Newsletter

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IN THIS ISSUE

From the Editor's Desk:

We are very pleased to release this issue of the INGENIC Newsletter since it contains articles on several topics of immediate concern and interest to our readership. The dilemma surrounding mis-labelling of cacao germplasm has taken precedence in most collections, and there is an urgent need to resolve this problem and avoid its occurrence in the future. An overview of the situation and a proposal for dealing with it are presented herein.

A very useful and timely treatise on germplasm collection is also presented. There are also articles on identifying suitable rootstocks for high density planting, tissue culture and evaluation of cacao germplasm. There is a novel and exciting paper on a related species of *Theobroma viz.*, *T. grandiflorum*.

Dr. Yoel Efron deserves special mention since he submitted five contributions on diverse topics for this issue. All of our contributors are gratefully acknowledged for allowing INGENIC to realise its goal of disseminating information on cocoa breeding strategies and methodologies, novel results on breeding and genetic studies, germplasm evaluation and collaborative research as well as promoting dialogue.

One of our esteemed scientists, and a contributor to this forum passed away in January. Rob Lockwood has eloquently summarised the contributions of Prof. Norman Simmonds. The INGENIC Committee extends condolences to his relatives and acquaintances.

We are pleased to announce that the Fourth INGENIC Workshop, entitled *Cocoa Breeding for Improved Production Systems*, is scheduled to coincide with the 14th International Cocoa Research Conference, in Ghana in October, 2003. Readers are invited to start working on presentations for this Workshop.

Once again, the INGENIC Committee thanks the newsletter readership for its interest, support, positive feedback, and, most of all, contributions. We look forward to your continued input. This issue was also distributed by e-mail. Please use that medium to submit articles, if possible, to louisebekele@hotmail.com by February, 2003. Figures should have minimal memory requirements.

Best wishes from the INGENIC Committee!

FrancesBekele

Is the Resistance to *Phytophthora* Pod Rot Mainly Polygenic and Additive?

Y. Efron, G.Blaha and P. Epaina

Introduction

A general consensus during the INGENIC International Workshop on the *Contribution of Disease Resistance to Cocoa Variety Improvement*, held in 1996 in Salvador Bahia, Brazil, was that the resistance to *Phytophthora* pod rot (Ppr) is polygenic and additive. This view was expressed by Zadoks and in few additional papers presented during the Workshop, including the paper from Papua New Guinea (1). This consensus was based mainly on the continuous variation in disease resistance, and on diallel crosses showing relatively high general combining ability (GCA) and low specific combining ability (SCA). Data, obtained recently in Papua New Guinea at the Cocoa and Coconut Research Institute (PNGCCRI), suggested that the hypothesis of additivity is too simplistic, and that some form of interaction between genes should also exist.

Cacao Plant Materials

The Trinitario clone, K82, is one of the most resistant clones to Ppr in PNG. It is used as a parent in several crosses of the polycross SG2 cacao hybrid. As K82 is a self compatible clone, it was self pollinated with the aim of obtaining more homozygous clones with possibly higher Ppr resistance levels from among which a replacement parent could be selected for it in the SG2 hybrid.

Seventy-five trees, derived from self-pollination of K82, were randomly selected and cloned by budding. Thirteen clones were rejected because of poor growth (inbreeding depression?). The remaining clones were planted in the field in two replications in 1999, 6 trees/rep together with two controls, K82 (resistant) and KA2 -101 (susceptible). Two years later, as pods became available, 57 clones were assessed for resistance to Ppr using a laboratory detached pod spray inoculation test.

Ppr Test Procedures

A detached pod spray inoculation test originally developed by CRU, in Trinidad, and modified by the CCRI's Pathology Section was used. Two mature, unripe pods per clone were collected in the morning and placed in plastic trays covered with polyethylene sheets. The trays were left overnight in a temperature controlled chamber at 24 ± 20° C. The detached pods were inoculated the following day by spraying with zoospore suspension (300,000z/ml), 3 ml/pod. The inoculated pods were incubated in the controlled chamber for 10 days. Two reference clones, 36-3/1 (resistant under the test conditions) and 38-3/4 (susceptible under the test conditions) were used in all the tests in the first and last sprayed trays.

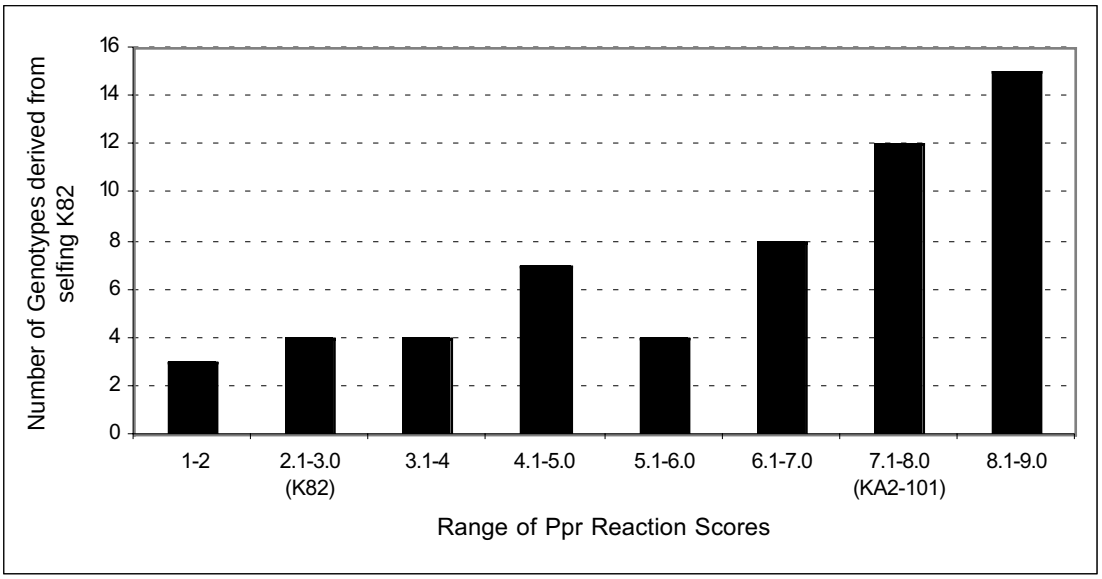
For inoculum preparation, pods of KA2-101, a highly susceptible clone, were inoculated by wounding with a sterile cork borer and inserting into the wound a plug of pure *Phytophthora* culture. The piece of husk previously removed was then replaced. The pods were incubated for 9 days in the dark chamber. The zoospores were then removed from the pods using a clean painting brush and sterile distilled water. Calibration was done to 300,000z/ml with the aid of haematocymeter.

Assessments of the pods were done 5,7 and 10 days after inoculation. The pods were assessed for the Type of Symptoms (severity) on a 1-5 rating scale whereby 1=only points/dots and 5=true necrosis. The pods were also assessed for *Extension Magnitude*, namely the relative pod surface area (%) covered with the disease. A scale of 1-9 was assigned to the clones in each test (2 pods) according to the judgement of the senior author, taking into consideration the data recorded 5,7 and 10 days after inoculation. However, emphasis was given to the data obtained on day 10 and for the more severely affected pod of the two inoculated. The *Extension Magnitude* was used as the first criterion to determine the approximate score. It was refined later according to the type of symptoms, usually by adjusting the score one unit up or down the scale, that is, if a clone should be scored as 3 or 4; 5 or 6; etc. Three tests of 2 pods/rep/clone were conducted.

Results and discussion

A total of 57 clones, derived from self-pollination of K82, were tested by the detached pod spray inoculation test for Ppr. The clones, grouped at 1 unit score scale intervals (Figure 1), ranged from highly resistant with an average score of 1.8 to highly susceptible with an average score of 9.0. However, there were fewer clones with low scores (resistant) than with high scores (susceptible). The control clones were classified as expected, K82 - resistant and KA2 - 101- susceptible.

Figure 1: Distribution of Ppr reaction scores among K82 self-pollinated clones



The clone, K82, was developed from an individual Trinitario mother tree of unknown pedigree probably derived from an open-pollinated pod. Therefore, it is possibly heterozygous for many genes, including genes for resistance to Ppr. Thus, K82 may be considered as an F1 plant, and the clones obtained by self-pollination as an F2 segregating population. If the resistance to Ppr in K82 is additive and polygenic, an approximately normal distribution of the clones with an average or midpoint towards the more resistant score should be obtained. However, the results obtained were completely different. The distribution was not normal, and there were high proportions of susceptible and highly susceptible clones (Figure 1). Therefore, the resistance to Ppr in K82 cannot be simply explained by a model of additive genetic control.

The number of clones tested could be too small for an accurate analysis of either the mode of inheritance of Ppr resistance in K82, or the number of genes involved. However, a simple model that can explain the results is based on complementary action of three major dominant alleles with the following assumptions:

- K82 is heterozygous for the 3 loci.
- The clones can be divided into 2 groups – resistant (1.0-6.0) and susceptible (6.1-9.0).
- The variation within each group is due to the effect of minor genes (modifiers) or experimental variability, or both.

The expected ratio of resistant to susceptible genotypes, according to a model of three complementary genes, is 27:37. The expected numbers of resistant and susceptible clones for the 57 clones tested are 24 and 33, respectively whilst the observed ratio was 22 to 35 resistant and susceptible clones, respectively. This observed ratio fits well with the model ($\chi^2 = 0.152$; $0.75 \geq P \geq 0.50$).

This article does not attempt to claim that the resistance to Ppr is generally controlled by a complementary action of three major genes. It may be a specific example of results obtained by self-pollination of one clone (K82), and tested by artificial inoculation in the laboratory with one pathotype of *Phytophthora spp*. Possibly, other explanations for the results may be found. The purpose of the article is rather to generate thought and discussion and to question the general consensus that resistance to Ppr is additive and polygenic.

During Zadoks' presentation at the Workshop, Van der Vossen made the following two comments (Workshop Proceedings p.21): (1). "... valuable inferences on the genetics of resistance require analyses based on sets of crosses (P1, P2, F1, F2, BC's etc.)." (2). "Diallel crosses are less useful because many of the preconditions for such an analysis are not met." It is believed that Van der Vossen's comments were very relevant. Additional research, with a wider range of genetic materials and a well-defined and planned set of crosses, is required to determine more accurately the genetic control of resistance to Ppr in cacao.

Reference

(1) Proceedings of the International Workshop on the Contribution of Disease Resistance to Cocoa Variety Improvement. November 24 – 26, 1996, Salvador, Bahia, Brazil. INGENIC, (1999).

Please send comments to:
Y. Efron
PNG Cocoa and Coconut Research Institute
P.O. Box 1846, RABAUL, E.N.B.P
Papua New Guinea
Fax: (675) 983 9115
e-mail address: ccri@datec.com.pg

Verification in Global Cacao Germplasm Collections

L. A. Motilal, D. R. Butler and V. Mooleedhar

Abstract

The *ex situ* conservation of cacao genetic resources is in the form of field genebanks, and the presence of mislabelled trees in national and international collections is beginning to be recognised. Misidentified trees represent a serious problem to:

- curators, who need to capture genetic diversity, and
- users of germplasm material, who must be certain of the identity of the accessions for proper evaluation of their results.

The presence of mislabelled trees was assessed from data compiled in the ICGD 2000 v4.1 CD-ROM, which contains the published records of cacao accessions in global holdings. Such circumstantial evidence revealed that many germplasm collections, but especially holdings in Brazil (CEPEC), Colombia (ICA), Costa Rica (CATIE), Côte d'Ivoire, Mexico, Malaysia, Togo, Trinidad (ICG,T) and Venezuela (EEC) possessed misidentified material. The severity of the problem may have a minimum value of 30%, and is suggested as an area for immediate resolution. Based upon the morphological data provided by the ICGD CD-ROM, several accessions were highly suspect, including EET 19 [ECU], EET 48 [ECU], EET 162 [ECU], ICS 1, ICS 6, ICS 43, ICS 89, ICS 100, IMC 67, PA 121 [PERU], POUND 7 [POU], SCA 6, SPA 9 [COL], UF 29, UF 613 and UF 667. However, the identification of mislabelled trees should ultimately depend on molecular analysis, and the use of voucher specimens for verification is recommended. The need for international collaboration is recognised, and the value of the concept of a public domain database for microsatellite analyses of germplasm holdings is underscored.

Introduction

The conservation of cacao genetic resources is primarily dependent on *ex situ* methods, and field genebanks are the only current practical means of conserving cacao germplasm. The presence of mislabelled trees is, however, commonly encountered in genebanks. Proper usage of germplasm material from any collection is dependent on the unambiguous identification of each tree. Genetic diversity can only be fully ascertained if the holdings in the respective genebanks are properly catalogued. Information on the genetic diversity within a genebank is critical for scientific planning of future prospections to capture primary germplasm in the wild. Another important aspect is the use of material for multi-locational international trials. If each tree of each accession planted has not been verified as true-to-type or authentic, mislabelled material may be used at any of the sites. Any data obtained from these trials would therefore be of limited use for analysis and interpretation. Unfortunately, dissimilarities among trees, purportedly representing the same accession in different cacao genebanks worldwide, have been recognised (Figueira, 1998; Anon., 2000). Detailed morphological studies to reveal off-types within and amongst cacao collections have not been conducted or reported. However, a molecular study (Risterucci *et al.*, 2001), using eight microsatellite markers for 28 different accessions from nine collections, has revealed that problems in identification occur in approximately 30% of these cases. Such misidentification is obviously a serious hindrance to ongoing and future research.

Wadsworth and Harwood (2000) have compiled the International Cocoa Germplasm Database on CD-ROM (ICGD 2000 v4.1), which contains information on cacao accessions held worldwide. The database comprises of records of 27,859 published clone names, which represent nearly 14,000 separate clones. For each clone, information is available on the names given to the clone, synonyms or homonyms, details of the origin, characteristics and presence in germplasm collections. The data given are mainly agronomic or morphological with little biochemical (isozyme, lipid

analysis) information. The objective of this paper is to determine whether misidentification exists within global cacao collections by using morphological information from the ICGD 2000 v4.1 CD-ROM, and identifying accessions and collections with markedly conflicting data.

