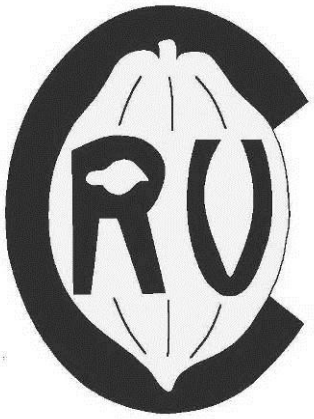
# Annual Report 2009




**Cocoa Research Unit**
**The University of the West Indies**
**St. Augustine, Trinidad and Tobago**
**2010**

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# Introduction

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

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

CRU is a research centre in the Faculty of Science and Agriculture of the University of the West Indies (UWI). Core activities in CRU are made possible by financial support from the Government of the Republic of Trinidad and Tobago (GORTT) and the Cocoa Research Association Ltd. (CRA). Sources of additional support for special projects and collaboration from other organisations are listed on the inside front cover of this report.

## Projects

The CFC/ICCO/Bioversity[1] project entitled *Cocoa productivity and quality improvement: a participatory approach* started in June 2004 and is referred to in this report as the "CFC/ICCO/Bioversity Cocoa Productivity Project". This project officially ended on 30th November 2009. An enormous amount of information has been generated on the accessions at the ICG,T during the five year duration of this project. Promising accessions have been identified and some used in the germplasm enhancement programmes at CRU, while others have been included in the Project Collection. Over 100 promising accessions with useful traits have also been transferred to the International Cocoa Quarantine Centre, Reading (ICQC,R), UK for future distribution to breeders in the various cocoa growing countries on request. These activities should facilitate the development of elite varieties combining high yield potential with an acceptable level of resistance to major cacao diseases. We recognise with gratitude the financial support received from the CFC and CRA, UK as well as the in kind contributions from other project collaborators. We also recognise major contributions by the late David Iwaro towards realising the objectives of this project

The second phase of the project *To evaluate cocoa germplasm for resistance to Witches' Broom disease* is continuing with support from the World Cocoa Foundation (WCF). During the 2009, emphasis was placed on propagation of new clones for confirmation screening. This was to facilitate the shift from the screening of seedling plants to screening clonally propagated plants (propagated using the technique of micro-grafting). A total of 46 new clones were propagated. In addition 24 promising clones were inoculated for confirmation screening to confirm and quantify their resistance. Evaluations were completed for 17 of these clones and from these 4

[1] United Nations Common Fund for Commodities/International Cocoa Organisation/Bioversity International

clones were confirmed to be resistant. Twenty clones were re-inoculated in December 2009 (spray inoculation for mass screening) for on-going evaluations.

CRU is continuing to participate in the project *To develop a DNA[1] fingerprinting database for all major cacao collections in the Americas* with the United States Department of Agriculture (USDA), through an agreement between USDA and CRU with inputs from CIRAD[2]. Since the start of the project in 2001, over 2,400 DNA samples from all the accessions held in the ICG,T have been sent to the USDA molecular biology laboratory in Beltsville, USA. At the start of 2009, 800 outstanding DNA samples were yet to be analysed and, to address this task, Ms. Antoinette Sankar (CRU Contract Officer I) spent six months at the Beltsville, Maryland ARS/USDA laboratory to perform genetic analysis. Approximately 400 of the 800 DNA samples were run with 12 SSR primers, and a subset of that (~300) was also run with the other 3 SSR primers of the list of 15 primers agreed for the global fingerprinting project.

The project entitled *DNA markers for cacao traits* is continuing with funding from the GORTT Research Development Fund. This work is being undertaken by Lambert Motilal, who was hosted by the USDA Molecular Biology Laboratory in Beltsville for two months of the year. This is part of a larger collaborative project between CRU and USDA; *Molecular characterisation of the cocoa germplasm in the International Cocoa Genebank, Trinidad (ICG,T).* The objective is to carry out association mapping to relate genes to specific traits in cacao.

The project entitled *Safeguarding the International Cocoa Genebank, Trinidad: a global resource for the cocoa industry*, supported jointly by the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector (administered by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV)) and the CRA, UK, ended on 30th November 2009. In this report it will be referred to as the "Dutch LNV Project to Safeguard the ICG,T". The project has allowed CRU to make significant progress in addressing four major factors that could increase the risk of genetic erosion in the ICG,T. The main aims to upgrade the irrigation facilities at the University Cocoa Research Station (UCRS), improve security of the site and re-propagate material at risk of genetic erosion and to propagate rooted cuttings from grafted trees in UCRS were accomplished fully or to a significant extent. In addition to improving the infrastructure and producing a critical mass of re-propagated plants, the project has aided the development of human capacity to address our ongoing cacao conservation efforts. It is with gratitude that we recognise the support from the LNV and CRA, UK in allowing CRU to realise its mission to provide support to the world cocoa industry.

A project *To assess the quality attributes of the Imperial College Selections* was approved by the Dutch LNV in June 2006 for funding by the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector. Good progress continues to be made in this project with the completion of the third and final crop year fermentations from the working collection of 30 ICS genotypes. Physical analyses on all project samples have been completed and cocoa liquors were prepared from the final crop year for flavour assessments. Analyses of other quality attributes and flavour related chemical compounds are ongoing. This project has attracted co-financing support and collaboration from several manufacturers of premium chocolate.

A collaborative project between CRU and Towson University (TU) entitled *Detection of misidentified plants in Theobroma cacao germplasm collections in Trinidad.* DNA extracted

[1] Deoxyribonucleic acid

[2] Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France

from leaves of replicated trees of Imperial College Selections are being analysed as part of the Dutch LNV project "To assess the quality attributes of the Imperial College Selections". The primary objective is to confirm the identities and assess the uniformity of the ICS material in their respective plots at the ICG,T from which pods are being harvested. Sarah Bharath was hosted again by TU for four months where she utilised the training received in 2008 for the completion of this ICS DNA fingerprinting exercise. The final results from TU are pending.

The project entitled *Development of a neutraceutical and flavour profiling system of cocoa beans in Trinidad and Tobago* funded by the GORTT Research Development Fund, is a joint project between the Department of Chemistry, UWI and CRU. The purpose is to acquire in-house expertise to perform analyses of flavour chemistry in cocoa, and involves the training of two post-graduate students. Good progress continues to be made in this project as the various protocols for carrying out the analysis of volatile and non-volatile compounds were successfully optimised.

A collaborative project between CRU and MALMR, *Improvement of resistance to Black Pod disease in Trinidad Selected Hybrids (TSH),* was approved by the GORTT to begin in 2007. The pollinations for the breeding design began in 2008 and due to its broad scope and the level of funding, a formal contract was prepared in December 2009 by CRU to implement the work plan of this project.

A World Bank Development Market Place project *Identification and promotion of ancient cacao diversity through modern genomics methods to benefit small-scale farmers*, began in 2009. The main aim of this two-year project is to develop a DNA fingerprinting based system to distinguish and trace cacao cultivars using modern genomics methods. The project will focus on ancient cacao varieties (predominantly Trinitarios) grown on small-holdings in Trinidad and Tobago as well as regional Trinitarios conserved at the ICG,T. These varieties are also being assessed for any unique flavours both as liquors and chocolates. The project initiation workshop was held at CRU from 29th March – 1st April 2009 with 14 participants from Bioversity International (Implementing Organisation), University of British Colombia, Canada, MALMR, CRU, a farmers' representative from PRISM Agri Estates Co. Limited (Trinidad) and a chocolate manufacturers' representative from MARS Inc. An observer from the Trinidad and Tobago Chamber of Industry and Commerce attended the first day of the workshop.

A Cocoa of Excellence Project (CoE) entitled *Unravelling and celebrating diverse flavour qualities of cocoas to promote market differentiation*, is being supported by the CFC through the ICCO. The goal of the CoE is to promote high quality cocoa origins by creating awareness among stakeholders in the national and international cocoa supply chain on producers of high quality cocoa origins whilst facilitating linkages between producers of quality cocoa origins and manufacturers of specialty chocolate products. The main organising institutions are Bioversity International, CIRAD and Event International (organisers of the "Salon du Chocolat"). The initiative is also supported by several chocolate manufacturers. The Cocoa Research Unit is part of the Sensorial Technical Committee responsible for initial blind screening of cocoa liquor samples to select the top 50 samples best representing the diversity and complexity of flavour from the various cocoa producing countries and regions of the world.

**Staff news**

*David Butler* (Director of CRU until 31 January 2009) demitted office on 31 January 2009 after

completing a 5-month extension contract as Director from 1 September 2008 to 31 January 2009. *Frances Bekele* and *Darin Sukha* were jointly responsible for overseeing the activities of CRU for the remainder of 2009.

*Surendra Surujdeo-Maharaj* (Plant Pathologist) assumed duties on 1 September 2009 as Plant Pathologist within the Pathology Section of CRU after completing his post-doc position in CIRAD, France.

*Eusebius Solozano* (Laboratory Assistant until November 2009) was promoted to Technical Assistant I on the basis of his key role and expansive list of responsibilities in running the University Cocoa Research Station (UCRS) and managing the UCRS field staff.

*Sarah Bharath* (Technical Assistant) was attached to the Department of Biological Sciences (under Dr. Roland Roberts) at TU to work on DNA verification for the collaborative project Detection of misidentified plants in *Theobroma cacao* germplasm collections in Trinidad from 1 July to 31 October 2009.

*Antoinette Sankar* (Contract Officer I) spent six months at USDA-ARS, Beltsville to perform Capillary Electrophoresis Genetic (CEQ) analyses on DNA in the project To develop a DNA fingerprinting database for all major cacao collections in the Americas. Whilst at Beltsville she also prepared plates of ICS and PNG samples for analysis with single-nucleotide polymorphisms (SNPs).

*Zainab Ali-De Freitas,* (Technical Assistant) who had been hired to assist Lambert Motilal, ended her service on 31 December 2009.

**Visitors**

The Cocoa Research Unit received close to 100 visitors in 2009 either individually or as part of group visits. The first group comprised executives from the company, Chocolates El Rey, Venezuela led by the President and CEO *Jorge Redmond*. Participants attending the Second Roundtable for a Sustainable Cocoa Economy (RSCE2) and the Regional Seminar on New Plant Variety Protection under the UPOV[1] Convention also visited CRU, as well as, a group of 12 French Chocolatiers who are members of the Confédération des Chocolatiers et Confiseurs de France.

The second meeting of the Roundtable for a Sustainable Cocoa Economy (RSCE2) took place at the Hyatt Regency Hotel in Port of Spain, Trinidad and Tobago from 24-26 March 2009. The new irrigation system for the ICG,T was commissioned by *Prof. Clement Sankat* (Pro Vice Chancellor of UWI and Principal of the St Augustine Campus), *Dr. Marcel Vernooij* (LNV Representative), *Dr. Michelle End* (CRA, UK Representative) and *Hon. Hans Horbach* (Ambassador of the Kingdom of the Netherlands) during the RSCE2 Field Trip which was held on 27 March 2009. *Prof. Sankat* later had an extended visit to CRU on 9 July 2009 together with representatives from the Business Development Office (*Dr. David Rampersad*) and The Faculty of Science and Agriculture including Dean (*Prof. Dyer Narinesingh*).

The Managing Director of the CFC (*His Excellency Ali Muchumo*) and the First Project Manager of the CFC (*Mrs. Eltha Brown*) paid a courtesy call to CRU on 15 May 2009 to discuss the work of the CFC/ICCO/Bioversity Cocoa Productivity Project and opportunities for new funding submissions to the CFC. They also visited the ICG,T to see the enhanced germplasm that resulted from the project.

[1] International Union for the Protection of New Varieties of Plants

**Freak storm damage at CRU**

On 10 August 2009 the offices and laboratories of the Cocoa Research Unit were completely flooded due to torrential rainfall from a tropical storm. Clean up was undertaken solely by CRU staff and we were able to resume experimental work within three days after the storm. The Unit was fully operational within one week after the flooding. It is with gratitude and great appreciation to all staff that we have now fully recovered from this freak weather event.

**Meetings and events**

RSCE2

Frances Bekele and Darin Sukha attended a series of planning meetings, sensitisation seminars (on January 22 2009) and group discussions (on 18 February 2009) as part of the local organising committee leading up to the RSCE2.

CacaoNet

Frances Bekele attended the 5th CacaoNet Steering Committee meeting at the Hyatt Regency on 23 March 2009 and Darin Sukha the 6th CacaoNet Steering Committee meeting in Bali on 17 November, 2009 where he made a presentation on "The role of International Cocoa Genebanks in the CacaoNet Global Strategic Active Collection".

The Ghana Fine Flavour Cocoa Project

Darin Sukha visited the Cocoa Research Institute of Ghana (CRIG) from 11 – 16 October 2009 and held meetings with staff at working on the Ghana Fine Flavour Cocoa Project to advise on fermentation and drying protocols used in this project. Whilst at CRIG he met with the Executive Director of CRIG to discuss opportunities for future collaborative work.

The 16th International Cocoa Research Conference (ICRC)

CRU was represented at the 16th ICRC by Drs. Darin Sukha and Surendra Surujdeo-Maharaj, and Sarah Bharath. Darin Sukha made an oral presentation during the 4th Session. Surendra Surujdeo-Maharaj made an oral presentation on behalf of Kamaldeo Maharaj, Patricia Maharaj and colleagues at the Cocoa Research Section, Central Experiment Station, MALMR, Frances Bekele, CRU and Dr. Isaac Bekele, Department of Food Production, UWI. Sarah Bharath presented a poster summarising the outputs and benefits of the CFC/ICCO/Bioversity Cocoa Productivity Project as well as a poster prepared by Balram Latchman and collaborators on the Dutch LNV Project to Safeguard the ICG,T.

Darin Sukha used the opportunity to hold meetings with the WCF and CRA representatives attending the 16th ICRC to discuss matters regarding project funding. Meetings were also held with the Reading University research teams, Ghana Fine Flavour Cocoa project team, University of Hamburg Cocoa Research Group, representatives from Chocolatier Barry Callebaut and Prof. David Guest from the University of Sydney, Australia to discuss opportunities for collaboration with CRU and FSA, The UWI.

The 6th International Group for Genetic Improvement of Cocoa (INGENIC) Workshop

Darin Sukha chaired the 4th session of the INGENIC meetings and participated in the discussions on "Breeding for quality attributes".

Sarah Bharath used the opportunity whilst in Bali to travel to Australia where she met with representatives from Queensland Department of Primary Industries and Fisheries to discuss progress on the collaborative project "Mapping compositional parameters of cocoa to sensory profiles", which is a sub project from the LNV funded ICS project. Ms. Bharath also visited the University of Sydney to continue discussions with Prof. David Guest on opportunities for collaboration.

Quality fora and other meetings

Darin Sukha was invited to attend and make presentations at cocoa quality and technical fora in Jamaica (6 - 11 July 2009) and in Belize (3 December 2009).

Surendra Surujdeo-Maharaj and Naailah Ali participated in a workshop organised by the National Institute for Higher Education, Research, Science and Technology, Trinidad, on Sector Best Bets Project-Value Chain held on 3 December, 2009.

Darin Sukha was invited to attend a meeting and tasting session at Hersheys, Pennsylvania (18 – 19 December, 2009) as part of the Ghana Fine Flavour Cocoa Project. He also used the opportunity to meet with research staff at Hersheys to discuss opportunities for collaborative research in cocoa quality.

**Participants of the World Bank Development Market Place project initiation workshop**

# The Cocoa Research Unit – an overview

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

Cocoa research began in Trinidad at the Imperial College of Tropical Agriculture (ICTA) in 1930 as the Cocoa Research Scheme and continued uninterruptedly until 1960, when ICTA was subsumed within the Faculty of Agriculture of the University of the West Indies. The Cocoa Research Unit (CRU) was established in 1965 to continue the research efforts of the ICTA. In the 1980's the CRU, with funding from the European Union, assembled the germplasm from different sites within Trinidad to a single site at Centeno to form the International Cocoa Genebank, Trinidad (ICG,T). The ICG,T is situated at UCRS, a 37 ha site, originally part of the La Reunion Estate at Centeno with individual accessions represented by 16 trees established from rooted cuttings. CRU is responsible for maintenance of the ICG,T around which on-going research activities in the Unit are centred. By 1994 over 2,000 accessions had been planted in the ICG,T and additional clones are added as they become available. The genebank contains one of the most diverse collections of cacao germplasm in the world and has been designated a Universal Collection by IPGRI[1] (now Bioversity International).

Cacao germplasm has to be conserved as a living collection, since seeds do not remain viable if they are frozen and other methods of cryopreservation are not yet widely available. Work to establish the ICG,T began in 1982 with support from the European Union by consolidating different collections established at different locations on the islands. These include:

a) The Imperial College Selections (ICS), which resulted from an exhaustive survey in Trinidad and Tobago carried out by F.J. Pound between 1930 and 1935. About 50,000 high-yielding trees were selected and those bearing small and thick-shelled pods were eliminated, resulting in approximately 1000 trees. The 100 most productive trees (ICS 1 to 100) were selected from the 1,000 trees using exact criteria from detailed observations.
b) A main source of original material for the ICG,T was Marper Farm at Manzanilla, east Trinidad, established by F.J. Pound following his expeditions to Ecuador and 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.
c) In addition, germplasm was available from other expeditions such as the Anglo-Colombian expedition in 1952-53 and
d) Chalmers' expeditions to Ecuador between 1968 and 1972.
e) Trinitario populations from other islands in the Caribbean and Central America (early 1990s)

[1] International Plant Genetic Resources Institute

f) Wild Criollo material from Belize
g) Lower Amazon material from French Guiana and Venezuela,
h) Genetically diverse Upper Amazon clones from the John Allen collection, Ecuador.

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

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

Research efforts at CRU over the last 10 years have been directed towards the task of characterising and evaluating all the accessions in the ICG,T, selecting those with desirable traits and undertaking pre-breeding to produce genetically enhanced populations for specific characteristics (such as disease resistance), while maintaining genetic variability. 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 and 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 (over 20 years after establishing the first 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. Cuttings are being taken from well established grafted plants and rooted to fill gaps in the ICG,T with plants on their own roots. It is important to make a concerted effort to raise plants from rooted cuttings if at all possible, to avoid potential confusion in the future with chupons from rootstocks.

### New introductions

The ICG,T is considered to be a dynamic germplasm collection. We are continuously adding accessions from collecting expeditions (when the opportunity arises) or from other national 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. Until 2003, new material was introduced through the Barbados Cocoa Quarantine Station however this activity has been suspended due to financial constraints. Material is now being introduced to Trinidad through the ICQC,R, UK.

Further acquisitions are proposed when funding permits, from Mexico (Criollo/Trinitario), Costa Rica (CATIE[1]) (Criollo), Guyana (Lower Amazon), French Guiana (Lower Amazon), Bolivia, Columbia, Ecuador and Peru (Upper Amazon) and Brazil (Lower Amazon). This would improve the representation of the known genetic groups of cacao in the ICG,T.

Documentation

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

Verification

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

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

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

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

[1] Centro Agronómico Tropical de Investigatión y Enseñanza

inconsistent, so it is possible that the 30% off-types identified by this technique is not accurate. Since 2001, we have adopted microsatellite analysis (otherwise known as Simple Sequence Repeats, SSR) for the verification work. In recent years the majority of DNA analysis has been carried out using a sequencer in the USDA-ARS laboratory in Beltsville through a collaborative agreement between CRU and USDA. SSR analysis for DNA fingerprinting is reported to be reliable, with consistent results between different laboratories.

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

## Characterisation

### Morphological characterisation

A significant proportion of the accessions in the ICG,T have yet to be fully described. To address this problem, a concerted effort is being made to systematically document each accession using morphological descriptors. Work started in 1990 using a complete list of 65 morphological descriptors developed by the International Board for Plant Genetic Resources (now Bioversity International) in 1981, but initial progress was slow and this was superseded by a short list of 22 morphological descriptors developed at CRU. The list includes detailed descriptions of leaves, flowers and fruit for traits that aid identification and/or affect economic yield. It remains a large task even with the short list of descriptors, and the work was further streamlined in 2000 by reducing the sample size of pods from 20 to 10 and that of flowers from 15 to 10. Full descriptions of 1,564 accessions and flower descriptions of 2,235 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, Reading, UK, for global distribution.

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

### Molecular characterisation

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

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

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

**Evaluation**

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

Disease resistance

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

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

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

Mass screening for resistance to WB is being undertaken using a spray inoculation method. This work was started in 1998 using young grafted plants, replicated up to five times to allow inoculations of the same clone to be repeated. The inoculation method had to be adapted for use with grafted plants (as opposed to seedlings) and to the environmental conditions in Trinidad, so early progress in this project was slow. However, almost 800 accessions have now been screened by spray inoculation. 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 is being used to confirm and quantify the WB resistance

of promising clones from spray inoculation. These results will also be combined with field observations in the ICG,T over a number of years.

Quality traits

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

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

The assessment of flavour traits is an expanding area of investigation in CRU, and there is an increasing demand for the CRU taste panel to assess flavour of cocoa liquors from a wide range of cocoa producing countries.

**Utilisation and application**

Distribution

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

Germplasm enhancement

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

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

A similar pre-breeding programme was initiated in 2004 for WB. Progeny from crosses between WB resistant clones are being screened with the agar-droplet inoculation method and promising seedlings are also being screened for BP resistance. Other work in CRU aims to develop alternative techniques for early screening of resistance to WB.

Marker assisted selection

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

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

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

## Conclusion

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

As the work of characterisation and evaluation continues, further selections of priority germplasm will be possible. In addition, practical results from the germplasm enhancement programme will soon be forthcoming after completing some basic field observations. A number of selections from BP resistant populations have already been 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 marker based selection techniques, together with well-documented information on genetic diversity, could lead to unprecedented progress in cocoa breeding in the foreseeable future.

[1] Association des industries de la chocolaterie, biscuiteries et confiserie de l'UE

# Conservation

# Safeguarding the International Cocoa Genebank, Trinidad: a global resource for the cocoa industry

**B. Latchman, Valmiki Singh and D.R. Butler**

The year 2009 marked the end of the Dutch LNV Project "Safeguarding the International Cocoa Genebank, Trinidad". The Project started in 2006 and its objectives were as follows:

- To upgrade the irrigation system at the University Cocoa Research Station.
- To establish a new propagation facility on the University of the West Indies St. Augustine Campus to propagate rooted cuttings.
- To re-introduce clones that were not represented in the UCRS, from historic genebanks (Marper Farm, San Juan Estate, and Campus fields) in Trinidad.
- To improve the security at the UCRS.

## Upgrading the irrigation system

When the ICG,T was originally established at the UCRS an irrigation system was installed that used water exclusively from the Caroni River which forms the northern boundary of the UCRS site. This irrigation system has proved to be inefficient in terms of water distribution and costly to operate, so it could no longer meet the growing demands of the genebank. In addition, in 2003 to the country's water authority imposed severe restrictions on the quantity of water that could be abstracted from the river for irrigation.

Through the Dutch LNV project the irrigation system was upgraded to include:

- Two large reservoirs capable of supplying 25,000$m^3$ of water, which meets half the annual irrigation requirement of the ICG,T at present;
- A connection to the Caroni River that will allow the abstraction of the remaining irrigation requirement;
- A network of subterranean pipes throughout the cocoa fields;
- A series of valves and hydrants to allow surface irrigation of every tree at UCRS.

The system now fully operational puts each plant at the UCRS within a 30m working radius of a hydrant (see Figure 1.) with a much reduced operating cost.

## Establishment of a facility to propagate rooted cuttings

Field 4A within at the UCRS was established in the 1990s as a nursery plot containing 554 clones that had been propagated with three or four replicates as grafted plants. To eliminate the risk of confusion between rootstock and scion, one component of the LNV project was to re-propagate these clones as rooted cuttings and re-establish them in the main plots of the ICG,T. To accomplish this, a propagation facility was built at the St. Augustine Campus of UWI and used in conjunction with a propagation facility of the Agricultural Services Division MALMR, at the La Reunion Estate. The propagation facility on campus comprised of 24 plastic covered propagation chambers with an automated misting system and MALMR used conventional concrete propagation bins. With both these facilities, it was possible to attempt a large number of cuttings.

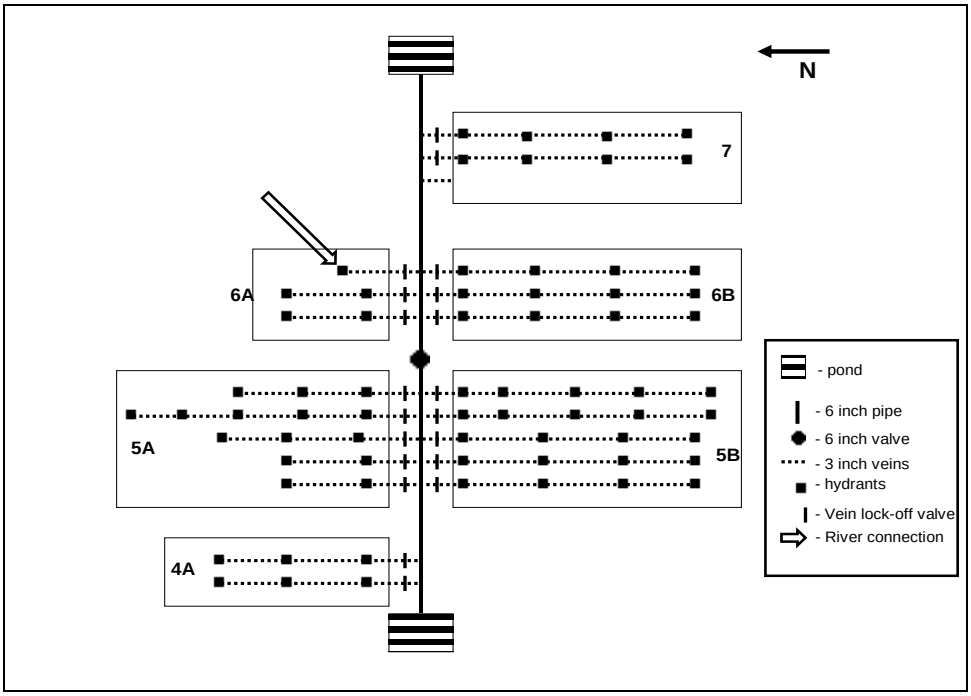


**Figure 1. Diagrammatic representation of the Irrigation System at UCRS.**

Over 53,000 rooted cuttings from 532 clones were attempted between 2006 and the end of 2009, repeating clones that proved difficult to root. On each collection date, 20 cuttings of each clone were collected for propagation on campus, and 25 cuttings were taken for propagation at MALMR. More than 52% of the clones were attempted more than twice and some were repeated up to six times. During the propagation exercise it was noted that root initiation for certain clones was extremely difficult and took as long as eleven weeks whilst others rooted within the expected four-week period. It was also noted that the survival during the hardening phase varied from clone to clone, giving an overall success after hardening of 12% at MALMR and 15% on campus in spite of better rooting success in the propagation chambers. Details of the number of cuttings attempted and survival after hardening are given in Table 1.

