# Newsletter

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## IN THIS ISSUE



## From the Editor's Desk:

The release of this issue of the *INGENIC* Newsletter coincides with significant developments in the international cocoa community. The international collaborative Project entitled ***Cocoa Germplasm Utilization and Conservation, a Global Approach*** has been officially in progress for almost a year. This Newsletter has assumed a more critical role because it provides a medium for the dissemination of information to participants in the Project and other interested parties.

The first article in this issue contains a report on the first Workshop of this CFC/ICCO/IPGRI Project. Also featured are articles of direct relevance to the implementation of the Project such as the problems of misidentification of cacao clones and with cacao germplasm nomenclature; mass multiplication/propagation of plants; and the use of a rapid screening technique for Black Pod disease.

For the first time in this newsletter, abstracts containing recent research results are presented. This is a welcome introduction, and we encourage scientists to consider making similar submissions for future issues.

The INGENIC Committee also encourages the publication of the Ostendorf Archive, described in this issue.

The cocoa community has lost prominent scientists in recent times, *viz.*, Dr. J.D. Amponsah, and Dr. C.A. Thorold. The passing of Dr. J. Robert Hunter is also regretted. Condolences are extended to the families and friends of the deceased.

The *INGENIC* Committee wishes its readership a happy Yuletide Season and best wishes for the New Year. We look forward to your continued support and contributions to the Newsletter. Contributions should be concise and related to cocoa breeding/genetics. Responses to previously featured articles are encouraged.

*Frances Bekele*

### Invitation

An open invitation is hereby issued for short articles on research or other issues of particular interest to cocoa breeders/geneticists. News on upcoming conferences and meetings would also be appreciated.

# Working Procedures for Cacao Germplasm Evaluation and Conservation

Note on the workshop of the CFC/ICCO/IPGRI project on "***Cocoa Germplasm Utilization and Conservation, a Global Approach***", held at Montpellier from 1-6 February, 1998.

*B. Eskes and J.M.M.Engels*

The project on "***Cocoa Germplasm Utilization and Conservation, a Global Approach***" (refer to INGENIC Newsletter No. **2**) officially commenced on December 9th 1997, the date of signing of the *Project Agreement* document by the Common Fund for Commodities (CFC), the International Cocoa Organization (ICCO) and the International Plant Genetic Resources Institute (IPGRI). This Project is an initiative involving the collaboration of 14 national and international cocoa research institutions, with a main objective to stimulate international collaboration in the field of cacao germplasm evaluation and utilisation. The total project budget of almost 10 million dollars is made up of approximately 30% CFC funding, 50% counterpart funding from all participating institutions and 20% co-financing from the American Cocoa Research Institute (ACRI), the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA), the Centre de Coopération Internationale en Recherches Agronomiques pour le Développement (CIRAD), and IPGRI. Research institutes in 10 cocoa producing countries (Brazil, Cameroon, Côte d'Ivoire, Ecuador, Ghana, Malaysia, Nigeria, Papua New Guinea, Trinidad and Tobago, and Venezuela) are actively involved in its implementation, as well as CIRAD and The University of Reading. ICCO is the Supervisory Body and IPGRI the Project Executing Agency. The latter has established a small coordination unit at the office of the *International Network for the Improvement of Bananas and Plantain* (INIBAP), one of IPGRI's three programmes in Montpellier, France.

The main objectives of the project include:

* promotion of germplasm evaluation and breeding (international clone and hybrid trials at different sites, as well as germplasm enhancement and population improvement activities);
* promotion of efficient conservation, characterisation and distribution of cacao genetic resources;
* improvement of active collaboration between cocoa researchers in the field of germplasm characterisation, evaluation and breeding, with emphasis on resistance to major diseases and pests.

**First Workshop of The CFC/ICCO/IPGRI Project**

The main objective of the first project Workshop, held in Montpellier in February, 1998, was to agree on the common working procedures for evaluation and selection of cacao germplasm to be applied in the Project. More than 40 specialists from 12 different countries participated in the Workshop, as well as representatives of ACRI, BCCCA, CFC, CIRAD, ICCO, and IPGRI. The Workshop was divided into three main subject areas, which also formed the themes of these Working Groups:

1. Agronomy and Breeding,
2. Pathology, and
3. Entomology.

Draft working procedures were presented by specialists, discussed and where necessary, modified by the Working Groups. Plenary sessions were held for reporting on the outcome of each of the specific Working Groups, for formal approval of the working procedures and for discussions on general topics, including organisational aspects. The Workshop was also used as an opportunity to distribute budwood for the International Clone Trial to all project partners.

**Working Procedures discussed**

***Agronomy and Breeding Working Group:***

The procedures discussed were:

* the choice of clones for the International Clone Trial,
* budding and pruning methods,
* field preparation and maintenance of young cacao trees,
* observations on yield, vigour, bean and pod traits and quality aspects,
* physiological traits,
* lay-out of field designs (for clone and hybrid trials),
* pollination techniques,
* use of molecular markers for identification of project clones,
* description and exchange of information on clones,
* methods for phenotypic selection of individual plants, and
* approaches for population improvement in the project.

***Pathology Working Group***

The working procedures concerning evaluation of resistance to *Phytophthora* pod rot were:

* early laboratory screening using leaf disc inoculations,
* rapid screening using detached pod inoculations,
* field evaluation using attached pod inoculations,
* field evaluation of natural infection, and
* implementation of a 'ring test' for study on interaction between fungal species isolates with project clones using leaf disc inoculations.

For Witches' Broom disease, the procedures included:

* detached leaf inoculation and apex inoculation,
* 'belt-spray' inoculation of young seedling progenies and clones,
* evaluation of natural disease incidence in the nursery and field and
* implementation of a 'ring test' for the study of interaction between fungal isolates and resistance of the project clones.

For resistance to vascular streak die-back (VSD), the procedures were:

* laboratory evaluation by the 'dual culture' method, and
* evaluation of resistance by natural infection in the nursery and field.

***Entomology Working Group***

Working procedures included:

* laboratory test on the preference of mirids for cacao,
* evaluation of resistance, tolerance and antibiosis to mirids,
* field observations on mirid incidence and damage,
* field incidence of insects on young cacao plants, and
* control of insects in project trials.

***Plenary Sessions***

During the Plenary Sessions, the following working procedures were discussed:

* a strategy for the germplasm enhancement programme supported by the project and implemented by CRU, Trinidad,
* a strategy to establish a project 'core' collection,
* a minimum descriptor list for the characterisation of germplasm, and
* opportunities and methods for the safe movement of germplasm between the project partners.

**Workshop Outcome**

Written reports were produced by the Working Groups on each working procedure that was officially approved during the plenary sessions. Finalisation and harmonisation of the procedures are underway. The procedures, including evaluation sheets, will be published shortly in a Workshop Report. This Report forms the basis for uniform recording of data in the CFC/ICCO/IPGRI project trials. It is expected that the Report will have a wider applicability thus facilitating international collaboration and producing comparable research results in cacao germplasm evaluation and selection.

Further information may be obtained from:
**Bertus Eskes**,
CFC/ICCO/IPGRI Project Coordinator,
c/o INIBAP
Parc Scientifique Agropolis 2,
34397 Montpellier Cx5, France.
(e-mail: b.eskes@cgiar.org; Fax: 33.467610334)

# Homonymous Genotypes and MisIdentification in Germplasm Collections of Brazil and Malaysia

*Antonio Figueira*

Cooperation among cocoa research groups from various countries appears to have increased in recent years, and it is a very desirable trend for the next century. Various cooperative international research projects, involving many cocoa producer and consumer countries, have been funded recently. Most fields of cocoa research, besides breeding, rely on the correct identification of the planting material used. Results obtained in one location can only be useful on a global scale if the results can indeed be transferred to other producing countries, and the identity of the original planting material can be trusted. As an example of problems that can occur, we have recently published a paper (Figueira *et al.*, 1997) dealing with the effect of genotype and environment on flavour development, and we detected differences in clone identification between important germplasm collections of two very active cocoa breeding programmes.

John Clapperton and collaborators from BAL Plantations Sdn Bhd, Malaysia, developed a test procedure, in which small amounts of wet seeds in net bags are inserted into fermentation batches of wet seeds from a single or mixed genotypes, allowing the flavour potential of a wide range of genotypes to be examined (Clapperton *et al.*, 1994c). Flavour profiles of cocoa liquors from genotypes grown at BAL Plantations differed consistently for various replicate preparations indicating a strong genetic contribution to flavour (Clapperton *et al.*, 1994a, b, c). Since various genotypes tested at BAL were available also at CEPLAC's germplasm collection in Ilhéus (Bahia, Brazil), Almirante Centro de Estudos de Cacau and CEPLAC conducted corresponding experiments in Brazil to see to what extent the effects on flavour observed in Malaysia apply to the same planting materials under the very different environmental conditions in Brazil (Figueira *et al.*, 1997). In general, similar flavour profiles were developed from cocoa from the same genotypes grown in Brazil and Malaysia and subjected to the same conditions of post-harvest processing, confirming the contribution of genotype to cocoa flavour. However, the key point of that paper was the confirmation that misidentification of genotypes in cacao germplasm collections is more common than is currently realised, and represents a problem for transferring results between various research programmes.

The genotypes tested included the whole range of cacao types (Upper and Lower Amazon Forasteros, Trinitarios, and Criollos). The Upper Amazon genotypes chosen were PA 137, NA 33, SPA 9, and AMAZON 2-1. The Lower Amazon types included SIAL 93, and "Amelonado", which in Malaysia originated from a seedling population from Ghana and is morphologically indistinguishable from the Ghanaian population, while the CEPLAC accession was introduced as a clone from the USDA Subtropical Horticulture Research Station at Miami, Florida in 1985. The Trinitario/Criollo genotypes included ICS 1 and ICS 6; Costa Rican selections (CC 10, CC 11, and UF 168), and 10P from Tabasco, Mexico.

There was a general agreement between the classification of genotypes based on flavour by the Almirante Panel with the published work by Clapperton *et al.* (1994c). The genotypes "Amelonado", ICS 6, NA 33, PA 137, SIAL 93 and SPA 9 were classified as having moderate to well developed cocoa flavour with low astringency and bitterness by Clapperton *et al.* (1994c), and medium to high cocoa flavour, with medium astringency and bitterness by the Almirante Panel. Similarly, the genotypes CC 10, CC 11, UF 168 and 10P presented low to medium developed cocoa flavour, with generally medium to high astringency and bitterness according to the Almirante Panel, and were classified as having low cocoa flavour intensity with high astringency and bitterness by Clapperton *et al.* (1994c). When the same genotypes from Brazil and Malaysia were taste tested by the same assessor (John Clapperton), there was a striking agreement between the scores from the two countries of origin. There were only two exceptions for both evaluations:

- The genotype AMAZON 2-1 was classified in the low cocoa flavour, with high astringency/bitterness, group of genotypes by Clapperton *et al.* (1994c), but was considered to have medium to high cocoa flavour, with reduced bitterness and astringency by the Almirante Panel;

- There was also a small disagreement between the scores for cocoa flavour for SIAL 93 from the trials in Brazil and Malaysia evaluated by John Clapperton.

The reasons for these discrepancies might derive from misidentification of the genotypes in one or both sites, thus it was decided to conduct an identity check on all genotypes tested using molecular markers.

Random Amplified Polymorphic DNA (RAPD) analysis was conducted with DNA obtained from trees sampled in Brazil and Malaysia. Leaves from all of the cacao genotypes included in the flavour tests were sampled from the germplasm collections at CEPLAC and BAL Plantations, and dried prior to shipment to the USA for DNA extraction. RAPD reactions were performed to amplify the DNA from the 11 genotypes from Brazil and Malaysia, and 358 fragments were scored. The average similarity among the 22 genotypes evaluated (11 from each country, excluding SPA 9) was 91.4%, ranging from 86.6 to 99.7%. In most cases, a pairwise comparison between the same genotypes present in BAL and at CEPLAC, had a high degree of identity (above 99%), except for "Amelonado", AMAZON 2-1, SIAL 93, and ICS 6 (Table 1). The divergence for AMAZON 2-1, ICS 6 and SIAL 93 clearly indicated that the genotypes are not identical in both countries, and some misidentification has occurred. In fact, pod morphology differed between Brazil and Malaysia for ICS 6 (Fig. 1), for SIAL 93 (Fig. 2), and for AMAZON 2-1 (Fig. 3). The variation observed for the "Amelonado" genotype was expected, considering that the Malaysian sample derives from an open-pollinated population originated from Ghana, while the Brazilian sample came from Miami, with no identification of country of origin, and might have been derived from any one of the cocoa producing countries in West Africa.