Methodology

Any accession should exhibit morphological features that uniquely identify it within the limits of environmental variation in the expression of each character. An exhaustive search of the ICGD 2000 v4.1 CD-ROM (Wadsworth and Harwood, 2000) database was therefore conducted for accessions with conflicting information for the same character(s) from multiple sources. Disease resistance was not considered because proper comparison could not be made since different isolates and pathovars at different sites would be involved. The data on lipid composition were also disregarded since there was only one published source for these data. In addition, apparent typographical errors (e.g. 19.0 mm as opposed to 190 mm) were disregarded. Entries, which were the result of non-standard measurements amongst sources, were also disregarded. An initial search yielded over 200 unique accessions for which information on any character had multiple entries. Characters included pod value; number of seeds per pod; seed dry weight; fruit dry weight; fruit shape; fruit colour; fruit rugosity; fruit ridges; fruit basal constriction; fruit apex; fruit husk hardness; genetics of fruit pigmentation; anthocyanin in various floral parts and fruit (including horns on petal pouch); dimensions of fruit, ovary, sepals and staminodes; length: breadth ratio of sepal; presence/absence of hairs on sepals and self-compatibility. This list was refined by selecting accessions with widely divergent data entries for any particular character. In the case of qualitative characters, this was taken as more than one class apart, except in the case of anthocyanin intensity where only the two ends of the scale were taken. In the case of quantitative characters, divergent values were taken when either a 50% difference was recorded between values or when the difference was greater than or equal to three times the standard error of the given values.

Results

One hundred and sixty-four accessions were short-listed as possibly mislabelled, and the majority of the accessions (70) exhibited differences for only one character, although this character was not the same for all of the accessions. Twenty-two percent of the short-listed accessions, however, were different for four or more characters (Figure 1). However, of these accessions, 52 (32%) had conflicting data for more than one character when only ovary dimension and the shape, colour, rugosity, ridge separation, basal constriction and apex form of pods were considered.

Figure 1: Distribution of cacao accessions with multiple distinct entries as obtained from the ICGD 2000 v4.1 CD-ROM (Wadsworth and Harwood, 2000). Classes represent the number of conflicting characters among 164 accessions

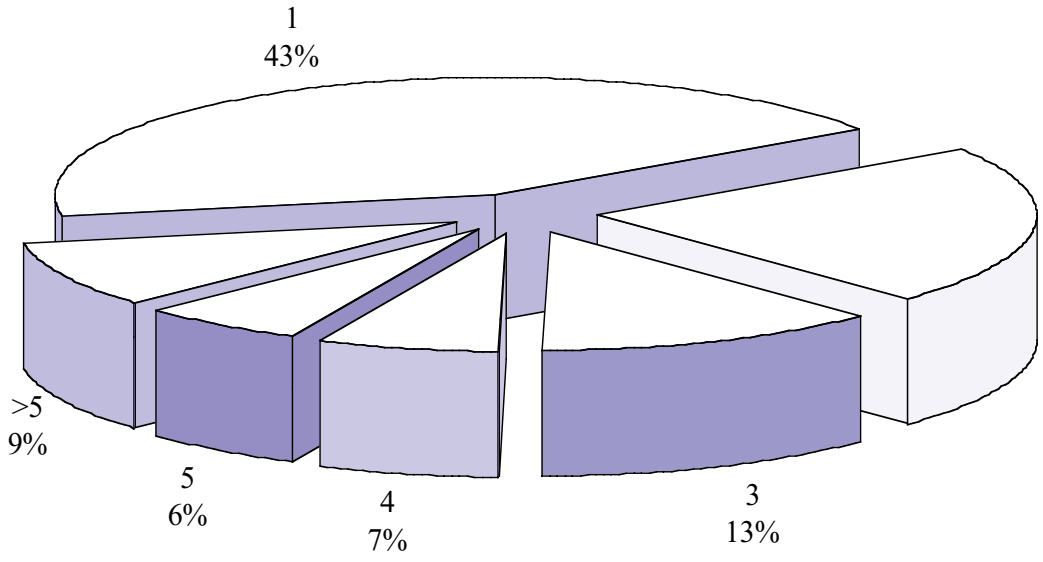


Table 1: Discrepancies amongst and within cacao (*Theobroma cacao* L.) collections as revealed by data compiled in the ICGD 2000 v4.1 CD-ROM (Wadsworth and Harwood, 2000)

Source 1	Source 2	Number of differing accessions	Total number of differing records
BAL	Posnette/Bartley/Coral & Soria	1	1
	Sime Darby Plantations, Malaysia	1	2
CATIE	Applied Agriculture Research Unit, Malaysia	1	1
	BAL Plantations, Malaysia	10	16
	Cacao Cultivars Registrar, IICA, Costa Rica	64	145
	CATIE, Costa Rica	55	117
	CEPEC, Brazil	63	76
	CIRAD-CP (Cote d'Ivoire & Togo)	17	44
	Dept. Agriculture, Trinidad	5	6
	EEC, Venezuela	3	5
	FELDA, Malaysia	2	3
	ICA, Palmira, Colombia	10	23
	ICG,T Trinidad	29	82
	LCTAP, Ecuador	3	3
	MCB, Malaysia	1	1
	Posnette /Bartley/Coral & Soria	2	2
	Rosario Izapa, Mexico	16	20
	Sime Darby Plantations, Malaysia	1	1
CEPEC	BAL Plantations, Malaysia	5	5
	Brazilian International Expedition 1965	3	3
	Cacao Cultivars Registrar, IICA, Costa Rica	8	8
	CIRAD-CP (Cote d'Ivoire & Togo)	12	15
	Dept. Agriculture, Trinidad	1	1
	EEC, Venezuela	1	1
	ICA, Palmira, Colombia	5	7
	ICG,T Trinidad	9	9
CIRAD-CP	BAL Plantations, Malaysia	8	8
	Cacao Cultivars Registrar, IICA, Costa Rica	7	10
	CIRAD-CP (Cote d'Ivoire & Togo)	2	2
	Dept. Agriculture, Trinidad	4	4
	Posnette/Bartley	7	7
EEC	EEC, Venezuela	1	4
	Cacao Cultivars Registrar, IICA, Costa Rica	3	4
ICA	BAL Plantations, Malaysia	1	1
	Cacao Cultivars Registrar, IICA, Costa Rica	3	8
	ICA, Palmira, Colombia	1	2
ICG,T	BAL Plantations, Malaysia	4	4
	Cacao Cultivars Registrar, IICA, Costa Rica	13	33
	Chalmer's Collection, Ecuador	2	2
	CIRAD-CP (Cote d'Ivoire & Togo)	13	15
	Dept. Agriculture, Trinidad	2	2
	EEC, Venezuela	4	6
	LCTAP, Ecuador	3	4
LCTAP	Cacao Cultivars Registrar, IICA, Costa Rica	2	2

Accessions, which were highly suspect, differed at the level of:

(a) 6 character levels - ICS 6, ICS 89, ICS 100, PA 121 [PERU], SCA 6, SPA 9 [COL], UF 29,
(b) 7 character levels - ICS 43, POUND 7 [POU],
(c) 8 character levels - EET 162 [ECU],
(d) 9 character levels - EET 48 [ECU], IMC 67
(e) 11 character levels - ICS 1, EET 19 [ECU], and
(f) 12 character levels - UF 667

These contentious multiple entries originated from a small number of sources, which formed a core group. This group was also the main source of contentious data entries for all other accessions, with records originating from Brazil (CEPEC), Colombia (ICA), Costa Rica (CATIE, IICA), Côte d'Ivoire and Togo (data compiled by CIRAD-CP), Ecuador, Malaysia (BAL Plantations), and Trinidad (ICG,T). Other genebanks that were not in this core group, but which also generated much conflicting data included Venezuela (EEC) and Mexico. Conflicting information for the same character have repeatedly arisen between records of holdings from Costa Rica (IICA vs. CATIE), Brazil (CEPEC vs. CATIE and CEPEC vs. CIRAD-CP (information compiled from Côte d'Ivoire and Togo)) and Trinidad (CATIE vs. ICG,T). Interestingly, conflicting data have also arisen from the same genebank for several characters over many accessions (CATIE), several characters for just a few accessions (ICA, EEC) or for only one character (ICG,T). However, suspect accessions were not different for all characters with multiple data entries among genebanks. For instance, for a contentious accession, the character presence or absence of horns on the petal pouch usually separated CATIE from IICA, but these collections would also possess similar data for fruit shape for that particular accession. These discrepancies amongst and within collection records are presented in Table 1.

Discussion

The ICGD 2000 v4.1 CD-ROM (Wadsworth and Harwood, 2000) was examined, and 164 accessions were found to have widely divergent entries for various morphological and agronomic descriptors. Such large deviations indicate one or more of the following:

- that improper germplasm materials were worked on,
- there is a need for standardisation of descriptor data,
- earlier records may need to be adjusted to fit current terminology or
- there is a likelihood of misidentified material in global cacao holdings.

This study found that 22% of the short-listed accessions were different for four or more characters. However, when characters involving anthocyanin intensity were disregarded and only ovary dimensions and pod descriptors were considered, 32% of the short-listed accessions had widely divergent entries.

This investigation revealed a misidentification level of 22%-32% in cacao germplasm collections. This compared favourably with the error rate recorded by other workers (Figueira (1998) – 27%; Christopher *et al.* (1999); Risterucci et al. (2001) and Sounigo *et al.* (2001) – 30%). Field cacao genebanks are distributed globally, and a large body of character information on their accessions are yet to be obtained. In addition, there were many accessions and character values with only a single data entry.

The detection of off-types should ultimately depend on molecular data rather than morphological characters so that the question of environmental variation would not arise. Thus, while a level of up to 30% may be taken as a reasonable estimate of the misidentification in major cacao germplasm holdings, the actual extent of misidentification remains to be assessed. Nonetheless, it would appear that several accessions are priority candidates for verification. These include EET 19 [ECU], EET 48 [ECU], EET 162 [ECU], ICS 1, ICS 6, ICS 43, ICS 89, ICS 100, IMC 67, PA 121, POUND 7 [POU], SCA 6, SPA 9 [COL], UF 29, UF 613 and UF 667. Many of these accessions are of major importance in trials in the areas of phytopathology, and molecular and genetic studies. It is therefore incumbent upon all curators of cacao genebanks to initiate or accelerate verification procedures for commonly used accessions as a priority.

It is recognised that misidentification, in any germplasm collection, can occur at any stage or be compounded at successive stages from collection to establishment and management of the genebank. In the first instance, proper nomenclature for all cacao genetic material collected in the wild must be adopted and implemented. Particular attention must be given to the cases when seeds are collected. Each seedling should be uniquely identified since it potentially represents genetically different material. The conservation protocol for *T. cacao* L. should include a process of comparing the characterisation data of recently introduced accessions with basic data that should accompany the material from its origin. This information can take the form of passport data and voucher specimens. Passport data should be collected for every sample collected in the wild or from another collection. More importantly, a recommended short list of botanical descriptors should be applied for characterisation of any collected sample. Basic information on pod shape, rugosity, ridging, and colour is useful as a reference for comparison with what exists in accessions planted in fields at some later date. In addition, some bean and flower descriptors are useful discriminating tools when combined with pod characteristics. In order to employ passport data in the verification process, collecting records should be accurate, stored safely and be readily available upon request. The use of herbarium samples or voucher specimens can also be useful in authenticating germplasm samples since they provide a replica of the plant organs. When collecting notes or passport data

accompany herbarium samples, an invaluable tool for germplasm verification analysis becomes available. In order to determine the source of cacao accessions in global holdings, their germplasm movements must be carefully traced. Again, proper documentation and passport data are critical to identify the point of origin of budwood, seed or live plants. Finally, in order to overcome quarantine problems, DNA can be extracted from the voucher specimens, frozen and shipped to the requisite laboratories for comparison with suspect trees using molecular techniques.

The verification work undertaken for any genebank should become increasingly reliant on SSR-PCR studies, as SSRs are known to be robust, informative, more reliable than RAPDs, and more cost-effective than AFLPs. This may lead to international collaboration since at some holdings the facilities for SSR-PCR work may be unavailable. Such a system of collaboration is necessary if source materials are to be obtained. The outcome of the verification work would serve to rationalise genebanks by enabling curators to decide whether to keep tagged off-types or remove and replace these off-types with other material. Replacements should be made using certified material from globally available sources. Voucher specimens of certified material would only be acceptable if all trees of any one accession throughout its global distribution are identical. The determination of authenticity would, in part, be feasible through the use of the same number and type of microsatellite primers at all verification laboratories. The creation of a website in the public domain for uploading of SSR-profiles into database sets would help to integrate information on the global holdings, and facilitate researchers seeking to obtain budwood material from certified accessions or for analysis within their own national verification programmes.

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Please send comments to:
L. Motilal
Cocoa Research Unit,
The University of the West Indies,
St. Augustine,
Trinidad and Tobago, West Indies

Cacao Germplasm: How and How Much to Collect?

