Newsletter

ISSUE NO. 6 **August, 2001**

IN THIS ISSUE

You are invited to contribute articles on research or other issues of interest to cocoa breeders/geneticists

FROM THE EDITOR'S DESK:

One of the major highlights for INGENIC since the last issue of the Newsletter was the Third INGENIC Workshop, held in Kota Kinabalu, Malaysia in October, 2000, which had as its theme "New Technologies and Cocoa Breeding. The deliberations at this Workshop were very fruitful, and the main conclusions and minutes of the INGENIC AGM are presented herein. Special tribute is due to Kevin Lamin, Dr. Lee Ming Tong and others of the Workshop Organising Committee for their superb organisation and hosting of this event.

During the International Cocoa Research Conference in Malaysia (October 9-14, 2000), the feasibility of launching a "Global Cocoa Programme" was discussed. Following the discussions, a survey was conducted by INGENIC to determine the consensus for priority setting in cocoa genetic improvement. Thirty-two responses from 16 institutions were received, and these were analysed. The results of this activity are also presented in this issue.

The other articles included in this issue represent a broad spectrum of activities pertinent to cocoa genetics and breeding. The INGENIC Committee wishes to thank the contributors for sharing these important findings and information.

INGENIC has undoubtedly reached a milestone in its existence as an international group endeavouring to promote collaboration in cocoa breeding and genetics. The Proceedings of the Third INGENIC Workshop are expected to be released shortly and we encourage readers to respond to the ideas presented there and in this issue of the Newsletter. The electronic mail addresses of contributors are included in the Newsletter articles to facilitate direct communication as well.

The INGENIC Committee thanks you for your continued support and looks forward to working with you in our effort to promote further collaborative approaches to solve our common problems. Please send contributions for publication in the next issue to me at louisebekele@hotmail.com or flbekele@trinidad.net before January, 2002.

Frances Bekele

INGENIC Inquiry on Priorities to be Considered in a 'Global Cocoa Programme'

B. Eskes

Scope

During the 13th International Cocoa Research Conference at Kota Kinabalu, Sabah, Malaysia, October 2000, a meeting was held to analyse the feasibility of setting-up a 'Global Cocoa Programme'. The participants of this meeting identified some major priority areas related to cocoa genetic improvement that would deserve further international collaboration, ie. conservation, characterisation and distribution of genetic resources and development of new varieties with improved disease resistance and yielding capacity. The organisers have requested INGENIC to provide a first list of general priority areas regarding cocoa genetic improvement to be considered in such a Programme. These priorities would relate to activities that require international or regional collaboration. So far INGENIC has provided a short list of recommendations put forward during the INGENIC Workshops (summary listed in Annex 1).

During the General Assembly of INGENIC held on 16 October, 2000 in Kota Kinabalu it was agreed that INGENIC wishes to play an active role in such a Global Cocoa Programme. The role of INGENIC could mainly be as a working group to propose certain priority areas with regard to the genetic improvement of cocoa. Furthermore, INGENIC itself, as an international group, might also be considered as a part of the Programme.

Regarding the possible priorities in a Global Cocoa Programme, an inquiry was sent around by e-mail to 34 INGENIC respondents from different research institutes involved in cocoa genetics or breeding research. The respondents were asked to give priority levels, with feedback from colleagues of the contact person working at the same institute, to the items identified in the Tables 1 and 2 and to add any other general research area or type of activity related to cocoa genetics that would need to be considered in a Global Cocoa Programme. The priority levels were identified as 1 = high priority, 2 = average priority and 3 = low priority.

In Table 1, the flowchart of activities required in cocoa breeding is indicated (genetic resources, breeding methods or tools, practical breeding to create new varieties, distribution of improved varieties and breeding objectives). In Table 2, some existing and new activities are indicated that could be considered to become part of a Global Cocoa Programme. The list of activities indicated was based on conclusions and recommendations formulated during the three INGENIC Workshops held so far.

Results

A total of 32 persons participated in the formulation of the 19 replies received, involving 16 different institutes or organisations. The average priority levels attributed as well as the standard deviations (SD) are indicated in Tables 1 and 2. The SD values can be considered as a uniformity measure in the replies.

With regard to the flowchart of activities required in cocoa breeding (Table 1), all were considered as important. Within genetic resources, highest priorities went to characterisation, conservation and evaluation. Support to pre-breeding also received high scores. Among new conventional methods to be developed and used through a collaborative activity, priority went to evaluation of disease and pest resistance. Within the new biotechnologies, more efficient regeneration, QTL studies and mass production methods of cocoa clones were given higher priority. In order to enhance collaboration for development of new cocoa varieties, a network system is preferred with exchange of base cocoa populations between partner institutes. Within international support for multiplication and distribution of new varieties, priority goes to clonal varieties.

Within the breeding objectives of global interest (Table 1, item 8), first and second priorities go to disease and pest resistance, respectively. More variable responses were obtained for physiological traits, adaptation and flavour quality, suggesting that these criteria are more of regional or local interest.

The respondents unanimously gave high priority to the ongoing CFC/ICCO/IPGRI Project on "Germplasm Utilisation and Conservation" and to INGENIC to be considered as an integral part of a Global Programme (Table 2).

Among ideas for new collaborative initiatives, listed according to recommendations put forward during the three INGENIC Workshops held so far, first priority goes to long-term support for germplasm conservation, utilisation (practical breeding) and improvement of disease and pest resistance screening methods. Secondary priority goes to pre-breeding, transfer and use of genetic marker technology in breeding and to establish replicated progeny trials for QTL detection. Priorities given to other areas were more variable, resulting in higher average scores and SD values, and suggesting wider variation in opinion.

Additional suggestions and remarks received

Additional suggestions and observations received from individual respondents are listed below.

- Training of young scientists involved in variety improvement should also receive priority.
- On-farm trials of new cocoa varieties is an activity that should receive international support.
- Linkage between international and national research institutes is to be reinforced.
- Among breeding for yield, the yield components are to be considered separately.
- Managers of germplasm collections should consider conservation of cocoa populations as random seed samples.
- Logistics required for carrying out cocoa breeding need to be reinforced.
- The sink relation between pod production and vegetative growth needs special attention.

Table1: Type of activities considered as potentially important to deal with in a collaborative manner in a Global Cocoa Programme to enhance cocoa variety improvement.*

Type of activity requiring increased international or regional collaboration	Priority (1=high, 2=average and 3=low) Average	SD
1. Management and research on internationally available genetic resources		
1.1. Collection of new germplasm	1.7	0.8
1.2. Conservation	1.4	0.6
1.3. Characterisation (including identification)	1.3	0.5
1.4. Evaluation	1.5	0.7
1.5. Distribution	1.6	0.7
1.6. Database	1.7	0.7
2. Germplasm enhancement (pre-breeding) using internationally available genetic resources	1.6	0.7
3. Development and application of new but 'conventional' tools in cocoa breeding		
3.1. More rapid/reliable measurement of resistance to diseases and pests.	1.2	0.5
3.2. Measurement of physiological traits (yield efficiency, development of dwarfing rootstock, ..)	1.8	0.8
3.3. Creation and use of inter-specific crosses	2.3	0.7
4. Development and application of new 'biotechnologies'		
4.1. DNA markers for characterisation and genetic diversity studies	1.7	0.7
4.2. Detection and use of reliable quantitative trait loci (QTL)	1.6	0.6
4.3. An efficient system for genetic modification of cocoa	2.2	0.9
4.4. More efficient regeneration system for cocoa	1.5	0.6
4.5. Mass production of cocoa clones (*in vitro* or *in vivo*)	1.6	0.8
5. Development of improved varieties (international support for practical breeding)		
5.1. Internationally organised breeding centre (collaborating with local centres)	2.1	0.9
5.2. Regionally organised breeding centres (collaborating with local centres)	2.0	0.9
5.3. Collaboration in a network of national programmes (with international support and no exchange of germplasm)	2.6	0.6
5.4. Collaboration in a network of national programmes (with international support and exchange of base populations)	1.5	0.5
5.5. Collaboration in a network of national programmes (with international support and free exchange of any germplasm)	1.6	0.8
6. Support to national systems for production and distribution of new varieties		
6.1. Seed gardens (based on hand-pollinations)	1.8	0.9
6.2. Systems for mass propagation of commercial clones	1.6	0.8
7. Other activities suggested by respondents (see text)		
8. Breeding objectives within internationally supported activities		
8.1. Yielding capacity (productivity)	1.5	0.7
8.2. Production efficiency, including physiological traits like dwarfism, improved distribution of assimilates, ..	1.8	0.9
8.3. Improved disease resistance	1.1	0.2
8.4. Improved pest resistance	1.5	0.7
8.5. Adaptation to different environments	2.0	0.8
8.6. Technological quality (bean size, fat content)	1.8	0.7
8.7. Organoleptic quality (flavour)	2.1	0.8

*Based on suggestions made at INGENIC Workshops

- The selection of trees reproducing a dwarf growing habit should receive special attention to increase efficiency of cocoa production.
- There is not much future in inter-specific crosses, except for academic reasons.
- The validity of any study on QTL in cocoa depends on the correct plant materials and procedures to be applied.
- The situation of each cocoa producing country in terms of cocoa breeding must be analysed for aspects such as: needs, objectives, interest in research, government priorities, suitability of research units, human and financial resources, production level and prospects, private and non-official research, germplasm base in the country and long-term prospects.

PHOTO

Table2: Specific projects or activities that might be considered as part of a Global Cocoa Programme

Activity	Priority (1=high, 2=average and 3=low) Average	SD
A. Existing projects/activities		
1. CFC/ICCO/IPGRI Project on Cocoa Germplasm Utilisation and Conservation, a Global Approach	1.1	0.3
2. INGENIC organisation and activities (Workshops, Newsletters)	1.2	0.4
B. Suggestions for other specific projects/activities*		
1. Long-term funding for germplasm conservation, characterisation, evaluation and distribution	1.2	0.4
2. Long-term funding for practical cocoa breeding	1.3	0.6
3. Studies on mis-identification in collections	2.0	0.9
4. Improvement of early screening tests for resistance to diseases and pests	1.4	0.6
5. Host x pathogen interaction studies outside cocoa producing countries	2.3	0.8
6. Development of dwarfing rootstock aiming at increased yield efficiency	2.2	0.8
7. Pre-breeding programmes	1.6	0.8
8. Evaluation of level of heterozygosity and genetic distance between important cocoa genotypes	2.1	0.9
9. Evaluation of wide crosses between homozygous populations	2.2	0.8
10. Replicated progeny trial for QTL detection	1.8	0.8
11. Transfer and integration of marker technology in breeding	1.7	0.7
12. Consortium for gene-expression studies using micro-arrays	2.3	0.8
13. Development of an efficient genetic transformation system in cocoa	2.1	0.9

*Based on suggestions made at INGENIC Workshops

- Support is to be given to those regions that present much genetic diversity, such as the Brazilian Amazon. Existing collections in the Amazon need to be evaluated and further collecting is required.
- Africa deserves a regional programme for cocoa breeding.
- INGENIC should set up an advisory panel with experienced members to further discuss the role of cocoa breeding in a Global Cocoa Programme.
- Distribution of germplasm should be based on valid criteria. In the past, some populations were distributed that do not present the required genetic variability for breeding (e.g. RIM clones).
- Extension of existing collaborative projects deserves full attention.

Conclusions

The objective of this inquiry was to assess priority areas to be considered in a Global Cocoa Programme according to INGENIC respondents. Thirty-two persons participated in the responses received from 16 different national and international institutions. This represents an important part of, though not the entire, cocoa genetics research community. Several respondents suggested additional areas to be considered in a Global Programme.

Higher priority was generally associated with more uniform responses, whereas for lower priority the scores were more variable. This suggests that these activities are likely to be more of regional and local interest.

Uniform high priority was given to long-term support for cocoa germplasm conservation/evaluation, practical breeding and pre-breeding initiatives. The idea was strongly supported that INGENIC as a group be part of a Global Cocoa Programme. Improvement of methodology and breeding for increased disease and pest resistance are considered breeding objectives of major global importance. Within the "new technologies", priority went to regeneration and mass multiplication of clones and extended QTL studies.

The additional suggestions and remarks received from several respondents suggest that some priority areas might have been left out of the inquiry. This appears to be the case for example with relation to support for on-farm variety trials. It is to be noted that this and other more "agronomic" aspects related to cocoa breeding were not covered specifically during the three INGENIC Workshops held so far. These aspects should receive due attention during the next INGENIC Workshop.

Annex 1. Summary of main recommendations for international collaboration in cocoa genetic improvement identified at INGENIC Workshops

1. INGENIC Workshop on Cocoa Breeding Strategies (1994)
   - 1.1. Improved methods to measure physiological traits at individual tree level
   - 1.2. Improved methodologies for rapid disease resistance measurements
   - 1.3. Studies on mis-identifications in germplasm collections
   - 1.4. Evaluation of Genetic Resources (International Variety Trials)
   - 1.5. Long-term funding for Cocoa Germplasm Conservation
   - 1.6. Long-term funding for Cocoa Breeding Programmes
   - 1.7. Support for INGENIC

2. INGENIC Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement (1996)

   2.1. Evaluation of real losses incurred by diseases and pests
   2.2. Improvement of early screening tests for disease resistance (witches' broom, VSD, moniliasis)
   2.3. Studies on resistance of cocoa genotypes with fungal pathogens outside cocoa producing countries
   2.4. Exchange of germplasm aiming at accumulation of disease resistance genes
   2.5. Development of new cocoa tree architecture to increase photosynthetic efficiency and facilitate disease control
   2.6. Establishment of multi-disciplinary approaches to cocoa breeding
   2.7. Global approaches to cocoa germplasm utilisation and conservation
   2.8. Pre-breeding programmes

3. INGENIC Workshop on New Technologies and Cocoa Breeding (October 2000)

   3.1. A co-ordinated effort in the area of identification and misidentification of cocoa genotypes in collections, including a ring-test to verify repeatability of results
   3.2. Continuous collaboration for further evaluation of the level of heterozygosity of important breeding materials and of genetic distance between these clones
   3.3. International collaboration to evaluate wide crosses between naturally occurring homozygous populations (Amelonado, wild French Guiana, Criollo, Nacional,..)
   3.4. Replicated progeny trials of large size to take full advantage of QTL studies
   3.5. Exploitation of genetic linkage disequilibrium between molecular markers and agronomic traits in breeding populations
   3.6. Further development and transfer of simple marker technology to user countries and integration of marker technology in cocoa breeding
   3.7. Introduction of molecular marker information in international databases
   3.8. Creation of a consortium for gene-expression studies using micro-arrays
   3.9. Development of an efficient genetic transformation system in cocoa

Please send comments to:

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Conclusions and Recommendations from the INGENIC Workshop on New Technologies and Cocoa Breeding (October 16–17, 2000)

Session 1. Introduction and application of new technologies in plant breeding

Introduction

- Progress in breeding will be accelerated if breeders can integrate their activities with those of scientists working in other disciplines. The purpose of this Workshop was to bring together traditional breeders and experts in molecular biology to stimulate discussions on the potential of new technologies in cocoa breeding.
- There is much to learn from research in other crops, where productivity has been dramatically increased, 50% by genetic improvement and 50% by agronomic measures. In view of the low overall increase in cocoa productivity, there is a lot to be gained through genetic improvement.
- Efforts in traditional breeding needs to be maintained and strengthened; if this does not happen there will be no good platform to benefit from the introduction of new technologies. Effective collaboration between traditional breeders and biotechnologists is required to strengthen cocoa breeding as a whole.

Management of genetic diversity

- Molecular markers can be effectively used to verify mislabelling (SSRs, CAPs, SCARs), to evaluate genetic diversity and develop core collections (AFLPs, ISSRs and SSRs), and to search for candidate genes in germplasm collections (gene-specific PCR).

Marker assisted breeding

- Selection efficiency can be improved through the use of DNA markers associated with QTL or with candidate genes, particularly in introgressive breeding strategies.
- Replicated progenies, made up of a large number of individuals and planted at different sites, are required to take full advantage of QTL analysis. These should enable minor QTLs to be mapped and the stability across environments to be verified.

Genetic modification

- Although no commercially grown cocoa has been genetically modified, there has been a ten-fold increase in the acreage of other genetically modified crops over the last three years. Although the public is gradually becoming more aware of GM technology and its benefits, it should be noted that in a significant proportion of the chocolate consuming world, current consumer preference is for non-GMO products.