The phenogram constructed based on the genetic similarity data separated the genotypes into statistically significant groups using a bootstrap procedure (Yap and Nelson, 1996), congruent with the traditional classification of cacao (Fig. 4). The first significant group (with bootstrap *P* values of 94.3%) included Trinitario/Criollo genotypes (CC 10, CC 11, UF 168, and 10P), while the other Trinitario genotypes, ICS 6 from Brazil and ICS 1 from both countries joined this group with bootstrap *P* values of 73 and 64.5%, respectively. Another significant group (bootstrap $P = 89\%$) contained both "Amelonados" and the SIAL 93 from Brazil, all three genotypes considered Lower Amazon Forasteros. These two significant groups joined to form a robust and significant cluster (bootstrap $P = 91\%$) containing all the "domesticated" cacao genotypes (Figueira *et al.*, 1994). The last two significant groups included only the wild genotypes from the Upper Amazon region, including PA 137, NA 33, and AMAZON 2-1, and presented the lowest genetic similarity (below 89%) between the various origins (Nanay river, Parinari, and AMAZON from Iquitos). Two genotypes (ICS 6 and SIAL 93 from Malaysia) were totally misplaced according to the expected classification. These genotypes were considered to be not identical to the Brazilian counterparts.

Considering that the ICS 6 from Brazil seemed to be the original genotype, according to its position in relation to the other Trinitario/Criollo in the phenogram (Fig. 4) and its morphology (Fig. 1), the genotype ICS 6 from Malaysia appeared to be either a hybrid between a Trinitario/Criollo or a Lower Amazonian Forastero with some Upper Amazonian Forastero. The genotype SIAL 93, according to its position in the phenogram (Fig. 4) seemed to be an Upper Amazonian genotype. The Malaysian SIAL 93 derives from an accession from Mayaguez, Puerto Rico, where, presumably, a bud from the rootstock (probably IMC 67 x PA 218) might have outgrown the canopy of SIAL 93 (B.G.D. Bartley, personal communication). In fact, according to B.G.D. Bartley, this misidentified SIAL 93 was detected in Mayaguez, and was introduced back into Brazil, where it was renamed CEPEC 533 at CEPLAC (B.G.D. Bartley, personal communication). This suspicion still needs to be confirmed by molecular analysis. The agreement in classification for flavour attributes between the Malaysian and Brazilian samples of SIAL 93 and ICS 6 represented more of a coincidence than a genetic effect. But for the AMAZON 2-1 sample, the discrepancy between the flavour profiles should have derived from genotypic differences between the clones from Brazil and Malaysia.

In conclusion, out of a very small sample of genotypes (11), at last least two (ICS 6 and SIAL 93) were not identical at both sites, a rate of 18.2% of misidentification! If AMAZON 2-1 is also considered, the rate rises to 27.3%! Therefore, misidentification may be more common than is currently realised and represents a serious problem for transferring results between various research programmes. The correct identification of at least the most commonly used and recommended clones is urgently required!

Send comments to:
**Antonio Figueira**
Centro de Energia Nuclear na Agricultura –
Universidade de São Paulo
Av. Centenário, 303 – CP 96 - Piracicaba, São Paulo, 13400-970, Brazil
e-mail: figueira@cena.usp.br

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**Table 1:** Degree of genetic similarity between accessions common to Brazil and Malaysia

| Genotype | Similarity |
|---|---|
| NA 33 | 99.7 % |
| UF 168 | 99.7 % |
| PA 137 | 99.4 % |
| ICS 1 | 99.4 % |
| CC 11 | 99.4 % |
| 10 P | 99.4 % |
| CC 10 | 99.2 % |
| AMELONADO | 97.6 % |
| AMAZON 2-1 | 94.6 % |
| ICS 6 | 93.3 % |
| SIAL 93 | 91.8 % |

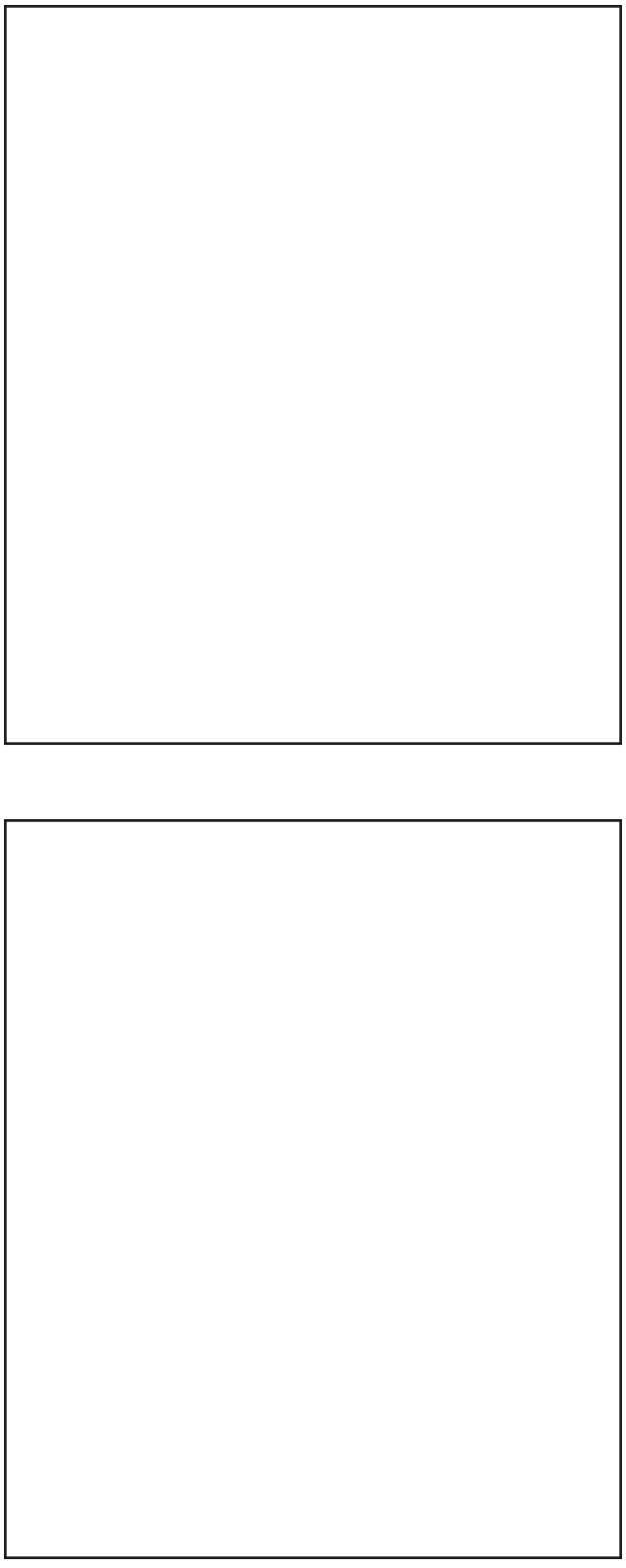

**Fig. 1.** Photographs of pods from genotype ICS 6 taken in BAL Plantations Sdn Bhd, Tawau, Malaysia (October 1994) (top) and CEPLAC (Estação Experimental Joaquim Bahia na, Itajuípe, November 1994) (bottom).

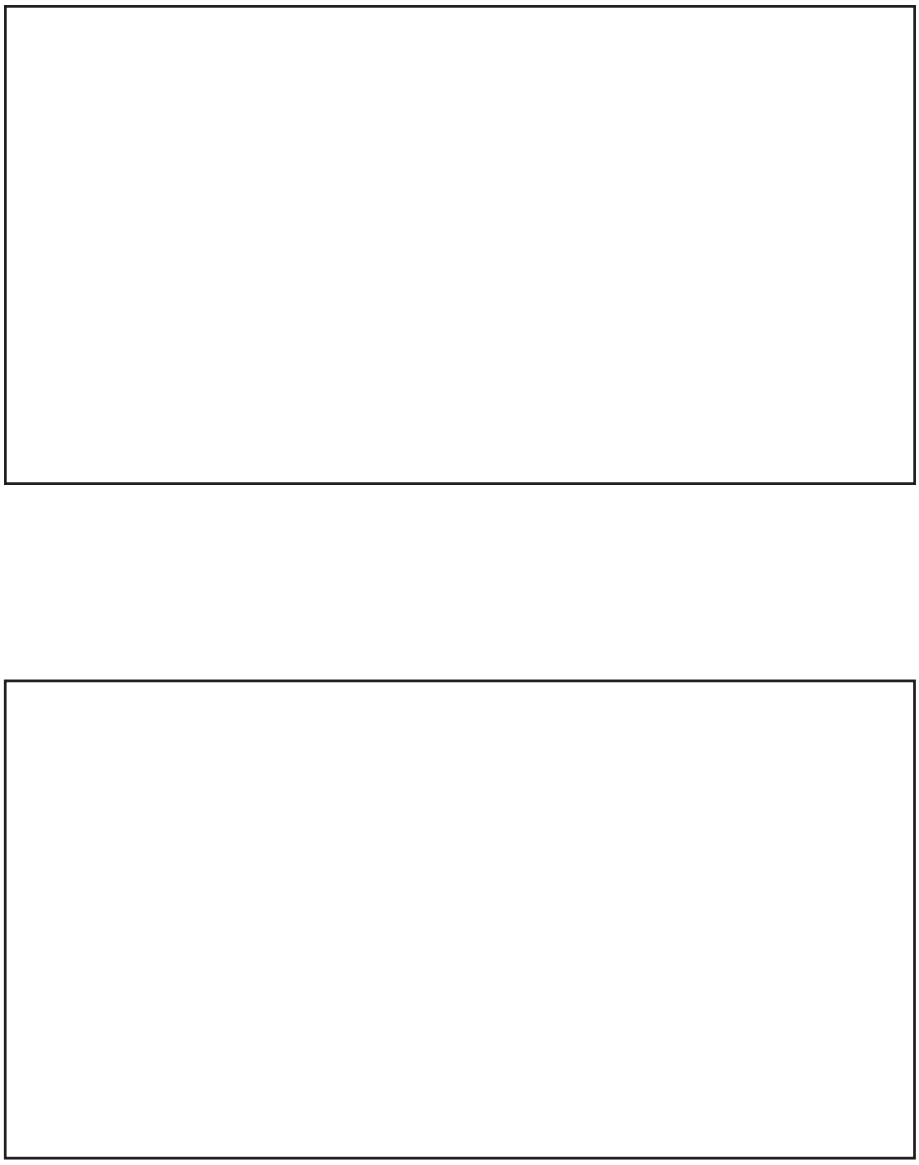

**Fig. 2.** Photographs of pods from genotype SIAL 93 taken in BAL Plantations Sdn Bhd, Tawau, Malaysia (October 1994) (top) and CEPLAC (CEPEC, Ilhéus, January 1995) (bottom).

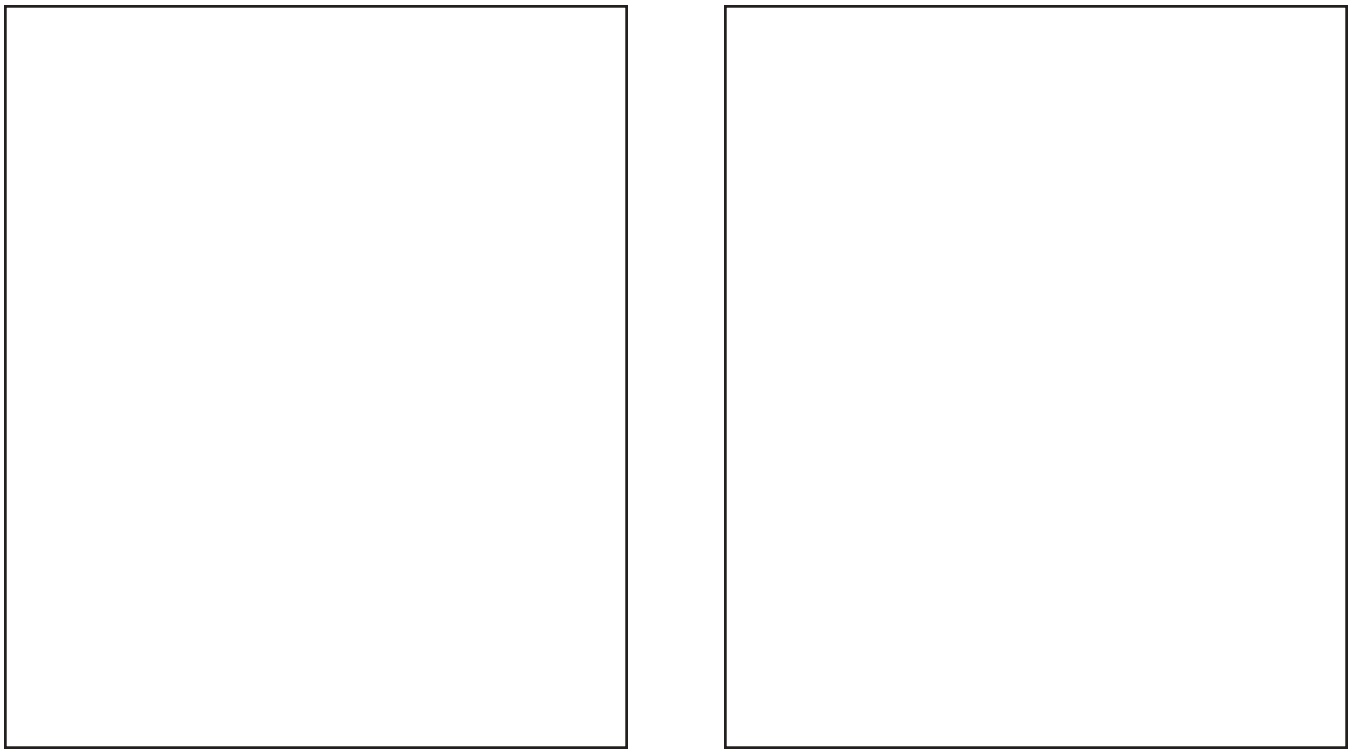

**Fig. 3.** Photographs of pods from genotype AMAZON 2-1 taken in BAL Plantations Sdn Bhd, Tawau, Malaysia (October 1994) (left) and CEPLAC, CEPEC (January, 1995) (right).

