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CONSERVATION, CHARACTERIZATION AND UTILIZATION OF COCOA GENETIC RESOURCES

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ABSTRACT

The International Cocoa Genebank, Trinidad (ICG,T) is one of two cocoa germplasm collections recognized by the International Plant Genetics Resources Institute (IPGRI). The ICG,T which has approximately 2,500 accessions and encompasses a wide range of germplasm collected in South and Central America and the Caribbean is managed by the Cocoa Research Unit (CRU). Sixteen plants of each accession are planted in a 35 ha site at La Reunion in Trinidad. Characterization of these accessions is underway by recording of morphological, biochemical (isoenzyme and Random Amplification of Polymorphic DNA), disease resistance (*Crinipellis* and *Phytophthora*) and physiological (drought tolerance and rates of photosynthesis) characters. For morphological characterizations 65 botanical attributes of leaf, flower and pod are recorded as recommended by the IPGRI. Research is now underway in an attempt to reduce this number to nineteen. CRU maintains a Quarantine Station in Barbados (where cocoa is not grown), through which incoming and outgoing accessions are quarantined for two years. There are two other quarantine stations in use for cocoa, one at Reading University in the United Kingdom and the other at Montpellier in France, which are also used for the transfer of cocoa materials. After passing through quarantine, these accessions are available to cocoa producing countries throughout the world, where they will be utilized in cocoa breeding programs to produce commercial varieties.

INTRODUCTION

The need for conservation of *Theobroma cacao* (cocoa) genetic resources arises from two causes, firstly the destruction of the Amazon forests which may remove the wild cocoa trees in centres of diversity and secondly the decline in cocoa production along with the introduction of superior clonal lines which may lead to the loss of diversity of fine flavored Trinitario types in the Caribbean.

The International Plant Genetic Resources Institute (IPGRI) formerly the International Board for Plant Genetic Resources) currently recognizes two international centres for cocoa germplasm conservation. These are the Tropical Agricultural Research and Training Center (CATIE) at Turrialba in Costa Rica and the International Cocoa Genebank, Trinidad (ICG,T) at the Cocoa Research Unit (CRU), St. Augustine, Trinidad and Tobago. The CATIE collection holds a valuable collection of Criollo material whereas the CRU collection has been developed mainly from introductions of Forastero from South America and Trinitario collected in the Caribbean.

The basic requirements for conservation of cocoa are a secure system for long-term maintenance of the gene pool; however if this material is to be useful to plant breeders then a reliable means of identification is essential and a knowledge of the occurrence of characters of economic importance is important for the utilization of the material. The ICG,T now holds some 2,500 accessions, and acquisition of new material by collection and exchange is continuing. Thus it is not possible or desirable that the full collection be obtained by every plant breeding program in the various cocoa producing countries. Thus selection of material by individual breeders is greatly facilitated by the characterization of the material in terms of morphology, disease and pest resistance, physiological characters (drought resistance, rates of photosynthesis).

THE INTERNATIONAL COCOA GENEBANK, TRINIDAD (ICG,T)

The ICG,T now comprises some 2,500 accessions of which approximately 2,000 have been established at the University Cocoa Research Station (UCRS) at La Reunion and some 500 are under quarantine (for two years) at the Quarantine Station maintained in Barbados by CRU. Since there is no cocoa in that country the accessions can be grown under natural conditions. The other quarantine stations (at Montpellier in France and Reading in the United Kingdom) have to grow the plants in heated greenhouses in the winter months.

At UCRS the ICG,T is planted on a 35 ha site. Each accession is to be represented by 16 trees as clonal plants derived from rooted cuttings. The site had been a cocoa plantation and the new plantings were made maintaining the original drainage system and can be put on a minimal maintenance basis if the need arises.

