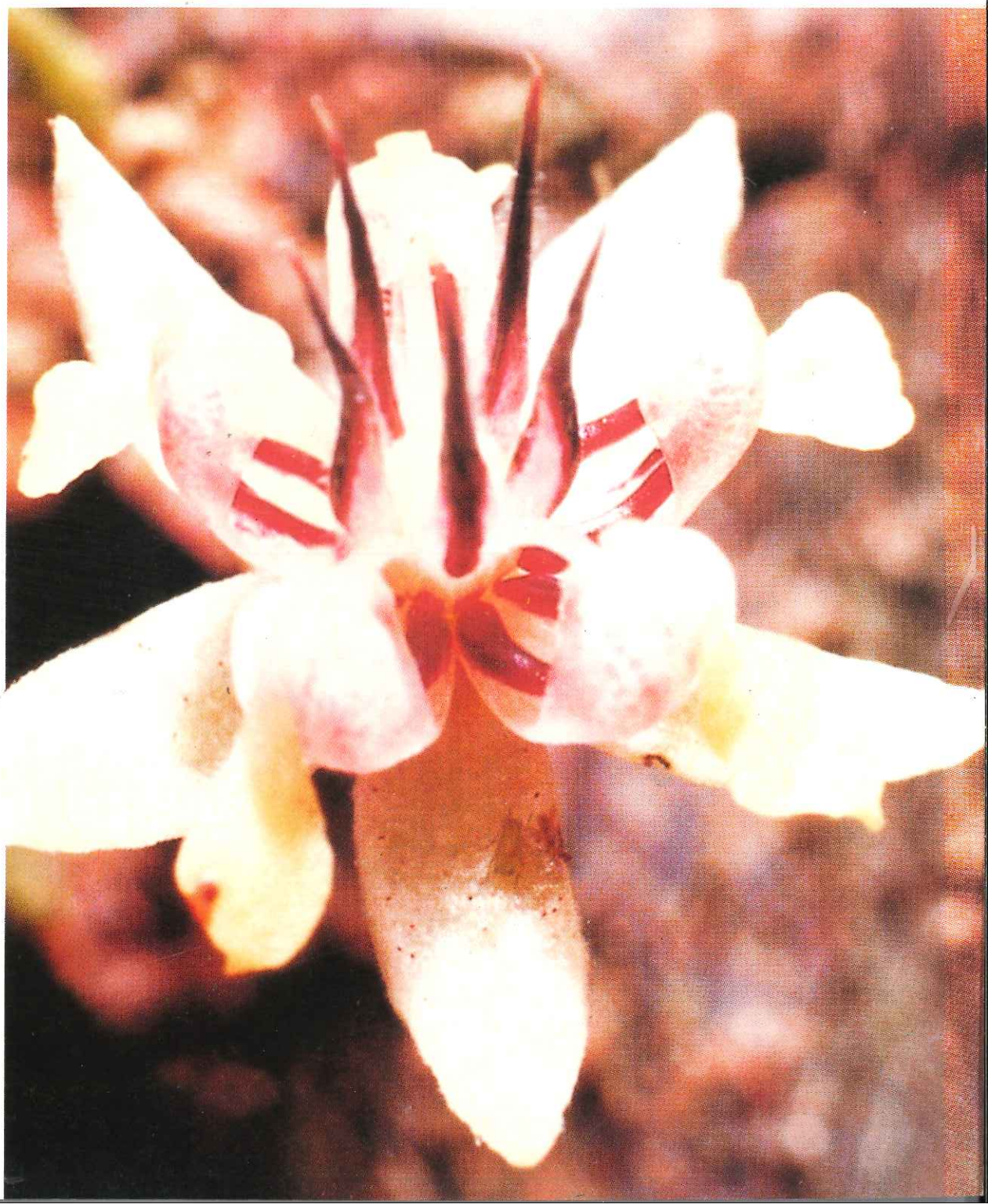
Working procedures for cocoa germplasm evaluation and selection

Proceedings of the CFC/ICCO/IPGRI Project Workshop
1 – 6 February 1998 – Montpellier, France

A.B. Eskes, J.M.M. Engels and R.A. Lass, *editors*



IPGRI is an institute of the Consultative Group on International Agricultural Research (CGIAR)

Working procedures for cocoa germplasm evaluation and selection

Proceedings of the CFC/ICCO/IPGRI Project Workshop
1-6 February 1998 - Montpellier, France
organized by IPGRI in collaboration with ACRI, BCCCA and CIRAD

A.B. Eskes, J.M.M. Engels and R.A. Lass, *editors*

The **International Plant Genetic Resources Institute** (IPGRI) is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI's headquarters is based in Rome, Italy, with offices in another 14 countries world-wide. It operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme, and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).

The international status of IPGRI is conferred under an Establishment Agreement which, by January 1998, had been signed and ratified by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mauritania, Morocco, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovak Republic, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

Financial support for the Research Agenda of IPGRI is provided by the Governments of Australia, Austria, Belgium, Brazil, Bulgaria, Canada, China, Croatia, Cyprus, Czech Republic, Denmark, Estonia, F.R. Yugoslavia (Serbia and Montenegro), Finland, France, Germany, Greece, Hungary, Iceland, India, Ireland, Israel, Italy, Japan, Republic of Korea, Latvia, Lithuania, Luxembourg, Malta, Mexico, the Netherlands, Norway, Pakistan, the Philippines, Poland, Portugal, Romania, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Thailand, Turkey, the UK, the USA and by the Asian Development Bank, Common Fund for Commodities, Technical Centre for Agricultural and Rural Co-operation (CTA), European Environment Agency (EEA), European Union, Food and Agriculture Organization of the United Nations (FAO), International Development Research Centre (IDRC), International Fund for Agricultural Development (IFAD), Interamerican Development Bank, United Nations Development Programme (UNDP), United Nations Environment Programme (UNEP) and the World Bank.

IPGRI is the Project Executing Agency of the **CFC/ICCO/IPGRI project** on 'Cocoa Germplasm Utilization and Conservation: a Global Approach'. For the implementation of this project, a Co-ordination Unit was established at INIBAP (International Network for the Improvement of Banana and Plantain), Montpellier, France. The CFC/ICCO/IPGRI project, developed under the aegis of the International Cocoa Organization (ICCO), London, is substantially funded by the Common Fund for Commodities (CFC), an intergovernmental organization with headquarters in Amsterdam, the Netherlands, with the involvement of a number of other funding organizations.

Citation: Eskes A.B., J.M.M. Engels and R.A. Lass, editors. 2000. Working Procedures for Cocoa Germplasm Evaluation and Selection. Proceedings of the CFC/ICCO/IPGRI Project Workshop, 1-6 February 1998, Montpellier, France. International Plant Genetic Resources Institute, Rome, Italy.

Cover: Flower of *Theobroma cacao* (D. Clement, CIRAD)

ISBN 92-9043-434-1

IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy

Contents

Foreword

Conservation and use of plant genetic resources are the core elements of the mandate of the International Plant Genetic Resources Institute (IPGRI). In the past, major attention was paid to food crops where considerable achievements were obtained through the institute's research and training activities. More recently, increased efforts have been devoted to crops such as banana, plantain and coconut, and the inclusion of cocoa in IPGRI's research agenda, as an important crop for smallholders, was a logical choice. In 1994 the European Economic Community commissioned to IPGRI an Evaluation Mission of their recent financial support to the Cocoa Research Unit (CRU), Trinidad. The resulting report identified a core of parties interested in developing a proposal on the conservation and use of cocoa genetic resources. Subsequently, the project entitled *"Cocoa Germplasm Utilization and Conservation: a Global Approach"* was developed in a co-operative effort between IPGRI and the International Cocoa Organization (ICCO) in consultation with numerous partners. After approval of the project proposal by the International Cocoa Council of ICCO, the final proposal was submitted to the Common Fund for Commodities (CFC) for financing. The project gained the approval of the CFC's Executive Board in 1997 and project implementation began on April 1st, 1998.

Because of the participation of scientists working in different disciplines and coming from all continents, the necessity arose to discuss and agree on standardized methods and approaches for the various activities of the project. Consequently, a workshop entitled "Working Procedures for Cocoa Germplasm Evaluation and Selection" was organized in Montpellier, France. Project scientists were invited to propose working procedures, which were discussed and agreed upon in specific working groups. The edited draft working procedures were distributed to all project sites and are now in use. The comments received have been used to finalize this document.

It is hoped that these agreed working procedures will find wide application and, thus, contribute to an increasingly more efficient conservation and use of cocoa genetic resources. However, since such procedures are dependent on knowledge and technologies they will have to be reviewed regularly and adjusted accordingly. Readers are cordially invited to send their comments and observations to IPGRI for this purpose.

Emile Frison
Director INIBAP

Acknowledgements

The Workshop was organized within the framework of the CFC/ICCO/IPGRI Project on "Cocoa Germplasm Utilisation and Conservation, a Global Approach". The following institutions are acknowledged for their specific support to the Workshop:

- The Common Fund for Commodities (CFC), as main sponsoring institution,
- The International Cocoa Organization (ICCO), as Project Supervisory Body,
- The American Cocoa Research Institute (ACRI), the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA) and the 'Centre de Coopération Internationale en Recherches Agronomiques pour le Développement/Département des Cultures Pérennes (CIRAD-CP), as project co-financing institutions,
- BCCCA and CIRAD for partly sponsoring the participation of some of the participants,
- CIRAD for its contribution to the organization of the Workshop,
- AGROPOLIS, Montpellier, for hosting the first day of the Workshop, and
- Cocoa Research Institutes for supporting their staff to come to the meeting.

Thanks go to all the participants who contributed to the very constructive debate which led to the success of the Workshop. Particular thanks go to Bernadette Sellers as workshop secretary, to Gillian Moffatt for the help in the final editing of this document, to Tom Thornton for dealing with the financial aspects of the Workshop and to Emile Frison for providing INIBAP support in organizing the Workshop and publishing this document.

It is appropriate to acknowledge the substantial contributions by a large number of organizations (both national and international) who are providing co-financing and counterpart contributions to the project, thus enabling the substantial work programme to move forward.

Welcome Addresses

Jan Engels
International Plant Genetic Resources Institute (IPGRI)

Ladies and gentlemen, I would like to welcome you on behalf of the Organizing Committee and I would like to take this opportunity to briefly introduce you to the front table. Our Chairman this morning will be Dr. Michel Nucé de Lamothe, President of Agropolis. There is also Dr. Mark Clayton from the Common Fund for Commodities (CFC), Dr. Jean-Marc Anga from the International Cocoa Organization (ICCO), Dr. Carol Shively-Knight from the American Cocoa Research Institute (ACRI), Dr. Emile Frison, Director of the International Network for the Improvement of Banana and Plantain (INIBAP) who also represents the Project Executing Agency i.e. IPGRI, Mr. Patrice de Vernou, Director of the Département des Cultures Pérennes du Centre de Coopération Internationale en Recherches Agronomiques pour le Développement (CIRAD-CP), and Mr. Tony Lass, representative of the Biscuit, Cake, Chocolate & Confectionary Alliance (BCCCA). With this brief introduction I would like to hand over the floor to the Chairman this morning, Dr. Michel Nucé de Lamothe, the convenor of this opening session.

Michel Nucé de Lamothe
Agropolis

On behalf of Agropolis, I would like to welcome you to our premises. Agropolis is a private association which counts among its members 17 French research institutes and higher education establishments specialized in agronomy, several foreign laboratories (Australian, American with a Brazilian one shortly to come), an international research centre (IPGRI's "Banana" programme), secretariats of large international networks such as the International Organization for Biological Control (OILB) and Burotrop (Bureau for the Development of Research on Tropical Perennial Oil Crops). In total there are almost 2 300 scientists and lecturers, and 5 000 employees spread over three campuses and a science and technology park where scientists and industrialists meet.

It seems to me that your meeting replies to a real need in agronomic research and in particular in research on perennial crops. Work on genetics but also on crop protection calls for continuity in terms of human and financial resources which is often beyond the capabilities of national programmes, even in major producing countries. Perennial crops are somewhat the orphans of international research; for CGIAR they are not part of their mandate. Fortunately, organizations, many of which are present here today, have done remarkable work, but in order to go further and to take up the challenge of the fight against poverty, all scientific resources have to make a contribution. Networking, or international consortia, are the best way to work, as INIBAP with its banana network has proved.

It seems to me that you have all the necessary ingredients for making good progress for cocoa: an international institute, IPGRI, research centres in the Northern and Southern hemispheres, in contact with small-holders, industrialists, financiers and, at the very end of the chain, the consumers forever demanding as we are.

The whole of the Agropolis community is at your disposal to help you wherever it can in research, training and technology transfer. I would also like to mention a project under way to create a platform - both physical and virtual – by which we will be able to invite foreign

teams of higher-calibre scientists in the field of the genome and animal and vegetal biotechnology. This platform will call for the involvement of our most competent teams here on site, including those working on the human genome.

I would like to conclude here by wishing you an excellent and fruitful workshop.

Patrice de Vernou

Director of the Département des Cultures Pérennes du Centre de Coopération Internationale en Recherches Agronomiques pour le Développement (CIRAD-CP)

Thank you Mister Chairman.

Ladies and gentlemen, on behalf of CIRAD's Director General, Mr Bernard Bachelier, I would like to welcome you to Montpellier where I hope you will enjoy your stay. As you noticed, CIRAD is part of the Agropolis complex, which houses numerous companies and organizations working in tropical agriculture. The CFC Workshop in which you are about to participate is of major importance for CIRAD. Firstly because cocoa is one of our prime concerns; consumers are very familiar with the product, but it remains mysterious in many ways. It is also of strategic economic importance. More than 30 CIRAD researchers work full or part-time on cocoa, on almost all the different biological aspects and in other fields, such as economics and sociology. The networks with which it is associated and its involvement in field operations in several producing countries makes the team one of our major assets. Secondly, because the Workshop follows on from the general spirit in which we operate. It is not only in our laboratories, but rather by talking to those involved in the sector, of course researchers but also producers, traders and industrialists, that we should be looking for research topics. Our aim is to provide answers to practical problems raised by the parties involved. The discussions due to take place this week and the questions raised will undoubtedly help to improve the effectiveness of our research. Lastly, the Workshop has been organized in close association with many research partners, and this joint cooperation is clear proof of the move towards dialogue between the different partners in the sector. I would like to thank IPGRI for organizing the Workshop, the speakers who I'm sure will be fascinating and all of you for taking time and trouble to come, a clear sign of the team spirit that we need in the cocoa sector. Thank you, Mister Chairman.

Mark Clayton

Common Fund for Commodities (CFC)

Mister Chairman, ladies and gentlemen, distinguished guests, I'm delighted to welcome you on behalf of the Managing Director of the Common Fund for Commodities to this, the first preparatory Workshop for the project, "Conservation and Utilization of Cocoa Germplasm: a Global Approach". The Workshop brings together leading research institutes involved in the development and promotion of cocoa research, a commodity that is the backbone of many countries' economic and foreign exchange earnings. I'd like to congratulate the International Plant Genetic Resources Institute for their excellent co-ordination and planning which has greatly facilitated the arrangements for the Workshop and the preparation and groundwork for project implementation. I hope and I trust that the deliberations over the forthcoming week will provide a sound basis and a clear direction for mutual co-operation and further development of project activities. The collaborative efforts necessary to develop new varieties of cocoa, with the desired market characteristics and resistance to the many pests and diseases which reduce productivity and threaten the sustainability of cocoa production are an essential element at the core of this project. Conservation and effective utilization of all available germplasm and genetic resources are clearly in the interest of both individual

countries but also for the wider benefit of the global cocoa economy, and in this respect the potential benefits of the project are highly significant in the context of the Common Fund's mandate. Finally, I'd like to thank you all for your co-operation in developing the project under the guidance of the International Cocoa Organization and IPGRI for submission to the Common Fund for financing. The project addresses fundamental issues for the cocoa industry and the multiplier effect is significantly enhanced through your co-operation and development, and I look forward to the next phase of implementation.

Thank you, Mister Chairman.

Jean Marc Anga
International Cocoa Organization (ICCO)

Thank you, Mister Chairman.

Ladies and gentlemen, I would like to welcome you here on behalf of the Executive Director of the International Cocoa Organization. We are indeed proud to be associated with this important project. It has been a long and arduous road to get this project started as representatives from IPGRI and the Common Fund will no doubt confirm to you. Anyway, we are all here now, ready to take this project forward and we are very much looking forward to its results, which I am sure, will help in solving the problems faced by many in the cocoa community. We are really hopeful that this week will see the initiation of all research activities which will result in a positive outcome at the end of the project, thus allowing us to report in a few years' time, to our various bodies at the International Cocoa Organization, that this important project has been a successful one. I will have the opportunity to contribute later today on the role of ICCO as the Supervisory Body for this project. We will, at that time, be able to discuss specifics. For the time being I would just like to thank you and welcome you here.

Tony Lass
Biscuit, Cake, Chocolate & Confectionery Alliance (BCCCA)

Thank you very much, Mister Chairman.

BCCCA is a somewhat difficult acronym for the trade association of UK cocoa users and chocolate manufacturers, and we, as an organization, have supported cocoa research since 1936. The fact that cocoa is an orphan commodity and the disparate nature of the cocoa industry has meant that progress really has been very limited. We still need better planting material for the smallholders of the world. We are involved as a co-financier in this project, in a number of the project elements, from the database to the quarantine facilities, through to support to the work of the Cocoa Research Unit in Trinidad. Therefore we are most anxious that this project should come to a successful conclusion. It is particularly important that we all work together as a team. It is not going to be very easy; there is a lot of work to be done, but let us hope that the results can be meaningful and can genuinely help the cocoa smallholders of the future. This project has been long in gestation. Talking to Dr. Engels this morning, we concluded that it really started during a European Development Fund consultancy mission, which IPGRI undertook to look at the results of the conservation programme for cocoa genetic resources in Trinidad. That was in 1993 and crystallized into a meeting in Malaysia in the autumn of 1994, when some of us had preliminary conversations. From then there have been long, and sometimes difficult, discussions between the various parties. So a long conception, or is it germination? I suspect that today is the birth of the project. We have a practical result of the deliberations in that we are all here together today. So, I wish us all well in our discussions, and a successful conclusion to this very interesting,

very worthwhile and enormously important project. Thank you all.

Carol Knight
American Cocoa Research Institute (ACRI)

Good morning.

As most of you know, I am new at ACRI, replacing Dr. Stillings. I think many of you worked with him. The American Cocoa Research Institute this year is celebrating fifty years of research. We started in 1948, so I guess that we are little newer than the BCCCA at it, but certainly dedicated to cocoa research. As I came here I got to know a lot of new people, and I feel like I'm really part of a family now as I look around the room and see people from literally all parts of the world. I think that we have come together on a project, which really makes us into a family. Cocoa is like a child, we are all the parents, we have to take care of it, and nurture it and grow it, watch after it and see it develop into its future. Cocoa germplasm evaluation, selection and conservation are certainly very important topics to ACRI. I think that probably the biggest thing that unites us as a family is that we have one common goal, that is to work together to achieve the very important objective of improving the yield of cocoa to increase productivity throughout the world; to really solve some of the devastating problems that face us with disease, such as witches' broom in Brazil and black pod in others; and also ultimately, as Tony said, to really help the small farmer of the future. So I am glad that ACRI is part of this project and I hope to get to know you all better and to work with you in the future. Thank you.

Emile Frison
International Network for the Improvement of Banana and Plantain (INIBAP/IPGRI)

Thank you Mister Chairman.

Ladies and gentlemen, on behalf of Dr. Geoffrey Hawtin, Director General of IPGRI, I would like to welcome all of you to this Workshop. Before I talk about cocoa, I think I should say a few words about IPGRI and how it comes that a network or programme dealing essentially with bananas is involved in this cocoa project.

IPGRI has three programmes. Its plant genetic resources programme is what IPGRI used to be in the past, before it broadened its scope, with its headquarters based in Rome. Now it also has a system-wide genetic resources programme which aims at bringing together and co-ordinating the effort of genetic resources programmes of all the centres of the Consultative Group on International Agricultural Research involved in the different commodities. In this programme IPGRI plays a convening and co-ordinating role. The third programme is INIBAP, the International Network for Improvement of Banana and Plantain, and which deals with, as its name says, the improvement of another important crop which is also to a large extent neglected in its research. The INIBAP programme is located here in Montpellier on the Agropolis campus and it is the INIBAP programme that also houses the co-ordinating office of the cocoa project. Therefore as a member of IPGRI Senior Management, I will be involved in the supervision of the project as one of the representatives of the Project Executing Agency.

This inaugural Workshop is the first major step in the project, which will involve a large number of collaborators for a period of five years. As was mentioned earlier, this major initiative did not just start a short time ago, but many years ago, and a lot of preparation has gone into it. During that period IPGRI has been working in very close collaboration with many institutions and individuals without whom this would not be possible. What the project has essentially been aiming at is to develop a set of partnerships which, through true

collaboration and creation of synergies, will develop into a major output which would not be possible without this true sense of partnership. I think that in any collaborative effort, and especially when you are talking about something of this size, what you will get out of it is directly proportional to what each individual will put into it. This is one way that the participants in this project will, I hope, look at the project. I think it is the two-way exchange in such a project which really will create the added value that collaboration can bring about.

Since I will not be here at the closing session of the Workshop, I would like to say a few words of thanks. I will start with the Common fund for Commodities which is the main funding agency and with the International Cocoa Organization as Supervisory Body. Of course, the participation of the co-financiers who are present here at the table, ACRI, BCCCA CIRAD, and, of all the participating institutions, which will make significant counterpart inputs, is fundamental to the project. The overall size of the project budget, adding the Common Fund funding, the co-financier funding and the counterpart funding is quite impressive. I think that this is one of the really large research initiatives and I hope that the outcome will be more than proportional to the input that will be made by everybody. I would also like to say a special word of appreciation to CIRAD. One of the reasons for locating the Project Co-ordinating Unit here in Montpellier is the close partnership with CIRAD. Without the very strong collaboration that we've been having with CIRAD, this project would never have come about. I would like everybody to be aware of that.

In addition, individuals have to be mentioned: my colleague, Jan Engels, has really been a driving force behind this initiative over all these years and I used to be his neighbour when I was located in Rome. The energy he's been putting behind this has been impressive. Dr. Bertus Eskes, who will be responsible for the co-ordination of the project, has also put a lot of time and energy so far into getting the first steps going and especially so in the last period of preparation for this particular Workshop. He was always there in the morning before I arrived and, at 8 p.m. when I left, he was still there and at the weekends as well. So he has put a tremendous amount of energy in making this Workshop successful. I am sure that he will continue to put as much energy, during this five-year period, into the co-ordination of the project. I would also like to say a word of thanks to Bernadette Sellers. She has also been putting in a tremendous effort in making sure that all the practical aspects of this Workshop and of your travel were without problem. Finally I would like to thank Dr. Michel Nucé de Lamothe, as Chairman of Agropolis, for hosting this inaugural session here. Agropolis in Montpellier is trying to create synergies between the different partners and to provide central facilities, such as this very pleasant meeting room in order to make the work of all the partners more efficient. So I thank you for hosting it today.

I would like to end by wishing you a successful Workshop. I hope that this first step will lead to many other significant steps that will make the whole effort a true success. Thank you for your attention.

Introductory Papers

Introductory note about the Common Fund for Commodities (CFC)

Mark Clayton

Assistant Project Manager, Common Fund for Commodities (CFC)

Objectives and operation of CFC

The primary focus of the Common Fund for Commodities (CFC) is on commodities and this has good reason. Many Developing and Least Developed Countries are heavily dependent on commodities, which form the backbone of their economies and account for the bulk of their export earnings and government revenue. With regard to the Least Developed Countries this dependency is generally much more severe. In addition, for each ton of commodity produced, exporting Developing Countries have received significantly less revenue over previous decades due to the deterioration in their terms of trade.

On the other hand, the number of people who rely on commodities for their livelihood has increased. It has been estimated that between 800 to 900 million people around the world depend on commodity production for exports. This figure would be substantially higher if one included commodity production for national consumption and subsistence needs. CFC, therefore, deals with the core issues for economic development in many regions of the world.

CFC's unique commodity focus has the advantage of seeking more generally applicable solutions for commodity problems benefiting many commodity producing countries. This contrasts with the traditional country orientation and focus which forms the essence of bilateral development aid and other multilateral development institutions. It is the role of CFC to assist commodity producing Developing Countries and Countries in Transition to meet the challenges of the liberalized global economy, and to facilitate the opening up of new opportunities.

CFC was negotiated under the United Nations Conference on Trade and Development (UNCTAD). The agreement establishing CFC was concluded in 1980 and became effective in 1989. A small Secretariat, comprising a total staff of 26, was established in Amsterdam, Netherlands and the first project was approved in 1991.

CFC forms a partnership of 104 countries (Annex 1 to this paper), both from the Developing and Developed Worlds. The European Union is also a member but without voting rights and the Organization of African Unity/African Economic Community (OAU/AEC) is due to become a member this year, under the same conditions as the European Union. Costa Rica and Trinidad and Tobago will also join the Fund during the forthcoming year and several more Latin and Central American countries have expressed their interest to become Members.

The objectives of CFC, as laid down in the Integrated Programme for Commodities (IPC) adopted by UNCTAD, include the achievement of stable conditions in commodity trade, the diversification of production, improvement of the real income of Developing Countries, market access, competitiveness, market structure, international trade and marketing. The full text of the IPC is reproduced as Annex 2 to this paper.

The commodity focus of the Fund makes it possible for the results of Common Fund projects to be applied across a number of Developing Countries. The impact of our projects, therefore, goes beyond the initial amount of money spent, through replication of project results. Therefore, every dollar spent on Common Fund projects usually has a large multiplier effect. Least Developed Countries, poverty alleviation, sustainable development and private sector involvement are among the priority areas for the Fund. CFC has a particular advantage in small to medium sized projects, which are more suited to

demonstrative and replicable measures and to the low absorption capacity of the poorest countries. The prime focus is on low-cost, high impact projects, which are sustainable beyond CFC's involvement.

The envisaged buffer stock supporting function of CFC, linked to its First Account, did not materialize. In the framework of the Fund's Five-Year Action Plan, consideration is given on how to use the resources of the First Account for actions other than buffer stocking within the spirit of the Agreement and, if possible, without amending the Agreement.

CFC now concentrates on measures under its Second Account, which are broadly defined in Article 18, 3(a) of the Agreement as "commodity development measures, aimed at improving the structural conditions in markets and at enhancing the long-term competitiveness and prospects of particular commodities." Such measures include research and development, productivity improvements, marketing and vertical diversification. This is complemented by the First Account Net Earnings Programme to assist Developing Countries to function more effectively in a liberalized global market. In its project work, CFC co-operates closely with 23 international commodity organizations (Annex 3 to this paper).

Private sector firms have been actively involved in many of the projects financed by the Fund. This has assisted in concentrating on commodity development issues, which will enhance the market competitiveness of commodities of interest to Developing Countries. The participation of private firms has also facilitated and accelerated project implementation and the dissemination of project results.

To date, CFC has approved 61 projects with a total financial value of about USD 164 million, covering 22 commodities of importance to Developing Countries. For more than half of the total project financing, CFC has attracted co-financing from other sources and institutions. This is testimony to the strong catalytic role played by CFC in addressing problems of commodity producers. CFC is expanding its operations to add loan-financed projects to its grant-financed project portfolio. In this context, more attention is being given to horizontal and vertical diversification.

CFC, with its small dedicated team, is an action-orientated organization. It is determined to work on practical solutions for the problems of commodity producing Developing Countries and Countries in Transition and to assist their integration into the global economic and trading system. This Workshop is part of CFC's effort to raise the awareness of the global nature of commodity problems, to stimulate an exchange of ideas and prioritise the use of scarce development resources. The objective of the Fund's work remains to improve the economic situation of the 800 to 900 million people in Developing Countries who are dependent, for a significant part of their income, on the production and export of commodities.

Types of projects financed by CFC

CFC's project financing operations are of two types. The first type comprises commodity development measures aimed at improving the structural conditions in markets and enhancing the long-term competitiveness and prospects of particular commodities. The second kind relates to supporting commodity market development actions designed to assist Developing Countries, especially Least Developed and land-locked countries, to function effectively in a liberalized global economy. The measures aimed at improving structural conditions of commodity markets and improving the competitiveness and long term prospects of particular commodities in the first category include:

Research and development and transfer of technology and know-how

In this framework, measures are directed towards finding new end-uses for commodities or their by-products, new processing methods, introducing new technologies and/or adapting existing technologies to new applications, as well as encouraging research and

development for processing natural products to add value and thereby improve their competitiveness.

Productivity and quality improvement

Under this category, all types of projects which aim to improve genetic materials for higher yields, disease resistance and tolerance to other natural adversities, reduction of production costs and post-harvest losses, improved quality and quantity in processing, storage or transportation are financed.

Marketing measures or initiatives

These involve promotional measures aimed at encouraging greater use of a commodity or introducing commodities to new geographic areas. The aim is to strengthen the competitive position of commodities facing structural difficulties such as falling market share in competition with synthetic substitutes, or changes in traditional export markets, as well as increasing developing countries' participation in commodity development activities and increasing their foreign exchange earnings from commodities.

Diversification (local processing, pilot and demonstration plants, feasibility studies, diversification of production)

In this area, the types of projects supported are: those which stimulate local processing of primary products and promote the industrialization of developing countries, including the introduction of pilot demonstration plants; feasibility studies and other types of technical assistance which could promote investment in commodity development and diversification of production, with a view to increasing export earnings of Developing Countries.

Improving productivity and efficient utilization of resources

The sustainable management of natural resources, including genetic diversity, is one of the policy objectives of the Fund. Projects which encourage the effective use of existing resources including optimal use of waste and by-products and renewable resources are among the types of projects favoured for CFC funding.

Sustainable commodity development

The Fund attaches particular attention to maintaining and promoting environmental conditions in commodity development and use. Stand alone projects or projects which contribute significantly to ongoing projects of CFC, addressing environmental concerns in commodity production and processing, are encouraged. Dissemination of environmentally-sound practices in commodity production or processing under CFC funded projects and dissemination of the results of projects which have environmental or sustainable development components will be pursued.

With regard to other projects aimed at commodity market development measures, support will be directed to, *inter alia*, the promotion of physical market development, enhancement of market infrastructure and support services to facilitate private sector initiatives; institution strengthening and training at all levels; enhancing commodity risk management and commodity trade financing; and micro-policy advice on commodity market development. In this framework, the following types of projects have been financed:

- Introduction of measures to minimize physical market and trade risks, including quality standards and grading measures, warehousing systems as well as information collection and dissemination;
- Improving the legal and policy framework, including a system of registration of warehouse receipts and casualty insurance. In this connection, efforts will be

supported which improve commodity trade finance by strengthening local financing systems creating a collateral system using an underlying commodity as security;
- Measures to introduce a transparent regulatory system that will enhance contract sanctity. These measures may include awareness-raising and training;
- Piloting market risk pooling through the organization of producer co-operatives and small trader organizations;
- The development of warehouse receipt systems that will permit the use of commodities as security for production, trade and marketing loans and/or credit.

Criteria applied for financing of projects

Project proposals which have qualified for CFC financing have met the following criteria:

- Primarily, all projects must be commodity related and should be sponsored by a recognized international commodity body (ICB) established on an intergovernmental basis comprising producers and consumers of the commodity in question.
- The objectives of the project must be achievable within a specified period of time and with due regard being paid to environmental concerns. The project must be designed in the context of an overall commodity development strategy of the ICB and should focus on countries, which are members of the Fund.
- The beneficiaries of CFC financed projects shall, primarily, be Developing Countries and/or Countries in Transition. Priority will, however, be accorded to projects of interest to the Least Developed and Low Income Countries, particularly small producers/exporters. Projects should also be beneficial both to the commodity itself and to several producing countries. However, a nationally based project can be considered if it is of interest to a number of countries, the results could be widely disseminated, if it is a pilot project or the nucleus of a multi-country project with potential for further development and replication, or if it is connected with vertical diversification.

The Fund will maintain a balance between the amounts allocated to projects for different commodities and between supply-side and demand-side projects.

The Fund, however, does not favour projects which:

- are predominantly, or exclusively, of a general training or extension nature;
- entail disproportionate overhead and administrative costs;
- have as their main objective institution building or basic research;
- cannot deliver results which will be sustained beyond the life of the project;
- require large-scale investments;
- are directed to general market studies;
- duplicate work elsewhere;
- form part of the core work of an ICB or implementing institution;
- aim at food production for local consumption; and
- concentrate on non-member countries of the Fund (see Annex 1 to this paper).

Annex 1

Members of the Common Fund for Commodities (February 1998)

1. Afghanistan
2. Algeria
3. Angola
4. Argentina
5. Austria
6. Bangladesh
7. Belgium
8. Benin
9. Bhutan
10. Botswana
11. Brazil
12. Bulgaria
13. Burkina Faso
14. Burundi
15. Cameroon
16. Cape Verde
17. Central African Republic
18. Chad
19. China
20. Colombia
21. Comoros
22. Congo
23. Côte d'Ivoire
24. Cuba
25. Democratic People's Republic of Korea
26. Democratic Republic of Congo
27. Denmark
28. Djibouti
29. Ecuador
30. Egypt
31. Equatorial Guinea
32. Ethiopia
33. Finland
34. France
35. Gabon
36. Gambia
37. Germany
38. Ghana
39. Greece
40. Guatemala
41. Guinea
42. Guinea-Bissau
43. Haiti
44. Honduras
45. India
46. Indonesia
47. Iraq
48. Ireland
49. Italy
50. Jamaica
51. Japan
52. Kenya
53. Kuwait
54. Lesotho
55. Luxembourg
56. Madagascar
57. Malawi
58. Malaysia
59. Maldives
60. Mali
61. Mauritania
62. Mexico
63. Morocco
64. Mozambique
65. Myanmar
66. Nepal
67. Netherlands
68. Nicaragua
69. Niger
70. Nigeria
71. Norway
72. Pakistan
73. Papua New Guinea
74. Peru
75. Philippines
76. Portugal
77. Republic of Korea
78. Russian Federation
79. Rwanda
80. Samoa
81. Sao Tome and Principe
82. Saudi Arabia
83. Senegal
84. Sierra Leone
85. Singapore
86. Somalia
87. Spain
88. Sri Lanka
89. Sudan
90. Swaziland
91. Sweden
92. Syrian Arab Republic
93. Thailand
94. Togo
95. Tunisia
96. Uganda
97. United Arab Emirates
98. United Kingdom of Great Britain and Northern Ireland
99. United Republic of Tanzania
100. Venezuela
101. Yemen
102. Yugoslavia
103. Zambia
104. Zimbabwe
105. European Commission

Annex 2

Objectives of the Integrated Programme for Commodities (IPC)

The section of the Integrated Programme for Commodities (IPC) covering the Objectives of the IPC reads as follows:

"With a view to improving the terms of trade in Developing Countries and in order to eliminate the economic imbalance between developed and Developing Countries, concerted efforts should be made in favour of the Developing Countries towards expanding and diversifying their trade, improving and diversifying their productive capacity, improving their productivity and increasing their export earnings, with a view to counteracting the adverse effects of inflation, thereby sustaining real incomes. Accordingly the following objectives are agreed:

1. *To achieve stable conditions in commodity trade, including avoidance of excessive price fluctuations, at levels which would:*
   - *(a) be remunerative and just to producers and equitable to consumers;*
   - *(b) take account of world inflation and changes in the world economic and monetary situations;*
   - *(c) promote equilibrium between supply and demand within expanding world commodity trade.*
2. *To improve and sustain the real income of individual Developing Countries through increased export earnings, and to protect them from fluctuations in export earnings, especially from commodities.*
3. *To seek to improve market access and reliability of supply for primary products and the processed products thereof, bearing in mind the needs and interests of Developing Countries.*
4. *To diversify production in Developing Countries, including food production, and to expand processing of primary products in Developing Countries with a view to promoting their industrialization and increasing their export earnings.*
5. *To improve the competitiveness of, and to encourage research and development on the problems of natural products competing with synthetics and substitutes, and to consider the harmonization, where appropriate, of the production of synthetics and substitutes in developed countries with the supply of natural products produced in Developing Countries.*
6. *To improve market structures in the field of raw materials and commodities of export interest to Developing Countries.*
7. *To improve marketing, distribution and transport systems for commodity exports of Developing Countries, including an increase in their participation in these activities and their earnings from them."*

Annex 3

List of Designated International Commodity Bodies (ICB'S)

1. The International Cotton Advisory Committee (ICAC)
2. The International Cocoa Organization (ICCO)
3. The International Coffee Organization (ICO)
4. The International Copper Study Group (ICSG)
5. The International Grains Council (IGC)*
6. The International Jute Organization (IJO)
7. The International Lead and Zinc Study Group (ILZSG)
8. The International Natural Rubber Organization (INRO)
9. The International Nickel Study Group (INSG)
10. The International Olive Oil Council (IOOC)
11. The International Rubber Study Group (IRSG)
12. The International Sugar Organization (ISO)
13. The International Tropical Timber Organization (ITTO)
14. FAO**Intergovernmental Group on Bananas
15. FAO Intergovernmental Group on Citrus Fruit
16. FAO Intergovernmental Sub-Committee on Fish Trade
17. FAO Intergovernmental Group on Grains
18. FAO Intergovernmental Group on Hard Fibres
19. FAO Intergovernmental Sub-Group on Hides and Skins
20. FAO Intergovernmental Group on Meat
21. FAO Intergovernmental Group on Oils, Oilseeds and Fats
22. FAO Intergovernmental Group on Rice
23. FAO Intergovernmental Group on Tea

* Formerly the International Wheat Council
** FAO: Food and Agriculture Organization of the United Nations

The role of ICCO as supervisory body of the project

Jean-Marc Anga
Project Officer, International Cocoa Organization (ICCO)

I am addressing you in the tradition of close co-operation and synergy which has always existed between the International Cocoa Organization (ICCO) and cocoa research institutions across the world.

As a scientist myself, I am only too aware of the important role played by scientists in solving the many problems encountered in the cocoa field. We at ICCO do take a special interest in scientific research. That is why the strategic plan of action to implement the International Cocoa Agreement 1993, has given prominence to the promotion of scientific research and development in the field of cocoa. In so doing, it recognizes the great importance of research in safeguarding the future of the cocoa trade and industry and in helping to improve the situation of farmers in cocoa-producing countries.

The project on '*Cocoa Germplasm Utilization and Conservation: a Global Approach*', for which we are gathered here today, is the biggest project ever sponsored by ICCO, in terms of its financial size, the number of countries involved and its potential implications in solving many of cocoa's diseases. We therefore take a particularly close interest in this project.

Before getting to the supervisory role to be played by ICCO, please allow me to give you an overview of other projects currently sponsored by ICCO, nearly all of which involve CFC.

The project on the generic promotion of cocoa in Japan, implemented by the chocolate and cocoa association of Japan is intended to promote increased consumption of cocoa-based products in Japan through a promotion campaign designed to counteract the negative image which these products had among the Japanese population. The project, which was a resounding success, was completed in November 1997 and the final evaluation will take place in the near future.

The project on pilot plants to process cocoa by-products in Ghana, currently being implemented by the Cocoa Research Institute of Ghana (CRIG) aims to develop and transfer to interested parties the technology for the commercial processing of cocoa by-products and cocoa waste. The project is showing encouraging results and these will be made available to interested parties. This should expand the income-generating capacity of the cocoa industry in cocoa-producing countries.

The project proposal on the use of molecular biology techniques in a search for varieties resistant to witches' broom disease, submitted by the *Comissão Executiva do Plano da Lavoura Cacaueira* (CEPLAC) and involving Brazil, Ecuador and Peru, was approved in October 1996 by the Common Fund for Commodities (CFC). All the necessary arrangements have almost been completed and we are expecting implementation of this project to start very soon.

The project profile to study the chemical and physical parameters to establish the difference between fine and bulk cocoa involving Ecuador, Papua New Guinea, Trinidad and Tobago, and Venezuela was submitted by the *Instituto Nacional de Investigaciones Agropecuarias* (INIAP) of Ecuador, and received an initial positive recommendation from CFC. Further discussions are currently taking place between the ICCO secretariat and the participating countries. It is planned to finalize the project proposal on the basis of these discussions and have the proposal ready for submission to CFC in July 1998.

The project proposal entitled "Contribution of cocoa-growing to the protection of the forest environment in West Africa" was submitted by the *Institut des Forêts-Département Café-Cacao* (IDEFOR-DCC, now CNRA) of Côte d'Ivoire. This project, also involving Ghana and Togo aims at developing and disseminating sustainable systems of cocoa cultivation in West Africa. The project profile was reviewed and accepted in May 1997 by the Consultative Committee of CFC. The ICCO Secretariat and CNRA are currently working on a full project

proposal.

The project proposal on the "Control of cocoa swollen-shoot in West Africa" was submitted by the Cocoa Research Institute of Ghana (CRIG) and also involves Nigeria and Togo. After initial analysis, it was agreed between the ICCO secretariat and CRIG to split the project into two separate components: the first one on integrated vector management and the second one on molecular biology. A project profile on the integrated vector management component is expected to be submitted by CRIG soon.

Another project of great importance to ICCO is the USD 17 million project on the improvement of cocoa marketing and trade in liberalizing cocoa-producing countries. The project aims to improve the quality of exported cocoa, facilitate the financing of the trade, provide market information and address trade and price risks. Just two weeks ago, the final project proposal was reviewed and adopted by the Consultative Committee of CFC. We now hope that the project will be approved by the Executive Board of CFC next April and that implementation can start soon after.

I am now coming to the point regarding the functions and responsibilities of ICCO as Supervisory Body for the germplasm project. I would like you to note that these are laid down in the project agreement signed in December 1997 between ICCO, CFC and IPGRI as well as in the rules and procedures of the second account of CFC.

Without going into too much technical detail and legal jargon, this consists in supervising and monitoring the implementation of the project. In so doing, we have a duty to examine all information submitted by the Project Executing Agency (or PEA, in this project that is IPGRI), with respect to the execution of the project, assess whether the actions undertaken, the expenditures made and the results achieved conform with the provisions of the project agreement and lastly consider the continued relevance of project activities as well as the prospects for its successful implementation.

Our main duties therefore are:

- to act as a focal point for discussions relating to the future development of the project, including possible modifications to be made;
- to receive and analyze the six-monthly and annual reports submitted by IPGRI and prepare regular supervision reports to CFC;
- to undertake the mid-term and final evaluations of the project, in conjunction with CFC;
- in the unlikely event of a suspension of the project, to convene a meeting in order to discuss possible remedies;
- finally, upon completion of the project, to approve the final report submitted by IPGRI and attach any comments we may have.

These are the main functions and responsibilities of ICCO as Supervisory Body for this project. I hope to further clarify these points through any questions you may have.

Mr Chairman, distinguished scientists, delegates, ladies and gentlemen, this project is an important one and we at ICCO regard our role mainly as a facilitator between IPGRI, the participating countries and CFC.

We aim at helping to solve any possible problem which may be encountered and in so doing, try our utmost to ensure a smooth and successful implementation of this project.

The international cocoa community is expecting a lot from this project and with the commitment of all, I am convinced that it will live up to this expectation. May I now conclude my address by wishing you a successful Workshop.

Thank you.

Project history, implementation and governance

Johannes M.M. Engels
Director Genetic Resources Science and Technology Group, IPGRI

Introduction

The project *'Cocoa Germplasm Utilization and Conservation: a Global Approach'* has been developed over an extensive period and is the result of wide consultations and discussions with experts throughout the world. In view of its complexity, its long-term nature (the first phase has a duration of five years), and the importance of linkages to be developed between conservation and use, a detailed account of the project history is presented as well as information on its implementation and governance. The latter two aspects are of interest since the project not only includes fourteen institutions spread around the world, but also involves institutes based in major cocoa-producing countries in America, Africa and Southeast Asia as well as institutions in cocoa-processing countries. Furthermore, public funded institutions work closely with those privately funded and significant parts of the project funds are being provided either as counterpart contributions or by the private sector. These aspects may be of general interest to stakeholders involved in the conservation and use of other commodity crops as well as other species or genepools.

The pre-project proposal stage

The original project idea was born during the European Union funded evaluation of their support to cocoa genetic resources project at the Cocoa Research Unit of the University of West Indies, Trinidad and Tobago (Engels and Dyce 1994). The following quote is taken from the final report:

"Based on the discussions with several institutes and individual scientists, the evaluation mission was able to identify a core of parties interested in developing a proposal on the use of cocoa genetic resources for submission to the Common Fund for Commodities (CFC). In order to leave space for other parties to join and be able to take advantage of rapidly developing technologies, especially with regard to molecular techniques, it is suggested to formulate this project (at least partly) as an umbrella project. New collaborating institutions will be added to the initial group of institutions participating in the project as they are selected during the course of the project implementation, these should be based on the submission of project proposals for which tenders are being requested. This type of approach is being successfully implemented with bananas and plantains.

During the course of finalizing this report it has been possible to draft a project proposal on the characterisation, evaluation and utilization of cocoa genetic resources, to be discussed with interested parties at an informal meeting at the International Cocoa Conference in Kuala Lumpur in 1994, and to finalize the proposal for submission to CFC, through ICCO.

In addition to the more global project approach described in the previous paragraph, it is assumed that an institution similar to the CRU, will be an attractive research partner for special projects which might have a less generic interest. This attraction might further increase when the Unit does not have to worry about "how to survive" and can channel all its energy into the support of research and training activities. Such efforts might be carried out bilaterally, or in close co-operation with other national or international cocoa germplasm collections.

With the implementation of international projects and with the establishment of an endowment fund, there will be an increasing need for some type of governance of these activities. This will be further stimulated through the provision of a legal status to the international collections by bringing them into the FAO International Network. A possible answer to how to structure such "governance" would be the formation of an international cocoa genetic resources network with its own technical steering committee. The latter could be established under the auspices of the ICCO Council. A small network secretariat will be required and a co-ordinator should be appointed to provide the necessary co-ordination as well as the day-to-day administrative and technical inputs while implementing the

agreed workplan. IPGRI could provide the administrative and scientific/technical infrastructure to the co-ordinator, possibly as his or her employer. The members of the network would be all interested countries or institutions that maintain cocoa genetic resources that produce cocoa or that are involved in trade, manufacture or even consumption. The first nucleus could be formed by the parties, which agree to co-operate in the suggested project to be submitted to an interested donor, for example CFC".

After consultation with several potential donors and based on expressions of interest, IPGRI took the initiative to develop a first general project outline in close consultation with Mr. Tony Lass (BCCCA representative) and Dr. Bertus Eskes (CIRAD).

Project proposal development

The aforementioned project outline, which had a strong focus on the conservation aspects but with suggested linkages to breeding and research, was extensively discussed at the occasion of the International Cocoa Conference in October, 1994 in Kuala Lumpur, and during the International Workshop on Cocoa Breeding Strategies, organized by INGENIC, the International Group for Genetic Improvement of Cocoa (End *et al.* 1995). Breeders, researchers, cocoa-producing countries' representatives and industry representatives discussed the proposed outline, made suggestions for changes and strongly supported the initiative. IPGRI was requested to co-ordinate the preparation of a draft proposal and to explore the interest of donors more formally on behalf of the participants in the one-day Workshop.

Informal discussions with CFC in Amsterdam in December 1994[1] resulted in a tentative indication of interest in funding a project on cocoa genetic resources but with a stronger focus on utilization and much less on conservation.

Based on these provisional indications, a first draft of the so-called Part I of the project proposal was developed in close co-operation with Mr. Lass and Dr. Eskes. This first draft, Part I of the CFC/ICCO/IPGRI project proposal, was formally submitted to the International Cocoa Organization (ICCO) in London in December 1994 for their endorsement (with an information copy being forwarded to CFC) after which it was then presented to the International Cocoa Council at its 51st Regular Session in March 1995. The author attended that session and in the relevant travel report[2] noted that the Council supported two suggestions put forward by IPGRI in a letter dated 17 February 1995, i.e.

- endorsed an appeal to the global community to financially support conservation of cocoa genetic resources, especially in its centre of diversity, and to advise on potential donors for this activity, and
- recommended using a proportion of the revenues from the sale of the Buffer Stock for supporting cocoa research activities, in general, and long-term conservation efforts in particular. Further, the Council endorsed, in principle, the objectives of the proposal and that a revised draft be sent to the Expert Working Group on Research for their assessment.

Following the receipt of informal comments and suggestions from the CFC Secretariat and discussions with ICCO a revised draft Part I was developed. This revised draft showed a further increased focus on the utilization aspects of cocoa genetic resources and less attention was given to purely conservation-related activities. CFC suggested that co-financing be secured for the conservation section of the project. This revised draft was forwarded to the ICCO Expert Working Group on Cocoa Research in June 1995 and subsequently discussed by the ICCO Council in September 1995. A slightly modified Part I was formally submitted

1 Travel Report by Jan Engels on visits with officers at the Common Fund for Commodities, Amsterdam. IPGRI, Rome (mimeographed).

2 Travel Report by Jan Engels on attendance at the 51st regular session of the International Cocoa Council in London. IPGRI, Rome (mimeographed).

by ICCO to CFC in November 1995. At this time a first general questionnaire was sent to twenty-three countries to clarify their interest in the project idea, other than the verbal expressions in Kuala Lumpur in 1994. The result was that eighteen countries clearly indicated their significant interest in participating in the project.

The institutions that had earlier indicated an interest were re-contacted with a second questionnaire in April 1996 to assess their specific needs, problems and preferred approaches to address and/or overcome these issues. In addition, specific questions were included to allow a more precise assessment of costs for the individual activities and other aspects which would facilitate the development of Part II of the proposal. Fifteen completed questionnaires were returned to IPGRI and provided the basis for developing a further draft. The latter was submitted to ICCO in February 1996 and thereafter this draft was discussed by the CFC Consultative Committee and suggestions for modifications were made to IPGRI.