**Fig. 4.** Cocoa phenogram generated using UPGMA clustering demonstrating the relationships among genotypes based on RAPD analysis. Bootstrap *P* values are given at the corresponding node for each cluster.

# A Proposed Methodology to Name Newly Collected Cacao

*O. Sounigo*

One quick survey of the ICGD clearly shows that the nomenclature for cacao needs improvement. In fact, all possibilities of confusion exist. It is very common that different cacao populations share the same name. One striking illustration of this situation is with the denomination "P", which indicates:

- cacao clones [1] from unknown origin, formerly held at BAL plantation, Malaysia (before the *oil palm invasion*) and at CATIE (Costa Rica),
- one cacao clone selected in Brazil,
- cacao clones selected in Mexico,
- cacao clones collected by Pound in Peru,
- cacao clones selected in Ghana,
- one cacao clone selected in Venezuela.

Not only do different populations share the same "P" name, but also different clones share exactly the same name:

P 8:
- clone selected in Venezuela, from a cross between OC 73 and SCA 6,
- clone collected by Pound in Peru,
- clone selected in Mexico,
- clone from unknown origin held at CATIE.

P 13:
- clone selected in Brazil, from unknown origin,
- clone collected by Pound in Peru.

Another source of confusion arises when one clone has several names, such as:

EET 3 = UF 650 and EET 11 = SCA 6.

Sometimes, it is not clear if a name designates a clone or a progeny, for example in the case of material collected in French Guyana.

It seems that some standard should be proposed to name cacao clones. In the case of clones selected in research institutes, the code indicating the name of the institute followed by a number is enough, assuming that a different type of name is applied to selected progenies and that every research institute uses a different code.

In the case of clones obtained from collection in the wild or in estates, a proposal is done in such a way that:

- a name would uniquely identify one cacao clone,
- this name would bring as much information as possible, and
- this name would be relatively easy to use.

It is suggested that each name starts with a three letter code to identify the country where the clone has been collected or selected (refer to Table 1 for the proposed standard codes for each country). This should be followed by a number code indicating the year the collection was performed. In cases where two collecting expeditions are performed in the same country during the same year, a letter should be added for each expedition, from the second one. Then, a capital letter code should indicate the mother tree from which budwood or pods were collected. The ordering of mother trees should continue in increasing order, even when material is collected from different populations. If material has been collected on more than 26 mother trees, a second letter will be added, and so on... Nothing would need to be added in the case of clones issued from budwood.

In the case of a clone issued from a seed, a seed number should be added, which should continue in increasing order when seeds are taken from different pods from the same tree. For example, ECU19S45 would identify a clone obtained from seed N°45 of a pod collected on the mother tree named "S", during the collecting expedition performed in Ecuador in 2019. ECU00aT would identify a clone obtained from budwood collected from a mother tree designated "T" during the second collecting expedition performed in Ecuador in 2000. MEX06AB298 would identify a clone obtained from seed N°298, extracted from a pod collected on the mother tree named "AB" during the collecting expedition performed in Mexico in 2006.

It is clear that the proposed scheme for nomenclature does not reveal all the useful information about the clone (the precise area of collection, whether seeds are from the same pod or different pods, whether the material is wild or cultivated...), but adding other codes would make the name much more difficult to deal with. Nevertheless, all this information is usually indicated in a report and communicated to the researchers in charge of the International Cocoa Germplasm Database (ICGD).

The appropriate time to adopt a new nomenclature has to be decided. If the system is adopted very soon, there will be some confusion for clones collected in 1998 and 1999 since the designated *year* codes could refer to 1998 or 2098 and 1999 or 2099, respectively. The solution could be to start using the new nomenclature in the year 2000.

It is suggested that before each collecting expedition, the team in charge of the ICGD is informed. That will enable the collectors to know if a collecting expedition has been performed the same year in the same country, in such a way that they would know how to name their material.

It is also suggested that the already named cacao clones keep their names, since changing them would most probably lead to more confusion.

All suggestions to improve this proposal are warmly welcomed, and it would probably be beneficial if a general agreement on the nomenclature of future cacao clones could be adopted and used by cocoa researchers worldwide.

Please send comments to :
**Olivier Sounigo.**
Researcher from CIRAD-CP (France)
Detached to C.R.U (Trinidad and Tobago).
Email: cru@cablenett.net
Telephone/fax: 1 868 662 8788

[1] In this context, a *clone* is a *genotype*, whether it originates from a seed or from vegetative propagation

**Table 1:** Proposed codes for countries in which cacao is collected.

| COUNTRY | CODE | COUNTRY | CODE |
|---|---|---|---|
| Angola | ANG | Indonesia | INO |
| Belize | BLZ | Ivory Coast | IVC |
| Benin | BNI | Jamaica | JAM |
| Brazil | BRA | Liberia | LIB |
| Cameroon | CMR | Madagascar | MAD |
| Colombia | COL | Malaysia | MAL |
| Congo | CGO | Martinique | MAR |
| Costa Rica | COS | Nicaragua | NCA |
| Cuba | CUB | Nigeria | NIG |
| Democratic Republic of Congo | DCO | Panama | PAN |
| Dominica | DOM | Papua New Guinea | PNG |
| Dominican Republic | DOR | Peru | PER |
| Ecuador | ECU | Philippines | PHI |
| El Salvador | SAL | Saint Lucia | SLU |
| Equatorial Guinea | EQG | Samoa | SMO |
| Fiji | FID | Sao Tome | SAT |
| French Guiana | FRG | Sierra Leone | SLE |
| Gabon | GAB | Solomon Island | SOL |
| Ghana | GHA | Sri Lanka | SRI |
| Grenada | GRE | Suriname | SUR |
| Guadeloupe | GDL | Tanzania | TAN |
| Guatemala | GUA | Togo | TOG |
| Guinea | GUI | Trinidad and Tobago | TRD |
| Guyana | GUY | Uganda | UGA |
| Honduras | HON | Vanuatu | VAN |
| India | IND | Venezuala | VEN |

# Orthotropic (chupon) Budding

*Y. Efron*

The Cocoa and Coconut Research Institute is presently concentrating its breeding efforts on developing hybrid derived clones. Clones are usually propagated by plagiotropic budding and their initial growth is different from a seed derived seedling. They may grow laterally with a variable branching habit depending on the genotype. Therefore, stacking and/or formation pruning is required. Unfortunately, the majority of farmers, particularly small-scale growers, are not familiar with the need and know-how of formation pruning. They may also be reluctant to stack their seedlings.

Budding can also use orthotropic (chupon) buds. The resulting seedling grows upright and forms itself by jorquetting. Stacking and formation pruning are not required. It is expected therefore that these types of seedlings will be familiar to growers and more easily accepted by them.

The major obstacle for orthotropic budding is the availability of budwood. A special budwood garden is required. CCRI is presently looking for the best possible way to establish and manage such a budwood garden. Our limited experience so far has shown a few additional possible problems as follows:

1) Availability of initial source of orthotropic budwood from a plagiotropic budded clone in the absence of the original mother tree. This can be solved by heavy pruning.

2) Unequal dormancy period of orthotropic buds resulting in non-uniform growth of seedlings in the nursery. The problem is somewhat less severe in “Juvenile” budding of two-week-old seedlings. Frequent removal of newly growing rootstock buds, and notching, can assist partially in breaking the dormancy.

3) Orthotropic budded seedlings jorquette very low at about 20-60 cm from the budding point. The jorquette height depends partially on the genotype of the clone and probably also on the root stock.

4) The number of jorquette branches in a budded seedling is usually 3-4.

### Induction of Orthotropic Growth by Heavy Pruning

The initial growth of cacao is orthotropic (chupon) followed by a jorquette of lateral plagiotropic (fan) branches. Generally, chupons produce only chupons and fan branches usually give rise to new fan branches. The same applies to the growth of budded seedlings.

A particular six-year-old clone trial was pruned heavily at the level of secondary main branches following a volcanic eruption in September 1994. A relatively high proportion of the newly growing branches was orthotropic. It was assumed that the stress caused by the volcanic ash and/or the heavy pruning had changed the hormonal balance of the trees to modify the behaviour of some dormant buds.

The effect of heavy pruning was later tested on ten-year-old trees of three clones - K82, KA2-101 (Trinitario) and KEE 43, (Upper Amazonian) that had to be replaced. The trees were heavily pruned at the level of secondary main branches and the newly growing shoots were counted as chupons or fan branches three months later (Table 1). A high frequency of chupons was induced by heavy pruning in all the clones ranging from 10.9% for K82 to 38.9% for KA2-101. The three clones differed also in their rates of recovery from the heavy pruning. The Upper Amazonian clone KEE 43 had produced many more new branches/tree as compared to the two Trinitario clones K82 and KA2-101.

**Table 1:** Frequency (%) of chupon type branches in heavily pruned K82, KA2-101 and KEE 43.

| Clone | No. of branches counted | Av. No. branches/ tree | Percent chupons |
|---|---|---|---|
| K82 | 4,654 | 19.3 | 10.9 |
| KA2-101 | 7,209 | 14.1 | 38.9 |
| KEE 43 | 15,474 | 69.0 | 22.2 |

### High Frequency of Orthotropic Growth in Seedlings Budded from Jorquette Primary Fan Branches

Progenies of 27 crosses introduced from Malaysia were used in an accelerated breeding scheme to shorten the time required for breeding new clones. Three clones - SCA 12, NA 33 and PA 13 were crossed as female with nine male clones (Table 2). The seedlings were planted in a well-shaded field at a very high density of 10,000 trees/ha (2 x 0.5 m) and budded as soon as budwood was available from the primary fan branches of the jorquette. Eight seedlings were budded from each mother plant using two-week-old (juvenile) rootstock.

A high frequency of orthotropically growing seedlings was observed. The number varied between and within crosses. Four seedlings from each clone were selected for planting with different ratios of plagiotropic: orthotropic seedlings per clone (4:0, 3:1, 2:2, 1:3, 0:4) depending on the availability of orthotropic seedlings. Whenever possible, the ratio of 2:2 was preferred. The ratios of 4:0 and 0:4 indicate that all the growing seedlings of the clone were plagiotropic or orthotropic, respectively. Similarly, the 3:1 and 1:3 ratios show that there was only 1 orthotropic or 1 plagiotropic seedling, respectively among the 4-8 growing seedlings of the clone. The ratios of 3:1, 2:2, 1:3 and 0:4 indicated very weak, weak, strong and very strong tendencies, respectively for orthotropic growth. Thus, an Orthotropic Strength Index was calculated for each cross by adding the proportion of each ratio as follows: (3:1) x 1 + (2:2) x 2 + (1:3) x 6 + (0:4) x 10 (Table 2). The index varied between 32 for (SCA 12 x NA 226) to 290 for (PA 13 x PA 300). Among the female

parents, SCA 12 showed the lowest tendency to produce orthotropic growing seedlings and PA 13 the strongest. The difference between the male parents was smaller ranging from 77.3 for NA 226 to 170.3 for ICS 1.

Seedlings of the same crosses were planted in a separate progeny (hybrid) trial. The average jorquette height and even more so the proportion of seedlings with jorquettes higher than 160 cm for the three female parents showed similar tendencies as the Orthotropic Strength Index (Table 3). The average jorquette height of progenies of the male parents was less variable and it was not possible to establish the same pattern of relationship.

The tendency for orthotropic growth is genetically determined whereby some clones, i.e. SCA 12 or NA 226, when used as parents, will have a low frequency of orthotropic growing seedlings and others (PA 13, ICS 1) a high frequency. It may be assumed that the differentiation of the buds into plagiotropic or orthotropic growth is controlled by the concentration of a growth hormone(s). Being close to the jorquette point, the first growing fan branches may have a residual hormonal effect derived from the orthotropic growing stem.

