



**IBPGR  
COCOA WORKING GROUP**

**Report of the Second Meeting**

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INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES

REPORT OF A SECOND MEETING OF THE COCOA WORKING GROUP

21-22 October 1983, Arlington, Va., USA

IBPGR Secretariat  
Rome 1984

The International Board for Plant Genetic Resources (IBPGR) is an autonomous international scientific organization under the aegis of the Consultative Group on International Agricultural Research (CGIAR). The IBPGR was established by the CGIAR in 1974 and its Executive Secretariat is provided by the Food and Agricultural Organization of the United Nations. The basic function of the IBPGR is to promote and coordinate an international network of genetic resources centres to further the collection, conservation, documentation, evaluation and use of plant germplasm and thereby contribute to raising the standard of living and welfare of people throughout the world. The Consultative Group mobilizes financial support from its members to meet the budgetary requirements of the Board.

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## INTRODUCTION

1. A second meeting of a Working Group on the Genetic Resources of Cocoa was held in Arlington, Virginia, USA, 21-22 October 1983, to consider an action plan for the Western Hemisphere. The membership included Dr. Q. Jones (IBPGR), Dr. A.J. Kennedy (Trinidad), Dr. R.E. Larson (ACRI), Dr. J. Leon (Costa Rica), Dr. G. Lockwood (UK), Dr. Williams (Nigeria) and Dr. J.T. Williams (IBPGR). Mr. J.B. Allen (Ecuador), Dr. E. (USA) and Dr. L.A. Withers (UK) attended as resource persons; Messrs. R.T. O'Connell and G.A. Trout represented ACRI, the latter also the IOCC. Dr. J. Soria (Brazil) and Dr. H. Toxopeus (IOCC) were unable to attend. The full addresses of the participants are shown in Appendix I and the agenda in Appendix II.
2. Dr. Larson, who chaired the meeting, informed the participants that the meeting had been convened to review the information that had been gathered in the past three years on the activities of individual national programmes, the needs for further collecting of germplasm and its maintenance in collections, to review any field work that had been carried out and to agree on priorities.
3. Dr. J.T. Williams reviewed activities since the first meeting of the Working Group. The IBPGR had organized collecting in Mexico, Nicaragua and Colombia, ACRI in Honduras; The London Cocoa Trade Commission had organized a major project in Ecuador and the IBPGR had recently provided assistance to the CRU, University of the West Indies, Trinidad to document the collection and to test out the agreed descriptor system.
4. Dr. J. Leon had visited the programmes in Rosario-Tzapa (Mexico), Pichilingue (Ecuador), Itabuna (Brazil), CRU (Trinidad), Caucagua (Venezuela) and CATIE (Costa Rica). In addition, detailed discussions were held at an ad hoc meeting in Miami, February 1983 attended by Drs. P. de T. Alvim (CEPEC, Brazil), B.G.D. Bartley (IICA/CEPEC, Brazil), G. Enriquez (CATIE, Costa Rica), A. Grobman (INIAP, Peru), R.E. Larson (ACRI), J. Leon (consultant), L. Lopez (ICA, Colombia), C. Molina (Caucagua, Venezuela), H. Reyes, Caucagua, Venezuela), L.R. Silber (Cocoa Programme, Mexico), P. Soderstrom (USDA), C. Suarez (Pichilingue, Ecuador), J.T. Williams (IBPGR) and L.R. Larson (CRU, Trinidad).

REPORT

Institutional framework

5. The first meeting of the Working Group recognized two universal collections. The present status is:

(i) The Cocoa Research Unit, University of the West Indies, Trinidad-Tobago. It is located at St. Augustine campus with other collecting sites at St. Joseph, Las Hermarnas and Marper Field. There are nine staff dealing with cocoa. The germplasm collection includes 1,250 entries as clones of very different origin, age and condition together with samples of 10 other species of Theobroma. However, a catalogue is lacking and the material has not been systematically evaluated. New activities include the consolidation of all material at a new site to form an International Field Genebank, development of a quarantine station in Barbados, planning of exploration and evaluation, strengthening of research in agronomy, breeding, pathology and outreach, such activities depending on outside funding from the Governments of Trinidad and Tobago and of Jamaica, the EEC-EDF and the CCCA.

(ii) The Cocoa Project, CATIE, Costa Rica is located at the Turrialba campus and La Lola Cocoa Station. There are three staff members. The germplasm collection includes 150 clones together with samples of 20 species of Theobroma and Herrania. A catalogue was issued in 1981; data scored according to a descriptor list are computerized. New activities include consolidation of research, training and outreach at La Lola, additional emphasis on pathology (especially Monilia); such activities depending on regular funds from the Costa Rican Government and outside funding from Federal Republic of Germany (GTZ) and ACR1 (for outreach in Central America).

6. The Working Group reaffirmed the request that the IBPGR designate these field genebanks as recommended by the first meeting of the Working Group. These two institutions are recognized for their long history of work in cocoa genetic resources and in breeding; their research and training activities in fields related to cocoa; their lack of any kind of restriction on the exchange of materials and information; and their geographic position which facilitates the transfer of germplasm material. International support to both collections has been sporadic but nevertheless, they have maintained their activities. Both have active outreach programmes of regional scope, each covering several countries. They merit continued support in the maintenance of collections and allied fields of research and training.

7. Recommendations made by the first meeting of the Working Group on the need for the two collections to duplicate their materials still stand.

8. Updated information was provided on national collections as follows:

(i) Brazil. The national centre for genetic resources (CENARGEN) has named CEPEC as the agency with responsibility for genetic resources with a network of operations centred in the Itabuna station, where the main collection and several research programmes are located. There is a second germplasm collection at Belem for Amazonian cocoa and a poorly equipped quarantine facility at Salvador which serves as an intermediate between Reconcavo, the main producing area which is free of witches' broom disease, and the other cocoa regions of the country. CEPEC and CENARGEN have

organized expeditions to collect Amazonian types, and have established temporary collections in the Amazon region at Manaus, Altamira, Aregue and other places. The information services on cocoa genetic resources are in a state of incipient development. (CEPEC also publishes the scientific journal fully dedicated to cocoa, the "Revista Theobroma") Brazil has its own resources of trained personnel, scientific expertise and logistic facilities, hence it is equipped to carry out germplasm maintenance and breeding programmes and the exploration of numerous spontaneous populations in the Amazon region. It is highly desirable that information on the Brazilian collecting expeditions receive more publicity and that documentation on the collections be made widely available. In addition, a wider interest in the interchange of germplasm with the rest of the world should be promoted.

A limiting factor may be Brazil's strict legislation on plant exploration. Therefore, planning or support of expeditions with outside funding merits careful consideration. On the other hand, CEPEC has always supplied, when properly requested, selected mutations or clones for making hybrids of advanced hybrids.