As a result of the discussions by the CFC Consultative Committee the eighteen interested partners were contacted once more with very focused questions on the availability of infrastructure, staff and germplasm as well as potential practical and/or legal constraints which might exist whereby they would be unable to participate in the various trials and/or research activities. The replies received allowed IPGRI to finalize a revised draft Part II which was submitted to ICCO in June 1996. Based on the suggestions made by ICCO, modifications were made in the final agreed draft which was formally submitted to CFC in July 1996. This draft was then discussed by the CFC Consultative Committee in September 1996 and recommendations were made to the CFC Executive Board which met in October 1996. Their approval of the proposal and allocation of the total contribution of the Fund to the project allowed the preparation of a draft Project Agreement and Appraisal Report by the CFC Secretariat which was received by IPGRI in November 1996. During the discussions with CFC, IPGRI was informed that countries which are not members of either/or CFC and ICCO cannot participate in the project. This ruling reduced the total number of cocoa-producing countries eligible to participate in the project to ten plus research institutes in Trinidad and Tobago, the UK and France.

During an informal meeting in Salvador de Bahia, Brazil in November 1996 the formally approved project proposal was discussed with all the partners. With the help of the individual co-ordinators general work plans and budgets were developed during the first half of 1997. In April 1997 CIRAD and IPGRI agreed on the part-time secondment of Dr. Albertus Eskes to IPGRI to act as Project Co-ordinator. Subsequently, a small co-ordination unit was established at the International Network for the Improvement of Banana and Plantain (INIBAP), one of IPGRI's three programmes, based in Montpellier, France. In 1997, during Dr. Eskes' visits to African, American and European institutes, the workplans and budget were discussed and amended. This allowed IPGRI, as the formal Project Executing Agency, to comment on the drafts, Project Agreement and Appraisal Report to the CFC Secretariat.

The Project Agreement and the Appraisal Report were finalized and agreed upon in November 1997 and the Project Agreement was formally signed by CFC, ICCO (as the project Supervisory Body) and IPGRI on December 9th, 1997, during the 96th Meeting of the Executive Committee of ICCO in London. Based on the agreed text of the Project Agreement, IPGRI developed the draft text of the Memorandum of Understanding (MOU) that was accepted by CFC and which IPGRI had to conclude with each individual partner to formally establish a legal basis for the contributions to be made by CFC, the co-financiers and the individual partners as well as for the work to be carried out. With the signed MOU's at hand, CFC can make the first budget-allocation to IPGRI and thus make the project effective. In order to make disbursements of project funds, IPGRI must conclude Letters of Agreement (LOA's) with each partner on an annual basis. The first LOA's were distributed to each partner during this Workshop in Montpellier. The project effectively commenced in February 1998.

Project partners

The project consists of research partners world wide, including institutes concerned with cocoa breeding specialized in agronomic, pathological or entomological research, cocoa germplasm programmes, intermediate quarantine centres as well as funding and co-ordination institutions. Details are presented in Table 1.

Organizational aspects

IPGRI, the Project Executing Agency or PEA, is responsible for the implementation of the project. In order to discharge this responsibility in the most efficient way, a small Co-ordination Unit was established at INIBAP. This Unit consists of the Project Co-ordinator (part-time, Dr. Bertus Eskes), a Finance Officer (part-time, Mr. Tom Thornton) and a Programme Assistant (part-time, Ms. Bernadette Sellers and reports to the INIBAP Director (currently Dr. Emile Frison). From an organizational administrative perspective, the cocoa project is one of the activities of the IPGRI project on "Integrating Conservation and Use", for which Dr. Jan Engels is the Activity Manager. Although not a formally recognized "body", an informal executive technical group evolved during the course of the project development consisting of Mr. Lass, and Drs. Eskes and Engels who were in constant e-mail contact and met regularly to handle critical developments.

Each of the partners nominated Technical and Administrative Co-ordinators who are responsible for the co-ordination of the respective activities at their institutes. The group of Technical Co-ordinators plus the Project Co-ordinator (*ex officio*) form the Technical Working Group which is the technical "parliament" of the project. Dr. Jeanne N'Goran was formally elected as its first Chairperson. Details of this group's duties and responsibilities, as well as its *modus operandi*, were discussed during this Workshop and indicated in Appendix I of these Proceedings.

In view of the fact that the co-financing contributions formed a substantial part of the total project budget (approximately 20% of the total of almost ten million US dollars) and since the administration of the co-financing did not fall directly within the responsibilities of the PEA, it was felt that some co-ordination between the co-financiers would be important. Consequently, a so-called Co-financiers Working Group was established under the chairmanship of Mr. Tony Lass. In addition, the chairperson of the Technical Working Group (*ex officio*) and the Project Co-ordinator (*ex officio*) form part of this Group. Details of the terms of reference and *modus operandi* of this Group are included in Appendix II of these Proceedings.

During the Workshop in Montpellier, the importance of efficient communication between all partners was felt to be critical for the progress of the project, and that this should therefore be facilitated as far as possible. It was proposed to ensure that all partners could be contacted via e-mail or fax and that, funds permitting, meetings should be planned on a regional or even global level. The Project Co-ordination Unit has endeavoured to facilitate such contacts between the various pathologists, entomologists and breeders, though funding is a limiting factor.

The legal framework of the project

The technical, administrative and legal aspects of the project were set out in the Project Agreement that was concluded between CFC, ICCO as the Supervisory Body and IPGRI as the Project Executing Agency. The Appraisal Report was an integral part of the Project Agreement and set out the technical details of the project, including a five-year work plan and budget. Within the context of the above documents, IPGRI concluded an MOU with each of the thirteen partners covering the mutual commitments for the entire duration of the project in the technical, administrative and legal sense. With the signed Project Agreement, concluded, MOUs and agreed first-year general work plan and budget at hand, the CFC then

declared the project effective in December 1997, and disbursed the first financial instalment to IPGRI in April 1998. Based on the annually signed LOA's, funds are being transferred by IPGRI following the timely receipt of progress and financial reports.

References

Engels J.M.M. and G.W. Dyce. 1994. Cocoa Research Unit - Trinidad. Phase II Evaluation. Final Report for the European Economic Community. 5th EDF Regional Programme. IPGRI, Rome, Italy.

End M.J., A.B. Eskes, M.T. Lee and G. Lockwood (Eds.). 1994. Proc. of the Int. Workshop on Cocoa Breeding Strategies, Kuala Lumpur, Malaysia 18th-19th October 1994. INGENIC.

Table 1. List of participating/contributing institutions

National cocoa research institutions

- Cocoa Research Institute of Ghana (CRIG), GHANA
- Cocoa Research Institute of Nigeria (CRIN), NIGERIA
- Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), BRAZIL
- Consejo Nacional de Investigaciones Científicas y Tecnológicas, (CONICIT) and the Fondo Nacional de Investigaciones Agropecuarias (FONAIAP), VENEZUELA
- Institut des Forêts, Département Café-Cacao et autres Plantes Stimulantes, (IDEFOR/DCC), now Centre National de Recherche Agronomique (CNRA), CÔTE D'IVOIRE
- Instituto Nacional de Investigaciones Agropecuarias (INIAP), ECUADOR
- Institut de Recherches Agronomiques pour le Développement (IRAD), CAMEROON
- Malaysian Cocoa Board (MCB), MALAYSIA
- Ministry of Agriculture, Land and Marine Resources (MALMR), TRINIDAD AND TOBAGO
- PNG Cocoa & Coconut Research Institute (CCRI), PAPUA NEW GUINEA

International research institutes

- Centre de Coopération Internationale en Recherches Agronomiques pour le Développement/Département des Cultures Pérennes (CIRAD-CP), FRANCE
- Cocoa Research Unit (CRU), University of the West Indies, TRINIDAD AND TOBAGO
- University of Reading, UK

Co-financing organizations

- American Cocoa Research Institute (ACRI), USA
- Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA), UK
- Centre de Coopération Internationale en Recherches Agronomiques pour le Développement/Département des Cultures Pérennes (CIRAD-CP), FRANCE
- International Plant Genetic Resources Institute (IPGRI), ITALY

Supervisory body

- International Cocoa Organization (ICCO), UK

Project executing agency

- International Plant Genetic Resources Institute (IPGRI), ITALY

Main financing institution

- Common Fund for Commodities (CFC), THE NETHERLANDS

Project and workshop objectives

Albertus B. Eskes
CFC/ICCO/IPGRI Project Co-ordinator, IPGRI/CIRAD

Project objectives

The general project objective, as defined in the Project Agreement, is as follows:

"The overall objective of the project is to contribute to the welfare of the large number of smallholders cultivating cocoa through the development and distribution of improved varieties of cocoa with good quality and higher and sustainable productivity levels. This type of cocoa can be produced at lower costs as expenditures on disease and pest control will be greatly reduced. The attainment of this objective will raise the competitiveness of cocoa in the market."

The specific objectives are:

1."*To strengthen national cocoa improvement programmes and increase international collaboration by carrying out co-operative evaluation, selection and breeding activities in number of countries".*
2."*To establish cost effective and efficient conservation, characterization and distribution efforts for available cocoa germplasm".*
3."*To strengthen cocoa germplasm utilization and conservation activities through scientific/technical backstopping, information exchange and human capacity building".*

Project activities

The project activities as identified in the Project Agreement, include the implementation of the following Project Components:

- **International Clone Trials:** A common set of 20-25 clones will be tested at ten project sites and compared to 20-25 clones selected locally. Another 80-150 promising local clones should be identified at each site and evaluated in Local Clone Observation plots. Resistance testing will be carried out for locally important diseases and pests and a 'ring test' be implemented to evaluate resistance of the 'international clones' against different geographical isolates of *Phytophthora* and witches' broom disease.
- **Internationally co-ordinated hybrid trials:** Approximately 40 hybrid progenies will to be created at five sites by inter-crossing selected local clones that were included in the International Clone Trials. This will to permit selection of superior hybrid varieties, comparison of the value of the parental clones with their progenies and increased knowledge on inheritance of traits.
- **Germplasm enhancement:** Resistance to black pod and witches' broom diseases will be enhanced by selecting within crosses between selected clones of the International Cocoa Genebank held at the Cocoa Research Unit (CRU) in Trinidad.
- **Population improvement:** Locally important populations will be selected for yield, quality and resistance to diseases and/or pests in four countries. Exchange of basic breeding material is stimulated between countries facing similar production constraints, thus promoting regional/international approaches to cocoa breeding.
- **Conservation, characterisation and evaluation of germplasm:** Interesting genotypes in international and local collections will be identified. Selected material in the CRU collection will be evaluated for its genetic diversity and agronomic interest. Based on the results, a 'CFC Project Collection' of about 100 accessions will be identified. All data obtained will be incorporated in the International Cocoa Germplasm Database.
- **Distribution and quarantine of germplasm**: This component specifically includes the distribution of the clones for the International Clone Trials, of the CFC Project Collection and of other improved populations.
- **Exchange of information and Workshops:** Reports on project achievements will be

exchanged between project partners. Relevant data will be introduced into existing databases and a compendium of results published as a final project publication. This first project Workshop was organised in order to discuss and agree on standardised procedures for evaluation and selection of cocoa genotypes in project trials. A second workshop will be held at the end of the five-year project.

- **Co-ordination and scientific/technical backstopping:** A Co-ordinating Unit dealing with technical and administrative matters has been established by IPGRI at INIBAP, Montpellier, in 1997. This Unit analyses work plans, budgets and reports prepared by project partners and is responsible for general technical and financial project reporting. Working visits of the Project Co-ordinator to all project sites are being carried out at regular intervals. Statistical analyses of the common data will be made CIRAD, Montpellier and feedback given to all participants. A 'Technical Working Group', including all local technical co-ordinators, and a 'Co-financiers Working Group', with representatives of co-financing institutes were created to follow-up project implementation (see Appendices I and II).

Objectives and organization of the Workshop to devise Working Procedures for Cocoa Germplasm Evaluation and Selection

As indicated above, many of the project activities are being undertaken at a number of sites and under a number of Project Components. Meaningful comparison of results and analysis of data can only be achieved if obtained by standardized methods. To agree upon such methods is not necessarily straightforward because different methods of cocoa germplasm evaluation and selection are practised in different countries. Discussions on standardized methods itself imply an exchange of information on the reliability of the different methodologies. Although the project will try to apply already well established methods, some methodologies are less well known and may need adaptation during the project implementation.

The following workshop objectives were identified:

- Obtain agreed working procedures for common project activities;
- Discuss administrative and financial aspects of project implementation;
- Discuss and agree upon the role of the Technical and Co-financiers Working Groups;
- Distribute budwood for the International Clone Trial; and
- Publish Workshop Proceedings, including adopted working procedures and recording sheets.

Draft working procedures were prepared in advance by experienced researchers. These were presented during the Workshop and discussed in three specific Working Groups: Breeding and Agronomy, Pathology and Entomology. The working procedures were adopted during Plenary Sessions and agreement was obtained as to the type of application for the procedures, as 'Standard' or 'Recommended' in the different project activities (see special chapter at the end of these proceedings). The Working Groups' reports were used to prepare advanced draft working procedures that were then distributed to all project sites. Comments received on the advanced drafts were used to finalize the working procedures. The names of the authors of the draft procedures are indicated in this document, although it was agreed during the Workshop that the finalized procedures should be seen as a collective project publication.

General Project Strategies and Approaches

Strategy for germplasm enhancement at CRU, Trinidad

Iwaro A.D[1] T.N. Sreenivasan[1], D.R. Butler[1], J.M. Thevénin[3], V.Mooleedhar[2] F. Bekele[1], O. Sounigo[3], P. Umaharan[1], and A.B. Eskes[4]

[1] *Cocoa Research Unit (CRU) of the University of the West Indies, Trinidad and Tobago*
[2] *Ministry of Agriculture, Land and Marine Resources (MALMR), c/o CRU , Trinidad and Tobago*
[3] *CIRAD-CP, c/o CRU, Trinidad and Tobago*
[4] *CIRAD/IPGRI, c/o INIBAP, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France.*

Introduction

Genetic enhancement of crops has become necessary to broaden the relatively narrow genetic base of modern crop cultivars and this should use the genetic resources in germplasm collections. Experience acquired by plant breeders over the past century has demonstrated that plant germplasm collections are rich sources of resistance to pests and pathogens. The lineage of many important resistant cultivars can be traced back to germplasm collections (Peterson 1975), yet most collections remain largely unexplored for their potential as reservoirs of resistance (Harlan 1977). The cocoa germplasm collection at the International Cocoa Genebank, Trinidad (ICG,T) is still under-utilized. It is yet to be fully exploited in breeding to reduce the vulnerability of cocoa cultivars to major diseases and pests. The susceptibility of most commercial varieties to black pod and witches' broom diseases poses a challenge to farmers as the cost of chemical control continues to rise. The ultimate solution is to breed resistant varieties and to use them for commercial cultivation. Under these circumstances, it is imperative that germplasm enhancement (pre-breeding) be initiated to provide new genes for resistance to major diseases (including black pod and witches' broom diseases), high yield and to broaden the relatively narrow genetic base of commercial cocoa cultivars.

The ICG,T contains approximately 3,000 cocoa accessions with a great degree of natural diversity. In addition, much of the original material was collected with disease resistance as the major criterion and hence is likely to be enriched in resistant genes. Furthermore, the Cocoa Research Unit (CRU) has been involved historically in the evaluation of field resistance to witches' broom disease in long-term trials and a considerable amount of information on the resistance status of some accessions has been accumulated. This is indeed an asset to the recently initiated germplasm enhancement programme at CRU, which is part of this CFC/ICCO/IPGRI project.

Objectives

A major objective of the germplasm enhancement programme is to develop a population with high levels of resistance to black pod and witches' broom diseases. Since the ICG,T contains diverse germplasm, it is intended to capture as much of this diversity as is consistent with the major objective of enhanced disease resistance. The programme will also give due attention to important agronomic traits.

Methodology

To achieve the above objectives, the germplasm collection at the ICG,T will be exploited as a source of genetic material. Since limited information is available on this collection, it is imperative for this programme to begin with evaluation of important characteristics such as resistance to black pod and witches' broom, and economically important agronomic traits. Information already available at CRU, together with new information acquired from the evaluation exercise, should permit identification of accessions with desirable traits relevant to the main objective of this programme. These promising accessions will be used as base

parents for the germplasm enhancement programme. By inter-crossing these base parents, a seedling population will be generated and later subjected to screening for resistance to *Phytophthora palmivora* and *Crinipellis perniciosa*. The resulting population of resistant genotypes should have a higher frequency of resistance genes than the parental population. Since no selection pressure is exerted on traits other than those of immediate interest (resistance to black pod and witches' broom), gene frequencies for other traits should be unchanged in the final population. It is important that the original population be large and genetically diverse so that the final population will contain ample variability for other traits. Breeders will then be able to find genotypes that combine some level of local adaptation, with other desirable traits in addition to disease resistance. The sequence of activities for the germplasm enhancement programme is outlined in Table 1 and discussed below.

Table 1. Sequence of activities

Stage	Activity
1	Evaluation of accessions for disease resistance (black pod and witches' broom) and agronomic traits (bean number and size)
2	Selection of base parents from the germplasm collection (ICG,T)
3	Establishment of bi-parental crosses
4	Evaluation of seedling progenies for disease resistance (black pod and witches' broom)
5	Establishment of promising seedling progenies in the field
6	Field observation for early flowering, vigour and disease resistance
7	Identification and transfer of promising genotypes/populations to user countries, applying the accepted standard phytosanitary and quarantine measures

Evaluation of germplasm collection

At CRU, considerable effort has been put into evaluating the germplasm collection at the ICG,T for black pod resistance, pod index (an indication of yield potential) and butter fat. Information is also available on some accessions which have been field screened for witches' broom at Marper Farm, Eastern Trinidad. Morphological and molecular characterization is also being undertaken to understand the genetic diversity within the ICG,T. Since the germplasm enhancement programme is primarily aimed at accumulating genes for disease resistance, the procedures adopted for the evaluation of resistance to black pod and witches' broom are being discussed.

Identification of useful sources of resistance to black pod and witches' broom diseases depends on the availability of effective and reliable inoculation methods. This requires the use of a standard inoculum concentration and conditions for the manifestation of infection and scoring for resistance. Several methods are presently available for inoculation, but the fact that we believe that pathogens cannot be introduced into the Genebank eliminates some of these. Other methods are plagued with discrepancies in results, limiting their validity. Available information on the nature of resistance also needs to be taken into consideration in developing new techniques.

Pathogen variation

The variation within and between species of pathogens causing specific diseases of plants is of significant importance in breeding for disease resistance. At least five species of *Phytophthora (P. palmivora, P. megakarya, P. capsici, P. citrophthora and P. megasperma)* have been identified as causal agents of black pod disease at different locations. Two of these species (*P. palmivora* and *P. capsici*) are present in Trinidad and Tobago. With restrictions on

the transfer of pathogens from one country to another, only *P. palmivora* and *P. capsici* can be used for screening in Trinidad, the location for the germplasm enhancement programme. So far, there seems to be constant ranking of resistance in relation to different isolates and even species of the black pod fungus. Van der Vossen (1997) reported that the ranking order for resistance to black pod caused by *P. megakarya* in Cameroon or Togo was very similar to that for black pod caused by *P. palmivora* in Côte d'Ivoire. Iwaro *et al.* (1998) also observed a similarity in the ranking of clones inoculated with *P. palmivora* and *P. capsici*. With respect to *C. perniciosa*, large differences in aggressiveness were observed between group A isolates from Ecuador (and also Bolivia and Colombia) and group B ones found in other areas such as Trinidad and Bahia, Brazil. However, in inoculation tests of such isolates on seedlings of a range of cocoa genotypes, no differential host-pathogen interaction could be detected (Van der Vossen 1997). Based on these observations, both Van der Vossen (1997) and Iwaro *et al.* (1998) indicated that the results of screening germplasm for resistance to *P. palmivora* and *C. perniciosa* at one location (e.g. Trinidad) would be relevant to breeding programmes at other locations for both black pod and witches' broom resistance. The International Clone Trials and the Ring Tests, being undertaken within this CFC/ICCO/IPGRI project, should provide more information on the stability of resistance with respect to variation in pathogen species and strains causing black pod and witches' broom diseases. Meanwhile, it is proposed that an aggressive isolate of *P. palmivora* and *C. perniciosa* be used for evaluation.

Screening procedure for resistance to black pod

Significant success has been achieved in developing a new inoculation method (spray technique) for the evaluation of black pod resistance at CRU (Iwaro 1996). This provides information on both penetration and post-penetration resistance which could enhance the selection of useful sources of resistance to black pod. This method will be adopted for further evaluation of accessions at the ICG,T.

Screening procedure for resistance to witches' broom

Some inoculation methods have been developed for the evaluation of witches' broom resistance in the field (Sreenivasan 1995), in the greenhouse (Frias 1987 and Andebrhan 1983) and in the laboratory (Fonseca and Wheeler 1990, Brownlee *et al.* 1990). Since field inoculation is not allowed at the Genebank, it is difficult to fully take advantage of the field inoculation method for the evaluation of the germplasm collection at ICG,T for resistance to witches' broom. Other inoculation methods are being assessed at CRU to identify a suitable one for the assessment of resistance to witches' broom.

Selection of base parents from the germplasm collection

Table 2 shows some criteria that could be adopted for the selection of parental material.

Table 2. Selection criteria for parents of base populations

	Characteristics
1	Resistance to black pod
2	Resistance to witches' broom
3	Yield characteristics (bean number and size, and pod index)
4	Representation of major groups and populations based on molecular data
5	Representation of major groups and populations based on geographic origin

Based on the currently available information at CRU from the evaluation of disease resistance and characterization activities, 38 accessions have already been selected as base parents for hybridization in the first year. It is intended that about 100 genotypes will be intercrossed over the first three years of the programme to generate new gene pools with an increase in the frequencies of desirable alleles.

Crossing scheme

Available literature on resistance to *Phytophthora* spp. and *C. perniciosa*, and yield in cocoa has shown that these traits are polygenically determined (Tan and Tan 1990, Rudgard *et al.* 1993, Iwaro *et al.* 1997c). Iwaro *et al.* (1997 a and b) have also indicated that *Phytophthora* resistance in cocoa is controlled by at least two different mechanisms. To accumulate genes for resistance, Toxopeus (1974) and Kennedy (1996) emphasized the need for a wide genetic base in cocoa breeding programmes. This requirement is satisfied by selecting a total of 100 diverse genotypes within the major cocoa groups for inter-crossing.

During the Workshop, breeders from different cocoa-producing countries have suggested that enhancement activities also be conducted separately for the major cocoa groups (Trinitario, Refractario and Forastero) as indicated in Table 3. Since little information is available on the genetic variation that could be generated within and between genetic groups, it is proposed that simple bi-parental crosses be conducted in the first year of the programme to assess genetic variation in the levels of resistance within and between groups.

Bi-parental crosses will also be conducted in the second and third year of the programme, using 30 to 50 accessions in each year, and giving emphasis to crosses within groups. This implies that 15 to 25 crosses will be conducted per year. Each cross will be represented by about 40 seedlings. In addition, the parental plants and two susceptible control clones will be grafted for evaluation along with the progenies. About ten grafts will be required per parental genotype. Approximately 1000 plants will be generated annually for evaluation.

Evaluation of seedling progenies for resistance to Phytophthora spp. *and* C. perniciosa

In view of cocoa's long generation time, it is imperative that efficient seedling tests be developed for the assessment of disease resistance. Such early screening tests will allow breeders to assess the genetic potential of the progeny population and conduct effective selection for resistance at the seedling stage.

The seedling populations generated from the bi-parental crosses and the grafted parental plants as well as the susceptible controls will be subjected to early screening tests using a leaf disc inoculation method (Nyassé *et al.* 1995) to determine their inherent genetic resistance to *P. palmivora*. Screening will be conducted for resistance to *Phytophthora* first. Promising resistant genotypes and the controls will be further screened for resistance to *C. perniciosa*. This will however depend on the availability of an efficient early screening test for witches' broom resistance.

The data obtained from the screening exercises will be analyzed to assess the degree of variability within and between populations and determine the relative transgression within crosses in relation to the parental clones. The selection procedure is expected to create two categories of genotypes among the progenies; those that show resistance to both *Phytophthora* and *C. perniciosa*, and those that are resistant to *Phytophthora* alone. The first category of genotypes is most desirable, however, genotypes with good resistance to *Phytophthora* alone will also be preserved and used in further studies.

Establishment of promising seedlings in the field

Promising seedlings with resistance to both *Phytophthora* and *C. perniciosa* or to *Phytophthora* alone will be established in the field. They will be further assessed for such characteristics as vigour, early flowering and field resistance. Confirmatory laboratory tests for black pod

resistance will also be performed, using leaves and/or pods. Although only one cycle in the germplasm enhancement programme is possible within the five-year period of this CFC/ICCO/IPGRI project, it is hoped that an opportunity will be created in the future for the realization of a second cycle, made up of intercrosses between the most resistant plants selected during the first cycle.

Identification and transfer of promising genotypes/populations to user countries

Promising genotypes/populations possessing usable levels of resistance to *Phytophthora* and *C. perniciosa* and showing good yield potential from the germplasm enhancement programme could be transferred to user countries following accepted standard phytosanitary and quarantine measures. These promising materials will be transferred in the form of budwood or as seed (crosses between selected parents).

Conclusion

It is expected that by the end of the programme, populations showing diversity and increased resistance to black pod and witches' broom diseases will have been developed. These will be made available to breeders for evaluation of disease resistance and agronomic characteristics of interest in their localities. This programme caters directly for all cocoa farmers confronted with the problem of black pod and witches' broom diseases. Moreover, it provides a most necessary insurance policy for those cocoa producers not currently affected by witches' broom disease.

Points arising from subsequent discussions

- The distinction was made between germplasm enhancement and population improvement. Germplasm enhancement is just one stage in the process of breeding, and improved germplasm (from pre-breeding) will be incorporated into local breeding programmes.
- Resistance genes are additive, and the aim is to accumulate genes to enhance disease resistance. Crossing with local germplasm is the responsibility of the local breeder.
- Within the five-year period of the project, only one cycle of the germplasm enhancement programme is possible. Given the opportunity (extension of the project), a second round of crosses will be undertaken.
- Concern was expressed that trying to combine resistance to more than one disease may require a compromise (diminishing the resistance of one to accommodate the other). However, it is thought that combining both black pod and witches' broom diseases would be cost effective and a good insurance policy for countries currently without witches' broom.
- It was made clear that, after initial crosses, F1 seedlings will be evaluated (not the pods). Reciprocal crosses will not be evaluated.
- Pre-breeding activities were started with open pollination. There was concern that progenies from pre-breeding should have the combining ability required in the local breeding programmes. It was agreed to request information from each member country on the genetic group preferred, Forastero, Refractario or Trinitario (see Table 3).
- Concern was expressed that the level of enhancement of desirable traits may be restricted for some "populations" if they are kept separate. The need to agree on what constitutes a population was noted.

- In the detached pod spray inoculation method for black pod resistance screening the exact age of the pod is not known. Full-size, unripe pods are selected. Initially, rapid mass screening is carried out to identify promising types. Resistance in these is confirmed by follow-up tests.
- RAPD and isozyme analysis are being used for diversity assessment and verification of the identity of clones. Not all the identities of promising clones have yet been confirmed.
- The importance of the dissemination of information on clones identified with desirable agronomic traits was pointed out. This will be done after confirmation of the results and the identity of the clones.

Table 3. Requested genetic groups for pre-breeding

Country	Trinitario	Refractario	Forastero	Wide crosses
Brazil	X	x	-	X
Cameroon	-	-	x	-
Côte d'Ivoire	X	-	x	-
Ecuador	X	x	x	-
Ghana	-	-	x	-
Malaysia	X	x	x	-
Nigeria	-	-	x	-
Papua New Guinea	x	-	x	-
Trinidad and Tobago	x	x	x	-
Venezuela	x	x	-	-

References

Andebrhan T. 1983. Witches' Broom disease of *Theobroma cacao* in Brazil. 1. Inoculation of ungerminated seeds. Revista Theobroma 13(1):47-49.

Brownlee H.E., A.R. Mc Euen, J. Hedger and I.M. Scott. 1990. Antifungal effects of cocoa tannin on Witches' Broom pathogen *Crinipellis perniciosa*. Physiol. Molec. Plant Pathology 36:39-48.

Fonseca S.E.A. and B.E.J. Wheeler. 1990. Assessing resistance to *Crinipellis perniciosa* using cocoa callus. Plant Pathology 39:463-471.

Frias G.A. 1987. An inoculation method to evaluate resistance to Witches' Broom disease of cacao. The University of Florida, Ph.D thesis.

Harlan J.R. 1977. Sources of genetic-defence. Annals New York Academy of Sciences 287:345-356.

Iwaro A.D. 1996. Cocoa Germplasm Enhancement for Resistance to black pod and Witches, Broom Diseases. Pp. 79-85 *in* Proc. of the Int. Workshop on the Utilisation of the Genetic Resources of the International Cocoa Genebank, Trinidad (ICG,T), 23-28 June 1996.

Iwaro A.D., T.N. Sreenivasan and P. Umaharan. 1997a. *Phytophthora* resistance in cacao (*Theobroma cacao*): Influence of pod morphological characteristics. Plant Pathology 46:557-565.

Iwaro A.D., T.N. Sreenivasan and P. Umaharan. 1997b. Foliar resistance to *Phytophthora palmivora* as an indicator of pod resistance in *Theobroma cacao*. Plant Disease 81(6):619-624.

Iwaro A. D., T.N. Sreenivasan and P. Umaharan. 1997c. Inheritance of Foliar resistance to *Phytophthora palmivora* (Butler) Butler. in cacao (*Theobroma cacao* L.). Euphytica 96: 377-383.

Iwaro A. D., T.N. Sreenivasan and P. Umaharan. 1998. Cacao resistance to *Phytophthora:* Effect of pathogen species, inoculation depths and pod maturity. Eur. J. of Plant Path. 104:11-15.

Kennedy A.J. 1996. The International Cocoa Genebank, Trinidad: Its potential for future improvements in the breeding of cocoa for the next century and the role of the Cocoa Research Unit in its efficient exploitation. Pp 86-89 *in* Proc. of the Int. Workshop on the Utilisation of the Genetic Resources of the International Cocoa Genebank, Trinidad (ICG, T), 23-28 June 1996.

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

Peterson C.E. 1975. Plant introductions in the improvement of vegetable cultivars. Hort Science, 10:575-579.

Rudgard S.A., T. Andebrhan, A.C. Maddison and R.A. Schmidt. 1993. Future prospects for improvements in disease management. Pp 213-220 *in* Disease Management in Cocoa-comparative Epidemiology of Witches' Broom (S.A. Rudgard A.C. Maddison and T. Andebrhan eds.).

Sreenivasan T.N. 1995. A versatile method for field inoculation of *Crinipellis perniciosa*. Ann. Report, Cocoa Research Unit, UWI, Trinidad and Tobago. 75pp.

Tan G.Y. and W.K. Tan. 1990. Additive inheritance of resistance to pod rot caused by *P. palmivora* in cocoa. Theor. Appl. Genet. 80:258-264.

Toxopeus H. 1974. Breeding for Black Pod resistance in *Theobroma cacao* L.. Pp 211-218 *in* Phytophthora Disease of Cocoa (P.H. Gregory, ed.). London, Longman.

Van der Vossen H.A.M. 1997. Strategies of variety improvement in cocoa with emphasis on durable disease resistance. An external review prepared for INGENIC. 32pp. Int. Group for Genetic Improvement of Cocoa.

Strategy to establish a CFC Project Collection

Sounigo O[1], V. Mooleedhar[2], A. D. Iwaro[3], F. Bekele[3], T.N. Sreenivasan[3], J.M. Thevenin[1], N. Khan[3] and D.R. Butler[3].

[1] *CIRAD-CP, Cocoa Research Unit (CRU) of the University of the West Indies, Trinidad and Tobago*
[2] *Ministry of Agriculture Land and Marine Resources (MALMR), Trinidad and Tobago, c/o CRU*
[3] *Cocoa Research Unit (CRU) of the University of the West Indies, Trinidad and Tobago.*

Introduction

One of the activities in the CFC project is the identification and distribution to cocoa breeders of a subset of accessions from the International Cocoa Genebank, Trinidad (ICG,T). The objective is to enrich local working collections with clones selected for a high level of genetic diversity and for specific agronomic traits. A strategy is proposed to identify such a subset that will be called the 'CFC/ICCO/IPGRI Project Collection' or in short CFC Project Collection. This involves the following four main steps:

- Selection within different 'populations' in the ICG,T of clones that show favourable agronomic traits. Each population will be represented by a number of selected clones proportional to the genetic diversity it contains and its expected value in breeding. Priority will be given to resistance to black pod and witches' broom diseases. About 500 clones (to be known as Sub-sample A) will be selected initially.
- After confirmation and further evaluation, multi-trait selection will be applied to identify Sub-sample B (250 clones). The genetic diversity of Sub-sample B will be assessed using RAPD markers.
- A consensus list of 100 genotypes (to be known as the CFC Project Collection) will be established based on agronomic value and genetic diversity.
- The level of heterozygosity of these clones will be evaluated, and they will be identified using a co-dominant molecular marker (RFLP and/or micro-satellites). The clones will be distributed to user countries.

The choice of clones to be included in the CFC Project Collection will depend on existing information and new information obtained during the course of the project, from activities carried out by the Cocoa Research Unit (CRU), on characterization and germplasm evaluation, as well as from other cocoa research institutes, whenever this becomes available. This paper describes the considerations and details of the strategy proposed to establish the CFC Project Collection.

Strategy regarding stratification

Stratification according to morpho-geographical groups

Traditionally, three major morpho-geographical groups are recognized in cocoa: Criollo (C), Forastero (F) and Trinitario (T). Genetic diversity studies, using morphological and molecular markers, have confirmed genetic diversification of these three groups (Engels 1986, Laurent 1993, N'Goran 1994, Lerceteau 1996, Motamayor 1996, Ronning and Schnell 1994). Furthermore, results obtained by Lanaud (1987), Laurent (1993) and N'Goran (1994) show a clear separation within the Forastero group, between genotypes originating from the Lower Amazon (LA) and the ones from the Upper Amazon (UA) basins. Trinitario appeared to be more closely related to LA than to UA, suggesting that this group is the result of hybridization between C and LA. Another hybrid group exists: the Refractario (R), which is derived from three different origins: Nacional from Ecuador, Trinitario from Trinidad and Upper Amazon Forastero from Peru. However, the separation between

representatives of this group and the two others (T and F) represented at the ICG,T is not very clear.

The different groups bring useful traits which are complementary (resistance to WB within Forastero and Refractario and cocoa quality within Trinitario). Based on these observations, we propose the morpho-geographical group as a first level of stratification, although genetic differentiation values between these morpho-geographical groups were rather low (Gst = 0.25) when calculated from RFLP data. Some doubts also remain about which group some populations belong to, such as GU from French Guiana and Nacional from Ecuador (Lachenaud 1997, Lerceteau 1996).

Stratification according to ÊpopulationÁ

The term 'population' designates here a group of clones sharing the same name (for example: MO, SCA, PA, LCT-EEN, ICS.....). This corresponds to various possible situations:

- population may refer to clones issued from one or several neighbouring mother trees, as is the case for the Forastero clones (IMC, NA, PA, MO, etc.) introduced from the collecting expedition by Pound in Peru.
- population designates clones obtained during the same collecting expedition, in a small area (as in the case of the GU) or in a very large area (as in the case of the LCT-EEN clones). The population can be composed of only spontaneous trees (as in the case of the GU) or of a mixture of spontaneous and cultivated trees (as in the case of the LCT-EEN).
- population may refer to clones selected in estates or in research centres. This is the case of the Trinitario and Refractario clones.

In out-breeding species, most of the genetic diversity is often found within, rather than between, population. This situation would lead us to consider a stratification according to population as useless, especially when one considers the imprecision of the term 'population' for Refractario and Trinitario groups. However, the available information on genetic diversity of cocoa suggests a more or less clear separation between some cocoa populations. For example, from RAPD data, it appears that the GU population is clearly separate from the others, and that IMC and NA are more closely related to each other than to the other Forastero populations. Another factor to consider is that selecting clones from different populations increases the chances of selecting alleles which contribute to the adaptation to different environments (e.g. resistance to different strains of a parasite). For these reasons, the proposed strategy will use the population as a second level of stratification.

Strategy regarding the number of accessions to be included

Size of the CFC Project Collection

An appropriate number of clones should be selected to allow a significant amount of the existing diversity to be represented, while still being manageable by breeders in terms of numbers. It has been calculated that there is a 95% probability of finding 80% of the total alleles in 10% of a genetically diverse collection (Brown 1989). In the case of the ICG,T this would mean a requirement of about 250 clones if chosen at random, but this number could be reduced by using an efficient sampling strategy. It is the objective of the CFC project to apply a sampling strategy in such a way that a more manageable sample of 100 clones will be identified as the CFC Project Collection.

Representation of the different morpho-geographical groups and populations

The level of representation of each morpho-geographiocal group and each population is estimated according to its degree of diversity and its expected value in breeding.

When using co-dominant markers, which allow direct observation of heterozygosity, one index for measuring the genetic diversity using biochemical and molecular data is defined by Nei (H), and combines allelic richness (number of alleles per locus) and evenness (distribution of allelic frequencies). The greatest number of genotypes should be selected from morpho-geographical groups and populations showing the highest H value. In the case of isozyme electrophoresis (IE), the use of this value alone may lead to an over-representation of those populations with a low allelic richness but with a high level of observed heterozygosity. It would therefore appear important to also consider the mean number of alleles per locus, the percentage of observed heterozygosity and the level of diversity, calculated on genotypic frequencies, in addition to the final H value. In the case of RAPD data, the level of genetic diversity can be calculated using the Shannon index which does not require any information on the level of heterozygosity. In the case of morphological data, the level of diversity can be estimated by coefficients of variation.

Sample size of morpho-geographical groups

The number of clones to be included in each morpho-geographical group was evaluated, using genetic diversity data obtained from IE (intra-group Nei diversity index (Hi), mean number of alleles/locus, percentage of polymorphic loci, level of observed heterozygosity). The values for Hi were found to be almost identical for three groups (T = 0.37, R = 0.40 and F = 0.38). A slightly higher mean number of alleles was found in the case of Forastero, which also showed a higher percentage of polymorphic loci and a much lower level of observed heterozygosity. These results imply the need for a higher number of Forastero to represent this group. A larger sample size for the Forastero is also required because this group is the main source of useful progenitors in existing cocoa breeding programmes. Another reason for a larger sample size for Forastero is that the Trinitario and Refractario groups are much more closely related to each other than to the Forastero. The Refractario will be represented by a slightly larger number of clones than the Trinitario, because of the slightly higher level of diversity found within the first group. We propose to represent the morpho-geographical groups by the following numbers of clones in the final subset of 100 clones:

- Forastero: 55
- Refractario: 25
- Trinitario: 20

Sample size of populations

Based on the above mentioned theoretical considerations, the numbers of clones (n) proposed to represent populations in the final CFC Project Collection are given in Table 1. For example, a large n value is proposed for LCT-EEN which has a high level of diversity. The individuals in this population, will have to be chosen in such a way that the subset represents most of the large geographical area from which the collection was made. On the other hand, the smallest n values are proposed for ACT, DOM, GU, MAR, R and SJ because of their low allelic richness.

Since the genetic diversity assessment of the ICG,T is far from complete (IE and morphological data are available on around 20% of the clones and RAPD data on around 10% of the clones), the levels of representation of the different populations proposed here will probably need to be modified, when data are available on other populations. For example, preliminary data suggest that cocoa recently collected in French Guiana Lachenaud *et al.* 1997) shows more diversity (than the GU population. This implies that more clones from French Guiana should be included in the CFC Project Collection than suggested earlier.

Table 1. Diversity of the different populations, estimated by several indexes calculated from isozyme data (the numbers in parentheses represent the standard errors) and suggested sample-sizes of these populations in the CFC Project Collection.

Population	Poly-morphic loci (%)	Heterozy-gosity (%)	Mean number of alleles per locus	Shanon diversity index (genotypic diversity	Net diversity index	Suggested sample size in the working collection (n°)
AM	83.3	40 (10)	2.2 (0.3)	0.84	0.40 (0.11)	3
B	83.3	36 (11)	2.3 (0.3)	0.88	0.41 (0.10)	3
CC	83.3	46 (16)	2.2 (0.3)	0.60	0.40 (0.11)	3
CL	83.3	33 (11)	2.5 (0.4)	0.92	0.39 (0.10)	3
DOM	66.7	18 (8)	1.8 (0.3)	0.38	0.16 (0.06)	1
EET	83.3	43 (13)	2.3 (0.4)	0.87	0.41 (0.11)	3
GS	66.7	30 (10)	2.0 (0.3)	0.58	0.26 (0.09)	2
GU	33.3	13 (9)	1.5 (0.3)	0.34	0.14 (0.10)	1
ICS	83.3	42 (11)	2.5 (0.3)	0.91	8.41 (0.08)	4
IMC	66.7	31 (10)	2.3 (0.4)	0.60	0.27 (0.08)	7
JA	83.3	35 (11)	2.3 (0.4)	0.93	0.40 (0.10)	1
LCT-EEN	100	29 (6)	2.7 (0.3)	1.04	0.44 (0.07)	12
LP	66.7	35 (12)	2.0 (0.4)	0.73	0.34 (0.12)	2
LX	66.7	37 (14)	2.0 (0.4)	0.61	0.35 (0.11)	1
MAR	33.3	6 (4)	1.3 (0.2)	0.16	0.06 (0.04)	1
MO	83.3	31 (10)	2.2 (0.3)	0.71	0.36 (0.11)	7
MOQ	83.3	11 (13)	2.3 (0.4)	0.89	0.42 (0.11)	3
NA	83.3	19 (8)	2.3 (0.2)	0.74	0.33 (0.07)	7
P	83.3	18 (10)	2.3 (0.3)	0.55	0.27 (0.05)	7
PA	83.3	18 (6)	2.0 (0.3)	0.67	0.29 (0.08)	7
R	83.3	67 (21)	1.8 (0.2)	0.08	0.40 (0.09)	1
SC	83.3	66 (18)	2.2 (0.3)	0.38	0.44 (0.09)	3
SCA	83.3	30 (9)	2.2 (0.3)	0.79	0.36 (0.10)	7
SJ	66.7	41 (13)	1.8 (0.3)	0.62	0.35 (0.11)	1
SLA	83.3	26 (10)	2.3 (0.4)	0.84	0.43 (0.10)	3
TRD	83.3	22 (8)	2.0 (0.3)	0.60	0.29 (0.07)	1
UF	100	50 (12)	2.5 (0.3)	0.77	0.43 (0.06)	4

Choice of the clones to be included in the CFC Project Collection

General strategy

Once the level of representation has been decided for each population, there remains the question of the choice of the most suitable clones to represent that population. A general strategy is proposed here to select clones within each population, according to their value for useful traits and to the level of genetic diversity that they bring. This strategy is also designed to fit the prevailing situation, since the evaluation of the ICG,T for useful traits is far from complete. Both the genetic diversity assessment and the evaluation of clones are on going.

A first Sub-sample of 500 clones (known as Sub-sample A) will be based on the relative levels of representation of the morpho-geographical groups and populations as described earlier. The choice of the clones to be included in this Sub-sample will be based on the data already available at CRU and in the International Cocoa Germplasm Database (ICGD) and on data from on-going evaluations.

Sub-sample A should then be composed of around 275 Forastero clones, 125 Refractario clones and 100 Trinitario clones. Clones will only be included if they carry at least one useful trait (these traits will be described later). This first selection of the most interesting clones will be made independently within each population.

A further selection step will then be applied to Sub-sample A, by completing information on all useful traits (described later) and choosing the clones with the best values for several of these traits; this selection will be applied independently within each population. This selection step will result in 250 clones, which constitute Sub-sample B, with the respective levels of representation of the different morpho-geographical groups and populations described earlier. This Sub-sample will contain 138 Forastero, 62 Refractario and 50 Trinitario. All the clones from Sub-sample B will be characterized using morphological traits as well as IE and RAPDs.

The last selection step from those in Sub-sample B will result in the CFC Project Collection containing 100 clones. The choice of clones will maintain the level of representation of each population in Table 1, and be based on the genetic diversity data, as detailed below. The 100 chosen clones will be characterized, using co-dominant molecular markers, such as RFLP and PCR-based SSR, to evaluate their level of heterozygosity (an important trait to be considered in further breeding).

Evaluation of useful traits

Resistance to black pod (BP) disease

In Trinidad, this disease is caused by *Phytophthora palmivora* and, to a lesser extent, by *P. capsici*. Two types of information will be used:

- data from laboratory inoculation of detached pods (680 clones already tested)
- data from field observations (171 clones already observed).

Resistance to witches' broom (WB) disease

In Trinidad, this disease is caused by pathotype B of *Crinipellis perniciosa*, which is less aggressive than pathotype A. Two types of information will be used:

- data from field observations (263 clones already observed)
- data from artificial inoculation tests, using the belt spray method (due to start in 1999).

Mean bean weight
Data are available on 234 clones in ICG,T. Newly selected clones for BP or WB resistance will be also evaluated for this trait.

Pod index
Defined as the number of pods needed to obtain 1 kg of dry cocoa, this index has already been calculated for 237 ICG,T clones. Newly selected clones for BP or WB resistance will also be evaluated for this trait.

Butterfat content
Measured as a percentage using Soxhlet extraction technique (solvent extraction and dry weight measurement). Only existing data (273 clones) will be used.

Other traits
The above traits are of priority interest. However, specific information available on other important traits (e.g. *Ceratocystis* resistance, rodent resistance, high yield/vigour ratio, good combining ability, etc.) will also be taken into account.

Priority
Priority will be given to disease resistance, as this is the main selection criterion in the CFC project. The other traits will be used to choose between clones with the same levels of resistance to disease.

Selection of clones according to diversity study data

One way to choose the clones and maintain a maximum genetic diversity within populations is proposed by Noirot *et al.* (1999), using the data of Principal Component Analysis (PCA) or of Factorial Correspondence Analysis (FCA). Clones are selected with the highest contribution to the variability of the studied germplasm, i.e. the clones with highest relative contribution to the general sum of squares, which correspond to individuals placed on the periphery of the graphical representation of groups in the PCA or the FCA. For this, all the genotypes of the ICG,T for which data are available (IE, RAPD and morphological data) will be used for the multivariate analyses. The relative contribution of each clone of Sub-sample B to the total genetic diversity will be determined by select genetically diverse clones and its position on the graphical representation of the multivariate analyses. Selection will aim to discard clones appearing to be closely related.

First steps carried out

CRU has collected data on a varying number of clones depending on the traits considered. Based on this information, a list of 100 clones was recently established. These clones can be considered to be part of Sub-sample A in the above-mentioned strategy of identifying the CFC Project Collection. The agronomic traits considered were:

- resistance to BP, evaluated by laboratory infection of detached pods
- resistance to WB, evaluated by field observations
- pod index
- % butterfat.

This list is presented in Table 2 and these clones.will be further evaluated to identify the ones to be included in Sub-sample B.

Table 2. First selection of candidate clones (part of Sub-sample A) for the CFC Project Collection

CLONE	GROUP *	ORIGIN	CLONE	GROUP	ORIGIN
IMC6	F	Peru	AM238	R	Ecuador
IMC11	F	Peru	B185	R	Ecuador
IMC20	F	Peru	B714	R	Ecuador
IMC47	F	Peru	CL1927	R	Ecuador
IMC57	F	Peru	CL1949	R	Ecuador
IMC67	F	Peru	EET59	-	Ecuador
IMC103	F	Peru	EET396	-	Ecuador
IMC107	F	Peru	EET397	-	Ecuador
NA3	F	Peru	EET400	-	Ecuador
NA26	F	Peru	JA55	R	Ecuador
NA176	F	Peru	JA631	R	Ecuador
NA246	F	Peru	JA934	R	Ecuador
NA387	F	Peru	LP3.4	R	Ecuador
NA 739	F	Peru	LP432	R	Ecuador
P16B	F	Peru	LV6	R	Ecuador
P18	F	Peru	LX48	R	Ecuador
P18A	F	Peru	LZ2	R	Ecuador
PA3	F	Peru	LZ8	R	Ecuador
PA13	F	Peru	MOQ4.28	R	Ecuador
PA27	F	Peru	MOQ116	R	Ecuador
PA30	F	Peru	MOQ524	R	Ecuador
PA46	F	Peru	MOQ655	R	Ecuador
PA125	F	Peru	SLA16	R	Ecuador
PA137	F	Peru	SLC17	R	Ecuador
PA194	F	Peru	SLC18	R	Ecuador
PA296	F	Peru	ICS1	T	Trinidad
SCA6	F	Peru	ICS6	T	Trinidad
SCA9	F	Peru	ICS15	T	Trinidad
SCA12	F	Peru	ICS29	T	Trinidad
SCA19	F	Peru	ICS39	T	Trinidad
SPEC138-11	F	Colombia	ICS41	T	Trinidad
SPEC194-15	F	Colombia	ICS43	T	Trinidad
GU114P	F	French	ICS46	T	Trinidad
GU175P	F	French	ICS60	T	Trinidad
GU219F	F	French	ICS68	T	Trinidad
GU243H	F	French	ICS69	T	Trinidad
GU255P	F	French	ICS70	T	Trinidad
GU285P	F	French	ICS72	T	Trinidad
GU277G	F	French	ICS75	T	Trinidad
GU307F	F	French	ICS84	T	Trinidad
GU310P	F	French	ICS85	T	Trinidad
GU322P	F	French	ICS88	T	Trinidad
CRIOLLO22	C	Costa Rica	ICS95	T	Trinidad
CERRO AZUL	C	Costa Rica	UF11	T	Costa Rica
POR3	C	Venezuela	UF12	T	Costa Rica
PLAYA ALTA	C	Venezuela	CRU70	U	U
R6	C	Mexico	CRU100	U	U
R41	C	Mexico	CRU104	U	U
VEN B 47	C	Mexico	CRU127	U	U
			CRU128	U	U
			CRU133	U	U

** F = Fortastero, C = Criollo, R = Refractario, T = Trinitario and U = unidentified group or origin*

Final discussion and conclusions

The purpose of this paper is to establish a strategy for obtaining a selected cocoa collection that is rich in agronomically promising clones and that shows a high level of genetic diversity. To fulfil these two conditions, the following strategy is proposed:

a) Use of genetic diversity data where available, to establish stratification according to:
   - morpho-geographical groups
   - populations

   Sample sizes are defined for each morpho-geographical group and population.
b) Identification of Sub-sample A (about 500 clones), containing promising clones with at least one favourable agronomic trait, selected within different populations of the ICG,T, according to a).
c) Completion of agronomic evaluation of Sub-sample A, by the end of 2000.
d) Select, from Sub-sample A, the most promising clones, using data from c), within each population, to give Sub-sample B of about 250 clones, by the end of 2000. The level of representation of each morpho-geographical group and population will be decided according to a).
e) Completion of morphological, biochemical and molecular characterization of the clones in Sub-sample B by mid 2002.
f) By mid-2002 select from Sub-sample B about 100 clones (the CFC Project Collection) that combine good agronomic traits with good representation of the genetic variability of each population and of the total variation in the ICG,T.
g) Evaluation of the CFC Project Collection for the level of heterozygosity and identification of the 100 clones with co-dominant molecular markers (RFLP, micro-satellites) to be finalized in early 2003.
h) Quarantine and distribution of clones to user countries to be initiated from mid 2002. It is suggested that all 100 clones for the CFC Project Collection be distributed to all project partners. However, some countries may be interested in more clones from specific populations and may request a broader sample of the populations in Sub-sample B.

