INGENIC Newsletter

ISSUE NO. 10 SEPTEMBER, 2005

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From the Editor's Desk:

The past year was again eventful for INGENIC, and its completion coincides with a significant milestone for the INGENIC Newsletter: the completion of a decade of publication! The INGENIC Committee is grateful to the readership and, particularly, contributors for making this possible. We look forward to a productive future, in which we will see the reward of this interaction redounding to the benefit of cocoa farmers, chocolate manufacturers and all stakeholders in the cocoa industry.

The INGENIC Committee is also pleased to announce the launch of its permanent website at http://ingenic.cas.psu.edu. This is the culmination of the diligent work of INGENIC Committee Member, Prof. Mark Guiltinan of Penn State University.

This issue of the newsletter has evolved into another compelling and informative compilation of research notes and news. Several articles are from first-time contributors. We hope the information shared will stimulate discussion and follow-up studies, and motivate other researchers to contribute articles to the newsletter as well.

We have lost many eminent cocoa researchers in the recent past, and many of us have felt the losses personally. We are grateful to those who have paid tribute to the deceased because their recollections have been illuminating, and highlight the outstanding quality of research that has preceded and fuelled our own. Two papers featured on the INGENIC website are by the late Drs. Schultes and Toxopeus. The former is entitled *The genus Herrania, a wild relative of the cultivated cacao. Agric Trop. Bogotá* 1951 **7 (7)**:43-8. The paper by Dr. Toxopeus is *The history of cocoa in Nigeria in the context of the 19th century socio-economic revolution of West Africa*. It was translated from *Landbouwkundig Tijdschrift'* number **83-12**: 485-490 in 1973. Both were graciously scanned and contributed by Dr. Rob Lockwood, and we thank him for his thoughtfulness.

The Next INGENIC Workshop is scheduled for October 16-17, 2006 after the ICRC in Costa Rica. The theme is *Cocoa Breeding for Farmers' Needs*. Readers are invited to start considering articles for presentation, indicate their interests to the Chairman and Secretary of INGENIC, and offer suggestions as to how we can make this Workshop a resounding success.

Please submit concise contributions (maximum 4 sides of A4 paper) for the next issue of the NL to me at fbekele@fsa.uwi.tt; louisebekele@yahoo.co.uk or louisebekele@hotmail.com on or before March 1st 2006.

Readers are invited to visit the INGENIC website to learn more about recent INGENIC activities including those of the *Cocoa Genomics Study Group*. A useful listing of relevant publications has also been posted.

We look forward to another year of useful discussions on further partnerships and collaborative research activities among our readership.

With best wishes from the editor of your INGENIC Newsletter and the other members of the INGENIC Committee!

Frances Bekele

Determination of Off-Types in a Cocoa Breeding Programme using Microsatellites

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Abstract

Microsatellite markers were used as a diagnostic tool to detect and label off-types from a cocoa breeding evaluation at CATIE, Costa Rica. Using 24 microsatellites, the genetic identity of parental trees and progeny was determined by capillary electrophoresis (Applied Biosystems). The microsatellites that detected differences between multiple trees of a parental clone were then used to fingerprint progeny made with off-type parental trees. The analysis resulted in the identification of two types of UF 273 parental clones involved in nine crosses. Among 285 offspring, 149 plants or 52.3% were identified as the offspring of type II (off-type) of UF 273. It was impossible to verify the genetic identity of four plants or 1.4% of all progeny. The findings from this work show that genotyping parental stock before proceeding on a large-scale breeding programme is an essential step.

Introduction

Conventional breeding methods used in *T. cacao* have resulted in a very narrow genetic base for commercial cultivars worldwide. The use of this narrow genetic base, confounded by mislabelling of breeding stock and germplasm collections, and the disruption in the continuity of breeding programmes has led to only marginal success in traditional cocoa breeding (Lockwood 2003; Motilal *et al.* 2002). Typically, it takes from 3 to 6 years to produce a sexually mature cocoa tree suitable for use in a breeding programme. Due also to the long breeding cycle, relatively little successful improvement of cocoa has been made since the 1930's (Saunders *et al.* 2004). The lack of a long-term recurrent selection programme for cocoa has resulted in the selection of heterozygous productive seedlings as parents. These seedlings are cloned and placed in seed gardens to serve as parental stock for multiplication of the clone. As a result of these factors, up to 80% of multiplied genotypes are unproductive on farms (Hunter 1990) due to genetic segregation. The improvement of *T. cacao* using modern breeding techniques can be greatly facilitated by correctly identifying accessions within existing cocoa collections (Young 1994). The challenge now is to integrate molecular marker technology into cocoa breeding schemes, especially for parental clone identification, marker-trait association, and marker-assisted selection.

Inconsistency in the performance of known clones when used as parents in field trials is, in part, due to incorrect genetic identity. It has been estimated that the mis-identification of cocoa accessions could be as high as 22 to 32% in some major germplasm collections (Motilal *et al.* 2002; Saunders *et al.* 2004). Mislabelling of clones is not limited to cocoa; misclassified clones have been observed in other tree species like Eucalyptus, Sitka spruce, oil palm, avocado, and crops like potato and enset (Schnell *et al.* 2003; Turnbull *et al.* 2004). The use of microsatellites as a tool for the identification of mislabelled accessions in field genebanks has been supported by several researchers in the cocoa community (Motilal and Boccara 2004; Turnbull *et al.* 2004). In a recent study, Schnell *et al.* (2004) reported pollen contamination and mislabelling of trees as the source of off-type trees in progeny. Off-types are undesirable, being unsuitable for scientific studies, and can hinder breeding progress when an incorrect parent is assigned for crossing (Schnell *et al.* 2004).

Molecular markers are an effective genomic strategy for fingerprinting, mapping, gene tagging, as well as for determining the genetic structure and relationships among cocoa genetic groups (Laurent *et al.* 1994; Motamayor *et al.* 2003). Simple sequence repeats (SSRs) or microsatellites are often preferred for reasons of cost, ease of scoring, and data analysis, as well as suitability for large-scale automation (Bredemeijer *et al.* 1998; Dreher *et al.* 2003). In this paper, we describe the use of SSRs to discard off-types from a cocoa breeding evaluation at the Tropical Agricultural Research and Higher Education Center (CATIE), Costa Rica. We determined the genetic identity of parental trees and progeny with the objective of classifying them correctly. This information will allow the calculation of more precise estimators of genetic parameters (published elsewhere), and more efficient plant selection.

Plant Material

Factorial crosses were made in an experiment designed by personnel of CATIE to widen the genetic base of

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resistance to major pathogens in Central America. Three clones: UF 273, UF 712 and ICS 95 formed parental Set 1, and ten clones: CC 137, ICS 6, CATIE 1000, Tree 81, CCN 51, SCA 6, ICS 44, CC 252, EET 75 and POUND 7 formed parental Set 2. All possible crosses between clones of set 1 and set 2 were made, with five exceptions (UF 273 x CC 252, UF 712 x CC 252, UF 712 x POUND 7, ICS 95 x SCA 6 and ICS 95 x EET 75). One or more trees per parental clone were actually used in making the crosses. Approximately thirty-two trees were produced from each cross. Molecular analyses were performed on all trees of the 13 clones used as parents to determine off-types. Molecular analyses were made only for progeny of crosses with off-type parental trees (UF 273).

Molecular Analyses

Leaf samples of candidate trees were harvested in Costa Rica, placed in brown envelopes and air-freighted to the USDA Miami laboratory, where they were stored at 4°C. DNA was extracted, quantified, and amplified by PCR as described by Schnell *et al.* (2004). Capillary Electrophoresis (CE) was carried out on an ABI Prism 3100 and ABI Prism 3730 genetic analyzers (Applied Biosystems) using the standard module for fragment analysis (Schnell *et al.* 2004). The ABI 3730 allows the high-throughput sample analysis of 16 plates of 96 wells per workday (about 16h running time) while the ABI 3100 is capable of 2 x 96 samples in 9 hours. The automated Capillary Electrophoresis system is computer-based and well suited for high-throughput work. The minimum number of samples the instrument (ABI 3730) will handle is 48, with a maximum of 1536.

The set of 24 microsatellite markers used to screen all parental clones is listed in Table 1. All microsatellite markers used in this work were developed by the Centre de Coopération Internationale en Recherches Agronomiques pour le Développement (CIRAD). An initial set of 13 SSRs was used to screen all trees of each parental clone. Eleven additional SSRs were used for analysis of trees of parental clones with off-types, as determined by the first set of markers. SSRs were chosen by their level of polymorphism and map position. The microsatellites generated either one or two alleles with associated PCR stutter bands in each

Table 1: Microsatellite loci, linkage groups and allele size

Microsatellites	Linkage group	Motif	Expected allele size (bp)
mTcCIR1	8	(ct)14	143
mTcCIR3	2	(ct)20(ta)21	249
mTcCIR6	6	(tg)7(ga)13	231
mTcCIR9	6	(ct) 8N15(ct)5N9(tc)10	274
mTcCIR12	4	(cata)4N18(tg)6	188
mTcCIR15	1	(tc)19	254
mTcCIR17	4	(gt7N4(ga)12	271
mTcCIR18	4	(ga)12	345
mTcCIR19	2	(ct)28	376
mTcCIR21	3	(tc)11N5(ca)12	157
mTcCIR24	9	(ag)13	198
mTcCIR25	6	(ct)21	153
mTcCIR26	8	(tc)9c(ct)4tt(ct)11	298
mTcCIR29	1	(ca)10	172
mTcCIR43	4	(tg)5(ta)(ga)15	206
mTcCIR69	5	(ct)20	203
mTcCIR85	9	(ag)16	211
mTcCIR102	1	(ga)9	124
mTcCIR150	-	(ag)13	135
mTcCIR177	7	(ca)7	128
mTcCIR244	1	(ta)4cata(ca)17(ta)4	264
mTcCIR256	5	(ac)13(atac)4	185
mTcCIR270	1	(ct)10(ca)9	224
mTcCIR285	9	(ag)16	216

Source: Pugh *et al.* (2004)

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accession as expected for co-dominant markers. The fragments amplified by primers were scored as alleles on the basis of molecular weight in comparison with internal standards using the ABI GeneMapper ver 4.0. A cluster of three or four discrete bands (stutter) was commonly observed. The program calls the most intensely amplified peak as the allele. The number of allele peaks depends on whether the individual tested is a heterozygote or homozygote. As an example, Figure 1 shows electropherograms of three parental clones analysed with the same primer, mTcCIR270. The top profile depicts a homozygous locus with one allele 223 bp (also denoted (223, 223) to make it amenable to computer analysis), the middle profile is heterozygous with two alleles 223, 235 bp, and the bottom panel is also heterozygous showing a different set of two alleles with sizes 234, 245 bp.

Results

One tree of the UF 273 clone was different from the other four trees of the same clone in 12 SSRs. Trees of UF 273 with identical genotype were labelled type I, and the off-type tree was labelled type II. Since only one allele of one tree of the CC 137 parental clone was found to be different among the 24 SSRs analysed, there was insufficient evidence to declare this tree an off-type of the CC 137 clone. Off-type trees were not detected in the rest of the parental clones with the set of 24 SSRs. Therefore, only progeny from crosses involving UF 273 as a parent were analysed with SSRs to discard off-types. The markers utilised to classify progeny were the 12 SSRs that detected differences between the two parental UF 273 types. Table 2 shows eight microsatellite genotypes of parental clones that were crossed with the UF 273 clone. Among the ten parental clones, three SSRs (mTcCIR12, mTcCIR102, mTcCIR270) amplified one parental clone as homozygote and the rest as heterozygotes, while another three (mTcCIR29, mTcCIR177, mTcCIR256) amplified three parental clones as homozygotes. However, one SSR (mTcCIR43) amplified five out of the ten clones as homozygotes, while one (mTcCIR150) amplified seven out of 10 clones as homozygotes. The differences displayed on electropherograms were unmistakably distinct and easily observable (Figure 1).

Table 2: Microsatellite genotypes of all parental clones

		mTcCIR12		mTcCIR29		mTcCIR43		mTcCIR102		mTcCIR150		mTcCIR177		mTcCIR256		mTcCIR270	
Clone	**Type**	**A1***	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**
UF 273	I	205	213	161	165	206	208	111	117	123	133	121	123	190	190	223	234
	II	205	221	165	169	208	208	111	109	123	123	123	131	184	190	245	234
CCN51	I	201	205	161	163	208	214	111	115	123	123	123	131	186	190	223	232
CC 137**	I	188	213	157	161	206	206	115	117	133	133	119	123	186	188	223	237
CATIE 1000	I	205	254	163	163	204	208	107	115	133	133	123	123	186	188	234	237
POUND 7	I	201	213	163	165	212	214	100	107	133	135	123	131	186	196	223	235
ICS 6	I	188	213	161	161	206	206	105	115	133	133	119	123	186	188	232	237
ICS44	I	188	213	161	161	206	206	117	117	123	133	119	123	188	188	223	223
SCA6	I	211	211	165	171	210	212	109	115	133	135	131	131	194	194	225	227
EET 75	I	188	221	165	169	206	206	109	111	123	133	131	131	184	184	234	245

*A1 = Allele 1; A2 = Allele 2
**Only one allele of this clone was found to be different using the 8 SSRs hence presence of off-type trees could not be confirmed

Molecular analysis of offspring

Off-type progeny were identified by fingerprinting progeny from crosses made with off-type parental trees. Interpretation of parentage data was relatively simple as SSRs are co-dominant markers inherited in a Mendelian fashion. The SSRs used had been previously identified to be at least partially informative (Table 2). Therefore the correct parental type has at least one allele in common with parental off-types. For example, the UF 273 type I yielded alleles 205, 213 bp and the UF 273 type II, had the alleles 205, 221 bp at locus mTcCIR12 (Table 2). Thus, the two types of UF 273 had the allele 205 in common and differed by the allele 213 and 221. The parental origin of an offspring can be determined if it has inherited a non-common allele.

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The expected marker genotypes of the offspring corresponding to the cross between the clone UF 273 and CC 137 are presented in Table 3. Analysing progeny with mTcCIR29, the observed genotypes grouped as follows: (161, 157), (161, 161) for type I (type I because allele 161 originated in UF273 type I); (169, 157), (169, 161) for type II (type II because allele 169 originated in UF 273 type II); and genotypes (165, 157), (165, 161), (165, 157) and (165, 161) for an undetermined category because these genotypes each contained allele 165, which is common to both type I and II (Table 3). Similarly, analysing with mTcCIR150, the observed genotypes grouped into two categories. Genotypes (133, 133) clearly identify with type I, and the only other genotypes (123, 133) elude classification.

It must be noted that each progeny can inherit one of eight genotypes available from a bi-parental cross, in the absence of pollen contamination. Each of these categories is represented in Table 3. The observed genotypes fell into one of three broad groups represented by the colors light blue for type 1, dark blue for type II, and yellow for unknown. By determining where each progeny belonged, we were able to sort all progeny into the three groups – type I, type II or undetermined. This repetitive analytical procedure was done, marker by marker, and cross by cross, with a computer program written in SAS 9.1.2.

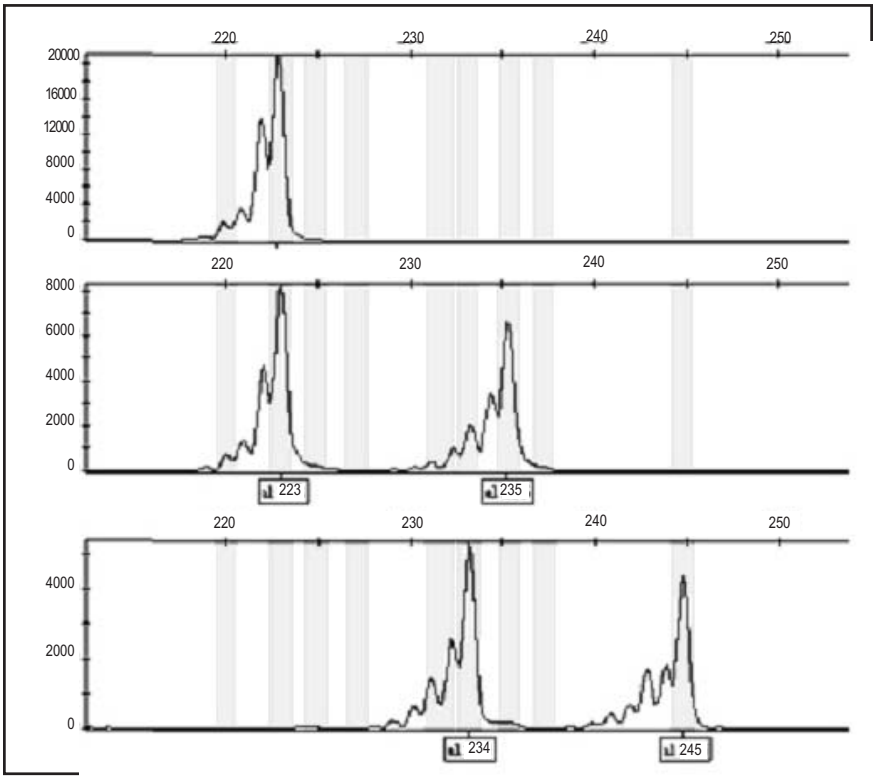

Figure 1: Allele sizes in base pairs detected at the mTcCIR270 microsatellite locus of three parental clones. The major peak in each electropherogram is the PCR amplified allele at this locus

A summary of the progeny types corresponding to the crosses involving the UF 273 clone as a parent is presented in Table 4. The use of microsatellites resulted in the identification of 149 plants or 52.3% among a set of 285 progeny that are the UF 273 parent type II at the genetic level. It was not possible to verify the genetic identity of four plants or 1.4% of all progeny. This will require further screening with an uncertain number of new primer pairs to identify these four trees. Thus, for the purpose of estimating genetic parameters and use in plant breeding, the time and cost involved to determine the origin of these progeny would not be justified.