**Table 1. Number of cuttings collected (C) and the number of surviving plants(S).**

| Clone | C | S | Clone | C | S | Clone | C | S |
|---|---|---|---|---|---|---|---|---|
| AGU 2 [CHA] | 120 | 22 | ICS 31 | 75 | 17 | NA 406 | 150 | 8 |
| AM 1/8 [POU] | 120 | 2 | ICS 35 | 50 | 17 | NA 423 | 95 | 31 |
| AM 1/12 [POU] | 120 | 0 | ICS 39 | 75 | 1 | NA 435 | 75 | 14 |
| AM 1/40 [POU] | 20 | 0 | ICS 42 | 125 | 9 | NA 471 | 100 | 0 |
| AM 1/53 [POU] | 120 | 9 | ICS 53 | 70 | 18 | NA 475 | 25 | 0 |
| AM 1/68 [POU] | 140 | 5 | ICS 56 | 50 | 25 | NA 507 | 150 | 17 |
| AM 1/70 [POU] | 70 | 22 | ICS 58 | 65 | 14 | NA 669 | 120 | 15 |
| AM 1/85 [POU] | 70 | 25 | ICS 62 | 45 | 10 | NA 678 | 25 | 0 |
| AM 1/87 [POU] | 190 | 15 | ICS 73 | 75 | 6 | NA 687 | 25 | 0 |

| | | |
|---|---|---|
| AM 1/88 [POU] | 45 | 1 |
| AM 1/96 [POU] | 70 | 24 |
| AM 1/97 [POU] | 140 | 6 |
| AM 1/107 [POU] | 115 | 6 |
| AM 1/109 [POU] | 75 | 9 |
| AM 2/1 [POU] | 115 | 6 |
| AM 2/3 [POU] | 115 | 4 |
| AM 2/6 [POU] (Plot 557) | 70 | 3 |
| AM 2/9 [POU] | 145 | 2 |
| AM 2/14 [POU] | 70 | 0 |
| AM 2/18 [POU] | 115 | 5 |
| AM 2/19 [POU] | 70 | 36 |
| AM 2/20 [POU] | 115 | 11 |
| AM 2/21 [POU] | 70 | 9 |
| AM 2/28 [POU] | 45 | 10 |
| AM 2/31 [POU] | 65 | 18 |
| AM 2/41 [POU] | 45 | 28 |
| AM 2/45 [POU] | 45 | 15 |
| AM 2/88 [POU] | 95 | 15 |
| AM 2/96 [POU] | 45 | 10 |
| AMAZ 5/2 [CHA] | 95 | 26 |
| AMAZ 6 [CHA] | 25 | 11 |
| AMAZ 10/1 [CHA] | 195 | 31 |
| AMAZ 11 [CHA] | 195 | 5 |
| AMAZ 15/15 [CHA] | 70 | 3 |
| B 5/11 [POU] | 95 | 7 |
| B 11/2 [POU] | 70 | 1 |
| B 13/5 [POU] | 70 | 6 |
| B 14/13 [POU] | 70 | 1 |
| B 14/14 [POU] | 70 | 1 |
| B 22/3 [POU] | 95 | 15 |
| C 96 [TRI] | 45 | 11 |
| C 97 [TRI] | 70 | 15 |
| C 99 [TRI] | 143 | 26 |
| CC 9 | 40 | 13 |
| CC 10 | 70 | 11 |
| CC 17 | 120 | 35 |
| CC 37 | 145 | 18 |
| CC 38 | 95 | 17 |
| CC 39 | 50 | 12 |
| CC 40 | 120 | 21 |
| CC 41 | 145 | 14 |
| CC 49 | 195 | 18 |
| CC 54 | 120 | 42 |
| CC 71 | 70 | 3 |
| CERRO AZUL 10 | 70 | 2 |
| CL 9/7 | 165 | 9 |
| CL 9/11 | 140 | 12 |
| CL 9/12 | 115 | 12 |
| CL 9/19 | 190 | 3 |

| | | |
|---|---|---|
| ICS 77 | 90 | 16 |
| ICS 81 | 95 | 3 |
| ICS 82 | 100 | 27 |
| ICS 88 | 75 | 17 |
| IMC 10 | 75 | 6 |
| IMC 18 | 100 | 4 |
| IMC 49 | 50 | 0 |
| IMC 50 | 50 | 0 |
| JA 1/5 [POU] | 75 | 5 |
| JA 1/9 [POU] | 125 | 26 |
| JA 2/12 [POU] | 45 | 8 |
| JA 3/4 [POU] | 70 | 11 |
| JA 3/37 [POU] | 70 | 20 |
| JA 5/11 [POU] | 70 | 24 |
| JA 5/27 [POU] | 25 | 9 |
| JA 8/42 [POU] | 50 | 23 |
| JA 9/1 [POU] | 125 | 4 |
| JA 10/34 [POU] | 150 | 0 |
| JA 10/35 [POU] | 125 | 18 |
| JA 10/51 [POU] | 175 | 2 |
| JA 10/58 [POU] | 125 | 2 |
| LCT EEN 6/S-1 | 50 | 10 |
| LCT EEN 15/S-3 | 200 | 22 |
| LCT EEN 20/S-10 | 150 | 12 |
| LCT EEN 21/S-4 | 175 | 16 |
| LCT EEN 23 | 100 | 25 |
| LCT EEN 37/F | 50 | 12 |
| LCT EEN 46 | 225 | 9 |
| LCT EEN 62/S-4 | 100 | 17 |
| LCT EEN 66 | 150 | 0 |
| LCT EEN 67 | 150 | 0 |
| LCT EEN 72 | 125 | 10 |
| LCT EEN 82 | 125 | 4 |
| LCT EEN 83/S-8 | 125 | 6 |
| LCT EEN 84 | 190 | 0 |
| LCT EEN 85 | 175 | 25 |
| LCT EEN 90 | 125 | 0 |
| LCT EEN 90/S-7 | 145 | 6 |
| LCT EEN 127 | 100 | 7 |
| LCT EEN 162/S-1010 | 115 | 13 |
| LCT EEN 163/A | 150 | 1 |
| LCT EEN 163/D | 150 | 10 |
| LCT EEN 201 | 125 | 0 |
| LCT EEN 202 | 175 | 16 |
| LCT EEN 203/S-3 | 75 | 4 |
| LCT EEN 212/S-4 | 100 | 4 |
| LCT EEN 246 | 100 | 9 |
| LCT EEN 250 | 100 | 0 |
| LCT EEN 251 | 125 | 0 |
| LCT EEN 261/S-4 | 150 | 22 |

| | | |
|---|---|---|
| NA 689 | 75 | 3 |
| NA 691 | 100 | 9 |
| NA 717 | 75 | 6 |
| NA 720 | 70 | 5 |
| NA 721 | 145 | 8 |
| NA 724 | 25 | 0 |
| NA 728 | 50 | 25 |
| NA 732 | 25 | 0 |
| NA 764 | 25 | 0 |
| NA 766 | 50 | 6 |
| NA 770 | 75 | 6 |
| NA 780 | 100 | 10 |
| NA 782 | 75 | 26 |
| NA 794 | 20 | 1 |
| NA 824 | 25 | 0 |
| NA 835 | 100 | 16 |
| OC 61 [VEN] | 25 | 0 |
| PA 20 [PER] | 120 | 8 |
| PA 45 [PER] | 125 | 10 |
| PA 72 [PER] | 75 | 0 |
| PA 81 [PER] | 100 | 11 |
| PA 90 [PER] | 100 | 10 |
| PA 105 [PER] | 100 | 4 |
| PA 118 [PER] | 125 | 11 |
| PA 124 [PER] | 100 | 8 |
| PA 125 [PER] | 100 | 1 |
| PA 134 [PER] | 45 | 0 |
| PA 135 [PER] | 70 | 12 |
| PA 139 [PER] | 100 | 4 |
| PA 176 [PER] | 100 | 4 |
| PA 187 [PER] | 45 | 1 |
| PA 189 [PER] | 100 | 16 |
| PA 194 [PER] | 125 | 5 |
| PA 200 [PER] | 95 | 1 |
| PA 207 [PER] | 75 | 0 |
| PA 275 [PER] | 50 | 0 |
| PA 289 [PER] | 70 | 5 |
| PA 293 [PER] | 50 | 0 |
| RIM 2 [MEX] | 70 | 32 |
| RIM 6 [MEX] | 50 | 19 |
| RIM 8 [MEX] | 125 | 25 |
| RIM 10 [MEX] | 25 | 12 |
| RIM 12 [MEX] | 50 | 12 |
| RIM 13 [MEX] | 50 | 23 |
| RIM 19 [MEX] | 45 | 12 |
| RIM 24 [MEX] | 75 | 15 |
| RIM 41 [MEX] | 50 | 21 |
| RIM 48 [MEX] | 45 | 19 |
| RIM 71 [MEX] | 25 | 8 |
| RIM 75 [MEX] | 75 | 23 |

| | | |
|---|---|---|
| CL 9/51 | 91 | 14 |
| CL 10/3 | 45 | 11 |
| CL 10/10 | 90 | 13 |
| CL 10/11 | 115 | 23 |
| CL 10/14 | 140 | 7 |
| CL 10/17 | 100 | 2 |
| CL 10/23 | 90 | 0 |
| CL 10/25 | 70 | 1 |
| CL 10/33 | 95 | 17 |
| CL 13/17 | 140 | 7 |
| CL 13/35 | 120 | 6 |
| CL 13/36 | 70 | 0 |
| CL 13/41 | 95 | 10 |
| CL 13/43 | 145 | 17 |
| CL 13/65 | 70 | 17 |
| CL 19/2 | 90 | 11 |
| CL 19/10 | 71 | 11 |
| CL 19/21 | 65 | 32 |
| CL 19/33 | 160 | 0 |
| CL 19/36 | 140 | 11 |
| CL 19/41 | 65 | 20 |
| CL 19/42 | 115 | 9 |
| CL 27/7 | 115 | 13 |
| CL 27/14 | 120 | 27 |
| CL 27/21 | 90 | 3 |
| CL 27/34 | 260 | 7 |
| CL 27/49 | 120 | 23 |
| CL 27/71 | 70 | 0 |
| CL 27/72 | 115 | 2 |
| CL 27/74 | 195 | 12 |
| CL 27/109 | 70 | 9 |
| CL 78/2 | 90 | 30 |
| CLM 6 | 45 | 0 |
| CLM 35 | 70 | 0 |
| CLM 65 | 70 | 2 |
| CLM 78 | 115 | 1 |
| CRU 4A/1 | 45 | 3 |
| CRU 4A/2 | 126 | 3 |
| CRU 4A/3 | 145 | 9 |
| CRU 4A/4 | 170 | 13 |
| CRU 4A/5 | 145 | 22 |
| CRU 4A/6 | 170 | 12 |
| CRU 4A/7 | 70 | 11 |
| CRU 4A/8 | 70 | 17 |
| CRU 4A/9 | 70 | 0 |
| CRU 4A/10 | 45 | 0 |
| CRU 4A/11 | 70 | 0 |
| CRU 270[1] | 70 | 11 |
| CRU 271[2] | 50 | 9 |
| DOM 1 | 70 | 15 |

| | | |
|---|---|---|
| LCT EEN 280 | 150 | 21 |
| LCT EEN 325 | 100 | 0 |
| LCT EEN 326 | 25 | 8 |
| LCT EEN 327 | 175 | 0 |
| LCT EEN 332 | 125 | 1 |
| LP 1/20 [POU] | 170 | 11 |
| LP 1/25 [POU] | 145 | 10 |
| LP 1/37 [POU] | 100 | 7 |
| LP 2/11 [POU] | 45 | 15 |
| LP 3/19 [POU] | 145 | 6 |
| LP 4/5 [POU] | 50 | 0 |
| LP 4/15 [POU] | 70 | 9 |
| LP 4/45 [POU] | 70 | 14 |
| LP 5/1 [POU] | 95 | 14 |
| LP 5/3 [POU] | 50 | 0 |
| LV 2 [POU] | 175 | 0 |
| LV 9 [POU] | 75 | 7 |
| LV 10 [POU] | 150 | 16 |
| LV 14 [POU] | 75 | 13 |
| LV 17 [POU] | 50 | 8 |
| LV 27 [POU] | 150 | 5 |
| LV 33 [POU] | 75 | 20 |
| LV 37 [POU] | 220 | 17 |
| LX 1 | 120 | 10 |
| LX 2 | 175 | 21 |
| LX 18 | 75 | 3 |
| LX 24 | 125 | 19 |
| LX 41 | 245 | 2 |
| LZ 4 | 75 | 3 |
| LZ 5 | 75 | 23 |
| LZ 7 | 125 | 9 |
| LZ 8 | 125 | 20 |
| LZ 17 | 200 | 7 |
| MAR 1 | 75 | 27 |
| MAR 3 | 75 | 27 |
| MAR 9 | 75 | 10 |
| MAR 10 | 100 | 2 |
| MAR 11 | 100 | 21 |
| MAR 12 | 75 | 20 |
| MAR 13 | 70 | 17 |
| MAR 14 | 75 | 15 |
| MAR 17 | 50 | 4 |
| MAR 19 | 150 | 15 |
| MAR 20 | 75 | 5 |
| MAR 21 | 100 | 14 |
| MAR 22 | 25 | 20 |
| MO 82 | 250 | 1 |
| MO 87 | 100 | 9 |
| MO 96 | 75 | 7 |
| MOQ 1/12 | 25 | 12 |

| | | |
|---|---|---|
| RIM 76 [MEX] | 75 | 8 |
| RIM 101 [MEX] | 50 | 26 |
| RIM 106 [MEX] | 50 | 22 |
| RIM 113 [MEX] | 45 | 12 |
| RIM 117 [MEX] | 75 | 22 |
| SC 1 [COL] | 20 | 9 |
| SC 3 [COL] | 100 | 29 |
| SC 4 [COL] | 75 | 16 |
| SC 5 [COL] | 50 | 8 |
| SC 6 [COL] | 25 | 14 |
| SC 7 [COL] | 75 | 10 |
| SC 11 [COL] | 45 | 7 |
| SC 12 [COL] | 50 | 14 |
| SC 15 [COL] | 145 | 14 |
| SC 17 [COL] | 25 | 11 |
| SC 19 [COL] | 75 | 15 |
| SC 20 [COL] | 75 | 25 |
| SJ 1/1 [POU] | 75 | 0 |
| SJ 1/10 [POU] | 100 | 11 |
| SJ 1/11 [POU] | 125 | 1 |
| SJ 1/18 [POU] | 175 | 5 |
| SJ 1/28 [POU] | 220 | 5 |
| SJ 1/29 [POU] | 75 | 7 |
| SJ 1/33 [POU] | 175 | 8 |
| SJ 1/37 [POU] | 200 | 3 |
| SJ 2/12 [POU] | 150 | 8 |
| SJ 2/17 [POU] | 100 | 12 |
| SJ 2/26 [POU] | 125 | 12 |
| SLA 10 | 75 | 2 |
| SLA 13 | 145 | 17 |
| SLA 48 | 175 | 8 |
| SLA 77 | 125 | 4 |
| SLA 95 | 100 | 19 |
| SM 1 [POU] | 50 | 0 |
| SM 5 [POU] | 100 | 19 |
| SM 9 [POU] | 75 | 1 |
| SM 10 [POU] | 25 | 7 |
| SPA 12 [COL] | 95 | 21 |
| SPA 16 [COL] | 100 | 9 |
| SPA 18 [COL] | 50 | 24 |
| SPA 20 [COL] | 50 | 9 |
| SPEC 41/6 | 125 | 26 |
| TRD 1 | 175 | 16 |
| TRD 2 | 25 | 11 |
| TRD 3 | 125 | 28 |
| TRD 5 | 25 | 11 |
| TRD 6 | 175 | 33 |
| TRD 7 | 150 | 11 |
| TRD 8 | 145 | 26 |
| TRD 9 | 175 | 22 |

| DOM 3 | 170 | 29 |
|---|---|---|
| DOM 4 | 70 | 9 |
| DOM 5 | 95 | 14 |
| DOM 7 | 70 | 23 |
| DOM 8 | 95 | 8 |
| DOM 9 | 66 | 11 |
| DOM 10 | 65 | 15 |
| DOM 13 | 120 | 17 |
| DOM 14 | 145 | 16 |
| DOM 15 | 170 | 19 |
| DOM 16 | 145 | 30 |
| DOM 18 | 170 | 18 |
| DOM 20 | 100 | 3 |
| DOM 21 | 95 | 17 |
| DOM 23 | 70 | 17 |
| DOM 24 | 145 | 15 |
| DOM 25 | 20 | 11 |
| DOM 27 | 95 | 14 |
| DOM 30 | 45 | 28 |
| DOM 31 | 95 | 16 |
| DOM 33 | 95 | 35 |
| DOM 34 | 120 | 23 |
| DOM 35 | 21 | 9 |
| FSC 13 | 25 | 9 |
| GNV 22 | 20 | 4 |
| GS 4 | 140 | 12 |
| GS 6 | 25 | 16 |
| GS 12 | 125 | 11 |
| GS 13 | 150 | 5 |
| GS 37 | 150 | 16 |
| GS 39 | 100 | 3 |
| GS 45 | 125 | 10 |
| GS 55 | 50 | 15 |
| GS 58 | 125 | 20 |
| GS 59 | 125 | 9 |
| GS 61 | 100 | 17 |
| GS 62 | 125 | 10 |
| GU 114/P | 20 | 14 |
| GU 151/F | 145 | 16 |
| GU 175/P | 120 | 16 |
| GU 195/P | 100 | 13 |
| GU 219/F | 120 | 21 |
| GU 222 | 25 | 18 |
| GU 241/P | 120 | 25 |
| GU 243/H | 45 | 25 |
| GU 255/P | 25 | 12 |
| GU 261/P | 65 | 13 |
| GU 265/P | 70 | 15 |
| GU 271/P | 25 | 15 |
| GU 277/G | 25 | 17 |

| MOQ 1/21 | 150 | 0 |
|---|---|---|
| MOQ 1/24 | 225 | 2 |
| MOQ 2/28 | 95 | 1 |
| MOQ 2/31 | 175 | 6 |
| MOQ 3/1 | 75 | 0 |
| MOQ 3/16 | 100 | 11 |
| MOQ 4/2 | 70 | 1 |
| MOQ 4/16 | 195 | 0 |
| MOQ 4/21 | 100 | 0 |
| MOQ 4/23 | 125 | 0 |
| MOQ 4/25 | 225 | 9 |
| MOQ 5/12 | 125 | 11 |
| MOQ 5/29 | 125 | 17 |
| MOQ 5/34 | 225 | 15 |
| MOQ 6/5 | 50 | 23 |
| MOQ 6/28 | 25 | 12 |
| MOQ 6/52 | 250 | 1 |
| MOQ 6/72 | 100 | 10 |
| MOQ 6/73 | 175 | 19 |
| MOQ 6/85 | 145 | 5 |
| MOQ 6/91 | 50 | 17 |
| MOQ 6/92 | 145 | 0 |
| MOQ 6/103 | 95 | 15 |
| MOQ 6/107 | 70 | 9 |
| MOQ 6/113 | 75 | 0 |
| NA 1 | 200 | 20 |
| NA 13 | 125 | 18 |
| NA 19 | 175 | 16 |
| NA 26 | 125 | 16 |
| NA 33 | 125 | 14 |
| NA 39 | 75 | 15 |
| NA 45 | 125 | 18 |
| NA 47 | 225 | 8 |
| NA 49 | 25 | 12 |
| NA 58 | 125 | 2 |
| NA 61 | 170 | 10 |
| NA 74 | 75 | 22 |
| NA 81 | 50 | 9 |
| NA 92 | 100 | 5 |
| NA 95 | 70 | 20 |
| NA 104 | 125 | 21 |
| NA 110 | 50 | 12 |
| NA 111 | 150 | 23 |
| NA 112 | 75 | 11 |
| NA 113 | 100 | 4 |
| NA 114 | 125 | 11 |
| NA 127 | 150 | 9 |
| NA 157 | 75 | 10 |
| NA 170 | 150 | 18 |
| NA 176 | 150 | 15 |

| TRD 13 | 170 | 51 |
|---|---|---|
| TRD 15 | 85 | 21 |
| TRD 16 | 25 | 10 |
| TRD 18 | 95 | 6 |
| TRD 19 | 175 | 31 |
| TRD 23 | 150 | 13 |
| TRD 24 | 150 | 21 |
| TRD 27 | 125 | 24 |
| TRD 28 | 150 | 14 |
| TRD 29 | 100 | 26 |
| TRD 30 | 70 | 19 |
| TRD 32 | 90 | 20 |
| TRD 33 | 90 | 22 |
| TRD 34 | 25 | 10 |
| TRD 35 | 45 | 13 |
| TRD 37 | 175 | 15 |
| TRD 38 | 200 | 28 |
| TRD 39 | 165 | 2 |
| TRD 41 | 20 | 12 |
| TRD 42 | 140 | 27 |
| TRD 43 | 90 | 15 |
| TRD 44 | 150 | 34 |
| TRD 45 | 25 | 13 |
| TRD 46 | 175 | 18 |
| TRD 47 | 25 | 13 |
| TRD 48 | 200 | 8 |
| TRD 49 | 125 | 13 |
| TRD 50 | 150 | 18 |
| TRD 52 | 150 | 14 |
| TRD 53 | 175 | 11 |
| TRD 58 | 150 | 9 |
| TRD 60 | 90 | 12 |
| TRD 65 | 90 | 18 |
| TRD 66 | 65 | 24 |
| TRD 71 | 100 | 24 |
| TRD 75 | 125 | 11 |
| TRD 77 | 150 | 23 |
| TRD 79 | 175 | 13 |
| TRD 81 | 175 | 22 |
| TRD 85 | 25 | 9 |
| TRD 86 | 125 | 17 |
| TRD 88 | 185 | 36 |
| TRD 90 | 50 | 8 |
| TRD 92 | 200 | 32 |
| TRD 93 | 125 | 5 |
| TRD 94 | 145 | 30 |
| TRD 95 | 145 | 23 |
| TRD 99 | 175 | 28 |
| TRD 108 | 175 | 23 |
| TRD 109 | 175 | 34 |

| | | |
|---|---|---|
| GU 286/P | 25 | 19 |
| GU 300/P | 20 | 14 |
| GU 305/P | 25 | 18 |
| GU 307/F | 50 | 17 |
| GU 310/P | 45 | 11 |
| GU 322/P | 25 | 12 |
| GU 335/P | 25 | 13 |
| GU 339/M | 45 | 27 |
| GU 351/P | 20 | 9 |
| GU 353/L | 25 | 3 |
| ICA 70 [COL] | 125 | 3 |
| ICS 2 | 75 | 20 |
| ICS 3 | 25 | 18 |
| ICS 12 | 120 | 43 |
| ICS 15 | 75 | 11 |
| ICS 20 | 120 | 21 |
| ICS 23 | 50 | 8 |
| ICS 28 | 75 | 3 |
| ICS 30 | 75 | 1 |

| | | |
|---|---|---|
| NA 178 | 100 | 11 |
| NA 191 | 50 | 16 |
| NA 204 | 100 | 10 |
| NA 214 | 100 | 33 |
| NA 229 | 50 | 9 |
| NA 241 | 5- | 7 |
| NA 244 | 175 | 28 |
| NA 246 | 100 | 17 |
| NA 251 | 150 | 1 |
| NA 271 | 125 | 10 |
| NA 277 | 75 | 1 |
| NA 326 | 75 | 9 |
| NA 327 | 50 | 13 |
| NA 331 | 75 | 22 |
| NA 339 | 150 | 16 |
| NA 370 | 100 | 5 |
| NA 372 | 125 | 16 |
| NA 395 | 100 | 5 |
| NA 399 | 75 | 1 |

| | | |
|---|---|---|
| TRD 110 | 150 | 18 |
| TRD 111 | 145 | 5 |
| TRD 112 | 125 | 8 |
| TRD 113 | 100 | 18 |
| TRD 114 | 175 | 25 |
| TRD 115 | 175 | 32 |
| TRD 116 | 20 | 11 |
| TRD 117 | 25 | 14 |
| TRD 118 | 175 | 20 |
| TRD 119 | 25 | 12 |
| UF 4 | 150 | 10 |
| UF 38 | 200 | 18 |
| UF 122 | 175 | 12 |
| UF 602 | 220 | 36 |
| UF 613 | 120 | 24 |
| UF 700 | 125 | 12 |
| UF 705 | 75 | 0 |
| UF 709 | 125 | 10 |

[1]Renamed clone CRU 270 (MIS_TTOICGT_CBO 177 [VEN])
[2]Renamed clone CRU 271 (MIS_TTOICGT_ICS 55)

**Establishing rooted cuttings in the field.**

In total 2,074 plants have been established from 403 clones in Fields 5A and 5B at the UCRS (Table 2). The improved irrigation system proved to be invaluable since the newly introduced cuttings needed regular irrigation. Re-designing of plots to accommodate 8 or 9 replicates (Latchman et al., 2008) has continued to make optimal use of limited land within the UCRS.