The above strategy has several difficulties and limitations. The value of resistane observations in Trinidad to other countries, with different environments and pathotypes, is to be further clarified (this will become apparent in the International Clone Trial). All the resistance tests need confirmation in order to give repeatable results. One of the limitations is that certain genetic populations are poorly represented in the ICG,T collection, e.g. Criollo, Nacional and wild Brazilian types are only present in low numbers. The exchange of data on individual country collections will be very beneficial to the process of identifying the CFC Project Collection. For example, a clone with recognized good field resistance to witches' broom in Brazil or to *Phytophthora* in Africa should be given special attention in the selection process in Trinidad. The collaboration of the project partners towards this common goal will be of utmost importance.

References

Brown A.H.D. 1989. The case for selected collections. Pp. 136-156 *in* The Use of Plants Genetic Resources. (A.H.D. Brown, O.H. Frankel, R.D. Marshall and J.T. Williams, eds). CUP, Cambridge, UK.

Engels J.M.M. 1986. The identification of cacao cultivars. Actae Horticulturae (La Haye) 182:195-202

Lachenaud P. 1997. Genetic/taxonomic structuring of the *Theobroma cacao* L. species. Fresh hypotheses. Ingenic Newsl. 3:10-11.

Lachenaud P., V. Mooleedhar and C. Couturier. 1997. Les cacaoyers spontanés de Guyane. Nouvelles prospections. Plantations Recherche et Développement 4(1):25-32.

Lanaud C. 1987. Nouvelles données sur la biologie du cacaoyer (*Theobroma cacao L.*): diversité des populations, systèmes d'incompatibilité, haploïdes spontanés. Leurs conséquences pour l'amélioration génétique de cette espèce. Doctorat d'Etat, Paris XI.

Laurent V. 1993. Etude de la diversité génétique du cacaoyer (*Theobroma cacao L.*) basée sur le polymorphisme de la longueur des fragments de restriction (RFLP). Doctoral thesis, Paris XI.

Lerceteau E. 1996: Diversité génétique, recherche de QTLs et analyse des profils protéiques de fèves de *Theobroma cacao L.* pendant la fermentation. Conséquences sur la qualité. Doctoral thesis. Paris XI.

Motamayor J.C. 1996. Etude de la variabilité génétique des cacaoyers Criollo du Venezuela (*Theobroma cacao L.*). Rapport de stage du Diplôme d'Ingénieur Agronome. Universidad Central de Venezuela, Maracay, Venezuela.

N'Goran J.A.K. 1994. Contribution à l'étude génétique de cacaoyers par les marqueurs moléculaires: diversité génétique et recherche de QTLs. Doctoral thesis. Montpellier, France.

Noirot M., F. Antony, S. Dussert and S. Hamon. 1999. Une méthode de constitution de core collections. Pp. 77-87 *in* Diversité génétique des plantes tropicales cultivées. Scient. Eds. P. Hamon, M. Seguin, X. Perrier and J.C. Glaszmann. Collection Repères CIRAD.

Ronning C.M. and R.J. Schnell. 1994. Allozyme diversity in a germplasm collection of *Theobroma cacao* L. J. of Heredity 85:291-295.

Use of cocoa populations for breeding purposes

Eskes A.B. and O. Sounigo
CIRAD, France

Introduction

Knowledge on genetic diversity in crop species has direct consequences on efficient utilization and conservation of genetic material. The species *Theobroma cacao* is, genetically speaking, very diverse. Recent studies with molecular markers have given a better insight into the genetic structure of the cocoa species.

The application of a 'population concept' in cocoa is important for the following activities:

Conservation and transfer of germplasm:

- efficient maintenance of diversity (Toxopeus 1997)
- easy transfer by seed or pollen
- 'core collection' strategy.

Utilization of germplasm:

- pre-breeding strategy
- hybrid selection (heterosis)
- population breeding (recurrent selection or reciprocal recurrent selection).

The population approach has been successfully applied in conservation and utilization of many plant species, including perennial crops such as coffee and oil palm. To date, conservation of cocoa has predominantly been as clones belonging to genetic groups (populations) of different geographic origin (of wild or cultivated origins). Breeding of cocoa has used heterosis in crosses between genetically unrelated genotypes. The objective of this paper is to analyze utilization of cocoa populations in the CFC/ICCO/IPGRI project in the light of the currently available knowledge on the genetic composition of *T. cacao.*

Use of populations in cocoa breeding

Indirect evidence of the potential importance of genetic groups (populations) in cocoa comes from observations on heterosis (hybrid vigour, yield precocity) and inbreeding depression. In cocoa it has been possible to trace back populations to some extent by knowledge of the geographic origin of wild, semi-wild or cultivated germplasm. This is an advantage that should continue to be explored in breeding programmes, as explained below.

Traditionally, the following populations are considered: Forastero from Lower Amazon (LA), Forastero from Upper Amazon (UA), Criollo and Trinitario (hybrid group between LA and Criollo). Data obtained with botanical and, more recently, molecular markers confirm the existence of these main genetic groups in cocoa. However, in addition, other groups are now considered as being genetically distinct such as wild French Guiana materials, wild Brazilian accessions and the Nacional variety from Ecuador. Sub-groups within main groups have also been identified, especially within UA. It has also become clear that intermediate forms exist between these populations and that a certain number of problems exist regarding the correct identification of many cocoa clones in collections.

For selection of new hybrid varieties in cocoa, the use of distinct parental base populations is necessary to maintain good levels of heterosis for yield. For example, the existence of distinct genetic sub-groups within UA might partly explain the high agronomic value of crosses between such sub-groups, as shown in a number of breeding programmes (e.g. the BAL breeding programme in Malaysia).

Heterosis for yield should not be confounded with heterosis for vegetative growth. The

wider the genetic distance between cocoa genotypes, the more vegetative vigour can be expected in the cross progeny. However, strong vegetative growth may be useful under difficult growing conditions (dry climate, low soil fertility) but may also have an adverse effect on the yielding capacity under more favourable growing conditions, due to increased competition between plants for space and nutrients.

If clone selection is envisaged, the genetic origin of the parental clones might be less important, because good clones might be found in cross progenies ('speculative crosses') between any two genotypes that show favourable traits. However, the use of genetically diverse parental clones would still be important in order to ensure a certain level of useful heterosis for yield and thus a bigger chance of finding interesting clones in the progeny.

Applications in the CFC/ICCO/IPGRI project

The cocoa breeders have identified the following main populations to be considered for local breeding trials in the CFC/ICCO/IPGRI project:

- two main base populations in the recurrent selection programme in Côte d'Ivoire: UA and LA (plus LA x Trinitario),
- sub-populations of UA and random UA progeny for hybrid selection in Ghana and Nigeria,
- variable populations for clone selection in Brazil, Malaysia and Papua New Guinea,
- the Nacional variety or related material for clone selection in Ecuador, and
- Trinitario or Trinitario x Criollo crosses for clone selection in Venezuela.

Molecular markers will be used to verify the identity of the clones for the International Clone Trial and to establish the CFC Project Collection (Sounigo *et al.* 2000). An attempt will also be made to apply molecular markers to speed up the introgression of favourable traits into base populations by backcrossing (Côte d'Ivoire).

Points arising from further discussions

- The application of the population concept was discussed in relation to the germplasm enhancement programme initiated by CRU in Trinidad. Based on the preference of the breeders participating in the CFC/ICCO/IPGRI project, the following three populations will be treated separately: Upper Amazon, Refractario and Trinitario. The interest of creating a very diverse population by crosses between these groups was also expressed. These approaches will facilitate the introduction of clones or seed populations selected in the germplasm enhancement programme into local breeding programmes.
- An important aspect of population improvement is the size of the base population and how to use these in further breeding. For practical purposes, it is proposed to use relatively modest sized populations (a maximum of 20-30 carefully selected clones for each). These could be inter-crossed in a pairwise manner (20-30 crosses) to allow for random intra-group recombination of favourable genes. It is expected that this approach would permit rapid progress in breeding for the more heritable traits and would maintain a manageable size of populations to be evaluated in the breeding programmes.
- The use of selfing in population breeding programmes has been discussed during the preparation phase of the project and also during working visits carried out in individual countries. Such an approach would be helpful to accumulate favourable genes, to obtain expression of recessive genes and to maintain the level of heterozygosity of parental material within acceptable limits. Too much heterozygosity is expected to decrease the average value of cross progenies and will make it more difficult to select homogeneous hybrid varieties from inter-group crosses. In some countries the value of progenies obtained by self and cross- pollination will be

compared in population breeding activities (e.g. in Ghana and Côte d'Ivoire).

References

Sounigo O., V. Mooleedhar, A. D. Iwaro, F. Bekele, T.N. Sreenivasan, J.M. Thevenin, N. Khan and D.R. Butler. 2000. Strategy to establish a CFC Project Collection. See page 49 in these Workshop Procedures.

Toxopeus H., 1997. What about populations? INGENIC Newsletter 3: 4-5.

Proposed short list of cocoa descriptors for characterization

Bekele F. and D. R. Butler
The Cocoa Research Unit (CRU), University of the West Indies, Trinidad and Tobago

Introduction

The value of characterization data for germplasm utilization is well documented. However, the collation and documentation of these data must be standardized so that they can be of relevance to international collaborative projects such as the CFC/ICCO/IPGRI project. This project, aiming at genetic enhancement/improvement in cocoa, will involve the use of standardized characterization data. The project creates an opportunity to systematically study the role of environment on the expression of phenotypic traits in cocoa.

Another useful output of the project would be information on possible Genotype x Environment (GE) interactions associated with not only agronomic/economic traits (of interest to breeders), but also morphological/botanical ones. The breeder's aim is often to find stable, relatively unresponsive genotypes (Simmonds 1981). It is important to know whether genotypes, identified as promising in the International Clone and Hybrid Trials included in the Project, are stable within and across environments as well as to determine their adaptive potential for the purpose of selection (Hill 1975). One of the objectives of this paper, therefore, is to propose a short list of cocoa traits/descriptors which can be used for such purposes. There is a potential contribution of GE interaction effects to the phenotype of all quantitative traits (Allard and Bradshaw 1964), and attention should therefore be focused on those traits.

For the purposes of characterization, identification and measuring diversity, a number of quantitative as well as qualitative descriptors is proposed. The genesis of this list is described along with the protocols for its use.

Development of a short list of cocoa descriptors

In 1981, the International Board for Plant Genetic Resources (IBPGR) (now the International Plant Genetic Resources Institute) Working Group on the *Genetic Resources of Cocoa* proposed an exhaustive list of 65 cocoa descriptors (Anon 1981). This descriptor list has been adopted internationally, and was used during the past decade to characterize cocoa germplasm at CATIE, Costa Rica (Engels 1981, Enríquez and Soria 1981, Phillips and Enríquez 1988); in the International Cocoa Genebank, Trinidad (ICG,T) (Bekele 1991a and b, and 1993) and at other germplasm collection centres around the globe, as can be seen from the International Cocoa Germplasm Database (ICGD) (End *et al.* 1992 and 2000). During the International Workshop on the *Conservation, Characterization and Utilization of Cocoa Genetic Resources in the 21st Century*, which was held in Trinidad in 1992, the consensus of opinion was that a concise descriptor list of 10-15 taxonomic descriptors was required to facilitate morphological characterization in the various centres. It was therefore decided that such a list would be generated by a core group of scientists with experience in this area and that the resulting list should be discussed, ratified and published.

Several scientists have made contributions to this area of research including Pound (1932), Cheesman (1944), Ostendorf (1957), Enríquez and J. Soria (1967 a and b), Engels (1981, 1983 a and b, 1986, 1993), Eskes (pers. comm.), Bartley (pers. comm.), and Lachenaud (pers. comm.), among others. A comprehensive list of descriptors was compiled by Engels *et al.* (1980), and this formed the basis for the IBPGR recommended descriptor list (Engels 1993). The discriminative values of both qualitative and quantitative descriptors were later assessed by Engels (1983a and b, 1986). A final set of traits for characterization and preliminary evaluation of accessions, based on discriminative value, genetic background and taxonomic and agronomic importance, was recommended by Engels in 1993 (Table 1) following procedures given by Engels (1986).

Table 1. List of ten descriptors selected for characterization by Engels (1993)

Descriptor	Order of taxonomic importance
Anthocyanin intensity in filament	1
Ridge pair separation	2
Anthocyanin intensity in ligule	3
Pod (fruit) apex form	4
Mesocarp hardness	5
Pod surface rugosity (texture)	6
Bean (seed) number per pod	7
Pod width to length ratio	8
Seed index (dry bean weight)	9
Ovary length	10

A limited number of descriptors have been used for characterization by other workers and their choice of descriptors may have been influenced by the nature and objectives of their work. Allen (1988) used pod surface texture, pod apex form, ratios between measurements such as the ratio of pod diameter to pod length, and anthocyanin intensity on unripe pod ridges to describe material collected in the wild during the London Cocoa Trade Amazon Project (LCTAP) expedition in Ecuador. Posnette (1945) found the following subset of descriptors useful:

- separation of pulvini on petioles,
- distribution of flower cushions on the tree,
- tree girth at a specified height,
- flowering patterns,
- age at which flowering starts, and
- pod production.

Phillips and Enríquez (1988) also used a somewhat shortened list of 26 descriptors (one leaf, five flower and 20 fruit descriptors) in their catalogue of accessions from the Centro Agronomico Tropical de Investigación y Enseñanza (CATIE) collection. CIRAD researchers in French Guiana employed 24 morphological descriptors for characterization (one leaf, eight flower and 15 fruit descriptors) (Lachenaud, pers comm.). Many of these were recommended by Engels (1986). CIRAD scientists have also observed that flower descriptors can be useful taxonomically, as has been found in the ICG,T (Bekele 1993, Bekele *et al.* 1994). At CRU, Trinidad, research to develop a short list of descriptors for routine characterization was undertaken from 1983-1985 (Bekele *et al.* 1994). In 1990, the ICG,T embarked on a project to systematically characterize its collection using the full complement of morphological descriptors from the list published by the International Board for Plant Genetic Resources (IBPGR, now IPGRI) (Bekele 1993, Bekele and Bekele 1995a, 1996). In the intervening years, data accumulated through routine morphological characterization were analyzed to identify the most useful descriptors for the short list (Bekele 1991a and b, Bekele *et al.* 1994, Bekele and Bekele 1995a, 1996).

Choosing the most discriminative subset of descriptors is a challenging statistical exercise. However, the role of biological information should not be underplayed, since in time it is possible to gain insight into the merits of descriptors, the ease of observation associated with them and their stability over season and differing environmental conditions. Indeed Beale *et al.* (1967) stated that "The question of how many variables should be used is delicate and is decided on grounds that are only partly statistical."

The collective findings of the aforementioned cocoa researchers resulted in the identification of a short list of cocoa descriptors, which was analyzed, modified and eventually adopted at CRU in February 1995 (Table 2).

Table 2. Short list of morphological and agronomic descriptors adopted by CRU in February 1995

	Descriptor	Category scales/units of measurement, [sample size]
Leaf	Flush colour	0=absent, 3=slight, 5=intermediate, 7=intense [n=4]
Flower	Ligule colour	0=absent, 3=slight, 5=intermediate, 7=intense [n=15]
	Filament colour	0=absent, 3=slight, 5=intermediate, 7=intense [n=15]
	Pedicel column colour	1=green, 2=reddish, 3=red [n=15]
	Style length	mm [n=15]
	Ligule width	mm [n=15]
	Sepal length to width ratio	length (mm), width (mm) [n=15]
	Number of ovules per ovary	[n=15]
Fruit	Pod shape	1=cundeamor, 2=angoleta, 3=amelonado, 4=calabacillo [n=20]
	Ridge colour of mature pod	0=absent, 3=slight, 5=intermediate, 7=intense [n=20]
	Ridge deposition	1=equidistant, 2=paired [n=20]
	Ridge pair separation	1=slight, 2=intermediate, 3=wide [n=20]
	Pod apex form	1=attenuate, 2=acute, 3=obtuse, 4=rounded, 5=mammelate [n=20]
	Pod basal constriction	0=absent, 1=slight, 2=intermediate, 3=strong [n=20]
	Husk hardness	3=soft, 5=intermediate, 7=hard [n=20]
	Pod rugosity	0=absent, 3=slight, 5=intermediate, 7=intense [n=20]
	Pod length to width ratio	length (cm), width (cm) [n=20]
	Dried bean weight	individual peeled beans (g) [n=60]
	Cotyledon colour	1=white, 2=grey, 3=light purple, 4= medium purple, 5=dark purple, 6=mottled [n=20]
	Bean number	[n=20]
	Bean length	peeled length (cm) [n=60]
	Bean width	peeled width (cm) [n=60]
Agronomic	Yield (based on pod index)	Total dry bean weight per pod (g) [n=20]

The selection of descriptors for inclusion in the short list at CRU

In the 80's, research funded by IPGRI was conducted at CRU to develop a short list of the cocoa descriptors (based on the full IBPGR Descriptor List for cocoa) to facilitate morphological characterization (Bekele 1993, Bekele *et al.* 1994). The descriptors selected were those found to be most useful for discrimination (taxonomic purposes). Less useful descriptors, which were correlated to more useful ones as well as those contributing little to the overall variance were discarded. Attention was also paid to the ease of observation of the traits, environmental stability and heritabilities (where known). Some descriptors of economic interest, which were taxonomically useful, were also included.

Many of the quantitative morphological descriptors are of agro-economic interest and some are also taxonomically useful (Bekele 1993, Bekele and Bekele 1993, Bekele *et al.* 1994). Conversely, the subjectivity involved in recording qualitative data may make them less reliable. However, many qualitative descriptors have been found to be highly heritable Engels 1986), and work in the ICG,T (Bekele and Bekele 1993, Bekele and Bekele 1995a, 1996) has demonstrated their taxonomic value. The reproductive traits, particularly those of the flower, were found to be more useful taxonomically than the vegetative ones. This could be explained by higher selection pressure and greater genetic control exerted on the reproductive traits in the process of evolution.

Over the years, efforts have been made to consult with experts, and to further analyze the data accrued (Bekele and Bekele 1995a, 1996) in order to determine the suitability of the short list for routine characterization and to ensure that enough useful information for identification, characterization and assessing diversity is recorded.

Recording morphological descriptor data at CRU

There are several general considerations that are relevant to morphological characterization, the primary ones being the effects of season and environment on the expression of descriptors. Practical constraints in sampling such as the availability of samples at a given time are also relevant. In ICG,T, the first two factors have a significant impact on most vegetative and some quantitative fruit traits. Consequently, data are collected during the same season for each group of descriptors; leaf, flower and fruit. Flush data are recorded mainly during the rainy season (June-December). Flower data are collated throughout the rainy season; mainly between July and December. The fruit (pod and bean) data are collected during the main harvest periods, which occur during the dry season. Most pods are available in late December to March and May to June. A higher incidence of diseased pods is common during the rainy season. Records on weather conditions and cultural and fertilization practices observed in the field collection are kept and distributed with the processed data, which are included in the International Cocoa Germplasm Database.

Some of the colour-related characteristics observed in the laboratory are assessed under uniform daylight conditions. The distinctness of the fruit categories, in most cases, makes the use of an artificial light source unnecessary. However, an artificial light source is used for observing pigmentation in the flower under a Carl Zeiss binocular dissecting microscope. Reference is made to the colour charts of The Royal Horticultural Society (Anon 1966).

Recording leaf descriptors

In the field, an average of four trees with flushes are selected for observation. The intensity of anthocyanin in the new terminal flushes is noted. Care is taken to ensure that the maximum expression of anthocyanin intensity is recorded since light intensity is known to affect this trait.

Recording flower descriptors

In the field, fifteen open flowers are collected, and only disease-free flowers with pearl-coloured thecae are used. Flowers open for more than 24 hours are

recognizable by the brown coloration of the thecae, which progressively moves to the base of the filament (Enríquez and Soria 1967a). Flowers are collected from the trunk and branches, at random, over the tree.

In the laboratory, a stamen from each flower is observed under a Carl Zeiss binocular, dissecting microscope (magnification x 10) to determine whether the filaments are pigmented. This is assessed on a scale of 0,3,5,7 depending on the pigment intensity. The presence of pigment on the pedicel column and on the ligule is also recorded.

One sepal is carefully removed with forceps from each of the fifteen flowers, placed with its abaxial surface facing upwards, and its length and width measured. A petal is then chosen at random and removed from each of the fifteen flowers. The hood is stripped from the petal and then the ligule is separated from the ribbon, cutting at the exact point of colour change or where the widening of the ligule begins. The ligule is then placed on a slide, covered with glycerol followed by another slide and its width is measured in millimetres under an Olympus binocular/compound microscope (magnification x 40). The length of the style is also measured under the compound microscope.

The number of ovules per ovary is counted according to the method prescribed by Lucas (Lucas and Reffye 1981) and Subali and Abdullah (1984). Each ovary is placed on a slide and covered with a drop of Analine blue and lactophenol and the slide is then covered with another slide. In this manner, the ovaries are squashed and the ovules can be detected as luminous, yellowish-green globules against a blue background under the binocular, compound microscope. The number of ovules per ovary can be easily counted four hours after staining.

Recording fruit and seed descriptors

Twenty healthy, well-developed, ripe pods are harvested per accession. If the complete sample is not obtained at one picking, every effort is made to obtain the full complement within one harvest period. Pods are considered mature when the pulp surrounding the seeds is semi-liquid and before the seeds sprout.

The pods are assessed in terms of shape, surface texture (rugosity), husk hardness, colour and disposition of ridges, basal constriction and apex form. The length and width are measured. They are then opened and the wet seed (bean) mass is removed and separated from the central placenta. The number of seeds per pod (excluding flats) and the number of flats are counted. Pods with an inordinately large number of flats are discarded. The mucilage surrounding the seeds of each pod is removed by washing with a high pressure hydro-jet after the seeds have been pre-treated with white lime (calcium oxide) (Bekele *et al.* 1992). The weight of the cleaned, air-dried seeds of each pod is recorded using a Sartorius balance (2,000 gm capacity). The seed samples are then dried in an oven at 105 C for twenty-four hours. The weights of three dried seeds from each of the 20 pods are determined thereafter using an Ohaus 40,000D balance. The testas or seed-coats are peeled off the dried seeds and the weight of the oven-dried, peeled seeds is found. The weight of the testas is then calculated and the percentage testa derived. The individual weights of the 60 peeled seeds are recorded, and their lengths, and widths measured using Vernier calipers.

The pod index is calculated by dividing 1,000 by the product of bean number and individual dried (peeled) bean weight.

Points arising from subsequent discussions

Collation of data at the CFC/ICCO/IPGRI project sites, based on the recommended short list, will allow an assessment of the stability of these traits. Data on some of the economically important traits included in the list such as bean weight, size, number and pod index can enhance the value of the project. The effect of environment on the expression of traits, such as bean weight and butterfat quality, has already been demonstrated by several workers, as outlined by Bekele and Bekele 1995b, and warrants detailed investigation. Wide-scale testing

of promising clones and new varieties developed by the project is recommended (Hill 1975). Genotypes which perform best over a range of environments may be identified. In addition, if all the genotypes respond similarly to all the environments tested, their relative performance in other environments may be predicted with some confidence (Freeman and Dowker 1973). Plots of the response under environments can be used to study specific adaptability.

It is recommended that data from at least four geographically distinct study sites be used for this assessment. In their study of the genetic and environmental variation in twoeconomic traits of white lupin (*Lupinus albus* L.), Julier *et al.* (1995) used four sites. In a cooperative breeding programme in timothy (*Phleum pratense* L.), the effects of site x year on the performance of cultivars were investigated at five locations over a three-year period (Helgadóttir *et al.* 1995).

Conclusion

For practical breeding purposes and for the purpose of assessing the effect of the environment on phenotypic expression in cultivars, characterization efforts need only focus on a few of the descriptors listed in Table 2. Under this CFC project the descriptors of particular interest would be the dried weight of individual beans, bean number, pod index and the percentage testa (shell). Breeders will also require agronomic (evaluation) data, not included in the list of morphological descriptors, such as butter fat content, vigour, precocity, productivity and drought tolerance as well as disease resistance.

This review suggests that a subset of cocoa descriptors may be identified which maintains the general classification structure obtained using the full set of descriptors. Such a concise list of internationally recognized (standardized) cocoa descriptors would be of enormous value to cocoa germplasm curators and users, and would facilitate collaborative programs such as this one. The use of this list should increase the rate of data accumulation considerably, allow a more efficient use of the existing resources and facilitate the preliminary evaluation of promising genotypes.

References

Allard R.W. and A.D. Bradshaw. 1964. Implications of Genotype-Environment interactions in Applied Plant Breeding. Crop Science 4:503-507.

Allen J.B. 1988. Geographical variation and population biology in wild *Theobroma* cacao. Doctoral Dissertation, University of Edinburgh, Edinburgh, Scotland, 198 pp.

Anon. 1966. The Royal Horticultural Society (in association with the Flower Council of Holland). R.H.S. Colour Chart. Leiden, London (reprint).

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

Beale E., M.G. Kendall and D.W. Mann. 1967. The discarding of variables in multivariate analysis. Biometrika 54:357-366.

Bekele F.L. 1991a. Determining the relationships among accessions in the International Cocoa Genebank, Trinidad (ICG,T) using flower descriptors. Pp. 5-17 *in* Ann. Report of the Cocoa Research Unit for 1991.

Bekele F.L. 1991b. The determination of correlations among leaf and flower descriptors for accessions in the International Cocoa Genebank, Trinidad (ICG,T). Pp. 18-24 *in* Ann. Report of the Cocoa Research Unit for 1991.

Bekele F.L. 1993. The use of botanical descriptors - CRU experiences. Pp. 77-91 *in* Proc. Workshop on the Conservation, Characterization and Utilization of Cocoa Genetic Resources in the 21st Century, Port of Spain, Trinidad, 13-17 September 1992. CRU, Trinidad.

Bekele F.L. and I. Bekele. 1993. A concise cocoa descriptor list. Paper presented at the Breeder's Meeting at the 11th Int. Cocoa Res. Conf., Yammoussoukro, Côte d'Ivoire, 17-24 July 1993.

Bekele F.L. and I. Bekele. 1995a. Further studies on a concise list of morphological descriptors for cacao. Pp. 155-165 *in* Proc. of the Int. Workshop on Cocoa Breeding Strategies, Kuala Lumpur, Malaysia, 18-19 October 1994.

Bekele F.L. and I. Bekele. 1995b. A preliminary study of the effects of environment on the expression of some fruit and seed characters in cacao from the international Cocoa Genebank, Trinidad. Pp. 13-32 *in* Ann. Report of the Cocoa Research Unit for 1995.

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

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

Bekele F., P. Birju, D. Nancoo, W. Maharaj, A. Richardson-Drakes, S. Ramnarine, G. Seow, R. Waheed and C. Sankat. 1992. Development of a machine for removing the mucilage from cocoa beans. Pp. 5-10 *in* Ann. Report of the Cocoa Research Unit for 1992. Trinidad.

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

End M.J., R.M. Wadsworth and P. Hadley. 1992. International Cocoa Germplasm Database. BCCCA/University of Reading. BCCCA, London, 355 pp.

End M.J., C.S. Ford, P. Hadley and R.M. Wadsworth. 2000. The role of ICGD in the CFC/ICCO/IPGRI Project. *In* Proc. CFC/ICCO/IPGRI Project Workshop, Montpellier, France, February 1998.

Engels J.M.M. 1981. Genetic resources of cacao. Catalogue of CATIE collection (Technical Bulletin No. 7), Turrialba, Costa Rica, CATIE Plant Genetic Resources Unit, 196 pp.

Engels J.M.M. 1983a. A systematic description of cacao clones. 1. The discriminative value of quantitative characteristics. Euphytica 32:377-385.

Engels J.M.M. 1983b. A systematic description of cacao clones. 11. The discriminative value of qualitative descriptors and the practical compatibility of the discriminative values of quantitative and qualitative descriptors. Euphytica 32:387-396.

Engels J.M.M. 1986. The systematic description of cacao clones and its significance for taxonomy and plant breeding. Doctoral thesis. Agricultural University, Wageningen, The Netherlands, 125 pp.

Engels J.M.M. 1993. The use of botanical descriptors - CATIE experiences. Pp. 69-76 *in* Proc. Workshop on the Conservation, Characterization and Utilization of Cocoa Genetic Resources in the 21st Century, Port of Spain, Trinidad, 13-17 September 1992. The Cocoa Research Unit, Port-of-Spain, Trinidad.

Engels J.M.M.; B.G.D. Bartley and G.A. Enríquez. 1980. Cacao descriptors, their states and *modus operandi*. Turrialba 30:209-218.

Enríquez C.G. and V.J. Soria. 1967a. Seleccion y estudio de los caracteres utiles de la flor para la identificacion y descripcion de cultivares de cacao. Cacao (Costa Rica) 12:8-16.

Enríquez C.G. and V.J. Soria. 1967b. A study of certain leaf characteristics of cacao. Trop. Agric. (Trinidad) 44:117-123.

Enríquez G.A. and V.J. Soria (Eds.). 1981. International Cacao Cultivar Catalogue. Turrialba, Costa Rica: CATIE Perennial Plant Program 1981. Technical Series. Technical Bulletin 6, CATIE, 156 pp.

Freeman G.H. and B.D. Dowker. 1973. The analysis of variation between and within genotypes and environments. Heredity 30(2):97-109.

Helgadóttir A., H. Bjornsson and A. Kristjansdóttir. 1995. Analysis of a site x year experiment with timothy polycross progeny. Euphytica 82:241-251.

Hill J. 1975. Genotype-environment interactions - a challenge for plant breeding. J. of Agri. Science (Cambridge) 85:477-493.

Julier B.; C. Huyghe; J. Papineau; C. Billot and C. Deroo. 1995. Genetic and environmental variation in architecture and yield components in determinate white lupin (*Lupinus albus* L.). Euphytica 81:171-179.

Lucas P. and P.H. Reffye. 1981. Assessment of genetic parameters for some cacao tree clones. Pp. 541-543 *in* Proc. of the 7th Int. Cocoa Res. Conf., Douala, Cameroon, 1979.

Ostendorf F.W. 1957. Identifying characters for cacao clones. Reuniao do Comite Tecnico Interamericano do Cacao 6a, Salvador, Bahia, Brasil. Pp. 89-110 *in* Trabajos presentados.

Phillips W. and G.A. Enríquez. 1988. Catalogo de cultivares de cacao. CATIE, Turrialba, Costa Rica. Programa Mejoramiento de Cultivos Tropicales. Oficina Nacional de Semillas. 1988 Serie Técnica, Boletin Técnico No. 18, 60 pp.

Posnette A.F. 1945. Incompatibility in Amazon cocoa. Trop. Agric. (Trinidad) 22:184-187.

Pound F.J. 1932. The genetic constitution of cacao crops 1. Pp. 9-26 *in* Annual Report of Cacao Research 1931-1945. Imperial College of Trop. Agri., Trinidad.

Simmonds N.W. 1981. Genotype (G), Environment (E) and GE components of crop yields. Exp. Agriculture 17:355-362.

Subali S. and I. Abdullah. 1984. Preliminary studies on cacao ovules in relation to bean number and bean size. *In* Proc. of the Int. Conf. on Cocoa and Coconuts, Kuala Lumpur, Malaysia, 1984.

Role of the ICGD in the CFC/ICCO/IPGRI project

End M.J., C.S. Ford, P. Hadley and R.M. Wadsworth
International Cocoa Germplasm Database (ICGD), The University of Reading, United Kingdom

Scope

The International Cocoa Germplasm Database (ICGD) has been designated as the vehicle for the exchange of information on germplasm traits for this project. The ICGD will build on its existing role as an information source for the cocoa research community and attempt to encourage further exchange of information in the most useful way for genebank curators and breeders. In this paper we set out to:

- establish how the ICGD can be developed to best satisfy the information needs of the project
- identify the types of information that should be included in ICGD and how they should be recorded
- propose ways to stimulate the flow of information into the ICGD.

Introduction

Efficient information exchange is vital for the success of any collaborative project. A central repository of information that will be easily accessible, helps:

- to increase the rate of exchange of data from different research centres
- to provide data for the assessment of environmental effects and genetic stability
- to minimize duplication of effort
- to minimize mislabelling problems in clone trials/genebanks, and
- to increase the potential value of individual accessions.

The University of Reading/LIFFE International Cocoa Germplasm Database (ICGD) project has the objective of providing a comprehensive source of information on germplasm for the international cocoa community. Thanks to the long-term funding commitment from the UK cocoa trade and industry (LIFFE and BCCCA) and the continuing support of research centres and researchers world-wide, the ICGD project is now in its tenth year. Outputs from the project include four releases of computerized databases and two printed versions which have been distributed free of charge to all those research institutes and organizations which have contributed to the project.

The success of the ICGD is largely due to the co-operation of research centres, and in particular, individual cocoa breeders and geneticists who have generously provided information in the form of publications, annual reports and personal communications for incorporation into the database. We always value the input from users of the database in improving the design and content of the database as it will ensure that the ICGD meets the needs of the CFC/ICCO/IPGRI project partners, and the wider cocoa research community.

The latest printed version of the ICGD, released in December 1997, gives information on over 17,000 clone names, arranged in two main tables:

- The "Clones" table which gives any available information on the origin, present location and characteristics for each clone
- The "Characters" table which groups similar types of information (for example, bean weight measurements or disease reaction data) in tabular format to facilitate searches for a particular characteristic or group of characteristics.

A coding system ensures that the original source of each piece of information can be determined and the full reference is given in the bibliography.

A brief examination of this printed version will indicate that some clones have been thoroughly characterized and evaluated in several research centres, whereas little information is available for others. Many research centres have active programmes to describe their germplasm and this information is forwarded to the ICGD managers as it becomes available. However, it will be many years before every clone has been fully characterized and evaluated. In the meantime, we hope that research centres will contribute any information they have on their germplasm, for example rough notes on pod size and colour, photographs and outline drawings of pod shapes, field observations on pest/disease reactions and agronomic performance, since this information may form a useful stopgap until the full descriptions are available.

Future

We are currently working towards the release of a new computerized version of the ICGD. We need information from ICGD users on the following:

The design of the ICGD

- Type of computing equipment and software available at your institute. This includes; computer processor (Pentium etc.), amount of RAM, hard disk capacity and amount of disk space available for ICGD, floppy disk and CD drives (type and capacity), operating system (DOS, Windows 3.1/95/98/NT).
- The current computerized version of the ICGD for DOS requires some 17Mb of disk space. Does this cause any problems and would you be interested in obtaining a "reduced version" containing a subset of the available information? If so, what would you regard as the essential information? Alternatively, we are producing a version of the ICGD on compact disk (CD) which will require very little hard disk space. This allows the incorporation of photographs and other graphical information which requires a large amount of storage space.
- If you are familiar with the Windows environment, would you prefer the ICGD to have a Windows "look and feel".
- We intend to produce a version of the ICGD which will allow users to enter their own data. We need to know whether you would find this useful, and how this could be integrated with your existing software which is used to store and analyze germplasm data.
- We are considering setting up a secure web-site for the ICGD and would be interested to find out whether you have access to the Internet and if you think this would be useful for accessing the ICGD or transferring data.
- The main language of the ICGD is English, although French and Spanish versions of the manuals have been produced. We would like your views on how we can make the software easier to use for people who do not have English as their first language.
- The ICGD managers at Reading have always offered an "information service" for those who do not have easy access to computing facilities and those who would like answers to complex or unusual searches. Do you think this is a useful service, and if so have you any suggestions as to how we can improve it?

Types of information/methods of recording

The ICGD includes all information on cocoa germplasm that is received, with priority given to information on clones held in international and national germplasm collections. Your views would be welcome on data that could usefully be included, such as information from breeding trials, local selections, including text, numeric data, photographs and diagrams.

The details of the information to be recorded by each of the project activities will be established in the appropriate sessions of this Workshop. Other useful information includes:

- the origins of clones (collection details for uncultivated material, parentage or selection details),
- the donor collection (where the material came from and when),
- characterization data (any morphological information such as colour, size and shape of the flower, pod or bean),
- evaluation data (any information on performance, disease/pest reaction),
- photographs and diagrams,
- genetic fingerprinting data (RFLP, RAPD, isozymes, micro-satellites, etc.).

The ICGD managers are pleased to accept data in any form, including publications, hand-written notes, spreadsheet or database tables. However, the following suggestions could make the process of entering data more efficient:

- Supply data in a tabular format wherever possible and ideally as files on diskette (dbase/access, lotus/excel or word processor)
- Use a standardized way of recording clone names. We would suggest the following:
  - Group or Family Name (15 characters).
  - Number (5 digits).
  - Suffix (10 characters).
  - Duplicate Identifier (three characters). This would ideally be the identifier used by the ICGD to distinguish similar clone names but could be some other information, such as country of origin or donor collection, which will help us to find out which clone is being referred to.
- Provide a full reference to the source of the information.
- Provide full experimental details (or refer to the appropriate CFC/ICCO/IPGRI project documentation)
- Provide a detailed key to any abbreviations used (including an English translation of any local terms where necessary).

Ways to increase the flow of information into the ICGD

Research centres already send their data for incorporation into the ICGD on a regular basis. However, we believe that there is considerably more information on cocoa germplasm that already exists in research centres but has not yet been included in the ICGD. This information could be extremely valuable to all those working on cocoa and we make the following suggestions to stimulate the flow of this information into the ICGD:

- Users of the ICGD should examine the bibliography of the ICGD to check that their publications referring to germplasm characterization or evaluation have already been included.
- Annual reports, internal reports, theses, student project reports and any other publications which do not have a wide circulation could be copied to the ICGD.
- Articles from journals which are not widely available in the UK (such as Agrotropica from Brazil and many more) would be gratefully received.
- Supply any information on germplasm and its history, including hand-written notes, packing lists, old accession lists, maps of genebanks and correspondence referring to germplasm exchange or availability.

Opportunities and procedures for the exchange of cocoa germplasm of common interest to the CFC/ICCO/IPGRI project

Engels J.M.M.
IPGRI, Italy

Introduction

The availability of sufficient and adequate genetic diversity of the cocoa (*Theobroma spp.*) genepool is of fundamental importance to cocoa breeders. Such diversity has naturally developed in Central and South America as well as through the interference of human beings since the domestication of *Theobroma cacao* L., the major cultivated species of the genus *Theobroma*. The diversity which resulted from human interference through breeding and selection has been widely spread and significant working and/or conservation collections have been established in all major cocoa producing countries. However, the diversity present in these collections is frequently rather limited and usually reflects the priorities of past improvement efforts and is seldom a due representation of the existing diversity of cocoa in its centre of diversity in the Americas. Consequently, ready access to germplasm in genebanks and the exchange of germplasm between breeding and research programmes is of vital importance in order to allow continuous improvement of the crop as well as to meet future problems and needs.

Apart from the considerable numbers of accessions present in international cocoa field genebanks at Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica and the Cocoa Research Unit (CRU), Trinidad and Tobago, as well as in national collections that exist in almost all the cocoa-producing countries, germplasm material is also maintained in the intermediate quarantine centres at Barbados, which is linked to the International Genebank at Trinidad and Tobago, the University of Reading (UK) and CIRAD in Montpellier, France. Although the total number of accessions is limited in these quarantine centres, they frequently represent material which is of known agronomic importance and free of diseases, as far as observations and applied methods allow.

In view of the above and since the objectives of the project are based on the availability of a wide range of genetic diversity at all partner sites, the exchange of germplasm is a vital component of the project. Presentation of the major legal implications on access and exchange of genetic resources are justified by the recent developments in the field of access and other regulations regarding biodiversity in general, largely triggered by the conclusion of the Convention on Biological Diversity (CBD), and Plant Genetic Resources for Food and Agriculture (PGRFA) in particular. This focus was predominantly caused by the negotiations of the International Undertaking on Genetic Resources of the Food and Agriculture Organization of the United Nations.

Legal frameworks for the exchange of germplasm

Through the ratification of the Convention on Biological Diversity (CBD), countries have collectively established a legal framework for the conservation and use of biodiversity. The CBD affirms that:

- *"States have sovereign rights over their own biological resources" and "the authority to determine access to the genetic resources";*
- *"States are responsible for conserving their biological diversity and for using their biological resources in a sustainable manner";*
- *"[...] the close and traditional dependence of many indigenous and local communities....on biological resources, and the desirability of equitably sharing benefits arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and the sustainable use of its components";*
- *"[...] economic and social development and poverty eradication are the first and overriding*

overriding priorities of developing countries";

- *"[...] each Contracting Party will endeavour to create conditions to facilitate access to genetic resources [...] and not to compromise restrictions that run counter to the objectives of the Convention";*
- *"access, where granted, will be on mutually agreed terms [...]" and "subject to prior informed consent of the Contracting Party providing such resources [...]".*

The Convention also recognizes "[...] *the importance of, and the need to promote, international, regional and global cooperation [...].for conservation [...].and use [...]".*

FAO International Undertaking

In 1983, the Food and Agriculture Organization of the United Nations (FAO) adopted the "International Undertaking on Plant Genetic Resources". This non-binding set of rules is aimed at halting the rapid and uncontrolled disappearance of crop plant diversity from genetic erosion by means of international and national conservation efforts, and at keeping the germplasm readily available for research and breeding purposes. The International Undertaking originally subscribed to the rule of free exchange of all plant genetic material. Article 1 states: "*This Undertaking is based on the universally-accepted principle that plant genetic resources are a heritage of mankind and consequently should be available without restriction*". Disagreement over the scope of intellectual property protection, and specifically over whether breeders' lines and material protected by plant breeders' rights should be available without restriction, led to the subsequent narrowing of the free exchange principle. In 1989, FAO adopted two resolutions providing an "*agreed interpretation*" according to which plant breeders' rights are not incompatible with the Undertaking, and that parties to the Undertaking may impose restrictions on the free exchange of material which is in the process of being bred. Such material would only be available at the discretion of the plant breeder. As a counterbalance to this concession, the concept of "*farmers' rights*" was endorsed, representing a commitment by the signatory countries to recognize and reward "*the enormous contribution that farmers of all regions have made to the conservation and development of plant genetic resources*". A 1991 FAO resolution, while recognizing the common heritage principle, subordinated it "*to the sovereignty of the states over their plant genetic resources," and modified the free availability rule by stating that "conditions of access to plant genetic resources need further clarification*".

The agreed legal framework for the exchange of germplasm and information in the project

Within the context provided by the CBD, influenced by the ongoing negotiations in the FAO Commission for Plant Genetic Resources for a multilateral exchange system as well as by regional/national access legislation, the partners have negotiated a legal framework for the project that is conducive to the exchange of cocoa germplasm. This framework respects the sovereign rights of states over genetic resources within their borders and facilitates the sharing of benefits that will derive from the breeding and research activities on the germplasm in the project. The relevant parts of the Project Agreement are cited below to illustrate the agreed framework.

"The Fund (CFC), the Supervisory Body or SB (ICCO) and the Project Executing Agency or PEA (IPGRI), in which capacity the latter shall represent the Participating Institutions and the countries participating in the Project, shall enjoy joint ownership of all results and technical outputs arising from the Project.

With regard to dissemination of technology, know-how and germplasm material:

a) The PEA shall procure that the Participating Institutions and the countries participating in the Project bindingly agree that the Fund, the SB and the PEA shall make available to Developing Countries at cost of production and dissemination, or, in the case of Least

Developed Countries, free of charge, the technology and know-how acquired during or as a result of the Project. This shall include printed or electronic databases, publications and documentation produced under the Project. Specific conditions may be set out in each case for the further use by third parties of technology or know-how thus acquired;

b) *In the event of any Participating Institution wishing to take out a property right on technology or know-how acquired during, or as a result of the Project, this shall be negotiated with the Fund, the SB and the PEA, in connection with which the latter shall represent the other Participating Institutions and the countries participating in the Project. The taking out of any such property right as referred to above, as well as the nature and extent of such right, shall be subject to the prior approval of the Fund, the SB and the PEA, the latter acting in the above referenced capacity;*

c) *Cocoa germplasm accessions distributed from the quarantine centres at Reading and Montpellier for inclusion in experimental trials are in the public domain and, not being subject to any property rights restrictions, may be used by any* bona fide *user;*

d) *Any selected genetic material and information derived as a result of the Project from public domain accessions, not being subject to property rights, will be made available to all Participating Institutions and countries participating in the Project without any restrictions;*

e) *Locally available cocoa germplasm to be included in project trials will be made available to any Participating Institution and country participating in the Project on mutually agreed terms, in compliance with the provisions of the Convention on Biological Diversity."*

The safe movement of cocoa germplasm

Apart from the legal conditions and requirements which need to be considered while exchanging cocoa germplasm there are also biological considerations which restrict the exchange. In order to illustrate the latter and to provide a general framework for the measures to be taken, the general recommendations from an expert meeting that developed the Technical Guidelines for the Safe Movement of Cocoa Germplasm (Frison and Feliu 1989) follow.

The guidelines set out below should be followed when transferring cocoa germplasm:

- Shipping pods or rooted plants is not recommended.
- Material should be moved as seeds whenever possible, according to the following procedure:
  1. Collect apparently healthy seed pods.
  2. Open pods and discard all immediately suspect pod and seed material.
  3. Remove pulp from seeds and reject all seeds from a pod if any seeds appear defective.
  4. Remove surface moisture from the seeds and treat them with appropriate fungicides and insecticides (see specific guidelines) according to manufacturers' instructions.
  5. Pack in a suitable medium, such as sterile sawdust. Outer packing material should be sterilized and treated with a contact insecticide. Packing should be carried out in a clear, covered area out of the wind.
  6. Seeds should be subjected to strict post-entry quarantine following arrival in the recipient country for the duration of at least three growth flushes.
- All vegetative material should be imported only through intermediate quarantine using the following procedures:
  1. Budwood should be selected from healthy plants and washed in soapy water. It should then be dipped in a water solution or a suspension of a combination of an appropriate fungicide and insecticide according to the manufacturers' instructions.
  2. The ends of the budsticks should then be dipped in paraffin wax to prevent desiccation.
  3. Each budwood stick should be labelled clearly so that no confusion may occur, and

each accession should be packed separately.

4. Budsticks should be incinerated in intermediate quarantine after removal of the buds.
5. Following arrival in the recipient country the budded material should be kept under close observation for at least three growth flushes.
6. Material originating in countries where virus diseases are known to occur should be indexed during intermediate quarantine (see specific guidelines for swollen shoot virus).
7. Material sent from intermediate quarantine to the recipient country should undergo post-entry quarantine for at least three growth flushes.

- Unpacking of seeds and budwood should be carried out in an enclosed area to contain any escaping insects. Appropriate protective clothing should be worn to prevent skin/eye contact and breathing of chemicals from the packing material. The packing material should be destroyed.

The following pests of quarantine importance are treated in detail in the above mentioned Technical Guidelines:

- *virus diseases*: cocoa swollen shoot, cocoa necrosis, cocoa yellow mosaic and other virus-like diseases;
- *fungal pathogens*: witches broom, *Moniliophthora* pod rot, vascular streak dieback, *Phytophthora* and other fungal diseases;
- *insects*: mirids, mealy bugs, thrips and termites.

