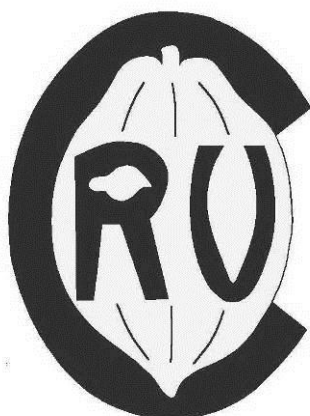


Annual Report 2009



Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
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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/Bioversity¹ 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 DNA¹ 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 CIRAD². 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 nutraceutical 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 UPOV¹ 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 uninterrupted 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 IPGRI¹ (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 (CATIE¹) (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 Investigación 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 CAOBISCO¹ 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 25,000m³ 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.

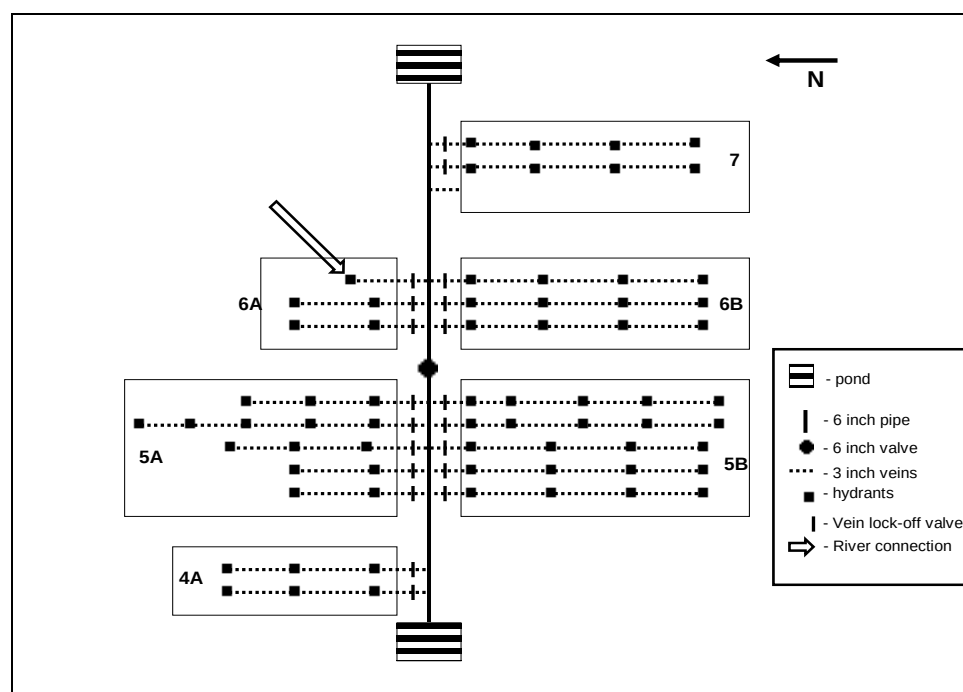


Figure 1. Diagrammatic representation of the Irrigation System at UCRS.

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	LCT EEN 280	150	21	RIM 76 [MEX]	75	8
CL 10/3	45	11	LCT EEN 325	100	0	RIM 101 [MEX]	50	26
CL 10/10	90	13	LCT EEN 326	25	8	RIM 106 [MEX]	50	22
CL 10/11	115	23	LCT EEN 327	175	0	RIM 113 [MEX]	45	12
CL 10/14	140	7	LCT EEN 332	125	1	RIM 117 [MEX]	75	22
CL 10/17	100	2	LP 1/20 [POU]	170	11	SC 1 [COL]	20	9
CL 10/23	90	0	LP 1/25 [POU]	145	10	SC 3 [COL]	100	29
CL 10/25	70	1	LP 1/37 [POU]	100	7	SC 4 [COL]	75	16
CL 10/33	95	17	LP 2/11 [POU]	45	15	SC 5 [COL]	50	8
CL 13/17	140	7	LP 3/19 [POU]	145	6	SC 6 [COL]	25	14
CL 13/35	120	6	LP 4/5 [POU]	50	0	SC 7 [COL]	75	10
CL 13/36	70	0	LP 4/15 [POU]	70	9	SC 11 [COL]	45	7
CL 13/41	95	10	LP 4/45 [POU]	70	14	SC 12 [COL]	50	14
CL 13/43	145	17	LP 5/1 [POU]	95	14	SC 15 [COL]	145	14
CL 13/65	70	17	LP 5/3 [POU]	50	0	SC 17 [COL]	25	11
CL 19/2	90	11	LV 2 [POU]	175	0	SC 19 [COL]	75	15
CL 19/10	71	11	LV 9 [POU]	75	7	SC 20 [COL]	75	25
CL 19/21	65	32	LV 10 [POU]	150	16	SJ 1/1 [POU]	75	0
CL 19/33	160	0	LV 14 [POU]	75	13	SJ 1/10 [POU]	100	11
CL 19/36	140	11	LV 17 [POU]	50	8	SJ 1/11 [POU]	125	1
CL 19/41	65	20	LV 27 [POU]	150	5	SJ 1/18 [POU]	175	5
CL 19/42	115	9	LV 33 [POU]	75	20	SJ 1/28 [POU]	220	5
CL 27/7	115	13	LV 37 [POU]	220	17	SJ 1/29 [POU]	75	7
CL 27/14	120	27	LX 1	120	10	SJ 1/33 [POU]	175	8
CL 27/21	90	3	LX 2	175	21	SJ 1/37 [POU]	200	3
CL 27/34	260	7	LX 18	75	3	SJ 2/12 [POU]	150	8
CL 27/49	120	23	LX 24	125	19	SJ 2/17 [POU]	100	12
CL 27/71	70	0	LX 41	245	2	SJ 2/26 [POU]	125	12
CL 27/72	115	2	LZ 4	75	3	SLA 10	75	2
CL 27/74	195	12	LZ 5	75	23	SLA 13	145	17
CL 27/109	70	9	LZ 7	125	9	SLA 48	175	8
CL 78/2	90	30	LZ 8	125	20	SLA 77	125	4
CLM 6	45	0	LZ 17	200	7	SLA 95	100	19
CLM 35	70	0	MAR 1	75	27	SM 1 [POU]	50	0
CLM 65	70	2	MAR 3	75	27	SM 5 [POU]	100	19
CLM 78	115	1	MAR 9	75	10	SM 9 [POU]	75	1
CRU 4A/1	45	3	MAR 10	100	2	SM 10 [POU]	25	7
CRU 4A/2	126	3	MAR 11	100	21	SPA 12 [COL]	95	21
CRU 4A/3	145	9	MAR 12	75	20	SPA 16 [COL]	100	9
CRU 4A/4	170	13	MAR 13	70	17	SPA 18 [COL]	50	24
CRU 4A/5	145	22	MAR 14	75	15	SPA 20 [COL]	50	9
CRU 4A/6	170	12	MAR 17	50	4	SPEC 41/6	125	26
CRU 4A/7	70	11	MAR 19	150	15	TRD 1	175	16
CRU 4A/8	70	17	MAR 20	75	5	TRD 2	25	11
CRU 4A/9	70	0	MAR 21	100	14	TRD 3	125	28
CRU 4A/10	45	0	MAR 22	25	20	TRD 5	25	11
CRU 4A/11	70	0	MO 82	250	1	TRD 6	175	33
CRU 270 ¹	70	11	MO 87	100	9	TRD 7	150	11
CRU 271 ²	50	9	MO 96	75	7	TRD 8	145	26
DOM 1	70	15	MOQ 1/12	25	12	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	NA 178	100	11	TRD 110	150	18
GU 300/P	20	14	NA 191	50	16	TRD 111	145	5
GU 305/P	25	18	NA 204	100	10	TRD 112	125	8
GU 307/F	50	17	NA 214	100	33	TRD 113	100	18
GU 310/P	45	11	NA 229	50	9	TRD 114	175	25
GU 322/P	25	12	NA 241	5-	7	TRD 115	175	32
GU 335/P	25	13	NA 244	175	28	TRD 116	20	11
GU 339/M	45	27	NA 246	100	17	TRD 117	25	14
GU 351/P	20	9	NA 251	150	1	TRD 118	175	20
GU 353/L	25	3	NA 271	125	10	TRD 119	25	12
ICA 70 [COL]	125	3	NA 277	75	1	UF 4	150	10
ICS 2	75	20	NA 326	75	9	UF 38	200	18
ICS 3	25	18	NA 327	50	13	UF 122	175	12
ICS 12	120	43	NA 331	75	22	UF 602	220	36
ICS 15	75	11	NA 339	150	16	UF 613	120	24
ICS 20	120	21	NA 370	100	5	UF 700	125	12
ICS 23	50	8	NA 372	125	16	UF 705	75	0
ICS 28	75	3	NA 395	100	5	UF 709	125	10
ICS 30	75	1	NA 399	75	1			