Conclusion

In this study, we performed microsatellite analysis to verify the identity of parental and offspring trees in a set of crosses made and evaluated at Turrialba, Costa Rica. The crosses studied are part of the cocoa breeding programme conducted at CATIE, Costa Rica to improve disease resistance and cocoa production. The objective of this research was achieved by tracking down off-type alleles into the progeny; a task which was virtually impossible until the advent of microsatellites. With the availability of microsatellite data, it would now be easier

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Table 3: Parental and offspring marker genotypes corresponding to the cross UF 273 x CC 137

			UF 273 Type I		UF 273 Type II	
SSR		**CC137**	**Allele 1**	**Allele 2**	**Allele 1**	**Allele 2**
			205	213	205	221
	Allele 1	188	205, 188	213, 188	205, 188	221, 188
mTcCIR12	Allele 2	213	205, 213	213, 213	205, 213	221, 213
			165	161	165	169
	Allele 1	157	165, 157	161, 157	165,157	169, 157
mTcCIR29	Allele 2	161	165, 161	161, 161	165, 161	169, 161
			206	208	208	208
	Allele 1	206	206, 206	208, 206	208, 206	208, 206
mTcCIR43	Allele 2	206	206, 206	208, 206	208, 206	208, 206
			167	204	204	204
	Allele 1	187	167, 187	204, 187	204, 187	204, 187
mTcCIR69	Allele 2	189	167, 189	204, 189	204, 189	204, 189
			197	207	199	207
	Allele 1	197	197, 197	207, 197	199, 197	207, 197
mTcCIR85	Allele 2	209	197, 209	207, 209	199, 209	207, 209
			117	111	109	111
	Allele 1	115	117, 115	111, 115	109, 115	111, 115
mTcCIR102	Allele 2	117	117, 117	111, 117	109, 117	111, 117
			123	133	123	123
	Allele 1	133	123, 133	133, 133	123, 133	123, 133
mTcCIR150	Allele 2	133	123, 133	133, 133	123, 133	123, 133
			123	121	123	131
	Allele 1	119	123, 119	121, 119	123, 119	131, 119
mTcCIR177	Allele 2	123	123, 123	121, 123	123, 123	131, 123
			261	259	259	259
	Allele 1	243	261, 243	259, 243	259, 243	259, 243
mTcCIR244	Allele 2	265	261, 265	259, 265	259, 265	259, 265
			190	190	190	184
	Allele 1	186	190, 186	190, 186	190, 186	184, 186
mTcCIR256	Allele 2	186	190, 186	190, 186	190, 186	184, 186
			234	223	234	245
	Allele 1	223	234, 223	223, 223	234, 223	245, 223
mTcCIR270	Allele 2	237	234, 237	223, 237	234, 237	245, 237
			202	202	202	217
	Allele 1	214	202, 214	202, 214	202, 214	217, 214
mTcCIR285	Allele 2	217	202, 217	202, 217	202, 217	217, 217

Colors: Light blue: offspring descendant of UF 273 Type I; Dark blue: offspring descendant of UF 273 Type II; Yellow: offspring with unknown of UF 273 type.

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Table 4: Fingerprinting of progeny using informative markers

Bi-parental crosses	No. of progeny	Progeny UF 273 type I	Progeny UF 273 type II	Progeny undetermined origin
UF 273 x CC 137	32	17	15	0
UF 273 x ICS 6	32	0	30	2
UF 273 x CATIE 1000	32	18	13	1
UF 273 x TREE 81	32	32	0	0
UF 273 x CCN 51	32	0	32	0
UF 273 x SCA 6	31	0	30	1
UF 273 x ICS 44	32	32	0	0
UF 273 x EET 75	30	1	29	0
UF 273 x POUND 7	32	32	0	0
TOTAL	**285**	**132**	**149**	**4**

to define the most appropriate crosses for a second phase of analysis with a much higher precision. With off-types from parental clones identified, a sound basis for a large breeding programme using microsatellites has been established. The advantages of doing this type of analysis before proceeding with a large-scale breeding programme cannot be over-emphasised. From their studies on disease resistance selection in a trial in Trinidad, Schnell *et al.* (2004) arrived at a similar conclusion: that it is essential to confirm the integrity of cross progeny, using molecular markers, until the crossing technique used by a research group is routinely accurate. In this situation, UF273 was a clone chosen as a parent because of its resistance to Moniliasis. Only after eight years of being planted and evaluated, it was realised that not all clones used as parents representing UF273 were correct; this meant unnecessary expenditures of time and resources.

A basic problem of incorporating molecular marker analysis in cocoa breeding programmes in producer countries, globally, is its cost. However, the high commercial value of cocoa and the role the crop plays in the economies of these countries justify deployment of microsatellites in their breeding programmes, a position unequivocally illustrated by the findings in this report.

Acknowledgement

This work was supported by a grant from the Kirkhouse Trust, UK to Jemmy Takrama. We thank Wilber Quintanilla, Nannette Langevin, Ashley Johnson and Cecile Olano, all of USDA ARS SHRS, Miami for technical assistance.

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Some Genes Expressed in *Theobroma cacao* L. Zygotic and Somatic Embryos

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Scope

Throughout the world, cocoa is mainly planted as seed. The yield of cocoa seedlings is highly variable mainly due to its heterozygous nature. In one field, 2-3% of the trees in a population of high-yielding families may generally account for 60% of the yield (Irizarry and Rivera 1998). One way of increasing yield, hence smallholder incomes, is somatic embryogenesis to multiply elite material and homogenise cocoa production. It is now possible to produce somatic embryos and plantlets from a large number of genotypes (Maximova *et al.* 2002). However, production is not sufficient for a scaling-up step, and many genotypes remain recalcitrant. Since the beginning of the genomic era, more information has become available about genes involved in the different phases of embryogenesis in the model plant *Arabidopsis thaliana*. Among them, *leafy cotyledon* (*LEC*) genes are central regulators of embryogenesis (Harada 2001). Transformation of *Arabidopsis* plants with *LEC1* (encodes a protein involved in maintaining embryonic cell fate) or *LEC2* (encodes a transcription factor) induces the formation of somatic embryos from vegetative cells, mimicking somatic embryogenesis (Lotan *et al.* 1998; Stone *et al.* 2001). *Lec1-like* (*L1L*), another gene of this family, has the same function as *LEC1* (Kwong *et al.* 2003). One of the challenges of plant developmental biology is now to extend the knowledge acquired from model plants - mainly annual plant species - to economically important crops, both annual and perennial plant species. One application of such research could be the use of *LEC* genes to increase the embryogenic capacity of recalcitrant genotypes. To that end, we launched a gene candidate strategy to isolate *LEC* genes

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homologues in *Theobroma cacao* L. This paper deals with the production of a few partial cDNA sequences, obtained upon the isolation of *LEC* with degenerated primers, from cocoa tree zygotic and somatic embryos. We identified 9 ESTs that had strong homologies with already characterised genes. This information may be of use to cocoa researchers and breeders, given recent and current interest in genomics (Jones *et al.* 2002; Bennett 2003).

Isolation of 9 ESTs from somatic and zygotic embryos

In order to isolate *LEC* genes homologues in cocoa, mRNA were first extracted from zygotic and somatic embryos. The zygotic embryos came from mature and immature pods from hand-pollinated *Theobroma cacao* L. IMC 67 x DHS 30 provided by CIAT in São Tomé. Embryos were separated into two batches: (i) immature, consisting of a mix of stages IIIz, IVz, and Vz, as defined by Alemanno *et al.* (1997) and (ii) mature, corresponding to stage VIIIz. Somatic embryos were produced according to a two-step protocol consisting of primary somatic embryogenesis and secondary somatic embryogenesis as previously described by Maximova *et al.* (2002). Reverse-transcribed cDNA first strands were obtained from the different mRNA samples. A PCR-based approach was used to amplify these cDNA using 9 highly degenerated oligonucleotide primers. These were designed from the known conserved regions of *Arabidopsis thaliana* LEC1 and LEC2 proteins (Lee *et al.* 2002; Stone *et al.* 2001). Various PCR products were obtained as described in Table 1. Cloning and sequencing of these PCR products were conducted in an attempt to identify expressed genes matching with known functions and, in particular, with *LEC* genes from *Arabidopsis*. Based on partial sequence analysis, it appeared that 1 out of 9 ESTs matched with *LEC1* or *LEC1-LIKE* sequences, and 1 with *ABI3*, which is a transcription factor with a region of close identity with *LEC1*. Low stringency in PCR amplification gave rise to other ESTs having homology with other functions.

Conclusion and perspectives

Partial sequences of *LEC1*-like homologous genes were isolated in *Theobroma cacao* L. As we used conserved regions of *LEC1* and *LEC2* genes to design primers, it was not surprising that we isolated other transcription factors such as *ABI3* (Monke *et al.* 2004). In *Arabidopsis*, *ABI3* is expressed throughout seed development (Parcy *et al.* 1994). This gene acts synergistically with *FUS3* and *LEC1* genes to control multiple elementary processes during seed development (Parcy *et al.* 1997). Some of the other genes found had homology with housekeeping functions, or with specific functions such as the *ERS* gene involved in ethylene perception. Full-length isolation of *LEC1-LIKE* is under way, by 5' and 3' RACE extension. Expression of this gene will be studied during both zygotic and somatic embryogenesis.

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Table 1: Explant source and putative function of the isolated and sequenced clones from zygotic and somatic embryogenesis, after blastn and blastx analyses

Explant sources for RNA extraction	Nucleic acid	Clone names	Putative identified gene functions	NCBI GenBank accession numbers
Somatic embryos	cDNA	TcES1	Protein kinase family (At5g57610) mRNA, *Arabidopsis thaliana*	DN237949
Somatic embryos	cDNA	TcES2	Expressed protein (At3g46920) mRNA, *Arabidopsis thaliana*	DN237950
Somatic embryos	cDNA	TcES3	Actin depolymerizing factor-related (At1g61660) mRNA, *Arabidopsis thaliana*	DN237951
Somatic embryos	cDNA	TcES4	Polyadenylate-binding protein, putative, *Arabidopsis thaliana*	DN237952
Mature zygotic embryos	genomic DNA	TcEZ1	AAA-metalloprotease Fts, *Pisum sativum*	DN237953
Mature zygotic embryos	cDNA	TcEZ2	Acetyl-CoA carboxilase biotin-containing subunit (CAC1) gene, nuclear gene encoding chloroplast protein, *Arabidopsis thaliana*	DN237954
Mature zygotic embryos	cDNA	TcEZ3	Leafy cotyledon 1-related L1L protein, *Arabidopsis thaliana* LEC1-LIKE protein mRNA, *Phaseolus coccineus*	DN237955
Mature zygotic embryos	cDNA	TcEZ4	AB13 mRNA for ABA insensitive 3, *Pisum sativum*	DN237956
Immature zygotic embryos	cDNA	TcEZIP1	Ethylene responsive sensor (ERS) gene, *Prunus persica*	DN237957

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The development of *T. cacao* flowers: a multi-level comparison to *Arabidopsis*: Summary of a Ph.D. thesis

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Comparative anatomical and developmental studies on *Theobroma cacao* L. flower development were conducted to provide insight into the general mechanisms that control floral development (Swanson, Ph.D. Thesis, 2005). These studies were also conducted to assess the level of conservation of these systems among plant species, especially with reference to *Arabidopsis thaliana*. This report will summarise some of the most important findings of this work.

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Arabidopsis, a small mustard plant, is evolutionarily closely related to *T. cacao*, both being members of the rosid group (APGII, 2003). *Arabidopsis* was the first plant to have its genome sequenced and is commonly used as a model for plant floral biology (Jack 2004; Ma 2005). Thus, *T. cacao* is in a unique position to benefit from the enormous amount of basic knowledge gained in *Arabidopsis*, although this idea had previously not been tested thoroughly. To this end, we conducted a study to compare the development of flowers of *T. cacao* to what is already known in *Arabidopsis* and other model species, at both the morphological and gene expression levels.

Morphological comparisons were made through the analysis of time-lapse photography and light and electron microscopy to create mathematical models of flower development for *T. cacao*. A time-lapse movie of cacao flower development can be viewed at http://guiltinanlab.cas.psu.edu/Research/Cocoa/flowers.htm. We obtained morphometric data for each of the floral organs as they developed inside the flower buds. With that data, we were able to build mathematical models that enable individual organ growth to be predicted based on the time and length of the developing flower bud (Figure 1). Furthermore, we were able to subdivide flower development into 12 stages corresponding to those previously defined in *Arabidopsis* (Smyth *et al.* 1990) (Figure 1). The duration of these stages were shown to have a similar relationship to the duration of those in *Arabidopsis* in terms of percent of total time.

We confirmed Bayer and Hoppe's earlier findings (1990) that it takes 30 days for the average 5.7mm x 3.6mm flower buds of *T. cacao* to develop and fully mature (~2x longer and larger than *Arabidopsis*). During development, the flower bud growth rate varies from a minimum of 0.088 mmd^{-1} to a maximum growth rate of 0.54 mmd^{-1} at different stages of development. Our observations suggest that the switch between the low to high growth rates correlates with the transition between net differentiation and net elongation of the developing cells at approximately 16 days of development. Finally, we determined that flowers begin opening nearly synchronously starting at 6pm on the 29th day of flower development, concluding at 6 a.m. the following morning.

As an additional approach to examine the similarities between the floral developmental programs of *Arabidopsis* and *T. cacao*, we examined the expression of several key floral regulatory genes. To precisely localise gene expression patterns during *T. cacao* flower development, we performed *in situ* hybridisations using the floral integrator *LEAFY* as a probe, as well as several ABC genes. The *LEAFY* gene is a key component of the flower developmental pathway that acts as both an integrator of the floral pathways and an activator of the floral organ identity genes (ABC genes) (Jack 2004; Ma 2005). The ABC genes are the basis of the ABC model for floral development that was first described using mutants in *Arabidopsis* (Jack 2004). The ABC model refers to the idea that combinations of three classes of gene products interact to determine the identity of the cells forming the four whorls of the flower. Simplified, the A class of genes are responsible for the formation of the sepals, the combination of the A and B genes are responsible

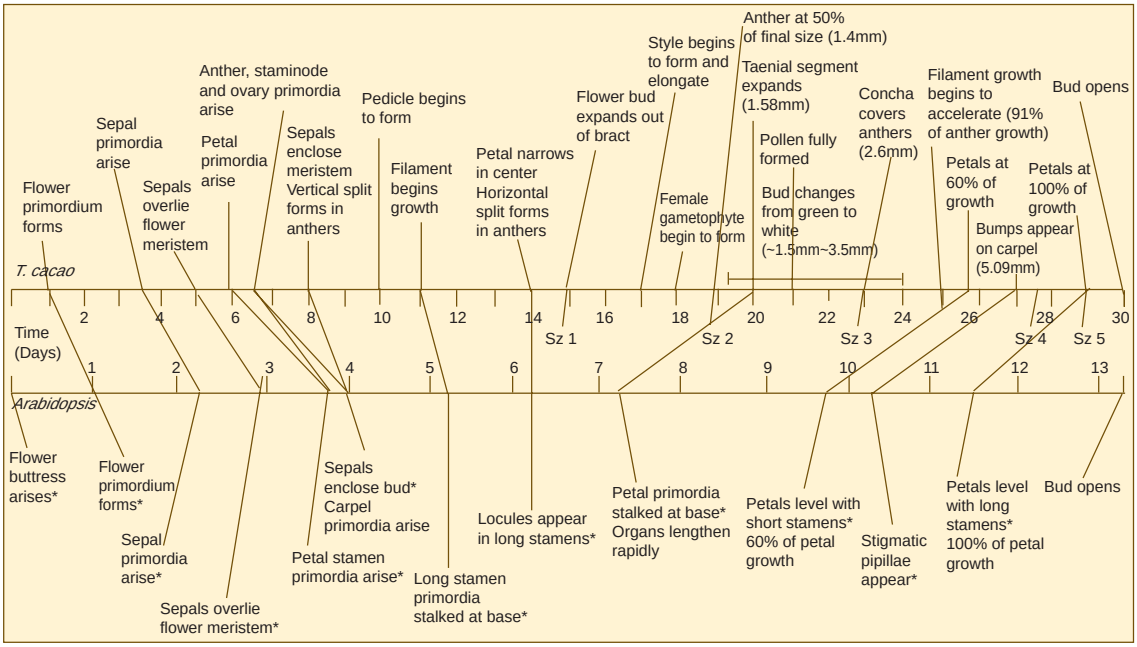

Figure 1: Comparison of *T. cacao* and *Arabidopsis* flower development. The major morphological events in the growth of *T. cacao* (top) and *Arabidopsis* (bottom) plotted along an axis of thirty and fourteen days, respectively. Bud sizes (Sz 1-5) refer to the average sizes of flower buds used in this experiment and are indicated along the axis. The dotted lines connecting the two diagrams represent stages as defined by Smyth *et al.* (1990)

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for the identity of petals, the combination of B and C genes are responsible for stamen identity, and the C genes alone are responsible for the identity of carpels. Finally, A and C genes are mutually repressive (Jack 2004; Ma 2005).

The expression of *LEAFY*, along with the *Arabidopsis* ABC genes *AP1*, *AP3*, *PI*, and *AG*, was observed in developing *T. cacao* floral tissue primarily during early floral development. In each case, the genes' temporal and spatial expression was highly restricted to specific locations in the early floral meristem, similar to what has been described in other species including *Arabidopsis* (Jack 2004; Ma 2005). These results may be summarised as follows: *LEAFY* expression is observed across the entire meristem during early developmental stages and expression reduces as organs form (Figure 2). *AP1* is initially expressed throughout the developing floral meristematic tissues, but dissipates to whorls 1 and 2 as AG expression inhibits AP1 (stage 6). AP3 and PI are observed to express in the meristematic tissue exclusively in whorls 2 and 3, and AG was observed to express in the central two whorls (3 and 4) only. Our data are very similar to that observed in *Arabidopsis* and thus support the hypothesis that the ABC model is also valid for *T. cacao*. Based on these results, we propose an ABC model for flowering in *T. cacao*, subject to further tests of its validity (Figure 3). The modified model incorporates the unique organs found in cacao but not *Arabidopsis*, the staminodes, which showed gene expression patterns similar to that seen for stamens.