CEPEC (Centro de Pesquisas do Cacão) is located at Itabuna, Bahia, and has four staff. The central collection at Itabuna contains 450 entries, mostly clones, and is being re-established in a new site. Other collections for Amazonian cocoa are in Belem, and for minor ones in several stations of the Amazonian basin. They include three other species of Theobroma. No catalogue or systematic evaluation of the collection is available. Future developments include: an increase in the exploration of critical areas in the Amazon, planned with CENARGEN; the establishment of a collection somewhere in the Amazon region in view of problems with the Belem station; and the evaluation of the collected materials, especially for disease resistance.

- (ii) Colombia. Germplasm activities are limited to the maintenance of an old collection in Palmira. Recently, short expeditions to the Amazon region and the Pacific slope have been carried out by the IBPGR, but it is too early to report whether these collections have been duplicated in other cocoa centres. Colombia has both official and private extension service for cocoa growers, but there has been a marked decline in germplasm activities in the last 20 years. Land is now available for additional collections at the Palmira station and at the substations of Chigorodo and Santa Marta on the Atlantic coast and at Florencia in the Amazon. However, the future of cocoa germplasm activities in Colombia depends on the development of a strong breeding programme. This will require special funds from the government or the cocoa producers and manufacturers, as well as trained staff.
- (iii) Ecuador. Germplasm activities include the large, mostly unduplicated and uncatalogued collection at Pichilingue, and the new explorations and genebank at Napo in the eastern part of the country. The latter has been partially duplicated elsewhere, and it seems that its future will depend mainly upon the continuation of external support. Pichilingue, on the other hand, will continue to supply planting materials for national needs.

Estacion Experimental Tropical (EET) is a national experiment station in the INIAP (Instituto Nacional de Investigaciones Agropecuarias) network located at Pichilingue, Quevedo with five breeders. The collection includes 400 clones and a few other Theobroma spp. but no catalogue has been published. Prepared future developments include strengthening, with outside help, of research in pathology and breeding and expansion of the collections with Amazonian cocoa.

- (iv) Mexico. The modest programme of cocoa germplasm in Mexico includes a collection at Rosario-Izapa, and duplication of clones mostly of local

origin at El Palmar substation in Veracruz. The importance of Mexico as a source of Criollos has been recognized and recently, with the aid of IBPGR, several expeditions have collected wild material in the Lacandon forests. To date there is no information or distribution of the materials to other cocoa centres.

Campo Experimental Rosario-Izapa, Secretaria de Agricultura y Recursos Hidraulicos de Mexico is located at Tapachula, Chiapas, Mexico with three staff. The collection includes 30 clones of local origin (highly uniform), some 20 other clones introduced from CATIE; and two species of Theobroma. All the materials are duplicated at El Palmar substation near Tezonapa, Veracruz; there is no catalogue. Future developments include further collection of Criollos in the Lacandon forest, with IBPGR support, and the production of new hybrids with a wider genetic base.

- (v) Peru. Although Peru has not had an active cocoa germplasm programme, the area is interesting. Peru is open to cooperation. (A detailed proposal for exploration and for the establishment of a cocoa research development programme is given in Appendix III.)
- (vi) Venezuela. Germplasm maintenance and breeding work is centred at the Caucagua station. The collections are well maintained; a catalogue was published in 1973 and up to date lists are available. Several expeditions for Criollo and other spontaneous populations are planned, with a separate station for the Criollas at Ocumare de la Costa. Venezuela seems to be more open to international cooperation in germplasm related activities than some other cocoa producing countries. The IBPGR could offer help for expeditions in the lower Orinoco, the Catatumbo basin and other pockets of Criollo diversity.

Estacion Experimental de Caucaagua, Venezuela, is an experiment station in the network of the Ministry of Agriculture devoted mainly to cocoa. It is located at Caucaagua, Miranda and carries seven staff. The collection includes 135 clones of local origin including a good number of Criollos, and 30 hybrid populations. Future developments should include further expeditions, especially for Criollo types and material to be established in a new collection site at Ocumare de la Costa, and of other local populations, in Delta Amacuro and other areas.

9. The Working Group sees no reason to change the recommendation that Primary Field Genebanks be designated by the IBPGR (see report of first meeting). However, the status of all national activities needs to be reviewed from time to time. For a summary of information see Appendix V.

This is necessary because:

- a. The collections contain mainly advanced selections (mostly clones) and have a poor representation of primitive landraces, mutants and wild relatives.
- b. The entries represent mostly local diversity. There appears to be a high degree of genetic repetition, although this cannot be proved because the majority of samples have not been subjected to analysis (see para. 27).
- c. The accessions are, in general, well identified in the field, but catalogues are available only for two collections, and some stations have been reticent to submit even simple lists.

A source of confusion is the change in clonal nomenclature begun after 1972. Traditional names were replaced by abbreviations representing countries of origin followed by numbers in consecutive order. Synonyms must be retained.

- d. The majority of clones/selections have not been evaluated.
- e. No concern appears to be demonstrated by the national programmes to duplicate material in other countries.
- f. Comprehensiveness of collections relates to fear of introduction of diseases. Most collections do not have quarantine facilities and those facilities which do exist are very inadequate. This is addressed in para. and also re-emphasizes the need for quarantine transfer collections which also serve as safe haven working collections for breeders. For instance, the USDA Miami/Mayaguez operation (Imle and Shrum, 1958) is of major importance and has not been sufficiently utilized by the national programmes.

#### Recommendations on the Current Programmes

10. A subcommittee was designated to make detailed recommendations on an action plan for 1984-90. This was chaired by Dr. Imle and included Mr. Allen and Drs. Kennedy, Larson, Leon and Lockwood. The following were subsequently agreed by the Working Group.

11. Collecting. Accelerated work on exploration is necessary and in some cases urgent, especially in areas where the survival of germplasm is immediately under threat. These areas include, but are not limited to, those listed below.

#### BELIZE

Southern section (Criollos, possibly autochthonous).

#### BOLIVIA

Departments of Pando and Beni: Upper courses of the Madre de Dios, Beni and Mamore rivers; centre for expeditions and germplasm bank at Riberalta.

#### BRAZIL

CEPEC and CENARGEN have detailed plans, manpower and other facilities for the organization of collecting expeditions. The critical areas, especially in Acre, are being collected in view of the threat of deforestation and the establishment of hydroelectric plants. The following areas are planned for exploration: Eixo da Rodovia, BR-152, Regiao do Baixo Tocantins, Eixo da E.F. Carajas-Itaqui, Regiao da Serra dos Carajas, Alto Xingo, Projeto Jari, Rio Trombetas, Area da Hidroelectrica de Balbina, Rodovia Perimetral Norte, Rio Braco, Alto Rio Negro, Medio Solimoes (Fonte Boa), Alto Japura (Cagneta), Alto Solimoes, Baixo Jurua, Medio Jurua, Regiao de Costa Marques, Area da Hidroelectrica de Samuel, Area de Colonizacao de Rondonia, Area de Colonizacao da Tendeco.

#### COLOMBIA

Amazonian region, basins of the rivers Yari, Caguan, Artegaça-Caqueta, Putumayo. Orinoco, upper region: headwaters of the Arauca, Casanare, Cusiana, Upia.

Pacific coast.