¹Renamed clone CRU 270 (MIS_TTOICGT_CBO 177 [VEN])

²Renamed 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	Clone	N	Clone	N	Clone	N
AGU 2 [CHA]	3	DOM 23	4	LV 37 [POU]	6	RIM 13 [MEX]	9
AM 1/8 [POU]	2	DOM 24	2	LX 1	7	RIM 19 [MEX]	9
AM 1/53 [POU]	2	DOM 25	12	LX 2	2	RIM 24 [MEX]	4
AM 1/68 [POU]	2	DOM 27	2	LX 18	1	RIM 41 [MEX]	9
AM 1/70 [POU]	9	DOM 30	8	LX 24	9	RIM 48 [MEX]	9
AM 1/85 [POU]	9	DOM 31	4	LX 41	4	RIM 71 [MEX]	8
AM 1/87 [POU]	2	DOM 33	3	LZ 5	7	RIM 75 [MEX]	4
AM 1/96 [POU]	9	DOM 35	12	LZ 8	6	RIM 76 [MEX]	3
AM 1/97 [POU]	2	FSC 13	9	MAR 1	4	RIM 101 [MEX]	9
AM 1/107 [POU]	2	GNV 22	4	MAR 10	2	RIM 106 [MEX]	9
AM 1/109 [POU]	6	GS 4	3	MAR 11	3	RIM 113 [MEX]	9
AM 2/1 [POU]	4	GS 6	8	MAR 12	2	SC 1 [COL]	9
AM 2/3 [POU]	2	GS 12	4	MAR 13	3	SC 3 [COL]	4
AM 2/6 [POU]	10	GS 37	2	MAR 14	1	SC 4 [COL]	6
AM 2/18 [POU]	4	GS 55	7	MAR 19	6	SC 5 [COL]	8
AM 2/19 [POU]	9	GS 58	1	MAR 21	1	SC 6 [COL]	9
AM 2/20 [POU]	4	GS 61	5	MAR 22	9	SC 7 [COL]	4

AM 2/21 [POU]	9	GS 62	4	MO 87	2	SC 11 [COL]	7
AM 2/28 [POU]	9	GU 114/P	9	MO 96	2	SC 12 [COL]	9
AM 2/31 [POU]	9	GU 151/F	2	MOQ 1/12	9	SC 15 [COL]	2
AM 2/41 [POU]	9	GU 175/P	5	MOQ 1/12	1	SC 17 [COL]	9
AM 2/45 [POU]	9	GU 195/P	1	MOQ 1/24	2	SC 19 [COL]	9
AM 2/96 [POU]	12	GU 219/F	6	MOQ 2/31	2	SC 20 [COL]	3
AMAZ 5/2 [CHA]	4	GU 222	9	MOQ 3/16	4	SIAL 330	4
AMAZ 6 [CHA]	9	GU 235/P	2	MOQ 4/2	8	SJ 1/10 [POU]	2
AMAZ 10/1 [CHA]	5	GU 241/P	4	MOQ 4/16	4	SJ 1/18 [POU]	2
B 5/11 [POU]	2	GU 243/H	9	MOQ 4/25	5	SJ 1/28 [POU]	2
B 13/5 [POU]	2	GU 255/P	9	MOQ 5/29	4	SJ 1/29 [POU]	7
B 14/13 [POU]	1	GU 261/P	9	MOQ 5/34	4	SJ 1/37 [POU]	1
B 22/3 [POU]	4	GU 265/P	9	MOQ 6/5	9	SJ 2/26 [POU]	2
C 96 [TRI]	9	GU 271/P	9	MOQ 6/28	9	SLA 10	1
C 97 [TRI]	6	GU 277/G	9	MOQ 6/72	1	SLA 11	1
C 99 [TRI]	9	GU 286/P	9	MOQ 6/73	4	SLA 13	4
CC 9	9	GU 300/P	9	MOQ 6/85	2	SLA 48	4
CC 10	9	GU 305/P	9	MOQ 6/91	9	SLA 95	3
CC 10	2	GU 307/F	2	MOQ 6/107	9	SM 5	7
CC 17	2	GU 310/P	9	NA 1	5	SPA 12 [COL]	5
CC 37	4	GU 322/P	9	NA 13	1	SPA 16 [COL]	1
CC 38	6	GU 335/P	9	NA 19	4	SPA 18 [COL]	9
CC 39	9	GU 339/M	12	NA 26	7	SPA 20 [COL]	7
CC 40	8	GU 351/P	9	NA 33	5	SPEC 41/6	3
CC 41	4	GU 353/L	9	NA 39	9	TRD 1	3
CC 49	3	ICA 70	1	NA 45	2	TRD 2	9
CC 54	4	ICS 2	5	NA 49	9	TRD 3	3
CC 71	3	ICS 3	9	NA 58	1	TRD 5	9
CL 9/7	1	ICS 12	12	NA 61	1	TRD 8	5
CL 9/11	4	ICS 15	4	NA 74	4	TRD 15	9
CL 9/12	1	ICS 20	3	NA 81	9	TRD 16	9
CL 9/19	1	ICS 31	9	NA 92	1	TRD 18	6
CL 9/51	8	ICS 35	9	NA 95	15	TRD 19	4
CL 10/3	9	ICS 42	6	NA 104	8	TRD 23	1
CL 10/10	6	ICS 53	9	NA 110	9	TRD 24	2
CL 10/11	9	ICS 56	3	NA 111	2	TRD 29	2
CL 10/14	4	ICS 58	9	NA 112	9	TRD 30	6
CL 10/25	4	ICS 62	9	NA 114	4	TRD 32	9
CL 10/33	9	ICS 73	1	NA 157	6	TRD 33	9
CL 13/17	2	ICS 77	2	NA 170	4	TRD 34	9
CL 13/35	3	ICS 81	3	NA 176	2	TRD 35	9
CL 13/36	1	ICS 82	4	NA 178	8	TRD 37	1
CL 13/41	8	ICS 88	8	NA 191	7	TRD 38	3
CL 13/43	2	IMC 18	1	NA 204	9	TRD 39	1
CL 13/65	8	JA 1/9 [POU]	5	NA 214	7	TRD 41	8
CL 19/2	2	JA 2/12 [POU]	8	NA 218	1	TRD 42	2
CL 19/10	7	JA 3/4 [POU]	9	NA 229	9	TRD 43	9
CL 19/21	8	JA 3/37 [POU]	9	NA 241	1	TRD 44	9
CL 19/33	3	JA 5/11 [POU]	9	NA 244	4	TRD 45	9
CL 19/36	4	JA 5/27 [POU]	9	NA 246	12	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		