- In other crops, genetic modification has sometimes been targeted at benefiting the farmer, and in others, the consumer. Currently, most applications relate to the correction of only those genetic weaknesses of the crop that are controlled by one or a few genes (e.g. susceptibility to pests, diseases or stress conditions).
- Traditional breeding will continue to be very important to create improved populations and to handle traits determined by several genes.
- There is a trend towards the introduction of more than one gene into genetically modified varieties through the use of tissue specific and inducible promoter sequences.
- An efficient genetic transformation system in cocoa is required for research and significant breeding perspectives in the long-term future. However, it is essential that any such work is carried out in conjunction with appropriate studies of the impact of the genetically modified organism on the environment and with due consideration to consumer opinion.

Synteny mapping and genome sequencing

- The application of new technologies in cocoa breeding can benefit from the advances made for other crops through synteny mapping; the use of anchored points on the genome which can be used to relate cocoa genetic maps with the maps of other species.
- There is no urgent need for the comprehensive sequencing of the cocoa genome; it is probably better to use information from model crops (candidate gene strategy).

Collaboration between producing and consuming countries

- It is essential to establish effective collaboration between scientists in producing countries and non-producing countries.
- There is a need to develop low-cost, low-tech methods to enable laboratories in producing countries to carry out their part in collaborative studies and to enhance their capacity for innovative research.

Session 2. Identification and characterisation of cocoa genotypes

Main results obtained to date

- So far, various markers have been used: isozymes, RFLP, RAPD, AFLP, I-SSR and SSR. Protocols for sample collection, shipment and analyses have been developed.
- The use of microsatellites (SSR) is the way forward for finger-printing, in the short term, to provide anchor points for mapping populations and for studies using linkage disequilibrium to investigate origins of stocks and gene flow between populations. Researchers at CIRAD have made much progress in this area and have already developed 69 microsatellite primers.
- The USDA is embarking on a large project to genetically characterise the cocoa accessions held in the gene banks of the Americas using an automated microsatellite analysis system, which has the capacity to analyse 1500 samples/person/year.
- It is expected that 15 well chosen SSR will be sufficient for clone identification and characterisation purposes. However, a much larger number of well-identified SSRs are needed for mapping studies (see below).

Applications in cocoa breeding

- Resolution of mislabelling is a major issue for efficient management and transfer of germplasm, for reliable exchange of information on germplasm accessions, for multi-locational trials and, thus, for any collaborative efforts in cocoa germplasm conservation and utilisation.
- Progress made to date is still very limited in view of the importance of the problem.

Recommendations

- A globally co-ordinated effort is required in the area of identification and characterisation of cocoa genotypes in collections. This should include a ring-test to establish the compatibility of the automated system with gel-based systems for SSR analyses.
- Reliable comparison of results between laboratories and between different visualisation techniques will require the use of a common homozygous control clone, e.g. Catongo.
- Additional experiments are needed to refine the techniques. These will include i) the adaptation of the various gel-based systems (including techniques which do not involve the use of radioactivity so that the research institutes holding the gene banks can carry out the analysis themselves), ii) determination of the power of resolution of the technique (through sib-analyses) and iii) determination of the frequency of mutations and null alleles.
- It will be essential to identify a "type" specimen for each clone which can be used as a reference to compare all other accessions with the same name. The "type" tree must be selected by an expert, ideally from the original source gene bank. Efforts should be made to ensure that it is safeguarded through careful documentation, labelling and possibly cryopreservation.
- Four different options have been presented, with different roles for the collaborative institutes, and advantages/disadvantages compared to a globally co-ordinated effort on cocoa germplasm identification and characterisation.
- If successful, this would allow the research institutes to carry out their own within accession testing using the same SSR primers and make comparisons with the international "type" fingerprint.

- Molecular marker information should be introduced in a standardised form into international databases.
- Strategies are needed for dealing with the off-types detected following molecular characterisation. Genebank managers will have the responsibility of discarding or assigning an appropriate new name to any genotypes which do not conform with the "type" specimen. This information should be disseminated to the cocoa community through the INGENIC Newsletter, the International Cocoa Germplasm Database and other means.

Session 3. Genetic Diversity Analysis

Results obtained to date

- Various molecular techniques and methods of data analyses have been of value in assessing the genetic structure and diversity of cocoa populations. There may be some advantages in using a variety of markers since they may each reveal different parts of the genome.
- Results obtained in different genetic diversity studies, involving in total more than 1000 cocoa genotypes, were analysed during the workshop and the estimated level of heterozygosity of more than 600 genotypes are presented in these proceedings.
- Studies carried out with RFLP, RAPD and microsatellites at CIRAD Montpellier have shown that cocoa populations differ widely in their levels of diversity and heterozygosity. Upper Amazon Forasteros contain high levels of diversity and medium levels of heterozygosity compared to Lower Amazon populations and to wild French Guiana material, both of which exhibit low levels of diversity and heterozygosity. 'Ancient' Criollo types form a very distinct, uniform and homozygous group. These results would suggest that founder effects or refuge areas have played an important role in the evolution of *T. cacao* populations. So called Trinitario and 'modern' Criollo types appear to derive from hybridisation between 'ancient' Criollo and Lower Amazon Amelonado.
- RAPD analyses carried out in Trinidad suggest the existence of sub-groups within the Upper Amazon populations: (LCT-EEN+MO), (PA) and (IMC+NA+AMAZ). Scavina genotypes are very distinct. French Guiana materials show a very different RAPD banding pattern from other Forastero types. RFLP analyses carried out by Nestlé also identified genetic affinity among IMC and Pound clones (which are NA and IMC types) and among PA types, but again showed the Scavina clones SCA 6 and SCA 12 to be very distinct.
- RFLP analyses carried out by Nestlé showed that the original Nacional variety is rather homozygous and very distinct from Forastero and Trinitario types. Molecular analyses appear to confirm that many of the cultivated Ecuador cocoa types derive from hybridisation between pure Nacional and introduced Trinitario types.
- RAPD analyses carried out on the CEPLAC collection in Bahia, Brazil have shown continuous variation among the 270 genotypes analysed. A large degree of variation appears among the Upper Amazon types (mainly Pound collections) and among accessions collected from the wild in Brazil. Lower Amazon Amelonado types (Comun variety) appear to be very closely related and at the extreme of the distribution of Forastero types, nearer to Trinitario types. Scavina types form a distinct group at one extreme of the range of genetic diversity, genetically distant from the Lower Amazon and Trinitario groups, and close to some of the accessions from the Ucayali river in Peru. Several unique RAPD bands were identified in the Scavina clones, indicating their distinctiveness. Cultivated and wild genotypes from Ecuador tend to group together between Trinitario and Scavina types, respectively. As expected, clones of hybrid origin, such as CCN 51, tend to be located between the putative parents on genetic diversity maps.

Applications in cocoa breeding

- Information from molecular studies is very useful in managing diversity in genebanks to establish base/core/working collections, ensuring that collections cover the full range of diversity without over-representation of certain types and avoiding duplications. This is particularly important with regard to evaluation. Establishment of small representative core collections allows more extensive and uniform evaluation data sets to be assembled.
- Information on the genetic structure of cocoa populations, such as the level of heterozygosity and genetic diversity, is directly useful in breeding. It can be used to guide population enhancement or population breeding programmes, including reciprocal recurrent selection based on recombinations between heterotic groups.
- The information generated can also be of value in attempts to maximise heterosis, and thus produce superior hybrids, since genetically distinct parental genotypes can be identified.
- Cocoa breeding programmes have started to integrate the new information obtained from molecular marker studies to ensure that the diversity of the germplasm is utilised effectively. However, much information is lacking, particularly for the material held in national genebanks.

Recommendations

- Current findings, using isozymes, RAPD and microsatellite analyses, indicate that a large part of the diversity of cocoa has not yet been exploited in breeding programmes. Collaboration in distributing this germplasm and evaluating its potential through field trials is urgently required.
- Genetic diversity studies need to be continued to give more information on the genetic diversity present in cocoa genebanks, with special emphasis placed on the identification of 'core' collections.

- Continuous collaboration for further evaluation of the level of heterozygosity of important breeding materials and of genetic distance between these clones is required.
- The information on the level of heterozygosity can be used immediately to create new speculative crosses between genetically distinct and homozygous genotypes, which have not yet been used in cocoa breeding (such as crosses between French Guiana, Amelonado, some Upper Amazon clones, Criollo and Nacional types). Such progenies can be expected to be uniform and exhibit good hybrid vigour (as observed in 'single crosses' between improved pure lines in hybrid maize selection). International collaboration is required to create and evaluate such promising 'wide' crosses.
- On the other hand, selection of new clones would be favoured in crosses between heterozygous and genetically distinct genotypes of high agronomic value, facilitating recombination of complementary traits.

Session 4. Correlation of Molecular Markers with Economically Important Traits

Results obtained to date

- QTL for resistance to *Phytophthora* were identified in a collaborative project ('CAOBISCO project'). Co-localisations of QTL were observed for a number of progenies on chromosomes 1, 4 and 9 (coded according to the reference map established at CIRAD).
- One strong QTL for resistance to *Crinipellis* has been identified in SCA 6.
- QTL for agronomic traits such as yield, pod and bean characteristics were identified mainly on chromosomes 1, 4 and 5 in a few different studies. For yield, some variation in QTL was observed over the years. One QTL on chromosome 4 appeared to explain 43% of the phenotypic variation for pod size. A number of co-localisations were observed, mainly for related genotypes, but in some cases also for unrelated genotypes of Trinitario or Upper Amazon origin. These results suggest a certain stability of QTL in cocoa.
- In one study, a major QTL for general agronomic value was found that explained 27.1% of the total phenotypic variation and was co-localised with a QTL for early flowering and trunk diameter. Pleiotropic and epistatic effects were both detected for these traits.

Applications in cocoa breeding

- The QTL studies have provided useful genetic information on the genetic basis of several selection traits in cocoa. For example, the different QTL identified for *Phytophthora* resistance suggest that breeding approaches allowing for accumulation of different resistance genes could be successful.
- It was generally recognised that the available information is generally not yet sufficient for the direct use of the QTL detected in cocoa breeding.
- After obtaining more robust and stable QTL, the markers associated with the QTL can be used for Marker Assisted Selection (MAS). The technique can be used to accelerate breeding progress, since those plants with QTL for one or more desirable traits could be identified at the seedling stage. It is anticipated that the QTL/MAS systems developed could be transferable for use on similar progenies in other countries.

Recommendations

- A common chromosome identification system for cocoa is essential and it was agreed that the system developed by Lanaud and co-workers at CIRAD should be adopted internationally.
- The identification of QTL should be optimised for a limited number of selection traits. There is a need to choose strong QTL, such as those for significant levels of resistance to several pathogens or strains of the same pathogen. Information from markers can be combined with phenotypic data to obtain a selection index.
- Clonally replicated progenies, containing a large number of individuals, will be needed to take full advantage of the QTL analyses. Each progeny should consist of at least 200 individual plants. It is very important to replicate these progenies in different locations to map minor QTL and verify QTL stability across environments. This can only be achieved through international collaboration.
- Another approach to identify stable QTL is to use the possible linkage disequilibrium that may persist in certain genetically related cocoa populations, such as Trinitario or related Amazon populations (IMC, GU, etc.). Studies have been recently initiated for the Trinitario/Criollo group.
- Further development and transfer of simple marker technology to user countries is required before it can be integrated into cocoa breeding.

Session 5. Other topics

Resistance gene homology and micro-array consortium

- There are good indications that gene sequences detected in cocoa are similar to known resistance genes in other plant species. Ten putative gene candidates have been identified that probably belong to three families of potential resistance (R) genes. The main objective is to screen germplasm for different resistance alleles.
- Microarrays are miniaturised systems which allow the simultaneous measurement of the comparative expression levels of

thousands of genes in experimental and control material. This technique could be used to monitor gene expression profiles during growth and development and in response to biotic and abiotic stresses. This can provide leads to understanding basic molecular mechanisms, for example which pathways are up regulated in response to a pathogen and which are turned off. It also provides a means to rapidly identify candidate genes involved in a target process.

- Microarray systems for cocoa are being set up in several institutions and progress could be accelerated if a cocoa gene expression microarray consortium can be established. A bioinformatics resource base is needed to link data obtained by different research teams.

Applications in CSSV resistance studies and indexing

- Molecular cloning methods have enabled the isolation of full-length infectious clones of severe isolates of CSSV from Togo and Ghana. Mild isolates of the virus, which have potential use in cross-protection, have also been isolated. Infection of cocoa beans and young seedlings by particle bombardment and/or *Agrobacterium*-mediated infection is now possible. With these tools, specific virus inoculum can be quantified in challenging new cultivars in resistance breeding or cross-protection programmes.
- New CSSV-specific primers have been designed for disease indexing by polymerase chain reaction (PCR). Further development of this method is required so that it can form part of a quarantine procedure and thus help prevent the spread of CSSV.

Session 6. Propagation methods

Somatic embryogenesis (SE)

- SE is a powerful tool for multiplication, germplasm conservation (cryopreservation), germplasm exchange and genetic modification.
- SE technology is not yet ready for commercial scale multiplication of improved cocoa genotypes for farmers usage.
- SE is expensive but can be used for fast multiplication of a limited number of genotypes and the establishment of clonal gardens for further use with conventional propagation methods.
- SE protocols have been developed and the technology applied in at least nine laboratories around the world. Floral parts are the explants of choice.
- The protocols are similar in the use of 2-4D and cytokinin but differ in the use of basal DKW, MS and Woody Plants Media. The majority of the laboratories use DKW.
- Close to 100 genotypes have been propagated by SE with high efficiency being achieved for a number of genotypes. Conversion was achieved at 55-60%.
- Secondary embryogenesis is more efficient and produces unified embryos.
- SE field tests and DNA tests to verify agronomic value and genetic uniformity are required.
- SE is a potential tool for cocoa germplasm exchange. However, it is not yet known if SE propagated material is guaranteed virus-free (as is the case for zygotic embryos with CSSV). If so, SE could be of great help in speeding up the time involved in transfer of germplasm.

Semi-industrial scale of production of rooted cuttings

- The largest propagation centre 'Biofabrica' was established recently in Bahia, Brazil, using traditional technology adapted from eucalyptus mass propagation systems.
- Currently, this centre is propagating 14 cocoa genotypes with resistance to Witches' Broom disease. The rooting house and nurseries have the capacity for a daily production of 50,000 rooted cuttings but at present a shortage of cuttings is restricting the daily production to approximately 10,000 rooted cuttings.

PHOTO

Witches Broom

You are invited to contribute articles on research or other issues of interest to cocoa breeders/geneticists

REFRACTARIO - An Explanation of the Meaning of the Term and its Relationship to the Introductions from Ecuador in 1937

B.G.D. Bartley

The INGENIC Newsletter No. 5, of April 2000, contains observations on the nomenclature of the genotypes introduced by F.J. Pound into Trinidad from Ecuador in 1937, and it is time to deal with a subject associated with this introduction. This concerns the practice, adopted in recent years, of grouping all of the genotypes attributable to this introduction under the heading of "Refractario". Although it is not known how this practice originated, the manner in which the term is used gives the impression that the genotypes concerned belong to a single variety. This is far from the case and, since the word cannot be applied to the vast majority, if any, of the genotypes, its use is unfortunate and misleading. The confusion that has been caused is apparent in the work associated with determining the genetic status of individual genotypes by molecular analysis.

Since Pound was responsible for the introduction of this material, it is customary to base its history on his report of the programmes of collection carried out by him in Ecuador and Peru, and published in 1938. However, the first contact with the work in Ecuador was initiated by F. Stell, also of the Trinidad and Tobago Department of Agriculture, and Pound only became involved when Stell became ill. Stell's report on his work in some ways contains more accurate information about the varieties that were present in Ecuador at the time, and some of these details are repeated in Pound's report. Pound also wrote about his work in Ecuador in other reports and articles. These articles contain statements that sometimes vary from the details that are found in his 1938 Report so it is necessary to look at all of the evidence, including the details given by Stell, in order to form a complete picture of the activities carried out in Ecuador and the origins of the material that was introduced into Trinidad.

The word "refractario", the specific meaning of which, in this context, implies absence of reaction to the pathogen, was employed in Ecuador to designate trees which were observed to be free of infection by Witches' broom disease [caused by *Crinipellis perniciosa* (Stahel) Singer] in the years following the outbreak of the disease in that country, identified around 1918, and its subsequent rapid spread. The detection of these uninfected trees (or those with slight infection) was initiated around 1923, according to Stell's report, and these were the trees which received the designation of "refractario". Seedlings from fruits produced by these trees were raised in nurseries and subjected to natural infection. The plants which were not infected at this stage were established on various farms, all of which, we are led to believe, belonged to the same proprietor. Presumably, it was in these properties that Stell and Pound collected the seeds which were to produce the progeny that belong to this group of introductions in Trinidad.

