INGENIC Newsletter

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IN THIS ISSUE

From the Editor's Desk:

In this issue of the INGENIC Newsletter, articles on the use of QTL's for mapping resistance and traits of agronomic interest are featured. This coincides with the theme of the Third INGENIC Workshop on *New Technologies and Cocoa Breeding* to be held in Kota Kinabulu, Malaysia, October 16-17, 2000. Please refer to page 23 for the Workshop announcement.

Recent initiatives at CRIG and CCRI are also included in this issue. There has been much discussion on the lack of information about the nomenclature of cacao. The account on the nomenclature of accessions collected in Ecuador by Dr. F. J. Pound is thus timely.

The INGENIC Committee looks forward to enthusiastic participation in the upcoming Workshop. The change in format is to allow increased dialogue to better address pertinent issues. The increasing cooperation among cocoa researchers, institutes and related organisations can only be strengthened by such approaches.

We look forward to continued collaboration among researchers working towards genetic improvement in cacao. Concise articles on relevant topics are welcome. Submissions should be made by October 31st of each year.

Best wishes to the readership and we anticipate a successful 13th International Cocoa Research Conference and INGENIC Workshop. Details of these upcoming events are provided on page 23.

Frances Bekele

• *Invitation* •

YOU ARE

INVITED

TO ATTEND THE

THIRD INGENIC WORKSHOP

(See details inside)

International Cocoa Breeding and Selection Initiatives*

B. Eskes

Scope

Good planting material is an essential feature of sustainable cacao cultivation. However, evaluation of cacao collections for such material is still incomplete and the potential of available genetic resources is still largely under-utilised. Only about 30% of the acreage under cacao is planted with selected varieties that show improved yield capacity. Most planting material is highly susceptible to diseases and pests though significant variation for resistance has been found in collections and breeding trials. Selection activities have suffered from the long period of low cocoa prices and some programmes have been discontinued.

International collaboration in the past has played an important role in breeding and selection of new cacao varieties. The CFC/ICCO/IPGRI project is currently reinforcing certain activities but more support will be required to ensure effective utilisation of cacao genetic resources. Regional selection programmes would be possible in the light of the similarity in production constraints within America, Africa and Asia. Interaction between different disciplines and with farmers is fundamental for obtaining results adapted to the needs of the producers and consumers.

During the last two years, discussions about '*Sustainable Cocoa Production*' have been intensified and meetings continue to be organised to develop plans of action. In these discussions, problems related to planting material have also been examined, especially the need to obtain and distribute better varieties with resistance to prevailing diseases and pests to cocoa producers. Use of selected varieties has been restricted in most producing countries for several reasons, including inefficient or non-existent systems for production and distribution of these varieties. Genetic erosion and inbreeding of existing planting materials have occurred in many countries by reproduction of hybrid varieties through open-pollination in seed gardens and by the way farmers reproduce their preferred trees. The challenges are to improve disease and pest resistance, yielding capacity and adaptation to a changing environment (degraded land, decreased rainfall, variable shade level, and associated tree crops).

The present note is extracted from a presentation given by the author during the USDA-CABI-ACRI *Collaborative Cocoa-Coffee Research Meeting*, held at ICCO, London, on 7th and 8th December 1999. It does not include the areas of germplasm collection, management, characterisation, evaluation and biotechnologies, which were covered by other speakers at the same meeting.

Past Collaborative Activities in Cocoa Breeding

International collaboration in cocoa breeding and selection probably began in the 1930's with the creation of a cacao selection and breeding programme at the University of the West Indies, in Trinidad (Eskes and Lanaud, 1997). This programme led firstly to the selection of the well-known 'ICS' clones and afterwards to the innovation of cocoa breeding methods in the 1950's, based on exploitation of hybrid vigour in crosses between genetically distant genotypes. Subsequently, hybrid cacao varieties were generated and selected in most national breeding programmes during the 1960's to 1980's. The continuation of the local selection programme in Trinidad resulted in the selection of the interesting 'TSH' clones. In Costa Rica, the 'Instituto Interamericano de Cooperación para la Agricultura' (IICA) and later CATIE carried out genetic studies from the 1970's to the 1990's and selected cacao varieties that were regionally distributed.

Amazon materials, collected in Peru during the 1930's by a team from Trinidad, were sent to West Africa in the 1940's. The so-called 'F3 Amazon' populations, with increased tolerance to the Cocoa Swollen Shoot virus (CSSV), were widely distributed by the West African Cocoa Research Institute (WACRI) in the 1950's and 1960's. In many Francophone West-African countries, the 'Institut Français du Café et du Cacao' (IFCC, now integrated within CIRAD – the Centre de Coopération Internationale en Recherche Agronomique pour le Développement) collaborated with the national research institutes in producing improved hybrid varieties and in carrying out genetic studies between 1955 and the 1990's. In Malaysia, the cocoa breeding programme of the Commonwealth Development Corporation, carried out at Bal Plantations in the 1980's and early 1990's, resulted in new hybrid selections and improved genetic knowledge (Lockwood and Pang, 1995).

International collaboration has also been important in developing early screening methods for disease resistance. The University of Florida has developed the 'belt spay' inoculation method for measuring resistance of seedling progenies to Witches' Broom disease. ACRI co-financed this research and provided the transfer of this method to Ecuador and Brazil. Two international research centres, CIRAD in France and CRU in Trinidad, have been involved in the recent development of a leaf inoculation method for early screening of resistance to *Phytophthora* spp. The development of these breeding tools should accelerate evaluation and selection procedures, an important aspect in breeding of the perennial cacao plant.

International collaboration in cocoa breeding was interrupted or slowed down sharply in the 1990's. Most cocoa producing countries have also been unable to maintain effective long-term breeding activities during the last decade with depressed cocoa prices. In addition, the number of cocoa breeders has decreased and many local breeders are operating under isolated conditions. These factors were the impetus for the creation of INGENIC, in 1993.

Pre-registration for the

Third INGENIC Workshop

on

New Technologies and Cocoa Breeding

to be filled out and faxed before

31 July, 2000

to:

Michelle End
Reading University

*Fax: **44.1256851082***

PRE-REGISTRATION FORM

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The CFC/ICCO/IPGRI project on 'Cocoa Germplasm Utilization and Conservation'

The CFC/ICCO/IPGRI project on *Cocoa Germplasm Utilization and Conservation* represents an internationally co-ordinated effort in the selection, evaluation and breeding of improved cacao genotypes aiming at sustainable cocoa production at lower cost (Eskes *et al.*, 1999). Selection for resistance to Black Pod and Witches' Broom diseases is a priority objective. Activities over the five-year duration of the project (1998-2002) include the establishment of international and local clone trials, hybrid trials and population breeding activities in 10, five and four cocoa producing countries, respectively. Standardised methods are applied to assess resistance to diseases and pests, yield and quality of the 'International Clones' in different environments. The project is promoting links between the International Cocoa Genebank, Trinidad (ICG,T), maintained by the Cocoa Research Unit (CRU) in Trinidad, and cocoa producing countries. The diversity of the Trinidad collection is being evaluated, especially for resistance to Black Pod and Witches' Broom diseases, and used to obtain improved populations. Interesting genotypes will be exchanged between participating countries.

The project executing agency is the International Plant Genetic Resources Institute (IPGRI) and co-ordination is ensured in collaboration with CIRAD. The International Cocoa Organisation (ICCO) is the Supervisory Body. National research institutes in Brazil, Cameroon, Côte d'Ivoire, Ecuador, Ghana, Malaysia, Nigeria, Papua New Guinea, Trinidad and Venezuela as well as the CRU, CIRAD and Reading University participate in the activities. The Common Fund for Commodities (CFC) is the main donor, supporting 30% of the project costs. Chocolate manufacturers from the UK (BCCCA) and from the USA (ACRI), CIRAD and IPGRI contribute 20%, and the national research institutes 50% of the project costs. IPGRI has created a Co-ordination Unit at its banana programme (INIBAP) in Montpellier, France.

Although the CFC/ICCO/IPGRI project has a wide approach, the following areas of activities are not covered:

- The project is only supporting the application of existing technologies in cocoa breeding.
- For resistance breeding, main attention goes to Black Pod and less to Witches' Broom resistance. Genetic improvement of resistance to other major diseases and pests is not covered (e.g. Moniliasis, VSD and insects).
- No multilocational or on-farm testing of new varieties is carried out (only single-site on-station variety trials).
- No interactions exist with the deficient or inoperative systems for multiplication and distribution of cacao varieties.
- Little institutional support is provided to the national research institutes.

Some Opportunities for New Collaborative Actions

In suggesting some opportunities for further collaborative initiatives, it is realised that important deficiencies occur all along the chain of activities that are required for effective use of cacao germplasm. As indicated earlier, the needs in the areas of germplasm collection and management, characterisation and evaluation, and development of biotechnologies will not be mentioned here because these will be covered in other papers presented at this meeting.

Some of the areas which require special attention would appear to be the following:

- Development or improvement of early screening methods, needed for rapid progress in accumulation of resistance to diseases and pests (e.g. Moniliasis and capsids).
- Increased regional collaboration in the selection of more productive and resistant varieties, which is justified by the regional similarities in production constraints.
- Increased emphasis on selection of new clones, which is the quickest way to obtain genetic progress.
- Direct involvement of farmers in evaluation, selection and possibly also multiplication of new varieties.
- Re-enforcement of multiplication and distribution systems of new planting materials to the farmers.

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The Nomenclature of the Accessions Derived from Dr. F.J. Pound's Collections in Ecuador in 1937

B. Bartley

The number of accessions represented by the progenies of trees selected by Dr. Pound in Ecuador is significantly large, but their use in breeding has been somewhat hampered by the absence of detailed knowledge regarding the origins and relationships of the individual progenies. Until recently, no information was available about the names given to these progenies. The ambiguities concerning what appeared to be a duplicate alphanumeric system of nomenclature and the absence of information about the significance of the names resulted in an inadequate understanding of the relationships among the progenies.