Identified germplasm for exchange

Several project activities depend to a larger or smaller extent on the exchange of identified or to-be-identified germplasm. This includes:

- International Clone Trial (ten countries)
  Approximately 25 clones from intermediate quarantine
  Approximately 25 clones selected locally
- International Hybrid Trial (six countries)
  Approximately 40 hybrid progenies using selected local clones as parents. Most of the material will be of local origin and would thus not fall into the category of germplasm which is meant for exchange.
- Pre-breeding activities
  This material might have to be distributed as seed
- Population breeding activities
  Possible exchange of parental genotypes or seed progenies
- Establishment of the CFC/ICCO/IPGRI Project Collection
  Approximately 100 selected clones representing the widest possible and economically relevant genetic diversity through intermediate quarantine facilities.

References

Frison E.A. and E. Feliu. 1989. FAO/IBPGR Technical Guidelines for the Safe Movement of Cocoa Germplasm. Food and Agriculture Organization of the United National Rome/International Board for Plant Genetic Resources, Rome.

Working Procedures for Cocoa Germplasm Evaluation and Selection

The Workshop developed 31 Working Procedures for measurements of parameters in the Project. These follow and are grouped in three sections:

- Implementation of Selection Trials and Data Collection (Working Procedures 1 to 13).
- Disease Resistance (Working Procedures 14 to 24).
- Insect Resistance and Control (Working Procedures 25 to 31).

These Working Procedures are now being used in the Project and may be modified in the light of field experiences. We hope that other researchers will also adopt them and propose possible amendments to us for further improvements.

The partners of the Project are encouraged to refer to the next chapter in these proceedings, entitled Application of the Working Procedures in the CFC/ICCO/IPGRI Project (page 164).

Implementation of Selection Trials and Data Collection

1. Choice of clones and rootstock for the International Clone Trial

Scope

The objective of the International Clone Trial is to compare a common set of about 20 clones in ten different countries. This will permit us to verify the stability of selection traits (yield, resistance and quality) in different environments. These clones will be compared to 20 local clones, either in the same trial or in a second trial, by using common control clones. The proposed method of multiplication of the clones is by hypocotyledonary budding onto a rootstock variety. The choice of the clones and type of rootstock is described here.

Clones from intermediate quarantine centres ('International Clones')

Choice of clones

The clones for the International Clone Trial were chosen among those available in the intermediate quarantine stations at Reading University and at Montpellier. The choice was based on existing information on black pod resistance and yielding capacity as well as on the genetic origin of the clones (see International Cocoa Germplasm Database, ICGD). A first list of 44 clones was prepared and circulated among the project partners to identify local preferences. Based on the replies from the countries a shortlist of 25 clones was identified (Tables 1 and 2). During the Workshop it was decided that, as far as possible, these 25 clones will be introduced into all ten countries participating in the trial.

Limitations

For phytosanitary reasons, South American countries decided not to introduce clones of African origin; this is reflected in Tables 1 and 2. These countries, as well as some other countries, requested additional clones from the list of 44 clones to meet local interest and as alternatives for the International Clone Trial if introduction and/or multiplication of clones of Tables 1 and 2 failed. Two clones (Na33 and Playa Alta 2) will not be distributed to all countries because of requirements for the duration of intermediate quarantine (two years). They will be used mainly in the countries where these clones are already locally available, if

the identity is confirmed.

According to local regulations, introduction of new clones into Trinidad and Malaysia requires post-entry quarantine of two years. These countries will use locally available clones (if identity confirmed) whenever possible to speed up the multiplication process and the initiation of the International Clone Trial.

It is anticipated that due to problems arising from transfer and multiplication, the effective number of common project clones established in each country will be generally lower than 25 (though probably above 15).

Verification of clone identity with DNA markers

Identity of the clones distributed from quarantine (Table 1 and 2) will be compared to that of clones with the same name that are available at the different project sites. During this Workshop it was decided that two types of DNA markers will be applied, i.e. 'conventional micro-satellites' at CIRAD, Montpellier, and 'anchored micro-satellites' at Reading University. If a DNA sample is found to be different by either one of these methods, the local source of the clone will not be used for the International Clone Trial.

Choice of local clones and control varieties

Each country will choose a set of 20 local clones that are to be compared to the 'International Clones'. The local clones should present potential commercial and/or breeding interest. They will be selected from outstanding hybrid progenies or from working collections.

During this Workshop, it was decided that each country would choose three standard clones to compare with the 'international' and 'local' clones. In the case where the 'international' and 'local' clones are tested in two separate trials, the same control clones will be in both trials. Well-known clones that have been largely used in breeding or commercial varieties should be chosen.

Choice of rootstock varieties

The choice of the rootstock variety will take into account local requirements and experience. Relative genetic uniformity is to be favoured to avoid undesirable between plant variation in the clone trials. An important criterion for Ecuador, Trinidad and Venezuela is resistance to *Ceratocystis* wilt. The originally proposed use of Amelonado or Catongo rootstock in African countries was modified because of expectedly poorer establishment ability provided by these varieties. The final choice of rootstock varieties is shown below. During discussions it was accepted that Côte d'Ivoire will use rooted cuttings instead of budded plants for the 'local clones', to be tested in a separate trial.

Country	Rootstock	Observations
Trinidad	IMC67xAmelonado	Resistance to Ceratocystis
Venezuela	IMC67xCatongo	Resistance to Ceratocystis
Ecuador	IMC67xCatongo	Resistance to Ceratocystis
Brazil	Catongo or Comun	Uniformity
Côte d'Ivoire	IMC67xAmelonado	Uniformity
Cameroon	IMC67xAmelonado	Uniformity
Nigeria	F3 Amazon	Establishment ability
Ghana	PA7 x T79/501	Establishment ability
Malaysia	Local hybrid seed	Local experience
Papua New Guinea	K82 x KEE12	Large bean size and vigour

Author of draft procedure

A. B. Eskes, IPGRI/CIRAD, c/o INIBAP, France

Table 1. International Clone Trial: choice of clones from the Reading Quarantine Centre

Clones	Brazil	Ecuador	Trinidad (MALMR)	Vene-zuela	Came-roon	Côte d'Ivoire	Ghana	Nigeria	Malaysia	Papua New Guinea	Total no.
AMAZ15-15	*	*	*	*	*	*	*	*	*	*	10
BE10	*	*	*	*	*	*	*	*	*	*	10
EQX3360-3	*	*	*	*	*	*	*	*	*	*	10
LCT-EEN46	*	*	*	*	*	*	*	*	*	*	10
MAN15-2	*	*	*	*	*	*	*	*	*	*	10
MXC67	*	*	*	*	*	*	*	*	*	*	10
PA107	*	*	*	*	*	*	*	*	*	*	10
Playa Alta 2	*	*	*	*	*	*	*	*	*	*	9
SCA6	*	*	*	*	*	*	*	*	*	*	10
SPEC54-1	*	*	*	*	*	*	*	*	*	*	10
Total (trial)	10	10	10	10	10	10	9	10	10	10	99

Table 2. International Clone Trial: choice of clones from the Montpellier Quarantine Centre

Clones	Brazil	Ecuador	Trinidad (MALMR)	Vene-zuela	Came-roon	Côte d'Ivoire	Ghana	Nigeria	Malaysia	Papua New Guinea	Total no.
APA4	*		*	*	*	*	*	*	*	*	9
CATIE1000	*		*	*	*	*	*	*	*	*	9
EET59	*	*	*	*	*	*	*	*	*	*	10
GU255-V	*	*	*		*	*	*	*	*	*	10
ICS1	*		*		*	*	*	*	*	*	8
IFC5			*		*	*	*	*	*	*	7
IMC47	*	*	*	*	*	*	*	*	*	*	10
Mocorongo	*		*	*	*	*	*	*	*	*	9
NA33						*			*		2
P7	*		*	*	*	*	*	*	*	*	8
PA120	*	*	*	*	*	*	*	*	*	*	10
PA150	*	*	*	*	*	*	*	*	*	*	10
SNK413			*		*	*	*	*	*	*	7
T85/799			*		*	*	*	*	*	*	7
VENC4-4	*	*	*	*	*	*	*	*	*	*	10
Total (trial)	11	6	14	9	14	15	14	14	15	14	126

Table 3. Additional clones requested for introduction into local collections (•) or as alternatives in the International Clone Trial (*)

Clones	Brazil	Ecuador	Trinidad (MALMR)	Vene-zuela	Came-roon	Côte d'Ivoire	Ghana	Nigeria	Malaysia	Papua New Guinea	Total no.
Reading:											
AMAZ5-2	*					*	*	*	•		5
ICS43		*			*				*		3
LCT-EEN37-I	*						•	•	•		4
MO20	*				*		*				3
SCA24	•	•		•	•	•	•	•	•		8
SIAL339									•	*	2
SIC5					*			*	*	*	4
UF676		*			*		*		•		4
Montpellier:											
GF24			•			*	*	*	•		5
GU175-V	*	*				*	*		•		5
GU307-V	*								•		2
K5	•								•	*	3
LAF1	*	*	•	*			*				5
N38	•				*		*	*	•		5
SNK64					*		*	*		*	4
VENC22-6	*	*		*					•	*	5
WA40			•	*			*	*	•	*	6
Total	10	6	3	4	7	4	11	8	14	6	73

2. Budding techniques

Scope

Budding is largely adopted in Malaysia for commercial reproduction of clones. However, in other countries rooted cuttings are used. The use of hypocotyledonary budding is proposed in the project for propagation of sufficient planting materials of selected clones. The propagation rate of budding can be higher than that of cuttings, which is important when starting material is available in limited quantities, as is the case for the International Clone Trial. Furthermore, buddings are expected to provide better field establishment than rooted cuttings, especially in drier areas, due to the more superficial root system of the latter planting material.

Budding techniques in cocoa have been well documented (Ramadasan 1976, Chong *et al.* 1981, Mohd. Yunus and Ramadasan 1986, Arasu, Lim and Yow 1994). The budding techniques used in cocoa propagation are conventional patch budding, green budding, chip budding and side-cleft budding. For most project applications, it is suggested to use conventional patch budding onto rootstock seedlings of 3 to 4 months old. However, countries that have developed another reliable hypocotyledonary budding technique, such as green patch budding, may wish to apply their own method.

Conventional patch budding

Equipment required:

- Polythene bags for raising rootstock: black and perforated with 250 gauge measuring 15 x 25 cm or 18 x 25 cm in width and length, respectively.
- As a source of rootstock, genetically uniform seedlings are to be preferred in order to obtain more uniform stands. The choice of rootstock varieties is dealt with elsewhere in this document.
- The recommended rootstock age for budding is three to four months. Older rootstock seedlings may result in root systems that grow out of the polybags and give poor development inside the bag when ready for field planting. The use of older rootstocks also increases the chances of rootstock sprouting at the cut, even with hypocotyledonary budding.
- Budwood materials: semi-brown budwood derived from the fan branches preferably collected on the day of budding.

Technique (see Figure 1)

- Three cuts should be made on the rootstock, i.e. one horizontal one of about 0.6 cm and from the end of this, two vertical ones of about 2.5 cm long. The cut should be made below the cotyledon scar to prevent sprouting of shoots below the bud-union.
- The cut strip is pulled back carefully.
- A corresponding cut is made on the bark of the budwood containing a bud.
- The bud patch is peeled off carefully.
- The bud patch is inserted into the rootstock's budding panel.
- The cut strip is cut to an appropriate length while ensuring that the bud-eye is exposed.
- Budding tape or parafilm is used to secure the bud union. It is recommended to apply the budding tape from the base upwards and to cover the bud completely so that the bud patch is best protected from accidental wetting.

- The bud patch should be protected from any wetting for four weeks and the environment in which the budded plants are placed should be aerated to reduce chances of infection of the bud patch. This can best be achieved by placing the budded plants under a roof, away from direct light, for four weeks. If this is done, there is normally no need to use fungicide on the wound.
- The growing point of the rootstock is removed soon after budding in order to reduce apical dominance.
- The budding tape is removed/opened two-three weeks after budding.
- The rootstock is cut back to 5 cm above the budding patch three weeks after budding to induce the bud to sprout. When the bud sprout is 10-15 cm long, the rootstock is further cut back just above the level of bud insertion. There is normally no need to treat the cut surface with any product.
- If the bud has not taken, the rootstock can be allowed to grow again and the opposite side of the stem can be re-used for budding.
- Each successfully budded plant is to be labelled individually. White typing error corrector ink can be used cheaply and conveniently for writing the clone name directly on the plastic bags. If this is not available, water and light resistant labels and ink should be used.
- After four weeks, the budded plant is placed again under normal nursery conditions with at least 50% shade. Watering of the pruned plant should be moderate while the new leaves have not yet completely expanded to avoid rotting of the roots.
- Fertilizer application of the budded plant should be undertaken when the first and second leaves of the scion have hardened.
- The budded plant is ready for planting four to six months after budding.

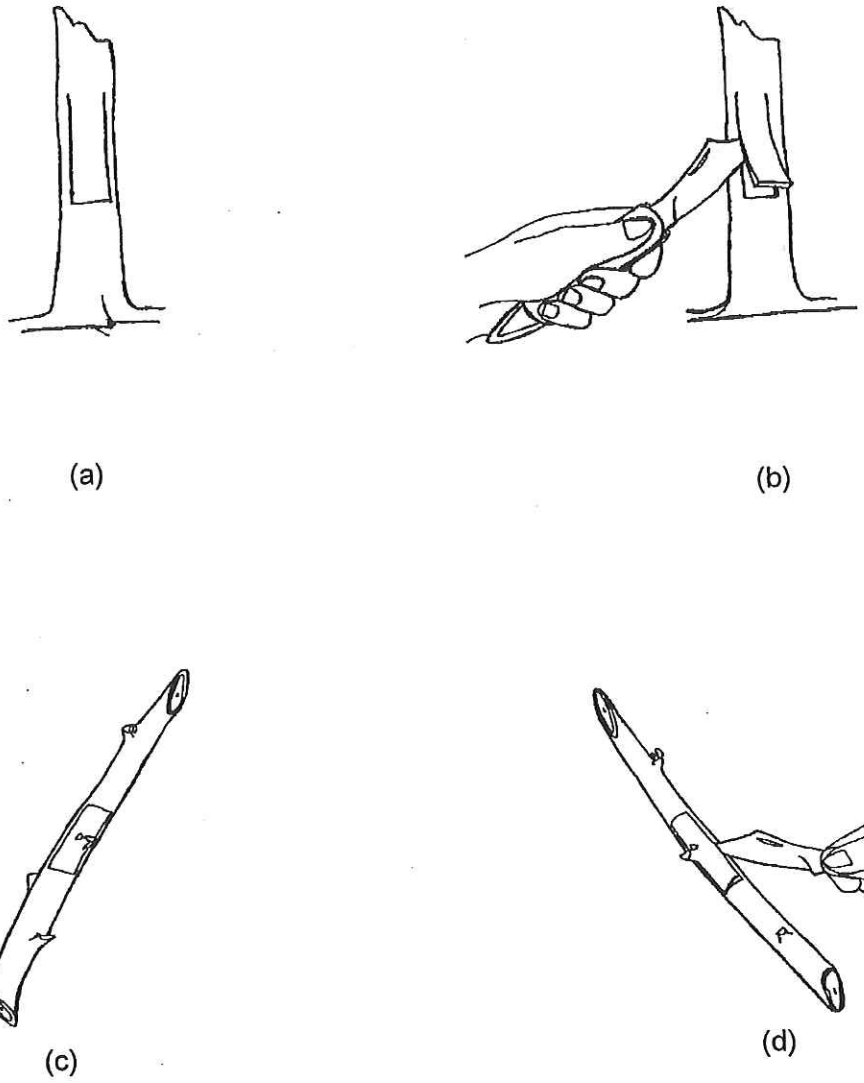
Factors to be standardized to obtain comparable results

- Polythene bag size.
- Rootstock type and age for budding.
- Transplanting age of the budded seedlings.

Points arising from subsequent discussions

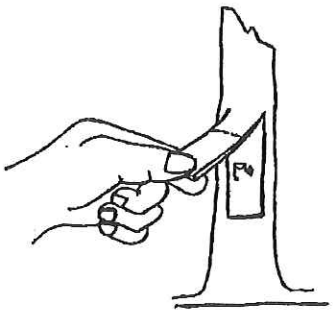
- Budwood derived from fan branches is recommended since it can be obtained in large numbers per tree. Semi-brown budwood should be used to ensure greater budding success.
- A sufficient amount of rootstock must be raised for the different multiplication phases, preferably 50-100% more than the number of budded plants needed.
- The cambium layer on both the budding panel and bud patch should not be damaged during budding. Only healthy budwood and rootstocks should be used for budding.
- The phytosanitary status of outgrowing budwood from newly introduced clones needs to be inspected at regular intervals. Plants showing any suspicious symptoms of fungal or virus infection should be destroyed.
- Budding for the International Clone Trial will be carried out in more than one phase. Firstly, clones received from abroad will be established on any available rootstock. Failed budwood needs to be re-supplied. Primary budded plants are to be established in a budwood garden. Once enough budwood of all the clones is available, mass budding will be carried out on the selected rootstock variety within a relatively short period of time (maximum two-three weeks), to ensure uniform age of the planting material.

Figure 1. Budding technique

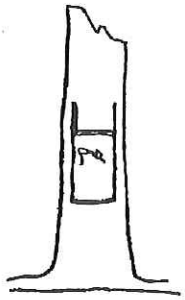


(a) Cuts of appropriate size are made on the budding panel
(b) The cut strip is gently pulled back
(c) Corresponding cuts made on the budwood that has a bud
(d) The bud patch is peeled off gently

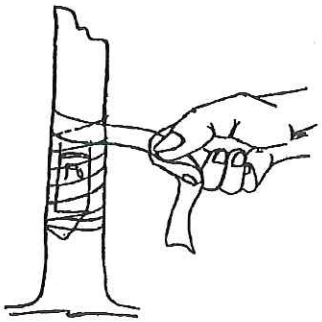
Figure 1 Budding technique cont.



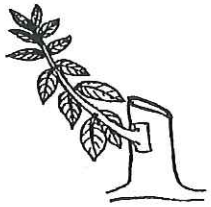
(e)



(f)



(g)



(h)

(e) The bud patch is gently inserted into the budding panel
(f) The cut strip is cut off to appropriate length
(g) The bud union is secured with the budding tape
(h) A successful budding with sprouted bud

Recording

The following data need to be recorded:

- Clone name.
- Budding date.
- Budder's name.
- Date of opening the budding tape.
- Number of seedlings budded per clone.
- Budding success at opening of the budding tape and monthly thereafter.
- The length of the outgrown buds three months after budding and before field planting (optional). This measurement might be useful to compare uniformity and vigour of the budded clones.

Authors of draft procedure

K. Lamin and M. T. Lee, Malaysian Cocoa Board, Malaysia

References for further reading

Arasu N.T. and K. Ramadasan. 1976. Vegetative propagation of *Theobroma cacao L.* and related problems. Pp. 23-24 *in* Proc. of the National Plant Propagation. Rubber Institute of Malaysia, Kuala Lumpur.

Chong C.F., R. Shephard and J.G. Taylor 1981. Experience with nursery budgrafting on cocoa estates in Malaysia. Cocoa Growers Bulletin 32:20-29.

Mohd. Yunus A. and K. Ramadasan. 1986. Recent advances in vegetative propagation techniques for *Theobroma cacao L.* under Malaysian condition. Pp. 133-146 *in* Proc. of the Int. Conf. on Cocoa and Coconuts: Progress and Outlook. Incorporated Society of Planters, Kuala Lumpur.

Lim D.H.K. and S.T.K. Yow. 1994. Green-patch budding on very young cocoa rootstocks and side grafting of mature tree. Cocoa Growers Bulletin 47:24-41.

3. Nursery maintenance

Scope

A comprehensive nursery maintenance work schedule is needed to ensure healthy seedlings for field planting.

Nursery establishment and maintenance

Site selection

- The site should be level and well-drained, near to a clean and adequate water source, and good top soil supply.
- Good road access is necessary.

Nursery house

- Clear plastic or fibreglass roofing is needed in an area where vascular streak dieback disease (VSD) or witches' broom disease pressure is high.
- The use of nylon wire sheet, 60-80% shade is recommended to obtain uniform light conditions. This will be a very important condition to successfully apply the leaf inoculation test for *Phytophthora* resistance.
- The nursery should preferably be fenced.
- It should be kept clean at all times.
- Diseased plants and waste should be discarded.
- The roofing should be repaired when needed.

Polybag size, soil filling and arrangement

- Recommended size is 15 x 25 cm or 18 x 25 cm (in width and length when flat) 250 gauge black perforated polythene 'polybags'.
- Good, well-drained, sieved top soil (not too clayish!) or an appropriate soil mixture (40% soil, 40% sand and 20% well decomposed organic matter) is recommended.
- Polybags should be filled to within 2-3 cm of the polybag top and its bottom folded to facilitate arrangement.
- It is recommended to incorporate 20 g of rock phosphate fertilizer into each polybag.
- Lime may be required depending on the soil's pH.
- Polybags are arranged in twin rows; a path of about 45 cm separating each twin row is recommended to facilitate movement of workers and maintenance.

Seed sowing

- Seeds are pre-germinated by spreading them into a thin layer between two moist jute sacks prior to sowing; the seeds should be kept moist by watering the sack daily.
- Germinated seed are collected every morning and planted immediately.
- The germinated seeds must be sown with the radicle downward and three quarters of the seed covered in soil.

Watering

- Watering is carried out daily, either manually or using an appropriate watering system such as a sprinkler system or drip-irrigation.
- Care is to be taken not to water too much as this waterlogging gives bad conditions for root development.

Weeding

- Regular manual weeding rounds are necessary to remove the weeds from each polybag.
- Herbicide spraying should be avoided.

Pest and disease control

- Regular application of insecticides and fungicides are carried out only when required. No fungicide is to be applied if the plants are to be used for resistance testing. Foliar fertilizer could be incorporated with each application.
- Pesticide spraying must be carried out after watering.
- Stringent culling should be undertaken to discard unhealthy, weak and diseased seedlings.

Manuring

- Monthly application of compound fertilizer at the rate of 2-4 g with a nutrient content of 15%N:15% P_2O_5 :6% K_2O: 4%MgO is recommended.
- The use of a fertilizer is more important when using poor top soil or poor organic matter in the soil mix for the polybags.
- A top dressing of fertilizer is applied after the first leaf flush of the seedlings have hardened and is interrupted one month prior to budding; it is resumed one month after removal of the budding tape.

Control of root development

- Monthly observation of main and secondary root development is to be carried out as root development in polybags is often less than optimal. Any eventual problems with root growth should be overcome by improved (possibly reduced) watering or by improving the soil mix.

Recording

- Number of polybags filled and planted.
- Seedling mortality.
- Number of graftings.
- Grafting success.
- Pest and disease incidence.
- Nutrient deficiency and treatments applied.
- Number of plants taken for field planting.

Authors of draft procedure

K. Lamin and M.T. Lee, Malaysian Cocoa Board, Malaysia

4. Pruning techniques for young plants

Scope

Pruning is the removal of unwanted/excess twigs and branches to ensure a well-balanced canopy at an optimum leaf area index and to avoid excessive competition between trial plots. Good pruning practice will contribute to a higher production of cocoa pods and either directly or indirectly to fewer losses due to pest and diseases.

Pruning of young budded plants (Figure 1)

Formative pruning

- Branching of the newly transplanted budded plant is induced by pruning the top of the terminal shoot. This is carried out six months after field planting.
- All side shoots below 15 cm from the ground must be removed.
- Allow and maintain three-four upward growing shoots to grow as main branches.
- The tips of the terminal shoot are removed once these three or four main branches have reached approximately 1.25 m, in order to induce further branching.
- No major pruning is undertaken until the budded plant is 16-24 months old in the field, when the smaller lower side branches are removed.

Maintenance pruning

- Etiolating and droopy branches are regularly removed.
- Diseased and infested branches are treated or removed.
- Keep the tree canopy at a convenient height of less than 3 m to facilitate pest and disease management and harvesting.
- Remove excess canopy when necessary to allow ventilation and to maintain sufficient leaf area for maximum sunlight interception.
- Prune lateral higher branches to prevent plants from growing over into neighbouring plots and competing with different genotypes (this treatment was agreed upon to decrease inter-plot interference in the International Clone and Hybrid Trials).

Pruning of young seedling plants (Figure 2)

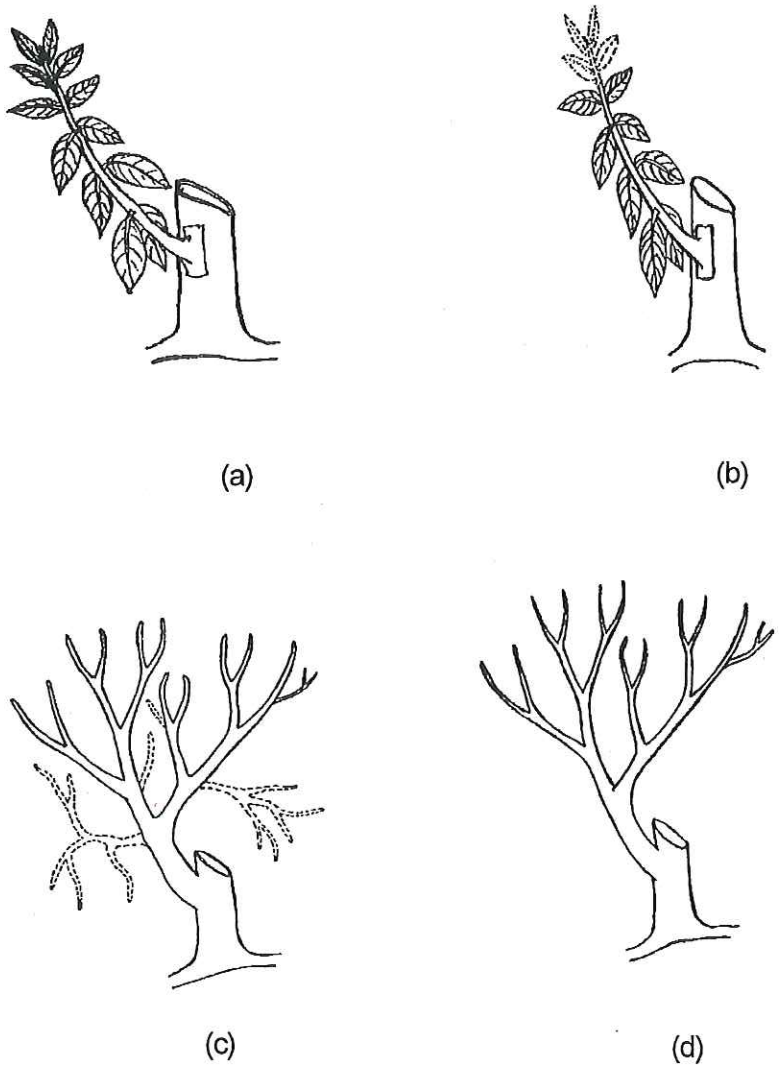
Formative pruning

- Trees are kept to the first jorquette; if the first jorquette is very low, a second jorquette is allowed to grow.
- All side shoots or chupons below the jorquette are removed.
- The main three or five branches are pruned to 1.0 m in length to induce side branching (if these are not induced naturally).
- A canopy with interlocking lateral branches is preferable. This allows maximum interception of the sunlight.

Maintenance pruning

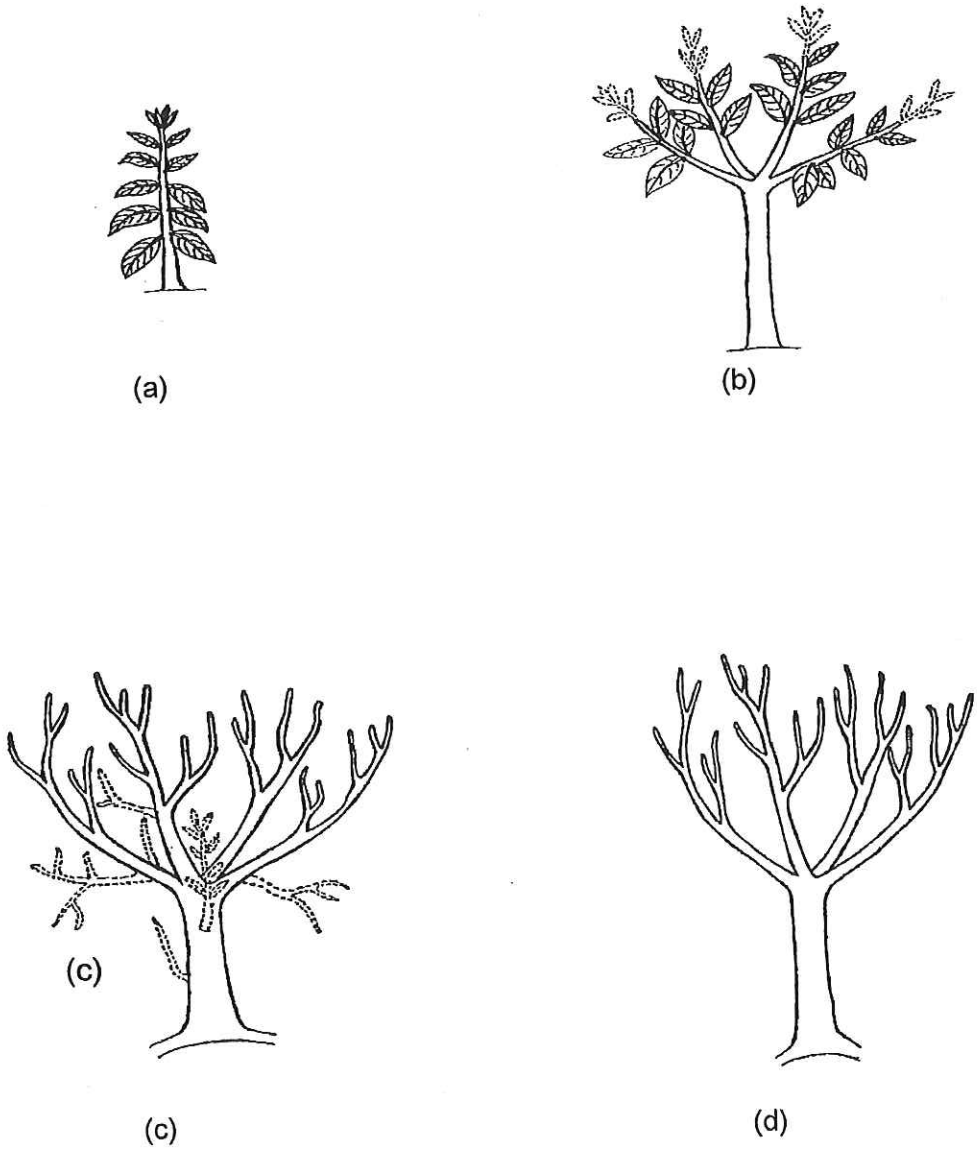
- All side-shoots and chupons below the jorquette are removed.
- Etiolated, droopy, diseased, dead twigs and branches are removed.
- The tree canopy is kept at a convenient height of less than 3 m to facilitate pest and disease management and harvesting.
- Remove excess canopy when necessary to allow ventilation and maintain sufficient leaf area for maximum sunlight interception.

Figure 1. Pruning of young budded cocoa tree



(a) - Six-month old budded seedling
(b) - Tipping of the terminal shoot
(c) - Removal of droopy, etiolating and other unwanted branches
(d) - An schematic drawing of well-formed young budded tree

Figure 2. Pruning of young hybrid cocoa tree



(a) - Removal of side shoot of hybrid seedlings (prior to jorquetting)
(b) - Cutting of terminal shoot of the main fan branches
(c) - Removal of chupons and etiolating branches
(e) - A well-formed hybrid tree

- Apply pruning of lateral higher branches to prevent plants from growing over into neighbouring plots and competing with other genotypes.
- Excess leaves are removed as necessary to avoid overcrowding.

Notes

- Keep tree height low to facilitate maintenance.
- Remove excess canopy while maintaining sufficient leaf area.
- Good sanitation is necessary.
- Avoid excessive pruning.

Recording

Working schedule for formative and maintenance pruning.

Authors of draft procedure

K. Lamin and M.T. Lee, Malaysian Cocoa Board, Malaysia

5. Planting and field maintenance of cocoa trials

Scope

The major consideration for breeding trials should be to establish and maintain the trials so as to permit the various genotypes in the trials to express as much of their true characteristics as possible. In order to achieve this objective, certain planting and maintenance practices should be adequately controlled so as to minimize variations that will mask the true performance of the genotypes. It should be emphasized that poor trial establishment and management increase residual variances which in turn reduce the sensitivity of treatment differences. The operations to be looked at carefully are:

- selection of trial sites;
- provision of temporary and permanent shade;
- planting practices;
- fertilization;
- management of plants till bearing;
- replacement of dead trees, and
- adult plant management.

Most practices described hereafter refer to sound agronomic recommendations, that may vary slightly from one site to another. However, it has been decided that the replacement of dead trees and pruning practices of adult cocoa plants are to be standardized in the International Clone and Hybrid Trials.

Selection of trial site

Since all participating countries are cocoa cultivating countries it is assumed that temperature and rainfall are about adequate for cocoa cultivation. Aside from these, natural vegetation, soil factors and access are to be considered. Easy access to the experimental site will aid all aspects of the establishment, maintenance and data collection of the trial. Cocoa is generally cultivated in areas covered by evergreen forest, semi-evergreen forest, deciduous forest, secondary forest and suitable lands previously cultivated with cocoa or other crops. Whatever the vegetation covering the land to be selected, homogeneity in the vegetation cover should be a criterion for selection of the site or land. Sites with heterogeneous soils as well as low soil fertility should be avoided and soil scientists should be involved in site selection so that the nature of soil variations in the site are documented. This is very important for the correct choice of the field layout of the trial (blocks should be located across any gradient in fertility or slope of the land). Once the trial site has been selected the area should be marked out and the undergrowth removed.

Shade

Temporary shade

Bananas, plantains, cocoyam, cassava, maize and some legumes are often used for temporary shade for young cocoa. The spacing of the temporary shade varies depending on the plant being utilized but efforts are made to ensure adequate and uniform temporary shade for the young cocoa seedlings. *Glyricidia sepium* has also been used to provide temporary shade on experimental plots. When *G. sepium* is used the spacing should be 10 x 10 m. Temporary shade should be established before field planting of cocoa or the shade plant should be a fast growing type so that if it is planted at the same time as the cocoa, it will grow fast enough to provide the shade needed during the dry season.

Permanent shade

Felling of all the indigenous trees depends on whether part of these will be used as a permanent shade. The type of shade trees to be used and the spacing should be such that uniformity of permanent shade on the trial is attained. Planted shade trees are used in some trials whilst in others no shade is provided. The commonest shade trees planted in cocoa trials in Ghana are *Terminalia ivorensis* and *T. superba* at a spacing of 40 m^2. In some cases however, *Glyricidia sepium* planted at 20 m x 40 m has also been used for permanent shade. Indigenous forest shade trees left on cocoa farms include *Milicia excelsa, Alstonia bonei, Ricinodendron heudlotii Spathodea camipanulata, Antiaris toxicaria, Neuboldia laevis, Funtamia elastica, Ficus sur, Khaya ivorensis* and *Entandrophragma utile.* This list can be expanded depending on local selection and preferences. The rule of thumb is to allow 15 to 18 mature trees per hectare. It is not known whether owing to their individual differences, micro-site variations can be created by the different forest tree species which will affect the performance of different genotypes of cocoa being evaluated under them. Forest trees not to be allowed on cocoa farms in Ghana include *Celtis spp, Glyphaea brevis, Ceiba pentandra, Piptadeniastrum africanum, Cola giganta, C. chlamydantha* and *Musanga cecropioides* as they are alternate hosts to cocoa diseases or show undesirable phenotypic attributes.

Planting of cocoa seedlings or budded plants

Lining and pegging should be carried out and planting holes should be dug at the planting site at least one week before transplanting. Spacing of 2.5 m x 2.5 m and 3 m x 3 m have been used for most breeding trials in Ghana but it is clear that in some of the trials in the CFC/ICCO/IPGRI project other densities will be required.

In the establishment of trials it is paramount that seedlings be planted and managed so as to permit the varieties to express their true characteristics. Strict control of planting material must be exercised. Errors in materials planted and other problems affecting individual plants must be identified early in the programme and appropriate remedial measures taken. Individual plants should have temporary labels and be inspected before, during and after planting in the field and the relevant observations recorded in field notebooks specially designed for this purpose. As far as possible, seedlings or budded plants of the same age and size should be selected for planting.

Fertilization

The type of fertilizer to use and the rate of application will depend on the nutrient status of the soil being used for the experiments. The advice of the soil scientists is very important and therefore they should be involved in the early stages of planting of the experiments especially during site and land selection. In Ghana, the recommendation for young and newly planted cocoa is 70 g/plant/year of sulphate of ammonia applied three months after planting with the rains for two to three years. For mature cocoa, 129 kg P_2O_5 and 76.5 kg K_2O/ha/year can be applied during the early rains (March - May).

Management after planting till bearing

This covers the early years up to canopy closure, i.e. two to four years after planting. Control of weeds, development of permanent shade and fertilization are the main operations at this stage. If there is a marked dry season, the provision of irrigation or mulch might be necessary. Mulch can be provided from cut bushes or grasses, tree loping or pruning etc. Overhead shade should be maintained at about 50% of direct sunlight. From the third and fourth years the temporary shade should be gradually removed as the permanent shade trees take over.

Replanting of dead trees

Records should be taken on the mortality of trees which occur in any stage of the trial. Any young cocoa seedlings or budded plants that die during the first two years should be replaced by reserve seedlings or budded plants to be kept in good conditions in the nursery for this purpose. Any trees that die afterwards will not be replaced. If the cause of the mortality is not related to the variety, this will then be a random factor affecting all varieties equally (if the layout of the trial is correctly chosen). If there is a genetic effect on mortality of adult trees, such as susceptibility to degenerative diseases, this is then to be considered as a characteristic of the variety involved. The plot means will be calculated without making any corrections for the number of surviving plants.

Pruning

Recommended procedures for formation and maintenance pruning of young trees are given in Working Procedure 4. There are three main objectives to pruning cocoa:

- to shape or form the young tree,
- to maintain the shape or form of the tree subsequently, and
- to renovate or rehabilitate a cocoa tree after it has been allowed to lose its shape by unrestricted growth.

The minimal pruning operations to be carried out in all CFC Project trials are:

- removal of diseased and dead wood,
- removal of undesired chupons,
- opening up the trees so as to facilitate spraying and harvesting,
- production of a not too dense canopy, and
- development of a tree of upright stature.

Pruning of adult trees in the International Clone and Hybrid Trials should be carried out in a standardized way, limiting tree height to the first canopy and eliminating lateral growth into neighbouring plots, in order to avoid excessive inter-plot competition for light and space. This precaution is necessary because of the relatively small plot size chosen for these trials (see also Working Procedure 7). For other project trials, local preferences and sound agricultural practices should be applied for maintenance pruning.

Weed control

In Ghana two main methods of weed control are practised in experimental plots, namely manual weeding and the application of herbicides.

High slashing of weeds is recommended at least four to six times during the year depending on the frequency of the rains. Clean weeding two times during the year results in cocoa seedling growth which is comparable to high slashing of weeds about four times a year. Best results in cocoa seedlings and temporary shade (plantain) growth and yield are usually obtained by clean weeding four times a year. However this practice is very laborious and expensive. The use of hedgerows of *Flemingia congesta* which is pruned twice during the year for mulching the cocoa provides very satisfactory weed control similar to clean weeding and also helps to improve the soil physical and chemical properties.

Under experimental conditions, cost savings of 10 to 15 percent have been obtained by using herbicides in cocoa establishment. The net benefit of a weed-free environment is faster growth of the cocoa, early flowering, early canopy closure and higher yields for three to six years. Another benefit is higher yield from the plantains used as temporary shade. Chemical weed control in Ghana has been achieved with the use of either a contact or systemic

weedkiller such as paraquat or glyphosate respectively. Paraquat can be applied at 1.5 litres per hectare four to six times a year during cocoa establishment using a polijet nozzle. Glyphosate is applied at the rate of 1.0 to 1.5 litres per hectare three to four times a year using a VLV nozzle. In mature cocoa with closed canopy, the frequency of application of the herbicides can be reduced by 50 percent.

Control of pests and diseases in experimental plots

The control of pests and diseases will depend on the objective of the trial. For example if the trial is for field assessment of say CSSV resistance of some varieties then control of the virus disease in the plot might not be necessary. However, in Ghana, the virus disease is generally controlled by eliminating all the infected and adjacent neighbouring trees.

Recommendations on control of insect pests attacking young cocoa in West Africa are given elsewhere in this publication (see Working Procedure 31). The cocoa mirids *Sahlbergella singularis* and *Distantiella theobroma* are the major pests that attack the young shoots of cocoa, cocoa pods and cherelles in West Africa. Various chemicals including organochlorine, carbamates and pyrethroids have been recommended. The choice of which chemical to use to control pests can be left to local experience and economic considerations though carbamates and pyrethnoids are to be preferred.

Since observations on field resistance to the disease are an important aspect of the project, generally no use of fungicides should be made in the project trials. The same applies to most other diseases and less destructive pests such as witches' broom, stem and pod borers, etc. However, if severe attacks of any disease or pest place the survival of the trees in danger, locally recommended control measures will have to be undertaken.

Data collection

Each single tree in the plot should be properly labelled. At the seedling stage, temporary labels should be provided. When the plants are established, normally after two years, each tree and plot should be identified with a permanent label. Individual trees should be inspected periodically and any relevant observations recorded in field notebooks which should be specially designed for this purpose.

Conclusion

Most of the activities mentioned above will take into account good agronomic practices adopted locally. Agreed exceptions, where standardization is required in the project, are formation pruning of the young budded plants and pruning of branches that grow into the space of neighbouring plots. Insect and disease control is needed if the survival of the tree is at risk. Recommendations for insect control are given elsewhere in this publication. In any case, the attacks of diseases and pests should be used to make observations on the degree of field resistance of the different varieties.

Authors of draft procedure

A.A. Afrifa, Y. Adu-Ampomah and K. Osei-Bonsu, Cocoa Research Institute of Ghana, Ghana

6. Evaluation of vigour, yield, pod and bean traits

Scope

The purpose of the international trials is to generate practical useful information on the genotype tested for the breeders, either for use as planting material or as a source of germplasm in the breeding program. As far as possible, the observations made or the measurements taken should be simple, accurate and inexpensive to collect.

Vigour

To minimize genetic vulnerability, cocoa varieties should be constructed as polycross hybrids or polyclonal varieties. The basic principle is to combine different genotypes, but to maintain similar characteristics such as vigour in order to minimize interplant competition. Knowledge of the potential vigour of trees is also important from the agronomic point of view, mainly for recommendations related to planting density and pruning.

Vigour is strongly affected by the environment, soil fertility, rainfall (amount and distribution) and temperature. Shade intensity, planting density and pruning practices are also important. Under similar environmental conditions, the size of the tree is determined by its genetic composition. When budded clones are concerned, an effect from the rootstock is possible. Thus, vigour is relative. Considerable differences are expected between sites. If GxE interaction occurs, it will be detected by the project activities.

Measuring the trunk circumference is the most commonly used method for assessing vigour. Height and width of the tree canopy are measured by some, but this is time-consuming and affected by pruning practices. Small differences in vigour are not of practical importance. Therefore, visual observation on a one to five rating scale can already be very useful in breeding to classify individual trees, families and clones into plant size categories (small, intermediate and large). In the nursery, if of interest, plant height is measured three times (30, 60, 90 days after sowing or budding).

The measurements proposed in the project are (see Recording Sheet 1):

- Trunk circumference annually at 30 cm for seedlings and at 15 cm for clones.
- Visual observation annually (1-5 rating scale, 1 and 5 refer to the largest and smallest trees, respectively) on individual plants.
- Jorquette height of individual seedling trees in the second year after planting.

For analyses, average data per hybrid or clone as well as within family or clone measure of variability (standard deviation within plots) will be considered.

Yield

Usually, there is a big gap between the yield reported by experimental stations and the actual yield obtained in farmers' fields. This is partly because of better management of experimental plots, but may also be due to a tendency to overestimate the experimental yield (extrapolation from small plots and sampling methods). A common practice for yield recording is to base this mainly on harvested pod counts. Other yield components are determined very few times or only once, based on a sample of pods taken from the harvest. This is a fast and easy way to record yield. However, it is open to various inaccuracies with a tendency to overestimate the yield. All the yield components are environment dependent and fluctuate during the year. Yield components therefore need to be estimated at regular intervals.

Dry bean yield per tree is a product of the following components: number of pods per tree, average pod weight, average wet bean/husk weight per pod and the percentage of dry beans obtained from wet beans after fermentation and drying. Alternatively, it can be obtained by multiplying the number of pods per tree (N) with the number of beans per pod

(n) and the dry bean weight (w). The measures used to obtain yield will also be used to calculate some pod and bean traits as well as for calculation of incidence of pod diseases at harvest.

Besides yield, some programmes are using "pod value" (number of pods required to obtain one kg of dry beans) and "yield index" (relationship between yield and vigour) as equally important selection traits.

The procedure proposes standard project records to measure yield, applicable to hybrid trials and clone trials.

Individual plant measurements (standard for hybrids only, optional for clones):

- Number of healthy, rodent-damaged and diseased pods per tree at fortnight intervals (except during long periods with no, or with negligible, pod production, when no counts are made). Only pods of mature size are considered (above 2-3 months old or longer than 10-12 cm). Those that have been partially or totally destroyed by pathogens are considered as diseased pods. *Phytophthora* pod rot (Ppr) will be observed at all project sites (see Recording Sheet 2), except in Ecuador where this disease is not common. Observations on Ppr incidence on cherelles is optional (see Working Procedure No 17) for countries where this disease is of great importance. When several important diseases occur simultaneously, such as witches' broom and Ppr, the pods destroyed by these diseases are to be recorded separately (see Recording Sheet 3).
- Weight of healthy pods per tree; twice annually during major harvesting peaks.

These observations are required for clone selection from hybrids and assessment of within family variability.

Plot measurements (standard for clones and hybrids):

- Number of healthy, rodent-damaged and diseased pods at fortnight intervals (as above).
- Weight of healthy pods twice annually (see Recording Sheet 4).
- Weight of wet bean (or husk), from healthy pods only, twice annually (see Recording Sheet 4).

Measurements on harvested samples (standard for clones and hybrids, see Recording Sheet 5):

- Percentage of dry bean from 5-6 kg wet bean samples after fermentation in mosquito nets, within large cocoa batches, and sun-drying; twice annually during the first three significant production years.
- The fermented and dried samples are to be used for observations on bean traits and shell content (see later) as well as for quality (butter fat, flavour: see Working Procedure 12). Fermentation of all genotypes should be carried out within the same cocoa bulk to allow for reliable quality observations.
- The average number of beans per pod (n) will be calculated as follows:
  $n = (A \times C) / (B \times D)$, with

  A = number of beans in the sample (observed most easily after drying)
  B= wet weight of this sample
  C = total weight of wet bean from all plots
  D = number of healthy pods used to obtain total wet bean of all plots

  This is possible as the ratio of the total number of beans (E) to the number of beans in the wet bean sample (A) is equivalent to the ratio of the total wet bean weight (C) to

the wet sample weight (B), or E/A = C/B and hence E = (A x C)/B. The number of beans per pod (n) is equivalent to E/D or (A x C)/(B x D).

The preparation of the dry bean samples should follow the same process as that for quality analyses (see Working Procedure 12). All samples are to be obtained simultaneously and fermented in mosquito net bags placed within the same cocoa fermentation mass of a minimum height of 50 cm and a fresh weight of 500 kg (0.5 m^3). The duration of fermentation should be standardized at six days (6 x 24h), with the beans turned over on day 1, day 3 and day 5. The wet beans should be obtained from the same trial or from another simultaneously harvested uniform cocoa lot.

Yield/vigour relationship (yield index)

Depending on the growing conditions and plot size, yield can be closely correlated to vigour. More vigorous trees will occupy more space and thus have access to more light and nutrients. The average level of young plant vigour can be more or less correlated with adult plant yield and can as such be considered as an early measure of yield heterosis. However, not all vigorous young trees will be productive when growing older. Vigorous trees in mono-culture (e.g. monoculture of a highly vigorous clone or mixture of vigorous hybrid trees planted at too high density) will compete strongly for space and nutrients and may therefore not be very productive.

The relationship between yield and vigour (or yield index) is a measure of yield efficiency. It is considered as a very important trait for individual tree selection (see Working Procedure 10). Yield index is normally calculated as dry bean yield divided by trunk circumference (or, better, by trunk cross-section, to make variation for vigour of similar magnitude to that of yield). Calculation of yield index should be done in all CFC Project trials and used for individual tree selection in hybrid trials.

Pod and bean traits

Beans and pods are the most important parts of *Theobroma cacao* for commercial uses. They vary in size according to the genetic origin and environment. Variability exists within clones due to growing conditions of the tree, pod load, season, etc. Bean and pod size are often correlated. Evaluation of most bean and pod traits is needed because they are components of yield, which itself is the most important trait in a breeding programme. The traits proposed here are to be evaluated in a standardized form in the International Clone and Hybrid Trials (see Recording Sheets 6, 7 and 8).

The following traits have been chosen:

- Pod weight
- Fresh bean weight per pod
- Number of normal beans per pod
- Percentage of badly filled or "flat" beans
- Pod length
- Pod diameter
- Fresh and dry bean weight
- Shell content
- Bean length
- Bean width, and
- Bean thickness.

In measuring quantitative traits, one needs to take into account the existing environmental variation in the trait in order to estimate the genetic variation with a certain level of precision. As the coefficients of variation (CV) for these traits have already been

observed in earlier experiments, the number of observations (N) indicated hereafter, is needed for obtaining a relative precision (dr) of the observation of 10% for pods and 5% for bean characteristics. The relationship between N and dr is as follows:

$$N = (4xCV^2)/dr^2$$

When this formula is applied to traits to be observed, the following N numbers are obtained.