CHARACTERIZATION

Morphological Description

The original descriptor list endorsed by IPBGR (now IPGRI) (Anon, 1981) consisted of 65 botanical characters of leaf, flower and pod. The first difficulty that arises is that flowers and pods are not available throughout the year. Thus the data has to be accumulated as and when material is available. The measurements involve linear measurements, color assessments, microscopic measurements such that progress with available resources would be very slow towards the objective of characterizing 2500 accessions. To meet this difficulty research has been conducted at CRU (Bekele, 1991) to produce a concise descriptor list of some 19 characters which would be considered adequate for practical purposes (Appendix I). In addition it is hoped that in the near future data of particular importance to the manufacturer (bean flavor, butterfat content and quality, proportion of shell to nib) would be recorded. The assessment of flavor awaits industry agreed standards which are now under discussion.

The data collected is stored in a computer data base at CRU and also sent to the International Cocoa Germplasm Database (ICGD) held at Reading University in the United Kingdom. To date 199 accessions have been fully characterized with a fewer number having been characterized in terms of leaf, flower and fruit descriptors.

It is expected that the concise description list will be put into use shortly, thus allowing for a much faster rate of description.

BIOCHEMICAL DESCRIPTION

1. Isozyme Analysis

Isozyme analysis offers a system with which a large number of individuals may be rapidly evaluated. Also isozyme descriptors are less likely to be influenced by the environment than morphological characters (Sirju-Charran *et al.*, 1991; Johnson *et al.*, 1991).

Currently acid phosphatase (ACP), malate dehydrogenase (MDH) isocitrate dehydrogenase (IDH), phosphogluco-isonerase (PGI), alcohol dehydrogenase (ADH) and Diaphorase are in use at CRU. It should be noted however that use of these six enzyme systems does not allow complete separation of all accessions tested into individual categories. Thus a combination of isozyme and morphological descriptors may have to be used for identification of each individual.

2. Random application of polymorphic DNA (RAPD)

This technique involves the extraction of DNA and assessment of variability. Studies on this

system were carried out at the Scottish Crops Research Institute (SCRI) in collaboration with CRU (Wilde *et al.*, 1991). Currently CRU is collaborating with CIRAD-CP (France) in refining the system. CIRAD-CP has stationed a molecular biologist (O. Sounigo) at CRU, thus collaboration takes place with their laboratories at Montpeller. Further work is required on this system; reproducibility at individual laboratories seems possible but differences are currently obtained in the results at different laboratories. Although the biochemical agents used are expensive this method should provide a rapid system for "finger printing" individual varieties.

DISEASE AND PEST RESISTANCE

Since new diseases cannot be introduced into Trinidad, testing is only possible against those diseases that already occur in that country - these are Black pod (caused by *Phyphthora palmivora*) and Witches' Broom (caused by *Crinipellis perniciosa*) and against the particular strains of these organisms which occur in Trinidad. Research has in the past concentrated on determining the best method for rapid testing of varieties and routine screening of the collection has now started.

COLLECTING OF GERMPLASM

The cocoa collection in Trinidad derives from Trinitario produced by natural hybridization and Forastero introductions from South America (Wood, 1991).

It is said that the first introduction in Trinidad was in 1525 - this would have been Criollo which was grown until the 18th Century when a "blast" disease decimated the plantations in 1727. In 1757 Forastero was introduced from Venezuela and this hybridized with the remaining Criollo creating the Trinitario population. Selections were made by the local planters from these populations. F.J. Pound collected 100 clones in Trinidad - the Imperial College Selections (ICS1-100).

Pound made introductions from Ecuador (1938) and the Amazon Valley (1943) of wild cocoa types - mainly for resistance to Witches' Broom which had then been introduced into Trinidad.

In the early 1940's F. Cope carried out a selection program in Grenada - the GS clones (Spence, 1991). In 1968 and 1972 Chalmers went on collecting expeditions in Ecuador, again seeking Witches Broom resistance (Warren and Kennedy, 1991). Then the joint Anglo Colombian expedition in the 1950's sampled germplasm using botanical criteria, thus providing a wider base for the genetic pool collected.

The London Cocoa Trade Amazon Project lead by J. Allen may be considered to be the most extensive systematic scientific collection. It had the defined objective of broadening the base of genetic material available to breeders and relied on collecting 25-50 plants from many populations rather than many plants from few populations. However material is still being transferred from Ecuador where it had been established after the initial collections.