Dr. Pound's report of the collecting visit to Ecuador indicates that several farms (haciendas) were involved in the material sent to Trinidad and that the names of some of them were related to the alphabetic component of the identifiers. However, this information was not available for the majority of these identifiers.

On a visit to Ecuador in 1967, I took the opportunity to ask Miguel Aspiazu to clear up this matter. The list that he gave me forms the basis of the definitions of these names in the draft *Catalogue of the ICTA/UWI germplasm collection* prepared by me in 1970. This information provided the following definitions of the hacienda names related to the alphabetic codes. In addition, there are a few that Sr. Aspiazu did not remember.

AM	=	Amalia
B	=	Bolivar
CL	=	Clementina
CLM	=	Clementina
JA	=	Javilla
MOQ	=	Moquique
LV	=	Limoncillo
LZ	=	"
LZ	=	"
SJ	=	San Juan (altered erroneously to San Javier)
SL	=	Santa Lucia

The other problem was that the numerical part appeared in two forms; for example, 1.12 and 112. Recently, the first report by Dr. Pound on the establishment of this material at Marper Farm in Trinidad has become available. This clears up the doubts about the nomenclature. The best way to explain the correct situation appears to be to reproduce the data given in the report, which gives the numbers of progenies "observed" (not necessarily all that were planted), as follows:

Table 1: Information on collections made by Pound in Ecuador in 1937

HACIENDA	TREE NUMBER	NUMBER OF PROGENIES
Clementina	9	36
	10	27
	13	36
	15	8
	19	43
	22	2
	27	47
	78	11
	91	7
	Mixed	78
La Paz	2	14
	3	30
	4	39
	5	19
	6	23
Santa Lucia	A	51
	C	16
Javilla	1	32
	2	20
	3	31
	4	12
	5	37
	6	11
	7	12
	8	37/39
	9	26
	10	41
Larga Vuelta		29
L	X	46
L	Y	17
Moquique	1	21
	2	38
	3	21
	4	23
	5	28
	6	94
Amalia	1	69
	2	67
Balao	1+2	15
	3	10
	4	4
	5	7
	6	18
	7	20
	8	5
	9+10	22
	11	2
	12	2
	13	6
	14	10
	15	1
	16	3
	17	15
	18	7
	20	2
	21	4
	22	16
	23	10
San Juan	1	33
	2	29
San Miguel A.S.		14 24

From the above Table, a correct and complete listing of the location codes may be made as follows:

AM	=	AMALIA
A.S.	=	This is uncertain as it appears in this form in the Table. It is probably = ANTONIO SOTOMAYOR, which is a place on the left bank of the Vinces river. The Sotomayor family owned several properties in the area including "El Porvenir" from which several EET clones - some important - originated.
B	=	BALAO (this should have been obvious since the farm gets special mention in the collecting report and also has the largest number of selected trees)
CL	=	CLEMENTINA
CLM	=	CLEMENTINA MIXED
J	=	JAVILLA
L	=	LARGA VUELTA (in fact this is an error on Pound's part since the farm is called Vuelta Larga; one of the EET clones was selected there - EET 145; it is near Limoncillo, both places being on the left bank of the Quevedo river. Although Pound indicated that V = Vuelta, the V is really the tree identifier)
LP	=	LA PAZ
MOQ	=	MOQUIQUE
SJ	=	SAN JUAN
SL	=	SANTA LUCIA (the letters following this are the tree identifiers)
SM	=	SAN MIGUEL

The second component of the genotype name is that of the tree selected. In most cases, this is a number, but for unexplained reasons, the trees on two farms were identified by letters. The third component refers to the progeny as probably identified in quarantine in Barbados.

According to the notion used by Pound in the report, when referring to individual progenies the name should be in the following form: X 1.11. Where the trees were identified by letters the least confusing system would be to write the name as, for example, SL A.15, L V.10.

The ambiguities have arisen because each genotype established at Marper Farm as budded plants would have been in a duplicate planting and one tree of a pair could have lost the "." that separated the tree number from the progeny number so that we now have, for example, X 1.11 and X 111. (In many cases only one tree survived which would be identified in one of these ways). It is now necessary to use a consistent method which would agree with the original; that is the "." (or other notion; "/", "-") should be added to the names where it is missing. In the two cases where only one parent tree was involved the tree number is not relevant.

When the clones are derived from a pair of trees of a progeny identified in both forms, the fact that they are the same genotype can be verified in the field.

The use of the original system of notation is important in that it tells us which clones are the progenies of the same tree. This would help in the utilisation of the clones in breeding. Presumably, where the numbers of progenies are low, these may have been descended from a single fruit and, therefore, closely related and perhaps similar. When a tree has numbers of progenies above 35 or 40, these would have resulted from several fruits. Since they were likely to have had more than one pollen parent it is expected that the scale of variability within a given female tree would be larger, possibly in identifiable groups according to the genetic constitution of the male parents involved. For this reason, the information given in the above table is important in determining the expectations regarding the scale of variability and the traits observed in each family of progenies.

It is evident from the above Table that, in some cases, the seeds or progenies from two fruits were mixed. This occurred especially in the Balao selections. The notation of such mixed groups of progenies would cause some confusion and to avoid this it would be appropriate to identify the genotypes from them in the following form: B 1+ 2.xx and B 9+10.xx. In the same report, Pound lists a genotype as CL 15+19.4. This may have been a mistake for B since a tree #19 is missing and there is only one progeny listed for tree #15. In any case, a situation such as this was probably a matter of being unable to distinguish between 15 and 19 on the label.

Taking these points into account, it will be simple to ascribe the correct identifiers to the genotypes in most cases. The only problems that may arise concern the selections from Balao and Clementina. Thus, a genotype labelled CL 9 11 could be CL 9.11 or CL 91.1 and doubts may arise in those families where the progeny numbers are above 100. Plants labelled B 231 and B 175, for example, will be B 23.1 and B 17.5 since there will not be B 2.31 or B 1.75. The Table provides a rough guide to the numbers of progenies expected from each female tree.

The total number of progenies listed in the Table is 1543. Since the numbers of the progenies identified in each family is usually higher than the numbers in the Table, it is obvious that the numbers of seeds and progenies raised from them were much higher than those of the progenies observed. The genotypes in existence at present, which resulted from these selections, are a small fraction of those originally established. One curious fact concerns the low numbers of progenies from the Balao trees, which are more numerous and received special attention with respect to the resistance to Witches' broom disease. This may have resulted from a low reproductive capacity and/or low survival ability of the type of cacao singled out for the supposed resistance. This is an important aspect to consider in the use of these types in breeding

but the possibility may be considered that the surviving genotypes represent the more valuable range of the variability perhaps through being hybrids with other types.

Another curious aspect of the data in the Table is the absence of Clementina selections with numbers up to eight since Pound describes them as having a resistant reaction, especially Clementina selection No. 8. It is possible that these selections are included in the "Mixed" group. It has been stated that the number of trees involved was "about" 80. From the report, the survival of families from 65 trees may be surmised and perhaps another five trees could be accounted for by the gaps in the sequences of tree numbers. Thus, the "CLM" group perhaps involved as many as eight trees. Another topic of interest refers to the relation between the trees chosen by Pound and the Clementina selections referred to by Muntzing and the EET clones derived from this farm.

One of the studies that could be conducted after the determination of the genotypes that belong to each family concerns the relationship of each family to the varietal types described by Pound as being of interest for the purpose of selection.

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Methodological Aspects of QTL Detection in Cacao for Traits of Agronomic Interest

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The principle of quantitative trait loci (QTL) mapping is based on the search for a linkage imbalance between marker loci and loci controlling the quantitative traits. The first map of the cacao tree was constructed from a cross between two heterozygote genotypes, an upper Amazon Forastero (UPA402) and a Trinitario (UF676) (Lanaud *et al.*, 1995; Risterucci *et al.*, in press). The cross between heterozygous parents was then considered as a double pseudo-testcross (Grattapaglia *et al.* 1994). Other maps have been constructed to detect QTL (Crouzillat *et al*, 1996; Flament, 1998). In our study, we used crosses similar to a testcross. The purpose of this article is to describe the procedure and methods of detecting QTL of agronomic interest for cocoa breeding and to give some general results. Three progenies derived from a factorial mating design in Côte d'Ivoire were used to detect QTL for traits of agronomic interest.

QTL Detection Methods

The populations

The choice of planting material was based on a compromise between the possibility of having agronomic data over several years and the advantages from studying different genetic backgrounds. The populations were obtained from crosses between three heterozygous female parents: DR 1, IMC 78 and S 52, and the homozygous Catongo male parent. The numbers of individuals analysed for the DR 1, S 52 and IMC 78 x Catongo progenies were 111, 112 and 128, respectively.

The molecular markers

Different types of markers were used: RFLP (Restriction Fragment Length Polymorphisms) from a bank of cDNA and genomic probes, microsatellites (Simple Sequence Repeats) and AFLP (Amplification Fragment Length Polymorphisms). The RFLP and microsatellites were codominant so their two allelic bands would be visible in a heterozygous individual. They were specific and therefore made it possible to identify the 10 linkage groups corresponding to the 10 chromosomes of *Theobroma cacao* L.. The dominant AFLP (no allele reading) enable generation of a large number of markers.

Mapping

The distance between two loci is estimated by the rate of recombination between those two loci. If the two markers are on different chromosomes, they will show independent segregation. If they are on the same chromosome, their co-segregation will be greater the smaller the distance separating them. Constructing a genetic map consists in carrying out statistical tests to detect any co-segregation between two markers. The linked markers are then grouped and ordered into linkage groups. Detection of a linkage between two loci can be achieved through two statistical tests: X^2 and LOD score. Mapping software usually tests for linkage between two loci using the LOD score (logarithm of the odds ratio). JOINMAP and MAPMAKER software were used. Marker ordering in each group was done by MAPMAKER's so-called multipoint statistical analyses. The Haldane and Kosambi functions are used to convert the rate of recombination into map distances. The Kosambi function was used for the genetic map of the cacao tree.