The impression is given that the attribution of the designation "refractario" to a genotype is equivalent to considering it to be "immune" to the pathogen. In fact, anyone who has had sufficient field experience with the host-pathogen relationship will know that absence of infection at a given time or during a given period is no indication of permanent resistance. There are many factors that enable a tree to escape infection during a certain period and also to appear to differ in appearance from neighbouring trees in respect to the presence of infected tissue. Such factors include age and growth patterns, which could have been influenced by the system of cultivation practised in Ecuador during the period the disease appeared, as Pound described in the various reports. It was also admitted that, since the "refractario" trees were growing amidst susceptible trees, the fruits produced by natural pollination could have resulted from pollination from the highly infected neighbours. The evident heterogeneity of the plantations in which the uninfected trees were located probably would have resulted in the occurrence of significant variability among the progeny descended from them.

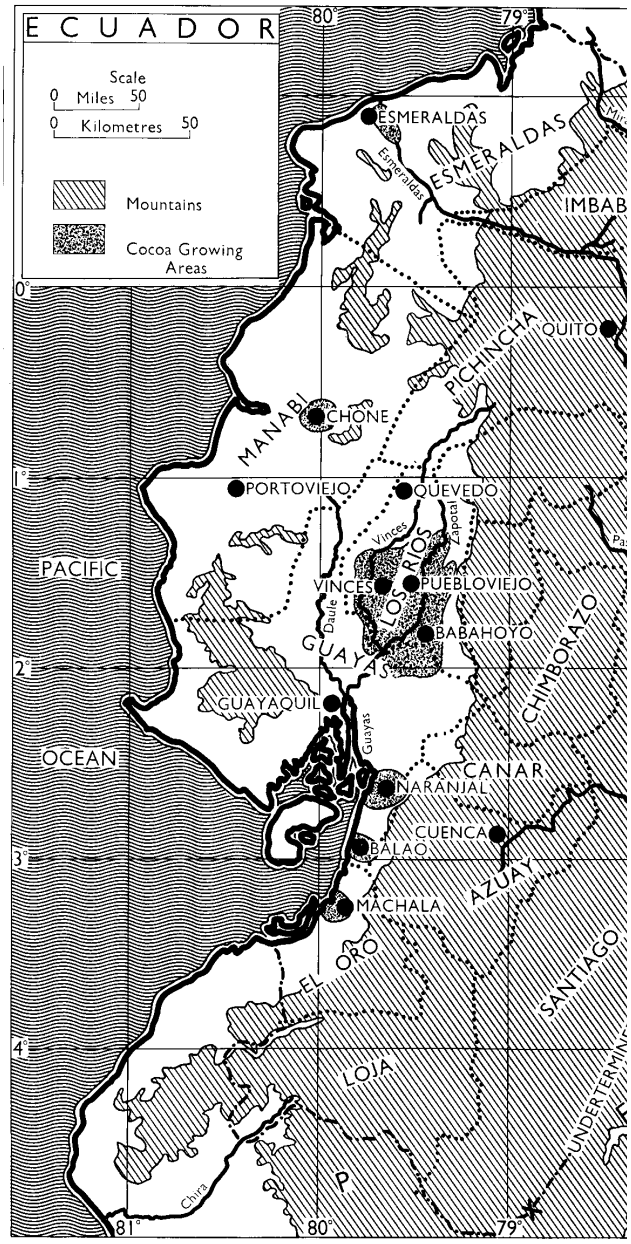
For our purposes, it is necessary to review the variety situation as it occurred in Ecuador at the time the search for uninfected trees was conducted. Some of this history is reviewed by Stell and Pound, but it may be helpful to add a further analysis of the situation as provided by a wider knowledge of the subject. The first published description of the situation in Ecuador was that of Preuss, who described the varieties he saw at the time that the original traditional population was effectively uncontaminated by introduced germplasm. Preuss indicates that the cacao cultivated (and also considered to be spontaneous) in the various regions of the Pacific Littoral of Ecuador belonged to a single variety. The application of the term "Nacional" to cover all of the cacao in the region would also give this impression.

However, this may not be a correct interpretation of the situation. The earliest photograph of cacao in Ecuador that I have seen dating from about 1882, perhaps from a place north-east of Babahoyo, shows fruits that differ in shape from those of the usual description of "Nacional". The trees depicted in a photograph of a field at Hacienda Clementina in 1911 also have fruits that differ essentially from the type shown to me at the same farm in 1963 as being genuine representatives of "Nacional".

In his 1938 Report, Pound refers to the narrow variability in the populations of "Nacional". He also hints at the differences between the types in the Balao region and those of the "Arriba" region. I concluded that the "Nacional" occurring around Balao and to the south were not exactly the same variety as those in the Guayas valley.

From about 1890, as stated in the Reports mentioned, other varieties were introduced into the region. This part of the history is not well documented and, consequently, there is much speculation as to what these may have been, the principal distinguishing influence of such introductions is the addition of types with red fruits. Preuss reported, "In recent years cacao was imported from Venezuela and Trinidad, but only in moderate amounts. I only came across the inferior Venezuelan varieties, Carupano and Trinitario. On the Hda. Elvira, on the Rio Caracol (a property of the Seminario family), I observed a very special variety.....I was told that it originated in Trinidad, but this must be a mistake, as this cacao does not exist in Trinidad."

Map of Ecuador showing Cocoa-growing areas



Source: "Cocoa-Growing in Venezuela, Colombia and Ecuador with notes on three cocoa diseases" by G.A.R. Wood, Cadbury Brothers Ltd, Bournville, England. 1959

Pound wrote, "..... a planter travelling back to Ecuador from Europe collected various pods from Trinidad and planted the seeds on his estate." The person is not named. The Annual Report for 1894 of the Royal Botanic Gardens, Trinidad, has an item referring to a visit in December by Baron Eggers. "He was accompanied by Señor Seminario from Ecuador who is largely interested in cacao in that country." The report mentions that the visitors described the "several varieties under cultivation (in Ecuador) which are not present in Trinidad." The existence at that time of introduced varieties is not mentioned, neither is there any reference to fruits or seeds being taken from Trinidad. If this had happened, the fruits may have been taken from the collection of cacao types at the St. Clair Station (which was the source of much of the genetic material supplied to the British Colonies and other countries during that period) but also, possibly, from private sources. However, it is more likely that seed was taken from Venezuela. The other possibility is that some seed may have come from neighbouring Colombia, especially as Preuss refers to Colombian overseers working at the Hda. Elvira.

There would also have been the preoccupation with obtaining what were considered to be the varieties which, during the period concerned, were famed as possessing the best qualities. This would have been the reason for introducing varieties from Venezuela and the appearance of references to the "Soconusco" type (as mentioned by Stell) would indicate the possible derivation of types from an introduction from Mexico or Guatemala. Preuss informs us that the Esmeraldas Criollo had been planted near Balao. The trees of these were short-lived but they would have survived long enough to have entered into hybridisation, the consequence of which would have been the appearance of other types, through recombination, which may have been included in the subsequent plantings referred to in the reports. Pound essayed a description of the Esmeraldas Criollo, but he obviously had not visited the region. He appeared to have been under the impression that the common variety occurring there was the same Nacional that he saw elsewhere. In this assumption, he was completely wrong. Up to fairly recent times, Esmeraldas was isolated from the main cacao growing zones of Ecuador and geographically, and in terms of cacao varieties, is connected with the Tumaco region.

In general, it would seem that the term "Venezolano" embraces a variety of types probably derived from different origins and, as the authors who knew the genotypes belonging to these introductions have described, it is not certain that they are directly connected with the Trinidad cacao population.

Another possible source of varieties is the Oriente or Amazonian region of Ecuador. The collections made in this region during the past fifty years provide a picture of its genetic diversity, knowledge that was not available to Stell and Pound. It is now possible to compare individual genotypes from the various populations with genotypes that are present in the cultivated populations. Thus, the comparisons made so far lead to the conclusion that the inclusion of Amazonian types in the populational complex, that existed in the littoral zone at the time the search for resistance to Witches' broom disease was being initiated, cannot be ruled out.

The appreciation of the existence of the complex varietal situation in the 1920s is essential to understanding the origins and characteristics of the trees found to be free of infection by *Crinipellis perniciosa* to which the term "refractario" was applied. Although Pound provides some description of the genotypes involved in the application of the term "refractario", the details given by Stell are less complicated and presentable in a succinct form. This identifies the following four varieties that may be presumed to have been the ancestors of the progenies concerned in the introduction to Trinidad.

Presumed ancestors of introductions into Trinidad from Ecuador (1937)

Type A

A tree identified by Sr. Carlos Seminario in 1923 - very healthy of a type of "Cojon de Toro". Seedling progeny practically immune, but of low market value. Unfortunately the characteristics of the tree concerned are never given. ("Cojon de Toro is a name used in Venezuela applied to types probably related to the Trinidad "calabacillo"). Preuss provides a description of the type to which the name was applied within the complex called Carupano or Trinitario, the variety also being classed as Trinitario amargo, fruit "a red or reddish brown, very smooth, blunt or short pointed". (However, we do not know if the Ecuador tree conforms to this description as the "calabacillo" types mentioned by Pound with white based fruits would also be named "Cojon de Toro").

Type B

In 1924, another tree was found to be quite immune, the type being called "Zambo" in Ecuador, being an importation from Venezuela. The leaves are quite different from other varieties being dull and very dark green, the bark of the tree is very rough and the cushions very pronounced. The "genuine" tree is immune, but progeny are attacked to some extent, moreover quality is poor (The name "Zambo" suggests a hybrid origin of the type, but Preuss lists among the Carupano complex a class called "Sambito").

Type C

Later another variety was detected which is not classified in normal terms. It has round leaves and very rough bark. When the pods are green, the colour is so light as to be almost white. Even when the pods are ripe, the yellow colour is lighter than the other varieties. The variety is called No. 5 and is highly resistant, and the quality is superior to the calabacillo and Zambo (Types A and B).

Type D

Another type was called "Soconusco" (a name of the region, which in pre-colonial times and during Spanish colonial times - formerly in Guatemala and now in Mexico, was renowned for its product and would belong to the Criollo group), the quality of which was considered to be the best discovered so far - but Stell does not refer to its reaction to the pathogen.

It is important to consider carefully these descriptions since, if the progenies imported by Pound are descended from these sources of "resistance", the characteristics should be identifiable in those cases where any of such progenies are the result of inbreeding.

After he concluded his explorations, Pound stated the following: "In searching for witchbroom resistant trees in Ecuador, planters have come to the conclusion that no very striking resistance is to found among the high quality "Nacional" types, but certain trees of the introduced types did show some resistance. I examined the fields of seedlings from some of these trees and found that some ten per cent of six-to nine-year-old seedlings were probably very highly resistant and also heavy bearers." *"Although I saw certain trees which bore pigmented pods exhibiting some resistance, the highly resistant trees in the young plantings all gave unpigmented pods and in some cases extraordinarily large pods for Trinidad types."* (The word "all" is of significant importance).

Stell sent to Trinidad, or brought with him on his return, seeds presumably from the varieties he mentioned. These were established at the Imperial College of Tropical Agriculture. I do not recall seeing the original progenies, but they were described by Posnette, who carried out some pollinations on the trees. I expect that these progenies were in the field to the west of the sugar factory. This field contained a number of different selections and introductions, some of which, in retrospect, would be valuable or interesting components of a germplasm collection. However, little attention was paid to most of this material, which is now irreparably lost.

It would appear that the plants established belonged to two families (from the different numbers attributed to the plants) each family of progenies being derived from a single fruit. Some of the individuals of one family were cloned and the resulting clones were established in another field on the Imperial College of Tropical Agriculture Campus, these being identified by the letter "E". I know of three such clones of which two have red fruits, the characteristics of which as far as I can remember would place them as progenies of Type A, that is, the "Cojon de Toro" or "Calabacillo". If the assumptions are correct, these clones should represent the best examples of material derived from "refractario" trees but they are, unfortunately, neglected when the subject of the "refractario" programme in Ecuador is considered.

Although we may have been given some idea of the genetic background of the genotypes introduced into Trinidad by Pound in 1937, the analysis of the genetic composition of this collection of genotypes is hampered by the existence of ambiguities in the reports and the absence of information regarding several important aspects of the general subject. It may be difficult to list all of the details that a plant breeder may require about the origins, relationships and characteristics associated with the families derived from the parent trees listed in the previous paper (INGENIC Newsletter, Issue 5), and the individuals belonging to these families. A few of the most important factors to be considered are given below, constituting an attempt to make sense of the material that we have inherited.

Some important factors pertaining to the genotypes introduced by Pound into Trinidad from Ecuador in 1937:

1. The locations where the uninfected "refractario" trees were growing are never given. Pound ventured the ambiguous statement of "one or two planters" noticed that occasionally a 'Venezuelan' tree would show very little infection." It would seem certain that the main location was the Hacienda Balao, a fact that is essential to understanding the genetic composition of the genotypes resulting from this work.
2. In addition, the types involved are not adequately described. Stell refers to "a plant" of the Calabacillo type while Pound stated "several trees of Calabacillo type (Cojon de Toro)" and "quite a number of these trees were located". The number of

trees involved has a bearing on the subsequent situation since, according to Pound, a small percentage of the seedling progeny submitted to infection in the "eliminatory nurseries" were used for planting. It is difficult to imagine how the small number of original "refractario" trees could have produced the quantities of fruits to give the one million plants that were established in the few years between the start of the selection programme and Stell's visit to Ecuador, unless the programme involved other, unknown, trees.

3. We do not know anything about the composition of the populations cultivated in the farms in which the parent trees were growing or the manner in which the introductions from which they are suspected of having been derived were planted; that is, for example, whether the "Cojon de Toro" trees were planted together or scattered among the older "Nacional" trees. In view of the fact that the seedlings planted in the eliminatory nurseries came from open-pollinated fruits, this consideration is important in determining the compostion of the progenies that were supposedly uninfected and planted on the other farms visited by Pound. Considering Points 2 and 3 it seems possible that these progenies included types other than those described above.

4. The evident uncertainty of the pedigrees of the trees from which Pound collected the material sent to Trinidad coupled with the fact that, except in rare cases, he never described the individuals involved, results in as much difficulty in the identification of the genotypes established at Marper Farm as that involved in the determination of the composition of the original introductions into Ecuador of the varieties grouped under the general title of "Venezolano" and others of unknown origin.

5. The exact status of the trees involved in this programme with regard to their reaction to *Crinipellis perniciosa* is often in doubt. The terms "immune" and "resistant" are used confusedly. While a casual reading of the reports may lead to the impression that the term "refractario" was applied to trees with absence of infection, consideration of the various statements that specifically relate to this situation leads to the conclusion that the truth seems to be quite different. Pound includes the mention that several other trees were considered to be "refractario", but later found to be just as susceptible to the pathogen as the general population, stating that these could no longer be called "refractario". From this we can conclude that the term cannot be applied to any plant which later became susceptible although once having been distinguished for the absence of infection.

6. I believe it is probable that most of the work of the resistance programme was carried out on the Hacienda Balao, the parent "refractario" trees being located there or on neighbouring farms. The question could be asked, "If these parent trees existed during Pound's visit, why were they not included in the collection sent to Trinidad?" Pound stated that all of the fruits involved came from seedling progenies. The fact was that these parent trees were infected at the time and, as Pound indicated, they should no longer be referred to as "refractario". The possibility has to be considered that some of the samples from Hda. Balao may have come from trees with a higher degree of resistance since I observed a promising degree of reaction to the pathogen in trees belonging to such families as B 13 and B 18. For this reason, such trees were incorporated into the breeding programme in the 1960s, and a few hybrids with them were planted in trials at Las Hermanas.

7. In the same way that no details are given regarding the composition of the population at the location at which the parent "refractario" trees were present, there are few details as to the actual composition of the plantations at which the progenies selected by Pound were established. It is indicated that in some cases they were planted in blocks but under the older "Nacional" trees. This situation is important in that fruits of any of the progeny trees selected, which were derived from hybridisation, may have been pollinated by the "Nacional" trees. Accordingly, the progenies of these fruits in Trinidad should express a greater similarity to the "Nacional" variety group than the other progenies whose fruits had resulted from pollination with trees descended from "Venezolano" or other introduced types. We must also take into account the statement Pound inserted into the 1938 Report, the importance of which could be overlooked, "....the few 'Nacional' refactario trees found." Could the progenies of these also have occurred in the fields examined?