**Table 2. Clones transplanted as rooted cuttings and the number (N) of plants per clone.**

| Clone | N |
|---|---|
| AGU 2 [CHA] | 3 |
| AM 1/8 [POU] | 2 |
| AM 1/53 [POU] | 2 |
| AM 1/68 [POU] | 2 |
| AM 1/70 [POU] | 9 |
| AM 1/85 [POU] | 9 |
| AM 1/87 [POU] | 2 |
| AM 1/96 [POU] | 9 |
| AM 1/97 [POU] | 2 |
| AM 1/107 [POU] | 2 |
| AM 1/109 [POU] | 6 |
| AM 2/1 [POU] | 4 |
| AM 2/3 [POU] | 2 |
| AM 2/6 [POU] | 10 |
| AM 2/18 [POU] | 4 |
| AM 2/19 [POU] | 9 |
| AM 2/20 [POU] | 4 |

| Clone | N |
|---|---|
| DOM 23 | 4 |
| DOM 24 | 2 |
| DOM 25 | 12 |
| DOM 27 | 2 |
| DOM 30 | 8 |
| DOM 31 | 4 |
| DOM 33 | 3 |
| DOM 35 | 12 |
| FSC 13 | 9 |
| GNV 22 | 4 |
| GS 4 | 3 |
| GS 6 | 8 |
| GS 12 | 4 |
| GS 37 | 2 |
| GS 55 | 7 |
| GS 58 | 1 |
| GS 61 | 5 |

| Clone | N |
|---|---|
| LV 37 [POU] | 6 |
| LX 1 | 7 |
| LX 2 | 2 |
| LX 18 | 1 |
| LX 24 | 9 |
| LX 41 | 4 |
| LZ 5 | 7 |
| LZ 8 | 6 |
| MAR 1 | 4 |
| MAR 10 | 2 |
| MAR 11 | 3 |
| MAR 12 | 2 |
| MAR 13 | 3 |
| MAR 14 | 1 |
| MAR 19 | 6 |
| MAR 21 | 1 |
| MAR 22 | 9 |

| Clone | N |
|---|---|
| RIM 13 [MEX] | 9 |
| RIM 19 [MEX] | 9 |
| RIM 24 [MEX] | 4 |
| RIM 41 [MEX] | 9 |
| RIM 48 [MEX] | 9 |
| RIM 71 [MEX] | 8 |
| RIM 75 [MEX] | 4 |
| RIM 76 [MEX] | 3 |
| RIM 101 [MEX] | 9 |
| RIM 106 [MEX] | 9 |
| RIM 113 [MEX] | 9 |
| SC 1 [COL] | 9 |
| SC 3 [COL] | 4 |
| SC 4 [COL] | 6 |
| SC 5 [COL] | 8 |
| SC 6 [COL] | 9 |
| SC 7 [COL] | 4 |

| | |
|---|---|
| AM 2/21 [POU] | 9 |
| AM 2/28 [POU] | 9 |
| AM 2/31 [POU] | 9 |
| AM 2/41 [POU] | 9 |
| AM 2/45 [POU] | 9 |
| AM 2/96 [POU] | 12 |
| AMAZ 5/2 [CHA] | 4 |
| AMAZ 6 [CHA] | 9 |
| AMAZ 10/1 [CHA] | 5 |
| B 5/11 [POU] | 2 |
| B 13/5 [POU] | 2 |
| B 14/13 [POU] | 1 |
| B 22/3 [POU] | 4 |
| C 96 [TRI] | 9 |
| C 97 [TRI] | 6 |
| C 99 [TRI] | 9 |
| CC 9 | 9 |
| CC 10 | 9 |
| CC 10 | 2 |
| CC 17 | 2 |
| CC 37 | 4 |
| CC 38 | 6 |
| CC 39 | 9 |
| CC 40 | 8 |
| CC 41 | 4 |
| CC 49 | 3 |
| CC 54 | 4 |
| CC 71 | 3 |
| CL 9/7 | 1 |
| CL 9/11 | 4 |
| CL 9/12 | 1 |
| CL 9/19 | 1 |
| CL 9/51 | 8 |
| CL 10/3 | 9 |
| CL 10/10 | 6 |
| CL 10/11 | 9 |
| CL 10/14 | 4 |
| CL 10/25 | 4 |
| CL 10/33 | 9 |
| CL 13/17 | 2 |
| CL 13/35 | 3 |
| CL 13/36 | 1 |
| CL 13/41 | 8 |
| CL 13/43 | 2 |
| CL 13/65 | 8 |
| CL 19/2 | 2 |
| CL 19/10 | 7 |
| CL 19/21 | 8 |
| CL 19/33 | 3 |
| CL 19/36 | 4 |

| | |
|---|---|
| GS 62 | 4 |
| GU 114/P | 9 |
| GU 151/F | 2 |
| GU 175/P | 5 |
| GU 195/P | 1 |
| GU 219/F | 6 |
| GU 222 | 9 |
| GU 235/P | 2 |
| GU 241/P | 4 |
| GU 243/H | 9 |
| GU 255/P | 9 |
| GU 261/P | 9 |
| GU 265/P | 9 |
| GU 271/P | 9 |
| GU 277/G | 9 |
| GU 286/P | 9 |
| GU 300/P | 9 |
| GU 305/P | 9 |
| GU 307/F | 2 |
| GU 310/P | 9 |
| GU 322/P | 9 |
| GU 335/P | 9 |
| GU 339/M | 12 |
| GU 351/P | 9 |
| GU 353/L | 9 |
| ICA 70 | 1 |
| ICS 2 | 5 |
| ICS 3 | 9 |
| ICS 12 | 12 |
| ICS 15 | 4 |
| ICS 20 | 3 |
| ICS 31 | 9 |
| ICS 35 | 9 |
| ICS 42 | 6 |
| ICS 53 | 9 |
| ICS 56 | 3 |
| ICS 58 | 9 |
| ICS 62 | 9 |
| ICS 73 | 1 |
| ICS 77 | 2 |
| ICS 81 | 3 |
| ICS 82 | 4 |
| ICS 88 | 8 |
| IMC 18 | 1 |
| JA 1/9 [POU] | 5 |
| JA 2/12 [POU] | 8 |
| JA 3/4 [POU] | 9 |
| JA 3/37 [POU] | 9 |
| JA 5/11 [POU] | 9 |
| JA 5/27 [POU] | 9 |

| | |
|---|---|
| MO 87 | 2 |
| MO 96 | 2 |
| MOQ 1/12 | 9 |
| MOQ 1/12 | 1 |
| MOQ 1/24 | 2 |
| MOQ 2/31 | 2 |
| MOQ 3/16 | 4 |
| MOQ 4/2 | 8 |
| MOQ 4/16 | 4 |
| MOQ 4/25 | 5 |
| MOQ 5/29 | 4 |
| MOQ 5/34 | 4 |
| MOQ 6/5 | 9 |
| MOQ 6/28 | 9 |
| MOQ 6/72 | 1 |
| MOQ 6/73 | 4 |
| MOQ 6/85 | 2 |
| MOQ 6/91 | 9 |
| MOQ 6/107 | 9 |
| NA 1 | 5 |
| NA 13 | 1 |
| NA 19 | 4 |
| NA 26 | 7 |
| NA 33 | 5 |
| NA 39 | 9 |
| NA 45 | 2 |
| NA 49 | 9 |
| NA 58 | 1 |
| NA 61 | 1 |
| NA 74 | 4 |
| NA 81 | 9 |
| NA 92 | 1 |
| NA 95 | 15 |
| NA 104 | 8 |
| NA 110 | 9 |
| NA 111 | 2 |
| NA 112 | 9 |
| NA 114 | 4 |
| NA 157 | 6 |
| NA 170 | 4 |
| NA 176 | 2 |
| NA 178 | 8 |
| NA 191 | 7 |
| NA 204 | 9 |
| NA 214 | 7 |
| NA 218 | 1 |
| NA 229 | 9 |
| NA 241 | 1 |
| NA 244 | 4 |
| NA 246 | 12 |

| | |
|---|---|
| SC 11 [COL] | 7 |
| SC 12 [COL] | 9 |
| SC 15 [COL] | 2 |
| SC 17 [COL] | 9 |
| SC 19 [COL] | 9 |
| SC 20 [COL] | 3 |
| SIAL 330 | 4 |
| SJ 1/10 [POU] | 2 |
| SJ 1/18 [POU] | 2 |
| SJ 1/28 [POU] | 2 |
| SJ 1/29 [POU] | 7 |
| SJ 1/37 [POU] | 1 |
| SJ 2/26 [POU] | 2 |
| SLA 10 | 1 |
| SLA 11 | 1 |
| SLA 13 | 4 |
| SLA 48 | 4 |
| SLA 95 | 3 |
| SM 5 | 7 |
| SPA 12 [COL] | 5 |
| SPA 16 [COL] | 1 |
| SPA 18 [COL] | 9 |
| SPA 20 [COL] | 7 |
| SPEC 41/6 | 3 |
| TRD 1 | 3 |
| TRD 2 | 9 |
| TRD 3 | 3 |
| TRD 5 | 9 |
| TRD 8 | 5 |
| TRD 15 | 9 |
| TRD 16 | 9 |
| TRD 18 | 6 |
| TRD 19 | 4 |
| TRD 23 | 1 |
| TRD 24 | 2 |
| TRD 29 | 2 |
| TRD 30 | 6 |
| TRD 32 | 9 |
| TRD 33 | 9 |
| TRD 34 | 9 |
| TRD 35 | 9 |
| TRD 37 | 1 |
| TRD 38 | 3 |
| TRD 39 | 1 |
| TRD 41 | 8 |
| TRD 42 | 2 |
| TRD 43 | 9 |
| TRD 44 | 9 |
| TRD 45 | 9 |
| TRD 47 | 9 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CL 19/41 | 9 | JA 8/42 [POU] | 9 | NA 271 | 2 | TRD 49 | 4 |
| CL 19/42 | 1 | JA 9/1 [POU] | 1 | NA 326 | 8 | TRD 52 | 1 |
| CL 27/7 | 9 | LCT EEN 6/S-1 | 7 | NA 327 | 4 | TRD 60 | 7 |
| CL 27/14 | 8 | LCT EEN 15/S-3 | 1 | NA 339 | 5 | TRD 65 | 10 |
| CL 27/21 | 2 | LCT EEN 20/S-10 | 4 | NA 370 | 3 | TRD 66 | 9 |
| CL 27/43 | 6 | LCT EEN 23 | 2 | NA 372 | 7 | TRD 71 | 4 |
| CL 27/49 | 9 | LCT EEN 37/F | 7 | NA 395 | 1 | TRD 79 | 2 |
| CL 27/71 | 1 | LCT EEN 46 | 1 | NA 406 | 2 | TRD 81 | 2 |
| CL 27/72 | 1 | LCT EEN 62/S-4 | 2 | NA 423 | 5 | TRD 85 | 9 |
| CL 27/109 | 6 | LCT EEN 82 | 1 | NA 435 | 4 | TRD 86 | 6 |
| CL 78/2 | 8 | LCT EEN 85 | 5 | NA 507 | 2 | TRD 88 | 7 |
| CLM 78 | 1 | LCT EEN 90/S-7 | 2 | NA 669 | 2 | TRD 90 | 8 |
| CRA 4A/4 | 2 | LCT EEN 127 | 2 | NA 689 | 2 | TRD 92 | 11 |
| CRU 4A/1 | 6 | LCT EEN 162/S-1010 | 4 | NA 691 | 5 | TRD 94 | 3 |
| CRU 4A/2 | 3 | LCT EEN 202 | 4 | NA 712 | 1 | TRD 95 | 4 |
| CRU 4A/5 | 2 | LCT EEN 21/S-4 | 6 | NA 717 | 1 | TRD 108 | 2 |
| CRU 4A/7 | 5 | LCT EEN 212/S-4 | 4 | NA 720 | 4 | TRD 109 | 2 |
| CRU 4A/8 | 6 | LCT EEN 246 | 4 | NA 728 | 5 | TRD 110 | 4 |
| CRU 4A/10 | 4 | LCT EEN 261/S-4 | 2 | NA 730 | 1 | TRD 111 | 5 |
| CRU 270[1] | 2 | LCT EEN 280 | 2 | NA 766 | 1 | TRD 112 | 1 |
| CRU 271[2] | 9 | LCT EEN 326 | 8 | NA 770 | 2 | TRD 114 | 4 |
| DOM 1 | 4 | LCT EEN 327 | 1 | NA 780 | 1 | TRD 115 | 1 |
| DOM 3 | 2 | LP 1/25 [POU] | 2 | NA 782 | 1 | TRD 116 | 12 |
| DOM 4 | 4 | LP 2/11 [POU] | 9 | PA 18 [PER] | 1 | TRD 117 | 9 |
| DOM 5 | 5 | LP 3/19 [POU] | 2 | PA 45 [PER] | 1 | TRD 118 | 3 |
| DOM 7 | 9 | LP 4/15 [POU] | 9 | PA 81 [PER] | 2 | TRD 119 | 9 |
| DOM 8 | 8 | LP 4/45 [POU] | 9 | PA 135 [PER] | 3 | UF 4 | 4 |
| DOM 9 | 2 | LP 5/1 [POU] | 9 | PA 194 [PER] | 2 | UF 38 | 4 |
| DOM 10 | 6 | LP 5/3 [POU] | 3 | PA 289 [PER] | 2 | UF 122 | 2 |
| DOM 13 | 1 | LV 9 [POU] | 2 | RIM 2 [MEX] | 9 | UF 602 | 3 |
| DOM 15 | 2 | LV 14 [POU] | 1 | RIM 6 [MEX] | 9 | UF 613 | 2 |
| DOM 16 | 2 | LV 17 [POU] | 8 | RIM 8 [MEX] | 2 | UF 700 | 4 |
| DOM 20 | 2 | LV 27 [POU] | 3 | RIM 10 [MEX] | 9 | UF 709 | 2 |
| DOM 21 | 2 | LV 33 [POU] | 8 | RIM 12 [MEX] | 9 | | |

[1]Renamed clone CRU 270 (MIS_TTOICGT_CBO 177 [VEN])
[2]Renamed clone CRU 271 (MIS_TTOICGT_ICS 55)

### Re-introduction of clones into the ICG,T

Attempts have been made to graft clones present only in historic genebanks and introduce these clones into nursery plots within the ICG,T at the UCRS. At the end of 2009, 827 plants from 241 clones had been introduced into nursery plots in Fields 5A and 5B within the genebank. Table 3 gives the number of grafted plants per clone introduced within the ICG,T.

**Table 3. Numbers of grafted plants (P) established in nursery plots at the UCRS.**

| Clone | P | Clone | P | Clone | P | Clone | P |
|---|---|---|---|---|---|---|---|
| ACT 3/6 [TTO] | 3 | GC T 998/60 | 5 | LP 1/23 [POU] | 8 | MOQ 6/66 | 2 |
| AM 1/39 POU] | 3 | GC T 998/76 | 7 | LP 1/31 [POU] | 3 | MOQ 6/70 | 5 |

| | |
|---|---|
| AM 1/42 [POU] | 5 |
| AM 1/55 [POU] | 4 |
| AM 1/63 [POU] | 4 |
| AM 2/7 [POU] | 1 |
| AM 2/42 [POU] | 5 |
| AM 2/46 [POU] | 6 |
| AM 2/68 [POU] | 4 |
| AM 2/94 [POU] | 5 |
| AS 2 [ECU] | 3 |
| AS 7 [ECU] | 4 |
| B 1/2-8 [POU] | 2 |
| B 3/4 [POU] | 3 |
| B 6/5 [POU] | 3 |
| B 9/10-2 [POU] | 2 |
| B 11/3 [POU] | 2 |
| B 18/8 [POU] | 2 |
| B 23/9 [POU] | 1 |
| CL 10/21 | 2 |
| CL 13/24 | 2 |
| CL 13/32 | 1 |
| CL 15/19-4 | 1 |
| CL 19/35 | 6 |
| CL 19/46 | 2 |
| CL 27/105 | 4 |
| CL 91/6 | 8 |
| CLM 3 | 3 |
| CLM 49 | 1 |
| CLM 61 | 2 |
| CLM 68 | 1 |
| CLM 88 | 5 |
| CLM 107 | 1 |
| CLM 111 | 3 |
| CLM 120 | 3 |
| CLM 122 | 1 |
| CRU 63 | 3 |
| CRU 89 | 4 |
| CRU 114 | 1 |
| CRU 130 | 4 |
| CRU 134 | 1 |
| CRU 135 | 7 |
| CRU 152 | 1 |
| CRU C2/1 | 3 |
| CRU C3/4 | 3 |
| CRU C4/3 | 6 |
| CRU C4/4 | 7 |
| CRU C4/5 | 5 |
| CRU C4/6 | 5 |
| CRU C4/7 | 7 |
| CRU C4/8 | 3 |
| CRU C4/9 | 4 |

| | |
|---|---|
| GC T 998/96 | 3 |
| ICS 6 | 1 |
| ICS 7 | 1 |
| ICS 9 | 1 |
| ICS 10 | 1 |
| ICS 11 | 3 |
| ICS 16 | 1 |
| ICS 26 | 2 |
| ICS 30 | 1 |
| ICS 34 | 3 |
| ICS 40 | 1 |
| ICS 46 | 1 |
| ICS 51 | 1 |
| ICS 60 | 3 |
| ICS 61 | 3 |
| ICS 63 | 3 |
| ICS 65 | 1 |
| ICS 69 | 2 |
| ICS 72 | 2 |
| ICS 73 | 1 |
| ICS 75 | 3 |
| ICS 77 | 3 |
| ICS 79 | 4 |
| ICS 80 | 4 |
| ICS 81 | 2 |
| ICS 82 | 2 |
| ICS 83 | 2 |
| ICS 84 | 1 |
| ICS 86 | 2 |
| ICS 87 | 1 |
| ICS 89 | 1 |
| ICS 90 | 1 |
| ICS 92 | 5 |
| ICS 93 | 2 |
| ICS 97 | 3 |
| ICS 100 | 3 |
| ICS 111 | 3 |
| IMC 67 | 4 |
| JA 1/28 [POU] | 1 |
| JA 2/8 [POU] | 5 |
| JA 2/11 [POU] | 3 |
| JA 2/2 [POU] | 4 |
| JA 2/26 [POU] | 3 |
| JA 3/3 [POU] | 5 |
| JA 3/29 [POU] | 5 |
| JA 3/38 [POU] | 5 |
| JA 3/39 [POU] | 3 |
| JA 4/2 [POU] | 4 |
| JA 4/21 [POU] | 4 |
| JA 5/4 [POU] | 2 |

| | |
|---|---|
| LP 1/34 [POU] | 5 |
| LP 1/36 [POU] | 1 |
| LP 1/42 [POU] | 1 |
| LP 1/47 [POU] | 10 |
| LP 1/56 [POU] | 4 |
| LP 2/2 [POU] | 2 |
| LP 2/10 [POU] | 3 |
| LP 2/16 [POU] | 3 |
| LP 3/38 [POU] | 5 |
| LP 4/2 [POU] | 4 |
| LP 4/41 [POU] | 7 |
| LP 5/23 [POU] | 4 |
| LP 6/12 [POU] | 2 |
| LP 6/16 [POU] | 3 |
| LV 36 [POU] | 3 |
| LX 15 | 1 |
| LX 21 | 4 |
| LX 49 | 3 |
| LZ 9 | 6 |
| LZ 11 | 4 |
| MARPER 2 | 9 |
| MARPER 5 | 5 |
| MARPER 9 | 3 |
| MARPER 18 | 6 |
| MARPER 19 | 1 |
| MARPER 22 | 7 |
| MARPER 27 | 4 |
| MARPER 29 | 5 |
| MARPER 31 | 1 |
| MARPER 33 | 2 |
| MARPER 34 | 3 |
| MARPER 43 | 3 |
| MARPER 46 | 5 |
| MARPER 47 | 7 |
| MARPER 48 | 5 |
| MARPER 50 | 4 |
| MARPER 51 | 3 |
| MARPER 54 | 3 |
| MO 84 | 3 |
| MO 122 | 5 |
| MOQ 1/2 | 2 |
| MOQ 1/6 | 1 |
| MOQ 1/9 | 4 |
| MOQ 1/16 | 4 |
| MOQ 2/7 | 2 |
| MOQ 2/8 | 1 |
| MOQ 2/16 | 4 |
| MOQ 2/37 | 5 |
| MOQ 2/38 | 3 |
| MOQ 3/19 | 5 |

| | |
|---|---|
| MOQ 6/97 | 3 |
| MOQ 6/108 | 7 |
| MX 14/20 [TTO] | 4 |
| NA 38 | 5 |
| NA 48 | 4 |
| NA 69 | 2 |
| NA 79 | 3 |
| NA 106 | 5 |
| NA 119 | 6 |
| NA 120 | 3 |
| NA 151 | 3 |
| NA 156 | 5 |
| NA 242 | 2 |
| NA 254 | 4 |
| NA 256 | 2 |
| NA 260 | 3 |
| NA 368 | 1 |
| NA 681 | 3 |
| NA 694 | 6 |
| NA 695 | 2 |
| NA 713 | 7 |
| NA 725 | 13 |
| NA 734 | 1 |
| NA 747 | 4 |
| NA 751 | 1 |
| NA 916 | 1 |
| PA 98 [PER] | 1 |
| PA 186 [PER] | 6 |
| PA 203 PER] | 2 |
| PA 246 [PER] | 1 |
| PA 288 [PER] | 3 |
| SAN JUAN WSC | 5 |
| SAN MIGUEL 3360/2 | 5 |
| SCA 7 | 2 |
| SCA 16 | 3 |
| SCA 27 | 1 |
| SI 22/1 | 5 |
| SI 22/4 | 5 |
| SI 45/2 | 5 |
| SJ 1/15 [POU] | 3 |
| SJ 1/20 [POU] | 4 |
| SJ 1/22 [POU] | 2 |
| SJ 1/36 [POU] | 5 |
| SJ 2/28 [POU] | 3 |
| SLA 23 | 4 |
| SLA 27 | 2 |
| SLA 28 | 6 |
| SLA 86 | 1 |
| SLA 97 | 2 |
| SLC 1 | 4 |

| | |
|---|---|
| CRU C4/10 | 7 |
| CRU C4/12 | 3 |
| CRU C4/13 | 5 |
| CRU C5/2 | 4 |
| CRU C5/7 | 6 |
| CRU C5/8 | 4 |
| EQX /JS | 3 |
| GC T 998/55 | 6 |
| GC T 998/59 | 4 |

| | |
|---|---|
| JA 6/12 [POU] | 5 |
| JA 8/8 [POU] | 2 |
| JA 8/18 [POU] | 5 |
| JA 9/9 [POU] | 1 |
| JA 10/29 [POU] | 5 |
| JA 10/31 [POU] | 2 |
| JA 10/40 [POU] | 2 |
| LCT EEN 28/S-1 | 4 |

| | |
|---|---|
| MOQ 3/20 | 5 |
| MOQ 3/22 | 2 |
| MOQ 4/5 | 5 |
| MOQ 4/17 | 2 |
| MOQ 5/18 | 4 |
| MOQ 5/35 | 4 |
| MOQ 6/34 | 7 |
| MOQ 6/55 | 4 |

| | |
|---|---|
| SLC 11 | 5 |
| SLC 25 | 3 |
| SM 2 [POU] | 5 |
| SM 4 [POU] | 2 |
| SM 18 [POU] | 4 |
| SPEC 194-15 | 4 |
| THY 1/105 | 3 |
| UF 676 | 3 |

## Future work

For clones that have proved to be difficult to root, selected trees will be frequently irrigated, pruned and fertilized to encourage new growth and desirable propagating material. Good quality cuttings will be collected from Field 4A and propagated in the chambers on the St. Augustine Campus.

For clones from historic genebanks that do not have sufficient grafted plants to establish at the UCRS, budwood will be recollected for grafting and eventual re-introduction into the ICG,T.

Further work on completing the security fence at UCRS will continue once new boundaries of the site have been agreed and confirmed by MALMR.

## Acknowledgements

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# Cacao germplasm management with microsatellites

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## Introduction

The ICG,T is the largest cacao germplasm collection in the public domain, and contains over 2,000 accessions. Formally planned in 1982, the genebank was assembled from diverse germplasm material brought to Trinidad from multiple collecting expeditions (1930 onwards) from Amazonian South America, Central America and the West Indies (Kennedy and Mooleedhar, 1993). The ICG,T consists of five fields (Fields 4A, 5A, 5B, 6A and 6B) that are each sub-divided into sections which are split into plots. Each plot was planned to contain a maximum of 16 replicates of an accession, with a core group of four trees surrounded by peripheral guard rows. Tree numbering is consistent in orientation for all plots. Each tree is given a unique identifier based on its field, section, plot and tree locations. For example, a tree of the accession IMC 67 may be found at Field 6B, Section A, Plot23, Tree number 12. The same accession may be present in (a) different plots within the same section of a field, (b) more than one section within the same field, (c) more than one field or (d) only one plot. The latter is the more common occurrence. In the majority of plots, each accession was replicated from rooted cuttings, however later introductions were established from grafted plants. An accession plot is therefore expected to contain clones of the named accession and where the accession is present in more than one plot, that all trees are identical to each other and belong to the stipulated accession group.

Mislabelled plants have been identified as a serious problem in germplasm collections (Hurka et al., 2004) including cacao (Figueira, 1998; Risterucci et al., 2001; Motilal and Butler, 2003). DNA fingerprinting using microsatellites has proved useful in resolving identity issues in cacao collections (Figueira, 1998; Risterucci et al., 2001; Saunders et al., 2004; Cryer et al., 2006; Zhang et al., 2006). At CRU, microsatellites are currently being used to reveal mislabelling issues and to confirm the homogeneity of plots within the ICG,T, and this report gives a current update of this work.

## Materials and Methods

Plant genomic DNA was obtained as previously described (Motilal et al., 2007), sampling across locations by field, by accession, and by trees within each accession plot (Tables 1, 2). Nine microsatellite loci were amplified using the primer pairs mTcCIR12, 15, 26, 33, 37, 42, 57, 243 and 244. Microsatellite amplification, separation and binning were carried out as described in Motilal et al. (2008). The allelic dataset was checked for binning errors with the microsatellite toolkit excel addin (Park, 2001). Match declaration was performed using the regroup option in the software GIMLET (Valière, 2002), and final declarations were given some flexibility by assigning questionable profiles as similar.

Mismatches were tabulated for plots within fields, within accession groups across locations and among accession groups irrespective of location. Comparisons were conducted only with groups that contained at least two trees. Contingency tables were tested with chi-square tests using the Contingency table programs v3.0 (Chang, 2001) according to the methodology of Siegal and Castellan (1998).

**Table 1. Number of accessions fingerprinted with nine microsatellite loci.**

| Field | Number of unique accessions present | Number of accessions analysed | Number of trees present | Number of trees analysed |
|---|---|---|---|---|
| Field 4A | 548 | 168 (30.7) | 1273 | 356 (27.9) |
| Field 5A | 351 | 65 (18.5) | 1408 | 161 (11.4) |
| Field 5B | 636 | 190 (29.9) | 3699 | 659 (17.8) |
| Field 6A | 100 | 44 (44.0) | 401 | 105 (26.2) |
| Field 6B | 374 | 59 (15.8) | 1939 | 199 (10.2) |
| Total | 1765 | 525 (29.7) | 8720 | 1479 (17.0) |

Numbers in parenthesis refer to percentages of the total.

Mislabelling within accessions groups was assessed by retaining accessions groups which contained at least seven accessions exhibiting errors. The remaining accessions were assigned into two groups (Other1 and Other2) on the basis of a coin toss. Contingency analysis was then performed as before.

**Table 2. Fingerprinting sampling by accession group at the University Cocoa Research Station (UCRS).**

| Accession Group | Total number of accessions at UCRS | Number of accessions analysed | Total number of trees in accession at UCRS | Number of trees analysed |
|---|---|---|---|---|
| AGU | 4 | 1 | 24 | 1 |
| AM | 73 | 30 | 350 | 109 |
| AMAZ | 10 | 4 | 28 | 10 |
| AMELONADO | 2 | 1 | 5 | 1 |
| B | 80 | 38 | 444 | 166 |
| CC | 13 | 3 | 48 | 9 |
| CL | 90 | 31 | 411 | 98 |
| CLEM | 1 | 7 | 16 | 15 |
| CLM | 24 | 1 | 73 | 15 |
| CRIOLLO | 1 | 1 | 2 | 2 |
| COCA | 6 | 1 | 12 | 1 |
| CRU | 116 | 6 | 626 | 33 |
| CRUZ | 3 | 2 | 19 | 6 |
| DOM | 26 | 3 | 82 | 10 |
| EET | 12 | 3 | 112 | 11 |
| FSC | 2 | 1 | 6 | 4 |
| GS | 32 | 5 | 109 | 10 |
| GU | 27 | 3 | 93 | 7 |
| ICA | 1 | 1 | 3 | 3 |
| ICS | 80 | 8 | 405 | 24 |
| IMC | 56 | 10 | 408 | 41 |
| JA | 141 | 65 | 819 | 177 |
| LCT EEN | 56 | 7 | 170 | 16 |
| LP | 79 | 28 | 357 | 112 |
| LV | 13 | 13 | 47 | 39 |

| | | | | |
|---|---|---|---|---|
| LX | 21 | 21 | 127 | 66 |
| LZ | 8 | 7 | 24 | 19 |
| MAR | 13 | 2 | 31 | 4 |
| MATINA | 2 | 1 | 6 | 2 |
| MO | 20 | 5 | 71 | 10 |
| MOQ | 80 | 47 | 312 | 105 |
| NA | 200 | 47 | 1276 | 110 |
| PA | 115 | 19 | 1003 | 74 |
| PENTAGONA | 2 | 2 | 13 | 2 |
| POUND | 27 | 2 | 159 | 5 |
| REDAMEL | 3 | 2 | 6 | 2 |
| RIM | 19 | 4 | 52 | 8 |
| SC | 16 | 3 | 39 | 8 |
| SCA | 13 | 4 | 52 | 14 |
| SJ | 32 | 14 | 124 | 41 |
| SLA | 23 | 5 | 89 | 21 |
| SLC | 8 | 5 | 40 | 26 |
| SP | 1 | 1 | 2 | 1 |
| SPEC | 30 | 4 | 122 | 7 |
| STAHEL | 1 | 1 | 2 | 2 |
| TRD | 68 | 12 | 107 | 23 |
| UF | 17 | 5 | 107 | 10 |
| Total | 1667 | 486 | 8433 | 1480 |

A reduced dataset containing 568 unique accessions (including reference samples) with 1.6% missing values for the same nine loci, was assessed for match declaration and summary statistics over the entire dataset with GIMLET (Valière, 2002) and the microsatellite toolkit excel addin (Park, 2001). Expected heterozygosity was calculated as unbiased gene diversity (Nei, 1987) and polymorphism information content according to Botstein et al. (1980). Probabilities of identity (Waits et al., 2001) was determined using the software GIMLET (Valière, 2002). Fourteen samples from nine accession groups (CRIOLLO, GU, MATINA, PENTAGONA, REDAMEL, POR, STAHEL and two references from Peru) were assessed for sibship using the software COLONY (Wang, 2004; Wang and Santure, 2009).

## Results

Accession and Plot heterogeneity

Heterogeneous plots (plots containing more than one genotype) averaged 28% in the ICG,T with maximal admixture (36%) being recorded in Field 5B (Table 3). However, the field identity did not significantly influence error scores ($\chi^2$ = 5.3, d.f.[1] = 4, $P$ = 0.26; $r_s$ = -0.0343, d.f. = 443, $P$ = 0.2356). The distribution of heterogeneous plots by field section ranged from 7.1% – 61.5% (Table 4). However, when sections were pooled to obtain valid size classes, chi-square analysis showed that the error score was also uninfluenced by section groupings (Table 5; $\chi^2$ = 6.1257, d.f. = 8, $P$ = 0.6332; Spearman's $r_s$ = -0.0359, d.f. = 443, $P$ = 0.2253).

