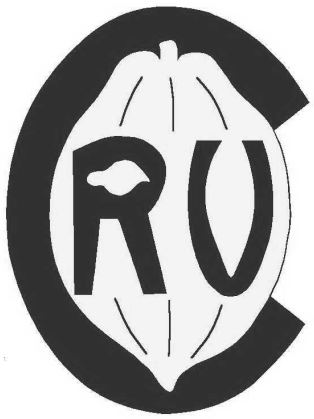
Annual Report 2007



Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
2008

Contents

Introduction

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

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

CRU is a department in the Faculty of Science and Agriculture of the University of the West Indies (UWI). Core activities in CRU are made possible by financial support from the Government of the Republic of Trinidad and Tobago (GORTT) and the Biscuit, Cake, Chocolate and Confectionery Association, UK (BCCCA, and now changed to 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/BI[1] project entitled *Cocoa productivity and quality improvement: a participatory approach* started in June 2004 and is referred to in this report as the "CFC/ICCO/BI Cocoa Productivity Project". Good progress continues to be made in two major components of this project (germplasm enhancement for Black Pod resistance and Witches' Broom resistance). The Black Pod resistance component that was being executed by David Iwaro, is demonstrating a significant increase in the proportion of resistant individuals in the enhanced population. The most promising individuals are being transferred to the International Cocoa Quarantine Centre, Reading (ICQC,R), UK. Second-round crosses have been made to further accumulate resistance genes for Black Pod and the progeny are currently being evaluated. The programme of germplasm enhancement for Witches' Broom disease is also progressing well, and selections from the first set of crosses were planted in Field 3A at Centeno in September 2007. Many of these individuals have resistance to both Witches' Broom and Black Pod diseases.

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). We are making good progress in compiling a comprehensive list of diverse accessions with confirmed resistance to Witches' Broom disease. These are being transferred to the ICQC,R for further distribution to end users.

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

CRU is continuing to participate in the project *To develop a DNA[1] fingerprinting database for all major cacao collections in the Americas* with the United States Department of Agriculture (USDA), through an agreement between USDA and CRU with inputs from CIRAD[2]. Since the start of the project in 2001, DNA samples from all the accessions held in the ICG,T have been sent to the USDA molecular biology laboratory in Beltsville, USA, and results for the Refractario group are discussed in this report.

A new project entitled *DNA markers for cacao traits* was approved for funding from the GORTT Research Development Fund in April 2007. This work is being undertaken by Lambert Motilal, who has been hosted by the USDA Molecular Biology Laboratory in Beltsville for much 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.

A project entitled *Safeguarding the International Cocoa Genebank, Trinidad: a global resource for the cocoa industry* was approved by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) in May 2005. The project is being jointly funded by the Support Scheme for Sustainable Development of the Cocoa and Chocolate Sector and the CRA, UK, and will be referred to in this report as the "Dutch LNV Project to Safeguard the ICG,T". The main aim is to upgrade the irrigation facilities in the University Cocoa Research Station (UCRS), improve security of the site and re-propagate material at risk of genetic erosion. In 2007 we concentrated on a component of the project to propagate rooted cuttings from grafted trees in UCRS. The work is being carried out both by CRU (on Campus) and by the Agricultural Services Division of the Ministry of Agriculture, Land and Marine Resources (MALMR), Centeno, and field planting is planned to begin in 2008.

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 was made in the 2006-2007 crop season in harvesting pods from a working group of 30 ICS genotypes, making cocoa liquors and assessing them for flavour. The second year harvests and on-farm processing are on-going. The project has attracted co-financing support and collaboration from several manufacturers of premium chocolate.

A new project entitled *Development of a neutraceutical and flavour profiling system of cocoa beans in Trinidad and Tobago* was approved for funding from the GORTT Research Development Fund in April 2007. This is a joint project between CRU and the Department of Chemistry, UWI. 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.

A new collaborative project between CRU and Towson University entitled *Assessment of the effect of the micro-floral succession during post-harvest cocoa fermentation on flavour* is in progress. Classical microbiological techniques are being used in UWI to study microflora succession during fermentation and, in addition, the micro-organisms are being analysed by molecular methods in Towson.

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

Staff news

I am saddened to report that David Iwaro passed away on 31st December 2007 following a prolonged illness. He remained cheerful and in remarkably good spirits to the end, but the deterioration of his condition was too advanced for him to win the fight. David was a real asset to CRU and the global cocoa community and will be greatly missed. A tribute to David is given at the end of the Introduction.

Sarah Bharath was appointed as a part-time Technical Assistant from November 2006 to December 2007. She was working on the Dutch LNV Project *To assess the quality attributes of the Imperial College Selections,* harvesting clones from the ICS working group, carrying out micro-fermentations and preparing samples for chemical analyses and organoleptic assessment.

Carlene Lakhan (Technical Assistant) was appointed from June 2007 to work on the GORTT Research and Development Fund project *DNA markers for cacao traits.* She has been recording morphological traits of designated cacao populations in UCRS.

Frankie Solomon (Technical Assistant) left CRU to take up a scholarship for full-time study towards a Masters degree when his contract expired on 31st August 2007. He had been working on the Dutch LNV Project to Safeguard the ICG,T, carrying out propagation of clones by rooted cuttings and grafting to prevent genetic erosion.

Naailah Ali was appointed as a part-time Technical Assistant from September 2007 to work on the Dutch LNV Project *To assess the quality attributes of the Imperial College Selections*. Her duties include preparation of cocoa liquor and the organisation of sensory evaluation of the samples.

Balram Latchman (Contract Officer I) was appointed in October to work on the Dutch LNV Project to Safeguard the ICG,T. His main activities are to propagate clones from aging trees in locations outside UCRS, and to raise rooted cuttings with budwood from grafted trees and to oversee the construction of irrigation facilities and security in UCRS.

Visitors

Cassandra Shaw and *Valmiki Singh* were hosted as placement students in CRU from 21 May to 27 July 2007. Ms. Shaw assisted with studies of morphological traits in selected genotypes at UCRS and Mr. Singh assisted with work on pre-breeding for resistance to Witches' Broom disease and screening for resistance to Black Pod disease.

Jim Saunders, Larry Wimmers, Roland Roberts and Kate Denniston from Towson Unisersity, Maryland, USA, visited CRU and held meetings with ourselves and other members of the Faculty of Science and Agriculture from 17 - 20 June 2007. Their interest was to explore avenues of possible collaboration between Towson and UWI.

Frazer Higgins is a placement student from the University of Bath, UK. He came to CRU at the beginning of September 2007 for a 9-month period, and is supported by Cocoa Research UK. He is undertaking two projects; one on compatibility studies of selected clones, and the other on variations in wet to dry weight conversions for cocoa beans.

Valmiki Singh is carrying out a final year undergraduate project in CRU. He is assessing clones with known resistance to Witches' Broom disease for their reaction to leaf inoculation with *Phytophthora palmivora*.

Meetings and events

David Butler was invited to speak on Fine or Flavour cocoa at the Chocolate Festival – Choco-Laté, Bruge, Belgium on 2 March 2007.

Surendra Surujdeo-Maharaj attended the World Intellectual Property Organization workshop on Successful Technology Licensing, Kingston, Jamaica, 30 April - 4 May 2007.

David Butler attended a CacaoNet meeting (22 May) and the World Cocoa Foundation Partnership meeting (23 - 24 May 2007) in Amsterdam, Holland.

David Butler and Annelle Holder participated in an international workshop on "Cocoa variety improvement in the Americas: Collaborative and farmer participative approaches" from 20-25 August 2007 in Guayaquil, Ecuador.

Darin Sukha participated in a Regional Round Table Meeting for Latin America and the Caribbean Basin, held by the Common Fund for Commoditites from 10 - 13 September 2007 in Lima, Peru.

Surendra Surujdeo-Maharaj participated in a theoretical and practical workshop on Molecular Approaches in Gene Expression Analysis for Crop Improvement at the International Center for Genetic Engineering and Biotechnology from 8 - 19 October 2007 in New Delhi, India.

David Butler participated in a Research Strategy Meeting organised by USDA on 26 October 2007 in Washington USA.

A tribute to David Iwaro

David first came to Trinidad in 1991 as a PhD student in the Cocoa Research Unit. He quickly made a mark in the University, publishing a research note in the 1992 Annual Report, a full research article in the 1993 CRU Annual Report and an oral and poster presentation at two conferences in 1994. He worked on Black Pod disease, which is without doubt the most important disease of cocoa globally, since it occurs wherever the crop is grown and accounts for an estimated crop loss of 30% in a multi-million dollar industry. The disease is of particular importance in David's native country (Nigeria) and throughout West Africa. He submitted his thesis and was awarded a PhD in 1995 however this was just the beginning of his career as an internationally respected research scientist. From 1996 to 1998 he held a post-doc Research Fellow position in CRU, during which time he developed a method for screening cocoa pods for resistance to Black Pod disease. This method has been adopted by cocoa researchers throughout the world and has resulted in an unprecedented increase in breeding for Black Pod resistance in the international cocoa community.

In 1998, David was appointed by UWI to be a member of the academic staff (Research Fellow) in CRU and took on the role of Technical Coordinator of a large international project ("the CFC project") involving 10 cocoa producing countries. His research contribution to this project was of great importance to the global cocoa research community, since he led the way in carrying out a "germplasm improvement programme" aimed specifically at Black Pod disease, but also including Witches' Broom disease resistance in yield-related traits. Progress in this research programme has been on or ahead of schedule from the outset, thanks to David's careful planning and thorough execution of his work-plan. Now, 10 years after the start of that ambitious

work programme, we have populations of resistant cocoa varieties growing in the fields at Centeno that can be utilised by cocoa breeders throughout the world. To follow up this important outcome in Trinidad, a joint project between CRU and the Ministry of Agriculture to utilise David's resistance populations was approved for Government funding and began in 2007. David wrote a detailed work-plan for this (covering the next 6 years) while in hospital at Mount Hope in November/December.

The quality of David's work was recognised by the American Phytopathological Society in 2005 when he was selected as the joint recipient of the *John and Ann Niederhauser Endowment research award* with Jorge de Silva in Brazil. These two scientists exchanged visit to plan a project for this award, but sadly it may not be possible for the project to reach fruition in the present circumstances.

From the time he came to Trinidad 17 years ago and throughout his career in CRU, David has been an enthusiast with clearly defined objectives that he followed through and achieved. As a scientist he was productive, writing papers for international journals of a high standard (he published seven papers in highly regarded scientific journal during his short career), and made valuable contributions to numerous international conferences and workshops. These included the 12^{th}, 13^{th}, 14^{th} and 15^{th} International Cocoa Research Conferences in Brazil, Malaysia, Ghana and Costa Rica between 1996 and 2006. In 2006, although he was already unwell he insisted (against my advice) on travelling to Costa Rica. The benefit of his work to cocoa producers throughout the world is still to be realised, but undoubtedly thousands of cocoa farmers will benefit from his diligence in the medium to long term. For this to happen, it is important that David's unfinished work programme is continued – this is a clear responsibility for CRU.

David was not only a respected scientist; he had a strong faith in God and was a light to his family and colleagues in his unfailing trust in Christ. He was a private person and kept his personal life to himself, yet we all knew of his dedication to the church and exemplary life that he led. He will be greatly missed by all of us, by his family in Nigeria and most especially by Wendy and their children to whom he was devoted; our prayers are with her at this difficult time and in the future. We should give thanks for his life and for his remarkable achievements, and can rest assured that he is at peace with his maker through his unfailing faith.

The Cocoa Research Unit – an overview

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

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

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

A main source of original material for the ICG,T was Marper Farm at Manzanilla, east Trinidad, established by F.J. Pound following his expeditions to 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. In addition, germplasm was

available from other expeditions such as the Anglo-Colombian expedition in 1952-53 and Chalmers' expeditions between 1968 and 1972. By 1994 over 2,000 accessions had been planted in the ICG,T and additional clones are added as they become available. The genebank contains one of the most diverse collections of cacao germplasm in the world and has been designated a Universal Collection by IPGRI[1] (now Bioversity International).

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

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

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

Conservation

Maintenance and propagation

If the ICG,T is not well maintained, research progress would become limited, so a balance is necessary between funds directed towards the genebank maintenance and research.

Apart from routine maintenance such as weed control, pruning, shade management, irrigation 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. Once established, cuttings can be taken from the grafted plants and rooted to fill gaps in the ICG,T with plants on their own roots. It is important to make a concerted effort to raise plants from rooted cuttings if at all possible, to avoid potential confusion in the future with chupons from rootstocks.

New introductions

The ICG,T is considered to be a dynamic germplasm collection. We are continuously adding

[1] International Plant Genetic Resources Institute

accessions from collecting expeditions (when the opportunity arises) or from other national collections. The objective of these inputs is to increase the representation of genetic groups that are currently under-represented in the genebank, thereby creating a balanced collection with maximum genetic diversity. Towards this end, recent acquisitions (since 1990) are Trinitario populations from other islands in the Caribbean and Central America, Lower Amazon material from French Guiana and Venezuela, wild Criollo material from Belize, and genetically diverse Upper Amazon clones from the John Allen collection, Ecuador. Until 2003, new material was introduced through the Barbados Cocoa Quarantine Station however this activity has been suspended due to financial constraints. Material is now being introduced to Trinidad through the ICQC,R, UK.

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

Documentation

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

Verification

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

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

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

[1] Centro Agronómico Tropical de Investigatión y Enseñanza

research or distribution should be verified by DNA fingerprinting to ensure their correct identity.

Initially, molecular verification was undertaken using random amplified polymorphic DNA (RAPD) analysis, this being the technique available in CRU when the work started in 1997. Results from the RAPD analysis showed that approximately 70% of the trees tested were true to type. However, more recently results from some RAPD analyses have been shown to be inconsistent, so it is possible that the 30% off-types identified by this technique is not accurate. Since 2001, we have adopted microsatellite analysis (otherwise known as Simple Sequence Repeats, SSR) for the verification work. We use two techniques to visualise SSR results; either agarose gels with ethyl bromide staining or polyacrylamide gel electrophoresis with silver staining, which gives much better resolution of bands, but is more costly. SSR analysis for DNA fingerprinting is reported to be reliable, with consistent results between different laboratories.

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

Characterisation

Morphological characterisation

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 1,370 accessions and flower descriptions of 2,010 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 gene association between specific traits (recorded as morphological characters) and well-identified parts of the cacao genome. Such information could lead to rapid advances in selection for desirable traits in plant breeding programmes of the future.

Molecular characterisation

From 1994 to 2001, molecular characterisation was carried out using RAPD analysis, with the completion of over 600 accessions. This technique provided information used to assess the genetic diversity within the germplasm collection. Genetic diversity studies can be used to

identify cacao types that are over- or under-represented in the ICG,T, to assess the degree of homogeneity within accession groups, and the genetic distances between them. For cacao, the term population is normally used to refer to accessions sharing the same collection name, but here the term "accession group" will be used. The geographic origin within an accession group can vary from a small estate to a large region. This would naturally affect its genetic diversity.

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

work in CRU aims to develop alternative techniques for early screening of resistance to WB.

Marker assisted selection

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

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

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

Conclusion

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

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

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

Conservation

New cacao introductions into the International Cocoa Genebank, Trinidad

D.R. Butler and J. Joseph

Cacao germplasm from collecting expeditions in Belize (Mooleedhar 1997, 1998) was introduced into Trinidad as seedlings in the 1990s via the Barbados Cocoa Quarantine Station. The majority of these plants have been kept in the greenhouse at UWI since early attempts to establish a selection of seedling plants in the field were not successful.

More recently, an area in the Campus 10 field at UWI was cleared and planted with banana to provide shade for young cacao. Forty of the seedling plants from Belize were propagated by grafting and these were planted in Campus 10 in June 2007, with seven other genotypes from the greenhouse (Table 1). The majority of these plants now look well established, and a similar method is recommended to transfer more of the Belize material to the field in future.

Table 1. Grafted plants established in the Campus 10 fields at UWI, St. Augustine.

Clone name	Number of trees
17/B-1 [BLZ]	1
17/B-5 [BLZ]	1
61/B-1 [BLZ]	1
61/B-2 [BLZ]	2
61/B-4 [BLZ]	1
61/B-6 [BLZ]	1
61/B-7 [BLZ]	2
74/6-1 [BLZ]	1
74/6-5 [BLZ]	2
74/6-6 [BLZ]	1
AC 1/1 [BLZ]	1
AC 1/2 [BLZ]	1
AC 2/1 [BLZ]	1
AC 2/7 [BLZ]	1
C/6-2 [BLZ]	1
C/B-3 [BLZ]	1

Clone name	Number of trees
C/B-4 [BLZ]	1
C/D-2 [BLZ]	2
Ex 1/(a)-1 [BLZ]	2
Ex 4/B-5 [BLZ]	1
ST 4/1 [BLZ]	3
BC 8/2 [BLZ]	2
BC 8/8 [BLZ]	2
BELIZE 1	3
BELIZE 10	1
BELIZE 15	1
BELIZE 2	1
BELIZE 20	2
BELIZE 24	1
BELIZE 28	2
BELIZE 29	2
BELIZE 32	2

Clone name	Number of trees
BELIZE 37	2
BELIZE 4	2
BELIZE 5	3
BELIZE 6	1
CL 27/43	2
DOM 2	2
HF 3/1 [BLZ]	2
IB 1/3 [BLZ]	3
IB 2/3 [BLZ]	2
LCT EEN 321/S1	2
LCT EEN 346	2
MORALES 2/2 [BLZ]	2
RIM 22 [MEX]	2
RIM 23 [MEX]	1
TAP 12 [CHA]	1

References

Mooleedhar, V. (1997) Conservation progamme (1996). Pages 9-18 in: *Annual Report 2006*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Mooleedhar, V. (1998) A study of the morphological variation in a relic Criollo cacao population from Belize. Pages 5-14 in: *Annual Report 2007*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Re-propagation of genotypes in the International Cocoa Genebank, Trinidad

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

Introduction

During 2007, most emphasis in the Dutch LNV Project to Safeguard the ICG,T was placed on the establishment of rooted cuttings from grafted trees in the University Cocoa Research Station (UCRS). Further progress was also achieved in propagating clones from ageing trees by grafting (reported in full by Solomon *et al.* (2007) in the Annual Report 2006) and this year many of the grafted plants have been established in Field 5A at UCRS.

Rooted cuttings

New introductions into the collection at UCRS from 1994 onwards were planted in Field 4A (Mooledhar *et al.* 1995). These genotypes were established as grafted trees and there is a need to re-propagate them as rooted cuttings to avoid the potential problem of confusion between scion and rootstock material in the future.

A new facility for propagating rooted cuttings, using plastic covered chambers with an automated misting system was constructed on campus. The facility consists of 24 chambers, each with a capacity of 200 cuttings. In addition, we obtained agreement from the Agricultural Services Division of MALMR to propagate cuttings using traditional concrete bins at Centeno. By using both these propagation facilities, it was possible to attempt the propagation of a large number of cuttings during the rainy season of 2007.

In April and May 2007, the trees in Field 4A were pruned, fertilized, treated with a systemic insecticide and heavily irrigated in order to promote new growth and the production of good quality cuttings. From June onwards, cuttings were collected on a regular basis, collecting 25 cuttings per accession for propagation at MALMR and 20 cuttings per accession for propagation at UWI. Where the success rate of rooting was poor, consignments of cuttings were collected repeatedly from the same accessions. A combined total of 22,280 cuttings from 511 clones (Table 1) were attempted. Of these, 192 clones have 10 or more survivors, 240 clones have between 1 and 9 survivors, and 80 clones had no surviving cuttings. Forty-four of those with no survivors were propagated more than once at either UWI and/or Centeno.

Table 1. Numbers of cuttings collected (C) and surviving plants with roots (R) from 511 clones in Field 4A of the University Cocoa Research Station.