Jorquette height is greatly influenced by the environment, particularly the amount of shade. But it is also under genetic control. The similarity between the Orthotropic Strength Index and the average jorquette height and the proportion of seedlings with jorquettes higher than 160 cm suggests that jorquette height may also be controlled by a hormonal effect.

**Table 2:** Orthotropic Strength Index in progenies of crosses between SCA 12, NA 33 and PA 13 as females and nine different male parents.

| | **Female Parent** | | | **Average** |
|---|---|---|---|---|
| **Male Parent** | **SCA 12** | **NA 33** | **PA 13** | |
| NA 226 | 32 | 59 | 141 | 77.3 |
| QH 441 | 33 | 77 | 137 | 82.3 |
| PBC 123 | 49 | 98 | 185 | 110.7 |
| NA 149 | 39 | 125 | 239 | 134.3 |
| BR 25 | 57 | 94 | 262 | 137.7 |
| KA2-106 | 100 | 154 | 159 | 137.7 |
| PA 300 | 94 | 57 | 290 | 147.7 |
| IMC 23 | 93 | 188 | 198 | 159.7 |
| ICS 1 | 91 | 186 | 234 | 170.3 |
| **Average** | **65.3** | **115.3** | **205.0** | |

**Table 3:** Relationships between Orthotropic Strength Index, and jorquette height in progenies of crosses of SCA 12, NA 33 and PA 13.

| | Female Parent | | |
|---|---|---|---|
| | **SCA 12** | **NA 13** | **PA 13** |
| Orthotropic Strength Index | 65.3 | 115.3 | 205.0 |
| Average Jorquette height | 110.5 | 116.3 | 129.1 |
| Jorquette higher than 160 cm (%) | 4.3 | 7.2 | 18.5 |

The purpose of this note is to share our experience and to exchange ideas with others. Your views and experience will be greatly appreciated.

Please send comments to:
**Y. Efron**
PNG Cocoa & Coconut Research Institute
P O Box 1846
Rabaul, ENBP
PAPUA NEW GUINEA
Fax: + (675) 983 9115
E-mail: ccri@datec.com.pg

# Sampling the Genetic Diversity of Criollo Cacao in Central and South America

J.C. Motamayor*****, P. A. Lopez*, C. Ortiz*, A. Moreno**, J. Zamora*** M. Pinto****, C. Lanaud*****

* *Colegio de Postgraduados de Tabasco, Cardenas, Tabasco, México.*
** *Estacion Experimental de Caucagua, FONAIAP, Venezuela.*
*** *Consejo Nacional del Hule, Villahermosa, Tabasco, México*
**** *Corpoica, Centro de Investigacion Caribia, Sevilla, Magdalena. Santa Marta, Colombia.*
***** *AGETROP/CIRAD, BP5035, 34032 Montpellier, France*

A preliminary study was carried out by the first author on the genetic diversity of Venezuelan Criollo types using DNA markers (Motamayor *et. al.*, 1997). Presently, this study is being enlarged to take into account representatives of the Criollo morpho-geographic group from Central American countries and Colombia. The authors collected material for this study during February and March, 1997 and 1998. This report gives a short description of this collection activity.

In Venezuela (as reported by Reyes, 1992), Criollo trees with different morphological pod types are found exclusively in specific regions (refer to Photo 1) :

1. Porcelana has amelonado type pods finishing in five angled points generally curved. The colour of the pod is white, green or red. It is found in small fields south of the Maracaibo lake.

2. Guasare has rough, green angoleta pods. It was collected at Guajira region in northern Venezuela and Colombia.

3. Andean Criollo has long cylindrical pods, with a smooth to rough surface and green to red colour. It is found in the Andean states of Tachira and Merida.

4. Pentagona has pods showing the pentagona form. This type was found in a small plantation in Tachira.

5. Criollos from the coastal area (Aragua State). These are a mixture of Criollo and Trinitario trees with pods morphologically close to those of Andean Criollo.

In Colombia, we found significant morphological diversity, as in Venezuela, in the Department of Magdalena not far from the border with Venezuela (Tayrona Park). However, the typical Porcelana type was not found in Colombia. On the other hand, the morphotype found in Central America was also present in Colombia, but not in Venezuela.

In Mexico, the Lacandon Forest in the state of Chiapas was visited. We found Criollo cacao close to the Bonampak ruins and along the Lacantun River (in the primary forest). In addition, several trees were sampled near to houses located on the Pacific coast in the state of Michoacan. In the state of Yucatan, we found Criollo cacao presumably cultivated by the Mayas in a " sinkhole " as reported by Gomez-Pompa (1990). In the state of Tabasco, the largest cocoa producing state, only Trinitario trees were observed. The pod morphology of all Criollo type trees observed in Mexico was very similar (see Photo 2), *viz.*, small angoleta pod types (15 cm long in average), shallow furrows, finished in five-angle points and generally curved. Only the roughness of pods and the colour tone of ridges varied slightly.

In Nicaragua, an exhaustive search was conducted along the Pacific coast over a period of fifteen days, but no Criollo trees were found. However, in a mining town called La Libertad de Chontalpa, where there is no cacao cultivation, four trees belonging to the Criollo group were found near to a house. The pods were very similar to those observed in Mexico.

In Guatemala, a collection of cacao germplasm at the " Estacion de Fomento Los Brillantes " was visited. An earlier expedition to the Petén forest (Rivera de Leon, 1989) had

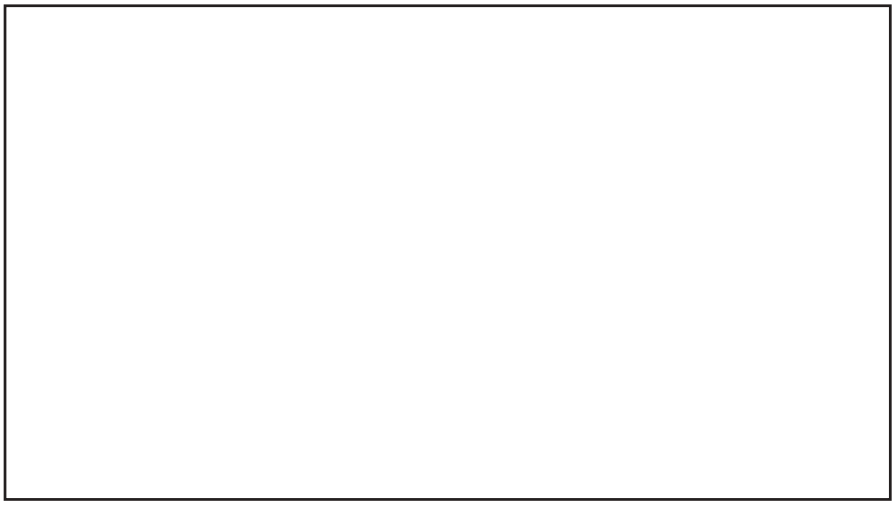

**Photo 1**. Pod morphological diversity of Criollo cacao from the west of Venezuela.

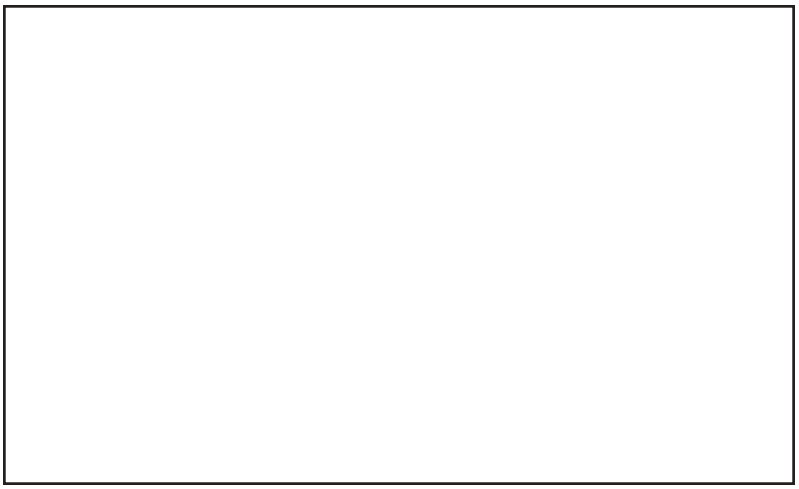

**Photo 2**. Pod morphology of Criollo type found in Mexico.

deposited Criollo germplasm there. However, it seems that this material was lost, as it was impossible to identify Criollo material in the collection during our visit. However, available photographs of the collected Criollo material showed a similar pod morphotype to that observed in Mexico and Nicaragua.

The first conclusion from the present research and collection activity is that pure Criollo types can still be found in South and Central America, either cultivated or in subspontaneous form. This is important, as the survival of pure Criollo types has been questioned until recently. Secondly, it is interesting that the widest morphological diversity inside the Criollo group is presently apparently found in South America (see Photo 1) and not in Central America (Photo 2). In Colombia, the predominant Central American morphotype was also present, but not in Venezuela. Conversely, cylindrical long pods, as those from Colombia and Venezuela were not observed in Central America. These morphological observations appear to contradict the observations and hypothesis about Criollo made by Cuatrecasas (1964), suggesting a Central American origin of this group.

Deforestation and substitution of Criollo trees in Central America may have caused the reduction of the diversity of Criollo in this subcontinent. On the other hand, it should also be considered that the same processes have occurred in South America. Therefore, the larger residual morphological variation found in Venezuela and Colombia would suggest a South American origin of Criollo. Molecular analyses are being performed at CIRAD (France) on the samples collected and the results are expected to give a clearer picture on the origin of this group and its phylogenic relationships inside the species.

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# Overview on the Ecuadorian cacaos

*D. Crouzillat, M. H. Evans and V. Pétiard*

The long reputed Ecuadorian cocoas, derived from "Nacional" cacao, are considered to be fine-flavoured. Cacao cultivation in Ecuador was poorly organised before the Spanish conquest and introduction of cocoa into Europe in the seventeenth century. With the opening of direct trade in the late eighteenth century, Ecuador began expanding cacao plantations, as cocoa became more popular and profitable. Ecuador thus became the world's leading cocoa producer, a position held until early twentieth century with a production of 23,000 tonnes of cocoa. Production continued to increase, and in 1915 reached 50,000 tonnes when Ecuador was second after Ghana. In the 1920s with the arrival of two mayor diseases, Witches' Broom and Moniliasis, production declined steadily until, by 1932, production was 10,000 tonnes (Wood and Lass, 1985). Harvest for the year 1996-97, a normal year, was just under 100,000 tonnes. This year, however, Ecuador's production has been affected by the climatic factor known as "El Niño". The harvest ending September is being estimated at 45,000 tonnes, a drop in production of more than a 50% and, more importantly, an estimated 80% reduction in exports (Sanchez, personal communication). As Ecuador produces half of the world's fine flavoured cocoa, this corresponds to a sharp drop in the availability of the raw material for the resulting specialty product.

The production of fine flavoured chocolate not only depends on genetic factors, but also on the post-harvest fermentation and drying methods used (Anon, 1991, 1994), and the processing in the factory. When the first two of these conditions are met, premium prices for the cocoa beans can be 10 to 40 % and, in exceptional cases, over 100% (Wood, 1978). Nestlé, France uses Nacional cacao to produce its Nestlé Noir and Eclat Noir brands.

The Nacional cacao as a genetic group has been difficult to define since it does not clearly belong to the Forasteros or Criollos. Van Hall (1932) indicated the uniqueness of this group found only in Ecuador. Later Pound (1945) indicated that the group was different from both Forasteros and Criollos. Cheesman (1944) and later Soria (1970) placed the Nacionals within the Forastero group due to their large green pods with rough surfaces and fairly deep ridges. More recently, Enríquez (1993) proposed to place them in the Criollo group due to their general agro-economic characteristics such as those of the seeds, and their flavour since fine flavour cocoas are traditionally placed as Criollo or Trinitario.