8. The programme carried out at Hacienda Clementina was started just after Señor Seminario's programme. It is important to pay attention to the fact that it was independent of the latter. The variety on which the selection of resistant trees was based was described by the manager, Mr. Schuldt, (according to Stell) as "Venezuelan Nacional", which implies that it was of hybrid origin. The description given by Pound also makes it clear that it is completely different from the Balao varieties, and also that there was a low level of infection on the selected trees, none of which could have been considered as justifying the application of the term "refractario". In my opinion, the Clementina selections should not be called "refractario". With regard to the selections made on this farm, Pound makes special reference to tree No. 8. Pound described the fruit of this tree as being, "long oval smooth white base type, more than partially pigmented" in contrast to the other seven selected trees that were of "a half blanco, long oval, warty type not very far removed from Nacional in quality". Pound then stated that "among the progeny (there exists) an unduly large proportion of types which show either pigmentation or pod type characters of No. 8, which suggests that seedlings of No. 8 are more resistant than seedlings of the other trees." These observations imply that the progenies of each of the parent trees were planted separately in the block of progenies, said to number 40,000, but we still do not know how many of the original selections were sampled. At least, with regard to the "CL" families, there is some indication of the characteristics that may be expected. Another aspect of this work at Clementina

is that we do not know how these plants are related to the selections reported later by Prof. Müntzing. Among the latter is one selection which had been planted, as a clone, in a fairly large block, which in 1963 showed a very low level of infection and had desirable traits for cultivation. The leaves of this plant appear to me to resemble those described by Stell regarding the variety referred to as Type C.

9. In the 1938 Report, Pound stated that fruits were collected from "some" eighty trees, each being a seedling progeny of a "refractario" type and was 5 - 10 years old. In another statement, reproduced above, the age range is given as six to nine years. In my opinion, if the fruits did come from trees that were uninfected, this situation may have been due the trees having escaped infection as a consequence of the combination of their early age and other factors that Pound himself described. The whole question as to whether the trees were really "refractario" is in doubt on account of the confused statements regarding the ability of trees to become infected in the circumstances he found. Although the purpose of the visit to Ecuador was to obtain material with potential resistance, Pound also made it clear that he also took productivity into account when making his selections, a trait that may have received greater emphasis. If the progeny have inherited the scale of productivity suggested, they would provide a potential use for breeding independently of the disease reaction.

10. Although Pound does make the recommendation that the apparently resistant trees should be propagated vegetatively, there is no evidence that this was ever done. Apart from three selections from Hda. Clementina (and not necessarily those described by Pound) and only one from Vuelta Larga, none of the locations of Pound's parent trees is to be found in the list of the clones in the EET series of selections made about 10 years later. The EET clones include several from Tenguel, the farm of the United Fruit Company, which would have started a selection programme about the time of Pound's visit to Ecuador. Among these selections are several outstanding ones such as EET 103, the fruits of which correspond in some respects to the large, unpigmented fruits described by Pound on trees "exhibiting some resistance". Several of the introduced genotypes answering to this description appear to be closely related. The connection between the UFCo. programme and Pound's visit is probable since Pound acknowledged the assistance given by the Company.

11. Besides the description of the Clementina programme, the only location of the Seminario properties he refers to is Hda. Amalia. In this case, he singled out one tree without infection of the few such trees among those he selected. However, he does not tell us which of the two trees selected was this tree. It is suggested elsewhere that some investigations on resistance were being conducted on this farm, but the sample is very small to reflect any positive results from such work.

12. In his report on the state of establishment of the introductions at Marper Farm, Pound listed about 25 clones that he considered to be worthwhile reproducing. However, although he refers to plans for planting blocks of these clones, there is no indication that such blocks were established but the possibility that some cloned progenies may be available in, as yet, unknown locations cannot be ruled out. Previously, Posnette included 10 of the Ecuador introductions in the material he sent to West Africa; these included three of those on Pound's list. Some descriptions of the clones despatched were given, which may help in the identification of the existing clones. The early performance of the Ecuador progenies at Tafo was very poor compared with the other varieties comprising the shipment. The Marper collection was disregarded until 1952, when Dr. Cope and I started evaluating the trees, work that was suspended on account of the Colombian expedition. In the 1960s, it was possible to return to this evaluation when unlimited access of UWI staff was allowed. During the subsequent years, 32 clones were established in The University of the West Indies Field Station Germplasm Collection and other clones were in the nursery plots on the UWI campus. Descriptions and evaluation records are available for most of these genotypes. A few selfed progenies were obtained and should have been established at the Las Hermanas Station.

13. There are two possible problems related to the identification of the Ecuador genotypes. One is that, since the original seedling progenies were established at Marper Farm as budded plants it is possible that some of the existing plants are really rootstocks which would belong to the Trinidad population. It may be impossible to distinguish these from any Ecuador hybrids descended from the "Venezolano" group. The other situation to be considered is that, although the Ecuador genotypes were planted exclusively in Blocks A and B, some of the Peru introductions were later planted in the same Blocks (to fill in gaps) and the possibility arises of misidentifications owing to the laxity of the labelling and recording systems, such as confusing "MO" and "MOQ".

It is hoped that there is enough evidence to to show the dubious nature of the association of the progenies introduced into Trinidad with the term "refractario", and it would be appreciated that the term should be forgotten.

Certainly, it should be clear that in terms of varieties there is no such thing as a "refractario" variety or genetic group. If the progenies that were established in Trinidad are descended exclusively from the original "refractario" trees or varieties, we are concerned with at least six types having distinct origins, not counting the male parents of the fruits produced during the two generations that would have produced the genotypes in Trinidad. For this reason, it is necessary to consider each family individually and attempt to relate it to the original varieties, as described in the article in INGENIC Newsletter No. 5. It would be impossible to provide an analysis of the genotypes involved without seeing the whole collection together. The molecular studies that have been reported give some indications of possible relationships. It seems that some types over several localities have a common constitution but there are others that appear to be very distinct, even in relation to the other elements of the cocoa diversity. However it is too early to arrive at any definite conclusions.

Possible assignments of some individual genotypes to original varieties

We can see a few possibilities for assigning the individual genotypes to the original varieties or pollen parents. If we are to accept Pound's account of the Clementina work and description of the progenies, it would be possible to find that the CL families are closely related. It would appear that AM 2 is more like a Nacional and the same applies to JA 5; both having probably two doses of pollen from this variety. They also have the large seeds and fruit characteristics that distinguish clones like EET 94, EET 96 and EET 103. The indications are that genotypes from other families also possess similar traits. A common trait of this collection of plants appears to be the very thick husk of the fruits and this would possibly have been inherited from the Nacional parents.

Plants from LP 3 and LP 4 have leaf characters that are often found in Criollo types. Criollo traits are also noticeable in the JA 1 family. It is possible that SJ 1/19 belongs to the Trinidad population.

Among the more outstanding families are those from Hda. Moquique (MOQ), and this location was the most represented in the original University of the West Indies collection. I suspect that this farm did not belong to those in the other group. There appears to have been a considerable influence of Criollo traits in the selected trees. The fruit of MOQ 4/17 is similar to some of the Colombian types. However, within the given family there are genotypes with characteristics far removed from those that approach the Criollo-like types. With such situations occurring, it is evident that the matter is more complex than the attribution of a single title to all of the genotypes represented.

Terminlogy

The readers whose knowledge of the subject is limited to the reports of Dr. Pound would have found that he consistently used the word refactario (which I have retained in the quotations) while in this article the term "refractario" has been used. The latter is the correct word and occurs in the Spanish translation of an article, of which Pound was the author, published in the magazine LA HACIENDA in April 1944, while refactario is not found in the dictionary.

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Estimation of the Level of Heterozygosity of 570 Cocoa Clones using Isozyme Electrophoresis

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The International Cocoa Genebank, Trinidad (ICG,T) contains approximately 2300 accessions, which are currently being characterised, evaluated and distributed to cocoa breeders worldwide. One of the techniques employed for characterisation is Isozyme Electrophoresis (I.E.). Five enzyme systems (ACP, ADH, PGI, IDH and MDH) are currently being used for characterisation of the accessions at the genebank. The genetic models of these systems were deduced by Lanaud (1986), and show polymorphisms at, at least, one locus each, representing a total of five heterozygous loci. To date, 570 clones have been completely characterised for these five Isozyme systems.

In a previous study, an assessment of the level of genetic diversity existing within and between the populations present in the ICG,T (Sounigo *et al.*, 1997) was undertaken using data obtained from I.E. analysis. In this paper, an attempt is made to estimate the level of heterozygosity present in the 570 characterised accessions. Although the study is somewhat limited due to the low number of loci studied, it is hoped that the results may still be of use to cocoa breeders. Indeed, the estimation of the level of heterozygosity of these accessions could guide breeders in their use as parents. Parents with a low level of heterozygosity are expected to produce rather homogeneous progenies, which is a desirable characteristic if the progenies are intended to be distributed as varieties. On the other hand, highly heterozygous parents are expected to produce rather heterogeneous progenies, which can be very useful for selection of individuals with particular traits.

The clones in this study are classified according to number of heterozygous loci, i.e. 0, 1, 2, 3, 4, and 5 (homozygous, poorly heterozygous, fairly heterozygous, heterozygous, highly heterozygous and very highly heterozygous, respectively), and are presented in Tables 1-6. The frequency (number and %) of clones from 8 groups, at each level of heterozygosity, is presented in Table

Table1: Homozygous accessions (no heterozygous locus)

AGUACARTEP1	LCTEEN67
AM1/87	LCTEEN127
AMAZ5/2	LCTEEN203/S3
AMELONADO3/4/6	LCTEEN327
BANANACREEK3P1	MAR3
BANANACREEK3P2	MAR9
BANANACREEK5P1	MAR10
BANANACREEK8P1	MAR11
BOB8	MAR12
CATONGO	MAR13
CERROAZUL10	MAR14
CERROAZUL11	MAR21
CL10/14	MAR22
CL19/2	MO109
CLM59	NA92
CRIOLLO22	NA118
CRU105	NA178
DOM21	NA286
DOM24	NA289
DOM27	NA311
DOM30	NA337
DOM31	NA359
DOM35	NA528
ELP21/10	NA534
ELP21/20	NA672
GS13	NA685
GS17	NA708
GU151/F	NA750
GU255/P	NA766
GU265/P	POUND16/B
GU286/P	PA70
GU307/F	PA13
GU322/P	PA134
IBHIGHFORESTP1	PA141
IBHIGHFORESTP2	PA189
IBHIGHFORESTP3	PA194
IBN°1P1	PA195
IBN°2P1	PA279
IBN°2P2	PA296
IBN°2P3	SCA3
IBN°2P4	SCA6
ICS8	SCA20
IMC38	SPEC41/6
IMC77	SPEC54/1
JA2/24	TRD6
JA9/16	TRD9
JA9/37	TRD16
LCTEEN31	TRD112

7. A comparison of the levels of heterozygosity for 25 common clones using I.E. and RFLP is depicted in Table 8.

The rather low numbers of accessions representing the Criollo, the French Guiana and the lower Amazon Forastero groups hinder us from making decisive conclusions about these. However, from the results presented (Table 7), all of the Criollo and two thirds of the French Guiana accessions appear to be completely homozygous (no heterozygous loci). The levels of heterozygosity for the remaining French Guianese accessions were poor or fair. More than half of the Upper Amazon Forasteros and about one third of the Lower Amazon Forasteros were either completely homozygous (21% and 22%, respectively) or poorly heterozygous (36% and 11%, respectively). The distribution of the accessions in the Refractario and Trinitario groups spanned the whole range of classes, with 73% and 61%, respectively, having heterozygosity levels ranging from fairy heterozygous to highly heterozygous.

The results indicate that accessions from the Criollo, French Guiana and most of the Upper Amazon groups could be useful parents for the production of rather homogenous progenies suitable for use as varieties. Most of the Trinitario and Refractario accessions would be more useful as parents for the production of heterogeneous progenies, suitable for individual selection. In addition, selection within the Refractario and the Trinitario groups for bean quality and resistance traits could yield very interesting results. Further selfing and crossing between them would then make it possible to accumulate quality traits inherited from their Criollo and/or Nacional genetic background and resistance traits inherited from their Forastero genetic background.

A comparison of the levels of heterozygosity observed in 25 accessions by I.E. (current study) and by RFLP (Laurent *et al*., 1995) is shown in Table 8. No correlation was found between the two sets of results (R=0.17). Some accessions, which were found poorly heterozygous with I.E. (ICS40, ICS89, PA20), were found to be highly heterozygous with RFLP, while accessions found heterozygous with I.E. were poorly heterozygous (OC61, ICS100) or even homozygous (SC6) with RFLP. However, all the accessions found very heterozygous in our study (GS29, GS36, ICS48, ICS84, RIM113, RIM19, SC5 and UF667), were also highly heterozygous when analysed with RFLP. In addition, both studies revealed very low levels of heterozygosity in the accessions from French Guiana. The Criollo accessions analysed in our study, most of which were collected in Belize, were all found homozygous. This agrees with the findings of Motamayor (1997), who also found very low levels of heterozygosity in Criollo accessions using RFLP analysis.

Discrepancies between the results of I.E. and RFLP may be due to the problem of mislabelling of trees (Figueira, 1998; Anon., 1999). For a valid comparison between both systems, the analysis would have to be carried out using the same tree or on trees where the same fingerprinting pattern has been confirmed.

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Table2: Poorly heterozygous accessions (1 heterozygous locus)

AM2/62	IMC58	NA771
AMAZ10/1	IMC59	POUND2/B
AMAZ12	IMC96	POUND15/A
AMELONADO	IMC98	POUND26/C
B7/38	IMC107	POUND27/C
B13/5	IMC60	PA16
B2/31	JA1/4	PA18
CC17	JA3/2	PA20
CL10/26	JA10/41	PA27
CL10/33	LCTEEN 46	PA44
CL13/2	LCTEEN 61/S-5	PA46
CL19/36	LCTEEN 83/S-8	PA51
CLM41	LCTEEN162/S-1010	PA84
CLM49	LCTEEN163	PA107
CRU50	LP1/9	PA12
DOM15	LP1/10	PA124
DOM16	LP4/5	PA125
DOM4	LV10	PA136
DOSHERMANOS	LV20	PA165
E34	LV33	PA171
EET397	LX38	PA200
ELP38/15	LX48	PA300
GS12	MO4	POR3
GS4	MO9	SCA5
GS48	MO28	SCA24
GS61	MO83	SLA8
GS62	MOQ5/23	SLA16
GS71	MOQ5/28	SLA20
GS78	MOQ6/30	SLA53
GU114/P	MOQ6/99	SLA66
GU219/F	NA3	SLC8
GU243/H	NA7/10	SLC18
GU261/P	NA12	SPA18
GU271/P	NA26	SPA8
GU277/G	NA40	SPEC138/8
GU300/P	NA45	SPEC138/15
GU305/P	NA46	THY2/12
GU310/P	NA90	TRD1
ICS24	NA168	TRD13
ICS28	NA170	TRD41
ICS40	NA181	TRD42
ICS41	NA186	TRD44
ICS58	NA191	TRD45
ICS82	NA283	TRD46
ICS89	NA326	TRD53
ICS99	NA342	TRD86
IMC2	NA399	TRD88
IMC14	NA471	TRD109
IMC16	NA730	TSH1077
IMC50	NA763	VENA58
IMC53	NA770	

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Table3: Fairly heterozygous accessions (2 heterozygous loci)