Pod traits:

Pod weight	N = 65
Fresh bean weight per pod	N = 80
Number of normal beans per pod	N = 100
Pod length	N = 16
Pod width	N = 7

Bean traits:

Fresh bean weight	N = 120
Shell content	N = 120
Bean length	N = 25
Bean width	N = 25
Bean thickness	N = 25
Dry bean weight	N = 145

Pod weight, fresh bean weight and bean number per pod will be calculated from the bi-annually harvested samples (see yield measurements). These measurements will be repeated over three years (six observations in total). This will involve a total sample size of far more than the 100 pods required to estimate within 10% accuracy for the pod traits. The six replicates will make statistical analyses possible.

The pod length and width will be measured twice in the first production year on the collected pod samples. Length and width of 24 randomly chosen healthy pods will be measured at each of the two observation dates (four pods per plot). This will ensure enough observations and replicates so that a reliable average for these traits can be obtained for statistical analysis.

Bean size measurements are taken twice using the dry bean samples prepared during the first year. Of each sample the length, width and thickness of 30 normal beans chosen at random will be measured.

The dry bean weight and shell content will be obtained by weighing samples of 100 beans, first with, then without the shells. Only normal beans will be chosen at random from the dried bean samples. These beans will be taken again from the dry bean samples prepared bi-annually, as indicated under yield measures. The total number of observations is therefore 3x2=6. The percentage of flat beans is also observed six times and calculated from the normal and flat bean counts in the dry bean samples.

Authors of draft procedure

Y. Efron, CCRI, Papua New Guinea; J. A. K. N'Goran, CNRA, Côte d'Ivoire and P. Lachenaud, CIRAD, c/o CNRA, Côte d'Ivoire

Recording Sheet 1. Tree vigour observations: jorquette height (Jh, in cm, observed only once), trunk circumference (Tc, in cm) and visual tree size observation (S, 1 to 5 scale)

Trial: _____ Planting date: _____

Block no.	Tree no. (row, tree)	Variety no.	Date			Date		Date		Remarks
			Jh	Tc	S	Tc	S	Tc	S	
Etc.,										

Recording Sheet 2. Yield observations on individual trees or plots (fortnightly intervals): number of healthy ripe harvested pods (HR), *Phytophthora* pod rot infected pods (Ppr, completely or partly rotten), rodent-eaten pods (Re) and other diseased or destroyed pods (Other)

Trial: _____ Planting date: _____

Block no.	Plot or tree no.	Variety no.	Date of observation				Date of observation			
			HR	Ppr	Re	Other	HR	Ppr	Re	Other
Etc.,										

Recording Sheet 3. Yield observations with recording of *Phytophthora* pod rot (Ppr) and witches' broom (Wb) infected mature pods. Number of healthy ripe pods harvested (H), Ppr infected pods (Ppr), witches' broom infected pods (Wb), rodent eaten pods (Re) and other rotten or damaged pods (Other).

Trial: _____ Planting date: _____

Block no.	Plot or Tree no.	Variety no.	Date of observation					Date of observation				
			HR	Ppr	Wb	Re	Other	H	Ppr	Wb	Re	Other
Etc.,												

Recording Sheet 4. Plot measurements on number of healthy pods (n), total weight of healthy pods (pw, in 100 g) and wet bean weight (wbw, in 100 g)

Trial: _____ Planting date: _____

Block no.	Plot no.	Variety no.	Date			Date			Remarks
			n	pw	wbw	n	pw	Wbw	
Etc.,									

Recording Sheet 5. Observations on wet and dry bean samples (bi-annual)

Trial: _____ Observation date: _____

Variety no.	Sample no.	Wet bean weight (in 100 g)	Dry bean weight (in g)	Bean number		No. of beans per pod (calculated)
				Normal	Flat beans	
Etc.,						

Recording Sheet 6. Pod length (pl) and width (pw) observations

Trial: _____ Planting date: _____

Block no.	Plot no.	Variety no.	Dates of observations								Remarks
			pl	pw	pl	pw	pl	pw	pl	pw	
Etc.,											

Recording Sheet 7. Weight of 100 normal beans with shell (100 bshw) and without shell (100 bw) of fermented and sun-dried samples, expressed in g

Trial: _____ Planting date: _____

ariety n	Sample no.	Dates of observations								Remarks
		100 bshw	100 bw	100 bshw	100 bw	100 bshw	100 bw	100 bshw	100 bw	
Etc.,										

Recording Sheet 8. Bean length (bl), width (bw) and thickness (bt) expressed in mm

Trial: _____ Planting date: _____

Variety no.	Sample no	Bean no.	Dates of observations									Remarks
			bl	bw	bt	bl	Bw	bt	bl	bw	bt	
Etc.,												

7. Layout of project trials

Scope

The main objective of cocoa genetic improvement is the selection of new clones or hybrid varieties. Field trials are therefore necessary to test these different clones or hybrid families. The families tested may represent independent crosses between clones (single pair crossing plan) or related to each other through specific crossing schemes (hierarchical, factorial or North Carolina II, diallel crossing scheme).

If early screening is possible, particularly for the selection of resistant material, experimental designs are needed in the nursery or the laboratory. This involves determining designs that are statistically effective and compatible with the use of inoculation and measurement techniques on the planting material in question.

Concepts on field designs

The choice of the experimental design depends on the objective and the restrictions of the trial. It involves determining the size of the plots (statistical units), which can vary from one to several plants, the layout of the trial (total randomization, blocks, incomplete blocks, lattice, etc.), and the number of blocks or replicates of the statistical units.

There are special restrictions for tree crops, such as the large area required for trials, interactions with environmental factors, the long duration of the trials and frequent presence of disruptive factors that reduce the efficacy of experimental designs.

Of the disruptive factors, soil heterogeneity is a major problem that cannot always be controlled by block or Latin square designs. There can be problems of micro-heterogeneity, resulting from patches of varying fertility, irregularly distributed throughout the trial site. Such heterogeneity is often not known before the trial has been set up and makes *a priori* control difficult. The duration of the trials entails accidental tree losses, resulting in further heterogeneity within the trial plots. With cocoa, average death rates of around 10% are recorded in the first two years.

In view of these problems, different experimental designs will be examined for cocoa selection trials, with special reference to trials in the CFC/ICCO/IPGRI project.

Plot size

Various studies have been carried out to determine the most suitable experimental designs for cocoa variety trials. The recommendation resulting from such studies was the use of designs based on total randomization of single-tree plots (Lotodé and Lachenaud 1988, Cilas 1995). From a practical point of view, designs with single-tree plots have a certain number of advantages and disadvantages.

Advantages of single tree plots are:

- With multi-tree plot designs, the plot means are not accurate if tree losses occur, since these are then calculated using a different number of trees. This problem does not occur with single-tree plot designs because the number of replicates per family (single trees) can vary; the analysis of variance can be carried out with unequal plot numbers per treatment.
- Distribution of the trees of the different crosses on the soil patches of different fertility is random with the single-tree plot design, taking into account the heterogeneity in the best possible way within the trial site. With multi-tree plot designs, the distribution of the plots is less random in relation to the within-trial heterogeneity which will increase the residual mean square in the analysis of variance and, hence, decrease the precision of the design.
- With single-tree plot designs, each tree of a given family is in contact with trees from other families. Competition between trees is therefore highly variable, as is the pollen

environment. Such a situation is close to reality, since the trees of the different selected hybrid families will be planted randomly in production plots.
- With single-tree plot designs, individual tree data are collected automatically allowing for combined individual-family selection. Hence, a certain number of trees that perform well can be selected and possibly used in a new selection cycle.
- When the hybrids involved were obtained using a particular crossing plan (North Carolina II or diallel), the genetic parameters such as narrow sense heritability are estimated at individual tree level. This is more useful for calculating selection progress than if one uses heritability estimates based on multi-tree plots.

Disadvantages of single-tree plots are:
- With single-tree plot designs the trees of different crosses are positioned in each of the blocks in a totally random way. Setting up such trials and gathering data is more laborious and more subject to errors than for multi-tree plot trials.
- With single-tree plot designs, visual checking of the families is difficult or impossible.
- With single-tree plot designs, competition between families of different growth habit or vigour will lead to an advantage for trees with high vigour. Such advantage may not be expressed in commercial varieties made up of hybrids of similar vigour. In fact, some breeding programmes wish to counter-select excessive vigour to reduce competition problems of adult cocoa stands.

On balance for these reasons, it seems preferable to use multi-tree plots in the clone and hybrid selection trials planned in the CFC/ICCO/IPGRI project. Plots with eight to ten trees would seem adequate for most of the CFC project trials.

Experimental design and number of replicates

Once the plot size and shape has been determined, the next step is to decide on their distribution in the trial plots. Between 20 and 50 clones or hybrids will be tested in each trial. The trial sites will therefore be large, probably with substantial heterogeneity. It would therefore seem preferable to adopt a randomized block design with a relatively small plot size and many replicates. At least six blocks are required to ensure sufficient precision of the trial.

Field designs adopted

International Clone Trials

The main objective of this trial is to assess yield as well as pest and disease incidence over a number of environments. For disease and pest incidence, an important consideration in the Workshop discussions was to limit inter-plot interference. This can be achieved by using large, square plots. As explained above, distribution of experimental error is however best obtained with smaller plots.

As a compromise, the following plot size and shape will be adopted: randomized block design with six replicates of eight tree plots (double rows of four trees per plot), totalling 6x8=48 trees per clone. To avoid inter-plot competition with these relatively small plots, it was decided that adult tree pruning would be carried out to eliminate branches that grow into the space of neighbouring plots. The blocks should always be placed across any gradient in soil fertility or slope of the land.

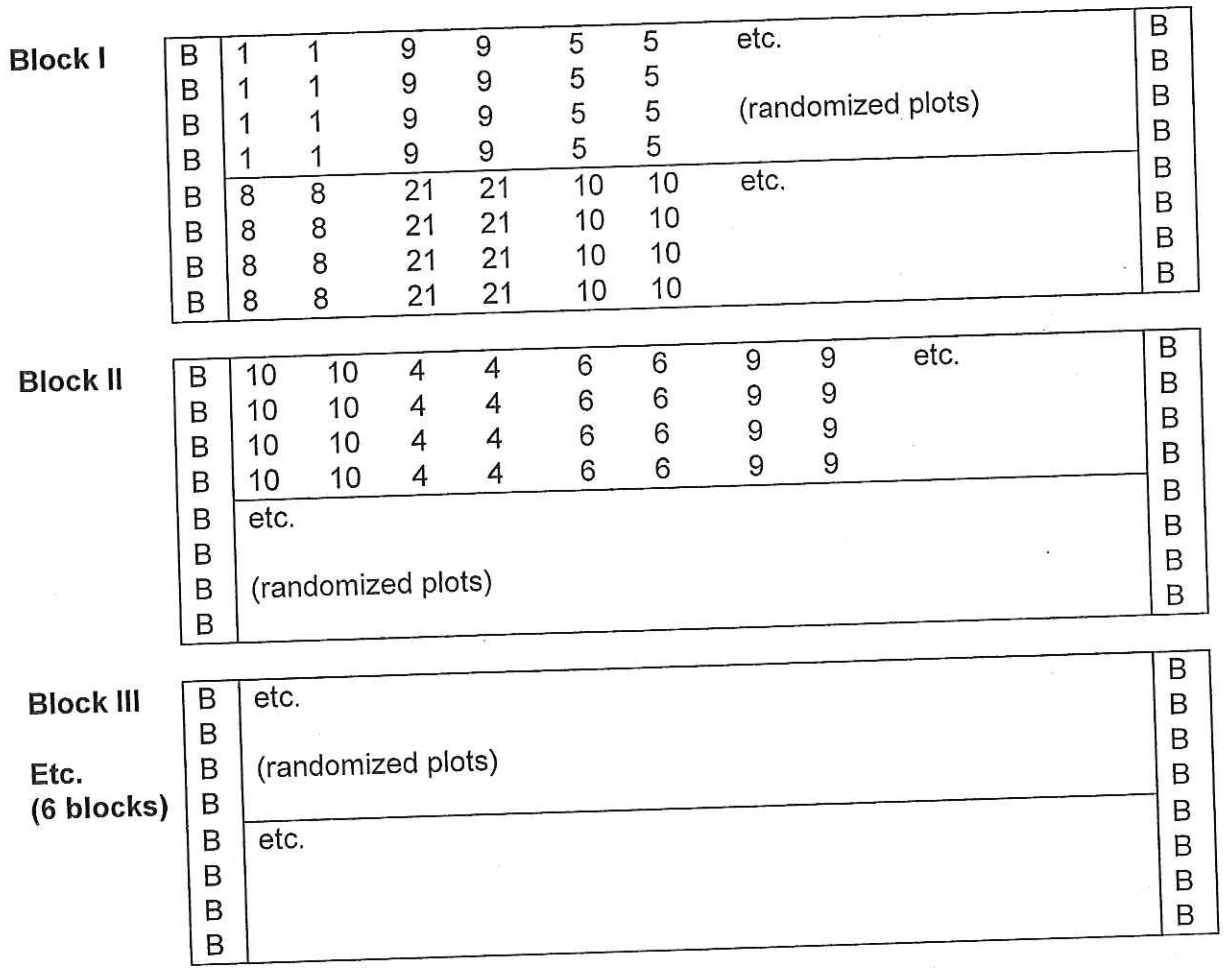
Depending on local recommendations, the spacing between trees may vary from one site to another. Planting density will be lower under more favourable growing conditions and higher under less favourable conditions. In general, spacing for clone trials can be slightly

less than is locally adopted for hybrid trials. For example, if the recommended spacing for seedlings is 3 x 3 m, the recommended spacing for clone trials in the project would be 2.5 x 3 m.

The blocks can be continuous or separated by a slightly larger spacing (i.e. 3.5 m instead of 2.5 m). The latter is recommended as it will permit easy plot identification, entrance for observations and appreciation of differences between plots. Border rows are required at the side edges of the trial. An example of a layout of the blocks and plots for the International Clone Trials is depicted in Figure 1.

The 'international' and 'local' clones may be compared within the same trial or in separate trials. If all clones are planted together the trial will be very large, which will increase heterogeneity in the trial. Furthermore, the 'local' clones can be multiplied immediately whereas the multiplication process for the 'international' clones will be more time-consuming. Therefore, it is generally better to plant the two types of clones in two separate trials. These can be installed in the same year or in two successive years. A common set of three standard clones is to be included in both trials to be able to compare the results. The statistical design and layout of the two trials should be identical (Figure 1).

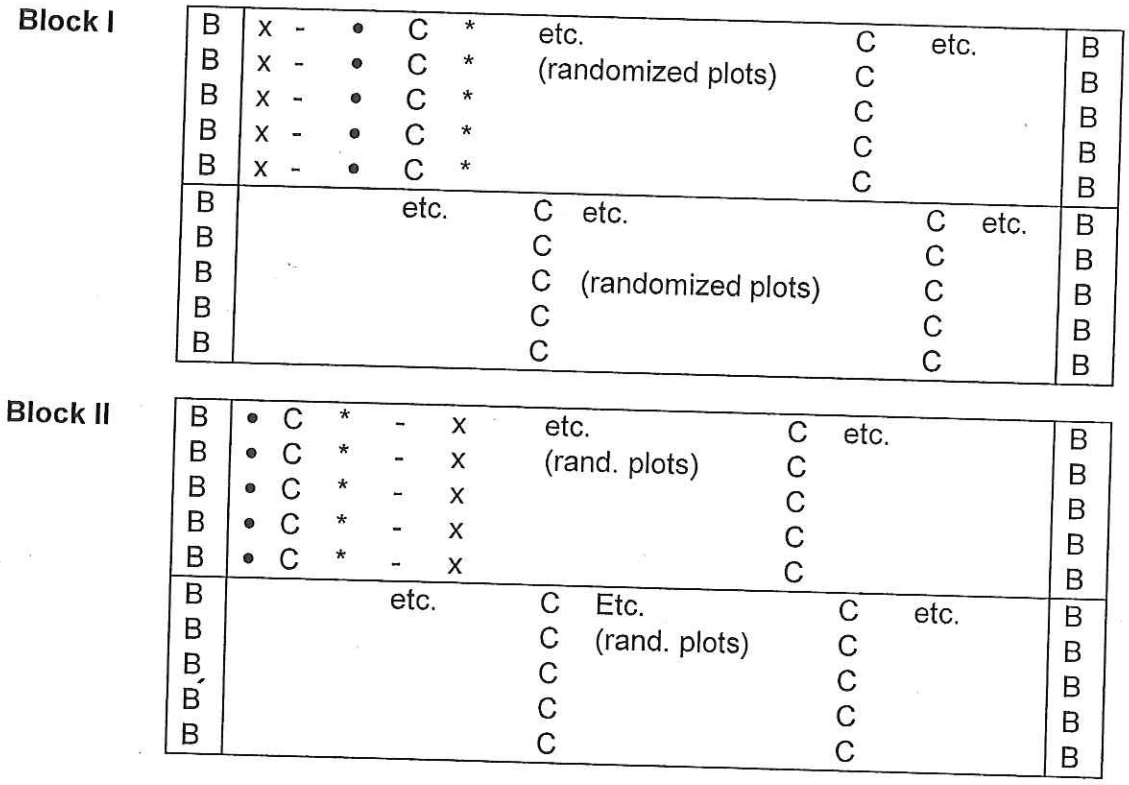
Figure 1. Example of a field design for the International Clone Trial with extra spacing between blocks. Trees of different varieties are indicated by different numbers. Border trees are indicated by the symbol B.



Local Clone Observation Plots

The objective of these plots is to make observations on clones to be selected for confirmation trials or for further breeding. At the same time, these plots serve as a collection in which useful observations on agronomic traits can be made. Experimental variation is controlled partially by using two replicates (blocks), with randomized plots of five trees each, and also by using of one or two control varieties planted at regular intervals (each ten or 15 rows, respectively, for one or two control varieties). The field layout is depicted in Figure 2.

Figure 2. Recommended field design for Local Clone Observation Plots. Test varieties are indicated by different symbols (x, -, etc.). B = trees in the border rows and C = trees of one or two control varieties, repeated at 10-15 row intervals.



Internationally Coordinated Hybrid Trials

A larger number of trees is needed for the hybrid trials because of the genetic variation within families. The recommended number of trees per plot is ten, planted in double rows of five trees, and the minimum number of replicates is six. However, the recommended number of replicates is eight, totalling 8 x 10 = 80 trees per family. The recommended field layout and use of border rows is basically the same as for the International Clone Trial (see Figure 1). As indicated above, spacing of the hybrid trials can be slightly wider than for the clone trials. Inter-plot competition is to be restricted by pruning of branches that grow into neighbouring plots (see Working Procedure 5).

Nursery designs

Some of the nursery plants (budded plants or seedlings) will be used for resistance screening, others will be used directly for the field trials. It is therefore necessary to provide uniform growing conditions in the nursery (especially uniform shade!). The use of an experimental design in the nursery will ensure that the environmental variation is randomly distributed over the test material. This also will allow for more accurate measurements

taken at that stage (such as vigour) and so the use of block designs is recommended. For example, random distribution of five plants in five or six blocks would be appropriate for application of the *Phytophthora* leaf disc test in the International Clone and Hybrid Trials. Similar designs will be required for screening of insect resistance or tolerance.

Laboratory designs

Experimental designs should be applied also in the laboratory tests to evaluate reaction of plant organs to diseases or pests. This will help to control sources of outside variation as effectively as possible and enable statistical analysis of the results. Requirements are indicated in the description of the working procedures for these tests (Pearce 1980).

Conclusion

Experimental designs are proposed for comparing cocoa clones and hybrids in the project. They are designs conventionally used for trials (Pearce 1980) and are relatively easy to set up in a trial network spread over several countries. Standard designs are defined here for field trials and some indications are given for evaluation of nursery materials and laboratory tests.

The standardized adoption of field trial design is required to obtain fully comparable results. This will facilitate compilation and joint statistical analysis of the data. It will provide knowledge of the genetic value of the material being tested, along with a reliable estimation of genotype x environment interactions. Little is known about such interactions in cocoa, yet they need to be estimated in order to fine tune cocoa genetic improvement strategies in the different producing countries.

Author of draft procedure

C. Cilas, CIRAD, France

References cited

Cilas C. 1995 Dispositifs expérimentaux adaptés aux essais de sélection chez le cacaoyer. Pp. 151-160 *in* Traitements statistiques des essais de sélection (CIRAD-CP).

Lotode R. and P. Lachenaud. 1988. Méthodologie destinée aux essais de sélection du cacaoyer. Café Cacao Thé 32: 275-292.

Pearce S.C. 1980. Randomized blocks and some alternatives: a study in tropical conditions. Trop. Agric. (Trinidad), 57, Pp. 1-10.

References for further reading

Lotode R. 1971. Possibilités d'amélioration de l'expérimentation sur cacaoyers. Café Cacao Thé 15: 91-104.

Nyassé S. 1998. Etude de la diversité de *Phytophthora megakarya* et caractérisation de la résistance du cacaoyer (*Theobroma cacao L.*) à ce pathogène. Thèse. Inst. Poly. de Toulouse. 145 pp.

8. Analysis of trial data

Scope

The main aim of cocoa genetic improvement is to select clones or hybrid families with good agronomic, resistance and quality traits. In addition, the stability of the desired traits in time and space is also a very important selection criterion. This latter aspect has only been taken into account in a very limited way in the selection operations of the different producing countries.

The trial network to be set up under the CFC/ICCO/IPGRI project provides a very good opportunity for obtaining reliable estimations of genotype x environment interactions. In order to do that, common experimental designs will need to be adopted and the data collected in the trials will have to be centralized for analyses. Procedures are proposed here for organizing data collection and their input into electronic datafiles. The possibilities for analyses are then discussed, aiming at optimum utilization of the data generated by the trial network.

Data collecting

Data should be collected using the agreed testing and observation procedures. If this is not done, comparison of data between countries will be impossible. The use of standardized recording sheets is a requirement for carrying out common analyses on the data gathered in different countries. The possibility of mistakes in the data observation and recording should be controlled at all stages.

Data processing

Standardized data processing and centralized data analyses are to be carried out for all data to be obtained on the International Clone and Hybrid trials and are also recommended to be applied for other data to be exchanged in a comparable manner between countries, such as data on the 'CFC Project Collection'.

The data gathered using the standardized procedures will need inputting in a standard format. The simplest method would be to use for all the data a spreadsheet type of software (Excel or Quattro Pro). The data entry will be undertaken locally. Mistakes in data processing must be strictly controlled at all project sites. This can be done by checking hard copies of the files and by calculating simple statistics on the data such as means, standard deviations, etc.

The mean data are to be used for reporting purposes. All electronic files will be forwarded for centralized analyses at six-month or yearly intervals, together with the progress reports.

For most trials, two type of tables will have to be produced: a table with data for each individual observation unit (tree, leaf disk, seedling, etc,) and a table with the observed or calculated average values for each statistical unit (plots within replicates). Both files are to be forwarded for centralized analysis.

Statistical analysis

The results of all project trials will have to be analyzed in each country, which will make it possible to class the different clones according to the traits observed.

Centralized analyses will be carried out on the common trials to compare results from different sites and calculate genotype x environment interaction. These are also required for comparison between project trials within the same country (e.g. local clone and hybrid trials, to calculate parent/offspring relationships) and/or to carry out more sophisticated analyses that cannot be made locally (as complete genetic analyses, multivariate analyses, etc., as required).

Analysis of variance will be carried out, followed by multiple comparison of means tests. The analysis of variance model for each trial is as follows:

$Y_{ij} = m + C_i + B_j + E_{ij}$;

Where

Y_{ij} is the variable to be analyzed (i.e. production of clone i in block j),
m is the trial mean,
C_i is the effect of clone i,
B_j is the effect of block j, and
E_{ij} is the error variance s^2.

Several genetic parameters will be estimated, such as heritability (h^2), general and specific combining ability, and phenotypic, genetic and environmental correlations between the different traits (Baradat *et al.* 1995). For this, it will be necessary to apply the multivariate analysis of variance model.

By grouping together the trials in the different countries, it will be possible to estimate genotype x environment interactions and study genotype stability in the different environments or countries taking part in the trial network. The model of variance for the centralized data is as follows:

$Y_{ijk} = m + C_i + L_k + B_j(L_k) + I_{ik} + E_{ijk}$;

Where

Y_{ijk} is the variable to be analyzed (production of clone i in block j in location k),
m is the mean of the trials,
C_i is the effect of clone i,
L_k is the effect of location k,
$B_j(L_k)$ is the effect of block j at location k,
I_{ik} is the clone x location interaction,
E_{ijk} is the error variance s^2.

The interaction effects can then be analyzed by different methods:

- estimation of ecovalences (Wricke 1962),
- separate regressions of genotypes on environments (Finlay and Wilkinson 1963), or
- joint regressions of genotypes on environments (Hardwick and Wood 1972).

An approach combining these different methods is proposed by Baradat and Yazdani (1987).

Conclusion

Procedures for data gathering and processing have been drawn up to enable centralization of the data from the trial network in the project. Adoption of common trial designs and identical data collection and processing by the different countries involved should permit optimization of the result analysis and interpretation phases. These procedures should make it possible to compare the results obtained in the different countries and study genotype x environment interactions, about which little is known in cocoa. Study on these interactions is essential for fine-tuning cocoa genetic improvement strategies and collaborative approaches between the different producing countries. The different possible models available for studying these interactions can be found on the INRA/CIRAD software known as OPEP.

Author of draft procedure

C. Cilas, CIRAD, France

References

Baradat P., J.M. Bouvet and T. Labbé. 1995. Conception d'index pour la sélection réciproque récurrente: aspects génétiques, statistiques et informatiques. Pp. 101-150 *in* Traitements Statistiques des Essais de Sélection. CIRAD-CP, Montpellier.

Baradat P. and R. Yazdani. 1987. Stability of genotypic expression for monoterpene synthesis in clones of Scots pine growing in different sites. Scand. J. Forest Res. 3: 21-36.

Finley K.W. and G.N. Wilkinson. 1963. The analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res. 14: 742-754.

Hardwick R.C. and J.T. Wood. 1972. Regression methods for studying genotype x environment interactions. Heredity 28: 209-222.

Lachenaud Ph. 1984. Une méthode d'évaluation de la production de fèves fraîches applicables aux essais entièrement randomisés. Café Cacao Thé 28: 83-88.

Wricke G. 1962. Uber eine Methode zür Erfassung der oekologishen Streubreite in Feldversuchen. Z. Planzen Schutz. 47: 92-96.

9. Physiological traits

Scope

One of the most important pieces of information that will be generated in any germplasm trial is the relative yield of each of the genotypes under study. Multilocational trials, such as the International Clone Trial, will generate information on the genetic stability of yield over different environments. It could be that certain genotypes perform better in certain circumstances than in others, and that certain sites may lead to generally higher or lower yield amongst all or some of the genotypes under study. The purpose here is to carry out, in addition to yield observations, relatively simple measurements on the physiology of the genotypes in the International Clone Trial which could help explain the yield variation that could emerge from the present investigation.

Cocoa yield can be considered within a relatively simple physiological framework that helps to explain yield capacity and growth of cocoa genotypes. For example, measurements of flower production, pollination rates and cherelle wilt on selected areas of trees will provide valuable information on the dynamics of pod production and could explain yield limitations occurring under certain conditions.

Measurements of changes in trunk circumference and the length of the main branches can provide non-destructive estimates of rates of biomass production. If combined with yield measurements, estimates can be made on the partitioning of carbon between structural and yield components of the tree; this can be an important measure of the efficiency of yield production. Measurements of canopy size and light interception by the canopy (using a ceptometer or a solarimeter) can provide useful information on the efficiency of the tree in converting light energy into yield. Sophisticated equipment, such as canopy analyzers, can provide more comprehensive information about canopy architecture.

Other measurements such as photosynthetic rates, may be beyond the scope of this study because of the lack of availability of this equipment, however, such measurements can provide valuable additional information on the efficiency of specific genotypes in converting light energy into yield.

Visual estimates of physiological traits

Flowering, fruiting and flushing rates (Recording Sheet 1)

Intensity of flowering, fruiting (presence of new cherelles), cherelle wilting and leaf flushing will be observed by applying a subjective 5-point observation scale: 1 is absence and 5 is abundant flowering, fruiting, cherelle wilting or leaf flushing. Observations will be made six times a year, three times at monthly intervals at the beginning of the main fruiting cycle and three times at monthly intervals at the beginning of the smaller fruiting cycle. In the International Clone Trial, these observations will be made on a plot basis, by estimating the average behaviour of all the plants of the clone in the plots (totalling about 150 observations, if 25 clones and six blocks). They will be carried out during the first two production years and repeated during the fifth and sixth production years to generate data on young and adult trees.

Branching pattern, canopy size and shape (Recording Sheet 2)

Branching pattern will be evaluated annually by a 5-point scale: 1 is low intensity of secondary branching and 5 is high intensity of secondary branching. Recommendations for canopy size evaluations are given elsewhere (see Working Procedure 6). The shape of a canopy is also evaluated by a 5-point scale: 1 is a horizontal, 'droopy' or 'spreading' branching pattern and 5 is an upwards growing canopy or 'erect' branching pattern. In clone trials, these observations will be made on a plot basis (estimates of average plot

behaviour for the trait) and carried out once a year during the first five years after planting.

Canopy density and light interception (Recording Sheet 3)

The canopy density (leaf density inside the canopy) is related to the leaf area index. It will be measured twice a year, in the middle of the rainy season and in the middle of the dry season. A 5-point scale will be used, with 1 indicating a very open leaf canopy and 5 a very dense leaf canopy. Please note that canopy density and size may not be correlated; small or large canopies may have a more open or more dense leaf layer.

Light interception of the leaf canopy is also related to leaf area index, though the nature of that relationship can vary between genotypes. Light interception by the canopy is visually determined in the middle of a sunny day by estimating the light transmission, i.e. the percentage of direct sunlight passing through the leaf canopy and reaching the soil (% sun-flecks in relation to shaded areas, without taking into account the light entering from open spaces between planting rows). A 5-point assessment scale is used for light transmission, with 5= more than 40% light transmission (sun-flecks), 4 = 20-40%, 3 = 10-20%, 2 = 5-10%, 1 = 1-5% and 0 = 0% transmission of light (no sun-flecks). Note that a dense canopy may intercept more or less light depending on the position of the leaves (more or less vertical). Both canopy density and canopy light interception will be initiated in the third year after field planting and observed twice a year during three consecutive years. For hybrid trials, observations will be on an individual tree basis and for clone trials on a plot basis.

It is realized that canopy density and light interception may be affected by insect attack or pruning techniques. They should therefore be observed before any major canopy pruning is undertaken (often done at the end of the dry season) and when no specific defoliation has occurred.

Canopy pruning intensity (Recording Sheet 4)

In many countries, good local agronomic practices include more or less severe canopy pruning. In the International Clone Trial it is recommended to apply canopy pruning to avoid inter-plot competition for canopy space. 'Canopy pruning intensity' is a measure of excessive vegetative growth, which might be unfavourable for pod production. For clone trials, this trait is again estimated on a plot basis on a 5-point scale by assessing the number of leaves/branches pruned. It should be observed any time a major pruning is applied during the first four production years.

Measurements involving equipment

Light interception can be quantified by using a relatively cheap light sensor (e.g. Skye Instruments SKE 500/510/140G) attached to a recording device with an extension lead. The method involves taking around ten measures per plot of alternate incident and transmitted radiation readings. Incident radiation is measured above the canopy, but below the overhead shade, and transmitted radiation is measured below the cocoa canopy, avoiding sun-flecks. Light interception is calculated as: (incident minus transmitted radiation)/incident radiation. It is proposed to apply quantitative measures of light interception by using equipment on at least some of the project sites. This should enable the verification of the value of the visual estimates of canopy density and light transmission.

Application of procedure

The above-described observations are strongly recommended for the International Clone Trials. However, these procedures may also be usefully applied to other project trials. For clone trials, the observation unit is a plot (with eight trees). For hybrid trials the same evaluation method is better applied at individual tree level.

Author of draft procedure

P. Hadley, The University of Reading, United Kingdom

Recording Sheet 1. Flowering, fruiting, cherelle wilting and leaf flushing intensity evaluated on a 1 to 5 point scale (1 = absence, 5 is abundance of the trait)

Trial: _____ Planting date: _____

Plot no.	Geno-type	Dates of observations								Remarks
		Flower-ing	Fruit-ing	Wilt-ing	Flush-ing	Flower-ing	Fruit-ing	Wilt-ing	Flush-ing	
Etc.,										

Recording Sheet 2. Branching intensity (bi) and canopy shape (cs), estimated on a 1 to 5 point scale (1 = low branching intensity or droopy canopy shape and 5 = high branching intensity and erect canopy shape, respectively)

Trial: _____ Planting date: _____

Block no.	Plot no.	Geno-type code	Dates of observations										Remarks
			bi	cs	Bi	cs	Bi	Cs	bi	cs	bi	cs	
Etc.,													

Recording Sheet 3. Canopy density (cd) and light transmission (lt), estimated bi-annually on a 1 to 5 point scale. For canopy density 5 = dense canopy and 1 = very open canopy; for light transmission 5 = more than 40% light transmission (sun-flecks), 4 = 20 – 40 % 3 = 10-20%, 2 = 5-10%, 1 = 1-5% and 0 = 0% transmission of light (no sun-flecks).

Trial: _____ Planting date: _____

Block no.	Plot no.	Geno-type code	Dates of observations										Remarks
			cd	lt	Cd	lt	Cd	Lt	cd	lt	cd	lt	
Etc.,													

Recording Sheet 4. Canopy pruning intensity, measured on a 1 to 5 point scale (1 = few leaves/branches pruned and 5 = many leaves/branches pruned)

Trial: _____ Planting date: _____

Block no.	Plot no.	Genotype code	Dates of observations				
Etc.,							

10. Criteria for visual selection of individual trees

Scope

The breeder is often confronted with the need to select individual trees in heterogeneous populations (farmers' fields, breeding trials), without availability of long term records on individual trees. In breeding trials, preference is to be given to selection within progenies that have good average performance. Within the CFC/ICCO/IPGRI project such pre-selection of adult trees is proposed for the local clone selection plots and for population breeding. The objective of this proposal is to indicate important selection traits that can be quite simply observed to increase efficiency of individual tree selection.

Selection criteria

Yield potential

Yield of individual trees has a low heritability. Therefore, it appears of little use to observe individual trees for several years for pod production. However, presence of a large number of protuberant flower cushions in relation to neighbouring trees can be an indicator for good yielding capacity of adult trees. Border trees or trees otherwise receiving more light tend to have more flower cushions than shaded trees in the middle of cocoa stands. This is an important factor to be considered when carrying out individual plant selection for yield.

Vigour

Generally, a medium level of vigour is to be preferred. Excessive vegetative vigour is undesirable when growing conditions are favourable, as this increases competition between adult plants and the chances of yield decline as the plantation ages. However, high vigour can be required under conditions that limit vegetative growth (e.g. poor soils, low rainfall areas, frequent high insect attacks in the canopy). Relatively low plant vigour is required for high planting densities and, in certain cases, canopy growth is to be limited (as this is often necessary for efficient disease and pest control measures, e.g. for witches' broom disease and the cocoa pod-borer).

Yield/vigour relationship (Yield Index)

Plants to be preferred should combine high yield with medium or low vigour. The yield index can be calculated by dividing the dry cocoa yield by the trunk cross section (cm^2). Studies carried out in Côte d'Ivoire (Sounigo *et al.* 1994), suggest that a favourable yield index for clones is correlated to high yield of the offspring (cross-progenies). Therefore, a high yield index is a very important trait for clone selection.

Canopy shape

For clone selection, the shape of the canopy is an important criterion. If a canopy shape is spreading or 'droopy', this may mean that the clone grown from such tree will also have a less favourable shape. A more erect upward growing canopy is generally preferable (see Working Procedure 9 for definition of terms).

Precocity

Early flowering and fruiting of young cocoa trees are favourable traits. These can be observed on an individual tree basis.

Self-compatibility

This trait is of importance for the selection of clonal varieties. Self-compatible clones tend to produce set fruit earlier in the season than self-incompatible trees. Confirmation by manual

self-pollination is required if this trait is to be a main selection criterion (for description of pollination method see Working Procedure 11).

Pod index components

Pod index is the number of pods required to obtain 1 kg of dry cocoa beans. A favourable pod index (low) is desirable, as this implies less laborious harvesting. A favourable pod index can be estimated from bean number, bean size and pod wall thickness. These traits can be visually estimated by opening five mature pods per tree (see Working Procedure 6).

Resistance to diseases and pests

In several countries resistance to diseases has become the most important selection criterion. Incidence of *Phytophthora* pod rot in the field can have relatively high heritability, when measured in uniform fields over several years. When such long-term data are not available, early screening by applying leaf inoculations (of adult trees or of budded nursery plants) or detached pod inoculations will be required (see Working Procedure 14). In areas with a high incidence of *Phytophthora* cushion canker, the number of active flower cushions in relation to neighbouring trees may be an indicator of resistance to this disease.

Resistance to witches' broom and VSD may be efficiently evaluated when sufficient natural infection occurs in the field, by comparing with neighbouring trees. Resistance or low attractiveness to insects is normally more difficult to evaluate by visual observations on a single tree basis. However, due to the importance of mirids it could be rewarding to try to select, within pest foci, individual trees that show less attack. Accumulated mirid damage (amount of affected bark tissue) can also be taken into account for individual tree selection.

Quality

Cocoa quality is an important selection goal in several countries. As indicators for quality, the bean size and shape (big and roundish for Criollo types), color (light cotyledon color) and taste of pulp (sweet) or beans (absence of bitterness for Criollo) can be considered (see Working Procedures 6 and 12).

Proposal for visual recording of individual tree selection criteria

All quantitative selection criteria (yield, vigour, field incidence of diseases and/or pests) should be observed in relation to neighbouring plants. For rapid selection purposes, a 3-point scale or 5-point scale is often sufficient to express the existing variation. Table 1, attached herewith, indicates the suggested way of recording.

Authors of draft proposal

D. Paulin, CIRAD, and A. B. Eskes, IPGRI/CIRAD, c/o INIBAP, France

References

Sounigo O., N'Goran J., Coulibaly N., Clement D. and P. Lachenaud. 1994. Evaluation de clones de cacaoyers pour la productivité, la résistance aux mirides et la résistance à la pourriture brune des cabosses. Pp. 375-381 *in* Proc. 11th Int. Cocoa Res. Conf., Cocoa Producers Alliance, Lagos, Nigeria.

Table 1. Criteria for visual selection of individual trees

Trait	Observation/Selection	Recording scale
1. Yield potential	Visual estimate of number of protuberant flower cushions	1 (low), 3 (medium), 5 (high)
2. Vigour	Size of tree and trunk diameter relative to neighbouring trees	1 (low), 3 (medium), 5 (high)
3. Yield index	Obtained by relating trait 1 (yield potential) to trait 2 (vigour). Plants to be selected should combine high yield potential with medium or low vigour.	
4. Canopy shape	Spreading or more erect	1 (horizontal growth of fan branches) and 5 (erect growth of fan branches)
5. Yield precocity	Intensity of flowering/fruiting of young trees (2-4 years old)	1 (low), 3 (medium), 5 (high)
6. Self compatibility	Precocity of fruiting within the season	1 (late), 3 (medium), 5 (early)
7. Pod index components	Visual estimate after opening 5 mature, normal-sized fruits	
7.1. Bean size		1 (small), 3 (medium), 5 (large)
7.2. Bean number		1 (low), 3 (medium), 5 (high)
7.3. Pod wall thickness		1 (thin), 3 (medium), 5 (thick)
8. Resistance to diseases	Incidence of infected organs (twigs, fruits, flower cushions, stems,..). relative to neighbouring trees. Confirmation to be sought by early screening method (if available)	1 (absence of symptoms), 3 (medium), 5 (high)
9. Pest resistance	Incidence or estimation of accumulated damage (mirids)	1 (low), 3 (medium), 5 (high)
10. Quality traits	Observed on 3-5 ripe, healthy pods	
10.1. Bean shape		1 (small, flat), 3 (medium), 5 (large, roundish)
10.2. Pulp taste		1 (acid), 3 (intermediate), 5 (sweet)
10.3. Raw bean taste		1 (very bitter), 3 (some bitterness), 5 (absence of bitterness)

11. Manual pollination techniques and verification of incompatibility

Scope

Pollination in cocoa must be controlled in order to obtain the expected result. In the past, errors in results from hand pollination were identified by using isozymes and more recently, DNA markers. Even with strict control of hand pollination, a small percentage of unwanted fertilizations can still take place (Bastide and Sounigo 1993).

Possible sources of errors are:

- Incomplete isolation of flowers (crawling insects may enter the pollination tubes)
- Emasculation of flowers before pollination: this unnecessary manipulation may induce selfing and contamination of the pollination tweezers
- Other manipulations of the flowers can contaminate the pistil with self-pollen
- Successive use of different types of pollen, without cleaning the tweezers with alcohol between manipulations.

A description is given of a manual pollination technique, including self and cross-pollination, the use of mentor pollen to overcome self-incompatibility, and comparison of this "standard" pollination procedure with locally applied methods.

Description of the manual pollination technique

Self or cross-pollination

- Identification of the flowers to be pollinated the previous day, from 16:00 onwards, according to their size and the beginning of the separation of sepals;
- Isolation of flowers with plastic tubes (no more than two per tube) after treatment with aerosol insecticide. The tube is cut from a soft, translucent water-pipe, about 7 cm long x 2-2.5 cm in diameter. The distal end is covered with gauze maintained by an elastic band or a metal wire, and the base is fixed to the tree with modelling clay.
- The following morning (at about 08.00) the tube or just the elastic band is taken off. Pollination is carried out without emasculation and without removing any staminodes, as these manipulations increase the chances of self-pollination. The pollination is carried out by rubbing two stamens of fresh flowers onto the style, using sharp tweezers. The pollen deposited on the style can be seen with a trained eye or with an magnifying glass. The tube is then quickly replaced or closed. When different pollen types are manipulated successively, it is recommended to clean tweezers and hands with diluted alcohol (70%) at each change of pollen.
- The tube is taken off two days later.

Comparison of manual pollination techniques

The objective is to compare the locally adopted method with the above-described "standard" method in order to estimate the pollination success and the risk of self-pollination with both methods. The procedure is the following :

Comparison of pollination success

This is done by evaluating the fruit-set ten days after pollination, comparing the local pollination method with the above procedure. In total, a minimum of 100 flowers must be pollinated, using the same compatible pollen, to obtain a reliable estimation of pollination success.

Percentage of selfing

Fifty to hundred pollinations will be undertaken with both methods, using Catongo as the female parent and pollen from an Upper Amazon clone, in order to obtain at least 20 ripe pods for each method. The number of white and purple beans are counted for each pod. The percentage of white beans is a measure of self-pollination. If no Catongo trees are available, the methods should involve pollen with a marker gene, such as "axil-spot" provided by clone K5. Seeds obtained from at least 20 pods are sown and seedlings observed for the dominant "axil-spot" trait.

Evaluation of self and cross compatibility

Cocoa trees can be partially or completely incompatible. Self or cross-compatibility should be assessed by the following methods :

The flower isolation technique

- Realization of 50 selfings or cross-pollinations per clone (as above) at the beginning of the main flowering period.
- Un-sticking of tubes two days later.
- Counting the fruit-set ten days after pollination.

The clone is considered as completely self or cross-compatible if the fruit-set is greater than 25% (Posnette 1945). However, many cocoa clones (e.g. Upper Amazon clones) can give a percentage set of between 0 to 25%, and should be considered as partially compatible.

Technique without flower isolation

Under unfavourable pollination conditions, such as in very wet weather, evaluation of compatibility can be initiated by 50 self-pollinations without isolation using 20 control pollinations with compatible pollen (Amelonado or Catongo) per pollination session. If the clone presents no fruit-set, it is considered self-incompatible (control pollination should show a good fruit-set). If fruit-set is present, the self-compatibility will need to be confirmed with the first method.

Self-pollination using mentor pollen

Mentor pollen is needed for obtaining seeds from "completely" self-incompatible clones. Two types of pollen can be used for this: pollen from clone K5 (carrying the "axil-spot" trait), as described by Glendinning (1960), and pollen from *Herrania sp.*

Author of draft procedure

Ph. Lachenaud, CIRAD, c/o CNRA, Côte d'Ivoire

References

Bastide Ph. and O. Sounigo. 1993. Production de semences hybrides de cacaoyer en Côte d'Ivoire. Essais de différentes pollinisations manuelles. Café Cacao Thé 37:121-128.

Glendinning, D. R. 1960. Selfing of self-incompatible cocoa. Nature 187:170. Trinidad.

Posnette, A. F. 1945. Incompatibility in Amazon cacao. Trop. Agric. (Trinidad) 22:10.

12. Evaluation of cocoa quality

Scope

So far very little is known about the stability of quality traits in cocoa. The International Clone Trial offers a unique opportunity to evaluate quality of the same clones in different environments.

Dry bean sample preparation

Each dry bean sample for quality testing should be at least 1 kg of fermented dried beans (or at least 5 kg of fresh beans). Samples are obtained during the main harvest period as described in the procedure for yield measures. It is very important that all samples be obtained simultaneously and fermented in mosquito net bags placed within the same cocoa fermentation mass of a minimum height of 50 cm and a fresh weight of 500 kg (0.5 m^3). The duration of fermentation should be standardized to six days (6 x 24h), with beans to be turned over on day 1, day 3 and day 5. The beans may be obtained from the same trial or from another simultaneously harvested uniform cocoa lot.

Quality traits to be observed

The traits to be considered are commercially important physical, chemical and aromatic components of cocoa quality. Analysis of some secondary compounds is also proposed as these vary according to genotype and little is known about environmental influence on these traits.

Physical traits

- Weight of 100 beans (this trait will be measured under yield observations)
- Shell content (this trait will be measured under yield observations)
- Butter hardness

Chemical traits

- Butter content
- Polyphenol, theobromine and caffeine content
- Content in aroma precursors (reducing sugars, free amino acids)
- Analysis of aromatic compounds

Organoleptic traits

- Flavour profiling

Application of quality analyses

The first samples from the International Clone Trial will become available only in the fourth or fifth project year. Samples collected during the first harvests may already be used for preliminary evaluation. Possibilities to carry out the quality analyses proposed here will depend on specific financing to be identified and probably also on project renewal.

It may be decided to start analyses in the first year with one sample per clone per country and three countries, each from a different continent (Africa, Asia and America). In the second year, the same may be carried out for three other countries.

Author of draft procedure

E. Cros, CIRAD, France

13. Documentation and photography of the commonly used project clones

Proposed activities

Over the past ten years the International Cocoa Germplasm Database (ICGD) has accumulated a great deal of data on many cocoa clones. Information from ICGD has been used to help select the most suitable accessions for the International Clone Trial.

Data-sheets for these 'International' clones will be produced and circulated as soon as the results of the molecular survey are available. The sheets will contain two parts; the first part will show passport data, agronomic characteristics, some basic morphological traits (i.e. large/small pods, large/small flowers) and include a picture with genetic fingerprinting information where available. The agronomic data should include traits such as vigour, yield, resistance to pests and disease, and bean weight. Details on the value of the clone as a parent, such as combining ability values, will also be included where these are available. The second part of the data-sheet will provide a more complete morphological description for use in confirming the identity of the germplasm. The original source of all these data will be clearly referenced.

The database files will also be used to produce a list of commonly used clones, i.e. clones present in several countries but which are not necessarily involved in the International Clone Trial, in the Local Clone Trials or in the 'CFC Project Collection'. This list will be circulated to the participants who will then be asked to respond with information on the utilization of these clones in their country and provide data where they are available. They will also be asked to provide data on the origin of these accessions in order that we may be able to trace any mislabelled material and look at which collections have clones from the same source. From this we will be able to make some assumptions as to the real identity of some of the more widespread material. Any questionable clones could then have their identity checked using molecular methods.

Once this process has been completed it will be possible to select 50-70 of the most widely used clones for subsequent documentation. Data-sheets will then be produced on these clones from the data available in the database and from that supplied by the participants and circulated among the group. As the project progresses these data-sheets can be updated when more information becomes available.

In the same way, during the course of the project, data-sheets will be created and circulated for the 'CFC Project Collection' (and probably used for subsequent publication). The type of data available for the clones for the 'International Clone Trial' and the widely used clones may not always be the same as for the core collection. The emphasis will be to distribute as much useful data as possible.

During the first project year it is hoped that the data-sheets for the international clones will have been produced and circulated. Also, by this time, the list of the most widely used clones will have been produced and sent out to participants for their response. In addition, images of all the various clones involved in the project will start to be produced. A BAL Plantations/BCCCA/INGENIC initiative in 1996 investigated various ways of photographing cocoa germplasm. They found that a light blue background with even lighting gave the best results for colour. A picture of a mature pod, whole and in transverse section, an immature pod, a sample of peeled beans, a mature leaf and some flush leaves were very useful. It is proposed that the project photographs be taken in the same way. A close-up photograph of the flower, whole and/or dissected to show distinguishing features would also be valuable. However, this may be a very time-consuming operation and it is suggested that photographs of the flower parts be an optional part of the photography project, to be decided by the project partners depending on their time and resources. As reference a scale bar, a colour bar and the date and location should also be included and, of

course the material should be clearly labelled. Specific outstanding features may also be photographed at the discretion of the project partners, for example unusual tree architecture or habit in the field.

Once the final lists of clones have been agreed using the existing database records and the results of the micro-satellite survey, the photographic survey will begin. It is anticipated that by the end of the third year images of all the clones in the project will have been produced. All such information will be redistributed, via the database network, among the group.