From the beginning of the 1930s, the quality of Ecuadorian cocoa has deteriorated due to the introduction of Trinidad

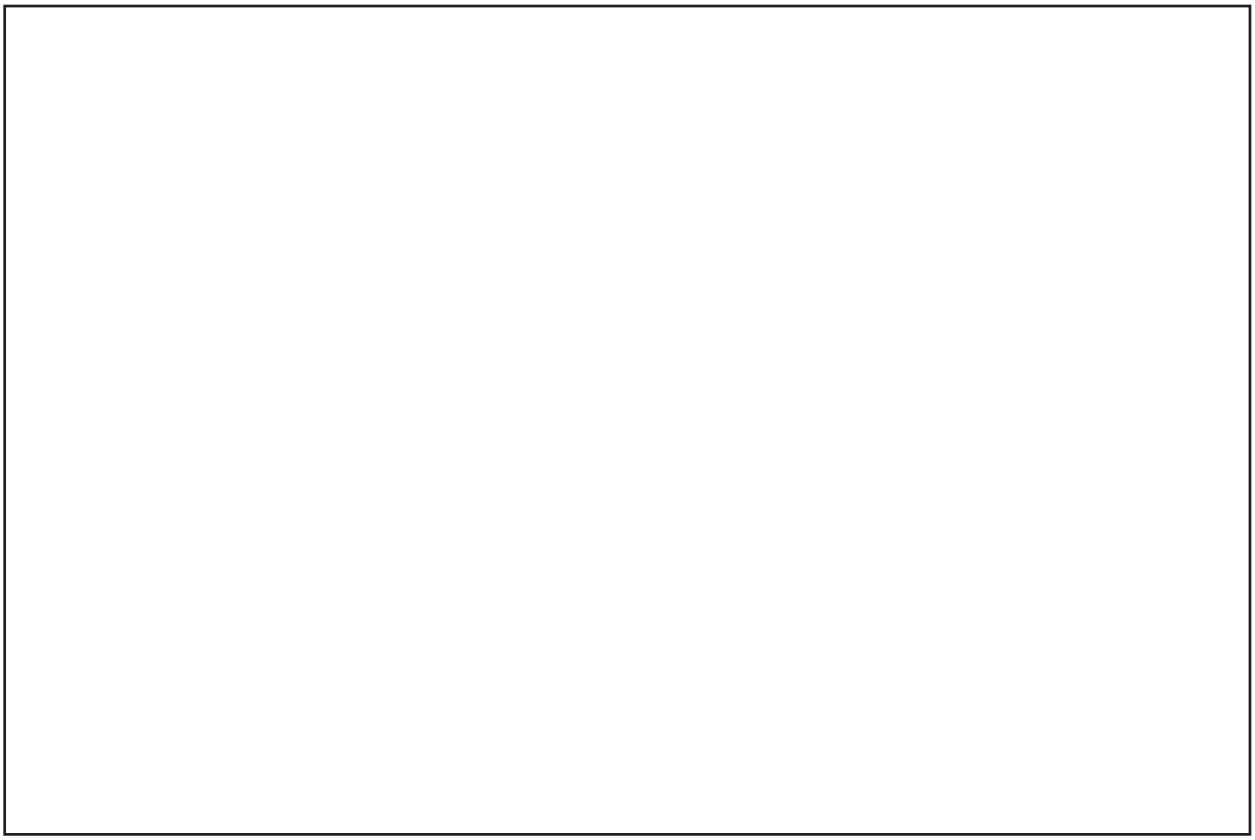

**Figure 1**: Principal Analysis Component based on an RFLP study using 50 RFLP probes and 416 genotypes. The first and second components accounted for 31.3% and 10%, respectively, of the variance for the RFLP analysis. ❑ Ecuador, ◆ Other countries.

clones collected by Pound for resistance to Witches' Broom disease, and lower market prices, which have de-motivated producers from fermenting properly. An increasing number of the trees planted today from seed are hybrids from these clones. This was reflected in ICCO's decision to consider Ecuador as a country that produced only 75% fine flavoured cocoa. Economically, this was a blow to the exporters as the premiums on cocoa prices decreased, and to the industry as good quality becomes harder to find.

Today, the fine cocoa flavour originates mainly from hybrids that continue to carry this trait. In order to assess the current genetic diversity of Ecuadorian material, a survey was carried out using molecular markers.

**Genetic assessment of the Ecuadorian cacaos using RFLP markers**

In order to better determine the genetic origin of Ecuadorian cacaos, 50 RFLP probes were used on 416 different genotypes from several collections (INIAP, INIFAP, CATIE). A total of 140 Ecuadorian clones were tested. Figure 1 shows the results of the Principal Component Analysis. Using these 50 RFLP probes, Figure 1 represents 41.3% of the total variance detected.

At first glance, the genetic diversity of the Ecuadorian material appears to be relatively large in comparison to the other non-Ecuadorian genotypes analysed. However, Ecuadorian diversity can be divided into two groups. The first group contains those clones with a high level of homozygosity (average 5%), an original (native) population with a low genetic diversity. This group is fenced in Figure 1. It is composed of 50 cacaos coming from old plantations. These genotypes represent a part of the original Ecuadorian stock and can therefore be considered pure "Nacional". The other group with a higher level of heterozygosity, and scattered in Figure 1, appears to be the result of the hybridisation between original "Nacional" material and imported genotypes at the beginning of the century. Thus genetic introgression has occurred giving rise to a range of variation between Nacional and hybrid forms. Previous molecular and agronomic studies (Lerceteau *et al.,* 1997a; Lerceteau *et al.,* 1997b) pointed out the specificity of the Nacional group and validated the definition of a natural genetic group as "Nacional". Lachenaud (1997) also validated these findings using Paleoclimatic, Paleogeographic and geobotanical arguments.

**Conclusions**

The use of molecular markers as an early screening tool will become increasingly important in the future due to the capability for testing genetic material while it is still in the nursery. It will also improve the efficiency of the breeders in the recovery of fine cocoa flavour while retaining disease resistance and field production, especially with the selection of the genetic stock used in the creation of new hybrids.

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Send comments to:
**Dominique Crouzillat**
Centre de Recherche Nestlé Tours
101, Avenue Gustave Eiffel
Notre-Dame D'Oé. B.P.9716
37097 Tours Cedex 2


# Negative Selection of Cacao Seedlings Highly Susceptible to *Phytophthora* spp. using the Leaf Disc Test

*Y. Efron and G. Blaha*

Black Pod (BP) caused by *Phytophthora* spp. is of Pan-tropical importance and causes a severe global loss of the cocoa crop. It is also the major constraint for cocoa production in Papua New Guinea. From the growers' perspective, breeding for disease resistance, if successful, is the easiest and most effective way to control a disease in an Integrated Pest Management (IPM) approach. In cocoa, however, much of the work on resistance breeding has given disappointing results (Kennedy *et al.*, 1987). The lack of sensitive and reliable methods for screening a large number of progenies was described as an undoubted cause for the poor progress. However, an inoculation test of detached young leaves or leaf discs was developed recently in France (Nyassé *et al.*, 1995) and Trinidad (Iwaro *et al.*, 1997). The test is based on the histological similarity of the lower leaf and pod surfaces. Results of inoculation tests of the two tissues are well correlated. It is a fast and inexpensive test that gives the breeder an opportunity to screen large breeding populations for BP resistance at an early seedling stage.

The severe problems presented by diseases have had major influences on breeding objectives. All too often, the search for resistance has been so dominant as to become the only objective and an end in itself, rather than a way to sustain high yield at an acceptable production cost (Kennedy *et al.*, 1987). Thus breeding for disease resistance should be an important component of breeding, but only as an integral part of a comprehensive breeding programme.

An accelerated breeding scheme to shorten the time required for breeding new cacao clones was proposed by Efron *et al.*, (1996). Starting with a large number of seedlings, population size is gradually reduced by negative selection. The proposed scheme, which is described in this article, is presently being implemented in PNG.

## Materials and Methods

Seeds of 29 crosses (Table 1) were produced at BAL Plantations, Malaysia and introduced into PNG in November, 1996. The seeds were planted initially in a quarantine screen house and transferred after four months to a shaded nursery for an additional three months. They were later transferred to a well-shaded field at a very high density of 10,000 seedlings/ha (2.0 x 0.5 m). Leaf disc testing was started in October, 1997 and ended in February, 1998. The number of seedlings varied between families (crosses) depending on the availability of seeds (Table 1). In total, 5,864 seedlings were tested.

Leaves from 320 seedlings, (20 seedlings from 16 families) were collected for testing twice a week. The selection of families was done randomly. One healthy leaf from a semi-hard, dark green portion of the stem was collected from each seedling early in the morning. Four discs of 1.5 cm diameter were cut from each leaf and placed on a sterile sponge soaked with distilled water in a deep baking tray (2 discs/plant/tray x 2). A total of 160 discs were placed in each tray (10 columns, 16 rows). The trays were covered with a plastic bag and left overnight in the dark at 24± 2°C.

The next day, zoospore suspensions of local *Phytophthora* spp. strain (approximately $2 \times 10^5$ z/ml) were prepared from artificially infected pods of the susceptible clone KA2-101 that were aseptically inoculated five days earlier in the laboratory and kept in the dark in a tray covered with plastic. Inoculation of the leaf discs was performed by placing 10μml of zoospore suspension at the centre. Assessment for reaction to the disease was done visually by two persons at three and five days after inoculation based on the following scale:

1 = few dark penetration points/dots
2 = many points with network
3 = necrotic network/oily appearance
4 = marbled necrosis with brownish centre
5 = brown/black necrosis

Three criteria were used to compare between families:

1. Percent rejected plants/family
2. Percent highly resistant seedlings selected per family
3. Average score of all seedlings tested/family at three and five days after inoculation.

## Results

Twenty-one tests were done during the period from 31/10/97 to 3/2/98. The average score of all tests at three and five days after inoculation was 3.03. However, the daily average fluctuated between 2.78 and 3.51. Two tests had a mean score below 2.50. The variability between tests was probably due to differences in the zoospore concentrations applied. As expected, the scores at three days after inoculation were lower than at day five by an average of 0.53 units of the 1-5 scale (2.77 and 3.30, respectively). This difference was very uniform in all the tests.

Rejecting seedlings highly susceptible to BP, as judged by the leaf disc test, was the major purpose with a pre-fixed target of 25-30%. The cut-off point varied between tests based on the average test score. In addition, when the average test score was low, the rejection was based on the scores taken five days after inoculation, while the scores taken after three days were the basis for rejection for the tests with high average scores. As a result, 28.8 per cent of the seedlings were rejected. The level of rejection varied between families (Table 1-b). The lowest percentage of seedlings was rejected from the progenies of PA 13 x NA 226 and PA 13 x NA 149 (21.9 and 22.3%, respectively) while the highest proportions were rejected from the families NA 33 x NA 226 and NA 33 x PBC 123 (36.3 and 35.8%, respectively). Among the female parents, the highest proportion of seedlings was rejected from progenies of NA 33. Similarly, more plants were rejected when PA 300, NA 226, and PBC 123 were used as male parents.

The range of highly resistant seedlings selected (Table 1-C) was between 2.8% (NA 33 x PA 300) to 12.7% (SCA 12 x QH 441). There were more highly resistant seedlings in crosses with SCA 12 and PA 13 as compared with NA 33.

Among the male parents, the highest proportion of highly resistant seedlings came from progenies of QH 441, KA2-106 and IMC 23 while the lowest was from progenies of PA 300, PBC 123, and NA 226.

Families varied also in their average score (Table 1-d). The lowest were found in the crosses PA 13 x NA 226 (2.61) and PA 13 x NA 149 (2.68) and the highest in progenies of NA 33 x NA 226 (3.48) and SCA 12 x PA 300 (3.31). NA 33 and PA 13 had the highest and lowest average scores, respectively among the female parents. PA 300, NA 226 and PBC 123 had the highest scores as male parents, while ICS 1 and NA 149 produced the lowest score.

**Discussion**

The leaf test, recently developed (Nyassé et *al*., 1995; Iwaro *et al*., 1997) was used at CCRI to test the reaction of a large number of cacao seedlings to *Phytophthora* spp. as the first step to reduce population size by negative selection. Seedlings showing the most susceptible reaction in each testing set (inoculation date) were rejected in the field (about 25-30%). Seedlings showing the highest resistance level, less than an average score of 2.0 at day five after inoculation, were marked separately in the field. Few very susceptible seedlings with an average score of 5.0 at day three were also maintained for a future follow-up under field conditions. It is probably the first example of large-scale practical application of the leaf disc test in a cocoa breeding project.

Encouraging results obtained on a limited number of genotypes showed that field resistance can be correlated with results from resistance leaf disc tests, particularly the ranking (Nyassé *et al*., 1996). The authors concluded that the leaf screening test can be used to increase the level of intrinsic resistance to BP in cacao populations. However, since the method was developed only recently and tested on a limited number of genotypes, caution is required in its practical use in breeding programmes. In addition, the test may not detect all the possible resistance/susceptibility mechanisms related to BP caused by *Phytophthora* spp. and the genes involved. Therefore, a rejection level of 25-30% of seedlings showing the most severe symptoms was set before initiating the test. Assuming random segregation of all other genes, unless closely linked, the most susceptible plants based on the leaf disc test would be eliminated without losing genotypes that may show other possible resistance mechanisms since they will be present at the same frequency within the remaining 70-75% of the plants.

The search for resistance should not become the only objective of a breeding project and an end in itself ( Kennedy *et al*., 1987). It should never be divorced from yield potential (Zadoks, 1996) or other required agronomic characteristics (Van der Vossen, 1996). Efron *et al*., (1996) had proposed that breeding for resistance should be addressed by an Integrated Resistant Breeding Approach (IRBA). Accordingly, the negative selection value of 25-30% was determined to ensure that highly productive trees with desirable agronomic characteristics would be maintained in a sufficient frequency within the remaining population.