Pockets of diversity: Antioquia; Huila; Atlantic coast (Criollos and mixtures); Cauca; Northern Santander.

#### COSTA RICA

Pacific coast (Criollos, from old plantations) Nicoya. El General. Burica. Northern section: Upala-Guatuso (Criollas and Pentagona).

#### ECUADOR

Amazonian region: The systematic exploration currently underway should be continued.

GUATEMALA

Pacific coast, Retalhuleu-Mazatenango Suchitepequez (Criollo, relics of former plantings) Alta Verapaz: Coban, Criollos and Pentagona in forests.

GUYANA, SURINAM

Forest areas in the southern section, for wild Calabacillo.

HONDURAS

Atlantic coast (Criollos, possibly autochthonous) Rio Aguan, Cuyamel, Olanchito.

MEXICO

Chiapas, Lacandon forest: Sinai, Nueva Zamora, Bonapak (autochthonous Pacific coast, from Tehuantepec populations) to Colima (isolated planting of Criollo).

NICARAGUA

Pacific coast, Azuero (Criollos, from abandoned plantings): Valle Menier, Mandaime, Masatepe.).

PANAMA

Pacific coast: Azuero (Criollas, from old plantations).

VENEZUELA

Orinoco, delta and medium and upper courses.

"Pockets of diversity": Rio Catataumbe; Santa Barbara, Chama; coastal area.

12. Collecting should be supported in other areas when the opportunity arises as it does in Peru at present (see Appendix III).

13. The improvement of collecting methodology requires a better estimate of genetic diversity and novel screening methods should be developed (see paras. 27, 28 below) which will assist in both new collecting and analysis of existing collections. In the meantime the Working Group noted that it would be helpful for a paper to be issued in the FAO/IBPGR Plant Genetic Resources Newsletter summarizing the methodology employed in the INIAP/LCTAP in Ecuador and recommended that Mr. Allen should prepare this as soon as possible.<sup>1/</sup>

14. There is a need for personnel from national programmes to receive training in collecting and the subsequent handling of materials and the IBPGR should support a number of scientists to visit INIAP/LCTAP for short on-the job training.

15. Management of Collections. The Working Group noted that little tangible progress was apparent in transferring material from national collections to the Universal Collections see also paras. 7 and 9c and there is an urgent need to promote the movement of materials between collections.

16. Since basic catalogues are not available, the IBPGR is asked to promote the preparation of inventories of the field genebanks in Brazil, Colombia, Ecuador, Mexico, Trinidad and Venezuela. This task should be combined with:

- (i) validation or revision of identifications. Curators should regard this as one of their primary responsibilities (if necessary, they could request the services of Dr. B.G D. Bartley through the Director General of IICA);

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<sup>1/</sup> This paper has been published in Plant Genetic Resources Newsletter No. 57.

- (ii) use of the IBPGR descriptor list;
- (iii) rationalization of the collections;
- (iv) eventual issuance of comprehensive catalogues for which the IBPGR could provide funds.

17. Wild species. There is an urgent need to increase the representation of the wild species in existing collections. Some should also be included in Botanic Gardens. The Working Group also recognizes the need for an up to date taxonomic monograph for Theobroma and Herrania species and for this to include new experimental taxonomic work. In addition there is a marked lack of ethnobotanical studies in relation to Theobroma (and part climatic changes) to explain distribution patterns. The IBPGR is asked to draw this to the attention of suitable institutions as a field for fruitful study.

18. Quarantine. The Working Group recognizes that the successful exchange of germplasm requires the maintenance of intermediate quarantine facilities. The IHPGR is asked to draw this to the attention of the Governments of the USA, UK and France and to point out the need for expanded and sustained support for the operations in Miami, Kew and Montpellier respectively. In addition the establishment of a regional (not inter-continental) quarantine station in Barbados is noted and endorsed.

19. The Working Group noted with dismay that material being transferred from the LCTAP has only a 5-15% budding success rate in the intermediate quarantine stations. The attention of the Directors of the Institutes concerned is drawn to this.

20. Exchange of information. The Working Group recognizes the need for a cocoa germplasm and breeding newsletter in English and Spanish.

21. Evolution gardens. There is some discussion on whether it would be feasible or not to expand some field genebanks into evolution gardens. On consensus it was felt that not enough is known about the genes which should be represented. However, it was felt that a number of primitive cultivars or wild species could be used as shade and wind breaks in some commercial plantations.

#### In vitro work on Theobroma

22. Dr. Withers tabled a report which represents a synthesis of current activities and opinions (Appendix VI). At a first meeting of the Working Group it was recognized that much more research is required to develop in vitro techniques. Since that time the IBPGR has supported work in Dr. Withers' laboratory and ACRI has supported work at the University of Florida, Homestead. In relation to the development of conservation techniques sensu stricto whatever method is adopted must be compatible with clonal propagation. The on going work is likely to result in enhanced distribution and conservation, rather than clonal enhanced in vitro hybridization between cultivars and wild species.

23. There have been few, if any, developments in pollen storage.

24. Following an initiative of the IBPGR In Vitro Committee, Dr. Withers outlined an in vitro collecting technique for Theobroma that has reached the stage of field testing. Such techniques could provide more secure collecting methods for cocoa and other crops.

#### Recommendations on research and development

25. A sub-committee was designated to make recommendations on specific areas of research that would help the genetic conservation efforts on Theobroma. This was chaired by Dr. J.T. Williams and included Dr. Jones, Dr. Withers, Mr. Trout and Dr. J.A. Williams. The following were subsequently agreed by the Working Group.

26. Disease indexing. There is an urgent need to develop biochemical techniques (e.g. ELISA, nucleic acid hybridization) for rapid indexing of Theobroma diseases. Pilot work is necessary in a competent laboratory in an area away from cocoa

production. Extracts of samples (not whole plants) could be sent from the areas of diversity. The IBPGR is asked to promote this work and to request a cocoa pathologist to advise on materials to be tested. It was noted that disease indexing could speed up transfer through quarantine where, at the moment, screening is a relatively slow process. A note of caution is also appended since some sap tests used for DNA hybridization have not always detected all races of a pathogen.

27. Estimates of diversity. The Working Group stressed that our knowledge of diversity is based on phenotypes. The IBPGR is urged to support the development of isozyme techniques for Theobroma. This has already started at the University of Nottingham with funding from the CCGRA. It could be accelerated and fully developed within two years for an additional cost of US \$30,000 per year.

28. If isozyme techniques are developed, collecting should be conducted as follows:

- (i) Pre-collecting survey with extended periods in the field to include the collection of small samples for analysis in a laboratory as proposed in para. 27.
- (ii) Analysis of samples and assessment of patterns of variability.
- (iii) Final targeted collecting.

29. The development of better estimates of diversity should help to rationalize collecting procedures as itemized above and also help to rationalize existing field genebank collections. Moreover transfer of the technology to active collections should help their use by permitting more rapid screening of hybridized materials.