As the project progresses it is hoped that our library of pictures will expand greatly and enable quick and efficient information exchange between participants. This widespread and comprehensive exchange of germplasm characterization data will minimize the risk of mislabelling problems in the CFC trials and address the wider issues of germplasm identification.

Publication

By the end of the five-year trial period a large amount of data will have been accumulated on the clones included in the clone trials, on the 'CFC Project Collection' and on other widely used clones, which it is anticipated will total between 250 and 300 clones. It is proposed that this information be presented in a document containing descriptors, information on agronomic traits, images and diagrams along with any other data deemed useful by the project partners. As a manual for breeders, agronomic data will be of most value. As a key for the identification and characterization of germplasm, the morphological detail will be of most use. The inclusion of pictures and diagrams will be valuable to all those working with cocoa germplasm. Any further suggestions for the type of data to include in this publication will be welcomed. A list will be circulated to allow project partners to suggest the type and amount of data they would find useful, bearing in mind that all the project data will be held in the ICGD. The project document would be complementary to any ICGD publications.

Authors of draft procedure

C. S. Ford and M. J. End, ICGD/The University of Reading, United Kingdom

Disease Resistance

14. Early screening of resistance to *Phytophthora* spp. by means of leaf disc inoculation

Scope

Resistance to cocoa black pod disease is quantitative in nature. Field resistance evaluation takes many years of observation in uniform, well-designed trials in order to be reliable. Broad sense heritability of the percentage of rotten pods on individual plants can be 0.6 for nine years of observations but is much lower (0.1 to 0.3) for experiments with only three to five years of observation (Kebe *et al.*, 1999). Broad sense heritability of the leaf disc inoculation method, by applying only one inoculation series, can be as high as 0.6 (Nyassé *et al. 1999*). Furthermore, it has been shown so far that, at progeny level, this test can give significant correlations with field attack (Kebe and Tahi, in press). Evaluation of individual plant resistance has so far given less conclusive results. The repeatability and effective application of this test will certainly depend on adequate standardization of environmental and physiological factors that influence leaf tissue resistance. These factors, as far as they are known, are indicated in the description of this test. This method is expected to speed up breeding and selection activities such as large scale evaluation of germplasm collections, breeding trials, early selection of individual plants or progenies growing in the nursery or field, and studies on host x pathogen interaction.

This working procedure has been adapted from the method applied by Nyassé *et al.* (1995).

Leaf sampling

Time of harvesting

In order to avoid the possible effect of time of harvesting on resistance level (Tahi, unpublished), leaves should best be harvested within the space of two hours, early in the morning (e.g. from 07.00 to 09.00). In large experiments, which take more than two hours for leaf harvesting, the order of harvesting is to be noted. This could be used in analysis of covariance to correct for any effect of harvesting time. Leaves are labelled and placed in an icebox containing humidified foam, to maintain 100% relative humidity.

Leaf age and exposure to light

Leaves should be in good physiological condition, without insect attacks, and of similar age and exposure to sunlight (the effects of leaf age and light intensity on resistance to *Phytophthora* are known: Tahi and Kebe, unpublished data). The leaves should be taken from twigs beginning to show a change in the colour of the cortex from green to brown, which corresponds to about two-month-old leaves. The exposure of the leaves to light should correspond to medium shade conditions (e.g. from the outer part of the canopy of a shaded cocoa tree, but from the inner lower part of the canopy of an unshaded tree with open canopy growth). For nursery plants, it is very important to provide uniform shading in order to obtain reliable results.

Number of leaves and inoculation series (replicates)

Replicate inoculation of a large leaf sample over a certain time, at regular intervals, will provide the best condition for obtaining reliable results. When more leaves of the right stage are available for one genotype at a given time, fewer inoculation series will be needed. When screening the average resistance of clones or seedling progenies, a sufficient number

of leaves of the adequate stage is normally available and fewer replicates will be needed. However, for individual tree or seedling selection, it is more difficult to find leaves of the right stage at one inoculation date, so more replicates will be required for accurate estimation of the average resistance level over a certain time.

For the evaluation of the average resistance of clones planted in the field a minimum of three leaves from four to six plants are to be used for each replicate. For budded plants in the nursery, two leaves from each of six to ten plants will be used for each clone (e.g. International Clone Trial). For seedling progenies (nursery seedlings for hybrid trials), it is recommended to use one leaf from each of 20 plants. Two inoculation series will normally be sufficient, but for the International Clone and Hybrid Trials three replicates are to be carried out: two series using nursery material and one series using leaves taken from field plants, one year after planting.

For reliable selection of individual plants (seedlings or field plants), the number of replicates will depend on the degree of repeatability observed. For evaluation of individual adult field plants, five to eight leaves are required per tree for each of a minimum of two inoculation series. For evaluation of individual seedling plant resistance (four to ten months old), the use of two leaves is recommended, with three to four replicates. If only one leaf is used per replicate, a larger number of replicates (four to six) will be required to obtain more reliable results.

Control variety

In general, it is recommended to use at least two control varieties (one resistant and one susceptible) growing under the same conditions as the test varieties. With cross progenies, it would be of interest to use the parental clones, grafted in the nursery, as controls. The controls make it possible to compare one inoculation series to another and to assess selection progress as compared with commercial varieties or parental genotypes. The control varieties should be sampled and treated in the same way as the test varieties (using 4-6 different plants per control variety).

Leaf disc preparation and number

Leaf disc preparation

Leaf discs (1.7 - 2.0 cm in diameter) can be obtained by using a cork borer or a semi-automated perforating device. Discs are placed with the underside of the leaf upwards on humidified foam in inoculation trays.

During leaf disc preparation, the relative humidity in the laboratory should be high to prevent the leaf discs from drying out; it may be necessary to spray water into the air to maintain high relative humidity. After distributing all the discs over the inoculation trays, it is recommended to atomize very fine droplets of sterile distilled water onto them. This will provide more uniform humidity of the discs at the time of inoculation (often several hours later). The trays are then closed and placed in the inoculation room.

Leaf disc number

The number of discs per genotype varies according to the number of leaves taken per genotype (individual nursery or field plants or progenies). If many leaves are available per genotype (as is the case for clones or seedling progenies) it is recommended to use relatively few discs per leaf. If few leaves are available (e.g. for selection of nursery seedlings), more discs per leaf will be used.

For each inoculation series, it is recommended to inoculate 8-16 discs for individual seedling screening and 40-60 discs for progeny screening. The discs of one genotype (individual plant or progeny) are to be distributed over three to four inoculation trays, which are required to even out the possible effect of the inoculation tray on disease severity.

All genotypes to be tested in one inoculation series should be present in each tray, each of which is then considered as a block. If this is not possible, for example when individual tree selection is carried out on a large number of trees or seedlings, the discs will still be distributed over three or four trays which then represent randomized blocks within the inoculation series.

For the International Clone Trial, it is recommended to use three discs per leaf (in total 36 to 48 discs per genotype) and these will be distributed over three or four trays at random. For estimation of the average resistance of seedling progenies (e.g. the International Hybrid Trials), three discs per leaf are also recommended (in total 45 to 60 discs per progeny), distributed at random over three to five trays. If the objective is to carry out individual field plant selection, three to four discs per leaf will be required (in total 15 to 32 discs per tree). For individual seedling selection, 8-12 discs are used if one leaf is available, and six to eight discs per leaf if two leaves are available per seedling. These will also be distributed over three or four trays.

Size of inoculation trays and number of discs per tray

Inoculation trays of about 30 x 50 cm are most suitable for the experiments. Each tray can contain 300-400 discs (or 30-50 genotypes). For example, an experiment with 110 trees (100 field plants and two control clones, five plants per clone) would have 110 x 36 = 3,960 discs and nine or 12 inoculation trays per inoculation series. The 36 discs from one tree will be randomly distributed over three or four trays (random blocks within the inoculation series). A minimum of three people are normally required in order to prepare such an experiment in one day.

Pathogen isolate, inoculation and incubation

Selecting the pathogen isolate

A moderately aggressive single-spore isolate is to be selected for the standard test. The level of aggressiveness is to be determined in preliminary tests using resistant, moderately susceptible and highly susceptible control varieties. Isolates with high or low aggressiveness will give, respectively, high and low disease severity scores for all genotypes, decreasing the discriminative capacity of the test. The selected isolate inoculated at the standard inoculum density should ideally have disease reaction scores, on the 5-point assessment scale, of around 1 for the resistant control variety, 2 to 3 for an intermediate susceptible control variety and 4 to 5 for a highly susceptible control variety.

Inoculum preparation

The standard method of inoculation is undertaken using zoospore suspensions obtained from ten-day-old cultures (i.e. four days in the dark and six days under alternate duration of light) on V8 agar medium, at 12 to 15 g agar per litre, and incubated at 26 ± 2°C. Zoospore liberation is induced by adding sterile distilled water at 4°C into cultures with sporangia. In case the above method cannot be applied due to insufficient laboratory equipment or in case of sporulation problems, spore production can alternatively be obtained by inoculating sterilized, rinsed, detached pods with a mycelium disc. After four to six days the spores are washed off with cold distilled water. The spore suspension is kept at room temperature for one hour. Before inoculation, zoospore concentration is adjusted using a Malassez haemocytometer.

Inoculation

Inoculation will generally be undertaken late in the afternoon of the same day on which the leaf discs were prepared or early in the morning the next day. The standard density of the zoospore concentration is 3 x 10^5 zoospores/ml. However, exceptionally, it may be

necessary to adjust the spore density according to the level of aggressiveness of the *Phytophthora* species involved. Automated repeatable dispensers, displaying 10 µl droplets in the centre of each disc are recommended. As an alternative, round filter paper patches (7 mm diameter) soaked in the suspension, could be used for inoculation. The inoculation should be carried out at right angles to the direction in which the discs of the cocoa genotypes are displayed in the trays.

Incubation

Incubation of the trays, which should be airtight closed with a plastic cover, must be in darkness, at a temperature of 26 ± 2°C (air conditioned room). In the light, symptom development may be slower. Large daily temperature fluctuations should be avoided, as these may cause evaporation (drying of discs) and condensation (formation of big droplets on the lower side of the lids) of the trays. After three days, the trays are inspected; inoculation droplets that have not yet been absorbed should be dried with filter paper before re-closing the trays.

Assessment scale and timing of observations

A five-point assessment scale is to be used, for scoring the size of the brown or necrotic infected leaf area:

0: no symptoms
1: very small localized brown or dark-brown penetration points
2: small penetration points with some connections between them
3: coalescence of brown spots forming intermediate-sized lesions
4: large, coalesced lesions containing lighter or darker brown coloured spots
5: large, uniformly expanding brown lesions.

Scoring should best be done by observing the leaf disc against the light (window). This is specially required to distinguish yellowish-brown water penetration spots from the darker fungal infected spots at the inoculation site. Depending on the speed of development of symptoms, disease severity is scored five, six or seven days after inoculation when the susceptible control variety reaches average scores of 3-4 (see Recording Sheet 1).

Repeatability

Before initiating large-scale resistance testing, it is recommended to check the repeatability of the leaf disc inoculation method under local test conditions. This is done by applying the same method three or four times, using a restricted number of standard clones or seedling progenies with different resistance levels.

In the CFC/ICCO/IPGRI project, the leaf disc test will be applied to evaluate resistance either of progenies (clones or seedling progenies represented by several plants), of individual plants (for individual plant selection) or both (combined family/individual plant selection). The repeatability of these tests is best evaluated by calculating the rank correlations for genotypes between inoculation series. If the coefficient of rank correlation is high, repeatability of the test can be considered good and no further replicates may be needed. However, if the rank correlation is low, more replicates will be required for accurate estimation of resistance.

Repeatability of the test is expected to be quite good when the average resistance level of progenies is evaluated according to the above indicated method. However, for individual field plants or nursery seedlings, repeatability is not expected to be so good due to the greater environment x genotype interactions and the availability of fewer leaves at the right stage for individual plants than for progenies. This means that a larger number of replicates are required to obtain reliable data with individual plant selection. It is possible that for one inoculation series no leaves at an adequate stage of development are available for certain

genotypes. If the experiment permits, it is recommended in such cases not to use such plants, or to use a leaf sample composed of some older and some younger leaves (with an average age of about two months).

Note: For individual field plants, a negative correlation between attached pod and leaf disc inoculation methods has been observed (Nyassé, unpublished data). A possible explanation is that the resistance of leaves and pods is adversely affected by light conditions: leaf resistance appears to increase and pod resistance appears to decrease with higher shade levels.

Statistical design and data analysis

Within inoculation series, the genotypes are distributed over at least three or four trays (blocks). This constitutes a randomized block design. When a large number of genotypes are tested within one replicate (inoculation series), the use of a common control genotype will help to calculate disease severity scores relative to the common control. These relative scores can then be analyzed as a complete randomized design, with three to four observations (average relative scores per tray) per genotype.

Analyses of more than one inoculation series (see Recording Sheet 2) are most correctly made as a randomized block design, each block being one inoculation series. The average score of one genotype in each replicate constitutes the basic statistical unit for analysis of variance.

Points arising from subsequent discussions

Subsequent observations of scientists involved in the project have indicated that care needs to be taken with the effect of aphid attack and insecticides in the nursery. Leaves that have suffered from aphid attack seem to become more susceptible, possibly due to the sugary aphid exudates that may favour fungal growth on the leaf discs. On the other hand, it appears that insecticides may inhibit the growth of the fungus and lower the disease reactions in the leaf disc test. It is hoped that these effects will be further quantified in the course of the project.

Authors of draft procedure

G. Blaha, CIRAD, c/o CCRI, Papua New Guinea; A.B. Eskes, IPGRI/CIRAD, c/o INIBAP, France; B.I. Kebe and G.M. Tahi, CNRA, Côte d'Ivoire and S. Nyassé, IRAD, Cameroon

References

Kebe B.I. and G.M. Tahi. In press. Evaluation of the cocoa tree resistance to *Phytophthora sp.*: correlation between sensitivity of pods and sensitivity of other plant parts. *In* Proc. 12th Int. Cocoa Res. Conf., Salvador, Bahia, Brazil, November 1996.

Kebe B.I., J.A.K. N'goran, G.M. Tahi, D. Paulin, D. Clement, and A.B. Eskes. 1999. Pathology and breeding for resistance to black pod in Côte d'Ivoire. Pp. 135-140 *in* Proc. Int. Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, Salvador, Bahia, Brazil, November 1996, INGENIC.

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

Nyassé S., L. Bidzanga Nomo, G. Blaha, M.H. Flament, D. Berry, C. Cilas, A.B. Eskes and D. Despréaux. 1999. Update on the work on selection of cocoa for resistance to *Phytophthora spp.* in Cameroon. Pp. 141-148 *in* Proc. Int. Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, Salvador, Bahia, Brazil, November 1996, INGENIC.

Recording Sheet 1. *Phytophthora* leaf disc test: recording of resistance scores (1-5 point scale) of individual leaf discs

Trial: _____ Name of observer: _____
Inoculation series: _____ Replicate (tray): _____
Inoculation date: _____ Observation date: _____

Treatment (code)	Leaf disc number										
	1	2	3	4	5	6	7	8	9	10	Mean
Etc.,											

Recording Sheet 2. *Phytophthora* leaf disc test: average resistance scores per inoculation tray or replicate (inoculation series)

Trial: _____ Name of researcher: _____
Inoculation Series: _____ Incubation period (no. of days): _____

Treatment (code)	Tray no. or Serial no.					Tray no. or Serial no.				
	1	2	3	4	Mean	1	2	3	4	Mean
Etc.,										

15. Rapid screening for *Phytophthora* pod rot resistance by means of detached pod inoculation

Scope

Spray inoculation of detached unwounded pods creates an opportunity for rapid assessment of pod resistance to *Phytophthora* infection. It provides information on resistance at the penetration and post-penetration stages of infection based on lesion frequency and size. The method is particularly useful for the evaluation of germplasm collections where direct inoculation of attached pods is restricted.

Collection and preparation of pods for inoculation

Fully grown, unripe pods of similar size to ripe pods (four to five months old) should be collected between 07.00 and 10.00 a.m., and must be harvested with care and kept in labelled transparent plastic bags. Not more than two pods should be kept in one bag to avoid surface damage which may occur when many pods are kept together in close contact with each other. Rinse harvested pods in two changes of sterile water and arrange them in plastic trays lined with moist paper towels. Cover each tray with another inverted tray aFs a lid and enclose in a polythene bag to maintain high humidity. Keep at 25 °C for a minimum of 12 hours or overnight to obtain a uniform condition before inoculation is performed.

Sample size

- Assessment of an individual tree: two to three pods in each of two trials are required.
- Assessment of a clone: two to three pods in each of two trials are required.
- Control: use at least one susceptible and one resistant clone in each experiment as standards.
- Experimental design: completely randomized design.

Inoculum preparation

A moderately aggressive isolate of *Phytophthora* should be used. Grow the isolate on a 20% V8 juice - calcium carbonate agar medium and from a ten-day-old culture, obtain a zoospore suspension by inundating each culture plate (9 cm diam.) with 10 ml sterile distilled water (chilled to 10 °C), refrigerate for 25 minutes (5 °C), to incubate in the dark at 25 °C for 30 minutes. Determine the zoospore concentration of the suspension using a haemocytometer and adjust to 100,000 ml^{-1}.

Inoculation

Uncover the pods prepared for inoculation to obtain a dry surface at room temperature. In a slanting position, spray half the surface area of each pod at a distance of 30 cm using a chromist atomizer (Cat. No 51901 spray unit, Gelman Sciences, Ann Arbor, Michigan). An average of 1 ml of zoospore suspension should be deposited on 150 cm^2 of pod area. This quantity of inoculum is normally retained on the pod surface. When too much inoculum is sprayed on the pod surface, run-off will occur. Place inoculated pods in the plastic trays lined with moist paper towels, cover with another inverted tray as a lid and enclose in a polythene bag.

Incubation

Incubate the covered trays containing the inoculated pods for four days at 25°C (air-conditioned room). Large daily temperature fluctuations, which cause evaporation and condensation in the trays, should be avoided.

Assessment of pod reaction to inoculation (Recording Sheet 1)

After four days of incubation, assess inoculated pods for their reaction to inoculation. Assessment is based on the frequency and size of lesions formed. The severity of infection is rated on an eight-point scale, as follows:

Disease rating	*Infection level*	*Susceptibility classification*
1	No symptom	Highly resistant to penetration
2	1-5 localized lesions	Resistant
3	6-15 localized lesions	Moderately resistant
4	> 15 localized lesions	Partially resistant/resistant to spread of lesion alone
5	1-5 expanding lesions	Partially resistant/resistant to penetration alone
6	6-15 expanding lesions	Moderately susceptible
7	> 15 expanding lesions	Susceptible
8	Coalesced lesions	Highly susceptible

Data analysis

The data collected should be entered on Recording Sheet 1 and be subjected to analysis of variance to assess significance in differences between the accessions tested. If there are differences between experiments / trials, data should first be adjusted using the values obtained from the standard clones.

Points arising from subsequent discussions

Age of pod

Care should be taken to identify pods at the right maturity stage recommended for spray inoculation. One option is to hand pollinate to determine pod age (four to five months).

Relationship with leaf test and field observation

Where possible, preliminary experiments should be conducted to determine relationships between the result of the spray inoculation of detached pods and of the leaf disc test with field observations.

Inoculum concentration

Since isolates and species of *Phytophthora* causing pod rot may differ in their aggressiveness, a preliminary test must be conducted at different locations to determine a suitable inoculum potential for inoculation by the spray method.

Replicate and trial

The number of replicates and experimental trials may be increased to more than those recommended in order to enhance the precision of results. This will depend on the availability of pods of the trees under test.

Authors of draft procedure

A.D. Iwaro, T.N. Sreenivasan, D.R. Butler and P. Umaharan, CRU, University of the West Indies, Trinidad and Tobago

References for further reading

Blaha G. 1974. Methods of testing for resistance. Pp. 179-195 *in Phytophthora* Disease of Cocoa. Ed. P.H. Gregory. Longman, London, United Kingdom.

Iwaro A.D, T.N. Sreenivasan and P. Umaharan. 1997a. Foliar resistance to *Phytophthora palmivora* as an indicator of pod resistance in *Theobroma cacao*. Plant Disease 81(6): 619-624.

Iwaro A.D, T.N. Sreenivasan and P. Umaharan. 1997b. *Phytophthora* resistance in cacao (*Theobroma cacao*): Influence of pod morphological characteristics. Plant Pathology 46:557-565.

Lawrence J. S. 1978. Evaluation of methods for assessing resistance to cacao cultivars, and hybrids to *P. palmivora*. Bol. Tec. 62, CEPLAC/CEPEC, Itabuna, Brazil.

Results

Results of three experiments carried out at CRU are given in Tables 1 to 3 (Iwaro, unpublished data).

Table 1. Reaction of pods from cocoa accessions to infection by varying zoospore concentrations of *Phytophthora* using the detached pod spray inoculation method. Two trials were conducted with four replicates (pods) in each trial.

Accessions	Zoospore Concentration (ml^{-1})				
	50,000	100,000	200,000	300,000	Mean
SCA 6	1.0	1.3	3.6	3.5	2.34
E 575	6.0	6.9	7.0	7.9	6.94
SAM 11-76	6.3	6.3	6.9	7.1	6.63
LX 39	6.6	7.6	7.9	7.9	7.50
IMC 67	7.8	7.9	8.0	8.0	7.91
Mean	5.53	5.98	6.68	6.88	6.26

LSD 0.05 (Zoospore concentration) = 0.24
LSD 0.05 (Accession) = 0.27

Table 2. Reaction of detached and attached pods of eight accessions to inoculation with *P. palmivora* using the detached pod spray inoculation method. Two trials were conducted with four replicates in each trial.

Accessions	Detached pod	Attached pod
SCA 6	1.13	1.00
M 8	6.75	6.63
POR 1	7.50	6.63
ICS 65	7.63	6.50
SPA 9	7.75	7.63
ICS 16	7.88	7.25
CATONGO	7.88	7.63
IMC 67	8.00	7.50

Table 3. Reaction of 30 cocoa accessions to inoculation with *P. palmivora* using the detached pod spray inoculation method

Accession	Disease rating		
	Trial-1	Trial-2	Mean
CRU 19	1.00	1.00	1.00
PA 136	1.00	1.00	1.00
PA 150	2.00	1.00	1.50
EET 59	2.00	1.00	1.50
NA 763	3.00	4.00	3.50
PA 151	4.00	4.00	4.00
P 25C	4.00	4.00	4.00
IMC 73	6.00	5.00	5.50
PA 72	5.00	6.00	5.50
CRU 37	6.00	7.00	6.50
CRU 47	7.00	6.00	6.50
PA 191	7.00	7.00	7.00
NA 45	7.00	7.00	7.00
CRU 40	7.00	7.50	7.25
CRU 132	8.00	6.50	7.25
EET 395	7.00	7.00	7.00
ICS 60	7.00	7.50	7.25
ICS 94	7.00	7.50	7.25
LP 313	7.00	7.00	7.00
JA 62	8.00	6.00	7.00
LP 45	7.00	7.00	7.00
NA 137	8.00	7.00	7.50
NA 528	7.00	8.00	7.50
LX 28	8.00	7.00	7.50
TRD 15	8.00	8.00	8.00
ICS 65	7.00	8.00	7.50
JA 518	8.00	8.00	8.00
JA 536	8.00	8.00	8.00
NA 258	8.00	8.00	8.00
SLA 44	8.00	7.50	7.75

L.S.D. $_{0.05}$ = 0.46, The means for trial-1 and trial-2 were not significantly different based on the paired t-test

Recording Sheet 1. Detached pod inoculation for evaluation of *Phytophthora* pod rot resistance

Inoculation date: _____ *Phytophthora* species: _____
Observation date: _____ Inoculum concentration: _____

Accession	Tree no.	Disease rating					Mean	Remarks
		Inoculated pods						
		1	2	3	4	5		
	1							
	2							
	3							
	4							
	1							
	2							
	3							
	4							

Disease rating	***Infection level***	***Susceptibility classification***
1	No symptom	Highly resistant to penetration
2	1-5 localized lesions	Resistant
3	6-15 localized lesions	Moderately resistant
4	> 15 localized lesions	Partially resistant/resistant to spread of lesion alone
5	1-5 expanding lesions	Partially resistant/resistant to penetration alone
6	6-15 expanding lesions	Moderately susceptible
7	> 15 expanding lesions	Susceptible
8	Coalesced lesions	Highly susceptible

16. Assessment of *Phytophthora* pod rot resistance by means of attached pod inoculation

Scope

To evaluate local and international accessions with a view to comparison of resistance to black pod disease employing a method which reflects intrinsic pod resistance.

The advantage of using point-inoculation with *Phytophthora spp.* zoospore suspension of unwounded attached pods is that it makes it possible to determine both types of resistance to penetration (infection frequency) and to colonization (lesion size). The inoculum quantification is precise and the inoculum is fully standardized. With this method, development of the lesion can be followed for a sufficiently long period. Measurements taken only once for lesion size mean that this screening test can be applied to large quantities of test materials. Another important aspect is that resistance is evaluated "in vivo" and "in loco". Results from this test have shown correspondence with the responses of natural infection in the field (Pires *et al.* 1994; Luz *et al.* 1999).

Method

Fully developed but unripe attached pods of four to five months old from the test accession must be selected in areas without fungicide applications. Five pods chosen on three to five different trees per accession and per *Phytophthora* species must be tested for each inoculation series (inoculation date at different times in the rainy season). All accessions and *Phytophthora* species or isolates to be tested should be inoculated on the same day in order to avoid differences due to changes in the environment. A minimum of six inoculation series are required for clones (30 inoculated pods) and 12 series for hybrid progenies (60 inoculated pods).

The standard method for inoculation uses zoospore suspensions obtained from ten-day-old cultures (i.e. four days in the dark and six days under alternate duration of light) on V8 agar medium incubated at 26 ± 2 °C. Zoospore liberation is induced by adding sterile distilled water at 4 °C into cultures with sporangia. In case the above method cannot be applied due to insufficient laboratory equipment or in case of sporulation problems, spore production can alternatively be obtained by inoculation of sterilized, rinsed, detached pods with a mycelium disc. After four to six days the spores are washed off with cold distilled water. The spore suspension is kept for one hour at room temperature. Before inoculation, zoospore concentration is adjusted using a Malassez haemocytometer.

Each pod will be inoculated without wounding at two places located laterally and opposite each other on the pod, using a standard volume (0.1 ml) and concentration (2 x 10^5 ml) of zoospore suspension. The inoculum drop will be retained by small cups made of plasticine or modeling-clay affixed to the pod surface. After inoculation, the pods must be enclosed in transparent polyethylene bags, containing distilled water and tied with string around the pod peduncles. Small holes must be made in the plastic bag with a needle approximately 2 cm above the lower-most points of bags to prevent them from filling up with rain water. Two days after inoculation the bottom part of the polyethylene bag is removed to allow free air flow.

It is preferable for all inoculations to be conducted in the afternoon and when the weather is cloudy and cool, to avoid harmful effects to the inoculum from heat or direct sunlight. The polyethylene bags will remain on the pods for the duration of the test to protect them from insects and provide a more uniform micro-environment.

To obtain stricter comparison between cultivars and between replicate tests, a standard highly susceptible cultivar will be included in each test and for Brazil, where more than one *Phytophthora* species occur, the standard susceptible cultivar must be inoculated with

P. palmivora because it has an intermediate virulence between *P. capsici* and *P. citrophthora*. If the average final lesion size of the susceptible cultivar is smaller than 4 cm the whole test will need to be repeated.

Observations

Lesion size will be observed when the average lesion diameter (average of length and width) of the most susceptible variety reaches about 5 cm (see Recording Sheet 1). Lesion size is assessed by measuring the lesion in two directions at right angles to each other, parallel to the longitudinal and transverse axes of the pod, and by calculating the mean of the two values (as criteria for internal pericarp resistance). It is optional to carry out more than one measurement; in that case it is recommended to take measurements at three, five and seven days after inoculation (Recording Sheet 2). In this way, the lesion growth rate can be calculated as an additional resistance parameter.

Observations on the number of infection sites per inoculation site are optional (see Recording Sheet 2). This is to be done two days after inoculation. The plasticine cups are removed and the number of infection points per inoculation site is evaluated on a 0-5 point scale (0 = no infection and 5 = maximum number of infection points found in the trial) for all inoculated pods.

The results of different tests will be compared based on average lesion size and on percentage of infection (Luz *et al.* 1996). As an option, the number of infection points per inoculation site and the rate of lesion growth will be considered.

Data analysis

For the analyses of variance and comparison of means, no zero values should be included for calculating the average lesion diameter of any accession. For cluster analysis on infection frequency and lesion size the SAS statistical package can be used.

Points arising from subsequent discussions

The method requires enough time for pod development in sufficient numbers to test each accession reliably with the *Phytophthora spp.* occurring in the countries. The need for a great number of pods from the susceptible cultivar at a given time can be a major limiting factor. However, within the project trials the accessions are identical and similar controls are present in the International and Local Clone trials that would eliminate the need for a second susceptible control.

In the absence of controlled climate conditions, results for the same accession may vary from test to test, therefore all accessions are to be tested at each inoculation date. If this is not possible, the accessions can be divided into two or three groups, using two control accessions in order to be able to compare between groups.

Uniform pod age would be best obtained by controlled pollination, but can also be obtained by marking newly formed cherelles each week.

All the inoculated and infected pods should be recorded and the breeder advised so that these can be counted in the yield observations of the accession.

All screening methods for assessing resistance to black pod need to consider differences in virulence among species or isolates occurring in the same region and different strains of a given species in different regions. For the aim of the CFC project, at least one isolate of the locally most important species is to be used.

Authors of draft proposal

E.D.M.N. Luz and S.D.V.M. Silva, CEPLAC/CEPEC, Brazil and G. Blaha, CIRAD, c/o CCRI, Papua New Guinea

References cited

Luz E.D.M.N., M.M. Yamada, S.D.V.M. Silva, J.L. Pires, U.V. Lopes, L.R.M. Pinto, W.R. Monteiro, J.L. Bezerra, G.R.P. Melo and M.I.B. Brugnerotto. 1999. Research on Cacao Resistance to Black Pod Disease in Bahia, Brazil - 1980 to 1995. Pp. 57-66 *in* Proc. Int. Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, Salvador, Bahia, Brazil, November 1996, INGENIC

Pires J.L., E.D.M.N. Luz, U.V. Lopes and B.G.D Bartley. 1994. Incidência natural de podridao parda em clones de cacaueiro na Bahia, Brasil. Pp. 871-877 *in* 11th Int. Cocoa Res. Conf., Yamoussoukro, Côte d'Ivoire, July 1994.

References for further reading

Blaha G. 1974. Methods of testing resistance. Pp. 179-195. *In* Gregory P.H., ed. *Phytophthora* Disease of Cocoa. P.H. Gregory (Ed.) Longman, London, United Kingdom.

Blaha G. and R. Lotodé. 1976. Un critère primordial de sélection du cacaoyer au Cameroun: la résistance á la pourriture brune des cabosses (*Phytophthora palmivora*). Café Cacao Thé 20:97-116.

Blaha G. and R. Lotodé. 1977. Contribution à la connaissance des modalités de la transmission héréditaire de la résistance du cacaoyer à la pourriture brune des cabosses (*Phytophthora palmivora*) au Cameron. Café Cacao Thé 21:179-196.

Lawrence J.S. 1978. Evaluation of methods for assessing resistance of cacao *Theobroma cacao* L. cultivars and hybrids to *Phytophthora palmivora* (Butler). CEPLAC/CEPEC, Ilhéus-BA, Brazil. Bol. Tec. 62. 47pp.

Lawrence J.S. 1983. Metodologia para avaliar resistência de cultivares e progênies híbridas de cacau a *Phytophthora spp* que causam podridão-parda na Bahia. Rev. Theobroma 13: 349-359.

Luz E.D.M.N. and M.M. Yamada, 1984. Indices para avaliar a reação de cultivares de cacau a *Phytophthora spp*. Rev. Theobroma 14(3):181-188.

Luz E.D.M.N., S.D.V.M. Silva, M.M. Yamada, U.V. Lopes, M.C.T. Braga and M.I.B Brugnerotto. 1996. Selection of cacao genotypes resistant to *Phytophthora capsici*, *Phytophthora palmivora* and *Phytophthora citrophthora* in Bahia, Brazil. Fitopatol. Bras. 21(1):71-79.

Recording Sheet 1. Evaluation of resistance to *Phytophtora* pod rot by attached pod inoculation

Inoculation date: _____ Observation date: _____

Replicate	Clone or hybrid	Pod number	Side of pod	Lesion size (cm)		
				Length	Width	Mean
			a			
			b			
			a			
			b			
			a			
			Etc.,			

Recording Sheet 2. Attached pod inoculation for detailed *Phytophthora* pod rot resistance evaluation (L = length of lesion, W = width of lesion)

Trial: _____ Name of observer: _____
Inoculation date: _____ Observation date: _____

Replicate	Clone or hybrid	Pod no.	Side of pod	Infection points (0-5 scale)	Lesion size (cm)						
					Date:		Date:		Date:		Mean
					L	W	L	W	L	W	
			A								
			B								
			A								
			Etc.,								

17. Field evaluation of *Phytophthora* pod rot and stem canker incidence

Scope

The objective is to describe methods for accurate evaluation of field resistance to *Phytophthora* pod rot (Ppr) in project trials. Firstly, the standard method for evaluation of incidence of *Phytophthora* in mature pods is described; this method is part of the procedure for yield assessment in project trials (see Working Procedure 6). Secondly, a method is described for complementary optional observations on *Phytophthora* attack on cherelles, immature pods as well as on ripe pods. Furthermore, suggestions are made for observations on *Phytophthora* stem canker incidence and on factors other than intrinsic resistance of the cocoa tree that may affect black pod incidence in the field.

Standard Phytophthora *pod rot assessment*

It was decided that for standardized observations in the CFC project, the attack of *Phytophthora* on cherelles will not be assessed because of interference with 'cherelle wilt' and other pathogens attacking young fruits. Cherelles are considered as young fruits that can still be affected by cherelle wilt, i.e. fruits that are normally less than two months old. This corresponds to cherelles that have a maximum length of about 10 cm (or two-thirds of the mature fruit size). All fruits bigger than the maximum cherelle size are considered here as 'pods' on which Ppr observations are to be made in project trials.

The field incidence of Ppr will be observed at bi-weekly intervals during the wet season at the same time the yield observations are made. Four types of pods are harvested and counted: healthy ripe pods, Ppr infected pods (partly or completely destroyed by the fungus), rodent eaten pods (ripe or green) and pods destroyed by other diseases or pests (see Recording Sheet 1, which is identical to Recording Sheet 2 of Working Procedure 6). Care should be taken to correctly distinguish Ppr from other pathogens that attack cocoa pods, such as *Botryodiplodia* causing soft pod rot. It is recommended that the pathologists train the field technicians involved in yield recording to be able to correctly identify the different type of pod rots that may occur in the project trials.

In countries where other pod diseases are also frequent, the main yield observation sheet must reflect the presence of these pathogens too.

Observations on Phytophthora *incidence including immature pods and cherelles*

This approach is recommended for countries in which Ppr is the major disease of economic importance. It requires weekly elimination of all wilting cherelles (partly or entirely yellowing, due to physiological causes or others except *Phytophthora*). These wilting cherelles should be eliminated by well-trained field workers. Cherelles affected by *Phytophthora* (yellow or green) should not be eliminated; these will be harvested and counted during the bi-weekly observations on yield and Ppr incidence. Recording Sheet 2 should be used for recording. This contains columns for three different sizes of Ppr affected fruits: pods of mature size (ripe or green), immature pods (green) and cherelles (green or yellow). These detailed observations should be carried out during two major epidemic periods.

Observations on Phytophthora *stem canker incidence*

In countries where stem canker is frequent, its incidence must be evaluated at a yearly interval using a 0 to 5 point assessment scale (0 = absence of symptoms and 5 is high incidence). Observations are to be made on all trees of the CFC project trials.

Observations on other factors that may affect field incidence of Ppr

Studies are continuing towards a better understanding of the environmental and physiological factors that may affect field incidence of Ppr. In recent years, the following significant correlations with field incidence have been observed:

- *Escape from infection.* This relates to the effect of the time of pod production in relation to the main rainy season. Significant correlations have been observed in Ghana and in Vanuatu between the percentage of pods harvested in periods of the year less conducive for the disease, and average yearly Ppr incidence. In these countries the escape phenomena would explain about 50% of the variation for natural Ppr incidence between cocoa genotypes (Lockwood and Dakwa 1978, Jagoret *et al.* 1994). The same phenomenon has been observed in other countries. Furthermore, cocoa varieties that show grouped harvesting periods (such as Amelonado) may carry over less inoculum to the next epidemic season than varieties with extended harvesting periods. In the CFC project trials, the relative importance of escape will be estimated by calculating the percentage of pods produced outside the main epidemic periods.
- *Number of pods per tree and Ppr incidence.* In countries with high black pod incidence, a positive environmental correlation between pod load and Ppr incidence has been observed for individual trees (r=0.4 to 0.5). This appears to be due to the increased chance of inter-pod contamination with Ppr when more pods are present on the tree, due to a smaller physical distance between the pods. The importance of this phenomenon will also be estimated in the CFC project trials and corrections applied, when needed, for more precise evaluation of genetic effect on Ppr incidence.
- *Other factors.* Other factors that are less well known may be related to natural incidence of Ppr. One example is variation in natural canopy density. It is known that intensive pruning of adult trees can help to control the disease effectively. Therefore, genotypes with a more open natural canopy should be less affected by Ppr than genotypes with a dense canopy. Pods growing in the canopy generally show less infection than those growing on the main trunk. The density of the canopy will be recorded as part of the measurement of 'Simple Physiological Traits' (see Working Procedure 9). It is considered optional in this CFC project to assess Ppr incidence separately in the canopy and on the main trunk.

Authors of draft proposal

G. Blaha, CIRAD, c/o CCRI, Papua New Guinea and A. B. Eskes, IPGRI/CIRAD, c/o INIBAP, France

References

Cilas C., D. Berry, D. Paulin, J.A. N'Goran and E. K. Djiekpor. In press. La résistance à la pourriture brune des cabosses au Cameroun, en Côte d'Ivoire et au Togo. Bilan d'évaluation au champ. *In* Proc. 12th Int. Cocoa Res. Conf., Bahia, Brazil. November 1996.

Jagoret P., P. Bastide, A. Pilecki, C. Besacier and A. B. Eskes. 1994. Performance et résistance à la pourriture brune d'hybrides du cacaoyer au Vanuatu (Pacifique Sud). Pp. 425-431 *in* Proc. 11th Int. Cocoa Res. Conf., Yammoussoukro, July 1994, Côte d'Ivoire.

Lockwood G. and J.T. Dakwa. 1978. The influence of self-incompatibility on the time of harvesting of cocoa in Ghana in relation to losses from black pod disease. J. Hort. Science 53:105-108.

Recording Sheet 1. Yield observations on individual trees or plots (fortnightly intervals): number of healthy ripe harvested pods (HR), *Phytophthora* pod rot infected pods (Ppr, completely or partly rotten), rodent-eaten pods (Re) and other diseased or destroyed pods (Other)

Trial: _____ Planting date: _____

Block no.	Plot or tree no.	Variety (no.)	Observation date				Observation date			
			HR	Ppr	Re	Other	HR	Ppr	Re	Other
Etc.,										

Recording Sheet 2. Yield observations with detailed recording of *Phytophthora* pod rot (Ppr) on mature pods, immature pods and cherelles. Number of mature healthy ripe pods harvested (HR), Ppr infected mature pods (PprM, green or yellow), Ppr infected immature pods (Pprl, green), Ppr infected cherelles (PprC), rodent eaten immature or mature pods (Re) and immature or mature pods destroyed by other pathogens or pests (Other).

Trial: _____ Observation date: _____

Block no.	Plot or tree no.	Variety (no)	HR	PprM	Pprl	PprC	Re	Other
Etc.,								

18. Ring test for black pod resistance

Scope

The standardized leaf disc inoculation test (see Working Procedure 14) will be carried out to evaluate resistance to black pod (*Phytophthora spp*) at all project sites, except in Ecuador where this disease is not important. This test will also be carried out at one place outside cocoa-producing countries (CIRAD, Montpellier, France) using a range of isolates of the pathogen from a variety of field sites. All tests together can be called the 'Ring Test' for black pod resistance in the project. The main objectives are to compare the stability of the resistance, to verify the repeatability of the test and to analyze possible interactions between host genotypes and pathogen isolates or species. The common aspects of this Ring Test, as carried out in Montpellier, are described hereafter.

Choice of cocoa clones

The clones that are most widely used in the "International Clone Trial" will be used for the Ring Test in Montpellier. These clones are the following:

From Reading (10 clones):
AMAZ15-5, MAN15-2, MXC67, SPEC54-1, LCT-EEN46, Playa Alta 2, PA107, SCA6, BE10, EQX3360-3.

From Montpellier (15 clones):
EET 59, GU255 V, IMC47, PA150, SNK413, APA4, ICS1, P7, VENC4-4, Mocorongo, NA33, CATIE1000, IFC5, T85/799, PA120.

Besides these clones, two control clones will be used: LAF1 (S) and SNK64(MR). The budded plants from the Montpellier clones are expected to be ready for testing early 1999 and from Reading a few months later.

Choice of isolates

The choice of the type and number of isolates is based on the genetic diversity of the pathogen species in the nine different countries affected by *Phytophthora*:

P. megakarya (5)	Ghana (1) Nigeria (2), 1 from Ibule Cameroon (2), 1 from border between Cameroon and Nigeria
P. palmivora (8)	Ghana (1); Trinidad (1); Brazil (1); Malaysia (1); Côte d'Ivoire (1); Venezuela (1); PNG (2)
P. capsici (5)	Trinidad (1); Brazil (1); Venezuela (1); PNG (1); Malaysia (1)
P. citrophthora (5)	Brazil (1); PNG (1); Venezuela (1); PNG (1); Malaysia (1)
P. nicotiana (1)	Malaysia (1)
P. megasporma (1)	Venezuela (1)

It is proposed that isolates also be obtained from Ecuador (at least two) for comparison, even if the resistance test will not be carried out in that country.

A total number of 27 isolates will be tested. The choice of each local isolate will be made in each research centre by the plant pathologist in charge. At least one of the local isolates sent to Montpellier is to be used as a standard in the local evaluation of the resistance of the CFC project trials. Possibilities of sending more isolates per country (up to ten) may be considered in order to offer possibilities of substituting an isolate if necessary.

The isolates should be purified before sending. All the isolates should be transferred to Montpellier before October 1998.

Preliminary evaluation of level of pathogenicity and confirmation of the species of the isolates will be carried out by isozyme analyses before starting large scale resistance testing at Montpellier. The pathogenicity of the isolates will be maintained in Montpellier by inoculation and re-isolation on pods from the greenhouse or from French Guiana.

Inoculation procedure

The inoculation method used in Montpellier will be the working procedure for leaf disc inoculations presented at this Workshop.

The isolate x clone combinations (25 x 25) will be tested in separate groups. Each series (date of inoculation) will contain all clones from one of the two quarantine centres and 10 isolates, including two control isolates. The statistical design for each series of inoculation (replicate or inoculation date) will be two randomized blocks (of five trays) for each isolate x clone combination. For each isolate x clone combination 10 discs per will be inoculated per series and 5 x 10 = 50 discs in total for the entire experiment. Twenty discs of each of five leaves will be used per clone for each series of inoculation.

Each series will then contain the following treatments:

- ten or 15 clones, from Reading and Montpellier respectively, plus the two control clones;
- ten isolates, including three control isolates;
- 2 x 5 trays per treatment of 12 or 17 clones inoculated with ten isolates;
- five leaves per clone and 10 discs per leaf per tray, total 20 discs per leaf;
- one disc per isolate per tray, in total (12 or 17) x 10 = 120 or 170 discs per tray;
- five discs per isolate x clone combination repeated twice.

Each of the above series will be carried out five times (at five different dates). For each clone a minimum of (5 x 3) x 5 = 75 leaves will be required for the entire test. For the control clones, 150 leaves will be required.

In order to classify the resistance of the Montpellier and Reading clones in one series, all 27 clones will be tested in the end against four isolates (one of each of the four main *Phytophthora* species). This test will be repeated three times.

Data to be recorded

The scale proposed in Working Procedure 14 will be applied four and six days (or five and seven days) after inoculation. In addition, it is proposed that the number of infection points be scored two days after inoculation. This would help to improve knowledge on the different levels of resistance: at penetration stage (number of infection points visible at two days) and post penetration stage (four and six days).

Points arising from subsequent discussions

It is proposed that the following additional isolates be obtained from stem cankers, e.g. from PNG and Malaysia. According to the availability of project funds, resistance of stem segments of the same clones could then be evaluated in relation to a certain number of fungal isolates from pods and stem cankers, aiming at correlation with stem canker susceptibility.

Author of draft proposal

M. Ducamp, CIRAD, France

19. Early screening for witches' broom resistance: belt spray inoculation of seedlings and clones

Scope

The objective is to present a standardized method for early evaluation of resistance to witches' broom disease (*Crinipellis perniciosa*) in local and international accessions. The method is adapted from the one developed by Frias (1987). It is proposed that this be used for seedling progenies as well as for clones.

Plant material

Seedling production and experimental design

To achieve uniform germination, peeled seeds must be left to germinate in water 24 hour before planting. Seedlings are raised in sterilized soil or a potting mix in plastic, cone-shaped tubes in racks, under greenhouse conditions. The tubes should be big enough to prevent the substrate from drying too quickly and to allow seedlings to grow freely for five to six months. Alternatively, seeds can be sown in boxes with a 10-15 cm layer of sterilized substrate. Seedlings will be inoculated when presenting a flush with the largest leaf up to 1.5 cm long (approximately three weeks after sowing). When using the tubes, in total one hundred and twelve seedlings of each accession are inoculated, divided into four replicates of 28 plants each. For each replicate, standard control seedling progenies are to be used in order to compare results between inoculation series. In the event of using germination boxes, the use of four replicates (with 25 to 30 seedlings per box) is also recommended.

Production of cloned plants and experimental design

When evaluating clones, grafted or rooted plants can be used for inoculation. The grafted plants are obtained by budding onto uniform two to three month-old rootstock seedlings. The budded plants or rooted cuttings are raised in polyethylene bags in a greenhouse or nursery and used for inoculation when they are two to three months old. Active outgrowth of buds is to be induced by pruning. Batches of ten uniformly growing budded plants or rooted cuttings of all genotypes to be tested are inoculated in one test series, together with the control clones. Inoculation may need to be repeated two or three times in order to obtain sufficiently high infection levels. This is repeated three or four times (totally 30 or 40 plants inoculated) in order to obtain reliable estimates of the susceptibility level for each clone.

Fungal material

Basidiocarp production

The only known source of the infective basidiospore stage of *Crinipellis perniciosa* is the flushes of basidiocarps which occur on dry "brooms" and mummified infected pods. Dry brooms are collected from the field, preferably those brooms produced in the foregoing epidemic season, that can be expected to produce basidiocarps quickly and for a longer time. Collections are made during the dry season, usually combined with the sanitary pruning of plantations to reduce labour requirements. It is important to collect brooms for an inoculation season all at the same location to avoid inoculum variation. Batches of brooms are collected and conditioned to start basidiocarps flushing approximately every six months, to ensure a continuous supply of basidiospores. When enough brooms are available, those about 20 cm long with a main axis and numerous side branches are selected.

The field-collected brooms are allowed to air dry for a few days before being soaked for five minutes in a benomyl (0.2% w/v) suspension and left to dry before being hung inside

"broom chambers" ('vassoureiros' or 'escoberos'). Inside the chambers, the brooms are wetted daily with an automatic atomizing device for 8 h and left to dry for 16 h, as described by Suarez-Capello (1977) and Rocha and Wheeler (1985).

Basidiospore collection, preparation and storage of stock solution

When basidiocarps become available they are collected in the afternoon. The pileus (= cap of basidiocarp) is stuck to the lid of a Petri-dish with petroleum jelly or vaseline, so that the gills face down, and placed over a beaker or similar containing 60 ml of a collecting solution. A stirring bar and stirring plate are used to agitate the solution in the beaker overnight during spore deposition. The basidiospore collecting solution is composed of glycerol at 16%, MES buffer (morpholino ethane-sulphonic acid) at pH 6.1 and at 0.01M, and Tween 20 at 0.01%. This stock solution can be used immediately or be stored for at least 1.5 years in liquid nitrogen (Dickstein, *et al.* 1987, Frias *et al.* 1995, Purdy *et al.* 1997).

Inoculation procedure

Preparation of inoculum

The stock solution can be obtained from fresh basidispores that can be kept for a few days in the refrigerator at 16% glycerol. However, generally the stock solution will be recovered from liquid nitrogen and left to thaw at room temperature. The day before inoculation the percentage of germination is determined, using only one vial of the stock solution, the rest being kept until preparation of the inoculum. Alternatively, the content of the stock solution from the different vials is mixed and a small amount diluted for germination. This latter approach would avoid possible mistakes in estimation of spore germination due to variation between vials.

Basidiospore suspension is calibrated with a haemocytometer (Newbauer) both before storage and later when diluted ready for inoculation. The percentage of basidiospores that germinate must be determined for each batch of inoculum. To allow basidiospore germination, glycerol concentration is reduced to 3%; this is accomplished by adding four parts of buffer with Tween 20, at 0.01%, to one part of the stock suspension. To further adjust the number of basidiospores to a desired concentration, a solution containing glycerol 3%, buffer, and a 0.01% solution of Tween 20 is used. Spore suspension is kept under agitation while diluters are slowly added.