L. A. S. Dias[1] & C. M. V. C. Almeida[2]

[1]DBG, BIOAGRO, Universidade Federal de Viçosa, Viçosa, MG, Brasil
[2] CEPLAC, SUPOC, SERPE, Porto Velho, RO, Brasil

The collection of cacao germplasm (*Theobroma cacao* L.) is aimed at rescuing natural or wild, cultivated or semi-cultivated populations, primitive cultivars and wild, closely related species. Despite their potential use for breeding, these genetic resources are not well represented in genebanks. The orchards of river margin dwellers of the Amazon colonisation project also represent gene depositories, which must be exploited. In such places, which are protected from genetic erosion, several different plant species are present, of which *T. cacao* L., *T. grandiflorum* and *T. speciosum* are among the most common. It should be remembered that germplasm collection offers an opportunity to

rescue new genes, which in future can contribute to genetic gains in breeding programmes. However, the high cost of this activity and the difficulty of returning to an already exploited region demand that the collector fully explores the different alternatives and possibilities offered by this activity. The following conceptual terms are used in this article:

- *population* refers to a community of potentially inter-crossing individuals at a determined site;
- *wild or natural* applies to a plant or population that has not undergone anthropic action through domestication or systemic cultivation;
- *cultivated*, for plants or populations, means systemic cultivation from seeds of wild cacao; and
- *semi-cultivated* refers to cultivation with little management technology (Almeida & Dias, 2001).

Samples should be randomly selected from the mother trees, which supply seeds and/or budwood, in order that any individual of a population can have the same probability of being represented. This principle of collecting a sample representing the variability of the exploited population has been in use since 1979, and was observed during the botanical expeditions carried out in the Brazilian (Almeida *et al*., 1995) and Ecuadorian (Allen, 1984) Amazon. Selective collection from mother trees is inefficient and therefore not justified because of the apparent low heritability of the main agronomic traits such as yield and resistance to pests and diseases. The expectation with randomised collection is that the sample contains the alleles common in the population, in their relative frequencies, minimising the detrimental effect of genetic drift, which results in allele loss. Random sampling and minimised genetic drift in the genetics of the population are contemplated in the context of effective population size (N_e), which measures the degree of genetic representativeness inherent in the sample (Dias & Kageyama, 1991). The expected outcome of randomised sampling and of achieving N_e is that maximum diversity is captured in a minimum quantity of the collected material.

Effective population size

Theobroma cacao L. is a perennial, monoecious, allogamous species, with hermaphroditic flowers pollinated by midges. The union of gametes occurs at random, and also includes self-fertilisation on a small scale. For species like this, the basic expression of N_e to quantify the genetic drift effect on the finite population samples is expressed by Crow & Kimura (1970) as:

$$N_e = \frac{2n}{\frac{s_k^2}{\bar{k}}(1+\alpha) + (1-\alpha)} \qquad (1)$$

Where n is the number of individual samples represented by seeds; α is the deviation from the panmmixia of the mother trees sampled (the quantity is negligible in some allogamous species); $\bar{k}$ is the average number of gametes contributed by the mother trees for the sample and; s_k^2 is the variance of $\bar{k}$. N, being the group formed by all the mother trees, leads to $\bar{k} = (2n)/N$. In turn s_k^2, whose complete composition was reported by Vencovsky (1978), has, as parameters, F which is the number of mother trees sampled randomly, and M that corresponds to the number of pollen-donor trees, whose male gametes participate in the generation of n individuals sampled. This variance is also composed of the quantities u = F/N e v = M/N. By neglecting α and substituting s_k^2 by its components in the equation (1), we obtain the equation (2) below, which was derived by Vencovsky (1987).

$$N_e = \frac{n}{\frac{1}{4}\left[\frac{n(1-u)-1}{F} + \frac{3n(1-v)-1}{M}\right] + 1} \qquad (2)$$

For germplasm collection of natural populations of allogamous species such as cacao, M is an unknown quantity and sufficiently small to be neglected. Similarly, the quantities u and v are equal and tend to be zero because F and M constitute a very small fraction of the group of N trees of the species. From this presumption, a derivative of N_e in (2) leads to equation (3).

$$N_e \cong \frac{n}{\frac{n-1}{4F} + 1} \qquad (3)$$

Equitative Sampling

Equation 3, however, neglects the control over the gametes contributed by F mother trees for generations of n descendents. For example, suppose that 200 seeds (n = 200) have been harvested from 20 mother trees (F = 20). The N_e in this situation would be equal to 57; i.e. the 200 sampled seeds genetically represent 57 mother trees of the original population. It must be noted that the N_e is defined in relation to the immediately preceding generation.

However, there are situations where the gametic control is obeyed and for this, an equitative seed sample (equal or approximately equal number of seeds from each mother tree) is collected. For this situation, the adequate equation of N_e is shown in (4)

$$N_e = \frac{n}{\frac{1}{4}\left[\frac{n(1-u)}{F} + \frac{3n(1-v)}{M}\right] + \frac{3}{4}} \qquad (4)$$

Where $u = v \cong 0$ and M is negligible leading to (5)

$$N_e \cong \frac{n}{\frac{n}{4F} + \frac{3}{4}} \qquad (5)$$

Let us suppose that 200 seeds have been collected in an equitative manner, picking 10 seeds from each of the 20 mother trees. Substitution of these values in (5) results in $N_e = 62$, resulting in a more effective value for the same sample size. In the case of cacao, the unit of collection is a fruit that contains, on an average, 30 seeds in natural populations. It is easy to sample 10 normal seeds per mother tree, collecting only one fruit from each of the 20 mother trees. It is important to remember that the sample composed of F mother trees should be random and not the seeds harvested from them.

As seen, the strategy of equitative sampling, also called gametic control, widens the genetic representativeness of the samples. Through the numerical evaluation shown in Figure 1, it is evident that a maximum possible number of mother trees should be equitatively sampled. For example, a total of 400 seeds randomly and equitatively harvested from 80 mother trees leads to $N_e = 200$. The simple fact of collecting the same sample in a non-equitative manner from the 80 mother trees reduces the effective size by 11% ($N_e = 178$, see Figure 1).

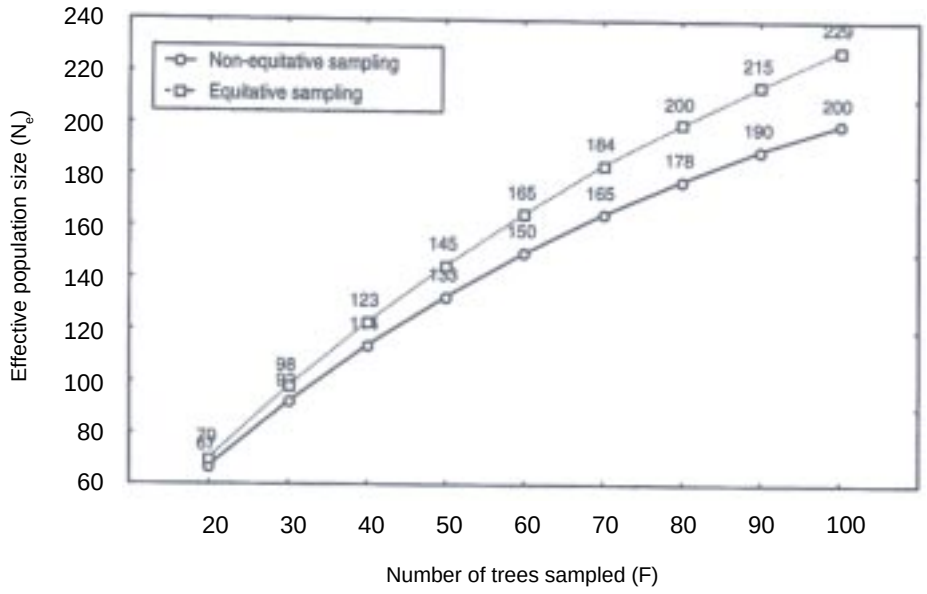
Number of seeds versus number of mother trees

Let us suppose now that four times as many seeds (1600) are equitatively collected from only one-fourth of the mother trees, that is 20 mother trees, the result would be $N_e = 77$ or 61.5% less. Therefore, it is highly recommended that fewer seeds be collected from a greater number of mother trees.

Number of mother trees for the sample

The number of mother trees to be sampled depends on factors like the percentage viability loss of the collected seeds, the quantity of accessions that can be maintained, and the rigour with which one wishes to maintain alleles above a given minimum frequency (p). The literature almost exclusively establishes that N_e

Figure 1: The effective size (N_e) for a sample of 400 seeds collected by the equitative and non-equitative methods from randomly selected F mother trees



greater than 150 provides good security for the conservation of germplasm.

With N_e = 150 and a minimum allelic frequency of for example p = 0.05 in the population, 95% of the collected samples will contain the alleles with frequency between 0.0283 and 0.0811, according to the binomial confidence limits table presented by Steel & Torrie (1960, p.456). This confidence interval, according to Vencovsky (1987), is a function of the standard deviation of the allelic frequencies (σ_p) in the samples, being $\sigma_p = [p(1-p)/(2N_e)]$. Again, under natural conditions, each population could be represented by an equitative sample of 400 seeds collected from 50 or 80 mother trees randomly selected (harvesting 8 or 5 seeds from each mother tree, respectively). This will ensure a N_e between 145 and 200 (refer to Figure 1). Curiously, Pound (1938) collected 320 fruits from 80 mother trees dispersed throughout various states during his travels to Ecuador in search of witches' broom resistant cacao. The advantage of sampling populations, as argued by Toxopeus (1997), is that its genes and cytoplasm may be transferred as packages of seeds. Some few hundreds of fresh cocoa seeds, weighing about 1 kg, contain the whole genetic variation of a population.

Alternatively, if budwood is being collected, N_e is equal to 1F, equivalent to the effective size for the self-pollinated progenies. Thus, if budwood is being collected from the same 50 mother trees, the sample has the same genetic representativeness as 50 individuals of the population. Therefore, this type of collection only allows sampling the genotype of the mother tree, while seed collection allows for sampling of alleles, and is therefore more efficient. In fact, in natural cacao populations, budwood collection is of limited value since no trait can be efficiently selected. However, such collection is justified if fruits are scarce and/or to complement seed sampling.

Sometimes it is necessary that before proceeding to collection, the collecting team must examine the wild cacao population; observe its geographic extension, the presence or absence of inter-connected populations and the variations in the characteristics of fruits, seeds, flowers, leaf etc. This *modus operandi* complements information on the theoretical number of individuals to be sampled and the distribution of the area to be exploited. The team can then proceed with the random selection of the mother trees. However, strict randomised sampling is not always possible because of the unavailability of sufficient propagules (seeds or budwood) from the mother tree at the time of collection. The other great difficulty lies in delimiting the exact extension of the natural cacao population especially when distributed in a continuous manner over an extensive area. Conversely, random sampling avoids collection biased by the breeder or botanist's point of view. The breeder looks for exceptional trees that can be useful for solving his breeding problems, while the botanist frequently concentrates on collecting a large quantity of botanical material for the herbarium. It is imperative that germplasm collection be seen and practised from the view of the conservationist.

Acknowledgement

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Please send comments to:
Dr. L. A. S. Dias
BIOAGRO
Universidade Federal de Viçosa
36.571-000 - Viçosa, MG
BRASIL
e-mail address: lasdias@mail.ufv.br

Callus and Embryo Formation in Different Cacao (*Theobroma cacao* L.) clones

J. J. Silva[1], L. Acosta[1], S. Montes[2], E. Arias[3] and E. Oliva[1]

[1] Centro de Estudios de Biotecnología Vegetal de la Universidad de Granma, Cuba;
[2] Instituto Nacional de Ciencias Agrícolas, Ciudad de la Habana, Cuba;
[3] Estación de Cuarentena de Café y Cacao de Velasco, Holguín, Cuba.

Introduction

The cacao (*Theobroma cacao* L.) tree was introduced from Mexico and Central America into Africa (into Sao Tome in 1822, and Ghana in 1850). The pre-Columbian people of Mexico used cocoa in sauce preparation, and the Aztecs used cacao seeds as currency. Cocoa is the third largest exported agricultural commodity in the world, after coffee and sugar, and had a commercial value in excess of 2, 000 million US dollars during 1988 (Sasson, 1993).

Pence (1989) listed the more important applications of cacao *in vitro* culture as the production of secondary metabolites and propagation using somatic tissues. Sondalh *et al.* (1993) stated that the use of biotechnological techniques in cacao culture must include the development of methods for propagation through somatic embryogenesis from somatic tissues, for both axillary shoots and anther culture, for protoplast fusion and for genetic transformation. The main objective of this research was to assess the effect of various 2-4D and kinetin combinations on somatic callus and embryo formation in three different cacao clones.

Materials and methods

Flower buds from SCA 6, Pound 7 and UF 650 were collected in the Quarantine Station of Coffee and Cacao in Velasco, Holguín (Estación de Cuarentena de Café y Cacao de Velasco, Holguín), transferred to laboratories in flasks containing distilled water, and disinfected using a 1 % sodium hypochlorite solution for 20 minutes. The buds were later washed with distilled water, excised and the staminodes were extracted. The flower buds were then sown horizontally on the culture medium surface. Fifty explants were used for each treatment. The 1962 Murashige and Skoog basal medium was used supplemented with Lopez-Baez vitamins, 400 mg/L myoinositol, 50 mL/L coconut water and 40 mg/L sucrose with a pH of 5.7. Different 2-4D and kinetin combinations were studied. Callus formation was evaluated after five weeks.

The callus formed was subcultured in the differentiation medium proposed by Silva *et al.* (2001). Embryo formation was evaluated five weeks later. The results were represented as percentages of the calculated rates.

Results and discussion

Table 1 shows that SCA 6 is one of the better performers in terms of callus formation, followed by UF 650 and POUND 7, respectively. There was a tendency for callus to form more quickly in media containing 2-4D and kinetin hormones. The best medium, based on the rate of callogenesis, was that with 3 mg/L 2-4D when used with POUND 7 and UF 650 explants.

All SCA 6 explants developed calli in media supplemented with 3 and 4 mg/L 2-4D and free of kinetin. In treatments with 2-4D and kinetin, the rate of success was greater than 90 %. In treatments where the media were free of hormones, the rate of callus formation was relatively low, but the calli developed in POUND 7 (40%) were viable.