Comparison of the *T. cacao* floral developmental program with that of *Arabidopsis* revealed that although the final sizes and morphologies of flowers in the two species differ, their developmental programs are strikingly similar both morphologically and genetically. Consistent with this analysis, a cross-species analysis of the current knowledge in this field indicates a high degree of conservation kingdom wide. These results indicate that *Arabidopsis* can be used as a model developmental system for understanding cacao molecular biology. We are expanding our research to test this concept for plant defense responses as well. The use of model plant systems to study cocoa biology could greatly accelerate the rate of progress on cocoa. Further details of these experiments are available at the Guiltinan lab website (http://guiltinanlab.cas.psu.edu/Research/Cocoa/flowers.htm).

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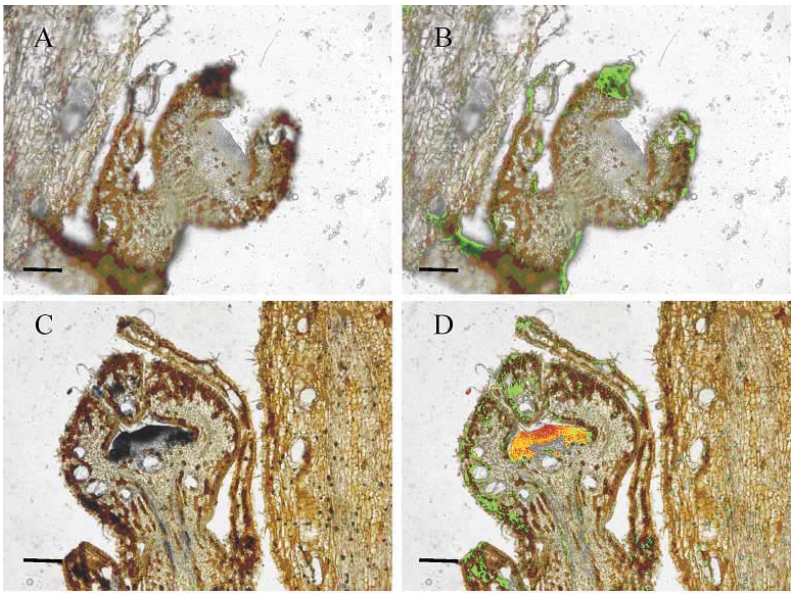

Figure 2: *In situ* hybridization of LEAFY probe in PSU-SCA6 plants. A-B, Negative control sense *LFY* probe hybridized to a stage 4 flower bud. C-D, Hybridization of anti-sense probe to a stage 5 flower bud. Left hand panels are raw images; right hand panels have had the hues of the NCB/NCIP precipitate selected in Adobe® Photoshop and replaced by green hues (see Methods). (Bar = 100 μ m)

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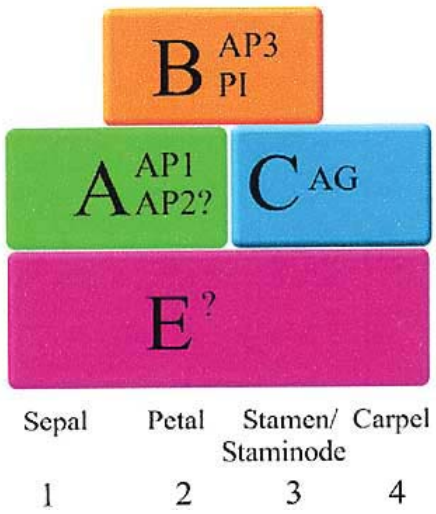

Figure 3: ABC model for *T. cacao* (modified from the model for *Arabidopsis thaliana*, Jack 2004)

The Compatibility – Yield Efficiency Relationship

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At the last INGENIC seminar (Accra, 19-21 October 2003), one of the questions raised concerned the relations between yield efficiency and compatibility: *"Do self-incompatible genotypes have lower yield efficiency than compatible genotypes?"* (INGENIC 2003).

Sounigo *et al.* (1994) had already mentioned the possibility of a positive response, since the self-compatible trees in their study were characterised by a higher ratio of productivity to bulk than the self-incompatible trees, though the difference was not significant. Self–incompatible trees are known to be generally more vigorous than self-compatible trees (Lockwood 1977), but as they are also more productive when mature, and as vigour and yield are well correlated, an appropriate protocol is required to find the answer, all the more so since yield efficiency is not a constant, but changes as plots grow older.

As a contribution to this knowledge, we describe results from a study of phenotypic correlations between the two descriptors, based on individual observation data from a hybrid comparative trial in Ivory Coast.

Material and Methods

The data came from a study on the effects of compatibility and flowering intensity on yield, conducted from 1983 to 1986 (unpublished data) in a hybrid comparative trial planted in 1979 at the IFCC station at Divo (Ivory Coast)[1]. This trial involved 50 families of Upper-Amazon (female) x Amelonado (male) crosses, plus a control family, at a rate of 20 trees per family in a totally randomised single-tree plot design. The trial came to a close in 1989 (Paulin *et al.* 1993), but the plot was monitored again from 1990 to 1994. In addition to the conventional vigour and individual yield observations (Lotodé and Lachenaud 1988), 42 trees were selected in 1983 for their yield and tested for their compatibility. Those trees were chosen exclusively on the basis of their production when young, not according to their genetic origin: 21 were high-yielding trees (from 5.0 to 6.5 kg of fresh beans in 1983, at only four years old) and 21 trees had no yield at all. Compatibility was measured by the conventional method of counting fruits set after self-pollination, with prior isolation of flowers (Lachenaud 2000), at a rate of 40 self-pollinated flowers per tree, in a suitable period.

The methodology used in the analyses had the following noteworthy features:

- compatibility was quantified by the successful fruit-set rate at 10 days (and no longer by the usual "all or none" method into "self-compatible" or "self-incompatible"),
- Yield Efficiency, which is basically the ratio of production to a cross-section of trunk (or a vegetative volume), was determined in two ways:
  - YE Cum: on the cumulative harvests of 9 seasons (1983-1994, with one missing year, 1989) and the cross-section in 1994 (*i.e.* the square of the 1994 radius),
  - YE3 Cum: ditto YE Cum, but with the cube of the radius of 1994.

[1] IFCC = Institut Français du Café et du Cacao; this station now belongs to CNRA, Centre National de Recherche Agronomique, in Ivory Coast.

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Results

The observations are summarised in Table 1, which gives the variations observed (average, extremes, coefficients of variation) for five variables in several groups: low yielders, high yielders, strictly self-incompatible trees, self-compatible trees (fruit set = 0.60) and all the assessed trees.

This table shows that:

- the two productivity groups identified in 1983 persisted in 1994, even though they were less clearly distinguished by the adult tree productivity values,
- the cross-sections in 1994 were similar for the two groups,
- the yield efficiency values were clearly and significantly higher in the high-yielding group and in the self-compatible group,
- compatibility was higher, on average, for the high yielders (X 2.7 compared to the low yielders); the range of values was large in both groups: trees with a zero fruit-set rate and trees with a high fruit-set rate were found in both groups.

Table 1: Averages (in bold), ranges (minimum---maximum) and coefficients of variation (in italics, as a %) observed for different variables in all the trees studies (36 trees surviving in 1994 with usable data), in the group of trees with initial zero production (16), in the group of initial high yielders (20), in the strictly self-incompatible group (14) and in the self-compatible group (15). The groups of trees with nil and very high initial yield were compared, letters indicating groups of means according to Newman and Keuls at a 5% threshold. The same type of comparison was performed between the self-compatible and strictly self-incompatible trees, seven trees with intermediate fruit set rates (>0 but < 0.60) were discarded from the analysis

	All the assessed trees (36)	Group with initial nil production (16)	Group of initially very high yeilding trees (20)	Strictly self-incompatible group (14)	Self-compatible group (15)
Fby 8394 (kg / tree)	**36.4** (7.5---109.7) *60*	**20.0** a (7.5---45.2 *57*	**49.6** b (21.4---109.7) *39*	**30.4** a (7.5---58.2) *54*	**42.9** a (7.5---68.6) *43*
Sec 94 (cm^2)	**221.9** (103.1---395.5 *37*	**227.1** a (103.1---389.9) *41*	**217.7** a (114.9---395.5) *35*	**237.3** a (103.1---389.9) *40*	**204.8** a (114.9---346.7) *28*
YE Cum ($kg.\ cm^{-2}$)	**0.17** (0.02---0.37) *54*	**0.09** a (0.02---0.18) *45*	**0.24** b (0.06---0.37) *29*	**0.14** a (0.02---0.34) *60*	**0.22** b (0.04---0.37) *42*
YE Cum ($kg.\ cm^{-3}$)	**0.07** (0.01---0.16) *60*	**0.04** a (0.01---0.9) *54*	**0.10** b (0.02---0.16) *37*	**0.06** a (0.02---0.15) *70*	**0.09** b (0.02---0.16)
Comp (%)	**38.1** (0.0---90.0) *93*	**19.5** a (0.00---72.0) *144*	**53.0** b (0.0---90.0) *64*	**0**	**74.8** (66---90.0) *10*

Where: FBY 8394= cumulative fresh bean production (1983-1994)
Sec 94: trunk cross section (in 1994)
Comp: compatibility (fruit-set percentage at 10 days)
(YE Cum, YE3 Cum: see text)

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The results are also displayed in the form of a matrix of total correlations (Table 2).

It can be observed that:

– Compatibility was significantly and positively correlated to the two YEs, with little variation in r, from 0.38 to 0.39 (probability of 1.8 to 2.2 %).
– Compatibility was not correlated to overall yield (FBY8394), and not to yield in the mature phase (data not shown). However, for the initial effects (data not shown), positive correlations were found between the compatibility rate and the two initial yields (1983 and 1984), with significant r values at 1% of 0.50 and 0.53, respectively. However, as early as 1985 there was no longer any significant correlation.
– Compatibility was not correlated to the cross-section in 1994 (and the same applied for the cross-section in 1989, data not shown).
– The two YEs were well correlated to each other (r^2 = 0.91).
– The YEs and yield were positively correlated and the general YE calculated with the trunk cross-section (YE Cum) was better correlated to cumulative yield than that calculated with R^3 (YE3 Cum).

Table 2: Matrix of Pearson's coefficients of correlation between different variables (see Table 1), on 36 trees surviving in 1994; only significant r values are shown (at 5% limit) and highly significant values are shown in bold (probability < 0.0001)

	FBY 8394	SEC 94	YE Cum	YE3 Cum
SEC 94				
YE Cum	**0.77**			
YE3 Cum	0.55	-0.49	**0.95**	
Comp			0.39	0.38

Discussion and Conclusion

The sample studied was not a random sample since the trees had been selected for their productivity (during the first harvest only), but might be considered as random as regarding the two descriptors studied (yield efficiency and compatibility). In terms of cumulative yield, it was found that the difference between the two study groups persisted: the “low yielders” displayed average cumulative yields of 20.0 kg of fresh beans (with extremes of 7.5 and 45.2) and the “high yielders” an average of 49.6 kg (and extremes of 21.4 and 109.7) for a plot average of 31.4. The different harvests were well correlated, particularly the 1983 harvest and the overall cumulative harvest (r = 0.732, data not shown). Consequently, with regard to the test sample, which concerns UA x Amelonado hybrid trees with a different yield level, it may be concluded that:

- Whilst compatibility did not favour cumulative yield, it did seem to favour the very first harvests significantly.
- Compatibility was correlated with yield efficiency, resulting in more efficient production. However, the coefficients of determination (r^2) between compatibility level and yield efficiency were low, with a maximum value of 0.15; this means that in our sample, the variations in compatibility (such as measured) only explained 15% of the observed variations in YE. Compatibility might provide a slight advantage for YE.
- The YE of the self-compatible group was significantly higher than that of the strictly self-incompatible group.
- Compatibility and “vigour” were not correlated.
- The trees with the highest yield-efficiency were also the highest yielders, which confirmed numerous results.

It needs to be said that these were phenotypic and not genetic correlations, and this affects the scope of the conclusions. Indeed, the environment affected YE (which is known in cocoa trees and in other tree crops subjected to competition between trees in plots: Lachenaud and Montagnon 2001), but it also affected the fruit-set rate. To obtain genetic coefficients of correlation, it would be necessary to work on a sufficient number of clones displaying good diversity for the two descriptors, ideally in the form of cuttings or grafted onto the same rootstock (as rootstocks may influence YE). Another possibility, which is less satisfactory as it is less rigorous, would be to work with progenies that

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are homogeneous (at least for the two descriptors studied), thereby having enough family data pairs (average YE values and compatibility rates), resulting from numerous within-family observations (at least 20 trees per family), which would obviously be a hefty task.

These initial results need to be confirmed by further work using appropriate protocols. If there proves to be a high genetic correlation between the two descriptors, selection for compatibility, which is easier and can be done earlier than for YE, might be worthwhile. Indeed, varieties with a high YE, moderate vigour and low aggressiveness (in terms of competition: Lachenaud and Montagnon 2002) appear to be the most suitable for the planting densities normally used. The discovery of such a correlation could therefore somewhat rehabilitate Amelonado-type planting material.

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Can We Select for Shorter Cocoa Plants?

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2 Masterfoods
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Breeding programmes for most crops have been mainly focused, during the first stages of selection, on the improvement of yield and on the introduction of resistance to the most important diseases. This is certainly also the case for cocoa breeding programmes, where most parents, used to obtain hybrids seedlings or to select clones, come directly from the wild. If selection is based mainly on yield, it is likely to result in taller and more vigorous plants, which do not perform well under high-density plantings or mixed cropping systems. If selection for yield is performed by also taking into consideration height and other aspects of plant architecture, it should be possible to produce more compact, shorter plants, and perhaps thereby increase the yield efficiency. This would allow the use of higher planting densities and mixed cropping of cocoa with other economically interesting plants, which would translate into higher incomes per hectare for the farmers.

Shorter plants and evidence of parents with shorter progenies

Two field trials, one in Costa Rica (CATIE) and the other in Brazil (Almirante, MARS Inc), are being carried out mainly to select for high yielding, disease resistant trees. The experiments were planted in an incomplete factorial design in 2003 and 2004, respectively. Parental clones were selected mostly for ability to confer resistance to Moniliasis in Costa Rica and Witches' Broom in Brazil. Parents were also selected with the objective of simultaneously recombining other favorable traits such as resistance to other diseases, bean quality, and combining ability for yield. A small number of the parents was also selected for their relatively short height (LF-1, Santa Clara-3 and SCA 24). Jorquette height was measured in Costa Rica in centimeters (cm) at 12 months after planting. In Brazil, the height of plants (cm) was measured after one year of being planted in the field; most of the plants had not yet jorquetted at the time of the measurement. Plants with jorquettes were not measured. Since pruning affects tree height, it was decided to perform preliminary analyses before pruning, at one year of development,

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to assess the relative height conferred by parental clones to their progeny. Analyses of variance for jorquette and plant height for data from Costa Rica and Brazil, respectively, were carried out. Results of these analyses are shown in Tables 1 to 3 and 4 to 6.

Table 1: Analysis of Variance for jorquette height 12 months post-planting in Costa Rica

Source	DF	Means Squares
Rep	4	70.5
Hybrid	30	741.1**
Female	4	2544.3**
Male	6	1936.9**
Female*Male	20	134.8**
Error	119	53.5

•, •• Significant differences at 0.05 and 0.001, respectively

Table 2: Means of crosses and parents (males and females) for jorquette height 12 months post-planting

Females/Males	Criollo 27 (T)	GU154 -L	IMC 67	LCTEEN 37/A	LF1 (T)	PA 150	Sta. Clara 3 (T)	$\bar{X}$ Females
ICS 43 (T)		110.1	86.4	97.8	91.0	91.9	93.9	95.2
IMC 60			104.5	94.4	115.4	111.6	117.7	108.7
PA 169	94.9	138.4	118.4	110.2	120.1	118.5	117.8	116.9
UF 273 (Type 1)	91.0	117.6	107.0	107.7	113.4	100.6	108.7	106.6
UF 273 (Type 2)	81.8	121.1	107.4			104.4	101.4	103.1
$\bar{X}$ Males	89.8	121.8	104.7	109.9	104.3	105.4	107.9	106.5

T: Trinitario origin (T x Nacional)

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Table 3: Specific Combinimg Abilities (SCA) and General Combining Abilities (GCA) for jorquette height 12 months post-planting

Females/Males (SCA)	Criollo 27	GU 154-L	IMC 67	LCTEEN 37/A	LF1	PA 150	Sta. Clara 3	GCA Females
ICS 43		3.70	-19.95**	-8.57*	-15.36**	-14.49**	-12.51**	-10.92
IMC 60			-1.85	-11.96**	9.03*	5.23	11.35**	2.64
PA 169	-11.50	32.05**	12.00**	3.84	13.69**	12.12**	11.39**	10.79
UF 273 (Type 1)	-15.33*	11.25**	0.61	1.30	7.05	-5.81	2.34	0.48
UF 273 (Type 2)	-24.60**	14.75**	1.00	-3.76		-2.01	-4.98	-2.99
GCA Males	-5.42	15.80*	-1.28	-3.47	3.96	-0.63	1.88	

Table 4: Analysis of variance for height in field12 months post-planting in Brazil

Source	DF	Means Squares
Rep	3	3258.5**
Hybrid	21	369.6**
Female	5	747.7**
Male	5	307.1**
Female*Male	10	254.7**
Error	54	122.0

*, ** Significant differences at 0.05 and 0.001 respectively

Table 5: Means of crosses and parents (males and females) for height in filed 12 months post-planting

Females\Males	LCTEEN 162/S-1010	ICS 95 T	SCA 24	H 56	37/A	LCTEEN IMC 47	$\bar{X}$ Females
COCA 3370/5	77.5	85.9	91.9	73.9	89.8		83.8
LCTEEN 37 A		89.8	95.8	85.7		94.8	91.5
GU 261	90.9	86.1		109.6	93.6		95.1
H 56		65.6	76.2			89.3	77.0
TSH 1188	93.9	81.7		82 .0	90.1		86.9
ICS 95 (T)			66.7				66.7
$\bar{X}$ Males	87.4	81.8	82.7	87.8	91.2	92.0	86.2

T: Trinitario origin

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Table 6: Specific Combinimg Abilities (SCA) and General Combining Abilities (GCA) for Height 12 months post-planting

Females\Males (SCA)	LCTEEN 162/S-1010	ICS 95	SCA 24	H 56	LCTEEN 37/A	IMC 47	GCA Females
COCA 3370/5	-8.7*	- 0.39	5.8	-12.3*	3.6		0.3
LCTEEN 37/A		3.6	9.5*	-0.55		8.6*	8.0
GU 261	4.7	- 0.14		23.4**	7.4		11.6*
H 56		-20.7**	-10.0*			3.0	-6.5
TSH 1188	7.6	- 4.5		-4.2	3.8		3.4
ICS 95			19.5**				-16.8**
GCA Males	0.27	-5.4	- 4.5	0.7	4.0	4.9	

*, ** Significant differences at 0.05 and 0.001, respectively

High heritability for jorquette height has been reported previously by several authors. In this study, we wanted to identify clones capable of transmitting relatively short height to their progeny. Even though the genotypes used in both trials were different, at both locations we were able to identify genotypes that, on average over their offspring, appeared to transmit relatively short height to their progeny. Significant GCA and SCA effects existed for the traits analysed. If we take into account that most of the genotypes used as parents were selected for disease resistance, we could assume that these genotypes constitute a random sample of the overall cocoa population for jorquette and plant height. This being the case, we can conclude that there is significant additive genetic variance for these traits, so it would be possible to obtain a response to selection for shorter cocoa plants. It would be optimal to perform more studies on these traits, especially using repeated measures over time (although avoiding the effect of pruning). The calculation of the correlation between vigour (stem girth) and the traits already measured should be performed as well on the trials studied here. The evaluation of yield of the shorter progeny would be necessary to obtain an estimate of gain in yield efficiency.