Screening for the purpose of negative selection was the main objective of the work described in this paper. Considering the large number of seedlings, it was possible to test only four discs from one leaf/ seedling without replications. Some inaccuracies due to different leaf age (Nyassé *et al*., 1995) or inoculation procedures were expected but acceptable considering the magnitude and nature of the work. Attempts were made to reject approximately the same number of plants from each one of the 29 families tested. Since the daily test average fluctuated, the cut-off point was modified accordingly. In this way, a desired level of 28.8% rejection was maintained. However, different proportions of plants were rejected from the 29 crosses ranging from 22.3-36.3%, suggesting differential family response to artificial inoculation of the leaf discs and possibly different levels of resistance/susceptibility to *Phytophthora* spp. Information on the 14 parental clones can also be obtained. Thus among the female parents, PA 13 showed the highest resistance level followed by SCA 12 while NA 33 was the most susceptible. The same ranking was obtained with the average score test and the percentage of selected seedlings showing highly resistant reactions. Among the male parents, PA 300, NA 226 and PBC 123 showed the highest susceptibility while ICS 1 and NA 149 showed the highest resistance levels in hybrid combinations as judged by the average family scores and the percentage of plants rejected.

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**Table 1:** Number of seedlings tested (a), percent rejected (b), percent highly resistant selected (c) and average score (d) of 29 families tested for *Phytophthora* spp. resistance/susceptibility by the Leaf disc test.

Number of Seedlings

| Male Parent | Female Parent | | | | | | | | | | | | Total/Average | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NA33 | | | | SCA 12 | | | | PA 13 | | | | a | b | c | d |
| | a | b | c | d | a | b | c | d | a | b | c | d | | | | |
| IMC 23 | 303 | 27.7 | 9.9 | 2.85 | 141 | 28.4 | 9.9 | 3.04 | 153 | 26.8 | 8.5 | 2.90 | 597 | 27.6 | 9.4 | 2.93 |
| PA 173 | 173 | 28.3 | 9.2 | 3.01 | - | - | - | - | - | - | - | - | 173 | - | - | - |
| KA2-106 | 236 | 33.1 | 7.2 | 3.24 | 135 | 28.9 | 11.1 | 3.02 | 149 | 27.5 | 11.4 | 2.97 | 520 | 29.8 | 9.9 | 3.08 |
| PA 107 | 326 | 28.8 | 7.1 | 2.98 | - | - | - | - | - | - | - | - | 326 | - | - | - |
| ICS 1 | 231 | 27.7 | 7.4 | 2.87 | 204 | 23.5 | 7.4 | 2.81 | 254 | 26.8 | 9.4 | 2.93 | 689 | 26.0 | 8.1 | 2.87 |
| BR 25 | 327 | 30.6 | 8.0 | 3.14 | 340 | 30.2 | 8.5 | 3.01 | 86 | 25.6 | 9.7 | 3.08 | 753 | 28.8 | 8.4 | 3.08 |
| NA 149 | 194 | 24.7 | 8.8 | 3.05 | 236 | 28.0 | 9.7 | 2.95 | 233 | 22.3 | 8.2 | 2.68 | 663 | 25.0 | 8.9 | 2.89 |
| QH 441 | 161 | 29.2 | 8.7 | 3.01 | 142 | 31.0 | 12.7 | 2.98 | 146 | 28.8 | 9.6 | 2.86 | 449 | 29.7 | 10.3 | 2.95 |
| NA 226 | 157 | 36.3 | 5.1 | 3.48 | 299 | 34.1 | 7.4 | 3.23 | 105 | 21.9 | 10.5 | 2.61 | 561 | 30.8 | 7.7 | 3.11 |
| PA 300 | 213 | 31.9 | 2.8 | 3.22 | 161 | 30.4 | 7.5 | 3.31 | 141 | 32.6 | 8.5 | 3.21 | 515 | 3.16 | 6.3 | 3.25 |
| PBC 123 | 106 | 35.8 | 6.6 | 3.23 | 225 | 27.1 | 8.4 | 2.99 | 287 | 28.9 | 6.6 | 3.07 | 618 | 30.6 | 7.2 | 3.10 |
| **TOTAL** | **2,427** | **30.4** | **7.3** | **3.10** | **1,883** | **29.1** | **9.2** | **3.04** | **1,554** | **26.8** | **9.0** | **2.92** | **5,864** | **28.8** | **8.5** | **3.02** |

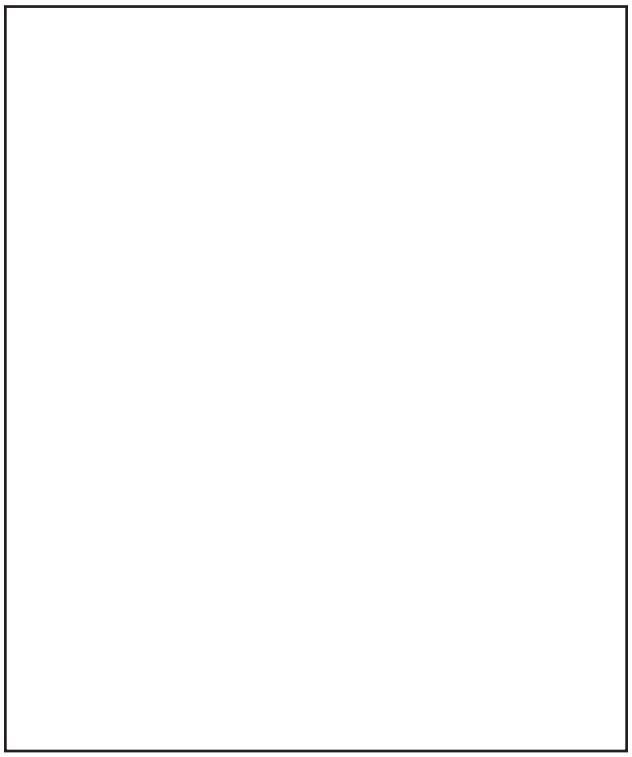

**Photo 1**. High density planting for leaf sampling and subsequent budding of non-rejected plants

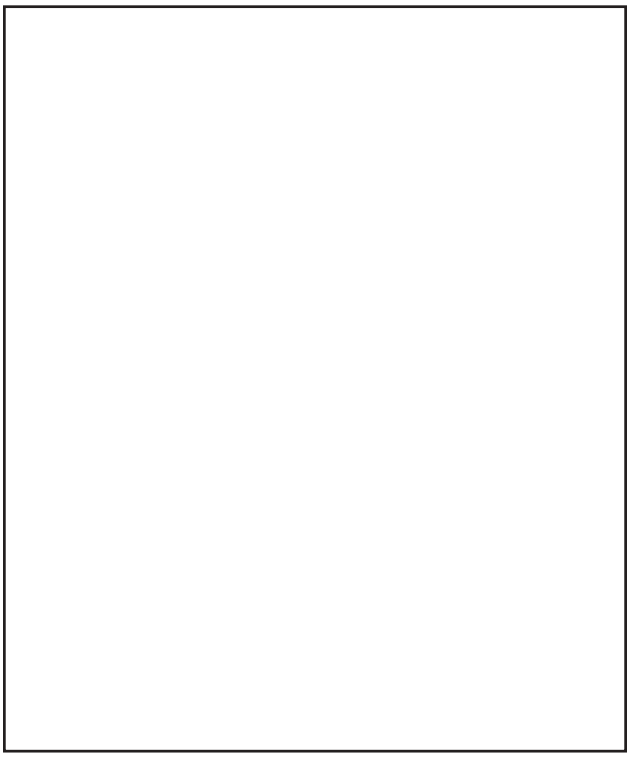

**Photo 2**. Differential reaction of leaf discs to inoculation by *Phytophthora spp.* zoospore

# Some Recent Research Findings from Brazil

L.A.S. Dias

Our investigations began with the analysis of a 5 x 5 complete diallel involving only self-compatible, lower Amazon Forastero cacao clones from Brazil ('SIAL 169', 'CEPEC 1' and 'SIC 19'), and Trinitario groups from Trinidad ('ICS 1'), and Costa Rica ('CC 41'). We addressed some major questions in cacao breeding, using the results from the analysis of that diallel, such as:

* Is the inheritance of yield components predominantly additive or dominant?
* Is it possible to examine the racial group classification using multivariate genetic divergence measures?
* Is it possible to assess superior cacao hybrids using a more scientific criterion, as those based on multivariate genetic divergence of parents?
* Is the divergence stable over time?
* What is the minimum period to conduct successive harvesting for evaluation of cacao genotypes?
* Are the multivariate methods of divergence equally consistent and in agreement with one other?

Answers to these questions can be found in the following articles:

**L.A.S. Dias & P.Y. Kageyama (1995).** Combining-ability for cacao (*Theobroma cacao* L.) yield components under southern Bahia conditions. ***Theoretical and Applied Genetics* 90(3/4): 534-41.**

The objective of this study was to assess five cacao cultivars (selfs) and 20 hybrids with regard to their general and specific combining abilities for yield components using method 1, model I, of the diallel analysis system. The selfings and the hybrids were obtained through controlled crossings, tested in the field in a random block design with four replications and plots containing 16 plants. The experiment was set up in the Centro de Pesquisas do Cacau, in Itabuna, Bahia, Brasil, in 1975. The characteristics studied were: the number of healthy and collected fruits per plant (NHFP and NCFP), the wet weight of humid seeds per plant and per fruit (WHSP and WHSF), and the percentage of diseased fruits per plant (PDFP), for 5 years (1986-1990). The F-test values, highly significant for general combining ability (GCA) and specific combining ability (SCA), demonstrated the existence of variability for both effects. However, the effects of SCA were greater than those of GCA, when compared in terms of average squared effects. This condition held for the characteristics NHFP, NCFP and WHSP, and demonstrates the relative importance of the non-additive genetic effects over the additive effects. The reciprocal effects were not significant. Breeding methods, which explore the additive portion of genetic variance, should be employed for obtaining higher-yielding cacao and high seed weight. For this, the segregant populations should involve cultivars CEPEC 1, SIAL 169 and ICS 1. Combinations involving the cultivar ICS 1 presented the most favourable results for the characteristics WHSP and WHSF, where the hybrid SIAL 169 x ICS 1 and its reciprocal were outstanding.

**L.A.S. Dias & P.Y. Kageyama (1997a).** Multivariate genetic divergence and hybrid performance of cacao (*Theobroma cacao* L.). ***Brazilian Journal of Genetics* 20(1): 63-70.**

Genetic distances among cacao cultivars were calculated through multivariate analysis, using the $D^2$ statistic, to examine the racial group classification and to assess heterotic hybrids. A 5 x 5 complete-diallel was evaluated. Over a five-year period (1986-1990), five cultivars of $S_1$ generation, pertaining to the Lower Amazon Forastero and Trinitario racial groups and 20 crosses between the corresponding $S_0$ Parents were analysed, based upon five yield components - number of healthy and collected fruits per plant (NHFP and NCFP), wet seed weight per plant and per fruit (WSWP and WSWF), and percentage of diseased fruits per plant (PDFP). The diversity analysis suggested a close relationship between the Trinitario and Lower Amazon Forastero groups. The correlation coefficient ($r$) was calculated to determine the association between genetic diversity and heterosis. Genetic distance of parents denoted by $D^2$ was found to be linearly related to average performance of hybrids for WSWP and WSWF ($r = 0.68$, $P < 0.05$ and $r = 0.76$, $P < 0.05$, respectively). The heterotic performance for the same components was also correlated with $D^2$, both with $r = 0.66$ ($P < 0.05$). A relationship between genetic divergence and combining ability effects was suggested because the most divergent cultivar exhibited a high general combining ability, generating the best performing hybrids. Results indicated that genetic diversity estimates might be useful in selecting parents for crosses and in assessing relationships among cacao racial groups.

**L.A.S. Dias & P.Y. Kageyama (1997b).** Temporal stability of multivariate genetic divergence in cacao (*Theobroma cacao* L.) in Southern Bahia conditions. ***Euphytica* 93(2): 181-87.**

The stability of the genetic divergence among five non-commercial cacao cultivars of advanced age was investigated over a five-year period (1986-1990). Cluster analysis was performed on five yield components measured on harvests from each crop year and on the data pooled over five years. The temporal stability was assessed by using a clustering procedure, which involved the calculation of the Mahalanobis distance ($D^2$) and of Tocher's algorithm applied to the distance matrix. The comparison of $D^2$ and of clusters based upon pooled analysis, and taken as the standard, with $D^2$ values and clusters obtained from each year showed a stable clustering pattern in the most favourable year. For the rest of the years, inconsistency in number and composition of clusters formed was observed. An optimum environment was shown to be important for better expressing divergence by $D^2$. Consequently, the evaluation of the genetic divergence among the studied cultivars may be conducted based on a single favourable year. This will result in considerable savings in manpower, financial resources, and time which would be wasted should the evaluation be extended for several years.