Results on the formation of somatic embryos are given in Table 2. Somatic embryogenesis was only achieved in SCA 6 and POUND 7. These results demonstrate the influence of genotype on somatic embryogenesis.

Table 1: Callus formation from staminodes in media with different auxin-cytokinin relations

Treatment	2-4D (mg/L)	Kinetin(mg/L)	Percentage of callus formation (%)		
			SCA 6	POUND 7	UF 650
1	0	0	7.5	40.0	12
2	1	0	86.6	26.6	41.6
3	2	0	90.0	25.0	56.6
4	3	0	100.0	15.0	80
5	4	0.25	100.0	13.3	81.08
6	5	0.25	57.5	30.0	8
7	6	0.25	97.4	53.3	55
8	7	0.25	95	30.0	46
9	8	0.25	92.5	74.0	82.05
10	9	0.25	93.3	40.0	80

Table 2: Somatic embryo formation in three different clones

Treatment	2-4D (mg/L)	Kinetin (mg/L)	Percentage of somatic embryos formation (%)		
			SCA 6	POUND 7	UF-650
1	0	0	0.0	0.0	0
2	1	0	0.0	7.1	0
3	2	0	0.0	14.2	0
4	3	0	11.1	22.7	0
5	4	0.25	0.0	0.0	0
6	5	0.25	0.0	0.0	0
7	6	0.25	0.0	0.0	0
8	7	0.25	26.3	20.0	0
9	8	0.25	40.0	21.2	0
10	9	0.25	0.0	54.5	0

The frequency of formation of somatic embryos is influenced by genotype and composition of culture medium. Alemanno *et al.* (1996) assessed the effectiveness of the procedure described by Lopez-Baez *et al.* (1993) for somatic embryogenesis induction, and demonstrated the appearance of genetic variability in relation to the clones used. Somatic embryogenesis has often been achieved in our laboratory with SCA 6 and POUND 7. Figures 1 and 2 show the calli obtained, and we can observe a plant regenerated from an embryo in Figure 3.

Conclusions

Callus formation was achieved in three different clones. The best results were obtained in media supplemented with 2-4D and kinetin, and somatic embryogenesis only occurred in SCA 6 and POUND 7.

Figure 1: Calli obtained

Figure 2: Calli obtained

Figure 3: Plant regenerated from an embryo

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Rootstocks for Cacao

L. H. Purdy and B. Eskes***

* Professor Emeritus, Plant Pathology Department, University of Florida, Gainesville, FL 32611
1519 NW 25th Terrace, Gainesville, FL 32605
** CIRAD-CP, TA 80/02, Av. Agropolis, 34398, Montpellier Cedex 5, France

Introduction

Rootstocks with known attributes have not been developed for cacao, and close tree spacings of cacao in the field failed because trees have grown too large and too rapidly to manage economically. Rootstocks that control tree size and possess additional attributes will permit cacao to flourish in new planting designs to increase the number of trees per hectare thereby intensifying production of cocoa beans.

Culture of cacao on its own roots from open-pollinated seed, seed from specific crosses, and rooted cuttings built the chocolate manufacturing industries of the world. Seed-derived cacao trees survived, produced pods from which cocoa beans were extracted, and to whatever degree and/or extent the genetics contributed by variable parents allowed, they survived the varied stresses induced by unfavourable edaphic, climatic, and biotic environments. However, too often trees became unmanageable, they produced poorly, and they occupied available planting sites.

The impact of stresses has been reduced by cacao breeding programmes. Crosses were made and progeny were evaluated for their responses to a wide array of stresses, followed by the selection of individuals that responded favourably in the selection environment. Obviously, cacao breeding programmes are essential to move cacao into the future, but that future will open more widely when cacao breeding embraces the additional responsibility to develop rootstocks to augment the characteristics of selected clones. Rootstocks for cacao have largely been ignored in the past, with the exceptions of rootstocks such as EET 400 and IMC 67 seedlings that may possess resistance to *Ceratocystis fimbriata* (Ceratocystis wilt).

Tree fruit rootstocks

Awareness of rootstocks for tree fruits has been extant for more than 200 years. A vast literature addresses rootstock attributes and the advantages they contribute to fruit trees that growth on the trees own roots will not provide (Rom and Carlson, 1987). Rootstocks augment scions of tree fruits by controlling tree size, reducing the period of juvenility, increasing tolerance to environmental stresses and adverse soil factors, providing disease/insect/nematode resistance, improving tree/soil water relations, assisting mineral nutrition, improving productivity, increasing fruit set, and more. Rootstocks may be seedlings, rooted cuttings, plants produced by layering, or they may result from other methods of propagation, such as somatic embryogenesis. Individual buds, or budsticks of cultivated varieties or clones (the scion) are applied to the rootstock by budding or grafting. A great advantage of propagating cultivated varieties on rootstocks is that fruit produced is true to the type that developed on the donor tree.

The history of citrus in Florida dates back to the 16th century when Spanish explorers introduced seed of citrus (sour orange, sweet orange, lemon, lime, and citron) from the Orient. Seedling trees of citrus were very thorny, fruit production took several years to begin, and the flavour and other characteristics of the fruit were not acceptable. The use of rootstocks for citrus production began about 1830, and rootstocks influence more than 20 horticultural and pathological characteristics of the tree and fruit (Castle *et al.*, 1993). Several species of *Citrus* as well as hybrids of *Citrus* spp. are used as rootstocks to meet the precise needs in the many and varied production sites in Florida. Serious rootstock development began in Florida in the 1970s, but tree-size-controlling rootstocks have been developed only recently. Rootstocks along with improved cultivars have made the Florida citrus industry what it is today, the leader in the world.

Status of cacao rootstocks

Cope and Murray (1952) established a rootstock evaluation experiment in the field in Trinidad in the early 1950s in which they used four clones of cacao in all combinations of scion vs rootstock. They compared growth of scions from the four clones on rootstocks of the same four clones to growth of rooted cuttings of each clone. They concluded that none of the clones functioned as an effective rootstock (Murray and Cope, 1955, and 1959). However, they prophesised that cacao trees could be managed more easily, and the witches' broom disease controlled more effectively if dwarf trees were available (Cope and Murray, 1952). Unfortunately, their prophecy failed to stimulate subsequent research that was published regarding dwarfing of cacao by size-controlling rootstocks.

Rootstock development, coupled with clone development during the past 5-6 decades could have provided rootstocks that possessed many specific attributes including rootstocks that control - more tree size for use today. At present, cacao clones on their own roots clause cannot be planted much closer than 3m x 3m because trees grow too large and too rapidly. With apples, for example, production related positively

to the numbers of apple trees per acre that in turn was associated with the planting design (Cummins and Aldwinckle, 1993). It is most likely that closely spaced, small-stature cacao trees will intensify production of cocoa beans, those trees can be managed more easily, and disease/pest management will be more effective as well as more economical.

Rootstock development for cacao

Present-day budding or grafting uses cacao seedlings with unknown attributes as rootstocks, and scions inserted into those rootstocks will grow as rapidly as, be precocious as, become as tall/large as, and be as unmanageable as the trees from which the propagating material was obtained. Top-working old trees, grafting or budding chupons of old trees, and any other methods to "save" or use the roots of old cacao trees are "quick fixes" to rectify poor performance.

Rootstock development for cacao will require continuity and a commitment of time to succeed, and will require inputs similar to cacao breeding. The large volume of literature devoted to rootstocks for other tree fruits contains a wealth of information that will help the formation of cacao rootstock programmes. In addition, planting designs for other fruit trees might function as models for new planting designs for cacao on newly available rootstocks.

For fruit trees such as apple, pear, peach, cherry, and others, the NC-140 Regional Rootstock Research Project was developed to observe candidate rootstocks selected by project participants in varied fruit-tree-growing environments in several locations in Canada, Mexico, and in 30 states of the United States. Within the NC-140 project there is an exchange of rootstock candidates with all participants.

An International Cacao Rootstock Research activity would advance cacao rootstock development significantly. Such activity might be considered as part of a new project on "*Cocoa Productivity and Quality Improvement, a Participatory Approach*" proposed for funding by CFC. Indeed, rootstock development has already begun at some sites in the present CFC/ICCO/IPGRI project, and additional locations may be added. Such a broad-scoped project probably should include the following objectives as well as other objectives, some of which might be location specific:

- Assess and improve asexual propagation techniques of cacao rootstocks.
- Develop new and better cacao rootstocks using any methods that might be appropriate.
- Develop tree size controlling (dwarfing) rootstocks for cacao.
- Acquire/exchange rootstocks within breeding/rootstock programmes in the cacao world.
- Evaluate the performance of cacao rootstocks in various environments and under different management systems.
- Determine biotic and abiotic stress tolerances of cacao trees in relation to new and existing rootstocks.

Tree fruit horticulturists and cacao agronomists together could formulate planting designs to evaluate and use cacao rootstocks to intensify production of cocoa beans.

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A Cacao Growth Mutant with Dwarfing Effect as Rootstock

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

Introduction

Improved harvest index is a major target of breeding programmes. In fruit trees, an improved harvest index can be achieved with the use of dwarfing rootstocks. Apples are probably the best-known example. In

cacao, to the best of our knowledge, a dwarfing rootstock has not yet been found.

A previous study at the Cocoa and Coconut Research Institute (CCRI) in Papua New Guinea (PNG) has shown that cacao hybrids of varying vigour did not affect the growth of budded clones when they were used as rootstocks. More recently, a mutant with abnormal growth characteristics was identified at CCRI. This mutant had a significant dwarfing effect when it was used as rootstock for budding three different clones.

Findings

The mutant was found among progenies of the cross SCA 12 x NA 149. Orthotropic buds from the mutant were grafted onto a normal rootstock to develop the clone MJ 12-226. All the grafted seedlings showed similar short growth with small and narrow leaves. Four trees were planted in the field for further observations. The trees continued to be shorter than other grafted clones from the same cross, with a high branching habit and compact dense growth. An average of 19.3 pods/tree were harvested from January to September 2001, which was similar to the two neighbouring clones (17.8 and 24.5 pods/tree). The pods were also of normal size of 412 g with 37.2 seeds/pod.

The clone, MJ 12-226, is probably self-incompatible. Over 100 self-pollinations were attempted, but all failed. However, it was cross-pollinated (male and female) with KEE 42, an Upper Amazonian local clone. All the seedlings derived from the cross KEE 42 x MJ 12-226 grew normally. However, seedlings that were obtained from open-pollinated pods segregated in variable proportions for normal and mutant phenotypes (Figure 1, Table 1). The mutant plants were significantly shorter with smaller leaves and a less developed root system. Most of the mutant seedlings also produced multiple stems, and developed into multi-stemmed, short and compact trees (Figure 2). The first fan branches after jorquetting also had a strong branching habit (Figure 3).

The presence of a smaller root system in the mutant seedlings suggested a possible effect on scion growth if they were used as rootstocks. This was tested by budding several clones onto mutant and normal seedlings derived from the same open-pollinated pods. The dwarfing effect of the mutant as a rootstock was clearly observed on scion height (Table 2, Figure 4), but less on leaf length and width.

Thus far, the dwarfing effect of the mutant as rootstock has been observed at the seedling stage. A larger number of mutant and normal segregants were recently used as rootstocks in buddings of three local clones (21-4-8, 17-3/1 and 37-13/1). The buddings will be planted in a replicated trial to test if the differences in growth rate persist with time and, if so, what the effect of dwarfing will be on the yield potential of the three clones.

The genetic control of the mutant condition has not yet been established. It appears that the mutant phenotype is due to a recessive nuclear allele since all the progenies of the cross with KEE 42 were normal. The appearance of mutant segregants in seeds from open-pollinated pods can be explained by partial self-pollination due to mixed self and foreign pollen carried by the pollinating insects. This hypothesis will be tested by self-pollinating progenies of the cross KEE 42 x MJ 12-226 as soon as they reach the reproductive stage. Considering the growth habit of the mutant, it is assumed that the mutation affects the quantity or the balance of growth hormones, or both.

Table 1: Average[1)] plant height, leaf length and leaf width of normal and mutant phenotypes derived from open-pollinated pods from the clone MJ 12-226

	Phenotype		Mutant/Normal (%)
	Normal	Mutant	
Seedling height (cm)	48.2 (43-54)	32.9 (24-37)	68.3
Leaf length (cm)	25.8 (23-29)	16.0 (11-19)	62.0
Leaf width (cm)	9.5 (9-10)	4.5 (3-5)	47.4

[1)] The range is given within brackets

Table 2: The effect of normal and dwarf mutant rootstocks on height, leaf length and leaf width of three budded clones

	Clone								
	21-4-8			17-3/1			37-13/1		
	N	M	M/N (%)	N	M	M/N (%)	N	M	M/N (%)
Height (cm)	24.8	16.2**	65.3	31.4	17.8*	56.7	29.4	22.2**	72.1
Leaf length (cm)	27.0	23.0*	85.2	25.2	19.8	78.6	26.2	21.0*	80.1
Leaf width (cm)	8.6	6.5**	75.6	936	7.5*	78.1	8.3	8.0	97.1

N - Normal rootstock M - Mutant rootstock
** - Highly significantly different ($P < 0.01$) from the normal.
* - Significantly different ($P < 0.05$) from the normal.