Clone name	C	R
AGU 2 [CHA]	70	6
AM 1/8 [POU]	70	5
AM 1/12 [POU]	70	1
AM 1/53 [POU]	70	0
AM 1/68 [POU]	90	3

Clone name	C	R
ICA 70 [COL]	50	4
ICS 2	25	11
ICS 3	25	18
ICS 12	70	11
ICS 15	25	5

Clone name	C	R
NA 271	50	2
NA 277	25	6
NA 326	50	18
NA 327	25	12
NA 331	25	9

AM 1/70 [POU]	70	30
AM 1/85 [POU]	70	26
AM 1/87 [POU]	70	2
AM 1/88 [POU]	45	21
AM 1/97 [POU]	65	5
AM 1/96 [POU]	70	27
AM 1/107 [POU]	65	6
AM 1/109 [POU]	25	10
AM 2/1 [POU]	70	7
AM 2/3 [POU]	45	3
AM 2/9 [POU]	45	1
AM 2/14 [POU]	70	0
AM 2/18 [POU]	70	2
AM 2/19 [POU]	70	35
AM 2/20 [POU]	65	15
AM 2/21 [POU]	70	29
AM 2/28 [POU]	45	28
AM 2/31 [POU]	45	17
AM 2/41 [POU]	45	44
AM 2/45 [POU]	45	17
AM 2/6 (543) [POU]	70	23
AM 2/6 (557) [POU]	50	11
AM 2/88 [POU]	45	0
AM 2/96 [POU]	45	19
AMAZ 5/2 [CHA]	45	6
AMAZ 6 [CHA]	25	11
AMAZ 10/1 [CHA]	70	7
AMAZ 11 [CHA]	45	7
AMAZ 15/15 [CHA]	45	16
B 5/11 [POU]	20	3
B 11/2 [POU]	20	3
B 13/5 [POU]	20	5
B 14/13 [POU]	20	1
B 14/14 [POU]	20	0
B 22/3 [POU]	20	9
C 96 [TRI]	45	30
C 97 [TRI]	45	24
C 99 [TRI]	70	5
CC 9	40	22
CC 10	70	11
CC 17	95	10
CC 37	45	4
CC 38	70	11
CC 39	25	16
CC 40	45	9
CC 41	70	6
CC 49	70	2
CC 54	45	8
CC 71	70	19
CERRO AZUL 10	45	16

ICS 20	45	2
ICS 23	25	20
ICS 28	50	12
ICS 30	50	16
ICS 31	50	6
ICS 35	25	8
ICS 39	25	9
ICS 42	25	7
ICS 53	70	13
ICS 56	25	12
ICS 58	65	29
ICS 62	45	11
ICS 73	25	6
ICS 77	65	31
ICS 81	45	18
ICS 82	50	14
ICS 88	50	12
JA 1/5 [POU]	25	1
JA 1/9 [POU]	50	6
JA 2/12 [POU]	45	8
JA 3/4 [POU]	70	18
JA 3/37 [POU]	70	34
JA 5/11 [POU]	70	29
JA 5/27 [POU]	25	14
JA 8/42 [POU]	50	21
JA 9/1 [POU]	50	3
JA 10/34 [POU]	50	0
JA 10/35 [POU]	50	1
JA 10/51 [POU]	25	0
JA 10/58 [POU]	25	0
LCT EEN 6/S-1	25	10
LCT EEN 15/S-3	50	0
LCT EEN 20/S-10	50	5
LCT EEN 21/S-4	25	7
LCT EEN 23	50	1
LCT EEN 37/F	25	10
LCT EEN 46	50	0
LCT EEN 62/S-4	50	4
LCT EEN 66	50	0
LCT EEN 67	50	0
LCT EEN 72	50	1
LCT EEN 82	50	1
LCT EEN 83/S-8	50	0
LCT EEN 84	90	3
LCT EEN 85	50	1
LCT EEN 90	50	0
LCT EEN 90/S-7	70	3
LCT EEN 127	25	0
LCT EEN 162/S-1010	90	2
LCT EEN 163/A	50	0

NA 339	25	8
NA 370	50	14
NA 372	50	15
NA 395	50	7
NA 399	25	0
NA 406	50	3
NA 423	45	7
NA 435	25	8
NA 471	50	0
NA 475	25	0
NA 507	50	3
NA 669	70	3
NA 678	25	1
NA 687	25	0
NA 689	25	6
NA 691	25	6
NA 717	25	2
NA 720	45	7
NA 721	70	1
NA 728	25	6
NA 732	25	0
NA 764	25	0
NA 766	25	1
NA 770	50	7
NA 780	50	1
NA 835	25	0
OC 61 [VEN]	25	3
PA 20 [PER]	20	2
PA 20 [PER]	20	1
PA 134 [PER]	20	3
PA 135 [PER]	20	7
PA 187 [PER]	20	4
PA 200 [PER]	20	6
PA 289 [PER]	20	3
RIM 2 [MEX]	45	22
RIM 6 [MEX]	25	4
RIM 8 [MEX]	25	7
RIM 10 [MEX]	25	12
RIM 12 [MEX]	25	1
RIM 13 [MEX]	25	9
RIM 19 [MEX]	45	23
RIM 24 [MEX]	25	18
RIM 41 [MEX]	25	6
RIM 48 [MEX]	45	20
RIM 71 [MEX]	25	18
RIM 75 [MEX]	25	18
RIM 76 [MEX]	25	17
RIM 101 [MEX]	25	0
RIM 106 [MEX]	25	7
RIM 113 [MEX]	45	20

CL 9/7	45	1
CL 9/11	65	7
CL 9/12	65	2
CL 9/19	65	5
CL 9/51	45	7
CL 10/3	45	12
CL 10/10	70	14
CL 10/11	70	19
CL 10/14	65	8
CL 10/17	25	0
CL 10/23	65	8
CL 10/25	45	0
CL 10/33	45	13
CL 13/17	65	3
CL 13/35	45	9
CL 13/36	45	12
CL 13/41	70	13
CL 13/43	45	2
CL 13/65	50	19
CL 19/2	45	13
CL 19/10	45	16
CL 19/21	45	28
CL 19/33	65	5
CL 19/36	65	7
CL 19/41	45	11
CL 19/42	45	0
CL 27/7	45	3
CL 27/14	25	23
CL 27/14	25	0
CL 27/21	20	3
CL 27/34	90	2
CL 27/43	45	11
CL 27/49	70	19
CL 27/72	90	16
CL 27/74	70	2
CL 27/109	70	19
CL 78/2	70	33
CLM 6	20	1
CLM 35	20	2
CLM 65	20	3
CLM 78	40	2
CRU 270[1]	45	0
CRU 271[2]	50	24
CRU 4A/1	20	7
CRU 4A/2	50	3
CRU 4A/3	45	2
CRU 4A/4	70	4
CRU 4A/5	70	21
CRU 4A/6	70	0
CRU 4A/7	45	12

LCT EEN 163/D	50	0
LCT EEN 201	50	1
LCT EEN 202	50	4
LCT EEN 203/S-3	50	0
LCT EEN 212/S-4	50	4
LCT EEN 246	50	5
LCT EEN 250	50	1
LCT EEN 251	50	0
LCT EEN 261/S-4	50	5
LCT EEN 280	50	3
LCT EEN 325	50	1
LCT EEN 326	25	10
LCT EEN 327	50	0
LCT EEN 332	25	0
LP 1/20 [POU]	70	11
LP 1/25 [POU]	45	15
LP 2/11 [POU]	45	15
LP 3/19 [POU]	45	5
LP 4/5 [POU]	25	0
LP 4/15 [POU]	70	21
LP 4/45 [POU]	45	16
LP 5/1 [POU]	70	12
LP 5/3 [POU]	25	0
LV 2 [POU]	25	0
LV 9 [POU]	25	2
LV 10 [POU]	25	0
LV 14 [POU]	25	1
LV 17 [POU]	25	10
LV 27 [POU]	50	6
LV 33 [POU]	50	20
LV 37 [POU]	70	8
LX 1	70	1
LX 2	50	11
LX 18	25	1
LX 24	50	14
LX 41	70	1
LZ 4	50	5
LZ 5	25	8
LZ 7	50	1
LZ 8	50	8
LZ 17	50	1
MAR 1	25	11
MAR 3	25	0
MAR 9	25	9
MAR 10	25	1
MAR 11	25	1
MAR 12	25	0
MAR 13	20	11
MAR 14	25	7
MAR 17	25	16

RIM 117 [MEX]	25	12
SC 1 [COL]	20	17
SC 3 [COL]	25	1
SC 4 [COL]	25	2
SC 5 [COL]	25	5
SC 6 [COL]	25	20
SC 7 [COL]	25	1
SC 11 [COL]	20	9
SC 12 [COL]	25	6
SC 15 [COL]	25	1
SC 17 [COL]	25	17
SC 19 [COL]	25	0
SC 20 [COL]	25	20
SJ 1/1 [POU]	25	0
SJ 1/10 [POU]	25	2
SJ 1/11 [POU]	25	8
SJ 1/18 [POU]	25	0
SJ 1/28 [POU]	45	2
SJ 1/29 [POU]	25	12
SJ 1/33 [POU]	25	0
SJ 1/37 [POU]	25	0
SJ 2/12 [POU]	25	0
SJ 2/17 [POU]	25	6
SJ 2/26 [POU]	25	4
SLA 10	25	3
SLA 13	45	9
SLA 48	25	8
SLA 77	25	5
SLA 95	25	16
SM 1 [POU]	25	1
SM 5 [POU]	25	15
SM 9 [POU]	25	3
SPA 12 [COL]	25	14
SPA 16 [COL]	25	10
SPA 18 [COL]	25	22
SPA 20 [COL]	25	18
SPEC 41/6	50	4
TRD 1	50	3
TRD 2	25	12
TRD 3	50	10
TRD 5	25	11
TRD 6	50	3
TRD 7	50	0
TRD 8	45	4
TRD 9	25	0
TRD 13	45	8
TRD 15	65	35
TRD 16	25	14
TRD 18	50	10
TRD 19	50	7

CRU 4A/8	45	26
CRU 4A/9	45	5
CRU 4A/10	45	9
CRU 4A/11	20	2
DOM 1	45	11
DOM 3	45	2
DOM 4	45	0
DOM 5	70	16
DOM 7	70	15
DOM 8	70	1
DOM 9	45	14
DOM 10	45	20
DOM 10	45	14
DOM 13	45	5
DOM 14	45	2
DOM 15	45	3
DOM 16	45	7
DOM 18	45	4
DOM 20	50	5
DOM 21	45	14
DOM 23	45	11
DOM 24	45	3
DOM 25	20	16
DOM 27	45	6
DOM 30	45	26
DOM 31	70	17
DOM 33	45	17
DOM 34	45	1
DOM 35	20	20
FSC 13	25	19
GS 4	90	10
GS 4	90	10
GS 6	25	10
GS 12	50	6
GS 13	50	0
GS 37	50	0
GS 39	50	0
GS 45	50	0
GS 55	25	11
GS 58	50	0
GS 59	50	0
GS 61	50	5
GS 62	50	5
GU 114/P	20	18
GU 151/F	70	8
GU 175/P	70	10
GU 195/P	25	0
GU 219/F	70	15
GU 222	25	15
GU 241/P	70	3

MAR 19	25	0
MAR 20	25	4
MAR 21	25	8
MO 82	50	1
MO 87	25	1
MO 96	25	3
MOQ 1/12	25	12
MOQ 1/21	50	1
MOQ 1/24	25	0
MOQ 2/28	45	1
MOQ 2/31	50	4
MOQ 3/1	25	0
MOQ 3/16	25	4
MOQ 4/2	45	9
MOQ 4/16	50	0
MOQ 4/21	50	0
MOQ 4/23	50	0
MOQ 4/25	50	8
MOQ 5/12	50	2
MOQ 5/29	50	5
MOQ 5/34	50	3
MOQ 6/5	50	34
MOQ 6/28	25	12
MOQ 6/52	50	0
MOQ 6/72	25	0
MOQ 6/73	50	8
MOQ 6/85	45	2
MOQ 6/91	50	18
MOQ 6/92	70	1
MOQ 6/107	50	14
MOQ 6/113	50	0
NA 1	50	9
NA 13	50	3
NA 19	50	12
NA 26	25	9
NA 33	50	12
NA 39	50	9
NA 45	50	3
NA 47	50	0
NA 49	25	15
NA 58	50	9
NA 61	70	6
NA 74	25	14
NA 81	50	9
NA 92	25	4
NA 95	70	29
NA 104	50	16
NA 110	50	23
NA 111	50	3
NA 112	25	0

TRD 23	50	2
TRD 24	25	5
TRD 27	25	0
TRD 28	25	0
TRD 29	25	2
TRD 30	45	14
TRD 32	90	29
TRD 33	90	31
TRD 34	25	11
TRD 35	45	13
TRD 37	50	1
TRD 38	25	0
TRD 39	45	3
TRD 39	45	2
TRD 41	20	15
TRD 42	65	5
TRD 43	90	15
TRD 44	50	8
TRD 45	25	16
TRD 46	50	2
TRD 47	25	13
TRD 48	50	0
TRD 49	50	5
TRD 50	50	0
TRD 52	50	0
TRD 53	50	0
TRD 58	50	1
TRD 60	90	19
TRD 65	90	35
TRD 66	65	36
TRD 71	50	10
TRD 75	50	11
TRD 77	50	1
TRD 79	50	0
TRD 81	50	4
TRD 85	25	12
TRD 86	50	3
TRD 88	90	14
TRD 90	50	10
TRD 92	50	3
TRD 93	50	0
TRD 94	50	8
TRD 95	70	8
TRD 99	50	2
TRD 108	50	1
TRD 109	50	3
TRD 110	50	4
TRD 111	70	9
TRD 112	50	0
TRD 113	50	1

GU 243/H	20	16
GU 255/P	25	20
GU 261/P	45	24
GU 265/P	70	13
GU 271/P	25	18
GU 277/G	25	17
GU 286/P	25	17
GU 300/P	20	20
GU 305/P	25	23
GU 307/F	25	19
GU 310/P	45	10
GU 322/P	25	25
GU 335/P	25	18
GU 339/M	45	23
GU 351/P	20	15
GU 353/L	25	23

NA 112	25	14
NA 113	50	11
NA 114	50	8
NA 127	25	4
NA 157	25	5
NA 170	50	4
NA 176	50	5
NA 178	50	16
NA 191	25	11
NA 204	50	11
NA 214	25	17
NA 229	25	15
NA 241	25	2
NA 244	25	11
NA 246	50	4
NA 251	50	0

TRD 114	50	1
TRD 115	50	2
TRD 116	20	17
TRD 117	25	17
TRD 118	50	4
TRD 119	25	12
UF 4	50	8
UF 38	50	5
UF 122	50	3
UF 602	70	3
UF 613	45	10
UF 700	50	8
UF 705	50	0
UF 709	50	2

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

Propagation by grafting of trees not represented in UCRS

The task of collecting budwood from ageing trees growing on Marper Farm, UWI campus and in the San Juan Estate, Gran Couva that are not represented in UCRS was continued in 2007. Where possible, 10 sticks were collected from each clone for grafting.

Inclusive of clones grafted in the previous year (Solomon *et al.*, 2007), all 245 clones from Marper Farm have been grafted at least once, with 236 having one or more surviving plants, giving a success rate for clones of 96%. By December 2007, 4 grafted clones had died, giving a balance of 13 clones that need to be recollected from Marper Farm.

Budwood from 149 clones on the UWI Campus and 11 ICS clones from the San Juan Estate where also grafted. Of these, at least one surviving plant was established from 131 clones on campus and nine ICS clones from the San Juan Estate.

Clones that have been established with less than five surviving plants will be multiplied until at least 5 plants are surviving. Where possible, multiplication will be done with budwood collected from grafted plants in the greenhouse but, in cases where this is not possible, material will be recollected from the original trees.

In October 2007, clones with at least 5 grafted plants established in the greenhouse, were planted Field 5A in UCRS. At least two plants were kept in the greenhouse to provide replacements in case of losses in the field during the establishment stage. A total of 595 plants from 135 clones have been established in Field 5A (Table 2).

Table 2. Numbers of grafted plants established in Field 5A at the University Cocoa Research Station.

Clone name	Trees in 5A	Clone name	Trees in 5A	Clone name	Trees in 5A
ACT 3/6 [TTO]	3	ICS 79	4	MOQ 3/19	5
AM 1/39 [POU]	3	CRU 272	4	MOQ 3/20	5
AM 1/42 [POU]	5	JA 10/29 [POU]	5	MOQ 4/5	5
AM 1/55 [POU]	4	JA 10/31 [POU]	2	MOQ 5/18	5
AM 1/63 [POU]	4	JA 2/11 [POU]	3	MOQ 5/35	4
AM 2/42 [POU]	5	JA 2/26 [POU]	3	MOQ 6/108	5
AM 2/46 [POU]	6	JA 2/8 [POU]	5	MOQ 6/34	7
AM 2/68 [POU]	4	JA 3/29 [POU]	5	MOQ 6/55	4
AM 2/94 [POU]	5	JA 3/3 [POU]	5	MOQ 6/66	3
AS 2 [ECU]	3	JA 3/38 [POU]	5	MOQ 6/70	4
AS 7 [ECU]	4	JA 4/2 [POU]	5	MOQ 6/97	3
B 1/2-8 [POU]	2	JA 4/21 [POU]	4	MX 14/20 [TTO]	4
B 11/3 [POU]	2	JA 6/12 [POU]	5	NA 106	5
B 6/5 [POU]	3	JA 8/18 [POU]	5	NA 119	6
CL 19/35	5	JA 9/9 [POU]	5	NA 120	3
CL 19/46	2	LCT EEN 28/S-1	4	NA 156	5
CL 27/105	4	LP 1/34 [POU]	5	NA 254	4
CL 91/6	6	LP 1/47 [POU]	10	NA 260	3
CLM 111	3	LP 1/56 [POU]	4	NA 38	5
CLM 120	3	LP 3/38 [POU]	5	NA 48	4
CLM 88	5	LP 4/41 [POU]	7	NA 681	3
CRU 130	6	LP 5/23 [POU]	4	NA 694	6
CRU 135	4	LP 6/16 [POU]	3	NA 713	7
CRU 63	3	LX 21	4	NA 725	7
CRU C2/1	3	LX 49	3	NA 747	4
CRU C3/4	3	LZ 11	4	NA 79	3
CRU C4/10	7	LZ 9	6	PA 186 [PER]	3
CRU C4/12	3	MARPER 18	3	SAN JUAN WSC	5
CRU C4/13	5	MARPER 2	8	SAN MIGUEL 3360/2 [CHA]	5
CRU C4/3	6	MARPER 22	7	SCA 16	3
CRU C4/4	6	MARPER 27	4	SCA 7	2
CRU C4/5	5	MARPER 33	2	SI 45/2	5
CRU C4/6	5	MARPER 43	3	SJ 1/15 [POU]	3
CRU C4/7	7	MARPER 46	5	SJ 1/20 [POU]	3
CRU C4/8	3	MARPER 47	6	SJ 1/36 [POU]	5
CRU C4/9	4	MARPER 5	9	SJ 2/28 [POU]	3
CRU C5/2	4	MARPER 50	3	SLA 23	4
CRU C5/7	6	MARPER 9	3	SLA 28	6
CRU C5/8	4	MO 122	3	SLC 1	4
EQX /JS [CHA]	3	MO 84	3	SLC 11	5
GC T 998/55	6	MOQ 1/16	3	SLC 25	3
GC T 998/59	4	MOQ 1/9	4	SM 18 [POU]	5
GC T 998/60	5	MOQ 2/16	4	SM 2 [POU]	11
GC T 998/76	7	MOQ 2/37	5	THY 1/105	3
GC T 998/96	3	MOQ 2/38	3	UF 676	3

Future work

Cuttings will continue to be collected from trees in Field 4A that are producing healthy shoots. We plan to fertilize, prune, irrigate and apply insecticide to the trees again in the 2008 dry season to stimulate new growth. Propagation will continue at both UWI and MALMR and clones that do not root after several attempts will be micrografted, ensuring that the union is below the cotyledonary node (Screenivasan, 1995).

Grafting will also continue from original trees not represented in UCRS, and grafted clones will be multiplied in the campus greenhouses. Grafted clones with at least 5 plants and genotypes with at least 10 rooted cuttings will be planted in UCRS in the next rainy season.