QTL detection

Several biometric methods can be used to detect a linkage imbalance between marker loci and loci controlling the quantitative trait. The QTL location method taking markers individually is based on an analysis of variance, or Student's test. However, this robust method does not provide a good estimation of the QTL positions or their effects. The detection method based on the existence of a QTL within an interval defined by two boundary markers linked to that QTL known as 'simple interval mapping', developed by Lander and Botstein (1989), is the one most frequently used. We carried out simple interval mapping with the following software: MapQTL following on from the use of JOINMAP, MAPMAKER/QTL and QTL/ Cartographer. The simple interval mapping method did not take into account the existence of multiple QTL, distributed over the genome. Several authors have proposed methods based on regression so as to take multiple QTL effects into account with several markers. The use of a mixed model combining interval mapping and multiple regression, known as composite interval mapping, was proposed by Jansen (1993). Jiang and Zeng (1995) proposed an addition to that method for simultaneous analysis of several traits, thereby testing the hypothesis of a QTL with a pleiotropic effect as opposed to the hypothesis of linked QTL. QTL/ Cartographer was used to carry out composite interval mapping analyses and the "Jzmapqtl" option enabled simultaneous composite interval mapping analysis for several traits.

Deviation from normality

Interval mapping requires a normal distribution of analysed trait residues. Most traits with continuous variation reveal distributions that are normal or close to normality. When distribution was asymmetrical, QTL detection was carried out using the Kruskal and Wallis non-parametric test analyses proposed by MapQTL software.

Significance thresholds

For the simple interval mapping analyses, a LOD score limit was defined below which the QTL found might be false, or would correspond to a putative QTL. Work by Lander and Bostein (1989) showed that the LOD score limit was 2.4 for a genome of around 1000 cM with one marker at every 15 cM. A method proposed by Churchill and Doerge (1994), based on permutation testing by random sampling of phenotypic data, can be used to estimate a relatively conservative and robust limit from a large number of permutations (at least 1,000). An option of the QTL/Cartographer software enabled us to test permutations on the variations analysed.

Results

The polymorphic markers were sorted from 210 RFLP probes, 22 microsatellites and 21 AFLP primer pairs. The polymorphism revealed by the RFLP markers and microsatellites revealed that DR 1 was the most heterozygous with 27% RFLP loci and 45% microsatellite loci in the heterozygous state. The microsatellite markers also made it possible to check the legitimacy of the individuals within each of the progenies. Less than 5% off-types were discovered.

The genetic maps for S 52, DR 1 and IMC 78 were constructed from 146, 195 and 223 markers respectively, with a LOD score limit of 3 or 4. The 10 linkage groups were identified for each of the three progenies. The mean distance between two markers was around 5 cM. The size of the maps was between 800 and 900 cM. A map bearing one marker every 15 cM on average was constructed for each cross for QTL analysis. A composite map was constructed with JOINMAP software using only specific markers positioned on the genetic maps of the test populations and on the reference map. Determination of the significance thresholds of QTL detection by the Churchill and Doerge methods showed that the LOD score limits for a risk of a = 0.05 were between 2.2 and 2.5, corresponding to the limit proposed by Lander and Botstein.

Several QTL were found for the agronomic traits studied. A year-by-year analysis of production (9 consecutive harvesting years) revealed QTL on different linkage groups. The unstable nature of those QTL suggested QTL x environment (year) interaction. The QTL had a LOD score of between 1.8 and 3. The QTL detected for mean pod weight were highly significant, and one QTL with LOD = 11.7 explaining 40% of the variation was located on chromosome IV of the cross with IMC 78. That trait encompasses the yield components and morphological characteristics of the pod.

The QTL linked to vigour, such as trunk circumference, were usually located near the QTL associated with yield. A statistical analysis of the traits revealed also strong correlations between production and vigour. On chromosome IV of the cross with IMC 78, there was co-location of QTL linked to mean pod weight and the percentage of rotten pods due to *Phytophthora*. A study of this co-location would be particularly worthwhile since it could suggest the existence of a relationship between the disease in the field and intrinsic pod characteristics. Co-location of the same type was found in the cross with DR 1 in virtually the same region of

chromosome IV although the QTL effects in DR 1 were less marked. Other QTL were also found for the morphological traits of the bean and mean number of ovules per ovary.

Discussion

The number of individuals studied is the factor that most affects QTL detection. For cacao, as for other tree crops, it is not easy to obtain ideal type of populations for QTL detection and problems linked to the number of individuals are often mentioned. The first prospective studies in this field (Crouzillat *et al.*, 1996; Flament, 1998) used segregating populations, which were genetically of interest despite this limiting factor. The number of individuals per progeny was also insufficient in our study, but several QTL were detected for certain agronomic traits. It appears fundamental to develop appropriate trials right away, in order to have the detection power required for efficient QTL location. When studying a single population, at least 200 individuals would seem necessary. Theoretical studies on forest trees (Muranty , 1996) and applications currently under way have shown that QTL detection is more effective when using connected populations discerningly chosen from factorial or diallel type mating designs. These designs can also be used for bulk segregating analyses for a given trait such as disease resistance.

Studies conducted on other progenies have shown that there may be a relatively large number of illegitimate individuals within populations. It is therefore strongly recommended that progeny conformity be checked before undertaking any QTL study. Almost all off-type individuals can be eliminated by using 10 microsatellites. Current enrichment of microsatellite banks will enable substantial completion of maps with codominant markers. Such markers are easier to use than RFLP's and have already taken over from them in recent mapping work. AFLP primer pairs produce a large number of polymorphic fragments that enable map completion. The average distance between two markers has little effect on the power of QTL detection. It is therefore necessary to have good marker distribution, rather than a very large number of markers. QTL confidence intervals are usually around 20 cM. Lastly, QTL detection in cacao should not be limited to simple interval mapping, but should take into account the effects of the other QTL. QTL detection softwares are evolving quickly and their development should be monitored in order to ensure the use of the best biometric detection tools available.

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Mapping of Quantitative Trait Loci (QTLs) for Resistance to *Phytophthora* in *Theobroma cacao* L.

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Black Pod disease of cacao is caused by several species of *Phytophthora*. Our study includes three of those species, *viz.*, P. *palmivora*, which has a worldwide distribution, *P. megakarya*, which is only present in Africa and *P. capsici*, which is only present in Latin America. Blaha and Lotodé (1976) classified a hundred clones according to their level of resistance using an artificial inoculation test on fruits still attached to trees and found genetic resistance to *P. palmivora* to be partial. Cilas *et al* (1996) studied a factorial trial planted in Côte d'Ivoire, and showed that genetic factors involved in resistance to *P. palmivora* are additive and could imply polygenic resistance. Recently, a new method for early Black Pod resistance screening using cacao leaves was developed by Nyassé *et al.* (1995) and results from Côte d'Ivoire have shown significant correlation of this test with field resistance to P. palmivora (Kebe *et al.*, 1999). A saturated genetic map was constructed by Lanaud *et al.* (1995). The objective of this study was to use the QTL approach to better understand the genetic determinism of the resistance to *Phytophthora* and to find markers for marker assisted selection.

The two parents of the mapping population were a hybrid tree, Scavina 6 crossed with an unknown clone X, and IFC 1, an Amelonado clone. Scavina 6 (SCA 6) is known to be highly resistant to *Phytophthora* and IFC 1 susceptible. The cross was made in Côte d'Ivoire and 151 seedlings were raised in a greenhouse in Montpellier, where resistance tests and the genetic linkage analyses were carried out. Leaf disc inoculations (Nyassé *et al* 1995) were carried out using three *Phytophthora* species and two strains for each species: *P. palmivora* (517 and TRI), *P. megakarya* (NS 269 and 309) and *P. capsici* (TR I3 and MHU 76.2). Each experiment consisted of ten trays each containing two discs of each genotype and one disc was inoculated for each strain in each tray. Symptom severity was scored at five and seven days after inoculation. AFLPs were revealed with the Gibco BRL AFLP analysis system II as recommended by the supplier. The primer EcoR 1 + 2 for AFLP and one primer for microsatellites were end-labelled with 33P. AFLP markers, selected to be present in SCA 6 and to segregate in the progeny. Only clearly scorable fragments were analysed; 5 to 15 fragments in a size range of 50 to 500 bases were used by primer combination. Linkage analysis was performed using the program JOIN MAP version 1.4. The segregation of 202 markers was studied using a LOD score of 4.0 to identify the linkage groups. The Kosambi mapping function was used to convert recombination frequencies into map distances. Each segregating marker was first tested with a X^2 test for goodness of fit to the expected Mendelian segregating ratio (1:1).

A total of 202 loci were assigned to ten linkage groups that putatively correspond to the ten gametic chromosomes of *T. cacao*. These loci correspond to 191 AFLPs and 11 microsatellites. Segregation was skewed for 12 loci (or 6 % of all loci). The total length of the map was 724 cM; individual linkage groups vary between 32 cM and 97 cM. The average distance between markers was 3.6 cM. Only three gaps were larger than 20 cM, the largest gap being 32 cM. Such distribution and density make this map a useful frame for QTL identification.

The interval mapping method was used for the QTL analyses (MapQTL 3.0 software). The theoretical significance threshold for the LOD score was 2.4. A search was conducted for QTLs for each strain of *P. palmivora*, *P. megakarya* and *P. capsici*. Eight QTLs were detected, four on chromosome 5 and one on chromosomes 1, 2, 4 and 6 (Table 1). For *P. palmivora*, six QTLs appeared significant and explained 57% of the variation of the character for the TRI strain; only four explained 35% of the variation of the character for strain 517. Three of them are common to the two strains. For *P. megakarya*, two QTLs appeared as significant for strain 309 and explained 16.8% of the variation. For *P. capsici*, only one QTL for the MHU 76-2 strain was significant and explained 12.4% of the variation.

In addition, several minor LOD score peaks were notable, although they were below the threshold. The results are summarised in Table 1, indicating also the minor peaks that fall in the same region as the significant QTL. Three QTLs were significant for more than one species, taking into account the minor peaks it appears that other similar cases may exist. On the other hand, some QTLs specific for one strain in one species were also encountered.