ACT2/6	ICS14	NA34
ACT2/13	ICS16	NA70
ACT2/18	ICS26	NA110
AM1/57	ICS30	NA149
AM1/107	ICS43	NA155
AM2/12	ICS53	NA176
AM2/21	ICS65	NA268
AM2/83	ICS75	NA327
AMAZ3/2	ICS95	NA717
AMAZ6	ICS83	NA780
AMAZ15/15	IMC12	NA794
AX11/89OP	IMC23	NA831
B2/34	IMC27	PA30
B7/3	IMC30	PA34
B7/14	IMC42	PA61
B8/5	IMC45	PA66
B14/9	IMC65	PA90
B17/17	IMC76	PA118
B18/4	IMC78	PA120
B18/5	IMC94	PA187
B20/2	IMC97	PA191
B22/3	JA5/28	PA211
B22/8	JA9/23	PA218
B23/6	LCTEEN15/S3	PA291
CC9	LCTEEN23	SCA9
CL13/4	LCTEEN72	SJ1/11
CL13/17	LCTEEN85	SJ1/37
CL19/22	LCTEEN201	SJ1/39
CL27/53	LCTEEN326	SJ1/40
CL27/58	LP1/58	SLA44
CL27/109	LP2/14	SLC7
CLM35	LP3/35	SLC24
CLM100	LP4/12	SPA9
CRUZ7/8	LP5/1	SPA12
CBO177	LX1	SPA20
DOM1	LX6	SPEC137/6
DOM3	LX28	SPEC138/17
EET156	LX32	SPEC138/21
EET272	LX45	SPEC184/2
EET338	LZ2	STAHEL
EET395	LZ5	THY23/111
EET401	LZ33	TRD1
AGU3339	MAR3	TRD2
AGU3339/8	MAR19	TRD3
AGU3339/12	MO14	TRD5
COCA3343/2	MO20	TRD28
SANMIGUEL3360/3	MO81	TRD37
GS77	MO125	TRD60
GU241/P	MOCO1/12	UF38
ICS1	MOQ6/73	UF700
ICS5	NA32	UF709

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Table4: Heterozygous (3heterozygousloci)

AM1/28	JA3/9
AM1/53	JA10/12
AM2/38	JA10/42
AM2/53	LCTEEN6/S-1
AX169/54	LP1/43
B2/16	LP1/61
B7/21	LP3/4
B13/7	LP3/29
C7/11	LP4/7
CC71	LP4/43
CC37	LV27
CC39	LV28
CC49	LX39
CL9/17	LX43
CL10/3	LX47
CL10/10	M33
CL10/11	MAN15/60
CL10/5	MATINA1/7
CL13/65	MOQ2/26
CL19/10	MOQ5/5
CL19/21	MOQ5/29
CLM11	NA68
CLM19	NA157
CLM99	NA246
CRU147	NA753
CRUZ7/11	NG3
DOM25	OC61
EET400	POUND4/A
SANMIGUEL3087/3	POUND9/B
EQX3164	POUND18
FSC13	POUND18/A
ICS10	PA3
ICS15	PA169
ICS29	RIM6
ICS45	RIM8
ICS57	SCA19
ICS67	SJ2/19
ICS76	SLA13
ICS92	SLA54
ICS100	SLA93
IMC3	SPEC194/39
IMC49	TRD43
IMC57	TRD66
IMC61	TRD81
IMC63	TRD85
IMC73	TRD118
IMC103	UF11
IMC104	UF12
JA3/7	UF705

Table5: Highlyheterozygous accessions(4heterozygousloci)

AM1/3	LZ8
AM2/1	MOQ3/8
AX122/72	MOQ6/102
EET19	NA258
EET59	NA406
EET95	PA184
EET399	RIM10
COCA3310/3	RIM19
GS26	RIM24
GS29	RIM39
GS36	RIM41
GS46	RIM48
ICS2	RIM71
ICS48	RIM102
ICS61	RIM106
ICS84	RIM113
IMC54	SC1
IMC105	SC4
JA1/19	SC5
JA5/2	SC6
JA5/5	SC15
JA5/7	SC17
JA5/9	SC19
JA5/24	SC20
JA5/25	SCA23
JA5/27	SJ2/2
JA5/36	SJ2/22
JA5/39	SPA10
LP4/10	TRD33
LX16	UF613
LX53	

Table6: Veryhighlyheterozygous accessions(5heterozygousloci)

C99
LCTEEN73/A
LCTEEN246
UF667

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Table7: Levelsofheterozygosityobservedinthedifferentgroupsof cocoa.

Numberof heterozygousI.Eloci	0	1	2	3	4	5	Total
Criollo	15	0	0	0	0	0	15
	100%	0%	0%	0%	0%	0%	
LowerAmazon	2	1	3	3	0	0	9
Forastero	22%	11%	33%	34%	0%	0%	
Refractario	7	32	47	37	20	0	143
	5%	22%	33%	26%	14%	0%	
UpperAmazon	40	68	54	20	7	2	191
Forastero	21%	36%	28%	10%	4%	1%	
Trinitario	21	36	33	27	28	2	147
	14%	25%	23%	18%	19%	1%	
FrenchGuiana	21	10	1	0	0	0	32
accessions	66%	31%	3%	0%	0%	0%	
Unknown	1	0	0	5	0	0	6
	17%	0%	0%	83%	0%	0%	
Hybrid	0	0	15	6	6	0	27
	0%	0%	56%	22%	22%	0%	

Table8: Comparisonofthelevelsofheterozygosityobservedwith I.E andRFLP

	Numberof heterozygousI.E loci	%of heterozygous RFLPloci
IMC78	2	24
LCT326	2	25
MO9	1	22
NA32	2	24
PA20	1	38
SCA6	0	15
SCA9	2	28
CC39	3	30
GS29	4	42
GS36	4	35
ICS16	2	31
ICS48	4	33
ICS53	2	33
ICS75	2	21
ICS84	4	38
ICS89	1	44
RIM113	4	43
RIM19	4	33
RIM8	3	37
SC5	4	39
SC6	3	0
UF667	5	37
ICS100	3	23
ICS40	1	56
OC61	3	13

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Cocoa Cultivar Distinctiveness and Hybrid Prediction using RAPD Markers

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Introduction

Cocoa (*Theobroma cacao* L.), despite its economic importance, lacks a formal committee that registers and documents new cultivars, in addition to supervising the supply of genetic material along with relevant information to cocoa producers. The establishment of such a committee would require the development, standardisation and definition of procedures to be used for cultivar differentiation. The cultivars should be protected by international laws, using the criteria of distinctiveness, homogeneity and stability (DHS). This study focuses on the first of these criteria, using information generated using molecular markers.

Cocoa cultivars planted in Brazil for the last three centuries belong to the Amazon Forastero racial groups known as 'Comum', 'Pará' and 'Maranhão'. Between 'Pará' and 'Maranhão', there are other types, such as 'Parazinho', probably derived from 'Pará', and 'Maranhão Liso' and 'Maranhão Rugoso', both derived from 'Maranhão'. The expansion of cocoa cultivation in the recent decades required the development of high yielding hybrid cultivars, and resulted in the development of hybrids involving crosses between local and introduced clones. Heterosis has been exploited commercially in Brazil and Trinidad since 1960. Nowadays, Malaysia and Indonesia use clonal plantings with great success. In Brazil, cocoa producers and breeders anxiously anticipate utilising this strategy. Cloning on a commercial scale allows the fixation of desirable hybrid mixtures. With clonal planting, the development of procedures and methods for differentiation and registration of clonal cultivars becomes necessary. The results of studies such as the one described here can contribute to such activities.

Until relatively recently, cocoa cultivars were differentiated mainly on the basis of morpho-agronomical characteristics that are highly influenced by environment. Therefore, the phenotypic distance may not consistently reflect the genetic relationships among cultivars. To overcome such a constraint, the use of DNA markers, which are uninfluenced by the environment, was introduced as a precise and accurate yardstick for quantifying the distance among cultivars thus revealing their distinctiveness. The genetic distance estimates express the difference between any two cultivars on the basis of the allelic variation of polymorphic loci, and their use in the differentiation of cultivars is growing (Staub *et al.*, 1996).

Heterosis for precocity, high bean yield, disease resistance and some other characters has been explored in cocoa. Although the hybrids have heterozygous clones as parents (non-conventional hybrids), commercially they are generally propagated through seed, and more recently using cuttings. While selecting the parent clones, attempts are made to overcome deficiencies by crossing parents from distinct geographical origins and racial groups. This strategy compliments selection based on phenotypic traits. Random crosses using the trial and error technique are also made, especially if information about the parent clones is not available. It must be noted that the cocoa tree has a long juvenile period (4 to 5 years) before coming into production. Thus, hybrid progenies can be selected only after the 8th or 9th year in field. Therefore, the development of a method that applies the estimates of genetic distance among possible parents becomes essential. Such a method can predict the performance of a hybrid without the need for crosses and progeny evaluation. The measures of distance are usually based upon pedigree information, such as the relationship coefficient, multivariate analysis of quantitative characters, or analysis of molecular markers.

Molecular markers directly and thoroughly sample the genome. The type of marker used is not as important as their number and the genomic cover that it can provide. The measures of genetic distance based on Restriction Amplified Polymorphic DNA (RAPD) markers (Williams *et al.*, 1992) appear very attractive. RAPD markers have the advantage of relatively low cost, simplicity, rapidity and operational automation for a large number of samples (Dias, 1995). This article demonstrates the application of information generated by RAPD markers for quantifying the genetic distance among cultivars and thus predicting the heterosis of the hybrids among them and allowing the differentiation of cultivars.

Distinctiveness of cultivars

The cocoa cultivars evaluated for differentiation, the field experiment and procedures for data collection were reported earlier (Dias *et al.*, 1998). The five cultivars used were 'Maranhão', 'Pará', 'Parazinho', an open pollinated 'ICS 1' and the commercial hybrid mixture. The experiment was set up in February of 1982, in a 5 × 5 latin square design, with 196–plant plots. The harvest was monitored monthly for over a period of 10 years (1984 to 1993).

For RAPD analysis, fresh and healthy mature leaves were collected from all five cultivars. For each cultivar, a bulk of DNA, constituted by 6 to 10 cocoa trees, was formed. Such leaves, properly identified, were taken to the Agricultural Applied Biotechnology Institute (BIOAGRO) of the Universidade Federal de Viçosa (UFV), and the DNA was extracted in agreement with Doyle & Doyle's (1990) method, with modifications. The amplification reactions were performed with a reaction volume of 13 µl. A total of 182 polymorphic bands, coded as 1 for the presence and 0 for the absence, were used for distinctiveness of cultivars. By using Jaccard's coefficient (Dias, 1998), the genetic similarities (GSij) were computed between all the pairs of cultivars i to j. The measure

of genetic distance (GDij) was obtained as the arithmetic complement of the genetic similarity, in other words, GDij = 1 - GSij. The GD matrix was submitted to cluster analysis following the UPGMA algorithm (Dias, 1998). To evaluate the robustness of the clusters formed, the binary dataset was submitted to bootstrapping. The data were reconstructed 2,000 times (replications) by resampling the bands with replacement. In that way, the number of times, expressed as a percentage, that a cluster is repeated can be considered using a non-parametric statistical test (with confidence limits), which checks the validity of the various clusters.

Hybrid prediction

Five cocoa cultivars from the S_0 (clones) and S_1 generations were studied. The clones belong to the germplasm collection of CEPEC. The S_1 cultivars, together with their 20 hybrids, belong to a 5x5 diallel and have been described earlier (Dias & Kageyama, 1997). In both cases, the cultivars used were 'CC 41', 'SIAL 169', 'CEPEC 1', 'ICS 1', and 'SIC 19'. The diallel was set up in 1975, in Itabuna, Bahia, in a randomised complete block design with four replications and 16-plant plots.

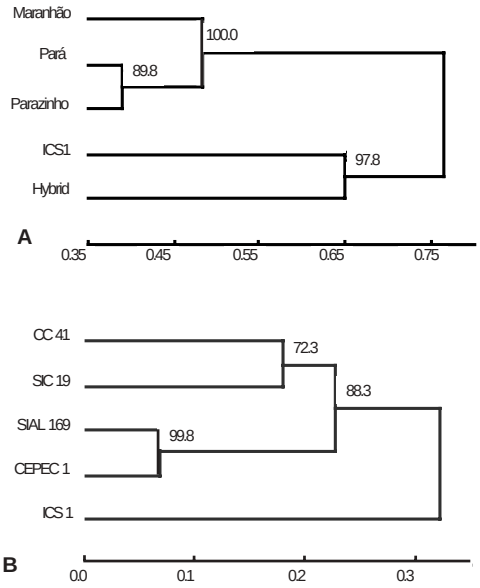
For analysis, 130 RAPD bands were used (Dias & Resende, 2001). The Nei & Li coefficient was used to compute genetic similarity between all the cultivar pairs (i to j) (Dias, 1998). The measure of genetic distance (GDij) was obtained as complement of genetic similarity as described above. Using the distance matrix, a dendrogram was constructed according the UPGMA algorithm (Dias, 1998). Rank correlation coefficients were computed between GDij and the Mahalanobis' distance (MDij), previously obtained by Dias & Kageyama (1997) with the employment of five yield components and, between these matrices and the average and heterotic performance of the hybrids for each yield component.

Results and Discussion

Distinctiveness of cultivars

The genetic distance among the cultivars varied from 0.39 for 'Pará' and 'Parazinho' to 0.83 between 'Maranhão' and 'ICS 1'. The dendrogram (Figure 1A) shows the formation of two clusters, one composed of traditional, unimproved cultivars 'Maranhão', 'Pará' and 'Parazinho', and the other formed by two improved cultivars (open pollinated 'ICS 1' and the commercial hybrid mixture). However, the Pará-Parazinho cluster was not confirmed because the bootstrap P value was less than 95%. The traditional cultivars belong to the Amazon Forastero racial group, whereas ICS 1 is a Trinitario - a hybrid group between Forastero and Criollo. The hybrid mixture possesses the genes from local and introduced Trinitario clones. ICS 1 and the hybrid mixture had a better performance and temporal stability of yield than the other three cultivars during the 10-year (1984–1993) study, as showed by Dias *et al.*, (1998). These results suggest that the RAPD markers are able to distinguish cocoa genotypes, which have undergone different degrees of improvement.

Figure 1: Genetic distance among cocoa cultivars based on RAPD data applied to differentiating cultivars (A) and hybrid prediction (B). *P* values of bootstrap are shown on the corresponding node of each cluster.



Hybrid prediction

The genetic distance (GD) among the five clones varied from 0.07 for SIAL 169 and CEPEC 1 to 0.36 for SIAL 169 and ICS 1. The maximum relative distance occurred in ICS 1 and the only confirmed cluster was that of SIAL 169 and CEPEC 1 (Figure 1B). The results (GD) obtained through RAPD were partially in concurrence with those obtained through yield components (MD). There was moderate correlation (r) between the GD and MD matrix (r = 0.67, P=0.03) (Dias & Resende, 2001). The GD was moderately correlated to the heterotic hybrid performance only for wet seed weight per plant and per fruit (0.73, P< 0.05 and 0.65, P< 0.05, respectively). This is similar to the values reported by Dias & Kageyama (1997) using MD for the same yield components (0.66, P< 0.05, for both). However GD did not correlate to mean hybrid performance considering the same yield components (0.61 and 0.62, respectively).

The agreement between distance matrices obtained from phenotypic data (MD) and RAPD (GD) was not perfect. A close correspondence between the two should not be expected, since the two datasets represent different genomic parts and are subjected different sources of error. In addition, GD and MD were just satisfactory predictors of heterotic performance of hybrids, indicating that divergence and heterosis do not only associate linearly. It appears that genetic distances based on molecular markers are good predictors of hybrid performance only under very specific situations. Moreover, since the association procedure worked for this small diallel, used as a model, it is expected that it should also work for the prediction of hybrid performance from crosses among a larger number of cultivars. The small diallel that could be analysed limits the scope of the conclusions, but the results do encourage more studies.

For cultivar distinction, the genetic distance calculated through RAPD appears to be adequate for use in differentiation, and also for the fingerprinting of cocoa genotypes. According to Wilde *et al*., (1992), only one primer of RAPD allowed unmistaken characterisation of 10 accessions of *T. cacao* L. Errors of identification of clones simultaneously planted in Malaysia and Brazil can be adequately verified by using RAPD markers (Figueira, 1998).

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Pre-Budwood Collection Twig Manipulation: A New Procedure For Increased Budding Success

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Introduction

The heterogeneity inherent in cocoa seedling populations makes the clonal propagation of materials with desirable qualities imperative. This is often achieved through vegetative methods such as stem cuttings, grafting, patch, green, or side-cleft budding. Budding techniques are preferred for the better field establishment of plants due to the well-developed taproots of rootstocks, and the higher multiplication rate compared to that for rooted cuttings. The rate of budding success is, however, very low; about 15%–30%. This is due to the low percentages of bud-take and sprouts of new growths from buddings. A trial was conducted at the Cocoa Research Institute of Nigeria (CRIN) to determine the effects of some pre-budwood collection treatments on budding success.