Acknowledgements

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References

Mooleedhar, V., Spence, J.A. and Bekele, F. (1995) The International Cocoa Genebank, Trinidad (ICG,T). Pages 11-17 in: *Annual Report 1994*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Screenivasan, T.N. (1995) grafting on very young cacao seedlings. Pages 45-48 in: *Annual Report 1994*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Solomon, F., Latchman, B. and Butler, D.R. (2007) Re-propagation of cacao germplasm for the ICG,T from original trees in Trinidad. Pages 14-16 in: *Annual Report 2006*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

Fingerprinting cacao trees in the International Cocoa Genebank, Trinidad with microsatellites

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

Introduction

Verification of tree identities within the ICG,T began in the late 1980s under the aegis of V. Mooleedhar, F. Bekele, F. Hosein, E. Johnson, Y. Christopher, and O. Sounigo. A methodology combining morphological description, isoenzyme characterisation and DNA amplification was used as a tool to match sample trees to a reference tree. Initially, DNA profiles were produced with randomly amplified polymorphic DNA (RAPD) products from several primers. However in 2001, RAPDs were phased out in favour of SSRs which give more informative and reliable amplification of small duplicated segments of DNA. Specific primers for these microsatellite loci were designed (Lanaud *et al.*, 1999) and many other primers are now available. More recently, the agarose system for fingerprinting was outmoded when the medium-throughput capillary sequencer system at the USDA, in Beltsville, Maryland became available.

Identity resolutions in cacao genebanks by multilocus microsatellite fingerprinting have commonly used 15 loci (Saunders *et al.*, 2004; Zhang *et al.*, 2006). Suggestions to increase the rate of output have been forwarded (Motilal *et al.*, 2007). The present contribution builds on earlier work and provides an early look into tree fingerprinting within plots in UCRS.

Materials and methods

DNA extraction, quantification, amplification and sizing of amplified products were carried out as described in Motilal *et al.* (2007).

Primer assessment

Table 1. Cacao accessions used for determination of best microsatellites for verification studies in field germplasm collections.

Accession	Location (fingerprinting code, fp)	Group	Country of Origin (Status)
AC 2 [BLZ]	Greenhouse, T1 (fp 1026)	Criollo	Belize (wild)
AC 20 [BLZ]	Greenhouse, T1 (fp 1032)	Criollo	Belize (wild)
B 9/10-25 [POU]	Marper Farm, C1078	Refractario	Ecuador (cultivated)
BC 3 [BLZ]	Greenhouse, T1 (fp 1019)	Criollo	Belize (wild)
COCA 3348/44 [CHA]	UCRS, Field 6B, E374 T2 (fp1047)	Forastero	Ecuador (wild)
CRIOLLO 22 [CRI]	UCRS, Field 4A C276 T3	Criollo	Costa Rica (cultivated)
EET 400 [ECU]	UCRS, Field 6B, F455 T1	Forastero	Ecuador (cultivated)
ELP 1	Greenhouse, T6 (fp 950)	Forastero	French Guiana (wild)
GU 241/P	UWI Campus Field 1A, x2y33 (fp 500)	Forastero	French Guiana (wild)
H 1	Not available	Forastero	Peru (cultivated)
HF 8 [BLZ]	Greenhouse, T1 (fp 987)	Criollo	Belize (wild)
IB 2 [BLZ]	Greenhouse, T1 (fp 1020)	Criollo	Belize (wild)

IB 9 [BLZ]	Greenhouse, T1 (fp996)	Criollo	Belize (wild)
ICS 75	San Juan Estate Block 2	Trinitario	Trinidad (cultivated)
ICS 97	San Juan Estate Block 1	Trinitario	Trinidad (cultivated)
ICS 100	San Juan Estate Block 2	Trinitario	Trinidad (cultivated)
IMC 3	UWI Campus Field 3 x1y3	Forastero	Peru (wild)
IMC 12	Marper Farm, C1056	Forastero	Peru (wild)
IMC 16	Marper Farm, D603	Forastero	Peru (wild)
IMC 67	La Reunion Estate	Forastero	Peru (wild)
JA 5/4 [POU]	Marper Farm, C526 (fp2307)	Refractario	Ecuador (cultivated)
JA 5/5 [POU]	Marper Farm, C324 (fp1351)	Refractario	Ecuador (cultivated)
LCT EEN 31	UCRS, Field 6A, A6 T3 (fp450)	Forastero	Ecuador (wild)
LCT EEN 162 S1010	UCRS, Field 5B, C216 T2 (fp2945)	Forastero	Ecuador
MO 9	Marper Farm, D835 (fp253)	Forastero	Peru (wild)
MO 20	Marper Farm, D809 (fp254)	Forastero	Peru (wild)
MOQ 6/95	Marper Farm, C1 (fp582)	Refractario	Ecuador (cultivated)
MXC 67	UWI, Campus Field 12, x3y6	Criollo	Mexico (cultivated)
NA 184	UCRS, Field 5B, G612 T1	Forastero	Peru (wild)
NA 241	UCRS, Field 4A, D383 T4 (fp2716)	Forastero	Peru (wild)
NA 244	UCRS, Field 5B, E400 T3 (fp16)	Forastero	Peru (wild)
NA 266	UCRS, Field 5B, G634 T3 (fp25)	Forastero	Peru (wild)
NA 331	Marper Farm, D477 (fp383)	Forastero	Peru (wild)
NA 406	UCRS, Field 5B, F447 T1 (fp23)	Forastero	Peru (wild)
NA 432	Marper Farm, D717 (fp271)	Forastero	Peru (wild)
NA 435	Marper Farm, D760 (fp260)	Forastero	Peru (wild)
NA 504	Marper Farm, D465 (fp167)	Forastero	Peru (wild)
NA 528	Marper Farm, D774 (fp112)	Forastero	Peru (wild)
NA 680	UCRS, Field 5A, D337 T3 (fp649)	Forastero	Peru (wild)
NA 702	Marper Farm, D104 (fp819)	Forastero	Peru (wild)
NA 705	Marper Farm, C102 (fp1280)	Forastero	Peru (wild)
NA 733	Marper Farm, D721 (fp274)	Forastero	Peru (wild)
NA 734	Marper Farm, D546 (fp377)	Forastero	Peru (wild)
NA 771	UCRS, Field 5B, F478 T4 (fp27)	Forastero	Peru (wild)
NA 773	UCRS, Field 5B, F547 T3 (fp1266)	Forastero	Peru (wild)
NA 831	Marper Farm, D741 (fp267)	Forastero	Peru (wild)
NA 833	Marper Farm, D640 (fp297)	Forastero	Peru (wild)
NAPO 2 [CHA]	UWI, Campus Field 7, x8y9 (fp1922)	Forastero	Ecuador (wild)
PA 279 [PER]	Marper Farm, D59 (fp426)	Forastero	Peru (wild)
PA 299 [PER]	Marper Farm, C936 (fp571)	Forastero	Peru (wild)
POR 1 [TTO]	UWI, Campus Field 2, x2y12 (fp1897)	Criollo	Venezuela
POUND 7/B [POU]	UCRS, Field 6B, F407 T3 (fp521)	Forastero	Peru (wild)
SCA 12	Marper Farm, D205	Forastero	Peru (wild)
SCA 24	Marper Farm, D569	Forastero	Peru (wild)
SPA 5 [COL]	UWI, Campus Field 2, x1y15 (fp1817)	Forastero	Colombia or Peru
U 1	Not Available	Forastero	Peru (cultivated)
UF 613	UCRS, Field 4A, A93 T2 (fp1237)	Trinitario	Costa Rica (cultivated)
YAL 6	Not Available	Forastero	French Guiana

The work reported here builds on that of Motilal *et al.* (2007) by including NA accessions that were not resolved by the fifteen recommended loci (Saunders *et al.*, 2004). The full set of 60 accessions is provided in Table 1. Summary statistics including the polymorphism information

content (PIC; Botstein *et al.*, 1980) were obtained with PowerMarker v3.25 (Liu and Muse, 2005). The probability of identity among full siblings (PID_{sib}; Waits *et al.*, 2001) from each SSR

Table 2. Information about microsatellite loci from sixty cacao accessions.

[1]Locus	Rank	[2]Sepn	[3]N_a	Allele range	[4]PID_{sib}	[5]PIC
CIR1	31	8 (13.3)	7	127-151	0.51	0.52
CIR3	1	21 (35.0)	15	211-279	0.33	0.85
CIR6	23	14 (23.3)	8	229-251	0.43	0.66
CIR7	29	11 (18.3)	6	148-162	0.50	0.55
CIR8	25	15 (25.0)	7	289-307	0.46	0.63
CIR9	12	15 (25.0)	9	258-296	0.39	0.73
CIR10	17	12 (20.0)	6	206-216	0.41	0.70
CIR11	13	20 (33.3)	13	282-320	0.39	0.73
CIR12	11	18 (30.0)	14	164-216	0.38	0.75
CIR15	4	27 (45.0)	14	232-260	0.35	0.80
CIR17	35	7 (11.7)	5	271-289	0.63	0.39
CIR18	14	17 (28.3)	9	331-355	0.39	0.73
CIR22	28	12 (20.0)	8	273-291	0.50	0.57
CIR24	33	11 (18.3)	7	186-204	0.55	0.49
CIR26	15	12 (20.0)	8	272-308	0.40	0.71
CIR29	21	15 (25.0)	9	159-187	0.42	0.68
CIR30	18	10 (16.7)	5	172-186	0.41	0.69
CIR33	3	25 (41.7)	15	273-347	0.35	0.81
CIR37	6	25 (41.7)	14	134-178	0.36	0.78
CIR40	16	21 (35.0)	12	258-296	0.41	0.71
CIR42	5	20 (33.3)	11	202-238	0.35	0.80
CIR43	8	17 (28.3)	8	202-216	0.38	0.75
CIR45	36	8 (13.3)	4	288-294	0.64	0.37
CIR55	34	5 (8.3)	3	240-252	0.60	0.40
CIR56	22	14 (23.3)	10	314-364	0.43	0.67
CIR57	24	10 (16.7)	5	247-257	0.46	0.62
CIR58	7	22 (36.7)	15	208-324	0.38	0.76
CIR60	10	18 (30.0)	10	189-215	0.38	0.75
CIR184	20	16 (26.7)	8	117-147	0.42	0.68
CIR210	26	10 (16.7)	7	138-152	0.47	0.60
CIR229	27	16 (25.0)	8	309-325	0.47	0.60
CIR243	9	16 (26.7)	7	125-141	0.38	0.75
CIR244	2	21 (35.0)	13	240-270	0.34	0.82
CIR274	19	21 (33.3)	11	186-224	0.42	0.70
CIR278	37	5 (8.3)	4	98-118	0.65	0.34
S012	32	9 (15.0)	6	264-285	0.53	0.52
S016	30	8 (13.3)	5	201-221	0.51	0.52
Average ± s.e.m.			8.8 ±0.6		0.44 ± 0.01	0.65 ± 0.02

[1]Microsatellite code; [2]Separation ability; [3]Number of alleles;
[4]Probability of identity of siblings (Waits *et al.*, 2001)
[5]Polymorphism information content (Botstein *et al.*, 1980);
N_a, range and PIC obtained from PowerMarker v3.25 (Liu and Muse, 2005).
PID_{sib} and separation ability obtained from GIMLET v1.3.3 (Valière, 2002).

was obtained with GIMLET v.1.3.3 (Valière, 2002).

Varying combinations of primers (244 sets) were prepared and the corresponding allelic datasets were analysed with GIMLET v.1.3.3 (Valière, 2002). The separation success, of each of the 244 primer sets, as a function of the separation ability of the full complement of the 37 loci was calculated. The 244 datasets were examined for the minimal combination of loci that would give resolution identical to the full complement of 37 loci.

Plot homogeneity assessment in UCRS

Fifty-two plots (51 accessions) containing at least two trees were assessed with six loci (mTcCIR1, mTcCIR6, mTcCIR7, mTcCIR8, mTcCIR33 and mTcCIR60). Trees with missing data were excluded from subsequent analysis. Genotype data were analysed with GIMLET v.1.3.3 (Valière, 2002) for individual plot homogeneity using the regroup option.

Results

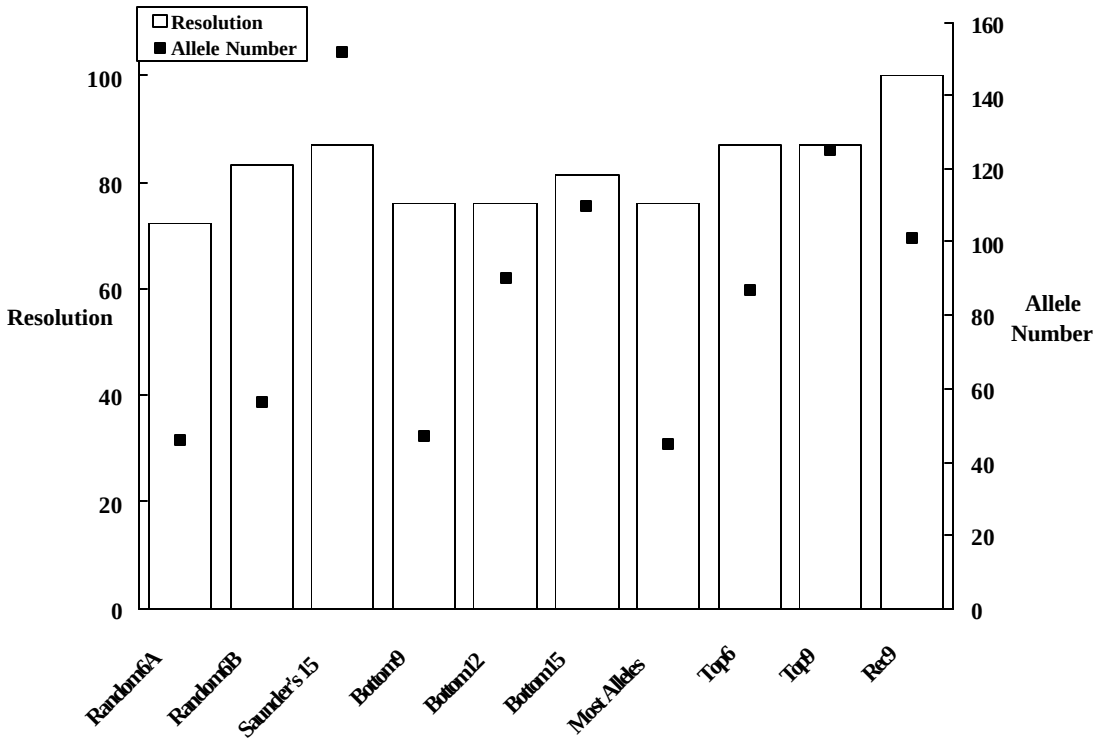

Figure 1. Comparison of resolution ability with allele number in ten primer combinations. Resolution ability was relative to that obtained with all (37) loci on sixty *Theobroma cacao* L. accessions. Top and bottom loci are as ranked with GIMLET v.1.3.3 (Valière, 2002). Saunder's 15 is the set recommended by Saunders *et al.* (2004). Most Alleles are three loci with 15 alleles each. Rec9 is the recommended set of nine primers from this study.

Primer assessment

Characteristics of the individual SSR loci based on the sixty accessions utilised in this study are provided in Table 2. A total of 326 alleles were obtained from 37 loci which resolved the 60 cacao accessions into 54 (90%) groups. Six pairs of accessions were unresolved: AC 20 [BLZ] vs. IB 9 [BLZ], BC 3 [BLZ] vs. HF 8 [BLZ], CRIOLLO 22 vs. IB 2 [BLZ], NA 184 vs. NA 331, NA 432 vs. NA 860 and NA 831 vs. NA 833. The set of primers currently in use for cacao fingerprinting (Saunders *et al.*, 2004) separated the 60 accessions into 47 (78.3%) groups. The accessions that were unresolved with the latter primer set were only NA accessions which are known to be comprised of several sib families: one additional pair was added (NA 406 vs. NA 528) and six other NA accessions (NA 266, NA 435, NA 504, NA 734, NA 773 and NA 860) were lumped into the same group as NA 184 and NA 331.

The separation ability of a primer set was influenced by its composition. Primer combinations comprising the most informative loci as ranked by GIMLET v.1.3.3 (Valière, 2002) performed as well as the set recommended by Saunders *et al.* (2004) even though the numbers of loci and alleles were fewer (Figure 1). An equivalent separation of the sixty accessions with nine loci as compared to that with 37 loci was achieved. These loci were: (a)

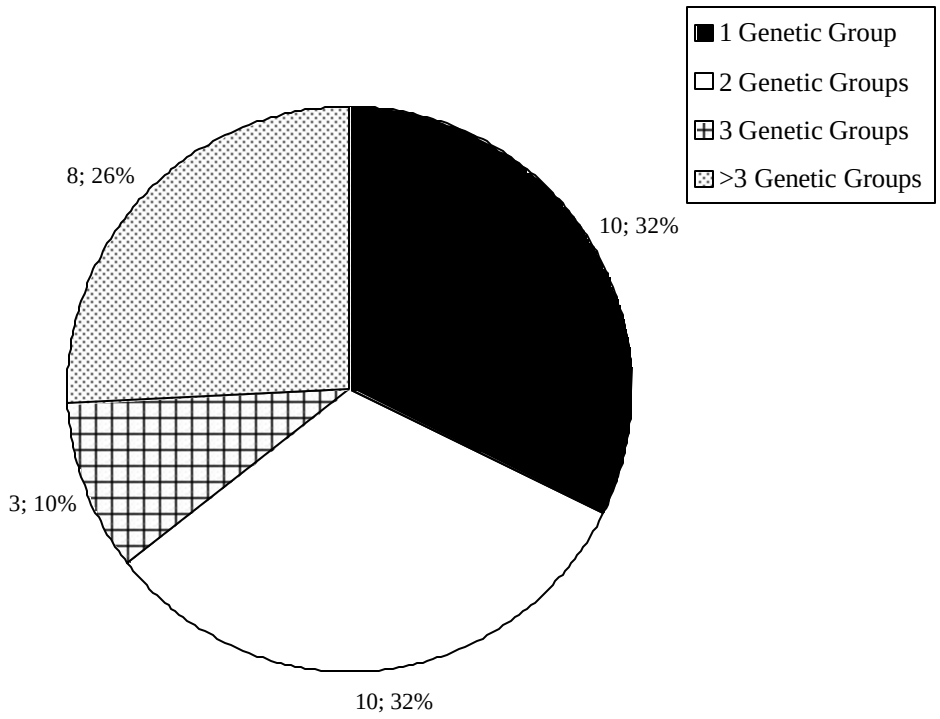

Figure 2. Plot homogeneity assessment in the Univeristy Cocoa Research Station of the International Cocoa Genebank, Trinidad. Thirty-one plots with genotype data from six microsatellite loci on at least four trees were evaluated with GIMLET v.1.3.3 (Valière, 2002).

Group 1 – mTcCIR15, mTcCIR26, mTcCIR37; (b) Group 2 – mTcCIR33, mTcCIR57, mTcCIR42 and (c) Group 3 – mTcCIR12, mTcCIR243, mTcCIR244. Each group represents a trio suitable for post-PCR[1] multiplexing based on allele ranges obtained in this study. This set of nine loci had a total of 101 alleles, a combined PID of 8.886×10^{-12} and a combined PID_{sib} of 1.437×10^{-4}; the latter being a hundred-fold increase to that obtained (2.233×10^{-6}) from the set of 15 recommended by Saunders *et al.* (2004).

UCRS plot homogeneity

The 52 plots examined contained 22 homogenous samples (42.3%) and 30 (57.7%) mixed plots with sixteen plots (30.8%) having two genetic groups (Table 3). Analysed plots which contained at least four trees had a mixed composition of genetic identities in 67.7% (21 of 31 plots) of the plots (Figure 2).

Table 3. Plot homogeneity of accessions in the International Cocoa Genebank, Trinidad.