Over the last five years CRU has been collecting cocoa material in the Caribbean, including Suriname and Belize and this process continues. Recently a very successful expedition was made by V. Mooledhar and W. Maharaj (both of CRU) to Belize where wild Criollo material was collected.

CONCLUSION

The greatest difficulty with the conservation of cocoa germplasm lies in the fact that the material has to be maintained as a field collection, at some considerable cost and this requires very long-term funding. Attempts to routinely tissue-culture cocoa material have met with limited success.

CRU has proposed an Endowment Fund as the only means of ensuring conservation in perpetuity. So far efforts to obtain such a fund have been unsuccessful and so the collection is maintained on the basis of project funding.

It should be noted, however, that the British Chocolate manufacturers and the Government of

Trinidad and Tobago have supported the work of the Cocoa Research Unit continuously for the last 64 years i.e. since 1930 when cocoa research was first started at the Imperial College of Tropical Agriculture (ICTA) which later (1960) became the University of the West Indies.

The present work of CRU is summarized in the Mission Statement (Appendix II) and detailed papers on the work are presented in the Annual Reports.

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Appendix I. Data recommended for a concise descriptor list for cocoa (CRU, 1994).

CHARACTERIZATION DATA

MORPHOLOGICAL
Leaf Descriptors
Flush Color
Flower Descriptors
Ligule color
Filament color
Pedicel column color
Style length
Ligule width
Sepal length

Number of ovules per ovary

Fruit Descriptors
Ridge disposition
Ridge pair separation
Pod apex form
Pod basal constriction
Husk hardness
Pod rugosity
Pod length to width ratio
Individual dried bean weight
Bean number
Bean length
Bean width

Appendix II. Mission Statement of the CRU.

1. To **conserve** on one site as a field genebank (to be known as ICG,T) all primary germ plasm existing in Trinidad.
2. To **enlarge** existing collection of primary germplasm by:-the acquisition (through exchange if appropriate) of material previously collected in the Caribbean and Latin America);
   -the conduct of expeditions to collect new material from the wild;
   -maintenance of a cacao quarantine facility in Barbados.
3. To **characterize** fully all the primary germplasm of ICG,T. This will include measure ment of:
   -heritable morphological characters (including leaf, flower pod and bean characteristics);
   -reproducible isozyme markers (using an appropriate number of enzyme systems);
   -relevant economic information (shell percentage, fat content, fat quality and flavor characteristics) of dry beans;
   -repeatable RAPD/PCR characteristics;
   -levels of tolerance to ***Phytophthora*** and ***Crinipellis*** pathogens.
4. To **catalogue** all the data collected on the primary germplasm in ICG,T and incorporate it into the International Cocoa Germplasm Database (or ICGD).
5. To **encourage** and **arrange** unrestricted international distribution through quarantine of primary germplasm from ICG,T on request. The material should be despatched with as much information as possible on the above-mentioned characters. Further, to develop and use tissue culture and/or micropropagation techniques for international distribution of germplasm under aseptic conditions.
6. To **produce** populations with enhanced levels of those genes which are of importance to plant breeders.
7. To **develop** and **adapt** appropriate scientific methodology to achieve the above-mentioned objectives.
8. To **train** scientists from cocoa-producing countries to higher degree standard by offering a variety of research projects on cocoa and to encourage the publication of the results of such work in the refereed scientific literature.
9. To **offer facilities** for visiting scientists to work on cacao subjects of relevance to the world cocoa economy. Research may be on topics not covered by the "Mission Statement", but should ideally utilize the wide range of primary germplasm at CRU and thus increase the knowledge of this material.

10. To **support** and **enhance** the cocoa research effort of Government of the Republic of Trinidad and Tobago (GORTT), in particular to identify the form of a "model cacao plant"[1] capable of significantly higher yields in an appropriate "orchard" system and furthermore to **develop** a strategy for enhancing pollination to ensure the realization of full yield potential of the model plant.
11. To **collaborate** with institutions and universities worldwide which have an interest in cocoa research.

[1] In terms of plant size, shape, habit of branching, flowering and photosynthetic potential in relation to appropriate levels of moisture, temperature and light intensity.