**L.A.S. Dias & P.Y. Kageyama (1998a).** Repeatability and minimum harvest period of cacao (*Theobroma cacao* L.) in Southern Bahia. ***Euphytica* 102: 29-35.**

Classically, selection for superior genotypes in cacao has been based on the successive harvest records across a number of years. Little information on the minimum duration of these harvest periods is available in the literature. The repeatability coefficient (rho) was used to estimate this period. Twenty-five cacao genotypes were assayed in a randomized block design with four replications and 16-plant plots. The following yield components were studied: number of healthy fruits per plant, number of collected fruits per plant, wet weight of seeds per plant and per fruit, and percentage of diseased fruits per plant, over 5 years (1986-90). Repeatability estimates were higher than 0.84 for all components, except percentage of diseased fruits per plant (rho equal 0.41). With such estimates, it is possible to select genotypes on the basis of only two years of successive harvests, with a determination coefficient of 90%. The advantages of applying the repeatability coefficient to the cacao breeding programme are discussed.

**L.A.S. Dias & P.Y. Kageyama (1998b).** Comparison between multivariate methods applied for the evaluation of genetic divergence in cacao (*Theobroma cacao* L.*).* ***Brazilian Archives of Biology and Technology*** (in press).

Several multivariate methods have been used in divergence analyses of populations. Consistency and relative association among four methods was assessed using a 5 x 5 complete-diallel data involving cacao cultivars. Over a 5-year period, five cultivars were analysed based upon five yield components - number of healthy and collected fruits per plant (NHFP and NCFP), wet weight of seeds per plant (WHSP) and per fruit (WHSF), and percentage of diseased fruits per plant (PDFP). In assessing the divergence of parents only the data obtained from five cacao cultivars were analysed. Four multivariate statistics presented close association when considered in pairs, in this case the Mahalanobis' ($D^2$) with the mean Euclidean distance obtained from canonical variates ($d_{cv}$), and mean Euclidean distance ($d_e$) with the mean Euclidean distance obtained from principal components ($d_{pc}$). In both cases, high correlations ($r > 0.95$) were obtained. However, a weak association was detected between $D^2$ and $d_e$ and between $d_{pc}$ and $d_{cv}$ (0.50 and 0.66, respectively). Thus, in studies on genetic divergence, statistics considering the error variance-covariance matrix should be preferred whenever its estimate is possible.

The performance of the hybrid in comparison to that of unimproved local cultivars was also studied. In a yield comparative trial set up in a 5 x 5 Latin square design, the relative performance of five cacao cultivars - 'Maranhão', 'Pará', 'Parazinho' (unimproved local cultivars), and open-pollinated 'ICS 1' and the commercial Hybrid (improved cultivars) was investigated. Our objective was to answer to some other questions such as:

* Is the hybrid superior in performance to the local cultivars?
* Under which conditions are the improved materials more temporally stable in comparison to those unimproved materials?
* What is the minimum period of successive harvests for evaluation of cacao genotypes?
* Are those materials distinct from each other?

The following articles answer these questions.

**L.A.S. Dias, C.A.S. Souza, S.G. Augusto, P.R. Siqueira, M.W. Müller (1996).** Desempenho de cultivares de cacau com relação a alguns componentes de produção em Linhares-ES, Brasil. {Performance of cacao cultivars in relation to some yield components in Linhares-ES, Brasil}. **In*: Proc. of the XII International Cocoa Research Conference* (in press).**

The presumed yield superiority of improved cultivars compared to that of unimproved local cultivars has been questioned by cacao growers. Thus, the relative performances of five cacao cultivars in a yield comparative trial were investigated at the Estação Experimental 'Filogônio Peixoto' (ESFIP), in Linhares, ES, Brasil. 'Maranhão', 'Pará', 'Parazinho' (unimproved local cultivars) and open-pollinated 'ICS 1' and the commercial Hybrid (improved cultivars) were evaluated in a 5 x 5 Latin square design containing 196-plant plots, over 10 years (1984-93). The number of healthy fruits per plant (NHFP) and the wet seed weight per hectare (WSWH) and per fruit (WSWF) were the yield components evaluated. Analyses of variance were performed in Latin square for each year and for the accumulated yield components over the decade. Joint analyses were performed on split plots in time, allocating the Latin square in plots, and years in sub-plots. Expressive genetic variability among cultivars for all yield components in the analyses involving annual yield and accumulated yield over the decade was revealed. Genotypic determination coefficient estimates ($\hat{h}$) were expressive and variable over the years due to variation in the genotypic expression of cultivars. Yield averages exhibited trends of increase with age of cacao trees and extensive variability among years, especially to NHFP and WSWH. The local adverse climatic conditions, particularly water deficit, resulted in large yield reductions. Latin square analyses on split plots in time showed that years constituted the main source of variation. Cultivar-year interaction occurred only with WSWH. The need to use improved cultivars in the plantation was reinforced, since the Hybrid and ICS 1 presented an outstanding performance for WSWH and WSWF, respectively, especially in the years with regular rainfall distribution.

This superior performance was due to the increase in WSWF, generating higher yield per area. In the case of perennial crops, the benefits from the use of improved hybrid cultivars are durable and without any cost to the growers since the seeds of these hybrids are provided by the Centro de Pesquisas do Cacau.

**L.A.S. Dias, C.A.S. Souza, S.G. Augusto, P.R. Siqueira, M.W. Müller (1996).** Estabilidade temporal de cultivares de cacau com relação à produção em Linhares-ES, Brasil. {Temporal stability of cacao cultivars in relation to yield in Linhares-ES, Brasil}. **In: *Proc. of the XII International Cocoa Research Conference* (in press).**

Great fluctuations in productivity of cacao trees have been verified in different regions and years, making difficult the recommendation of cultivars. In Linhares, due to climatic adversity, particularly water deficit, there is a need to select genotypes stable over time. Thus, the adaptability and temporal stability of five cacao cultivars were investigated at Estação Experimental 'Filogônio Peixoto' (ESFIP), in Linhares, ES, Brasil. 'Maranhão', 'Pará', 'Parazinho' (unimproved local cultivars) and open-pollinated 'ICS 1' and the commercial Hybrid (improved cultivars) were evaluated in a 5 x 5 Latin square design containing 196-plant plots, over 10 years (1984-93). The wet seed weight per hectare (WSWH) was the yield component evaluated, for which cultivar-year interaction was found. Adaptability and stability analyses, applied to describe the performance of cultivars over a series of favourable and unfavourable years, were performed by using segmented bilinear regression. This methodology revealed that the improved cultivars - ICS 1 and Hybrid - were the most fit and stable. Hybrid and ICS 1 were also identified as the cultivars with the best performance, suggesting that the genetic improvement used to increase productivity also contributed to a larger cultivar stability. This fact reinforced the importance of using improved cultivars in the planting. ICS 1 was the most responsive to improvement of the years ($\beta_{1i} + \beta_{2i} = 1.85$). The hybrid showed good stability during the unfavourable years ($\beta_{1i} = 1$), associated with the highest mean productivity during these years and over the decade. However, it was not responsive during the favourable years ($\beta_{1i} + \beta_{2i} = 1$). Since the hybrid presented general adaptability, its use can be recommended for an extensive period of years. The local cultivars presented lower mean productivities during the decade and the unfavourable years, and were less responsive to improvement over the years ($\beta_{1i} + \beta_{2i} < 1$). Another methodology of phenotypic adaptability and stability analysis was also applied to investigate the contribution of each cultivar to the overall interaction. The hybrid-year interaction (2.9%) was lower than the interactions of the other cultivars. Data on temporal stability are important for the cacao grower interested in obtaining income stability by exploring a stable cultivar over the years. This information will also be useful in the future, whenever recommendations of cultivars take into consideration heterogeneity over the years, for a given region.

**L.A.S. Dias, C.A.S. Souza, S.G. Augusto, P.R. Siqueira, M.W. Müller (1996).** Evaluation of cacao cultivars in Linhares, Brasil: minimum harvest period. ***Brazilian Journal of Genetics* 20(1): 206.**

The research interest in cacao breeding is evaluating, as early as possible, the genetic potential of a cultivar. This has been done based on successive harvest records, for some years. Nevertheless, little information on the minimum duration of these harvest periods is available in the literature. Thus, the relative performances of five cacao cultivars in a comparative yield trial were investigated. 'Maranhão', 'Pará', 'Parazinho' (unimproved local cultivars) and open-pollinated 'ICS 1' and the commercial Hybrid (improved cultivars) were evaluated in a 5 x 5 Latin square design containing 196-plant plots, over 10 years (1984-93). The number of healthy fruits per plant (NHFP) and the wet seed weight per hectare (WSWH) and per fruit (WSWF) were the yield components evaluated. The evaluation was performed on these three yield components measured on harvests from each agricultural year and on the data pooled over ten years. The combination of the first six and eight years of successive harvests for NHFP and WSWH, respectively, showed this to be necessary for cultivar evaluation, when correlation analyses between annual yields and cumulative yield over the decade were performed. For WSWF, a single year proved to be sufficient. Extensive variability, superior performance and cultivar trends towards presenting interaction with post-climax years indicated that evaluation should be conducted in Linhares cacao trees after the 8th year from planting.

**L.A.S. Dias, C.A.S. Souza, S.G. Augusto, P.R. Siqueira, M.W. Müller (1996).** Evaluation of cacao cultivars in Linhares, Brasil: distinctness analysis. ***Brazilian Journal of Genetics* 20(1): 206.**

Distinctness among five cacao cultivars set up in a yield comparative trial was investigated. 'Maranhão', 'Pará', 'Parazinho' (unimproved local cultivars) and open-pollinated 'ICS 1' and the commercial Hybrid (improved cultivars) were evaluated in a 5 x 5 Latin square design containing 196-plant plots, over 10 years (1984-93). The number of healthy fruits per plant (NHFP) and the wet seed weight per hectare (WSWH) and per fruit (WSWF) were the yield components evaluated. This evaluation was performed on these three yield components measured on harvests from agricultural years. The distinctness of cultivars was studied through multivariate analysis, using $D^2$ statistic of Mahalanobis. $D^2$ is a robust measure of difference between two cultivars since it allows for both different scales of measurement and correlations between the characters. The critical $D^2$ value required for distinctness has been generalized from the $T^2$ statistic of Hotteling, at the 1% probability level. A significance test validates the null hypothesis that the cultivar means are equal over all characters to be tested against the alternative by which the cultivar means are different and hence the cultivars are distinct. The $D^2$ values among the five cultivars

ranged from 0.62 between Parazinho and Pará to 63.62** between Pará and ICS 1. Maranhão and the commercial Hybrid were very close ($D^2$ = 2.12*), demonstrating that the improved cultivar retains genes of the unimproved local cultivar in high proportion. Maranhão and Pará were shown to be distinct cultivars ($D^2$ = 6.69**) while Parazinho, derived from Pará, was not confirmed to be one.

The cacao populations from Bahia are considered homogeneous and uniform. Is this true or not? The answer is found in this article:

**L.A.S. Dias, P.Y. Kageyama & G.C.T. Castro (1997).** Divergência fenética multivariada na preservação de germoplasma de cacau (*Theobroma cacao* L.). {Multivariate phenetic divergence in germplasm preservation of cacao (*Theobroma cacao* L.)}. ***Agrotrópica* 9(1): 29-40.**

The evaluation of local populations is an important phase of the cacao breeding programme. Therefore, the multivariate phenetic divergence among SIC and SIAL series clones was quantified by cluster and principal components analyses. These Forastero Amazon clones were selected in the populations from Bahia and set up at the CEPEC's germplasm bank. Quantitative descriptor data consisted of mean vectors of 13 fruit and seed traits measured in 26 clones. SIC 17 and SIAL 244 clones showed the highest divergence (3.05), while SIC 18 and SIC 765 clones formed the most similar pair (0.33) according to the observation of the Euclidean distance matrix. Tocher's and single linkage methods applied to this matrix identified four clusters. Cluster I was composed of 16 clones, cluster II was formed by 8 clones, and the clusters III and IV were composed of a single clone each. The maximum Euclidean distance interclusters were recorded between clusters II and IV (2.66), and between III and IV (2.34). Cluster IV, formed by the SIAL 244 clone, combined the highest distance with the best performance.

The plot from the first two principal components (71.4% of the total variance) coincided with the clusters formed by Tocher's method. The presence of divergence among local clones contradicted the uniformity paradigm of cacao populations from Bahia; suggesting that selection and breeding in such populations are feasible. Among 13 traits analysed, four were discarded (31%) due to redundancy, without loss of information. The multivariate methods proved to be useful for identifying heterotic groups, duplicate accessions, and optimizing germplasm collections of cacao.

In the following article, the author revised the literature of the modern techniques introduced into cacao research and discussed their impact on the cacao breeding. There are some questions to ask in this regard:

* Are biotechnological methods more suitable for application in cacao producer countries?
* Is cacao breeding sufficiently flexible to accommodate such techniques?