30. In vitro maintenance. In vitro propagation of somatic material will provide for the rapid multiplication of clones. Ovules and pollen should store the genetic diversity as base collections, but gamete storage needs considerable developmental work. The Working Group points out that ACRI and the IBPGR need to be receptive to relevant proposals; it is suggested that the In Vitro Committee of the IBPGR should screen the proposals.

31. Collection and exchange of germplasm using in vitro techniques. In vitro collecting techniques appear to be feasible. The Working Group would welcome the final development and implementation of these techniques and emphasize that exchange could be accelerated in this way. When the techniques are ready there will be a future need to train scientists from recipient institutes in how to handle the material.

32. Technical support to the research projects. The IBPGR is asked to provide 2-3 interns to meet evident needs for research and development by scientists in the field for long periods. These interns would carry out ecogeographical surveys, provide specific materials on request and start the collecting work. They could, if agreement were reached, be located at CATIE, Costa Rica; Manaus, Brazil; or the Inciva Botanic Garden, Cali, Colombia. It is important that they have access to areas where there is maximum diversity in the field and especially the wild species.

33. The Working Group points out that support of the research proposed above (paras. 26-31) should rate much higher priority for funding than any areas of biotechnology. It is important that the IBPGR aims to see that the collections encompass a well-evaluated gene pool, adequately conserved and readily exchanged.

#### Miscellaneous

34. The attention of the IBPGR is drawn to the need for accelerated coordinated work on the genetic resources of Theobroma and it is recommended that a standing committee or working group provide technical advice to the IBPGR and the relevant institutions.

35. This report lays stress on action needed in the Western Hemisphere, as agreed by the first meeting of the Working Group - the IBPGR should note that any future meetings will take a global view.

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AGENDA

1. Introduction of participants and adoption of agenda (R.E. Larson)
2. Review of first Working Group meeting (J.T. Williams)
3. Activities pursued by Dr. J. Leon
  - (a) National contacts
  - (b) Meeting with national Government representatives  
- February 2-4, 1983 (Florida)
4. Alternative methods of conservation (L.A. Withers)
5. Interim activities funded by:
  - (a) IBPGR (J.T. Williams)
  - (b) ACRI Honduras (J. Leon)
  - (c) LONDON COCOA TRADE COMMISSION Ecuador (J.B. Allen)
  - (d) Trinidad documentation (A.J. Kennedy)
6. Discussion and recommendations (J.T. Williams)
9. Closing comments (R.T. O'Connell)

PROJECT ACTION IN PERU

1. Peru offers special conditions for exploration and the subsequent exchange of germplasm. There are no active research programmes at present, except for the maintenance of a small collection at Yurimaguas; the cocoa industry is still developing, and the country has enough land to produce for the national market and even to export.

An active programme in exploration, research and training and transfer of technology would be of mutual benefit to the country and for other cocoa producing areas.

2. The richness of the flora and fauna of the upper Amazon, in contrast to the rest of the Amazon basin, is already well known. The Amazonian area of Peru has been known to be of basic importance for its cocoa genetic resources, since Pound's expeditions. As part of the Upper Amazon, it is a centre of genetic diversity for many species and according to Cheesman (1944), the area of origin of Theobroma cacao. In detailed studies of other species, like Hevea (Seibert, 1947), the Peruvian Amazon proved to be very rich in total number and diversity, with high diversity at subspecific level.

3. The area is too large and difficult for a systematic exploration. A practical approach would be to follow the steps of Pound by prospecting one by one the areas between the large rivers, as there are indications, according to Bartley (1978), that the cocoa populations of a river basin differ genetically from the neighbouring populations. (The same situation seems to exist in other Amazonian crops.) The expeditions should be carefully planned after a study of factors including peak times of pod maturation, high flooding of the rivers and transportation systems. The large rivers of Putumayo, Napo, Tigre, Pastora and Marañon and its smaller tributaries, like the Nanay and Chambira, have settlements up to the Ecuadorian border, which may facilitate collecting operations. In the large rivers coming from the south, the Huallaga and Ucayali, the lower courses form part of the Amazonian cordilleras. The southern end of the area to be explored may be the lower Urubamba, where wild cocoa is collected and sold by the Indians. Huber (1906) found stands of wild cocoa in the high Purus.

- a) A separate area is the Department of Madre de Dios. The upper tributaries may contain cocoa populations similar to the ones now being collected by CEPEC in the Acre department in Brazil. As bases of operations, Puerto Maldonado and Iberia offer good transportation facilities.
- b) One or more places have to be chosen as centres for the expeditions and at the same time for establishment of temporary collections. Iquitos offers the best conditions in the whole area, as a trade centre where information may be obtained from travellers and Indians, on the location of wild stands of cocoa with good national and international transportation facilities. Iquitos is also a centre of operations of the Peruvian navy, an agency which may be very helpful in the explorations.
- c) The centre for the lower Huallaga is Yurimaguas, where a small cocoa collection is maintained. Eventually, the national cocoa germplasm bank may be established in a permanent form at Tingo Maria, in the upper Huallaga, as the research and training facilities there are the best in the Peruvian Amazon. Tingo Maria could be also the centre for cocoa breeding, evaluation and seed production. The international support given eventually to exploration, should later be directed to reinforce the national cocoa programme with the introduction of the most developed technology in cultural practices and processing.

4. In a preliminary approach, the interest of the Government of Peru in this project should be evaluated. If the response is positive, a detailed plan of operation could be defined in cooperation with Peruvian scientists and authorities. Outside support will be indispensable in this common effort to save cocoa germplasm and to strengthen the overall cocoa programme in Peru.

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THE MAJOR COLLECTIONS

		CLONES		PRIMITIVE CULTIVARS	OTHER SPP OF <u>THEOBROMA</u>	DETAILED CATALOGUES
		LOCAL	INTRODUCED			
CEPEC	(1)	128	121	-	2	NO
CATIE	(2)	151	248	41	8	YES
CAUCAGUA	(3)	182	108	-	-	YES
PICHILINGUE	(4)	403	163	-	4	NO
ROSARIO-IZAPA	(4)	30	21	-	2	NO
PALMIRA	(4)	90	160	-	-	NO
U.W.I.	(5)	363	790	-	4	Work started
LCTAP(INIAP)	(6)	1800	-	-	3	YES

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Summary of cocoa research projects involving genetic resources and/or in vitro techniques

	<u>COLLECTING</u>		<u>PROPAGATION</u>		<u>CONSERVATION</u>					<u>BREEDING</u>					<u>PATHOLOGY</u>		<u>BIOTECHNOLOGY</u>					<u>EVALUATION OF GERMLASM</u>	
	1	2	1	2	3	4	5	6	7	1	8	9	10	11	1	2	12	13	14	15	1	16	
<u>BRAZIL</u> CEPLAC	C	F	T	C	C	C	C	F		C	C	F	F		C	C	F	F	F	I			
<u>CAMEROON</u> IRCC Nkoemvone/IRA	C		C		C		F			C	F	F	F		C	F						C	
<u>CANADA</u> University of British Colombia																	T	T	T				
<u>COLOMBIA</u> ICA Palmira	C	T/F	T	T/F	C	C	F			C					T							T	
<u>COSTA RICA</u> CATIE	C	F	T	F	C	T	F	F		C	C				C							C	
<u>ECUADOR</u> INIAP, including LCTAP	C		C	C	C					C					F								
<u>FIJI</u> Koronivia Research Station, Nausori										C					C								
<u>FRANCE</u> GERDAT, Montpellier	C	F	C	F	C		F					F			C	F						C	
<u>GHANA</u> Cocoa Research Institute, Tafo				T	C					C	C				C							C	





