to the 10 participating cocoa-producing countries of the CFC/ICCO/IPGRI Project for use in national breeding programmes. The selection criteria were resistance to Black Pod, Witches' Broom, Ceratocystis and Moniliasis diseases, and favourable pod index, mean bean weight and butterfat content.

Some of the characteristics of the germplasm contained in the ICG,T in terms of diversity, and some morphological, agronomic and disease resistance traits have been presented by Bekele *et al*., 1994; Bekele and Bekele, 1996 and Iwaro *et al*., 2003. Genetic diversity was assessed by Sounigo and Ramdahin (2000) and Sounigo *et al.* (2001).

Materials and methods

The accessions were characterised in terms of 24 morphological traits listed in Table 1, which are from the IBPGR descriptor list for cacao (Anon., 1981; Bekele *et al*., 1994; Bekele and Butler, 2000). In December 2003, when data for this analysis were collated, 82 clones, representing 26 accession groups (Iwaro *et al*., 2003), from the 115 clones of the provisional CFC Project Collection had been fully characterised[1]. At the same time, 966 accessions from the ICG,T, representing 70 accession groups, had been fully characterised. The phenotypic diversity and inter-relationships among clones in the Project Collection were assessed using Cluster Analysis (CA) – UPGMA[2] method (Mardia *et al*., 1979), and Principal Component Analysis (PCA) (NTSYSpc 2.1 and MINITAB ver. 14), and that of the larger sample using PCA (MINITAB). Descriptive statistics, variances for quantitative traits and the Shannon Weaver Diversity Index (SWDI) for qualitative (ordinal) traits were generated using Microsoft EXCEL and MINITAB.

[1] The Project Collection (115 clones) was finalised in March 2004 after this analysis had been conducted, but changes after 2003 were minor.

[2] Unweighted pair-group method using arithmetic averages.

Table 1. Descriptors for morphological characterisation - their states and sample sizes (n).

Descriptor	State
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, 5= other [n=10]
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, length (cm) [n=10]	
Fruit, width (cm) [n=10]	
Seed, number [n=10]	
Seed, shape	1=oblong 2=elliptic 3=ovate [n=40]
Seed, cotyledon colour	1=white, 2=grey, 3=light purple, 4=medium purple, 5=dark purple, 6=mottled [n=40]
Cotyledon length (cm) [n=20]	
Cotyledon width (cm) [n=20]	
Cotyledon weight (g) [n=20]	
Seed weight (including testa) [n=20]	
Pod index (the number of pods required to produce 1 kg of dried cocoa) [n=10]	

Results

Cluster Analysis (NTSYSpc) of 82 accessions from the Project Collection

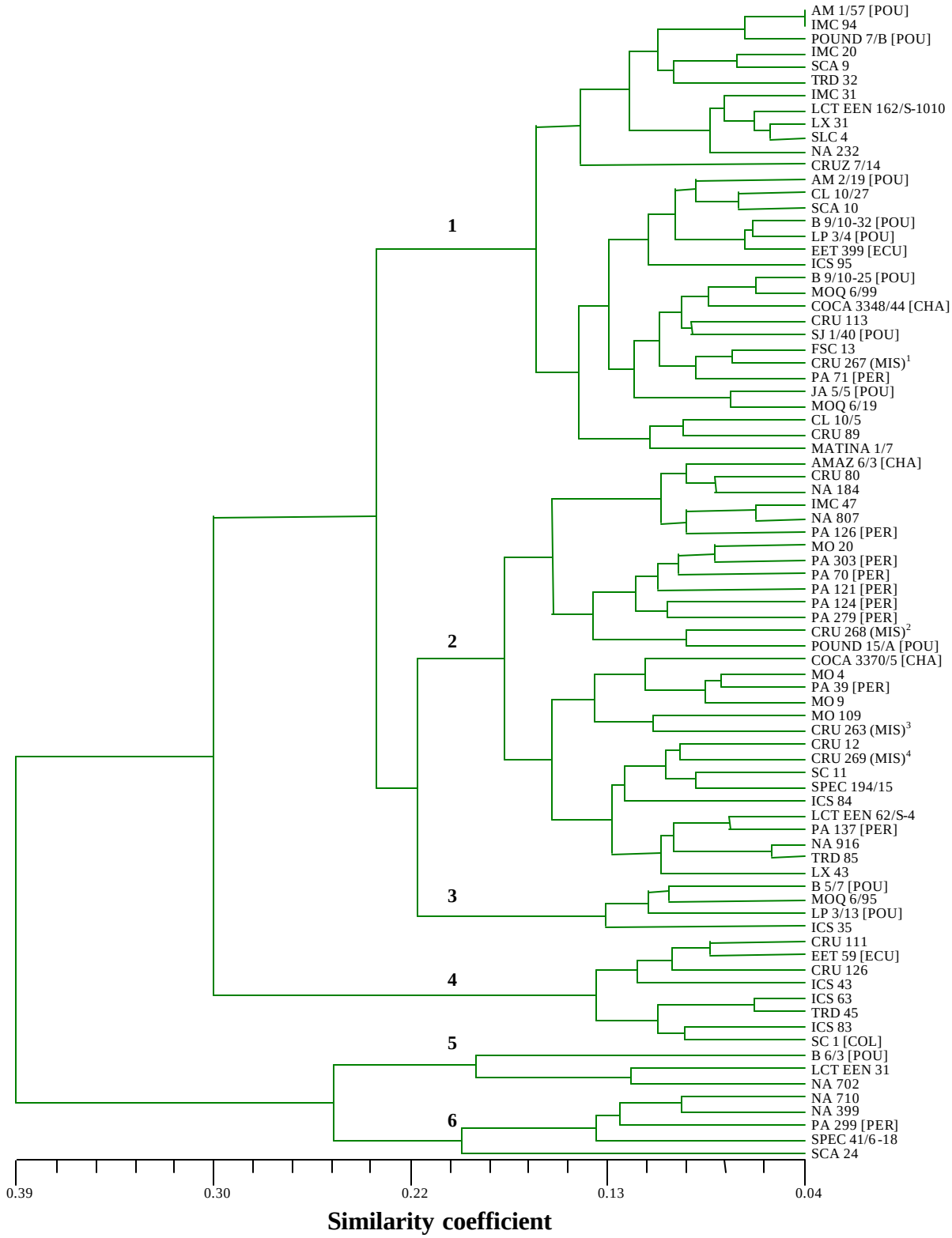
Two accessions (AM 1/57 [POU] and IMC 94) were phenotypically identical at the 96 % level of similarity (similarity coefficient of 0.04) (Figure 1).

At the 93.5% level of similarity (similarity coefficient of 0.065), several clusters comprised mainly of pairs of accessions were formed (Figure 1) including the following:

LX 31 & SLC 4 (Refractarios) & LCT EEN 162/S-1010 (Forastero);
B 9/10-32 [POU] & LP 3/4 [POU] (Refractarios);
B 9/10-25 [POU] & MOQ 6/99 (Refractarios);
IMC 47 & NA 807 (Forasteros);
NA 916 & TRD 85 (Forastero & Trinitario);
ICS 63 & TRD 45 (Trinitarios).

At this level, many of the accessions were ungrouped.

Figure 1. Dendrogram showing the inter-relationships of 82 clones from the CFC Project Collection.



[1]CRU 267 (MIS_TTOICGT_ICS 40); [2]CRU 268 (MIS_TTOICGT_PA 150 [POU]); [3]CRU 263 (MIS_TTOICGT_POUND 12/B [POU]); [4]CRU 269 (MIS_TTOICGT_ICS 25)

Six clusters, numbered in Figure 1, were formed at the 80% level of similarity (similarity coefficient of 0.20). Cluster 1 was comprised of 32 clones and was a heterogeneous assemblage based on geographic origin and genetic grouping. Cluster 2 consisted of 30 clones; mainly Forasteros, and 3 Trinitarios; CRU 269 (MIS), ICS 84 and TRD 85. Cluster 3 contained 4 clones; 3 Refractarios and 1 Trinitario. Cluster 4 included 8 clones; Trinitarios (ICS, TRD), EET accessions, and CRU 111 and 126. In Cluster 5, there were 3 clones: B 6/3 [POU], LCT EEN 31 and NA 702 while Cluster 6 contained 5 clones, all Forasteros, including SCA 24. Three accessions, *viz*., CRUZ 7/14, B 6/3 [POU] and SCA 24 remained ungrouped at the 86.5% level of similarity. SCA 24 remained ungrouped up to the 81% level of similarity (similarity coefficient of 0.19). All of the Collection accessions formed one group at the 61% level of similarity (similarity coefficient of 0.39).

The PA accession group has been identified as a good source of genes for BP resistance (Iwaro *et al*., 2003), and some of the PA clones included in the Project Collection also have acceptable Pod Index values (less than 30). These are PA 39 [PER] (26.4), PA 70 [PER] (29.7), PA 71 [PER] (19.5), PA 124 [PER] (29.8), and PA 137 [PER] (28.1). At the 88% level of similarity (similarity coefficient of 0.12), the following PA accessions were grouped together (Figure 1): PA 70 [PER], PA 279 [PER] and PA 303 [PER] (Type 1); PA 124 [PER] (Type 3); and PA 121 [PER] (Type 4). The classification according to 'type' is based on Pound's observations, which are reported in the International Cocoa Germplasm Database (Wadsworth and Harwood, 2000). Five other PA clones included in the Project Collection (PA 39, 71, 126, 137 and 299 [PER]) fall outside this cluster.