To determine the full capacity of basidiospore germination, 0.12 ml of the collecting solution is placed onto 1.2% water agar in 60 mm Petri-dishes. After three to five hours at 27 °C, the percentage of germination is determined by counting 150-200 basidiospores in randomly selected microscope fields in each of five different Petri-dishes.

The percentage of germination of basidiospores in the collecting solution is used to obtain a known number of viable basidiospores/ml. It is not recommended to use spore batches with germination rates below 80%, as such would be indicative of storage problems. Diluted spore batches should be used immediately for germination tests or for inoculation, as stored diluted batches will gradually lose their germination capacity. For inoculation, the concentration of the spore suspension should be at least 60,000 viable spores per ml. However, depending on the aggressiveness of the isolate, it may be be necessary to increase the inoculum density to 200,000 spores per ml.

Host inoculation

The final basidiospore suspension is placed in an Erlenmeyer flask with a magnetic stir bar, the flask is covered with aluminium foil to protect it from sunlight, and the flask is placed on a magnetic stir plate and stirred slowly. The pick-up tube from a peristaltic pump is immersed in the inoculum suspension. The peristaltic pump is calibrated to deliver 30 ml/min to a solid, cone-shaped, stainless steel, air-liquid atomizing spray nozzle that

receives air at 7.0 lb/sq inch from an air compressor. The spray nozzle is located about 15 cm above the cocoa flushes to be inoculated. Two spray nozzles apply inoculum to plants in two rows of 14 conical plastic tubes in each rack placed on a conveyor belt that travels at the rate of 2.7 ml/min. Four racks of plants (112 plants) are inoculated per min, and then the inoculation racks are placed in an incubation chamber. A similar procedure is applied if boxes with seedlings progenies or with clones are used. However, clones may need to be inoculated more than once in order to obtain satisfactory infection levels. At the end of the application, the sprayed basidiospore suspension is caught on water agar in small (60 mm) Petri-dishes to verify the percentage of basidiospores that are likely to germinate during the infection period.

Incubation

Inoculated plants must remain wet for 14 to 24 hours on order to achieve germination of basidiospores, penetration of the host plant, and establishment of infection. This requirement is met by placing racks or boxes of inoculated plants in an incubation chamber for 24 hours. The incubation chamber consists of a wooden block with 2 m x 2 m sides, its internal walls covered with spirofoam, and a polythene cover. An irrigation system runs through three lateral walls to maintain moisture (95 to 100%) and temperature (27 °C $\pm$ 2 °C). The inoculation area is covered with muslin to avoid drifting of spore suspension particles due to air currents.

Handling plants before and after inoculation

As indicated above, two kinds of plants may be used to evaluate disease incidence: seedlings and vegetatively propagated plants (clones).

Cocoa seeds to be evaluated are preferably chosen from the central area of the pod, eliminating the very small ones, as these are less vigorous. The testa are removed to obtain a more uniform germination, soaked in water for 24 hours and placed on plastic cones (4 cm in diameter x 21 cm in length) containing a garden substrate composed of peat, organic matter and supplemented with nitrogen, phosphorus and potassium. These cones are arranged in parallel rows on racks.

For vegetatively propagated material, rooted cuttings or grafted plants are to be placed under favourable growing conditions. Outgrowth of new buds before inoculation is best induced by pruning.

After inoculation, plants are kept in the greenhouse for evaluation. Temperature and relative humidity in the greenhouse should be near to optimal conditions for development of the disease. Too high temperatures or dry growing conditions will affect infection success and symptom expression. All the plants in the greenhouse are regularly irrigated. Every two months a complete fertilizer (20N-20P-20K) or a foliar fertilizer is to be applied. During dry months, insecticides are required to control mites and thrips. Foliar spraying of fertilizers and insecticides should not to be undertaken, however, on batches of plants during the three weeks before inoculation, as this may affect spore germination.

Evaluation of plant responses to infection

Individual seedling responses are recorded 60 days after inoculation (see Recording Sheet 1). The most relevant symptoms to be recorded for seedlings are terminal brooms, axillary brooms, cotyledonary brooms, petiole swelling, pulvinus swelling and stem or hypocotyl swellings. 'Secondary' variables are calculated from these primary records, such as the frequency of plants with each kind of symptom or by combining different symptoms (frequency of any type of brooms or any type of swellings). The length of the terminal broom (mm) and number of axillary brooms greater than 1 cm might also be observed.

Observations of symptoms on clones are somewhat different from those on seedlings. An adapted recording sheet is provided for clones (see Recording Sheet 2), for observations on the number of growing points per plant and number of different symptoms observed (swellings, cankers and terminal or axillary brooms).

Studies conducted at CEPLAC, sponsored by the American Cocoa Research Institute, have indicated that the most important variables are the percentage of plants with brooms (terminal, axillary or cotyledonary) and percentage of plants with swellings (petiole and pulvinus or stem swelling).

Resistant and susceptible standard cultivars

In all inoculation tests, at least one standard resistant and susceptible cultivar must be included, such as Catongo and open pollinated SCA6 seedlings (or a resistant and susceptible clone for clone evaluations). Tests are considered valid if at least 30% of the plants of the susceptible cultivar presents brooms or swellings. In order to be able to compare inoculation series, data need to be calculated relative to the average results obtained with the control cultivars. The use of control cultivars is not necessary if, as proposed for the International Clone Trial, all clones will be inoculated in all inoculation series. The average reaction of the inoculation series will then be indicative of infection success.

Data analysis

Analyses of variance and Duncan multi-range tests will be used to identify differences among families for each variable. The statistical analyses are performed separately for each test series. Combined analyses can be made using data relative to the standard control varieties. An F test is used to verify the occurrence of differences between treatments or inoculation seasons (for those experiments with more than one inoculation season) and interactions between these two sources of variance.

The correlation matrix among all variables for each experiment is estimated to identify relationships among variables and select the best ones to assess disease resistance. Multivariate analyses can be useful to estimate the relationships between variables observed for a given pathogen isolate and set of cocoa genotypes. Principal component analyses can be performed to classify the variables with high loading using the Eigen vector of the correlation matrix and a dendrogram constructed using similarity measures to cluster variables. The variables selected for each experiment as the ones having high positive loading are used to group progenies within a dendrogram using Euclidean distances. The canonical analysis, based on the covariance matrix among the variables offers the possibility of creating a graphic image of the genotype dispersal evaluated in relation to the first two canonical variables that best explain the total variation among those assessed for each set of progenies. Tocher's method can also be used to group the families based on genetic dissimilarity coefficients, expressed by Mahalanobis distances. Anderson's discriminating analysis is performed to confirm the grouping of the families of each trial using the frequencies of the principal variables.

Authors of draft procedure

E.D.M.N Luz and S.D.V.M. Silva, CEPLAC/CEPEC, Brazil and C. Suarez-Capello, INIAP, Ecuador

References cited

Purdy L.H., R.A. Schimdt, E.R. Dickstein and G.A. Frias. 1997. An automated system for screening *Theobroma cacao* for resistance to witches' broom. Agrotropica 9:119-126.

Rocha H.M. and B.E.J. Wheeler. 1985. Factors influencing the production of basidiocarps and the deposition and germination of basidiospores of *Crinipellis perniciosa*, the causal fungus of witches' broom on cocoa (*Theobroma cacao*). Plant Pathology 34:319-28.

Suarez-Capello C. 1977. Growth of *Crinipellis perniciosa* (Stahel) Singer *in vivo* and *in vitro*. PhD

Thesis, University of London, 1977. 134 pp.

References for further reading

Dickstein E.R., L.H. Purdy and G.A. Frias. 1977. *Crinipellis perniciosa*, the cacao witches' broom fungus: Inoculum production and storage. Phytopathology 77:1747.

Frias G.A. 1987. An inoculation method to evaluate resistance to Witches' broom disease of cacao. PhD Diss., Plant Pathology Dept., Uni. Fla., Gainesville, 111 pp.

Frias G.A., L..H. Purdy and R.A. Schmidt. 1995. An inoculation method for evaluating resistance of cacao to *Crinipellis perniciosa*. Plant Disease 79:787-91.

International Witches Broom Project. 1989. Detailed description of symptoms of witches' broom disease of cocoa caused by *Crinipellis perniciosa*. Cocoa Growers Bulletin. No. 41. England, 32 pp.

Holliday P. 1954. A test for resistance to *Marasmius perniciosus*. Stahel. Report on Cocoa Research. Imperial College of Tropical Agriculture, St. Augustine, Trinidad.

Instituto Nacional Autónomo de Investigaciones Agropecuarias. Informes anuales técnicos, 1986, 1989, 1991, 1992, 1996. Departamento de Fitopatología. Estación Experimental Tropical Pichilingue. (Mecanografiados).

Luz E.D.M.N., S.D.V.M. Silva, P.S.B. de Albuquerque, L..R.M. Pinto, H.P. Gramacho, U.V. Lopes, J.L. Pires, J.L. Bezerra, M.I. Brugnerotto, M.C.A. Paim, L..H. Purdy and R.A. Schmidt. In press. Evaluation of cacao progenies in Bahia, Brazil, for resistance to *Crinipellis perniciosa*. *In* Proc. Int. Cocoa Res. Conf., Salvador, Bahia, 1996.

Purdy L.H., E.R. Dickstein and R.A. Schimdt. 1998. Relative resistance of cacao clones to *Crinipellis perniciosa* determined by artificial inoculation using an automated basidiospore spray technology. Agrotropica 10:9-12.

Rivera J. L. 1995. Evaluación de la reacción de material promisorio de cacao de origen nacional a Escoba de Bruja, *Crinipellis perniciosa* (Stahel) Singer. Thesis Ingeniero Agrónomo, Guayaquil, Ecuador, Universidad Agraria del Ecuador, 84 pp.

Silva S.D.V.M. 1997. Histologia e seleção de variáveis para avaliar resistência de cacaueiro a *Crinipellis perniciosa*. Viçosa, MG, Brazil. UFV, 1997, 105 pages. Dissertação (Doctorado em Fitopatologia), Universidade Federal de Viçosa, 1997.

Recording Sheet 1. Evaluation of young cocoa seedlings for witches' broom by means of the belt spray method

Trial: _____ Hybrid: _____
Sowing date: _____ Evaluation date: _____
Inoculation date: _____ Observer: _____

PLN	S	TB	AB	NAB	CB	HS	ES	PS	PCS	SR	HP	CNS	Remarks
1													
2													
3													
4													
Etc.													

PLN - Plant No.; S - Symptom; TB - Terminal Broom; LTB - Length of terminal broom; AB - Axillary broom; NAB - No. of axillary broom >1 cm; CB - Cotyledonary broom; HS - Hypocotyl swelling; ES – Epycotyl swelling; PS – Pulvinus swelling; PCs – Petiole swelling; SR – Stem rugosity; HP – Hypersensitivity; CNS – Cotyledonary node swelling.

Recording Sheet 2. Recording sheet for witches' broom resistance evaluation by inoculation of clones (grafted plants or rooted cuttings).

Trial: _____ Clone: _____
Grafting date: _____ Inoculation date(s): _____
Observer: _____

Plant no.	Total no. of growing points	Number and type of infections per observation date*								
		Date				Date				Remarks
		S	C	TB	AB	S	C	TB	AB	
Etc.,										

S – Swellings; C - Stem canker; B - Terminal (T) or Axillary (A) brooms, TB - Terminal brooms AB - Axillary brooms

20. Evaluation of witches' broom incidence in the nursery and field

Scope

The objective of this proposal is to record comparable quantitative data at project sites on cocoa genotypes that show degrees of resistance/susceptibility to witches' broom (WB) disease.

Witches' broom is an airborne disease and, for infection, relies on sources of spore production on dead tissue of infected trees, and on suitable meteorological conditions (*i.e.* temperature >20-28 °C, relative humidity >80% and alternate cycles of rainy and dry weather). Therefore to evaluate resistance at field level it is necessary to consider the available source of inoculum and its coincidence with cocoa flushes as well as any factor that may influence disease expression such as vigour of the trees, shade, etc. As incidence may vary between years and sites, observations during two growing cycles with average or high infection rate are to be considered for reliable evaluations.

Considerable knowledge is available on the epidemiology of the witches' broom disease (Rudgard *et al.* 1993) carried out for more than five years in six countries, four of which participate in this project: Brazil, Ecuador, Venezuela and Trinidad. Cocoa trees may express resistance to WB in any of the following ways: a small number of brooms (either or both vegetative and cushions), smaller brooms and brooms producing fewer basidiocarps per unit. The disease cycle in most countries varies from nine to thirteen months, however where conditions for infection are suitable all year round as in Brazil, this period may be shorter.

The information on disease incidence, as described below, should be best completed with information on yield and phenological characteristics (fruiting and flushing cycles) of the material to be evaluated. This is because trees flushing or fruiting outside the main epidemic period will help escape the disease to a variable degree.

In this CFC/ICCO/IPGRI project, cocoa materials of different ages will be compared. The following method considers field planted materials (clones and hybrids). Observations of young field plants are qualitative, by using visual assessment scales, whereas observations in the nursery and on adult field trees are quantitative (counting number of infected plant organs or basidiocarps). In case heavy infection occurs in the trials, normal management will include removal of excessive infected tissues, to avoid weakening of the trees. In this case, observations of the pathologists should precede the time of routine removal of infected tissue.

Nursery observations

Nursery observations are to be made for the International Clone Trial and other project trials, every time enough planting material can be obtained for these observations and infection level is high enough to make reliable results possible.

Nurseries are established nearby or under naturally infected trees. If this is not possible, dry brooms should be hung on wires over the nursery to increase chances of infection. The minimum number of plants used is 25 for clones (budded plants of four to five months old before exposure to the disease) and 50 for seedlings (three to four month old). The plants should be observed for six months after initiation of exposure to infection. The plants should be distributed in randomized blocks with a minimum of five plants per plot for clones and of ten plants per plot for seedling progenies. The following observations are made at monthly intervals after appearance of the first symptoms:

Clones

- Number of growing points per plot (each budded plant may have one or more growing points).

- Number of infected growing points, to be subdivided into:
  (i) terminal brooms,
  (ii) axillary brooms,
  (iii) stem or twig swellings/cankers without broom formation.

Seedling progenies

- Number of growing points per plot (equal to number of plants, if no multiple stems are formed).
- Number of infected growing points (plants), with identification of terminal brooms, axillary brooms and swellings/cankers (as above).

Field observations

General information

General information on field plot to be recorded is:

- Area of the trial.
- Soil (type and quality).
- Shade (type and quantity).
- Spacing.
- The area of trial containing the plot is to be shown on a plan indicating shade and potential witches' broom sources, and extent of shaded canopy on adult trees.
- Meteorological data (rain, temperature and relative humidity) from the nearest station will be desirable.
- Distance to other cocoa (plantations and/or wild cocoa).
- History (of cultivation of the plot).
- Ground cover and litter.
- Cultural practices carried out throughout the evaluation period.
- Genetic material.
- Any other peculiarity regarding the trees.

For young trees, all trees in the trial should be observed (in total 40-60 trees). For adult trees, the minimum number of trees per genotype is two per plot, repeated five or six times (in total 10-12 trees per genotype). Trees will be individually assigned and marked with a number.

Assessment scale for vegetative broom incidence on young trees

The use of a subjective assessment scale is recommended for evaluation of vegetative broom incidence of young cocoa clones or seedlings, before the trees enter into bearing. This is based on an estimation of the percentage of infected growing points (apexes) in relation to the total number of growing points per tree. Observations are to be made individually for all trees in the trial, at six-month intervals, when detectable infection levels are present in the field. The scale includes 6 points: 0 = no infection, 1 = up to 5%, 2 = 5 to 10%, 3 = 10 to 25%, 4 = 25 to 50% and 5 = more than 50% infected growing points.

Assessment of adult tree infection level

For **vegetative broom incidence**, a total of five branches per plot are marked, each containing at least ten healthy growing points (a minimum of 50 growing points per plot). Branches are to be well distributed over the plot and representative for the canopies. The field trials will normally have five or six replicates (blocks), so of each genotype (clone or hybrid) a total of 25 or 30 branches will be observed, which should be sufficient for a reliable estimate of vegetative broom incidence.

The following data are recorded on these branches at bi-monthly intervals:

- Number of axillary or terminal growing points (healthy or infected, young or old) per branch.
- Total number of new flushes per branch (healthy or infected).
- Number of:
  (i) Terminal brooms
  (ii) Axillary brooms
  (iii) Swellings/cankers.

Cushion infection is observed on 1 m segments of the same branches observed for vegetative broom incidence. The following data will be recorded bi-monthly during epidemic periods (e.g. over six successive months per year).

- Total number of active healthy cushions showing only normal flower buds (closed or open).
- Number of infected flower cushions with presence of dead flowers, 'chirimoyas', or cushion brooms.

Each infected cushion is marked with pins of a different colour to denote when the infection is first observed (i.e. six different colours for an infection period of six months). Only newly infected cushions are identified in successive months of observation. At the end of the observation period (epidemic period) all chirimoyas and cushion brooms are removed. In trials where the number of flower cushions is very high, the above observation method may become impractical for use on a large number of trees. In this case, it is recommended that an estimate is made of the relative number of infected flower cushions compared to the total number of active cushions (with normal and/or infected tissues) by using a 0 to 5 visual assessment scale (with 0 = no infection, 1 = up to 5%, 2 = 5 to 10%, 3 = 10 to 25%, 4 = 25 to 50% and 5 = more than 50% infected active flower cushions).

The **incidence of fruit infection** is to be recorded every two to four weeks, at the same time as the ripe pods are harvested by the breeders. The following records need to be made (this work should be done with the breeders):

- Number of healthy ripe fruits harvested.
- Number of diseased yellowing pods (small or big) indicating presence of *Crinipellis*, *Moniliophthora*, *Phytophthora* or other. For correct diagnosis of the type of disease, it may be necessary to consider the shape of the fruit (malformations are often due to witches' broom) or to open the fruit.

Data analysis

The WB incidence observed will be considered as any other trait to be taken into account for characterizing and selecting material. The type of analysis will be as described in the working procedure on this item.

Author of draft proposal

C. Suarez-Capello, INIAP, Ecuador

References for further reading

Enriquez G. and V.J. Soria. 1981. Mejoramiento genético para resistencia a cinco enfermedades de cacao. Pp. 41-56 *in* Proc. 6th Int. Cocoa Res. Conf. 1977. Caracas, Venezuela.

Purdy L.H. and R.A. Schmidt. 1996. Status of cacao witches' broom: Biology, epidemiology and management. Ann. Rev. Phytopathol. 34:573-94.

Rudgard S.A., T. Andebrhan, A.C. Maddison and R.A. Schmidt. 1993. Future prospects for improvements in disease management. Pp. 213-220 *in* Rudgard, S.A., T. Andebrhan and A.C. Maddison (eds.). Disease Management in Cacao: Comparative epidemiology of witches' broom. Chapman and Hall. London, 249 pages.

Wheeler B.E.J. and R. Mepsted. 1988. Pathogenic variability amongst isolates of *Crinipellis perniciosa* from cocoa (*Theobroma cacao*). Plant. Path. 37:475-488

21. Evaluation of resistance to witches' broom by means of bud inoculation

Scope

The bud inoculation test is presented here as an alternative to the belt spray method for comparisons between clones (budded plants or rooted cuttings). During the Workshop a leaf inoculation method, developed by M. Ducamp in Trinidad, was also presented. The leaf method associates the effect of basidiospore germination/penetration and the phytotoxic effect of glycerol. However, subsequent work carried out with this method showed that it needs further development before it can be recommended in the project. The description of this method is not included in these Proceedings.

Bud inoculation test

This method has been used in Trinidad and has been adapted from Evans and Bastos (1978). Around 30 cuttings or grafts of similar size (three to six month-old) are to be obtained per clone. The growing conditions should be favourable; a shaded nursery is to be preferred to avoid extreme temperatures and plants may also need to be protected from rain to avoid natural infection. To obtain a synchronized formation of sufficient vegetative buds, the growth of the plants should be stimulated by fertilizing and followed by pruning or bending of the main shoots two weeks later.

All buds which are in the right physiological stage on three to six plants per clone are labelled one day before inoculation. Four plants of a resistant and susceptible control clone are also included. Inoculations are carried out in the afternoon of the following day, to avoid exposure to high temperatures. Depending on the average number of buds available, four to six series of inoculations are to be carried out (total minimum number of inoculated buds is 50). The lay-out of the experiment is randomized blocks, with 8-12 buds inoculated for each clone in each inoculation series (blocks).

The inoculation technique is based on the one described by Evans and Bastos (1978). One drop (15 µl) of a suspension of basidiospores (in 0.75% water agar) is applied to each young bud (about 0.5 cm in length). Inoculation should be carried out at the end of the afternoon in order to avoid high temperatures and evaporation of the inoculum. Preliminary tests are required to determine the optimum inoculum density, which may vary between 60 and 400,000 per ml according to the aggressiveness of the isolate. Alternatively, inoculation may be carried out by manually spraying a basidiospore suspension in distilled water onto the buds. Plants are incubated at moderate temperatures for 48 h in a humid chamber or by placing humidified plastic bags over the growing points. During incubation, the plants should be placed in the shade or darkness to avoid overheating. After incubation, the plants must be placed back in the shaded greenhouse or nursery avoiding exposure to extreme temperatures.

The following observations are made 12-16 weeks after inoculation:

- percentage of infection (presence of swellings, number of terminal and axillary brooms per inoculated bud), and
- the diameter and the length of the brooms.

Two parameters will be used for resistance evaluation: infection frequency (% of shoots developing brooms or swellings) and severity of infection (size of broom). Statistical differences are noted on the percentage of infection and severity data.

Points arising from subsequent discussions

The bud test is proposed as an alternative for testing clones in the nursery. It is recognized that this test is more laborious than the leaf disc test and, as such, may be less suited to the need for a large number of comparisons. However, this test is better developed and known to give more reliable results. One aspect that deserves further investigation concerns the way to obtain more uniform young buds (pruning trials are recommended).

Authors of draft procedure

M. Ducamp, CIRAD, France and J.M. Thevénin, CIRAD, c/o CRU, Trinidad

Reference

Evans H.C. and C.N. Bastos. 1978. Witches' broom disease of cocoa (*Crinipellis perniciosa*) in Ecuador I: The fungus. Ann. Appl. Biol. 89:185-192.

22. Ring test for witches' broom resistance

Scope

The aim is to evaluate the stability of resistance of the clones to isolates from different countries, by means of a standardized comparative technique. The primary purpose is to allow cross-comparison of the resistance values reported from the centres using spray-based inoculation methods. The early screening tests with sprayed basidiospores in producer countries should provide most of the information required, but to test isolates from all regions on all material requires the work to be done in a non-cocoa-growing country. The secondary purpose is to provide carefully standardized and reproducible data on the clones in the trial.

Methods

In the first year, a test method that is reliable, repeatable and easy to use will be developed, based on previous work. Two possible methods will be tested: bud inoculation of clonal material, and leaf necrosis assay. We shall also compare the convenience and reproducibility of different sources of basidiospores. Initial work will establish a successful infection system using an Amelonado host and a few *Crinipellis* isolates. Subsequently, the dose and conditions required to distinguish clearly between resistant (Scavina 6) and susceptible (Amelonado) reactions will be established. The work will be carried out at the University of Reading, at a site 4 km from the quarantine station, and will be done by separate individuals, who will not visit both sites in one day. Culture growth and spore production is done in a specially reserved, closed room with access only to authorized people, and with clear warnings to maintenance staff. Tests on the longevity of basidiospores on clothing and dry tests on slides will be done as soon as spores are produced, and used to reassess the risks in the work.

Leaf test

This builds on the work discussed by Dr. Ducamp and referred to in Working Procedure 21. We have attempted to optimize the characteristics of droplets, leaves and glycerol concentration required. Droplet size tested varies (as 5, 10, 20 µl), and concentration (as 0.5, 1, 2, 4, 8 and 16%). Results are scored at 2-3 day intervals. It is clear that the age of leaf is absolutely critical and a precise description of this is a problem. So far, we have not successfully identified a class of leaf which will survive long enough under our controlled conditions to let a result be read, but to which glycerol is phytotoxic. Therefore, we have to consider seriously whether to use alternative methods.

Differences in colonization, on which the rapid leaf test is based, can be visualized by clearing and staining leaf discs. A number of staining techniques will be tested.

Clonal bud infection test

We shall test methods directly equivalent to the Silwood Park seedling test (removal of the tip of growing stem of budded wood, followed by inoculation of buds released from dominance with agar containing a known number of spores). We shall also try inoculation soon after budding, or after induction of new bud formation on larger plants. The reasoning is that an expanding bud represents rapidly dividing tissue ideal for infection. First we will bud, wait about 1 week for callus production, then inoculate at the same time as the seedling bud and the tape over the clonal bud is removed. We may try a further wait before inoculation. The advantage of this would be that it is quite fast, since it would not be necessary to wait for the expanding branch to break. Discussions at the Montpellier meeting suggested that this material might be very susceptible. Standardization of spore age, source, concentration and plug size is needed. This will be done by dose-response experiments on

Scavina 6 and Amelonado clones or seedlings to determine a suitable dose to distinguish susceptible and resistant reactions. Inoculations at two doses may be needed to give a clear indication of susceptibility. It will also be necessary to compare germination and infection by freshly collected spores with germination and infection by spores stored frozen in aqueous glycerol/MES. We are using the technique developed at Silwood Park for spore production *in vitro*. Scoring will initially involve broom development after two to three fixed times, using a photographic key of size and form. Later on, if time permits, we will also explore the possibility of looking at the extent of mycelial development in the developing bud at, say, three weeks after inoculation. We will also investigate the possibility of using biotrophic phase cultures of the pathogen on potato callus as inoculum. This would simplify inoculum production and ensure much higher throughput in the later phases.

We will choose the method for the large-scale tests based on the results of this work.

Germination tests

The feasibility of methods involving germination of basidiospores in plant extracts will also be considered. This method will use extracts obtained after grinding tissues, intercellular fluids obtained after centrifugation and exudes from stem tissue.

Clonal material

Spore viability and pathogenicity of different inoculum types will be checked using Amelonado seedlings or clones. As indicated above, Scavina 6 and an Amelonado clone (SIAL 339 and/or SIC5 available at Reading) are proposed as control clones, with which further development of the methods will be tried. Once operational, the test will be applied to the clones most used in the International Clone Trial, with priority to those being tested in witches' broom affected countries:

Origin	*Priority 1*	*Priority 2*
Reading	AMAZ15-15	BE10
	LCT-EEN46	EQX3360-3
	MAN15-2	
	MXC67	
	PA107	
	Playa Alta 2	
	Scavina 6	
	SPEC54-1	
Montpellier	EET59	APA4
	GU255	GU175
	ICS1	GU307
	IMC47	
	NA33	
	LAF1	
	PA150	
	SNK413	
	VENC22-6	
	VENC4-4	

Number of genotypes (clones, seedlings or hybrid varieties) tested in one replicate

The advantage of the leaf disc inoculation method is that many cocoa genotypes can be tested simultaneously in one inoculation series (replicate). In the CFC/ICCO/IPGRI project,

the number of genotypes is about 25 for the International and Local Clone trials. It is recommended that all genotypes be represented in each inoculation tray at one inoculation date. For example, if one tray can contain 200 discs and 25 clones need to be tested, eight discs of each clone can be placed in each tray.

However, the number of genotypes can be much higher for mass screening of clones in large collections or of individual field trees or nursery seedlings. In such cases, it is recommended to distribute the genotypes randomly over different trays, using a control variety to compare between trays. For example, if 50 clones need to be tested at one inoculation date, these could be subdivided into two groups of 25 genotypes plus a control variety, and the leaf discs distributed randomly over five trays (total 5 x 8 = 40 leaf discs per genotype and 5 x 2 inoculation trays for one inoculation date).

Fungal Isolates

The isolates to be used in Reading should be as genetically homogeneous as possible. If possible, they will be monospore isolates multiplied *in vitro* in Reading. If culturing proves impractical, bulk populations from single brooms/pods imported under an appropriate import licence from Brazil, Venezuela, Ecuador and Trinidad will be used instead. Spores will be obtained to start cultures, or create a bulk population from live material provided by these countries. At Reading, brooms from Trinidad have been used to produce basidiocarps easily.

A minimum of one isolate per country will be tested. However, two will be tested if time and resources permit. Priority will be given to the use of an extra isolate obtained from Sca 6 progeny in Ouro Preto, Rondonia, Brazil, so as to provide an indication of the stability of Scavina resistance.

Standard recording sheet

This is illustrative only: it assumes that the bud inoculation method with frozen spores works. Scoring will be by comparison with a standard series of photographs or descriptions, in a similar way to the belt spray inoculation method. At the time of inoculation, a sheet A (see below), cross-referencing pot codes to date of inoculation, spore source and concentration, isolate and clone will be prepared. This has the desirable effect of partly blinding the assessment process. Results are then recorded on B. Swelling could be measured semi-objectively by using calipers to measure the diameter of the extending stem at its narrowest and widest parts.

A. Date:
Experimenter:

Clone	State	Isolate	Source	Concentration	Pot codes	Comments

B. Date:
Recorder:

Pot code	Plant state	Broom development		Comments
		Swelling	Branching	

Author of draft procedure

M. Shaw, The University of Reading, United Kingdom

23. *In vitro* method for preliminary screening of cocoa germplasm for resistance to vascular streak dieback

Scope

The *in vitro* or dual culture method described below is for preliminary large-scale screening of cocoa germplasm for resistance to vascular streak dieback (VSD). The method is non-destructive, faster and cheaper and means that screening can be undertaken on a larger scale than the nursery or field evaluation method described elsewhere. However it is less accurate. It entails the inoculation of cocoa callus with the pathogen, *Oncobasidium theobromae*, the quantitative and qualitative assessment of the growth of callus and colonization of the callus by the pathogen respectively. The method was first described in 1995 (Bong and Puad 1995) and further elaborated subsequently (Bong *et al.* in press a and b; Bong and Lee 1999).

Experimental design

Standard susceptible and resistant control clones are to be included in the screening. Candidate clones for controls are those which are proven in the field. In Malaysia available susceptible controls include NA32, PA7 and PBC130, the resistant controls include ICS95, NA33, PBC123 and DESA1. Five to six replicates of each of the following treatments are included per clone:

- inoculated callus (five to six flasks)
- uninoculated callus (control) (five to six flasks).

Any number of clones can be included in each batch of screening and is limited only by the capacity of the incubators.

Initiation and maintenance of cocoa callus

Callus can be initiated from stem, leaf or petiole and maintained on MS (Murashige and Skoog) medium supplemented with kinetin (0.05 mg/l) and IAA (indole-acetic acid) (10.0 mg/l). Callus is initiated and subcultured in universal bottles each containing 10 ml of the medium. Culture medium is sterilized by autoclaving at 121 °C for 15 minutes.

Selection and sterilization of explants

Suitable explants are the young green stems or petioles or young hardening green leaves. The stem, petiole or leaf is washed under running tap water and wiped dry before surface sterilization. The stem or petiole can be adequately sterilized by swabbing with 70% ethanol. The bark is peeled or the outer layer pared off and pieces of the inner tissue or the wood part are aseptically cut and cultured on the MS medium. The leaf is cut into pieces of about 1-2 x 1-2 cm squares and sterilized with hypochlorite solution (1:4, hypochlorite:distilled water) for a duration of 10-40 sec, and rinsed three times in sterile distilled water before being aseptically cut into pieces of about 0.5 x 0.5 cm square for culture on the medium.

Culture conditions and subculture of callus for screening

The culture is incubated in the dark within the temperature range of 25 °C to 28 °C, preferably at the latter temperature. After four to five weeks, the callus initiated is subcultured to remove the original explant tissue. The same medium and culture conditions apply. After four to five weeks, the callus is of sufficient size to be divided into pieces of about 2 x 5 x 5 mm in size for use in screening.

Preparation of inoculum

The inoculum of the pathogen, *Oncobasidium theobromae* Talbot and Keane, is freshly isolated on 1% water agar from infected twigs obtained from the field. Seven to ten day old

inoculum is used. Inocula used are mycelial discs of 5 mm in diameter taken from the margin of the colony.

Procedure for screening and data to be taken

For screening, the same medium and culture conditions are applied. Conical flasks of 100 ml containing 50 ml medium are used. These are sterilized by autoclaving. One piece of the callus is placed into each flask. The callus is inoculated with one centrally placed mycelial disc (dual culture). For the control (single culture), no inoculum is introduced to the callus. These are incubated for a duration of four to six weeks after which the calli are harvested. Mycelium on the surface of the calli is removed, and fresh and dry weights of the calli taken (see Recording Sheet 1).

Nine cm plates with 20 ml MS medium are also prepared for the culture of the pathogen as a control, to check on the viability and vigour of the pathogen inoculum. An inoculum disc is placed on the centre of each plate. The plates are incubated under the same conditions.

Analyses of data

The statistical difference between the mean weights of inoculated (i) and healthy (h) calli from each clone can be tested via the t-test. The i/h ratios of the clones give an indication of their resistance or susceptibility by comparison with the i/h values of the susceptible and resistant control clones.

Correlation of laboratory results with field data

The results of screening of more than 30 clones using the dual culture method show fairly good correlation with field data and some of these have been reported (Bong *et al.*, in press a and b). However variance and variability can be expected to be high in tissue cultures.

Features of the method

The method described is cheaper and faster and there is a higher screening capacity with this one, rather than the nursery or field method although it is less accurate. However, the method can be further extended to comparison of different isolates of the pathogen and their interactions with different genotypes as reported by Bong *et al.* (in press a).

Points arising from subsequent discussions

The method is only recommended for use in preliminary screening when a large number of clones needs to be screened and resources such as funds and land area are limited or not available for the more accurate and also more expensive field or nursery evaluation method. The potential of this method of dual culture and assessment can conceivably be explored further for use in the screening for resistance to witches broom disease. This was also suggested by Fonseca and Wheeler (1990) in their study using different methodology in culture and in the type of data recorded.

Author of draft procedure

C.L. Bong, Malaysian Cocoa Board, Malaysia

References

Bong C.L. and M.T. Lee. 1999. Resistance to vascular streak dieback: Research and applications in breeding and disease management. Pp. 195-204 *in* Proc. Int. Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, Salvador, Bahia, Brazil, November 1996, INGENIC.

Bong C.L. and M.A. Puad. 1995. Influence of temperature on the growth of *O.theobromae*, cocoa callus and host-pathogen interaction. P. 26 *in* Abstracts of 18th Malaysian Microbiology Symposium, 23-

24 Aug 1995, Kuching, Sarawak, Malaysia.

Bong C.L., M.T. Lee and M.N. Yahya. In press (a). Interaction between cocoa clones and isolates of *Oncobasidium theobromae*, causal pathogen of vascular streak dieback. *In* Proc. 12th Int. Cocoa Res. Conf., 17-23 Nov, 1996, Salvador, Bahia, Brazil.

Bong C.L., M.T. Lee and M.N. Yahya. In press (b). Interaction between explant tissue sources and cocoa genotypes in the expression of resistance to vascular streak dieback. *In* Proc. 12th Int. Cocoa Res. Conf., 17-23 Nov, 1996, Salvador, Bahia, Brazil.

Fonseca S.E.A. and B.E.J. Wheeler. 1990. Assessing resistance to *Crinipellis perniciosa* using cocoa callus. Plant Pathology 39:463-471.

Recording Sheet 1: Screening for resistance to VSD by dual culture: weights of cocoa calli

Batch of clones: _____ Date started: _____ Date harvested: _____

			Callus weight determinations					
			Fresh weight			Dry weight		
Clone	Inoculated or control (uninoculated)	Flask number	Filter paper	Callus + filter paper	Callus	Filter paper	Callus + filter paper	Callus
Etc.,								

24. Evaluation of vascular streak dieback resistance in the nursery and field

Scope

In Malaysia, there are two methods being used for screening for resistance to vascular streak dieback (VSD). One is the in vitro method using dual culture and the other is the field method. These two methods were outlined recently (Bong 1989a and 1989b, Bong and Lee 1999). The *in vitro* method is used for preliminary screening or detection of resistance in cocoa germplasm. The field method is used for confirmatory assessment, generally applied in the evaluation of resistance of clones and progenies in breeders' trials and also in fungicide screening and other studies in the nursery and field. It has been in routine use in breeders' trials in Malaysia since 1983. It is a non-destructive method. The method has been found to be consistently sensitive and reliable for comparing relative resistance among planting materials and in other VSD studies (Bong and Phua 1989, Bong *et al.* 1994, Yapp and Hadley 1994).

Resistance of cocoa to VSD is relative. Some materials are more susceptible or resistant than others. The rating of relative resistance is based on the measurement and comparison of VSD disease severity of the plants on exposure to natural inoculum over time. The details of the method and procedure used are indicated hereafter.

Experimental layout

The experimental layout generally used in the nursery and field is the randomized complete block design. Split-plot design has also been used in field experiments when other factors are also being considered (Bong *et al.* 1994). There would be a minimum of three replicates, (preferably four) per clone or hybrid for each set of materials to be screened. Included in each set of materials to be screened are the susceptible and resistant control materials. For hybrids, the standard controls most commonly used and acceptable by the breeders are PA7xNA32 for susceptible control, PA138xSCA9 for resistant control and UIT1xNA33 for moderately resistant control. Suitable candidates for susceptible control clones include QH968, PA7 or NA32, PBC130, PBC140, KKM20 and KKM27. The resistant control clones that can be used are PBC123, DESA1, DESA2, and KKM25. The number of plants per replicate varies from 25 to 50 plants per hybrid or 16 plants per clone. The number of plants on which VSD is assessed is usually eight plants per hybrid or clone per replicate or a minimum of 20-24 plants per treatment. The plants used for VSD assessment are located in the centre of the replicate plots.

Inoculum source

The pathogen, *Oncobasidium theobromae* (Talbot and Keane) is an obligate parasite. It is not easily cultured. Successful induction of sporulation has been reported but the quantity of spores produced is not predictable or easily reproducible (Prior 1982, Lam *et al.* 1988, Zainal Abidin 1996). Therefore screening for resistance has relied on a natural inoculum source from the surrounding infected mature trees. The nursery is usually on a site surrounded by mature infected fields. Natural inoculum builds up easily in the field over time when susceptible hybrids are planted as guard rows. Inoculum pressure in the nursery can be built up and standardized by doing this or by planting susceptible hybrids within the nursery or field trial plot.

Rating host resistance to VSD

With this method, the host resistance to VSD is determined by measuring disease severity over time that covers both the period of disease development (5-6 months) and recovery of the plants (4-6 months) from the disease (Bong 1989b, Bong and Phua 1989). The values used

for rating resistance are apparent rate of infection and/or ADPC (area under disease progress curve). These are derived from measuring two parameters. The two parameters used for measuring VSD disease severity are the incidence and disease symptom severity or extent of damage.

Incidence of infection and measurement

Incidence of infection as a proportion of the population infected is an adequate parameter to use on seedlings or young plants which are not yet jorquetted. Infection here is defined as positive infection on one or more leaves found on the plant. The incidence is recorded at monthly intervals over a period of six to nine months (see Recording Sheet 1). It is generally adopted for use in screening at nursery level. From the incidence data taken over time, the apparent rate of infection is calculated and used as a measure of relative resistance. The rate is obtained by regressing ln{1/(1-X)} or ln{X/(1-X)} against time in months where X is the incidence of infection (van der Plank 1963). This has been found to be reliable and sensitive for screening cocoa progenies and clones or fungicides in the nursery (Bong and Phua 1989, Bong and Seow 1989b).

Disease symptom severity and measurement

This is used generally in the field as well as the nursery. The extent of disease symptom severity or damage is quantified by using a disease severity or damage scale as shown in Table 1. The exact procedure, as detailed by Bong (in 1989a), is summarized here. This method of measurement incorporates both the incidence and the extent of damage caused by the disease.

To determine the extent of infection and damage from VSD on a plant, the disease severity of each of the terminal three flushes of a stem or twig is scored (see Recording Sheet 2). The assessment is conducted on the three terminal flushes because these bear the most photosynthetically active leaves. Ten to 15 branches (depending on the size of the canopy) per tree are randomly selected. Each flush is examined for infection and scored according to the scale. Positive identification that the damage is due to VSD rests on the presence of the characteristic discrete brown discoloration of the vascular traces on leaf scars or base of the petiole or midrib of the leaf. The branches are left intact and not destroyed. The assessment is carried out at one to ten-month intervals over a period of 12 months, or one complete epidemic cycle.

The disease severity scores are used in the calculation of the disease severity index for each plant. The indices (Y values) are then used for the calculation of ADPC or area under disease progress curve for each replicate as follows:

$$\text{ADPC} = \sum_{i=1}^{n} \{[(Y_{i+n1} + Y_i)/2]\,(t_{i+1} + t_i)\}$$

where Y= disease severity index at i^{th} observation, t = time in months, n = total number of observations (Shaner and Finney 1977).

The ADPC values are then analyzed using ANOVA or in accordance with the experimental design.

VSD symptom or damage severity scale

The scale used for scoring disease severity on a branch (three flushes long) is from 0 to 6 (Table 1) on progressive damage from chlorosis to defoliation to dieback. The severity scores obtained for each flush are weighted differently. The weighting factors are arbitrarily set at 1, 0.5 and 0.25 for the scores of first (terminal), second and third flush respectively of each the branches assessed. These are necessary to reflect the greater damage observed on the

younger flushes or branch given the same scores for each flush. For example, a branch with a score of 6 for the first flush is one that is dying back whereas a branch with a score of 6 on the third flush does not show dieback.

Table 1. VSD disease symptom severity scale

Severity scale	Primary symptom severity of a flush	Associated symptoms
0	Apparently uninfected or healthy	Leaves glossy, healthy
1	One or two infected leaves; infected leaves showing early signs of symptoms – loss of glossiness or shine; discrete brown vascular bundles on petiole or leaf scar or midrib of the leaf lamina	Smooth bark of twigs or stems, no swollen lenticels
2	Few infected leaves, with one or more showing chlorosis in progress	Lenticels on bark may or may not be swollen
3	Some leaves infected; one infected leaf abscised; one or more of the leaves showing chlorosis and necrosis in progress	Lenticels on bark may or may not be swollen
4	Two infected leaves abscised, some or all of the remaining leaves showing chlorosis and/or partial necrosis	Lenticels on bark may or may not be swollen
5	Three or more infected leaves abscised; remaining leaves infected, chlorotic or necrotic; apparent cessation of growth (of first flush)	Lenticels usually swollen; fruit bodies may or may not be present
6	Near complete or complete defoliation from abscission of infected leaves; dieback (first flush) in progress as indicated by the drying of the twig/stem	Lenticels usually swollen; fruit bodies may or may not be present; there may or may not be any proliferation of axillary shoots

Note: the word 'infected' or 'uninfected' refers to VSD and not to any other diseases. The presence of infection is confirmed by the discrete brown vascular traces on the leaf scars or petiole (revised from Bong 1989a).

Applications and results of using the method: some examples

As mentioned above, the method described has been in use in Malaysia since 1983 in screening for host resistance and in other studies. Specific examples of the applications in resistance screening can be found in the list of references given (Bong and Phua 1989, Bong *et al.* 1994).

Features of the method

The use of the above method in VSD disease resistance screening takes time and manpower and these are to be expected in field screening. The accuracy of the results increases with the number of assessments over time that includes both the epidemic and recovery phases. An adequate duration would be 12 months in field screening. A period of six to nine months in nursery screening would be sufficient as seedlings when infected rarely recover. The method described above has several features as mentioned in the past (Bong and Lee 1999).

These are attractive to the pathologists, breeders and others involved in field work with VSD:

- Both incidence and disease damage are included in the assessment.
- Extent of infection is also indicated from assessing three flushes on the randomly selected branch.
- Disease severity or damage is scored on flushes of leaves that are productive – the photosynthetically active flushes.
- The method is non-destructive in that the assessed branches are left intact and not cut off or destroyed. The method allows normal growth or development of the canopy.
- The method is free of the confounding factors expressed by Tan (1992) that are present in the use of the parameters, number of infected branches and extent of infection determined by destructive means.
- The method is sensitive and reliable.

Factors and links in international collaborative trials

The following important factors are to be considered for obtaining informative results in international collaborative trials:

Susceptible and resistant control materials

For the selection of standard susceptible or resistant controls to be used in international trials, the following need to be considered:

- the susceptibility or resistance of the materials to VSD have been proven under as wide an environmental range as possible.
- the control materials should have agronomic characteristics acceptable to the breeders for use as the standard controls in the trial (to reduce the area required for the trial).
- the susceptibility or resistance to other diseases such as black pod should also be known.

Each participating country should include standard control materials that have been proven to be susceptible or resistant in the respective countries. Furthermore, these materials should also be included in participating countries as link materials for comparison - an example is given in Table 2.

Table 2. Standard control and link hybrids/clones

	Malaysia		Papua New Guinea	
	susceptible	Resistant	susceptible	resistant
Standard controls	PA7xNA32	PA138xSCA9	PNG1	PNG2
Link controls	PNG1	PNG2	PA7xNA32	PA138xSCA9
Standard controls	PBC130	PBC123	PNG3	PNG4
Link controls	PNG3	PNG4	PBC130	PBC123

Other clones with good agronomic characteristics selected by the breeders in Malaysia and that are suitable candidates for use as controls include:

- susceptible controls: QH968, PBC130, KKM20, KKM27, PBC140.
- resistant controls: QH37, PBC123, KKM25, DESA1, DESA2.

PBC123 would serve well as resistant VSD and black pod control. PBC130 would serve well as susceptible control for both VSD and black pod.

Inoculum source

It is proposed that susceptible hybrids be planted as guard rows to provide and standardize the source and distribution of inoculum to the trial plot or nursery.

Sample size for VSD assessment

In the nursery, with incidence as the parameter to measure, the number of plants to be assessed ranges from 25-50 plants per replicate for hybrids, or 16 plants per replicate for clones. For the assessment of disease severity of jorquetted or older plants in the field, the number of plants can vary from 4-8 per replicate with a minimum total of 20-24 plants per treatment. The number of branches (twigs) to be assessed can vary from 10-15 branches/twigs per plant.

Frequency and duration of VSD assessment

Accuracy increases with frequent and longer screening periods. Incidence or disease severity scores can be recorded monthly or at intervals of six weeks for 6-12 months (to include a minimum of one epidemic phase, and the recovery phase) to obtain good derivation of apparent rates of infection or values of ADPC (area under disease progress curve). Raw data or disease severity scores alone would give an indication of the relative resistance but would be less informative than the values on apparent rate of infection or ADPC.

Author of draft procedure

C.L. Bong, Malaysian Cocoa Board, Malaysia

References

Bong C.L. 1989a. A method for quantifying and indexing VSD disease severity. *In* 'Research on Cocoa VSD in Sabah', Sabah State Department of Agriculture, Technical Bulletin 9:8-11.

Bong C.L. 1989b. VSD epidemic development and weather factors in Sandakan. *In* 'Research on Cocoa VSD in Sabah', Sabah State Department of Agriculture, Technical Bulletin 9:50-56

Bong C.L. and P.K. Phua. 1989. Screening of hybrid materials for resistance to VSD. *In* 'Research on Cocoa VSD in Sabah', Sabah State Department of Agriculture, Technical Bulletin 9:43-49.

Bong C.L. and S.T. Seow. 1989a. Chemical control of VSD on mature cocoa. *In* 'Research on Cocoa VSD in Sabah', Sabah State Department of Agriculture, Technical Bulletin 9:23-42.

Bong C.L. and S.T. Seow. 1989b. Field and laboratory screening of fungicides for the control of VSD. *In* 'Research on Cocoa VSD in Sabah', Sabah State Department of Agriculture, Technical Bulletin 9:15-22

Bong C.L. and M.T. Lee. 1999. Resistance to vascular streak dieback: Research and applications in breeding and disease management. Pp. 195-204 *in* Proc. Int. Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement, Salvador, Bahia, Brazil, November 1996, INGENIC.

Bong C.L., E.B. Tay, M.T. Lee, and T.C. Chong. 1994. Comparative studies and cultural practices and planting materials in the management of vascular streak dieback and productivity of cocoa. Pp. 197-203 *in* Proc. 11th Int. Cocoa Res. Conf., 18-24 July, 1993, Yamoussoukro, Cote d' Ivoire.

Lam C.H., G. Varghese and M.A. Zainal Abidin. 1988. In vitro production of *Oncobasidium theobromae* basidiospores. Trans. Br. Mycol. Soc. 90:505-507.

Prior C. 1982. Basidiospore production by *Oncobasidium theobromae* in dual culture with cocoa callus. Trans. Br. Mycol. Soc. 78:571-574.

Shaner G. and R.E. Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

Tan G.Y. 1992. Cocoa breeding in Papua New Guinea and its relevance to pest and disease control. *In* 'Cocoa Pest and Disease Management in Southeast Asia and Australasia' (P.J. Keane. and A.A.J. Putter, eds.). FAO Plant Protection Paper 112:117-128.

Yapp J.H.H. and P. Hadley. 1994. Inter-relationships between canopy architecture, light interception, vigour and yield in cocoa: implications for improving production efficiency. *In* Proc. Int. Cocoa Conf.: Challenges in the 90s, 25-28 September 1991, Kuala Lumpur, Malaysia.

Zainal Abidin M.A. (In press). A method for inducing sporulation of *Oncobasidium theobromae* Talbot and Keane in axenic cultures. *In* Proc. 12th Int. Cocoa Res. Conf., 17-23 Nov. 1996, Salvador, Brazil.