Figure 1: Mutant (L) and normal (R) phenotypes of seedlings

Figure 2: Multi-stemmed, short, compact habit of the mutant plant

Figure 3: Branching habit of the first fan branch of the mutant seedling

Please send comments to:
Y. Efron, D. Nideson, P. Epaina and M. Faure
PNG Cocoa and Coconut Research Institute
P O Box 1846
RABAUL
East New Britain Province
Papua New Guinea
Ph: + (675) 983 9131
Fax: + (675) 983 9115
e-mail address: ccri@datec.net.pg

Figure 4: Dwarfing effect in mutant (L) versus normal rootstocks

Performance of Experimental Cacao Hybrids from Bal Plantation, Malaysia in Papua New Guinea

Y. Efron, P. Epaina and J. Marfu

Introduction

Results obtained in BAL Plantation, Malaysia (Lockwood, *pers. comm.*) have shown that Upper Amazonian x Upper Amazonian hybrids produced higher yields than the Upper Amazonian x Trinitario hybrids, the type of hybrids that are being used in Papua New Guinea (PNG). BAL plantation is situated in Sabah, East Malaysia. The soil and rainfall are favourable for cacao cultivation, similar to the conditions in East New Britain (ENB) in PNG. Accordingly, a joint GxE trial was designed to test the same hybrids both in Malaysia and in PNG. Unfortunately, BAL Plantation was sold to another company that replaced all the cacao trees with oil palms. Therefore, the trial was conducted only in PNG. Most of the clones used as parents are well known and internationally available. Therefore, the results obtained in PNG might be of interest and use for other cocoa breeding programmes in other parts of the world.

Materials and methods

The design of the crossing scheme and the crosses were done by BAL Plantation. Upper Amazonian (UA), Trinitario (T) and commercially released clones in Malaysia were used as parents. Seeds of 32 hybrids between 3 female and 11 male parents (Table 2) were introduced into PNG in November, 1996. The seeds were planted first in a quarantine screen house, then, they were transferred to the nursery for hardening under close supervision.

Field planting was done in July 1997, after a final inspection and release by the Quarantine Officer, in a Randomised Complete Block design with five replications and 20 trees/replicate at 833 trees/ha. Four commercially grown SG2 hybrids from PNG (Table 1) were included as controls.

Dry bean yield was calculated based on the number of pods produced/plot, the average pod weight and the percentage of wet bean weight from the pod weight. A uniform conversion rate of 0.3 was used for all hybrids to convert wet to dry beans.

Results and discussion

Pod production started in 1999, about 18 months after planting. This is a usual time for initial production of hybrids in PNG. The results obtained by the end of 2000 did not support the observation from BAL Plantation that UA x UA hybrids produce higher yield than UA x T hybrids (Table 1).

Table 1: Yield and yield components of the 10 highest yielding Experimental hybrids and four SG2 Control hybrids

Hybrid	1999-2000 Dry Bean Yield (Kg/ha)	Rank	Average Dry Bean Weight (g)	Average Pod Weight (g)	Wet Bean (%)	Shell (%)
PA 13 x NA 226	2416	1	0.99	399	26.4	17.8
SCA 12 x KA2-106	2399	4	1.08	388	28.5	16.9
SCA 12 x PA 107	2385	5	0.99	404	27.2	17.1
SCA 12 x ICS 1	2305	6	0.97	438	26.8	16.7
PA 13 x NA 149	2298	7	1.01	381	26.1	15.8
SCA 12 x NA 226	2223	8	0.86	352	26.4	16.8
SCA 12 x NA 149	2124	9	0.77	350	26.0	15.4
PA 13 x PA 300	2066	10	0.89	394	24.0	16.2
PA 13 x ICS 1	2061	11	1.15	447	27.2	14.4
PA 13 x IMC 23	2034	12	1.04	383	26.6	16.1
SG2 CONTROLS						
KEE 42 x K82	2408	2	1.38	555	28.4	17.4
KEE 12 x KA2-106	2403	3	1.11	390	32.3	14.5
KEE 43 x KA2-106	2020	13	1.50	615	29.2	15.1
KEE 42 x K82	1981	16	1.03	331	29.7	15.8

The yield obtained from the various hybrids was not significantly different. Two of the control hybrids (UA x T) ranked 2nd and 3rd. Other UA x T hybrids, i.e. SCA 12 x KA2-106 and SCA 12 x ICS 1 were also among the highest yielding hybrids. The hybrids produced with NA 33 or the local Malaysian clones (QH 441, PBC 123 and BR 25) were not included among the 10 highest yielding hybrids (Table 1).

The hybrids produced in BAL Plantation usually had lower percentages of wet beans and similar shell percentages as compared to the SG2 control hybrids. Two of the control hybrids (KEE 42 x KA2-106 and KEE 43 x KA2-106) had particularly bigger pods of 555 and 615g in weight, respectively. However, the major draw- back of these hybrids was their very small bean size. Most of them had an average bean weight below 1.0g, which is usually not desired by the industry. All the four SG2 control hybrids had bean weights above 1.0g.

The experiment provided some information about the combining abilities of the parents used (Table 2). Among the female parents, NA33 was clearly a donor for lower yield as compared with SCA 12 and PA 13. PA 13 was a donor for larger pods and beans but a lower percentage of wet beans. Similarly, NA 149 had the highest average yield, ICS 1 the heaviest pods, PA 173 the highest percentage of wet beans and KA2-106 the largest beans among the male parents. Interestingly, the local Malaysian clones, QH 441, PBC 123 and BR 25, were among the worst performers as donors for high yield potential.

Table 2: Average yields and yield components of the parental female and male clones of the Malaysian produced hybrids

Clone	Number Hybrids[1)]	1999-2000 Yield (kg/ha)	Average Pod weight (g)	Average Wet Bean (%)	Bean weight (g)
SCA 12 (F)	11	2050	364	26.7	0.90
PA 13 (F)	9	2010	399	25.2	1.06
NA 33 (F)	11	1750	367	27.6	0.99
NA 149 (M)	3	2113	365	26.9	0.87
PA 107 (M)	2	2090	379	28.0	0.99
ICS 1 (M)	3	2060	417	26.9	0.87
KA 2-106 (M)	3	2014	399	26.9	1.09
NA 226 (M)	3	2006	381	25.9	0.95
PA 300 (M)	3	1907	364	26.0	0.90
QH 441 (M)	3	1862	339	26.4	0.99
IMC 23 (M)	3	1858	392	25.7	0.94
PBC 123 (M)	3	1781	387	26.0	1.01
PA 173 (M)	2	1795	335	28.9	0.94
BR 23 (M)	3	1779	344	26.2	0.92

[1)]Number of hybrids tested with the parental female (F) and male (M) clones in their pedigrees.

The following conclusions were drawn from the experiment:

- The experimental hybrids produced by BAL Plantation, Malaysia did not have higher yields than the locally produced hybrids in PNG.
- The experimental hybrids had very low bean weight when tested in PNG.

Considering the above information, the experimental hybrids tested are of no value as hybrids to cocoa production in PNG. Therefore, the experiment was terminated, but the germplasm is maintained as clones. There was no support for the observation made in BAL Plantation that UA x UA hybrids are higher yielding than UA x T hybrids. Under PNG conditions, both UA x UA and UA x T hybrids have similar yields.

Please send comments to:
Y.Efron or P.Epaina
PNG Coconut & Cocoa Research Institute
P.O. Box 1846
RABAUL, ENB
Papua New Guinea
Fax: (675) 983 9115
e-mail address: ccri@datec.com.pg

Utilisation of the Cacao Genetic Resources at the International Cocoa Genebank, Trinidad

A.D. Iwaro, F.L. Bekele and D.R. Butler

Introduction

The conservation of cacao genetic resources and distribution of promising genotypes or populations are of fundamental importance to cacao breeders worldwide. The Cocoa Research Unit (CRU), Trinidad maintains one of the largest (approximately 2,000 accessions) and diverse collections of cacao germplasm in the public domain, which is designated the *'International Cocoa Genebank, Trinidad'* (ICG,T). Since the value of conserved germplasm depends on information available for each accession, CRU gives high priority to systematic characterisation of the collection for important agronomic and economic traits.

Evaluation of some characteristics of economic interest

In this article, information is presented on bean number, bean weight and pod index for 581 genotypes evaluated using the methods of Bekele *et al*. (1993) and Bekele *et al*. (2000). Five hundred accessions were also evaluated for resistance to *Phytophthora* pod rot (black pod disease) using the method of Iwaro *et al.* (2000).

A wide range of variation was observed in bean number, bean weight and pod index (the number of pods required to produce 1kg of dry beans) among the 581 accessions evaluated. Bean number varied from 17 to 58 with a mean of 40 ± 0.26, while bean weight ranged from 0.44g to 1.84g with a mean of 0.96g ± 0.008. Pod index on the other hand, ranged from 13.9 to 66.1 with a mean of 27.9 ± 0.28. Ninety-nine (17.0 %) of the 581 genotypes evaluated had large bean number (> 45), while 64 accessions (11.0%) possessed large bean weight (> 1.20g). These genotypes are good sources of genes for the genetic improvement of cacao bean number and bean weight. Fifty-six of the 581 accessions evaluated had low pod index (< 20.1) and are potentially good sources of genes for yield improvement. Among the 500 accessions evaluated for *Phytophthora* resistance, 15.6% were found resistant (disease rating 1-3), 21.6% moderately resistant (disease rating 4-5) and 62.8% susceptible (disease rating 6-8). The resistant genotypes (seventy-eight) are potential sources of genes for the development of new varieties with an acceptable level of resistance to *Phytophthora* pod rot. Favourable combinations of low to intermediate pod index (< 35.0) and resistance to *Phytophthora* pod rot were observed in some genotypes (Table 1a and b). Previous investigations indicated that some of these accessions also had resistance to witches' broom disease.

Utilisation of the genetic material in ICG,T

The results reported on bean weight and bean number, pod index and resistance to *Phytophthora* pod rot showed a wide variation among the accessions assessed in ICG,T. Such diversity provides an opportunity for effective selection and exploitation of the desirable variants within the ICG,T as a source of genes for the genetic improvement of cacao yield and resistance to *Phytophthora* pod rot. Those accessions with a low pod index and resistance to *Phytophthora* pod rot are potential candidates for inclusion in international clonal trials to assess their adaptability to different environments. Other genotypes that combine low to moderate pod index with resistance to *Phytophthora* pod rot and which also show resistance to witches' broom disease are invaluable in cocoa breeding programmes in the Americas and the Caribbean where black pod and witches' broom diseases are major threats to cocoa production. These accessions are also potential candidates for inclusion in national working collections.

Table 1a: Genotypes with moderate to low pod index and resistance to *Phytophthora* pod rot

Genotype Value	Category	Pod Index	*Rating for *Phytophthora* pod rot
CL 10/5	a	19.3	1
AM 282	a	19.5	2
CRU 89	a	20.4	2
LP 3/5†	b	20.9	2
IMC 94	b	21.1	2
CRU 48	b	21.2	2
MOQ 218	b	21.4	2
CRU 78	b	22.9	3
POUND 7/A	b	23.1	2
IMC 20	b	23.2	1
EET 59	b	23.5	2
SLC 4†	b	24.0	1
PA 157	b	23.1	3
LP 3 / 4	b	24.4	2
IMC 76†	b	24.4	2
AMAZ 12	b	24.6	3
NA 672†	b	24.6	3
EET 272	b	25.0	1
JA 6/4	b	25.1	3
NA 168	b	26.1	1
ICS 70‡	b	26.4	1
CRU 72	b	26.5	1
POUND 4/A	b	26.8	1
IMC 47	b	27.4	1
NA 3	b	27.4	2
PA 30	b	27.5	2
NA 312	b	29.1	2
CRU 19	b	29.6	1
PA 70	b	29.7	3
CL 19/49	b	29.8	3
PA 124	b	29.8	2

a : Low pod index and resistant to *Phytophthora* pod rot
b : Intermediate pod index and resistant to *Phytophthora* pod rot
† Tolerant/resistant to Witches' Broom disease (Wadsworth *et al.*, 1997)
‡ Highly resistant to Witches' Broom disease (Laker, 1987)

*Rating	Infection level
1	No visible lesion
2	1-5 localised lesions
3	6-15 localised lesions

Detached pod inoculation by spray method
Inoculum concentration: 100,00mL^{-1}

Table 1b: Genotypes with moderate to low pod index and resistance to *Phytophthora* pod rot

Genotype Value	Category	Pod Index	*Rating for *Phytophthora* pod rot
PA 27	b	29.9	3
NA 235	b	30.0	3
PA 95	b	30.2	2
NA 534	b	30.2	3
PA 136	b	30.3	2
SPEC 18/6	b	30.5	3
CRU 80	b	31.1	2
MOQ 6/82	b	31.3	1
DE 52B	b	31.4	3
NA 399	b	32.7	1
NA 7/10	b	33.1	3
ICS 41†	b	33.2	1
CL 19/10	b	35.0	3
PA 120	b	35.9	3

b : Intermediate pod index and resistant to *Phytophthora* pod rot
† Tolerant/resistant to Witches' Broom disease (Wadsworth e*t al.*, 1997)
‡ Highly resistant to Witches' Broom disease (Laker, 1987)

*Rating	Infection level
1	No visible lesion
2	1-5 localised lesions
3	6-15 localised lesions

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Please send comments to:
A.D. Iwaro
Cocoa Research Unit,
The University of the West Indies, St. Augustine,
Trinidad and Tobago, West Indies.
e-mail address: iwaro@hotmail.com

CUPUASSU (Theobroma grandiflorum) Genetic Resources and Breeding in the Brazilian Amazon

R. Moysés Alves and A. Figueira

Introduction

Theobroma grandiflorum (Willd ex Spreng) Schum. (cupuassu) is native to the Brazilian Amazon region, with its putative center of diversity located in the south/south-eastern region of Pará state (Cuatrecasas, 1964). Apart from being considered a potential source of useful genes for cacao (*Theobroma cacao* L.), cupuassu is a promising new crop (Figure 1). The strong tropical flavour from the pulp coating the seeds is highly appreciated in juices, ice cream, jams, candies, desserts and liquors (Figure 2) (Cavalcante, 1991; Barbosa *et al.*, 1978; Calzavara *et al*., 1984). The pulp comprises up to 40% of the fruit weight (Figure 1). A product similar to cocoa powder (called "cupulate") can be obtained from fermented seeds (Ribeiro *et al*., 1993).