This study revealed several genome regions involved in resistance to *Phytophthora* in cacao. In comparable studies, Leonard-Schippers *et al.* (1994) used a leaf test to estimate the resistance of potato to *P. infestans* and they found 11 QTLs involved in the resistance. Lefebvre and Palloix (1996) found 13 QTLs for resistance to *P. capsici* in pepper with a test applied to stems and roots.

The substantial percentage of variance explained here by the regions involved in the resistance and the molecular markers surrounding them offer scope for marker assisted selection. The detection of several QTLs significant for more than one species of *Phytophthora* is of particular interest for application of resistance tests in different countries. In conjunction with sound agricultural practices, it will be important to tag resistance parameters apart from leaf reaction to ensure efficient management of genetic field tolerance.

Table 1: QTLs detected in the progeny (Sca 6 x X) x IFC1 for resistance to different *Phytophthora* strains evaluated by leaf disc inoculation at five days. Low score values are indicated as well as the % of the trait explained by the QTL (between brackets).

		P. megakarya		P. palmivora		P. capsici	
Chromosome	Marker	NS269	309	517	TRI	MHU75.2	TRI3
Chr 1	AF14/8			2.5 (7.4 %)	1.5 (4.6 %)	1.7 (5.0 %)	
Chr 3	AF1/5		2.5 (7.6 %)				
Chr 4	AF13/8				2.5 (7.8 %)		
Chr 5	AF1/5		3.1 (9.2 %)	1.6 (4.9 %)	2.5 (7.7 %)		
Chr 5	MTC 2	1.3 (4.0 %)		2.0 (6.6 %)	2.7 (7.9 %)		
Chr 5	AF14/3 and AF6/14	2.0 (7.0 %)	1.5 (5.2 %)	2.9 (10.7 %)	3.4 (12.7 %)	3.1 (12.2 %)	1.8 (7.9%)
Chr 5	AF 13/19	2.3 (6.6 %)	1.7 (5.2 %)	2.5 (7.8 %)	3.2 (9.6 %)	2.0 (6.5 %)	
Chr 6	MTC 28 and AF 9/9	1.0 (4.1 %)		2.6 (8.9 %)	3.1 (11.0 %)	2.4 (8.4%)	

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Factors Affecting Jorquette Height in Orthotropic Buddings

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Jorquette height of cacao trees derived from seeds is under genetic control and greatly influenced by the environment. Information about jorquette height in orthotropic budded cacao seedlings is, however, very limited. This article describes information obtained on several factors affecting jorquette height in orthotropic budded cacao at the Papua New Guinea (PNG) Cocoa and Coconut Research Institute (CCRI).

Source of Budwood

Orthotropic budwood was obtained from three sources:

1) Primary orthotropic buds derived from the stems of growing seedlings about four months after planting;
2) Secondary orthotropic buds were derived from regrowths after cutting the main stem of a seedling at 40 cm from the ground;
3) Secondary orthotropic buds were derived from an orthotropic budwood garden; and
4) Jorquette height was also measured in orthotropically growing trees derived spontaneously from plagiotropic (fan branch) budded seedlings.

Jorquette height differed greatly depending on the source of budwood (Table 1). The greatest average height was obtained from primary orthotropic buds. It was more than twice that of plants budded with secondary orthotropic buds. The lowest jorquette height was found in the plants spontaneously derived from plagiotropic buds. All the buddings were done on four month-old rootstocks and grown in the same nursery under very similar conditions.

Table 1: Average jorquette height of orthotropically growing budded plants from different sources of budwood

SOURCE OF BUDWOOD (Material Measured)	AVERAGE HEIGHT (cm)	RANGE (cm)	NO. OF CLONES [1]
Orthotropic – Primary	119.7	105.1-140.1 [2]	289 (4)
Orthotropic – Secondary (regrowth)	45.8	34.2-81.5 [2]	488 (4)
Orthotropic – Secondary (budwood garden)	46.5	42.9-57.5	8 (40)
Plagiotropic – Spontaneous	28.9	23.7-41.6	8 (5)

1) A range of average heights/cross with various numbers of clones per cross.
2) Numbers in brackets indicate number of plants measured per clone.

Age of Rootstock

Secondary orthotropic buds after re-growth, as described before, were budded onto four-month and two-week old (juvenile) rootstocks. Budwood was obtained from the same crosses. The average jorquette height for the older rootstocks was 23.5% greater than when juvenile rootstocks were used (Table 2). This is probably because of the larger food reserves that were available to the growing budded shoots in the older rootstocks.

Table 2: The effect of rootstock age on jorquette height of orthotropic buddings

AGE OF ROOTSTOCK	AVERAGE JORQUETTE HEIGHT (cm)	RANGE (cm) [1]	NUMBER OF CLONES [2]
4 months	45.8 (123.5%)	34.2-81.5	488
2 weeks	37.1 (100.0%)	26.1-58.8	1,216

1) A range for average jorquette height/cross with various numbers of clones/cross
2) Four plants/clone

Effect of Rootstock Vigour

Eight Trinitario x Upper Amazonian clones derived from hybrids in an orthotropic budwood garden were budded onto eight different rootstocks. The rootstocks used were crosses of two commercial hybrids - SG2-B (big) and SG2-S (small) that differ in their potential vigour (Table 3). The budded plants were maintained in the nursery until the stage of jorquette development. Then the heights of the five tallest plants for each clone and each rootstock were measured.

Table 3: Effect of rootstock vigour on jorquette height of orthotropic buddings

ROOTSTOCK VIGOUR AS HYBRIDS [1]			AVERAGE [3] JORQUETTE HEIGHT (cm)	AVERAGE PARENTAL EFFECT	
PEDIGREE	TRUNK CIRCUMFERENCE (cm)	VIGOUR [2] INDEX		TRINITARIO	JORQUETTE HEIGHT (cm) [3]
KA2-106 x KEE 43 (SG2-B)	42.6	129	45.6 d	K82	47.4 a
KA2-106 x KEE 42 (SG2-B)	41.9	111	47.7 bc	KA2-106	45.6 b
KA2-106 x KEE 5 (SG2-B)	41.1	112	43.7 e	Upper Amazonian	
K82 x KEE 42 (SG2-B)	38.1	108	48.2 b	KEE 43	48.4 a
KA2-106 x KEE 12 (SG2-S)	35.1	80	45.7 b	KEE 42	47.9 a
K82 x KEE 5 (SG2-S)	34.0	73	43.5 e	KEE 12	44.3 b
K82 x KEE 43 (SG2-S)	33.9	70	51.2 a	KEE 5	43.4 c
K82 x KEE 12 (SG2-S)	31.7	60	46.9 c		

1) Source - CCRI Annual Report 1994
2) Relative vigour based on trunk circumference, tree width and height
3) Average of 8 clones, 5 plants/clone. Means sharing the same letters are not significantly different at $P = 0.05$

The average jorquette height of plants budded onto the eight rootstocks differed significantly and ranged from 51.2 cm for K82 x KEE 43 to 43.7 cm for KA2-106 x KEE 5, a difference of 18.2%. However, it was not related to the potential vigour of the rootstock as hybrid (Table 3). It was more related to the parental Trinitario and Upper Amazonian clones. Significant differences were found in the two groups. The hybrids with K82 as rootstocks gave higher jorquette height than hybrids with KA2-106. However, the two clones had an opposite effect as donors for tree vigour in hybrid combinations. Among the Upper Amazonian clones, KEE 5 contributed to the lowest jorquette height.

Effect of Scion Genotype

Genetic differences in jorquette height were found between clones derived from hybrids of different origins in several experiments. Eight clones derived from Trinitario x Upper Amazonian hybrids were budded onto eight different rootstocks, as described above. Their average jorquette heights were significantly different (Table 4), ranging from 42.9 cm (63-7/3) to 57.5 cm (33-8/3), a difference of 34.0%. The difference between the two clones was larger (32.0 and 61.0 cm, respectively) when 63-7/3 was budded onto KA2-106 x KEE 5 and 33-8/3 onto K82 x KEE 43 as rootstocks.

Different genetic materials were used in another experiment (Table 5). Secondary orthotropic buds were budded onto four month-old rootstocks. The number of clones established per cross varied between 5 and 40. Average jorquette height/cross ranged between 34.2 (NA 33 x PA 300) to 81.5 (PA 13 x KA2-106). The differences were much larger (22.0 and 130.3) between the lowest and highest jorquettes of clones derived from the two hybrids, respectively.

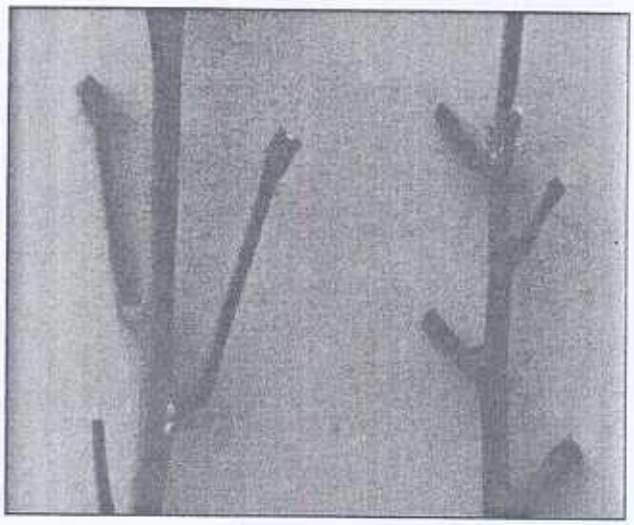
Table 4: Average jorquette height of Trinitario x Upper Amazonian derived clones budded with secondary orthotropic buds

CLONE	PEDIGREE	JORQUETTE HT. (cm)[1]
33-8/3	KA2-106 x KEE 12	57.5 a
73-3/3	K24-102 x KEE 12	47.1 b
36-3/1	KA2-106 x KEE 42	46.9 b
24-4/4	KA2-101 x KEE 22	45.1 c
23-3/1	KA2-101 x KEE12	44.8 c
73-6/1	K24-102 x KEE 12	44.6 c
17-14/4	K82 x KEE 43	43.2 d
63-7/3	KA6-101 x KEE 12	42.9 d