Manipulation Methods

Semi-brown twigs, from which budwood was to be collected, were subjected to four different treatments on different days before collection on the day of budding. The conventional method of budwood collection on the same day of budding was used as a control. Budwood was collected at 2, 4, 6, 8 and 10 days after treatment (DAT). The trial was a 5 × 5 factorial experiment laid out in a randomised complete block design (RCBD) with five replications.

The treatments were:

a. whole leaf clip - entire leaf blade clipped leaving only the petiole.
b. half leaf clip - half the size of the leaf clipped.
c. apical bud clip - terminal bud portion of the twig clipped.
d. wounding - ringing the twig by making a shallow cut round the circumference into the wood, but not so much as to interfere with the xylem flow.
e. Control - semi-brown twig left intact and budwood collected on the day of budding.

Bud-take was assessed on opening of the budding tape 14 days after budding

Results and Discussion

The mean count of bud-take due to each treatment expressed as a percentage is presented in Table 1. The bud-take ranged from 24%–88% over all the treatments. However, bud-take

was always highest for the wounding treatment across all the days of study, ranging from 74%–88%. It was always lowest for the whole leaf clip treatment, ranging from 24%–52%.

Analysis of variance (ANOVA) in Table 2 showed that methods of twig manipulation had a significant effect ($P<0.001$) on bud-take. Bud-take was also significantly affected by the number of days after treatment. The interaction effect of the method and days after treatment was not significant ($P<0.609$).

Budding success was assessed by the number of sprouts and green buddings four weeks after opening (Figure 1). The wounding treatment had a significantly higher budding success (46%–72%) than the control (26%–54%) over all the days studied. The highest budding success was recorded for budwood collected 2 days after treatment.

The results of this study showed that the wounding treatment applied to twigs, even 2 days before budwood collection, greatly enhanced cambial activity necessary for successful bud union with the rootstock. It is suggested that the break in phloem transport downstream and accumulation of photosynthates and endogenous substances on the twig to be severed initiated and increased cambial activity and processes associated with callus formation, which are necessary for successful union and consequently, budding success.

Table 1: Mean 'bud take' (%) resulting from treatments at different days before budding

	Days before budding				
Method	2	4	6	8	10
Whole leaf clip	52c	50b	30b	24c	40b
Half leaf clip	78ab	56ab	26b	42bc	58b
Apical bud clip	62abc	54ab	38b	50b	50b
Wounding	82ab	74a	68a	76a	88a
Control	72abc	62ab	48ab	40bc	56b

Means not followed with the same letters are significantly different.
l.s.d. ($p<0.05$) = 22.0

Table 2: Analysis of variance for bud-take

Source	df	MS	F	P
Method	**4**	**47.66**	**15.01*****	**0.0001**
DAT	4	31.06	9.80***	0.0001
Block	4	89.04	28.10***	0.0001
Method*DAT	16	2.745	0.87^{ns}	0.609
Error	96	3.169		
Total	124			

DAT = days after treatment
Method = treatment

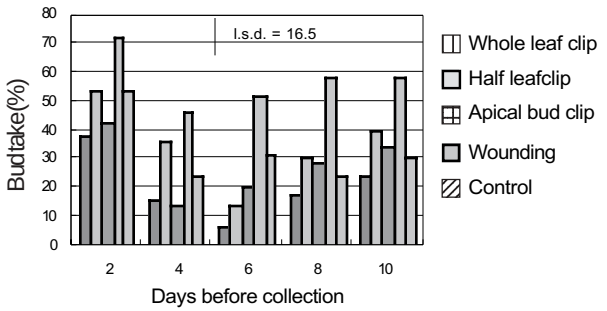

Figure 1: Sprouts and green buddings (%) of different pre-budwood collection treatments

Four weeks after opening

Other researchers have reported success with pre-treatments applied before grafting. Mishra (1982) found that defoliation of scion shoots 2 weeks before grafting resulted in better union and active division of the cambial tissue. Dhuria *et al.* (1977) also achieved 88.9% successful grafts in persimmon, *Diospyros lotus* L., as a result of incubator storage of grafts for 2 weeks before planting.

Conclusion

From this study, it is shown that pre-treatment of twigs before collecting budwood from cocoa trees resulted in greater bud-take hence, budding success than the conventional method of patch budding. Wounding the twig by making a shallow cut round the circumference through the bark into the wood, even 2 days before collecting budsticks for budding, resulted in a higher level of bud-take hence, budding success than the conventional method of budstick collection on the day of budding. This underscores the advantage of pre-treatment of twigs before budwood collection in cocoa.

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***Editor's note**: The following article, written by Dr. Bartley in 1969, is presented below in its original form (with few amendments) as it covers a subject of considerable interest to cocoa researchers. It was written shortly after POUND 31 became available for research at the Cocoa Research Unit, having been established at the University of the West Indies Field Station Collection. Dr. Bartley was unable to photograph the fruits of SCA and POUND types referred to in the text, as initially planned, due to his departure from Trinidad in 1970.*

The Origin and Compatibility Relationships of the Scavina Variety of *Theobroma cacao* L.

B.G.D. Bartley

Following the occurrence and spread of Witches' Broom disease, caused by *Marasmius perniciosus* Stahel (now *Crinipellis perniciosa* (Stahel) Singer) in cocoa plantations in Trinidad in 1928, a search was instituted for resistant trees in an effort to control the disease. Reports of resistant ("Refractario") trees in the cocoa-growing areas of Ecuador where the disease was present in epiphytotic proportions since 1918, encouraged the Department of Agriculture of Trinidad and Tobago to take steps to introduce the resistant material into Trinidad in order to alleviate the problem.

In 1937, F.J. Pound visited Ecuador and collected material from several plantations, which appeared to be resistant. He observed that one resistant type was not typical of the cocoa varieties cultivated in Ecuador, and hypothesised that this type was an introduction originating in the Amazon Basin to the east of the Andes. In the following year, he travelled up the Amazon River to Iquitos and studied the cocoa growing in the area including the tributaries of the Rio Marañon. Pound (1938) described the background to the disease problem and descriptions of the cocoa varieties seen in Ecuador and Peru.

The material collected in both countries was established (after quarantine) at Marper Farm, in the eastern part of Trinidad, for observation of the disease reaction under conditions of severe incidence of the fungus. All material collected was in the form of seed, and the plantings consisted of the vegetative progeny of the seedlings. The progenies of each type or location collected were separately designated, but were planted at random in the field. No records are available to indicate the exact origin or parentage of each family and, in some instances, the group designation gives no clues as to the identification of the material described in Pound (1938). The characteristics of the offspring of parent types frequently deviate from the corresponding descriptions in the report since they are descendants of the open-pollinated fruits of largely self-incompatible trees.

Exposure to natural infection for several years indicated that resistance to Witches' Broom disease was present in one family referred to as SCAVINA (SCA). Attempts to utilise this resistance in clonal populations of the selections from this family, SCA 6 and SCA 12, were not successful as the very small seeds were not acceptable commercially. Hybridisation of the two clones with susceptible clones was initiated with the objective of combining disease resistance with larger seed size. These hybrids exhibited hybrid vigour for yield and combined a certain level of resistance with a satisfactory seed size. The good combining ability for yield factors of the SCAVINA family led to the widespread use of its segregants as parents of hybrid varieties, which have been planted on a wide scale in many cocoa-growing areas. Much breeding work in progress is based on the use of this source of genes.

The clones SCA 6 and SCA 12 were shown to be self-incompatible (Cope, 1959; 1962). Tests of Witches' Broom infection on the clones, their hybrid progeny and advanced generations show that the clones are heterozygous for resistance factors. Because of the considerable attention which this family commands and the heterozygosity exhibited by its members, the origin and collection of the parent type is of great interest. The family to which this designation was given is one for which no record of origin is available and it was not clear whether it was collected in Ecuador or Peru, although some reports refer to it as being Ecuador. Posnette (1951) stated that "Those called "Upper Amazon" were the progeny of types collected by Pound [13] from the Iquitos, Nanay, Morona, Parinari, and Scavina regions of Ecuador[1]." It is obvious that he made a mistake and wrote Ecuador instead of Peru.

While the material collected in 1937 and 1938 were under observation at Marper Farm, a further visit was made to the Iquitos area in 1943, when the trees seen during the 1938 exploration were re-examined for their status of infection by *Marasmius perniciosa*. On this occasion, trees, which continued to be free of infection, were sent to Trinidad via quarantine. The budded progeny of each selection was planted at River Estate, another station of the Department of Agriculture. The planting appears to have been neglected until the middle of the 1950's during the period when River Estate was the experiment station of the Cocoa Research Scheme of the Imperial College of Tropical Agriculture (ICTA, now the University of the West Indies). The conditions under which the trees were growing precluded any proper identification and study, especially as they were buddings onto unknown rootstocks. Cuttings were made of each tree and planted in the cocoa plots at ICTA. These trees have been kept under observation and have been included in the breeding programme.

Again, the report of the 1943 trip (Pound, 1943) contains no information which connects the selections made at that time with any of the material introduced into Trinidad in 1937 and 1938. The author observed that one of the types in this collection was different in appearance, tree habit, as a cutting and flowering pattern from the other selections. This outstanding variety is Pound 31, which is described by Pound (1943) as being a large tree, which grew at Contamana on the Rio Ucayali.

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[1] Presumably, the juxtaposition of "Scavina" and "Ecuador" has led various authors to interpret the sentence as stating that the Scavina family originated in Ecuador. It is obvious, from its inclusion among the "Upper Amazon" types, that the origin of Scavina was in Peru. This is definitely confirmed by the report of Pound cited by me in INGENIC Newsletter 5.

The observation was also made that the characteristics of this variety resembled those of the Scavina family, which is also distinctive in foliage, flowering and fruit characters. The cuttings of SCA trees are relatively slow growing and, in the first years, grow as compact, low bushes, eventually tending to produce longer branches. The leaves are relatively small and are dark green in colour being quite distinctive in this respect. The flowers are borne in fairly compact cushions and the buds are almost unpigmented and small with a blunt apex. Flowering tends to be heavy in May, which is not usual with other cacao varieties in Trinidad. The fruit also is distinctive with a very intense green colour when unripe with some anthocyanin pigmentation occurring on the ridges. The fruits are elongate with a longish but blunt point. The seeds are small and cylindrical in shape with darkly pigmented cotyledons.

Comparison of some of the Scavina types and Pound 31 showed that, in most cases, the descriptions of the two types matched. Pound 31 has a slightly larger fruit than SCA, which is more sculptured and narrower.

It was thought that the best indication of relationship would be demonstrated by the compatibility between the 13 sibs which exist in the Scavina family, of those which were first introduced, and Pound 31. Cross pollinations have been carried out and so far have given the following results (Bartley, 1968):

- Pound 31 compatible with SCA 6, 12, 9, 11, and 19
- Pound 31 incompatible with SCA 23

Selfing of Pound 31 indicates that the clone is probably self-incompatible. Although SCA 6, 12, 9, 11 and 19 may not be progenies of the POUND 31 tree used, SCA 23 and other SCA incompatible with it are progenies of it or related to it as male or female parent. If it is assumed that Pound 31[2] possesses two S alleles, as in the case of SCA 6 and SCA 12, it is probable that all of its offspring must possess one allele in common with it and that they must be the products of fertilisation by one or more neighbouring trees, so that the other allele is contributed by the male parent. Since there is no evidence to show that all the SCA sibs are descended from the same male parent, it is evident that more than three incompatibility alleles are involved in the Scavina family of which one is contributed by Pound 31.

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[2]Slight morphological differences were found between POUND 31A, B and C, which may also differ with regard to their compatibility alleles.

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Agronomic Assessment of Wild Cocoa (*Theobroma cacao* L.) Trees from the Camopi and Tanpok Basins (French Guiana)

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Introduction

Numerous studies of the wild cocoa trees from south-eastern French Guiana (Lachenaud and Sallée, 1993; Lachenaud *et al.*, 1997) have revealed the distinctiveness of this germplasm within the "Forastero" group (Lanaud, 1987; Lachenaud and Sallée, 1993; Laurent *et al.*, 1994; N'Goran *et al.*, 1994, Lachenaud *et al.*, 1997; Sounigo *et al.*, 1997, 1999; Lachenaud *et al.*, 1999). Nevertheless, before it can be used for genetic improvement, it must be described, characterised and evaluated, particularly for selection criteria.

Work on the characterisation of wild Guianese material has been underway since 1988 in French Guiana (Paracou-Combi reference collection). The aim is twofold:

- to gain further knowledge of the variability in this material, and
- to identify origins that are potentially useful in breeding to provide practical indications for breeders who wish to use them.

Only the latter aspect is covered in this article along with

results of ten years' agronomic assessment of progenies from wild cocoa trees collected in 1987 in the basins of the Tanpok and Camopi Rivers. The traits used, which are all selection criteria, were vigour, earliness of production, yield, the yield:vigour ratio (i.e. "cropping efficiency"), pod size and resistance to rot in the field (caused by various species of *Phytophthora*).

Material and methods

Planting material: 144 open-pollinated progenies (around 1600 individual trees) from wild cocoa trees originating from two basins in the far Southeast of the French Guiana and belonging to the following wild populations (Lachenaud and Sallée, 1993):

- River Tanpok: population 5;
- River Camopi: populations 1, 3, 6, 7, 8, 9, 10, 11, 12, 13.

Planting design: 7 blocks, planted in 1988 (0.99 ha, 3 m × 2 m), monitored for 10 years. Analyses of variance (unbalanced incomplete blocks) were carried out on the 7 most represented populations (1, 3, 5, 7, 9, 12 and 13, i.e. 97 % of the trees at 10 years). Progenies with fewer than 5 trees were taken out of the analyses.

Agronomic descriptors (per tree basis)

- Juvenile growth, i.e. increase in stem cross-section (15 cm from the ground) between 1 and 2 years in the field.
- Adult vigour, i.e. stem cross-section at 10 years, 50 cm from the ground.
- Yield, expressed as the weight of healthy pods, or as a potential, if rotten pods are taken into account.
- Earliness of production and overall yield were obtained by cumulating the figures for 5 and 10 years.
- The yield: vigour ratio (i.e. "cropping efficiency" ratio of potential yield cumulated at 10 years to the cross-section 50 cm from the ground at 10 years).
- The occurrence of rotten pods, taking a minimum yield of 50 pods per tree.
- The average weight of one pod, taking a minimum of 20 healthy pods per tree.

Results

Analyses of variance revealed significant "population" and/or "progeny" effects in all traits. Although our results (Tables 1 and 2) were obtained in a single environment, they should help geneticists who have Guianese material at their disposal or who wish to acquire it to make a choice. To facilitate that choice, Table 3 provides a list of the populations and progenies, and Table 4 indicates the correspondence between the material mentioned in this study and the sib material (half or full sibs, derived from pods collected from the same mother-trees) already supplied to several producing countries.

Table1: Means (adjusted to the blocks) of the main 7 populations (and plot mean) for 7 traits. The values indicated for potential yield, potential yield/cross-section and earliness are given in kg of pods (* = population taken out of the analysis to satisfy homogeneity of within-population variances)

Population	Juvenile growth (cm^2)	Adult vigour (cm^2)	Yield (Kg/tree)	Yield: vigour (Kg/cm^2)	Earliness (Kg/tree)	Average pod weight(g)	% rotten pods
1	13.8 a	87.7 a	18.2 a	0.17 a	1.2 ab	435 a	1.3 bc
3	13.5 ab	87.4 a	11.1 a	0.11 a	0.8 ab	322 d	1.4 bc
5	13.4 abc	85.2 ab	14.9 a	0.14 a	2.6 *	351 c	3.6 a
7	10.2 d	75.7 b	20.4 *	0.21 *	3.0 *	380 b	1.6 b
9	11.6 cd	83.2 ab	15.7 a	0.15 a	1.2 ab	359 c	1.0 bc
12	12.1 bc	88.1 a	16.1 a	0.17 a	0.6 b	316 d	1.2 bc
13	12.7 abc	82.6 ab	16.9 a	0.17 a	1.3 a	386 b	0.6 c
Mean	12.3	85.1	16.4	0.16	1.2	367	1.2

Table2: Range of individual means per progeny and ranking of some GU progenies for 7 traits.