Principal Component Analysis (PCA) of the Project Collection clones

The contribution of the first four Principal Components (PC) to the total phenotypic variation expressed in the Project Collection was as follows:

PC 1 accounted for 19.6 %; PC 1 & 2 accounted for 30.6 %; PC 1, 2 & 3 accounted for 40.3 % and PC 1, 2, 3 & 4 accounted for 48.2 % of the variation.

The descriptors that accounted for most of the variation were as follows:

PC1 – Total wet bean weight, bean length, pod index, pod width, cotyledon weight

PC 2 – Ovule number, bean number, bean shape, cotyledon colour, pod surface texture

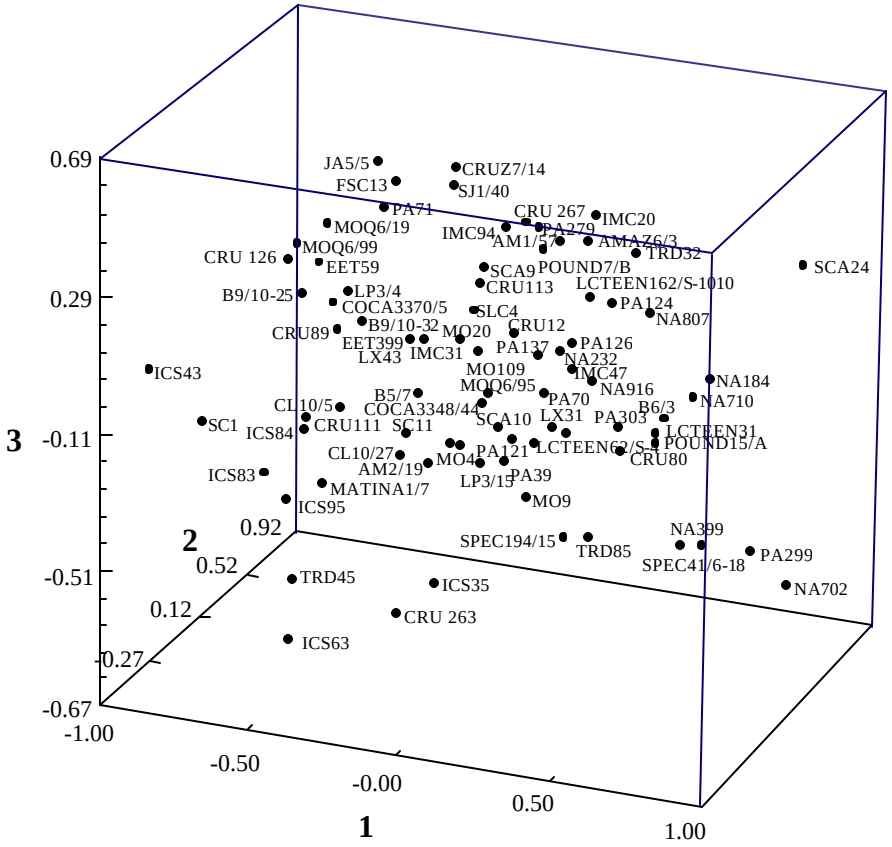
PC 3- Style length, pod basal constriction, pod length, mature pod colour, cotyledon width, sepal width.

Main findings of PCA

Some of the patterns of association among clones revealed by PCA (NTSYSpc) (Figure 2) were similar to those evident in the results of Cluster Analyses (Figure1).

The accessions were dispersed with relatively little clumping. SCA 24, which has a small cotyledon weight of 0.65g, was very distinct, almost an "outlier". CRUZ 7/14 and NA 702 were also distinct. ICS 43, 83, 95, 84, 63, 35, SC 1, MATINA 1/7, and TRD 45 grouped together in the same region of the 3-dimensional PCA plot (with CRU 263 (MIS)), but were fairly widely dispersed by PC 3. The Forasteros and Refractarios were interspersed with one another and distinct from the aforementioned Trinitarios. AM 1/57 [POU] and IMC 94

Figure 2. Three-dimensional PCA Plot of the Project Collection.



were in close proximity as in Cluster 1 of the CA. They were also close to POUND 7/B [POU], IMC 20, SCA 9 and TRD 32 as in Cluster 1. PA 303, 121, 124, and 70 [PER] were also close to each other as in Cluster 2. Accessions, which were closely associated in the PCA plot, included:

AM 1/57 [POU] & IMC 94*	AM 1/57 [POU] & POUND 7/B [POU]*
CL 10/27 & SC 11	COCA 3348/44 [CHA] & MO 4*
CRU 89 & B 9/10-32 [POU]	CRU 80 & POUND 15/A [POU]
CRUZ 7/14 & SJ 1/40 [POU]*	CRU 111 & ICS 84
EET 59 [ECU] & CRU 126*	EET 339 [ECU] & LX 43*
ICS 95 & ICS 83*	SCA 9 & CRU 113
NA 232 & PA 126 [PER]	NA 232 & IMC 47*
NA 399 & SPEC 41-6/18*	NA 710 & NA 184
LCTEEN 62/S-4 & LX 31*	MO 109 & MOQ 6/95
SCA 10 & PA 121 [PER]*	SPEC 194/15 & TRD 85

*Similar relationship revealed by Cluster Analysis (Figure 1).

Principal Component plots (MINITAB) for the Project Collection and larger sample

Figure 3. Principal Component plot of 82 clones from the CFC Project Collection.

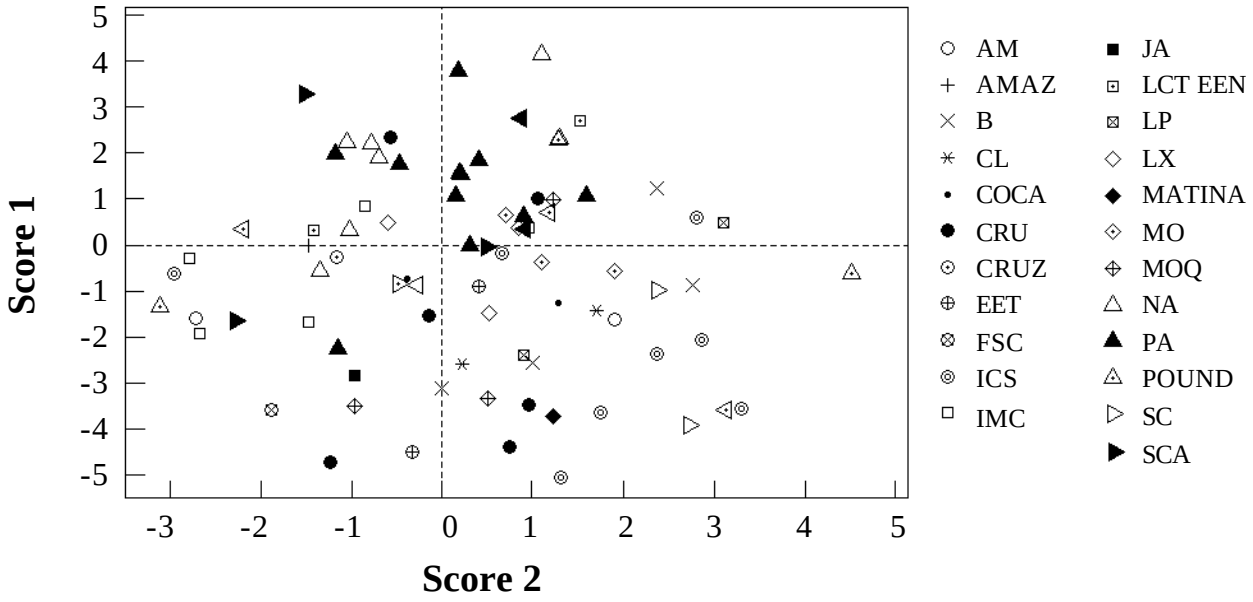
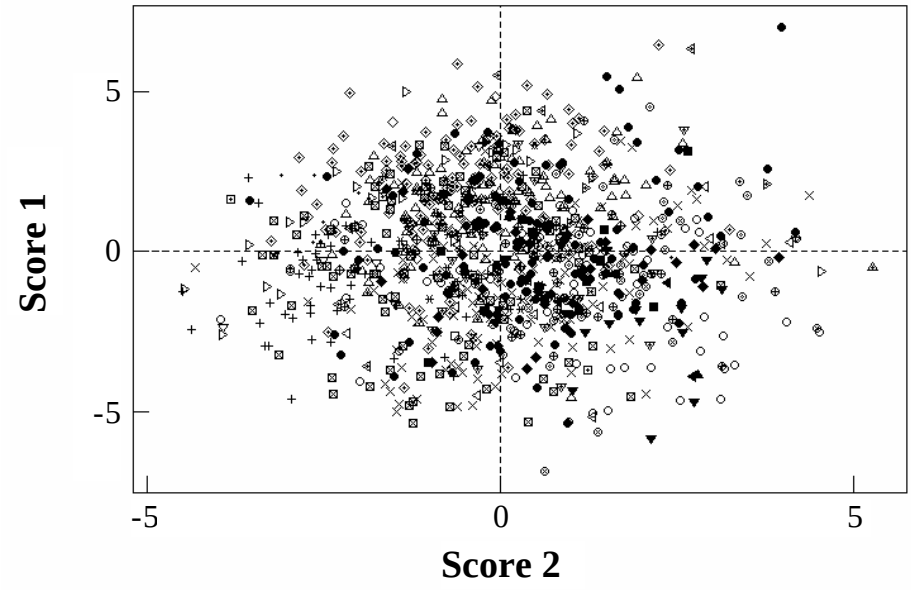


Figure 4. Principal Component plot of 966 clones from the ICG,T.