Accession	Field, plot in UCRS	# Trees in plot	Trees studied	# Genetic groups
AM 1/19	5B, I771	8	8 (T1-8)	1
AM 1/28	6A, A1	8	7 (T2,3,4,7,9,12,14)	1
AM 1/53	6A, A2	7	5 (T1,2,13,15,16)	1
AM 1/54	5B, I811	11	4 (T1,2,6,7)	1
AM 1/60	5A, A26	3	3 (T1,3,6)	1
AM 1/70	4A, F549	2	2 (T2,3)	1
AM 1/85	4A, F538	3	2 (T1,2)	1
AM 2/12	5B, B95	4	4 (T1,4,5,8)	1
AM 2/18	5B, H679	2	2 (T2,15)	1
AM 2/61	5B, H716	3	2 (T7,9)	1
AM 2/62	5B, B105	13	5 (T2,4,6,10,15)	2 (T2,4,6,10); (T15)
AM 2/65	5B, I810	8	5 (T5,7,8,11,14)	2 (T14); (T5,7,8,11)
AM 2/82	5B, I806	4	3 (T1,3,4)	1
AM 2/83	5B, B108	15	9 (T1,2,3,4,6,8,9,11,12)	1
AM 2/96	5B, I819	8	3 (T3,5,7)	2 (T3,T5); (T7)
B 12/1	6B, F461	9	9 (T1,2,6,10-15)	4 (T2,13); (T12); (T1,10); (T6, 11,14,15)
B 13/7	5B, I728	12	9 (T3,4,5,6,7,8,11,12)	2 (T3,4,5,6,712,14); (T8,11)
B 17/17	5B, I784	10	7 (T2,3,5,6,7,9,10)	2 (T3); (T2,5,6,7,9,10)
B 18/4	6B, F457	14	10 (T1,2,5,6,8,12-16)	3 (T6); (T1,5,15); (T2,8,12,13,14,16)
B 4/8	6B, F439	5	3 (T1,3,7)	3 (T1); (T3); (T7)
B 7/21	6B. F438	9	8 (T2,3,5,6,7,12,13,14)	7 (T2); (T5); (T6); (T7); (T13); (T14); (T3,12)
CL 10/5	5B, A4	4	4 (T3,4,5,6)	2 (T3,4); (T5,6)
CL 10/14	5A, A1	11	7 (T2,4,5,6,7,13,14)	4 (T7); (T13); (T5,6); (T2,4,14)
CL 13/27	5B, A24	9	6 (T2,10,11,12,13,14)	2 (T12); (T2,10,11,13,14)
CL 27/50	5B, I743	12	9 (T2,3,4,6,9,11,12,13,14)	1
CL 91/5	5B, A64	2	2 (T4,7)	2(T4); (T7)
CL 9/17	5B, A24	12	12 (T1-10,12,16)	4 (T6); (T9,12); (T2,3,4,8);(T1,5,7,10,16)

[1] Polymerase chain reaction

CRUZ 7/8	6B, B83	6	3 (T1,9,10)	2 (T1,9)*; (T10)*
DOM 27	4A, B203	2	2 (T1,2)	2 (T1); (T2)
ICA 70	4A, C290	3	3 (T1,2,3)	1
JA 1/9	6A, A51	3	3 (T3,6,12)	1
JA 5/27	5B, F483	6	5 (T1,4,6,8,9)	5 (T1); (T4); (T6); (T8); (T9)
JA 5/39	5B, D234	14	11 (T1-8,10,12,15)	2 (T6,8); (T1,2,3,4,5,7,10,12,15)
JA 10/16	5B, E411	2	2 (T1,2)	1
LP 1/21	5B, I746	4	4 (T4,6,8,13)	2 (T4); (T6,8,13)
LP 1/21	5B, I779	5	4 (T3,4,5,8)	3 (T4); (T8); (T3,5)
LP 3/4	5B, A33	16	14 (T1-4, T6-9, T11-16)	4 (T12); (T13); (T1,2,14); (T3,4,6,7,8,9,11,15,16)
LP 4/12	5B, I803	10	9 (T1-7,9,10)	1
LP 4/48	5B, B140	10	9 (T1-3, T5-10)	8 (T2); (T3); (T5); (T6); (T7); (T8); (T10); (T1,9)
LP 5/19	6A, B95	3	3 (T2,8,9)	1
LX 38	5B, C206	8	7 (T2,3,4,5,6,8,9)	4 (T4); (T5); (T9); (T2,3,6,8)
LX 43	5B, C201	16	12 (T1, T3-9, T11, T14-16)	2 (T9); (T1, T3-8, T11,T14-16)
MOQ 6/95	5B, C221	5	3 (T4,6,8)	3 (T4); (T6); (T8)
NA 176	4A. D389	3	3 (T1,2,4)	1
NA 669	4A, D418	4	3 (T1,2,4)	3 (T1); (T2); (T4)
PA 169	6B, C180	11	6 (T1,4,7,10,12,15)	3 (T7)*; (T12)*; (T1,4,10,15)
PA 293	4A, F516	4	3 (T1,2,4)	2 (T4); (T1,2)
SLA 16	5B, D242	8	5 (T1,3,7,8,14)	1
SLC 4	5B, A39	6	4 (T1,2,5,6)	1
SLC 18	5B, A13	9	5 (T5,6,7,8,9)	2 (T5,7,8,9); (T6)
TRD 15	4A, A43	2	2 (T1,3)	2 (T1); (T3)
TRD 111	4A, A87	3	3	1

*May be one group as one difference of 2 base pairs is responsible for the separation

Discussion

Fingerprinting a germplasm collection with the aim of detecting mislabelling errors relies on the use of loci that can differentiate among present holdings and future acquisitions. These loci should be able to maximise differences amongst accessions. The present study demonstrated that the composition of the set of loci used for fingerprinting will affect the resolution efficiency. Furthermore, a set of nine loci (mTcCIR12, 15, 26, 33, 37, 42, 57, 243 and 244) was identified that was superior to that of Saunders *et al.* (2004) and supersedes those recommended in an earlier report (Motilal *et al.*, 2007).

This study found a high level (58%) of plots in UCRS that putatively contained replicated clonal material but instead contained more than one genetic group. This is nearly twice the percentage of off-types reported previously for cacao germplasm collections (Figueira, 1998; Risterucci *et al.*, 2001; Motilal and Butler, 2003) including the ICG,T (Sounigo *et al.*, 2001). However, this is still a relatively small sample from the 2,300 accession in the ICG,T, so it can only serve as an approximate estimate of the error rate in the whole collection. Nevertheless, the importance of recording the tree number when samples are taken and maintaining up-to-date records for information on individual trees cannot be over-emphasised.

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References

Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Amer. J. Hum. Genet.* **32**: 314-331.

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

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

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

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

Motilal, L.A., Umaharan, P., Boccara, M., and Zhang, D. 2007. Evaluation of microsatellites for verification of identities in cacao field genebanks. Pages 17-24 in: *Annual Report for 2006*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

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

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

Sounigo, O., Christopher, Y., Bekele, F., Mooleedhar, V., and Hosein, F. (2001) The detection of mislabelled trees in the International Cocoa Genebank, Trinidad (ICG,T). Pages 34-39 in: *Proceedings of the 3rd International Group for Genetic Improvement of Cocoa (INGENIC) International Workshop on the New Technologies and Cocoa Breeding*, 16–17 October 2000, Kota Kinabalu, Malaysia: INGENIC, UK.

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

Waits, L.P., Luikart, G., and Taberlet, P. (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol. Ecol.* **10**: 249-256.

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

Identity assessment of Refractario origin cocoa accessions held in Trinidad: the contribution of the collaborative USDA/CRU project

M. Boccara and D. Zhang

Introduction

"Refractario cacao" originated from a large group of germplasm collected during the 1920s from the coastal region of Ecuador and selected for its potential resistance to Witches' Broom disease. Seedlings from fruits of these trees were raised in nurseries, screened for resistance to the disease and then established in various farms in Ecuador. Dr F. J. Pound collected pods from 9 farms selected from "some" 80 trees free of Witches' Broom disease symptoms, and seeds were planted in Barbados for quarantine purposes (Table 1). After a suitable period, healthy budwood from the seedlings was forwarded to Trinidad, budded onto rootstock and subsequently planted mostly in Marper Farm and a few on the ICTA Campus.

Records available in CRU show that more than 1,250 Refractario clones were present at one time in Marper Farm: 1,000 in Block C and 250 in Block D. Other Refractario trees were also planted on the ICTA campus and among them 6 clones that were not represented in Marper.

When the ICG,T was established from 1986 to 1994, 746 plots of Refractario origin trees were planted in UCRS.

Currently, in Marper Farm, 716 Refractario trees are still alive, 599 in block C, 117 in Block D and in UCRS, 544 clones replicated in 746 plots.

An international collaborative project on DNA fingerprinting of cocoa germplasm was started in 2001, and priority was given to the analysis of material collected by Pound such as the "Refractarios" since they are of special interest to the international cocoa community.

Table 1. Refractario accessions inventory and their origin.

		Trees planted		Remaining trees		
Farm	**Group name**	**Marper C**	**Marper D**	**Marper C**	**Marper D**	**UCRS only**
Amalia	AM	116	12	79	11	4
Balao	B	67	96	37	41	11
Clementina	CL, CLM	179	32	116	11	19
Javilla	JA	203	30	130	14	12
Large Vuelta	LV, LX, LZ	27	13	13	9	2
La Paz	LP	140	15	83	8	14
Moquique	MOQ	188	24	86	10	15
Santa Lucia	SLA, SLC	37	22	23	11	5
San Juan	SJ	47	5	32	2	6
Total		**1004**	**249**	**599**	**117**	**88**

Achievements

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

A total of 802 samples were collected, including 716 from original trees from Marper fields, 67 from UCRS trees now absent from Marper as well as 41 samples from replicated trees in UCRS to check their conformity.

DNA was extracted in CRU and samples were sent to the USDA Beltsville laboratory for analysis with 15 selected SSR primers, following the recommended protocol and guide-lines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS[1] Beltsville laboratory are available for 1,200 clones from CRU, including 590 Refractario clones and have been used for different purposes:

- To assess the population identity of the Refractario group
- To detect off-type clones of the population
- To verify that duplicate trees are identical
- To place individual trees in appropriate half-sib families
- To assess population admixture
- To discover potential mislabelling and to find conceivable explanations

Methods

Genetic diversity of the 590 Refractario clones was assessed in relation to the 1,200 clones sampled in the ICG,T, using dissimilarity analysis (DARwin software, 5.0.142) and Principal Component Analysis (GENETIX software, v.4.03).

Duplicate trees were assessed by identifying matching multilocus genotypes among Refractario accessions.

Mislabelled trees were identified by comparing their multilocus profile to the reference tree or a putative replicate.

The identities of off-types were sought from matching profiles, and by using all the information available in historical records, publications and maps.

Results

Genetic diversity of the Refractario clones and potential mislabelling

The principle component analysis (PCA) (Figure 1) shows clearly that the Refractario accessions form a group that is distinct from the rest of the clones analysed. It also shows that some accessions labelled Refractario belong to other accession groups mainly Trinitario (Table 2), but

[1] USDA – Agriculture Research Service

Figure 1 here

Table 2. List of off-type Refractario accessions that group with Trinitario genotypes.

Accessions clustered with Trinitario accessions					
Clone name	**Fingerprint code**	**Location**	**Clone name**	**Fingerprint code**	**Location**
AM 1/28 [POU]	fp702	Marper C189	AM 2/61 [POU]	fp1336	Marper C 278
AM 1/73 [POU]	fp2147	Marper C925	AM 2/64 [POU]	fp1565	Marper D739
AM 1/96 [POU]	fp412	Marper D28	AM 2/90 [POU]	fp264	Marper C911
B 1/2-24 [POU]	fp1126	Marper D155	B 13/6 [POU]	fp73	Marper D42
B 21/7 [POU]	fp371	Marper D580	CL 10/14 [POU]	fp 2005	Marper C370
CL 10/27 [POU]	fp214	Marper D296	CL 13/17 [POU]	fp569	Marper C67
CL 13/36 [POU]	fp2148	Marper C930	CL 13/4 [POU]	fp1321	Marper C299
CL 19/2 [POU]	fp603	Marper C36	CL 19/21 [POU]	fp2244	Marper C707
CL 19/22 [POU]	fp1360	Marper C279	CL 19/41 [POU]	fp2039	Marper C519
CL 27/21 [POU]	fp2024	Marper C461	CL 27/58 [POU]	fp1322	Marper C224
CL 9/13 [POU]	fp1305	Marper C43	CL 9/16 [POU]	fp2241	Marper C666
CL 78/3 [POU]	fp1362	Marper C277	CLM 3 [POU]	fp2015	Marper C394
CLM 43 [POU]	fp2025	Marper C454	CLM 64 [POU]	fp2177	Marper C768
CLM 99 [POU]	fp2159	Marper C906	JA 1/14 [POU]	fp2437	Marper C1001
JA 1/8 [POU]	fp1304	Marper C42	JA 10/5 [POU]	fp2248	Marper C630
JA 3/20 [POU]	fp2444	Marper C699	JA 3/22 [POU]	fp1853	Campus 11
JA 3/30 [POU]	fp2084	Marper C1106	JA 4/9 [POU]	fp2441	Marper C717
JA 5/28 [POU]	fp1852	Campus 11	LP 1/24 [POU]	fp2252	Marper C724
LP 2/17 [POU]	fp2169	Marper C925	LP 3/15 [POU]	fp2467	Marper C307
LP 3/20 [POU]	fp2048	Marper C691	LP 3/48 [POU]	fp2393	Marper C821
LP 4/32 [POU]	fp2256	Marper C752	LP 6/16 [POU]	fp2055	Marper C538
LP 6/19[POU]	fp1193	Marper D317	LX 18 [POU]	fp685	Marper C138
LX 25 [POU]	fp814	Marper D105	LX 31 [POU]	fp353	Marper D113
LX 45 [POU]	fp68	Marper D78	MOQ 1/14 [POU]	fp249	Marper D793
MOQ 1/25 [POU]	fp1592	Marper C956	MOQ 4/17 [POU]	fp2174	Marper C984
MOQ 4/2 [POU]	fp697	Marper C144	MOQ 4/20 [POU]	fp136	Marper D799
MOQ 5/34[POU]	fp2198	Marper C795	MOQ 6/102[POU]	fp2635	Marper C846
MOQ 6/103[POU]	fp2156	Marper C931	MOQ 6/19 [POU]	fp1297	Marper C116
MOQ 6/36 [POU]	fp2335	Marper C462	MOQ 6/41[POU]	fp1332	Marper C182
MOQ 6/67[POU]	fp684	Marper C140	MOQ 6/77 [POU]	fp2315	Marper C756
MOQ 6/87 [POU]	fp558	Marper C4	MOQ 6/93 [POU]	fp2450	Marper C839
SJ 1/29 [POU]	fp2061	Marper C410	SJ 2/16 [POU]	fp577	Marper C5
SJ 2/17 [POU]	fp589	Marper C33	SJ 2/19 [POU]	fp1369	Marper C337
SJ 2/30 [POU]	fp2376	Marper C909	SLA 13 [POU]	fp1370	Marper C230
SLA 20 [POU]	fp1340	Marper C231	SLA 64 [POU]	fp2071	Marper C1163
SLC 3 [POU]	fp2066	Marper C508	SLC 18 [POU]	fp822	Marper D183

also Parinari, IMC, Nanay, or Scavina. (Table 3).

The Cluster analysis of the 590 DNA samples of the Refractario labelled accessions was performed using the DARwin software (Figure 2) and provided additional information:

- ? 480 accessions labelled Refractario are grouped together in a cluster
- ? Some Refractario accessions share the same profile but were labelled differently
- ? Some Refractario accessions have a duplicate which is an off-type

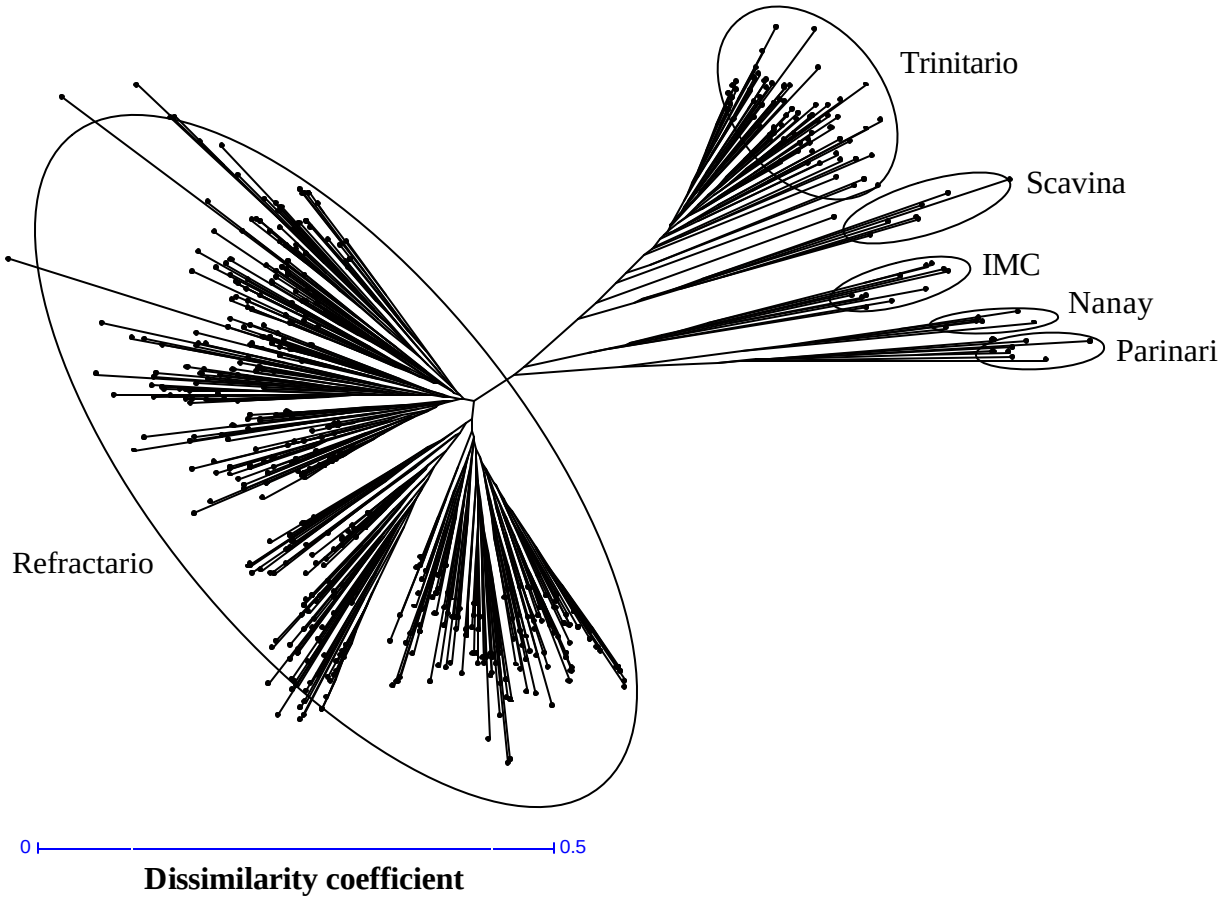

Figure 2. Cluster analysis of 590 DNA samples from trees with labels of the Refractario group.

Mislabelling analysis

Refractario trees presenting a Trinitario profile.
The DNA profiles of 72 accessions with Refractario labels show that they belong to the Trinitario group, implying that the tree or portion of it still alive in the field is constituted of rootstock.

Trees presenting a Refractario profile
The analysis of AM 1/19 [POU] (Marper C922) and AM 2/92 [POU] (Marper C923) profiles show that these trees are identical; this is also the case for AM 2/70 [POU] and LX 20, established just opposite and in the next row, inferring that labels were misplaced.

Table 3. List of identified off-type Refractario accessions.