[1] Degrees of freedom

**Table 3. Plot heterogeneity at the University Cocoa Research Station.**

| Field | Number of accession plots with at least two trees | Number of plots assessed[1] | Number of mixed plots | % of mixed plots |
|---|---|---|---|---|
| 4A | 376 | 111 (30%) | 25 | 22.5 |
| 5A | 315 | 39 (12%) | 10 | 25.6 |
| 5B | 548 | 129 (24%) | 47 | 36.4 |
| 6A | 77 | 29 (38%) | 4 | 13.8 |
| 6B | 298 | 38 (13%) | 11 | 28.9 |
| Total plots | 1614 | 346 (21%) | 97 | 28.0 |

[1]percentage of total number of accessions with at least two trees

$\chi^2$ = 5.3, d.f. = 4, *P* = 0.26, Spearman's $r_s$ = -0.0343, d.f. = 443, *P* = 0.2356

**Table 4. Plot heterogeneity by field section.**

| Field | Section | No. plots assessed with at least 2 trees | No. of plots with errors | % error |
|---|---|---|---|---|
| 4A | A | 19 | 3 | 15.8 |
| | B | 11 | 2 | 18.2 |
| | C | 12 | 3 | 25.0 |
| | D | 35 | 6 | 17.1 |
| | E | 14 | 5 | 35.7 |
| | F | 20 | 6 | 30.0 |
| 5A | A | 9 | 1 | 11.1 |
| | B | 10 | 4 | 40.0 |
| | C | 8 | 2 | 25.0 |
| | D | 12 | 3 | 25.0 |
| 5B | A | 13 | 8 | 61.5 |
| | B | 21 | 7 | 33.3 |
| | C | 19 | 6 | 31.6 |
| | D | 14 | 4 | 28.6 |
| | E | 15 | 6 | 40.0 |
| | F | 11 | 3 | 27.3 |
| | G | 7 | 4 | 57.1 |
| | H | 9 | 2 | 22.2 |
| | I | 20 | 7 | 35.0 |
| 6A | A | 15 | 3 | 20.0 |
| | B | 14 | 1 | 7.1 |
| 6B | A | 6 | 1 | 16.7 |
| | B | 4 | 1 | 25.0 |
| | C | 5 | 1 | 20.0 |
| | D | 8 | 2 | 25.0 |
| | E | 0 | 0 | Not valid |
| | F | 15 | 6 | 40.0 |
| Σ | | 346 | 97 | 28.0 |

Four accessions (JA 5/47 [POU], LCT EEN 162/S-1010, LP 1/21 [POU] and NA 471) were each represented by two plots in one field. The former three accessions all contained errors while NA 471 was deemed homogenous due to missing values. The resulting match declarations and the average matching probability of identity ($P_{ID}$) and probability of identity for full siblings $P_{(ID)sib}$ are presented in Table 6. Thirty-eight accessions were present in two fields and 58% of these contained differential groups. Accessions with complete concordance (among sampled trees) were AM 2/31 [POU], AM 2/38 [POU], AM 2/65 [POU], CL 9/6, IMC 50, JA 5/34 [POU], LP 3/29 [POU], LP 4/5 [POU], LV 17, LV 28, MOQ 4/23, MOQ 5/5, NA 176, NA 471, PA 124 and SLA 77. The accessions AM 1/53 [POU], AM 2/96 [POU], B 14/9 [POU], B 22/3 [POU], B 23/2 [POU], CL 7/82, CL 10/3, CL 10/14, CL 19/10, CL 19/49, CLM, CRU 47, JA 1/9 [POU], JA 3/4 [POU], LCT EEN 162/S-1010, LP 3/48, LP 4/12, LP 5/1, LV 10 NA 246, NA 406 and SLA 20 all contained mixed plots. Nine accessions (AM 1/53 [POU], CL 10/3, CL 19/10, CL 78/2, CLM 78, JA 1/9 [POU], JA 3/4 [POU], LCT EEN 162/S-1010 and LP 5/1) were distinctly dissimilar between fields.

**Table 5. Heterogeneity error from pooled field sectors.**

| Field | Sections | Approx. Size (ha) | Number of plots assessed with at least 2 trees | Number of plots with errors | % Error |
|---|---|---|---|---|---|
| 4A | A-C | 4.00 | 42 | 8 | 19.1 |
| | D-F | 3.50 | 69 | 17 | 24.6 |
| 5A | A-B | 1.63 | 19 | 5 | 26.3 |
| | C-D | 3.10 | 20 | 5 | 25.0 |
| 5B | A-D | 2.61 | 67 | 25 | 37.3 |
| | E-I | 3.60 | 62 | 22 | 35.5 |
| 6A | AB | 1.34 | 29 | 4 | 13.8 |
| 6B | A-C | 1.63 | 15 | 3 | 20.0 |
| | D-F | 2.84 | 23 | 8 | 34.8 |
| Σ | | | 346 | 97 | 28.0 |

$\chi^2 = 6.1257$, d.f. = 8, $P = 0.6332$
Spearman's $r_s = -0.0359$, d.f. = 443, $P = 0.2253$

The presence of mislabelling within accession groups varied according to the number of accessions analysed. For instance, the three AMAZ accessions analysed (AMAZ 3/2 [CHA], AMAZ 12 [CHA] and AMAZ 15/15 [CHA]) exhibited 100% within-group homogeneity. However, the POUND group contained only one entry with at least two trees (POUND 18/A [POU]). This accession comprised of two genotypes, resulting in 100% error for the POUND group. Contingency analysis of a constructed dataset with appropriate class sizes revealed that mislabelling error may be affected by the accession groups (Table 7). Chi-square testing returned a non-significant result ($\chi^2 = 9.5610$, d.f. = 7, $P = 0.215$) unlike Spearman's rank correlation coefficient ($r_s = -0.1024$, d.f. = 428, $P = 0.017$).

**Table 6. Match declarations for accessions at two plots in same field.**

| Accession | Field | Plot | # trees analysed | Match declaration | $^{1}P_{(ID)}$ | $^{2}P_{(ID)sib}$ |
|---|---|---|---|---|---|---|
| JA 5/47 [POU] | 5B | D300 | 4 | Same to each other but differ from F454 | $2.46 \times 10^{-3}$ | $4.45 \times 10^{-2}$ |
| | | F454 | 3 | Same to each other but differ from D300 | $3.73 \times 10^{-5}$ | $5.87 \times 10^{-3}$ |
| LCT EEN 162/S-1010 | 4A | A60 | 1 | Differs from A99 | $1.69 \times 10^{-9}$ | $5.74 \times 10^{-4}$ |
| | | A99 | 4 | Same to each other | $5.50 \times 10^{-4}$ | $1.13 \times 10^{-2}$ |
| LP 1/21 [POU] | 5B | I746 | 4 | Same to each other and those of I779 except T8 | $5.83 \times 10^{-3}$ | $3.35 \times 10^{-2}$ |
| | | | | I746 T8 | $9.30 \times 10^{-5}$ | $1.77 \times 10^{-2}$ |
| | | I779 | 5 | I746 T4, T5, T7 similar to I779 | $8.2 \times 10^{-3}$ | $4.0 \times 10^{-2}$ |
| NA 471 | 6A | B86 | 4 | Same to each other and to B92 | $3.1 \times 10^{-1}$ | $4.5 \times 10^{-1}$ |
| | | B92 | 3 | Same to each other and to B86 | $2.5 \times 10^{-1}$ | $3.9 \times 10^{-1}$ |

$^{1}P_{(ID)}$ = probability of identity, $^{2}P_{(ID)sib}$ = probability of identity among full siblings separately calculated for each accession with GIMLET (Valière, 2002).

**Table 7. Heterogeneity levels within accession groups.**

| Accession group | Total number of accessions in UCRS[1] | Number of fingerprinted accessions | Number of accessions analysed with at least two trees | Number of analysed accessions with errors | % Error |
|---|---|---|---|---|---|
| AM | 73 | 30 | 22 | 7 | 31.8 |
| B | 80 | 38 | 26 | 14 | 53.8 |
| CL | 90 | 31 | 22 | 8 | 36.4 |
| JA | 141 | 65 | 42 | 16 | 38.1 |
| LP | 79 | 28 | 20 | 7 | 35.0 |
| NA | 200 | 47 | 27 | 9 | 33.3 |
| Other1 | 558 | 94 | 72 | 11 | 15.3 |
| Other2 | 427 | 144 | 100 | 25 | 25.0 |
| Total | | | 331 | 97 | 29.3 |

[1]University Cocoa Research Station

$\chi^2 = 9.5610$, d.f. = 7, $P = 0.215$; $r_s = -0.1024$, d.f. = 428, $P = 0.017$.

The dataset of unique accessions contained 141 alleles across all nine loci, a mean number of alleles of 15.7 ± 5 and an estimate of 0.75 ± 0.02 for gene diversity. Polymorphism estimates ranged from 0.636 (mTcCIR57) to 0.784 (mTcCIR15). Probabilities of identities (random) ranged from $1.78 \times 10^{-29}$ (GU 300/P Field 4A, Plot B197, T4) to $3.71 \times 10^{-3}$ (AM 2/43 [POU] Field 5A, Plot A18, T5). Probabilities of identities based on siblings ranged from $1.16 \times 10^{-5}$ (GU 310/P Field 4A, Plot B233, T3) to $2.17 \times 10^{-3}$ (JA 9/37 [POU] Field 5B, Plot E394, T16). Matching loci resulted in 536 groups yielding a 94.4% identification rate.

Case Studies

*GU 241/P*

In this accession the three trees (T1-T3) in Field 4A, Plot B258 appeared to differ at two loci (mTcCIR12 and mTcCIR244) from that in Campus Field 1A, X2Y33. This accession together with GU 300/P and GU 310/P were declared full-sibs ($P$ = 0.99).

*IMC 47*

For this accession, located in Field 6B, Plot F401, there were two genotype groups. One group was comprised of trees T1, T3, T4, T9, T11 and T12 and the other group consisted of trees T6, T10, T13 and T14. This latter group was identical with the plant located in Campus Field 11, X5Y12.

*IMC67*

The reference plant at La Reunion Estate was different at just one locus (mTcCIR12) being homozygous instead of heterozygous like the 13 trees in Field 6B, Plot A23. The size difference was four base pairs and the homozygous allele was common to both groups.

*LCT EEN 162/S-1010*

Two trees in Campus Field 11 (X13Y26 and X8Y26) were identical with T1 in Field 4A, Plot A60 and perhaps T2 in Field 5B Plot C216. The plants in Field 4A, Plot A99 were genetically distinct, as mentioned earlier.

*MATINA 1/7*

Prior to 2003, this accession was represented by five trees in Field 6B, Plot D236 and DNA from T1 was analysed under the USDA/CRU fingerprinting project. This tree died in the 2003 dry season and only three trees remain in the field. Two trees (T12 and T15) were present in the current study for comparison. Based on five common loci, T1 was similar to T12 but T15 differed from T12 at all nine loci. The two trees, T12 and T15 were not likely half-sibs ($P$ = 0.007). However, half-sib relationships existed between T12 and CRIOLLO 22 ($P$ = 0.3 – 0.4), T15 and POR 1 [TTO] Campus Field 22, X2Y12 ($P$ = 0.58) and between T15 and a Peruvian reference H 1 ($P$ = 0.52). Interestingly, the POR representative was not matched to the CRIOLLO tree.

*PENTAGONA*

This accession group contains two accessions at the UCRS: PENTAGONA 1 and PENTAGONA 2. These were distinct from each other. However, CRU records have both linked under the preferred name of PENTAGONA. Each accession was represented in the analysis by one tree. The two trees were found to be full sibs ($P$ = 0.96). Half-sib relationship with PENTAGONA 1 ($P$ = 0.12) and PENTAGONA 2 ($P$ = 0.14) with CRIOLLO 22 was possible. The PENTAGONA accessions were also putative half-sibs with MATINA 1/7 T12 ($P$ = 0.11 ). POR 1 [TTO] campus Field 22, X2Y12 was a more likely candidate half-sib for MATINA 1/7 T15 ($P$ = 0.56)

than for MATINA 1/7 T12 ($P$ = 0.01). PENTAGONA 2 Field 6B, Plot F492, T8 was the paternal choice and REDAMEL 1/30 Field 6B, Plot D266, T4 as the maternal choice for the reference sample U 1 ($P$ = 0.98) that was collected in Peru.

*STAHEL*

This accession group consists of only one accession: STAHEL. There are only two trees at UCRS with a single representative on Campus. The Campus tree was not fingerprinted. Both trees at UCRS were distinct from each other but together with REDAMEL 1/30 Field 6B, Plot D266, T4 were matched as full sibs ($P$ = 0.88). Half-sib matching at $P$ = 0.16 for the STAHEL trees and REDAMEL1/30 Field 6B, Plot D266, T4 with CRIOLLO 22 was observed. The tree in Field 6B, Plot D267, T4 was assigned as the maternal parent for the offspring GU 241/P, GU 300/P and GU 310/P with the paternal parent as POR 1 [TTO] campus Field 22, X2Y12 ($P$ = 0.99).

## Discussion

The error rate at the University Cocoa Research Station site of the International Cocoa Genebank, Trinidad was estimated at 28% overall, with a maximum rate per field of 36% (Field 5B), and a range from 7 – 62 % within field sections (or from 14 – 37 % when sections were grouped). When accession groups were considered, the value was 29%. These figures were lower than those estimated earlier (Motilal et al., 2009) and represent the effect of a larger sample size and some flexibility in matching. The average of the maximal values of the latter four estimates is 41 % and this value may represent the upper limit for the mislabelling error at the UCRS site.

There was a 94.4% identification rate in the reduced dataset with nine loci. However, this level may be further increased if additional microsatellite primers are employed. The redundancy level in the genebank is therefore probably less than six percent.

The case studies illustrated the need to protect and fingerprint accessions contained in Campus fields. Some of these plants were probably used as the source of propagation material when the UCRS site was established. IMC 47 and LCT EEN 162/S-1010 are two such examples. The STAHEL accession on campus is recommended for fingerprinting as the two representatives at UCRS were dissimilar. The full sib allocation of the two UCRS trees illustrate the need for proper passport documentation. If STAHEL was shipped as seedling material, it could account for the sibship found. PENTAGONA 1 and 2, for instance were sent as seedling material from Reading, UK and as such the accession names should be kept unique. Bartley (2008) mentioned that MATINA 1/7 was sent as seedling material to Trinidad. However, while T1 (now dead) seemed to be identical to T12 (faithful propagation of a type), T12 and T15 could not be considered as half-sibs ($P$ = 0.007). This may be confirmatory of the report by Bartley (2008) that ‘MATINA was a name given to the plants that grew in the area known as Matina in Costa Rica’ and that ‘in 1898 a quantity of fruits were imported into Trinidad from an unknown farm in Matina; seed from the progenies raised were distributed to other Trinidad farms’ (Pittier, 1902 in Bartley, 2008). A heterogeneous mix of plants may represent the MATINA 1/7 or the MATINA group and further fingerprinting should be assigned to these accessions in order to confirm whether wrong plants were planted in the MATINA 1/7 plot.

A Criollo background was indicated for MATINA 1/7 Field 6B, Plot D236, T12 but not T15 further supporting the different origins of these two trees. Low Criollo background was indicated for the POR 1, PENTAGONA (1 and 2) and the STAHEL trees. This is contrary to that expected according to the information gleaned from the ICGD (Turnbull et al., 2004). However, Motilal et al. (2010) established the negligible Criollo background of the aforementioned MATINA 1/7 T15 and STAHEL trees but did find about 30% Criollo ancestry for the PENTAGONA accessions. These results show the value of combining different analyses.

The present study found an overall mislabelling rate of about 30% in the genebank which directs the Cocoa Research Unit to prioritise the fingerprinting of accessions and to ensure that the fingerprinted trees are securely tagged. The dissemination of the identity results as in-house information is essential to assist the other sections of the unit in planning and conducting their research.

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# Characterisation

# Examining phenotypic relationships among Trinitario and Refractario cacao clones conserved in the International Cocoa Genebank, Trinidad

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## Introduction

Approximately 236 Trinitario and 716 Refractario clones (accessions) are held in the ICG,T. They comprise approximately 42 percent of the germplasm conserved in this Universal cacao depository and warrant careful study.

Trinitario is a recognised cacao genetic group (class) that originated in Trinidad and Tobago and is considered to be a hybrid of Criollo and Forastero (Cheesman, 1944). Criollo and Trinitario beans are collectively known as "fine or flavour" cocoa that is used to manufacture fine chocolates throughout the world, and commands premium prices on the world market. The reputation of Trinidad and Tobago as a producer of 100% fine or flavour is well-known (Bekele, 2004). Grade I cocoa beans exported from Trinidad and Tobago currently command approximately US $4,500 per tonne compared to US $2,838 per tonne paid for bulk cocoa (New York Futures market, 10 March, 2010).

The Refractario group is comprised of an assemblage of genotypes. It was not considered a genetic group per se until the findings of Zhang et al. (2008) revealed that it is indeed a genetic group. The Refractarios were selected in Ecuador based on apparent field resistance to Witches' broom disease (Preuss, 1901; Stell, 1934; Pound, 1938; Bartley, 2001), and introduced by the late Dr. F.J. Pound into Trinidad in 1937. From 1923 onwards, the word "refractario" (stubborn) was employed to designate trees without apparent infection by the Witches' Broom pathogen based on field observations in Ecuador. Seedlings generated from these trees were raised in Ecuadorian nurseries and subjected to natural infection in the 1930s. Uninfected plants were established on various farms or "haciendas" (owned by the Seminario family of Ecuador) from which Pound collected seeds to produce the progeny that constitute this group of introductions into Trinidad. According to Bartley (2001), there are "at least six types having distinct origins, not counting the male parents of the fruits produced during the two generations that would have produced the genotypes in Trinidad." This germplasm includes genotypes of "Nacional" origin. Flavour beans produced in Ecuador are known as "Nacional" and are regarded as unique. Botanically, "Nacional" has been assigned to the "Forastero" class of cacao. "Nacional" cocoa beans are distinguished by their strong floral flavour, which is designated as "Arriba".

The objective of this report is to provide information on phenotypic and agronomic traits of 186 Trinitario and 479 Refractario clones conserved at the International Cocoa Genebank, Trinidad and to elucidate the phenotypic relationship between these two groups of cacao germplasm.

## Materials and methods

### Fruit characterisation

One thousand five hundred and thirty-four accessions, including the aforementioned Trinitario and Refractario clones, have now been characterised using morphological descriptors according to the standard protocol described by Bekele et al. (1994; 2006). These

descriptors were selected based on the findings of Bekele and Bekele (1996) and Bekele and Butler (2000), and are listed in Table 1. They were found to be the most discriminative and taxonomically useful and to preclude redundancy (Bekele et al., 1994). In addition, they were also selected for ease of observation, reliability of scoring, and, in the case of seed characters, agronomic/economic value.

**Table 1. Descriptors used 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 |
| Fruit, basal constriction | 0=absent, 1=slight, 2=intermediate, 3=strong, 4=wide shoulder [n=10] |
| Fruit, apex form | 1=attenuate, 2=acute, 3=obtuse, 4=rounded, 5=mammillate, 6=indented [n=10] |
| Fruit, surface texture (rugosity or degree of wartiness) | 0=absent, 3=slight, 5=intermediate, 7=intense [n=10] |
| Fruit, anthocyanin intensity in mature ridges | 0=absent, 3=slight, 5=intermediate, 7=intense [n=10] |
| Fruit, ridge disposition | 1=equidistant, 2=paired [n=10] |
| Fruit, primary ridge separation | 1=slight, 2=intermediate, 3=wide [n=10] |
| Fruit, pod wall hardness [n=10] | 3= ≤ 2 MPa, 5 = > 2 to □ 4.99 MPa 7= □ 5.0 MPa |
| Fruit, length (cm) [n=10] | |
| Fruit, width (cm) [n=10] | |
| Seed, number [n=10] | |
| Seed, shape | 1=oblong 2=elliptic 3=ovate |
| Seed, cotyledon colour | 1=white, 2=grey, 3=light purple, 4=medium purple, 5=dark purple, 6=mottled [n=40] |
| Wet bean weight (total) (g) [n=10] | |
| Cotyledon length (cm) [n=20]. | |
| Cotyledon width (cm) [n=20]. | |
| Cotyledon weight (g) [n=20] | |
| Pod index (the number of pods required to produce 1 kg of dried cocoa) [n=10] | |

Statistical analysis

Data for 186 Trinitario (Table 2) and 479 Refractario (Table 3) clones were analysed to assess the phenotypic diversity among the various accession groups and between the two aforementioned classes/designations of cacao, viz. Trinitario and Refractario.

Descriptive statistics based on morphological descriptors (Table 5) were generated and analysis of variance (ANOVA) following the General Linear Model was performed using MINITAB 15. Pairwise comparison of means was done using the Tukey-Kramer test (Dunnett, 1980).

**Table 2. Trinitario germplasm characterised in this study.**

| Accession code | Name represented by code | Country of origin | Number of accessions in ICG,T | Number of accessions characterised |
|---|---|---|---|---|
| DOM | Dominica | Dominica | 25 | 19 |
| DR | Djati Roenggo | Indonesia | 1 | 1 |
| FSC | | Colombia | 1 | 1 |
| GA [HAI] | Grande Anse | Haiti | 1 | 1 |
| GDL | Guadeloupe | Guadeloupe | 4 | 2 |
| GS | Grenada Selection | Grenada | 32 | 27 |
| ICS | Imperial College Selections | Trinidad, Nicaragua and Venezuela | 93 | 48 |
| LAFI | | Western Samoa | 1 | 1 |
| MAR | Martinique | Martinique | 13 | 9 |
| RED AMEL | Red Amelonado | Brazil | 3 | 3 |
| RIM | Rosario Izapa | Mexico | 23 | 13 |
| SNK | Selection N'koemvone | Cameroon | 1 | 1 |
| TRD | Trinidad | Trinidad | 68 | 44 |
| UF | United Fruit Company | Costa Rica | 19 | 12 |
| VEN | | Venezuela | 5 | 4 |

**Table 3. Refractario germplasm from Ecuador characterised in this study.**

| Accession code | Name represented by code | Number of accessions in ICG,T | Number of accessions characterised |
|---|---|---|---|
| AM | Amalia | 99 | 47 |
| B [POU] | Balao | 125 | 58 |
| CL | Clementina | 148 | 52 |
| CLM | | 37 | 20 |
| CLEM | | 4 | 1 |
| JA [POU] | Javilla | 198 | 103 |
| LP [POU] | La Paz | 118 | 61 |
| LV [POU] | Large Vuelta | 15 | 10 |
| LX | Limoncillo | 25 | 13 |
| LZ | Limoncillo | 11 | 7 |
| MOQ | Moquique | 131 | 54 |
| SJ [POU] | San Juan | 47 | 30 |
| SLA | Santa Lucia | 29 | 16 |
| SLC | Santa Lucia | 11 | 6 |

*Phenotypic relationships among accessions*

Principal Component Analysis (PCA) (MINITAB 15) was used to examine the level of diversity expressed by the germplasm studied and to compare the grouping of the Trinitario relative to the Refractario germplasm. Data for the 25 descriptors used for characterisation were first standardised to eliminate the effects of different scales of measurement.

Discriminant analysis (MINITAB 15) was performed, using data for the 25 morphological traits (Table 1), to verify the assignment of accessions to their respective groups (Trinitario or Refractario) based on the phenotypic profiles associated with each group.

## Results

Descriptive statistics for the descriptors of economic importance are presented in Tables 4 and 5. All of the descriptors in Table 5 were normally distributed ($P < 0.001$) except wet bean weight for which the data were log transformed prior to being subjected to ANOVA to compare the means recorded for the two genetic groups. There were significant differences in the mean values for Refractarios and Trinitarios in terms of pod index (an indicator of yield potential), individual cotyledon weight, total wet bean weight (without mucilage) and cotyledon colour (Table 5). According to Wellensiek (1931), pale cotyledon colour is indicative of Criollo ancestry. Both genetic groups were typified by purple cotyledons (Table 5) that could be attributed to genetic constitution as well as the pollen donor effect.

**Table 4. Mean pod index values for the accession groups studied.**

| Accession Group | Sample size | Mean | Standard Error | Coefficient of variation | Range |
|---|---|---|---|---|---|
| AM | 47 | 25.8 | 0.79 | 21.0 | 25.4 |
| B [POU] | 58 | 31.5 | 1.52 | 36.7 | 75.6 |
| CL | 52 | 30.9 | 1.23 | 28.6 | 41.2 |
| CLEM | 1 | 20.7 | * | * | 0 |
| CLM | 20 | 27.1 | 1.58 | 26.0 | 25.4 |
| DOM | 19 | 28.0 | 1.16 | 18.0 | 17.2 |
| DR | 1 | 20.9 | * | * | 0 |
| FSC | 1 | 19.9 | * | * | 0 |
| GA | 1 | 20.4 | * | * | 0 |
| GDL | 2 | 21.5 | 1.66 | 10.9 | 3.3 |
| GS | 27 | 23.7 | 0.73 | 16.0 | 14.7 |
| ICS | 48 | 23.7 | 0.74 | 21.6 | 23.2 |
| JA [POU] | 103 | 24.4 | 0.50 | 20.9 | 26.5 |
| LAFI | 1 | 19.6 | * | * | 0 |
| LP [POU] | 61 | 27.6 | 0.80 | 21.7 | 25.8 |
| LV [POU] | 10 | 29.4 | 0.90 | 9.9 | 9.6 |
| LX | 13 | 28.4 | 1.86 | 23.6 | 23.9 |
| LZ | 7 | 26.0 | 1.60 | 16.2 | 10.8 |
| MAR | 9 | 28.2 | 0.99 | 10.6 | 9.4 |
| MOQ | 54 | 25.6 | 0.69 | 19.9 | 22.3 |
| PLAYA ALTA | 1 | 22.1 | * | * | 0 |
| RED AMEL | 3 | 32.3 | 5.74 | 30.8 | 19.4 |
| RIM | 13 | 26.4 | 1.69 | 23.1 | 21.0 |
| SJ [POU] | 30 | 26.9 | 1.05 | 21.4 | 24.5 |
| SLA | 16 | 26.8 | 1.52 | 22.7 | 21.6 |
| SLC | 6 | 25.4 | 2.52 | 24.4 | 16.3 |
| SNK | 1 | 20.5 | * | * | 0 |
| TRD | 44 | 26.1 | 0.77 | 19.6 | 23.4 |
| UF | 12 | 22.2 | 1.28 | 19.9 | 14.6 |
| VEN | 4 | 26.0 | 2.73 | 20.9 | 11.6 |

In addition, as found by Bekele et al. (2006), style length was significantly longer for the Refractarios (mean = 2.52 mm) compared to the Trinitarios (mean = 2.16 mm) ($F = 199.5$, $P < 0.0001$).