The Amazon region has been suffering from intense colonisation pressure, with extensive deforestation and timber exploration, which is threatening the conservation of the natural cupuassu populations (Figures 3 and 4). The incorporation of forested areas containing natural populations of cupuassu into agricultural land can cause irreversible losses of valuable genetic resources (Homma *et al*., 2001).

Until the 1980s, most of the cupuassu production was extractive, gathered from natural stands. This practice helped to preserve some areas where cupuassu occurred naturally together with Brazil nut trees (*Bertholletia excelsa* Humb. & Bonpl). More recently, cupuassu plantings have been established, leading to a decrease in fresh fruit prices. Cupuassu fruit gathering is becoming economically less attractive, leading to an irreversible trend for technical cupuassu cultivation (Homma *et al.*, 2001), and a consequent loss of protection for some cupuassu natural populations, endangering their conservation. Due to the absolute lack of improved selected cultivars, the commercial plantings have been established mainly using seeds collected from natural populations, resulting in a great heterogeneity in stands, with low yields, but preserving an important sample of natural genetic diversity. The renewal of plantings with clones and other selected cultivars will result in improved yields, but will contribute to genetic erosion of natural germplasm. To limit this problem, *in situ* and *ex situ* conservation strategies are being implemented, and some of the current activities will be presented in this article.

Economic importance

Cupuassu products were once restricted to local Amazonian markets. The recent increase in production made it possible to reach other markets with the consequent economic and social benefits to the region. The transition from a mainly extractive activity to commercial plantings had a reduced environmental impact since new plantings were mainly established in areas already cultivated with other crops, and further deforestation was avoided. Cupuassu is an ideal crop for agro-forestry systems because it requires shade during the juvenile stage, as does cacao, but stands unshaded at the bearing stage thus favouring the preservation of forested areas. There are more than 20,000 ha cultivated with cupuassu in the Amazon, with at least 14,000 ha in Pará state alone, the major producer in Brazil (Homma *et al.*, 2001).

Classification and geographic distribution

Cupuassu is a *Malvaceae s.l.* (Alverson *et al.*, 1999), classified as a member of section Glossopetalum, which contains 12 of the 22 species of *Theobroma* (Cuatrecasas, 1964). *Theobroma grandiflorum* is diploid ($2n = 20$), but Moraes *et al*. (1994) reported that a seedless mutant of cupuassu, collected in Cametá (Pará), was triploid.

Cupuassu's natural geographic distribution is limited to native forests south of the Amazon River, west of the Tapajós River, including the south and south-eastern region of Pará state and the Amazon of Maranhão state [Figure 3] (Ducke, 1953). This region was considered to be the centre of diversity of *T. grandiflorum* by Cuatrecasas (1964). Cupuassu was spread throughout the whole Amazonian region, probably in association with the migration of native people in pre-Columbian times (Clement, 1999).

The strong association of cupuassu with human migrations confounds the identification of real spontaneous or semi-spontaneous populations (Moraes *et al*., 1994). Tree density is an important indicator since in natural populations trees are found at very low density. For instance, a natural area with a higher density of cupuassu plants near Marabá (Pará state) had an average of 2 plants ha^{-1}, reaching 3.75 plants ha^{-1} (Homma *et al.*, 2001). Under natural conditions, cupuassu can reach up to 30 m in height (Figure 4), but under cultivation the trees are kept between 6 to 10 m high by pruning. Pod production is

highly irregular, with spontaneous trees bearing an average 25 pods tree-1, with an estimated loss of 10% due to damage during harvesting and animal attack (Homma *et al.*, 2001).

Ecology

Cupuassu occurs naturally in hot and wet climates with annual mean temperatures between 21.6ºC to 27.5ºC, relative humidity between 77 to 88% and average rainfall ranging from 1,900 to 3,100 mm (Diniz *et al*., 1984). Cupuassu occurs naturally as an understorey species in dry Amazon soils, but it can be found on low wetlands associated with heart-of-palm or açai (*Euterpe oleraceae*).

Reproduction system

Cupuassu is a self-incompatible, cross-pollinated species, with hermaphrodite flowers. Physical barriers isolate the stigma from the anthers, and the complex self-incompatibility system makes cupuassu an obligate cross-pollinated species (Venturieri, 1993; Venturieri and Ribeiro, 1995). The flowers are commonly visited by various insects such as bees, beetles and ants, making it difficult to distinguish between pollinators and pests or pollen eaters. Venturieri *et al*. (1997) proposed that seven beetle species (Chrysomelidae; subfamily Eumolpinae) were effective cupuassu pollinators based on studies in two experimental areas.

Under natural Amazonian conditions, cupuassu blooms during the dry season (July to December) and produces fruits from August to April (Prance and Silva, 1975), with a major peak from January to March (rainy season). In plantings under more favourable conditions (fertilisation, pruning, etc.), cupuassu bears fruit continuously with short intercrop periods.

Natural dispersal agents

Animals, mainly large rodents such as agouti (*Dasyprocta* sp.) and paca (*Cuniculus paca*) perform natural seed dispersion of cupuassu. In addition, fruit abscission from tall trees facilitates self seed-dispersion, since a high proportion of fruits break open while hitting the ground, spreading seeds. However, seedlings are not commonly found around a mature tree, possibly because of the intense attack by animals. Incidentally, cracked pods are a major factor contributing to lower yields in the extractive production system.

Population conservation

Cupuassu seeds are recalcitrant and do not tolerate desiccation or cold temperatures. *In vitro* conservation and cryopreservation are attractive technologies for long-term germplasm storage, but both are not yet available for cupuassu, since little progress has been reported on cupuassu *in vitro* culture, lagging behind the recent progress achieved for *T. cacao*. Thus, the only alternatives are *in situ* conservation or through an active *ex situ* germplasm collection.

***In situ* conservation**

In situ conservation is the method of choice for long-term germplasm preservation since when properly managed and implemented, it can maintain the evolutionary potential of the species especially for perennials (Debouck, 1993). Another advantage for *in situ* conservation includes lower costs of maintenance than for *ex situ* germplasm collections. Since cupuassu is endemic to a limited region, which coincides with an area under the strongest anthropic and deforesting pressure in Brazil, there is an urgent need to define zones that better represent the species diversity and convert these into legal conservation areas. The major difficulty is to transform areas containing wild cupuassu populations into legally recognised and respected areas of conservation.

In 2001, a project was launched to identify populations based on studies of the genetic diversity and structure between and within wild *T. grandiflorum* populations at the centre of maximum diversity proposed by Cuatrecasas (1964). After the populations are defined, studies will be conducted to elucidate mechanisms associated with the maintenance of genetic diversity such as gene flow, pollinators, and seed dispersal to allow sustainable management of the genetic resources.

***Ex situ* conservation**

The establishment of *ex situ* collection has been the first measure to support breeding programmes. Almost all Amazonian research institutes keep a small cupuassu germplasm collection for breeding. However, these *ex situ* collections appear to represent only a small fraction of the species diversity, resulting from selections rather than a systematic sampling of the diversity. Collection of areas under strong colonisation pressure is urgently needed, such as the region of Marabá in Pará and the Southern region of the state (Alves *et al*., 1996) (Figure 3).

The lack of adequate methods for conservation in field collections and trained human resources, as well as losses due to biotic and abiotic stresses associated with high costs of maintenance, have contributed to small interest in the establishment of new germplasm collections in the region. To minimise pest and disease attacks in germplasm collections, a new model for Amazonian perennial species conservation was

proposed, combining diversity, density and genetic variability (Paiva, 1994; 1998). In this model, cupuassu accessions, now maintained as a single species collection, would be combined with other species in order to mimic natural conditions, making the trees less vulnerable mainly to pathogen attacks (such as by *Crinipellis perniciosa* (Figure 7)), and allowing conservation and evaluation (Dantas *et al*., 1986). Similarly, large growers cultivate cupuassu together with passion fruit, papaya, black pepper or banana, but only at the juvenile stage. Small growers tend to keep various species together in culture as a house garden.

The *ex situ* conservation approach presents the great advantage of allowing through characterisation and evaluation of the accessions, a preliminary knowledge of the potential of each genotype, kept in a more protected site.

Cupuassu genebanks

The cupuassu genebank comprises of various germplasm collections established at many research institutes in the Amazon region (Figure 3). The collections contain both clonal material and half-sib progenies established in Belém, Pará state (PA); Manaus, Amazonas state (AM); Porto Velho, Rondônia state (RO) and Rio Branco, Acre state (AC). The accessions were collected from spontaneous populations, commercial plantings and house gardens (Moraes *et al.*, 1994).

The Embrapa Amazonia Ocidental, at Manaus, AM contains 128 clonal accessions along with 119 half-sib families, originally collected at the Upper Solimões (AM), Medium Amazonas (AM), and Bragantina (PA) (Souza, 1996). Another germplasm collection is located in Manaus, at the Instituto de Pesquisa da Amazonia (INPA), and contains 132 accessions collected from the flooded area of the hydroelectric dam of Tucuruí (PA) (Figure 3).

In Belém, PA, at the Embrapa Amazonia Oriental, there are 46 cupuassu clones conserved in the local collection, originally sampled from areas in Pará, Amazonas, and Amapá, shown in Figure 3. This genebank is complemented with another 50 clones and 49 half-sib families selected in commercial areas in Pará and established at Tomé-açu, PA (Alves *et al.*, 1997a), and another 34 clones from Belterra, PA (Figure 3).

In Rondônia, a collection was started in 1992 by Embrapa Rondônia, 64 trees were selected and identified in various regions of the state, and 36 were established in the field as families (Ribeiro, 1997). A similar exercise was conducted in Acre, where selections were made in commercial plantings, and the 12 most promising clones were planted at Embrapa Acre (Cavalcante and Costa, 1997).

Agronomic evaluation and characterisation

Morphological and agronomic characterisation of the cupuassu germplasm collections commenced a decade ago. Most efforts have been concentrated on the establishment of a list of descriptors to enable the classification and identification of the various accessions. The objectives are to estimate the variability for those characters; to define the need for increasing the variability present in the collections; and to define the genetic relationships among accessions to maximise diversity and eliminate duplicates.

Characterisation has been routinely conducted for pod yield (Alves *et al.*, 1997a; Souza and Silva, 1997, Cruz and Alves, 2001) and witches' broom resistance (Alves *et al*., 1998c; Bastos *et al.*, 1999; Cruz *et al.*, 2000), and on phenology (Araujo and Alves, 1995; Alves *et al.*, 1997a) in various germplasm collections (Figure 5). Detailed morphological description has been conducted for leaf descriptors (Alves *et al.*, 1997b); flowers (Alves *et al*., 1998a); pods (Guimarães *et al.*, 1992; Alves *et al*., 1996; Souza, 1996; Araújo, 2000), and pulp quality (Alves *et al.*, 1998b). Examples of morphological variability are shown in Figure 5.

Molecular characterisation

The development of microsatellite primers for *Theobroma cacao* (Lanaud *et al.*, 1999) has enabled more efficient evaluation of genetic diversity using co-dominant, multi-allelic, and highly reproducible genetic markers. The objective is to optimise the conservation of genetic resources by establishing core collections (Brown, 1989) since a single cupuassu tree requires a space of 36 m^2. The Belém germplasm collection, with 46 accessions, was analysed using 19 microsatellite loci, and exhibited 93 alleles with an average of 5.1 alleles per locus (Alves *et al*., 2001), and each accession could be distinguished from the others (Figure 6).

Breeding strategies

A major objective for cupuassu breeding is the development of cultivars resistant to witches' broom disease caused by *Crinipellis perniciosa* (Alves *et al*., 1997a) (Figure 4). Similar to what occurs with *T. cacao* L., witches' broom disease cannot be efficiently controlled using phytosanitary pruning or fungicide sprays, and growers have abandoned cupuassu plantings because of this scourge. There are plans to release five witches' broom resistant clones to renew plantings in the near future. Pod production and yield stability are other important breeding objectives (Souza *et al*., 1992a). Similar to cacao, cupuassu has a low pod set rate, with average values as low as 0.55% under natural conditions in Manaus, AM (Falcão and

Lleras, 1983). Preliminary studies indicate that phenotypic selection for pulp fresh weight can result in larger gains in pod weight than direct selection for pod weight, probably because of selection for thinner pod husk (Fonseca *et al*., 1990).

Due to the large natural variability available for cupuassu, the first breeding strategy has been to directly select genotypes from spontaneous, semi-spontaneous populations or from heterogeneous commercial plantings (Alves *et al.*, 1998d). After evaluation, the selections should be clonally propagated and distributed to growers. Medium and long-term strategies might involve the development of hybrid varieties to combine important characters.

Conclusions

Cupuassu, a species native to the Brazilian Amazon became an important crop for the northern region of Brazil, spreading to other regions and countries, with increasing economic significance. A major constraint to the further development of cupuassu as a crop is the lack of improved cultivars with resistance to witches' broom disease.

The establishment of many development projects in southern Pará during the 1980s, such as a large mining project (Carajás), a hydroelectric dam (Tucuruí), large agriculture and pasture projects, and road openings, has resulted in a strong migration and land exploration pressure. The putative center of diversity of cupuassu has been suffering from this intense colonisation pressure with large deforestation and timber exploration threatening the conservation of the native cupuassu populations. These remaining spontaneous populations are important sources of genes. Furthermore, cupuassu genebanks contain a very limited number of genotypes, mostly collected based on breeding objectives and not for germplasm conservation.

There is an urgent need to rescue populations under threat and to survey and identify areas with the greatest genetic diversity for the establishment of *in situ* conservation zones. Genebanks should also be enriched with new collections to support breeding programmes.

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Send comments to:
A. Figueira
Laboratório de Melhoramento de Plantas, Centro de Energia Nuclear na Agritultura, Universidade de São Paulo, Piracicaba, SP, 13400-970, BRAZIL
e-mail address: figueira@cena.usp.br

Figure 1: A) Young cupuassu tree starting production; b) cupuassu immature pod; c) open cupuassu pod.