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**VENEZUELA**

Caucagua

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**WEST INDIES**CRU Univ. W. Indies,  
including ICG, T

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**Key:**

- |                    |                                |   |                                 |
|--------------------|--------------------------------|---|---------------------------------|
| 1. Conventional    | 5. Embryo storage              | 9. Ovule culture                          | 13. Secondary product synthesis |
| 2. <u>In vitro</u> | 6. Shoot tip/internode culture | 10. Haploid production/anther culture     | 14. Cocoa butter production     |
| 3. Plantation      | 7. Cryopreservation            | 11. <u>In vitro</u> /genetic manipulation | 15. Lipid metabolism            |
| 4. Seed storage    | 8. Embryo culture/rescue       | 12. Cell/callus culture                   | 16. Novel                       |

T = terminated, C = current, F = future

A Report on the In Vitro Culture of Cocoa  
A Synthesis of Current Activities and Opinions

by

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An assessment of current activity in and future potential for the application of in vitro culture to problems in the agriculture and genetic conservation of Theobroma cacao. Based upon published work, and correspondence with scientists active in appropriate areas and representatives of the cocoa industry.

Background

Theobroma cacao is an important economic crop in several areas of the world, notably Latin America, West Africa and Southeast Asia. The genotypes currently used in cocoa production and in breeding are derived from a very limited number of specimens collected in the field, largely between 1937 and 1943. Thus, the genetic base of cultivated cocoa is very narrow. Much material remains to be collected, particularly in the Amazon Basin. The IBPGR Working Group has identified the urgent need to collect, conserve and evaluate this material.

Cocoa is susceptible to a number of diseases, not all of which have spread to all growing regions. Accordingly, strict quarantine precautions are observed in the movement of germplasm between sources, genebanks and users.

Propagation of cocoa is carried out by cuttings, grafting, marcotting or by seed. Because of the high degree of heterozygosity, the latter is unsuitable for the propagation of much elite material. No conventional method of vegetative propagation is entirely satisfactory. Storage of seeds for conservation is not possible because they are short-lived. Thus, at present, genebanks take the form of plantations ("field genebanks" in the terminology of the IBPGR).

Problems are met in the areas of pathology, breeding, propagation, germplasm collecting, conservation, evaluation and distribution. It would seem that genuine practical and biological problems are compounded by shortfalls in implementation of available techniques, utilization of currently available germplasm and by political/human factors. This is a realistic proposal since in vitro culture is a broad discipline covering many different approaches and techniques. However, it is least well developed for woody species such as cocoa.

In the following, an assessment will be made of the genuine potential for application of in vitro techniques, the level of appropriate activity world-wide and areas in which effort must be increased. Any dangers which are perceived will be identified. Particular attention will be given to aspects involving germplasm per se, although it is felt that the question of the handling of germplasm cannot and should not be addressed in isolation from all other aspects of cocoa agriculture.

Collecting Germplasm

Sampling in the field results in the collection of budwood, wood for rooting cuttings and, if possible, seed pods. Material is transported back to a base and handled appropriately. Once established at the field base, clones can be transported to

end users or genebanks via quarantine. Where feasible, newly collected material may be transported directly to quarantine, usually as budwood. Several quarantine stations exist, although not all are universally recognized (Kew; Barbados; Tropical Crop Husbandry; Wageningen; GERDAT, Montpellier; USDA, Florida). Clearly, transport over some very long distances may be involved at this stage.

A number of problems arise in collecting, primarily in relation to the nature of the wild material encountered and its maintenance in a healthy condition in readiness for later processing. The latter factor limits the duration of collecting missions and potentially, therefore, their completeness. Cuttings for rooting must be processed within hours and budwood within days.

Loss of material at this stage (and at later stages where similar propagation techniques are called upon; see "Germplasm Distribution" below) is widely perceived as a problem in the genetic conservation of cocoa. In vitro techniques are commonly, but not universally seen as a possible solution. Data relating to recent collecting expeditions underline the problem, not necessarily in direct terms but the fact that collecting missions are limited in duration to avoid excessive loss.

It would seem that in vitro methods may be able to help in three particular ways: i) widening the choice of material for collection; ii) sustaining healthy samples for longer periods of time; and iii) providing a superior means of transporting collected material.

In theory, in vitro culture permits propagation from virtually any part of the plant. For cocoa, this is not yet feasible but we should take a long-term view. As a direct translation from the current method of collecting, "mini-budwood sticks" could be collected in tissue culture vessels and in contact with a nutrient medium. Various somatic and floral tissues including shoot apices, leaves, immature and mature seeds and pods and floral primordia could be handled in a similar way. The major limitation at present is in the inadequacy of propagation technology (see below), and less seriously, under-exploration of the necessary collecting techniques.

Deterioration at the collecting stage, under conventional conditions, is attributed to fungal pathogens. However, physiological deterioration should not be ruled out and physiological stress may enhance susceptibility to pathogen attack. In one current investigation, attempts are being made to identify the particular pathogens responsible for fungal attack and develop prophylactic applications to prevent deterioration of the budwood. To date Diplodia spp., Fusarium spp. and Colletotrichum spp. have been identified and appropriate fungicides sought. The relationship between climatic factors at the time of collecting and subsequent deterioration is also being investigated. Variability in survival in relation to physiological condition and genotype are other aspects worthy of investigation.

In another study, attempts are being made to bridge the gap between budwood collecting and sophisticated axenic culture by developing semi-sterile culture methods which are practicable under field conditions. The rationale is similar to that with the exception that anti-fungal and anti-bacterial treatments are being used to both reduce microbial attack and consequent decomposition of the specimen and prevent overgrowth and depletion of nutrients in the supplied culture medium. The supply of the medium is aimed towards forestalling physiological deterioration but may also promote development consistent with later propagation stages. The composition of the culture medium is being investigated with a view to determining minimal, common nutrient requirements and optimal anti-microbial additives. Preliminary investigations suggest that control of contaminants will only be achieved if some surface sterilization be carried out. Simplified methods for so doing are under investigation, and have been welcomed by an IBPGR In Vitro Sub-committee meeting, 14-16 September, 1983. The above approaches represent a new departure in in vitro plant culture in that they contradict the dogmas that sterility should be absolute and that anti-microbial compounds should not be used in culture media.

### Germlasm Distribution

This is considered to be an area with great potential for improvement, possibly by the introduction of in vitro approaches, even giving the opportunity to circumvent existing quarantine procedures (see "Pathology and Quarantine", below).