Recording Sheet 1. Observations on VSD disease incidence

Project code: _____ Station/region: _____ Sheet no: _____

Hybrid/Clone code			
Replicate no.		Replicate no.	
Date		Date	
N_t (total)=		N_t (total)=	
N_x (X) =		N_x (X) =	
N_0 (0)=		N_0 (0)=	
N_k (k)=		N_k (k)=	
VSD incidence		VSD incidence	
Plant no.	X/0/k	Plant no.	X/0/k
Etc.			

Hybrid/Clone code			
Replicate no.		Replicate no.	
Date		Date	
N_t (total)=		N_t (total)=	
N_x (X) =		N_x (X) =	
N_0 (0)=		N_0 (0)=	
N_k (k)=		N_k (k)=	
VSD incidence		VSD incidence	
Plant no.	X/0/k	Plant no.	X/0/k
Etc.			

X = infected; 0 = healthy; k = any casualty other than from VSD

Recording Sheet 2. Observations on VSD disease severity

Project code: _____ Clone/hybrid: _____

Station/region: _____ Replicate no: _____

Date: _____ Sheet no.: _____ Field no.: _____

Tree no:			
Branch / twig no.	Disease severity score		
	Flush 1	Flush 2	Flush 3
01			
02			
03			
04			
Etc.			
sum	x 1.0	x 0.5	x 0.25
ws =	s1=	s2=	s3=
DSI =(s1+s2+s3)/(6x10)=			

Tree no:			
Branch / twig no.	Disease severity score		
	Flush 1	Flush 2	Flush 3
01			
02			
03			
04			
Etc.			
sum	x 1.0	x 0.5	x 0.25
ws =	s1=	s2=	s3=
DSI =(s1+s2+s3)/(6x10)=			

Sum = flush totals; ws = weighted scores, s1, s2 and s3 of 1st, 2nd and 3rd flush respectively;

DSI = disease severity index of the tree

Insect Resistance and Control

25. Laboratory microtest for cocoa tree attractiveness to mirids

Scope

The aim of this activity is to quantify the attractiveness levels of cocoa cultivars to mirids in the laboratory under controlled conditions. The method is proposed as a standard method for evaluation of adult plants for the International and Local Clone trials in countries where mirid attacks are common. It is recommended that this be applied as an early evaluation method, using nursery plants, provided that enough plant material is available. The method is as described by Nguyen-Ban (1993 and 1998).

Material and methods

Entomology equipment: collection and preparation

Mirid larvae of 4th and 5th instars should be collected by a team of three observers at daybreak. The larvae should belong to the locally predominant mirid species, although comparison in attractiveness of a number of cocoa clones to different mirid species could be the subject of a specific study. Larvae are collected individually using a haemolysis tube and a brush, then placed in a collecting box with green cocoa twigs for food.

After collection, the larvae are left to rest and fast overnight, to encourage their feeding on the plant material to be provided during the experiment the following day.

Plant material and experimental layout

Green twigs of expanded young flushes, about two weeks old, are used for the test. These are cut into 50-mm sections. Care should be taken to use twigs of the same diameter. The layouts to be used are triangles, squares, etc. depending on the number of branches that can fit into one Petri dish. The twigs are stapled together. The sides of the triangles, squares, etc. should be of different cocoa genotypes.

Care must be taken to ensure that each of the selected genotypes is represented at least twice in the series of tests, keeping the same control in all the series. The control can be a moderately attractive field clone of which abundant young twigs are available. For the triangular layout this would give the following series of tests:

e.g.: Series 1: Genotypes A, B and control: 20 replicates
Series 2: Genotypes B, C and control: 20 replicates
Series 3: Genotypes C, D and control: 20 replicates
etc.,
Series n: Genotypes X, A and control: 20 replicates

Once the twigs have been assembled they are placed in 120-mm diameter Petri dishes, the bottom lined with filter paper. One fourth or fifth instar larva is placed in each, representing a replicate; each test series comprises a minimum of 20 replicates. This means that each genotype is tested 2 x 20 = 40 times. The dishes are placed in darkness for 24 hours. The best would be to carry out five replicates of each of the series on the same day and repeat this four times until all 20 replicates have been carried out.

Test results and interpretation

The dishes are checked 24 hours later. Mirid feeding punctures are easily recognizable on the twigs through the dark patches left at the feeding sites. The wound is formed by stylet penetration and subsequent removal of abundant amounts of food. The insect first injects toxic saliva containing digestive enzymes at high pressure between the cell walls of the

epidermis. Destruction of the cortical zone causes the underlying tissue to collapse, forming a depression at the penetration site. A water soaked area with a clearly defined edge appears. It is initially colourless, but gradually turns dark brown, even during mirid feeding.

If the insects find the material particularly succulent bites can overlap on the same twig so that by the end of feeding there is a large brownish patch with a scalloped edge. Close examination of the dead zone can provide a fairly reliable measure of the number of feeding punctures and generally reflect the attractiveness of the cultivar. The bites on the twigs are counted, grouped by cultivar and marked on the record sheet (see Recording Sheet 1). An analysis of variance is carried out to class genotypes in increasing or decreasing order of attractiveness.

Alternative layout

The above-proposed layout can be adapted according to the availability of materials and the size of the receptacles. More than two genotypes could be compared in the same type of set-up, if the laboratory receptacle is sufficiently large. It is also possible to compare the genotypes without using a control variety (as such a variety may not always be available in large enough quantities). The arrangement would then be as follows:

- Series 1: Genotypes A, B and C: 15 replicates,
- Series 2: Genotypes B, C and D: 15 replicates,
- Series 3: Genotypes C, D and E: 15 replicates,
- etc.,
- Series n-1: Genotypes W, X and A: 15 replicates,
- Series n: Genotypes X, A and B: 15 replicates.

Each genotype is tested 3 x 15 = 45 times. This is comparable to the minimum of 40 times in the arrangement previously described with a standard control variety.

Author of draft procedure

J. Nguyen-Ban, CIRAD, France

References

Nguyen-Ban J. 1993. Nouvelle technique de sélection du cacaoyer tolerant aux attaques de ravageurs. Pp. 229-235 *in* Proc. 11th Int. Cocoa Res. Conf., Yammoussukro, July 1993.

Nguyen-Ban J. 1998. Nouvelle technique de criblage et de selection de cacaoyers pour la resistance aux mirides. Insect. Sci. Applic. 18:119-127.

Recording Sheet 1. Laboratory microtests for attractiveness to mirids: example of triangular layout for test

Date: _____ Site: _____ Plot: _____ Country: _____

Replicate	Number of feeding punctures			Total
	Genotype A	Genotype B	Genotype C	
1				
2				
3				
4				
Etc.,				

26. Evaluation of tolerance, antixenosis and antibiosis to cocoa mirids in the nursery

Tolerance

Objective
To assess the ability of selected cocoa genotypes to recover from mirid damage

Method
Experiments will be conducted in simulated greenhouse conditions. Plantlets to be used will be either grown from seed or regenerated by budding or grafting in appropriate greenhouse pots. Genotypes to be evaluated and a susceptible genotype (control) will be grown individually in appropriate greenhouse pots for a total of 15 plants per genotype.

A nylon mesh sleeve cage will be used to confine one fourth or fifth instar nymph to each of the plants. Both ends of the cage are securely tied to prevent the insects from escaping. The mirids will be allowed to feed for a period of 24 hours before being removed from the plants. The number of feeding punctures will be determined for each plant. The plants will be observed over time to assess their ability to recover from damage by examining the evolution of the branches and feeding wounds. The survival of the plant will be assessed in relation to the number of feeding wounds. The observations will be made weekly for the first month and monthly for another six months (see Recording Sheet 1).

Antixenosis

Objective
To assess the ability of selected cocoa genotypes to attract or reject mirids in simulated greenhouse condition

Method
The experiment will be conducted in simulated greenhouse conditions. Genotypes to be evaluated will be either grown from seed or multiplied by budding or grafting in appropriate greenhouse pots. Six pots containing plants to be compared will be laid out in a circle within a cage comprising a nylon metal screen folded in a hemisphere (height 50-100 cm and diameter about 50 cm). A susceptible standard genotype will be included as a control.

Ten newly emerged adult mirids which have been starved for 24 hours will be released into the cage. The mirids will be allowed to feed for a period of 24 hours before being removed. There will be about 15 cages or replicates. The number of feeding punctures will be determined to assess feeding preference (see Recording Sheet 2).

Antibiosis and tolerance

Objective
To assess mirid survival/development and tolerance of selected cocoa genotypes

Method
The experiment will be conducted in simulated greenhouse conditions. Plants to be used will be either grown from seed or regenerated by budding or grafting in appropriate greenhouse pots. A total of 25 plants (five plants for each five replicates) for each genotype to be evaluated and a susceptible genotype (control) will be grown. Once the plants are about one year old, a nylon mesh sleeve cage will be used to confine two thirds instar

nymphs on the five seedlings or buddings and, in total, ten mirids will be observed per genotype (see Recording Sheet 3).

Both ends of the cage will be securely tied to prevent the insects from escaping. These nymphs will feed on the plants until they become adults or until the plant dies. The mortality of the nymphs will be recorded every two days. The subsequent development of the plants will be followed for six months, with monthly observations on the reaction of the twigs, using the same recording system as for tolerance (see Recording Sheet 1).

Points arising from subsequent discussions

The procedure is a proposal and has never been used before to assess cocoa resistance to mirids. Improvements may therefore be required before large scale application of the method can be undertaken.

On several occasions, it has been discussed that the method using mirids is laborious and requires large amounts of host tissue. Within the possibilities of the CFC project, it is strongly recommended to develop resistance tests using smaller insects (e.g. aphids which are easier to handle and may give quicker results), including their relationship with resistance to mirids. The development of such a quick method would present great advantages for rapid screening for insect resistance, including the possibility of evaluating such resistance in any country where aphids occur.

Authors of draft procedure

K.F. N'Guessan, CNRA, Côte d'Ivoire and J. M. Mpé, IRAD, Cameroon

Recording Sheet 1. Assessment of mirid tolerance in the nursery

Observer: _____ Trial: _____

Clone or hybrid	Replicates or cage	Number of feeding punctures	Vegetative status of the plants*					Remarks
			Observation date					
Etc.,								

* The average vegetative status of the test plants is observed on a 0 to 4 point scale: 0 = death of all twigs and 4 = no damage.

Recording Sheet 2. Assessment of mirid antixenosis in the nursery

Observer: _____ Date: _____

Replicates or cage	Clone or hybrid	Number of feeding punctures	Remarks
Etc.			

Recording Sheet 3. Assessment of mirid antibiosis in the nursery

Observer: _____ Date: _____

Clone or hybrid	Replicates or cage	Total number of mirids	Number of mirids alive	Number of dead mirids	Remarks
Etc.,					

27. Evaluation of resistance and tolerance to mirids under field conditions

Scope

This methodology is a complementary approach to susceptibility tests in the laboratory as described by Nguyen-Ban (1993). The objectives are to study the development and survival of mirids on some cocoa clones (antibiosis) and to measure the tolerance of clones by studying the physiological reaction of branches to injuries.

Study of the tolerance of clones

Actively growing branches with soft leaves, which are beginning to turn green, are used. They are covered with sleeves made of nylon mosquito mesh. After 24 hours of starvation, one mirid of the third instar is allowed to feed on the branch. Five sleeves are placed on one tree. Such a tree constitutes one replicate and for each variety six trees (for clones) or 12 trees (for hybrid varieties) are used. After a feeding period of 24 hours the mirids are removed but the sleeves are left on to avoid other insect damage to the twigs. The physiological reaction of the shoot is observed for three months. The following data are recorded (see Recording Sheet 1):

- feeding punctures after 24 hours (number)
- the physiological reaction of the twigs once every week during the first month and every four weeks during three months thereafter. A 5-point scale is to be used, with 0 = dead and 4 = completely healthy twigs.

Antibiosis test

Branches are covered with sleeves as described above. One second instar mirid is placed within each sleeve and observations are made on its development and survival as well as on the reaction of the branches. To avoid the negative effect of necrosis of the twig on the development of the insect, the mirids should be transferred to new twigs every time the twigs start withering. The study is composed of three to six series of five branches taken at random from different trees of one clone or hybrid variety. The following data are recorded (see Recording Sheet 2):

- development stage and survival
- physiological reaction of the twigs (as above).

The observations are made every two days for antibiosis. For the reaction of the branches, observations will be made every week for the first month, then once a month for the next three months.

Authors of draft procedure

J.M. Mpé, IRAD, Cameroon and K.F. N'Guessan, CNRA, Côte d'Ivoire

Reference

Nguyen-Ban J. 1993. Nouvelle technique de sélection du cacaoyer tolerant aux attaques de ravageurs. Pp. 229-235 *in* Proc. 11th Int. Cocoa Res. Conf., Yammoussukro, July 1993.

Recording Sheet 1. Field study of the tolerance of clones to mirids

Observer: _____

Clone	Tree and branch number	Number of injuries	Branch status (0-4)* Observation dates						
Etc.,									

* 0 = dead, 4 = green, growing normally

Recording Sheet 2. Antibiosis / tolerance test for mirids in the field

Observer: _____

Clone or hybrid	Tree and branch number	Development stage and survival of mirids* Dates					Branch status(0-4)** Dates						
Etc.,													

* Indicate L1 to L5 (first to fifth instar), A (adults) or D (dead)

** 0 = dead, 4 = green, growing normally

28. A method for assessing mirid damage in the field

Scope

The objective is to identify the level of mirid attack and damage caused by this insect on different cocoa genotypes in the field. The method has been used by several researchers in Côte d'Ivoire (Decazy and Coulibaly 1981; Sounigo *et al.* 1993; IDEFOR 1994; Brun *et al.* 1997). It consists in a visual rating of the trees based on:

- presence of cankers on the trunk and on the main branches
- dieback of twigs, and
- damage to pods.

Accumulated old damage on the trunk and branches

The rating takes into account the cankers that have accumulated on the trunk and branches over several years. The rating scale goes from 0 (no damage) to 4 (severe damage), with 0 = no damage, 1 = 1/4 of the trunk and branch surface showing cankers, 2 = 2/4 of the trunk and branch surface showing cankers, 3 = 3/4 of the trunk and branch surface showing cankers and 4 = almost the entire trunk and the branches are covered with cankers.

New damage on twigs

For the new damage resulting in dieback of twigs and drying up of leaves, a 5-point scale is also to be used. The scale points indicate the following degrees of damage: 0 = no dieback, 1 = 1/4 of the canopy shows dieback, 2 = 2/4 of the canopy shows dieback, 3 = 3/4 of the canopy shows dieback and 4 = almost the entire canopy shows dieback.

Damage to pods

For the fruits, a 5-point scale is also proposed. The scale points are: 0 = no damage on pods and cherelles, 1 = 25% of the fruits show mirid attack, 2 = 50% of the fruits show mirid attack, 3 = 75% of the fruits are attacked, 4 = almost all the fruits show mirid attack.

The above scoring (see Recording Sheet 1) should be undertaken by four well-trained technicians. The average of the records of these four technicians will be used for further calculations. All trees of a trial are observed. It is recommended to record one score at the start of the swarming period and another one immediately after the swarming period. The rating scores are analyzed statistically as quantitative data. This method is fast and the most susceptible genotypes can quickly be eliminated.

Points arising from subsequent discussions

Visual appreciation of damage varies from one person to another and from one country to another. So only the general classification at the end of the evaluation can be relied on. It means that one genotype may be identified as resistant in several countries, but the exact score will not be the same.

Authors of draft procedure

K.F. N'Guessan, N. Coulibaly and J.A.K. N'Goran, CNRA, Côte d'Ivoire and J. M. Mpé, IRAD, Cameroon

References

Brun L.A., Sounigo O., Coulibaly N. and Cilas C. 1997. Methods of analysis for studying cocoa (*Theobroma cacao* L.) susceptibility to mirids. Euphytica 94: 349-359.

Decazy B. and Coulibaly N. 1981. Comportement de cultivars de cacaoyers à l'égard de quelques insectes déprédateurs: possibilité d'une sélection précoce des cacaoyers tolérants. Pp. 685-688 *in* Proc 8th Int. Cocoa Res. Conf. Cocoa Producers Alliance, Lagos, Nigeria.

IDEFOR-DCC. 1994. Rapport bi annuel 1993-1994. Côte d'Ivoire. 48pp.

Sounigo O., N'Goran J., Coulibaly N., Clement D. and P. Lachenaud. 1993. Evaluation de clones de cacaoyers pour la productivité, la résistance aux mirides et la résistance à la pourriture brune des cabosses. Pp. 375-381 *in* Proc 11th Int. Cocoa Res. Conf., Cocoa Producers Alliance, Lagos, Nigeria.

Recording Sheet 1. Field assessment of damage due to mirids

Trial: _____ Observer: _____ Date: _____

Line no.	Tree no.	Hybrids/ clones	Scores (0-4)		
			Old cankers on branches and trunk	New damage on twigs and leaves	Damage on pods
Etc.,					

29. Evaluation of insect attacks on young cocoa trees in the field

Scope

Replanting in cocoa zones is often seen to fail in the absence of effective phytosanitary protection due to the combined effect of climate (drought) and pest attacks. For project trials, such protection is required in order to guarantee healthy development of young cocoa plants. At the same time, it is important to assess the susceptibility of cocoa cultivars to insect attacks especially the entomofauna that lives on the terminal buds of cocoa trees. Attacks can be expected even in the presence of control measures. This study is therefore applicable in all the project countries where insect attacks on young cocoa plants are frequent. Observations should be made right from planting until the canopy formation is complete (about two years after planting).

Insects living on terminal buds

The description of this procedure is based on the experience in African countries. The two insect species responsible for early abortion of terminal buds on young cocoa trees in Africa are the psyllid *Mesohomotoma tessmanni* (Aulm) and the cocoa bollworm *Earias biplaga* (WLK). In Cocoa Swollen Shoot virus zones, mealybugs are vectors of the virus disease leading to rapid tree death.

When the terminal bud of the orthotropic (seedlings) or plagiotropic stems (clones) is destroyed by the action of insects, growth of the young tree slows down, affecting increase in stem diameter.

With severe attacks, the axillary buds may also suffer, resulting in malformed plants, of a "bayonet" shape. Field observations have shown that a more susceptible hybrid progeny forms 50 % and 90 % of the canopies at 14 and 22 months after planting, respectively. Peaks in bollworm outbreaks corresponded to a delay in canopy formation (Nguyen-Ban 1977). For a more tolerant hybrid progeny, canopy formation was continuous throughout the year, resulting in 50 % and 90 % of canopies at ten and 20 months after planting, respectively. The average numbers of bollworms counted on the trees varied less for the more tolerant hybrid than for the susceptible one. The working procedure proposed here has been based on these studies.

Observation methods

A minimum of 20 plants for clones and 40 for hybrid progeny are needed and the observation frequency should ideally be every month, and at least every three months. The following observations must be made on field-planted hybrid progenies or clones (see Recording Sheet 1):

- Number of terminal meristems attacked and aborted, before canopy formation.
- Number of neoformed axillary shoots (vegetative compensation).
- Time taken for canopy to form.
- Number of plants to be observed: all plants of the trial.
- Observation frequency: if possible monthly, at least every three months.

Other pests

The recommendation is made to observe the incidence of any other pests that may occur on a 0 to 5-point scale (0 = absence and 5 = abundance of the insect).

For example, young cocoa trees can suffer from defoliation attributable to noctuid caterpillars (*Anomis* sp., *Lophocrama* sp., *Ascotis* sp., etc.) or Curculionidae beetles (*Chelyophes* sp., *Scolochirus* sp.). These leaf-eaters usually require little chemical intervention unless outbreaks are intense. On the other hand, given their egg-laying behaviour, female longhorn beetles can cause worrying damage in young plant. Such is the case with

Tragocephala beetles in Côte d'Ivoire and with *Phosphorus* sp. which is rife in the Ntem Department of Cameroon. These insects are controlled by collecting adults and mining larvae by hand and spraying the treetops with insecticide.

In Latin America and Asia, the same types of observation are required on the pests most harmful to young cocoa trees, especially *Selenothrips* sp.

Author of draft procedure

J. Nguyen-Ban, CIRAD, France

References

Nguyen-Ban J. 1977. Contribution à l'étude biologique et écologique d' *Aerias biplaga* (WLK) Lepidoptera -noctuidae- ravageur du cacaoyer. PhD thesis, University Paul Sabatier de Toulouse, France. 178pp.

Recording sheet 1. Evaluation of insect attack in young cocoa trials (H = health, A = attacked alive, AD = attacked dead)

Trial/Rep: _____ Recorder: _____

Observation date: _____

Clone	Repli-cate	Plant no.	Pest incidence (0-5)				Terminal shoot			No. of axillary shoots			Canopy** (0, 1)
			Insect code*				H	A	AD	H	A	Total	
Etc.													

* e.g.: a= aphid, m = mealy bugs, p = psyllid, c = caterpillar, etc.

** Canopy: 0 = no canopy; 1= canopy formed

30. Evaluation of field resistance to cocoa thrips (*Selenothrips rubrocintus*)

Scope

Thrips, *Selenothrips rubrocintus* (Giard), is an important pest on cocoa. The objective of this study in the International Clone Trial of the CFC project is to evaluate varietal resistance of cocoa to this pest and to compare it with resistance to other insects. The parameter of observation is the average incidence or degree of attack.

Method

Observations are made yearly when natural attacks occurs in the field. The degree of attack of each plant is visually estimated on a three-point scale and the number of insects on attacked leaves is counted (see Recording Sheet 1).

Field observations

For each field plant, the degree of attack is observed on all trees by means of the following assessment scale:

* 0 = completely healthy, no visible attacks;
* 1 = average attack, signs of chlorosis on some leaves but no defoliation;
* 2 = severely attacked, defoliation is observed.

Counts of insects on detached field leaves

For the International Clone Trial, two leaves with most severe visible attack are taken from each plant which is 16 leaves per plot (8 trees per plot). The total number of leaves sampled per clone is 6 x 16 = 96. The numbers of thrips (adults and larvae) are counted after washing the leaves in alcohol diluted to 10%, and filtering the solution through fine-mesh gauze for one night.

Author of draft procedure

J. Nguyen-Ban, CIRAD, France

Recording Sheet 1. Observation on field attack of thrips

Site: _____ Plot: _____ Planting date: _____
Recorder: _____ Observation date: _____

Clone	Tree number (row/ line)	Degree of attack (*)			No. of leaves sampled	No. of thrips counted (adults and larvae)	Parasites (presence=1, absence=0)	Observations
		0	1	2				
Etc.,								
Total								

* 0 = healthy tree, no visible attack ; 1 = signs of chlorosis on leaves, no defoliation ; 2 = tree severely attacked, defoliation.

31. Recommendations for chemical control of insects

Scope

The objective is to indicate chemical control measures that can be applied to fight effectively insects which are harmful to young cocoa plants (nursery and in the field) and adult trees in the field trials in the CFC Project.

Control of insects on young cocoa plants

The fight against most insects which are harmful to young cocoa trees in the nursery and in a young cocoa plantation (defoliating caterpillars, psyllids, thrips, hoppers, chrysomelids, curculionides, locusts etc.) is ensured by treating systematically, every 45 days with a sprayer. The following products are recommended:

- Acephate (Orthene 50 PM)
- Deltaméthrine (Decis)

In order to protect nurseries from small mammals, these must be surrounded by fine fencing or netting (0.5 to 1 m high).

Mirid control for adult cocoa trees

Timing of treatment

The fight against mirids involves systematic treatment and depends on the seasonal variation in the populations of these insects. Outbreaks of mirids vary with time and according to the regions and even from one country to another.

Generally speaking, mirids appear in June-July in cocoa fields in African countries. The population then increases to reach a peak in outbreak in August-September (Côte d'Ivoire) or October-November (Cameroon). In some countries (Côte d'Ivoire) re-infestation may occur at the beginning of the dry season (December).

With this kind of population development, chemical treatment comes into play as early on as June-July (Cameroon) or July-August (Côte d'Ivoire) with two sprayings at a one-month interval. A second treatment is necessary in December-January in Côte d'Ivoire.

This treatment is effective on larvae and adults, but does not kill the eggs and does not therefore prevent subsequent hatching of these eggs. The second spraying in each period of treatment enables quite a thorough check to be made. The products must be alternated every two years to limit any problems of resistance to insecticides.

Recommended products depending on the local situation might include:

- Organochlorine: endosulfan (Thiodan 50 EC), 200 to 300 g of active ingredient (ai) per ha;
- Organophosphorous: diazinon (Basudine 600 EC), 350 to 450 g ai per ha;
- Carbamates: fenobucarb ou BPMC (Bassa 500 EC), 500 g ai per ha, and propoxur (Unden 200 EC ou Unden 75 PM), 200 to 300 g ai per ha;
- Pyrethroids + organophosphorous: bifenthrine + diazinon (Diastar 420 EC), 210 g ai per ha.

Treatment devices or means of spreading the products

Treatment devices vary from one country to another:

- atomisers (Echo or Solo) are used in en Côte d'Ivoire, Ghana, Togo, Cameroon and Sao Tomé;
- thermonebulisers are used in Togo;
- smoke-producing pots (Fumivap) are used in Côte d'Ivoire.

Authors of draft proposal

K.F. N'Guessan, CNRA, Côte d'Ivoire; J. Nguyen-Ban, CIRAD, France and J. M. Mpé, IRAD, Cameroon

Application of the Working Procedures in the CFC/ICCO/IPGRI Project

The type of application of the Working Procedures in the CFC/ICCO/IPGRI project was decided in Working Groups during the Workshop and presented in the Plenary Sessions. Tables 1 and 2 indicate the application of the Working Procedures in the different Project Components and Actions in three possible ways:

- *"Standard (S)"*: this indicates that the working procedure is to be applied in a standardized way in the Project Component/Action involved.
- *"Recommended (R)"*: this indicates that the Working Group recommends using the proposed method. This is generally applicable for Project Components (Actions) that are only undertaken locally.
- *"Not applicable (-)"*: this indicates that the use of the method is not applicable to the project activities.

The standardized way of experimental procedure and reporting (S) is a basic requirement to be able to carry out comparative analyses of results (yield, disease resistance, etc.) for Components 1 and 2 (International Clone and Hybrid Trials of the Project). For other Project Components, most Working Procedures are 'Recommended' rather than 'Standard'.

These Working Procedures were agreed at the first Workshop and are intended for application during the life of the project. However, suggestions for improvements or refinements may be made by project scientists based on new research findings. Accordingly, some of these Working Procedures may need revision and such could be decided by the Technical Working Group at any time during project implementation.

Table 1. Application of working procedures in the CFC/ICCO/IPGRI project: selection trials and data collection (S = standard method; R = recommended method, - = not applicable)

WORKING PROCEDURE (Number)	PROJECT COMPONENTS/ACTIONS								
	1. International Clone Trial			2. International Hybrid Trials	3. Germplasm Enhancement	4. Population Breeding	5. Characterization and Evaluation		6. Information Exchange
	International clones	Local clones	Observation plots				CFC Project Collection	Local collections	
Choice of Clones (1)	S	R	R	-	-	-	-	-	-
Choice of Rootstock (1)	R	R	R	-	-	-	-	-	-
Budding Techniques (2)	R	R	R	-	-	-	R	-	-
Nursery Maintenance (3)	R	R	R	R	R	R	R	-	-
Pruning of Young Plants (4)	R	R	R	R	R	R	R	R	-
Pruning of Adult Plants (5)	S	S	R	R	R	R	R	R	-
Field Maintenance (5)	R	R	R	R	R	R	-	R	-
Vigour/Yield Observations (6)	S	S	R	S	R	-	-	-	-
Pod and Bean Traits (6)	S	S	R	S	R	-	-	-	S
Layout of Field Design (7)	S	S	R	S	-	-	-	-	-
Data Analysis (8)	S	S	R	S	-	-	R	-	-
Physiological Traits (9)	S	R	R	R	R	R	R	-	-
Individual Tree Selection (10)	-	R	R	R	R	R	R	R	-
Pollination Techniques (11)	-	-	-	R	R	R	-	-	-
Cocoa Quality (12)	S	R	-	-	-	-	-	-	-
Documentation (13)	S	R	-	R	-	-	S	R	S

Table 2. Application of working procedures in the CFC/ICCO/IPGRI project: evaluation of disease and pest resistance (S = standard method; R = recommended method, - = not applicable)

WORKING PROCEDURE (Number)	PROJECT COMPONENTS/ACTIONS								
	1. International Clone Trial				2. International Hybrid Trials	3. Germplasm Enhancement	4. Population Breeding	5. Characterization and Evaluation	
	International clones	Local clones	Observation plots	Ring test				CFC Project Collection	Local collections
Phytophthora Resistance:									
Leaf Disc Inoculations (14)	S	S	S	S	S	R	R	R	R
Detached Pod Inoculations (15)	R	R	R	-	R	S	R	R	R
Attached Pod Inoculations (16)	S	S	R	-	S	-	-	R	-
Field Evaluation (17)	-	-	-	-	-	-	-	-	-
Witches' Broom Resistance:									
Belt Sprayer (19)	S	S	R	-	S	R	R	R	R
Natural Infection Nursery (20)	R	R	R	-	R	R	R	-	-
Natural Infection Field (20)	S	S	R	-	S	R	R	R	R
Bud Inoculation (21)	-	-	-	R	-	-	-	-	-
VSD Resistance:									
Dual Culture Test (23)	R	R	-	-	-	-	-	-	-
Natural Infection Nursery (24)	R	R	R	-	R	-	R	-	-
Natural Infection Field (24)	S	S	R	-	S	-	R	-	R
Insect Resistance and Control:									
Mirid Laboratory Test (25)	S	S	-	-	R	-	-	-	R
Mirid Nursery Test (26)	R	R	-	-	R	-	-	-	-
Mirid Field Test (27)	R	R	-	-	R	-	-	-	-
Mirid Field Damage (28)	S	S	S	-	S	-	R	-	-
Insects on Young Cocoa (29)	S	S	S	-	R	-	-	-	-
Thrips Field Evaluation (30)	R	R	R	-	R	-	-	-	-
Insect Control in the Field(31)	R	R	R	-	R	-	R	-	-

Appendix I

Terms of Reference for the Technical Working Group (TWG)

The TWG acts within the framework of the project to provide a linkage between the Project Partners and the Project Implementing Agency (PEA) for discussion on technical matters of mutual concern.

Membership

Members will be the Technical Co-ordinators of all participating institutions, who will elect a chairperson. A representative of IPGRI and the Project Co-ordinator will be *ex-officio* members of TWG.

Terms of Reference

Important roles of the TWG are to:

- agree on standard procedures,
- recommend additional related research to the project financing institutions,
- be central to the Mid-Term Project review,
- be central to the final reporting process,
- act as a focal point for dissemination of information and to supply feedback to the PEA on technical matters, and
- share opinion on the general progress of the project.

Modus Operandi

The Technical Project Co-ordinators for each participating institution will represent all technical interests of that institute. They will transmit opinions of scientists in each discipline to the Project Co-ordinator and provide feedback to scientists concerning matters of interest common to all countries, including distribution of all project documents. The approval of local annual work plans and budgets will be done at institute level. This will be used by the Project Co-ordinator to elaborate the General Annual Work Plan and Budget for the Project. The TWG will be consulted by the Project Co-ordinator to approve any common technical aspects of project implementation, including any modification of standard working procedures. Members of the TWG will maintain contact with each other mainly through e-mail. The elected chairperson will be the focal point for exchange of information among TWG members and with the Project Co-ordinator. Advantage will be taken of workshops and cocoa conferences to organize TWG meetings. The participants of the First Project Workshop strongly recommended that mid-term technical meetings be organized on a regional basis to discuss progress among project scientists.

Appendix II

Terms of Reference for the Co-financiers Working Group (CWG)

The CWG acts within the framework of the project to provide a linkage between the co-financiers, the project partners and the Project Implementing Agency (PEA) for discussion on matters of mutual concern.

Membership

Members will be representatives of the co-financing bodies, (ACRI, BCCCA, CIRAD and IPGRI), the Chairperson of the Technical Working Group and the Project Co-ordinator will be *ex-officio* members of CWG.

Terms of reference

The CWG will:

- provide advice to the PEA on relevant matters related to the project implementation;
- ensure that contributions of co-financiers are supportive in meeting the project objectives and outputs and activities, and advise on possible remedial action in the event that this should become necessary;
- comment on drafts of general progress reports and project publications;
- assist the PEA in the identification of complementary research activities and of possible funding opportunities to enhance the achievements of the project objectives, in particular as regards continuation of the work of the project beyond its initial term.

Note: In no way does the above affect the requirements for reporting by the individual co-financiers on co-financed activities to the Project Co-ordinating Unit and the required provision of budget specifications at intervals indicated in the Project Agreement. Co-financed activities are spelt out at country level in the recipient institute's work plans and budgets. Specific matters concerning one co-financing institution alone will be dealt with directly between this institution, the PEA and the recipient institution.

Chairperson

Members will elect a chairperson who will ensure the implementation of the TOR of the CWG by maintaining regular contacts with members. All relevant project correspondence related to co-financiers' activities will be sent by the Project Co-ordinator to the Chairperson with copies to all members of the CWG.

Modus operandi

The CWG will make opportune use of conferences and other events to meet. Otherwise, the PEA may organize "virtual" electronic meetings, either by conference calls or e-mail. The Project Co-ordinator will act as the secretary to the CWG.

Appendix III

Workshop Programme

Monday 2 February

Opening Session: *Convener: Engels, IPGRI; Chairperson: Nucé de la Mothe, AGROPOLIS*

8.30 Welcome addresses (Representatives of ACRI , AGROPOLIS, BCCCA, CFC, CIRAD, ICCO and IPGRI)

Plenary Session 1: *Chairperson: Frison, IPGRI; Rapporteur: N'Goran, CNRA*

10.00 Introductory note by CFC (Clayton, CFC)

10.30 Project Implementation and Governance (Engels, IPGRI)

11.30 Role of ICCO as Supervisory Body of the project (Anga, ICCO)

Plenary Session 2: *Chairperson: Owusu, CRIG; Rapporteur: Butler, CRU*

13.15 Role and constitution of the 'Project Technical Working Group' (N'Goran, CNRA; Engels, IPGRI)

14.00 Role of the 'Project Co-financiers Working Group' (Lass, BCCCA)

14.30 Workshop Objectives and Oganization (Eskes, IPGRI/CIRAD)

15.45 Strategy to be applied to the germplasm enhancement (pre-breeding) activity carried out in Trinidad (Iwaro, Sreenivasan, Butler, Bekele and Umaharan, CRU; Thevénin and Sounigo, CIRAD; Mooleedhar, MALMR)

17.30-18.30 Welcome cocktail offered by CIRAD-CP

20.30-21.30 Discussion on plenary sessions and method of approval of working procedures

Tuesday, Wednesday and Thursday 3-5 February

Group Sessions on Specific Working Procedures

These occurred simultaneously for three technical areas: A. Breeding and Agronomy, B. Pathology, and C. Entomology. After presentation and discussions, agreement of the individual Working Groups was obtained on each procedure. During plenary evening sessions (17.30 to 18.15) reports were given by the Working Group and agreement was obtained on the procedures.

Group A: Breeding and Agronomy

Tuesday 3 February

Group A, Session 1: *Chairperson: Monteiro, CEPLAC; Rapporteur: Efron, CCRI*

8.15 Choice of clones and rootstocks for the International Clone Trials (Eskes, IPGRI/CIRAD)

9.15 Budding of clones, nursery maintenance and young plant pruning techniques (Lamin and Lee, MCB)

10.45 Field preparation for planting of cocoa, including temporary shade, fertilisation and maintenance of young and adult cocoa (Adu-Ampomah, Osei-Bonsu and Afrifa, CRIG)

Group A, Session 2: *Chairperson: Adu-Ampomah, CRIG; Rapporteur: Lamin, MCB*

13.15 Vigour and yield observations (Efron, CCRI)

14.30 Simple physiological traits to be observed in the International Clone Trial (Hadley, Reading University)

16.00 Observations on bean and pod traits (N'Goran, CNRA; Lachenaud, CIRAD)

Wednesday 4 February

Group A, Session 3: *Chairperson: Badaru, CRIN; Rapporteur: Gonzalez, CONICIT*

8.15 Layout of field and laboratory experiments: international clone and hybrid trials, resistance tests (Cilas, CIRAD)

10.30 Collecting and analysis of data (Cilas, CIRAD)

11.30 Evaluation of quality traits in the International Clone Trial (Cros, CIRAD)

Group A, Session 4: *Chairperson: N'Goran, CNRA; Rapporteur: Sounigo, CIRAD*

13.15 Use of molecular markers for identification of 'international' clones (Wilkinson, University of Reading)

14.30 Description and photographs of clones used in the International Clone Trial and in the core collection (Ford and End, LIFFE/BCCCA/ University of Reading)

16.00 Criteria for phenotypic individual plant selection in the project (Eskes, IPGRI/CIRAD; Paulin, CIRAD)

Thursday 5 February

Group A, Session 5: *Chairperson: Amores, INIAP; Rapporteur: Mooledhar, MALMR*

8.15 Common aspects of population breeding activities in the project (Eskes, IPGRI/CIRAD; Sounigo, CIRAD)

10.30 Manual pollination techniques and evaluation of self-incompatibility (Lachenaud, CIRAD)

GROUP B: Pathology

Tuesday 3 February

Group B, Session 1: *Chairperson: Opuku, CRIG; Rapporteur: Blaha, CIRAD*

8.15 Early screening for *Phytophthora* pod rot resistance: leaf disc test (Nyassé, IRAD; Blaha, CIRAD; Kebe and Tahi, CNRA)

10.30 Rapid screening for *Phytophthora* pod rot resistance: detached pod inoculation test (Iwaro, Sreenivasan, Butler and Umaharan, CRU)

Group B, Session 2: *Chairperson: Berry, CIRAD; Rapporteur: Bong, MCB*

13.15 Evaluation of *Phytophthora* pod rot resistance: attached pod inoculation test (Luz and Silva, CEPLAC; Blaha, CIRAD)

16.00 Field evaluation of *Phytophthora* pod rot incidence in project trials (Blaha, CIRAD; Eskes, IPGRI/CIRAD)

Wednesday 4 February

Group B, Session 3: *Chairperson: Suarez, INIAP; Rapporteur: Nyassé, IRAD*

8.15 Evaluation of resistance to VSD in the nursery and field (Bong, MCB)

10.15 Evaluation of resistance to WB by means of detached leaf and apex inoculation tests (Ducamp and Thevénin, CIRAD)

Wednesday 4 February

Group B, Session 4: *Chairperson: Shaw, University of Reading; Rapporteur: Iwaro, CRU*

13.15 Early screening for WB resistance: belt spray inoculation of seedlings and clones (Luz and Silva, CEPLAC; Suarez, INIAP)

16.00 Evaluation of WB incidence in project trials in the nursery and field (Suarez, INIAP)

Thursday 5 February

Group B, Session 5: *Chairperson: Owusu, CRIG; Rapporteur: Luz, CEPLAC*

8.15 Organization of and procedures to be applied in the 'Ring Test' for black pod resistance (Ducamp, CIRAD)

10.30 Organization and procedures to be applied in the 'Ring Test' for witches' broom resistance (Shaw, University of Reading)

GROUP C: Entomology

Tuesday 3 February

Group C, Session 1: *Chairperson: Lass, BCCCA; Rapporteur: Nguyen-Ban, CIRAD*

13.15 Mirids: observation on field damage (N'Guessan, Coulibaly and N'Goran, CNRA; Mpé, IRAD)

16.00 Mirids: laboratory test on preference (Nguyen-Ban, CIRAD)

Wednesday 4 February

Group C, Session 2: *Chairperson: N'Guessan, CNRA; Rapporteur: Mpé, IRAD*

8.15 Mirids: nursery/field tests for resistance, tolerance and antibiosis (Mpé, IRAD; N'Guessan, CNRA)

10.30 Insect attack on young cocoa trees in the field (Nguyen-Ban, CIRAD)

Group C, Session 3: *Chairperson: Lass, BCCCA; Rapporteur: Nguyen-Ban, CIRAD*

13.15 Recommendations for chemical control of insects (N'Guessan, CNRA; Nguyen-Ban, CIRAD; Mpé, IRAD)

14.00 Field study of cocoa tree resistance to *Selenothrips rubrocintus* (Nguyen-Ban, CIRAD)

Thursday 5 February

Group C, Session 4:

8.15-15.00 Finalisation of entomology working procedures

Thursday 5 February

13.15-15.30 Visit to CIRAD by workshop participants

Plenary Session 3: *Chairperson: Lass, BCCCA; Rapporteur: Eskes, IPGRI/CIRAD*

16.00-18.30 General Reporting of Working Group results to Plenary Session and official adoption of Procedures

Friday 6 February

Plenary Session 4: *Chairperson: Lass, BCCCA; Rapporteur: Badaru, CRIN*

8.15 Conservation and characterisation activities in the national and international germplasm collections: Proposed Short list of Cocoa Descriptors (Bekele and Butler, CRU)

9.00 The role of the International Cocoa Germplasm Database (End and Ford, Hadley and Wadsworth; LIFFE/BCCCA/University of Reading)

10.00 Strategy for the constitution of a 'CFC Project Collection' (Sounigo and Thevénin, CIRAD; Mooledhar, MALMR; Iwaro, Bekele, Sreenivasan, Khan and Butler, CRU)

11.30 Opportunities and forms of exchange of germplasm of common interest in the project (Engels, IPGRI)

Plenary Session 5: *Chairperson: Despreaux, CIRAD; Rapporteur: Anga, ICCO*

13.15 Administrative and procedures of project implementation (Engels, IPGRI)

14.30 Financial procedures of project implementation (Thornton, INIBAP/IPGRI)

16.00 Closing session (Representatives of the Technical Working Group, Co-financiers Working Group, IPGRI and ICCO)

16.30 Distribution of budwood for the International Clone Trials (Paulin, CIRAD; Parker, University of Reading; Eskes, IPGRI/CIRAD)

Appendix IV

List of Participants and Working Addresses

Wilson Monteiro, Breeder
Edna Dora Luz, Pathologist
Stela Silva, Pathologist
CEPEC/CEPLAC
Centro de Pesquisa de Cacau
Comissao Executiva de Recuperaçao
da Lavoura Cacaueira
CP7, 45600 Itabuna, BA
Brazil

Jean Bosco Zangue-Cheuka, Breeder
Salomon Nyassé, Pathologist
Jean-Michel Mpé, Entomologist
IRAD
Institut de Recherches Agronomiques
pour le Développement
BP 2067, Yaoundé, Nkolbisson
Cameroon

Jeanne N'Goran, Breeder
Ismaal Kebe, Pathologist
François N' Guessan, Entomologist
Philippe Lachenaud, Breeder (CIRAD)
CNRA
Centre National de Recherche
Agronomique
B.P 1827, Abidjan 01
Côte d'Ivoire

Freddy Amores, Agronomist
Carmen Suarez, Pathologist
INIAP
Instituto Nacional de Investigaciones
Agropecuarias, EET – Pichilingue
Casilla Postale 24, Quevedo
Ecuador

Emile Frison, Director
Tom Thornton, Finance Officer
Bertus Eskes, Project Coordinator
Bernadette Sellers, Programme Assistant
INIBAP
International Network for the
Improvement of Banana and Plantain
Parc Scientifique Agropolis II
34397 Montpellier Cedex 5
France

Didier Paulin, Breeder
Jean Nguyen-Ban, Entomologist
Michel Ducamp, Pathologist
Christian Cilas, Statistician
Dominique Berry, Pathologist
Michel Barel, Head Cocoa Programme
Patrice de Vernou, Director
Juan Carlos Motamayor, FUNDACITE
Geneticist
CIRAD-CP
Centre de coopération internationale en
recherches agronomiques pour le
développement – Département des
cultures pérennes
Av. Agropolis
34398 Montpellier Cedex 5
France

Georges Owusu, Director
Yaw Adu-Ampomah, Breeder
Isaac Yaw Opuku, Pathologist
CRIG
Cocoa Research Institute of Ghana
New Tafo, c/o Private Mail Bag
International Airport
Accra
Ghana

Jan Engels, Director Genetic Resources Science and Technology Group
IPGRI
International Plant Genetic
Resources Institute
Via delle Sette Chiese 142
00145 Rome
Italy

Kelvin Lamin, Breeder
Chui Lian Bong, Pathologist
MCB
Malaysian Cocoa Board
Wisma Muis
Jalan Tengku Abdul/Rahman
Beg Berkunci 211
88999 Kota Kinabalu, Sabah
Malaysia

Mark Clayton, Project Officer
CFC
Common Fund for Commodities
Postbus 74656
1070 Amsterdam
Netherlands

Kolawole Badaru, Breeder
O. A. Olunloyo, Pathologist
CRIN
Cocoa Research Institute of Nigeria
Idi- Ayunre, PMB 5244
Ibadan
Nigeria

Yoel Efron, Breeder
Georges Blaha, Pathologist (CIRAD)
CCRI
Cocoa and Coconut Research Institute
P.O Box 1846
Rabaul
Papua New Guinea

David Butler, Head
David Iwaro, Pathologist
Olivier Sounigo, Geneticist (CIRAD)
CRU
Cocoa Research Unit
University of the West Indies
St. Augustine
Trinidad & Tobago

Vish Mooleedhar, Geneticist
MALMR
Ministry of Agriculture,
Land and Marine Resources
Research Division
Central Experimental Station
Centeno, P.O. Arima
Trinidad & Tobago

Tony Lass, Cocoa Specialist
BCCCA
Cocoa Dept., F3
Cadbury International Ltd
Franklin House, Bourneville
Birmingham B30 2NB
United Kingdom

Jean-Marc Anga, Project Officer
ICCO
International Cocoa Organization
22 Berners Street
London WIP 3DB
United Kingdom

Paul Hadley, Physiologist
Mike Wilkinson, Molecular Biologist
Michelle End, Physiologist
Michael Shaw, Pathologist
Caroline Ford, Biologist
Yvonne Charters, Molecular Biologist
Ann Parker, Pathologist
Alice Davies, Technical Assistant
The University of Reading
Plant Science Laboratories
Whiteknights
P.O. Box 221
Reading RG6 2AS
United Kingdom

Carol Knight, Vice President Scientific Affairs
ACRI
American Cocoa Research Institute
7900 Westpark Drive
Suite A320 McLean
Virginia 22102-4203
USA

Ventura Gonzalez, Geneticist
FUNDACITE/ARAGUA
Av. Las Delicias
Maracay
Venezuela

Argelio Moreno, Breeder
Dercy Parra, Pathologist
FONAIAP
Fondo Nacional de
Investigaciones Agropecuarias
Miranda Research Station
Caucagua, Miranda
Venezuela

Appendix V

List of Abbreviations and Acronyms

ACRI	American Cocoa Research Institute, USA
AEC	African Economic Community
BCCCA	Biscuit, Cake, Chocolate and Confectionery Alliance, UK
BP	Black pod
Burotrop	Bureau for the Development of Research on Tropical Perennial Oil Crops
CATIE	Centro Agronomico Tropical de Investigación y Enseñanza, Costa Rica
CBD	Convention on Biological Diversity
CCRI	Cocoa and Coconut Research Institute, Papua New Guinea
CEPLAC	Comissão Executiva do Plano da Lavoura Cacaueira, Brazil
CFC	Common Fund for Commodities
CGIAR	Consultative Group on International Agricultural Research
CIRAD-CP	Centre de Coopération Internationale en Recherches Agronomiques pour le Développement/Département des Cultures Pérennes, France
CNRA	Centre Nacional de Recherche Agronomique (formerly known as IDEFOR-DCC), Cote d'Ivoire
CONICIT	Consejo Nacional de Investigaciones Científicas y Technológicas, Venezuela
CRIG	Cocoa Research Institute of Ghana
CRIN	Cocoa Research Institute of Nigeria
CRU	Cocoa Research Unit, Trinidad
CTA	Technical Centre for Agriculture and Rural Co-operation
CWG	Co-financiers Working Group, CFC/ICCO/IPGRI Project
FAO	Food and Agricultural Organization of the United Nations, Italy
FCA	Factorial Correspondence Analysis
FONAIAP	Fondo Nacional de Investigaciones Agropecuarias, Venezuela
ICB	International Commodity Body
ICCO	International Cocoa Organization
ICG,T	International Cocoa Genebank, Trinidad
ICGD	International Cocoa Germplasm Database, Reading University, UK
IDRC	International Development Research Centre
IFAD	International Fund for Agricultural Development
INGENIC	International Group for the Genetic Improvement of Cocoa
INIAP	Instituto Nacional de Investigaciones Agropecuarias, Ecuador
INIBAP	International Network for the Improvement of Banana and Plantain
INTAS	International Association for the promotion of co-operation with scientists from the New Independent States of the former Soviet Union
IPC	Integrated Programme for Commodities
IPGRI	International Plant Genetic Resources Institute (formerly known as IBPGR)
IRAD	Institut de Recherches Agronomiques pour le Développement, Cameroon
LCTAP	London Cocoa Trade Amazon Project
LOA	Letter of Agreement
MALMR	Ministry of Agriculture, Land and Marine Resources, Trinidad
MCB	Malaysian Cocoa Board
MOU	Memoradum of Understanding
OAU	Organization of African Unity
OILB	Organization for Biological Control
PCA	Principal Component Analysis

PEA	Project Executing Agency
PGRFA	Plant genetic resources for food and agriculture
Ppr	*Phytophthora* pod rot
RHS	Royal Horticultural Society
SB	Supervisory Body
TWG	Technical Working Group CFC/ICCO/IPGRI Project
UNCTAD	United Nations Conference on Trade and Development
UNDP	United Nations Development Programme
UNEP	United Nations Environment Programme
VSD	Vascular streak dieback
WB	Witches' broom disease

ISBN 92-9043-434-1