¹Renamed clone CRU 270 (MIS_TTOICGT_CBO 177 [VEN])

²Renamed 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	GC T 998/96	3	LP 1/34 [POU]	5	MOQ 6/97	3
AM 1/55 [POU]	4	ICS 6	1	LP 1/36 [POU]	1	MOQ 6/108	7
AM 1/63 [POU]	4	ICS 7	1	LP 1/42 [POU]	1	MX 14/20 [TTO]	4
AM 2/7 [POU]	1	ICS 9	1	LP 1/47 [POU]	10	NA 38	5
AM 2/42 [POU]	5	ICS 10	1	LP 1/56 [POU]	4	NA 48	4
AM 2/46 [POU]	6	ICS 11	3	LP 2/2 [POU]	2	NA 69	2
AM 2/68 [POU]	4	ICS 16	1	LP 2/10 [POU]	3	NA 79	3
AM 2/94 [POU]	5	ICS 26	2	LP 2/16 [POU]	3	NA 106	5
AS 2 [ECU]	3	ICS 30	1	LP 3/38 [POU]	5	NA 119	6
AS 7 [ECU]	4	ICS 34	3	LP 4/2 [POU]	4	NA 120	3
B 1/2-8 [POU]	2	ICS 40	1	LP 4/41 [POU]	7	NA 151	3
B 3/4 [POU]	3	ICS 46	1	LP 5/23 [POU]	4	NA 156	5
B 6/5 [POU]	3	ICS 51	1	LP 6/12 [POU]	2	NA 242	2
B 9/10-2 [POU]	2	ICS 60	3	LP 6/16 [POU]	3	NA 254	4
B 11/3 [POU]	2	ICS 61	3	LV 36 [POU]	3	NA 256	2
B 18/8 [POU]	2	ICS 63	3	LX 15	1	NA 260	3
B 23/9 [POU]	1	ICS 65	1	LX 21	4	NA 368	1
CL 10/21	2	ICS 69	2	LX 49	3	NA 681	3
CL 13/24	2	ICS 72	2	LZ 9	6	NA 694	6
CL 13/32	1	ICS 73	1	LZ 11	4	NA 695	2
CL 15/19-4	1	ICS 75	3	MARPER 2	9	NA 713	7
CL 19/35	6	ICS 77	3	MARPER 5	5	NA 725	13
CL 19/46	2	ICS 79	4	MARPER 9	3	NA 734	1
CL 27/105	4	ICS 80	4	MARPER 18	6	NA 747	4
CL 91/6	8	ICS 81	2	MARPER 19	1	NA 751	1
CLM 3	3	ICS 82	2	MARPER 22	7	NA 916	1
CLM 49	1	ICS 83	2	MARPER 27	4	PA 98 [PER]	1
CLM 61	2	ICS 84	1	MARPER 29	5	PA 186 [PER]	6
CLM 68	1	ICS 86	2	MARPER 31	1	PA 203 PER]	2
CLM 88	5	ICS 87	1	MARPER 33	2	PA 246 [PER]	1
CLM 107	1	ICS 89	1	MARPER 34	3	PA 288 [PER]	3
CLM 111	3	ICS 90	1	MARPER 43	3	SAN JUAN WSC	5
CLM 120	3	ICS 92	5	MARPER 46	5	SAN MIGUEL 3360/2	5
CLM 122	1	ICS 93	2	MARPER 47	7	SCA 7	2
CRU 63	3	ICS 97	3	MARPER 48	5	SCA 16	3
CRU 89	4	ICS 100	3	MARPER 50	4	SCA 27	1
CRU 114	1	ICS 111	3	MARPER 51	3	SI 22/1	5
CRU 130	4	IMC 67	4	MARPER 54	3	SI 22/4	5
CRU 134	1	JA 1/28 [POU]	1	MO 84	3	SI 45/2	5
CRU 135	7	JA 2/8 [POU]	5	MO 122	5	SJ 1/15 [POU]	3
CRU 152	1	JA 2/11 [POU]	3	MOQ 1/2	2	SJ 1/20 [POU]	4
CRU C2/1	3	JA 2/2 [POU]	4	MOQ 1/6	1	SJ 1/22 [POU]	2
CRU C3/4	3	JA 2/26 [POU]	3	MOQ 1/9	4	SJ 1/36 [POU]	5
CRU C4/3	6	JA 3/3 [POU]	5	MOQ 1/16	4	SJ 2/28 [POU]	3
CRU C4/4	7	JA 3/29 [POU]	5	MOQ 2/7	2	SLA 23	4
CRU C4/5	5	JA 3/38 [POU]	5	MOQ 2/8	1	SLA 27	2
CRU C4/6	5	JA 3/39 [POU]	3	MOQ 2/16	4	SLA 28	6
CRU C4/7	7	JA 4/2 [POU]	4	MOQ 2/37	5	SLA 86	1
CRU C4/8	3	JA 4/21 [POU]	4	MOQ 2/38	3	SLA 97	2
CRU C4/9	4	JA 5/4 [POU]	2	MOQ 3/19	5	SLC 1	4

CRU C4/10	7	JA 6/12 [POU]	5	MOQ 3/20	5	SLC 11	5
CRU C4/12	3	JA 8/8 [POU]	2	MOQ 3/22	2	SLC 25	3
CRU C4/13	5	JA 8/18 [POU]	5	MOQ 4/5	5	SM 2 [POU]	5
CRU C5/2	4	JA 9/9 [POU]	1	MOQ 4/17	2	SM 4 [POU]	2
CRU C5/7	6	JA 10/29 [POU]	5	MOQ 5/18	4	SM 18 [POU]	4
CRU C5/8	4	JA 10/31 [POU]	2	MOQ 5/35	4	SPEC 194-15	4
EQX /JS	3	JA 10/40 [POU]	2	MOQ 6/34	7	THY 1/105	3
GC T 998/55	6	LCT EEN 28/S-1	4	MOQ 6/55	4	UF 676	3
GC T 998/59	4						

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.

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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
STAHHEL	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, STAHHEL 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

¹percentage 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

$$\chi^2 = 6.1257, \text{ d.f.} = 8, P = 0.6332$$

$$\text{Spearman's } r_s = -0.0359, \text{ d.f.} = 443, P = 0.2253$$

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	2.46×10^{-3}	4.45×10^{-2}
		F454	3	Same to each other but differ from D300	3.73×10^{-5}	5.87×10^{-3}
LCT EEN 162/S-1010	4A	A60	1	Differs from A99	1.69×10^{-9}	5.74×10^{-4}
		A99	4	Same to each other	5.50×10^{-4}	1.13×10^{-2}
LP 1/21 [POU]	5B	I746	4	Same to each other and those of I779 except T8	5.83×10^{-3}	3.35×10^{-2}
				I746 T8	9.30×10^{-5}	1.77×10^{-2}
		I779	5	I746 T4, T5, T7 similar to I779	8.2×10^{-3}	4.0×10^{-2}
NA 471	6A	B86	4	Same to each other and to B92	3.1×10^{-1}	4.5×10^{-1}
		B92	3	Same to each other and to B86	2.5×10^{-1}	3.9×10^{-1}

¹P_(ID) = probability of identity, ²P_{(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

¹University 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 1.78×10^{-29} (GU 300/P Field 4A, Plot B197, T4) to 3.71×10^{-3} (AM 2/43 [POU] Field 5A, Plot A18, T5). Probabilities of identities based on siblings ranged from 1.16×10^{-5} (GU 310/P Field 4A, Plot B233, T3) to 2.17×10^{-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 ICGT. 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 $\square$ 4.99 MPa 7= $\square$ 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.