Traits	Range	Best progenies	5 last progenies
Juvenile growth	0.4 – 21.9 cm^2	264, 287, 340, 238, 186	291, 313, 350, 354, 126
Adult vigour	11.8 – 131.5 cm^2	178, 323, 285, 174, 163	350, 304, 344, 345, 313
Yield (Potential)	0.0 – 68.4 kg	285, 303, 143, 280, 134	244, 250, 222, 113, 282
Yield: vigour ratio	0.0 – 0.50 kg/cm^2	134, 303, 285, 139, 325	313, 194, 250, 113, 282
Earliness of production	0.0 – 8.7 kg	134, 143, 139, 116, 146	***
Average pod weight	200 – 510 g	285, 212, 274, 161, 157	311, 162, 218, 205, 230
% rotten pods	0.0 – 9.2 %	240, 134, 325, 321, 252	186, 157, 129, 146, 116

(***: 21 progenies yielded 0)

Table3: Distribution of GU XXX progenies per population

Populations	GU progenies
1	116, 156 to 161 and 250 to 286
3	218 to 241 and 347 to 349
5	113 to 116 and 123
7	126 to 152
9	162 to 198, 242 to 249, 297 to 330 and 350 to 355
12	203 to 217
13	287 to 295 and 331 to 346

Yield: vigour ratio

Population 7 is the most worthwhile for this paramount selection criterion in cocoa improvement. Population 3 was the least promising in terms of this trait.

Earliness of production

The most precocious population was 7, followed by 5. The best progeny produced the equivalent of 1,275 kg of dry cocoa per hectare, cumulated over 5 years. Population 12 seemed to be particularly late.

Table 4: Correspondence between the GU XXX progenies studied (A) and those supplied to certain producing countries and quarantine centres (B), where Δ = no corresponding material outside French Guiana.

A	B	A	B	A	B	A	B	A	B
GU113	GU114	GU161	Δ	GU218	GU219	GU274	GU275	GU313	GU314
GU116	Δ	GU162	Δ	GU222	Δ	GU280	GU281	GU321	GU322
GU126	Δ	GU163	GU164	GU230	GU231	GU282	Δ	GU323	GU324
GU129	Δ	GU174	GU175	GU238	GU239	GU285	GU286	GU325	Δ
GU134	Δ	GU178	GU179	GU240	GU241	GU287	GU288	GU340	GU341
GU139	GU140	GU186	Δ	GU244	Δ	GU291	Δ	GU344	Δ
GU143	GU144	GU194	GU195	GU250	GU251	GU303	Δ	GU345	GU346
GU146	GU147	GU205	Δ	GU252	GU253	GU304	GU305	GU350	GU351
GU157	GU158	GU212	GU213	GU264	GU265	GU311	GU312	GU354	GU355

Yield

The best populations were 7 and 1, and the worst 3. Over 7 seasons, the best progeny produced an annual mean of 1,426 kg of dry cocoa per ha.

Average pod weight

Population 1 produced the heaviest pods, followed by 13 and 7. Some trees (and even one progeny) were found to produce an average pod weight of over 500 g (up to 600 g).

Performance with respect to rot diseases

The study material has a particularly interesting overall tolerance level. The population from Tanpok was significantly more susceptible than the Camopi populations.

Conclusion

Agronomic descriptors remain worthwhile and necessary in practice when choosing parents to be incorporated into breeding programmes. The wild cocoa trees from French Guiana, which form a special group, have yet to be used in cocoa breeding. As they have been distributed to numerous countries, it is important to facilitate their use through characterisation and assessment and to make these data accessible to researchers. The agronomic characteristics of wild material from the Camopi and Tanpok Basins reveal the noteworthy performances of certain progenies, or even populations, particularly as regards yield, the yield:vigour ratio and resistance to rot diseases. Based on these three criteria, we propose their use in genetic improvement programmes, and the practical indications provided (populations and progenies) should assist breeders in their choices.

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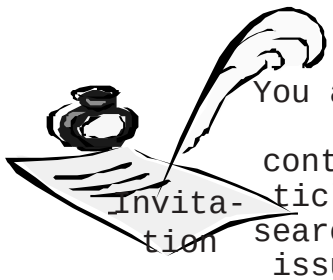

You are invited to contribute articles on research or other issues of interest to cocoa breeders/ge-

Overview of the Cocoa Breeding Activities in Indonesia

S.Mawardi

Introduction

From the end of the 1980's onwards, cocoa has played an important role in generating foreign exchange for Indonesia, as well as in alleviating poverty in the villages. The commodity is widely grown by estate companies and smallholder farmers. Recently, the total area under cocoa cultivation was estimated at approximately 600,000 ha, and cocoa production at up to 450,000 tonnes.

Indonesia produces fine-flavoured as well as bulk cocoa. Fine-flavoured cocoa is only grown in the Province of East Java so that this commodity is well known as Java Cocoa or Java "A" Fine Cocoa in the world market. It is categorised as Trinitario and is propagated clonally by grafting.

Smallholder farmers on Sulawesi Island are the main producers of bulk cocoa in Indonesia. Sulawesi cocoa is also well known for producing hard butter. The planting material used by Sulawesi farmers is mainly open-pollinated hybrids of Upper Amazonian (UA) clones, however a limited amount is derived from the progeny of West African Amelonado.

The other main producing areas of bulk cocoa are North Sumatra, West Papua, Maluku and Bali. The farmers in these areas also plant seeds of open-pollinated hybrids of Upper Amazonian clones.

Over the last two decades, farmers in many areas of Indonesia have rapidly expanded cocoa growing. It is predicted that cocoa cultivation will expand continuously in the coming years. Naturally, the farmers will request superior planting material, developed by the research institutes, in order to rehabilitate their old plantings. This report provides an overview of the current status of cocoa breeding in Indonesia.

Breeding Objectives

The cocoa industry in Indonesia faces several potential problems in every sphere of activity from production to post harvest handling and manufacture of chocolate. Some of the problems should be solved through breeding. Currently, the main objective of cocoa breeding in Indonesia is to identify hybrid varieties and clones with the following characteristics:

1. Higher yield,
2. Better bean quality
   - large size (> 1.0 g/bean),
   - uniform size,
   - high fat content (> 55 percent), etc.
3. Tolerance to cocoa pod borer - CPB (*Conopomorpha cramerella*) attack,
4. Tolerance to Black Pod disease (*Phytophthora palmivora*),
5. Tolerance to vascular streak dieback disease - VSD (*Oncobasidium theobromae*),
6. Less vigorous canopy suitable for higher density plantings.

Cocoa Breeding Activities

Currently, the Indonesian Coffee and Cocoa Research Institute (ICCRI) at Jember (East Java) has a major cocoa breeding programme. ICCRI conducts breeding research on fine as well as the bulk cocoa. Most of this research is done in East Java, which is characterised by a dry climate.

Another institution that consistently does cocoa breeding work is The Bah Lias Research Station (BLRS) located in North Sumatra, which is characterised by a wet climate. A private estate company of PT PP London Sumatra Indonesia is the owner of this research station. BLRS only works on bulk cocoa breeding especially designed to maximise profit.

The Indonesian Oil Palm Research Institute (IOPRI) at Medan (North Sumatra) and the Gadjah Mada University (GMU) at Yogyakarta (Central Java) also do research in cocoa breeding. Both institutions also work only on bulk cocoa.

Breeding with fine cocoa

In 1912, Dr. C.J.J. van Hall initiated cocoa breeding in Java. He started his work by doing individual selection of open-pollinated Trinitario progenies at Djati Roenggo (DR) Estate in Central Java. The progenitors of the Trinitario were Java Criollo that had been introduced from Venezuela in 1888, and a Forastero type introduced from Venezuela in 1880.

This initial work resulted in several clones being recommended for commercial planting, *viz.*, DR 1, DR 2 and DR 38. To date, DR 1 and DR 2 clones are still being used for polyclonal commercial planting in East Java to produce Java Fine Cocoa. DR 38, however, has not been recommended yet due to the significant quantity of necrotic tissue inside the beans. In terms of quality, this unfavourable characteristic has been a significant problem.

Selection activities were continued using the open-pollinated progenies of DR 1. This resulted in several mother trees such as DR 48, DR 53 and DR 67. Selection was also continued with the progenies of the latter clones (open pollinated). In 1952, the promising clone, DRC 16, was found among the progenies of DR 53.

In 1995, the government released the clone DRC 16 for commercial planting in order to replace DR 38. DRC 16 is superior to previous clones in terms of yield, tolerance to Black Pod disease and bean size. It is also self-compatible. Currently, the clone is being used mainly to rehabilitate old plantings by side grafting. Some new planting is also being done using this clone.

ICCRI is still working on breeding with fine cocoa in order to support the sustainability of Java Fine Cocoa production. The main objective is to find new clones with better bean quality, higher yield capacity, more tolerance to Black Pod disease and self-compatibility.

The quality of Java Fine Cocoa must be paid special attention in the breeding programme because this cocoa has a special market in several countries. Consequently, ICCRI is main-

taining several experiments in order to select promising individuals from among the Trinitario population, make new Trinitario hybrids and test the genotype x environment interaction. Two promising clones, KWM 108 and KWM 117, have just been identified through these activities.

Breeding with bulk cocoa

Breeding activities involving bulk cocoa were intensified since the end of the 1970's when the government decided to accelerate cocoa growing in Indonesia. At that time, it was also predicted that the growers would need an abundance of cocoa seeds for new planting and rehabilitation.

ICCRI

ICCRI has undertaken breeding of bulk cocoa together with fine cocoa. The research was done at Getas estate (Central Java), and resulted in the production and release of the GC 7 clone for commercial planting.

The institute intensified bulk cocoa breeding activities by introducing UA clones from many other countries in the early 1970's. The introduced clones were crossed with each other artificially, and the F1 progenies were tested at several locations. This work was aimed at finding superior F1 hybrids as quickly as possible in order to supply the cocoa seeds needed by the growers. The crossings were not only made between UA clones but were also done between UA and Trinitario (DR and DRC) clones and Forastero (Getas) clones collected by the institute. This resulted in the recommendation and release for commercial planting of a number of F1 open-pollinated hybrids such as DR 1 x SCA 12, ICS 60 x SCA 12, GC 7 x SCA 12 and TSH 858 x SCA 9. The seeds of the hybrids were produced in several isolated seed gardens.

ICCRI still continues its search for better hybrids. Many F1 hybrids are being tested at Kaliwining station and other cocoa estates. Two promising F1 hybrids of NW 6261 x SCA 12 and NIC 7 x SCA 6 were recently identified, and a seed garden for these is being prepared at Kaliwining station.

ICCRI has been conducting clonal selection with bulk cocoa more intensively since the end of the 1980's. Trials involved several clones from other countries along with local selections. Consequently, GC 7, ICS 60 and ICS 13 have been recommended for commercial planting.

Selection activities have also resulted in the identification of 14 promising clones within the accession series KW, namely from the Kaliwining station. A multi-locational trial to study the adaptability of each clone is still in progress. After the first crop, KW 23 and KW 26 were found to perform better than the standard, GC 7.

Preliminary work on the selection of genotypes resistant to CPB commenced three years ago. Selections were made at smallholders' fields, which were severely attacked by CPB in West Kalimantan (Borneo), East Kalimantan and Southeast Sulawesi. A number of mother trees have now been selected, which are expected to be tolerant or resistant to CPB. Resistance will be confirmed by establishing trials in the infected areas in due time, since East Java is still free from CPB attack.

ICCRI is also conducting breeding research on resistance to VSD. Selection among progenies of KEE 2 (hand pollination) resulted in the identification of several mother trees resistant to the disease. The mother trees will be propagated through grafting in further trials to confirm resistance.

In order to support future cocoa breeding strategies, ICCRI is conducting genetic studies on subjects such as dominance, mode of inheritance, combining ability, and genetic x environment interaction. Molecular approaches to cocoa breeding have also been formulated in collaboration with other relevant institutes.

BLRS

BLRS conducts cocoa breeding research in order to support plantations of PT PP London Sumatra, a private estate company, in obtaining high productivity. The objective of the breeding programme on cocoa is to produce high-yielding progenies and clones, which have good bean quality and tolerance to pests and diseases. The activities are done in North Sumatra and East Java.

The breeding trials in East Java will also allow the selection of progenies and clones, which are potentially tolerant to drought conditions. There is also commercial interest in planting cocoa at a higher density, thus increasing yield per hectare. It is thought that for this to be successful high-yielding clones with low vegetative vigour will have to be identified and evaluated.

In trials involving hand pollination, four progenies have been identified, which have displayed low vegetative vigour. These progenies are promising for producing clones for testing in high-density trials whilst two progenies gave higher yields compared to the standard cross. Progenies of the female parent, PA 7, continuously gave the lowest percentage yield loss due to CPB.

In clonal trials, 35 clones (13%) had higher yields than the standard clone, GC 29, and 20 clones (7.5%) had yields 25% higher than that of GC 29. Five of these clones were also less vigorous.

Preliminary results on resistance breeding to CPB indicate that there are differences between clones in terms of the ability of the CPB larvae to penetrate and exit the pod, although the scoring of this character is difficult. Observations of CPB incidence on different clones at the R. Sialang estate (North Sumatra) revealed that there were no significant differences between clones for CPB infested pods, which had extractable beans. However, the clones PBC 113 and PBC 128 have significantly lower incidences of damage in terms of pods with beans, which cannot be extracted.

Cocoa planting materials recommended by BLRS are as follows:

Recommended clones:

- Vegetative vigour (North Sumatra 926 trees/ha and East Java 1,025 trees/ha):
  BL 703, GC 29, PA 4, PA 191, PA 310, PBC 139 and PBC 140.
- Moderately vegetative vigour (North Sumatra 1,010 trees/ha and East Java 1,212 trees/ha):
  BL 496, IMC 49, POUND 7C, PA 20, PBC 123, PBC 128 and TSH 858.

Recommended crosses:
-Open pollinated seed:
PA 310 × UF 713 (not reciprocal) AMAZ 3-2 × PA 137 (also reciprocal)
PA 300 × UF 713 (not reciprocal) PA 191 × P4C (also reciprocal)
-Hand pollinated seed:
PA 310 × IMC 85 TSH 858 × IMC 30
PA 7 × NA 32 NA 32 × PA 107
PA 20 × BLC 439A-Rootstock crosses
PA 191 × POUND 4C PA 137 × AMAZ 3-2
UIT 1 × SCA 9.

IOPRI

IOPRI, formerly the Research Institute for Estate Crops of Medan, had initiated breeding work on bulk cocoa at Pabatu and Adolina Estates (North Sumatra) of PTP VI (a government owned estate company) before 1975. In the preliminary research undertaken, the breeder conducted a systematic mass selection over cocoa populations derived from seeds, which had been imported from Sabah, Malaysia between 1972 and 1973. This population consisted of several F_1 open-pollinated hybrids of NA 33 x ICS 60, NA 32 × PA 35, NA 32 × Amelonado, NA 33 × PA 7 and NA 33 × Amelonado.

This work resulted in four types of seeds for commercial planting, viz., PTP VI improved F_2 (illegitimate), PTP VI synthetic no. 1, PTP VI synthetic no. 2 and PTP VI synthetic no. 3 varieties. The institute also conducted hybridisation and progeny trials during the 1980's. This resulted in a number of clones with accession code RCC. RCC 70, RCC 71, RCC 72, and RCC 73 have been recommended for commercial planting.

The cocoa breeding programme at IOPRI was terminated in the mid 1990's to allow the institute to concentrate on oil palm research. Therefore, valuable genetic material has been transferred to ICCRI.

GMU

The Agriculture Faculty of GMU undertook cocoa breeding in the mid 1990's. The main goal of the breeding programme is to find clones with high yield potential and high fat content. The programme was initiated by doing selection over hybrid populations. Reports on the progress of this work are not yet available.

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Analysis of Experiments with Repeated Measures

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Introduction

The main characteristic of experiments with repeated measures is that the same subject is measured repeatedly. In a comparative yield trial of a perennial crop, for example, where the yield is distributed throughout the year and over many years, yield increase can be evaluated by measuring it at regular time intervals. In such circumstances, the subjects are the trees in the plots competing for yield and being measured repeatedly to determine the yield response over the years. In such a situation, the data can subjected to two types of statistical analysis (Dias & Resende, 2001). The first type can be applied to data collected in one year or in one productive season or to data accumulated over the years. In this case, the analysis is similar to that performed for annual crops, and follows the format defined by the experimental design used for the trial. In the second type of analysis, the data collected over years or seasons are analysed in conjunction. In this case, different approaches can be taken, such as factorial or split-plot in time analysis, where the focus is on the yield-year interaction thus dealing with the data of repeated-measures.