Variances and Shannon Weaver Diversity Index values calculated for the two samples

The variances calculated for the quantitative data of the two samples of germplasm under consideration are presented in Table 2. These were similar for most of the variables, however there was a noticeable difference with cotyledon weight. The increases in mean cotyledon weight, length and width and wet bean weight, and the decrease in pod index values in the Project Collection (Table 2) attest to the success of the selection process.

Table 2. Variances and Shannon Weaver Diversity Index (SWDI) values associated with each descriptor assessed for the two samples of germplasm studied.

	Project Collection		Larger sample from the ICG,T	
Descriptor	**Mean**	**Variances**	**Mean**	**Variances**
Sepal length (cm)	7.55	0.89	7.59	0.76
Ligule width (mm)	2.43	0.10	2.44	0.09
Ovule number	43.4	33.0	43.7	30.0
Style length (mm)	2.26	0.33	2.25	0.11
Bean number	38.1	41.0	39.5	33.9
Cotyledon weight (g)	1.04	0.09	0.97	0.76
Cotyledon length (cm)	2.22	0.035	2.16	0.039
Cotyledon width (cm)	1.25	0.015	1.21	0.015
Pod length (cm)	16.3	3.10	15.9	3.38
Pod width (cm)	8.2	0.48	8.1	0.56
Wet bean weight (total) (g)	58.2	205.7	56.5	175.3
Pod index	26.7	41.4	27.8	50.49
		SWDI		**SWDI**
Ligule colour		1.11		1.15
Filament colour		1.28		1.31
Pedicel colour		0.75		0.82
Mature pod colour		0.78		0.67
Pod shape		0.86		0.95
Husk hardness		0.58		0.84
Pod basal constriction		1.15		1.19
Pod apex form		1.46		1.55
Pod surface texture		0.99		1.00
Pod furrow disposition		0.29		0.15
Pod furrow separation		0.77		0.74
Cotyledon colour		0.78		0.85
Cotyledon shape		0.95		0.96

As expected, the values for the SWDI were larger for the large germplasm sample for the majority of qualitative traits studied (10 of 13) (Table 2). However it is noteworthy that these differences seem very small, except in the case of pod wall hardness, and suggest that phenotypic diversity, in terms of these traits, has been maintained in the Project Collection. However, all of the Project Collection accessions have moderately hard or hard pod walls, which are thought to avert cocoa pod borer attack (Azhar, 1988). These results indicate that selection for a particular condition such as hard pod wall or large bean weight results in loss

of diversity for the specific trait, but the diversity of unselected traits is maintained.

Conclusion

The results of CA and PCA (Figures 1 and 2) were complementary in displaying the diversity and inter-relationships in the Project Collection. The spread of diversity displayed in the PCA plots (Figures 3 and 4) for the two samples of germplasm studied is similar. Furthermore, the spread of phenotypic diversity in the Project Collection is similar to that of genetic diversity revealed by molecular markers (Sounigo *et al*., in press). In addition, a comparison of the variances and SWDI values for the Project Collection and the larger sample (Table 2) revealed that the general level of diversity in the former compares favourably with that of the latter. These findings have positive implications for breeding, and confirm the objective to maintain diversity in selecting this working collection.

The results also show that it is possible to detect mislabelled accessions through studies such as this. In Figure 2, CRU 263 (MIS) grouped correctly with the Trinitarios since it was a red-pod off-type. This clone was included in the analyses since it had some traits of interest, but was subsequently withdrawn from the Project Collection.

Future Direction

It would be useful to examine the bases for the observed phenotypic similarities between certain clones. Are they due to potential redundancy or duplication? It would also be important to compare the observed phenotypic diversity patterns with those of the genetic diversity, and determine whether the phenotypic similarities correspond to genetic affinities. In the case of CRU accessions (unknown identity), molecular techniques will ascertain whether we have indeed revealed their identities or affiliations or identified putative groups to which they belong. For example, is:

CRU 89 = B 9/10-32 [POU]; CRU 80 = POUND 15/B [POU];
CRU 111 = ICS 84; CRU 126 = EET 59; or CRU 113 = SCA 9?

These analyses will be repeated in the future using data for the finalised Project Collection.

Acknowledgements

Financial support from the Government of Trinidad and Tobago and BCCCA is gratefully acknowledged along with useful discussions with Dr Isaac Bekele and Olivier Sounigo. We thank Eusebius Solozano and Timal Jugmohan for assistance in collecting samples over the past two years.

References

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

Azhar, I. (1988) Host plant resistance to cocoa pod borer – a research in progress. Paper presented at MARDI Senior Staff conference, Kuala Lumpur, Malaysia.

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.L. 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.

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.

Mardia, K.V., Kent, J.T. and Bibby, J.M. (1979) Pages 213-228 in: *Multivariate Analysis*. London: Academic Press.

Sounigo, O. and Ramdahin, S. (2000) Assessing genetic diversity in the ICG,T using RAPD. Pages 28-32 in: *Annual Report 1999*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Sounigo, O., Christopher, Y., Ramdahin, S., Umaharan, R., and Sankar, A. (2001) Evaluation and use of the genetic diversity present in the International Cocoa Genebank (ICG,T) in Trinidad. Pages 65-71 in: *Proceedings of the International Workshop on New Technologies and Cocoa Breeding*. 16-17 October 2000, Kota Kinabalu, Malaysia. London: INGENIC.

Sounigo, O., Bekele, F.L., Iwaro, A.D., Thévenin, J-M., Sukha, D.A., Singh, V. Umaharan, R., Sankar, A., Boccara, M. Butler, D.R. and Eskes, A.B. Description of the CFC/ICCO/IPGRI project collection. Paper presented at the *14th International Cocoa Research Conference*. 13-18 October 2003, Accra, Ghana (proceedings in press).

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

Evaluation

Evaluation of cacao germplasm for resistance to Witches' Broom disease under controlled conditions

R. Umaharan, J-M. Thévenin, A. Holder and J. Bhola

Introduction

A five-year project with the aim of screening the cacao accessions held in the ICG,T for their resistance to Witches' Broom disease was initiated in July 1998 and was financed by the American Cocoa Research Institute (ACRI), now the World Cocoa Foundation (WCF).
The goal of the project to screen 400 accessions per year was perhaps over-ambitious, but it kept the team motivated in spite of numerous set backs and challenges during the project. Details of steps taken to overcome problems with the methodology have been described in previous reports (Umaharan *et al*. (2000), Umaharan *et al*. in press), and here, it suffices to list the various challenges:

- The need to raise and maintain a large number of rootstocks in order to propagate accessions selected for screening, taking into consideration the frequent high percentage of losses during the grafting exercise.
- The necessity of adapting the inoculation method to match the variable size and weight of grafted plants.
- The need to adjust and fine tune the inoculation method of Purdy *et al.* (1997) in terms of concentration and quantity of basidiospores to be delivered per plant to allow for the conditions in Trinidad to obtain appropriate measurements to indicate the level of resistance to WB.
- The need for the accessions to produce synchronized flushes prior to inoculation.
- The necessity to create incubation conditions close to ideal (temperature of 25-27°C and relative humidity close to saturation) for a good infectious process where the dry season can last up to four months and during which outside temperatures can reach 38°C.
- The need to develop greenhouse conditions favourable to the development of symptoms and to identify the most appropriate variables to measure.

Overall results

In spite of the limitations listed above, five years after the beginning of this project, the total number of accessions grafted reached 1,065. To achieve this, over 22,000 rootstocks were established and utilised.

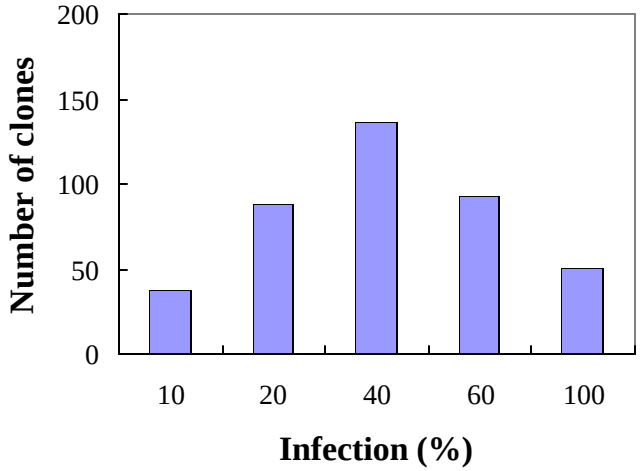
Over 700 accessions were inoculated and screened for WB resistance in green house experiments using spray inoculation techniques (Umaharan *et al.*, in press). The analysis of results was based on the percentage of the total number of inoculated shoots with symptoms and on the broom-base diameter (analysis of variance using the general linear model, MINITAB ver. 13.1 software).

For the purposes of this report, results from 553 accessions were selected and assessed for percentage infection and broom-base diameter, since they fulfilled the criteria of having at least 3 replications with four shoots per replicate. These accessions belong to 53 accession groups and represent a good cross-section of the accessions held in the ICG,T.

Percentage infection

Overall, a normal distribution (Figure 1) of resistance to WB was obtained (r = 0.99, *P* <0.01, Ryan-Joiner test for normality). Approximately 32% of the clones assessed showed less than 20% symptoms after being inoculated with *Crinipellis perniciosa*.

Figure 1. Distribution of resistance to Witches' Broom disease for 533 clones from the ICG,T, based on percentage infection.