**Table 5. Descriptive statistics for economically important descriptors based on genetic groups.**

| Variable | Genetic Group | Mean and standard error | Comparison of means | COV | Range |
|---|---|---|---|---|---|
| Ovule number | Refractario | 42.4 ± 0.20 | F = 3.28 ns | 10.53 | 35 |
| | Trinitario | 41.7 ± 0.36 | | 11.72 | 30 |
| Husk Hardness | Refractario | 5.2 ± 0.09 | $\chi^2$ = 0.11 ns | 38.95 | 7 |
| | Trinitario | 4.9 ± 0.18 | | 49.97 | 7 |
| Cotyledon Colour | Refractario | 4.5 ± 0.03 | $\chi^2$ = 11.52** | 12.66 | 3 |
| | Trinitario | 4.4 ± 0.05 | | 13.99 | 3 |
| Wet Bean Weight | Refractario | 58.7 ± 0.57 | F = 26.29 *** | 21.36 | 77.2 |
| | Trinitario | 64.2 ± 0.90 | | 19.27 | 81.5 |
| Bean Number | Refractario | 37.6 ± 0.25 | F = 2.1 ns | 14.31 | 37 |
| | Trinitario | 38.2 ± 0.34 | | 12.28 | 30 |
| Cotyledon Weight | Refractario | 1.04 ± 0.008 | F = 10.88** | 18.10 | 1.27 |
| | Trinitario | 1.09 ± 0.02 | | 19.32 | 1.15 |
| Pod Index | Refractario | 27.2 ± 0.33 | F = 12.78*** | 26.88 | 77.2 |
| | Trinitario | 25.1 ± 0.38 | | 20.85 | 29.4 |

Legend: **, *** Significant at $P < 0.001$ and $P < 0.0001$, respectively, based on analysis of variance using the General Linear Model to compare means using the Tukey-Kramer t-test to allow for unequal sample sizes (Minitab Inc., 1997; Dunnett, 1980)
COV – Coefficient of variation
ns – not significantly different

The first two principal components (PCs) accounted for 27.1 percent of the phenotypic variation expressed in the germplasm under study. Although the first two PCs did not account for a large amount of the total variation expressed by this large germplasm sample, a score plot of the first two principal components revealed that while there is considerable overlapping between the Refractario and Trinitario accessions, two discernible clusters comprising mainly Refractarios or Trinitarios, respectively, are evident (Figure 1). Furthermore, a high proportion of the accessions studied (0.86 or 573 of the 665 accessions) were correctly assigned to their respective groups when discriminant analysis was performed (Table 6). This conforms to previous findings based on molecular studies by Zhang et al. (2008) that revealed the distinct genetic nature of the Refractarios.

The overlapping of the Trinitarios and Refractarios observed in this study is to be expected since several Refractarios may be of Trinitario or Venezuelan origin.

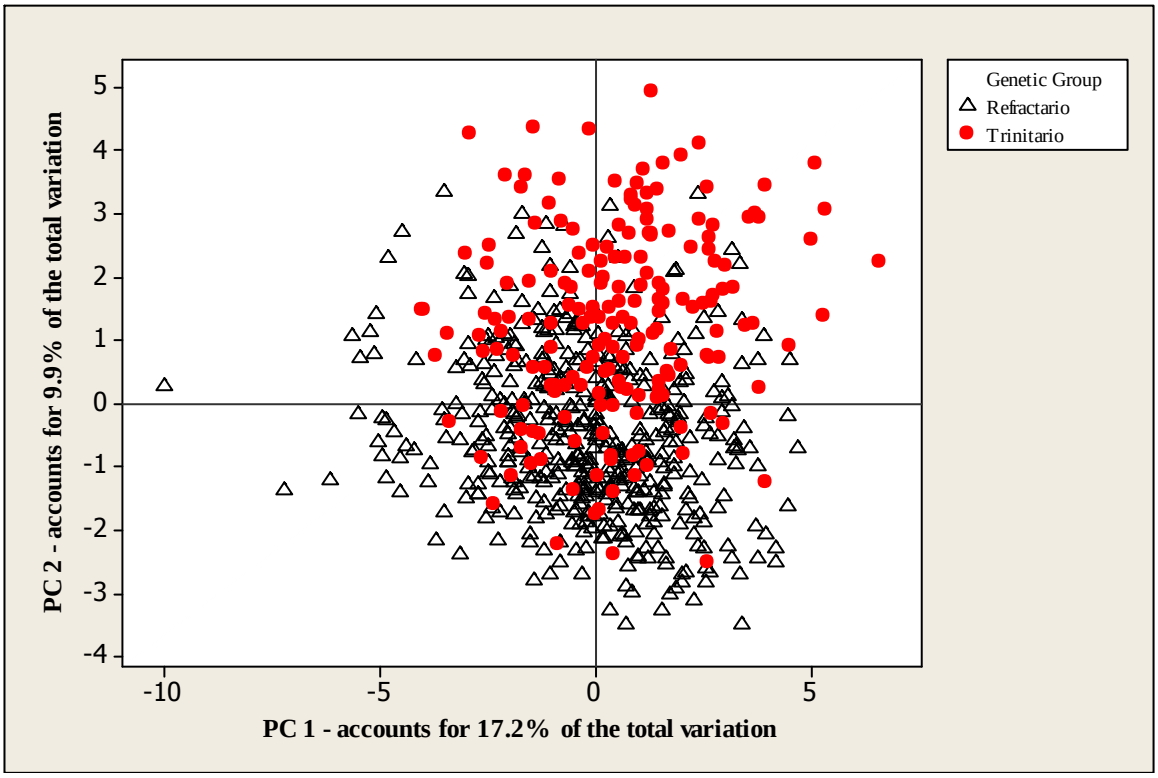
The heterogeneous distribution of accessions in Figure 1 can be ascribed to the hybrid and diverse origin of all of the genotypes studied.

Wet bean weight, pod index, cotyledon weight, length and width, pod width and pod length accounted for the most phenotypic variation expressed by Principal Component (PC) 1 while filament colour, mature pod wall colour, pod apex form, style length, ovule number, pod width, cotyledon colour and bean number accounted for the most variation expressed by PC 2 (Figure 1). It is noteworthy that many traits of economic interest are also useful taxonomically.

**Table 6. Results of Discriminant Analysis.**

| | True group | |
|---|---|---|
| **Put into group** | Refractario | Trinitario |
| Refractario | 408 | 21 |
| Trinitario | 71 | 165 |
| Total N | 479 | 186 |
| N correct | 408 | 165 |
| Proportion | 0.852 | 0.887 |
| N = 665 | N correct = 573 | |

**Figure 1. Principal Component (PC) plot showing the distribution of Refractario and Trinitario accessions studied using 25 morphological descriptors.**




In terms of yield potential, the Trinitarios had significantly better ($P < 0.05$) Pod Index (PI) values than the Refractarios (25 as opposed to 27) (Table 5). However, among the 75 accessions that had PI less than or equal to 19.99 (favourable), 49 (65 per cent) were Refractarios. This is closely aligned with the proportion of Refractarios studied relative to Trinitarios (72 per cent of the accessions under study were Refractarios). This suggests that the two groups are comparable in value as sources of potentially high-yielding genotypes that can be exploited in germplasm enhancement. The good agronomic potential of some of the Refractarios may be explained by the fact that Dr. Pound also took productivity into account when making his selections in Ecuador.

**Table 7. Accessions with the most favourable pod index values, bean size (in terms of cotyledon weight) and bean number.**

| Accession | "Genetic" Group | Pod Index | Bean number | Cotyledon weight | Pod wall hardness | Cotyledon colour |
|---|---|---|---|---|---|---|
| UF 11 | Trinitario | 13.94 | 39 | 1.84 | 7 | 4 |
| UF 12 | Trinitario | 14.87 | 38 | 1.77 | 7 | 4 |
| TRD 35 | Trinitario | 15.34 | 41 | 1.59 | 5 | 5 |
| JA 5/36 [POU] | Refractario | 15.53 | 46 | 1.40 | 7 | 4 |
| ICS 60 | Trinitario | 15.63 | 39 | 1.64 | 7 | 4 |
| JA 5/7 [POU] | Refractario | 15.76 | 45 | 1.41 | 7 | 5 |
| ICS 68 | Trinitario | 15.87 | 50 | 1.26 | 7 | 4 |
| ICS 43 | Trinitario | 16.05 | 38 | 1.64 | 7 | 4 |
| AM 1/85 [POU] | Refractario | 16.65 | 39 | 1.54 | 5 | 5 |
| CLM 59 | Refractario | 16.67 | 40 | 1.50 | 5 | 5 |
| MOQ 2/29 | Refractario | 16.85 | 43 | 1.38 | 5 | 5 |
| JA 5/35 [POU] | Refractario | 16.98 | 43 | 1.37 | 7 | 4 |
| JA 2/21 [POU] | Refractario | 17.11 | 35 | 1.67 | nd | 4 |
| B 17/20 [POU] | Refractario | 17.20 | 34 | 1.71 | 5 | 5 |
| GS 10 | Trinitario | 17.23 | 45 | 1.29 | 5 | 3 |
| JA 5/31 [POU] | Refractario | 17.23 | 45 | 1.29 | 7 | 5 |
| AM 1/38 [POU] | Refractario | 17.23 | 43 | 1.35 | 7 | 5 |
| AM 2/91 [POU] | Refractario | 17.24 | 40 | 1.45 | 7 | 4 |
| ICS 16 | Trinitario | 17.25 | 42 | 1.38 | 5 | 3 |
| ICS 6 | Trinitario | 17.49 | 43 | 1.33 | 7 | 4 |
| JA 5/21 [POU] | Refractario | 17.51 | 42 | 1.36 | 5 | 5 |
| GS 29 | Trinitario | 17.55 | 37 | 1.54 | 5 | 4 |
| LP 3/40 [POU] | Refractario | 17.66 | 38 | 1.49 | 5 | 4 |
| MOQ 2/36 | Refractario | 17.77 | 42 | 1.34 | 5 | 4 |
| SLA 30 | Refractario | 17.80 | 41 | 1.37 | 3 | 4 |
| JA 5/38 [POU] | Refractario | 17.90 | 44 | 1.27 | 5 | 5 |
| JA 4/7 [POU] | Refractario | 17.90 | 44 | 1.27 | 7 | 4 |
| JA 5/47 [POU] | Refractario | 17.92 | 45 | 1.24 | 7 | 5 |
| JA 2/2 [POU] | Refractario | 18.15 | 38 | 1.45 | nd | 5 |
| GS 4 | Trinitario | 18.15 | 38 | 1.45 | 7 | 4 |
| SLA 68 | Refractario | 18.21 | 45 | 1.22 | 3 | 4 |
| MOQ 3/20 | Refractario | 18.32 | 42 | 1.30 | nd | 5 |
| JA 1/17 [POU] | Refractario | 18.33 | 44 | 1.24 | 7 | 5 |
| JA 3/35 [POU] | Refractario | 18.46 | 43 | 1.26 | 7 | 5 |
| JA 5/24 [POU] | Refractario | 18.48 | 44 | 1.23 | 7 | 4 |
| LP 1/44 [POU] | Refractario | 18.53 | 38 | 1.42 | 7 | 5 |

Legend: nd - no data available

NOTE: None of these clones were included in the CFC\ICCO\IPGRI Project Collection (Sounigo et al., 2005 ).

## Discussion and Conclusion

The agronomic and economic value of the Trinitarios is well known and apparent from this study and others such as that done by Johnson et al. (2004) and Bekele et al. (2007). This study has revealed that the Refractarios are also a good source of potentially high-yielding genotypes. They have also demonstrated favourable Witches' Broom and Black Pod resistance/tolerance (Iwaro et al., 2003).

The adaptive advantage of the longer style lengths observed in Refractarios is worth investigating (Bekele et al., 2006).

The two groups studied are phenotypically similar, but yet distinct (Tables 5 and 6 and Figure 1). This coincides with the findings of Johnson et al. (2008; 2009), who reported that ICS and TRD cacao of Trinidad and Tobago is an admixture of ancestral germplasm groups from Venezuela, Brazil and Peru, but distinct from the Nacional cacao of Ecuador. Furthermore, Zhang et al. (2008) reported on the distinct genetic grouping of the Refractarios among a diverse germplasm sample including Parinari accessions (Upper Amazon Forasteros) and Imperial College Selections (Trinitarios) as well as Nacional cacao.

Bartley (2001) stated that the Refractarios LP 3 and 4, JA 1 and some of the MOQs such as MOQ 4/17 are thought to possess Criollo ancestry, while some selections from Hacienda Clementina are reported to be of Venezuelan Nacional origin, and AM 2 and JA 3 appear to be derived from Nacional cacao. SJ 1/19 was described as belonging to the Trinidad population. These groups may possess good flavour potential and organoleptic assessments are warranted.

The superior bean size and pod index values (yield potential) of many of the genotypes included in this study (Table 7) justify the promotion of promising Trinitarios and Refractarios in future local germplasm enhancement programmes once the flavour profiles and disease resistance or tolerance levels have been found to be favourable.

In addition, accessions with pod wall hardness ratings of 7 or more (Table 7) are recommended for assessment for resistance to Cocoa Pod Borer in South-East Asia in keeping with the recommendation of Day (1985).

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# DNA fingerprinting of *Theobroma cacao* accessions with SSR primers

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## Introduction

Participation by CRU in the USDA Fingerprinting Project, with the goal to fingerprint *Theobroma cacao* accessions held at genebanks located in the Americas, was initiated in 2001. It was a project of enormous scope involving the use of 15 simple sequence repeat (SSR) primers to establish a genotypic fingerprint for each of the accessions to be analysed. In accordance with the project mandate, a representative sample of DNA from the most original tree of each accession in the ICG,T was sent to Beltsville, Maryland (USA) for analysis. The chosen mode of SSR analysis using a capillary electrophoresis system is well-designed for high-throughput processing; however the results require a considerable investment of time to be transformed into usable data. It is not remarkable, therefore, given the constraints of time and budget that not all of the approximately 2,300 DNA samples sent from CRU, Trinidad to Beltsville have yet been completed. In 2008, several hundred samples remained to be fingerprinted. Some of them represented accessions that had not yet been processed, while others were samples that proved challenging to analyse, possibly due to variable DNA concentration or poor sample quality. The materials and resources available at the Beltsville Agricultural Research Center (BARC) Sustainable Perennial Crops Lab (SPCL) headed by Dr. Lyndel Meinhardt make it an ideal location for processing the outstanding DNA samples at minimal cost to generate the important data required to continue characterisation, verification and genetic diversity studies. At the invitation of SPCL geneticist Dr. Zhang, with the approval of Dr. Meinhardt, Antoinette Sankar was therefore assigned to spend six months in Beltsville, to learn to operate the Beckman Coulter CEQ 8000 Genetic Analysis System, to perform Polymerase Chain Reaction (PCR) and to conduct analyses of as many samples as possible. Upon arrival in Beltsville, the list contained 866 samples to be analysed (D. Zhang pers comm). Some accessions on the list were classified as "missing" which was an indication that they could have been misplaced during laboratory relocation.

The CEQ Genetic Analysis system was used to perform capillary electrophoresis and analysis of PCR fragments produced by the amplification of the cacao DNA. PCR amplification was done using the SSR primers officially established for the USDA Fingerprinting Project (Saunders et al., 2004). The resultant fingerprinting profiles were exported as numeric allele data. Approximately 400 accessions were processed and data obtained for a subset of that number will be used for verification and genetic diversity studies. The work program consisted of training to use the CEQ system, cataloguing of samples, trial runs to optimise the DNA dilution factor, followed by systematic analysis of the cacao DNA samples alongside known cacao DNA controls.

## Materials and Methods

All available DNA samples were sorted to separate those that had been completed from the ones which needed to be analysed. All samples that were not included on the outstanding list of 866 were classified as completed and separated from the rest. Trial runs were done to determine the

DNA dilution required for optimal results after which serial dilutions (up to the working concentration of 1:1000) of the stock DNA were prepared in 96-well PCR sample plates. From these plates, aliquots of DNA were removed by pipetting into new PCR plates for PCR amplification, after the samples had been vortexed and spun down to mix and collect the DNA at the bottom of the well. A sub-optimal number of positive DNA controls were used in the PCR due to short supply of this material.

**Table 1. Fluorescent-labelled SSR primer pairs.**

| SPCL title* | CIRAD nomenclature | Expected Range |
|---|---|---|
| **Primer 9** | **mTcCIR1** | 122-156 |
| **Primer 3** | **mTcCIR6** | 218-246 |
| Primer 1 | mTcCIR7 | 150-167 |
| **Primer 7** | **mTcCIR8** | 276-322 |
| Primer 10 | mTcCIR11 | 286-322 |
| **Primer 11** | **mTcCIR12** | 187-273 |
| **Primer 12** | **mTcCIR15** | 221-256 |
| Primer 16 | mTcCIR18 | 329-354 |
| **Primer 4** | **mTcCIR22** | 271-288 |
| Primer 5 | mTcCIR24 | 184-201 |
| **Primer 21** | **mTcCIR26** | 267-313 |
| **Primer 15** | **mTcCIR33** | 270-344 |
| **Primer 14** | **mTcCIR37** | 132-184 |
| Primer 22 | mTcCIR40 | 261-288 |
| **Primer 25** | **mTcCIR60** | 187-210 |

*Primer pairs numbered according to the system used at SPCL

PCR with Fluorescent-labelled SSR primers (Table 1) was done in accordance with SPCL protocol (S. Pinney, pers comm). The PCR master mix was prepared on ice using the composition shown in Table 2. All of the components, except the polymerase (Amplitaq Gold®) were thawed and vortexed briefly to collect the tube contents before pipetting. The reaction mix was pulsed briefly before addition of the polymerase. Both before and after adding primers to the PCR mix, they were protected from light to prevent primer degradation. The polymerase was the last component to be added and was spun down in an Eppendorf Centrifuge 5417R (refrigerated) for one minute then added to the reaction mix. The mix was incubated on ice while being added to the DNA template.

For PCR, 96-well plates of DNA were amplified according to the program shown in Table 3 (or a slight variant of it) using one of three thermal cyclers; these were two MJ Research PTC-200 Peltier Thermal Cyclers (one a Gradient cycler) and one AB (Applied Biosystems) GeneAmp PCR System 9700 cycler. An annealing temperature of 51$^{o}$C was used for all primers as recommended.

PCR products were stored at -5 $^{o}$C until they were prepared for electrophoresis in the Beckman Coulter CEQ 8000 Genetic Analysis System (CEQ 8000 System) to separate PCR fragments. To prepare samples for loading into the instrument, a Sample Loading Solution (SLS)

**Table 2. PCR mix composition.**

| Component | Volume (µl) |
|---|---|
| Water | 0.02 |
| 10X Amplitaq Gold® Buffer | 1.0 |
| 10 mg/ml BSA | 1.0 |
| 25mM $MgCl_2$ | 1.0 |
| 10mM dNTPs | 0.2 |
| 20µM Forward primer | 0.2 |
| 20µM Reverse primer | 0.2 |
| 5U/µl Amplitaq Gold® Polymerase | 0.08 |
| DNA template | 6.3 |
| Total volume | 10 |

**Table 3. PCR program.**

| Step | Temperature | Time |
|---|---|---|
| Step 1 | 94°C | 7 min |
| Step 2 | 94°C | 30 sec |
| Step 3 | 51°C | 1 min |
| Step 4 | 72°C | 1 min |
| Step 5 | Go to Step 2 | 34 times |
| Step 6 | 60°C | 15 min |
| Step 7 | 4°C | ∞ |
| Step 8 | End | |

consisting of 1.5ml Hidi Formamide (Applied Biosystems - Genetic Analysis Grade) and 51µl of GenomeLab™ DNA Size Standard Kit - 400 (Beckman) was prepared, vortexed for at least 90 seconds and aliquots were transferred into 96-well CEQ sample plates. PCR plates were thawed and spun down, then 1.0 or 1.5µl aliquots of PCR product were mixed (and multiplexed in the CEQ plate) with the SLS for loading into instrument. The volume of 1.0 µl was used for blue dye primers because their signal strength was always much higher than that of the other two primers.

Separation buffer (Beckman) was pipetted into a separate 96-well buffer plate and the two plates loaded into the instrument. The PCR products were pulled from the plates through a Separation Gel in the DNA Separation Capillary of the CEQ system. Raw colour-coded fragment data output from the CEQ system were "read" by the CEQ software to generate the allele data for the 15 SSR primers. The output was assessed using the system software to determine which samples needed to be repeated. Regular assessment was necessary to ensure the CEQ system was running optimally. Rainin EDP Electronic pipettes and Gibson multichannel pipettes were crucial for pipetting and a Sigma 4-15C (Qiagen) centrifuge was used for spinning down and mixing samples in 96-well plates.

## Results

The complete set of 15 SSR primer pairs was used to amplify DNA from 310 accessions. An additional 71 accessions were run with 9 SSR primer pairs. Peaks were only trusted to be used to "call" the alleles if the standard resembled a typical standard profile. Sometimes atypical standard profiles could be improved by re-analysing the data using system spectra (an option provided by the CEQ analysis software), thus allowing allele peaks to be called. Some samples had to be re-run because they either gave "un-callable" peaks/alleles, no alleles, or had poor standards that could not be improved using the system spectra. Usually problematic samples were re-run through the CEQ system as a first option and if this failed, the PCR was redone. In some cases, there was no improvement in the result obtained. Although the SLS (and DNA standard before addition to Hidi formamide) was vortexed as required and used in the recommended ratio (of Hidi formamide to DNA standard), most of the standard peaks (see

Figure 1) were low in comparison to many of the primer peaks. For some accessions for which results were not obtained, DNA may need to be re-extracted. Samples of ICS and PNG accessions were not included due to being earmarked for SNP analysis. Some samples initially classified as missing were found after an initial search, and updated in the catalogue of all available samples. The profile displayed in Figure 1 is a screen capture of an actual (annotated) profile obtained from three multiplexed primers.

**Figure 1. SSR profile: Multiplexed primer pairs P15, P9 and P25.**

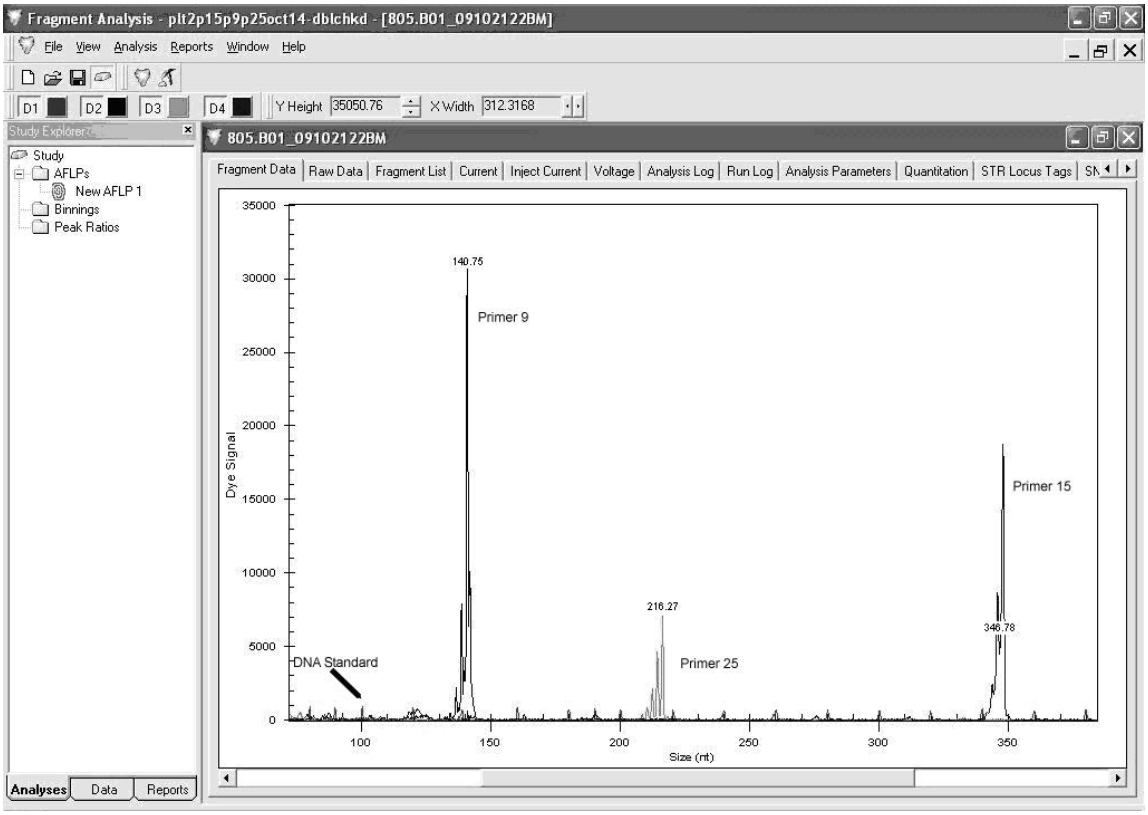


**Table 4. Summary of data generated.**

| No. of primer pairs | No. of accessions with data |
|---|---|
| 1-3 | 38 |
| 4-6 | 34 |
| 7-9 | 53 |
| 10-12 | 74 |
| 13 | 7 |
| 14 | 23 |
| 15 | 102 |

The expected success rate for accessions that were difficult to analyse was estimated at <30% (S. Pinney, pers comm) however, when the CEQ system was running well, with a new capillary system, up to 70% success was achieved (allele peaks were "called"). Primer stock quality may have affected success. Table 4 shows the number of accessions for which new data were

generated. Data are available for 331 accessions however not all of the accessions have a complete dataset for all of the SSR primer pairs used. Complete data from all 15 primer pairs is available for 102 accessions.

## Conclusions

The assessment of SSR profiles is very time intensive. Although most of the DNA samples were of sufficiently good quality for processing using this method, cacao DNA integrity (especially of samples that were "tried" previously) is one factor that could have adversely affected the output. Also, primer integrity (only old stock was available), DNA size standard quality and capillary system "life" could all potentially have affected the results obtained. Atypical standard profiles that did not improve with the system spectra and occurrences of "pull-up" during the late stages of the capillary "life" led to frequent loss of data, although these occurrences were sometimes ignored in the attempt to prolong the "life" of the capillary. It was not possible to test the efficacy of the primer stock since no new stock was available.

## Future Prospects

Data have been compiled in a Microsoft® Excel workbook and this file has been made available for verification work although some accessions are incomplete. Problematic DNA samples will need to be redone, either at a lower concentration, or it may be necessary to re-extract DNA from leaf samples. For those samples tagged as "missing" and that could not be located, DNA aliquots housed at CRU can be used to complete the project. It is possible the project could be completed at Beltsville during another six month period, or if the new sequencing system at UWI has been set up, tested and shown to be functional and the necessary reagents supplied, an attempt could be made to finish the remaining samples with that machine. The one caveat is that with the SSR protocol, completion of the project on the same system is preferred to avoid differences in allele scoring that may exist between the two different though similar types of instruments.

## Acknowledgments

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## References

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Boccara, M., Johnson, E.S., Butler, D.R., Bekele, F.L., Motilal, L.A., Sankar, A., Sounigo, O., O'Connor, C. and Williams, C. (2001) Sampling strategies for DNA Extraction in the USDA fingerprinting project. Pages 18-22 in: *Annual Report for 2000*. St. Augustine, Trinidad: Cocoa Research Unit, the University of the West Indies.

Saunders, J.A. (2001) USDA DNA Fingerprinting programme for identification of *Theobroma Cacao* accessions. Pages 108-114 in: *Proceedings of the international workshop on new technologies and cocoa breeding*, 16th-17th October 2000, Kota Kinabalu, Sabah, Malaysia. London, UK: INGENIC.

Saunders, J.A., Mischke, S., Leamy, E.A., Hemeida, A.A. (2004) Selection of international molecular standards for DNA fingerprinting of *Theobroma cacao*. *Theoretical and Applied Genetics* **110:** 41-47.

# New insight of genetic diversity and genotype identification using SNP-gene based markers

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Conservation of genetic resources is one of the priorities of the Cocoa Research Unit in Trinidad. Mislabelling, however, is a major problem in germplasm collections all around the world, and the ICG, T is no exception. Estimates of the size of the problem vary, and some estimates indicate that it could reach up to 40% of the trees (Motilal and Butler, 2003).

A joint USDA/CRU collaborative project that aims to fingerprint each original accession held in the ICG,T with microsatellite markers started in 2001.The results of the DNA profiles obtained with SSR markers are currently available for 1,400 accessions from UCRS and Marper Farm.