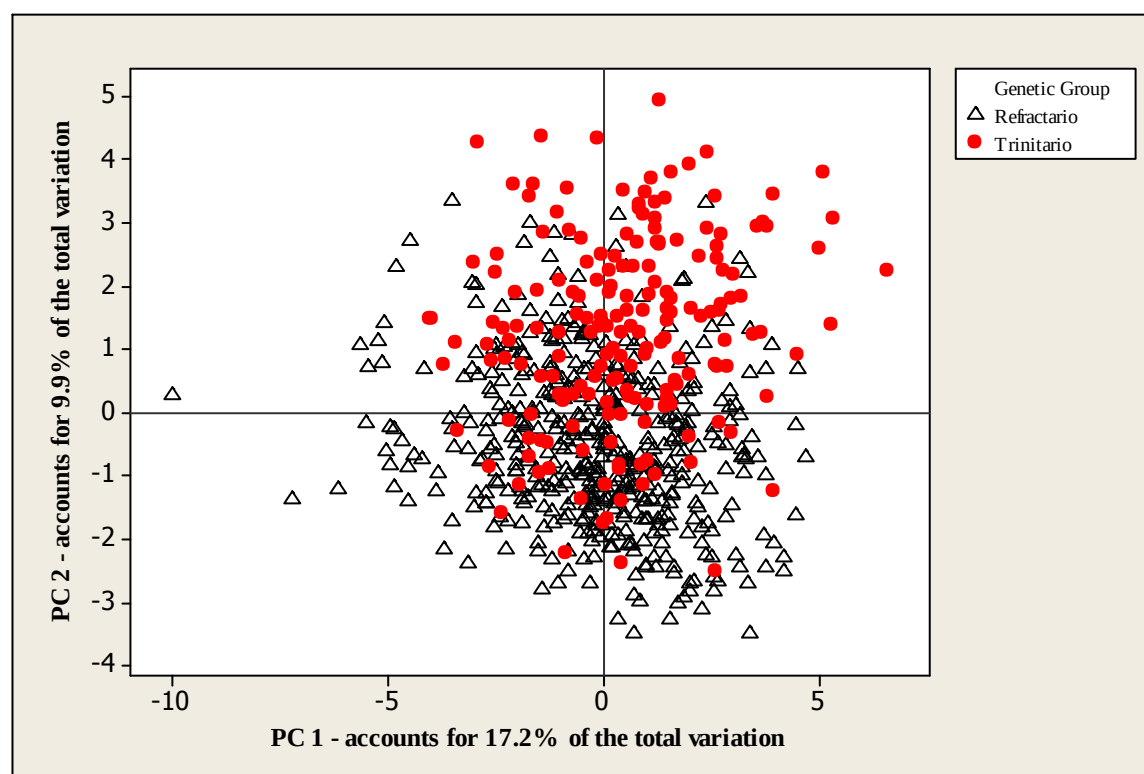
The overlapping of the Trinitarios and Refractarios observed in this study is to be expected since several Refractarios may be of Trinitario or Venezuelan origin.

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.

Put into group	True 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

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

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 ICGT 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°C was used for all primers as recommended.

PCR products were stored at -5 °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.

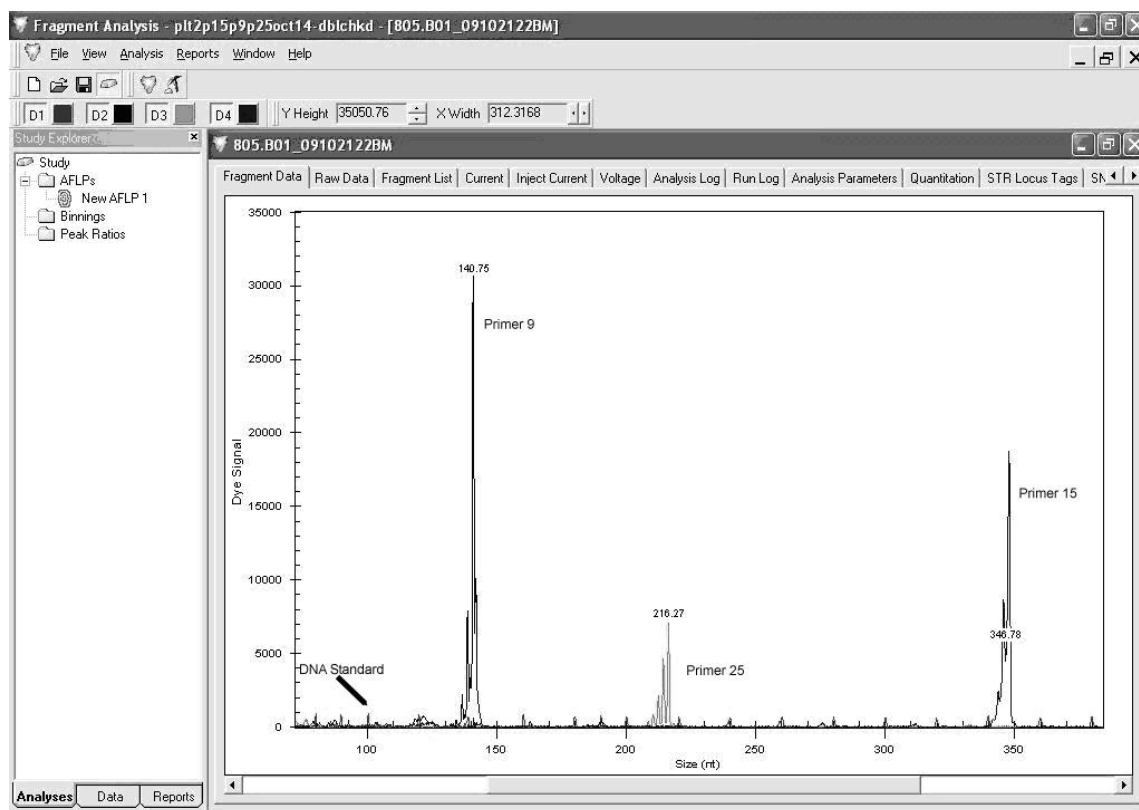


Table 4. Summary of data generated.

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.

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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 CIRAD¹ and CNG³ 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.