At present, this is largely linked to collecting techniques and is carried out by the transportation of budwood, cuttings and seeds from genebanks or other sources to users. Exceptionally, seedlings may be used. Material is transported as air freight or hand-carried baggage. Experience with the transportation of material from collections in Mayaguez to Florida, for example, would suggest that hazards here parallel those in field collecting in terms of the risks of deterioration in transit. Important differences are that there may be less pressure of time in preparing material for transportation, greater opportunity for replication and the security of clones being readily available at source. However, material still has to be isolated from the parent tree and suffered against deterioration for a number of days.

All of the comments made earlier in relation to germlasm collecting using in vitro approaches apply here with the possibilities of carrying out a more thorough surface sterilization of material, application of more orthodox in vitro methodology and the incorporation of in vitro clonal propagation prior to distribution. The material for distribution may, in fact, have passed through a number of in vitro transfers and have had the opportunity to be screened for pathogens and in vitro genetic characterization (see "Pathology and Quarantine" and "Evaluation" below).

There are precedents (cassava, potato and others) to suggest the transportation of aseptic cultures to be feasible over long distances with potentially high rates of success. Factors to be taken into account include preparation, packaging, conditions in transit and handling upon arrival.

### Propagation

A number of reasons can be presented in support of the need to develop alternative methods of propagation. Seeds are appropriate as propagules in some circumstances but they are not always available. The time to maturity in seedling material can be an impediment to rapid utilization. Material taken for vegetative propagation requires careful and speedy handling as detailed earlier. Further, varietal differences are observed in these techniques, some material being entirely recalcitrant. Studies are under way to improve conventional propagation techniques and the project in prospect will examine the scope for applying alternative horticultural methods such as the nutrient film technique.

Rates of propagation by conventional means range from 400 per annum by grafting to 2,000 per annum by seed from an individual (male) plant. An in vitro method would have to compete with these rates, be relatively inexpensive, reliable and technically undemanding. On present knowledge, all of these are reasonable expectations. Further, in vitro methods could be used in hybrid seed production to propagate favourable crosses rapidly. The potential of using in vitro propagation to multiply selected clonal material is widely recognized.

Two main approaches can be taken to in vitro clonal propagation: i) non-adventitious, via pre-existing meristems, or ii) adventitious via the induction of de novo development of organized structures (shoots and roots or embryos). The former is favoured since it is thought to confer greater genetic stability. However, if in the latter, adventitious development were to occur directly from the tissue explants rather than via an intervening unorganized callus stage, the risks of instability should be relatively low.

Studies of in vitro propagation of cocoa have lacked all approaches with differing degrees of success. Shoot-tip and internode culture have featured in a number of past and present studies. In what is probably the most successful, 50-80% outgrowth of axillary buds occurred on nodal explants (2-6 shoots per explant), of which 70-90% could be rooted. Transfer to independent growth has not been clearly demonstrated but

should not present serious problems in rooted material. Notable features of this work include the use of liquid medium and the application of the growth hormones BAP (benzyl-amino-purine; shoot outgrowth stage) and IBA (indole-butyric-acid, plus NAA (naphthyl-acetic-acid; rooting stage). In a similar study it was found that shoot outgrowth could be induced but that it showed periodicity (relating to flushing cycles?). BAP alone was more successful in inducing outgrowth than a combination of BAP and IBA, with which callusing was severe. Some success in the induction of a second phase of shoot outgrowth from nodal segments of the in vitro induced shoots has been recorded in both studies above.

Several other studies have taken a similar approach to propagation. In the foregoing, both fan and chupon explants have been used. The habit of regenerating trees has not been specified but should be checked in order to comply with requirements for orthotropic growth.

In many studies of shoot tip and internode culture, deterioration with time in culture has been cited as a problem, often insurmountable. In some cases, very healthy growth and bud break is followed by progressive deterioration. Frequently, deterioration is attributed to either microbial contamination or overproduction of phenolic (browning) compounds. Observations that careful selection of explant material and heat and/or shade pretreatment of parent plants may influence survival are worthy of further exploration. The superiority of liquid culture medium along with the observation of latent deterioration may indicate that availability of nutrients could be critical. This is supported by observations that attachment of a leaf (potential nutrient supply?) will forestall deterioration in nodal cuttings.

Problems in the implementation of the culture of nodal cuttings for mass propagation would appear to be some lack of reproducibility, a rather low rate of propagation making the process laborious, and a failure to demonstrate repeated cycles of propagation.

In view of the problems in achieving mass propagation by non adventitious means, efforts have turned to adventitious development. Accepting the reservations which must be taken into account, as mentioned earlier, it is still considered to be worthy of pursuit since the potential rate of propagation offered may well offset disadvantageous aspects (which could perhaps be screened for in any case). This approach has been found satisfactory for oilpalm and coffee.

Several projects have included attempts to propagate cocoa by means of regeneration of shoots and roots from callus. It is widely found that rooting may be initiated (although not necessarily at will) but that shoot regeneration cannot. This parallels experience with other difficult species wherein rooting capacity may remain after the capacity to regenerate shoots has been lost. Difficulties are found with callus from many sources (but see reference to secondary embryos below). Mass propagation via shoot or somatic embryo development from leaf discs has been reported for a number of species including coffee and potato. Attempts to reproduce this with cocoa have met with limited success. As yet, only roots have been regenerated erratically. Shoot-like protruberances have been observed on some material and the inductive conditions are currently under investigation. Difficulties in handling this material include microbial contamination, failure of tissue to survive in culture for adequate periods of time and callus overgrowth. Drawing upon observations with callus and leaf material, it is felt that success in adventitious regeneration may require control of development such that explant material can survive long enough to embark upon de novo organogenesis (a certain amount of callusing possibly helping here) without the tissue becoming completely committed to an unorganized callus growth pattern. Physiological studies of the whole plant should aid the development of in vitro propagation systems involving somatic tissues.

Having dealt with the most problematic systems involved in propagation studies, it is encouraging to turn to one which is giving some success, namely secondary embryogenesis in zygotic embryos. This system has been developed to the extent that mass propagation, virtually at will, can be achieved from immature zygotic embryos. Stock material can be maintained in culture without apparent decline in embryogenic

competence. It has even been possible to isolate embryogenic callus from embryos. Plants derived from embryos can be transferred to independent growth. (It has been noted that such material may be morphologically abnormal at the mature embryo stage).

In this work, the key to success would appear to be the immaturity of the embryos, since the capacity to proliferate is lost as the original zygotic embryos age. Thus the potential application to the clonal propagation of trees of proven performance must be limited. However, the system provides a useful model and we should not rule out the possibility of inducing a state receptive to induction in mature material. A direct application of the work would be in the clonal propagation of hybrid seed from controlled crosses and the rescue and propagation of seed which would otherwise abort. Although an obvious application in propagation for plantation growth can be seen, the motivation for the embryogenesis work was the development of an *in vitro* method for cocoa butter production. This is dealt with under "Biotechnology" below.

Propagation has also been attempted by the use of anther and ovule culture. This is dealt with under "Breeding" below.