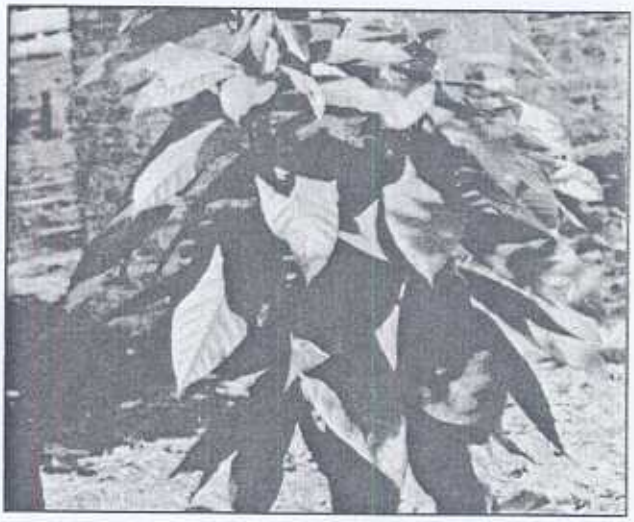
1) Average of budding onto eight different rootstocks, five plants/rootstock. Means showing the same letter are not significantly different at $P = 0.05$

Table 5: Average jorquette height of orthotropic budded clones from 21 different crosses

MALE PARENT	JORQUETTE HEIGHT (cm) FEMALE PARENT			
	PA 13	NA 33	SCA 12	Average
KA2-106	81.5	36.7	39.3	52.5
NA 226	64.3	41.8	41.5	49.2
NA 149	56.7	46.3	44.4	49.1
BR 25	53.9	45.5	40.3	46.6
PBC 123	51.2	42.4	43.9	45.8
IMC 23	51.7	43.1	41.8	45.5
PA 300	49.4	34.2	41.1	41.6
Average	57.7	41.4	41.7	

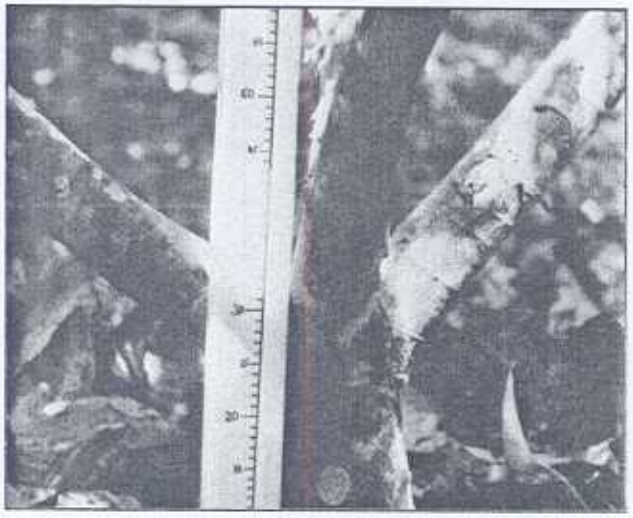


• Orthotropic (left) and plagiotropic (right) budwood. Note the spiral and alternate petiole arrangements, respectively.

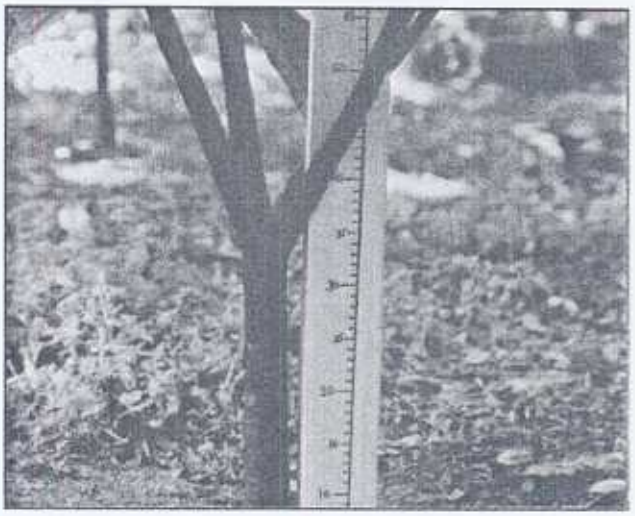


• Cacao tree in an orthotropic budwood garden.

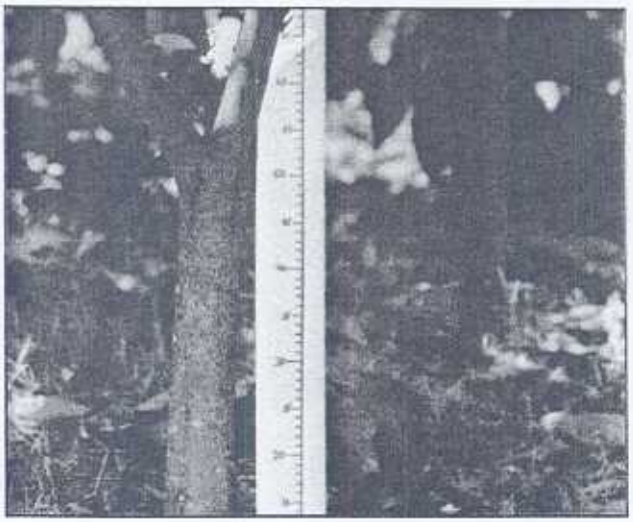
JORQUETTE HEIGHT OF BUDDED COCOA TREES



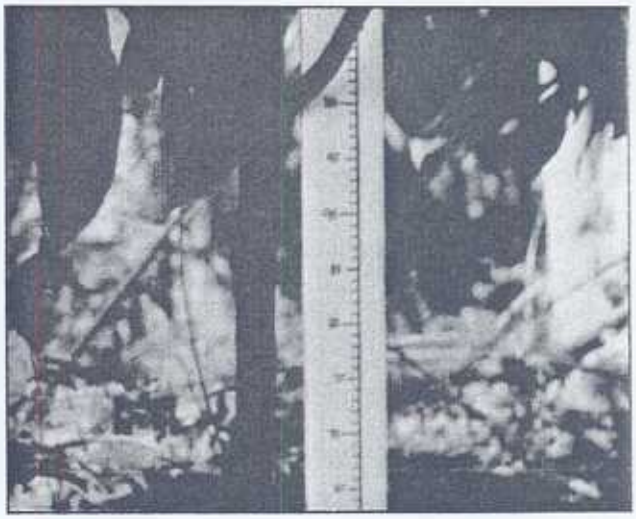
• Spontaneous orthotropic growth from plagiotropic bud.



• Secondary orthotropic budding on two week-old root stock.



• Secondary orthotropic budding on four month-old root stock.



• Primary orthotropic budding on four month-old root stock.

Clone PA 13 clearly had a major effect as a parent among the females. The average jorquette heights for NA 33 and SCA 12 were very similar. This was well correlated with the average jorquette height in trees derived from seeds of the same parents and hybrids (129.8, 116.8 and 110.1 cm, respectively). Clone PA 300 was a donor for the lowest jorquette height among the male parents.

Similar genetic differences in jorquette height were found in clones derived from different sets of crosses that were budded with primary orthotropic buds. The highest and lowest average jorquette heights/cross were 140.1 (110-172) and 105.1 (82-125) cm for clones derived from the crosses K-6 x KEE 12 and K-4 x 36-3/1, respectively.

Several factors affecting jorquette height in budded plants were described. The source of budwood had a very noticeable effect. However, it is practicable to use only secondary orthotropic buds from a budwood garden commercially. This results in trees with an average jorquette height of about 50-cm, which is much lower than that of the trees grown directly from seeds. This may prove to be advantageous since it lowers the canopy thus allowing more efficient use of the surface available for pod production, easier harvesting and pruning activities. At this level, jorquette height can be manipulated to a large extent by breeding and to a lesser extent by the choice of type and age of rootstock. Though not yet tested, jorquette height of budded plants can probably be manipulated also by the level of shade in the nursery, and soil fertility.

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Finding New Female and Male Parents for the Production of Better Hybrid Cacao Planting Material for Farmers in Ghana

B. Adomako and Y. Adu-Ampomah

Two breeding programmes were initiated in Ghana in the early 1980's to identify better parents than those that were being used for hybrid seed production for farmers. In the first programme, which identified superior female parents, 10 Upper Amazon cacao selections were evaluated for their breeding value in a field trial by estimating their combining abilities for yield and its components, incidence of Black Pod disease (*caused by Phythophthora palmivora*) and vegetative characteristics. In the second programme, which focused on the identification of better pollen (male) parents, the agronomic characters of inter-Amazon top crosses with two female parents, T60/887 and T85/799, were compared with Upper Amazon x Upper Amazon and Upper Amazon x Amelonado controls in two progeny trials. All the trials were established under farm conditions for growing cacao in Ghana as the cacao was grown under shade of mixed species of forest trees and insect damage controlled with insecticides, but neither fungicides nor fertilizers were used.

In all the trials involved in the two programmes, early yield and stem girth at three years were both significantly correlated with yields in later years. Progenies with the most favourable yield-vigour relationships were generally the best for net yield. All the hybrids obtained bean weights of not less than 1.0g. Bean weight, bean number, conversion ratio and pod values varied markedly between crosses.

PA 150, POUND 7 and PA 7 (Table 1) were the best female parents identified. In the trials involving the inter-Amazon top crosses, some of the Upper Amazon parents produced hybrids which are as good as, and sometimes better than the control hybrids in terms of net yield and losses due to Black Pod disease (Tables 2 & 3). Out-crossed progenies were superior to within-group crosses in yield. Recommendations are made as to which of the parents should be used to produce seeds for farmers' use.