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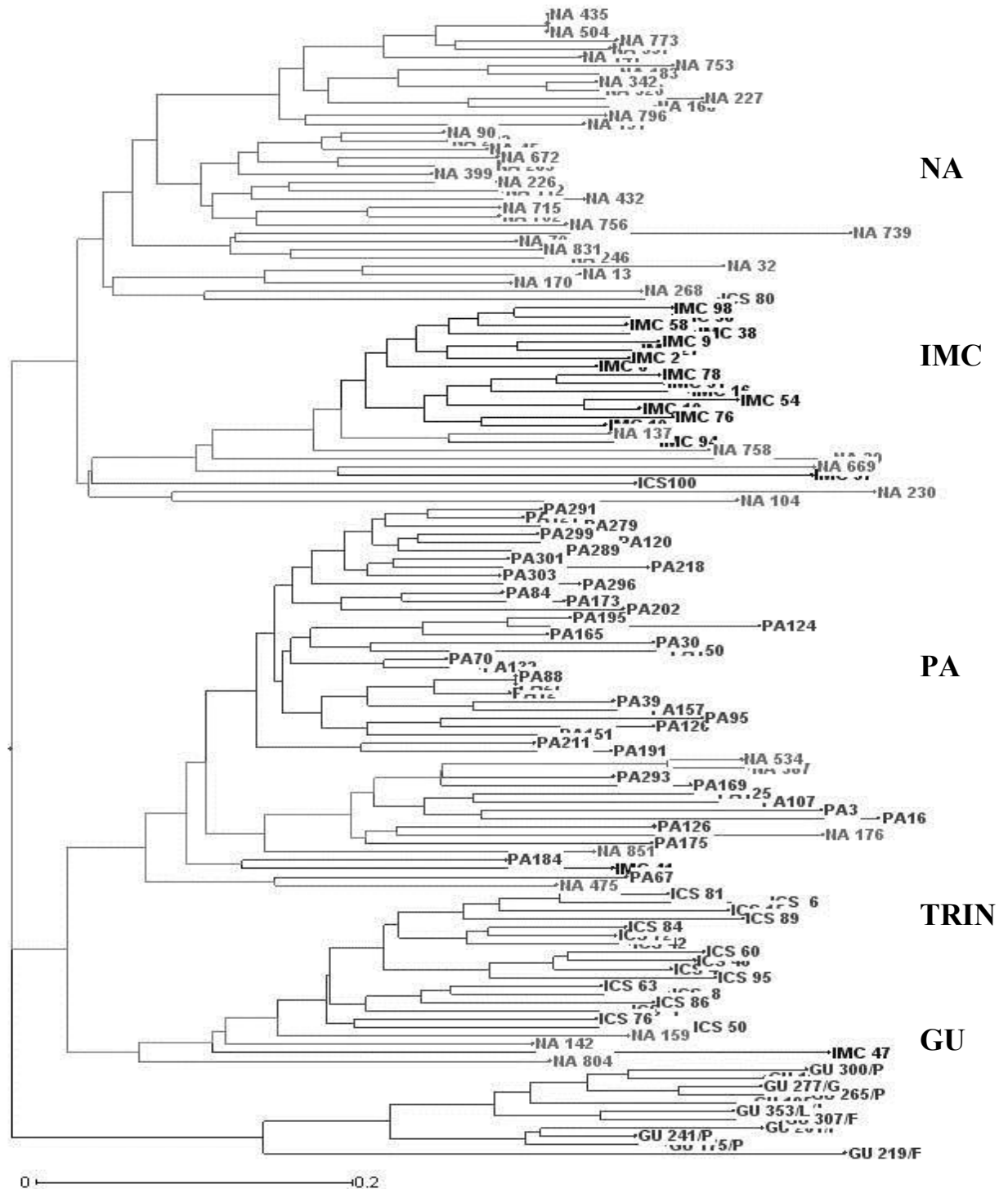


Figure 1. Dendrogram of dissimilarity analysis run with 15 SSR markers.

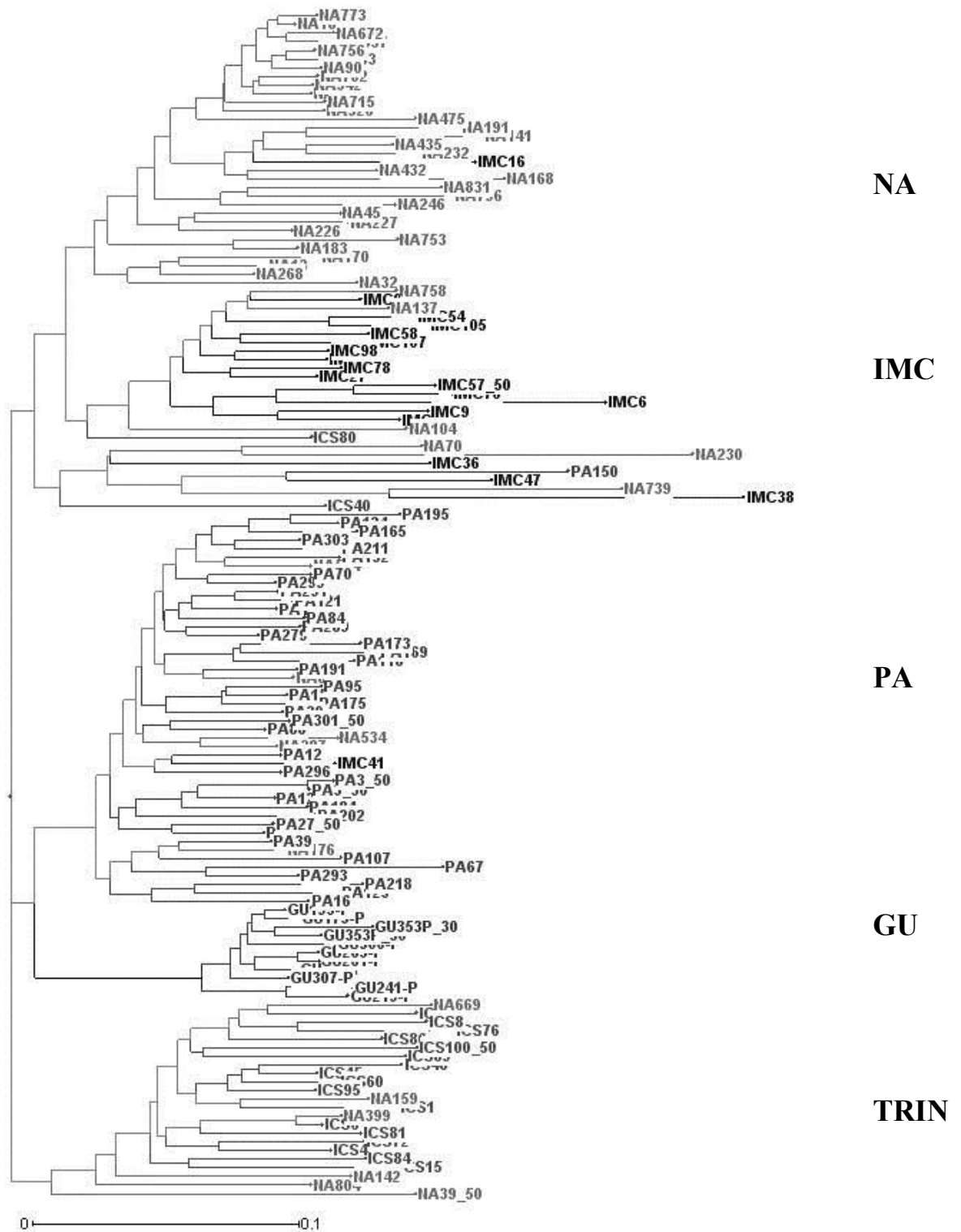


Figure 2. Dendrogram of dissimilarity analysis run with 100 SNP markers.

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.

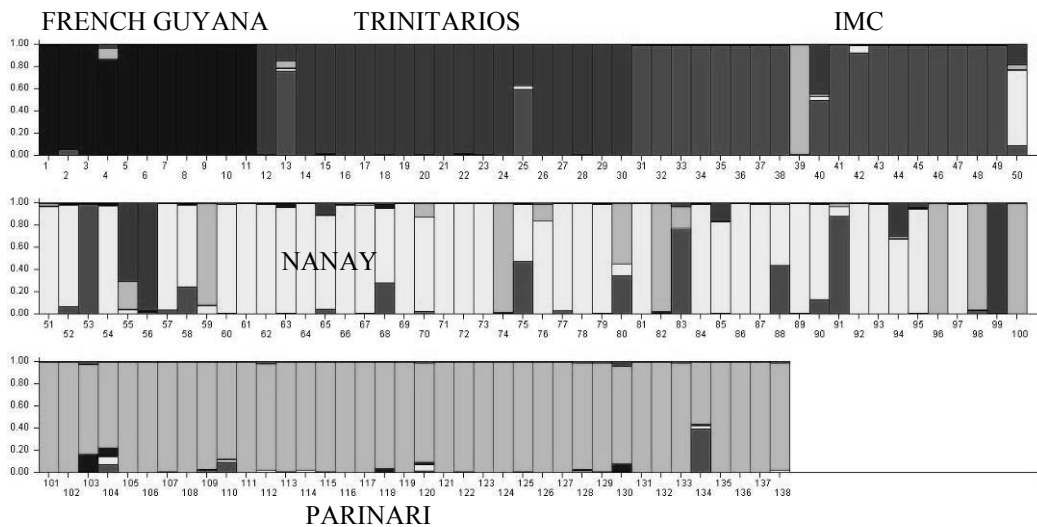


Figure 3. Representation of clusters using STRUCTURE with K=5 : 138 accessions run with 15 SSR markers.