#### Genetic Conservation

There is a need to conserve both vegetative material and seeds of cocoa. Seeds are of value since they reflect the heterozygosity of the crop, whereas vegetative material is of obvious value to conserve clones. Seed storage is not feasible in the long term so genebanks take the form of plantations consisting of rooted cuttings, budded plants and seedlings. A number of collections (either genebanks *sensu stricto* or local collections) are in existence, including: ICA; CATIK; ICG,T; CEPLAC; Marcor Field, Trinidad; St. Joseph's Nursery, Trinidad; San Carlos; Pichilingue; IICA Turrialba; USDA Mayaguez; Kew; Sabah; Tafo; Ghana; Ivory Coast; Nigeria. An alternative, or rather supplement, to plantations would be attractive, in view of practical problems in plantation management, their finite lifetime and geographical/climatic restrictions. *In vitro* culture is an obvious approach for exploration, doubly useful since it may interface with collecting, exchange and vegetative propagation. Its potential value is widely acknowledged.

However, before expanding upon this, it is important to examine the possibility of improving seed storage. It is generally experienced that storage at low temperature and/or in the desiccated state is unsuitable for cocoa seeds. Several studies have examined (or are currently examining) the problem of developing alternative storage procedures by maintenance of the seeds in the imbibed state. In one study, seeds have been stored in the pod for periods of weeks to several months, or as individual seeds stored fully imbibed in PEG for ca. 25 weeks. In another similar study, "sub-imbibed" seeds were stored for periods of up to eight months by which time viability had dropped to 24%. In this and other cases, control of relative humidity and fungal overgrowth are essential to maintenance of viability.

Embryonic axes dissected from otherwise inviable seeds, damaged by cold/or physiological deterioration in the pod, can be rescued and germinated *in vitro*. This opens up the possibility of further manipulating the environment of the embryo. Accordingly, attempts have been made to use manipulation of the culture conditions to extend the storage period. Inhibition of germination and arresting development post-germination by application of chemical inhibitors have been attempted. By this means, storage for periods of one or more years should be feasible. However, it would not be a long term answer unless cryopreservation could be introduced (see below). As in all works involving large seeds, storing genetically representative quantities may present practical problems and a drawback of this approach is that each embryo/seed requires individual handling and the provision of an individually protected environment. However, deterioration due to microbial contamination should present only a slight risk and be easily controllable.

The introduction of a cloning stage (see "Propagation" above) prior to storage, would reduce the risk of losing single examples of useful genotypes, permitting duplication of collections and evaluation of germplasm whilst replicates are safely stored. This, and the possibility of extending the storage period would appear to be

the useful directions in which to take the work. The development of seedling banks has been suggested (J.G. Hawkes). The foregoing is perhaps an in vitro version of the idea.

For a number of herbaceous and rather fewer woody species, storage of vegetative material in the form of in vitro cultures has been carried out successfully. Over 50 species have been stored by growth limitation and over 40 by cryopreservation (freezing to the temperature of liquid nitrogen:  $-196^{\circ}\text{C}$ ). The former offers a means of storing material in increments of one, or at most, two years. At these intervals it is necessary to transfer the culture to fresh medium. Some risk of contamination is introduced at each transfer and it is advisable to monitor for contamination and deterioration in condition more frequently than the transfer intervals. However, the IBPGR recognizes these as active (breeder's) collections, not to be confused with base stores. In contrast, cryopreservation offers the possibility of indefinite storage in which the only attention required is to replenish the supply of liquid nitrogen coolant in the storage refrigerator. Arguments may be made which question the feasibility of this approach to storage in remote locations or under relatively unsophisticated conditions. However, neither of the latter two constraints need apply; a frozen genebank can be located virtually anywhere, needing very simple technical back-up. The important factors to take into account in determining desirable approaches should be based on users needs and scientific constraints (such as suitability of the approach for the species in question, or the level of technical development at the time).

Although a number of studies have been initiated with the expressed objective of developing in vitro methods of genetic conservation, most of these have not been able to proceed to a close examination of storage techniques. This is partly due to the low level of technical development in general, but more importantly, due to the frequently poor performance in culture of cocoa and its recalcitrance to in vitro clonal propagation techniques. Attempts at growth limitation of cultures other than the zygotic embryos cited above, have been restricted to callus cultures and have been largely unsuccessful. In one case, a deterioration in secondary product synthesis was observed in stored material.

Cryopreservation has received very little attention, and only for fine cell cultures of cocoa has any success been achieved. All other types of culture aggregated cell suspensions, callus, shoot tips, zygotic embryos, somatic embryos and dissected embryo apices are lethally damaged by freezing. Examination of the cryopreservation requirements of organized tissues is continuing. It is clear that the material will need very careful handling at all stages and a very high level of survival cannot be anticipated. Serious freezing damage would threaten genetic stability in storage and introduce risks of selection for freeze tolerant genotypes, demanding that methods permitting a high standard of genetic characterization be developed (see "Evaluation", below).

Several culture systems can be examined as potential candidates for storage. Cell and callus culture may have their uses in biotechnology (see below) but even if not disregarded because of risks of genetic instability, they are of little direct relevance to genetic conservation because of our current inability to regenerate plants from them. Whilst shoot tips, internodes and embryos, as organized systems present better subjects for storage, their gross nature suggests suitability for growth limitation but not cryopreservation. They are perhaps best considered as axenic plantlets than strictly in vitro cultures. However, early stage embryos embody the potential for development to a mature stage and independent growth, yet can in theory be isolated at a very early, immature stage. Incorporation of clonal propagation as described above, would permit replication of genotypes. This principle also applies to shoot apical meristems where a very small explant, preferably cloned in vitro would be suitable for cryopreservation.

It is emphasized that the storage method eventually adopted must be compatible with clonal propagation, thereby necessitating prior development of competence in this aspect. Despite the latter point, it is noted that there may be potential for developing cryopreservation for dormant buds excised directly from the plant and then recovered either in vitro or by grafting.

## Breeding

In terms of crop improvement (yield, disease resistance, sunlight tolerance, precocious bearing, stress tolerance etc.), there is vast scope for useful breeding work with cocoa. It has been commented that material now available for breeding is not being utilized to the full; hence a current population improvement programme is welcomed. (Underlying the problem in application of conventional methods is a widely recognized inadequacy in evaluation of available germplasm see below). Therefore, until conventional breeding methods are applied to make full use of germplasm which is available at present, the development of novel in vitro breeding methods may be unjustified. Further, there is a danger that the apparent 'glamour' of in vitro techniques may attract attention away from perfectly adequate conventional methods.

An area of in vitro culture which is developing very rapidly now, but which should be treated with such caution, is the production of new genotypes apparently spontaneously via "somaclonal variation" or induced variation. The former has attractions in that no toxic, mutagenic compounds are involved. Its application to several crops including sugarcane and potato has been demonstrated already. The potential for producing new genotypes of cocoa has been realized and several projects are under way or in prospect with the aim of developing high yielding, disease resistant or otherwise valuable varieties.