In recent years, a new DNA fingerprinting technique using SNP (single nucleotide polymorphism) markers has been developed allowing a rapid and accurate identification of an accession. The technique has been used in a collaborative project with CIRAD[1] and CNG[3] with a sample of the ICG,T collection. Although SSR is the marker of choice because they are well characterized with respect to the number of alleles, data collection and analysis are time consuming. On the other hand, because SNP markers typically are bi-allelic, analysis is easier. However being less informative, the number of SNP makers needed would be significantly higher.

Here, results obtained with the two markers are compared to examine their respective suitability and precision.

## Materials and methods

DNA samples sent to the USDA-ARS Beltsville laboratory were analyzed with 15 selected SSR primers, following a recommended protocol and guide-lines (Saunders, 2000). Out of the 1,400 accessions analysed so far, 138 accessions were selected among 5 diverse groups: Trinitarios, Iquitos (IMC), French Guyana, Nanay and Parinaris. (Table 1).

DNA samples used by CIRAD/CNG were analyzed with 835 SNP markers; however for the purpose of this study, a selection of 100 of these markers spread over the genome, was used for data analysis.

[1] CIRAD (Centre de coopération Internationale en Recherche Agronomique pour le Développement), - URM DAP TA A96/03-34398 Montpellier cedex 5 – France
[2] CRU (Cocoa Research Unit) The University of the West Indies, St Augustine, Trinidad and Tobago
[3] INRA-EPGV, CEA/Institut de Génomique - Centre National de Génotypage, 2 rue Gaston Crémieux, CP 5724, 91057 Evry Cedex – France
[4] United States Department of Agriculture- Agriculture Research Service, PSI SPCL,10300 Baltimore Avenue, Bldg. 001, Rm. 223, BARC-W, Beltsville, MD 20705, USA

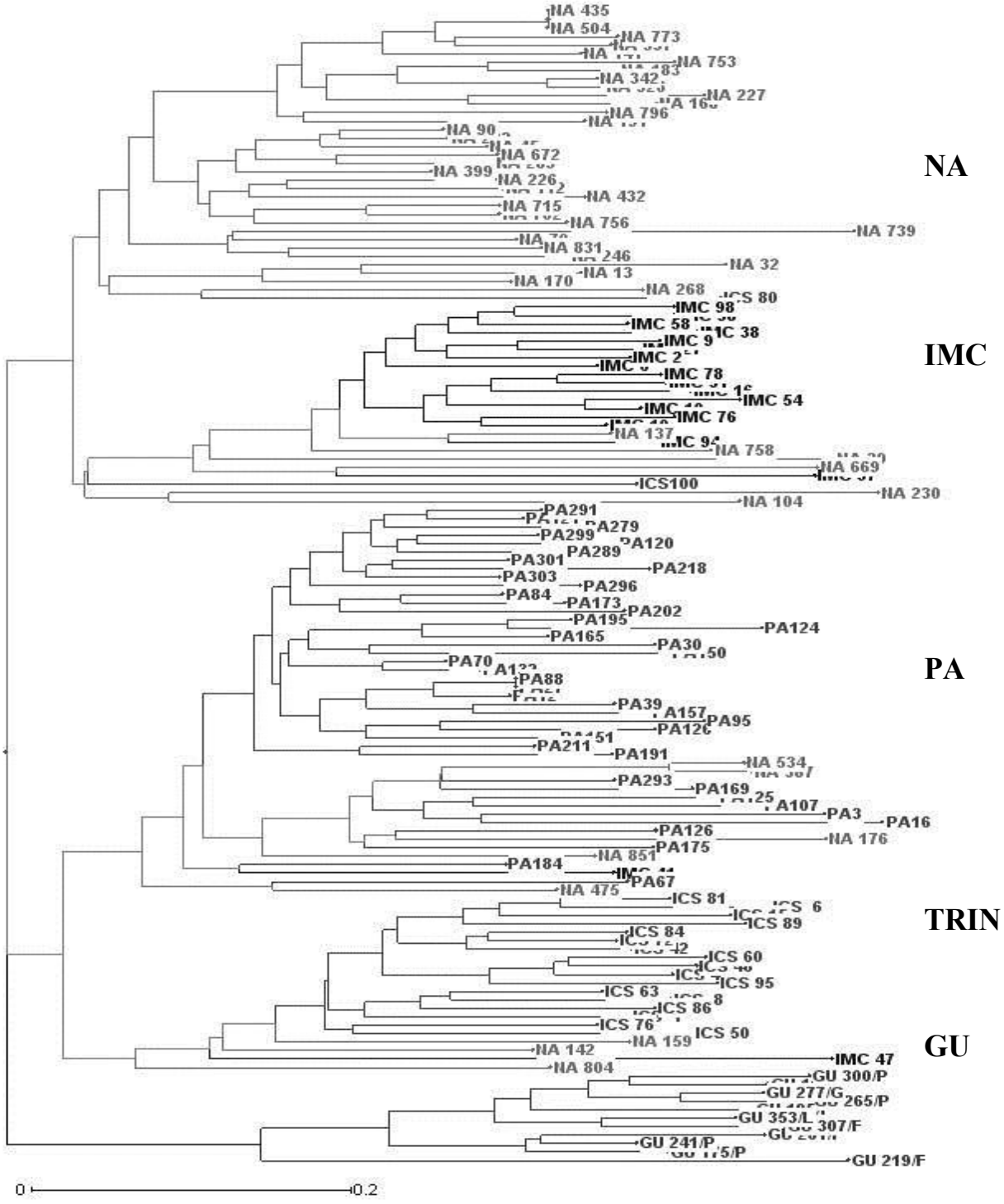


**Figure 1. Dendrogram of dissimilarity analysis run with 15 SSR markers.**

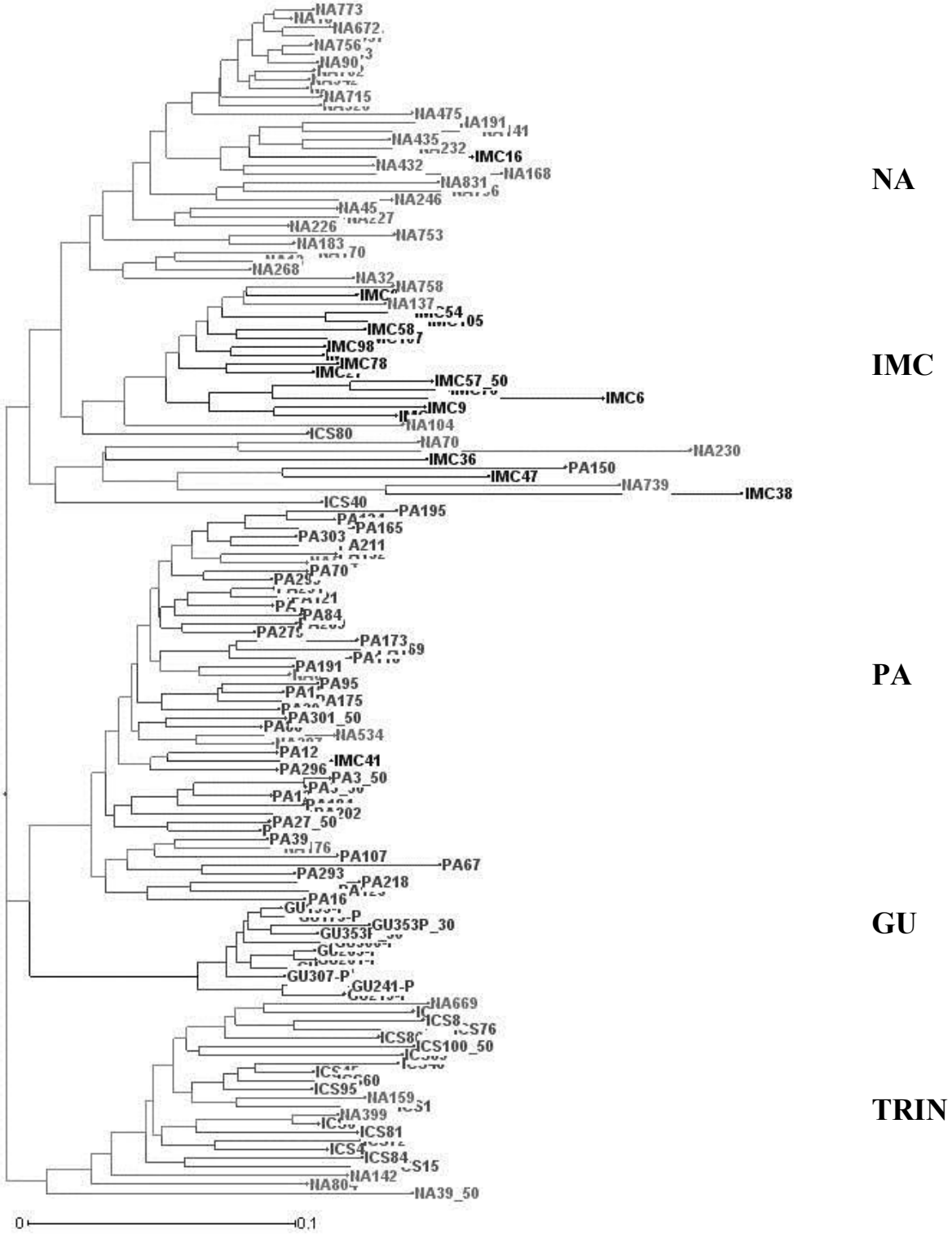


**Figure 2. Dendrogram of dissimilarity analysis run with 100 SNP markers.**

**Data analysis**

Results of DNA fingerprints obtained with SSR and SNP markers were compared to verify the accuracy of both techniques for:

- assessing genetic relationships
- detecting mislabelling

Fingerprints generated with 15 SSR markers and 100 SNP markers were run with DARwin software (DARwin5, version 5.0.158) for cluster analysis (displayed as dendrograms) and off-type detection, by generating Weighted Neighbour-Joining trees. The program Structure v2.3 was also used, with the number of clusters set to 5. All Structure runs used an admixture model with 100,000 iterations after a burn-in period of 100,000 and reiterated 10 times.

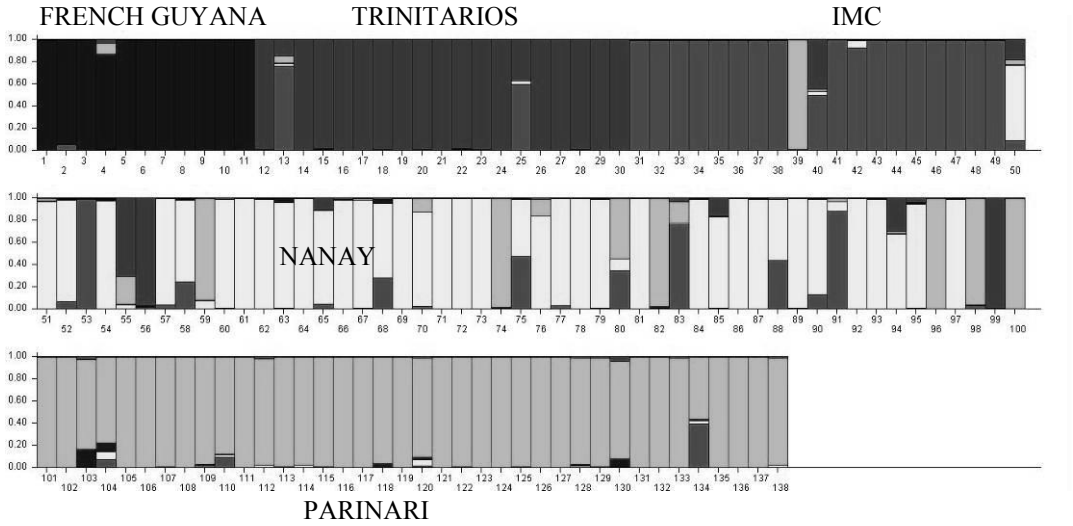


**Figure 3. Representation of clusters using STRUCTURE with K=5 : 138 accessions run with 15 SSR markers.**

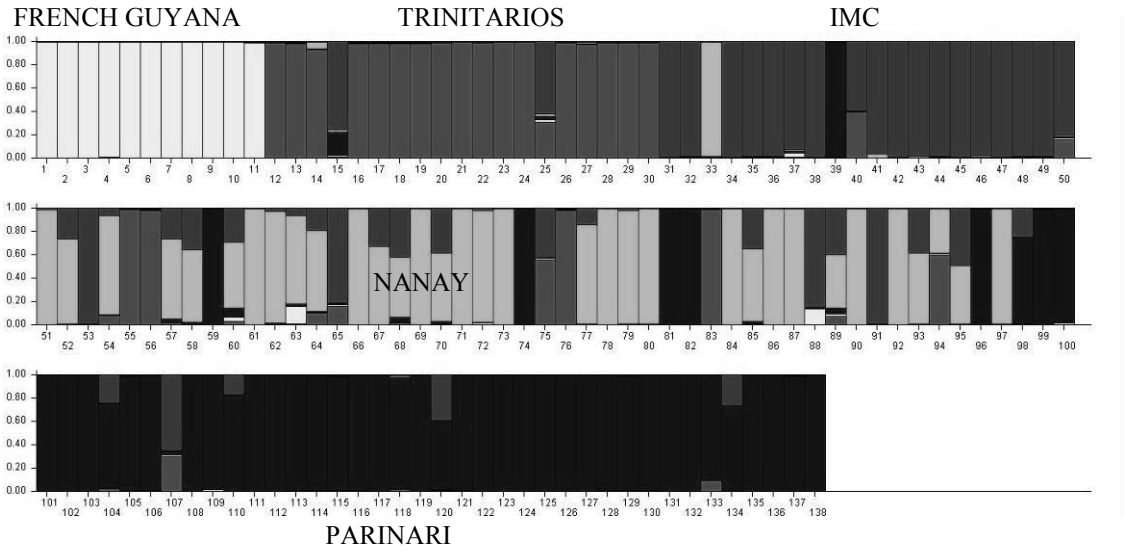


**Figure 4. Representation of clusters using STRUCTURE with K=5 : 138 accessions run with 100 SNP markers.**

## Results

Analysis of diversity

The dendrogram of dissimilarity for 138 accessions analyzed with the 15 SSR markers showed the expected clustering into 5 groups according to their origin: Trinitario, Guyanese, IMC, Nanay and Parinari. (Figure 1). Very similar clustering was obtained from the results of fingerprints given by the selected 100 SNP markers (Figure 2).

The results of Bayesian clustering analysis (Structure software) confirmed the above results, with a similar outcome for SSR and SNP markers when K was set to 5 (Figures 3 and 4). Out of the 138 samples, 102 were assigned in expected group with a 90% confidence threshold, and off-type trees were detected with the remaining samples (Tables 2 & 3).

**Table 2. List of true-to-type accessions identified with SSR markers and confirmed with SNP markers.**

| Accessions run with SSR markers | | | Accessions run with SNP markers | | |
|---|---|---|---|---|---|
| **Code** | **Clone name** | **Location** | **Code** | **Clone name** | **Location** |
| 1 | GU 114/P | CAMPUS 1A | 1 | GU 114/P | Field 4A B195 T1 |
| 2 | GU 175/P | CAMPUS 1A | 2 | GU 175/P | Field 4A B228 T2 |
| 3 | GU 195/P | CAMPUS 1A | 3 | GU 195/P | Field 4A B229 T2 |
| 4 | GU 219/F | CAMPUS 1A | 4 | GU 219/F | Field 4A B237 T1 |
| 5 | GU 241/P | CAMPUS 1A | 5 | GU 241/P | Field 4A B258 T2 |
| 6 | GU 261/P | CAMPUS 1A | 6 | GU 261/P | Field 4A B231 T2 |
| 7 | GU 265/P | CAMPUS 1A | 7 | GU 265/P | Field 4A B230 T2 |
| 8 | GU 277/G | CAMPUS 1A | 8 | GU 277/G | Field 4A B259 T2 |
| 9 | GU 300/P | CAMPUS 1A | 9 | GU 300/P | Field 4A B197 T2 |
| 10 | GU 307/F | CAMPUS 1A | 10 | GU 307/F | Field 4A B232 T2 |
| 11 | GU 353/L | CAMPUS 1A | 11 | GU 353/L | Field 4A B199 T1 |
| 12 | ICS 1 | S.J.E | 12 | ICS 1 | Field 6B B122 T9 |
| 14 | ICS 15 | S.J.E | 14 | ICS 15 | Field 4A C302 T1 |
| 16 | ICS 42 | S.J.E | 16 | ICS 45 | Field 6B B113 T6 |
| 17 | ICS 48 | S.J.E | 17 | ICS 48 | Field 6B E318 T6 |
| 18 | ICS 50 | S.J.E | 18 | ICS 49 | Field 6B B121 T4 |
| 19 | ICS 6 | S.J.E | 19 | ICS 6 | Field 6B E281 T15 |
| 20 | ICS 60 | S.J.E | 20 | ICS 60 | Field 6B E332 T6 |
| 21 | ICS 63 | S.J.E | 21 | ICS 63 | Field 6B E317 T2 |
| 22 | ICS 72 | S.J.E | 22 | ICS 72 | Field 6A A72 T9 |
| 23 | ICS 76 | CAMPUS 11 | 23 | ICS 76 | Field 6B B109 T3 |
| 24 | ICS 8 | S.J.E | 24 | ICS 8 | Field 6B B111 T2 |
| 26 | ICS 81 | S.J.E | 26 | ICS 81 | Field 4A C281 T1 |
| 27 | ICS 84 | S.J.E | 27 | ICS 84 | Field 6B E329 T3 |
| 28 | ICS 86 | S.J.E | 28 | ICS 86 | Field 6B E330 T5 |
| 29 | ICS 89 | S.J.E | 29 | ICS 89 | Field 6B E344 T14 |
| 30 | ICS 95 | S.J.E | 30 | ICS 95 | Field 6B B84 T4 |
| 31 | IMC 105 | MARPER | 31 | IMC 105 | Field 6B A24 T6 |
| 32 | IMC 107 | Field 6B A28 T3 | 32 | IMC 107 | Field 6B A28 T3 |
| 34 | IMC 2 | Field 6B A41 T8 | 34 | IMC 2 | Field 6B A41 T8 |
| 35 | IMC 27 | MARPER | 35 | IMC 27 | Field 6B A20 T3 |
| 36 | IMC 31 | MARPER | 36 | IMC 31 | Field 6B A32 T9 |
| 37 | IMC 38 | MARPER | 37 | IMC 36 | Field 6B A62 T1 |

| | | | | | |
|---|---|---|---|---|---|
| 38 | IMC 38 | Field 6B A21 T15 | 38 | IMC 38 | Field 6B A21 T3 |
| 41 | IMC 54 | MARPER | 41 | IMC 54 | Field 6B A9 T3 |
| 42 | IMC 57 | MARPER | 42 | IMC 57 | Field 6B A22 T7 |
| 43 | IMC 58 | MARPER | 43 | IMC 58 | Field 6B A47 T5 |
| 44 | IMC 6 | MARPER | 44 | IMC 6 | Field 6B A1 T6 |
| 45 | IMC 76 | MARPER | 45 | IMC 76 | Field 6B A45 T4 |
| 46 | IMC 78 | MARPER | 46 | IMC 78 | Field 6B A14 T2 |
| 47 | IMC 9 | MARPER | 47 | IMC 9 | Field 6B A40 T3 |
| 48 | IMC 94 | MARPER | 48 | IMC 94 | Field 6B A17 T7 |
| 49 | IMC 98 | Field 6B A63 T3 | 49 | IMC 98 | Field 6B A63 T3 |
| 51 | NA 112 | Field 5B F491 T1 | 51 | NA 112 | Field 5B F491 T1 |
| 52 | NA 13 | MARPER | 52 | NA 13 | Field 6B C174 T2 |
| 54 | NA 141 | Field 5B G620 T4 | 54 | NA 141 | Field 5B G620 T4 |
| 60 | NA 183 | Field 5B G603 T2 | 60 | NA 183 | Field 5B G603 T2 |
| 61 | NA 184 | Field 5B G612 T1 | 61 | NA 184 | Field 5B G612 T8 |
| 62 | NA 191 | Field 5B F433 T3 | 62 | NA 191 | Field 5B F433 T3 |
| 63 | NA 226 | Field 6B E292 T6 | 63 | NA 226 | Field 6B E292 T6 |
| 64 | NA 227 | Field 5A D312 T1 | 64 | NA 227 | Field 5A D312 T1 |
| 66 | NA 232 | Field 5B G628 T7 | 66 | NA 232 | Field 5B G628 T7 |
| 69 | NA 283 | Field 5B G618 T3 | 69 | NA 283 | Field 5B G618 T3 |
| 71 | NA 326 | MARPER | 71 | NA 326 | Field 5B E416 T1 |
| 72 | NA 337 | MARPER | 72 | NA 337 | Field 5B G617 T2 |
| 73 | NA 342 | Field 6B C168 T6 | 73 | NA 342 | Field 6B C168 T6 |
| 77 | NA 432 | MARPER | 77 | NA 432 | Field 6B E293 T9 |
| 78 | NA 435 | Field 5B F531 T3 | 78 | NA 435 | Field 5B F531 T3 |
| 79 | NA 45 | MARPER | 79 | NA 45 | Field 5B F510 T10 |
| 84 | NA 672 | MARPER | 84 | NA 672 | Field 5B F477 T3 |
| 86 | NA 702 | MARPER | 86 | NA 702 | Field 5B G631 T3 |
| 87 | NA 715 | MARPER | 87 | NA 715 | Field 5A D338 T5 |
| 90 | NA 756 | MARPER | 90 | NA 756 | Field 6A B101 T9 |
| 92 | NA 773 | Field 5B F547 T3 | 92 | NA 773 | Field 5B F547 T3 |
| 97 | NA 90 | MARPER | 97 | NA 90 | Field 5B F550 T12 |
| 98 | PA 107 [PER] | MARPER | 98 | PA 107 [PER] | Field 5A D247 T3 |
| 100 | PA 12 [PER] | MARPER | 100 | PA 12 [PER] | Field 6B D200 T2 |
| 101 | PA 120 [PER] | Field 6B D188 T2 | 101 | PA 120 [PER] | Field 6B D188 T13 |
| 102 | PA 121 [PER] | MARPER | 102 | PA 121 [PER] | Field 6B C166 T10 |
| 103 | PA 124 [PER] | MARPER | 103 | PA 124 [PER] | Field 6B D192 T8 |
| 104 | PA 125 [PER] | MARPER | 104 | PA 125 [PER] | Field 5B F527 T8 |
| 105 | PA 126 [PER] | Field 6B D198 T14 | 105 | PA 126 [PER] | Field 6B D198 T4 |
| 106 | PA 132 [PER] | MARPER | 106 | PA 132 [PER] | Field 5B D275 T3 |
| 108 | PA 151 [PER] | MARPER | 108 | PA 151 [PER] | Field 5B F437 T1 |
| 109 | PA 157 [PER] | Field 5B F466 T3 | 109 | PA 157 [PER] | Field 5B F466 T11 |
| 110 | PA 16 [PER] | Field 6B D186 T1 | 110 | PA 16 [PER] | Field 6B D186 T13 |
| 111 | PA 165 [PER] | Field 5B F451 T1 | 111 | PA 165 [PER] | Field 5B F451 T1 |
| 112 | PA 169 [PER] | MARPER | 112 | PA 169 [PER] | Field 6B C180 T3 |
| 113 | PA 173 [PER] | Field 5B F480 T8 | 113 | PA 173 [PER] | Field 5B F480 T3 |
| 114 | PA 175 [PER] | MARPER | 114 | PA 175 [PER] | Field 5B F473 T6 |
| 115 | PA 184 [PER] | MARPER | 115 | PA 184 [PER] | Field 5B F490 T8 |
| 116 | PA 191 [PER] | MARPER | 116 | PA 191 [PER] | Field 5B F536 T4 |
| 117 | PA 195 [PER] | Field 6B C165 T1 | 117 | PA 195 [PER] | Field 6B C165 T1 |
| 118 | PA 202 [PER] | Field 5A D309 T1 | 118 | PA 202 [PER] | Field 5A D309 T1 |
| 119 | PA 211 [PER] | MARPER | 119 | PA 211 [PER] | Field 5B F528 T2 |
| 121 | PA 27 [PER] | Field 5B E423 T4 | 121 | PA 27 [PER] | Field 5B E423 T3 |

| | | | | | |
|---|---|---|---|---|---|
| 122 | PA 279 [PER] | MARPER | 122 | PA 279 [PER] | Field 6B D197 T2 |
| 123 | PA 289 [PER] | Field 5B F535 T12 | 123 | PA 289 [PER] | Field 5B F535 T1 |
| 124 | PA 291 [PER] | Field 6B C167 T6 | 124 | PA 291 [PER] | Field 6B C167 T13 |
| 125 | PA 293 [PER] | MARPER | 125 | PA 293 [PER] | Field 5A D308 T7 |
| 126 | PA 296 [PER] | Field 6B D207 T1 | 126 | PA 296 [PER] | Field 6B D207 T6 |
| 127 | PA 299 [PER] | Field 5B E398 T6 | 127 | PA 299 [PER] | Field 5B E398 T2 |
| 128 | PA 3 [PER] | MARPER | 128 | PA 3 [PER] | Field 5B E355 T2 |
| 129 | PA 30 [PER] | Field 6B C144 T1 | 129 | PA 30 [PER] | Field 6B C144 T7 |
| 130 | PA 300 [PER] | Field 5B E407 T14 | 130 | PA 300 [PER] | Field 5B E407 T14 |
| 131 | PA 301 [PER] | MARPER | 131 | PA 301 [PER] | Field 5A D320 T7 |
| 132 | PA 303 [PER] | MARPER | 132 | PA 303 [PER] | Field 6B D211 T3 |
| 133 | PA 39 [PER] | Field 5A D264 T1 | 133 | PA 39 [PER] | Field 5A D264 T3 |
| 135 | PA 70 [PER] | Field 5B F489 T14 | 135 | PA 70 [PER] | Field 5B F489 T10 |
| 136 | PA 84 [PER] | Field 5B E388 T2 | 136 | PA 84 [PER] | Field 5B E388 T7 |
| 137 | PA 88 [PER] | MARPER | 137 | PA 88 [PER] | Field 5B F443 T1 |
| 138 | PA 95 [PER] | MARPER | 138 | PA 95 [PER] | Field 5B F460 T11 |

**Table 3. List of off-type accessions identified with SSR markers and confirmed with SNP markers.**

| **Accessions run with SSR markers** | | | **Accessions run with SNP markers** | | |
|---|---|---|---|---|---|
| **Code** | **Clone name** | **Location** | **Code** | **Clone name** | **Location** |
| 53 | NA 137 | Field 6B C155 T15 | 53 | NA 137 | Field 6B C155 T10 |
| 55 | NA 142 | MARPER D682 | 55 | NA 142 | Field 6A B89 T3 |
| 56 | NA 159 | MARPER D650 | 56 | NA 159 | Field 5B G635 T14 |
| 59 | NA 176 | Field 4A D389 T4 | 59 | NA 176 | Field 5B E403 T2 |
| 74 | NA 387 | Field 5A D251 T2 | 74 | NA 387 | Field 5A D251 T2 |
| 75 | NA 39 | MARPER D138 | 75 | NA 39 | Field 4A D370 T1 |
| 76 | NA 399 | MARPER D456 | 76 | NA 399 | Field 4A D408 T3 |
| 82 | NA 534 | Field 5B G630 T1 | 82 | NA 534 | Field 5B G630 T2 |
| 83 | NA 669 | MARPER C733 | 83 | NA 669 | Field 4A D418 T2 |
| 39 | IMC 41 | Field 6B F418 T1 | 39 | IMC 41 | Field 6B F418 T15 |
| 40 | IMC 47 | Field 6B F401 T1 | 40 | IMC 47 | Field 6B F401 T9 |
| 13 | ICS 100 | S.J.E | 13 | ICS 100 | Field 6B B100 T1 |
| 15 | ICS 40 | S.J.E | 15 | ICS 40 | Field 6B E287 T4 |
| 25 | ICS 80 | S.J.E | 25 | ICS 80 | Field 6A A72 T9 |
| 107 | PA 150 [PER] | MARPER D697 | 107 | PA 150 [PER] | Field 6B C179 T1 |
| 134 | PA 67 [PER] | Field 5B E346 T4 | 134 | PA 67 [PER] | Field 5B E346 T11 |

## Detection of mislabelling

Some accessions used in the sample had been previously identified as mislabelled or rootstock in the CRU/USDA Fingerprinting Project (Table 4, Boccara and Zhang, 2005, 2008).