The results of these preliminary analyses suggest that it is possible to select for shorter plants, a trait that could allow us to obtain higher yield efficiency if combined with other traits related to tree architecture (*i.e.*, compact canopy) and high productivity. This should enable the use of higher planting densities in the field, thus making the crop easier to handle. It is interesting to note that, in both trials, the parents that produced the taller progeny were of French Guianan origin (GU 154-L and GU 261 showed significant positive GCA), while the progeny with shorter height (Criollo 27, ICS 43 and ICS 95) were of Trinitario origin. However, although the Trinitarios studied showed a narrow genetic diversity in the observed trait, one cannot assume that the results are a consequence of inbreeding depression. In most crosses with Criollo 27, ICS 43 and ICS 95 as one parent, we observe relatively shorter progeny (including those from Trinitario x Forastero) compared to other crosses having a Trinitario parent (as those having LF-1 and Santa Clara 3 as one of the parents). If inbreeding depression were the cause of the relatively shorter stature of the progeny observed, only Trinitario x Trinitario crosses should have shorter progeny. In other words, although Criollo 27, ICS 43 and ICS 95 did not show significant GCA for the trait studied, all three clones had a relatively high (negative) GCA for shorter height (Tables 3 and 6). It is also interesting to note that the parents included in the trials for their relative shorter height, LF-1, Santa Clara 3, and Scavina 24, did not produce families with shorter height (Tables 2 and 5). It is possible, however, that the clones, LF-1 and Santa Clara 3, were shorter in stature due to inbreeding depression, and this was not transmitted except when they were crossed with another Trinitario. As mentioned above, significant SCA estimates were also found, indicating the presence of non-additive genetic variance.

The results presented in this note suggest that it is possible to find clones with positive and negative GCA for jorquette and plant height. The height of the parental clone in the germplasm collections is no indicator of the capacity of that clone to transmit its height to its off-spring. Further study of Trinitario clones is warranted to facilitate the identification of parents that transfer short jorquette and seedling height to their progeny.

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Compatibility and Duration of Pod Maturation in Guianan Wild Cocoa Trees - Preliminary Results

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Compatibility and duration of pod maturation are among the traits studied at CIRAD's Paracou-Combi station (Sinnamary, French Guiana) on Guianan wild cocoa trees. These two descriptors belong to the "secondary agronomic" category, since they appear to affect, in an as yet poorly documented way, net yields: the first insofar as self-compatibility is sometimes considered to be an advantage (nothing more, except in monoclonal plantations), and the second is involved in disease escape phenomena. We report here on the preliminary results obtained in an initial phase of research.

Material and Methods

Seventy-two Guianan wild ortets belonging to ten populations from the basins of the Camopi (Cam), Tanpok (Tan) and Kérindioutou (Ker) rivers, surveyed between 1985 and 1995 (Lachenaud and Sallée 1993; Lachenaud *et al.* 1997) planted at Paracou-Combi, were tested for their compatibility (Table 1). The clones providing compatible pollen and used as controls were the Amelonado, IFC 1 (clone selected in Ivory Coast), GF 21 and GF 23 (the latter two being Amelonado-type clones selected in French Guiana). Of the 72 clones surveyed, 19, which were planted in the same plot, were also observed for the duration of pod maturation, along with another two clones (of Camopi origin). In this case, the pollen used was variable, and two clones from a neighbouring plot were used as controls, GF 23, and the well-known Upper Amazon clone, IMC 67.

The hand-pollination techniques used have already been described (Lachenaud 2000) and recommended in the 1998-2003 CFC-ICCO-IPGRI project entitled "Cocoa Germplasm Utilization and Conservation: a Global Approach". Initially, flowers were isolated in sleeves, and 20 to 27 self-pollinated flowers were used per tree. When it turned out that most of the germplasm being studied was self-incompatible or only very slightly self-compatible, the technique without isolation, which is simpler in a very wet country like French Guiana and takes less time, was also used. In this case, 30 to 45 self-pollinations were carried out and if the success rate after a month exceeded 15%, the clone had to be tested again with isolation because of the possibility of natural fruit-setting. Fruit-setting was observed after 10 and 30 days. Simultaneously, on some of the trees observed, pollinations were carried out (with isolation) using compatible pollen from the three Amelonado controls to ensure that the general environmental conditions effectively enabled fruit-setting to take place. The healthy pods obtained in this case, which were all harvested at a similar stage of ripeness (same uniform yellow colour), along with the few pods resulting from self-pollination, were used to determine the duration of maturation. The pods obtained after hand-pollination of clones GF 23 and IMC 67 were used as controls.

Results

The results of the compatibility tests are shown in Table 1, and a histogram of the fruit-setting observed after ten and thirty days is shown in Figure 1.

It can be seen that after ten days 43 clones (*i.e.* 59.7%) proved to be self-incompatible and 29 slightly compatible. Only two clones displayed fruit-setting rates after ten days of between 20 and 25%, with isolation. Three clones displayed fruit-setting rates without flower isolation of between 16.6 and 20%, needing to be tested again with isolation (to be done). At the same time, pollinations carried out with the control pollen displayed fruit-setting percentages of 53.5, on average, after ten days.

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Table 1: Distribution of the 72 clones studied (from ten populations) in three compatibility classes, based on the fruit-setting percentages observed ten days after self-pollination (the figures in brackets indicate the number of clones to be tested again with flower isolation)

Population	Number of clones	Self-Incompatible clones (0%)	Very slightly compatible clones (1-14%)	Slightly compatible clones (15-25%)	Maximum value observed (%) with isolation
Cam 1	14	9	5	0	5.0
Cam 3	7	2	4	1	20.0
Cam 7	8	3	4	(1)	12.0
Cam 8	1	1	0	0	0.0
Cam 9	26	17	6	3 (2)	25.0
Cam 10	1	1	0	0	0.0
Cam 12	6	5	1	0	4.5
Cam 13	3	1	2	0	13.6
Tan	1	0	1	0	14.3
Ker	5	4	1	0	-
Totals	72	43	24	5 (3)	

For those populations with a sufficient number of pods reaching ripeness without damage, the durations of pod maturation observed are shown in Table 2. It can be seen that they were similar for the four Guianan populations and the two controls.

Table 2: Duration of pod maturation observed in four populations and two control clones

Population	Number of clones	Average time (days)
Cam 1	5	145.6
Cam 3	6	147.7
Cam 7	2	145.3
Cam 9	4	149.7
Controls		
IMC 67		150.5
GF 23		141.8

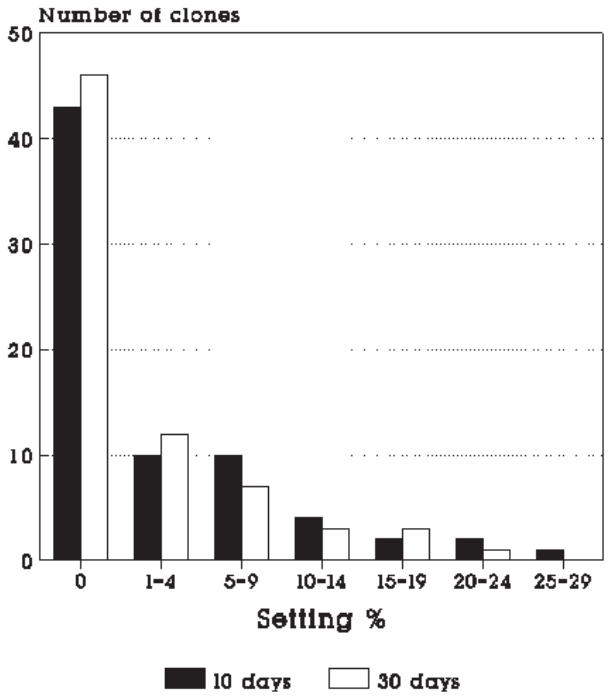

Figure 1: Histogram of fruit-setting recorded 10 and 30 days after self-pollination for 72 Guianan clones

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Discussion and Conclusion

Despite the preliminary nature of these results (some populations were not represented in the study and a few clones need confirmation), it can be seen that the maximum fruit-setting percentage observed after 10 days, 25%, placed the Guianan wild cocoa trees in the self-incompatible category, at least if one adheres to the widely accepted definition (Posnette 1945). However, we prefer to consider that this is mostly self-incompatible material that could prove to be slightly self-compatible under certain circumstances, just as Upper Amazon cocoa trees (Posnette 1945) and probably like all wild Forastero cocoa trees (Allen 1988). As Voelcker (1937) showed that incompatible pollinations led, in some cases, to initial pistil growth and maintenance of the flower for up to approximately 14 days, we proceeded with a second inspection after 30 days. In practice, the results were identical to those obtained after ten days.

Although this point was not systematically examined, it was found that all the cross-pollinations carried out at Paracou-Combi between Guianan clones were followed by fruit-setting. Like Upper Amazons, the Guianan wild cocoa trees would therefore seem to be inter-compatible. In addition, the study plots planted with Guianan wild cocoa trees were productive, indicating overall inter-compatibility (Lachenaud 2000).

The duration of pod maturation (Table 2) for Guianan wild cocoa trees is short since ripening of cocoa pods is generally acknowledged to take between 150 and 210 days (Braudeau 1969; Enríquez 1985). Under conditions at Paracou-Combi, some pods ripened in 131 days, *i.e.* barely after four months.

These initial results will have to be confirmed and extended to other populations and, if possible, under different environmental conditions.

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The CFC/ICCO/INIAP Cocoa Flavour Project - Investigating the Spectrum of Fine Flavour within Genotypes and between Origins

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Background and Introduction

In 1998, the International Cocoa Organisation (ICCO) submitted a project proposal with its recommendation for financing through the Second Account of the Common Fund for Commodities (CFC). This was a "Project to establish the physical, chemical and organoleptic parameters to differentiate between fine and bulk cocoa" (referred to as the "CFC/ICCO/INIAP Cocoa Flavour Project") sponsored by the ICCO, and executed through Instituto Nacional Autonomo de Investigaciones Agropecuarias (INIAP), Ecuador. The project was initiated in 2001 with participants from four fine or flavour producing countries, *viz*. Ecuador, Papua New Guinea (PNG), Trinidad and Tobago and Venezuela.

The central objective of the CFC/ICCO/INIAP Cocoa Flavour Project is to develop universally accepted criteria to differentiate between fine and bulk cocoas through a series of scientific evaluations of physical, chemical and organoleptic parameters. The project

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also aims to provide methodologies to enable the evaluation of cocoa quality in relation to genotype and the environment, as well as to provide and disseminate methodologies, standards and instruments to be used in the evaluation of cocoa quality.

Three main outputs expected from this project include: 1) reliable information on physical, chemical and organoleptic characteristics, which differentiate fine from bulk cocoa; 2) methodologies to measure and compare the main variables that define quality of fine cocoas and 3) standards and instruments to evaluate the quality of fine cocoa thereby contributing towards improving the competitive position of fine cocoa as a distinctive product.

The CFC/ICCO/INIAP Cocoa Flavour Project was originally approved for a three-year period, but it has been extended (with its original budget) for an additional year. We are therefore in the fourth and final project year and the majority of results from the different analyses for each quality parameter have been generated. This article will focus on a subset of organoleptic results, which have provided new and useful insights into the spectrum of what is traditionally considered fine or flavour cocoa.

Materials and Methods

Coding

The identities of the commercial Trinidad Selected Hybrids (TSH) used in this project have been coded with "CRU" accession codes in compliance with an agreement between The Ministry of Agriculture, Lands and Marine Resources (MALMR), Trinidad and Tobago and the Cocoa Research Unit (CRU) governing the presentation of results for these accessions. These accessions will be referred to as local Trinidad clones in this article.

Organoleptic evaluation

The methods used to prepare the cocoa bean and subsequent liquor samples have been adapted and modified from Clapperton *et al.* (1994) and are outlined in Sukha (2001) and Sukha *et al.* (2004). Evaluations using trained sensory panels were conducted under controlled conditions with appropriate experimental designs, reference samples and statistical analyses. Cocoa liquors were evaluated via profiling, descriptive and differentiation techniques in accordance with the American Society for Testing and Materials (ASTM), (1992).

Panel training

After completing an initial pre-screening questionnaire, panellists were trained in the areas of basic tastes identification, taste sensitivity via threshold concentration, introduction to cocoa flavour attributes and vocabulary generation, cocoa off-flavours, scoring and ranking of flavour attributes by paired comparison tests and finally profiling with panellist calibration and hidden references.

Liquor evaluation

Liquors were assessed by a trained panel of at least six persons in a sensory design that incorporated hidden reference liquors to check panellist consistency between repetitions. Randomly selected three-digit codes were assigned to cocoa liquors and the order of tasting liquors was randomised over three repetitions to minimise carry-over effects. No two panellists received liquors in the same order in any given evaluation session. Sensory profiles were recorded for eight cocoa flavour attributes using 10-cm line scales with a possible range of scores from 0 to 10, the higher numbers denoted stronger flavour intensities.

Data analysis

Data from the three repetitions (each crop year represents a repetition with time) were pooled, and analysis of variance (ANOVA) conducted using MINITAB Release 13.1 (Minitab Inc.). The significance of treatment effects and interactions as well as mean flavour profiles and the standard errors of the mean (SE) were calculated. Principal Component Analysis (PCA) was performed on the pooled data using GenStat 7.0 (VSN International), and graphical representation was carried out in MS Excel.

Results and Discussion

Figures 1 and 2 present respective PCA plots of average flavour profiles for the local Trinidad clones over three crop years and average flavour profiles from other country clones (Ecuador, PNG and Venezuela). Both plots include a Ghana "bulk" reference sample, which serves as a flavour standard against which the other flavour profiles can be compared.

Trends in the pooled sensory data, presented in Figure 1, reveal that two principal components explained 84.9% of the variation in the pooled sensory data for the local clones and Ghana reference sample. Principal

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component 1 represented 66.9% of the variation and principal component 2 represented 18.0% of the variation over eight flavour attributes, *viz.* cocoa flavour, acidity, astringency, bitterness, fruity, floral, nutty raw/beany/green and other flavours.

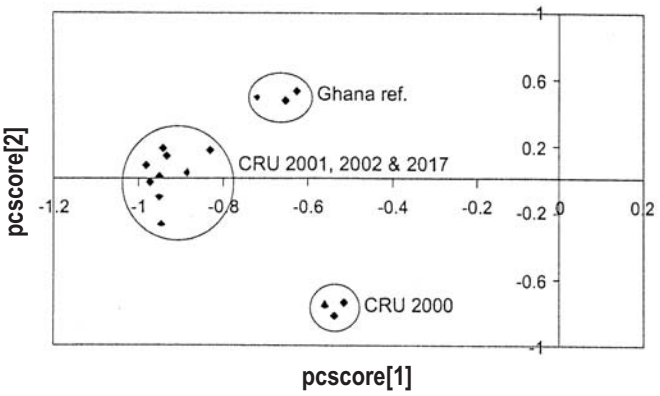

Figure 1: PCA plot of average flavour scores over three crop years for local clones and the Ghana reference sample

Results presented in Figure 1 highlight the differences in organoleptic attributes of the local Trinidad clones compared to the Ghana reference sample, and the separation of local clone "CRU 2000" from the rest of the samples. The actual flavour profiles of these samples reveal that all the local clones had consistently higher fruity and acid scores than the Ghana reference sample, which displayed the highest cocoa and nutty scores. CRU 2000 possessed a very strong floral note that was significantly different ($p \leq 0.001$, data not presented) from the other three local clones. These profile trends have been observed consistently and outweigh any seasonal effects (Sukha *et al.* 2005). The results presented in Figure 1 show strong genotypic differences in "fine or flavour" attributes within the local Trinidad clones that ranged from floral to fruity. These strong and consistent differences in the local Trinidad clones highlight the genetic differences in the "fine or flavour" attributes of related clones, effectively differentiate between "bulk" (Ghana) and "fine" (local Trinidad clones) cocoa, and demonstrate the use of organoleptic analysis as a reliable technique to discriminate between fine and bulk cocoa.

Average flavour profiles of local country clones from Ecuador, PNG, Trinidad and Tobago and Venezuela, with the reference samples from Ghana are presented in Figure 2. The first two principal components explained 77.8% of the variation. Principal component 1 represented 55.7% of the variation and principal component 2 represented 22.1% of the variation over the eight flavour attributes assessed. Principal component 1 clearly separated the Venezuelan Criollo varieties from the rest of the samples whilst principal component 2 distinguished four groups; 1) the Ecuador Nacional types, 2) CRU 2000, 3) the Ghana "bulk" reference sample and 4) the remaining local Trinidad clones and the country clones from PNG.