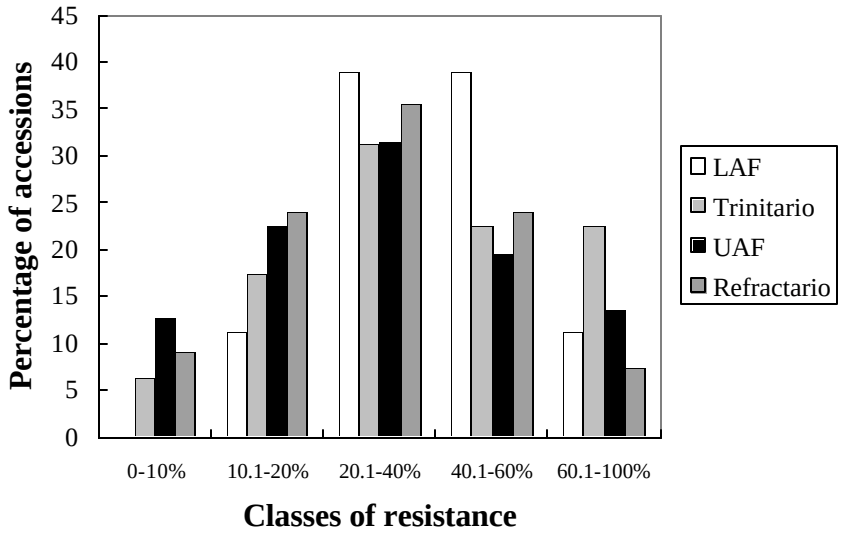


The results were further analysed according to the main genetic groups, the Forastero (subdivided into Upper and Lower Amazon types), Trinitario and Refractario types (Figure 2). When the Forastero group was evaluated, the Upper Amazon Forastero (UAF) had approximately 35% of the accessions showing less than 20% symptoms. The Refractario group also showed a similar high percentage of accessions (33%) showing less than 20% infection. In contrast, the Lower Amazon Forastero (LAF) and Trinitario populations had 11% and 24%, respectively.

This indicates that a large percentage of resistant clones screened from the ICG,T belong to the UAF group, which concurs with Pound's observation (Pound, 1938), that resistant trees appeared frequently in the Upper Amazon area around and to the west of the Iquitos. Furthermore, the accession groups showing the largest percentage of clones (58.8%) with less than 20% symptoms was IMC (Iquitos Mixed Calabacillo), which is a wild UAF type from Peru, originally selected for lack of symptoms to WB. In addition, while the UAF accessions were predominantly collected from Peru, the Trinitario types are more diverse in their origin and were not collected/selected solely for their reaction to WB but for other traits such as yield and/or resistance to BP, as well as for the uniqueness or diversity of their germplasm.

Some individual accession groups within the Refractario genotypes also yielded especially promising results e.g. the B [POU] (47.1%), LX & LV [POU] (41.2%) and LP [POU] (45.2%) accession groups. This agrees with the observation of Pound (1938), that there were trees showing little or no disease symptoms from highly diseased areas in Ecuador.

Figure 2. Distribution of resistance to Witches' Broom disease for Forastero, Trinitario and Refractario groups from the ICG,T, based on percentage infection.



Symptom severity

Due to the problems of repeatability and disease escapes, we endeavoured to conduct experiments where most of the inoculated plants developed symptoms, including the resistant controls. This was a reliable indicator that the experiment was successful, and permitted resistance/susceptibility to be assessed according to symptom severity, in addition to percentage infection.

Broom-base diameter was found to effectively discriminate between levels of resistance (Surujdeo-Maharaj *et. al*., 2003) regardless of screening techniques used (spray inoculation using Preval Sprayer and agar droplet technique). However, no significant correlation ($P > 0.05$) was found between percentage infection and broom-base diameter since accessions with large broom-base diameters did not necessarily have high percentage infection and vice versa. This suggests that there may be more than one mechanism of resistance influencing infection and disease development. Additionally, percentage infection is influenced by the number of shoots inoculated, which is highly variable in clonal plants and is difficult to standardise.

Analyses of broom-base diameter was found to be highly significant (Table 1), indicating that there were useful differences among the accessions screened and therefore selections could be made based on this variable.

Table 1. Analysis of variance for broom-base diameter.

Source	Degrees of freedom	Seq SS	Adj SS	Adj MS	F	*P*
Clone	138	1428.1	1428.1	10.35	2.87	<0.001
Error	300	1080.9	1080.9	3.60		
Total	438	2509.0				

From the results, a total of 90 accessions (Table 2) from the 553 accessions analysed were selected for further confirmation using the agar droplet screening technique. These accessions either developed few symptoms (total percentage of symptoms less than 20% or absence of brooms) or when brooms developed, they were small and thin, with an average diameter less than 6 mm, which is the average broom-base diameter observed in resistant controls.

Table 2. Accession groups with promising clones.

Accession group	No. of clones	Accession group	No. of clones
AM [POU]	4	LV [POU]	2
B [POU]	10	MATINA	1
CL	2	MO	1
CRU	3	MOQ	3
CRUZ	1	NA	4
CBO [VEN]	1	POUND [POU]	2
DOM	1	PA [PER]	11
EET [ECU]	2	SCA	3
GU	5	SJ [POU]	3
ICS	8	SLA	1
IMC	10	SLC	1
LCT EEN	2	SPA [COL]	1
LP [POU]	6	UF	2

Continuation of the project

Before the project came to an end in July 2003, a proposal was submitted to WCF to continue the activities with a change of emphasis, giving particular attention to the confirmation and quantification of the level of resistance of clones found to be putatively resistant during the preliminary screening exercise. Revised targets were set, based on the projected level of financing and the technical difficulties encountered in the first phase of the project: screening of 60 accessions per year and confirmation of resistance of 30 accessions per year. The first year of this proposal was approved with the possibility of extension and the new phase of the project started in August 2003.

A total of 33 clones were screened in the period August to December 2003, out of which 17 were selected for further confirmation.

From the list of 90 accessions selected for confirmation during the first phase of the project (Table 2), 50 clones have been grafted, 10 of them have been inoculated using the agar droplet technique and they are currently undergoing symptom evaluation.

Conclusions

Results obtained from screening have shown that there is considerable variation for resistance to WB within the collection of cacao held at the ICG,T. Apart from the selection of promising types, the project has identified those accessions which are definitely susceptible to the disease. Also inferred from the observations, was the need for additional criteria to that of percentage infection, as measures of resistance or susceptibility to WB.

The results also suggest that a suitable protocol to screen for resistance to WB is a two-tiered system. In this, initial mass screening is carried out by spray inoculation, which is convenient for screening a large number of plants at the same time, followed by confirmation of selected clones and quantification of their level of resistance using the agar droplet technique.

The results from this screening for WB resistance, after being cross-checked with data on natural infection in the field, will be combined with data on other traits like BP resistance, pod index etc. This information will provide a comprehensive list of accessions from the ICG,T which will be used as a guide for the selection of parents for germplasm enhancement and breeding work in the future.

Acknowledgements

We gratefully acknowledge financial support from ACRI and the WCF, and assistance from CIRAD in making this project possible.

References

Purdy, L.H., Schmidt, R.A., Dickstein, E.R. and Frias, G.A. (1997) An automated system for screening *Theobroma cacao* for resistance to Witches' Broom. *Agrotropica* **9**(3): 119-126.

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.

Umaharan, R., Thévenin, J-M., Surujdeo-Maharaj, S. and Latchman, B. (2000) Evaluation of cacao germplasm for resistance to Witches' Broom disease: preliminary experiments. Pages 34-38 in: *Annual Report 1999*. St Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Umaharan, R., Thévenin, J-M., Surujdeo-Maharaj, S. and Butler, D.R. Identification of resistance to Witches' Broom disease in the International Cocoa Genebank, Trinidad. Presented at the *14th International Cocoa Research Conference.* 13-18 October 2003, Accra, Ghana (proceedings in press).

Utilisation

Progress report on the germplasm enhancement programme for resistance to Black Pod disease

A.D. Iwaro and V. Singh

The main objective of the germplasm enhancement programme (GEP) is to accumulate resistance genes for Black Pod disease in populations that can be exploited in national cacao breeding programmes for the development of high yielding, resistant varieties. During the first five years of this programme, resistant and moderately resistant genotypes were selected, intercrossed and progenies were evaluated for resistance to *Phytophthora* using the leaf disc method (Iwaro and Singh, 2003). Following this exercise, data were analysed to assess the level of progress in the programme. Higher proportions of resistant and moderately resistant plants were observed among the Forastero progenies than among the Trinitario, Refractario or mixed progenies (Figure 1). A similar trend was observed among the accessions evaluated for resistance to Black Pod disease in the ICG,T (Iwaro *et al*., 2001). This shows that resistance is heritable and improved levels of resistance could be achieved in breeding with careful selection of promising resistant parents. While many inoculation tests conducted in the past had shown that most cacao accessions were susceptible to *Phytophthora* infection (Iwaro, 1997; Iwaro *et al*., 2001), a higher percentage of moderately resistant genotypes (52.5%) was observed among the 3,486 seedlings evaluated in the germplasm enhancement programme (Iwaro and Singh, 2003). Resistant and moderately resistant genotypes together form 63.7% of the tested population. This implies an increase in the frequency of resistance genes in the new population compared to the results of previous inoculation tests on other cacao populations, in which the proportion of susceptible types is higher. This improvement confirms the effectiveness of the selection criteria and other strategies adopted in this programme.

Establishment of field trials

Replicates of the year 3 population (176 seedlings) and year 4 population (177 seedlings) were established in September 2003 in Field 14 at the La Reunion Estate, Centeno.