Both marking techniques were able to detect mislabelling of trees.

**Table 4. List of accessions previously identified as off-types.**

| Clustered with Trinitario accessions | Clustered with IMC accessions | Clustered with PA accessions |
|---|---|---|
| NA 159 | NA 137 | NA 176 |
| NA 142 | NA 758 | NA534 |
| NA 804 | NA 39 | NA387 |
| PA 114 [PER] | NA 669 | NA 851 |
| IMC 47 | NA 230 | IMC 41 |
| | ICS 80 | |
| | ICS 100 | |

French Guyana

No mislabelled tree was recorded with SSR markers with samples collected from Campus Field 1B; SNP markers showed that all the trees tested were correctly propagated when they were established in Field 4A at UCRS.

Trinitario trees

The analysis with SNP confirmed that ICS 80, detected as an off-type at the San Juan Estate with SSRs, doesn't belong to the Trinitario group.

The SNP results suggest that ICS 100 (detected as non-Trinitario in the San Juan Estate with SSRs), could be correctly labelled in Field 6B, Plot B100 at UCRS. In the case of ICS 40, the tree sampled from the genebank (Field 6B, Plot E287, T4) doesn't match the original tree, confirming earlier morphological observations (Bekele et al., 2004). Structure analysis suggests with a probability of 80% assigning this accession to the IMC group (Figure 4).

IMC trees

The SNP markers confirm without ambiguity that IMC 41 belongs to the Parinari group. It had previously been suggested that the trees in Field 6B, Plot F418 were propagated from one of the neighbour trees in Marper Farm, PA 200 [PER] or PA 207 [PER] (Boccara, 2006; Bekele et al., 2005).

The analysis confirms that neither of the two IMC 47 samples (trees 1 and 9) from Field 6B, Plot F401, conform to the IMC group. Previous results (Boccara et al., 2004) suggested that trees 1, 3, 4, 11, 12 were identical, but were different to both the Marper tree and the Campus tree, and were similar to IMC 57 (IMC 57 not falling in the IMC group) .

The SSR profile IMC 16 in Marper Block D603 showed that it belongs to the IMC group; however the same technique revealed that Tree 2, in Field 6B, Plot A11 has a NA profile; SNP marker analysis of this tree gave the same information, inferring that budwood could have been taken from NA 105, the adjacent tree in Marper Farm (now recorded as dead).

Nanay trees

All the accessions already identified as off-type (Table 4), were confirmed with SNP markers, and furthermore each can be assigned to a specific accession group.

*Case of NA 475*
Tree 9 in Field 5B, Plot F534 was identified as an off-type with SSR markers; the SNP profile however, suggests that tree 3 in Field 4A, Plot D415 was correctly re-propagated from the original tree in Marper Farm.

Parinari trees

As for Nanay group, SNP markers confirmed the identity of the correct labelled accessions and identified the mislabelled ones.
*Case of PA 150*
Whereas the original tree in Marper Farm had been identified as correct, the SNP profile confirms that Tree 1 in Field 6B, Plot C179 is not identical. The result confirms earlier pod morphology observations and microsatellites markers results (Motilal et al., 2008).

## Conclusion and future perspectives

The results of the analysis of the genetic diversity with SNP markers are in complete agreement with those obtained with SSR markers. Comparison with original reference trees and assignment tests demonstrated the efficiency and accuracy of the 100 selected SNP markers for the detection of mislabelling.

More verification of mislabelled trees will be needed to reduce the risks of erroneous duplication and distribution of trees from UCRS.

A technical problem of the use of SSR markers is that it is not easy to compare data produced by different laboratories: discrepancy in allele size calling mainly due to the large variety of automatic sequencing machines and software used; SNP markers would be a suitable tool for use at CRU for future identification work. Preliminary tests have shown that a subset of 65 selected markers was efficient for the unambiguous recognition of mislabelled trees.

## Acknowledgements

We thank Antoinette Sankar for DNA sample preparation, Frances Bekele for sharing her knowledge on morphological traits, and L.A. Motilal for advice. USDA-ARS Beltsville, CIRAD and CNG were responsible for the efforts made to run the samples and collect the data.

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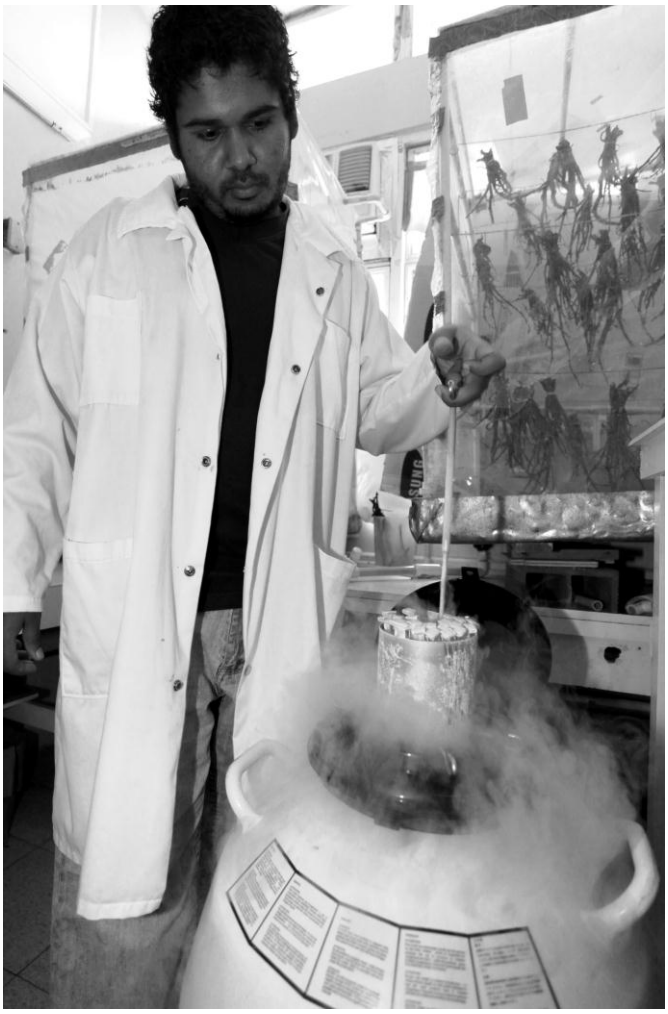
# Evaluation



# Proposals for new areas of research in pathology post CFC germplasm enhancement for Black Pod resistance at CRU

**S. Surujdeo-Maharaj**

Over the five year duration (2004-2009) of the CFC/ICCO/BIOVERSITY Cocoa Productivity Project, significant advances were made in the area of germplasm enhancement for black pod (GEP-BP) disease.

## Germplasm Enhancement Programme

During the first-cycle of crosses in the GEP-BP, 25 cycles of observations were carried out on 866 progeny in Field 14 (located at La Reunion Estate, Centeno) and 768 in Field 7 (located at UCRS) for BP and WB resistance, vigour and precocity. Seven hundred and sixty-six genotypes were screened for BP resistance using the detached pod test and confirmatory testing was done for 367 of these. Bean number and bean weight for 762 genotypes with 1 – 10 pods were recorded. Data were collected on 5 – 10 pods for 451 of these genotypes. In the second-cycle GEP-BP 1,017 seedlings were generated from 24 crosses using first-cycle progeny as parents. These plants were established in the greenhouse and early screening for resistance to BP was successfully completed on 1,013 progeny and 411 (41%) were found to be highly resistant. Plants with high levels of resistance to BP were selected and established in Field 7.

## Breeding

It was proposed by the late A.D. Iwaro that some of the highly resistant GEP-BP derived material be used to incorporate genes for resistance into the local commercial Trinidad Selected Hybrid (TSH) types. The proposal was formalized into a new joint project - 'Improvement of resistance to black pod disease in Trinidad Selected Hybrids' between MALMR and CRU and started in May 2009. Thirty bi-parental crosses were carried out between enhanced GEP material from CRU and TSH types (Table 1) and their progenies were established at Cocoa Research, MALMR. Optimisation experiments in CRU on progeny generated by MALMR started in November 2009. The project involves; *early screening* for resistance of progenies generated by MALMR, using the punch-inoculation test on leaves; *selection* of promising individuals for establishment in field trials; *assessment* of pod production, bean number and wet bean weight on genotypes established in the field; *detached pod* testing on trees established in the field trial; *assessment of WB resistance* through laboratory screening; *assessment of flavour* for the best 200 genotypes; *selection of promising genotypes* combining good yield potential with resistance to BP and WB diseases; establishment of clonal *on-farm trials*; training of MALMR staff; *dissemination of project results*; commencement of second cycle.

## Host-pathogen interaction between *Phytophthora palmivora* and *Theobroma cacao*

Recent preliminary studies suggest that aggressive strains of *P. palmivora* exist in Trinidad and that pathogen interaction is occurring among different plant organs. Based on this it is proposed that pathogenicity testing be done to investigate variability in aggressiveness of *P. palmivora*

**Table 1. Bi-parental cross made between germplasm enhanced clones from CRU and TSH clones from MALMR.**

| Cross No. | Female | Male |
|---|---|---|
| 1 | TSH 730 | GEBP 82/B-F [ADI] |
| 2 | TSH 730 | GEBP 303/B-M [ADI] |
| 3 | TSH 730 | GEBP 706/B-T [ADI] |
| 4 | TSH 730 | GEBP 1322/A-T [ADI] |
| 5 | TSH 730 | GEBP 1348/A-T [ADI] |
| 6 | TSH 1076 | GEBP 82/B-F [ADI] |
| 7 | TSH 1076 | GEBP 303/B-M [ADI] |
| 8 | TSH 1076 | GEBP 706/B-T [ADI] |
| 9 | TSH 1076 | GEBP 1322/A-T [ADI] |
| 10 | TSH 1076 | GEBP 1348/A-T [ADI] |
| 11 | TSH 1102 | GEBP 82/B-F [ADI] |
| 12 | TSH 1102 | GEBP 303/B-M [ADI] |
| 13 | TSH 1102 | GEBP 706/B-T [ADI] |
| 14 | TSH 1102 | GEBP 1322/A-T [ADI] |
| 15 | TSH 1102 | GEBP 1348/A-T [ADI] |
| 16 | TSH 1347 | GEBP 82/B-F [ADI] |
| 17 | TSH 1347 | GEBP 303/B-M [ADI] |
| 18 | TSH 1347 | GEBP 706/B-T [ADI] |
| 19 | TSH 1347 | GEBP 1322/A-T [ADI] |
| 20 | TSH 1347 | GEBP 1348/A-T [ADI] |
| 21 | TSH 1364 | GEBP 82/B-F [ADI] |
| 22 | TSH 1364 | GEBP 303/B-M [ADI] |
| 23 | TSH 1364 | GEBP 706/B-T [ADI] |
| 24 | TSH 1364 | GEBP 1322/A-T [ADI] |
| 25 | TSH 1364 | GEBP 1348/A-T [ADI] |
| 26 | TSH 1188 | GEBP 442/B-F [ADI] |
| 27 | TSH 919 | GEBP 1079/A-T [ADI] |
| 28 | TSH 1350 | GEBP 1095/A-T [ADI] |
| 29 | PA (TSH 1076 × PA 296 [PER] × PA 171 [PER]) | GEBP 1109/A-T [ADI] |
| 30 | TSH 1352 | GEBP 1131/A-T [ADI] |

isolates collected in different hydrological zones of Trinidad and Tobago. Subsequently, a subset of these isolates will be tested on selected clones having a wide range of levels of resistance to BP under field conditions to confirm host-pathogen interactions. It would be useful to know if isolates within a region compete with each other to establish themselves in the field. To answer this it is proposed that some inoculations be carried out under laboratory conditions, combining two or more isolates (of known aggressiveness) in the inoculum mixture and noting the response of the host tissue. Such an activity will allow for the determination of the average resistance of clones to a number of different isolates, which will guide breeders in the selection of clones with good levels of resistance to many isolates of *P. palmivora*. The behaviour of Trinidad *P. palmivora* isolates will be compared with isolates from different countries using clones in the CFC/ICCO/Bioversity International Clonal Trial. Here it is proposed that each country will carry out pathogenicity tests on common clones using a standardized protocol with their local isolates. Partners could include Trinidad, Costa Rica, Ecuador, Brazil, and Cameroon. Isolates will be collected and screened annually from selected fields to determine if those from

the different regions are evolving.

**Cocoa Trinidad Virus (CTV)**

In May 2009, the coordinator of the International Cocoa Quarantine Centre, Reading alerted CRU that virus-like symptoms were seen on ICS 76 plants from Trinidad during routine virus indexing activities. Since then, investigations were carried out in Trinidad by Dr. T.N. Sreenivasan to confirm if virus symptoms can be detected on ICS 76 in its natural environment. To date, he has prepared a comprehensive report. His findings report, based on the symptoms observed on ICS 76 trees in UCRS, that the disease in question is most likely caused by Cocoa Trinidad Virus (CTV). CTV was first detected in Trinidad in 1943 and reported in 1944 by Posnette although it was probably here before that time. The disease was passed on to new generations of plants through successive vegetative multiplication. Surveys in the 1940's showed that the disease was confined to Diego Martin, Santa Cruz, Sangre Grande and Maracas. On the basis of symptoms induced on the differential host, ICS 6, two strains (A and B) were identified. Strain A produces feather-like red banding of few or all the main veins on flush leaves. As the leaves mature the red vein banding disappears. In some clones a mosaic type symptom persists on mature leaves. The mosaic is associated with red mottling on the flush leaves of some varieties, but in other varieties the mottling is absent. The first leaf of a new flush is most likely to show symptoms. Strain B produces a continuous vein banding extending to fine veins, which persists after the leaves have matured. A red vein banding was seen for a short period on young leaves of some varieties. CTV is not sap transmissible and the agent involved in its transmission is the citrus mealy bug *Planococus citri*, which infests cacao and is endemic to Trinidad. Under prevailing conditions there is no threat of CTV spreading at a rapid rate either within or outside UCRS, nor is there a threat of an epidemic of CTV in Trinidad and Tobago. However, in an effort to manage its spread, an action plan has been prepared by Dr. Sreenivasan which includes: systematic survey of the ICG,T for CTV symptoms by qualified individuals - inspecting trees during flushing cycles; label trees with symptoms, removing them from ICG,T and destroying them either through burning or burying. Scientific considerations for studying the disease include: propagation of virus free ICS 76 plants through tissue culture; proper characterisation of the virus using molecular biology tools; maintenance of the virus in live plants within an insect proof enclosure at CRU for further studies. It is important to note that CTV does not negatively affect cacao production in Trinidad and Tobago. It was first noted in Trinidad by Posnette in 1943 (Kirkpatrick, 1945) and has been latent for many years and would have gone unnoticed if it were not for the virus indexing work at the ICQC,R. Additionally, there is no reason for alarm by end users of clones sent from the ICG,T.

**Establishment of working collection of clones in the GH**

In order to carry out ongoing scientific investigations done by the various sections at CRU (Molecular Biology, Pathology, Descriptors and Flavour), it is proposed that a working collection of at least 20 clones be maintained in the greenhouses at CRU. The intention is to propagate and keep these plants under standardized conditions for long-term studies, at least 10 years. The plants will be micrografted from trees of confirmed identity and grown in large UV-protected pots. An initial list of clones proposed by the various sections include: AM 2/91

[POU], AMAZ 15-15 [CHA], Belize 1*, Belize 2*, Belize 3*, COCA 3370/5 [CHA], CRU 51, CRUZ 7/8, EET 58 [ECU], EET 59 [ECU], GU 261/P, ICS 1, ICS 15, ICS 46, ICS 57, ICS 61, ICS 67, ICS 68 , ICS 70, ICS 84, ICS 95, IMC 10 , IMC 11, IMC 14, IMC 57, IMC 6, JA 5/36 [POU], LCT EEN 162/S 1010, LCT EEN 37/A, MATINA 1/7 , MXC 67, NA 68, NA 33 , NA 45, NA 719, PA 71 [PER], SCA 6, SPEC 194/75 and WAA.

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# Evaluation of cocoa germplasm for resistance to Witches' Broom disease

**Romina Umaharan**

The protocol for screening for resistance to Witches' Broom disease is a two tiered system, involving the use of two methods of screening. Cacao clones are collected from the ICG,T as budwood and top-grafted in the greenhouse. The plants are then screened manually, using a spray inoculation technique. Clones are then evaluated for symptom expression and potentially resistant clones are selected and re-screened to confirm this resistance. The selected clones are re-collected, re-grafted and inoculated using the agar-droplet technique.

During January – December 2009, emphasis was placed on clonal propagation of clones previously selected for potential resistance, for re-screening or confirmation screening. Clones were collected as budwood from the ICG,T and micrografted onto TSH rootstocks. Each clone was replicated 10 times, with the aim of obtaining 3-5 plants per clone. A total of 46 clones have been propagated so far.

In addition to the propagation of clones for screening, 24 promising clones were inoculated to confirm and quantify their resistance. These clones were inoculated using the agar-droplet technique and the results confirm that four of these were resistant to Witches Boom disease.

# Utilisation

# Preliminary insight into the biochemical composition, aroma volatile fingerprints and sensorial profiles of the Imperial College Selections over two crop years

**D.A. Sukha, F. Davrieux[1], R. Boulanger[1] P. Alter[1], S. Assemat[1], S. Bharath, N. Ali, and D.R. Butler**

## Introduction

The Imperial College Selections (ICS) accession group represents the origins of desirable flavour attributes for which Trinitario germplasm has gained its "fine or flavour" reputation and is a historically important and valuable genetic asset. Although ICS germplasm has been distributed globally across continents and used in many international breeding programmes, it still remains under utilised and is potentially a useful source of cacao material with quality traits of economic importance for the global cocoa industry. A project funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) and co-financed by three chocolate companies one in France, one in Switzerland and the other in USA was initiated in 2006 with the following aims:

- to identify ICS accessions that have potentially interesting flavour, near infrared (NIR) and volatile aromatic attributes (via solid phase micro extraction (SPME) coupled with gas chromatographic mass spectrometry (GC-MS));
- to highlight their potential for use in breeding programmes throughout the world.

The benefits of this project therefore accrue to a wide range of stakeholders in the production chain from farmers to final consumers.

This report follows on from Sukha et al., (2009) where we presented first impressions from physical and organoleptic quality attributes of selected Imperial College Selections (ICS) assessed on samples from the first crop year of the project. In that article we indicated that as results from the chemical analyses, such as chemical aroma profiles and polyphenols, become available in this project, these will be linked to the organoleptic results will provide a clearer picture of the potential value of the ICS clones. In this report we are providing a preliminary insight into linkages between the biochemical composition, aroma volatile fingerprints and sensorial profiles of the Imperial College Selections over two crop years.

## Materials and methods

Beans were harvested from trees of ICS clones growing in the ICG,T. The beans were all processed according to standardised protocols in the same location; therefore any differences observed would be primarily due to genetic effects and not due to growing environment or processing activities or location.

### Preparation of bean and cocoa liquor samples and organoleptic assessment

Micro fermentations were carried out according to Sukha et al. (2008) on 27 ICS clones from the working group, yielding 47 samples (27 samples in 2006/2007 and 20 samples in 2007/2008).

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Preparation of cocoa liquor samples and organoleptic assessments were carried out on 45 samples (25 samples in 2006/20071 and 20 samples in 2007/2008 crop years) according to Sukha et al. (2008) using a trained sensory panel of nine panellists. Liquor samples were tasted blindly over three repetitions. Individual flavour attribute scores were entered into a data template in Microsoft® Excel where mean flavour profiles and the standard errors of the mean (SE) were calculated. Principal Component Analysis (PCA) was performed on the pooled data (from both years) using XLSTAT version 2008.1.01 (Addinsoft, USA). Graphical representations of organoleptic results were carried out in Microsoft® Excel.

NIR assessments

Near infrared spectroscopy (NIRS) acquisitions were obtained on a Foss-Perstorp 6500 analyser using a spin cell. Spectral data were collected and processed using Winisi 1.5 software (InfraSoft International, Port Matilda, USA). A 3 g sub-sample of cocoa taken from 100 g of shelled, ground and sieved beans (0.5 mm) was analysed by diffuse reflectance from 400 nm to 2,500 nm in 2 nm steps. Data were saved as the average of 32 scans and stored as log(1/R) where R is the reflectance at each wavelength and 1 the reflectance of a standard ceramic reference. Spectrum acquisitions were done randomly, each sample was duplicated (two filled cells) and the average spectrum was stored. Statistical analyses were performed using Win-ISI II software and XLSTAT version 2008.1.01 (Addinsoft, France).

Theobromine and caffeine

*Wet chemical method*
After reflux extraction in water, caffeine and theobromine contents were determined by high-performance liquid chromatography (HPLC) using an Agilent system series 1100 with a UV-VIS diode array detector. The detection and quantification were performed at the maximum absorption wavelength (280 nm).

*Near Infrared method*
Chemical data and NIR fingerprints of 47 ICS clone samples were added to the CIRAD database (Davrieux et al., 2007) and Partial least square models were used to establish quantitative relations between NIR spectral bands (900 nm to 2,500 nm) and both caffeine and theobromine contents. Calibration statistics include the following parameters: standard deviation of the population, coefficient of determination ($r^2$), standard error of calibration, and the standard error of cross-validation (SECV). Caffeine and theobromine contents were then predicted for the whole set of samples using the new models. Assignment of samples to type (Criollo-like or Forastero-like) was then based on caffeine and theobromine contents. As part of the calibration development, PCA was used to extract the relevant information from the spectral matrix (n = 47). The generalised Mahalanobis distance (H) was calculated on the extracted PCs for each sample. This statistical distance is useful for defining boundaries of the population and a similarity index between spectra (Schenk, et al., 1996).

[1] There were insufficient quantities of beans from two ICS clones to make cocoa liquors for organoleptic evaluation. Due to sample availability, ICS 15 was also only present for the 2006 – 2007 crop year.

SPME assessments

A 3 g sub-sample of shelled and ground cocoa powder was placed into a 10 mL vial, which was capped with a PTFE/silicon septum. It was heated to 50°C for 15 minutes before the SPME fibre (DVB/Carb/PDMS) was introduced into the headspace surrounding the cocoa powder and left in at the same temperature for 45 minutes. Each extraction was done in triplicate. After every 20 extractions, a SPME fibre control was performed using a mixture of standards, during this control, extractions were done at 50°C with a pre-incubation duration of 15 minutes and an adsorption duration of 30 minutes (Laguerre et al., 2007).

*Mass Spectrometer conditions*

Mass spectra were registered using an Agilent 6980 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled with an Agilent 5973N quadrupole mass spectrometer (Agilent Technologies). The volatiles were thermally desorbed from the fibre in the GC-MS injector at 250°C operating in split/splitless mode for 4 minutes. They were quickly transferred to the mass detector through a DB-5MS fused silica capillary column operating at 220°C with helium (2 mL/min) as the carrier gas. The MS source temperature was 150°C, and the mass spectra were scanned in Electron Ionization (EI)+ mode with an excitation of 70 eV. The m/z[1] range used was from 45 to 190 at a rate of 8.17 scans/s. The global signal registered between 2.8 and 8 minutes was transformed by using Pirouette software v 3.1. (Infometrix Inc., Woodinville, WA). The global area was the mean abundance values of the mass fragments recorded between 2.8 and 8 minutes.

**Results and Discussion**

Organoleptic assessments

The composite average score for each flavour attribute over all samples of the 25 ICS clones revealed that the ICS clones in general terms were balanced in their cocoa and fruity flavour attributes with some acidity and floral notes and a moderate basal astringency. There were no significant crop year effects (data not presented).

Results from the PCA showed that the first three principal components explained 73.1% of the variation in the samples. A PCA plot of the first two principal components for the average flavour profiles over three repetitions for the 45 samples assessed over two crop years (Figure 1) revealed that principal components 1 and 2 accounted for 40.1 and 20.7% of the variation in the samples respectively.

For clarity, the "ICS" accession code has been omitted from the clone names in Figure 1 and the crop years are indicated by the suffix "1" and "2" respectively after the ICS clone number. Figure 1 shows a spread of the ICS clones over the main aromatic flavour attributes (fruity and floral), as well as, cocoa and nutty flavours. No distinct clustering around a particular flavour attribute was observed, however, a minority of samples were associated with astringency, bitterness and raw/beany/green (R/B/G) flavours. Clone ICS 15 was only available from the 2006-2007 crop year and appeared to be an outlying sample but closer examination of the individual average profile scores (data not presented) revealed that a combination of bitterness,

[1] Mass to charge ratio

astringency, cocoa and moderate fruity flavour, more than any specific aromatic attribute, accounted for the uniqueness of ICS 15 from the other samples.

The percentage contributions of the nine different flavour attributes to the first three principal components derived from XLSTAT are presented (with dominant contributions in bold) in Table 1. Nutty, acidity, floral and R/B/G flavours had the highest percent contribution to principal component 1, whilst bitterness, astringency and fruity flavours had the highest percent contributions to the principal component 2. Cocoa, "other" flavours and bitterness contributed most to principal component 3.

**Table 1. Percentage contribution of different cocoa liquor flavour attributes to the first three principal components from the PCA analysis. Average flavour scores over three repetitions were used for each of the 25 ICS clones.**

| | Contribution of flavour attributes to the principal components | | |
|---|---|---|---|
| **Flavour attribute** | **PC 1 (%)** | **PC 2 (%)** | **PC 3 (%)** |
| Cocoa | 8.78 | 10.78 | **29.61** |
| Acidity | **18.26** | 0.04 | 2.40 |
| Astringency | 1.15 | **24.91** | 6.01 |
| Bitterness | 0.29 | **27.00** | **20.34** |
| Fruity | 10.55 | **17.30** | 0.14 |
| Floral | **14.74** | 7.22 | 12.18 |
| Nutty | **21.54** | 0.60 | 0.39 |
| Raw/Beany/Green (R/B/G) | **13.55** | 12.10 | 4.59 |
| Other | 11.14 | 0.07 | **24.35** |

A summary of the percentage contributions of the different ICS clones to the first three principal components derived from XLSTAT shows that ICS 15 from the 2006-2007 crop year which was the outlying sample in Figure 1 contributed most to principal components 1 and 2 in Figure 1 with 12.8 and 13%, respectively. ICS clones 48, 61, 62 and 65 from the 2007-2008 crop year contributed 10.3, 11 and 12% respectively to principal component 2. ICS 45 from the second crop year made the second highest contribution (18%) to principal component 3. By linking the trends from Table 1 and the percentage contributions of the different ICS clones to the first three principal components we can associate nutty, acid, floral and R/G/B flavours with ICS 15, as well as, astringent, bitter and fruity flavours with ICS 15 (from the 2006 – 2007 crop year) and ICS 48, 61, 62 and 65 from the 2007-2008 crop year. Clone ICS 45, also from the 2007-2008 crop year, was associated with cocoa, "other" flavours and bitterness.

A correlation matrix of the flavour attributes from the different ICS clones (with significant ($P \leq 0.05$) values in bold) (Table 2) revealed that cocoa was significantly correlated with nutty flavour (r = 0.586). Fruity and floral flavours were significantly correlated with acidity (r = 0.573 and 0.490 respectively). Additionally, nutty and R/B/G flavours were positively correlated with "other" flavours (r = 0.585 and 0.482 respectively). Cocoa flavour was negatively correlated (inversely related) with floral flavour (r = -0.674) whilst acidity was negatively correlated with nutty, R/B/G, "other" and cocoa flavours. Bitterness and fruity flavour were also

negatively correlated with each other (r = -0.358).