Clone name	Fingerprint code	Location	Clone name	Fingerprint code	Location
Accessions clustered with IMC accessions					
B 7/1 [POU]	fp1382	5A B80 T15	B 17/33 [POU]	fp1378	5A B78 T8
B 18/8 [POU]	fp41	Marper D173	CL 13/43	fp1254	5B A63 T9
JA 7/11 [POU]	fp1710	5B F521 T2	LP 4/7 [POU]	fp2257	Marper C624
MOQ 6/28	fp2699	4A D357 T1	MOQ 6/88	fp1850	Campus 11
SJ 1/39 [POU]	fp2294	Marper C428	SJ 2/20 [POU]	fp1695	5B B131 T5
Accessions clustered with NA accessions					
CL 10/21	fp578	Marper C9	JA 10/35 [POU]	fp1596	Marper C1002
LP 1/45 [POU]	fp291	Marper D385	LP 3/5 [POU]	fp2357	5B B86 T6
MOQ 1/1	fp1390	6A B83 T13	MOQ 2/33	fp2193	Marper C781
SLA 45	fp1957	5A D299 T10	SLA 48	fp666	Marper D88
SLA 64	fp2695	Marper C1165			
Accessions clustered with PA accessions					
B 9/10-33 [POU]	fp299	Marper D632	B 21/6 [POU]	fp1204	Marper D395
CL 19/49	fp1603	Marper C1100	CL 19/51	fp66	Marper D27
MOQ 6/29	fp2103	Marper C765	SLA 16	fp2707	5B D242 T8
Accessions clustered with SCA accessions					
B 6/29[POU]	fp1381	5A B81 T14	CL 19/35	fp1361	Marper C328
JA 4/21 [POU]	fp416	Marper D2	JA 1/25 [POU]	fp1413	5B D287 T4
JA 5/8 [POU]	fp1874	Campus 11	LP 3/4 [POU]	fp2311	Marper C523
MOQ 4/6	fp1563	5B H680 T13			
Other Refractario off-type accessions					
B 14/14 [POU]	fp1572	5B A44 T11	JA 7/25 [POU]	fp1414	5B G567 T6

Trees presenting a non-Refractario profile

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

SLA 48 (Marper C86) shows a Nanay profile: it had been planted next to NA 22, believed to have died, which could be its true identity. Similar situations are found for B 18/8 [POU] (Marper D173) which could be either IMC 75, IMC 64 or IMC 38; LP 1/45 [POU] (Marper D385) which could be NA 163 or NA 332; CL 19/51 (Marper D27) which could be PA 281 [PER]; B 9/10-33[POU] (Marper D632) which could be PA 72 [PER], and LP 3/4 [POU] (Marper C523) which could be PA 35 [PER].

Some duplicate trees in UCRS were propagated from the wrong original tree. It happened usually in the case where the original tree was already dead when the ICG,T was established: budwood for cuttings was taken from an adjacent tree. The analysis shows that this occurred for accessions B 7/1 [POU], SJ 2/20 [POU] and LP 3/5 [POU].

B 6/29 [POU] tree 14, from Field 5A shows a Scavina profile whereas the DNA analysis of the original tree, still alive in Marper, matches a Refractario identity. Verification of the other trees duplicated in the same plot must be performed.

Mislabelling of plots in UCRS

Matching of some DNA profiles allows us to detect some incorrect labelling of UCRS plots. This is the case of plot B83 in Field 5A labelled B 10/28 [POU] instead of B 9/10-28 [POU], plot A45 in Field 5B labelled B 2/34 [POU] instead of B 23/4 [POU], plot B104 in Field 5A labelled CL 7/89 instead of CL 78/9, plot E473 in Field 4A labelled LP 4/45 [POU] instead of LP 4/41 [POU] (the original LP 4/45 [POU] tree in Marper Farm being dead), as well as plot D350 in Field 4A labelled MOQ 1/24 instead of MOQ 1/22.

Discussion and conclusion

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

The use of 15 markers has been efficient in completing the unambiguous identification of accessions amongst the group.

The analysis confirmed the membership of 480 clones to the Refractario group, and revealed that 72 trees in Marper Farm with Refractario labels were rootstock. It has been reassuring that not more than 40 other accessions were identified as off-types.

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

More verification of duplicate trees will be needed to reduce potential errors in material distributed from UCRS.

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References

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

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

Pound, F.J. (1938) Cacao and witches' broom disease (*Marasmius perniciosus*) of South America. Pages 20-72 in: *Archives cacao research, Vol. 1* (Toxopeus, H. Ed.). American Research Institute, Washington DC and International Office of Cacao and Chocolate, Brussels, Belgium

Pound, F.J. (1943) First report on the selection of cacao trees for resistance to Witches' Broom disease. Unpublished report, Ministry of Agriculture, Trinidad.

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

Characterisation

Selecting superior cacao accessions from the ICG,T based on phenotypic traits including pod wall hardness

F. L. Bekele, G.G. Bidaisee, J. Bhola & N. Barnwell

Introduction

Measures of yield potential and agronomic traits observed at ICG,T that are of special interest to breeders are pod index (PI), which is the number of pods required to produce 1 kg of dried cocoa, cotyledon weight, bean number, cotyledon size (length and width), pod wall hardness and cotyledon colour (pale or violet cotyledons are associated with Criollo and Trinitario germplasm, respectively). Wellensiek (1931) reported that "single selection on white cotyledon colour" was sufficient to maintain the superior quality and high market value of Java cocoa (originally Criollo). Since an absence or low concentration of anthocyanin pigment in cotyledons is associated with fine or flavour cocoa, accessions with no or very little pigment are being identified.

Selection of accessions with low pod index and other desirable traits

Low PI values are associated with high yield potential. For breeding purposes, a PI of less than 15 is very desirable (Pound, 1932). Selection of promising genotypes in terms of PI at the Cocoa Research Unit has been based on values that are equal to or less than 20 (Bekele, 1999; Bekele *et al.*, 1996; 1999; 2000**)**.

Pod wall (sclerotic layer) hardness has been identified as a phenotypic factor associated with resistance to the cocoa pod borer, *Conopomorpha cramerella* (Snellen), (Mumford 1988; Roepke, 1912; Wessel, 1983; Wood, 1980) due to increased mortality of the larvae with harder pod walls (Azhar, 1988; Azhar & Long, 1996). Based on the likely association between resistance to cocoa pod borer (CPB) and pod wall hardness, routine measurements with a penetrometer have been undertaken as part of the morphological characterisation project at CRU.

Methods

Fruit characterisation

One thousand three hundred and sixty-one accessions have been characterised in terms of fruit descriptors and were observed according to the standard protocol described by Bekele *et al.* (1994; 2006). These descriptors were selected based on the findings of Bekele and Bekele (1995; 1996) and Bekele and Butler (2000), and are listed in Table 1. They were found to be the most discriminative and taxonomically useful and precluded redundancy. In addition, they were also selected for ease of observation, reliability of scoring, and, in the case of seed characters, agronomic value.

Pod wall hardness

Between January 1996 and June 2006, the hardness of pod walls of mature, ripe, healthy pods was determined using an Effigi penetrometer, model FT327 (David Bishop Instruments,

Heathfield, United Kingdom), which was firmly mounted on a BOSCH S2 drill stand. A pointed plunger or probe, with a tip 8mm in diameter, was used to facilitate a slow penetration into the pod wall, and hardness was measured as the pressure (in MPa) required to penetrate the pods (Bekele *et al.*, 1997). Ten mature pods were assessed immediately after harvesting at two points per pod (on the ridges at the equator or median of the pod). If the first two readings were not consistent, more were taken at different points along the median of the pod, and the mean was derived. To date, the pod wall hardness of 901 accessions has been assessed using this penetrometer, and these data are summarised below.

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 [n=10], 5= other.
Fruit, basal constriction	0=absent, 1=slight, 2=intermediate, 3=strong, 4=wide shoulder [n=10]
Fruit, apex form	1=attenuate, 2=acute, 3=obtuse, 4=rounded, 5=mammillate, 6=indented [n=10]
Fruit, surface texture (rugosity or degree of wartiness)	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, anthocyanin intensity in mature ridges	0=absent, 3=slight, 5=intermediate, 7=intense [n=10]
Fruit, primary ridge separation	1=slight, 2=intermediate, 3=wide [n=10]
Fruit, length (cm) [n=10]	
Fruit, width (cm) [n=10]	
Seed, number [n=10]	
Seed, shape	1=oblong 2=elliptic 3=ovate
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]	

As of January 2, 2007, a different drill stand, penetrometer and probe are being used to assess pod wall hardness at CRU. This equipment is the same as that used in South-East Asia by Dr. Smilja Lambert (Masterfoods Australia/NZ (Mars, Inc.)) and colleagues, and was kindly donated to CRU by Dr. Lambert. The drill stand is a Model 5023, (Wolcraft Gmbh (www.wolfcraft.de)). The penetrometer is also an FT 327 (0.5-13 kgs force) model, but was manufactured by ACE Industrial Supplies Ltd, Tonbridge, United Kingdom. However, the probe is not pointed and measures 2.5mm in diameter. From initial observations, it appears to penetrate cocoa pod walls

with the application of significantly less force than that used with the other penetrometer. Data collated using the first penetrometer are presented in this report since only 24 accessions have been measured with the new penetrometer.

Statistical analysis

Descriptive statistics for the descriptors studied were generated using MINITAB 15 (Minitab Inc., 1997). Accessions that combined favourable PI with hard pod walls were identified. Their phenotypic relationships were then examined.

Phenotypic relationships among accessions combining favourable pod index and pod wall hardness

In order to display the phenotypic relationships among accessions combining these desirable traits, 3-dimensional PCA was performed on a correlation matrix of the data recorded for twenty-three descriptors (Table 1) using NTSYS-pc (NTSYS, 2000). The disposition of ridges on the pods was not included since it was the same for all of the accessions in this subset of germplasm (*i.e*. paired).

Data for the 23 descriptors used were first standardised to eliminate the effects of different scales of measurement.

Results

The mean bean number for the 1,361 accessions characterised was 38.9 ? 0.16 (Table 2) with a minimum of 17 for B 5/11 [POU] and CL 27/96 and a maximum of 59 for IMC 39. The mean cotyledon weight was 0.98g with a minimum of 0.44g for B 9/10-28 [POU] and the maximum of 1.84g for UF 11. The statistics for cotyledon length and width are presented in Table 2. The mean pod index (PI) for the accessions characterised is 28.1 ? 0.19. The minimum value is 13.94 (UF 11) and the maximum is 92.7 (B 9/10-35 [POU]).

There are now **122** accessions with PI ? 20 from 28 accession groups (Table 3) out of 77 characterised to date. Two of these (denoted by asterisks in Figure 1) have been included in sub-sample A of the CFC Project Collection (Sounigo *et al.*, 2005).

Some accessions, including UF 11, have low pod indices, but are susceptible to Witches' Broom disease (WB). ICS 60 also has a PI ? 21, but is susceptible to Black Pod disease (BP).

As has been shown before (Bekele *et al.*, 1999), there is a wide range in PI values within some accession groups as well as highly significant differences between groups. AGU, AM, B, CC, CL, CLM, CRU, EET, FSC, GS, ICS, IMC, JA, LAFI, LCT EEN, LP, MATINA, MOQ, NA, PA, POUND, SC, SD, SILECIA, SJ, SLA, TRD and UF all have one or more accessions with desirably low PI (? 20). The accession groups JA, CRU, ICS and IMC contain 54 % of the 122 accessions observed with PI ? 20:

JA	17.2%
CRU	16.4 %
ICS	13.9 %
IMC	6.6 %

Thirty-four accessions had bean numbers ? 45, 98 accessions had an optimum bean size of at least 1.2 g and 16 accessions had cotyledon weight ? 1.5 g. The latter are UF 11, UF 12, B 17/20 [POU], ICS 60, ICS 43, CRU 111, EET 58 [ECU], AGU 3339/8 [CHA], CRU 153, TRD 35, AM 1/85 [POU], GS 29, ICS 88 (mislabelled), CRU 5B/25, CLM 59 and CRU 105.

Ninety-six accessions (10.7 % of those studied) had maximum pod wall hardness (? 2.5 MPa) that is associated with resistance to penetration by the CPB larvae. Within these, 72 accessions also had PI values ? 25.

Fifty of the 1,361 accessions characterised had pale-coloured beans (unpigmented, gray or violet). Twenty-nine of these were Refractarios, 10 were Trinitarios, and the others were either hybrids or of unknown origin (such as CRU accessions). Two were suspect "Forasteros" (NA 462 and IMC 63), and two others were "SPA 5" (mislabelled with red pods) and the mutant, CATONGO.

Table 2. Descriptive Statistics for pod and bean traits of economic interest.

Descriptor	Mean	Standard error of the mean	Minimum value	Maximum value
Pod Index	28.1	0.19	13.9	92.7
Bean Number	38.9	0.16	17	59
Cotyledon Weight (g)	0.98	0.006	0.44	1.84
Cotyledon Length (cm)	2.16	0.005	1.37	2.72
Cotyledon Width (cm)	1.21	0.003	0.63	1.61

Table 3. Statistics for accession groups with favourable pod index (PI).

Accession groups	Mean PI	Mean bean number	Representation among 122 accessions with PI ? 20 (%)
JA	18.4	43	17.2
CRU	17.0	43	16.4
ICS	18.1	41	13.9
IMC	18.5	53	6.6
AM	18.0	41	4.9
LP	18.6	39	4.1
B	18.8	41	3.3
NA	17.1	42	3.3
CL	18.9	39	2.5
CLM	18.1	41	2.5
EET	19.1	39	2.6
MOQ	18.0	44	2.5
TRD	18.2	42	2.5
UF	16.0	41	2.5
GS	17.8	38	1.6
PA	18.0	43	1.6
POUND	18.4	54	1.6
SJ	19.2	41	1.6
SLA	18.0	43	1.6

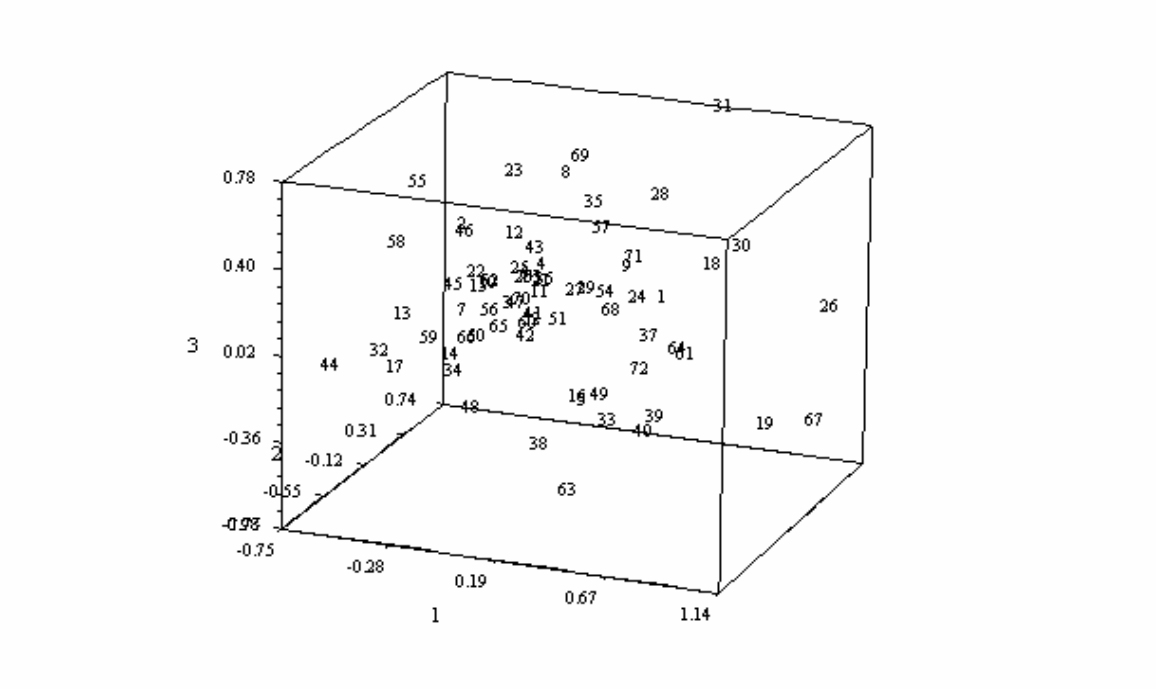

Figure 1. Principal component plot, based on 23 phenotypic traits, of the 72 accessions combining favourable pod index (? 25) with hard pod walls (? 2.5 MPa).

1. AM 2/43 [POU]	19. CRU 5B/25	37. LAFI 7	55. NA 104
2. AM 2/31 [POU]	20. CRU 5B/2	38. LP 4/13 [POU]	56. PA 310 [PER]
3. AM 2/21 [POU]	21. DOM 4	39. LP 1/58 [POU]	57. SC 6 [COL]
4. AM 1/70 [POU]	22. DOM 30	40. LP 1/44 [POU]	58. SD 1/6
5. AM 1/39 [POU]	23. GC T 998/2 (pale beans)	41. LX 24	59. SIAL 339
6. AM 1/19 [POU]	24. GS 78	42. LX 17	60. SJ 2/6 [POU]
7. AMAZ 15/15 [CHA]	25. GS 61	43. M 252 [ICT]	61. SJ 2/16 [POU]
8. CC 17	26. GS 4	44. M 116	62. SJ 1/40 [POU]**
9. CL 78/3	27. GS 12	45. MOQ 6/88	63. SJ 1/39 [POU]
10. CL 27/53	28. ICS 80	46. MOQ 6/41	64. SJ 1/37 [POU]
11. CL 10/27**	29. ICS 75	47. MOQ 6/33	65. SJ 1/1 [POU]
12. COCA 3310/3 [CHA]	30. ICS 15	48. MOQ 6/28	66. SLC 1
13. CRU 86	31. ICS 1	49. MOQ 6/10	67. SM 9 [POU]
14. CRU 21	32. IMC 83	50. MOQ 5/1	68. SPEC 194/48
15. CRU 142	33. JA 9/13 [POU]	51. MOQ 3/12	69. TRD 30
16. CRU 133	34. JA 5/37 [POU]	52. MOQ 2/35	70. UF 4
17. CRU 13	35. JA 4/7 [POU]	53. NA 71	71. UF 38
18. CRU 122	36. JA 3/20 [POU]	54. NA 444	72. UF 191

** Included in the CFC/ICCO/IPGRI Project Collection (Sounigo *et al*., 2005). The accession underlined is potentially mislabelled.

The first three principal components (Figure 1) account for 39% of the total phenotypic variation expressed in this sample of germplasm. The first principal component (PC) 1 accounted for 17.3%, the second for 12.7% and the third 9%. Cotyledon weight, length and width accounted for the most variation expressed by PC 1 while pod index, ovule number and bean number accounted for the most variation expressed by PC 2.

Future direction

All future assessments of pod wall hardness will be made with the new penetrometer. However, an investigation is being undertaken to determine how the readings obtained using the original equipment compare to those obtained with the new. At least 30 accessions (10 pods per accession) will be assessed using each probe in turn so that the results may be compared. Two readings at the median of each pod will be obtained using each probe.

Further work on how pod wall hardness varies with pod age is recommended. Pods that are older than seven weeks before ripening appear to be preferred for oviposition by *Conopomorpha cramerella* (Snellen) (Azhar and Long, 1996). However, Day (1985) found that mortality of the larvae increased at pod maturity, implying that younger pods may be more susceptible.

Accessions with pod wall hardness ratings of 2.5 MPa or more (based on the readings with CRU's original equipment) are recommended for assessment for resistance to CPB in South-East Asia. Direct assessment of resistance to CPB should be made on accessions common to the 901 accessions already assessed in Trinidad and those held in Asia. Providing accessions with a good range of hardness values are available in Asia, this would allow firm relationships between wall hardness and resistance to be established.

Conclusion

Accessions combining low pod index, large bean size and bean number with a hard pod wall and high levels of resistance to BP and WB are potential candidates for germplasm enhancement and for inclusion in international clone trials to assess their adaptability in different environments as stated by Iwaro *et al.* (2003).

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References

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

Azhar, I. and Long, G.E. (1996) Effect of cocoa pod age on egg distribution and egg parasitism of the cocoa pod borer in Malaysia. *Entomologia Experimentalis et Applicata* **81**:81-89.