Field observations

Results of field observations on flower and pod production, and the presence/absence of WB symptoms are presented in Table 1. Among the year 1 population (246 plants) established in 2000, 129 plants (52%) had flowers, while 78 (32%) were bearing pods during a survey conducted in October 2003. In the year 2 population, planted in 2001, 71 plants (27%) were flowering and 42 of these (16%) had pods. Very few plants had flowers and pods in the year 3 and year 4 populations established in 2002 (Table 1). To increase pod production for the evaluation of BP, it may be necessary to carry out hand pollinations. This would facilitate further evaluation of the progenies, parental genotypes and controls, which depends on the availability of pods. About 16% (39 plants) of the year 1 population and 10% (26 plants) of the year 2 population had WB (Table 1). There was no evidence of WB attack in the newly established

populations (year 3 and year 4 populations). Information on WB incidence provides an opportunity for negative selection against the disease. Further field observations should provide useful information for the selection of promising genotypes resistant to both BP and WB.

Figure 1. Distribution of scores for resistance to *P. palmivora* among groups of progenies.

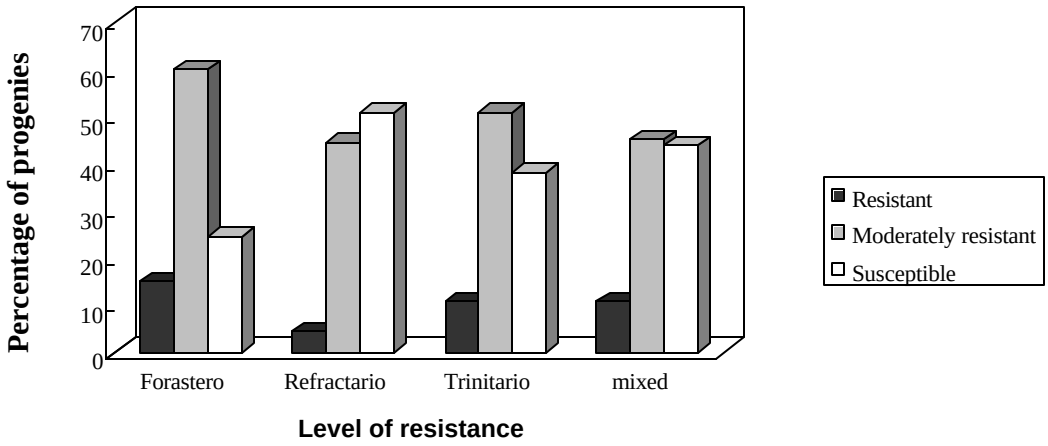


Table 1. Field observations of numbers of plants flowering, fruiting and with WB symptoms. Percentage values are given in parentheses.

Population	Year of establishment	No. of plants	No. of plants With flowers	No. of plants with fruit	No. of plants with WB
Year 1	2000	246	129 (52%)	78 (32%)	39 (16%)
Year 2	2001	263	71 (27%)	42 (16%)	26 (10%)
Year 3	2002	268	7 (3%)	2 (1%)	0
Year 4	2002	230	11 (5%)	3 (1%)	0

Constraints

Drought

The maintenance of plants in the field was very challenging during the severe dry season of 2003. Irrigation was carried out on a regular basis to maintain adequate soil moisture for plant survival.

Future direction

Confirmation of resistance to BP in the progeny populations in the field will be obtained using the detached pod inoculation method. A second cycle of the GEP will be carried out as part of the new CFC/ICCO/IPGRI project, *Cocoa productivity and quality improvement: a participatory approach*. The following strategy will be adopted.

- ? Selection of 100 resistant genotypes from the first cycle.
- ? Intercrossing of the selected genotypes.
- ? Establishment of 50 progenies (1250 seedlings).
- ? Evaluation of progenies for leaf resistance.
- ? Establishment of 20% (200 seedlings) in the field.
- ? Field observations for vigour, early flowering and resistance to BP and WB.
- ? Selection of 110 resistant genotypes for distribution (Forastero: 30, Refractario: 30, Trinitario: 30, mixed: 20).

The various selection criteria adopted in this programme should facilitate not only effective selection of promising genotypes/populations with enhanced levels of resistance to BP, but also selections with good yield potential and field resistance to WB.

References

Iwaro, A.D. (1997) Cocoa germplasm enhancement for resistance to Black Pod and Witches' Broom diseases. Pages 79-85 in: *Proceedings of the International Workshop on the Utilisation of the Genetic Resources of the International Cocoa Genebank, Trinidad (ICG,T).* 23-28 June 1996, St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Iwaro, A.D., Singh, V., Bharath, S.M. and Jugmohan, N. (2001) Germplasm evaluation at the International Cocoa Genebank, Trinidad for resistance to *Phytophthora* pod rot. Pages 34 - 40 in: *Annual Report 2000*. St. Augustine, Trinidad: Cocoa Research Unit, the University of the West Indies.

Iwaro, A.D. and Singh, V. (2003) Enhancing cacao germplasm for resistance to Black Pod disease. Pages 54 -59 in: *Annual Report 2002*. St. Augustine, Trinidad: Cocoa Research Unit, the University of the West Indies.

Flavour profiles of samples evaluated over two growing seasons for the CFC/ICCO/INIAP Flavour Project

D.A. Sukha, S.M. Bharath, S.S. Straker and D.R. Butler

Introduction

We have evaluated a range of samples for different quality attributes under the purview of the CFC/ICCO/INIAP Flavour Project *To establish physical, chemical and organoleptic parameters to differentiate between fine and bulk cocoa*. This report compares and contrasts the flavour profile trends of various accessions evaluated over two crop years (2002 and 2003) since the project was initiated in 2001.

Materials and methods

Coding

The identities of the commercial Trinidad Selected Hybrids (TSH) used in this project have been coded with "CRU" accession identities in compliance with an agreement between MALMR and CRU governing the presentation of results for these accessions. Samples taken from commercial estates have also been coded to protect the true identities of these estates.

Sample preparation

Detailed accounts of the protocols followed for sample preparation *viz.* fermentation and drying (primary processing) and roasting and milling (secondary processing) as well as the organoleptic assessment procedure can be found in the proceedings of the workshop to establish working procedures for the CFC/ICCO/INIAP Flavour Project (Sukha, 2001a, 2001b) and in a previous report, (Sukha *et al*., 2002). The following is a summary of the sample preparation and assessment procedure.

Primary processing

The identities of trees used in the Trinidad and Tobago component of this project were verified locally using both morphological and molecular (RAPD and SSR) identification techniques.

Fully mature, undamaged and healthy pods were harvested and the beans were extracted from the pods. These beans were placed in labelled nylon net bags in preparation for batch insert fermentations at the Manickchand Estate in East Trinidad. The bags were inserted 30 cm from the top of a sweatbox containing approximately 2,000 kg of wet cocoa and left to ferment for 7 days with turning on the second and fifth days. On the seventh day, the nylon bags with the bean samples were removed from the sweatbox and the fermented beans were placed in labelled wooden drying trays for sun drying which lasted 5 days. If the moisture content of the beans was not between 6-7% after the fifth day of sun drying, drying was completed in a mechanical convection oven set at 35ºC.

After drying, the beans were stored in quarter-sized jute sacks, similar to the ones used for commercial bean shipments. To avoid problems with moths, mould and other infestations these jute sacks were placed in plastic bags that were then sealed in plastic buckets with airtight lids. This

method of storage proved to be effective in reducing the incidence of mould and moth infestation compared to previously used storage methods.

Secondary processing
Secondary processing involves roasting, breaking and winnowing, coarse grinding and milling the bean samples into cocoa liquors for organoleptic evaluation. Roasting was done on 330 g of beans from a coned and quartered bean sample from the particular accession. Beans were roasted at 140ºC for 30 minutes in a mechanical convection oven and the broken and shelled nibs were coarsely ground with a coffee grinder. The coarsely ground beans were then milled for 90 minutes into liquor in an end runner mill. The liquors were stored at -6 to -8ºC prior to organoleptic evaluation.

Organoleptic evaluation

Evaluations using trained sensory panels were conducted under controlled conditions with appropriate experimental designs, test methods and statistical analyses. Cocoa liquors were evaluated by profiling, descriptive and differentiation techniques (Sukha, 2001a and American Society for Testing and Materials (ASTM), 1992).

Panel training
After completing an initial pre-screening questionnaire, panellists were trained in the areas of basic taste identification, taste sensitivity using threshold concentration, introduction to cocoa flavour attributes and vocabulary generation, cocoa off-flavours, scoring and ranking of flavour attributes by paired comparison tests and, finally, profiling with panellist calibration and hidden references.

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

Data analysis

Data from the three repetitions were pooled and analyses of variance (ANOVA) conducted using MINITAB Release 13.1 (Minitab Inc.). The significance of treatment effects and interactions as well as mean flavour profiles and the standard errors of the mean were calculated.

Results

In this report only flavour profile results from the coded TSH country clones and commercial estates for crop years 2002 and 2003 will be presented and discussed. The results presented in

Figure 1. Average flavour profiles for coded TSH country clones compared to the Ghana reference sample for the 2002 crop. Bars indicate standard errors.