Figure 2: Sample of manufactured products derived from cupuassu.

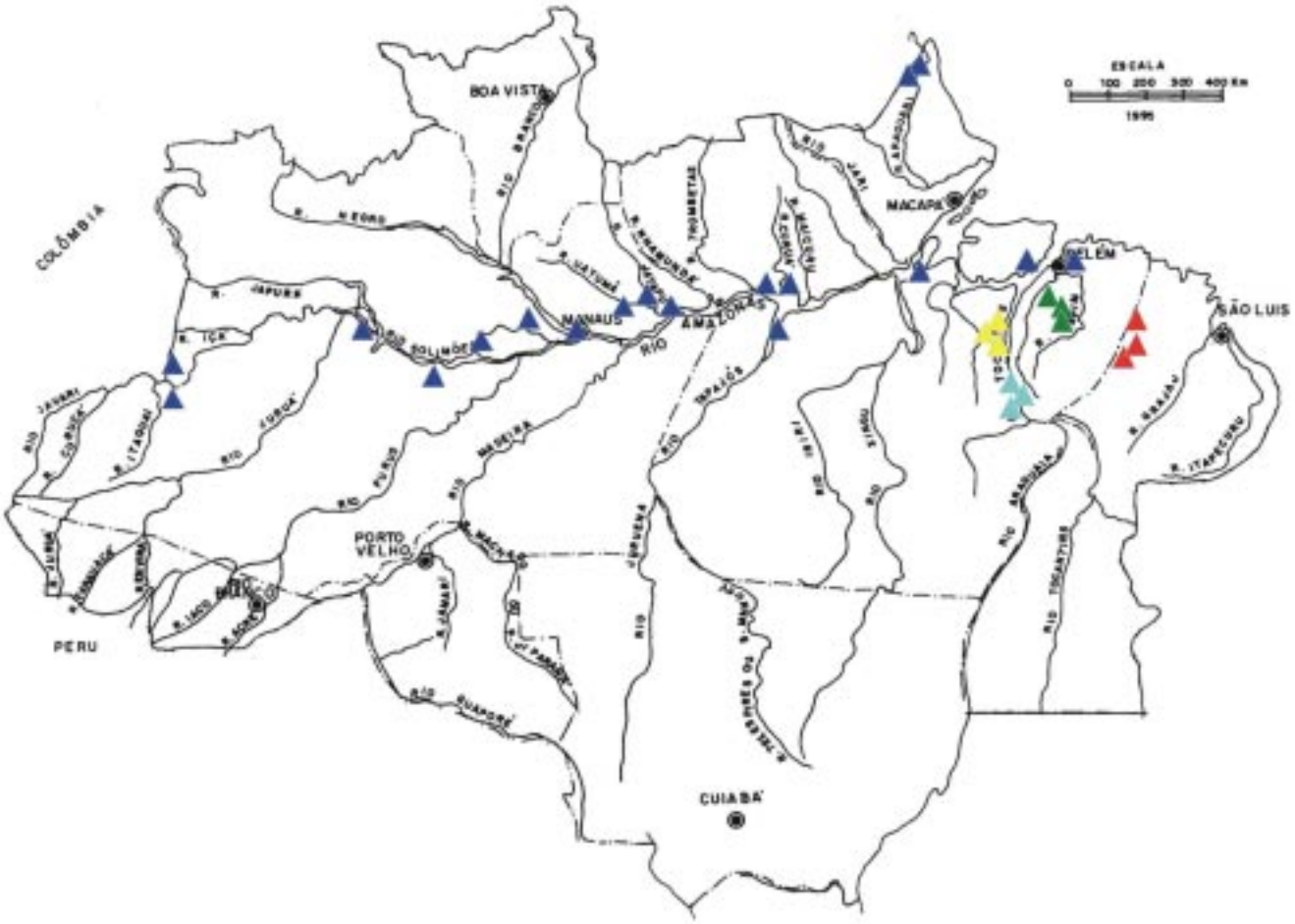

Figure 3: Map of Brazilian Amazonia. Populations sampled and conserved at EMBRAPA, Belém germplasm collection; Tucuruí and Maranhão conserved at INPA, Manaus, AM; spontaneous population collected at Nova Ipixuna, Pará; Tomé Açú, PA.

Figure 4: Spontaneous cupuassu tree at Nova Ipixuna, Pará.

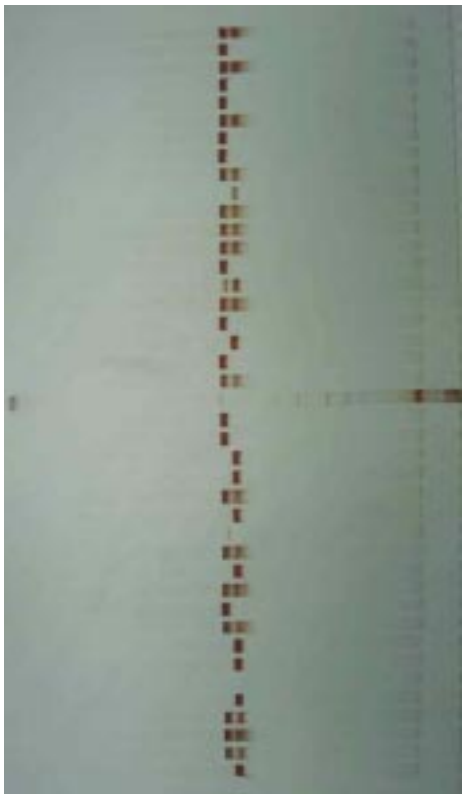

Figure 6: Microsatellite analysis of accessions from EMBRAPA Belém germplasm collection.

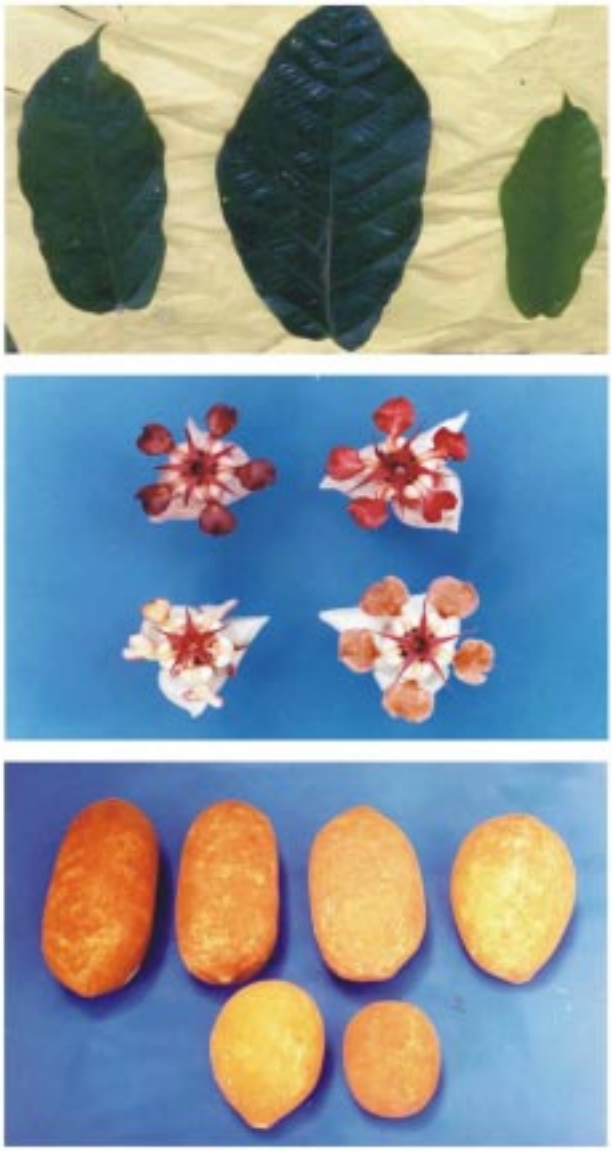

Figure 5: Sample of morphological diversity of cupuassu at the EMBRAPA Belém collection.

Cacao Fish Bone Disorder in Papua New Guinea

Y. Efron, P. Epaina, J. Saul and J. Konam

A cacao growth disorder was identified recently at the Cocoa and Coconut Research Institute (CCRI) in Papua New Guinea (PNG). It was first observed on a Trinitario clone, KA2-106, in a rootstock x scion interaction trial (Figure 1). All the trees in 24 different plots (9 trees/ plot) showed the same disorder symptoms. At the same time, another Trinitario clone, K82, which was also included in the trial, showed only very minor symptoms and only on few trees. It was later observed also on other genotypes.

The disorder in KA2-106 was expressed as bare and proliferated branch tips (Figure 2). The axillary branches were compressed, short, poorly developed with very small leaves only at the growing point. The compacted scars of the leaves resemble a spinal structure of a fish. Therefore, the condition was named Fish Bone Disorder (FBD). Symptom expression of the disorder varied between genotypes. In the clone KA2-101 (Figure 3), the axillary branches were shorter and less compacted.

The severity of the symptoms was associated with the amount of shade. They were more severe when the trees were more exposed to the sun. Thus, in the root stock x scion interaction trial, the average score of 89 KA2 -106 trees located nearby a *Gliricidia* shade tree was 4.4 (1–9 visual rating scale) as compared with an average score of 6.3 for 112 trees that were located further away from the shade tree. Furthermore, trees of KA2-106, planted in a nearby heavily shaded block, did not show any FBD symptoms.

During the vegetative flush period, buds in the infected areas started to sprout (Figure 4). However, soon after, black dots or holes appeared on the very young leaves. Later, these leaves shriveled and fell off. Larger leaves remained on the trees, but had holes (shot holes), necrotic edges and were curled and twisted (Figure 5). Inspection of samples of infected leaves revealed the presence of a large number of *Colletotrichum* conidia. No insects were found to be associated with the damage.

There were clear genetic differences between genotypes in their relative resistance/susceptibility to FBD. KA2-106 was one of the most susceptible clones. K82, under the same conditions, was much more resistant. The effect of shade, age and probably other factors that affect plant vigour prevented an accurate assessment of different genotypes. However, a survey conducted in several breeding trials showed the following:

- Only few highly susceptible clones, similar to KA2-106, were identified.
- The Trinitario germplasm tended to be more susceptible than the Upper Amazonian germplasm.
- The highest proportion of clones with FBD (26.6% moderate and 3.5% severe) was found in a sunlight tolerance trial planted without shade.
- Resistance is under genetic control and, if required, the problem can be solved by breeding.
- Among international introduced clones, POUND 30, MOQ 5/28 and UF 11 showed clear FBD symptoms.

The symptoms of FBD were not persistent. The first symptoms of FBD were observed in November, 2000. The number of trees showing symptoms increased with time during 2001, until September. From September 2001, most of the trees, but not all, developed normal growing shoots out of the FBD affected areas (Figure 6), giving the trees a normal appearance.

The causal agent for FBD is not yet known. The most probable explanation is that FBD was caused by *Colletotrichum*. It is a common pathogen in PNG, and may also cause cherelle wilt in cacao, particularly during dry periods (Blaha, per. comm.). Usually, *Colletotrichum* is considered as a weak pathogen. However, somehow, due to unknown reasons, the conditions were changed to favour infection with the disease or to increase the vulnerability of the cacao trees to the disease. Similar symptoms to those attributed to *Colletotrichum* were reported in East Java, Indonesia (Yohanes and Sri-Sukamto, 1992).

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Please send comments to:
Y.Efron or P.Epaina
PNG Coconut & Cocoa Research Institute
P.O. Box 1846
RABAUL, ENB
Papua New Guinea
Fax: (675) 983 9115
e-mail address: ccri@datec.com.pg

Figure 1: A tree of KA2-106 with Fish Bone Disorder symptoms.

Figure 2: Branches of KA2-106 with Fish Bone Disorder

Figure 3: Fish Bone Disorder in the clone KA2-101.

Figure 4: Sproting of leaves on branches with Fish Bone disorder

Figure 5: Older leaves of KA2-106 with advanced symptoms. Note the necrotic edges, the holes and the curling of the leaves.

Figure 6: A normal branch growing from a fish Bone Disorder affected area.

Proposal for the Creation of an "International Working Group on Cocoa Genome studies"

USDA and INGENIC jointly organised a discussion session at the end of the Cocoa Research Review Meeting organised by USDA and Mars Inc. in Miami from January 21 to 23, 2002. A report on this discussion session, and on possible further involvement of INGENIC in this group is presented hereunder.

Firstly, Bertus Eskes led off the session with an introduction on the activities of the International Group for the Genetic Improvement of Cocoa (INGENIC) over the first seven years of its existence. The INGENIC mailing list currently contains the addresses of 350 interested persons. Three workshops were organised so far, and six Newsletters were released. During the last workshop, held in Malaysia in October, 2000, a proposal to set up a consortium for cocoa genomic studies was launched by Mark Guiltinan. The INGENIC board endorses this important initiative and has received positive reactions from INGENIC e-mail correspondents informed on the intention to create a group dealing with genomic studies. INGENIC is grateful to USDA for having provided the possibility for discussions on this matter during the present Cocoa Research Meeting.

As an example, the Banana Genome Consortium was briefly described. Two meetings were organised to set up the banana consortium, which has the following objectives:

- Unite forces to obtain common, non-competitive research objectives of global interest;
- Release results into the public domain;
- Concentrate on main activities, which relate to the development of tools and use of genome knowledge from other species;
- Facilitate coordination through a unit at INIBAP, Montpellier;
- Obtain additional funding (EU and Brazilian sources are proposed) although participants have already initiated their activities.

A document will soon be published on the banana initiative, which can be requested from Claudine Picq at INIBAP (c.picq@cgiar.org) by any interested person.