Individual flavour profiles of the different country clone samples provide interesting insight into the spectrum that exists under the designation "fine or flavour". The Venezuelan Criollos (Guasare and Criollo Merideño) are organoleptically the most distinct set of samples with very nutty and some raw/beany flavour notes, however, they possess a unique caramel/malt/fudge-like flavour attribute that no other set of samples assessed under the purview of theThe CFC/ICCO/INIAP Cocoa Flavour Project possessed.

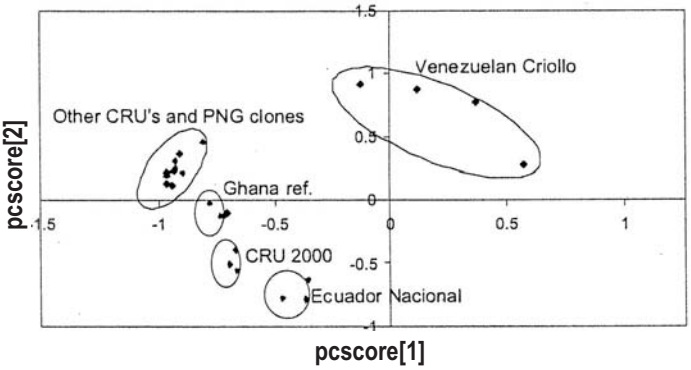

Figure 2: PCA plot of average flavour profiles from different country clones (Ecuador, PNG, Trinidad and Tobago and Venezuela) and the reference sample from Ghana

Ecuador Nacional types (CCAT clones) all contain elements of nutty flavour, but also a unique aromatic "floral" attribute that tends to be a combination of "herbal", "forest green" and moderate "fresh flower" aromas. This was quite distinct from the very sweet and pungent perfume and citrus-like floral attribute associated with CRU 2000. The other coded Trinidad clones, CRU 2001, 2002 and 2017 possessed a mixture of moderately acid "raisin" and "brown fruit" flavour notes, which were different from the fresh fruit, almost "banana"-like fruitiness, of the "KA" accession group from PNG. The aromatic flavour attributes from the different country clones were, in all instances, found to contrast sharply to the dominant cocoa flavour and nutty attributes found in the Ghana reference sample.

The diverse range of flavours that emerged from the different country clones assessed for the CFC/ICCO/INIAP Cocoa Flavour Project provides strong evidence that "fine or flavour" producers do not compete with each other in this small, specialised market segment, but rather each producer occupies a unique flavour niche within the market.

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Conclusions

Findings from organoleptic analysis of the flavour attributes of different country clones reveal clear differences between these samples and the Ghana "bulk" reference sample. We have therefore demonstrated via systematic study that there are clear organoleptic differences between fine and bulk cocoas. Additionally, we have demonstrated genotypic differences in the ancillary flavours characteristic of "fine" cocoas (as in the case of CRU 2000). Finally we have found a broad range of flavours that vary between countries growing fine or flavour cocoa.

The CFC/ICCO/INIAP Cocoa Flavour Project set about the task of finding clear classifications for "fine" and "bulk" cocoa. The results presented in this article from the organoleptic component of the project have moved us closer towards this goal. However, with each question answered we raise other equally intriguing ones such as those dealing with the relative contribution of growing environment (climatic and edaphic) on the flavour and quality attributes of different cocoa genotypes.

We have examined cocoa quality in an unprecedented manner in each of the participating countries under the purview of the CFC/ICCO/INIAP Cocoa Flavour Project. In so doing, we have created awareness of the value of cocoa quality to the producing countries and we have built human capacity to objectively assess cocoa flavour in each country. The project has highlighted the importance of optimal processing techniques to the development and expression of the genetic flavour potential that exists within each project member country.

Current marketing trends with premium dark chocolates have already begun to exploit the origin specificity of fine or flavour cocoa, and the spectrum of ancillary flavours allows for niche marketing in this highly specialised and discriminating market. The results from the CFC/ICCO/INIAP Cocoa Flavour Project can therefore be used to bolster these efforts towards improving the competitive position of fine cocoa as a distinctive product.

Acknowledgements

The ongoing financial support, assistance and collaboration of the United Nations Common Fund for Commodities, ANECACAO, Ecuador, ACIAR, Australia, CONICIT, Venezuela, Guittard Chocolate Co., California, USA, Lindt & Sprüngli, Switzerland, Masterfoods UK, The Ministry of Agriculture Land and Marine Resources, Nestlé Product Testing Centre, York, UK, Paul Manickchand Estates Ltd. (Trinidad), Produce Marketing Associates Ltd. (Trinidad) and all sensory panellists are gratefully acknowledged for their participation in this project. The collaboration of INIAP, Ecuador, INIA, Venezuela and CCI, PNG in providing country clone cocoa liquors for sensory assessment is also gratefully acknowledged.

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Evaluation of Resistance of Cocoa Genotypes to Witches' Broom Disease (*Crinipellis perniciosa*) using Phloem Sap

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Abstract

Witches' broom disease, caused by *Crinipellis perniciosa*, is the major limiting factor in the development of cocoa (*Theobroma cacao*) in Brazil. Effective control is only achieved through phytosanitation and the use of resistant cocoa genotypes. Brazil has the world's largest cocoa germoplasm collection established at the Station of Genetic Resources of Cocoa, Marituba, Pará, but identification of resistant genotypes has been hampered by several factors. This report is based on a study of the feasibility of identifying cocoa genotypes resistant to *C. perniciosa* through basidiospore germination on cocoa phloem sap extracted from adult trees. Phloem sap of cocoa genotypes with field resistance such as CAB 270, CAB 208, CAB 214 and CCN 51 totally inhibited basidiospore germination wheras there was no effect with the sap from susceptible genotypes (ICS 39, PA 195, CAB 155, CAB 0112 and Catongo). These results indicate that this technique provides a simple and quick alternative method for screening for resistance of cocoa to *C. perniciosa*.

Introduction

Witches' broom disease (WBD), caused by the basidiomycete *Crinipellis perniciosa* Singer, is the most important disease of cocoa (*Theobroma cacao* L.) in the Amazon region and in the State of Bahia, Brazil. Although *C. perniciosa* is endemic to the Amazon basin, the disease is now widespread on cocoa plantations throughout South America and parts of Central America and the Caribbean (Baker and Holliday 1957). The pathogen infects actively growing (meristematic) tissue and induces a range of symptoms on vegetative shoots, flower cushions, flowers and pods (Baker and Holliday 1957). Annual losses due to the disease may reach 50-90% in many parts of the Amazon region (Andebrhan 1985; Laker and Mota 1990).

Genetic resistance to diseases is the main component of integrated plant protection systems, and this remains the unique long-term control measure against WBD. Although there is a large and genetically diverse cocoa germplasm collection, the results of identifying resistant genotypes through artificial or natural inoculation have been inconsistent (Andebrhan and Furtek 1994). Different artificial inoculation techniques have been used for screening cocoa genotypes such as dipping germinated seeds into a basidiospore suspension (Holliday 1955); applying agar blocks agar blocks containing basidiospores to hypocotyls (Wheeler and Mepsted 1982); evaluating spore germination in extracts of young shoots (Evans and Bastos 1980) and in cocoa callus (Fonseca and Wheeler 1990); and spraying basidiospore suspension on the terminal shoots of the seedlings (Frias 1987).

The objective of this paper was to evaluate the reaction of cocoa genotypes for resistance to *C. perniciosa* based on the degree of basidospore germination in sap extracted from the phloem.

Materials and Methods

The cocoa genotypes used in this study are from CEPLAC's Centre for *Theobroma* Germplasm Collection from the Station of Genetic Resources of Cocoa, Marituba, Pará, Brazil. Holes (6 x 1 cm) were made in cocoa trunks using a manual drill. The phloem sap was extracted in a 30 ml vial fitted with a resistant plastic hose (15 x 6 cm) wherein the free extremity of the hose was introduced into the hole of the trunk and

Explanation of acronyms used in this issue

BCCCA	Biscuit, Cake, Chocolate and Confectionery Association (United Kingdom)
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza
CEPLAC	Commissao Executiva do Plana da Lavoura Cacaueira (Brazil)
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CRIG	Cocoa Research Institute, Ghana
CRU	Cocoa Research Unit
IITA	International Institute of Tropical Agriculture
MCB	Malaysian Cocoa Board
UESC	Universidade Estadual de Santa Cruz
USDA	United States Department of Agriculture
WCF	World Cocoa Foundation

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fixed by moulding clay (Figure 1). After 48 hours, the vials were collected and the sap was filtered through Whatman No. 1 filter paper and stored at 5-8°C until use.

Brooms collected from the Germplasm Collection were used as sources of basidiospores. Drops of 100 µl of sap were pipetted onto glass cavity slides in glass Petri dishes lined with moist filter paper. Agar blocks containing freshly-deposited basidiospores were placed face down in test solutions and incubated at 25°C in the dark. Three replicates were used for each sample. Distilled water was used as a control. The percentage of basidiospore germination was calculated after 24 hours. Based on the differential inhibitory effects, the genotypes were categorised into: highly susceptible - over 50% basidiospores germinated; susceptible - 25 to 50% basidiospores germinated; moderately tolerant - 10 to 25% basidiospores germinated; tolerant - less than 10% basdiospores germinated; resistant - 0% basidiospores germinated.

Results and Discussion

This current study shows that an assay based on phloem sap, extracted from the trunks of cocoa trees, and incubated with basidiospores of *C. perniciosa*, may be used in screening for resistance to WBD. The results obtained in a sap test showed ample correlation with those obtained from the evaluation of the natural disease in the field (Fonseca and Albuquerque 2000)). Sap from genotypes selected as resistant to *C. perniciosa* in the field and in the greenhouse markedly reduced basidiospore germination as compared to sap from susceptible genotypes. Among 20 listed genotypes (Table 1), which caused low germination or total inhibition of basidiospore germination are two TSA (774 and 654), three CCN (10, 12 and 51), two SCA (6 and 12) and some clones of the series CAB (197, 214, 208, and 271) that presented a low level of brooms in a field evaluation of brooms carried out in Belém, Pará, Brazil (Fonseca and Albuquerque 2000). A positive correlation was also found between results obtained from the sap test and seedling inoculation of cocoa genotypes (Albuquerque *et al.* 1999). Despite the fact that SCA 6 and SCA 12 clones were formerly considered originally resistant to *C. perniciosa* (Pound 1938), and were incorporated into the breeding programme (Baker and Holliday 1957), in the cocoa germplasm collection of Ceplac in Marituba, Pará, Brazil, these genotypes are now infected by *C. perniciosa* according to a field evaluation (Fonseca and Albuquerque 2000). In SCA 6 and SCA 12 sap, basidiospores germinated with short, apically swollen germ-tubes, and most spores ceased germination at this stage. In saps of highly susceptible genotypes, such as ICS 39, UF 296 and others, the basidiospores germinated normally, producing thin and long germ-tubes as in sterile water.

Evaluations carried out in the field and greenhouse showed low levels of infection by *C. perniciosa* in the CAB 208, CAB 214 and CAB 270 genotypes (Albuquerque *et al.* 2005). The results obtained in this study show that the saps of the aforementioned genotypes caused total inhibition of basidiospore germination.

During the evaluation in field, some promising genotypes were selected, and crosses were made between resistant and susceptible genotypes. From these crosses, seedlings were established in the greenhouse and they were evaluated for *C. perniciosa* resistance. Following evaluation, the plants were established in the field. When they were 3-4 years old, phloem sap was extracted and basidiospore germination tests were conducted. Table 2 shows that the saps of CAB 270 x CAB 214 and CAB 214 x CAB 270 caused total inhibition of basidiospore germination. Progenies of these materials, evaluated in the greenhouse for resistance to *C. perniciosa*, had very low levels of infection (Albuquerque *et al.* 2005).

Use of resistant cultivars remains an attractive and effective means of reducing WBD and hence losses due to *C. perniciosa*. Greater efforts should therefore be directed towards identifying new sources of resistance for incorporation into breeding programmes.

Conclusion

The expression of resistance by phloem sap could theoretically be an economical and rapid alternative screening method to the conventional screening at the nursery stage, which takes up 4-6 months, and allows the study of the underlying resistance mechanisms. These preliminary results have been consistent. However, during collection and storage of saps, special care should be taken to avoid contamination since it could confound the results of the basidiospore germination. Screening phloem sap of different cultivars for their capacity to avoid or stop fungal growth must clearly differentiate between susceptible and resistant or tolerant genotypes. The identification of the substance(s) responsible for the inhibitory activity of basidiospore germination is in progress.

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Table 1: Effect of phloem sap of cocoa (*Theobroma cacao*) genotypes on basidiospore germination of *C. perniciosa*

Cocoa genotypes	Basidiospore germination (%) [1]	Category [2]
CAB 155	> 50,0	HS
CAB 012	> 50,0	HS
CAB 079	> 50,0	HS
CAB 069	> 25,0	S
CAB 158	< 25,0	MT
CAB 270	0,0	R
CAB 271	<1 0,0	T
CAB 197	0,0	R
CAB 208	0,0	R
CAB 214	0,0	R
SCA 6	<10,0	T
SCA12	<10,0	T
ICS 39	> 50,0	HS
PA 195	> 50,0	HS
TSA 654	< 25,0	MT
TSA 774	0,0	R
CAB 324	> 25,0	S
Catongo	> 50,0	HS
CCN 51	0,0	R
RB 36	> 25,0	S

[1] Means of three replicates, 100 basidiospores per replicate
[2] Category: HS = highly susceptible, S = susceptible, MT = moderately tolerant, T = tolerant, R = resistant.

Table 2: Effect of phloem sap extracted from crosses of genotypes of cocoa (*Theobroma cacao*) on basidiospore germination

Cross	Basidiospore germination (%)[1]	Category[2]
CAB 270 X CAB 214	0,0	R
CAB 214 X CAB 270	0,0	R
CAB 324 X CAB 214	< 25,0	MT
CAB 324 X ICS 39	> 50,0	HS
ICS 39 X CAB 27	< 25,0	MT
ICS 39 X CAB 214	< 10,0	T
PA 195 X ICS 39	> 50,0	HS

[1] Means of three replicates, 100 basidiospores per replicate;
[2] R = resistant; MT = moderately tolerant; T = tolerant; HS = highly susceptible; S = susceptible.

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Figure 1: Apparatus for collecting sap from trunks of cocoa (*Theobroma cacao*) genotypes

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Editor's note: This is a reprint of a letter from G.A.C Herklots and a note by the late D.B. Murray on the **'Comparison of Seedlings and Cuttings as Planting Material'**, which were scanned and kindly submitted by Dr. Rob Lockwood. They are reproduced here because of the resurgence of interest in this topic in recent times, particularly at the last INGENIC Workshop.

Cacao Yields from Clones and Seedlings

G.A.C. Herklots

Director, Regional Research Centre
The Imperial College of Tropical Agriculture, Trinidad. B.W.I.

26th January, 1956.

A remarkable view seems to be held in some quarters in England that there is considerable doubt as to whether at this stage of knowledge and with the range of clonal material available the planting of rooted clonal cuttings can be certain to give significantly higher yields than seedling cacao.

This view is completely erroneous. No one on the research staff here has ever said or ever written anything to this effect for our evidence is entirely the reverse.

It must be noted that I am not speaking of Amelonado cacao as grown in West Africa, but of selected Trinidad clones as compared with Trinidad seedlings,

What has been said and emphasised by me and others at the recent London Conference is that Trinidad clones have not yielded as highly as some people expected and predicted but we hastened to add that this was because - to put it simply - they had been grown in marginal land. Even under such conditions, clones yield much more than seedlings.

Where advice is sought as to which clones to plant under specified conditions, we can guarantee a far higher yield than from chance seedlings. Moreover, if our published advice as to the right balance of shade and fertiliser treatment is adopted, we can guarantee greatly increased yields.

It must be remembered, and we do emphasise this, that the cost of raising rooted cuttings is greater than the cost of seedlings. These costs are small compared with the increase in yields and with reasonable care deaths of transplants can be kept down.

In order to dispel this bogey of considerable doubt, a paper has been prepared by Dr. Jolly which will be published in due course. Mr. Murray, with Dr. Jolly's and Mr. Cope's co- operation, and following discussions with other members of the research staff, has prepared a summary, which is attached.

Comparison of Seedlings and Cuttings as Planting Material

D.B. Murray

26th January, 1956

It is now generally accepted that the yields from selected cuttings are higher than from unselected seedlings. However, the cost of producing a rooted cutting is far higher than for a seedling partly because the capital outlay required for the annual production of large numbers of cuttings is considerable. The question has arisen - are the yields from selected cuttings sufficiently greater than from seedlings to recoup the higher capital outlay? Moreover, the situation has recently been complicated by the suggestion that use should be made of "clonal seedlings" which it has been suggested, "could be as efficient producers as, and could cost very much less than, rooted cuttings"[1].

Botanically, a clone is a new individual plant formed by separating a vegetative part from the parent plant and its subsequent independent growth; its genetic factors are thus exactly the same as the parent plant. The term clonal seed is therefore a contradiction but it has become established in the Literature, largely in connection with rubber in the East, to describe seed obtained from a number of trees of a single clone planted in isolation or from a mixture of several clones.

To argue by analogy that because some success has been achieved in planting rubber from such material that similar results could be obtained for cacao is completely unscientific. Only by experimental work can the value of such seed be assessed and the results of a trial at Airbase Field, San Juan Estate Trinidad (Jolly 1953)[2] are relevant.

In this field, cuttings from three clones ICS 1, 45 and 95 are compared with seedlings from open-pollinated pods taken from the same three clones. Though the male parents are unknown, the parent trees were growing in a plot of selected ICS clones and they therefore represent what is meant by clonal seed. These field yields have been brought up to date in Figure I.

[1] The reference is to the report of the Cocoa Conference held at Grosvenor House 13-15 September 1955. The discussion of Planting Material on pages 83-85 includes recommendations of "clonal seed" although the exact words quoted by Dennis Murray do not appear.

[2] Jolly, A.L. 1953. Notes on the performance of ICS clones at San Juan Estate, Report on Cacao Research 1945-51. Imperial College of Tropical Agriculture.