Bekele, F.L. (1999) The role of characterisation and preliminary evaluation data in cocoa improvement. Pages 18-26 in: *Proceedings of Agriculture in the Caribbean: Issues and Challenges. Volume II.* 19-21, August 1998, St. Augustine, Trinidad: The University of the West Indies, Faculty of Agriculture and Natural Sciences-CEPAT, Trinidad.

Bekele, F.L. and Bekele, I. (1995) Further studies on a concise list of morphological descriptors for cacao. Pages 155-165 in: *Proceedings of the International Workshop on Cocoa Breeding Strategies*. 18-19, October 1994, Kuala Lumpur, Malaysia: INGENIC, U.K.

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

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

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

Bekele, F.L., Iwaro, D. and Bidaisee, G. (1996) Evaluation of some economic characters of germplasm from the International Cocoa Genebank, Trinidad. Pages 19-33 in: *Annual Report for 1995*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Bekele, F., Bidaisee, G. and Rampat, R. (1997) A preliminary study of pod husk hardness of accessions in the International Cocoa Genebank, Trinidad. Pages 67-76 in: *Annual Report for 1996*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Bekele, F., Bidaisee, G. and Badall, V. (1999) Morphological characterisation- some characters of economic interest. Pages 21-24 in: *Annual Report for 1998*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

Bekele, F., Bidaisee, G., Polar, P. and Mollineau, W. (2000) In search of interesting phenotypes for breeding in the new millennium. Pages 14-18 in: *Annual Report for 1999*. St. Augustine, Trinidad: Cocoa Research Unit, The University of the West Indies.

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

Day, R.K. (1985) Control of the Cocoa Pod Borer, PhD. Thesis, University of London.

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

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

Mumford, J.D. (1988) Control of the Cocoa Pod Borer (*Conopomorpha cramerella*). *Cocoa Growers Bulletin* **40:** 19-29.

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

Pound, F.J. (1932) The genetic constitution of the cacao crop. Pages 25-28 in: First *Annual Report on Cacao Research for 1931*. Trinidad and Tobago: Government Printery.

Roepke, W. (1912) Voorloopige mededeeling omtrent het optreden van mot in verschillende typen van den Djati-Roenggo cacao. Meded, Proefstat, Midden Java, No. **3**, 1-3, cited by Wessel, P.C. (1983) in: *The Cocoa Moth Pod Borer (*Acrocercops cramerella *Sn.)*, Review and Translation of Research in Indonesia 1900-1918. Wageningen: Druk Co. Pudoc. 36pp.

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

Wellensiek, S.J. (1931) The genetics of cotyledon-colour of cocoa as a basis for quality selection. *Archief voor de Koffiecultuur* **5**: 217-232. (Translated into English by Toxopeus, H. and Wessel, P.C. (Eds.) (1983) in: Archives of Cocoa Research Volume II, Wageningen, The Netherlands: ACRI/IOCC.)

Wessel, P.C. (1983) The Cocoa Moth Pod Borer (*Acrocercops cramerella* Sn.). Review and Translation of Research in Indonesia 1900-1918. Wageningen: Druk Co. Pudoc. 36pp.

Wood, G.A.R. (1980) The Cocoa Pod Borer - A Menace to South East Asia. *Cocoa Growers Bulletin* **30:** 25-27.

Evaluation

Focusing on early-screening methods of Witches' Broom resistance

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Introduction

A component of the CFC/ICCO/BI project *Cocoa productivity and quality improvement: a participatory approach* was to investigate and develop the methods of screening for WB resistance. Three experiments were performed either in the greenhouse or in the field. The experiments performed in the greenhouse were also scheduled to be compared with similar experiments in two others countries (Brazil and Ecuador) participating in the project.

Materials and methods

Experiment 1

Experiment 1 was a comparative study between two inoculation methods, spraying and agar droplet (Surujdeo-Maharaj *et al.*, 2003), and three types of planting material (open-pollinated seedlings, hand-pollinated seedlings and clonal material). Clones studied were SCA 6 and NA 289 (resistant to WB), MAN 15/2 [BRA] and UF 668 (susceptible to WB) and LX 25 (moderately resistant to WB). To obtain the hand-pollinated seedlings, the clone BE 10 was used as the male donor which is homozygous and susceptible to WB. The experiment was performed in the greenhouse. Seven-month-old seedlings were inoculated using an inoculum concentration of $3x10^5$ basidiospores/mL. About 6 grafted plants, 30 open-pollinated seedlings and 30 hand-pollinated seedlings were inoculated, the exact number depending on the availability of plants. To assess the WB resistance level, the variables recorded were the time to the appearance of first symptoms (TFS) and the maximum broom diameter (MBD).

Experiment 2

Experiment 2 was a ring test between Trinidad, Brazil and Ecuador to assess the degree of resistance to WB of genotypes in the International Clone Trial (ICT) and to compare the level of resistance obtained in 3 different sites. Open-pollinated progeny populations were generated from ten ICT clones EET 59 [ECU], LCTEEN 46, MXC 67, PA 150 [PER], PA 120 [PER], NA 33, AMAZ 15/15 [CHA], PLAYA ALTA 2, GU 255/V and IMC 47. These clones displayed various level of resistance in the field under natural conditions of infection. The experiment was performed in the greenhouse. Seven-month-old seedlings were inoculated by the agar droplet method using an inoculum concentration of $3x10^5$ basidiospores/mL. One replication contained between 19 and 30 open-pollinated seedlings per progeny population. The seedlings were inoculated using the agar-droplet method, and two replications were performed. To assess the WB resistance level, the variables recorded were TFS and MBD.

Experiment 3

Experiment 3 focused on developing a method of screening for resistance to WB on attached pods in field. Three clones were selected in UWI Campus fields according to their different level

of WB resistance: ICS 95 (susceptible to WB), IMC 67 (resistant to WB) and LCT EEN 162/S-1010 (resistance level unknown). Open-pollinated pods were generated from each clone. Young growing pods were inoculated when they were estimated to be 8 weeks old. The length of each cherelle was measured at inoculation time. Two inoculation methods were performed: i) the spray method (1 mL of suspension of inoculum manually sprayed on the whole pod) and ii) the agar-droplet method (30 µl of the inoculum suspension in 1% agar placed on the convex surface of the pod). The inoculum concentration was 10^6 basidiospores/mL. A control treatment was also performed with sterile water. For each inoculation method including the control treatment (3 treatments performed), about 15 cherelles were inoculated. Filter paper plus tape were used to cover the site of inoculation on cherelles inoculated with the agar-droplet method. After inoculation, cherelles were enclosed in a plastic bag containing wet tissue paper. The bags, filter papers and tapes were removed after different incubation times of 72, 36 or 18 hours. The status of the pods was recorded weekly as "Wilt", "Black Pod disease", "WB disease", "other damage (damage due to rodent or to human influence)" and "No Symptom". After 3 months, internal symptoms were recorded as the proportion of necrosis in pods showing external WB symptoms.

Data analysis

The analysis of variance was performed using the general linear model (GLM) procedure (SAS Software).

Results and Discussion

Experiment 1

Time to first symptoms (TFS, days)

Analysis of variance showed that there was a significant interaction between the method of inoculation and the planting material ($F = 12.82$; $P < 0.0001$). There was also a significant interaction between the method of inoculation and the clone tested ($F = 3.79$; $P = 0.005$), therefore data analysis was performed for each inoculation method:

(i) *Agar-droplet method.* No interaction was found between planting material and clone ($F = 1.30$; $P = 0.27$). The analysis of variance showed that there were significant differences among the clones tested ($F = 4.52$; $P = 0.0017$) and among the planting material ($F = 48.69$; $P < 0.0001$). According to the TFS measure, the clones tested were separated into 2 statistical groups with the period for NA 289 being significantly shorter than all the other clones (Table 1). The planting materials were also separated in 2 statistical groups, with significantly longer TFS in the clonal material and than either of the seedling teatments (Table 2).

(ii) *Spray method*. No interaction was found between planting material and clone ($F = 0.16$; $P = 0.9772$). The analysis of variance showed that there were no significant differences among the clones tested ($F = 1.14$; $P = 0.3393$; Table 1), but significant differences were found among the planting materials ($F = 15.55$; $P < 0.0001$). According to the TFS measure of resistance, the planting materials were separated into 3 statistical groups showing the longest TFS in the clonal material and the shortest one in the hand-pollinated seedlings (Table 2).

Maximum broom diameter (MBD, mm)
Analysis of variance showed that there was no interaction between the method of inoculation and the planting material (F = 0.20; *P* = 0.82) or between the method of inoculation and the clone tested (F = 2.32; *P* = 0.057). There was also no interaction between the clone tested and the planting material (F = 1.37; *P* = 0.23). No significant differences were found among the two methods of inoculation (F = 3.14; *P* = 0.066) while significant differences were found among the planting materials (F = 33.95; *P* < 0.0001) and among the clones tested (F = 5.57; *P* = 0.0002). According to the MBD measure of resistance, the clones tested were separated in 2 statistical groups the value for UF 668 (known to be susceptible to WB) being statistically greater than the other clones (Table 1). The planting materials were separated into 3 statistical groups with the clonal material having the smallest MBD and the hand-pollinated seedlings having the largest one (Table 2).

Table 1. Measures of symptom severity in five cocoa genotypes following inoculation by the agar droplet and spray methods.

	TFS (days)				MBD (mm)	
	Agar droplet method		Spray method			
Genotype	**Mean***	**SE**	**Mean***	**SE**	**Mean***	**SE**
UF 668	13.6 a	0.39	14.7 a	0.38	13.8 a	0.43
NA 289	12.1 b	0.25	13.6 a	0.35	12.4 b	0.35
LX 25	13.4 a	0.72	15.3 a	0.74	12.1 b	0.61
MAN 15/2 [BRA]	14.6 a	0.47	15.5 a	0.53	11.7 b	0.47
SCA 6	14.6 a	0.52	14.8 a	0.41	11.0 b	0.42

Table 2. Measures of symptom severity in three types of plant material representing cocoa genotypes.

	TFS (days)				MBD (mm)	
	Agar droplet method		Spray method			
Plant material	**Mean***	**SE**	**Mean***	**SE**	**Mean***	**SE**
Grafted	17.2 a	0.22	16.7 a	0.31	8.9 c	0.38
Open-pollinated	12.9 b	0.22	14.7 b	0.26	12.3 b	0.23
Hand-pollinated	12.5 b	0.37	12.8 c	0.32	14.5 a	0.43

TFS = incubation period; MBD = maximum broom diameter; SE = standard error.
*Mean scores followed by the same letter are not significantly different according to the Student Newman-Keul's test at 5% probability.

Experiment 2

Time to first symptoms (TFS)
The analysis of variance showed that there were no significant differences among the clones tested (F = 1.86; *P* = 0.057). Mean values varied between 14.38 days for PA 150 [PER] and 16.84 days for GU 255/V (Table 3)

Maximum broom diameter (MBD)
The analysis of variance showed that there were highly significant differences among the clones

tested (F = 4.94; *P* < 0.0001). According to the MBD measure of resistance, the clones tested were separated in 4 statistical groups showing PA 120 [PER], IMC 47 and GU 255/V clones with the smallest MBD and EET 59 [ECU] and LCT EEN 46 clones with the largest ones (Table 3). These results confirmed both a recent study on the evaluation of the WB resistance of ICT clones on grafted plants (collaboration between MALMR and CRU) and results obtained under natural conditions of infection in the UCRS, both conducted within the frame of the first CFC/ICCO/BI project.

Table 3. Measures of symptom severity in 10 open-pollinated progeny populations from genotypes in the International Clone Trial.

Genotype	TFS (days)		MBD (mm)	
	Mean*	SE	Mean*	SE
EET 59 [ECU]	15.2 a	0.41	12.1 A	0.37
LCT EEN 46	16.7 a	1.12	11.4 Ab	0.42
MXC 67	14.5 a	0.39	11.3 abc	0.46
PA 150 [PER]	14.4 a	0.35	10.9 abc	0.37
NA 33	15.0 a	0.50	10.9 abc	0.47
AMAZ 15/15 [CHA]	15.0 a	0.43	10.7 abc	0.37
PLAYA ALTA 2	16.3 a	0.91	9.9 bcd	0.50
GU 255/V	16.8 a	0.82	9.8 cd	0.39
IMC 47	15.5 a	0.51	9.6 cd	0.52
PA 120 [PER]	14.4 a	0.56	9.0 d	0.38

TFS = incubation period; MBD = maximum broom diameter; SE = standard error.
*Mean scores followed by the same letter are not significantly different according to the Student Newman-Keul's test at 5% probability.

Experiment 3

Eighty pods were inoculated and compared with the control treatment (sterile water as the inoculum suspension). No WB symptoms were recorded on pods inoculated with sterile water (control pods). A high proportion of cherelles died because of wilt and other damage and few due to the black pod disease.

Only one pod showed WB disease-like symptoms on the susceptible clone ICS 95 inoculated with the agar-droplet method: one month after inoculation, multiple necrotic spots covered a large area of the pod; three months after inoculation, distortion of the pod was recorded; the pod then stopped growing (the ratio of initial to final pod length over 3 months was 1.25 for the inoculated pods and 2.58 for control pods) and 50% of the internal fruit was necrosed.

Conclusion and perspective

The results of the experiments 1 and 2 revealed that for both TFS and MBD, the effect of plant material was greater than the effect of clones. This is true for both methods of inoculation. This provides evidence that results of inoculation can only be compared if the planting material is the same throughout, it may explain some anomalies in results from earlier work. For TFS, the agar droplet inoculation method was able to discriminate between clones, but no clone effect was found with spray inoculation, suggesting that agar droplet may be a better method for screening. The results also showed that measurements of MBD gave better discrimination between

susceptible and resistant clones than TFS. This finding is in contrast to the results of Surujdeo-Maharaj *et al.* (2004) which suggest that TFS is the most discriminatory measure of resistance. The most apparent difference between the experiments described here and those of Surujdeo-Maharaj *et al.* was the age of the plants at the time of inoculation. Surujdeo-Maharaj *et al.* (2004) used seedlings that were at least 12 months old,

The most promising ICT clones showing a high level of WB resistance were PA 120 [PER], IMC 47 and GU 255/V. It will be interesting to compare the results of experiments 1 and 2 with those from similar experiments carried out in Ecuador and Brazil.

The results of the experiment 3 performed in Campus field revealed that it was possible to induce WB like symptoms on attached cherelles, however Koch's postulate should be verified if there is any doubt whether the symptoms are characteristic of the disease. Previous work reported a weak correlation (Thévenin *et al.*, 2005) between vegetative brooms and WB symptoms on pods ($r = 0.39$). We need therefore to focus on the development of a screening test for pods. The next step will be to get the right conditions to repeatedly obtain symptoms. It may therefore necessary to work in UCRS where susceptible clones can be more easily found.

References

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

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D. R. (2004) Assessment of resistance to Witches' Broom disease in clonal and segregating populations of *Theobroma cacao*. *Plant Disease* **88:** 797-803.

Thevenin J.-M., Umaharan, R., Surujdeo-Maharaj, S., Latchman B., Cilas, C. and Butler, D.R. (2005) Relation between Black pod and witches'-broom diseases in *Theobroma cacao*. *Phytopathology* **95 (11):** 1301-1307.

Assessing drying rates of cacao beans using small samples

S. Bharath and C. Bowen-O'Connor

Introduction

Drying forms a very important part of post-harvest processing in the cocoa production chain. The water content of the bean must be reduced from about 60% at the end of fermentation to less than 8% to obtain beans in good condition for storage and transport (Mossu, 1992). Drying also facilitates a reduction in the bitterness and astringency of the beans, and it encourages the development of the chocolate brown colour characteristic of well-fermented beans. Proper drying also ensures that off-flavours do not develop within the beans (Mossu, 1992).
Extremes of drying rates must be avoided because of the negative impacts they tend to have on the beans. If drying is done too slowly, moulds may develop. This can cause serious problems for industry because of the off-flavours created if the moulds penetrate the testa. If the drying is too rapid however, the oxidation of acetic acid can be prevented and this leads to excess acid trapped within the beans. This acid content will ultimately adversely affect the flavour of the nib.

Drying rate depends on three factors:

- Heat transfer into the bean
- Movement of water vapour into the surrounding air from the bean and,
- The surface area of beans exposed to the air.

To investigate flavour of small samples of beans from a single genotype or treatment, micro-fermentations are carried out in an effort to expose the samples to the same conditions as the larger fermenting mass. In micro-fermentation the bean samples are kept physically separate from the fermentation mass by the use of netted nylon bags. In this way, both sets of beans experience similar conditions. Similarly, for the drying regime, similar conditions should ideally be experienced by both the small samples and the larger fermented mass. To date, the micro-fermented samples have been dried in small wooden trays on the floor of the cocoa house adjacent to the beans from the fermentation mass that are spread out on the drying floor.
In a 2005 study, drying rate measurements done at San Juan and Manickchand Estates showed that tray dried micro-fermented samples dried twice as fast as those of the floor-dried fermented mass. This creates the possibility for case hardening and acid trapped in the beans of the small samples. This is cause for concern when there are few pods available from the accession used, the quantity of beans is quite small and the drying mass may be only one bean layer thick. The evidence gathered so far has shown that the rate of drying in trays – especially of very small samples – is not representative of the rate of drying of the fermented mass on the floor of the cocoa house.

Two experiments were therefore designed to explore the extent of the problem of drying small samples, and to test possible solutions to it.

Methodology

Experiment 1

Aim: To compare the drying rates on the cocoa house floor of beans in nylon net sacks with

those of the bulk fermentation mass.

1. Nine 3kg samples of well-fermented wet cocoa (mixed Trinitario beans) were placed into nylon net bags (30cm × 20cm), and labelled according to their location on the drying floor.
2. Three bags were placed at each of 3 locations on the drying floor (one at each end and the other in the middle) within the fermentation mass from which the samples were taken. Beans in the bags were turned in a similar way to the fermentation mass (agitated both laterally and vertically to move the lower beans to the top of the drying mass and upper beans to the bottom layer). The beans were spread out along the length of the bag in such a way as to imitate the thickness of the surrounding layers. In this case, it was about 7 bean layers thick.
3. Sampling from the bags was done at the same time every day (14.00 h).
4. For each day of drying starting from Day 0, 10 beans were removed from each sample bag by emptying the contents into a large plastic bag, shaking it thoroughly for 1 minute, and removing 10 beans in random fashion.
5. For each day of drying starting from Day 0, 10 beans were randomly sampled from the floor at each of the drying positions of the floor (both ends and the middle).
6. This sampling procedure was continued until the 6^{th} day of sun drying.
7. All the samples were removed and placed in labelled (sample id and day of drying) snap-seal plastic bags, immediately sealed and put into a mini-cooler to avoid direct sunlight.
8. The samples were then transported to the laboratory at CRU with minimal delay.
9. At CRU, foil boats were prepared and weighed (in grams to 3 decimal places) using an analytical balance (Sartorius).
10. Individual bean samples were then added to the foil boat and the combined weight of container and sample recorded.
11. The weighed samples were then placed in a mechanical convection oven (Shel Lab 1350 FX) set at 135ºC.
12. The foil container and sample were weighed every day at the same time until a constant weight was reached. The final weight was recorded.
13. On the final day of sun drying, the moisture content of a 150g sample was measured with a moisture meter (Burrows Digital Moisture Computer 700) using the recommended manufacturer's procedure.

The moisture content of each oven-dried sub-sample was calculated using equation 1:

$$MC = WWS/IWW \times 100 \qquad (1)$$

where

Initial weight of wet beans (IWW) = Initial weight of wet beans and container (A) – Weight of empty container (B)
Final weight of dried beans (FWD) = Final weight of dried beans and container (C) - Weight of empty container (B)
Weight of water in sample (WWS) = IWW - FWD

Moisture Content (MC) is expressed as a percentage.

Experiment 2

Aim: To compare response curves for drying rates with different amounts of cocoa in the drying tray.

Two experiments where carried out, one (2a) with large samples (2 and 3 kg) in individual square drying trays, and the other (2b) with small samples (ranging from 50 to 800 kg) in multi-celled drying trays.