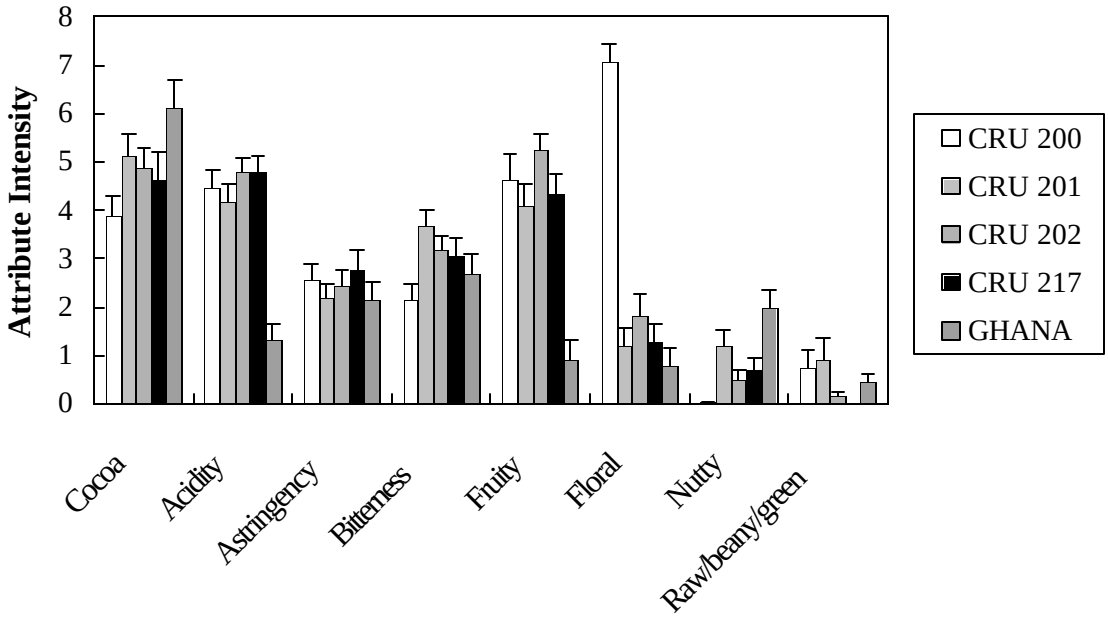
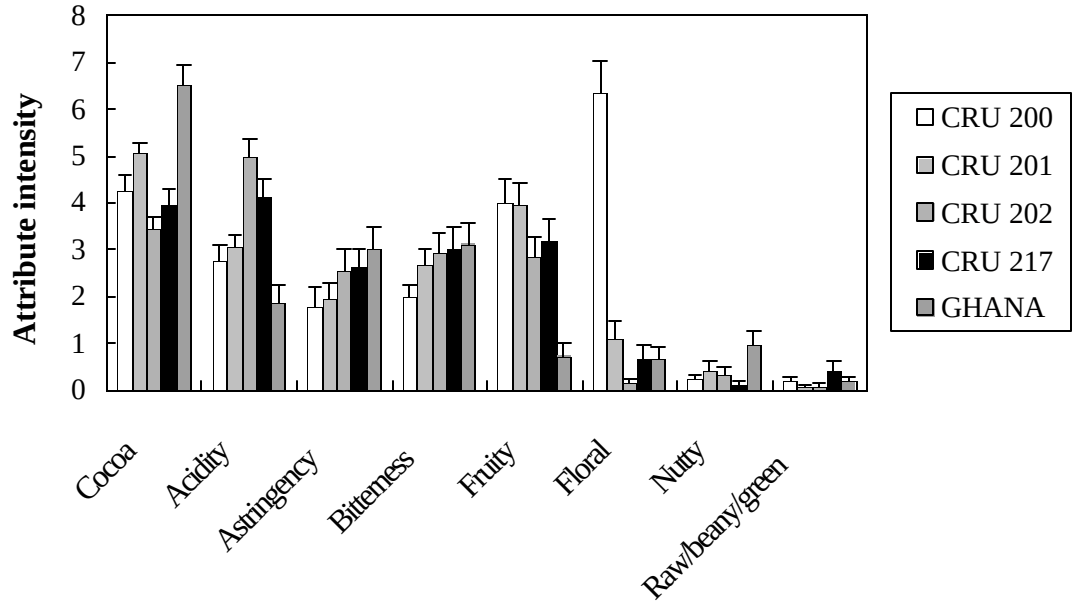


Figure 2. Average flavour profiles for coded TSH country clones compared to the Ghana reference sample for the 2003 crop. Bars indicate standard errors.



Figures 1 and 2 show the average flavour profiles for the coded TSH country clones compared to the bulk reference from Ghana for crop years 2002 and 2003 respectively.

The flavour profile trends over the two crop years combined showed that acidity, astringency, bitterness, floral and raw/beany flavours varied significantly (P =0.05 to P =0.001) among the accessions (Table 1). The ANOVA in Table 1 also revealed that some flavour attributes varied significantly within one particular crop year and not the other and that floral was the only flavour attribute to vary significantly (P =0.001) for both individual crop years. It must be noted that all the coded TSH country clones had consistently higher fruity and acid scores than the Ghana reference, which displayed the highest cocoa and nutty scores (Figures 1 and 2). Within a particular crop year, the coded TSH country clones all varied significantly (P =0.001) in their cocoa, acid, bitter, floral and nutty flavour attributes. Whilst CRU 200 consistently showed the highest floral score of any sample over the two crop years.

CRU 202 differed somewhat between the two crop years and the average flavour profile for this clone showed that cocoa, fruity and floral flavour attributes were all higher in the 2002 crop year.

Table 1. ANOVA of flavour profiles for coded TSH country clones for 2002 and 2003 crop years and both years combined.

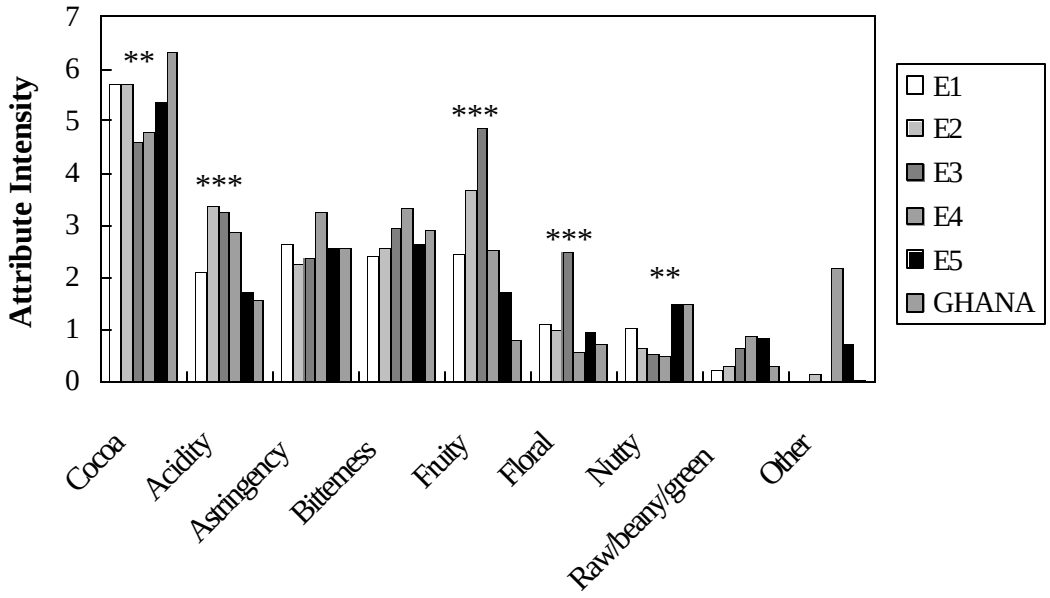
Flavour attribute	Significance		
	2002 crop year	2003 crop year	2002 and 2003 combined
Cocoa	NS	***	NS
Acidity	NS	***	*
Astringency	NS	NS	**
Bitterness	*	NS	**
Fruity	NS	NS	NS
Floral	***	***	***
Nutty	*	NS	NS
Raw/beany/green	NS	NS	*

* P ?0.05 ** P ?0.01 *** P ?0.001 not significant (NS) P ? 0.05

The average flavour profiles for commercial estate samples compared to the Ghana reference sample for the two crop years combined are presented in Figure 3. Flavour profiles for each crop year averaged over all the commercial estates are presented in Figure 4. Cocoa, acid, fruity, floral and nutty flavours varied significantly (P =0.01 to P = 0.001) amongst the commercial estates (Figure 3) whilst bitter, fruity, nutty raw/beany and other flavour attributes differed significantly (P =0.05 to P ?0.001) between the two crop years (Figure 4). The commercial estate samples had more variable flavours with generally diminished fruitiness and acidity compared to the coded TSH samples. However, all the commercial estates differed significantly (P =0.001) from the Ghana reference especially in acidity and fruitiness.