Analysis of experiments with repeated measures has been used rarely in plant breeding probably due to its complexity and the unconventional procedures involved, despite its elegant and interesting properties. However, it must be noted that such an approach involves uni- and multivariate (Danford *et al.*, 1960; Cole & Grizzle, 1966) analysis of variance with concurrence between the two (Littell, 1989) to validate F-tests for time (years) and for its interaction. Thus, when the measures are taken over time on the same subject, a covariance structure is generated, which can inflate probabilities of type I error for F-tests. In such cases, a chi-square test is used to check for covariance structure. The data are also

tested for compliance with the Huynh-Feldt (H-F) condition by establishing if any orthogonal contrasts between repeated measures have equal variance and zero covariance. If the data do not violate H-F condition and the chi-square is not significant, the usual univariate F-test for time and its interactions are valid.

In cases where the H-F condition is violated, multivariate analysis will be justified because it does not require any premise about covariance structure of repeated measures. Another interesting alternative can be the Greenhouse-Geiser (G-G) adjustment of probability value (*P* value) associated with the univariate-F, which involves adjusting the degrees of freedom of the numerator and the denominator for the F-test. Significant *P* values for the H-F condition, G-G adjustment and multivariate-F can be calculated using SAS software (SAS Institute, 1989). In fact, SAS performs analysis of variance of repeated-measures with great robustness. For testing the significance of the difference between the vectors of averages of time and its interaction with the subject, the statistics of Wilks' Λ transformed to a corresponding F value can be used. Polynomial transformation is frequently used due to the time required to represent a factor with quantitative levels.

On the other hand, this type of analysis has been treated commonly and erroneously as split-plot in time (Steel & Torrie, 1980), with the time factor allocated to the sub-plots. The time is not an experimental factor whose levels can be randomly allocated to sub-plots (Littell, 1989). Therefore, it is desirable to further investigate the potential and applicability of the repeated-measures analysis of variance in plant breeding programmes. This article reports on the development of procedures for analysis of experiments with repeated measures and its numerical illustration through application in a cocoa breeding trial.

The repeated-measure analysis was applied to data of a comparative yield trial involving five cocoa cultivars (Dias *et al.*, 1998). The cultivars were 'Maranhão', 'Pará', 'Parazinho', open-pollinated 'ICS 1' and the commercial hybrid mixture. The trial was laid down in February of 1982 in a 5 × 5 latin square design with 196-plant plots. The harvest was monitored on a monthly basis for ten years (1984–1993). The wet seed weight data (kg/ha), recorded in the agriculture years (April/March), were used for statistical analysis.

Results and Discussion

Traditional analysis in split-plot

Initially, the traditional split-plot analysis was done according to Dias *et al.* (1998), where the Latin square was allocated to the plots and the years to the sub-plots (Table 1). Note that the LINE-COLUMN-CULTIVAR interaction is the ERROR 'a' in the split-plot that was used to test the source CULTIVAR (Table 1). The differences among the CULTIVARS, YEARS and the interaction CULTIVAR × YEAR were highly significant (see also Dias *et al.*, 1998). However, to determine the extent of validity of these univariate tests for YEAR and CULTIVAR × YEAR, it is necessary to re-analyse the data as repeated-measures, because the yield was measured monthly and accumulated annually over a 10-year period. The split-plot model assumes that the pairs of measures taken in the same plot are equally correlated. This is not always true with repeated-measures. The measures taken in a narrow time interval tend to be more correlated than those taken in a wider time interval. The solution to such a problem is to perform repeated-measures analysis of variance.

Table 1: Univariate analysis of variance of cacao wet yield (kg/ha).

Sources	DF	SS	MS	F	Pr > F
LINE	4	4339083.26		10.33	0.0001
COLUMN	4	910072.85	1084770.81	2.17	0.0746
CULTIVAR	4	3513308.40	227518.21	7.63	0.0027
ERROR	12	1381934.89	878327.10	1.10	0.3657
YEAR	9		115161.24	143.76	0.0001
CULTIVAR*YEAR		135901335.67			
	36		15100148.41	2.71	0.0001
ERRORb	180	10246138.40			
		18906681.46	28461496		
CV(%)			105037.12		
MEAN		50.69			
		639.42			

Analysis of repeated-measures in split-plot

The SAS command "REPEATED" is used in ANOVA and GLM procedures for repeated-measures analysis for testing the covariance structure of repeated-measures in order to validate the split-plot in time analysis. While using this command, it is necessary to quote the repeated factor in time, in this case the year, followed by their number and level, all between parentheses. In its syntax, the command admits several specifications or options. In this study, the year being a quantitative factor, the option "POLYNOMIAL" was used to analyse for orthogonal contrasts. The option "SUMMARY" generate tables of analysis of variance by contrast. "PRINTE" and "PRINTH" list the matrices E and H of variance and covariance associated with the error and the treatments, respectively. The option "PRINTE" also processes the test for sphericity of the covariance structure among the repeated-measures.

The partial correlation coefficients (Table 2) within plot show that the degree of association, in fact, decreased as the time interval between measure increased, i.e. for year 1 and 2 the correlation is 0.88, for year 1 and 3 it is 0.68, and 1 and 4 it is 0.79. Again, it is common in repeated measures on the same character and means that the correlation of yield trait with itself is not 1. The test for sphericity, known as Mauchly's criterion, of the error covariance matrix checks for the H-F condition with an approximated chi-square test for orthogonal contrasts, e.g., if they have same variance and zero covariance. Since the chi-square value of 362.8 for 44 degrees of freedom is significant at $P < 0.0001$, the validity of the F-test for CULTIVAR × YEAR interaction becomes doubtful, bearing in mind that the sphericity test examines the hypothesis that univariate F-tests are valid. Certainly, the *P* value obtained with the split-plot analysis is very small (Pr > F = 0.0001, Table 1).

The multivariate test, on the other hand, does not require compliance with the H-F condition to test for the effect of the YEAR

Table2: Partial correlation coefficients for annual wet yield of cocoa overtime.

	84/85	85/86	86/87	87/88	88/89	89/90	90/91	91/92	92/93	93/94
84/85	1.00	0.88	0.68	0.79	0.66	0.22	0.08	-0.24	0.35	0.48
85/86		1.00	0.83	0.83	0.75	0.43	0.15	-0.10	0.47	0.50
86/87			1.00	0.61	0.55	0.38	0.22	0.20	0.57	0.36
87/88				1.00	0.84	0.58	0.23	-0.05	0.48	0.55
88/89					1.00	0.75	0.55	0.19	0.43	0.55
89/90						1.00	0.68	0.52	0.50	0.29
90/91							1.00	0.77	0.51	0.44
91/92								1.00	0.52	0.09
92/93									1.00	0.58
93/94										1.00

and of the CULTIVAR × YEAR interaction. This test produced *P* values equal to 0.0001 and 0.0005 associated with multivariate-F for YEAR and CULTIVAR × YEAR interaction, respectively, in contrast to P equal to 0.0001 from univariate-F, for both the effects (Table 3). In practice, it is non-compliance with the H-F condition that reduces the *P* value associated with univariate-F for CULTIVAR × YEAR interaction. In fact, the H-F condition was not complied with (*P* = 0.0001) (Table 3), therefore in such cases multivariate or the adjusted univariate tests are recommended. Although both tests reveal the significance of probability associated with effects of YEAR and CULTIVAR × YEAR interaction, the *P* values (*P* = 0.0001) of the univariate and multivariate tests and G-G adjustment agreed only with the former. Obviously, the sum of squares, the mean square and F values are same as for the split-plot analysis (Table 1). The additional columns of G-G and H-F represent adjusted probabilities for correction of unequal correlations between pairs of repeated-measures. These new probabilities associated with the F-tests are obtained by multiplying the epsilon estimate for degrees of freedom from both the numerator and denominator. Normally, the epsilon value ranges from 0 to 1, and the values found in this analysis (G-G epsilon = 0.22 and H-F epsilon = 0.30) indicate the necessity of adjustment for correction of the probability levels considering the correlation among the measures within the plots.

Table3: Significance probabilities for univariate and multivariate F tests.

Sources	DF	Pvalue (univariate F)	Pvalue (G-G adjustment)	Pvalue (H-F condition)	Pvalue (multivariate F)
CULTIVAR	4	0.0027			
ERRORa	12				
YEAR	9	0.0001	0.0001	0.0001	0.0001
CULTIVARx YEAR	36	0.0001	0.0164	0.0073	0.0005
ERRORb	180				

The univariate tests, in principal, are more powerful than their multivariate counterparts, but the consistent rejection of the sphericity for the correlation matrix among the repeated-measures (*P* < 0.0001) suggests that these tests should be interpreted with care. In such situations, the use of multivariate tests together with adjusted univariate tests is justified. However, it must be emphasised that in all the procedures used there were highly significant differences between the effects of YEAR and CULTIVAR × YEAR interaction.

Acknowledgement

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INGENIC General Assembly Meeting

Minutes of the Meeting held

16th October, 2000 in Kota Kinabalu, Malaysia

Chairman: Bertus Eskes

Secretary: Michelle End

Minutes of previous INGENIC General Meeting on 24th November, 1996 were read and agreed.

1. The Chairman reported briefly on the structure and recent activities of INGENIC. Regarding the structure of INGENIC, he confirmed that membership was open to anyone who wished to be on the INGENIC mailing list and that INGENIC continued to operate as an informal group. The Committee is currently composed of a Chairman, Vice-Chairmen representing Africa, the Americas and Asia, a Secretary/Treasurer and an Editor. Support of the institutions in contributing with staff time to INGENIC Committee members' activities was acknowledged. Since the last GA meeting, INGENIC has edited and published the Proceedings of the Workshop on the Contribution to Disease Resistance by Variety Improvement and the two external reviews commissioned for this Workshop. These publications have been very well received by the cocoa research community; approximately 250 copies have been distributed. INGENIC has also compiled and distributed two Newsletters and is preparing a third Newsletter due to be published in the middle of 2001. INGENIC is pleased to note that its activities have contributed to new collaborative breeding efforts and the initiation of programmes on the effective measurement of resistance to diseases. The financial and other types of support received for INGENIC activities since the last Workshop from many institutions was acknowledged, namely ACRI, BCCCA, Bundesverband der Deutschen Susswarenindustrie, CIRAD, CPA, CRIG, CRU, CR (UK) Ltd., CTA, LIFFE, MCB, and Universidade Estadual de Santa Cruz.
1. The Treasurer gave the financial report and presented a summary of INGENIC accounts (1994-2003). She estimated that INGENIC had sufficient funds to publish the Proceedings of the 2000 Workshop and cover running costs for the next three years, including annual issues of the Newsletter. She reported that ACRI had kindly offered to audit these accounts and that she would be sending the full documentation to their offices in due course.
2. The Chairman delivered the Editor's report in Mrs. Bekele's absence. Five issues of the INGENIC Newsletter have been produced since 1994 containing a variety of articles, letters and news items of interest to cocoa breeders and geneticists. It was agreed that the Newsletter was a good vehicle for communication and that ways be sought to stimulate contributions. It was suggested that one way would be to identify individuals who would be responsible for collating articles from their institutes and/or countries and communicating these to the Editor on a regular basis. The Chairman commented that some time ago, there had been a suggestion that the INGENIC Newsletter merge with other Newsletters focusing on cocoa germplasm such as those produced by the Cocoa Research Unit, Trinidad and the Reading Quarantine Facility. It now seemed that these sources were no longer producing their own Newsletters and were content to use the INGENIC Newsletter without the need for a formal merger or name change.
3. The Chairman introduced the topic of Committee Structure. Currently the Committee consists of regional representatives and specific task members. The Committee is flexible and welcomes rotation, although there are presently no regulations governing the rotation/lifespan of the Committee. Although some participants supported a move towards more formal regulations, it was generally agreed that an informal structure was still more appropriate to a small group such as INGENIC and that no changes to the Committee's structure were required at present. The Chairman agreed that the issue of rotation should be discussed again at the next meeting.
4. The Vice-Chairman for Asia, Dr. Lee Ming Tong announced that he wished to delegate his position to Mr. Kelvin Lamin with immediate effect. On behalf of the INGENIC Committee, the Chairman thanked Dr. Lee for his major contribution to the group since its foundation in 1994, and in particular for his efforts which had ensured the success of the INGENIC Workshops held in Malaysia in 1994 and 2000. The Chairman thanked Mr. Lamin for the contribution he had already made to INGENIC as co-ordinator of the Local Workshop Organising Committee and welcomed him to the INGENIC Committee.
5. The Chairman introduced a discussion on forthcoming INGENIC activities. It was agreed that the next workshop should coincide with the next International Cocoa Research Conference. A suggested theme for this workshop was "Cocoa Breeding for Improved Production Systems". This would cover agronomic and physiological aspects such as high density planting, ideotype, genotype x environment interactions, and intercropping. It was left to the committee to work out details for the next workshop.
6. The Chairman invited Dr. Sona Ebai to inform the participants on a discussion held during the 13th International Cocoa Research Conference on the feasibility of setting-up a "Global Cocoa Programme". Dr. Sona Ebai reported that the discussions had focused on the content of the programme and that some priority areas had been identified which related to cocoa genetic improvement that would benefit from further international collaboration. He invited INGENIC to participate in the development of the Global Programme since our group could have an important role in identifying priorities and gaps in current breeding/genetics research. INGENIC agreed that it should be involved in the Global Programme and wished to be represented at future meetings. INGENIC agreed to initiate an activity to establish a first list of general priority areas for cocoa genetic improvement, particularly those that required international or regional collaboration.

ERRATUM

FOR ISSUE 5 OF THE INGENIC NEWSLETTER

In the article

"Factors Affecting Jorquette Height in Orthotropic Budding"

The full list of authors should read:

Y. Efron, M. Faure and P. Epaina.

The editor apologises for this oversight.

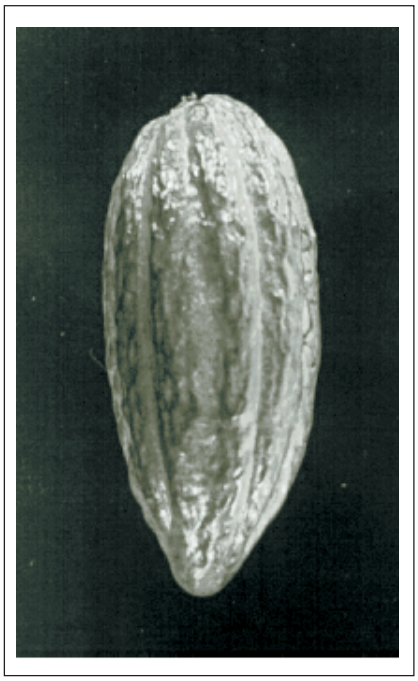
FORTHCOMING EVENTS

THE SECOND VENEZUEALAN CONGRESS OF COCOA AND ITS INDUSTRY

October 15th-19th, 2001
Carúpano, Sucre State
VENEZUELA

BOOK RELEASE

Some of the most widely used Cocoa Clones (according to the ICGD, January 1999) photographed at the International Cocoa Genebank, Trinidad



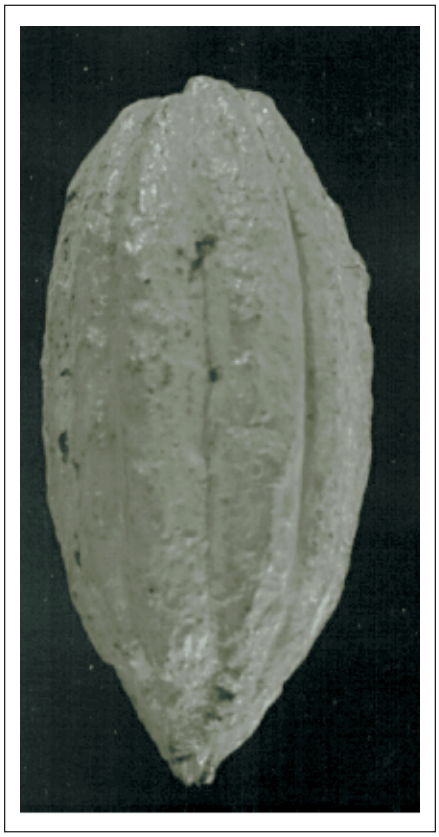
MARPER FARM, Trinidad SCA 6

San Juan Estate, Trinidad ICS 6

International Cocoa Genebank, Trinidad IMC 67

ST. Augustine, Campus ICS 1

International Cocoa Genebank, Trinidad UF 667



International Cocoa Genebank, Trinidad GS 36

International Cocoa Genebank, Trinidad SPA 9

International Cocoa Genebank, Trinidad RIM 6

International Cocoa Genebank, Trinidad PA 169

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