Procedure for experiment 2a

1. Triplicate sets of 2 kg and 3 kg samples were weighed, and placed in 60 × 60 cm trays the beans were spread out one layer thick.
2. Replicate sets of 2 kg and 3 kg were placed in the halved trays, and the bean samples were left in heaps.
3. The trays were placed in a convenient location on the floor of the drying house.
4. On each day of drying start from Day 0, 10 beans were randomly removed from all four treatments.

Procedure for experiment 2b

1. Samples of well-fermented wet cocoa were weighed to give ten sets each of 50g, 100g, 200g, 400g and 800g. Each set was laid out in a cell 14 cm × 14 cm and 5 cm deep.
2. The bean samples for each mass were spread such that for the smaller masses, the beans were laid out one layer thick and for the larger masses, the beans filled the entire volume of the cells evenly.
3. The trays were placed in a convenient location on the floor of the drying house.
4. On each day of drying starting from Day 0, a 10-bean sub-sample was taken from one of the ten sets for each mass, and labelled according to the mass and the day of drying.

Common procedure for experiments 2a and 2b

1. For each day of drying starting from Day 0, 10 beans were randomly sampled from the floor at each of three locations (one at each end and one in the middle).
2. The sampling procedure was continued until the 6^{th} day of sun drying.
3. All the treatment and floor samples were removed and placed in labelled (sample id and day of drying) snap-seal plastic bags, immediately sealed and put into a mini-cooler to avoid direct sunlight.
4. The samples were transported to the laboratory at CRU with minimal delay.
5. In the laboratory, foil boats were prepared and weighed (in grams to 3 decimal places) using an analytical balance (Sartorius).
6. Individual bean samples were then added to the respectively labelled foil boat and the combined weight of container and sample recorded.
7. The weighed samples were then placed in a mechanical convection oven (Shel Lab 1350 FX) set at 135ºC.
8. The foil container and sample were weighed every day at the same time until a constant

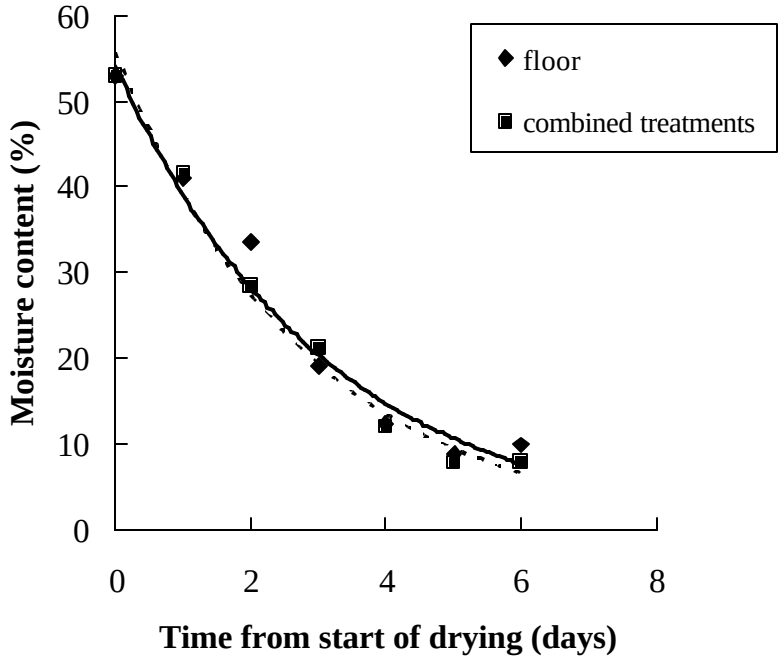
weight was reached. The final weight was recorded.

9. On the final day of sun drying, the moisture content of a 150g sample was measured with a moisture meter (Burrows Digital Moisture Computer 700) using the recommended procedure.
10. The moisture content of each oven-dried sub-sample was calculated using equation 1 (above).

Results

Experiment 1

There was no day by location interaction in the moisture content of samples ($P = 0.513$), and there were no significant difference between bulk floor samples and beans dried in net bags ($P = 0.243$). Since there were no statistically significant differences among the samples from different locations on the floor, the mean moisture content of these samples was calculated for each day. These are plotted together with the moisture content of the fermentation mass in Figure 1.



Figu **of**

The moisture content of both the bulk beans and the bagged samples decreased exponentially, reaching a value of about 8% on day 5. For all locations there was no significant change in moisture content from day 5 to day 6 ($P = 0.686$).

Experiment 2a.

Although all the treatments and the bulk floor samples had reached a similar moisture of 7-8% content by day 6, clear differences were observed in the rate of drying during the first two days (Figure 2). Between the start of drying (day 0) and day 2, the decrease in moisture content was approximately linear, and linear regression lines, with a fixed intercept of the initial value, were fitted to the points over this restricted period. Lines for the bulk floor sample, 2 kg spread and 2 kg heaped are shown in Figure 2 (the other lines are omitted for the sake of clarity), and the slopes of all the lines are given in Table 1 with the coefficients of determination (r^2).

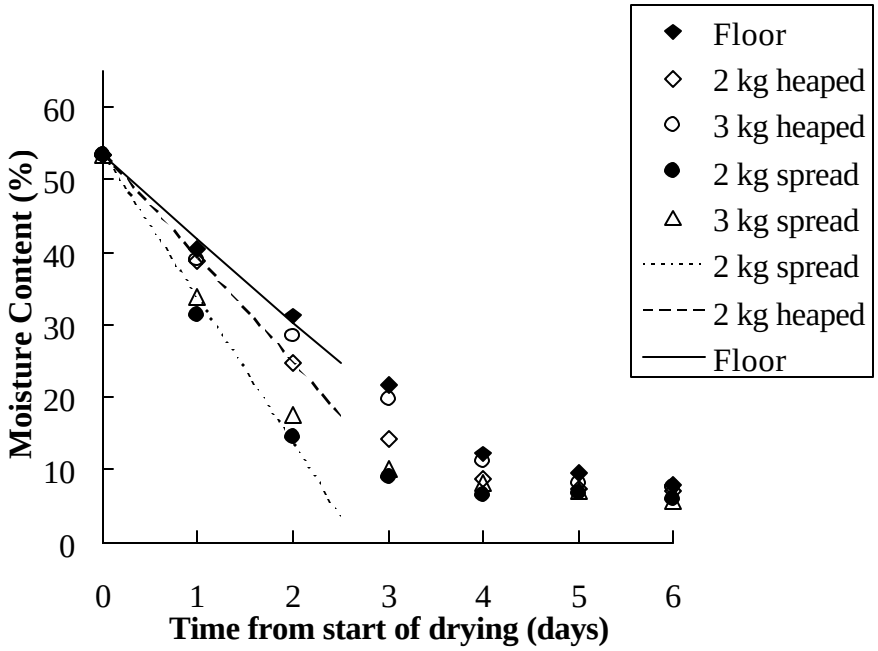


Figure 2. The change in moisture content with time for the bulk fermentation mass and weighed samples in 60 × 60 cm drying trays on the floor on a cocoa house. Lines were fitted by linear regression of days 0-2 with a fixed intercept of the starting value.

Significant differences were observed between the drying rates of the heaped and spread samples compared to the floor mass. As seen in Table 1, the 2kg and 3kg spread samples showed the steepest slopes over the first 2 days of drying. Compared to the floor sample, these dried at rates that were 1.7 and 1.6 times faster, respectively. This led to differences of 16.4% and 13.5% between the moisture content of the floor sample and those of the 2kg and 3kg spread samples on day 2, respectively. Drying rates of the 2kg and 3kg heaped samples were much closer to the floor sample (1.3 and 1.1 times faster, respectively). This led to differences of 6.3% and 2.6% between the moisture content of the floor sample and those of the 2kg and 3kg heaped samples on day 2, respectively. Results for the 3kg heaped sample were very similar to the bulk floor sample.

Table 1. The rate of change of moisture content for cocoa bean samples from day 0-2, given by the slope of regression lines. The treatments compare the bulk floor sample with heaped and spread samples in drying trays.

Treatment	Slope of line (% per day)	Coefficient of determination	Moisture content on day 2 (%)
Floor	-11.59	0.988	31.0
2kg spread	-20.03	0.992	14.6
3kg spread	-18.33	0.996	17.5
2kg heaped	-14.49	0.9996	24.7
3kg heaped	-12.98	0.990	28.4

Experiment 2b.

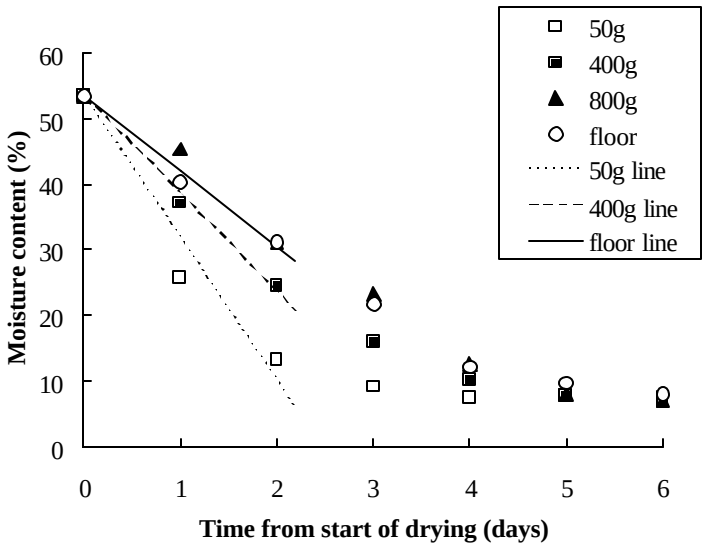


Figure 3. The change in moisture content with time for the bulk fermentation mass and weighed samples in a multi-celled tray on the floor on a cocoa house. Lines were fitted by linear regression of days 0-2 with a fixed intercept of the starting value.

The final moisture contents for all treatment and the bulk floor samples were also similar moisture (7-8%) by day 6 of experiment 2b. However, clear differences between treatments were observed in the rate of drying during the first two days (Figure 3). Between the start of drying (day 0) and day 2, the decrease in moisture content was approximately linear, and linear regression lines, with a fixed intercept of the initial value, were fitted to the points over this restricted period. Lines for the bulk floor sample, 400 g and 50 g treatments are shown in Figure 3 (the other lines are omitted for the sake of clarity), and the slopes of all the lines are given in Table 2 with the coefficients of determination (r^2).

Table 2. The rate of change of moisture content for cocoa bean samples from day 0-2, given by the slope of regression lines. The treatments compare the bulk floor sample with samples of different sizes in multi-celled drying trays.

Treatment	Slope of line (% per day)	Coefficient of determination	Moisture content on day 2 (%)
Floor	-11.59	0.988	31.1
50g	-21.73	0.944	13.2
100g	-20.67	0.928	15.1
200g	-19.53	0.982	16.1
400g	-14.99	0.994	24.3
800g	-10.64	0.973	31.1

Clear differences were observed between rate of drying of the floor sample and those for the 50 g, 100 g, 200 g and 400 g samples (they dried 1.9 to 1.3 times faster than the floor sample, Table 2). On the other hand, the 800g sample dried at a similar rate to the floor sample, resulting in almost identical values of moisture content on day2.

Discussion

These results demonstrate the possibility of large differences between the drying rate for the bulk floor and small samples, especially during the first two days of the drying process. The smaller the bean masses and the thinner the bean layer, the faster the drying rate. Rapid drying rates early in the process are likely to lead to case hardening that would trap acetic acid in the beans (Jinap and Thien, 1994).

The results of Experiment 2a confirm the advantage of arranging the beans in a heap when 2-3 kg samples are being dried. Both the heaped treatments were fairly similar to the bulk floor fermentation mass. It is clear from Figure 2 that, for any sample size, thin bean layers result in drying rates that diverge significantly from that of floor the early in the drying process.

For situations in which small bean samples (<1000g) must be dried, small cells that ensure that the beans are arranged in thick layers would help to achieve optimal drying rates. However, for samples of less than 800g it would be better to avoid the use of small cells.

Experiment one demonstrated that samples in netted bag and placed within the bulk floor fermentation mass dried at a similar rate to the surrounding beans throughout the drying process. This desirable situation would also minimise the disruption of normal activities when drying samples in a commercial estate. The space occupied by the micro-fermentation samples on the house floor would be kept to a minimum, so the use of netted samples instead of drying trays is recommended.

References

Jinap, S. and Thien, J. (1994) Effect of drying on acidity and volatile fatty acids of cocoa beans. *Journal of Science of Food and Agric.* **65:** 67-75.

Mossu, G. (1992) Drying. Pages 73-77 in: *Cocoa. The Tropical Agriculturalist*. London: Mac Millan.

Utilisation

Germplasm enhancement for resistance to Black Pod disease: progress achieved in ten years

A.D. Iwaro, V. Singh, Bharath, S. and D.R. Butler

Background

A Germplasm enhancement programme was initiated in 1998 as part of the CFC/ICCO/IPGRI project on conservation and utilisation of cacao germplasm. The main objective of this programme is to accumulate genes for resistance to Black Pod disease (BP) in small populations that that will provide material to cacao breeders for the improvement of BP resistance in new cacao varieties. Between 1998 and 2001, 136 resistant/moderately resistant genotypes were selected and used in 96 bi-parental crosses (36 Forastero, 17 Refractario, 20 Trinitario and 23 mixed). Progenies (3,486 seedlings) were raised in the greenhouse and screened for resistance to *P. palmivora* using a leaf disc test (Nyassé *et al.*, 1995). Between 2000 and 2003, 1,026 plants including resistant, moderately resistant and susceptible genotypes (control) were established under old cacao trees in Field 14 at the La Reunion Estate, Centeno. In addition, 70 parental genotypes involved in the first two batches of crosses in 1998 and 1999 were planted in Field 14. A replicate of the same population was planted in a newly established field (Field 7) at UCRS as a backup and to increase pod production for the evaluation of pod resistance and bean traits (bean number and bean weight). Since establishing the field trials, genotypes bearing pods have been assessed for resistance to BP under field conditions and in the laboratory using the detached pod test (Iwaro *et al.*, 2003). Genotypes have also been evaluated for bean number and bean weight, as well as resistance to Witches' Broom disease (WB) under field conditions. The assessment and evaluation of these traits are on-going, and the most recent results are presented here.

Methodology

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

From October 2006 to July 2007, field observations were made on 883 genotypes in Fields 14 and the replicates (760 plants) in Field 7, recording the following characteristics.

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

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

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

Fully grown, unripe pods (2-4 pods per genotype) were used for the evaluation of resistance to *P.*

palmivora with the detached pod test (Iwaro *et al.*, 2003). A total of 441 genotypes have now been assessed by this method, and are compared here with a sub-population of 500 genotypes from the ICG,T.

Evaluation of bean number and bean weight

Five to ten healthy, well-developed, ripe pods were harvested from 334 genotypes for the determination of bean number and bean weight. Average bean number per genotype was determined by counting the number of seeds (excluding flat beans) in the available pods and dividing the total number of seeds by the number of pods. Average wet bean weight per genotype was determined by dividing the wet bean weight of the available pods by the total number of beans. Average dry bean weight per genotype was estimated using a 40% conversion ratio (Freeman, 1969).

Results and Discussion

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

Black Pod symptoms were observed in 152 (38%) of the 398 genotypes bearing pods in Field 14 (Table 1), and 81 (14%) of the 571 genotypes bearing pods in Field 7 (Table 2). The higher level of infection in Field 14 (old cacao field) as compared to Field 7 (newly established field) is likely to be due to greater inoculum pressure in Field 14 from the time of establishment. However, the percentage of genotypes with *Phytophthora* infected pods in Field 7 has increased 7-fold since the previous report two years ago (Iwaro *et al.*, 2006). Taking the results from Fields 7 and 14 together, over 60% of the progeny population in pod production are still free from *Phytophthora* infection.

Table 1. Field observations conducted in Field 14 (La Reunion Estate).

Batch	Date of establishment	No. of genotypes established	No. of genotypes alive	No. of genotypes with pods	No. of genotypes with BP	No. of genotypes with WB
1	2000	314	246	202	70 (35%)	178 (72%)
2	2001	339	268	181	81 (45%)	233 (87%)
3	2003	258	194	6	0 (0%)	85 (44%)
4	2003	202	175	9	1 (11%)	75 (43%)
Total		**1,113**	**883**	**398**	**152 (38%)**	**571 (65%)**

BP - Black Pod disease WB - Witches' Broom disease

Witches' Broom symptoms were observed in 571 (65%) of the 883 genotypes in Field 14, but only in 22 (3%) of the 760 plants in Field 7. In the last two years, the percentage of plants with Witches' Broom symptoms in Field 14 increased from 43% to 65%, but in Field 7 the percentage was similar (2-3%) on both assessment dates. Field observations in Field 14, where the inoculum pressure is maintained by the presence of old cocoa, provide an opportunity for a negative selection against WB. However, it is important that the field results are confirmed independently in controlled conditions using a technique such as the agar droplet inoculation (Surujdeo-Maharaj *et al.*, 2003).

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

Batch	Date of establishment	No. of genotypes established	No. of genotypes alive	No. of genotypes with pods	No. of genotypes with BP	No. of genotypes with WB
1	2001	204	168	149	54 (36%)	5 (3%)
2	2002	171	134	96	5 (5%)	4 (3%)
3	2002	310	244	156	12 (8%)	8 (3%)
4	2002	274	214	170	10 (6%)	5 (2%)
Total		**959**	**760**	**571**	**81 (14%)**	**22 (3%)**

BP - Black Pod disease WB - Witches' Broom disease

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

Among the 411 genotypes (progeny population) evaluated for resistance to BP using the detached pod test, 192 genotypes (47%) were found to be resistant (disease rating 1 - 3) (Table 3). No visible lesions (disease rating 1) were observed on 38 genotypes, 98 genotypes had 1 - 5 localised lesions (disease rating 2), and 56 genotypes had 6 - 15 localised lesions (disease rating 3). The combined disease ratings 4 and 5 gave 99 moderately resistant genotypes (24%) and the remaining 120 genotypes (29%) were classified as susceptible (Table 3).

Table 3. Distribution of scores for resistance to *P. palmivora* in the progeny population (411 genotypes) and ICG,T sub-population (500 genotypes).

	Disease Rating							
Population	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Progeny	38	98	56	38	61	88	27	5
(411 genotypes)	9%	24%	14%	9%	15%	21%	7%	1%
ICG,T*	20	34	24	47	61	109	142	63
(500 genotypes)	4%	7%	5%	9%	12%	22%	28%	13%

*Iwaro *et al.* (2003)

Comparing the progeny population in Table 3 with a sub-set of genotypes from the ICG,T, shows a considerably higher frequency of resistant genotypes (47% versus 16%) and consequently a higher level of resistance alleles than the base population. The subset of the progeny population shows a significant reduction in the frequency of susceptible genotypes (29%) compared to the subset from ICG,T which had 63 % susceptible genotypes. This shows a significant improvement in BP resistance in the composition of the new population. It further confirms the effectiveness of the selection criteria imposed on the base population for the selection of the parental genotypes and confirms that resistance is heritable.

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

Assessments for bean number and bean weight were completed on 334 genotypes of the progeny

population (Table 4). The genotypes were classified into three groups (large, intermediate and small) for bean number, bean weight and pod index.

Table 4. Percentage of genotypes in three categories of bean number, bean weight and pod index in the progeny population (334 genotypes) and ICG,T sub-population (581 genotypes).

Character	Category	Rating	Progeny population (334 genotypes)		*ICG,T population (581 genotypes)	
			Number	%	Number	%
Bean number	Large	> 45	30	9	99	17
	Intermediate	36 - 45	223	67	302	52
	Small	< 36	81	24	180	31
Bean weight (g)	Large	> 1.20	64	19	64	11
	Intermediate	0.81 - 1.20	226	68	395	68
	Small	< 0.81	44	13	122	21
Pod Index	High	> 35.0	86	26	80	14
	Intermediate	20.1 - 35.0	218	65	445	77
	Low	< 20.1	30	9	56	10

* Iwaro *et al*., 2003

Seventy six percent (76%) of the progeny population had large to intermediate bean numbers (compared to 69% in the ICG,T) (Table 4). This confirms that selection for large to intermediate bean number will be effective in the progeny population.