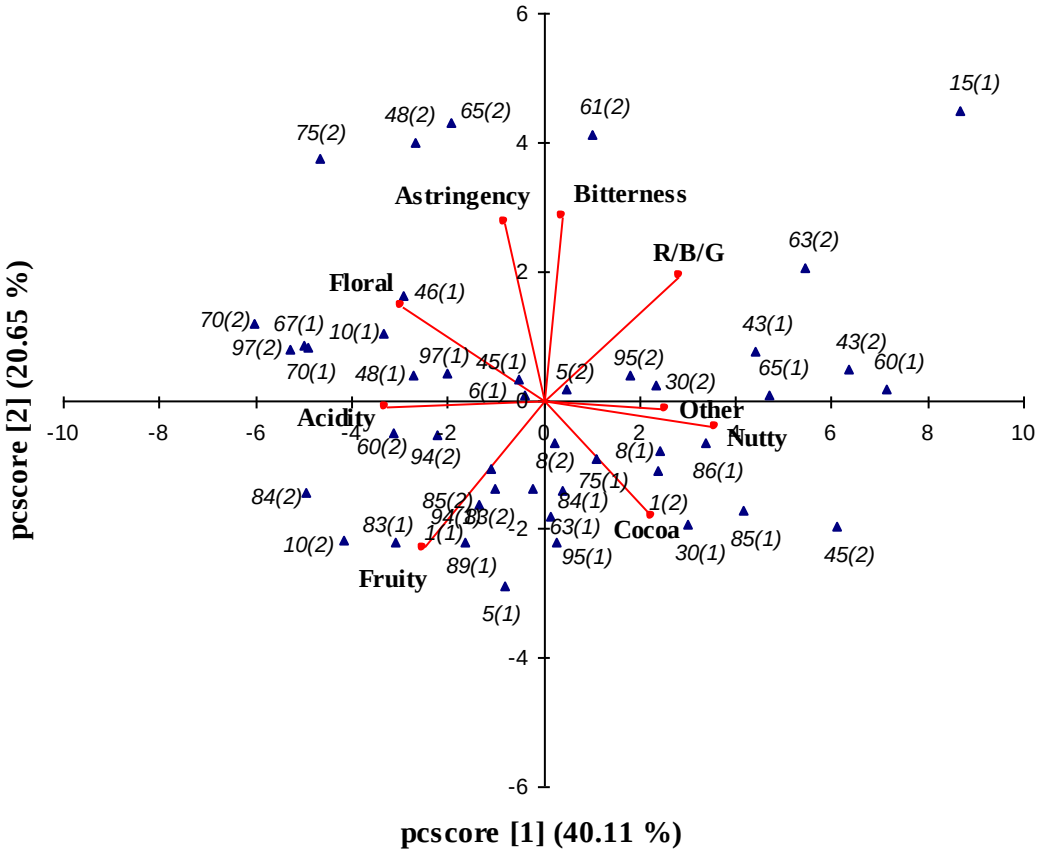


**Figure 1. Principal component analysis plot of flavour scores averaged over three repetitions of tasting for ICS clones in the working group from the 2006-2007 (suffix 1) and 2007-2008 (suffix 2) cocoa crop years of the project.**

**Table 2. A correlation matrix of the flavour attributes from 25 ICS clones presented with significant (P ≤ 0.05) values in bold.**

| Variables | Cocoa | Acidity | Astringency | Bitterness | Fruity | Floral | Nutty | R/B/G[1] | Other |
|---|---|---|---|---|---|---|---|---|---|
| Cocoa | 1 | **-0.309** | -0.144 | -0.091 | -0.075 | **-0.674** | **0.586** | 0.087 | 0.130 |
| Acidity | **-0.309** | 1 | 0.285 | -0.051 | **0.573** | **0.490** | **-0.598** | **-0.529** | **-0.431** |
| Astringency | -0.144 | 0.285 | 1 | **0.366** | -0.136 | **0.301** | -0.172 | 0.125 | -0.089 |
| Bitterness | -0.091 | -0.051 | **0.366** | 1 | **-0.358** | -0.010 | 0.054 | 0.248 | -0.159 |
| Fruity | -0.075 | **0.573** | -0.136 | **-0.358** | 1 | 0.210 | **-0.410** | **-0.609** | -0.242 |
| Floral | **-0.674** | **0.490** | **0.301** | -0.010 | 0.210 | 1 | **-0.618** | -0.273 | **-0.313** |
| Nutty | **0.586** | **-0.598** | -0.172 | 0.054 | **-0.410** | **-0.618** | 1 | **0.529** | **0.585** |
| R/B/G[1] | 0.087 | **-0.529** | 0.125 | 0.248 | **-0.609** | -0.273 | **0.529** | 1 | **0.482** |
| Other | 0.130 | **-0.431** | -0.089 | -0.159 | -0.242 | **-0.313** | **0.585** | **0.482** | 1 |

[1]Raw/beany/green

Near infrared reflectance spectroscopy (NIRS) assessments

Principal components 1, 2 and 3 extracted from a PCA done on the 47 samples, explained respectively 85.6, 7.0 and 2.1% of total variation in the samples. No sample presented a distance H higher than 3 which indicated that no sample was atypical due to post-harvest process, growing conditions or measurement bias. No significant year effect on spectral fingerprint was observed in agreement with the organoleptic results.

New calibration models for caffeine and theobromine were developed by adding data from these ICS samples to the database previously established in CIRAD. The $r^2$ of regressions were 0.91 and 0.89 respectively and SECV were 0.048% for caffeine and 0.090% for theobromine. The distribution of the 25 ICS clones according to their caffeine and theobromine:caffeine ratio showed a wide distribution of the clones from Forastero-like to Criollo-like (Figure 2) confirming the expected diversity within these Trinitario clones. When these data were compared to the entire database at CIRAD that includes a wide variety of cocoa types sampled over many years (Figure 3) we see the ICS clones distributed across full range of the Trinitario genetic group. This confirms the efficiency of the relationship between caffeine and the theobromine:caffeine ratio to classify cocoa in the Forastero, Trinitario and Criollo genetic groups.

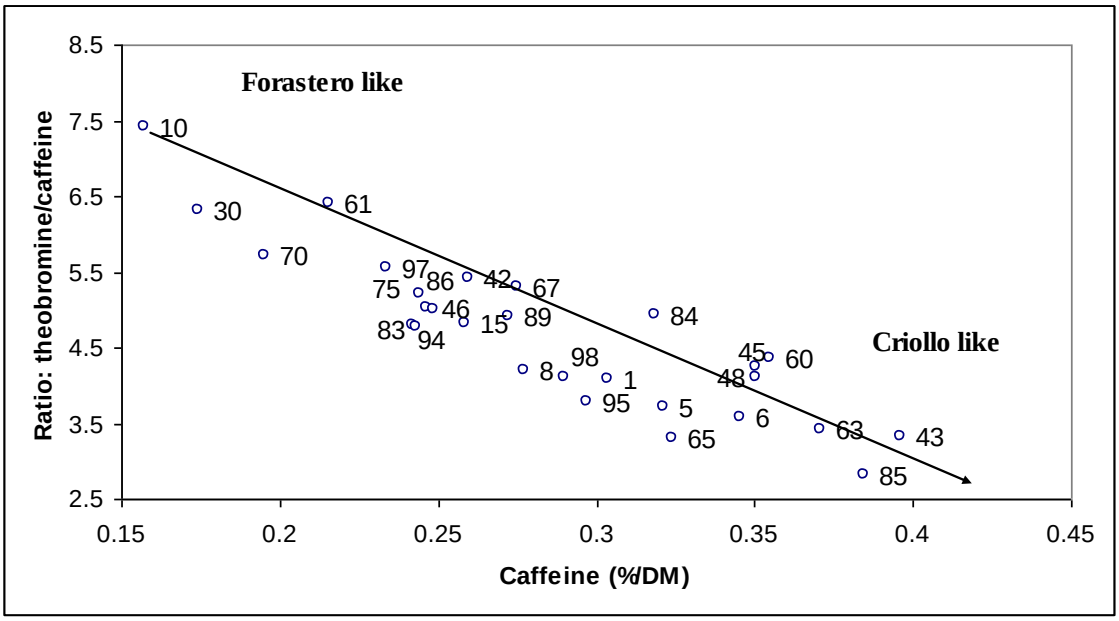


**Figure 2. Distribution of 25 ICS clones according to their caffeine content and theobromine:caffeine ratios.**

The maximum values observed for the theobromine:caffeine ratio were for ICS 10, 30, 61 and 70, typically indicating cocoa genotypes close to the Forastero group. At the opposite end of the spectrum, ICS 63, 43 and 85 presented caffeine and theobromine:caffeine ratios close to the Criollo group. The remaining ICS clones could be considered classical Trinitario genotypes. The NIR predicted values were year independent which was a satisfactory result that confirmed the

potential of NIR to accurately assess genetic traits related to cocoa quality when robust prediction models are set up on a representative database.

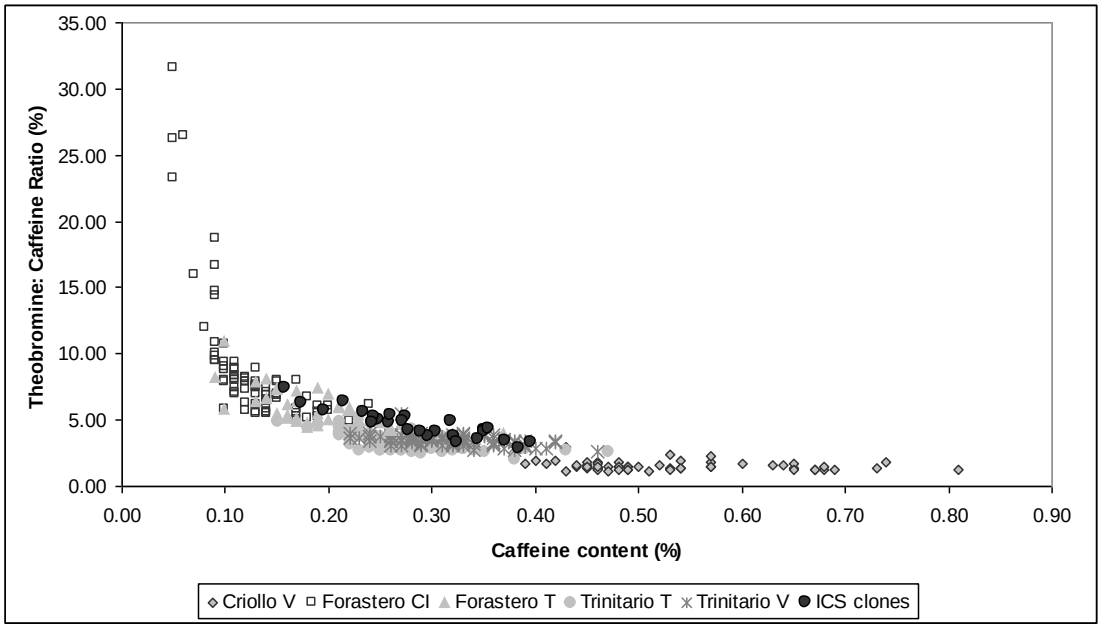


**Figure 3. Separation of ICS clones according to their caffeine and theobromine:caffeine ratios among the entire data base of cocoa types analysed in CIRAD.**

Solid Phase Micro Extraction (SPME) assessments

Principal component analysis performed on the entire database of 141 volatile fingerprints revealed that the first three principal components accounted for 55.9% of the variance in the data set. Since residual acetic acid at the end of drying contributes to most of the acidity found in cocoa liquors (Jinap and Dimik, 1990 and Jinap, 1994) this could cause discrimination in relation to the fermentation process. To avoid this, the ion m/z 60 (which is linked to the acetic acid content) was excluded from the PCA of volatile fingerprints. Contrary to the sensory and NIR analyses, a significant year effect on volatile fingerprint was observed (data not presented). Consequently, PCA for samples from each crop year separately was performed with 74 fingerprints for the first crop year and 60 for the second crop year. The first three principal components explained 65 and 62 % of the variance for the first and the second crop years respectively.

Some specific ICS clones formed distinct groups based on their volatile fingerprint analysis. For the first crop year, ICS 5, 30, 45, 63, 67, 70, 85, 97 and 98 were grouped; for the second year ICS 5, 10, 30, 65, 70, 85 and 97 were grouped. There were five ICS clones (data not presented) that seemed to have a specific fingerprint since they were discriminated from other clones each crop year.

It was not always possible to find associations between the volatile fingerprints of ICS clones and their sensory results. However clear examples of distinct sensory notes were found, such as ICS 70 with a floral note, ICS 43 and 45 with a nutty note and for ICS 30 with nutty and cocoa

flavour notes. Since the aim of this exercise was limited to differentiation of the ICS clones using different attribute assessments to find similar groups, we did not quantify different volatile compounds. As a result, linkages to specific aromatic compounds were not made; this forms another component of the project.

Nevertheless, the literature shows that cocoa and nutty flavours are developed mainly during roasting via the Maillard (non-enzymatic) reaction (Cros 1996). Flavour precursors such as peptides, free amino acids and reducing sugars derived from cocoa bean acidification and proteolysis participate in this reaction (de Brito et al. 2001 and 2004). Trimethylpyrazine and tetramethylpyrazine compounds noted for their roasted cocoa and nutty flavour attributes are also thermally derived both from roasting and during fermentation and drying from thermally initiated biochemical reactions, or by microbial synthesis (Reineccius et al. 1972 and Sukha et al. 2005). Fruity flavours on the other hand are generally based on the presence of esters derived from organic acids and alcohols which are themselves derived from sugar metabolism of the pulp whilst floral flavour has been linked to terpenes (Ziegleder, 1990; Biehl and Voigt, 1999 and Pino and Roncal, 1992).

The organoleptic and spectral (NIR) assessment results suggested that good diversity exists in the ICS accession group. These trends were also observed in the analysis of volatile fingerprints which showed an effect of crop year on certain volatile fingerprints whilst there was a specificity of ICS clones according to cocoa, nutty and floral flavours. Organoleptically, many of the ICS clones had a dominant fruity flavour characteristic associated with some acidity which can be considered typical of Trinitario beans from Trinidad (Sukha et al., 2008).

## Conclusion

The literature does not cite any results, specific to these clones, of screening for organoleptic attributes and chemical attributes linked to aromatic properties despite the global distribution of ICS clones and their use in many international cacao breeding programmes. The impressions from the results presented here suggest that the ICS clones have potential value in terms of diversity within the Trinitario group showing a good range of interesting organoleptic and aromatic traits. The future direction of this work will be to identify specific ICS clones to highlight their potential for use in breeding programmes throughout the world. These results so far confirm that the ICS accession group provides a useful source of cacao material with quality traits of economic importance to the global cocoa industry.

## Acknowledgements

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# Cocoa Research Advisory Committee

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Vindra Singh

Vinosh Jadoo

# Cocoa Research Unit staff 2009

**Research Staff**

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**Frances Bekele** *MPhil* Research Fellow (joint acting Director, February – December)

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**Balram Latchman** *MSc* Contract Officer I

**Lambert Motilal**[1] *MPhil* Contract Officer I

**Antoinette Sankar** *MSc* Contract Officer I

**Darin Sukha** *PhD* Research Fellow (joint acting Director, February - December)

**Surendra Surujdeo-Maharaj** *PhD* Research Fellow (September – December)

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

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**Naailah Ali**[1] *BSc* Technical Assistant (part-time, January - September)

**Sarah Bharath**[1] *BSc* Technical Assistant (part-time)

**Junior Bhola** Laboratory Assistant

**Zainab Ali-De Freitas** *BSc* Technical Assistant (June – December)

**Annelle Holder** *MPhil* Technical Assistant

**John Joseph** Laboratory Assistant

**Gangadeen Ramdhanie** Senior Laboratory Assistant

**Valmiki Singh** *BSc* Technical Assistant

**Vindra Singh** *BSc* Technical Assistant

**Eusebius Solozano** Laboratory Assistant (January – June)/Technical Assistant (July – December)

**Visiting scientists**

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

**Administrative staff**

**Sophia Thompson** Secretary (June – December)

**Phulmatee Hetai** Messenger/cleaner

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

# Publications and presentations

## Refereed Journals

Mollineau, W., Bekele, F. and G. Garcia (2008) An examination of the Neo-tropical red squirrel (*Sciurus granatensis*) as a pest of cacao (*Theobroma cacao* L.) in the International Cacao Genebank, Trinidad, Trinidad. *Tropical Agriculture* (Trinidad) **85(2):** 149-160 (released in December 2009).

Motilal, L.A., Zhang, D., Umaharan P., Mischke, S., Boccara, M., and Pinney, S. (2009) Increasing accuracy and throughput in large-scale microsatellite fingerprinting of cacao field germplasm collections. *Tropical Plant Biology* **2(1):** 23-37, DOI 10.1007/s12042-008-9016-z.

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## Conference Proceedings

Bekele, F.L. and Bidaisee, G.G. (2009) Assessment of morphological traits in 1180 accessions from the International Cocoa Genebank, Trinidad. Pages 67-77 in: *Proceedings of the 15th International Cocoa Research Conference*, San Jose, Costa Rica, 9 - 14 October 2006. Nigeria: COPAL.

Iwaro, A.D., Bekele, F.L., Butler, D.R., Singh, V., Holder-John, A., Bharath, S., Surujdeo-Maharaj, S., Thévenin, J.-M., Deberdt, P. and Bidaisee, G.G. (2010) Recent progress in breeding for specific traits in cocoa to meet challenges to production. Pages 43-52 in: *Proceedings of the International Congress on Tropical Agriculture: Overcoming Challenges to Developing Sustainable Agri-Food Systems in the Tropics*, Port of Spain, Trinidad, 30 November – 5 December 2008, Hyatt Regency, Trinidad. Trinidad: The University of the West Indies.

Iwaro, A.D., Bharath, S. Bekele, F.L. and Butler, D. R. (2007) Assessment of genetic gain in a germplasm enhancement programme for resistance to black pod disease. Pages 33-39 in: *Proceedings of the 15th International Cocoa Research Conference* Vol. I, San Jose, Costa Rica, 9 - 14 October 2006. Nigeria: COPAL.

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## Conference and Workshop presentations

### Papers presented

Davrieux, F., Assemat, S., Boulanger, R., Sukha, D.A., Eskes, B., Paulin, D. and Cros, E. Characterization of cocoa clones from different origins for purine contents predicted by NIRS. Presented at the 16th International Cocoa Research Conference. Bali, Indonesia. 16 – 21 November 2009.

Eskes, A.B., Adu-ampomah, Y., Aikpokpodion, P., Butler, D., Amores, F., Daymond, A.J., Efombagn, M.I.B., Engels, J.M.M., Gonzalez V.V., Iwaro, D., Garcia, L., Gilmour, M., Maharaj, P., Motamayor, J-C., Lamin, F.K., Marfu, J., Monteiro, W., N'Guessan, F.K., Paulin, D., Phillips W., and Seguine, E.S. Results from collaborative and farmer participatory approaches to cocoa variety selection and breeding. Presented at the 16th International Cocoa Research Conference. Bali, Indonesia. 16 – 21 November 2009.

Maharaj, K., Maharaj, P., Bekele, F.L., Ramnath, D., Bidaisee, G.G., Bekele, I., Persad, C., Jennings, K., Sankar, R. Trinidad Selected Hybrids: An investigation of the phenotypic and agro-economic traits of 20 selected cacao cultivars. Presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Sabau, X., Loor, G., Boccara, M., Fouet, O., Allègre, M., Argout, X., Legavre, T., Risterucci, A.M., Boulanger, R., Lanaud, C. Expression of genes involved in the terpenoid biosynthetic pathway during cocoa seed development and fermentation. Presented at the 16th International Cocoa Research Conference. Bali, Indonesia. 16 – 21 November 2009.

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Sukha, D.A. Chocolate manufacture at the Cocoa Research Unit. Presented at the 2009-2012 Tobago Cocoa Sub-sector Re-structuring Initiative. Argyle Community Centre, Tobago. 24 April 2009.

Sukha, D.A. Innovations in Solar Drying of the Fine or Flavour Cocoa Bean. Presented at the Cocoa Technical Forum. Kingston, Jamaica. 6 - 11 July 2009.

Sukha, D.A. Evidence for "terroir" effects on cocoa quality. Presented at the 6th INGENIC Workshop. Bali, Indonesia. 22 – 24 November 2009.

Sukha, D.A. The concept of quality along the cocoa supply chain. Key note address presented at the forum on Cacao Quality: Inputs for a national strategy. Punta Gorda, Belize. 3 December 2009.

Sukha, D.A., Davrieux, F., Boulanger, R., Alter, P., Assemat, S., Bharath, S.M.., Ali, N.A. and Butler, D.R. Characterisation of the Imperial College Selections according to their biochemical composition, aroma volatile fingerprints and sensorial profiles. Presented at the 16th International Cocoa Research Conference. Bali, Indonesia. 16 – 21 November 2009.

Surujdeo-Maharaj. S. The future of cocoa research. Invited paper presented at the Caribbean Regional Science Technology and Innovation Workshop - Building a Critical Mass for Science and Innovation: Identifying the Value Proposition for Caribbean Young Professionals and Entrepreneurs. Kingston, Jamaica September 23-25.

## Posters presented

Allègre M., Sabau X., Boccara M., Fouet O., Argout X., Thevenin, J-M., Berard A., Brunel D. and Lanaud C. Mapping and expression of candidate genes involved in the metabolism of compounds related to cocoa qualities, during seeds development and fermentation. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Boccara, M., Argout, X., Allegre, M., Fouet, O., Thevenin, J.M., Pot, D., Brunel, D., Bérard, A., Arevalo-gardini, E., Zhang, D., Pires, J.L., Wallace da Silva J., Mota, E., Loor, G., Butler, B., and Lanaud, C. New insight into cocoa diversity using SNP gene-based markers. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Chang Yen, I., Ramtahal, G., Bekele, I., Wilson, L.A., Bekele, F., Sukha, B. Application of cost-effective methodologies for certification of heavy metals in cocoa beans in Trinidad and Tobago. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Chang Yen, I., Robert, J., Bekele, I., Wilson, L.A., Bekele, F., Sukha, B. Development and application of an improved method of determination of Ochratoxin A in cocoa beans in Trinidad and Tobago. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Iwaro, A.D., Singh, V., Bharath, S.M., Perez, C., Ali, L. and Butler, D.R. Germplasm enhancement for resistance to Black Pod disease – final stages and achievements. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Latchman, B., Singh, V., Joseph, J. and Butler, D.R. Safeguarding the international cocoa genebank, Trinidad: a global resource for the cocoa industry. Poster presented at the 16th International Cocoa Research Conference, Bali,

Indonesia, 16 – 21 November 2009.

Legavre, T., Ducamp, M., Sabau, X., Argout, X., Surujdeo-Maharaj, S., Paulin, D. and Lanaud C. Identification of *Theobroma cacao* genes differentially expressed during *Phytophthora megakarya* infection. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Legavre, T., Sabau, X., Surujdeo-Maharaj, S., Gramacho, K., Cascardo, J., Argout, X., Fouet, O., Allegre, M., Guiltinan, M., Butler, D. and Lanaud, C. Towards the understanding of the cocoa transcriptome: a microarray dedicated to studies of disease resistance and cocoa quality. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Ramroop, D.V.; Maharaj, K.; Ramnath, D. and Sukha, D. Farmer participatory approaches for sustainable cocoa production in Trinidad and Tobago. Poster presented at the Second Roundtable for a Sustainable Cocoa Economy, Port of Spain, 24 – 26 March 2009.

Risterucci, A.M., Blois, H., Boccara, M., Sabau, X., Fouet, O., Sounigo, O. and Lanaud, C. DArT (diversity array technology): a new high throughput and cost effective genotyping tool for diversity and mapping analyses. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Thevenin, J.-M., Holder-John, A., Butler, D.R. and Cilas, C. Creating cocoa populations with greater resistance to witches' broom disease in Trinidad. Poster presented at the 16th International Cocoa Research Conference, Bali, Indonesia, 16 – 21 November 2009.

Zhang, D., Boccara, M., Motilal, L., Mischke, S., Johnson E.S., Butler, D.R., Bailey B., and Meinhardt L. Characterization of an original cacao collection using microsatellite markers. Poster presentation at the *Mid-Atlantic Plant Molecular Biology Society 26th Annual Meeting*, 20-21 August 2009, Patuxent National Wildlife Refuge, Beltsville, Maryland.

# Visitors to CRU in 2009

| | |
|---|---|
| Patrick Van Damme | University of Ghent, Belgium |
| Marie Louise Norton-Murray | Centre for Development of Enterprise Regional Field Office, Dominican Republic |
| Krishna Sirinathsingh | National Canners Ltd., Trinidad |
| Jorge Armas | Chocolates El Rey, Venezuela |
| Jorge Redmond | Chocolates El Rey, Venezuela |
| Beatriz Escobar | Chocolates El, Rey, Venezuela |
| Randall Turner | Chocolates El Rey, Venezuela |
| Sandra Julien | Dominica |
| Sandra Williams | Antigua |
| James Housel | US Patent and Trademark, Office, USA |
| Kerri-Faye Campbell-Rushton | Ministry of Agriculture, Jamaica |
| Lily-Claire Bellamy | Jamaica Intellectual Property Office, Jamaica |
| Herman Adams | CARDI, Trinidad |
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| Deron Thompson | Attorney General Ministry, Belize |
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# Acronyms and abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| BARC | Beltsville Agricultural Research Center |
| BP | Black Pod disease |
| CAOBISCO | Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE |
| CATIE | Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica |
| 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 |
| CoE | Cocoa of Excellence Project |
| COV | Coefficient of variation |
| CRA | Cocoa Research Association Ltd., UK |
| CRU | Cocoa Research Unit, Trinidad and Tobago |
| d.f. | Degree of freedom |
| DNA | Deoxyribonucleic acid |
| EI | Electron ionization |
| FP | Frosty pod disease |
| GEP | Germplasm enhancement programme |
| GC-MS | Gas chromatographic mass spectrometry |
| GORTT | Government of the Republic of Trinidad and Tobago |
| H | Mahalanobis distance |
| HPLC | High-performance liquid chromatography |
| ICCO | International Cocoa Organisation, London, UK |
| ICGD | International Cocoa Germplasm Database |
| ICG,T | International Cocoa Genebank, Trinidad |
| ICRC | International Cocoa Research Conference |
| ICQC,R | International Cocoa Quarantine Centre, Reading, UK |
| ICTA | Imperial College of Tropical Agriculture |
| INGENIC | International Group for Genetic Improvement of Cocoa |
| IPGRI | International Plant Genetic Resources Institute, Rome, Italy |
| LNV | Ministry of Agriculture, Nature and Food Quality, Holland |
| MALMR | Ministry of Agriculture, Land and Marine Resources, Trinidad and Tobago |
| NIR | Near infrared |
| NIRS | Near infrared spectroscopy |
| *P* | Probability |
| $P_{(ID)}$ | Probability of identity |
| $P_{(ID)sib}$ | Probability of identity for full siblings |
| PC | Principal component |
| PCA | Principle component analysis |
| QTL | Quantitative trait loci |
| r | Correlation coefficient |
| $r_s$ | Spearman's correlation coefficient |
| $r^2$ | Coefficient of determination |
| RAPD | Random amplified polymorphic DNA |
| SE | Standard error |
| SECV | Standard error of cross-validation |
| SNP | Single-nucleotide polymorphism |
| SPCL | Sustainable Perennial Crops Laboratory |
| SPME | Solid phase micro extraction |
| SSR | Simple sequence repeats |
| TSH | Trinidad Selected Hybrid |
| TU | Towson University |

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|---|---|
| UCRS | University Cocoa Research Station |
| UE | Union Européenne |
| UPOV | International Union for the Protection of New Varieties of Plants |
| USDA | United States Department of Agriculture |
| USDA-ARS | United States Department of Agriculture – Agriculture Research Service |
| UWI | The University of the West Indies |
| WB | Witches' Broom disease |
| WCF | World Cocoa Foundation, USA |