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Even though one-third of the cuttings consist of ICS 45, a rather poor clone no longer recommended for planting, the marked superiority of the cuttings as a whole is obvious. This is particularly so in the early years, the cuttings giving nearly 500 lbs, dry cacao per acre in their fourth year, a figure reached by the seedlings only in their seventh year. By their fifth year, the cuttings have already outyielded the seedlings by 1,000 lbs. per acre or over 3 lbs. dry cacao per tree. At the figure of 2 shillings per lb. for dry cacao, the cost of the original rooted cuttings at say 4 s each has already been recovered. No fertilisers have been used in this field, the trees receiving only routine estate attention.

There are no trials of cuttings versus seedlings at River Estate of comparable age, but at least a dozen clones have been shown capable of yields of 1,000 lbs. dry cacao per acre and higher, despite the lack of manuring or any special treatment. That these yields can be secured over a reasonable acreage is shown by the 11-acre shade and fertilizer trial where ICS 1, averaged over all treatments, has given in its third, fourth and fifth years, yields of 264, 408 and 1,028 lbs. per acre. The net return from this area for 1954-55 was over £1,000 for eleven acres.

River Estate soils cannot be classed as particularly good and under better conditions, in Grenada, clones like ICS 29 and GS 36 have yielded at the rate of 2,200 lbs, per acre in the Ashenden trial in their fourth year in the field, which again cannot be equalled by seedling trees.

Why then do not all estate plantings of cuttings produce 1,000lbs cacao per acre or more? The answer is that the plant can do no better than its environment permits. If, for soil physical or chemical reasons, nutrient uptake is unsatisfactory, the clones least suited to such conditions will perform poorly. Clones better adapted to such conditions may yield reasonably well, but still at less than they are capable of. Under such conditions, only a narrow range of clones is available and work must continue both on environmental improvement and on increasing by selection and breeding the range of outstanding clones.

The fact that trees of a given clone have been found to vary in yield from spot to spot in the field does not mean that the **average** yield is not much better than that of un-selected seedling cacao. The enormous range shown by seedling cacao has been amply demonstrated in the past: we are now better able to distinguish genetic from environmental variability and the point of using clones is that their genetic properties are known and can be separated from environmental factors. Thus, because we have control over the genetic factors with cuttings we can advise planters on certain clones which they should plant and certain others which they should avoid. We can do this yet with seedling material from these same clones other than to say that some of the progeny will be good, some indifferent and probably many worthless.

Some information is becoming available on the performance of the progenies of controlled clonal crosses *i.e.* crosses made between proven high-yielding clones. Certain specific crosses, ICS 1 x ICS 6 in particular, have given progenies whose **average** yield based on only a handful of trees does approach ICS1 and ICS 6 as cuttings. This means that some of the seedlings are superior to either of the parents and in due course new and better clones may be developed by taking cuttings from these trees. It is too early yet, however, to say that open pollinated pods from any particular planting of mixed clones will produce progenies averaging anything like the original clones.

The planting policy in any area must depend upon the economics of the planting programme. Clearly, to get large areas of undeveloped land under cacao rapidly, recourse must be made to seedlings. Where, however, in a well-developed area with high land values it is intended to rehabilitate or extend the cacao industry, the optimum cash return per acre must be envisaged. This can only be assured by the use of clonal cuttings and it has been shown that the higher cost of establishing cuttings can be recovered by the fifth year of planting. The cost of producing a tree and bringing it into bearing has to spread over the whole of its economic life. This cost becomes negligible against the higher yields of selected clones.

It is of course difficult to establish propagating stations to turn out large numbers of cuttings overnight largely because of the time required to establish adequate nurseries. Also their capacity cannot be such as to produce the estimated requirements of cuttings in a short time; they must be designed to meet a fairly permanent annual demand at a more or less steady level. This may mean that seed must be planted in undeveloped areas and such seed should be the best available. In due course, when they come into bearing, such seedling fields can be rehabilitated by cuttings but it would be a false economy to delay the production of rooted cuttings until then. The use of seedlings should be considered only as a stopgap until cuttings are available. If seedlings are planted, one practical consideration might be planting at double density, i.e. 12' x 6'. This would permit drastic thinning when they come into bearing and reduce the number of cuttings needed for rehabilitation.

Cacao must follow the lead of other tropical crops such as rubber, tea, citrus where intensive and often expensive systems of culture must be employed in order to obtain high yields per acre. On a long term view, the higher capital outlay for the production of rooted cuttings should undoubtedly lead to a higher

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return per acre and in consequence a better return for the territory on the money invested.

Finally, should the world price of cacao fall in the future, it is the producer who is getting a high yield per acre who will survive. The grower with similar cultivation expenses, who is getting lower yields from unsuitable planting material, may not weather the storm.

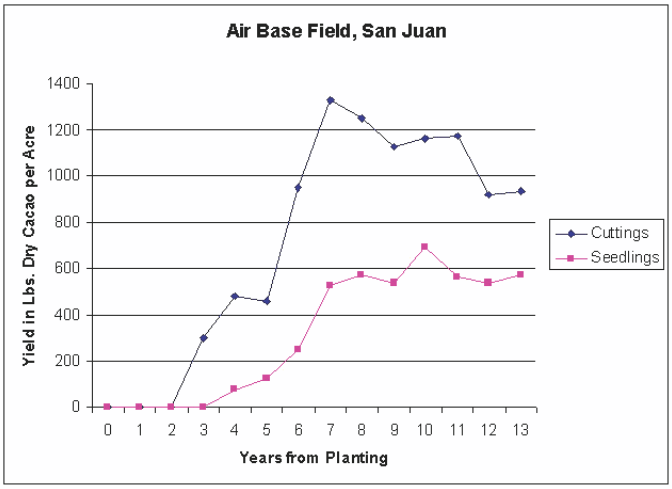

Figure 1: Comparison of the yields of cuttings and seedlings over time

FORTHCOMING EVENT

Fifth INGENIC WORKSHOP
Theme:
Cocoa Breeding for Farmers' Needs,
October 16-17, 2006
and the ICRC in Costa Rica

Cocoa Breeding in the Dominican Republic

M. Ventura López

Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF)

Introduction

Cocoa is one of the traditional export crops in the Dominican Republic. Currently, the area planted with cocoa is 152,262 ha, and there are approximately 40,000 farmers. Over the last ten years, exports of cocoa amounted to 44,448 metric tonnes annually, which represented an average of 55 million USD per year. Internal consumption is around 6,700 tonnes per year. Average productivity of dry cocoa varies between 430 - 580 kg ha^{-1}.

Cocoa research was initiated in 1962 with the introduction of parental clones for the production of seed of hybrid cocoa varieties. In the 1980's, progress in cocoa research included results from studies on post-harvest technology, identification of pests and diseases, planting density and the effect of permanent shade on cocoa production. Despite the research progress obtained, it is very important to further improve quality traits (bean size, organoleptic quality) and also post-harvest technology. These appear to be limiting factors precluding cocoa produced in the Dominican Republic from penetrating the fine-flavour cocoa markets.

Cocoa plantations are mostly comprised of hybrid varieties derived from Trinitario x Forastero crosses (about 70%). To a lesser extent (about 30%), pure Trinitario and some "Criollo" type plantations still exist. Areas planted with "Criollo" material have been deceasing progressively due to renovation of cocoa plantations as well as to natural disasters (hurricanes).

The genetic erosion of the "Criollo" cocoa plantations is considered a deterrent to the genetic improvement of the crop, as these populations may contain the quality traits for which the international markets are looking. Accordingly, it is necessary to evaluate these materials to estimate their yield and quality traits. Cocoa breeding may therefore have an important impact on yield, quality of the produce and income of the farmers.

History of cocoa selection and breeding in the Dominican Republic

In 1962, 61 clonal accessions were introduced and planted in a collection at San Francisco de Macorís (see also under section 3). These were later transferred to the IDIAF Experimental Station of Mata Larga.

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After a few years of observation, the most promising clones were used for production by manual pollination of hybrid seed that was used for new plantings and to renovate old plantations. In 1987, a trial comparing 17 hybrid varieties was established at the Mata Larga Station. Simultaneously, 21 selections were made in farmers' fields, intended for use as clonal selections.

Since the commencement of activities at IDIAF, in 2001, studies have been undertaken on the production of seed of hybrid varieties and also of clonal propagation methods (budding). IDIAF is now selecting 10 superior hybrid varieties. It has also identified 28 superior mother trees, which were selected in farmers' fields.

Genetic Resources Available

The existing germplasm collection in the Dominican Republic contains 58 introduced clones: 11 UF, 3 SIAL, 1 Catongo, 2 SIC, 2 Pound, 3 Parinari, 8 ICS, 1 TSH, 5 RIM, 11 EET, 1 SPA, 1 SNK, 1 SGU, 2 CC, 1 IMC, 1 Nanay (NA), 1 GS, 1 TSA and 2 Scavina (SCA) clones. The local clone collection contains 49 accessions: 21 ML, 18 IML and 10 Rizek clones.

Current Breeding Activities and Perspectives

The IDIAF selection and breeding activities were initiated in 2000. Currently, two researchers, trained at the MSc. level, are involved.

The objective of the programme is to find solutions to the problems identified and to take advantage of opportunities. In order to be pertinent, the programme has to be based on the correct paradigm with all stakeholders involved in the production chain. IDIAF has consulted with cocoa producers, agronomists, extension officers and with local and international experts to decide on the research agenda. The problems identified as a result of this consultation process are as follows:

- Low productivity and quality of the product;
- Genetic erosion of potentially interesting cocoa populations;
- Mistakes in identification of clones in the clonal garden of the Mata Larga Station;
- Low profitability of the cocoa plantations.

As an important opportunity, the increasing demand for good quality cocoa was identified.

The main objectives of the IDIAF breeding programme are therefore the selection of genotypes that provide good quality cocoa and that are high-yielding. Since 2001, the evaluation of new clonal and hybrid varieties is being conducted in farmers' fields to encourage the direct involvement of farmers, and quicker adoption of new varieties.

The following activities are currently being carried out:

- Evaluation of vigour, yield and quality of 10 bi-parental crosses (introduced UF and ICS clones crossed with local ML selections, which are Trinitario or Forastero types) on the experimental station and in farmers' fields,
- Evaluation of vigour, yield and quality of 21 clones at the research station,
- Evaluation in farmers' fields of 10 local selections, 15 Criollo selections and 12 Trinitario selections for quality and agronomic traits,
- Selection of new Trinitario and Criollo mother trees in farmers' fields for yield and quality traits,
- Correction of identification errors in the clone collection at the Mata Larga Station,
- Study on cross-compatibility among Trinitario and "Criollo" clones,
- Evaluation of resistance of 21 clones to *Phytophthora* through field observations and artificial laboratory inoculations.

IDIAF has selected local clones based on two years of observation on yield (kg dry cocoa per tree), pod index, bean weight, cotyledon colour and number of pods affected by *Phytophthora* sp. The most promising selections are the following:

- Among Trinitario selections, the IML-44 clone appears to be most productive,
- Among "Criollo" clones, IML-11 and IML-32 were selected because they possess 100% white beans,
- Among other local material, the Rizek-12 and Rizek-43 clones appeared to be most productive.

Despite the efforts of IDIAF in supporting cocoa selection and breeding activities, the following limiting factors have to be recognised:

- decrease of financial resources, and
- lack of training.

The IDIAF is therefore trying to identify other sources of financing and training opportunities, within the country and externally.

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Cocoa Breeding at CPCRI, India

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Cocoa was introduced into India around the late eighteenth century, and was mainly grown in homestead gardens. Systematic cultivation of cocoa in arecanut and coconut plantations commenced only after the late 1960s. Cocoa research at the Central Plantation Crops Research Institute (CPCRI) began in the early 70's, and CPCRI has been pioneering with its mandate of introduction, selection, evaluation and hybridisation of cocoa. The germplasm holding at CPCRI, Regional Station, Vittal, Karnataka consists of 146 accessions, which were introduced from the Cocoa Research Institutes in Nigeria and Ghana, Malaysian plantations and Kew Gardens. These were collected through various primary and secondary agencies such as Reading University (United Kingdom); Lalbaugh Garden, Bangalore; Horticulture College, Kerala Agriculture University,Vellanikara; and farmers in Wynad and other cocoa growing areas of Kerala (Bhat 1999; Nair and Bhat 1990).

The following strategies have been adopted in the cocoa improvement programme:

(i) germplasm collection
(ii) selection for high yield and large bean size
(iii) vegetative propagation
(iv) hybridisation
(v) establishment of progeny trials and
(vi) establishment of clonal orchards with objectives of high yield, large bean size of more than 1.0g, drought tolerance and resistance to black pod disease.

Germplasm evaluation

Evaluation of germplasm was carried out for yield (number of pods/tree/year), pod index, bean weight and number of beans per pod. The collections showed wide variation for all the characters studied. Seven trees with high bean yield and good bean size were selected from the Malaysian and Nigerian collections. The compatibility status of these selections was identified (Nair and Rekha 1996; Table 1).

Table 1: Characteristics of selected high-yielding trees

S. No	Tree No.	Genotype	Bean wt. (g)	SI/SC
1	I-56	PA 7 x NA 32	1.20	SI
2	I-14	Jarangau Red Axil	1.17	SI
3	II-67	Landas 364	1.34	SI
4	III-35	Amelonado x NA 32	1.09	SI
5	III-105	Amelonado x PA 7	1.06	SI
6	IV-20	Landas 357	1.06	SC
7	NC 42/94	T 86/2	1.08	SI

SC - Self Compatible
SI - Self Incompatible

The evaluation studies revealed that the most efficient lines have been those that produced more pods and dry beans from a small or moderate-sized canopy, and have superior foliage, spread over the width of the canopy, and more branches providing space for cushion development and pod bearing (Bhat and Rajan 1997). Plants belonging to accessions EET 272 attained a maximum height of 3.5 m while the smallest plants belonged to ICS 6 (2.3 m). Widest stem girth was noted with plants of V-1 (23.95 cm) while the smallest stem girth was noted for ICS 95 (15.96 cm).

Laboratory screening of the majority of the available germplasm against the black pod disease using isolates of prevailing *Phytophthora* spp., *viz. P. palmivora*, *P. capsici* and *P. citropthora* indicated a few lines with a certain degree of tolerance (Chandramohanan 1982; 1994).

Progeny Trials

Hybridisation work was intensified from 1983 onwards, and 58 hybrids were evaluated in separate progeny

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trials. The best yielders were identified (Nair *et al.* 1990; 1996) and recommended for cultivation in Kerala (I-14 x I-56, I-14 x NC 42/94, I-56 x NC 42/94) and Karnataka (III-105 x I-56). The four parents were vegetatively propagated and established in one poly-clonal and six bi-clonal seed gardens at Seed Farm, Kidu, Karnataka for quality seed distribution among farmers.

The softwood wedge-grafting method used for vegetative propagation was standardised. The method was used to produce grafts from the high yielders selected among the released hybrids mentioned in the previous paragraph.

The hybrids and selected clones were evaluated in five statistically (RBD) laid-out field trials designated Progeny Trials I to V. The total numbers of hybrids and clones tested as well as the best performers are given in Table 2.

In the clone trial, eight high-yielding trees of Nigerian origin were evaluated. Pooled analysis of six years of data for stable yield and stability indices showed that NC 45/53 had the highest yield (0.94 - 1.73 kg dby/plant/year) with a lower value for its coefficient of variation followed by NC 38/119 (1.40 kg dby/plant/year).

Based on the results, four hybrids and one clone with high yield and desirable bean characters were identified, and will be released for cultivation during 2005. The characteristics of these are given in Table 3.

Table 2: Progeny trials and best performers

Progeny trial	Hybrids/clones tested	Best varieties	Yield (dry bean kg/tree/year)
I	5	NA 33xICS 89	1.01
II	24 + 1 (Check)	I-56xII-67 I-14xI-56	1.48 1.55
III	13	ICS 6xSCA 6 ICS 6xSCA 12 IMC 67xICS 6	1.60 1.30 1.20
IV	14	II-67xNC29/66 II-67xNC42/94	1.65 1.30
V Clonal trial	18 8 + 2 (Checks)	Under evaluation NC 45/53	- 1.11

Table 3: Performance of hyrbids and clone selected for release

Varieties	Yield potential (kg/plant/year)	Compatibility	Bean weight (in g)
I-56 x II-67	1.48	SI	1.0
ICS 6 x SCA 6	1.15	SI	1.0
II-67 x NC42/94	1.13	SI	1.0
II-67 x NC29/66	1.48	SI	1.1
NC 45/53 (clone)	1.15	SC	1.1

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Drought tolerance

The growth and yield of cocoa are influenced by a number of environmental factors, particularly rainfall, temperature and water stress. Cocoa is very sensitive to drought. Water stress affects the most important physiological determinants of yield- canopy architecture, photosynthesis and partitioning of assimilates. The intensity of the drought is more pronounced in the northern regions of Kerala and coastal Karnataka (dry spell extending up to 3 - 6 months). The plants are subjected to severe stress in the rainfed coconut gardens. However, the situation is slightly better in arecanut gardens, which are irrigated, but non-availability of water towards the end of summer exposes the plants to stress in these gardens also (Balasimha 1999; Balasimha *et al.* 1988). Consequently, efforts have been made to identify drought-tolerant characteristics in cocoa accessions under field conditions (Balasimha *et al.* 1985; 1988). All the germplasm accessions were screened for physiological and morphological parameters. The results have shown that a thick leaf, higher epicuticular wax content and efficient stomatal closure under drought reduce transpirational water loss, and this is responsible for better drought adaptation. Such increases in stomatal resistance did not affect net photosynthesis significantly, and it was found that the water use efficiency was enhanced in tolerant accession types (Balasimha *et al.* 1991). It has been possible to identify drought-tolerant accessions, *viz.* NC 23 (P3 x P), NC 29(P6 x P4) and NC 42 (T 86/2), which are introductions from Nigeria. These were used as parents in crosses with high-yielding clones in the breeding programme. The hybrids and parents were evaluated for drought-tolerant traits and yield under field trials. The hybrids, I-21 x NC 42/94 and I-29 x NC 23/43, retained a higher water potential and stomatal resistance, indicating their drought tolerance (Balasimha *et al.* 1999).