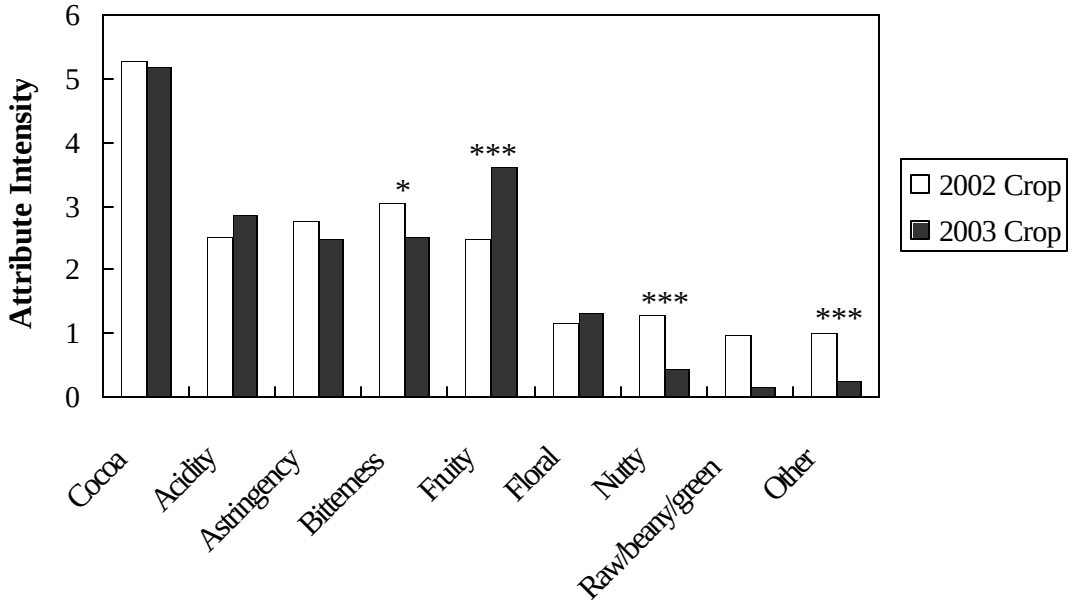
The commercial estate E3 consistently produced the most fruity and floral samples over both crop years, whilst commercial estate E1 consistently had the lowest acid scores and the most balanced flavour profile in terms of all the major flavour attributes.

Figure 3. Average flavour profiles for coded commercial estate samples compared to the Ghana reference for the two crop years combined.



** *P* ?0.01 *** *P* ?0.001

Figure 4. Average flavour profiles for the commercial estate samples combined over each of the two crop years.



* *P* ?0.05 *** *P* ?0.001

In the 2002 crop three estates had off-flavours that included mouldy/earthy for estate E5 and a mild sacky flavour note for estate E2. A smoky off-flavour note was detected for estate E4 in both crop years but this, and some raw/beany/green flavour notes, were less pronounced in the 2003 crop. Additionally, a mild floral flavour note was detected in estate E4 in the 2003 crop year but not in 2002.

Estates coded E2 and E5 differed the most between the two crop years. A more detailed examination of the flavour profiles revealed that fruity and acid flavour attributes were stronger at both these estates in the 2003 crop. In addition, some floral flavour notes were detected in 2003 in estate E5.

Discussion

The comparison of flavour profiles of selected samples from the CFC/ICCO/INIAP Flavour Project over two crop years has revealed that the impact of growing season varies depending on different cocoa varieties and the fermentation and drying regimes used. Flavour profiles of samples from a third and final growing season remain to be added to the analysis before conclusive statements can be made, however, the following are some preliminary inferences that one can make from the data generated so far.

The Ghana bulk reference sample used for the CFC/ICCO/INIAP Flavour Project came from a single batch of beans set aside for use by the project participating countries in 2002. The liquors of this sample over the two crop years therefore came from the same batch of beans and were not subject to the influence of the growing season. Therefore the Ghana bulk reference serves as a flavour control against which the other liquors are compared as well as a standard to check sensory panel consistency between the two crop years. The flavour profile results for the Ghana bulk reference over the two crop years show that the sensory panel was consistent in identifying the typical flavour attributes of well-fermented "bulk" cocoa, generally characterised by a dominant well-developed cocoa flavour with low acid, astringent and floral flavour attributes.

The coded TSH country clones showed clearly identifiable differences between these samples and the Ghana bulk reference. The TSH varieties are reputed for their "fine or flavour" attributes which are fruity, sometimes floral with moderately acid notes. The results over the two crop years revealed that there was little effect of growing season on fruity and floral attributes.

In addition to the genetic flavour potential of the TSH varieties, another factor that has possibly contributed to the consistency in the flavour profiles of the TSH varieties over the two crop years is the fact that the fermentation and drying protocol for these samples were perhaps more uniform than is commonly the case on commercial estates.

The flavour profiles for the five commercial estates follow a similar trend to that of the TSH country clones in having clearly identifiable differences to the Ghana bulk reference. However compared with the TSH country clones, there was more variation between estates and between crop years for fruity flavour.

This variation may be a function of many factors such as the different mixtures of cocoa germplasm ranging from almost pure ICS clones to pure TSH clones, with some estates having a mixture of the two types of germplasm. The flavour profiles of liquors from estate E3 showed definite fruity and floral flavour notes, which can be attributed directly to the cocoa germplasm grown there. This estate contains some ICS germplasm but the TSH clone CRU 200 predominates

and this clone is characterised by strong fruity and floral flavour notes (Figures 1 and 2). The influence of this single clone affects the overall character of the beans from this estate.

Fermentation practices are fairly consistent among commercial estates and this is reflected in the relative similarity of the profiles for astringency and bitterness for the commercial estates between the two crop years. The mild off-flavours detected from some of the commercial estates from crop year 2002 were primarily due to re-humidification during storage, probably on the estates concerned. In many instances this is a function of the prevailing ambient weather conditions during storage and inappropriate storage practices.

Drying practices vary more widely among the commercial estates than fermentation, ranging from purely sun drying to a mixture of initial sun drying followed by artificial drying to purely artificial drying. The drying regime in terms of temperature and drying time also varies somewhat among commercial estates and this has a direct effect on the acidity of samples dried at different estates. At estate E1 pure sun drying at a very gradual rate is practised and this probably contributes to the considerably lower scores for acidity observed over both crop years. In addition, the smoky off-flavour note detected at the estate E5 in both years was a direct result of a mal adjusted diesel-fired artificial dryer. Attempts were made to rectify this problem in the 2003 crop but a mild smoky off-flavour note was still detected.

Conclusion

Organoleptic evaluation using trained sensory panels with appropriate sensory designs have so far proven to be effective in realising the main aim of the CFC/ICCO/INIAP Flavour Project, that is, to differentiate between fine or flavour and bulk cocoa. The differences in the flavour attributes of both the TSH germplasm and cocoa from commercial estates in Trinidad compared to the bulk reference sample from Ghana had been clearly identified and were consistent in some previous studies. This study over two crop years has reaffirmed this view.

In addition to satisfying the main aim of the CFC/ICCO/INIAP Flavour Project the sensory panel results have provided more detailed information about the “fine or flavour” characteristics of the TSH clones. These range from predominantly fresh or “brown” fruity flavours to a mixture of fruity and floral flavours with moderate acid notes. Also, we have been able to characterise the unique flavour attributes of the selected commercial estates.

All the information generated from this body of research provides a good opportunity to investigate quality and flavour issues locally on an unprecedented detailed scale. Such information has far reaching benefits and implications for the local cocoa industry by providing a means to investigate new marketing approaches that target origin and estate specific cocoas for premium dark chocolates.

Acknowledgements

The ongoing financial support, assistance and collaboration of the CFC, Guittard Chocolate Co., California, USA, Lindt & Sprüngli, Switzerland, Masterfoods, UK, MALMR, Paul Manickchand Estates Ltd. (Trinidad), Produce Marketing Associates Ltd. (Trinidad), WCF, USA (formerly ACRI) and all sensory panellists are gratefully acknowledged in this study.

References

American Society for Testing of Materials (ASTM) (1992) The flavour profile and quantitative descriptive analysis. Pages 1-21in: *ASTM Manual on descriptive analysis and testing for sensory evaluation*. (R. Hootman Ed.) Philadelphia, USA: ASTM.

Sukha, D.A. (2001a) Strategy for the post-harvest processing of cocoa samples and physical assessment factors. Pages 41-43 in: *Proceedings of the initial workshop on the project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa*. 31 January – 2 February 2001, Macoya, Trinidad: Cocoa Research Unit, The University of the West Indies (limited distribution).

Sukha, D.A. (2001b) Strategy for the organoleptic assessments of cocoa samples. Pages 44-48 in: *Proceedings of the initial workshop on the project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa*. 31 January – 2 February 2001, Macoya, Trinidad: Cocoa Research Unit, the University of the West Indies (limited distribution).

Sukha, D.A., Butler, D.R., Bowen-O'Connor, C. and Straker, S.S. (2002) Suggested flavour profiles for selected cacao (*Theobroma cacao* L.) accessions. Pages 49-54 in: *Annual Report 2001*. St. Augustine, Trinidad: Cocoa Research Unit, the University of the West Indies.

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. Charles McDavid,
Dean, Faculty of Science and Agriculture,
The University of the West Indies,
St. Augustine, Trinidad and Tobago

Mr Trevor Murray, *Representative*
Permanent Secretary,
Ministry of Agriculture, Land and Marine Resources (MALMR)
Port of Spain, Trinidad and Tobago

Mr Tony Lass, *Representative*
Biscuit, Cake, Chocolate and Confectionery Association (BCCCA),
London, UK

Dr Phillipe Petithuguenin, *Representative*
Centre de coopération internationale en recherche agronomique
pour le développement, Departement cultures pérennes (CIRAD-CP),
Montpellier Cedex 1, France


88
NIKE

Cocoa Research Unit staff 2003

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 *MPhil* Contract Officer I

Antoinette Sankar *MSc* Contract Officer I

Darin Sukha[1] *MSc* Junior Research Fellow

Romina Umaharan *MPhil* Contract Officer I

Support staff

Sarah Bharath *BSc* Technical Assistant

John Joseph Laboratory Assistant

Naveta Persad[1] *BSc* Technical Assistant

Anil Roopchand *BSc* Research Technician (Jan to July)

Gangadeen Ramdhanie Senior Laboratory Assistant

Herman Ramjewan Laboratory Assistant (Jan to March)

Vindra Singh *BSc* Technical Assistant

Eusebius Solozano Laboratory Assistant

Sharlene Straker *BSc* Technical Assistant

Visiting scientists

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

Jean-Marc Thévenin *Ingenieur Agronomie*
Plant Pathologist CIRAD-CP, France

Administrative staff

Claudia Lyons Secretary

Jan Rodriguez Secretary (August to September)

Phulmatee Hetai Messenger/cleaner

Carolyn Timothy Messenger/cleaner (February to April)

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

Publications and Presentations

Conferences and Workshops

Papers presented

Bekele, F.L. The history of cocoa production in Trinidad and Tobago. Presented at the Seminar/exhibition on revitalization of the Trinidad and Tobago cocoa industry: targets, problems and options, the Association of Professional Agricultural Scientists of Trinidad and Tobago. 20 September 2003, St. Augustine, Trinidad.