Results and Discussion

In the first programme, which focused on the identification of superior female parents, net yield was generally low (Table 1). This was mainly due to the fact that the trial conditions were adopted to approximate conditions on farms where the cacao is grown under shade and insect damage controlled with insecticides, but neither fungicides nor fertilisers were used. In Ghana, new farms are being established with mainly replantings and shade is normally provided since replanting in full sunlight under such conditions is very difficult. Paulin and Eskes (1995) noted that growing cacao in full sunlight would ensure higher yield but would require fertiliser and regular phytosanitary treatments. Trials in West Africa have also shown that potential yields can be doubled by removing permanent

shade, provided fertilisers are applied (Perithuguenin, 1998). If cacao is grown in full sunlight, without fertilisers, yields are satisfactory in the short term, but fall in the medium-term (Ahenkorah *et al.*, 1974). The use of fertilisers on farms in Ghana is not common because of the high cost involved.

Net yields were again more variable during the first three years of cropping as indicated by the high coefficient of variation (27.7%), but the variation decreased during the later years of cropping. In Malaysia, Lockwood and Pang (1994) also observed higher coefficients of variation in 'younger' trials than in 'older' ones.

The female parents with the highest and significantly positive general combining ability (GCA) values for the first three years of cropping were T60/887, PA 150 and POUND 7 whilst for the latter eight year and entire 11-year cropping periods PA 150, POUND 7 and PA 7 were the most promising parents with the largest GCA effects (Table 1). Among the male parents, SCA 9 gave significantly positive GCA values for the first three years of cropping but not so for the other cropping periods. In a multilocation trial in Cote d'Ivoire, T60/887 and PA 150 were among the best female parents for yield (Paulin *et al.*, 1994) and in a similar study in Malaysia, progenies of SCA 9 were the highest yielding (Lockwood *et al.*, 1994). PA 150 and POUND 7 combined high yields with low Black Pod disease incidence in the present study.

Table 1: Mean, range, coefficient of variation and GCA values for yield (kg/ha) and percentage Black Pod for female and male parents of Upper Amazon cacao

	GCA values			
PARENT	YIELD (11 yrs)	YIELD (first 3 yrs)	YIELD (last 8 yrs)	BLACK POD Incidence
Female				
PA 150	139.35***	66.73	166.59***	-1.88**
POUND 7	61.29*	47.76	66.36*	-1.41
PA 7	23.65	-23.94	41.52	-0.08
T60/887	13.25	78.43***	-11.21	0.92
IMC 60	-1.11	-40.71*	13.72	0.79
T85/799	-18.01	-6.01*	-22.51	1.16**
ALPH. B36	-20.41	-26.17	-18.28	1.58***
POUND 26	-57.18*	-52.34**	-58.98*	2.92***
T65/238	-64.76***	-16.13	-95.11**	-0.28
T17/524	-76.05***	-59.87**	-82.11**	-0.54
S.E.	21.63	16.64	25.58	0.49
Male				
SCA 9	18.00	103.94*	-14.23	0.36
P30	-3.63	-38.60	9.48	-0.44
ICS 6	-14.37	-65.33	4.75	0.07
S.E.	8.77	42.90	6.92	0.22
Grand mean	392.4	271.2	437.8	11.0
Range (2)	266.3-586.5	103.8-638.9	242.1-714.4	7.5 – 14.9
C.V.	15.1	27.7	18.6	16.8

(1): *,**,***: GCA values significantly different from zero at the 0.05, 0.01, and 0.001 probability level respectively

C.V.: Coefficient of variation

(2): Range for means of individual crosses

In the second programme which evaluated candidate pollen (male) parents in two field trials, crosses of BE 8, POUND 10 and EQX 3356 with T60/887 in the 25th progeny trial area (PTA) had the highest net yields for the entire 10-year and the later 5-10-year cropping periods (Table 2). In the 26th PTA, crosses of T85/799 with the Parinari pollen parents appeared to be the best for yield. This may be due to heterosis. The crosses with the lowest yields were T85/799 crosses with IMC 23, NA 440 and IMC 78 (Table 3). The low yields may be due to some level of inbreeding resulting from intra-population hybridisation since the female parent T85/799 is a cross between IMC 60 and NA 34.

Table 2: Net yield (kg/ha) and percentage Black Pod levels of progenies in the 25th Progeny Trial Area (P.T.A)

PROGENY	YIELD (1-10 years)	YIELD (1-5 years)	YIELD (6-10 years)	BLACK POD Incidence
T60/887 x BE 8	965.8 h	766.0 c	1105.6 bc	4.8 bcdef
" x POUND 10	912.5 fgh	671.7 abc	1092.3 bc	5.3 cdef
" x EQX 3356	904.3 efgh	693.8 bc	1129.0 c	5.7 ef
" x T17/524	857.6 cdefg	640.3 abc	1019.3 bc	6.0 fg
" x CATONGO	857.2 cdefg	700.0 bc	1016.9 bc	7.7 g
" x MA 12	846.8 cdefg	622.7 abc	1023.8 bc	2.8 a
" x EQX 3338	816.7 cdef	613.4 abc	1016.9 bc	3.0 a
" x ICS 6	813.9 cdef	699.0 bc	955.3 abc	6.5 fg
" x AMEL	813.5 cdef	616.7 abc	1047.5 bc	5.6 def
" x AMAZ 3 2	804.7 cde	655.9 abc	926.8 abc	3.9 abcd
" x POUND 15	801.7 cde	633.9 abc	938.7 abc	5.1 cdef
" x IMC 60	793.8 cd	623.4 abc	938.1 abc	4.1 abcde
" x POUND 7	793.4 cd	542.9 ab	1047.5 bc	3.4 abc
" x IMC 78	757.3 cd	606.6 ab	905.7 ab	4.9 cdef
" x RB 49	756.7 c	579.1 ab	911.6 ab	3.7 abc
" x POUND 21	753.0 bc	528.7 a	976.7 bc	4.2 abcde
" x C SUL 7	627.7 a	543.5 ab	916.6 ab	6.5 fg
" x EQX 3364	612.4 a	523.6 a	765.1 a	3.1 ab
T85/799 x AMEL (C_1)	943.7 gh	665.0 abc	1108.5 bc	5.6 def
T85/799 x T79/501 (C_2)	831.0 cdef	589.4 ab	1010.0 bc	3.6 abc
Mean	813.9	625.6	988.8	4.8
S.E.D.	52.1	79.6	103.7	0.85

C_1, C_2 Standard (Control) crosses included for comparison

For each column, figures followed by the same letter are not significantly different at the 0.05 level of significance.

Table 3: Net yield (kg/ha) and percentage Black Pod levels of progenies in the 26th PTA

PROGENY	YIELD (1-10 years)	YIELD (1-5 years)	YIELD (6-10 years)	BLACK POD INCIDENCE
T85/799 x PA 150 704.9 g	405.4 h	829.8 h	6.0 c	
" x PA 7	646.9 fg	280.6 fg	777.4 gh	4.8 abc
" x MA 12	594.1 efg	325.6 h	726.2 fgh	3.9 a
" x SCA 9	544.7 def	293.6 g	661.3 efg	5.8 c
" x PA 107	523.8 de	280.5 fg	751.9 gh	6.6 cd
" x ALPH.B36	494.3 cde	258.7 ef	596.4 def	4.6 ab
" x GA 11	384.1 bc	182.2 c	479.4 abcd	10.6 f
" x T44/547	312.3 ab	190.6 cd	403.8 abc	7.1 d
" x IMC 78	279.3 ab	130.9 ab	393.7 ab	5.6 bc
" x NA 440	269.7 ab	144.4 bc	393.0 ab	5.7 c
" x IMC 23	189.7 a	113.6 a	331.6 a	7.5 de
T85/799 x AMEL (C_1)	438.7 cd	238.7 de	530.0 bcde	8.3 e
T85/799 x T79/501 (C_2)	379.4	229.8 d	551.9 cde	5.9 c
Mean	443.2	234.0	571.2	6.3
S.E.D.	68.9	25.3	74.2	0.52

C_1, C_2: Standard (Control) crosses included for comparison

For each column, figures followed by the same letter are not significantly different at the 0.05 level of significance.

Conclusions

Two of the 10 candidate female selections, T85/799 and T60/887, are already being used widely for the production of hybrid seeds for farmers in Ghana. Results from the present study indicate that PA 150, POUND 7 and PA 7 are better female parents than the two predominant ones. Thus, a new series of superior hybrids can be obtained by using the parents, identified as being better, in crosses with unrelated Upper Amazon pollen parents or with Upper Amazon material not collected by Pound.

Results from the 26th PTA indicated that inter-group crosses were superior in yield to within-group crosses. In the 25th PTA, however, there was no conclusive evidence to suggest that inter-group crosses were better than the intra-group progenies in yield. This may have resulted because T60/887 has good GCA for yield. CRIG has initiated a new breeding programme with support from the Common Fund for Commodities (CFC) project. Field trials have been set up, as part of a recurrent selection scheme, intended to evaluate in more detail the value of inter- and intra-group crosses. This will confirm which type of crosses are the best to employ in the breeding programmes to bring about increased cocoa production in the 21st century.

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Breeding as a Subject in the *Cocoa and Chocolate Bibliography*

W. Gerritsma

*This is a collaborative project

The *Cocoa and Chocolate Bibliography* (Gerritsma, 1997-1999) is a continuously growing collection of cocoa and chocolate literature references and abstracts that is proving to become a substantial product of the 'Collective research for agro-technological growth and quality model for cocoa' project. This is a collaborative project between Wageningen University and five leading cocoa and chocolate processing companies in The Netherlands.

The bibliography is intended to serve students, scientists and others concerned with all aspects of the growing, processing, grinding, manufacturing and sales of cocoa and chocolate products. It includes annotated references on agronomy, botany, crop-protection, breeding, processing, manufacturing, biochemistry, consumption and economic aspects of the cocoa and chocolate industry worldwide. For cocoa breeders, the bibliography is a useful research compendium providing additional information on the breeding aspects of the crop complementary to the *International Cocoa Germplasm Database*.