**L.A.S. Dias (1995).** Biotecnologia e melhoramento genético do cacaueiro (*Theobroma cacao* L.) {Biotechnology and cacao (*Theobroma cacao* L.) breeding}. ***Agrotrópica* 7(1): 1-14.**

Biotechniques recently introduced into cacao research have been examined. Implications of technological advances in cacao breeding are discussed in some detail. The techniques considered are: *in situ* hybridization, production of dihaploids, tissue culture, electrophoresis of isoenzymes, RFLP and RAPD. *In situ* hybridization proved to be a powerful technique, and when combined with conventional cytogenetic techniques, determines the cacao tree ploidy level. Research with dihaploids should be continued so that hybrid production of inbred lines can be made possible. Hybrids from lines with partial levels of inbreeding should be tested in comparison with nonconventional hybrids. However, special attention should be given to clonal propagation of nonconventional, superior hybrids as an alternative way to maintain the uniformity and high performance of these hybrids. With regard to tissue culture, micropropagation should be applied as a routine technique to hasten large-scale multiplication of superior hybrids and elite germplasm. The use of isozyme analysis should be intensified in cacao, especially in the genetic structure of natural populations and in germplasm characterization studies. RFLP and RAPD should be more intensively applied to cacao genetic mapping to make possible the early selection of material. All revised techniques should be integrated into cacao breeding programmes and carried out with representative samples.

# The Ostendorf archive: Cocoa since 1502

*W. Gerritsma*

The cocoa project of WAU and the Dutch cocoa industry recently acquired the *Ostendorf Archive*. Dr. F.W. Ostendorf was a breeder, who started work on cocoa around 1933 in the Dutch East Indies. He continued research on cocoa until the late 1960s. His archive is a substantial set of notes, memoranda and unpublished reports mainly on cocoa, but including some information on rubber, coffee and tea.

The most valuable part of this collection is the extensive cocoa bibliography, which covers the earliest publications on cocoa up until 1960. A crude estimate is that this collection concerns about 2500 meticulously annotated cards. Of about 400 references, he made very extensive extracts of a couple pages length. The cards and extracts are written in a very clear and punctual handwriting. It appears from the subjects covered by the extracts, that Ostendorf had a special interest for botanical aspects of cocoa, the explorers of the South American continent and their description of cocoa in the wild and the early history of cultivation. It is clear from all the notes that he had the intention to publish a book on the botany and agronomy of cocoa, which he never completed. He started writing this book during the camp years in Indonesia. Some chapters of this book have been typewritten already, whilst other chapters are drafted in steno. It appears that he continued this work until his death in the early 1970s.

The current status of the archive is not good. Time and the tropical environment have done their damage. Experts estimate that the paper will last only another 20-30 years before it is completely disintegrated. We are now seeking support and advice to preserve the archive and unlock the wealth of information for a larger audience. Electronic publishing on the Internet is one of our objectives. The inclusion of the bibliographic information along with our cocoa and chocolate bibliography will give a comprehensive overview of cocoa science and technology- a valuable resource base for cocoa scientists and the industry.

For further information contact:
**Wouter.Gerritsma@users.agro.wau.nl**
Wageningen Agricultural University
Dep. of Crop Science
Haarweg 333 fax: +31-317-484575
6709 RZ Wageningen voice: +31-317-483074
The Netherlands

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# Obituaries

*IN MEMORY OF THE LATE J.D. AMPONSAH*

Mr. John Darkwa Amponsah, affectionately called J.D. by colleagues and friends, joined the then West African Cocoa Research Institute, WACRI (now Cocoa Research Institute of Ghana), CRIG on the 20th of May, 1957 as a Technical Assistant. He was appointed Research Assistant in Plant Breeding by CRIG in1963 after completing a degree in Science at the University of Ghana in the same year. J.D. obtained the M.Sc. degree from The University of the West Indies, Trinidad in 1967, and returned to work at CRIG as a plant breeder. He was the second Ghanaian Plant Breeder to be employed at CRIG and was held in very high esteem. He made a substantial contribution to the development of quick screening procedures for the selection and breeding of Black Pod resistant cacao varieties in Ghana. He had several publications in this area and, in fact, was considered as an expert in the selection and breeding for fungal disease resistance in cacao, having earlier worked on Witches' Broom disease in Trinidad. He trained and supervised the work of a number of breeders in Ghana especially in his area of expertise.

For his experience in cocoa breeding, Mr. Amponsah was made Chairman of a special Co-ordinating Committee for cocoa seed gardens in Ghana and was subsequently seconded by CRIG to take up an appointment as Officer-In-Charge of the then newly created Seed Gardens Unit, from 1978-1985. He returned to CRIG to head the Plant Breeding Division and continued to make significant contributions to cocoa, sheanut (*Vitellaria Paradoxa*) and kola (*Cola nitida* & *Cola Acuminata*) breeding. However, Mr. Amponsah will be most remembered for his pioneering work on the development of improved kola planting materials for Ghana.

After his retirement from CRIG in 1994, he was engaged on contract for two years to continue work on kola in view of the great interest and importance of the results of his kola research. Even after the expiry of his 2-year contract, J.D. voluntarily continued to work at CRIG and thus unofficially became the first 'Research Officer Emeritus' at CRIG.

Mr. J.D. Amponsah died on 23rd June, 1998 after a short illness. By his death, Ghana and the cocoa world have lost a dedicated plant breeder.

May his soul rest in peace.

**G.K. Owusu & Y. Adu-Ampomah**,
Cocoa Research Institute of Ghana,
P.O. Box 8, New Tafo, Akim, Ghana

*IN MEMORY OF THE LATE J. Robert HUNTER*

We also note with regret the passing of Dr. J. Robert Hunter on January 3rd, 1997 at the age of 75. He was considered an expert on tropical ecology, and contributed significantly to the Milwaukee Public Museum's work with the Costa Rican Rain Forest. He lived in Costa Rica for 35 years.

Dr. Hunter introduced new crops into the rain forest, hoping to establish diversified agriculture that could thrive on the land with minimal impact. He planted rubber trees and introduced black pepper, vanilla and nutmeg. He was a business partner of a cocoa farm, and spent his latter years in Costa Rica managing the La Tirimbina and Las Vegas farms, south-east of San Jose, where spices, peach palm and cacao were raised.

Hunter may be best remembered by those involved with cocoa for his article, published in 1990, entitled "**The status of cocoa (*Theobroma cacao*, Sterculiaceae) in the Western Hemisphere"**, in *Economic Botany* **44** (4) 425-439.

(*Information extracted from the* ***Milwaukee Journal-Sentinel****, January 8th, 1997).*

**MICC 98** (The third **Malaysian International Cocoa Conference**) was held in Kuala Lumpur from November 26-27, 1998.

Most of the presentations were related to the post-harvest process. There were four oral presentations on cocoa breeding. The subjects covered were:

- Molecular markers linked to resistance to Vascular Streak Dieback;
- A genetic diversity study;
- Molecular finger-printing;
- Double-cross progeny evaluation.

In addition, there was one poster on cocoa breeding activities at Sabah.

For further information check the website:
micf98htm@www.koko.gov.my


INGENIC Newsletter Issue No. 3

Please note the following changes in:

O. Sounigo's paper in Table 2, page 14 in the row corresponding to the GU accessions, replace:

| | |
|---|---|
| 151 with 151/F | 286 with 286/P |
| 21g with 213/F | 300 with 300/P |
| 241 with 241/P | 305 with 305/P |
| 243 with 243/H | 307 with 307/P |
| 255 with 255/P | 310 with 310/P |
| 261 with 261/P | 322 with 322/P |
| 265 with 265/P | 114 with 114/P |
| 271 with 271/P | 351 with 351/P |

## FORTHCOMING EVENTS

The third INGENIC Workshop is scheduled to be held in Malaysia in 2000. It will coincide with the CPA International Cocoa Research Conference. The dates will be announced subsequently. The Proposed Topics for the Workshop are:

- Biotechnology
- Genotype x Environment interaction.

Suggestions on possible themes are invited.

## INGENIC Committee

| | |
|---|---|
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## Request for Updating of The INGENIC Newsletter Mailing List

Please assist us in updating our mailing list by filling out the form below to indicate your continued interest, and return to:

**Frances Bekele**, Editor - INGENIC
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**OR**

**Michelle End**, Secretary/Treasurer - INGENIC
Dept. of Horticulture, School of Plant Sciences
University of Reading
Whiteknights, P.O. Box 221
Reading RG6 2AS
UNITED KINGDOM

Name _____

Title _____

Research Interest _____

Institute _____

Postal Address _____

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e-mail address _____

# WORKSHOP ANNOUNCEMENT

## *Second and Final Announcement*
## *International Workshop on the Contribution of*
## *Disease Resistance to Cocoa Variety Improvement*

1. **Organizing Institutions**

   The International Group for the Genetic Improvement of Cocoa (INGENIC) and CEPLAC/CEPEC.

2. **Scope and Objectives**

   Worldwide cocoa production is affected by a number of diseases, several of which cause serious crop losses. The workshop objectives are to analyse the possibilities for increasing the profitability of cocoa cultivation through utilization of more resistant varieties, to identify the problems which have been encountered in this endeavour, and to find ways of overcoming them. Invitations to this meeting have been entended to both cocoa breeders and pathologists. The scope of the Workshop includes pathology research related to host-pathogen interactions, the nature and expression of resistance in the field as well as in laboratory tests, and the reliability of early screening tests. Breeding research to be discussed includes genetic variation for the resistance observed (germplasm collections, existing varieties, breeding trials), inheritance of resistance, stability of resistance in different environments, efficiency of early selection methods (for individual plant or progeny testing) and breeding strategies applied.

   Two external reviews have been commissioned by the INGENIC committee; one of which covers pathology aspects and the other breeding aspects. These external reviews should provide a general analysis of the progress obtained, the problems faced and a comparison with the progress obtained with similar type diseases in other crops.

3. **Time and Place**

   **Please note the Workshop dates have been changed to 25-26 November**. The Workshop has been rescheduled to take place after the 12th International Cocoa Research Conference (12th ICRC) due to the fact that there is a public holiday in Brazil on 15 November. The workshop will be held at the same hotel as the 12th International Cocoa Research Conference (17 to 23 November). In addition, a field trip for workshop participants is planned for Sunday 24 November.

4. **Programme**

   Based on the reactions received in response to the First Announcement, the organizing committee is endeavouring to organise the workshop in such a way as to allow for a comprehensive overview of cocoa disease resistance studies and breeding whilst allowing sufficient time for discussions. There will be presentations on economic aspects, general pathology and breeding aspects (externally commissioned reviews), and approximately fifteen presentations which will cover the research being carried out in America, Africa and Asia (country or institutional reviews).

5 **Language**

   Presentations will all be in English. Simultaneous translation into Portuguese/Spanish may be provided depending on demand. Questions in other languages will be translated.

6. **INGENIC General Meeting**

   INGENIC will hold its General Meeting on Monday 25 November. This will provide an opportunity to discuss INGENIC's current and planned activities, discuss the proposed regulations for INGENIC and, possibly, to elect new committee members. Anyone interested is welcome to attend.

7. **Accommodation**

   Participants who are also attending the 12th International Cocoa Research Conference will be able to reserve their accomodation, at the same Conference rates, on the Conference accomodation booking form. If you require any further assistance in making your accomodation arrangements please contact the INGENIC secretariat at the address below.

8. **Registration**

   The registration fee of US$150/UK£100 (US$100/UK£70 student rate) includes participation at the Workshop, coffee/cocoa breaks and lunches on both days and a copy of the Proceedings. Registration forms and fees must be received before 31 August, 1996.

9. **Travel**

   Participants are advised to book their tickets with Ilheus as their final destination since internal travel within Brazil is expensive.

10. **Supporting Organizations**

    INGENIC would like to thank the organizing committee of the 12th International Cocoa Research Conference and the following organizations for their contributions towards this Workshop: ACRI, Almirante Centro de Estudos de Cacau, BCCCA, CTA, Dutch Ministry of Foreign Affairs (DGIS) and FAO.

# REGISTRATION FORM

## INGENIC Workshop on the Contribution of Disease Resistance in Cocoa Variety Improvement

**Personal Details**

Name _____

Position _____

Institution _____

Business Address _____

_____

_____

Tel. _____

Fax _____

E-mail _____

**Registration Fee** Standard US$150 Sterling£?? or Student US$100 Sterling£??

☐ ☐

**Language** ☐ I am interested in simultaneous translation into Portuguese/Spanish

**Hotel reservations** ☐ Please make a reservation at the Cana Brava Hotel and receive my additional payment for one daily rate

Single US$100 / Double US$120 No. and type of room(s): _____

Arrive on _____ Depart on _____

☐ I will make my own reservations in another hotel (please indicate which hotel):

_____

Please note reservations can only be guaranteed upon receipt of one daily arte.

Cheques for registration and hotel reservations should be made payable to INGENIC and sent to:

Dr. Michelle End,
INGENIC Secretariat,
c/o School of Plant Sciences,
The University of Reading,
Whiteknights, PO Box 221,
Reading, RG6 6AS, U.K.

Registration forms, hotel reservations and cheques must be received before 31st August 1996.