Comparative Yield Trial

A comparative yield trial of the hybrids and clones, selected from earlier progeny trials, showed that performances of the best hybrids and clones are comparable with higher dry bean yields (Elain Apshara 2005; Table 4).

Table 4: Comparative yield of hybrids and clones

Hybrids	dby kg/tree/year	Hybrids	dby kg/tree/year
ICS 6x SCA 6	1.54	I-56 x III-35	1.42
PA 7 x NA 32	1.79	III-35 x IV-20	1.40
IMC 67 x ICS 6	1.75	I-14 x NC 42/94	1.39
NA 31 x ICS 1	1.42	III-105 x NC 42/94	1.37
Amel x PA 7	1.22	I-56 x IV-20	1.36
SCA 6 x ICS 6	1.10	1-14 x IV-20	1.33
NA 33 x ICS 89	1.01	**Clones**	**dby kg/tree/year**
SCA 6 x IMC 67	1.25	NC 39/102	1.15
I-56 x II-67	1.48	NC 34/113	1.02
I-14 x I-56	1.47	NC 45/53	1.75

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Biotechnology

Studies of germplasm using RAPD markers have been initiated at CPCRI. Genomic DNA was extracted from 76 cocoa accessions using a standardised protocol. The PCR amplification reaction components were optimised after elaborate trials to estimate optimal Mg++ concentration, dNTP's, primer and template DNA concentrations. In addition, temperature profile conditions were determined by testing the primer annealing temperature in a gradient of 100°C from 35°C to 55°C. A primer survey was done on 40 primers from primer kits OPB and OPC using three accessions, *viz.* Jaranagau Red Axil, NA 33 and EET 272 in order to identify the ones suitable for analysis. Of the 40 primers screened, 18 were found to be polymorphic for the accessions tested. RAPD analysis was carried out using 10 polymorphic primers to distinguish cocoa accessions (Sane *et al.* 2002). The results indicated that accessions can be effectively identified with RAPD markers. Band polymorphism was lowest in the Nigerian accessions and highest in the KAU collections. The UPGMA algorithm, based on Jaccard's coefficient, grouped 76 accessions into six groups based on genetic distance, and revealed four highly divergent accessions, *viz.* BE 10, EQX 78, I-56 and SCA 12.

Conclusions

The main concerns in cocoa breeding programmes in India are high yield, resistance to biotic and abiotic stresses and bean quality. In view of the threats from diseases like vascular streak die-back (VSD), black pod disease (BPD) and Ceratocystis wilt to the cocoa industry, the thrust at CPCRI is on resistance breeding using biotechnological approaches. In addition to this, efforts to widen the genetic base of the existing germplasm are also being pursued through introductions.

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American Regional Cocoa Breeding Initiative

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At the end of the INGENIC Workshop in Ghana (October 2003), a meeting was held to discuss how best the different national breeding programmes in the different regions of the world could integrate to identify and solve regional problems. Representatives of CEPLAC, UESC, CRIN, CATIE, CCRI, CRU and IPGRI were present. The meeting lasted three hours, and Bertus. Eskes (Chairman of INGENIC) dubbed it the 8th INGENIC session. Several mechanisms were discussed. The original proposition of Yoel Efron, put forward during the INGENIC workshop, was to co-ordinate efforts to establish a type of regional centre for cocoa breeding research, but eventually it was agreed rather to adopt a network approach to promote further regional collaboration. The idea behind this network is co-ordinating efforts to achieve clear common objectives in the different regions.

A first meeting to discuss each of the three regional initiatives was held at the University of Reading (UK) in April 2004 after discussions on the second CFC project. At that time, there was little time for the members of each region to discuss, in detail, the scope and activities of each regional programme. For the Latin-American region, a second meeting was held in February 2005 in CATIE, Costa Rica, to identify the scope, organisation and activities of what could be the Latin American breeding programme. It was suggested during that meeting to change the name of this initiative to the "American Breeding Programme" (ABP), given that countries from South, Central and North America are involved.

Following are some keynotes results from the said meeting:

1. A vision of what should be the goal of the ABP:

A virtual programme where members representing different institutions could lead different activities or projects based on comparative/competitive advantages.

2. Problems identified that could be solved through genetic improvement in America:

- Low yields
- Losses due to Moniliasis, Witches' broom, Black Pod and Ceratocystis wilt
- Quality
- Size and shape of trees

3. Problems associated with the implementation of breeding programmes in America:

- Difficulties associated with exchange of germplasm (laws and methods)
- Lack of training
- Lack of coordination
- Lack of standardised methods for selection
- Lack of improved planting material
- The small proportion of resistant material available

4. List of on-going activities with regional interest:

- Population improvement for Moniliasis in Costa Rica
- Population improvement for WB in Ecuador and CRU, Trinidad
- Population improvement for BP in CRU
- CFC-Biomol Programme (CEPLAC, INIAP, ICT)
- CFC Regional variety trial
- CFC and USDA replicated QTL trial
- Molecular characterisation of collections (USDA)

It was agreed that coordination between these activities through ABP could improve their efficiency.

5. Priority area of immediate concern to the ABP:

Recognising the present impact of Monilia on cocoa production within the region and the future risk if not controlled, participants at the meetings unanimously agreed that the control and prevention strategies for this disease through breeding should be given high priority on the agenda of the American Breeding Programme.

The participants at the meeting agreed to support breeding activities related to the control and prevention strategies of Monilia as the first project to launch the regional collaboration.

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The objectives of this first project would be:

- To create a coordination mechanism for all activities on Moniliasis resistance in the region
- To support CATIE and NARS Moniliasis resistance programmes (through coordination)
- To improve the regional germplasm exchange situation (including a possible upgrading of the Barbados quarantine facility)
- To support local trials within the region to validate data on Moniliasis resistance
- To support the utilisation of the introduced material in national breeding programmes (enhancement of current activities and preventive breeding)
- To support research to establish new methodologies for early screening of the disease (molecular markers, phloem sap, etc.)

6. Other related priority activities:

- The collection of germplasm in Peru is important for the region, and an effort should be made to evaluate the collected germplasm
- It was agreed that various collections within the regions should be evaluated for resistance to Moniliasis
- The search for resistance to Moniliasis in Colombia was proposed as one of the activities that should be carried out within the ABP
- The participation of the farmers in the search for resistance to Moniliasis was noted.

7. Organisation of the ABP:

It was agreed that a Working Group mainly comprising geneticists would take decisions on the direction to be taken within the programme. The following are the institutions and persons comprising the said working group: CEPLAC (Uilson Lopes, Jay Wallace), CRU (David Iwaro), CATIE (Wilbert Phillips), INGENIC (Bertus Eskes), INIAP (Freddy Amores), USDA/ Masterfoods (Juan Carlos Motamayor).

The first area agreed to be developed within the American Breeding Programme was, as mentioned above, the coordination of breeding activities related to Moniliasis resistance. The coordinator of this project is Wilbert Phillips.

Once the frame of the ABP is officially established and the first project of the regional collaboration is prepared in detail, research for obtaining funds will be conducted to implement the programme. Several institutes are being considered to approach for funding. Funds are necessary to support at least the coordination of activities and implementation of priority activities: introduction of germplasm, evaluation of germplasm, development of methodologies of evaluation, *etc.* through visits of the coordinator or technical workshops.

If resources are available, an ABP meeting will be convened to coincide with the next CFC/ICCO/IPGRI Project Coordination Meeting, planned for February 2006. Significant progress on the establishment of the programme is expected in the interim.

Asia/Pacific Regional Cocoa Breeding Initiative

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During the INGENIC meeting in Accra in October 2003, Dr Yoel Efron from the Papua New Guinea (PNG) Cocoa and Coconut Institute proposed regional approaches to cocoa breeding, including the establishment of regional breeding centres. As a follow up, Africa and Asia held Regional Meetings during 2004, and the Americas held theirs in early 2005.

The Asia/Pacific meeting gathered together cocoa breeders from Indonesia, Malaysia, PNG and Vietnam on 11 and 12 June 2004 in Singapore. The objectives were to:

- identify the problems, threats, opportunities and critical gaps in knowledge in regional cocoa breeding;
- define which of them are the priorities;
- recognise which of these priorities could benefit from being addressed in the regional context and would bring the most benefit to the regional cocoa farmers.

The main problem in the region is cocoa pod borer (CPB, *Conopomorpha cramerella*) that, together with *Phytophthora* pod rot (PPR) and vascular streak dieback disease (VSD),causes large losses to cocoa production. Development of material with high yields in the presence of these pest/diseases is the highest priority in the

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Region. Thus far, there is no confirmed report of CPB[1] in PNG, but the threat of infestation is real due to its well-established presence in West Papua, the Western part of the New Guinea Island. CPB is the crucial issue for the whole Asia/Pacific Region. It is not yet in Vietnam, but the threat of its introduction also exists.

Currently, no cocoa planting material that yields well in the presence of CPB is available. The Malaysian Cocoa Board (MCB) breeding programme is addressing the problem, and the Indonesian Cocoa and Coffee Research Institute (ICCRI) also works on this issue, with emphasis on selection on cocoa farms. However, this is still the critical issue in the Region that needs more attention and action.

The second priority identified during the meeting was the assessment of CPB resistance/tolerance, and estimation of losses in the field. Current screening methods are unsatisfactory because they lack both precision and accuracy. Several factors that affect the degree of the CPB infestation have been tested and reported, including pod smoothness, thickness and hardness of the sclerotic layer in the pod wall, and ovipositional preference. The majority of these factors are crucially influenced by the age of pods that are being infested. This complexity of factors influences the susceptibility of a cocoa pod to CPB infestation, making it difficult to define screening methods and assure their reliability.

Hardness of the sclerotic layer is one, but possibly not the only factor that affects the infestation rate of CPB. It can be used to predict susceptibility to CPB infestation. A standardised method of measuring hardness repetitively would be very valuable as a screening technique, especially if it would allow sharing of data between countries in the region and could be used in PNG before CPB becomes a problem. Projects to identify a suitable technique are underway at ICCRI, MCB and Reading University.

Threats of new encounter diseases, especially Witches' broom and Monilia, were also discussed. It was agreed that introduction to the region of material with resistance to these diseases would be a sensible preparation for possible future problems. In general, it was also observed that cocoa genetics and physiology are global issues that should not be addressed as priorities in the regional context.

Dr. Efron's proposed regional breeding centre was discussed. It was agreed that a regional breeding centre linked to strong national and private institutions would be the ideal approach, but one that might be prohibitively difficult to fund in the long term, and be less cost-effective than co-ordinated activities in the existing and prospective institutions. As it is, lack of long-term funding is hampering breeding activities. The conclusion was that any regional collaborative activities should be based on existing centres.

The mechanism for the co-ordination of activities was agreed as a Regional Coordinating Group with one representative from each of the contributing institutions. This group will meet at least once a year and will plan/manage activities, budget them and seek external funds.

A second Asia/Pacific regional cocoa breeding meeting is planned for July 2005, immediately after the Malaysian Cocoa Conference in Kuala Lumpur. In addition to cocoa breeders from Indonesia, Malaysia, Papua New Guinea and Vietnam, a breeder from the Philippines has been also invited to attend, and a cocoa scientist from India, who has expressed interest, might participate. The meeting will define a regional activity that would initiate the common efforts to identify some planting materials that will be high-yielding in the presence of CPB/PPR/VSD. Region-wide testing of the best regional clones and exchange of relevant seedling populations to be tested in all regional centres are two possible activities to be considered at the meeting. Related to this will be defining the best legal and practical ways for exchanging planting material among the countries in the region. Activities on screening for resistance to CPB will be reviewed, and their continuation coordinated during the meeting.

Any regional activities will start in the frame of the existing cocoa breeding activities in participating national institutions, due to lack of external funds. However, a plan for a larger regional breeding project could be instigated with just some of the activities started in the framework of current funding of national institutions. For the rest of the activities, some external funding would have to be procured.

[1] Two pod boring moths, *Crytophlebia encarpa* and *Olethreutes* sp. that are referred to as pod borer in PNG are minor pests.

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Global Network on Cacao Genetic Resources Conservation for Use

Concept note prepared by IPGRI in consultation with CIRAD, INGENIC and USDA, and presented at the World Cocoa Foundation meeting held in Brussels on May 4, 2005 (1)

Summary

The creation of a network is proposed to optimise the conservation and utilisation of cacao genetic resources (GR) worldwide for the benefit of breeders, researchers and farmers. Such a network, identified for present purposes as 'CacaoNet' (2), would bring together national and international players in both public and private sectors, in order to carry out shared conservation and utilisation activities such as targeting collection actions, maintaining key field collections, carrying out joint characterisation and identity studies, evaluating accessions for important traits, managing shared databases, promoting the exchange of germplasm and information, enhancing germplasm for specific traits, distributing improved populations and carrying out collaborative research on topics related to more effective conservation and use of cacao GR. Proposals are offered for further discussion on the *modus operandi* and likely activities of such a network.

Background and Justification

The future of the world's cocoa economy depends on the preservation and use of a broad genetic base to breed for disease and pest resistance, for quality and for adaptation to local environments. Effective long-term conservation, evaluation and utilisation of cacao genetic resources (GR) should therefore be accorded specific attention.

Cacao GR are conserved in a number of national genebanks and in two international genebanks: one in Trinidad (ICG,T), managed by the CRU, and one in Costa Rica, managed by CATIE. Safe movement of germplasm at the global level, including virus indexing, is achieved through the intermediate quarantine facility at the University of Reading, UK. The USDA/ARS facility in Miami, USA, offers quarantine facilities for regional transfer. Molecular characterisation is currently carried out at a number of institutions (CIRAD, CRU, IITA, University of Reading, USDA). Phenotypic data on cacao GR are stored in the International Cocoa Germplasm Database (ICGD), which is maintained at the University of Reading. Recently, molecular data have been included in the combined CocoaGenDB database, which was created and is maintained at CIRAD, France. Additional activities contributing to the conservation of cacao GR are occurring on an individual institute basis around the world.

Cacao, a so-called 'orphan-crop', has not benefited from an internationally coordinated approach to conservation and improvement of its GR. The conservation of cacao GR has been carried out with insufficient and unstable funding. This situation has made optimal management of cacao GR impossible, has allowed genetic erosion to continue unchecked, and has limited the linkages between genebanks and potential end-users. National institutions (such as those in Brazil, Ecuador, and Peru) maintain with difficulty valuable unique collections for which they do not receive any, or very little, international support. Furthermore, potentially high-return activities, such as collecting new cacao GR and research on more efficient methods of transfer of GR, have not been carried out.

The area of genetic identification of germplasm accessions requires a globally coordinated and prioritised approach. The existing international databases need more support to become fully effective and possibly more user-friendly. Currently, information exchange on cacao GR relies largely on the International Group for the Genetic Improvement of Cocoa (INGENIC), which is an *ad-hoc* group created and maintained by the initiative of individual scientists and without stable funding.

International collaboration on the evaluation and improvement of cacao GR has been stimulated by the implementation of the CFC/ICCO/IPGRI projects, initiated in 1998. The multi-sector collaboration in these projects demonstrate how shared priorities can help to set the agenda at a national and international level, aiming at more efficient use of GR to achieve common goals. However, the informal network created by the CFC/ICCO/IPGRI projects is limited in scope and in time. No support is provided for conservation and characterisation of cacao GR, and collaborative evaluation and selection activities will come to an end upon completion in 2008.

Discussions on the needs for stable funding of cacao GR have been held on numerous occasions. Two years ago, INGENIC identified this problem for priority action in the context of the Global Coordination Group on Sustainable Cocoa Economy. The subject was presented and discussed to some extent at the WCF meeting in Washington in October 2004. IPGRI has also initiated discussions on the need for stronger networking in cacao GR conservation as part of its recently created "Commodity for Livelihoods" Programme.

The present initiative proposes to establish a stable global framework to increase the efficiency and

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coherence of cacao GR conservation and utilisation efforts. Until an appropriate name is identified, such a framework or network might be tentatively called 'CacaoNet'.

Goal and Objectives

The overall goal of CacaoNet is to optimise the conservation and utilisation of cacao genetic resources worldwide for the benefit of breeders, researchers and farmers.

The objectives are:

- To ensure cost-effective long-term conservation and management of cacao GR in the global public domain;
- To enhance the value of cacao GR for breeding, through effective characterisation, evaluation and pre-breeding efforts;
- To provide a platform for the coordination and implementation of priority research related to cacao GR (e.g. virus indexing, improved methods for germplasm exchange, genetic identity studies, studies on diversity in farmers' fields, etc.);
- To prioritise and implement collecting missions to ensure conservation and access to poorly-known gene pools, especially where the natural habitat is threatened;
- To develop priorities for, and coordinate regional approaches to cacao GR conservation, exchange and utilisation;
- To ensure effective management and exchange of information on cacao GR;
- To promote capacity building on GR conservation and utilisation for national breeding programmes;
- To facilitate access by breeding programmes to useful germplasm;
- To promote access to and the adoption of superior cultivars by farmers, including participatory selection approaches, and ensuring maintenance of genetic diversity at farm level; and
- To promote the use of genetic diversity in cacao-based cropping systems to improve the livelihoods of farmers.

Potential Approaches for Establishing and Functioning of CacaoNet

A consultation process with stakeholders and potential partners on the feasibility and functioning of a network for cacao GR will be carried out. Until this process is completed and a more precise plan of action is agreed, it is envisaged that:

- CacaoNet will be a private-public sector partnership to ensure the effective implementation of global and regional objectives for cacao GR conservation and utilisation;
- CacaoNet will build on existing collaborative efforts on cacao GR conservation and utilisation;
- CacaoNet will operate through a Global Coordination Unit (possibly as part of the Commodity for Livelihoods programme of IPGRI), requiring a full-time coordinator and programme assistant;
- CacaoNet will also establish an Information Service, responsible for database update and management, production of newsletters and other publications;
- Representatives of CacaoNet will meet regularly to discuss progress made and agree on global priorities for cacao GR conservation, evaluation, research and exchange;
- CacaoNet will establish regional coordination mechanisms, including regular meetings, for the planning and implementation of collaborative activities on cacao GR at a regional level;
- The implementing partners of CacaoNet will be responsible for carrying out collectively identified priority activities;
- Funding of the long-term CacaoNet activities (Coordination Unit, Information Service, collaborative conservation and utilisation efforts) will be sought through the establishment of long-term commitments from public and private donor/supporting partner institutions;
- Additional funding of specific short and medium-term priority activities will be sought where required;
- Collectively identified priority activities or projects carried out within the framework of CacaoNet will be supported through in-kind or direct contributions from implementing and supporting partner institutions.