The cocoa and chocolate bibliography is not only useful as a first introduction to certain aspects of the cocoa and chocolate science and its industry. It also serves as a tool for checking references and conducting reviews of the literature. Analysis of the database provides views on the priorities and changes in the focus of cocoa and chocolate research. The current version of the database serves as an important starting point for any discipline of cocoa and chocolate research.

To date, approximately 10,200 cocoa literature references have been collected, of which about 1600 are from the pre-W.W.II literature. The earliest references are from the 1500's onwards, but the majority cover the 20th century. The value of this bibliography lies in its specialisation in all aspects of a single crop and its products, and the inclusion of many references from the period before computerised bibliographies became widely available. The inclusion of a large amount of 'grey' cocoa and chocolate literature and news items is also an important contributing factor to the importance of the bibliography.

Software and installation

The bibliography was compiled using Cardbox® and is published on the Internet using the same software. The database is accessible free of charge. Users with web access only need to install the free Cardbox® client software once. Instructions for the installation and downloading of the software can be found at the website given below, and its related links. Once properly installed, the database is fully functional for browsing and complex searches.

Breeding references

Of the current collection of 10,200 references, 780 are on the subject of cocoa breeding. It is therefore one of the more important

subjects in the cocoa literature, ranking 3rd after crop protection and economics. The very first references related to cocoa breeding are concerned with the taxonomy and botany of the crop. In 1901, the first cocoa breeding programme was started in Indonesia, and a report of the first cacao germplasm collection expedition by Preuss was published. Since then, cocoa breeding is a much-published subject in the cocoa literature. In Figure 1, an overview of the articles on breeding published throughout the years is presented.

Within the category of breeding, the keyword 'germplasm' occurs 177 times. This is the most important keyword, followed by 'resistance'. Selection for resistance to pest and diseases is covered by 97 references. Resistance to *Phytophthora* is the most widely investigated (46 references) disease resistance followed by Cocoa Swollen Shoot Virus. The three authors with the most publications on the subject of cocoa breeding in the database are G. Lockwood, V.J. Soria and B.G.D. Bartley. Of course, this collection is not complete, but it is currently the largest collection of references in this field.

Newsletter and Updates

At present, about 150 users receive an electronic newsletter with information on updates and progress with the cocoa and chocolate bibliography. Interested breeders can send me an e-mail with a request to be included on this list. Currently, the frequency of updates (of the bibliography and the newsletter) is about four per year.

When you are missing some important references or wish to provide some references to include in the database, please feel free to contact me. The last update was in December,1999. The emphasis of the update was mainly on references from French sources.

Acknowledgements

The financial support for this project by ADM Cocoa, Barry Callebaut, Dutch Cocoa, Gerkens Cacao and Mars is gratefully acknowledged.

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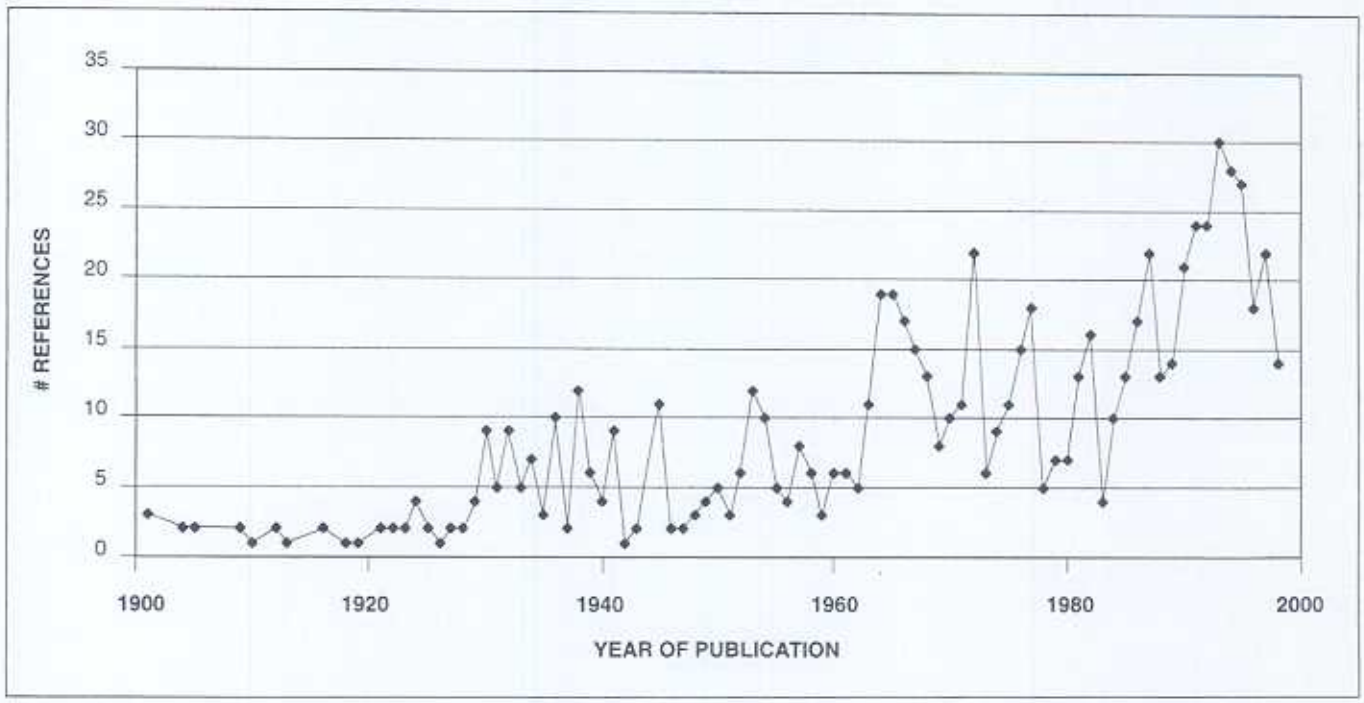
Figure 1. Breeding references



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Announcement of the THIRD INGENIC WORKSHOP

'New Technologies and Cocoa Breeding' was chosen by the plenary session of the second INGENIC Workshop in Bahia, Brazil as the theme for the third INGENIC Workshop to be held in conjunction with the XIII International Cocoa Research Conference in Malaysia in October 2000. The Workshop aims at reviewing advances in new technologies and their possible applications to cacao variety improvement. The provisional scientific programme includes presentations on novel methods to improve tree crops and progress in molecular markers, genome sequencing, micro-propagation and cryopreservation in cacao. During the event, any proposals for new collaborative research projects will also be discussed. It is expected that the general topics will be introduced by a key-speaker. INGENIC will invite speakers to review the specific topics applied to cacao and is welcoming any suggestions to improve the scientific programme or to include additional review topics.

The Workshop will be held on October 16 and 17, 2000, in Kota Kinabalu, Sabah, Malaysia, just after the 13th International Cocoa Research Conference. Local organisation of the workshop will be done through the kind collaboration of the Malaysian Cocoa Board. The *modus operandi* of the Workshop will be the same as of those held before, except that more time should be available for discussions. The registration fee will be US$125 for the two-day meeting, including conference materials, four cocoa breaks and two lunches. This fee will be due during the final registration for the Workshop on 15 September, 2000. Pre-registration for participation should be done before 31 July, 2000, by filling out the registration slip and sending it by fax or e-mail to the INGENIC Secretariat (see below). Only pre-registered persons will receive the second Workshop announcement with more details on the venue.

For more information and any suggestions on the scientific programme, please contact the INGENIC Secretariat:

Michelle End
Plant Science Laboratories
The University of Reading
Whiteknights
P.O Box 221
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UK

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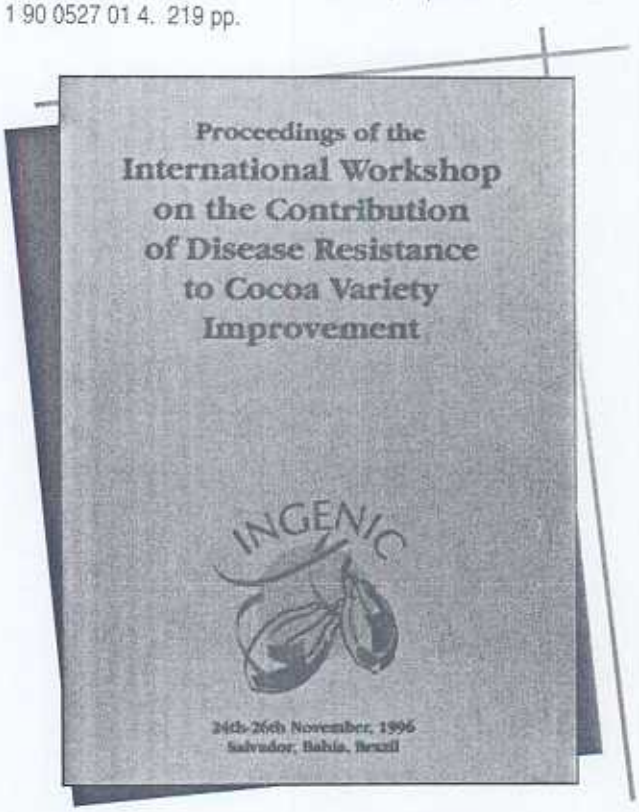
BOOK RELEASES

PROCEEDINGS OF THE INTERNATIONAL WORKSHOP ON CONTRIBUTION OF DISEASE RESISTANCE TO COCOA VARIETY IMPROVEMENT

November 24-26, 1996. Salvador, Bahia, Brazil. F. Bekele, M. End, and A. Eskes, editors. INGENIC, September, 1999. ISBN 1 90 0527 01 4. 219 pp.



Copies of these Proceedings may be obtained from the INGENIC Secretariat (address given above).