Classification of bean weight in the progeny population was generally similar to that in the ICG,T. A total of 87% of the progeny population had intermediate to large bean weights, again confirming that selection for this trait would be effective in a breeding programme. It also indicates that resistance to BP can be combined with large to intermediate bean weight.

The percentage of genotypes with a low pod index is similar in the progeny population (9%) and the ICG,T (10%). There is a higher percentage in the progeny population with a high pod index (26% compared to 14% in the ICG,T), however a confounding factor in comparing the two populations may be tree age (Table 4).

Conclusion

The germplasm enhancement programme for resistance to BP is proving to be an effective way of accumulating genes for this trait. An increase in the frequency of resistant individuals confirms the effectiveness of the selection criteria and the overall strategy being adopted in the programme. As the evaluation exercise progresses, more promising genotypes are being identified which combine good yield potential with resistance to BP. The most promising genotypes have been used as base parents for the second cycle of the germplasm enhancement programme. In addition, some of the promising genotypes from the first cycle have been transferred to the ICQC,R for distribution as valuable sources of genes for resistance to BP.

Based on the results obtained in the first cycle, one can be optimistic that a more substantial improvement could be achieved in the second cycle of the programme. Crosses have already been made between 24 selected genotypes from the first cycle progeny, and 1,075 seedlings have

been raised. These are being screened for resistance to *P. palmivora* using the leaf disc test (Nyassé *et al.*, 1995), and selections will be made in 2008.The end product of the programme will allow cacao breeders to combine good yield potential with an acceptable level of resistance to BP in new cacao varieties.

References

Freeman, W.E. (1969) Some aspects of the cocoa breeding programme. Pages 507-527 in: *Proceedings, Agricultural Society*, Trinidad and Tobago, December 1968.

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

Iwaro, A.D. and Singh, V. (2006) Progress report on germplasm enhancement for resistance to Black Pod disease. Pages 63–67 in: *Annual Report 2005*. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.

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

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

Pre-breeding of cacao for resistance to Witches' Broom and Black Pod diseases

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

Introduction

A pre-breeding programme for resistance to Witches' Broom disease (WB) was initiated in July 2004 as an activity in the CFC/ICCO/BI Cocoa Productivity Project. Pollinations at UCRS were completed using an incomplete diallele Kempthorne and Curnow (1961) model in year one and a combination of incomplete factorial experimental designs and bi-parental crosses in years two and three. A total of 5,300 seedlings from 50 crosses were screened for WB resistance between 2005 and 2008.

Methodology

Screening

Witches' Broom disease

Analysis of variance of the measures; MBD (maximum broom diameter), TFS (time to first symptoms) and TBI (time to broom initiation) for year two progeny was prepared with the General Linear Model using Statistical System Analysis (SAS). Classification of WB resistance was based on TFS and MBD values (Table1). Depending on the time first symptoms (TFS <20 or TFS =20) the MDB values were submitted to GLM statistical analysis and classified into 3 statistical groups (resistant, moderately resistant or susceptible). This classification took into the fact that some plants took a very long time to show symptoms (TFS =20), but eventually developed large brooms. It is possible that these individuals showed partial resistance. Surujdeo-Maharaj *et al.*, (2004) highlighted the importance of time to first symptoms as a measure of resistance and this was also taken into account when selecting individual plants.

Table 1. Classification scale of resistance to Witches' Broom disease based on time to first symptoms and maximum broom diameter.

Time to first symptoms (days)	Maximum broom diameter (mm)		
	Resistant	Moderately resistant	Susceptible
< 20	= 7.8	> 7.8 = 9.7	> 9.7
= 20	= 8.5	> 8.5 = 12.0	>12.0

Progeny from year three crosses between the ages of 6-7 months together with their parents (seedlings from open pollinated pods) were screened in four batches (June, August, September and October 2007) using the agar droplet technique (Surujdeo-Maharaj *et al.*, 2003). Percentage of plants infected (incidence), MBD (severity) as well as TFS and TBI were observed. Plants which were determined to have low incidence or severity will be tested for black pod resistance together with a few WB susceptible crosses.

Black Pod disease

Selected crosses from the first pollination year together with the controls ICS 46, CAS 1, IMC 57 and PA 124 [PER] were screened for resistance to Black pod using the leaf disc test (Nyassé *et al.*, 1995). Results were analysed via ANOVA (General Linear Model of SAS) and plants were classified according to their score (degree of necrosis scale developed by Nyassé *et al.*, 1995). Resistant plants scored = 2.5, moderately resistant plants 2.51 – 3.0 and susceptible plants scored > 3.0. Plants classified as resistant or moderately resistant to WB and BP were planted at a 1.8 × 1.8 m spacing in Field 3A, La Reunion Estate, Centeno in October 2007.

Results and Discussion

Witches' Broom disease screening

Four hundred and sixty nine (469) seedlings spanning 20 families from year two crosses were selected for WB resistance (Table 2) from 1,373 seedlings screened for WB resistance.

Screening for WB resistance of 1,238 seedlings of year three crosses is complete, but analysis of data and selections for screening for BP resistance is still to be completed.

Table 2. Number and percentage of seedlings selected as resistant to Witches' Broom disease from each family of year two crosses and the mean time to first symptoms (TFS) and maximum broom diameter (MBD) for each family.

Cross	Total plants	No. of resistant plants	% plants	TFS	MBD
CC 71 × NA 33	28	12	42.9	15	10.1
TRD 45 × NA 471	58	24	41.4	15	9.7
PA 171 [PER] × TRD 109	132	42	31.8	14	9.9
PA 126 [PER] × AMAZ 6/3 [CHA]	72	42	58.3	15	9.8
CRU 80 × MATINA 1/7	50	22	44.0	16	9.3
MO 9 × PA 150 [PER]	83	29	34.9	14	9.5
CL 10/15 × [ICS 84 × TSH 1077(T64)]	75	21	28.0	15	9.8
IMC 47 × [NA 45 × B 7/21 [POU] (T83)]	89	27	30.3	16	9.8
NA 399 × [SCA 6 × IMC 67 (T12)]	104	39	37.5	15	8.9
TRD 32 × NA 471	26	17	65.4	13	9.5
B 9/10-25 [POU] × CL 10/5	92	29	31.5	14	9.2
LP 3/15 [POU] × CL 10/5	10	3	30.0	13	11.9
PA 195 [PER] × [ICS 1 × GU 175/P(T28)]	25	13	52.0	13	10.3
CRU 89 × [ICS 1 × GU 175/P(T28)]	36	20	55.6	14	9.6
CRU 89 × SJ 1/40 [POU]	66	22	33.3	13	9.7
AM 2/19 [POU] × SJ 1/40 [POU]	20	15	75.0	14	8.6
AM 2/19 [POU] × NA 232	101	26	25.7	14	9.9
MOQ 6/95 × NA 232	20	5	25.0	14	11.3
MOQ 6/95 × [IMC 67 × GU 353/L (T64)]	35	21	60.0	15	8.7
B 9/10-25 [POU] × [IMC 67 × GU 353/L (T64)]	78	40	51.2	14	8.7

Black Pod disease screening and field planting

One hundred and thirty-four (134) plants from 26 families of the year 1 crosses were selected as resistant / moderately resistant to BP and WB from 355 WB resistant plants screened for BP

resistance (Table 3). These plants together with 55 WB susceptible seedlings showing varying levels of resistance to BP, grafted parents (29) and WB resistant and susceptible controls (IMC 57 (6) and UF 29 (6)) controls were planted in the field. Screening of year 2 WB resistant progeny for BP resistance is approximately 20 % completed.

Table 3. Seedlings from year one crosses selected as resistant and moderately resistant to Witches' Broom and Black Pod diseases.

Cross	Resistant to WB	Resistant to WB &BP
PLAYA ALTA 2 [VEN] × RB 29 [BRA]	1	0
ICS 46 × CATONGO	2	1
JA 3/4 [POU] × SLC 4	5	1
CRUZ 7/8 × RB 29 [BRA]	5	2
GU 114/P × SLC 4	6	2
LP 1/45 [POU] × CATONGO	8	2
SLC 4 × GU 114/P	14	2
GU 114/P × CRUZ 7/8	4	3
ICS 46 × LP 1/45 [POU]	21	3
PLAYA ALTA 2 [VEN] × CATONGO	8	3
JA 3/4 [POU] × SPA 9 [COL]	6	4
RB 29 [BRA] × PLAYA ALTA 2 [VEN]	5	4
PLAYA ALTA 2 [VEN] × LCTEEN 90/S-7	9	5
PA 303 [PER] × LP 1/45 [POU]	14	5
RB 29 [BRA] × CRUZ 7/8	8	6
RB 29 [BRA] × CATONGO	10	7
SPA 9 [COL] × PA 303 [PER]	15	8
LP 1/45 [POU] × ICS 46	17	9
SPA 9 [COL] × JA 3/4 [POU]	23	10
SPA 9 [COL] × CATONGO	16	10
CRUZ 7/8 × CATONGO	13	11
GU 114/P × CATONGO	19	11
IMC 57 × CATONGO	12	11
PA 303 [PER] × CATONGO	16	11
LP 1/45 [POU] × PA303 [PER]	28	14
PA303 [PER] × SPA 9 [COL]	25	14
CRUZ 7/8 × GU114/P	35	21

Conclusion

Field planting of 134 seedlings showing resistance to WB and BP from year one crosses was completed in October 2007. Several other seedlings from different families were kept to replace any which may die during establishment. A proposal to replicate those seedlings in the field by micro-grafting is also being planned to ensure that this important germplasm is not lost.

Witches' Broom disease screening of seedlings from years two and three is virtually complete and next year the focus will be on screening for Black Pod disease resistance.

References

Kempthorne, O. and Curnow, R. N. (1961). The partial diallele cross. *Biometrics* 17: 229-250.

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

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

Surujdeo-Maharaj, S., Umaharan, P. and Butler, D. R. (2004) Assessment of Resistance to witches'-Broom disease in Clonal and Segregating Populations of *Thebroma cacao*. *Plant Disease* **88 (8):** 797-803.

Cocoa Research Advisory Committee

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Cocoa Research Unit staff 2007

Research staff

David Butler *PhD* Director

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Antoinette Sankar *MSc* Contract Officer I

Darin Sukha[1] *MSc* Junior Research Fellow

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

Balram Latchman *MSc* Contract Officer I (October– December)

Support staff

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

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

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Annelle Holder *MPhil* Technical Assistant

John Joseph Laboratory Assistant

Carlene Lakhan *BSc* Technical Assistant (June – December)

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Eusebius Solozano Laboratory Assistant

Surendra Surujdeo-Maharaj[1] *BSc* Technical Assistant

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

Peninna Deberdt *PhD* Phytopathologist
CIRAD-CP France

Administrative staff`

Claudia Lyons Secretary

Phulmatee Hetai Messenger/cleaner

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

Publications and presentations

Refereed Journals

Ali, N., Badrie, N. and Sukha, D.A. (2008) Effects of adding cocoa (*Theobroma cacao* L.) pulp nectar to stirred yoghurts on physicochemical and sensory properties. *Journal of Food Technology* **6 (2):** 51-56.

Deberdt, P., Mfegue, C.V., Tondje, P.R., Bon, M.C., Ducamp, M., Hurard, C., Begoude, B.A.D., Ndoumbe-Nkeng, M., Hebbar, P.K. and Cilas, C. (2008) Impact of environmental factors, chemical fungicide and biological control on cacao pod production dynamics and black pod disease (*Phytophthora megakarya*) in Cameroon. *Biological Control* **44(2):** 149-159.

Khan, N., Motilal, L.A., Sukha, D.A, Bekele, F.L, Iwaro, A.D, Bidaisee, G.G., Umaharan, P., Grierson, L.H. and Zhang, D. (2008) Variability of butterfat content in cacao (*Theobroma cacao* L.): combination and correlation with other seed-derived traits at the International Cocoa Genebank, Trinidad. *Plant Genetic Resources: Characterization and Utilisation* (in press).

Sukha, D.A., Butler, D.R., Umaharan, P. and Boult, E. (2008) The use of an optimised organoleptic assessment protocol to describe and quantify different flavour attributes of cocoa liquors made from Ghana and Trinitario beans. *European Food Research and Technology*. **226 (3):** 405-413.

Zhang, D., Boccara, M., Motilal, L., Butler, D.R., Umaharan, P., Mischke,S., Meinhardt, L. (2007) Microsatellite variation and population structure in the "Refractario" cacao of Ecuador. *Conservation Genetics*. Available online at DOI 10.1007/s10592-007-9345-8

Zhang, D., Boccara, M., Mische, S., Motilal, L., Leamy, E. and Butler, D.R. (2008) Assessing genetic identity, sibship structure, and population admixture in an Upper Amazonian cacao germplasm group using microsatellite markers. *Tree Genetics and Genomes* (in press).

Conference and Workshop presentations

Papers presented

Butler, D.R. Germplasm enhancement in Trinidad with emphasis on fungal diseases. Presented at The workshop on the *Prevention and management of the global spread of cocoa pests and pathogens*, 17-20 July 2007, Abidjan, Côte d'Ivoire.

Butler, D.R. The history and use of the International Cocoa Genebank in Trinidad to obtain varieties with resistance to diseases. Presented at the international workshop on *Cocoa variety improvement in the Americas: Collaborative and farmer participative approaches*, 20-25 August 2007, Guayaquil, Ecuador.

Butler, D.R. Challenges and progress of cocoa pre-breeding activities carried out in Trinidad and Tobago. Presented at the international workshop on *Cocoa variety improvement in the Americas: Collaborative and farmer participative approaches*, 20-25 August 2007, Guayaquil, Ecuador.

Holder, A. Comparative studies on resistance testing methods carried out at CRU. Presented at the international workshop on *Cocoa variety improvement in the Americas: Collaborative and farmer participative approaches*, 20-25 August 2007, Guayaquil, Ecuador.

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

Sukha, D.A. General comments on sensory analysis. Presented at: INIAP/PRONORTE Workshop on *Application of fermentation and liquor tasting practices to improve cocoa quality*, 18 - 22 June 2007, Pichilingue, Ecuador.

Sukha, D.A. The development of flavour in cocoa. Presented at: INIAP/PRONORTE Workshop on *Application of fermentation and liquor tasting practices to improve cocoa quality*, 18 - 22 June 2007, Pichilingue, Ecuador.

Sukha, D.A. Protocols to evoke, measure, analyse and interpret sensory responses in cocoa. Presented at: INIAP/PRONORTE Workshop on *Application of fermentation and liquor tasting practices to improve cocoa quality*, 18 - 22 June 2007, Pichilingue, Ecuador.

Sukha, D.A. Specific flavours in cocoa and their association with different origins. Presented at: INIAP/PRONORTE Workshop on *Application of fermentation and liquor tasting practices to improve cocoa quality*, 18 - 22 June 2007, Pichilingue, Ecuador.

Sukha, D.A. Organoleptic testing and quality in cocoa – implications for the local industry. Presented at: The Cocoa and Coffee Industry Board buying agents' meeting. Caroni, Trinidad. 20th July 2007.

Sukha, D.A. The potential of cocoa varieties to increase cocoa flavour. Presented at the international workshop on *Cocoa variety improvement in the Americas: Collaborative and farmer participative approaches*, 20-25 August 2007, Guayaquil, Ecuador.

Posters presented

Roberts, R.P., Wimmers, L., Jonnalagadda, K., Campbell, S., Butler, D.R., Sukha, D.A., Bekele,

F.L. and Saunders, J.A. Detection of misidentified plants in *Theobroma cacao* germplasm collections in Trinidad. Poster presented at *World Cocoa Foundation Partnership Meeting*, 24 October, 2007, Washington, DC.

Zhang, D., Boccara, M., Motilal, L., Butler, D.R., Umaharan, P., Mischke, S., Meinhardt, L. Population structure and chocolate trees in Ecuador. Poster presentation at *Mid-Atlantic Plant Molecular Biology Society 24th Annual Meeting* August 16-17 2007, Patuxent National Wildlife Refuge, Beltsville, Maryland.

Visitors to CRU in 2007

Elizabeth McCullough (née Baker)	Jacksonville, FL, USA
Judy Hayes (née Baker)	Templestowe, Victoria, Australia
Ted Hayes	Templestowe, Victoria, Australia
Jesse Guittard	Guittard Chocolate Co. Burlingame, CA, USA
Keith Holmes	CABI, Trinidad
David Preece	BCCCA/Cadbury Schweppes plc. UK
Christian Cilas	CIRAD Montpellier, France
Cynthra Persad	Ministry of Agriculture, Research Division, Centeno
Pierre Emmanuel Hublet	Museum of Cacao and Chocolate, Brussels
Elisabete Cunha Pessoa	Museum of Cacao and Chocolate, Brussels
Benoit Vandervelde	Museum of Cacao and Chocolate, Brussels
Sue Hainsworth	Green Ink, Bridgewater, Somerset, UK
Judith Cavey	Norwich, Norfolk, UK
Linda Heath	Dept. of Psychology, Loyola University, Chicago, IL, USA
Lindsay Nichols	Loyola University, Chicago, IL, USA
Aisha Leverett	Loyola University, Chicago, IL, USA
Lisa Sandberg	Loyola University, Chicago, IL, USA
J.A. Nicholas Wallis	Daklow, UK
Nazeer Ahmad	Food Production, UWI
Cheryl Ryan-Mohammed	Chaguanas
Monica Jane Clement	T&T Embassy, Brazil
Victoria Mendes-Charles	T&T High Commission, Nigeria
S.N. Gordon	T&T High Commission, Ottawa, Canada
Roanna Gopaul	Ministry of Foreign Affairs, Trinidad
Larry Wimmers	Towson University, MD, USA
Roland Roberts	Towson University, MD, USA
Jim Saunders	Towson University, MD, USA
Kate Denniston	Towson University, MD, USA
Pete Bishop	The Shop, London, UK
Biki Khurana	Rausch Chocolates, Mainz, Germany
Ina Vanderhoof	Toronto, Canada
Claude Vuillaume	CIRAD,Capesterre Belle Eau, FWI
Philippe Godon	CIRAD, Capesterre Belle Eau, Guadaloupe
Alessio Tessieri	Amedei, Italy

Acronyms and abbreviations

BCCCA	Biscuit, Cake, Chocolate and Confectionery Association, London, UK
BI	Bioversity International
BP	Black Pod disease
CAOBISCO	Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
CATIE	Centro Agronómico Tropical de Investigatión y Enseñanza, Costa Rica
CFC	United Nations Common Fund for Commodities
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement, France
CIRAD-CP	Centre de Coopération Internationale en Recherche Agronomique pour le Développement -Culture Pérennes, France
CPB	Cocoa pod borer
CRA	Cocoa Research Association, UK
CRU	Cocoa Research Unit, Trinidad and Tobago
DNA	Deoxyribonucleic acid
fp	DNA sample number (fingerprint number)
FP	Frosty pod disease
GLM	General linear model
GORTT	Government of the Republic of Trinidad and Tobago
ICCO	International Cocoa Organisation, London, UK
ICG,T	International Cocoa Genebank, Trinidad
ICQC,R	International Cocoa Quarantine Centre, Reading, UK
ICTA	Imperial College of Tropical Agriculture
INIAP	Instituto Nacional Autonomo de Investigaciones Agropecurias, Ecuador
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
MBD	Maximum broom diameter
P	Probability
PC	Principle component
PCA	Principle component analysis
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PID	Probability of identity
QTL	Quantitative trait loci
r	Correlation coefficient
RAPD	Random amplified polymorphic DNA
SSR	Simple sequence repeats
TFS	Time to first symptom
TBI	Time to broom initiation
UCRS	University Cocoa Research Station
UE	Union Européenne
USDA	United States Department of Agriculture
USDA-ARS	United States Department of Agriculture – Agriculture Research Service
UWI	The University of the West Indies
WB	Witches' Broom disease
WCF	World Cocoa Foundation, USA