Potential Partners of CacaoNet

- Implementing partner institutions of CacaoNet are national, regional and international cacao research and development institutions (NARS in cacao producing countries in America, Africa and Asia, CATIE, CIRAD, CRU, IPGRI, IITA, University of Reading, USDA, etc.)

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- Donor/supporting partner institutions of CacaoNet might include those already directly involved in funding of cacao GR, such as BCCCA, Masterfoods, USDA and WCF. Potential new donors will be actively identified.

Resources Required for the Operation of CacaoNet

CacaoNet will promote more efficient and better-targeted use of existing resources. Additional resources required for the operation of CacaoNet will depend on the type of activities that can be agreed upon.

(1) For further information or comments on the development of the proposal please contact Charlotte Lusty, Commodities for Livelihoods Programme, IPGRI (c.lusty@cgiar.org).

(2) The name of the network, CacaoNet, is very much preliminary and open to discussion.

A Farewell to INGENIC Members

Yoel Efron

Please be informed that by January 31st, 2005 after 11 years of intensive practical cocoa breeding research with the Cocoa and Coconut Institute (CCI) of Papua New Guinea (PNG), I will no longer be actively involved with cocoa breeding. However, my heart and interest will always remain with this fascinating tree.

The establishment of INGENIC, its Newsletter and the Workshops organised in conjunction with the International Cocoa Conference were significant and very important milestones in the history of cocoa genetics and breeding research. INGENIC provides an excellent opportunity to overcome the local isolation and to bring international perspectives into one's work. It is a forum to interact, exchange ideas and personally meet with other scientists, people from the industry and donors. Moreover, INGENIC is a dynamic Network that takes initiatives. The two CFC/ICCO/IPGRI projects could not have materialised without the involvement and efforts of INGENIC. I believe that we should all be proud of our network, and be more actively involved with it. My wish is that it would further expand with its role, resources and activities. At least, it should be maintained at the same level of operation as it is now.

I am retiring from my work in PNG with a sense of accomplishment. During the last 11 years, the cocoa breeding team has developed a balanced comprehensive breeding programme with short and long-term objectives. We were able to achieve the ultimate goal of any breeder by releasing two modified versions of the SG2 hybrid and, a first in PNG, two poly-clonal varieties, HC1-B and HC1-S, which are now being grown commercially. New promising hybrids and clones were also identified. They may be released to growers in 2-3 years time. In a way, I am also glad that through our practical cocoa breeding research we were able to generate valuable information of general interest to cocoa breeders, which was presented in the INGENIC Newsletter or the INGENIC Workshops.

Cocoa is really a fascinating tree with a tremendous amount of existing genetic variability to be explored by breeders in order to achieve further progress in cocoa breeding. I wish you all productive exploration in the years to come.

My address will be as follows:

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Obituaries

Kolawole Badaru (Kola)
April 24, 1945 to October 21, 2004

by Bertus Eskes and Peter Aikpokpodion

It was only one year ago when Kola helped to write an obituary for Hille Toxopeus. Kola has been at the forefront of the "renaissance" of cocoa breeding in Nigeria at the end of the 1990's, of which Toxopeus had been one of the founders in the 1960's. Kola had just retired from the Cocoa Research Institute of Nigeria (CRIN), and was busy creating an NGO (Agroserve International), concerned with the well-being of the Nigerian cocoa farmers. We were not aware of the extent of advancement of the illness that was then already affecting Kola's health. His optimistic nature and forceful, spiritually-based willingness to look forward would not allow him to show any doubt about the future.

Born in the ancient town of Abeokuta, the capital of Ogun State in 1945, he attended the popular Abeokuta Grammar School from where he graduated in 1966. He obtained his university degree in General Agriculture in 1975 and an M.Phil in Plant Science (Plant Breeding) in 1980 from the University of Ife (O.A.U), Ile-Ife. He received post-graduate training in Plant Breeding Research Methods, Genetic Resources Management and Technology Transfer. He was a beneficiary of several scholarship awards including the IITA Scholarship Award (1973), Western Nigeria Marketing Board Scholarship Award (1974) and Ford Foundation Scholarship (1976).

Kola was involved with breeding of the cola tree at CRIN for a long time. He became the main cocoa breeder of CRIN by 1995, after his fellow cocoa breeder, Dr. Simeon Akinwale, passed away in a road accident. His first participation in an INGENIC workshop was in 1996, where he made a presentation on the current state of cocoa breeding in Nigeria. From then onwards, he would never fail to attend any event organised by INGENIC. All of us will remember him for his blunt and humorous manner of speech and warm personality! He considered himself an intimate part of the big INGENIC family, and he was one of the driving forces behind the initiatives taken by INGENIC over the last ten years.

Thanks largely to his enthusiasm and decisive attitude, he was able to attract external funding, for example through the CFC/ICCO/IPGRI Project that allowed him to pursue his priority activities. He was a good manager, and able to achieve results even under difficult conditions. Thanks to his honest and straightforward nature, he was fully trusted by everyone. As a cocoa breeder, Kola was primarily deeply interested in recovering the diversity of West-African Amelonado. The relatively small tree size and low incidence of black pod in this population impressed him. However, the disadvantages of the Amelonado type also became clear to him when the material collected in different farmers' fields failed to establish well at the CRIN station. He then channelled his energy towards recuperationg the gains made in the three successive CRIN breeding programmes. This included the re-selection and further use in breeding of the accessions already selected in the 1960's for black pod resistance and high-yielding capacity. He then proceeded to resume the recurrent selection approach based on selection of interesting trees within NA x NA and PA x PA crosses, which had been introduced into Nigeria by Toxopeus at the end of the 1960's. The CFC/ICCO/IPGRI project team, led by him, was able to establish the International Clone Trial, local hybrid trials, and local clone trials and observation plots. He carried out innovative studies to overcome one of the major constraints in the programme, which was the low success of vegetative multiplication. Before retiring, he prepared for the future of the breeding programme by adding the participatory component to the selection process. He has personally been visiting hundreds of smallholder cocoa farmers, for whom he nurtured deep respect. When he retired, he left a very active programme in the hands of his successor.

Life is fragile, especially in Nigeria. CRIN has been hindered in its development by the premature death of leaders and skilful staff. It requires the energy and forcefulness of people like Kola to build a better future by focussing primarily on the opportunities, rather than contemplating the constraints that often seem insurmountable. Kola was a marvellous person with a great personality, who will continue to be our exemplar and whom we will miss a lot! Kola left behind his beloved wife, Adeola whom he cherished dearly, and three children, Kasopef'Oluwa, Anjola-Oluwa and Tanmole-Oluwa. To them we offer our heartfelt sympathy.

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Professor A.F. Posnette CBE, DSc, FRS

January, 1914 to July 17, 2004

Rob Lockwood

Peter was posted to the Botanic Garden at Aburi in the Gold Coast in 1937 as specialist botanist with instructions to "breed for resistance to whatever was killing a great many of the trees". His greatness was to achieve so much when so young in an age when little was known about plant virus diseases, certainly nothing about such diseases in trees and very little was known about the genetics or even reproductive biology of cocoa.

Peter was borne in January, 1914 in Birmingham and died on 17 July 2004. He was educated at Cheltenham Grammar School and Christ's College Cambridge, where he intended to become a zoologist. His membership of the University air squadron was to have far-reaching consequences. He had an interest in genetics and accepted a Colonial Agricultural Studentship that gave him a year each in the Cambridge University School of Agriculture and the Imperial College of Tropical Agriculture in Trinidad. In Trinidad, he wrote two dissertations, one of which was on the pollination of cocoa. He came under the influence of Cheesman and Pound.

During his twelve years in what is now Ghana, Peter laid the foundations of cocoa research in West Africa - including literally of the Central Cocoa Research Building at Tafo. Means were modest: when he wrote his first official letter requesting some simple equipment as an "urgent necessity" to the Director of Agriculture in Accra, he received a reply stating that "in this country nothing is urgent and very little is necessary". Against this background, Peter undertook a large-scale on-farm selection programme more than half a century before this became fashionable, he planted clone and progeny trials and he elucidated the pollination of cocoa. He recognised the limitations of the genetic base long before this too became fashionable and made a huge introduction of seedling germplasm from Trinidad - despite the submarine war in the Atlantic. It is the basis of cocoa variety production throughout West Africa and to quite an extent Malaysia and Indonesia too. Former colleagues from the University air squadron flew the seed pods from Port-of-Spain to Takoradi. Typically, Peter didn't waste time while in Trinidad where he worked on the incompatibility of Upper Amazon cocoa - the legitimate T types were the successful crosses. He identified swollen-shoot disease for what it was, characterised the virus, undertook pioneering work on resistance and cross protection and devised field control measures based on cutting out and replanting infected trees - that led to riots. Some have said that Peter's work on swollen shoot saved the Ghana cocoa industry. Peter's output was remarkable. Even more remarkably, it has stood the test of sixty years and more.

In 1949, Peter was seconded from the Gold Coast to East Malling Research Station so that he could develop a fruit tree virus research programme. His PhD. thesis was based on this work, not on cocoa, but later the cocoa work was presented for his doctorate. Peter remained at East Malling for the rest of his career, becoming Director in 1972 until his retirement in 1979. Cocoa was always close to his heart and he maintained a keen interest in the crop until he was well into his eighties.

And Peter the professional? I first met him in 1966 when I was an undergraduate at Wye College. Peter gave a lecture on fruit tree viruses. His waistcoat made as much of an impression on me as his lecture: little did I know of how he was to influence my life. A year later, he interviewed me for a scholarship and shortly after I was at Wye College doing an MSc. in applied plant sciences. John Purseglove, who was running the course module on the ecology of tropical crops, asked East Malling colleagues to lecture on crops where they had first hand knowledge, so Peter covered cocoa. I saw more of Peter as I was doing the virus module too. When the course finished, I was attached to the East Malling Fruit Breeding section while waiting for an overseas posting. One day at lunch Peter asked me if I would like to go to CRIG to breed cocoa for virus resistance. I got to know him even better as he was one of three advisors to the project.

What sort of man was Peter? His wife and family provide part of the answer. Bunny was a larger than life American lady, with an outsized heart of gold. Legend has it that when she arrived in the Gold Coast as the new wife before Peter was senior enough to have his wife in country, she challenged the authorities to deport an American citizen. They had three children - two girls and a boy. The Posnette home was a wonderfully warm and stimulating place with remarkable hospitality. Any cocoa person was most welcome to visit. Peter was generous to a fault.

Part of Peter's success was due to his intellect, his hard work, a phenomenal visual memory, a great ability to improvise, his inspiration to his staff, his insights and his self-criticism. He was a brilliant teacher and collaborator, provided that you had done your homework. If he discovered that you hadn't, he wasn't interested, because he assumed the same standards of others as he set himself. His attitude was that if something was going to take time you had better get on with it. When writing, his motto was "when in doubt, cut

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it out", leading to an economy with words. People spoke of Peter's luck: not a bit of it, when he saw an opportunity he ran with it, and some of his insights were inspired. Of his many honours, he was proudest of his FRS because that was awarded by his peers. These are some of the reasons why his work was so extensive, so influential and has proved to be so durable.

The other part of Peter's success was due to Bunny. We can only guess at the challenges of the living conditions including malaria and other health problems during those twelve years, combined with the shortages and uncertainties of war. Remarkably, their two daughters were borne during this period. Peter always said that his achievement was as much Bunnie's as his, for she was his strongest supporter and sternest and most constructive critic.

In the last *INGENIC* Newsletter, I speculated on how cocoa breeding might have developed in Malaysia had Eric Rosenquist not moved to oil palm. Even more so, how far might Peter have taken cocoa breeding in Ghana if swollen shoot had not of necessity dominated his work? This great man was and will remain an inspiration to everyone involved in cocoa research and cultivation.

• • • • • • • • • • • • • • • • • •

Maro Ran-ir Söndahl

July 29, 1943 - January 10, 2005

Antonio Figueira

I regret to inform the INGENIC Newsletter readership that Dr. Maro Söndahl died early this year in a tragic car accident in Brazil. Maro was not a frequent attendant of cocoa meetings, and probably most of our cocoa research community did not know him well, except for the biotechnologists. Maro was more popular with the coffee community since he dedicated 35 years of his life to this crop, working in many aspects of physiology, breeding and biotechnology.

Maro is widely recognised for his great contributions and pioneering work on tissue culture of various tropical crops. He developed the first protocol for somatic embryogenesis of coffee in the late 1970s, followed by other great achievements in maize, oil palm and roses during the 1980s. He was also a pioneer in cocoa tissue culture. In the 1980s, his team at the DNA Plant Technology Corp. (Cinnaminson, NJ, USA), with support from a chocolate manufacturer, developed the first protocols to obtain cocoa somatic embryos from sporophytic tissues (nucellus and floral parts). Before that, somatic embryos had only been obtained from immature zygotic embryos, with obvious limitations for propagation and genetic transformation. He was granted a US patent for somatic embryogenesis and plant regeneration of cocoa in 1994. His protocol opened the possibility for further developments. In fact, improved somatic embryogenesis protocols were published in 1993 by Nestlé and CIRAD, culminating with the advances developed in the Penn State group in 1998, all derived from Maro's pioneering work.

I first met Dr. Maro Söndahl in 1982 in Rio de Janeiro, Brazil during my last year in college, when he gave a talk about the use of tissue culture in plant breeding. His seminar definitively helped to direct my career to biotechnology. Maro had attended the same school (Brazilian Federal Rural University of Rio de Janeiro), graduating 15 years earlier (1968). He got his Masters degree in 1972 at the Center for Nuclear Energy in Agriculture of the University of São Paulo, where I currently work. His PhD. degree in Cell Biology was from the Developmental Biology Program of Ohio State University (1978).

He started his successful scientific career in 1970 as a researcher at the Agronomic Institute of Campinas, a state owned research center of São Paulo, where he worked mainly with coffee physiology. After concluding his PhD. in the US, he returned to the same Institute, where he became the chairman of the Department of Plant Genetics, and later he was indicated to be the Director of the Biology Division. In 1983, he moved to the US, joining DNA Plant Technology Corporation in Cinnaminson (NJ), as research manager, supervising work with somaclonal variation (coffee, popcorn), protocol development for somatic embryogenesis (cocoa), anther culture (rice, sweet corn) and breeding (sweet corn, popcorn). He became Senior Research Director in 1987 with technical and business responsibilities in cell genetics and breeding on the following crops: coffee, cocoa, oil palm, pineapple, banana, corn, sweet corn, popcorn, rice, oats, watermelon and rose. He later became Director of New Business and Product Development of DNA Plant Technology. In 1993, he started his own company, Fitolink Corp. More recently (1997), he started a new company in Brazil, Bionova (www.bionova-mudas.com.br), working with commercial micropropagation of sugarcane, banana, and pineapple. The tragic accident occurred during a business trip to establish new contracts to provide micropropagated plants to growers in Mossoró. Maro will be remembered for his great contribution to biotechnology of tropical crops and to plant sciences in general.

During his career, Maro was very successful in combining science, publishing important breakthrough articles, with a business-oriented entrepreneur perspective. Maro had a great sense of humor, and was an entertaining person to have around during

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meetings. He was born in Brazil, but his family was originally from Iceland. He was an Honorary Consul for Iceland in Curitiba, Brazil since 2000. He was survived by his wife Dr. Clemencia Noriega, who continues to run their business in Brazil, and three children.

BOOK RELEASE

From CABI Publishing: The Genetic Diversity of Cacao and its Utilization,
Author: B.G.D. Bartley, Consultant, Portugal. April 2005. 368 pages.
Special Discount Price: £60.00 / US$112.00 (Normal Price: £75.00 /US$140.00)

This book provides a comprehensive review of current knowledge of the diversity of the cacao (*Theobroma cacao*) plant. It starts by examining the diversity and inheritance of the characteristics of primitive populations in the Amazonian and Caribbean regions. It then looks at the evolution of diversity within cultivated populations first in South America and around the Caribbean, and then beyond the Americas. The book describes the inter-relationships between populations based on morphological and molecular markers. It also examines the conservation of genetic resources and how these genetic resources can be utilized to produce new cultivars. To obtain a discount simply quote reference JCX20 when placing an order by phone, fax, email or via our online bookshop: www.cabi-publishing.org/bookshop <http://www.cabi-publishing.org/bookshop.

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Melhoramento Genético do Cacaueiro
by Dias L.A.S. (Ed.).
Translation by Abreu-Reichart C.E., Viçosa, M.G., Brazil; aided by the Editor, and FAO
EcoPort version by Peter Griffee, FAO.

The Brazilian book 'Melhoramento Genético do Cacaueiro' by Luiz Antônio dos Santos Dias, 2001 (xii + 578 pp.) has been translated into an English Internet version in EcoPort (http://www.ecoport.org) as 'Genetic Improvement of Cacao'. The translation is by Cornelia Elisabeth Abreu-Reichart, Viçosa, M.G., Brazil; aided by the Editor and Peter Griffee, Senior Officer for Industrial Crops, of FAO's Crop and Grassland Service (AGPC) and supported by FAO. Please note, that it is not available as a hard copy, as was in inferred by many, who learnt of it under **Book Release** in INGENIC Newsletter 9.

For further information, please contact Luiz (lasdias@ufv.br) and Peter (peter.griffee@fao.org).

For any comments on the EcoPort technology involved please contact Tonie Putter (Supervisor@ecoport.org), the creator of EcoPort. The following URL takes you to the cover - just click on "Table of Contents" and choose the section of interest or use the yellow scroll arrow. Each section is a maximum of 32 KB to facilitate those with slow connections. N.B.: Care with "Get full eArticle" as it is over 1.4 MB. At the end of the Table of Contents, the Entities (plant, arthropod, fungus, location etc.), References and Glossary terms are listed as well as being linked in the text.

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Printed by: The Multimedia Production Centre, School of Education,
The University of the West Indies,
St. Augustine, Republic of Trinidad & Tobago

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