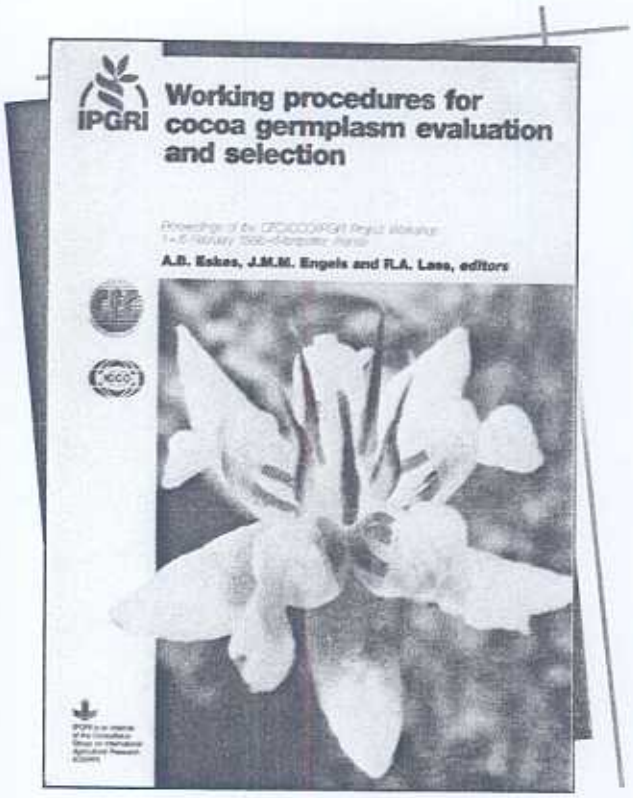
WORKING PROCEDURES FOR COCOA GERMPLASM EVALUATION AND SELECTION

Proceedings of the CFC/ICCO/IPGRI Project Workshop. February1-6 1998. Montpellier, France. A.B. Eskes, J.M.M. Engels and R.A. Lass, editors. International Plant Genetic Resources Institute, Rome, Italy. 2000. 176 pp.

These proceedings are a result of the first Workshop of the CFC/ICCO/ICCO Project on 'Cocoa Germplasm Utilisation and Conservation; A Global Approach.' This project, developed under the aegis of the International Cocoa Organisation (ICCO), is substantially funded by the Common Fund for Commodities (CFC), an intergovernmental organisation with headquarters in Amsterdam. The International Plant Genetic Resources Institute (IPGRI) is the Executing Agency. The main objective of the project is the production of better cacao planting material, including increased resistance to pests and diseases, allowing for reduced costs of production and

increased competitiveness of cocoa in the market. The project supports collaborative evaluation, selection and conservation activities in ten cocoa producing countries using the same methodology.

Scientists involved in the project met for the first project Workshop, from 1-6 February 1998 in Montpellier, to discuss and agree on procedures for evaluation and selection of cacao genotypes. The draft procedures produced during the Workshop have been circulated to all participants for comments and have been amended over the last two years. The Proceedings of the



Workshop contain Introductory Papers, papers on General Project Strategies, and 31 Working Procedures on Evaluation and Selection of Cacao Germplasm.

The General Project Strategy papers include articles on the germplasm enhancement programme carried out at CRU in Trinidad, the establishment of a 'CFC Project Collection', the use of cacao populations in breeding, a short list of cacao descriptors for characterisation, the role of the International Cocoa Germplasm Database (ICGD) and opportunities and procedures for exchange of cacao germplasm in the Project.

The Working Procedures are the following:

- Choice of clones and rootstock for the International Clone Trial
- Budding techniques
- Nursery maintenance
- Pruning techniques for young plants
- Planting and field management of trials
- Evaluation of vigour, yield, pod and bean traits
- Layout of project trials
- Analysis of trial data
- Physiological traits
- Criteria for visual selection of individual trees
- Manual pollination techniques and verification of incompatibility
- Evaluation of cocoa quality
- Documentation and photography of the commonly used project clones
- Early screening of resistance to *Phytophthora* spp. by means of leaf disc inoculation
- Rapid screening for *Phytophthora* pod rot resistance by means of detached pod inoculation
- Assessment of *Phytophthora* pod rot resistance by means of attached pod inoculation
- Field evaluation of *Phytophthora* pod rot and stem canker incidence
- Ring test for Black Pod resistance
- Early screening for Witches' Broom resistance: belt spray inoculation of seedlings and clones
- Evaluation of Witches' Broom incidence in the nursery and field
- Evaluation of resistance to Witches' Broom by means of bud inoculation
- 'Ring Test' for Witches' Broom resistance
- *In vitro* method for preliminary screening of cacao germplasm for resistance to Vascular Streak Dieback
- Evaluation of Vascular Streak Dieback resistance in the nursery and field
- Laboratory microtest for cacao tree attractiveness to mirids
- Evaluation of tolerance, antixenosis and antibiosis to cacao mirids in the nursery
- Evaluation of resistance and tolerance to mirids under field conditions
- A method for assessing mirid damage in the field
- Evaluation of insect attacks on young cacao trees in the field
- Evaluation of field resistance to cacao thrips (*Selenothrips rubrocintus*)
- Recommendations for chemical control of insects.

Although these Working Procedures will be used in the CFC/ICCO/IPGRI project, it is expected that they will have a much wider usage in any programme or activity on cacao characterisation, evaluation and selection. Further information and copies of the Proceedings can be obtained from:

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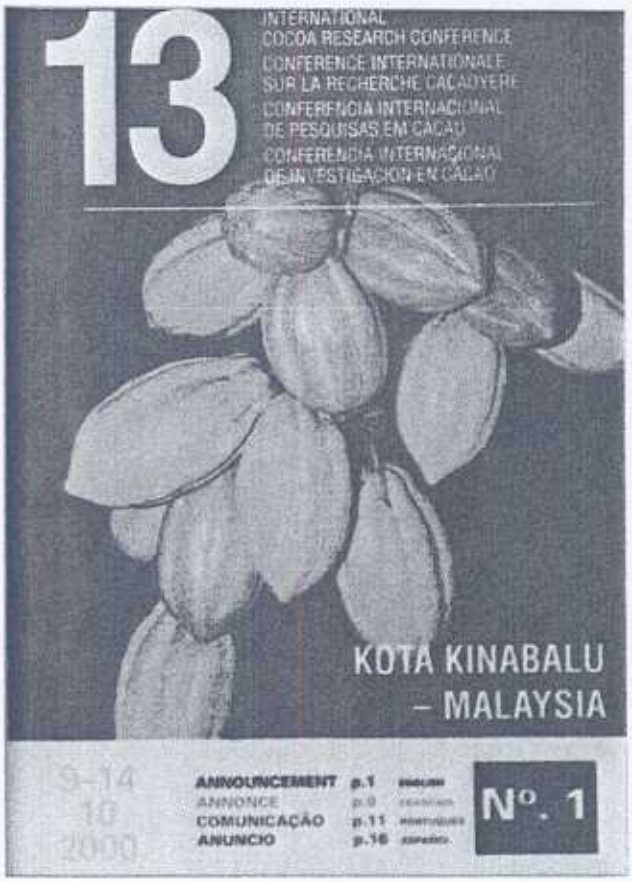
34397 Montpellier Cedex 5
FRANCE
Fax: 33.467610334
e-mail: b.eskes@cgiar.org

ANNOUNCEMENTS OF UPCOMING EVENTS

THIRD INGENIC WORKSHOP

16-17 October, 2000
Kota Kinabalu
MALAYSIA



List of acronymns

ACRI:	American Cocoa Research Institute (USA)
BCCCA:	Biscuit, Cake, Chocolate and Confectionery Alliance (London UK)
CABI:	Centre for Agriculture and Bioscience International (Ascot, UK)
CAOBISCO:	Association des industries de la chocolaterie, biscuiterie et confiserie de l'UE
CATIE:	Centro Agronómico Tropical de Investigación y Enseñanza (Turrialba, Costa Rica)
CCRI:	Cacao and Coconut Research Institute (Papua New Guinea)
CFC:	Common Fund for Commodities
CIRAD:	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CRIG:	Cocoa Research Institute, Ghana
CRU:	Cocoa Research Unit (Trinidad & Tobago)
ICCO:	International Cocoa Organisation
IPGRI:	International Plant Genetic Resources Institute
USDA:	United States Department of Agriculture

You are invited

to contribute

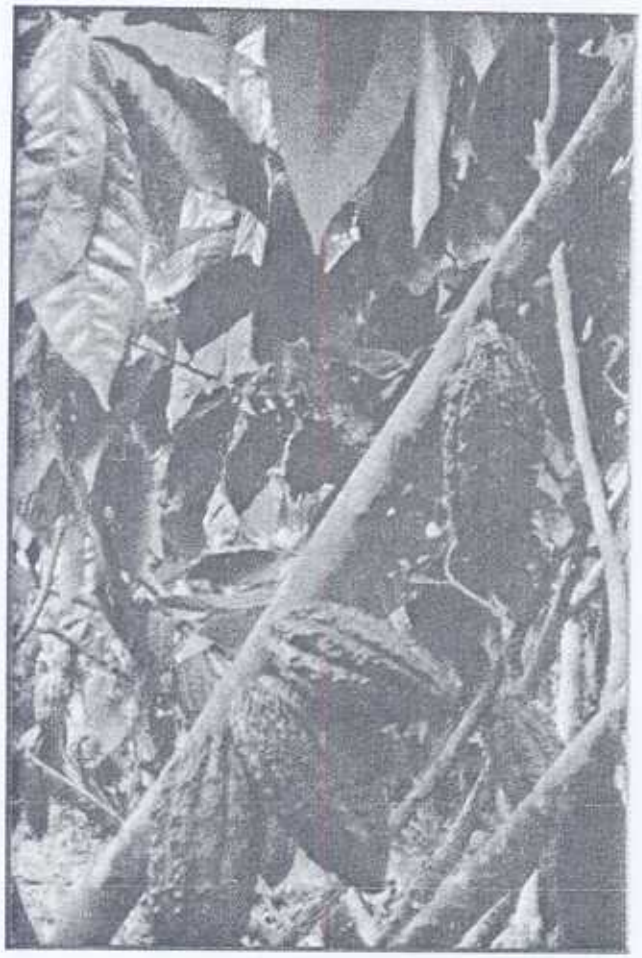
articles on research

or other issues

of interest to

cocoa breeders/

geneticists.



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