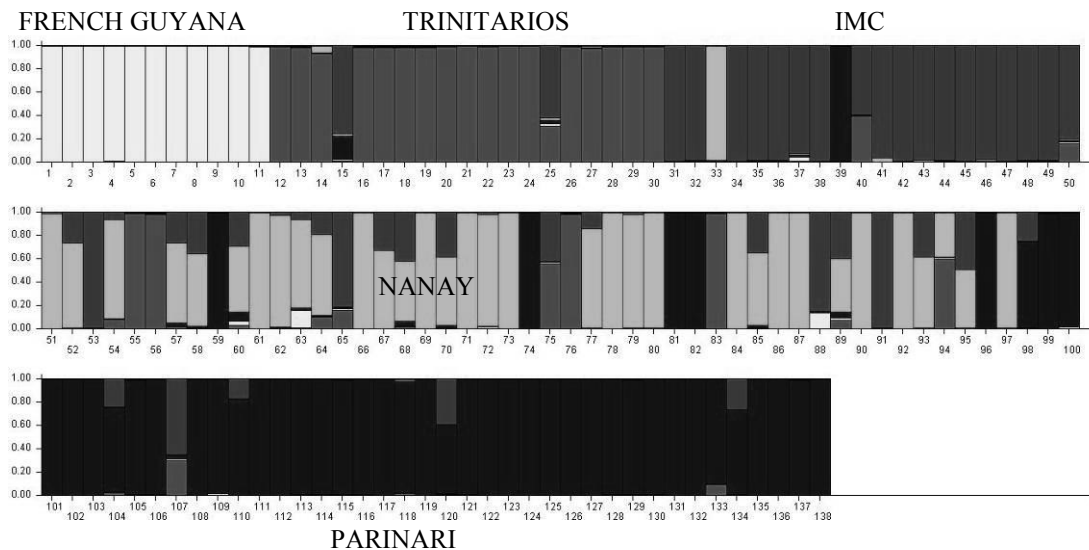


Figure 4. Representation of clusters using STRUCTURE with K=5 : 138 accessions run with 100 SNP markers.

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] (Boccaro, 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 (Boccaro 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

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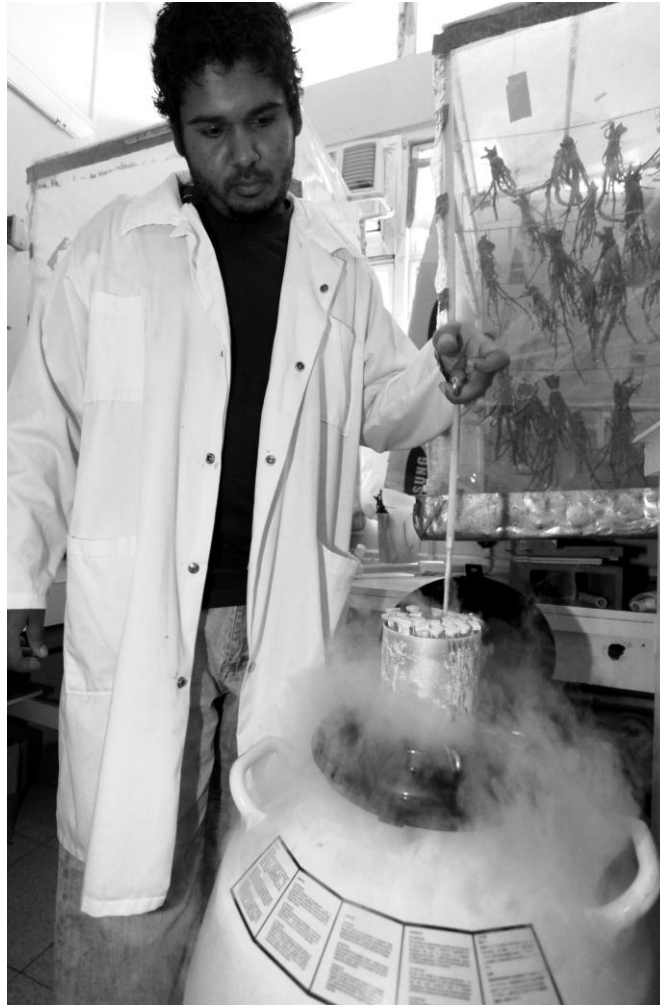
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Evaluation



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 *Planococcus 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.

Reference

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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. Davrieux¹, R. Boulanger¹, P. Alter¹, S. Assemet¹, 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.

Flavour attribute	Contribution of flavour attributes to the principal components		
	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 ($r = -0.358$).

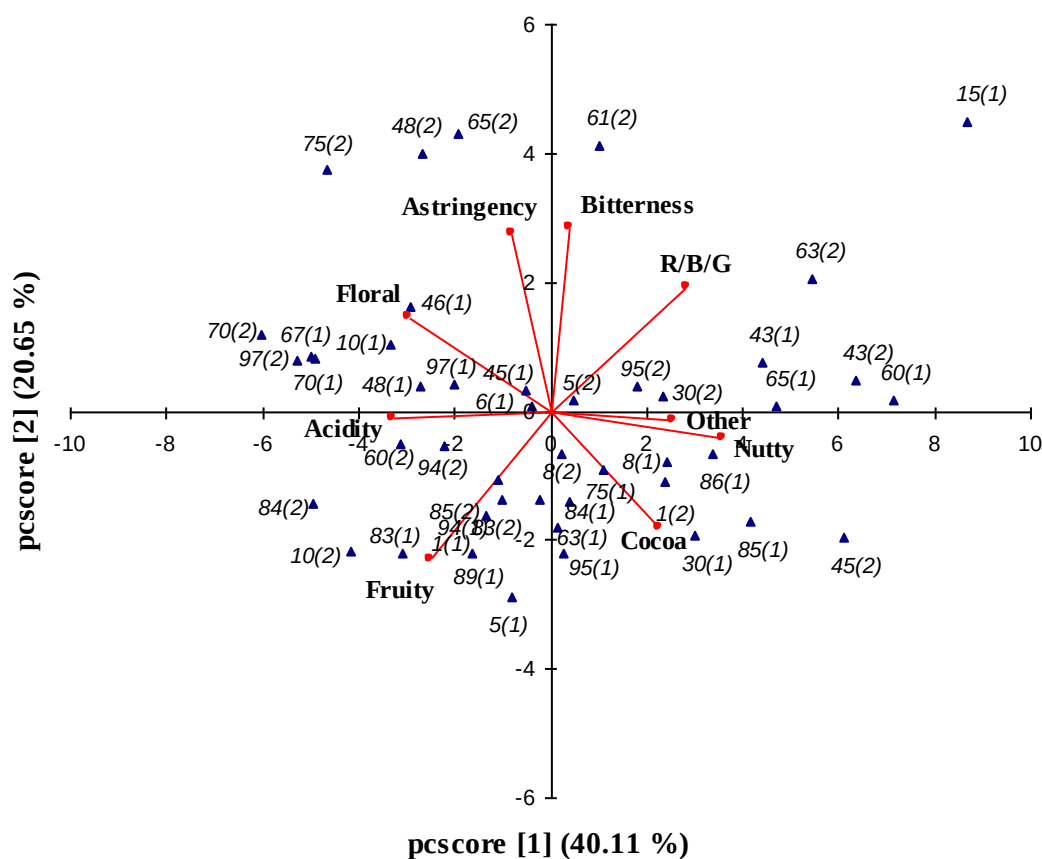


Figure 1. Principal component analysis plot of flavour scores averaged over three repetitions of tasting for ICS clones in the working group from the 2006-2007 (suffix 1) and 2007-2008 (suffix 2) cocoa crop years of the project.