Two techniques which come under the heading of "novel approaches to plant breeding" but for which immediate, relevant applications can be seen, are anther or ovule culture and embryo culture. By conventional methods, the production of homozygous plants is lengthy and arduous. In several past, current or proposed projects attempts are being made to induce haploid cultures from anthers and ovules as well as to take advantage of spontaneously occurring haploidy. Regenerant plants would then be chromosomally doubled using colchicine, yielding material which could be introduced into breeding programmes. This would accelerate breeding as well as providing reference genebank material. However, as yet, only callus has been regenerated. Considerable development of the system is required. To date, haploid and homozygous diploid plants have only been produced from polyembryonic seeds.

As mentioned earlier, embryo culture may be used to rescue otherwise inviable embryos. Recent attempts to cross T. cacao with T. grandiflora or T. angustifolia provide illustrative examples of its potential application. This provokes a general comment on the role of in vitro work. There is no doubt that the ability to clone material rapidly would permit much more rapid realization of the benefits of any breeding programme.

## Pathology and Quarantine

Cocoa is susceptible to a number of diseases including Witches' Broom, Monilia Pod Rot, Canopy Wilt, Swollen Shoot, Vascular-streak Dieback and Black Pod. Resultant losses may be very severe. As already mentioned, it is necessary to carry out strict quarantine precautions for cocoa to avoid spread of disease to geographical areas which have escaped infection so far. Clearly, as a priority, breeding for resistance must be carried out, but in vitro culture has a part to play here in several respects: (i) by facilitating study of the relationship between the causative organism and cocoa tissue; (ii) screening for pathogens; (iii) presenting ways of eradicating pathogens from diseased material; and (iv) permitting the storage and exchange of "clean" material.

In work directed towards the development of a screening method for Vascular-streak Dieback, spores of the causative organism Oncobastidium theobromae, are required. In the past, these could only be obtained from naturally occurring basidiophores. However, it is now possible to produce them in dual culture of the fungus with callus of cocoa. Similar work has been carried out with Crinipellis pernicioso (the causative organism of Witches' Broom). Direct study of disease processes in vitro has not been carried out.

As yet, little work appears to have been carried out on either disease indexing in vitro or pathogen eradication. The former might involve truly in vitro systems which

give rapid indications of disease, or biochemical tests (nucleic acid hybridization; ELISA technique) involving identification of the pathogen related compounds. A limited amount of work has been carried out on the application of the ELISA technique to the detection of Swollen Shoot Virus. Meristem-tip culture is relatively widely used to eradicate viral pathogens from various plant species and might be appropriate to cocoa. Further, in vitro culture might perhaps be used for the eradication of bacterial or fungal pathogens by a combination of exclusion (as in meristem tip culture) and disinfection/chemotherapy.

The development of methods for distributing clean material must await progress in in vitro clonal propagation and germplasm storage, as well as transportation. It is acknowledged that there may be resistance to this by users and there is some genuine cause for concern should there be a lack of good screening methods. The apparent cleanliness of cultures should not be taken as an indication of absence of all pathogens. Thus it would be wise to develop methods for screening cultures as well as plants.

### Biotechnology

The propagation of embryos in vitro (see "Propagation", above) was conducted with a view to producing cocoa butter in vitro as an alternative to agriculture. Considerable progress has been made here in understanding and inducing the process of embryo maturation. It would appear that the in vitro system mimics the in vivo situation to a large degree but, as yet, not sufficiently closely to substitute for it (ignoring economic aspects). The only points which are perhaps of relevance here are that the system may provide useful spin-off in the form of a well developed propagation system and secondly, a reminder to us that if such approaches are to be taken in the future, the genotypes of cocoa in use will be very restricted. Hence, the case for genetic conservation is strengthened.

The production in vitro of cocoa flavour has been attempted by some workers. Only limited success has been achieved. The flavour compounds may, in the plant, be strictly associated with organized structures, quite different from the cell or callus cultures used in work on secondary product synthesis. Again, we should have some interest in this work in that it may aid the general development of in vitro competence with cocoa.

### Evaluation

As stated above, if cocoa germplasm is to be exploited to the full, it must be evaluated more thoroughly. Biochemical techniques applied to in vivo samples or in vitro cultures may be of assistance in permitting the screening of large quantities of material. Similarities and differences between accessions, obscured in assessments on the basis of conventional descriptors, may be revealed. Characterization ("fingerprinting") by means of isoenzyme analysis as one of several potential techniques is being examined in two projects which have recently been initiated. As well as aiding evaluation in the conventional sense, this should also help to reveal the stability of material maintained in culture, the stability of cultures subjected to various storage procedures and describe the degree of genuine variability generated by unconventional (biotechnological) breeding techniques.

### Problems and potential in the application of in vitro techniques

Of all of the potential in vitro applications with cocoa, as set out above, the judgement is made here that germplasm collecting and distribution are probably the ones with the most pressing need for development. Materials could then be collected in the field and established in genebanks with a much higher rate of success than at present, with explorations being carried out more thoroughly. Propagation can be carried out adequately by conventional means, but it would be short-sighted to adopt in vitro collecting and distribution methods whilst retaining less than compatible propagation methods for associated stages. No doubt both in vivo and in vitro approaches will have a place in future practice.

Conservation in the medium term would appear to be adequately satisfied by plantations, except where seed material is concerned. Clearly, seed storage methods must be improved and in vitro storage is offered as the most appropriate. It is felt that attempts to overcome seed recalcitrance have only limited potential and are unlikely to reward effort. Long-term conservation must be tackled by alternative, safe methods and the IBPCR recognizes that in vitro storage by cryopreservation is the most obvious. Again, as in the case of collecting and distribution, it would be short-sighted to associate this exclusively with conventional propagation methods when in vitro ones may link in so much more satisfactorily. However, having stated that satisfactory in vitro propagation methods will be needed to support the recommended areas for technical development, it is clear that attention must be given to overcoming the persistent problems encountered there. Whereas other techniques are underdeveloped as a result of neglect, propagation cannot be judged so. Effort must therefore be reinforced further.

Germplasm evaluation, pathogen screening, and pathogen eradication all may succumb to in vitro approaches in the future. However, in connection with disease and quarantine, a note of caution is sounded. It would be disastrous to assume in vitro cultures always to be clean simply because no obvious symptoms are displayed. The international exchange of cultures should only be adopted if and when appropriate disease indexing methods have been developed adequately.

Finally, turning to biotechnology: Given sufficient investment and interest, no doubt useful work could be carried out on the in vitro synthesis of cocoa products; similarly for the generation of new genotypes. However, these are seen as lower priorities than the aspects of in vitro culture which relate to conservation of germplasm and should not attract funds, interest and effort away from them (although preparative work to develop culture systems in cocoa such that biotechnological methods for developing new genotypes can be applied once they have been refined using other, more amenable crops would be justified). It is better that efforts and resources first be devoted to the application of conventional methods (supported by novel ones such as anther culture only when appropriate) to the germplasm which is available in genebanks and in the field. Finally, it should be understood that all aspects of breeding, be they by conventional or novel approaches, will require the availability of a broadly based, well evaluated gene pool, which is under efficient conservation.

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