The discussions continued with presentations by Claire Lanaud and Julio Cascardo on how genomic tools work, and what can be expected from genome studies in cacao. After the break, an open discussion was initiated on the creation of a group on cocoa genome studies. It was recognised that during the fruitful discussions that had been carried out the

previous day, a common research goal related to EST libraries and bio-informatics had already identified. The present discussion session was aimed at following up on these earlier discussions within a larger group, and with broader objectives.

The following name was proposed for the consortium: 'International Working Group on Cocoa Genome Studies'. The main objective suggested was to "identify and share molecular genetic information to improve cocoa varieties". Results should be in the public domain. The following main activities were identified:

- Creation of EST libraries,
- Micro-array studies,
- Increase of SSR markers,
- BAC mapping,
- Genotype identification studies,
- Creation and management of segregating populations, and
- Database management.

The institutions involved in the group would initially be: CEPLAC, CIRAD, MARS Inc., Penn State University, USDA, UESC and possibly the new CFC project (for the creation and management of segregating populations). Other possibly interested institutions (Reading University, Unicamp, CNRA, MCB) have been contacted by INGENIC.

During the discussions on the organisation of the group, it was agreed that the first step was the possible creation of a 'scientific committee', comprised of one representative from each participating institution. This committee should take initiatives to further develop the activities of the group. The following persons accepted nominations to be part of this committee during the meeting: Julio Cascardo, Mark Guiltinan, Martin Gilmour, Philippe Petithuguenin, Ray Schnell and Uilson Lopes. It was suggested that the group could act as a sub-group of INGENIC. The INGENIC chairman has discussed the proposal with the INGENIC Board members. The board members agreed that the chairperson of such a group could become part of the INGENIC Board, and as such inform a wider INGENIC audience on the progress of the group and possibly use the INGENIC workshops for discussion meetings.

A chairperson for the group still has to be identified by direct contacts between the group members. Possibly, the chairmanship could be done on a rotation basis.

One of the first activities of the group would be to prepare a strategy document, aimed at a larger audience, which would explain the main objectives and working procedures of the group. As a second stage, the need for additional funding of activities will be analysed. The group would tentatively meet within one year to discuss further developments and activities.
It was recognised that the collaborative group on EST libraries, created earlier during the week in the Miami discussion sessions, is specifically looking into collaboration on EST activities and bio-informatics. The relationship between this group and the broader 'International Working Group on Cocoa Genome Studies' should be clarified.

The INGENIC chairman has already informed e-mail respondents of INGENIC of the outcome of this meeting, and requested reactions. Early reactions, related to this initiative, are reproduced below. INGENIC hopes that this will lead to further constructive discussions, by e-mail and through further notes in the Newsletter, on the relationship between traditional breeding and cocoa genomic studies.

Bertus Eskes
INGENIC Chairman

Reactions to the proposal to create an international group on cocoa genome studies

The King is Naked

Thank you for sharing with us the report on the joint USDA/INGENIC discussion meeting on the creation of an "International Working Group to Study the Theobroma cacao Genome". Frankly, I am very disturbed and concerned. To start with, I would like to cite a sentence from Dr. Philip Keane's (La Trobe University, Australia) review on the paper "Screening segregating cocoa genotypes for resistance to Vascular-Streak Dieback (VSD) under natural conditions in Papua New Guinea" that we have submitted for publication in the journal Australasian Plant Pathology: *"In this day and age when pathologists (and breeders) are becoming more and more obsessed with molecular biology and sophisticated technology, and are conducting research which often bears little relationship to the realities of agriculture in the field, it is refreshing to see a piece of research which, while apparently simple in methodology, goes right to the practical heart of the matter and produces such stunningly informative and useful results."* His statement is very well written and says it all!

Yes, genome studies are very fashionable. Almost everybody wants to be associated with it, including donors. It is a very good "milking cow". It had an enormous value in *Arabidopsis* as a model plant to expand our basic biological knowledge and understanding. Genome analysis is also an important tool for very well researched crops like wheat, maize, tomatoes, etc. in which tremendous progress was already achieved by "conventional" methods. In these crops, it can provide the basis for a new "breakthrough". However, in cocoa, at the present time, it is like a "gold ring in a pig's nose". Most of the National Programmes do not have sufficient resources, and probably also sufficiently trained personnel to carry out a decent breeding programme. Then, how will the study of the cocoa genome assist them in providing better planting materials to the growers? There is so much that can be done and should be done in cocoa in a more practical way with more relevance to farmers' needs and faster pay-off before using the very limited resources available in order to study the cocoa genome. However, the latter requires proper and more sustainable financial support, more training and possibly some direction.

My major concern is that the financial resources available for cocoa research are extremely limited. Genome studies of cocoa will compete for these limited resources and I have no doubt about whom is going to win in this competition. Please note the institutions involved. They are much more powerful and influential than any small national programme. They are more experienced and knowledgeable in the preparation of research proposals, and they have better links with the donor community. I wonder if they ever thought that pursuing their own interest would be at the expense of the badly needed, practical breeding research required by the producing countries. Moreover, since genome studies is a very fashionable subject, it will be more attractive research to attract the support of donors. After all, why is it necessary to invest US$50,000 to develop the VSD resistant cocoa varieties rapidly and by simple methodology, if they can invest $500,000 to identify a few QTL's for VSD resistance on the chromosomes?

I am sorry to say it but *"The king is naked"*.

Yoel Efron
Practical cocoa breeder
CCRI, Papua New Guinea

Is the King Really Naked?

The establishment of an international working group on the cocoa genome was based on the need to coordinate the efforts of institutions involved in this research. The major goal of the group is to reduce duplications, and to ensure that the limited resources available for cocoa research are utilised in the most efficient manner. Laboratories working on molecular studies in tropical species, including cacao existed long before the discussions to create this international initiative. These labs are funded and will continue receiving funds from their respective governments, independently of the working group. Therefore, the sharing of molecular genetic information to improve cacao varieties can only help classical breeding programmes. The working group will allow more efficient production and dissemination of information generated by researchers.

Molecular analyses can greatly improve classical breeding programmes. For instance, molecular marker analyses performed on several families from different countries conclusively demonstrated that a large proportion of progeny from 'controlled crosses' were off-types. Genetic parameters estimated using these families are seriously biased and this may also apply to many other families studied, but not analysed molecularly. The notion of genetic groups in cacao, on which the recurrent selection methods utilised in several countries is based, has also been shown to be seriously compromised through molecular analyses. The value of conventional breeding without molecular support could be considered questionable.

Finally, we would like to emphasise that molecular analyses are not receiving more attention than practical breeding and field phytopathology. For example, the USDA breeding programme has as its objectives the recovering and evaluation of germplasm collections, the improvement of disease screening methods and the selection of hybrids and clones from different trials. These objectives are being accomplished through collaborative agreements with INIAP (Ecuador), CEPLAC (Brazil) and CATIE (Costa Rica). Other participating institutions in the working group on cocoa genome studies such as CIRAD and CEPLAC also have extensive field-based programmes.

J.C. Motamayor, J. S. Brown, D. N. Kuhn, M. Heath, and R. J. Schnell
Miami Cacao Genetics Group

Announcement

The LIFFE/University of Reading

International Cocoa Germplasm Database

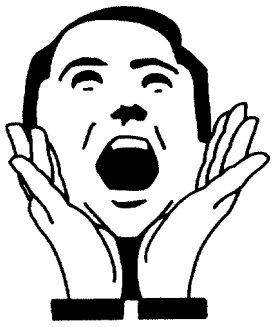
I C G D

The International Cocoa Germplasm Database is pleased to announce the launch of ICGD Online, a searchable version of the ICGD on the internet. The site offers a set of standard search routines that enable the user to create lists of clones using one or a combination of three search criteria (disease resistance/susceptibility, current location, country/area of origin) or to access all ICGD data on individual clones.

www.icgd.reading.ac.uk

Coming soon!

The site will continue to develop to allow much broader access to ICGD data and to include new data capabilities such as microsatellite data and photographs.



OBITUARY

Norman Simmonds:
5 December 1922 to 4 January 2002

Rob Lockwood

Norman was one of the giants of post-war tropical agriculture. A Cambridge Graduate, he started his tropical career with the Imperial College of Tropical Agriculture (ICTA) quickly making his name with bananas and sugar cane. On leaving ICTA in 1959, he joined the John Innes Institute as head of potato genetics. He was Director of the Scottish Plant Breeding Station (SPBS) from 1965-1976, where he further developed his interest in the genetics of potatoes. From 1976, he was with the Edinburgh School of Agriculture. Norman's interest in tropical agriculture extended well beyond bananas and sugar cane into rubber: for many years he was a highly valued member of the Rubber Research Institute of Malaysia advisory panel, publishing several authoritative papers on the crop. The origin of crops was a special interest, leading to his book "The Evolution of Crop Plants" (1976, updated by Smartt and Simmonds, 1995) (with over 80 authors!)

Norman met cocoa in Trinidad in 1945. Athough he had no formal involvement with the crop, he got to know Basil Bartley and Frank Cope. There were some lively debates. His interest was strengthened when Dalton Glendinning joined his SPBS staff, fresh from breeding cocoa in Ghana. The UK Confectionery Alliance brought Norman into cocoa in the seventies, when he chaired several meetings. It was an inspired move. His involvement became closer when Tony Kennedy joined the Cocoa Research Unit in Trinidad: they already knew each other through sugar.

I first met Norman at SPBS in 1969, when visiting Dalton Glendinning prior to going to Ghana. We met several times more when I was at the Plant Breeding Institute at Cambridge and Norman visited for various reasons. It was natural to turn to him when, at BAL Plantations in Sabah, we came to the question: "How do we select clones?" There was no clear answer and Norman put considerable effort into developing a genetic theory. In November 1991, he spent a week at BAL, working on cocoa when we could distract him from remnant abaca (BAL means Borneo Abaca Ltd) and more so the abundant seedling bananas (the centre of diversity of the crop extends to Sabah), and eating tiger prawns. An evening of karaoke was not to his taste!

Norman set out clear guidelines for the selection of clones. Two principles are worth repeating here: firstly, he argued, on theoretical grounds, that in the cocoa

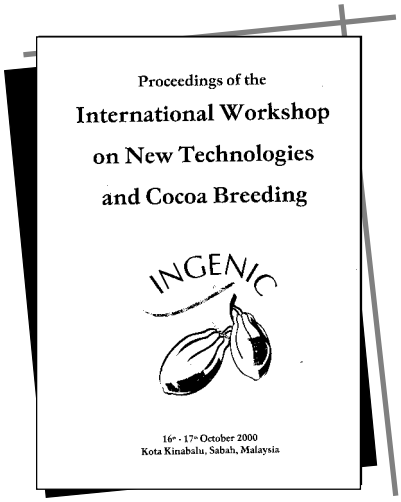
situation the variances within and between families are approximately equal. This means that when selecting clones, it is only worth starting with families which are themselves an advance. Secondly, he argued that single plant selection for yield was wholly ineffective, though it will work for other traits, like bean weight. A clone trial with two selected and ten randomly chosen seedlings each from ten families, plus all their parents and commercial controls, was planted to test his theory. Unfortunately, the Trial was terminated prematurely following change of ownership of the company, but the preliminary results showed that for yield Norman was right on both counts.

His advice on further breeding was simple. It is an economic activity with yield the key trait in cocoa. The inheritance of yield in cocoa is largely additive, so the better clones will be the better parents, and until we have better clones we will not have better seedling families. Quite large populations of clones - say 200 per family - must be evaluated to give a reasonable prospect of getting better ones: the numbers in the trial were far too small but it was not planted with a view to selecting clones. The slow rate of improvement of clones is at the heart of the slow progress in cocoa breeding.

I found Norman a generous and stimulating person, interested in all aspects of tropical agriculture. He was quick to recognise and demolish a weak argument, and I was the better for it. Equally, he was endlessly patient with what to him might well have been simple questions, recognising their importance, not just to the people who asked them but more so for the people - third world farmers - who stood to benefit from the right answers. He was never too busy to provide advice and open doors when asked.

Norman's patience extended to his great hobby - fishing - whether in the sea or preferably with a fly on the remote Scottish lochs. His interest in the ecology of the trout extended even to study of the crayfish that, he was convinced, were eaten by the largest ones. He invented flies to mimic them. I have no information on whether he was successful with such an outlandish lure.

Norman married Christa while he was at the John Innes. She died in December 2000. There were no children. Norman was proud of his Scottish heritage and even prouder of Edinburgh University. He belonged to the tradition of Scots, who served with great distinction in the former British colonies and then returned to equally distinguished careers in UK science. Tropical agriculture and the many people who depend on it owe a great deal to Norman. Cocoa's debt will become apparent as breeding develops successfully on the lines he set out. Personally and professionally I miss this eminent scientist, whom I was privileged to know.



BOOK RELEASES

Proceedings of INCOPED 3rd International Seminar. 16-17 OCTOBER, 2002, Kota Kinabalu, Sabah, Malaysia. (C.L. Bong, C.H. Lee, F.S. Shari, Eds.) INCOPED/MCB 2001. ISBN 983-2433-00-2

Proceedings of the Third INGENIC International Workshop on New Technologies and Cocoa Breeding. 16-17 October 2000, Kota Kinabalu, Sabah, Malaysia. INGENIC 2001. ISBN 1 900527022.

The New Taste of Chocolate: a cultural and natural history of cacao with recipes. Maricel Precilla, 2001. Ten Speed Press, 2001. ISBN 1-58008-143-6.
www.tenspeed.com.

FORTHCOMING EVENTS

- The Fourth INGENIC Workshop
  Cocoa Breeding for Improved Production Systems
  Ghana, October, 2003. (Coinciding with the 14th International Cocoa Research Conference) Tentative date: October 14th

- The 14th International Cocoa Research Conference
  Towards a Sustainable Cocoa Economy - What Strategies to this End?
  Secretariat: Cocoa Producers' Alliance
  e-mail: copal@alpha.linkserve.com

INGENIC Committee