Table 2. A correlation matrix of the flavour attributes from 25 ICS clones presented with significant ($P \leq 0.05$) 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

¹Raw/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.

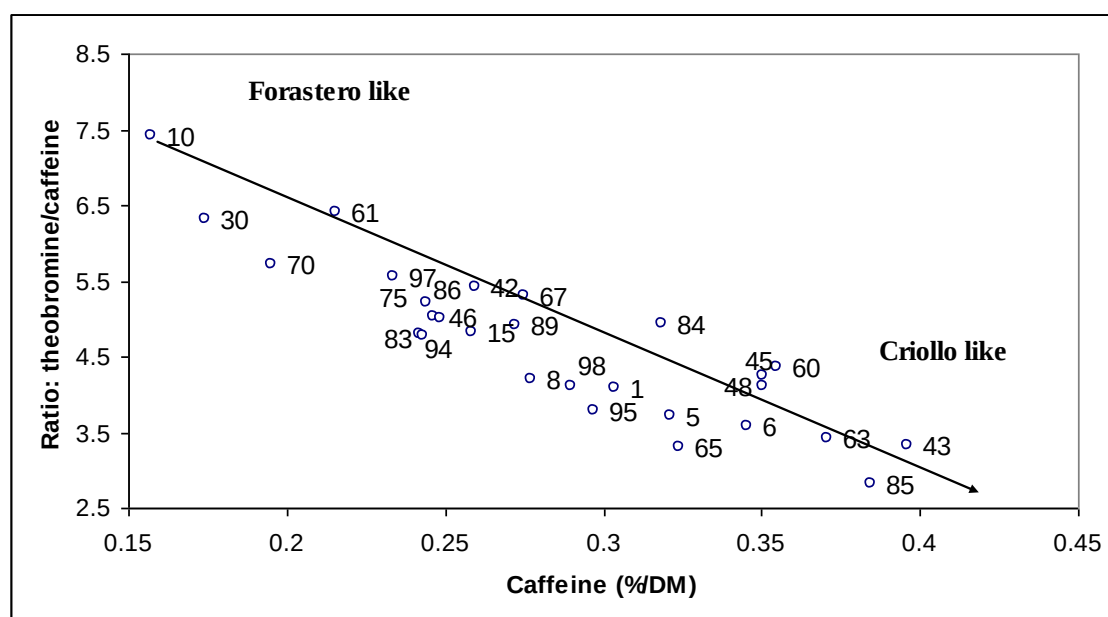


Figure 2. Distribution of 25 ICS clones according to their caffeine content and theobromine:caffeine ratios.

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.

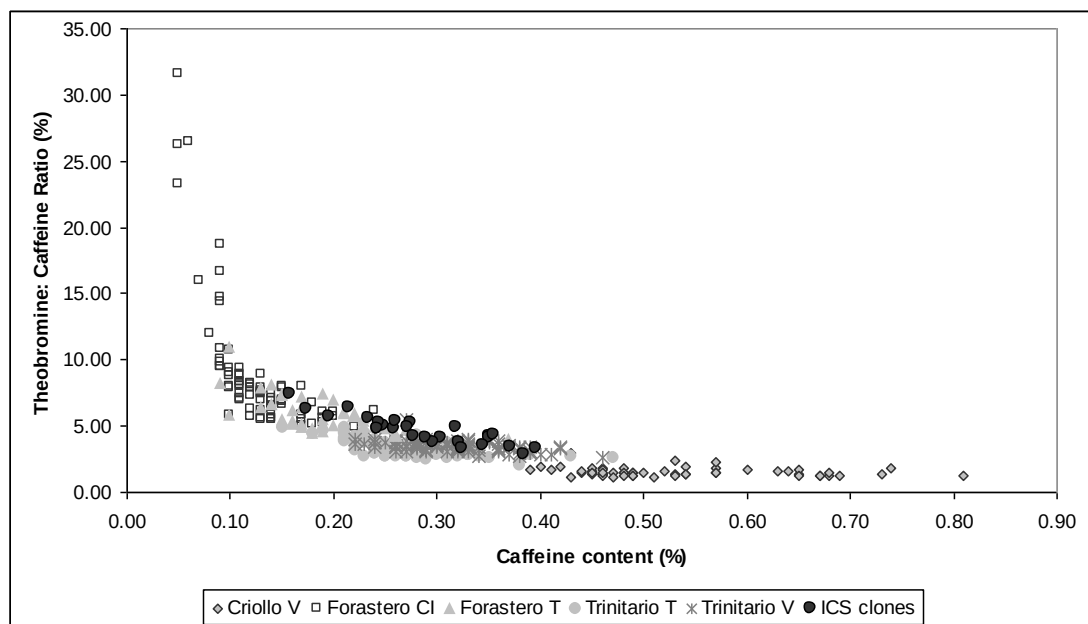


Figure 3. Separation of ICS clones according to their caffeine and theobromine:caffeine ratios among the entire data base of cocoa types analysed in CIRAD.

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 (Ziegler, 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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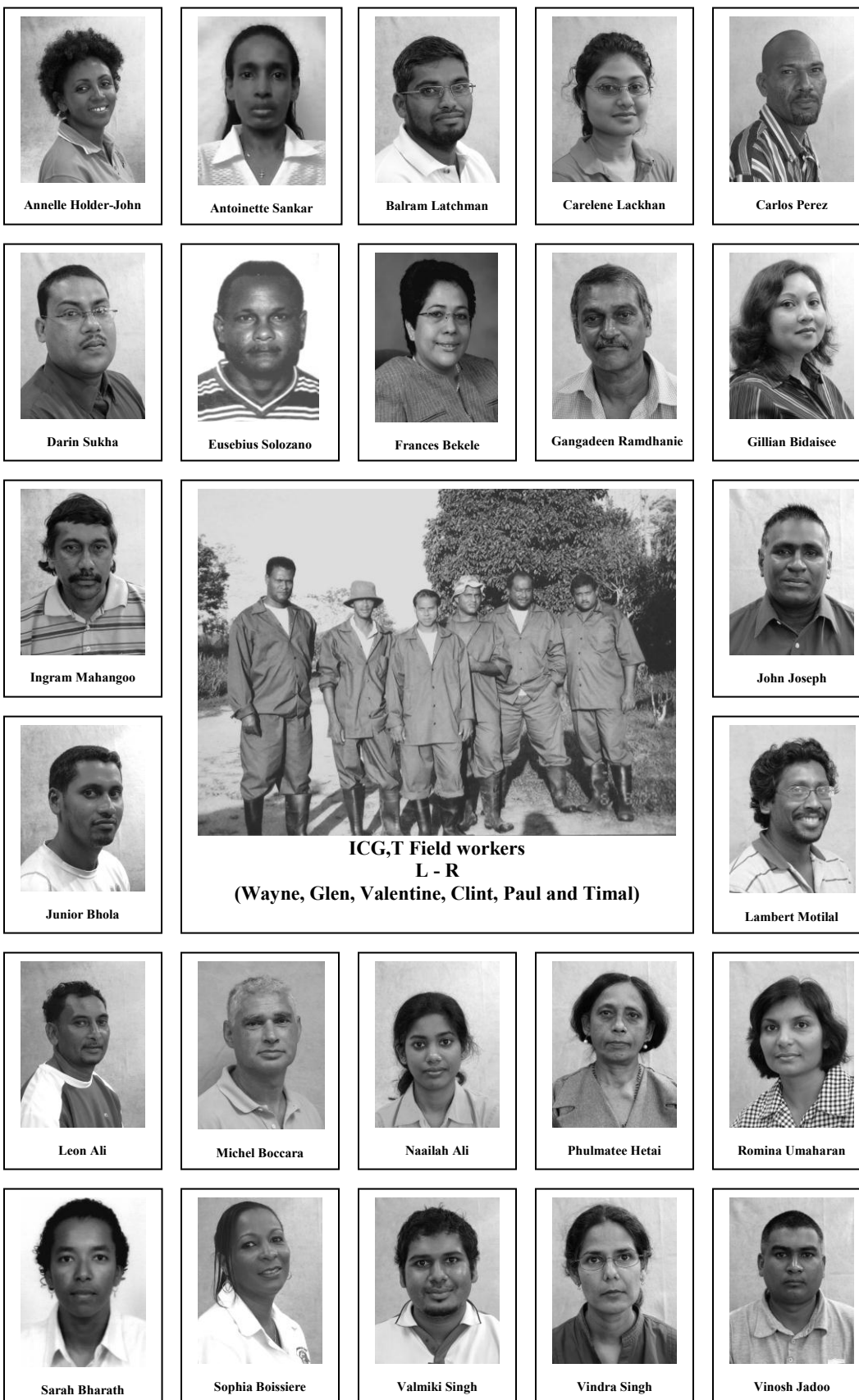
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Valmiki Singh *BSc* Technical Assistant