Butler, D.R., Sukha, D.A. and Maharaj, K. The agro-ecology of cocoa in Trinidad and Tobago. Presented at the Seminar/exhibition on revitalization of the Trinidad and Tobago cocoa industry: targets, problems and options, the Association of Professional Agricultural Scientists of Trinidad and Tobago. 20 September 2003, St. Augustine, Trinidad.

Iwaro, A.D. and Butler, D.R. Sources of resistance to *Phytophthora* pod rot at the International Cocoa Genebank, Trinidad. Presented at the 14th International Cocoa Research Conference. 13-18 October 2003, Accra, Ghana.

Iwaro, A.D., Bharath, S.M., Bekele, F.L. and Butler, D.R. Germplasm enhancement for resistance to black pod disease: strategy and prospects. Presented at the 14th International Cocoa Research Conference. 13-18 October 2003, Accra, Ghana.

Mollineau, W.M., Bekele, F.L. and Garcia, G.W. A wildlife pest affecting cacao (*Theobroma cacao* L.): the status of the neotropical red squirrel (*Sciurus granatensis*) at the International Cacao Genebank, Trinidad. Presented at the 4th INCOPED Workshop. 20-21 October 2003, Accra, Ghana.

Sounigo, O., Bekele, F.L., Iwaro, A.D., Thévenin, J-M., Bidaisee, G., Umaharan, R., Sankar, A., Sukha, D.A., Boccara, M., Butler, D.R. and Eskes, A.B. Description of the "CFC/ICCO/IPGRI Project Collection". Presented at the 14th International Cocoa Research Conference. 13-18 October 2003, Accra, Ghana.

Sukha, D.A., Butler, D.R., Umaharan, P., Straker, S.S. and Bharath, S.M. A preliminary investigation into possible processing location and growing environment influences on the flavour attributes of cocoa (*Theobroma cacao* L.). Presented at the 14th International Cocoa Research Conference. 13-18 October 2003, Accra, Ghana.

Sukha, D.A. Potential value added products from Trinidad and Tobago cocoa. Presented at the Seminar/exhibition on revitalization of the Trinidad and Tobago cocoa industry: targets, problems and options, the Association of Professional Agricultural Scientists of Trinidad and Tobago. 20 September 2003, St. Augustine, Trinidad.

Sukha, D.A. Primary processing of high quality Trinidad and Tobago cocoa beans – targets, problems and options. Presented at the Seminar/exhibition on revitalization of the Trinidad and Tobago cocoa industry: targets, problems and options, the Association of Professional Agricultural Scientists of Trinidad and Tobago. 20 September 2003, St. Augustine, Trinidad.

Sukha, D.A. Organoleptic quality assessments and data handling techniques. Presented at the mid-term review of CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. 5-9 May 2003, Guayaquil, Ecuador.

Sukha, D.A., Bharath, S.M., Straker, S.S. and Butler, D.R. Cocoa liquor preparation and organoleptic quality assessments – activities and results. Presented at the mid-term review of CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. 5-9 May 2003, Guayaquil, Ecuador.

Sukha, D.A., Bharath, S.M., Straker, S.S. and Butler, D.R. Chemical quality assessments – activities and results. Presented at the mid-term review of CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. 5-9 May 2003, Guayaquil, Ecuador.

Sukha, D.A., Bharath, S.M., Straker, S.S. and Butler, D.R. Fermentation and drying trials – activities and results. Presented at the mid-term review of CFC/ICCO/INIAP Project to establish the physical, chemical and organoleptic parameters to establish the difference between fine and bulk cocoa. 5-9 May 2003, Guayaquil, Ecuador.

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D.R. Using an optimized agar drip inoculation method of identifying and measuring resistance to Witches' Broom disease of cacao. Presented at the 14th International Cocoa Research Conference. 13-18 October 2003, Accra, Ghana.

Journal Articles

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

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

Motilal, L.A. and Butler, D.R. (2003) Verification of identities in global cacao germplasm collections. *Genetic Resources and Crop Evolution* **50**: 799-807.

Ratnadass, A. and Butler, D.R. (2003) Abundance of sorghum panicle-feeding buds (Hemiptera Miridae) in Mali and empirical relationships with weather. *Insect Science and its Application* **23**: 239-250.

Other Articles

Motilal, L.A. and Butler, D.R. (2003) Management of cacao genetic resources: streamlining a field genebank. *Research and Graduate Studies,* Volume 5, the University of the West Indies, Trinidad.

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. *Research and Graduate studies*, Volume 5, the University of the West Indies, Trinidad.

Newsletters edited

Bekele, F.L. INGENIC Newsletter Issue 8, July 2003, St. Augustine, Trinidad.

Visitors to CRU in 2003

Claude Guillerer	Frêterive 73250 France
Sylvie Toulet	Maraval, Trinidad
Maris Berthas	Nordansjo, Kovland, Sweden
Ia Orre Montan	Bragevagen, Stockholm, Sweden
Carl Montan	Bragevagen, Stockholm, Sweden
Maja Berthas	Stockholm, Sweden
Duane Dove	Styrmansgatan, Sweden
Richard Bingham	Riale Co., Slipgatan, Stockholm, Sweden
Peter Andersson	Stockholm, Sweden
Wendell Mottley	Maraval, Trinidad
Claude Voillavit	University of Hamburg, Hamburg, Germany
Christina Rohsius	University of Hamburg, Hamburg, Germany
Nicole Leighton	Tiburon, California
Robert Steinberg	Berkeley, California
Kobayashi Tadashi	Nishi-Sugamo, Toshima-ku, Tokyo, Japan
Masataka Nishi	Yakumo, Megroku Tokyo, Japan
Akaboshi Chiaru	
Masako Chen	Cascade, Trinidad
Rennie Dumas	Minister of Environment, Plymouth, Tobago
Gervon Abraham	D'Abadie, Trinidad
Jo Draps	Museum of Cocoa and Chocolate, Brussels
Shirley Jeffrey Sinanan	Mabaruma/Hosororo Organic Cocoa Growers' Association, Guyana
Sherene Soyer	Agricultural Development Bank, Port of Spain, Trinidad
Alan Pomella	Almirante Cacao, Itajuipe, Bahia, Brazil
John Rahael	Minister of Agriculture, Land and Marine Resources, Trinidad and Tobago
H. Sona Ebai	Cocoa Producer's Alliance, Lagos, Nigeria
R. Urlin-Knights	University of Guyana
Prof. J. Burley	Oxford Forestry Institute, Yale School of Forestry and Environmental Studies
Fedna Stoll	Organic Juice Produce
Nick D. Brown	Oxford Forestry Institute, UK
Brit Leoseth	Ambassador of Norway, (Oslo)
Stuart Jardine	Royal Norwegian Consulate General, Trinidad
Juan C. Motamayor A.	USDA, Miami

Acronyms & Abbreviations

ACRI	American Cocoa Research Institute, USA
ANOVA	Analysis of variance
APASTT	Association of Professional Agricultural Scientists of Trinidad and Tobago
BCCCA	Biscuit, Cake, Chocolate and Confectionery Alliance, London, UK (2003) Biscuit, Cake, Chocolate and Confectionery Association, London, UK (from January 2004)
BCQS	Barbados Cocoa Quarantine Station
BP	Black Pod disease
CA	Cluster analysis
CAOBISCO	Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
CATIE	Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica
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
CRU	The Cocoa Research Unit, Trinidad and Tobago
DNA	Deoxyribonucleic acid
ESTs	Expressed sequence tags
EU	European Union
FSA	Faculty of Science Agriculture, UWI, St. Augustine, Trinidad and Tobago
GEP	Germplasm enhancement programme
GORTT	Government of the Republic of Trinidad and Tobago
IBPGR	International Board for Plant Genetic Resources, Rome, Italy
ICCO	International Cocoa Organisation, London, UK
ICGD	International Cocoa Germplasm Database
ICG,T	International Cocoa Genebank, Trinidad
INIA	Instituto Nacional de Investigaciones Agricolas, Venezuela
INIAP	Instituto Nacional Autonomo de Investigaciones Agropecurias, Ecuador
INCOPED	International permanent working group for cocoa pests and diseases
INGENIC	International group for genetic improvement of cocoa
IPGRI	International Plant Genetic Resources Institute, Rome, Italy
LAF	Lower Amazon Forastero
MALMR	Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago
NL	No label
P	Probability
PAGE	Polyacrylamide gel electrophoresis
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
r	Correlation coefficient
RAPD	Random amplified polymorphic DNA
SJE	San Juan Estate, Trinidad
SSR	Simple sequence repeats
TSH	Trinidad Selected Hybrid
UAF	Upper Amazon Forastero
UCRS	University Cocoa Research Station
UE	Union Européenne
UPGMA	Unweighted pair-group method using arithmetic averages
USDA	United States Department of Agriculture

UV	Ultra-violet
UWI	The University of the West Indies
WB	Witches' Broom disease