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.

Zhang, D., Boccara, M., Motilal, L., Mischke, S., Johnson, E.S., Butler, D.R., Bailey B., and Meinhardt Lyndel. (2009) Molecular characterization of an earliest cacao (*Theobroma cacao* L.) collection from Upper Amazon using microsatellite DNA markers. *Tree Genetics & Genomes* **5(4)**: 595-607, DOI 10.1007/s11295-009-0212-2.

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.

Motilal, L. A., Khan, N., Sukha, D.A., Bekele, F.L., Iwaro, A.D., Bidaisee, G.G., Umaharan, P., Grierson, L.H. and Zhang, D. (2007) Cocoa (*Theobroma cacao* L.) bean traits for the identification of superior accessions for germplasm enhancement. Pages 533-538 in: *Proceedings of the 15th International Cocoa Research Conference* Vol. I, San Jose, Costa Rica, 9 - 14 October 2006. Nigeria: COPAL.

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 Seguire, 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.

Susilo, A.W., Zhang, D., Motilal, L. and Meinhardt, L.W. Genetic diversity and population structure of Java fine-flavor cocoa (*Theobroma cacao* L.) by simple sequence repeat (SSR) markers. Presented at the 16th International Cocoa Research Conference. Bali, Indonesia. 16 – 21 November 2009.

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 Cannery 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
Neville C. Mc Andrew	Ministry of Agriculture, Belize
Deron Thompson	Attorney General Ministry, Belize
Claudette Jenkins	Intellectual Property Office, St. Kitts
Barry Mahabir	Forestry Division, Trinidad
Susan De Shong	Intellectual Property Office, St. Vincent
Ralph Farnom	Ministry of Agriculture, Barbados
Thomas Jackson	Department of Agriculture, St. Kitts
Alejandro F. Barrientos	Universidad Autónoma Chapingo, México
Germeus Kerby	Haitian Copyright Office, Haiti
Daphnis Emmanuella	Haitian Copyright Office, Haiti
Jennifer Avery	Kairi Blossom, Trinidad
Shiva Maharaj	Intellectual Property Office, Trinidad and Tobago
Michael Edwards	Intellectual Property Office, Trinidad and Tobago
David Chadee	MALMR, Horticultural Division, Trinidad
Richard Aching	Intellectual Property Office, Trinidad and Tobago
Laloo Ramlal	Intellectual Property Office, Trinidad and Tobago
Maudvere Bradford	St. John's, Antigua
Raimundo Lavignolle	UPOV, Geneva, Switzerland
Rolf Fordens	UPOV, Geneva, Switzerland
John Scharffenberger	Scharffenberger Chocolate Maker, USA
Brad Kintzer	Scharffenberger Chocolate Maker, USA
Vernon Barrett	International Partnership Initiatives, UK
Denise Thompson	UTT, O'Meara, Trinidad and Tobago
Julie Reneau	Nespresso, Switzerland
Lawrence A. Wilson	UWI/FSA/CRAC, Trinidad
David Preece,	CRA Ltd/Cadbury, UK
Michelle End	CRA Ltd, UK
Marcel Vernooij	Ministry of Agriculture, Nature and Food Quality, The Netherlands
Peter Bretting	USDA-ARS, USA
Yaw Adu Ampomah	CRIG, New Tafo, Ghana
Jan Engels	CacaoNET Coordinator, Bioversity International, Italy
Stephen Weise	Sustainable Tree Crops Programme/IITA, Accra, Ghana
Martin Gilmour	MARS, UK
Lyndel Meinhardt	Sustainable Perennial Crops Lab/USDA-ARS, USA
Dapeng Zhang	Sustainable Perennial Crops Lab/USDA-ARS, USA
Hannes Dempewolf	University of British Columbia, Canada
Christopher N. Taylor	Taylor Gentles Group, Florida, USA
Bill Guyton	World Cocoa Foundation, Washington, USA
Ed Seguine	MARS Chocolate North America, USA

Philippe Bastide	CIRAD, Montpellier, France
Victoria Henson-Apollonio	CGIAR CAS-IP, Italy
Elisabeth Gotor	Bioversity International, Rome, Italy
Rianna E. Paul	T&T Chamber of Industry & Commerce, Trinidad
Egli Anton	Nespresso, Switzerland
Trevor and Susan Hetenyi	Dawson Creek, British Colombia, Canada
Bonnie Tyler	Department of Chemical Engineering, UWI, St. Augustine
Rishi Sookraj	Department of Chemical Engineering, UWI, St. Augustine
James H. Small	USAID, Washington, USA
Scott Tiffin	IDRC, Ottawa, Canada
René Lamielle	Lamielle Chocolatier, Epinal, France
Francis and Christine Boucher	France
Gérard and Francine Joly	France
Carlos and Elaine Mota	France
Marie Danielle Berthoud	France
Jean-Paul Raffin	Switzerland
Boucher Francis	Président de al Confédération des Chocolatiers et Confiseurs de France, France
Loiza Rauzduel	Guadeloupe
Jean Claude and Brigitte Breton	Chocolatier JC Berton, France
Ambassador Ali Mchumo	Managing Director, Common Fund for Commodities, The Netherlands
Fatima S. Mchumo	The Netherlands
Eltha Brown	Project Manager, CFC, The Netherlands
Arlington Chesney	Executive Director, CARDI, Trinidad
Steve Hallett	Botany Department, Purdue University, USA
Michel Jacquet	CIRAD, Montpellier, France
Sephra Rampersad	Department of Life Sciences, UWI
Eric Rosenquist	World Cocoa Foundation, Washington, USA
Gina Singh	Maraval, Trinidad
Clement Sankat	Pro-Vice-Chancellor and Campus Principal, UWI
Fazal Karim	Business Development Office, UWI
John Warren	Aberystwyth University, UK
Michel Jacquet	CIRAD, France
Lorenza Robinson	St. Joseph, Trinidad
Peter Laderach	CIAT, Cali, Colombia
Vanessa Butler	Brazil
Ian Ivey	Next Corporation, Trinidad
Anton Doldron	Cocoa and Coffee Industry Board, Trinidad
Pierre Costet	Chocolaterie Valrhona, France
Margaret Taylor	CARIRI, Trinidad
Karen Camejo	Food Science and Technology Unit, UWI
Gail Baccus-Taylor	Food Science and Technology, Unit, UWI
Liaquat Ali Shah	CARIRI, Trinidad
Megnath Gosein	CARIRI, Trinidad
Barbara Whittington	CARIRI, Trinidad
Ramin Ganeshram	New York, USA
Markus Grob	Walter Matter SA, Geneva, Switzerland

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 Investigación 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

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